Recent infection by *Wolbachia* alters microbial communities in wild *Laodelphax striatellus* populations

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**Abstract**  
**Background:** Host-associated microbial communities play an important role in the fitness of insect hosts. However, the factors shaping microbial communities in wild populations, including genetic background, ecological factors, and interactions among microbial species, remain largely unknown.  
**Results:** Here, we surveyed microbial communities of the small brown planthopper (SBPH, *Laodelphax striatellus*) across 17 geographical populations in China and Japan by using 16S rRNA amplicon sequencing. Using structural equation models (SEM) and Mantel analyses, we show that variation in microbial community structure is likely associated with longitude, annual mean precipitation (Bio12), and mitochondrial DNA variation. However, a *Wolbachia* infection, which is spreading to northern populations of SBPH, seems to have a relatively greater role than abiotic factors in shaping microbial community structure, leading to sharp decreases in bacterial taxon diversity and abundance in host-associated microbial communities. Comparative RNA-Seq analyses between *Wolbachia*-infected and -uninfected strains indicate that the *Wolbachia* do not seem to alter the immune reaction of SBPH, although *Wolbachia* affected expression of metabolism genes.  
**Conclusion:** Together, our results identify potential factors and interactions among different microbial species in the microbial communities of SBPH, which can have effects on insect physiology, ecology, and evolution.  
**Keywords:** *Laodelphax striatellus*, Microbial community, *Wolbachia*, Endosymbiont, Microbial interactions

**Background**  
The fitness of insects can be affected by their interactions with associated microbiomes [1–3]. Fitness traits affected by host microbiomes include development [4], fecundity [5], resistance to natural enemies [6], climate adaptation [7], and synthesis of essential amino acids [8, 9]. In addition, disturbing an insect’s bacterial population can change host fitness [10], such as producing enhanced sensitivity to bacterial pathogens in bees [11] and altering fecundity in mosquitos [12, 13].  
The microbial communities of hosts are influenced by diverse factors that include diet [14], pH [15], host [16], life stage [17], temperature and humidity [18], and genetic background [19]. Evidence for effects of genetic background on microbial communities is mostly based on correlations between microbial structure and phylogenetic relationships at the macro-evolutionary level [20, 21], although such correlations might reflect factors like geographic isolation that drive speciation rather than genetic backgrounds per se. Apart from external factors, changes in microbial communities can also be driven by interactions between different microbial...
species [22]. For example, Wolbachia has been shown to compete against pathogens in Drosophila [23] and Aedes [24, 25]. Similarly, Spiroplasma reduces the density of Wolbachia in Drosophila [26] and Asaia impedes the vertical transmission of Wolbachia in Anopheles stephensi mosquitoes [27]. Mechanisms involved in these microbial interactions are often not clear.

To understand factors influencing the microbial distribution within hosts, investigations are needed at the population level when there are likely to be fewer confounding effects than in interspecific comparisons across hosts. Here, we undertake such an investigation on the small brown planthopper (SBPH, Laodelphax striatellus), a notorious agricultural pest that damages rice plants by sucking rice sap and spreading rice stripe virus (RSV) [28]. The SBPH has a strong migratory ability but also shows population genetic differentiation [29, 30], providing a suitable model for studying the impact of genetic background on microbiomes. Previous studies of the microbiota of SBPH have relied on laboratory samples [31–33]. However, stable laboratory rearing conditions are likely to alter the original microbial community structure which might be shaped by their original environmental conditions, with a homogenizing effect on the microbial community [34–36]. Moreover, genetic drift can occur, affecting the genetic background of both the host and the microbial community during rearing, generating potential differences between the microbial communities observed in the lab and the field. Given these concerns, our current study focuses on natural populations. We combine 16S rRNA amplicon sequencing with a transcriptome analysis to test factors shaping the microbial community in their host at the population level, and we explore the nature of the interactions between different microbial species.

**Methods**

**Sample collection**

SBPH individuals were collected from rice plants at 17 locations in China and Japan during the summers (May to September) of 2010–2018 (Fig. 1, left panel; Additional file 1: Table S1). We haphazardly collected about 60–100 adult female individuals at each location. To avoid sampling siblings, we collected only one SBPH per host plant and selected host plants that were at least 1 m apart. All samples were preserved in 100% ethanol and stored at –20 °C until DNA extraction.

