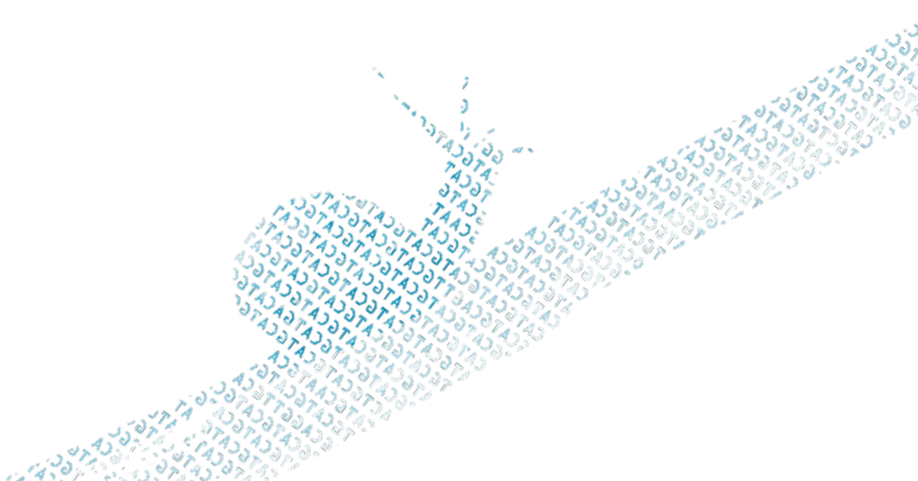
GDC Genetic Diversity Centre Zurich

Genetic Diversity: Analysis

Literature Club

Thursday, 1. July 2021



frontiers in Microbiology

An Apple a Day: Which Bacteria Do We Eat With Organic and Conventional Apples?

Investigating the **apple fruit microbiota** resulted in profound differences between the tissues, applicable for microbiota diversity, composition and abundance. A significant management effect on the microbiota was furthermore apparent for all tissues, even for seeds. Organic and conventional apples are occupied by a similar quantity of microbiota; consuming the whole apple includes an approximate uptake of 100 million bacterial gene copy numbers. However, freshly harvested, organically managed apples harbor a significantly more diverse, more even and distinct microbiota, compared to conventional ones; the abundance of almost 40% of bacterial genera and orders differed significantly between organically and conventionally managed apples. Moreover, organic apples conceivably feature favorable health effects for the consumer, the host plant and the environment in contrast to conventional apples, which were found to harbor potential food-borne pathogens.

Swiss Gourmet Arlet

Wassermann et al. (2019) An Apple a Day: Which Bacteria Do We Eat With Organic and Conventional Apples? Frontiers in Microbiology. Volume 10 | Article 1629.

- What do you like about the article?
- It there anything you dislike about the article?
- What are the main findings according to the authors?
- Do you understand the sampling design?
- Would you be able to reproduce the data analysis?
- Where can you find the raw data?
- Do you agree with the statistical tests applied?
- Do you agree with the conclusions?
- Do you understand figures and tables?
- ! or ?

frontiers in Microbiology

An Apple a Day: Which Bacteria Do We Eat With Organic and Conventional Apples?

Investigating the apple fruit microbiota resulted in profound differences between the tissues, applicable for microbiota diversity, composition and abundance. A significant management effect on the microbiota was furthermore apparent for all tissues, even for seeds. Organic and conventional apples are occupied by a similar quantity of microbiota; consuming the whole apple includes an approximate uptake of 100 million bacterial gene copy numbers. However, freshly harvested, organically managed apples harbor a significantly more diverse, more even and distinct microbiota, compared to conventional ones; the abundance of almost 40% of bacterial genera and orders differed significantly between organically and conventionally managed apples. Moreover, organic apples conceivably feature favorable health effects for the consumer, the host plant and the environment in contrast to conventional apples, which were found to harbor potential food-borne pathogens.

Wassermann et al. (2019) An Apple a Day: Which Bacteria Do We Eat With Organic and Conventional Apples? Frontiers in Microbiology. Volume 10 | Article 1629.

An apple carries about 100 million bacteria. Good luck washing them off.

According to the study, which was published this month in the journal Frontiers of Microbiology, a single apple contains about **100 million bacterial cells — but if you toss out the core, you're only consuming about 10 million of these precious cells.**

If you've been eating an apple a day to keep the doctor away but haven't been consuming the **core**, you are likely missing out on some of the **most beneficially nutritious parts of the apple**.

Escherichia-Shigella – a group of bacteria that includes known pathogens – was found in most of the conventional apple samples, but none from organic apples.

What do you like about the article?

- 👍 biological replicates (n=4)
- 👍 quantification with qPCR
- 👍 simple design, clear question
- 👍 some nice and appealing figures
- 👍 conclusion are clearly formulated

Abstract

(off to a bad start)

Apples are among the most consumed fruits world-wide. They represent a source of direct human exposure to bacterial communities, which is less studied. We analyzed the apple microbiome to detect differences between tissues and the impact of organic and conventional management by a combined approach of 16S rRNA gene amplicon analysis and qPCR, and visualization using fluorescence in situ hybridization and confocal laser scanning microscopy (FISH-CLSM). Each apple fruit harbors different tissues (stem, peel, fruit pulp, seeds, and calyx), which were colonized by distinct bacterial communities. Interestingly, fruit pulp and seeds were bacterial hot spots, while the peel was less colonized. In all, **approximately 10^8 16S rRNA bacterial gene copy numbers were determined in each g apple.** Abundances were not influenced by the management practice but we found a strong reduction in bacterial diversity and evenness in conventionally managed apples. In addition, despite the similar structure in general dominated by Proteobacteria (80%), Bacteroidetes (9%), Actinobacteria (5%), and Firmicutes (3%), significant shifts of almost 40% of bacterial genera and orders were monitored. Among them, especially bacterial signatures known for health-affecting potential were found to be enhanced in conventionally managed apples. **Our results suggest that we consume about 100 million bacterial cells with one apple.** Although this amount was the same, the bacterial composition was significantly different in conventionally and organically produced apples.

In all, approximately 10^8 16S rRNA bacterial gene copy numbers were determined in each g apple.

$$190\,g \rightarrow \frac{190 \times 10^8}{4} = 4{,}750{,}000{,}000 \text{ bacteria/apple}$$

Our results suggest that we consume about 100 million bacterial cells with one apple.

100,000,000 bacteria/apple

The Figures

(and it gets worse)

Fig.1 - Bacterial Abundance

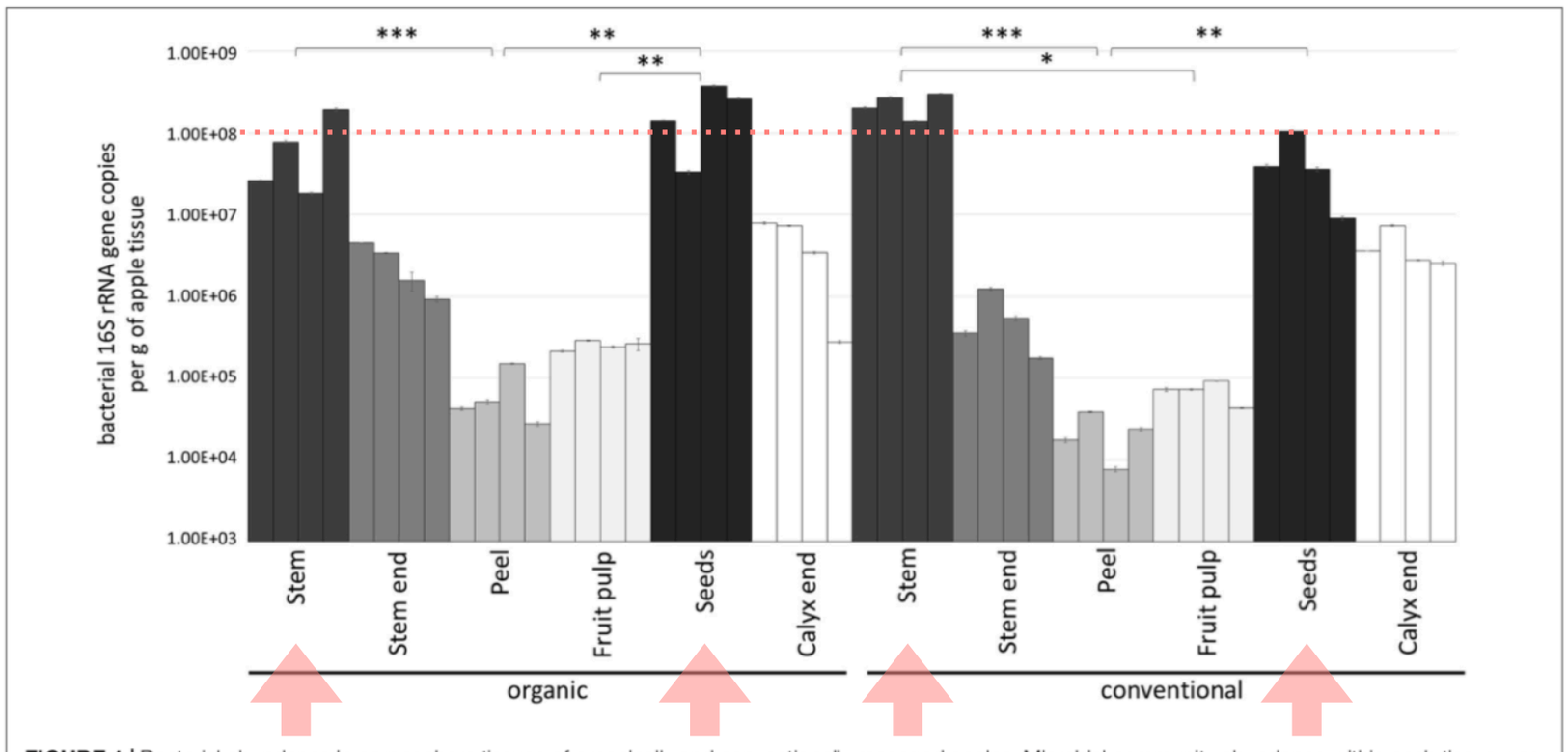


FIGURE 1 | Bacterial abundance in carposphere tissues of organically and conventionally managed apples. Microbial community abundance within each tissue was measured in four replicates by qPCR using PNAs to block mitochondrial and plastid 16S DNA. Asterisks indicate significant differences in 16S rRNA gene abundance (calculated per g of apple tissue) between the tissues within a management group.

