

014 Characterization of Soil and Lettuce Resistomes from Harvest Through Storage in Modified Atmosphere Packaging



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Introduction

- Leafy vegetables carrying bacteria that harbor antimicrobial resistance genes may provide a pathway for those genes to enter the human microbiome.
- Both lettuce phyllosphere and soil microbiomes are impacted by weather factors and agricultural practices and differences in the microbiome may result in differences in the resistome.
- Soil microbial communities contribute to the lettuce microbiome.
- In this study, antimicrobial, metal, and biocide resistance genes (ARGs, MRGs, and BRGs) in soil and lettuce phyllosphere microbiomes were profiled and compared to determine possible transfer between soil and lettuce as well as persistence during lettuce storage.

Materials and Methods

- Romaine lettuce was grown, harvested, processed (cut, washed in chlorinated water, and rinsed 3x), and stored at 5±1°C in modified atmosphere packaging (MAP) for 7 to 14 days [1, 2]. Three fields in Salinas, CA were harvested, an experimental USDA field in fall 2017, fall 2018, and spring 2018 (field 1), and a plot in a conventional grower's field in fall 2018 (field 2) and spring 2018 (field 3). Including the five harvests, a total of 225 samples consisting of surface soil, harvested lettuce heads, processed lettuce, and stored MAP lettuce were collected.
- Shotgun metagenomic sequencing was performed on DNA obtained from sample microbiomes using an Illumina NextSeq platform generating paired-end 150 bp reads.
- Classification of resistance determinants in the sequence datasets was accomplished using MEGARes 2.0. [3].

References:

- Leonard, SR, et. al. 2021. Environ. Microbiome, doi: 10.1186/s40793-021-00393-y
- Brandl, MT, et. al. 2023. Food Microbiol., doi: 10.1016/j.fm.2023.104260
- Doster, E, et. al. 2020. Nucleic Acids Res., doi: 10.1093/nar/gkz1010

Conclusion

- A greater absolute number of resistance genes were found in processed lettuce after cold storage compared to pre-storage or in soil. Although resistance genes were not detected in every lettuce sample, resistance genes were found in all lettuce sample types: harvested, processed, and cold-stored.
- Soil, harvested lettuce, processed lettuce, and lettuce stored at 5±1°C in MAP have different resistance gene profiles. The relationships between resistance gene classes and sample types differed among the five harvests. However, overall, the relative abundance of BRGs was highest in lettuce after processing and before storage. MRGs were prevalent in both soil and lettuce, with copper resistance particularly high in lettuce.
- While resistance gene alleles were detected in common between soil and lettuce samples for each harvest, there were also resistance gene alleles, including many ARGs, found in lettuce resistomes that were not found in soil. Omitting genes conferring both drug and biocide resistance, and including all samples, the greatest percentage of ARGs belonged to the beta-lactam class (30%) followed by aminoglycosides (6.7%), and specific allele sequences from both classes were observed in common between soil and lettuce.