**16S rRNA amplicon sequencing**

For each of the 17 locations, three female adults were pooled to provide a biological replicate and three biological replicates were established per location. Total genomic DNA was extracted with a DNeasy blood and tissue kit (QiaGen, Hilden, Germany) according to the manufacturer’s protocols. A two-step PCR approach recommended by Illumina [37] was performed to generate amplicon libraries. Briefly, the PCR amplification of the bacterial 16S rRNA genes involved universal primer sets 338F (5’-ACTCCTACGGGAGGCAGCAG-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’). The PCR products were purified on a 2% agarose gel, and extracted with an AxyPrep DNA Gel Extraction Kit (Axyprep, Nanjing, China). Final PCR products were purified with Hieff NGS DNA selection Beads (YEASEN, Shanghai, China), and equalized and normalized using the dsDNA HS assay kit for Qubit (YEASEN, Shanghai, China). Samples were quantified and pooled in equimolar ratio using a Qubit 4 Fluorometer (Invitrogen, Waltham, MA, USA) and then were submitted to Majorbio Bio-Pharm Technology Company Limited (Shanghai, China) for high-throughput sequencing on an Illumina MiSeq PE300.

After sequencing, raw fastq files were demultiplexed, quality-filtered by Trimmomatic, and merged by FLASH [38] (http://www.cbcb.umd.edu/software/flash/). OTUs were clustered with 97% similarity cutoff using UPARSE [39] (version 7.1, http://drive5.com/uparse/) and sequences were then phylogenetically assigned to taxonomic classifications using an RDP classifier [40] (http://rDP.cme.msu.edu/). To normalize sequencing depth, the samples were rarefied to 34135 sequences (the lowest coverage sample) to ensure a random subset of OTUs for all samples.

**Mitochondrial COI gene PCR**

In SBPH, no significant differentiation among populations exists for nuclear genes but mitochondrial genes that are passed down from mother are differentiated [29]. To determine the degree of genetic differentiation, 20 to 46 female adults were haphazardly selected from each population (Fig. 1, left panel) for mitochondrial COI gene amplifications and sequencing according to Sun et al. [29]. The PCR products were sent to Tsingke Biological Technology Company (China) for sequencing.

**Diagnostic PCR**

To measure infection frequencies of Wolbachia, an additional eight to 46 female adults were haphazardly selected from each population. The specific primers [41] are listed in Additional file 1: Table S2. DNA extraction and PCR were done as described above. Positive controls (known sample with Wolbachia) and blank controls were also run. PCR products of 599 bp size were run on
1.0% agarose gels stained with ethidium bromide at 150 volts and visualized by GenoSens 1860 (Clinx, Shanghai, China). The number of samples showing bright DNA bands compared with the DL 2000 DNA mark (Tsingke, China) was used to calculate the infection rate.

Transcriptome analyses
To investigate the effects of Wolbachia infection on the SBPH transcriptome, we compared Wolbachia-free and Wolbachia-infected SBPH strains. The uninfected strain was obtained by treating the infected strain with tetracycline for 10 generations according to the method of Guo et al. [42]. Briefly, approximately 30 abdomens of SBPH as a biological replicate were dissected from 3-day-adults of both Wolbachia-infected and Wolbachia-free females. The female abdomens contain a large quantity of fat body and blood cells which are the basis of innate immunity. Total RNA was extracted from three biological replicates using TRIzol Reagent (Invitrogen, CA, USA) according to the manufacturer’s instructions. RNA purity was measured with a NanoPhotometer® spectrophotometer (IMPLEN, CA, USA). RNA concentration was measured with a Qubit® RNA Assay Kit in a Qubit® 2.0 Fluorometer (Life Technologies, CA, USA). Finally, RNA was pooled for Illumina MiSeq sequencing (BGI, Wuhan, China) according to a standard protocol [43].