- What primer pair was used for the qPCR?
- Did the PNAs block or reduce coamplification?
- The indicated significant differences between tissue seem arbitrary.
- Why would there be bacteria in the seeds?

Fig. 2 - Alpha diversity

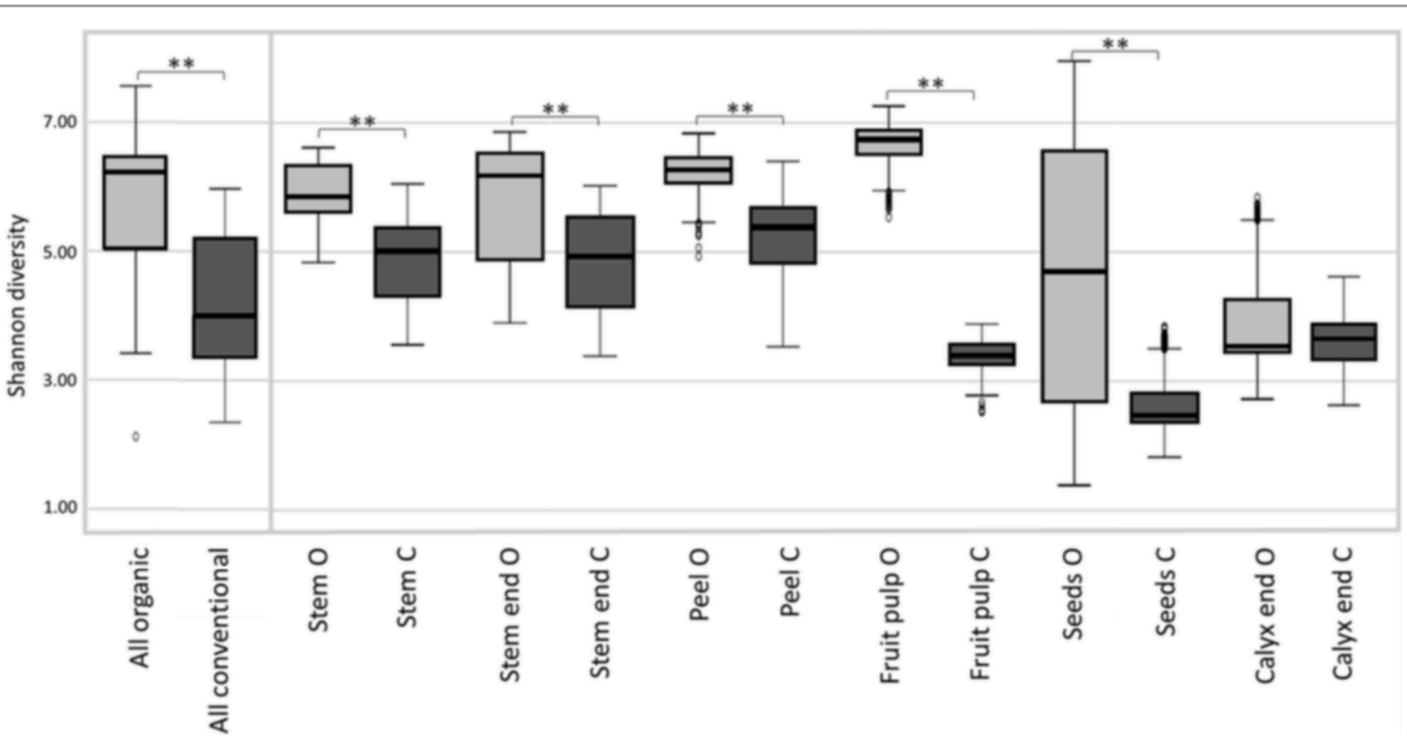


FIGURE 2 | Microbial diversity estimates of organically and conventionally managed apples and apple tissues. Suffixes O and C of carposphere tissue in the bottom legend, denote for organic and conventional management, respectively. Significant differences in Shannon diversity estimates of the apple management analogs are indicated by brackets and asterisks.

- Combined boxplot? The tissue samples are not independent!
- Why are there outliers?

Fig. 3 - PCoA plots

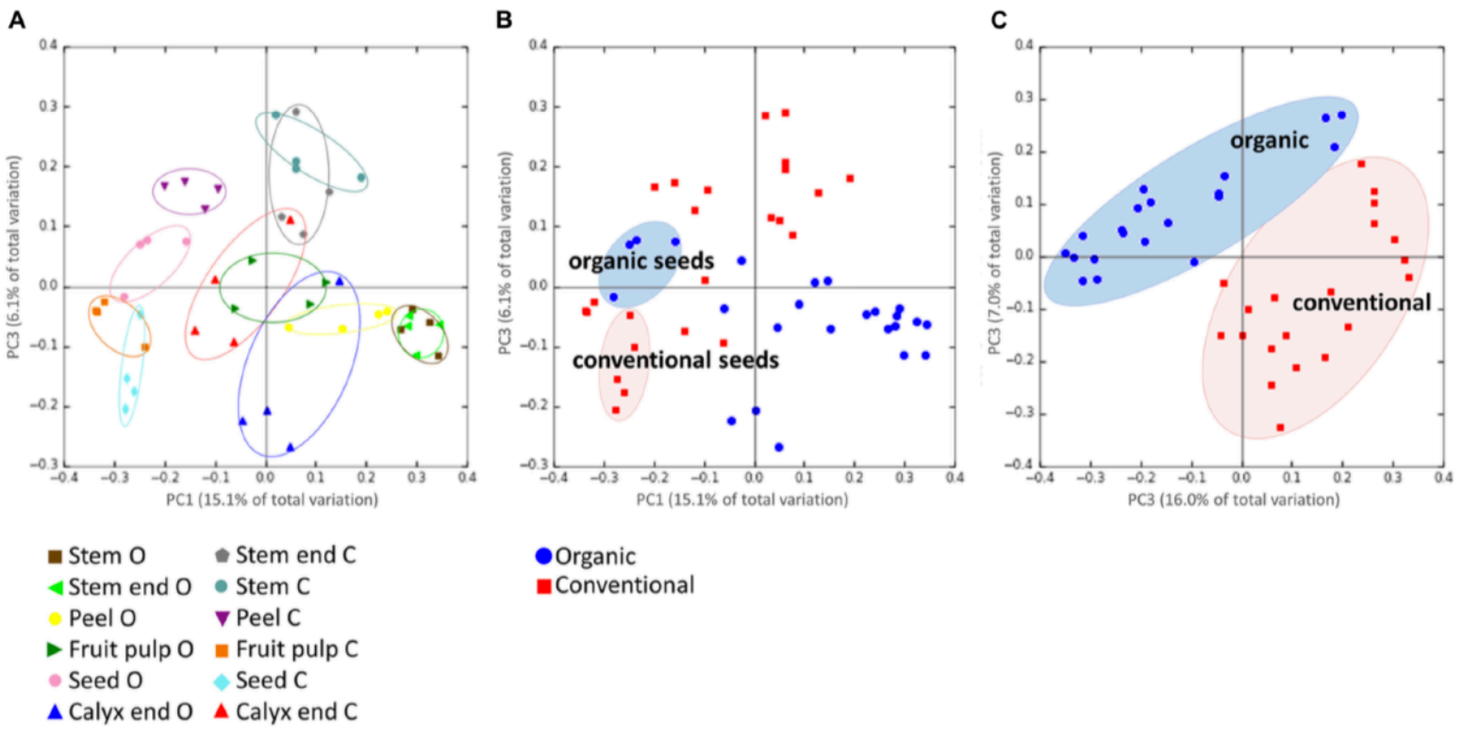


FIGURE 3 | Beta-diversity analysis on microbiota composition dependencies. Panel **(A)** shows the microbiota composition grouped by the tissue of the respective management group, where O and C in the bottom legend denote for organically and conventionally managed apples, respectively. Panel **(B)** visualizes composition of all tissue replicates, colored by organic (blue circles) and conventional (red squares); seeds of organically and conventionally managed apples are highlighted. In Panel **(C)**, same dataset is shown but seed samples of both management groups were excluded. PCoA plots are based on unweighted UniFraq distance matrix.

- Where are the clusters
- Why PC1 & PC3?
- Typos in the figure?