Results and Discussion

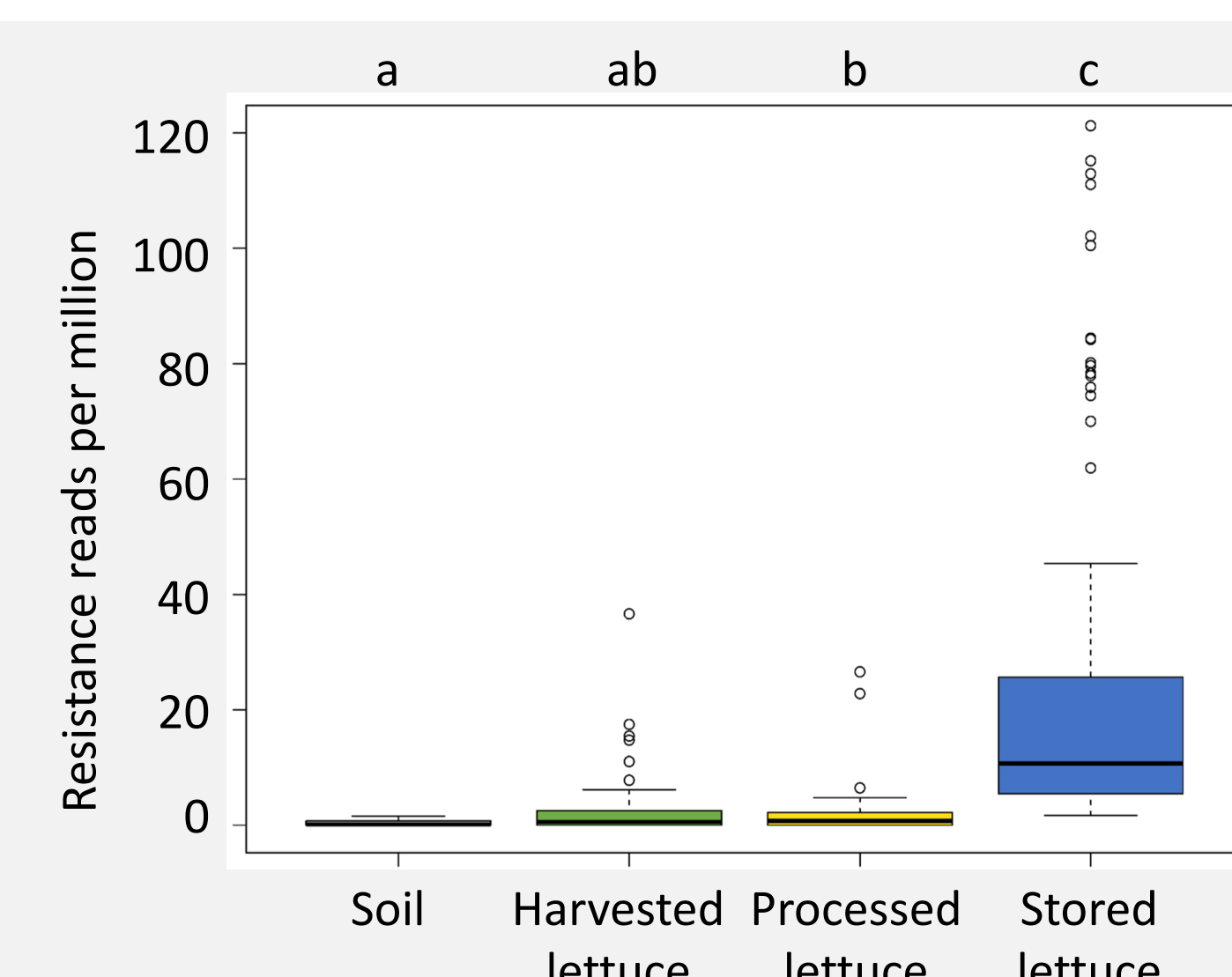


Figure 1. Absolute number of reads matching resistance genes in the different sample types normalized to reads per million total bacterial reads. Statistical differences based on Wilcoxon rank sum method denoted by letters (P<0.05).