The sequencing generated 6.6 Gb per biological replicate. Clean reads were obtained by removing reads with adaptors, poly-N, and having a low quality. Gene expression levels were estimated by RSEM software package [44] (http://deweylab.biostat.wisc.edu/rsem). Immune-related genes of SBPH were obtained from Zhu et al. [45], which were generated by alignments with immune genes of D. melanogaster, A. gambiae, Aedes aegypti, and Culex quinquefasciatus by using BLASTX [46]. In addition, sequences were annotated to the KO database with the KEGG Automatic Annotation Server.
Statistical analyses
Bray–Curtis dissimilarity metrics among all samples were constructed using beta_diversity.py in QIIME [47] (http://qiime.sourceforge.net/) and were visualized with a principal coordinate analysis (PCoA). The difference of microbial communities among the populations was calculated by ADONIS. The population genetic differentiation value ($F_{ST}$) was calculated in Arlequin 3.5 [48]. The annual mean temperatures (Bio1) and the annual mean precipitation (Bio12) of the 17 locations were obtained from DIVA-GIS 7.5.0 [49] (https://www.diva-gis.org), which is a geographic information system for the analysis of species distribution data. A structural equation model (SEM) [50] was used to estimate the relative contributions of $F_{ST}$, Bio1, Bio12, latitude, and longitude (Additional file 1: Table S3; Table S4; Table S1) on microbial community structure with communities based on Bray–Curtis dissimilarity metrics. The SEM tests were performed in the R “SEM” package (https://cran.r-project.org/web/packages/se�/index.html), and the path diagram for the SEM tests is shown in Fig. 4. As non-normal distribution of variables may compromise SEM analyses results, we also undertook Mantel tests using the Spearman method with 1000 permutations to determine the associations between microbial community structure variation and the five aforementioned factors.

The relative abundance of a given phylogenetic group was estimated by examining the number of reads of that group for each population. In order to analyze the evenness and richness of the microbial community, we calculated several α diversity indexes including the Sods, Shannon, Simpson, Ace, Chao, and Coverage indexes. Spearman's rank correlations were calculated between the proportion of Wolbachia and the α diversity indexes (Shannon indexes and Simpson indexes) of the populations. The significance of differences in read proportions of bacterial 16S rRNA genes at the genus level was assessed by Mann–Whitney U tests. The significance of differences in α diversity indexes between Wolbachia-infected and -uninfected populations was calculated by a t test. All statistical analyses were carried out in R 3.5.2 [51].

Probabilistic features recognition for the OTU distribution
Components of collective ecological and biological systems presented an obvious probabilistic similarity in their aggregation, in which only several species made up a relatively high share of the whole sample, while most species accounted for much less. By looking into our datasets, we noted that the abundance data of OTUs explicitly met this property. Therefore, the power-law function that satisfied the mathematical characterization of such distribution behavior was considered as an appropriate function to recognize the probability distribution features of OTUs. Given the type of power-law function, the abundance had the probability density function (pdf):

$$p(x) = ax^{-\varepsilon}, x > x'$$

where $x'$ was the threshold that ensured a robust fitting for the power-law distribution. We probabilistically characterized the distribution of abundance of OTUs by calculating the exceedance probability distribution function [52] that was given by:

$$P(X > x) = x^{1-\varepsilon} f \left( \frac{x}{x'} \right)$$

where $\varepsilon$ was the scale exponent of power-law distribution underlying the statistical patterns of data considered. This scale factor implied the property of mean and variance of data: when $\varepsilon \leq 2$, the mean and variance were both infinite; when $2 < \varepsilon < 3$, the mean existed, while the variance was still infinite; and when $\varepsilon \geq 3$, both mean and variance existed. Additionally, $f \left( \frac{x}{x'} \right)$ was introduced to give a general formulation for the homogeneity function. The probabilistic features for the OTU distribution for each population were given in Fig. 2.

To assess the microbial community variations between populations in terms of probabilistic distributions of OTUs, we calculated the Kullback–Leibler divergence (KL divergence) by using the R package “LaplacesDemon” (https://cran.r-project.org/web/packages/LaplacesDemon/index.html). Probability density functions of OTUs used as the arguments for KL divergence calculation function were computed by using the R package “histogram” (https://cran.r-project.org/web/packages/histogram/index.html). The KL divergence was used as a surrogate index of microbial community structure and was also used for SEM and Mantel tests to analyze the relationships between microbial community structure and five putative predictor variables as mentioned above.

