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Regional and Management-driven Variation in Apple Microbiome and Resistome

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Abstract

Background

Antimicrobial resistance (AMR) in plant-associated microbiomes is increasingly recognized as One-Health concern. Apples are widely consumed, yet little is known about how agricultural practices and geography shape its microbiome and resistome. Using shotgun metagenomics, we compared the bacterial, plasmid, and phage communities, and antibiotic resistance genes (ARGs) in apples from organic and conventional orchards in Israel and the USA.

Results

Microbiome composition analysis revealed that geographical regions explained the largest proportion of variation in microbial composition, whereas management had a smaller effect but statistically significant across the domains ($p \leq 0.003$). Pairwise PERMANOVA analyses further showed that most regional contrasts explained substantial variation, however, when USA orchards were pooled, the contrasts were statistically significant ($p = 0.003–0.006$). In contrast, within-region management showed a sizable effect ($R^2 = 0.34–0.56$), however the differences were not statistically significant. Bacterial diversity was significantly higher in organic orchards compared to conventional ones. Phage diversity showed no significant differences, while plasmid diversity was consistently higher in conventional compared to organic orchards, with multiple significant differences. Five genera were identified as the main carriers of ARGs, with *Pseudomonas* being the predominant contributor to ARG transmission, accounting for 14–32% of the microbes. ARG diversity varied across the management, the majority of ARGs encoded for multidrug resistance. Smaller but consistent resistance were observed in Macrolide-Lincosamide-Streptogramin (MLS), aminoglycoside, β-lactam and glycopeptide. Efflux pumps, antibiotic inactivation and target alteration were the most dominant resistance mechanisms. Additionally, conventional presented a higher burden of mobile ARGs compared to organic orchards.

Conclusion

Our findings reveal that apples carry unique regional microbial signatures and management practices contribute to the context-dependent variation in the resistome composition. Overall, our study highlights an integrated ecological framework linking microbiome, resistome, and horizontal gene transfer events, providing critical insights into AMR management in plant associated microbes.

Background

The increasing occurrence and prevalence of antibiotic resistance (AR) in agricultural settings has raised concerns about its potential impact on public health, environmental sustainability and food safety (1–3). Antibiotic resistant strains and their associated ARGs are widely distributed in the natural environment,

including water, soil, (wild) animals, and plant microbiomes, where they significantly shape the environmental resistome (4, 5). Although clinical and livestock production settings are often linked to AMR, growing evidence points to anthropogenic activities including agricultural practices, playing a key role in the selection and spread of ARGs (2, 6, 7). In this context, the plant-gut microbiome axis may be particularly significant to human health (8, 9), as raw, fresh produce is increasingly emerging as an important reservoir and silent vector for AMR transmission. Apple fruits for example are a potential source of AMR. However, little is known on how agricultural management practices in apple farming influence fruit microbiome as well as the associated resistome, particularly in relation to different geographical regions.

Organic and conventional farming systems vary substantially in their use of antibiotics, pesticides, manure, and other synthetic inputs, all of which can influence the crop resistomes. Mechanistic insights into how, when and where agricultural practices substantially impact AMR is crucial in understanding food safety inquiries. For instance, conventional farming systems often depend significantly on synthetic inputs such as chemical fertilizer, herbicides, pesticides and hormones (10). The presence, degradation and persistence of these synthetic compounds in the soil depends on soil physicochemical properties and microbial activity, exerting a continuous selective pressure on soil and plant microbiomes (11, 12). In contrast, organic farming is a holistic approach to sustainable agriculture which restricts or exclude the use of synthetic inputs, while integrating soil health management, crop rotation, implementation of cover crops and promotion of biodiversity (13, 14). Although organic farming is generally associated with reduced environmental pollution and a production of food without synthetic chemicals, studies have documented that organic farming systems can harbor diverse ARGs (15).

Previous studies have detected diverse profiles of antibiotic resistance in apples, revealing variations between epiphytic and endophytic bacteria (16) as well as arrays of resistome in stored apple fruits (17). Moreover, studies showed different compositions of the apple microbiota from organic and conventional orchards (18). However, comprehensive comparative analysis of the apple microbiome and the associated resistome from different agricultural management practices remain scanty. Therefore, we systematically investigated apple microbiome, resistome, and HGT differences across regions and management practices. Overall, our study addresses a largely overlooked dimension of the environmental AMR crisis, by providing insight into potential exposure pathways of AMR at the human-food-environment interface.

Experimental design

Site description and sampling

The samples were collected from two separate regions (USA and Israel). In USA, apples at maturity stage, Golden delicious Reinders were collected from 12-year-old trees in two (organic and conventional) orchards i.e., Kearneysville and Rellim Orchard in West Virginia, USA. The management measures were in accordance with the WVU Extension Service's annual guidelines found at (https://

www.virginiafruit.ento.vt.edu/SprayGuide/TreeFruitSprays.html). To protect plants from pests and diseases, USA Orchards used Vintrazol Super as a dormant paraffinic oil spray for managing apple scabs, which are mostly caused by the fungus *Venturia inaequalis*. Altacor, a pesticide belonging to a class of anthranilic diamide was used to prevent the Apple-coddling moths and other insects from attacking the apples. Moreover, pyrethroids were used to control brown marmorated stinkbugs. Premature fruit drops were prevented by applying Retain (NAA).