Fig. 4 - Taxonomic network

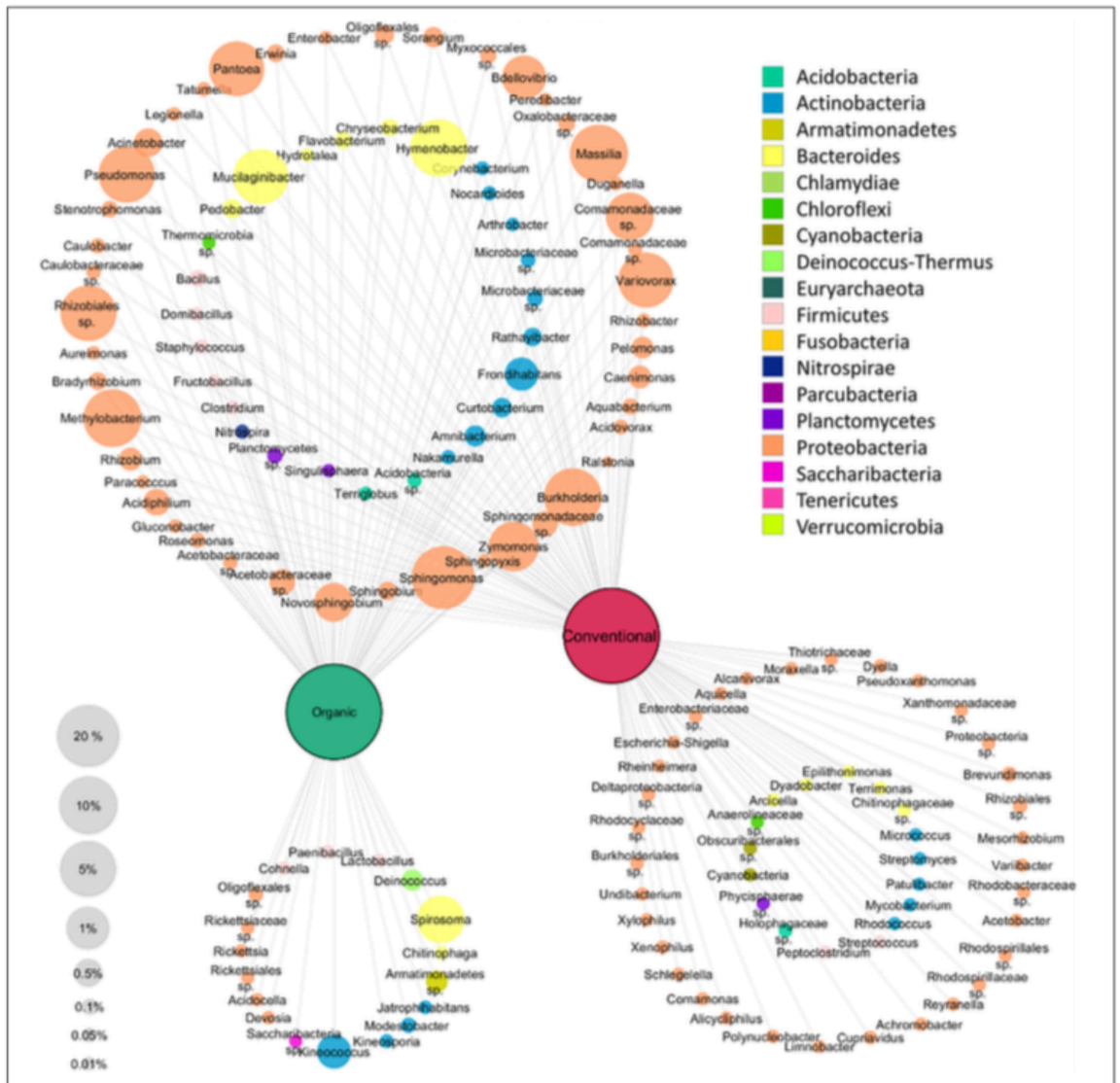


FIGURE 4 | Core and specific microbiota for organic and conventional apples. Core microbiota (taxa occurring in 75% of all replicates) of each management group (conventional and organic) were combined for network analysis. To be included, taxa had to exhibit at least 0.01% abundance in the whole dataset. Node size correspond to relative abundance in the dataset as denoted in the legend on the bottom left, node labels display taxonomic identification of OTUs on genus level wherever possible and node color indicates appropriate phylum, as described in the legend on the top right.

- Where is the network?
- Why are we looking at pooled samples?

Fig. 5 - Taxonomic composition

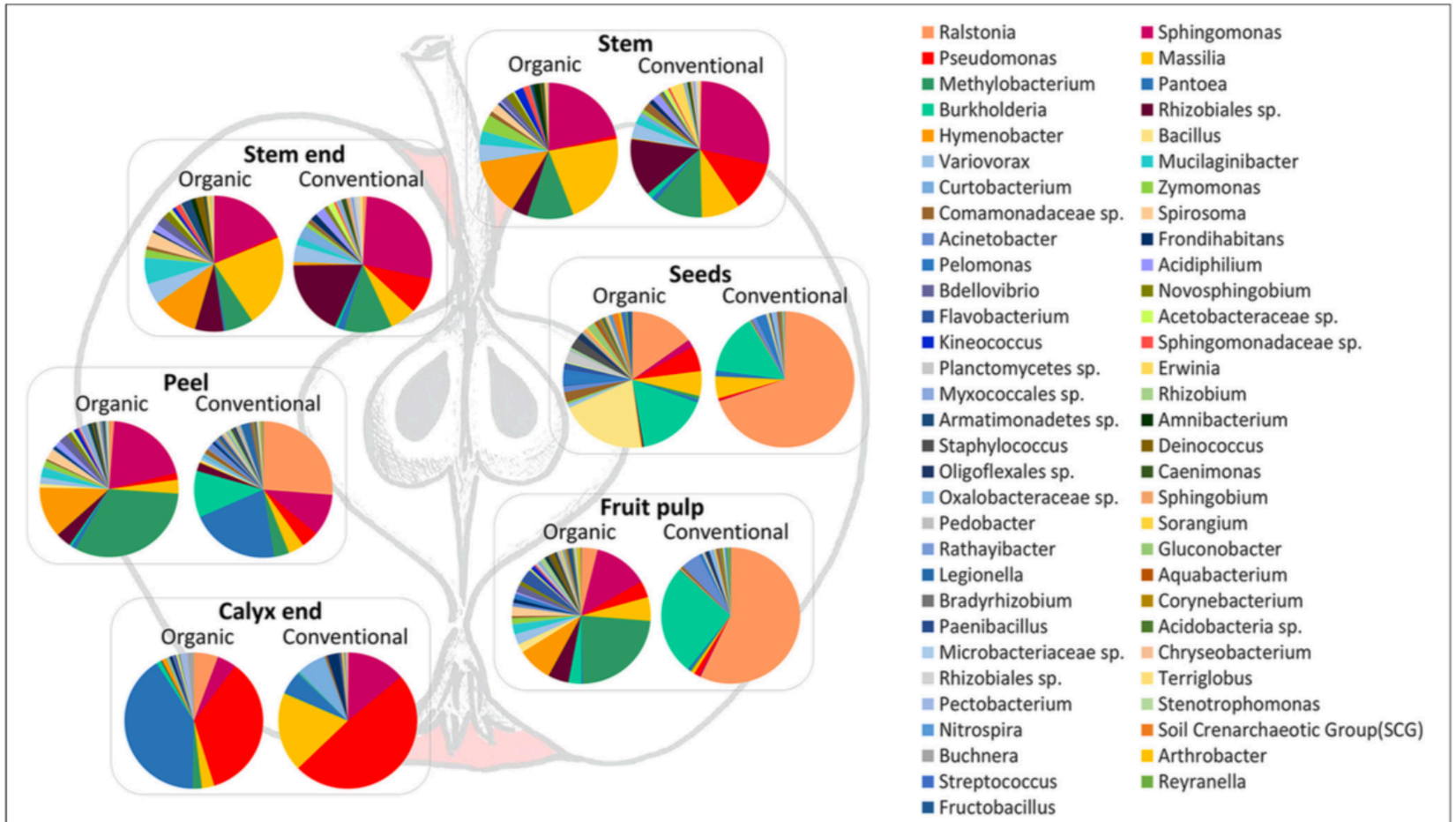


FIGURE 5 | Taxonomic composition of organic and conventional apple tissue microbiota. Pie charts visualize taxa occurring in the core microbiomes of each tissue, with at least 0.1% abundance in the whole dataset, and visualize differences between conventional and organic apples.

- Pie charts with pooled replicates?