Results
Microbial diversity and environmental factors in the absence of Wolbachia
Based on the infection frequencies of Wolbachia, only the SAP population was found to have Wolbachia-uninfected individuals. And a notable difference in microbial community structure was found between SAP and the remaining populations as showed by the probabilistic features of the OTU distribution (Fig. 2). To eliminate the potential influence of Wolbachia on pooled samples, the SAP population was excluded for testing the impact of other factors on the microbial community. Among the 48 samples from the remaining 16 SBPH populations, the RDP classifier identified a total of 314 OTUs (Additional file 2: Table S5). Wolbachia were the most abundant bacteria, accounting for 87.9% of the 16S
Fig. 2 Exceedance probability distribution function of OTU abundance for each population. A power-law function is used as the model to estimate the pdf of abundances. Population codes are given in Additional file 1: Table S1.
The abundance and distance of microbial communities of SBPH across 16 populations. **a** Relative abundance of bacterial 16S rRNA genes at the genus level. Dashed line separates the Chinese and Japanese microbial community abundance. Blocks of populations were arranged by origin sites (south to north). Other genera ("others") account for <5% of the classified sequences. **b** Principal coordinate analysis (PCoA) of SBPH samples collected from different locations. PCoA was generated by the Bray–Curtis dissimilarity method.
showing a relatively higher Wolbachia incidence in China [53], the frequency of Wolbachia infection observed in the present study was higher than it was in the previous studies, especially in Japan [54]. This showed that Wolbachia has increased in recent decades.

The correlations between the α diversity indexes (Shannon and Simpson indexes) [55, 56] and the proportion of Wolbachia in all samples were examined by Spearman’s rank correlation analyses (Fig. 5a, b). The results revealed that the proportions of Wolbachia were significantly correlated with the Shannon \((r = -0.940, P < 0.001)\) and Simpson \((r = 0.979, P < 0.001)\) indexes, suggesting that the presence of Wolbachia in SPBH decreased the richness and evenness of microbial communities.

**Wolbachia infection and the relative abundance of bacterial taxa in SPBH**

To further test the impact of Wolbachia infection on the microbial communities, 10 female adults infected with Wolbachia and 9 female adults uninfected with Wolbachia, both from the SAP population, were used to compare the microbial communities by 16S rRNA ampli-con sequencing. After the samples were rarefied to 39,872 sequences (the lowest coverage sample), 1985 OTUs were obtained between the two groups (Additional file 2: Table S8). Wolbachia predominated in the microbial communities of Wolbachia-infected females (Fig. 6a). The relative abundances of 154 genera in the Wolbachia-infected adults were significantly reduced (Additional file 2: Table S9). PCoA analysis based on Bray–Curtis dissimilarity (Fig. 6b) clearly separated the Wolbachia-infected individuals from the Wolbachia-uninfected individuals, indicating that the microbial community structures of the two groups were significantly different. Compared to the Wolbachia-infected group, the Wolbachia-free group possessed high microbial diversity as suggested by the Sobs, Shannon, Simpson, Chao, and Ace indexes (Table 2: Welch two-sample t test: \(p = 0.003\) for Sobs, \(p < 0.001\) for Shannon, \(p < 0.001\) for Simpson, \(p = 0.001\) for Chao, \(p = 0.002\) for Ace). Furthermore, Mann–Whitney U tests.
revealed that the abundances of seven genera that dominated the communities found in the Wolbachia-free adults were very low in the Wolbachia-infected adults (Fig. 7). These results provided further evidence that Wolbachia decreased the relative abundance and diversity in the microbial community of SBPH.