In Israel, apples were collected from two orchards (organic and conventional), in the Northern Galilee region and in Baram respectively. In organic orchard, plant protection measures included two sprays of Serenade ASO (*Bacillus subtilis*; Bayer Crop Sciences) against apple scabs. Neem oil (TOTACH, Botanix) and food-grade mineral paraffinic oil (MCW Eos, ADAMA) were used against insect pests. For the conventional orchard, plant protection measures were applied to prevent codling moths, powdery mildew, apple scabs, mites and fruit flies. The following insecticides were used: Armada (Spinetoram + Methoxyfenozide), Lintex (Cyhexatin), Vectra (Abamectin), Meteor (Fenpyroximate), Maverick (Tau-fluvalinate), Sparta-Supe (Spinetoram), Tersip (Cypermethrin), Kurmoran (Acetamiprid + Novaluron) and Tsabar (Lufenuron). Phantom (Trifloxystrobin), Skiper (Trifloxystrobin), and Merpan (Captan) fungicides were used to treat powdery mildew and apple scabs. Data in Additional file ***Table 1*** provides information on orchard spraying schedules. In every orchard, ten trees were randomly selected. To create three biological duplicates, 60 apple fruits were pooled and split into three groups of 20 fruits. The DNA from fruit replicates were extracted and sequenced as described by (19).

Preprocessing of metagenomic data

We assessed the quality of the raw reads using FastQC v0.12.1 (20). We later trimmed the adapter and low-quality bases using fastp v1.0.1 (21), with a sliding window mean Q30, tail trimming at Q≥25, minimum read length of 50 bp, and poly-G/X trimming.

Recovery of Metagenome-Assembled Genomes (MAGs)

We assembled high-quality reads for each sample using metaSPAdes algorithms implemented in SPAdes v4.2.0 (22). Contigs shorter than 1 kb were removed. We then used QUAST v5.3.0 (23) to compute assembly statistics (N50, L50, GC content, contig count, and total assembly size) and validated using BBTools v38.98 (24). To confirm the quality of the reads, we aligned trimmed reads back to their assemblies using BWA-MEM2 v2.3 (25). Contigs ≥ 1.5 kb were binned using MetaBAT2 v2.17 (26), Binny v2.2.15 (27), and COMEBin v1.0.3 (28). We performed bin refinement using MAGScoT v1.1 (29). Genome completeness and contamination were assessed using CheckM2 v1.1.0 (30) and validated using BBTools v38.98 (24). Subsequently, we identified rRNA genes using Barrnap v0.9 (https://github.com/tseemann/Barrnap) and predicted tRNAs using tRNAscan-SE v2.0.12 (31).

Taxonomic annotation

For taxonomic identification, we classified MAGs using GTDB-Tk v2.4.1 (32) and dereplicated using dRep v3.4.2 (33) at ≥ 95% ANI and ≥ 30% alignment coverage. The taxonomic composition of the assemblies was assessed by Kraken2 v2.1.6 (34) using the PlusPFP database (release 14 July 2025). We generated coverage profiles for downstream binning using the JGI script jgi summarize bam contig depths (minimum MAPQ 10, alignment identity ≥ 97%).

Plasmid sequence detection and typing

We identified plasmids using PLASMe v1.1 (35) and metaPlasmidSPAdes (36), with both outputs filtered using ViralVerify v1.1 in plasmid mode to exclude uncertain plasmids. In parallel, we included plasmid predictions from geNomad v1.11.1 (37). We later dereplicated filtered plasmid predictions (both from PLASMe and metaPlasmidSPAdes) and geNomad results using Stampede-ClusterGenomes at 95% ANI and 85% alignment coverage. We analysed the dereplicated plasmids with HOTSPOT v1.1 for host prediction and MOB-suite v3.1.9 (38) for plasmid typing and mobility classification.

Detection and characterization of bacteriophages

We identified bacteriophage using VirSorter2 v2.2.4 (39), VIBRANT v1.2.1 (40), and geNomad v1.11.1 (37). Subsequently, we evaluated phage sequences with CheckV v1.0.3 (41), and discarded low-quality genomes. The remaining phage sequences, we dereplicated using Stampede-ClusterGenomes at 95% ANI and 85% alignment coverage. We inferred host associations using iPHoP v1.4.1 (42) and classified phage lifestyle (temperate or virulent using DeePhage (43).

Identification of ARGs

We predicted ARGs using DeepARG (44) and aligned the predicted proteins to CARD 2023 database (45) using MMseqs2 (46) with sensitivity of 7.5, matches ≥ 90% identity and ≥ 80 amino acid alignment length. Subsequently, we re-annotated the proteins without direct CARD matches through accession- or gene-name-based mapping, followed by manual classification into resistance mechanisms and drug classes. We labelled all the annotations based on their evidence type to ensure consistency across MAG, plasmid, and viral datasets (Supplementary ***Table 1,*** Additional file ***Table 3***).