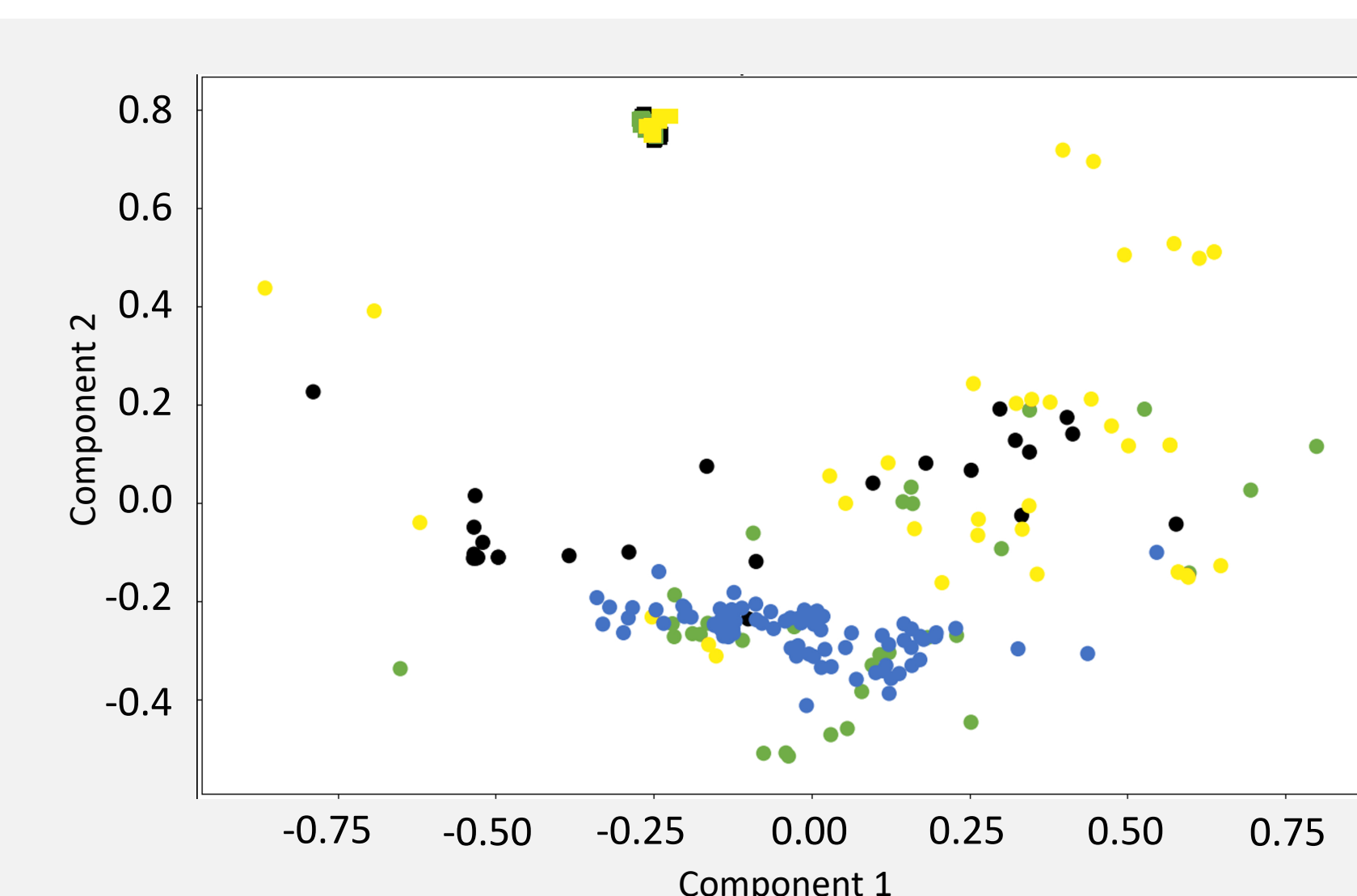


Figure 2. Non-metric multidimensional scaling plot depicting the beta diversity of resistance gene classes among sample types. The resistance gene profiles among the four sample types are statistically different (PERMANOVA, P<0.001).