Fig. 6 - Relative abundance for the order Enterobacterials

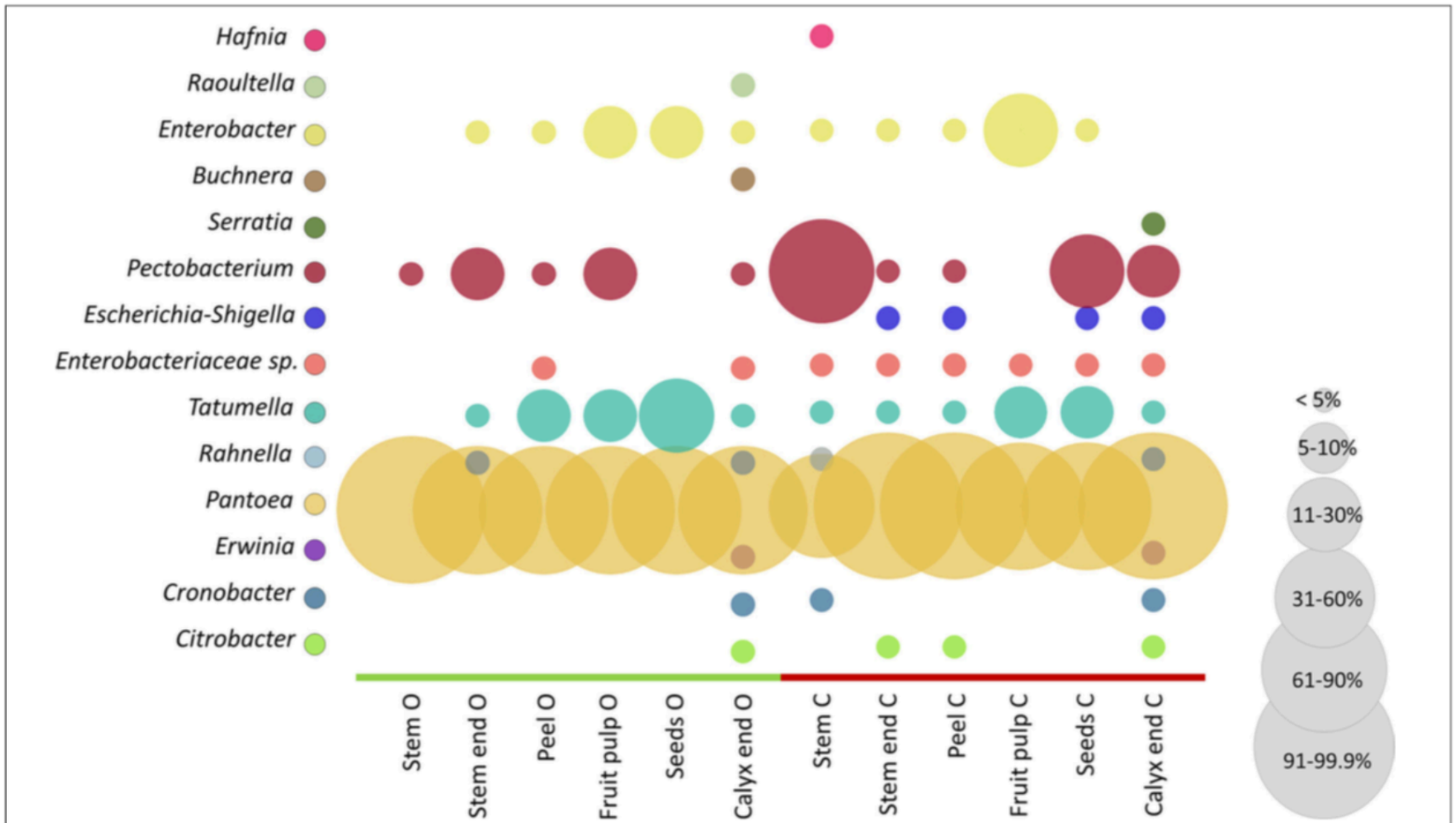


FIGURE 6 | Comparison of conventional and organic apple tissues regarding *Enterobacteriales* abundance. Color code for bubbles is depicted in the legend on the left and bubble size indicates relative abundance of taxa within total *Enterobacteriales* microbiota, as explained in the legend on the right. The abbreviations O and C denote for organically and conventionally managed apple tissues, respectively.

- This is diffcult to read?
- Where are the differences?

Fig. 7 - Bacterial Colonization

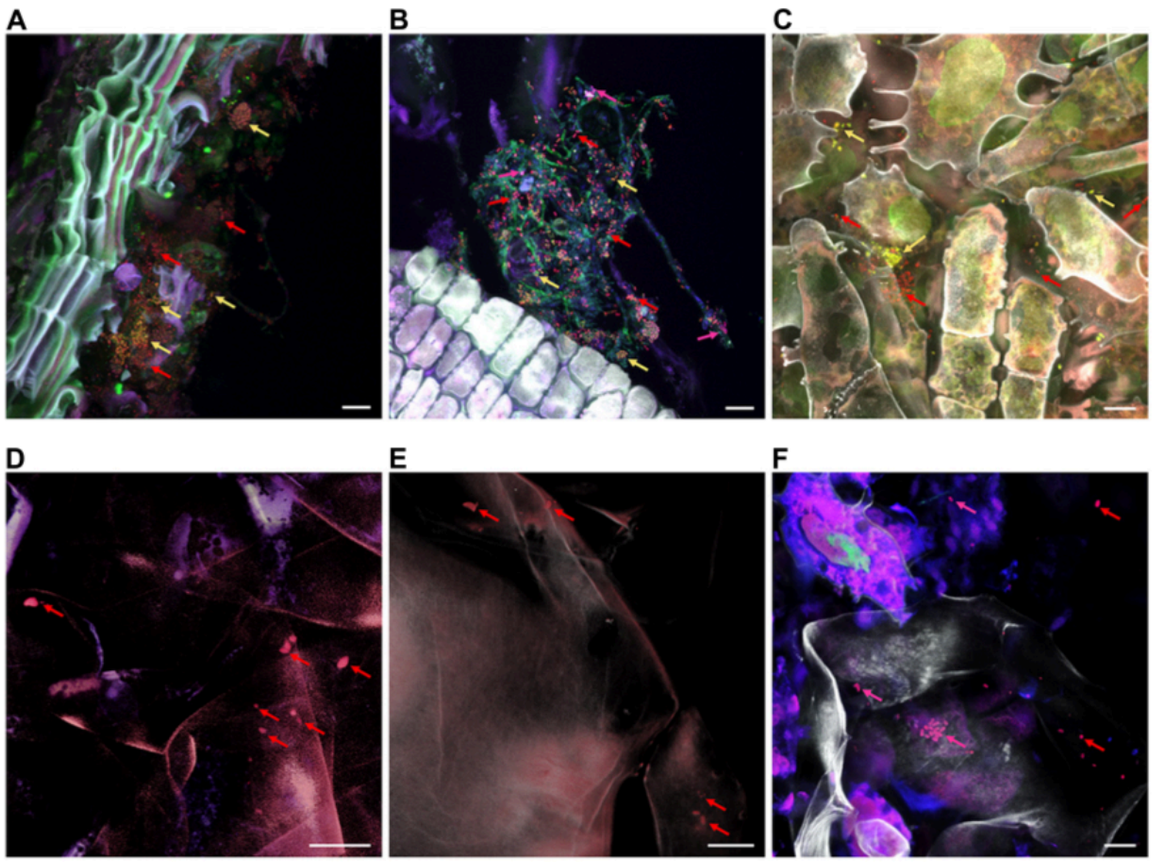


FIGURE 7 | FISH-CLSM micrographs showing bacterial colonization of organic apple tissues. Panels **(A–F)** visualize stem, stem end, peel, fruit pulp, seeds and calyx end samples, respectively. Bacteria were stained with FISH probes specific for *Gammaproteobacteria* (fluorescing pink and indicated by pink arrows), *Firmicutes* (yellow) and remaining bacteria of other classes (red); host structures are fluorescing white. Bar on the bottom right of each panel denotes for 10 μm.

- What should I see here?

Sample Design

Sample Preparation

Organic versus Conventional ?

Organically managed apples originated from an organic orchard, which follows the international “demeter” guidelines for organic farming, using sterile gloves and instruments. Conventional apples originated from a conventional orchard in Styria. In contrast to the organically produced apples, they underwent the following post-harvest treatments: directly after harvest, apples were short-term stored under controlled atmosphere (1–2°C, 1.5–2% CO2), washed and wrapped in polythene sheets for sale. Both apple management groups (“organic” and “conventional”) were transported to laboratory immediately and processed under sterile conditions.

Freshly Picked versus **Supermarket**

GDC Genetic Diversity Centre Zurich

An Apple a Day: Which Bacteria Do We Eat With **Organic** and **Conventional** Apples?

Organinc

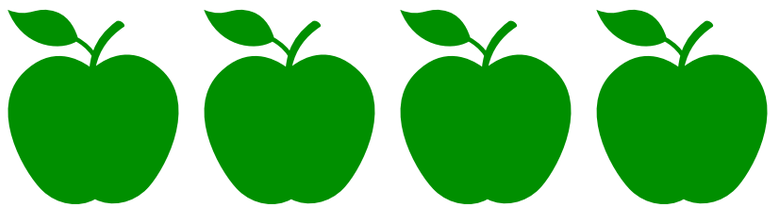
?

Non-Organinc

Are you kidding me?

Material and Methods

Four apples, weighing 190 ± 5 g, were selected from each of the **two management groups** and each apple was divided into **six tissues** with the following weights: stem: 0.2 g, stem end: 2 g, peel: 9 g, fruit pulp: 12 g, seeds: 0.2 g, and calyx end: 3 g. Thus, each tissue was represented by four replicates, where each replicate consists of the respective tissue of one apple.



stem

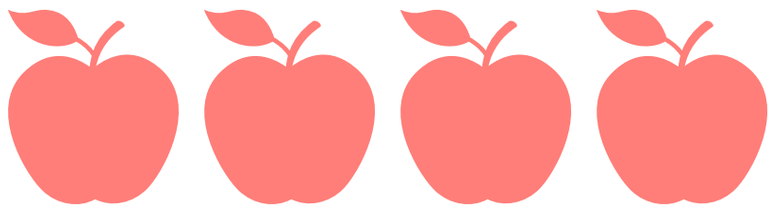
stem end

peel

fruit pulp

seeds

calyx



stem

stem end

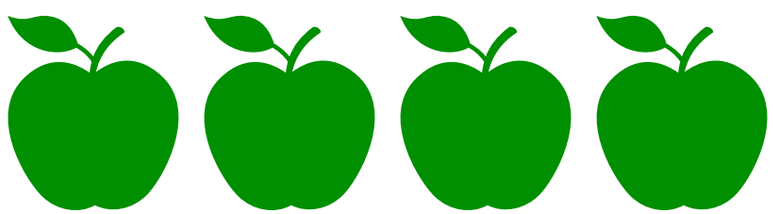
peel

fruit pulp

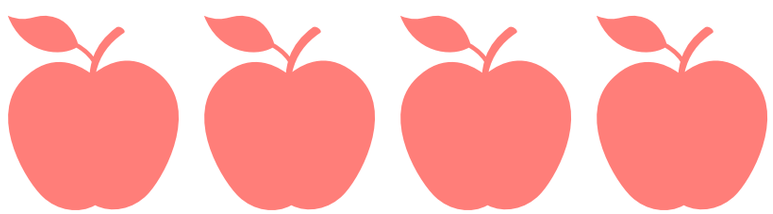
seeds

calyx

$$6 \text{ tissue} \times 2 \text{ treatments} \times 4 \text{ replicates} = 48 \text{ samples}$$



stem (0.2g)
stem end (2g)
peel (9g)
fruit pulp (12g)
seeds (0.2g)
calyx (3g)