Statistical analyses and visualizations

We analysed between-sample compositional differences (beta diversity) for bacteria, phage, and plasmid and contrasted management practices within each region (Israel (ISR): Org vs Conv; USA: Conv1 vs Conv2). Group separation was tested with permutational multivariate analysis of variance (PERMANOVA; *vegan::adonis2*) using the model distance ~ Region * Management, with 999 permutations and within-region contrasts were performed separately using stratified permutations. To verify the PERMANOVA assumption, we tested for the homogeneity of multivariate dispersions using betadisper followed by permutest. For alpha diversity, we calculated Shannon per sample and visualized the results using box and jitter plots with a unified region×management color scheme. Differences between management

practices within each region were evaluated using two-sided Wilcoxon rank-sum tests. All analyses were performed in R (47) using *vegan* package (48) and *ggplot2* (49) for visualization.

To investigate the impact of agricultural practices on the resistome, we calculated Shannon alpha-diversity indices and total ARG abundance, expressed as normalized counts per sample (e.g., CPM or relative abundance). These metrics were visualized using plots stratified by both region and management practice. For each region-management combination, we summarized unique ARG counts and determined the mean abundance per mechanism per sample. Finally, to identify shared and unique ARGs across bacteria, phages, and plasmids, we constructed an ARG presence-absence matrix and visualized the overlaps using an UpSet plot. Within each region×management practice, we plotted class-level proportions. The counts were summed across replicates and converted to relative abundance across bacteria, phage, and plasmid. To further determine which ARGs are mobilized (plasmid-/phage-linked) and act as horizontal gene transfer (HGT) hubs, the network analysis was computed using *Gephi version 0.10.1* (50).

Results

Overview of metagenome-derived MAGs, plasmids, and phages

From the shotgun metagenomic data, we obtained between 10 and 55 million high-quality reads per sample, summing to over 350 million reads across all libraries, with an average read length of 150 bp. From the high-quality reads, (of which 98 - 99.9% passed quality filtering), we assembled contigs, retaining 73 - 96% of sequences, and identified contigs ≥1,000 bp representing 28 - 86% of reads per sample. Using binning and annotation approaches, we reconstructed metagenome-assembled genomes (MAGs) that captured 7 - 68%, plasmid accounted for 4 - 28%, and phages represented 0.5 - 3.8% of the reads across samples. Detailed sequencing statistics, and read mapping efficiencies of all samples are provided in Additional file ***Table 2***.

Effects of of geographical regions and management practices on the composition and diversity of bacteria, phages and plasmids

To determine whether geographical region and management interactively shape microbial composition, we performed a PERMANOVA on Bray–Curtis distances across the bacteria, phages and plasmids. The results showed that geographical region explained the largest proportion of variation in microbial composition ($R^2 \approx 0.42$ - 0.50, $p \leq 0.001$). Management also had a significant but smaller effect across domains ($R^2 \approx 0.27–0.31$, $p \leq 0.003$). Pairwise PERMANOVA analyses showed that most regional contrasts explained substantial variation but the differences were not statistically significant (all $p > 0.05$). However, when USA orchards were pooled, the contrasts were statistically significant ($p = 0.003$ - 0.006) (Supplementary ***Table 2***). In contrast, within-region management (ISR: Org vs Conv and USA: Conv1 vs Conv2) showed moderate proportion of variation sizable effect ($R^2 = 0.34$ - 0.56) but did not reach statistical significance (all $p > 0.05$) (***Figure 1A*****,** Supplementary ***Table 2***). The Alpha diversity further supported the observed differences across regions and management practices. Bacterial diversity was

significantly higher in Israel organic compared to conventional orchards (p = 0.0495), while all other bacterial contrasts were not significant (p > 0.05). Phage diversity showed no significant differences across the majority of comparisons. Conversely, plasmid diversity was consistently higher in USA compared to Israel orchards, with multiple significant differences (p =0.020137 - 0.0495) (**Figure 1B,** *Supplementary* ***Table 3***).

Impact of geography and management practices on resistome profiles

To determine how environmental and anthropogenic factors shape resistome, we analyzed the impact of geographical locations and management practices on the resistome profiles. The resistome composition across agricultural management practices showed clear host-specific and ecological patterns in ARGs distribution. A total of five genera were identified as the main carriers of ARGs. Among these, *Pseudomonas* emerged as the most dominant genus, accounting for 14 - 32.4% of the microbes carrying ARGs. Other frequently detected genera included *Pantoea*, *Sphingomonas* and *Methylobacterium,* each contributing to 5 - 17% of the microbes depending on the management systems (Supplementary ***Figure 1A, Figure 2***). Moreover, a pairwise comparisons of ARG diversity showed significant differences across management practices. The ARG diversity in Israel conventional was significantly higher compared to USA conventional_1 orchards (p = 0.0495), while no significant differences (p > 0.05) were observed between Israel conventional and USA-conventional_2 or between Israel conventional and pooled USA conventional orchards. Within-region management analysis, Israel-conventional had higher ARG diversity compared to organic orchards, but this difference was not statistically significant (p > 0.05) (Supplementary ***Table 4***). Conversely, USA-conventional_1 exhibited significantly higher ARG diversity compared to Israel-organic orchards (p = 0.0463). However, comparisons between Israel organic and USA conventional_2 orchards showed no statistically significant (p > 0.05) (***Figure 2A*****,** Supplementary ***Table 4***).