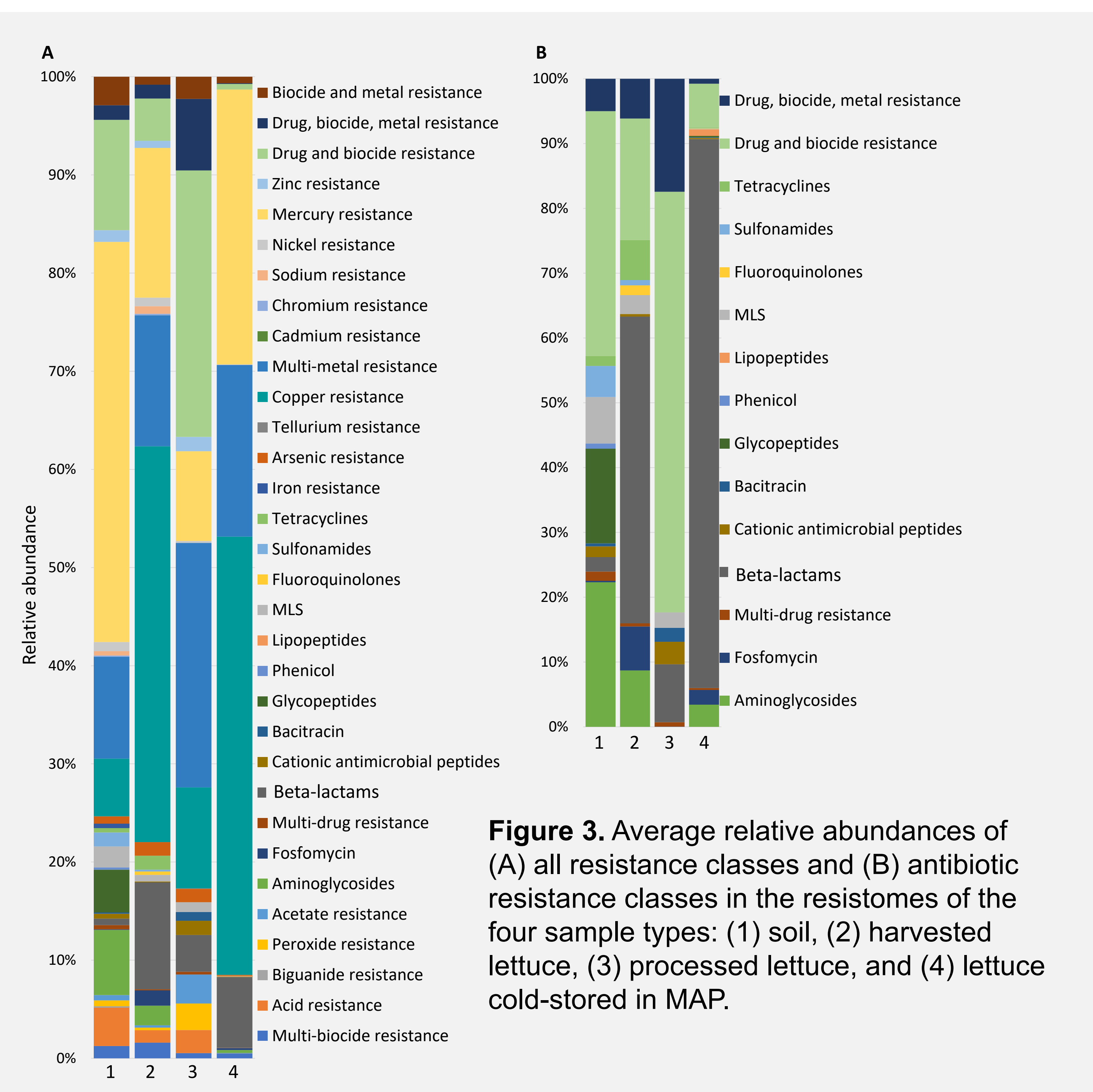


Figure 3. Average relative abundances of (A) all resistance classes and (B) antibiotic resistance classes in the resistomes of the four sample types: (1) soil, (2) harvested lettuce, (3) processed lettuce, and (4) lettuce cold-stored in MAP.

FDA Mission Relevance: These results provide insight into the transfer of antimicrobial resistance genes from soil to the lettuce phyllosphere and ready-to-eat packaged lettuce, thus their potential to enter the human food chain.

Table 1. Number of unique resistance gene alleles in the resistomes

Harvest	Gene alleles in all samples ¹	ARGs in lettuce but not soil ²	Genes shared in soil and lettuce ¹	ARGs shared in soil and lettuce ²	ARGs shared in soil and lettuce ³
Fall 2017 Field 1	156	57	16	2	Aminoglycoside: ANT(3 ^{II}), APH(3 ^{II})
Fall 2018 Field 1	208	29	30	12	Aminoglycoside: APH(6); beta-lactam: CTX, PBP4b; bacitracin: bacA; multi-drug resistance efflux pump: msbA; cationic antimicrobial peptides: eptA
Fall 2018 Field 2	277	68	59	16	Aminoglycoside: APH(6); beta-lactam: PBP2; bacitracin: bacA; cationic antimicrobial peptides: pmrF
Spring 2018 Field 1	116	52	3	1	Not identified
Spring 2018 Field 3	124	34	11	0	Not identified

¹ARGs, BRGs, and MRGs, ²ARGs including categories of drug and drug plus biocide and/or metal resistance, ³Genes listed exclude category of drug plus biocide and/or metal resistance