Changes in microbial communities by Wolbachia infection
To detect the effect of Wolbachia infection on the structure of the microbial community, we compared microbial taxon abundance between the Wolbachia-infected and Wolbachia-free individuals sampled from the SAP population. To normalize sequencing depth, we haphazardly extracted 1144 reads for each sample (based on the minimum number of reads after removing Wolbachia reads in the Wolbachia-infected samples, Additional file 1: Table S10) for these analyses. Our results showed that the structures of the microbial communities were different between Wolbachia-infected females (after excluding Wolbachia reads) and Wolbachia-uninfected females (Fig. 8a; Additional file 1: Table S11). Both the Shannon and Simpson indexes indicated that the Wolbachia-free group possessed higher microbial diversity than Wolbachia-infected group (excluding Wolbachia reads) (Additional file 1: Table S11).
Table 2: Measures of species richness and evenness of SBPH from 10 Wolbachia-infected females and 9 Wolbachia-free females from the SAP population.

| Samples | Sobs | Shannon | Simpson | Ace | Chao | Coverage |
|---------|------|---------|---------|-----|------|----------|
| w+1     | 693  | 1.594   | 0.532   | 816.372 | 766.533 | 0.996    |
| w+2     | 356  | 0.707   | 0.832   | 434.097 | 409.182 | 0.998    |
| w+3     | 76   | 0.378   | 0.829   | 206.037 | 126.647 | 0.999    |
| w+4     | 161  | 0.210   | 0.949   | 234.636 | 206.217 | 0.998    |
| w+5     | 139  | 0.406   | 0.850   | 188.332 | 178.200 | 0.999    |
| w+6     | 132  | 0.206   | 0.943   | 314.430 | 235.542 | 0.998    |
| w+7     | 107  | 0.561   | 0.771   | 199.706 | 152.000 | 0.999    |
| w+8     | 95   | 0.475   | 0.787   | 209.990 | 159.688 | 0.999    |
| w+9     | 96   | 0.337   | 0.877   | 148.918 | 133.625 | 0.999    |
| w+10    | 93   | 0.965   | 0.466   | 149.309 | 143.167 | 0.999    |
| w−1     | 247  | 1.232   | 0.459   | 342.134 | 312.632 | 0.998    |
| w−2     | 402  | 1.490   | 0.412   | 431.463 | 443.143 | 0.998    |
| w−3     | 399  | 3.987   | 0.040   | 461.191 | 457.400 | 0.998    |
| w−4     | 636  | 5.063   | 0.013   | 689.927 | 684.838 | 0.998    |
| w−5     | 528  | 3.990   | 0.052   | 628.688 | 643.000 | 0.997    |
| w−6     | 516  | 3.962   | 0.052   | 630.807 | 621.726 | 0.997    |
| w−7     | 640  | 4.590   | 0.027   | 709.431 | 722.787 | 0.997    |
| w−8     | 534  | 3.915   | 0.052   | 632.849 | 651.018 | 0.997    |
| w−9     | 497  | 3.817   | 0.065   | 599.893 | 629.255 | 0.997    |

Table S12; Welch two-sample t-test: *p < 0.035 for Shannon, p = 0.020 for Simpson. PCoA analysis based on Bray–Curtis dissimilarity (Fig. 8b) also clearly separated the two groups, except for two samples of the Wolbachia-uninfected females. Two samples contained very few Wolbachia reads (accounting for 0.04% of their microbial communities), which might lead to a distorted pattern. However, it appears that even Wolbachia infections at low titers can significantly change the microbial community.

In addition to decreasing bacterial diversity, we also found that the Wolbachia infection changed bacterial taxon abundance, with 25 genera significantly increasing and 65 significantly decreasing in Wolbachia-infected individuals (Fig. 9; Additional file 2: Table S13; Table S14; Table S15). Most of these bacteria have widespread distributions in insect tissues, including the gut, ovary, and head. Notably, four genera occurring in high proportions (with log (read percent) > 1) in both Wolbachia-infected and Wolbachia-free groups were also significantly different, with Thermus, Spiroplasma, and Ralstonia enriched in the Wolbachia-infected group, in contrast to Prevotella_9 which was enriched in the Wolbachia-uninfected group (Fig. 9). Apart from these changes, Wolbachia infection seems associated with the existence of particular bacterial taxa, with 160 genera specifically existing in relative low abundance in the Wolbachia-infected group (Additional file 2: Table S13).