To further confirm the number of shared and unique ARGs across the bacterial hosts and MGEs, we plotted the ARGs in an UpSet plot. We observed that majority of the unique ARGs were found in bacteria (with 314 unique ARGs). A smaller subset was shared between bacteria and plasmids (31 ARGs) while three resistance genes were identified as unique plasmid-encoded ARGs. Only one ARG was present in bacteria - phage - plasmid combination and one unique phage-encoded ARG. Set-size bars confirmed that the bacterial pool dwarfs MGEs (***Figure 2B***). Across management practices, we noticed that the ARG hosts determined the resistance patterns. The bacterial ARG profiles carried the bulk of resistome, with majority encoding multidrug resistance (with ~ 45 - 70% of each sample). Smaller but consistent resistance shares were observed in MLS, aminoglycoside, β-lactam and glycopeptide. Plasmid-encoded ARGs varied, with multidrug being common, however, some samples showed sizable fraction of sulfonamide and glycopeptide. Phage-encoded ARGs appeared in only one sample (USA, conventional_1) driven by very low absolute counts (***Figure 2C***). Additionally, we observed that antibiotic efflux pumps, antibiotic inactivation, and target alteration were the most dominant resistance mechanism across the management practices. Resistance by absence was essentially negligible (***Figure 2D***)

Influence of management practices on HGT proxies

The constructed HGT network comprised 79 nodes, (representing bacterial hosts, MGEs and ARGs) connected by 180 edges corresponding to predicted gene transfer events. Thereinto, 175 (97.22%) were mobilizable interactions, while 5 (2.78%) represented conjugative connections (***Figure 3A***). The weighted degree distribution showed that a small number of highly connected hubs, especially plasmid, played the role of central intermediaries between several bacterial hosts and ARGs. The bacterial hosts, especially from the genera *Pseudomonas*, *Pantoea,* and *Methylobacterium*, had strong connections with the conjugative ARGs whereas mobilizable ARGs were distributed more uniformly on the less connected bacterial hosts (***Figure 3A*****,** Supplementary ***Figure 2***). Glycopeptide resistance genes (*vanH*), tetracycline resistance genes (*tetR*), multidrug reistance gene (*acrB*) along with MLS resistance gene (*vatB*), were frequently co-located with conjugative transfer genes. Additionally, we noted the variation of mobilizable and conjugative ARGs among the management practices, Israel conventional orchards presented the highest burden of mobile ARGs with a total 38 ARGs. In contrast, Israel organic orchards exhibited a marked reduction in the number of mobile ARGs (with only 6 ARGs). Notably, no conjugative ARGs were detected in USA - conventional_2 orchards (***Figure 3B***).

Discussion

In this work, we aimed to explore how agricultural management practices, i.e., organic vs conventional, from two distinct geographical regions (USA and Israel) shape the microbiome and resistome of apples. Our results showed that, regional and agricultural practices significantly influenced the composition and diversity of bacteria, phages, plasmids, and their respective resistome profiles. The key findings were: (1) The results showed that geographical regions explained the largest proportion of variation in microbial composition while management had a significant but smaller effect across across the domains; reflecting how ecological niches, environmental factors, and farming practices all interact to shape apple microbial communities. (2) ARG diversity was higher in conventional than organic orchards, with *Pseudomonas* being the predominant ARG carrier. Lastly, (3) Mobile ARG burden was highest in conventional, and remarkably reduced in organic orchards. No conjugative ARGs were detected in USA-conventional_1 orchards, underscoring strong management-driven differences in horizontal gene transfer potential.

The metagenomic data generated in this study provides a comprehensive overview of the apple-associated microbiome. Leveraging deep sequencing coverage enables the robust detection of bacteria and mobile genetic elements (MGEs), as well as the detailed characterization of functional resistance traits. Nevertheless, these findings should be interpreted in light of certain methodological limitations. In this study, the analysis was based on short-read metagenomic profiling. This technique inherently limits the recovery of low-abundance microorganisms and complicate the assembly of repetitive, GC-rich genomic regions or strain-level, potentially biasing genome and reconstruction of MGEs (51, 52). Additionally, the reliance of solely one method (metagenomics) for AMR detection, is not widely accepted within the scientific community. The limited sequencing depth is offset by the sample size,

which provides sufficient statistical power for making generalizable predictions and differentiating between the effects of various potential confounders. Despite these limitations, which are often seen in most short-read metagenomic studies, our dataset remains sufficiently enough to support comparative analyses of microbial community structure, and resistome within the apple microbiome.