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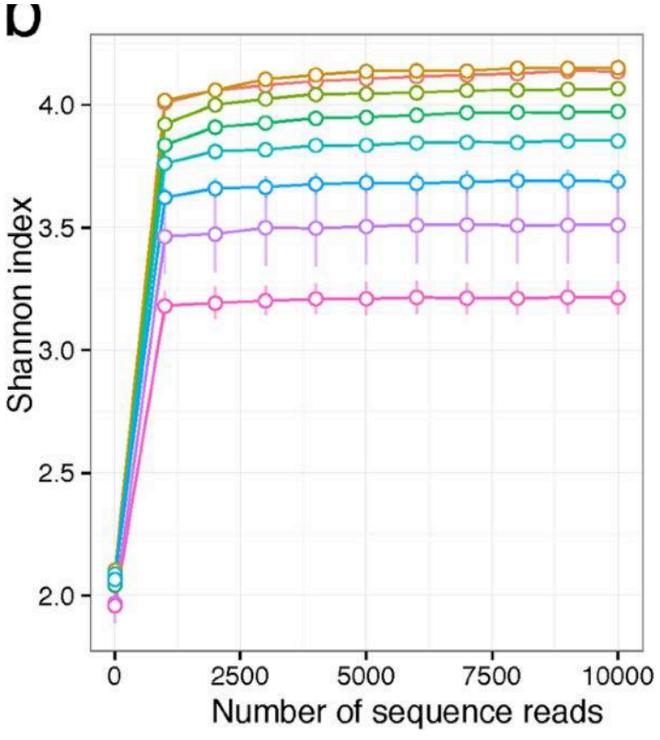

Multinu et al. (2018). Systematic Bias Introduced by Genomic DNA Template Dilution in 16S rRNA Gene-Targeted Microbiota Profiling in Human Stool Homogenates. mSphere, 3(2).

contaminants

stem end
peel
fruit pulp

+4ml NaCl
blender

2ml

Negative Controls?

calyx

stem
seeds

+?ml ?NaCl
mortar

2ml

contaminants

Correction for Unequal Sequencing Depth

Results

After removing chimeric, mitochondrial and chloroplast sequences, the overall bacterial community of all apple samples, assessed by 16S rRNA gene amplicon sequencing, contained **6,711,159 sequences** that were assigned to 92,365 operational taxonomic units (OTUs).

Expected number of sequences (counts) per sample

$$\frac{6'711'159 \text{ counts}}{48 \text{ samples}} = 139'815 \text{ counts/sample}$$

Material and Methods

OTU tables were rarefied to **1,525** sequences per sample, according to the sample with lowest amount of sequences. Rarefied OTU tables served as input matrix for upcoming alpha and beta diversity analyses and according statistics were calculated in QIIME. Beta diversity, based on unweighted UniFraq distance matrix, was visualized by Principle Coordinates Analysis (PCoA) and statistical significance was calculated by Analysis of Similarity (ANOSIM).

Used number of sequences

$$1'525 \times 48 = 73'200 \rightarrow \frac{100}{6'711'159} * 73'200 = \ 1.09(\%)$$

Well done !

You succefully removed 99% of the data.

Raw Data

GDC Genetic Diversity Centre Zurich

Microbial DNA Extraction and Amplicon Library Construction

For culture-independent **Illumina MiSeq** v2 (**250 bp paired end**) amplicon sequencing, the primers 515f – 806r (Caporaso et al., 2010) were used to amplify the 16S rRNA gene using three technical replicates per sample.

DATA AVAILABILITY

The raw sequence files supporting the findings of this manuscript are available from the European Nucleotide Archive (**ENA**) at the study Accession Number: **PRJEB32455**.

ENA Beta
European Nucleotide Archive

PRJEB32455 Search

Examples: histone, BN000065

Enter accession View

Examples: Taxon:9606, BN000065, PRJEB402

Home | Submit | Search | Rulespace | About | Support

You are using the new ENA Browser. To see the corresponding view in the old ENA Browser, please click https://www.ebi.ac.uk/ena/data/search?query=PRJEB32455

Text Search

Uses EBI Search to perform a free text search across ENA data. For more detailed usage please refer to the help & documentation section.

Search term: PRJEB32455 Search

Search results for PRJEB32455

- **Read**
  - Experiment (48)
  - Run (48)
- **Study**
  - Study (1)
  - Study (Sequence) (1)

Experiment View all 48 results.

ERX3372260	Illumina MiSeq sequencing

Run View all 48 results.

ERR3347719	Illumina HiSeq 1000 sequencing

Study

ERP115147	Investigating the apple microbiome and the impacts of organic and conventional management practices

Study (Sequence)

PRJEB32455	Investigating the apple microbiome and the impacts of organic and conventional management practices

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ERX3372263: Illumina MiSeq sequencing

1 ILLUMINA (Illumina MiSeq) run: 158,404 spots, 45.9M bases, 15.8Mb downloads

Submitted by: Graz University of Technical

Study: Investigating the apple microbiome and the impacts of organic and conventional management practices

PRJEB32455 • ERP115147 • All experiments • All runs

show Abstract

Sample: Calyx end Organic 4

SAMEA5670130 • ERS3474137 • All experiments • All runs

Organism: plant metagenome

Library:

Name: unspecified

Instrument: Illumina MiSeq

Strategy: AMPLICON

Source: GENOMIC

Selection: PCR

Layout: SINGLE

Runs: 1 run, 158,404 spots, 45.9M bases, 15.8Mb

Run	# of Spots	# of Bases	Size	Published
ERR3347689	158,404	45.9M	15.8Mb	2019-08-19