Wolbachia does not appear to strongly affect immune-related genes of SBPH but affects metabolism genes

To test if Wolbachia promotes the expression of immune-related genes in SBPH, we compared the transcriptomes of pooled abdomens from Wolbachia-infected and Wolbachia-free females. Of 330 immune-related genes in SBPH identified by Zhu et al. [45], 306 genes representing 25 gene families were identified (Additional file 2: Table S16). Most of these genes were not differentially expressed (Fig. 10; Additional file 2: Table S16), which suggests that Wolbachia had little or no impact on the immune systems of SBPH. However,
through an analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) terms, we found 141 differentially expressed genes in metabolism processes including oxidative phosphorylation-related and glycolysis-related genes (Fig. 10; Additional file 2: Table S17), which suggests that the effect of Wolbachia on microbial community is likely mediated through changing the overall metabolism and physiology of SBPH.

Discussion
Effects of environmental factors and genetic background on the microbial community of SBPH
Our analyses suggest that, based on Bray–Curtis dissimilarity, longitude and precipitation may impact microbial communities, and these effects appear separate because precipitation did not associate with longitude. To date, any effects of precipitation on insect microbiome have rarely been considered. Our previous study in spider mites found that precipitation can influence the incidence of Spiroplasma [57], a facultative endosymbiont which can manipulate host production. As the SBPH is polyphagous, any effects of longitude and precipitation may reflect effects of these variables on vegetation and food resources for SBPH, which could alter the physiology and metabolism of SBPH hosts and in turn influence microbial communities. SBPH might acquire some bacteria directly from plant sap, as has been suggested for the cochineal insect Dactylopius [58], and different bacteria present in different environments could contribute to variation in microbial communities. For example, Pantoea was abundant in the MDJ population of SBPH (Fig. 3a) and is thought to have been acquired from the environment in Ae. albopictus [59]. It is also possible that microbial communities are responding directly to environmental factors rather than being acquired from the

environment, and they might even provide a fitness advantage to hosts under certain conditions, although this remains speculative in the absence of experimental data. Future studies should also consider the impacts of variability in climatic variables on microbial communities, whereas we have only considered the average estimates available to us from the tested locations.

Our results based on both Bray–Curtis dissimilarity and KL divergence suggested an association between mtDNA variations and microbial community structure. Previous studies at the macro-evolutionary level have suggested associations between mtDNA variation and microbial communities, but these might reflect geographic isolation that drive speciation rather than genetic backgrounds per se [20, 21], whereas our findings from the population level with shallow divergence in the mitochondrial genome [30] provide relatively more direct evidence of an association. Some bacterial groups that are maternally transmitted and living inside cells (like Wolbachia) might be expected to be associated with mtDNA variants which can hitchhike along with spreading endosymbionts [60]. A more recent study in mice found that different mitochondrial genotypes can alter ROS productions, which modulates microbial structure in the host gut [61]. In SBPH, two mitochondrial haplogroups thought to be associated with altered functions exist in natural populations [30], and their impacts on microbial communities could be explored in future work.

The effects of Wolbachia on the microbial community of SBPH
Maternally inherited Wolbachia endosymbionts are common in insects. They can manipulate host reproduction, facilitating Wolbachia’s rapid spread in a host population.
In SBPH, *Wolbachia* can induce strong cytoplasmic incompatibility (CI), resulting in no offspring when uninfected females mate with infected males [62]. Comparison of the microbial communities of *Wolbachia*-free and *Wolbachia*-infected SBPH individuals clearly shows that *Wolbachia* infection severely decreases the diversity and abundance of bacteria in SBPH. The abundance of the seven other main genera in *Wolbachia*-infected adults was very low (Fig. 7). A similar phenomenon has been observed in *Aedes aegypti*, in which a large proportion of bacterial taxa disappeared when *Wolbachia* was induced by artificial injection [63]. Bacterial diversity was also found to be very low in the gut of *Drosophila melanogaster*, which is naturally infected with *Wolbachia* [64].