Our findings showed a strong effect of both region and management practices on microbiome composition, and the clear separation of conventional versus organic orchards indicate that regional environmental factors including climate, soil type, cultivar genotype, and local microbial pools set the baseline community (39, 53–57). Consistent with the findings of Yurgel et al. (2025), our results demonstrate that root-associated microbiomes are primarily structured by site-specific environmental factors, upon which management practices impose additional selective pressures(58–60). In contrast, compositional structuring within-region subsumes most variance, while management exerts subtle but context-dependent shifts possibly requiring larger dataset sizes. The alpha diversity further indicated that conventional systems generally exhibited higher Shannon diversity, which can potentially arise because of several non-exclusive processes i.e., (1) conventional management practices increases microbial evenness where minor taxa rise in relative abundance while dominants are suppressed possibly by chemical inputs (61–63); (2) inputs (fertilisers, organic amendments, irrigation) boost resource heterogeneity and thus niche availability (60, 64, 65); or (3) introduction of taxa via inputs, machinery, or irrigation water (66, 67). Organic farming systems are known to promote more diverse bacterial community (68, 69), which is consistent with our findings. The diversity and variability of MGEs may instead reflect the selective pressures for possible HGT events. The observed phage diversity patterns are especially interesting, given their roles as ecological regulators while carrying out HGT (70). Phages adapt rapidly to changes in their environments, and the diverse phage communities present in the management practices may perhaps point to a 'larger' or more dynamic underlying host, or more opportunities for transduction events. Recent studies on large-scale viral surveys and rhizome-associated phages in soil ecosystems have found that, land management have a profound effect on viral diversity (71–73).

Resistome composition in orchards showed pronounced host-specific and management-associated patterns, underlining the impact of agricultural management on ARG mobilization patterns. *Pseudomonas* was prevalent in ARG carriage, followed by *Pantoea*, *Sphingomonas*, and *Methylobacterium*, mirroring their taxonomic predominance, metabolic versatility, and resilience to management practices (15, 17, 74, 75). ARG diversity was significantly higher in conventional than organic orchards, signifying selective pressure on ARG-rich bacteria, probably influenced by co-selection with chemicals or nutrient-mediated competitive strategies (76). Efflux pumps, inactivation, and target alteration are known to confers broad-spectrum antibiotic resistance in diverse array of environments (77–80), a pattern that is evident in our findings. The ARGs were skewed towards bacteria compared to MGEs, signifying that plasmid or phages may play a role in HGT (81), while bacteria remains the major reservoir for intrinsic resistance (2) as previously confirmed in apples microbiome and its resistome in post-harvest period (17) and an exemplary comparison of resistome in undomesticated bog environments (79). Phages rarely encode for ARGs (82) while transduction probability remains

stochastic (70, 83, 84). Additionally, we observed that majority of the ARGs encoded resistance to multidrug, a common characteristics in environmental context (79), that in turn support the bacteria to colonise the host (85). MGEs harbored additional sulfonamide and glycopeptide resistance, suggesting the prevailing HGT events for clinically-relevant ARGs (86–88).

HGT proxies indicated that plasmids act as hubs for ARG spread. The majority of interactions were mobilizable, while conjugative interactions were few. Though conjugative transfer is efficient for ARGs transfer, most were due to mobilizable elements, which rely on integrative elements for spread, independent of conjugation mechanisms. Plasmids showed connectivity to several hosts, such as *Pseudomonas*, *Pantoea*, and *Methylobacterium*. These bacteria are generally metabolically versatile, and often observed dominating apple microbiomes (18, 53, 74) as well as disrupted environments (89), which could increase their possibility to act either as donors or as acceptors for ARGs (90). Moreover, resistance genes such as glycopeptide (*vanH*), tetracycline (*tetR*), MLS (*vatB*), and Multidrug resistance (*acrB*) were generally mediated by conjugative plasmids, indicating their roles in spreading to multiple microbial taxa, when selective pressures favor HGT events (87). Differences in mobile ARGs across management practices represent joint interaction between selective pressure and microbial taxonomic composition. Conventional showed the higher counts of mobile ARGs than organic orchards. This pattern demonstrates that in conventional orchards, chemical inputs may subsequently promote selection of resistant microbial taxa (13, 91), thereby increasing potential for HGT proxies.

Conclusion

Our findings demonstrate that geography, in conjunction with agricultural management practices, significantly shapes the apple microbiome, resistome, and potential HGT proxies. Conventional orchards exhibited higher antibiotic resistance gene (ARG) diversity. Furthermore, the increased co-occurrence of ARGs and mobile genetic elements (MGEs) suggests enhanced pathways for HGT events. Conversely, organic orchards supported more diverse bacterial communities while maintaining lower ARG diversity. Notably, four genera: *Pseudomonas*, *Pantoea*, *Sphingomonas*, and *Methylobacterium* emerged as the primary ARG reservoirs, reflecting their adaptation to environmental stressors. Phage-mediated ARGs were rare, likely due the prevalence of temperate and low-virulent lifestyles, suggesting that viruses play a limited role in shaping the resistome. Overall, our findings highlight that reducing of selective pressure through organic practices may mitigate the expansion of environmental resistome, providing critical insights into AMR management in plant-associated microbial communities.