ID: 8872040

Boxplots

Quantitative Records of Diversity Estimates of Apple Microbiota

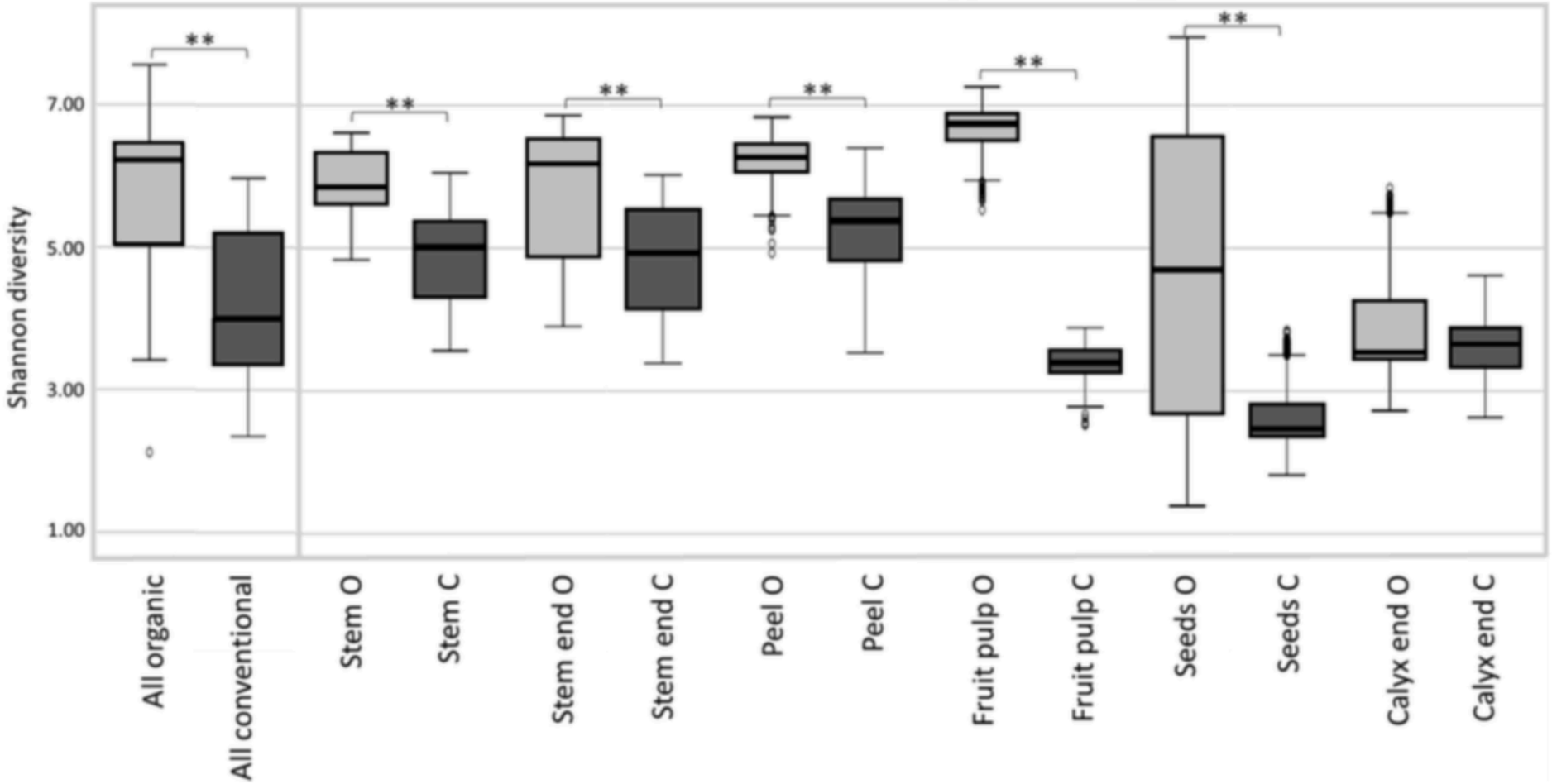
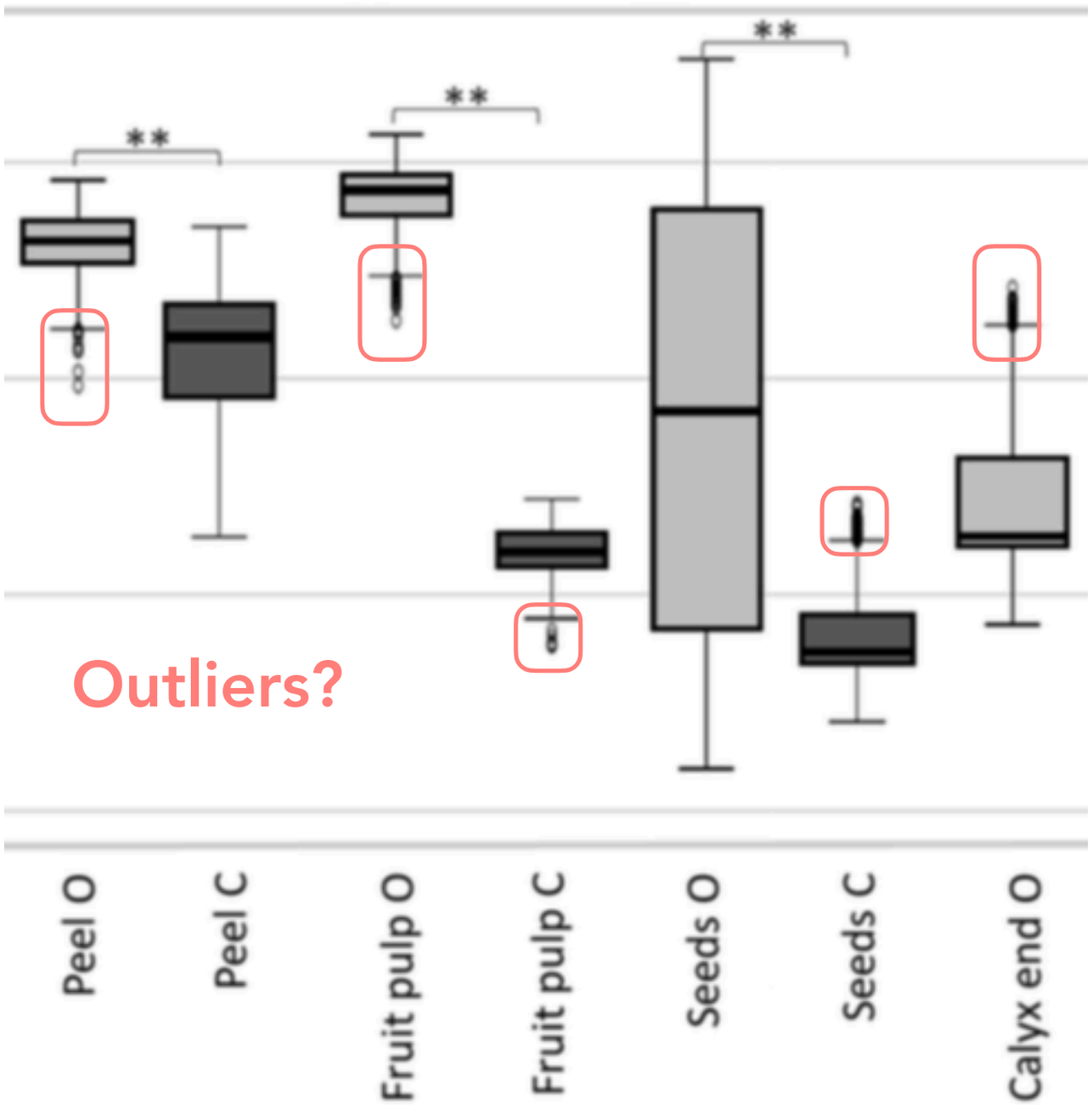
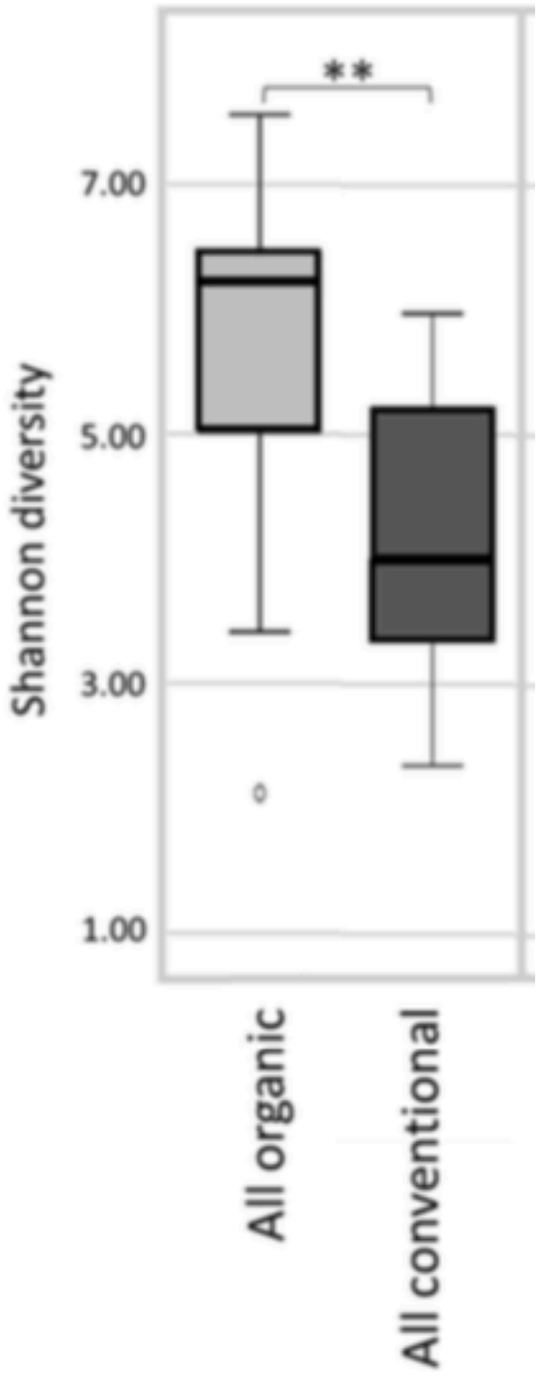


FIGURE 2 | Microbial diversity estimates of organically and conventionally managed apples and apple tissues. Suffixes O and C of carposphere tissue in the bottom legend, denote for organic and conventional management, respectively. Significant differences in Shannon diversity estimates of the apple management analogs are indicated by brackets and asterisks.



How many samples per tissue were used?



- The combined samples are not all independent. The different tissue can originate from the same apple.
- The distribution of the samples is not the same.

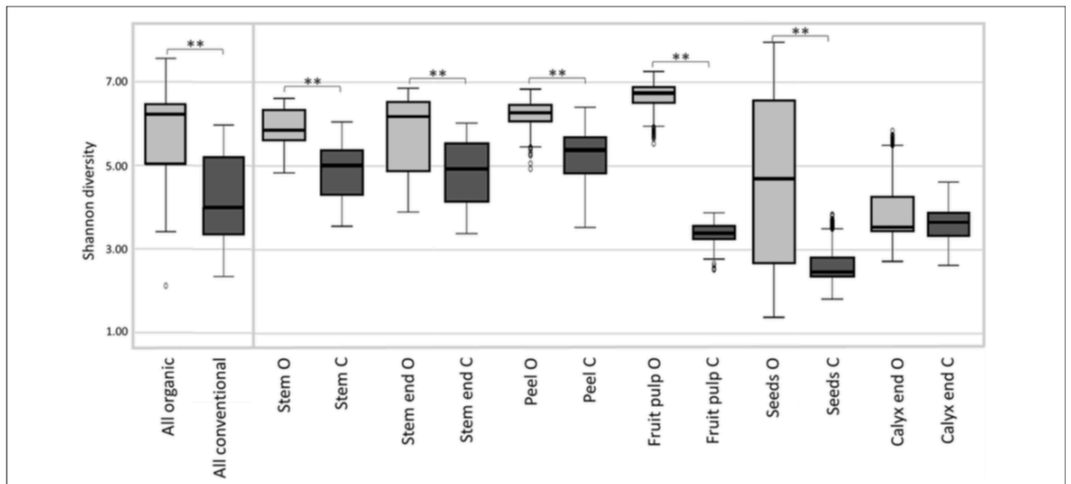


FIGURE 2 | Microbial diversity estimates of organically and conventionally managed apples and apple tissues. Suffixes O and C of carposphere tissue in the bottom legend, denote for organic and conventional management, respectively. Significant differences in Shannon diversity estimates of the apple management analogs are indicated by brackets and asterisks.

We recommend always **indicating the sample size** and avoiding notches unless they fall entirely within the IQR.

Krzywinski_ & Altman (2014) Visualizing samples with box plots. Nature Methods. Vol.11 No.2.