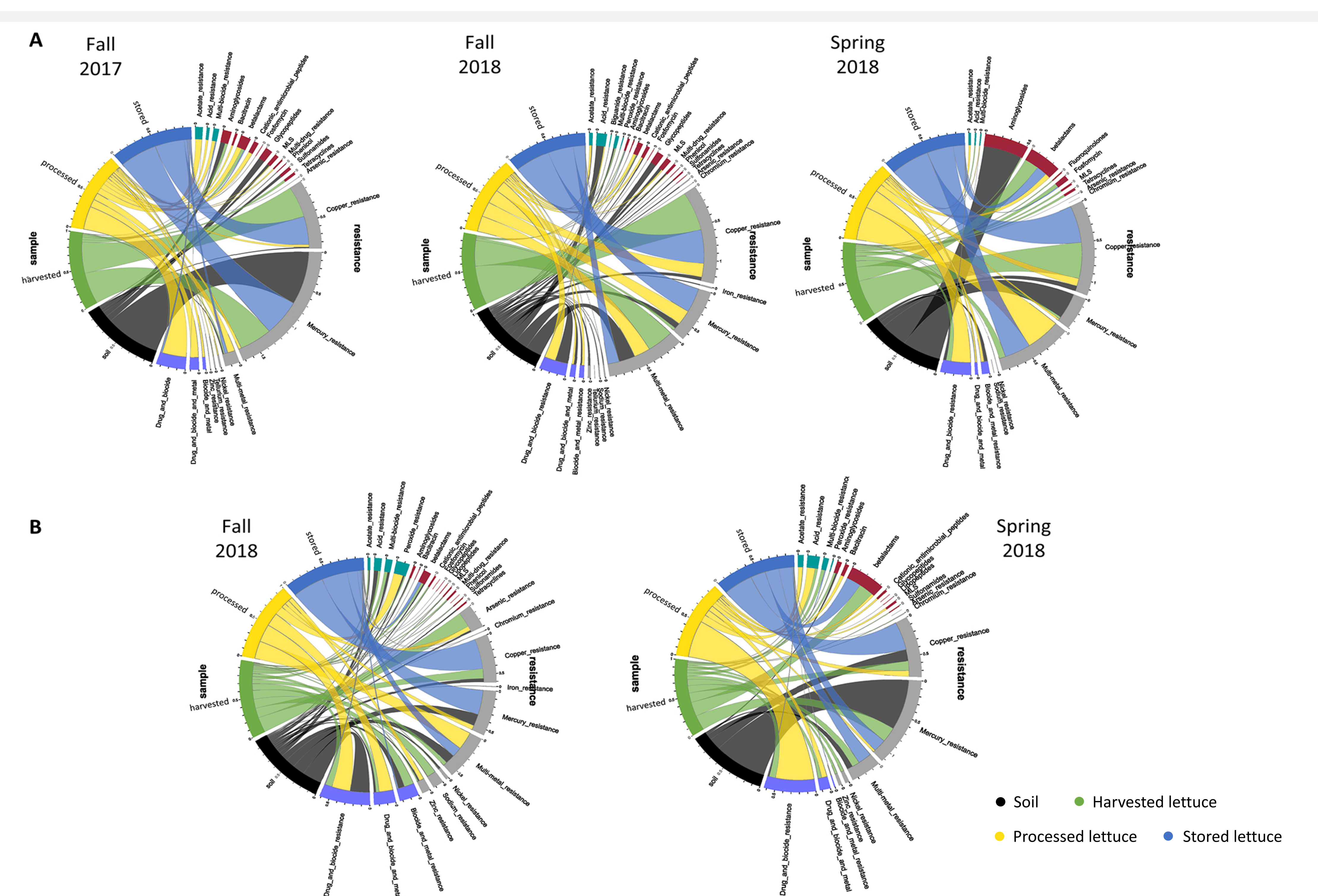


Figure 4. Chord diagrams displaying the inter-relationships between resistance gene classes in the resistomes and sample type. The five harvests are depicted separately and include three harvests in the (A) experimental field and two harvests in a (B) conventional grower's field.