Significant differences in microbial communities were observed between the Chinese and Japanese populations of SBPH (Fig. 3b). The present results, together with previous studies, suggest that *Wolbachia* has rapidly spread in SBPH populations during recent decades in both China and Japan. The incidence of *Wolbachia* has increased from around 90% in Chinese populations [53] to 100% [29], and from around 65% in Japanese populations [54] to more than 90%. The strong CI of *Wolbachia* and the high migratory ability of SBPH likely contribute to this rapid spread. The spread of *Wolbachia* seems to have pushed the infection to fixation in the Chinese populations, while the invasion is still ongoing in the Japanese populations. In Japan, spread is most noticeable in high latitude

**Fig. 9** Common logarithm values of the read percentage of bacterial 16S rRNA genes for each genus across the microbial communities between *Wolbachia*-infected females (excluding *Wolbachia* reads) and *Wolbachia*-uninfected females and comparisons by Mann–Whitney U tests. Significant differences in the genera existing in *Wolbachia*-infected females (excluding *Wolbachia* reads) and *Wolbachia*-uninfected females are indicated by different colors. Proportions where genera in *Wolbachia*-infected females (excluding *Wolbachia* reads)/*Wolbachia*-uninfected females = 1 is shown as a dotted line.
regions where Wolbachia was previously rare. The difference in histories of Wolbachia between China and Japan may be contributing to divergence in their SBPH microbial communities, but this remains to be tested directly, such as through comparisons of the communities when hosts are reared in a common environment.

By removing Wolbachia reads from the Wolbachia-infected females in SAP populations, we further analyzed the effect of Wolbachia on the other bacteria and found that Wolbachia infection changed microbial evenness and other measures of microbial diversity. Three bacteria (Thermus, Spiroplasma, Ralstonia, Fig. 9; Additional file 2: Table S14) were highly enriched in the Wolbachia-infected samples. Vitamin B can be synthesized by Thermus [65], as well as by Wolbachia where it can lead to an increase in host fertility [66]. Thermus associated with Wolbachia may provide an intermediate for the synthesis of vitamin B. In Drosophila neotestacea, Wolbachia can promote the abundance of Spiroplasma [67], pointing to the possibility of direct interactions among microbes. On the other hand, the effect of Wolbachia on Spiroplasma may lead to different tissue tropisms [26] and asymmetrical interactions between the two bacteria where Spiroplasma negatively affects the population of Wolbachia, but Wolbachia does not influence the population of Spiroplasma [26]. In SBPH, Spiroplasma was found to induce late male killing [68] which is predicted to have advantages not only in facilitating maternal transmission, but also in promoting horizontal transmission of Spiroplasma. This is based on the notion that dead males could burst and release Spiroplasma spores into the environment [69]. Whether the bursting of dead males also promotes the spread of other microbes such as Wolbachia in nature is unclear. If so, it could partly contribute to the rapid spread of CI inducing Wolbachia in SBPH populations without decreasing mitochondrial DNA diversity [29]. Ralstonia is a devastating soil-borne plant pathogen and affects growth and development of 200 host species belonging to more than 50 botanical families [70]. For SBPH, we speculated that Ralstonia may have been obtained from food resources, but the function of Ralstonia in insect hosts is unknown. We also note that many bacteria were reduced by Wolbachia infections (Additional file 2: Table S15), and most of them were located in the gut, ovary, and head
where *Wolbachia* exist [33]. *Wolbachia* may interact competitively with many components of the microbial community of SBPH but this remains to be investigated.

The main mechanisms by which *Wolbachia* are thought to decrease the microbial diversity are immune system modulation and resource competition [63]. Other mechanisms may include *Wolbachia*-induced changes in ROS, transcription/posttranscription, and pH [64]. Because no significant difference in the expressions of immunity-related genes was detected in the transcriptomes of *Wolbachia*-infected and *Wolbachia*-free female adults, it appears that immune modulation is not involved in SBPH. The only effect detected in this study was a decrease in the expression of the gene encoding defensin in the *Wolbachia*-infected females (Additional file 2: Table S16), the opposite of what might be expected. Through KEGG analysis, we showed 141 differentially expression genes involving metabolic processes including oxidative phosphorylation and glycolysis (Additional file 2: Table S17), which may suggest that intracellular localized somatic *Wolbachia* affect the overall metabolism and physiology of the insect to suppress the diversity/abundance of bacterial populations.