Abbreviations

DNA: Deoxyribonucleic Acid

AMR: Antimicrobial resistance

rRNA: Ribosomal Ribonucleic Acid

MGEs: Mobile genetic elements

ARGs: Antibiotic resistance genes

NAA: Naphthaleneacetic Acid

MAGs: metagenome-assembled genomes

HGT: Horizontal gene transfer

QUAST: Quality Assessment Tool for Genome Assemblies

CARD: Comprehensive Antibiotic Resistance Database

ANI: Average Nucleotide Identity

PERMANOVA: Permutational Multivariate Analysis of Variance

Declarations

Ethics approval and consent to participate

Not applicable

Availability of data and materials

The normalized counts of contigs, MAGs, plasmids, and viral reads per one million reads dataset is newly generated and is detailed in **Supplementary file *Table 1***. The PERMANOVA results comparing bacteria, phage, and plasmid composition Dataset is newly generated and is referenced in **Supplementary file *Table 2*.** The pairwise comparisons (Shannon diversity) of bacteria, phage, and plasmid Dataset is newly generated and can be found in **Supplementary file *Table 3***. The pairwise comparisons of ARG Shannon diversity Dataset is newly generated and is referenced in **Supplementary file *Table 4*.** The spraying regime in orchards Dataset is newly generated and is referenced in **Additional file *Table 1*.** The sequencing output, assembly statistics, and read mapping efficiency Dataset is newly generated and is referenced in **Additional file *Table 2*.** The distribution of ARGs across contigs, MAGs, plasmids, and viruses Dataset is newly generated and is referenced in **Additional file *Table 3*.**

The metagenomic data have been deposited in the [SRA NCBI] repository (Accession number: PRJNA948808) and can be accessed from the following link: https://www.ncbi.nlm.nih.gov/bioproject/?term=(PRJNA948808)%20AND%20bioproject_sra[filter]%20NOT%20bioproject_gap[filter]

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

A.A. conceptualized and directed the study. M.W. and S.D. coordinated the sample collection and sequencing. D.K., S.S., and M.K.N. performed the data analysis. M.M.-C.H., R.M., and G.B. provided supervision and resources. D.K., S.S., M.K.N., and A.A. interpreted the results. D.K. wrote the first manuscript draft. All authors read, reviewed, edited, and approved the final manuscript.