The Kruskal–Wallis test does NOT assume that the data are normally distributed; that is its big advantage. If you're using it to test whether the medians are different, it does assume that the observations in each group come from populations with **the same shape of distribution**, so if different groups have different shapes, the Kruskal–Wallis test may give inaccurate results. If you're interested in any difference among the groups that would make the mean ranks be different, then the Kruskal–Wallis test doesn't make any assumptions.

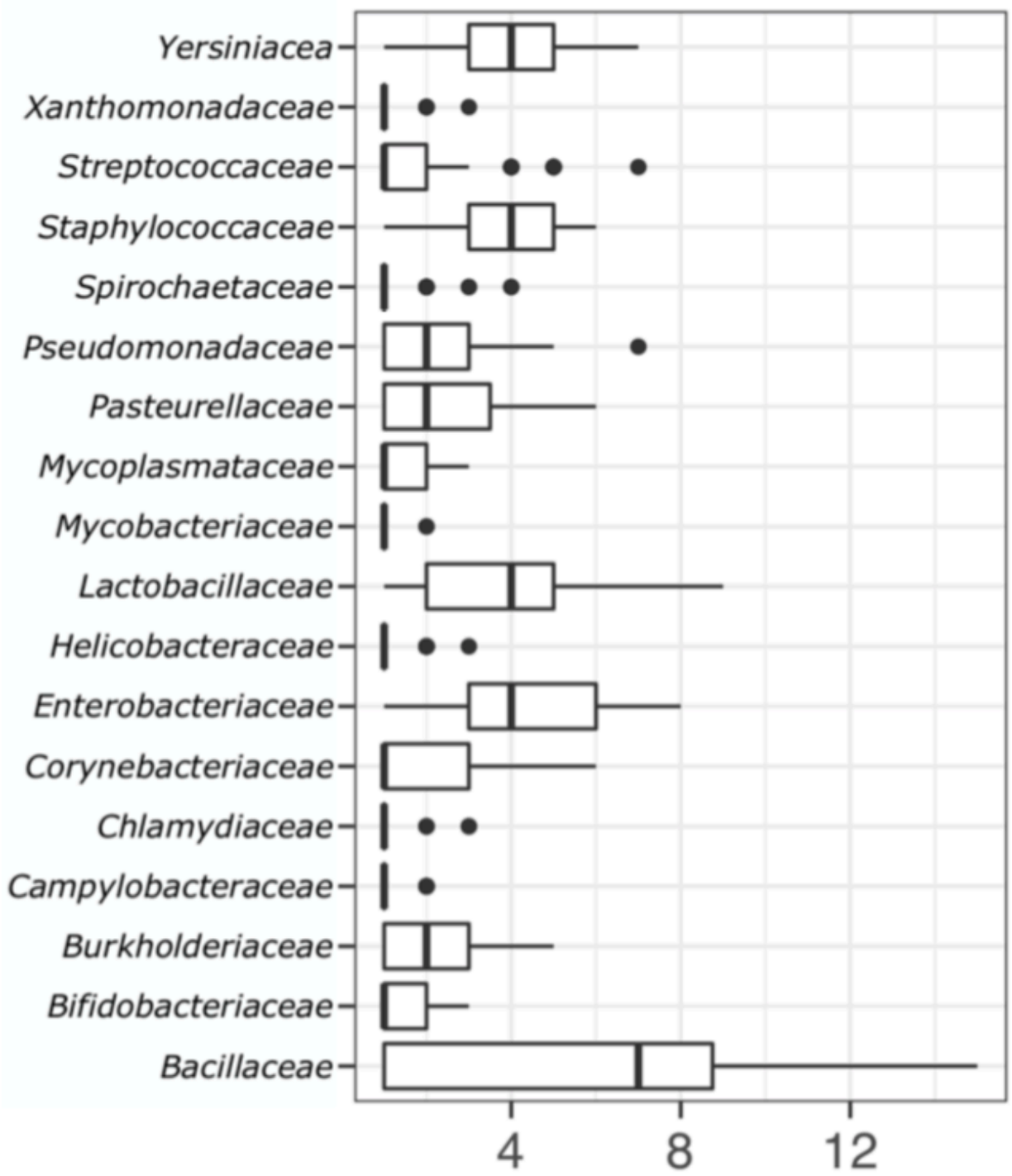
McDonald, J.H. 2014. Handbook of Biological Statistics (3rd ed.). Sparky House Publishing, Baltimore, Maryland.

Gene Abundance

≠

Bacteria Abundance

16S rRNA gene copy numbers vary among the bacterial species.



Koehorst et al. (2018) Expected and observed genotype complexity in prokaryotes: correlation between 16S-rRNA phylogeny and protein domain content. DOI:10.1101/494625

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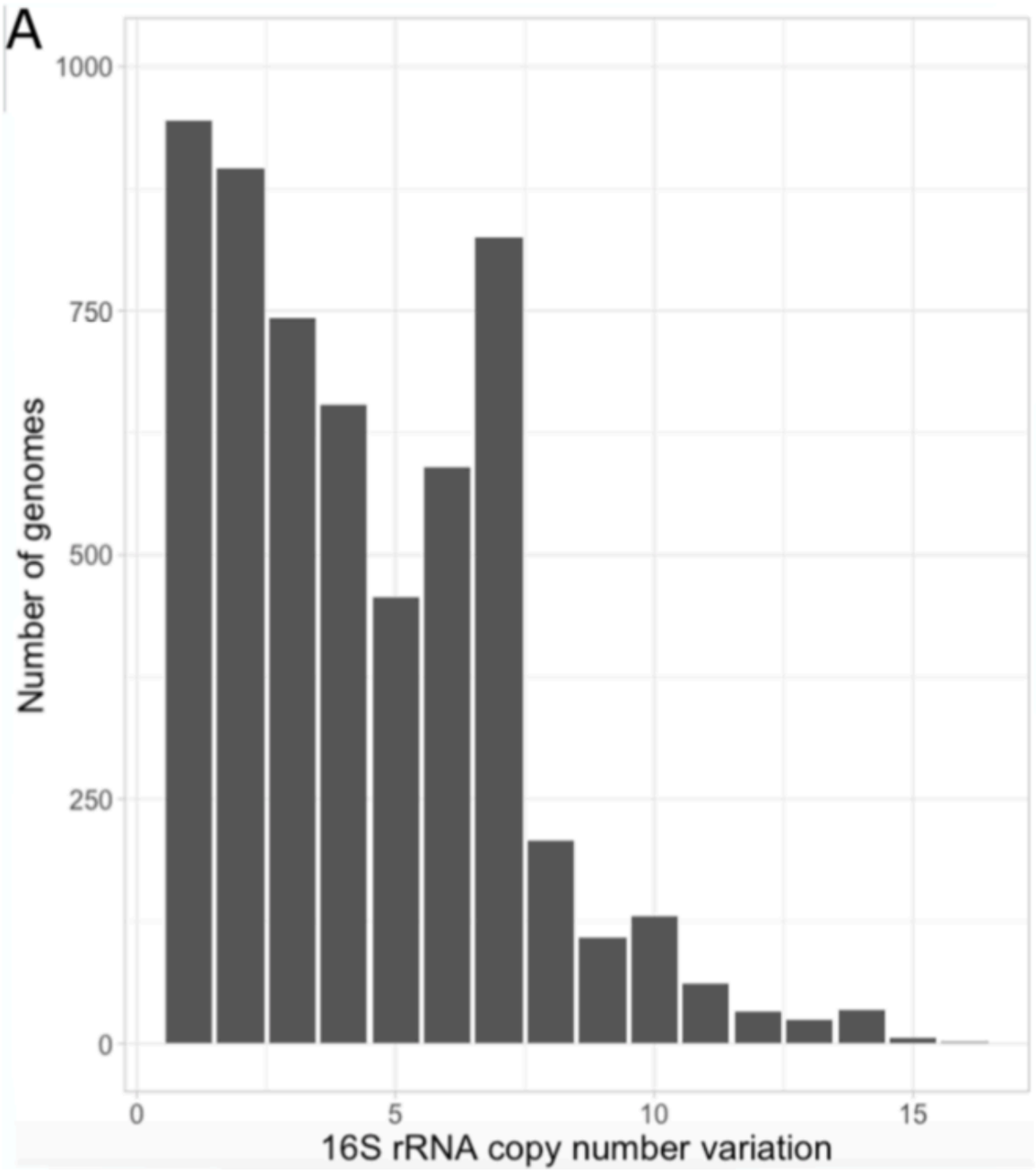
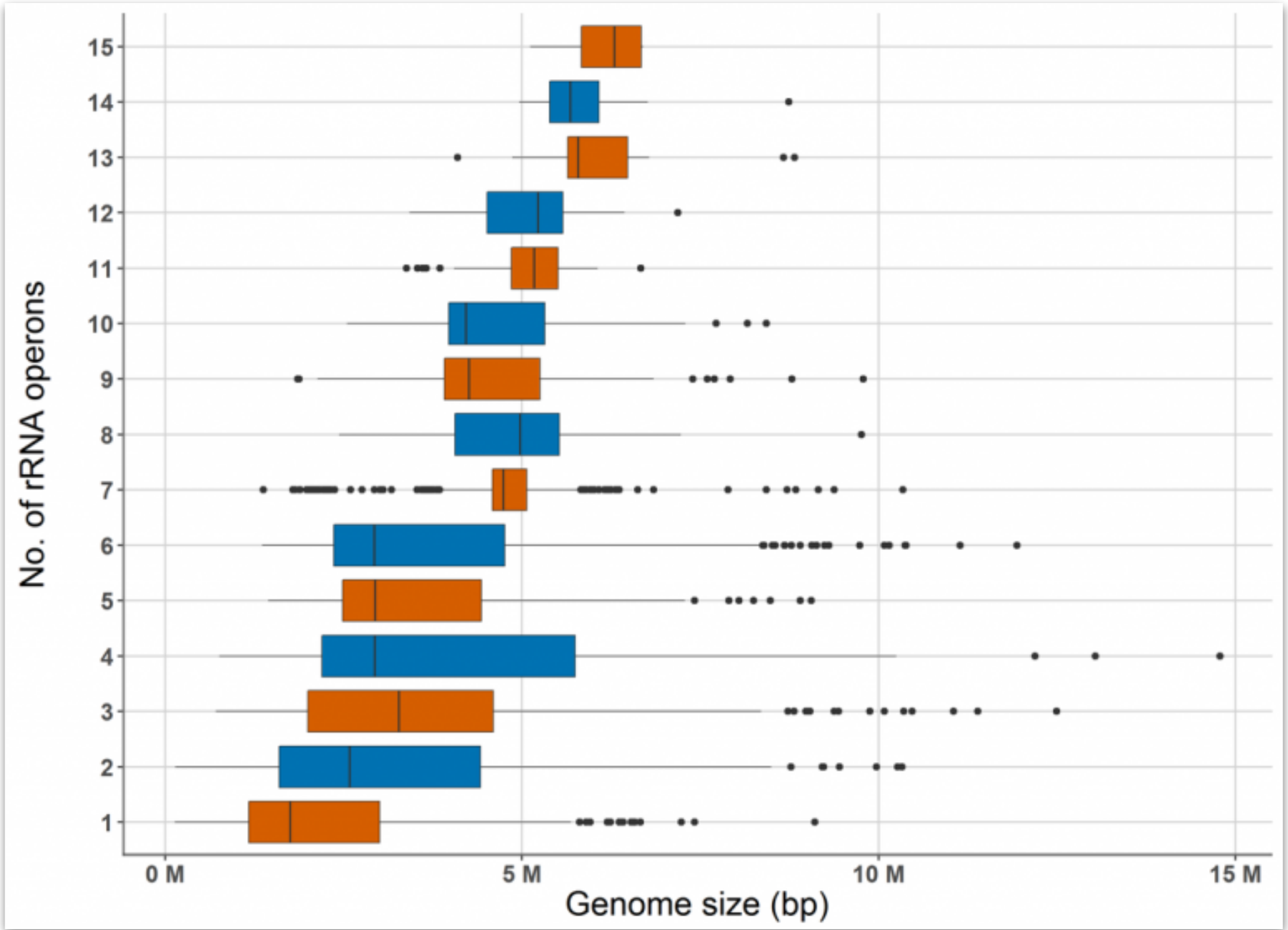