*Wolbachia* infection-associated immune regulation has been reported in organisms in which *Wolbachia* was artificially introduced [24, 25, 71], but not in organisms that are naturally infected with *Wolbachia* [23, 72]. It seems that immune regulation mediated by *Wolbachia* is lost with a long history of *Wolbachia* colonization. The initial colonization of *Wolbachia* may trigger an immune response in the host, which then changes after long-term co-evolution between *Wolbachia* and its host. If that is the case, managing insect pests by releasing insects artificially infected with *Wolbachia* should be undertaken with caution because the “pathogen blocking” efficiency of insect vectors may eventually be lost in nature as *Wolbachia* and its host co-evolve. We agree with Simhadri et al. [64] who argue that future studies of *Wolbachia*-associated phenotypes should consider the effects of *Wolbachia* on the microbial community.

Conclusions

In this study, by profiling 16S rRNA genes using next-generation sequencing, we explored the relative contributions of genetic background, ecological factors, and interactions among microbial species on the microbial communities of natural populations of SBPH. Our results suggest that *Wolbachia* infection has a stronger role in shaping the microbial community than ecological factors and genetic (mtDNA) background. When *Wolbachia* is introduced into the community, it seems to become the dominant species and decreases microbial diversity. Comparative RNA-Seq analyses between *Wolbachia*-infected and -uninfected strains indicate that the *Wolbachia* do not seem to alter the immune reaction of SBPH, although *Wolbachia* affected expression of metabolism genes, suggesting *Wolbachia* affect the overall metabolism and physiology of the insect to suppress the diversity/abundance of bacterial populations.

Supplementary information

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**Additional file 1:** Table S1 Summary of collection details. The population code (ID), province, city, county, latitude, longitude, and date of the field collections assessed here are provided. Table S2 Specific primers used in PCR for this study. Table S3 Pairwise Fst estimates between populations based on a sequence of the mitochondrial CoI gene. Population codes are given in Table S1. Table S4 Annual mean temperatures (Bio1) and the annual mean precipitation (Bio12) of the 17 locations obtained from DIVA-GIS 7.5.0. Table S5 Relative abundance of bacterial 16S rRNA genes at the genus level observed for Chinese, Japanese, and all populations. Table S6 Effects of factors in the structural equation model (SEM) analysis undertaken on 16 populations where *Wolbachia* was fixed in the population. Table S7 Relative abundance of bacterial 16S rRNA genes at the genus level observed for *Wolbachia*-infected females (after removal of *Wolbachia* reads). Table S8 Abundance of OTUs among the 48 samples. Table S9 Read proportions of bacterial 16S rRNA genes among *Wolbachia*-infected females and *Wolbachia*-free females at the genus level by Mann-Whitney U tests. Table S10 Differences of bacterial 16S rRNA genes for *Wolbachia*-infected females (excluding *Wolbachia* reads) at the genus level by Mann-Whitney U tests. Table S11 Significantly increased read proportions of bacterial 16S rRNA genes and the tissue in which their corresponding bacteria were distributed for *Wolbachia*-infected females (excluding *Wolbachia* reads) at the genus level by Mann-Whitney U tests. Table S12 Significantly reduced read proportions of bacterial 16S rRNA genes and the tissue in which their corresponding bacteria were distributed for *Wolbachia*-infected females (excluding *Wolbachia* reads) at the genus level by Mann-Whitney U tests. Table S13 Expression differences of immune genes between *Wolbachia*-infected and *Wolbachia*-cured female adults expressed in abdomens. Table S14 Expression differences of metabolism genes between *Wolbachia*-infected and *Wolbachia*-cured female adults expressed in abdomens.

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Authors’ contributions

JTS, XHY, and XZD designed the research. JTS, XZD, and MK collected samples. XZD, LTW, and YG performed the research. XZD and XHS conducted the data analyses. YXZ, AAH, and XLB advised on interpretations. XZD, JTS, AAH, and XHY wrote the paper. The authors read and approved the final manuscript.
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Availability of data and materials
The raw reads of 16S rRNA sequencing have been deposited in the NCBI Sequence Read Archive (SRA) database (accession number SRP195568). The raw reads of transcriptomes have been deposited in the NCBI SRA database (accession number SRP195568).

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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