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Figures

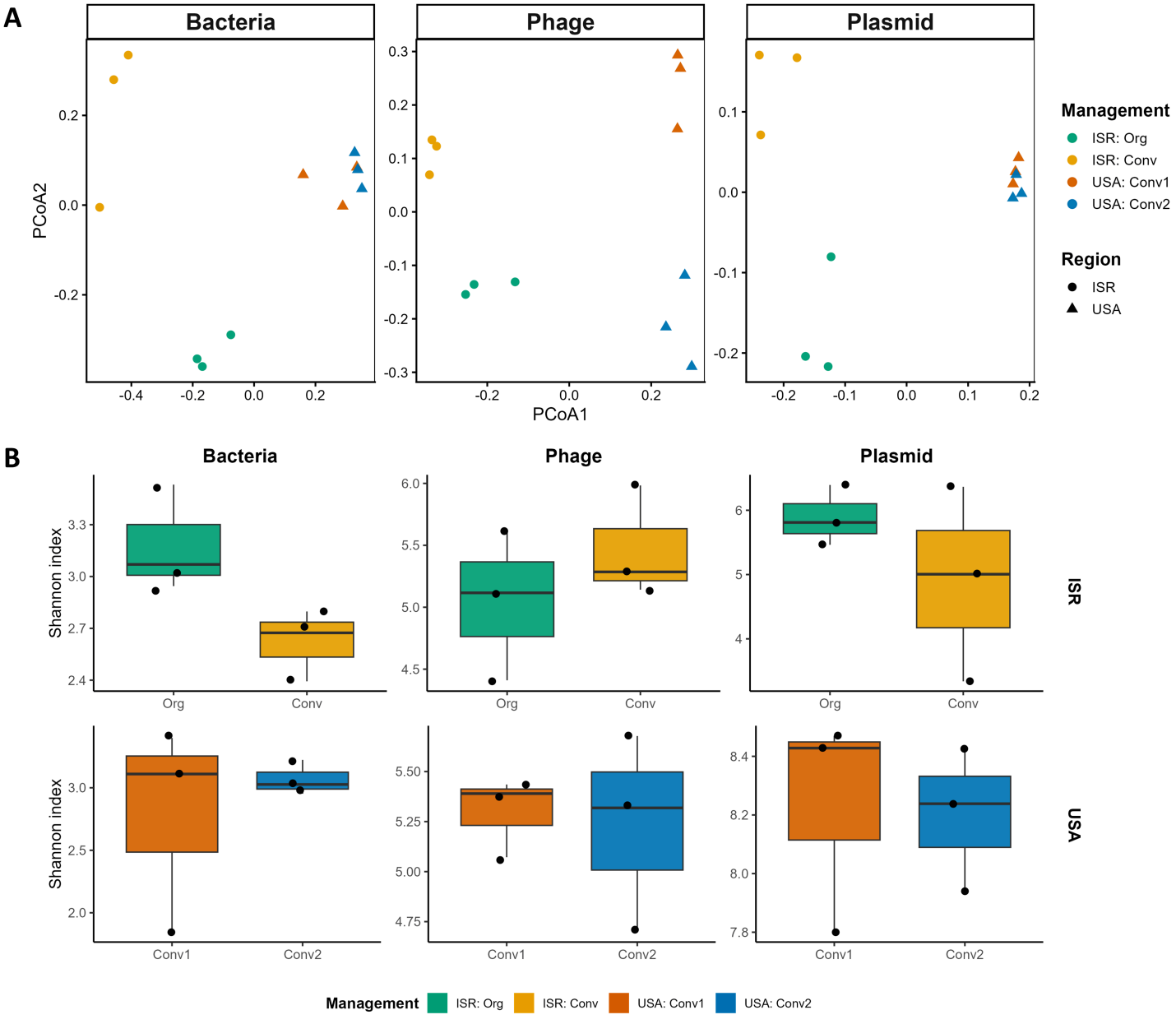


Figure 1

Effect of geographical regions and agricultural practices on the composition and diversity of bacteria, phages, and plasmids. (**A**) Ordination plot based on Bray-Curtis distances of the community composition across the bacteria, phages, and plasmids. Each points represents an individual sample. Colors indicate management practices: Israel organic (green), Israel conventional (yellow), USA conventional_1 (orange), and USA conventional_2 (blue). Point shapes differs between both regions: Israel (circles) and USA (triangles). (**B**) Box plot illustrating Shannon α-diversity by management and region across bacteria, phages and plasmids. Boxplot shows median (line), IQR (box), and whiskers (1.5×IQR). The colors correspond to management practices in (**A**).

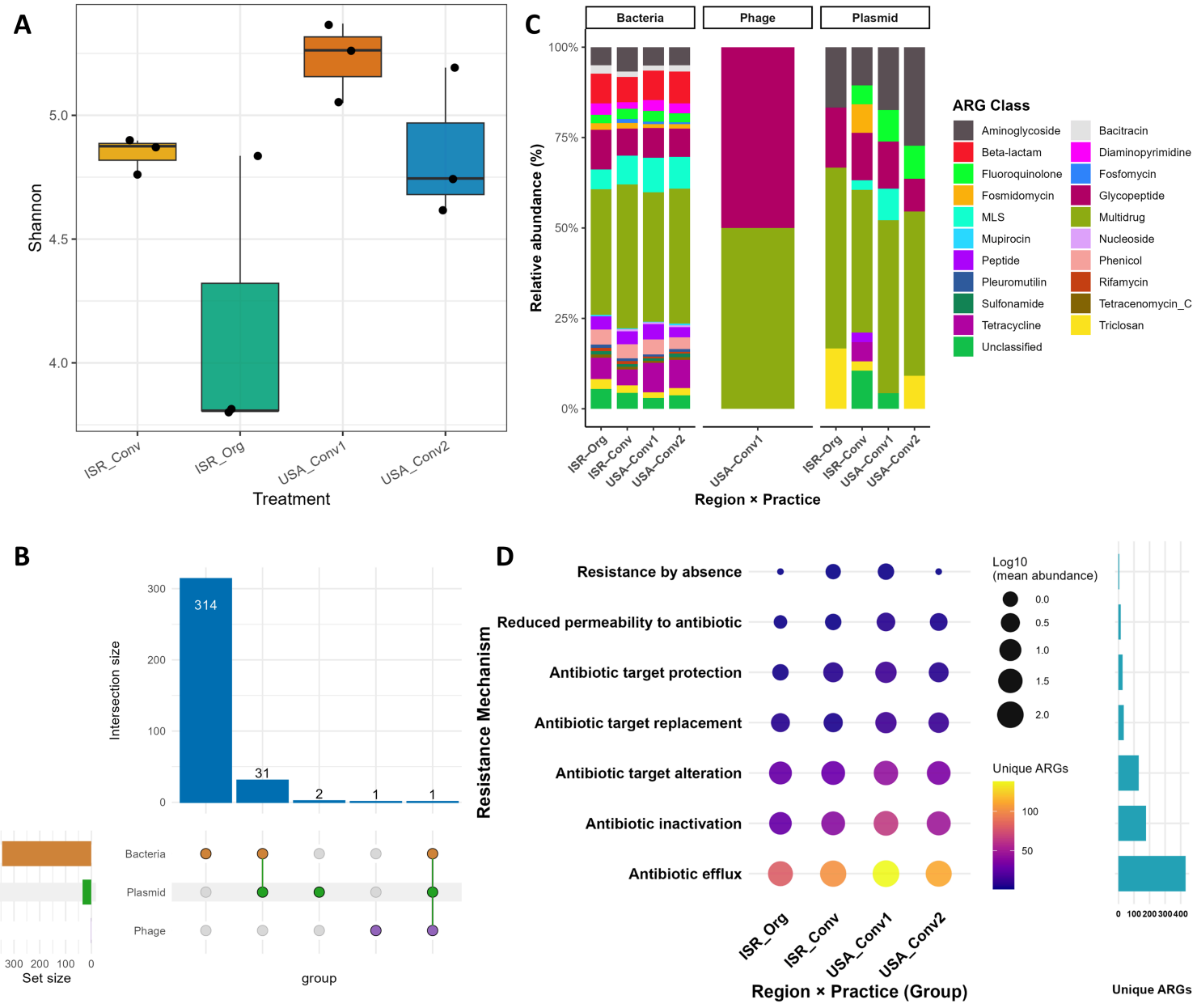


Figure 2

Effect of geographical regions and agricultural practices on resistome composition. (**A**) ARG diversity across management practices. Boxplot shows median (line), IQR (box), and whiskers (1.5×IQR). The

colors denote management practices: Israel organic (green), Israel conventional (yellow), USA conventional_1 (orange), and USA conventional_2 (blue). (**B**) Upset plot illustrating the number of shared versus unique ARGs. The bars indicate intersection sizes of ARGs in bacteria, plasmid, and phage. Lower-left bars indicate the total set size, highlighting larger bacterial ARG repertoire compared to mobile genetic elements (plasmids and phages). (**C**) Relative abundance of ARG class composition across the management practices. The coloured segments (legend) denote ARG class. (**D**) Resistance mechanisms by management practices. The point size refers to the log10 mean abundance and colour denotes the number of unique ARGs per sample.

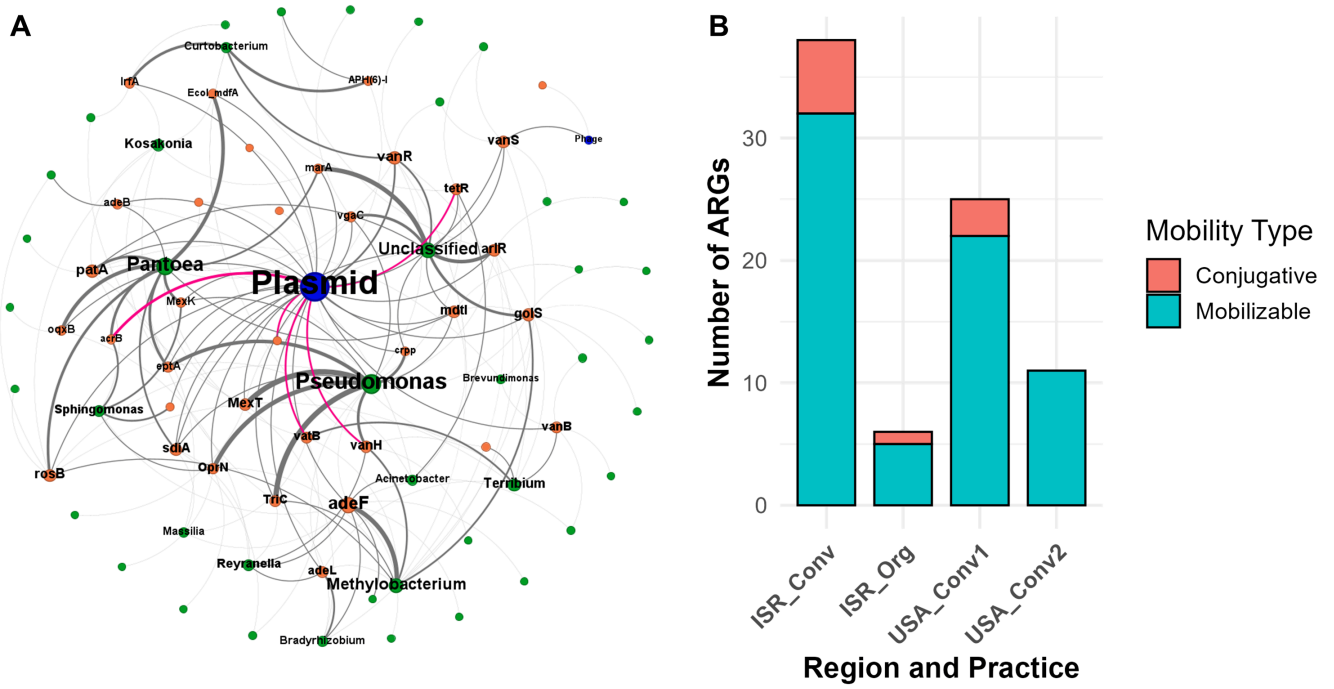


Figure 3

Horizontal gene transfer (HGT) proxies. (**A**) Horizontal gene transfer (HGT) proxies between bacterial hosts and MGEs. The nodes were colored green, purple and orange to denote bacterial hosts, MGEs and ARGs respectively. The edges denote inferred potential co-occurrence-based mobility, where red edges correspond to conjugative connections and gray edges represent mobilizable interactions. The node size is proportional to the number of connections and edge thickness corresponds to the interaction weight. (**B**) Distribution of mobilizable ARGs across the management practices. The y-axis represents the number of ARGs per sample while x-axis denotes samples across the regions.

Supplementary Files

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