Koehorst et al. (2018) Expected and observed genotype complexity in prokaryotes: correlation between 16S-rRNA phylogeny and protein domain content. DOI:10.1101/494625

16S copy numbers of bacteria in EzBioCloud database



https://help.ezbiocloud.net/user-guide/microbiome-basics/16s-copy-number-correction/

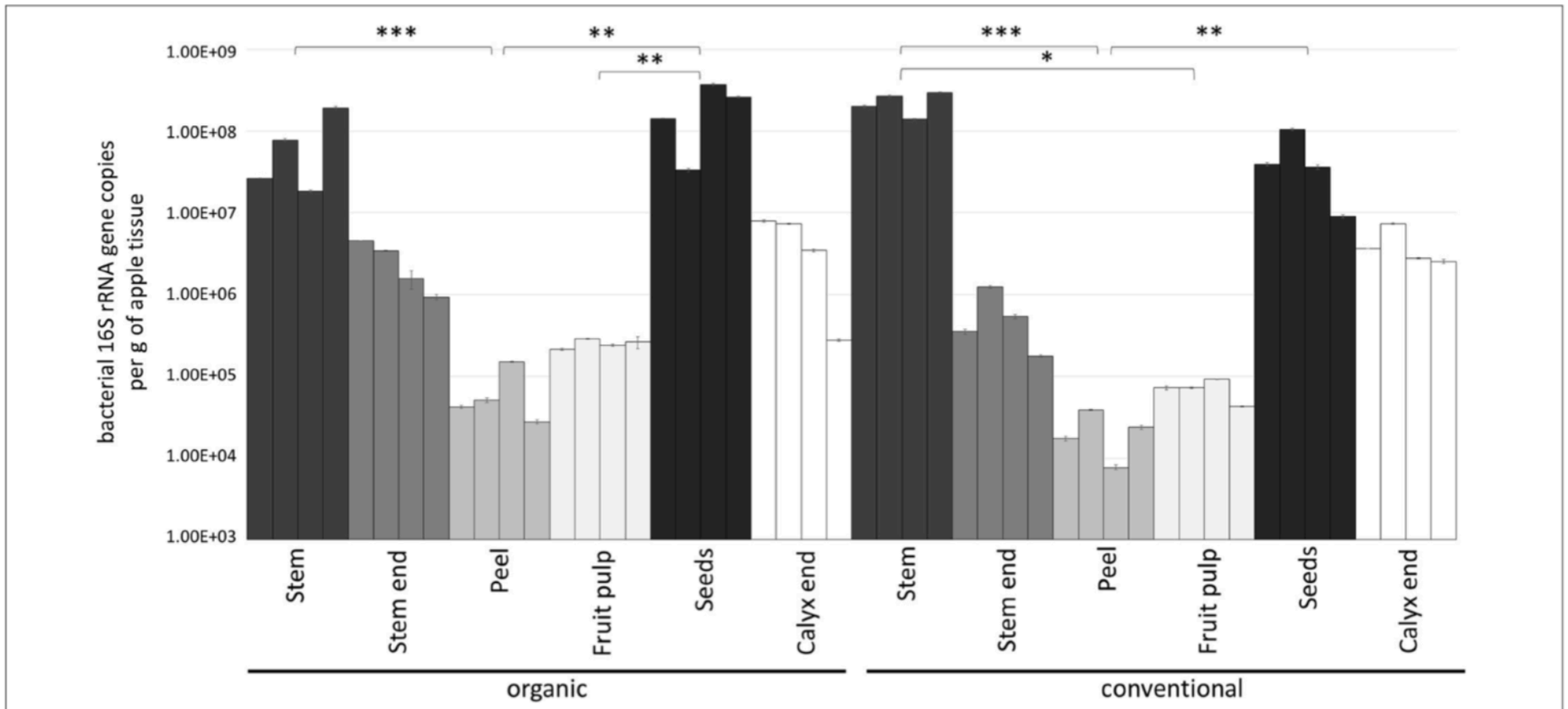


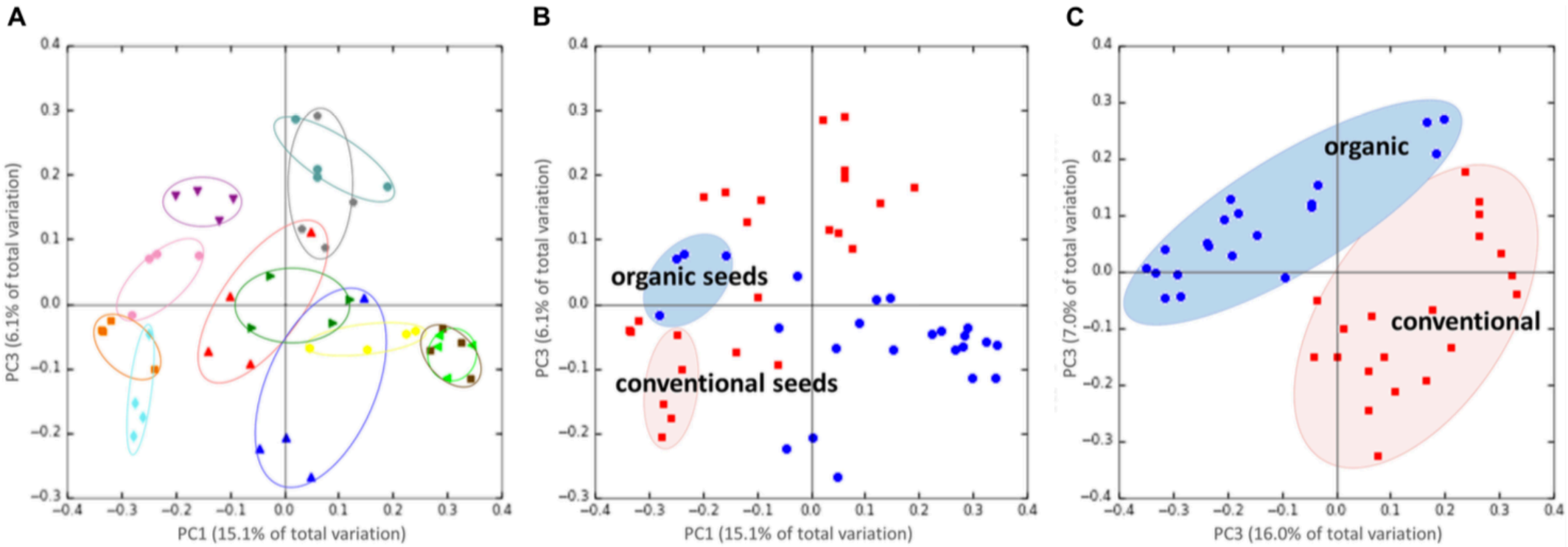
FIGURE 1 | Bacterial abundance in carposphere tissues of organically and conventionally managed apples. Microbial community abundance within each tissue was measured in four replicates by qPCR using PNAs to block mitochondrial and plastid 16S DNA. Asterisks indicate significant differences in 16S rRNA gene abundance (calculated per g of apple tissue) between the tissues within a management group.

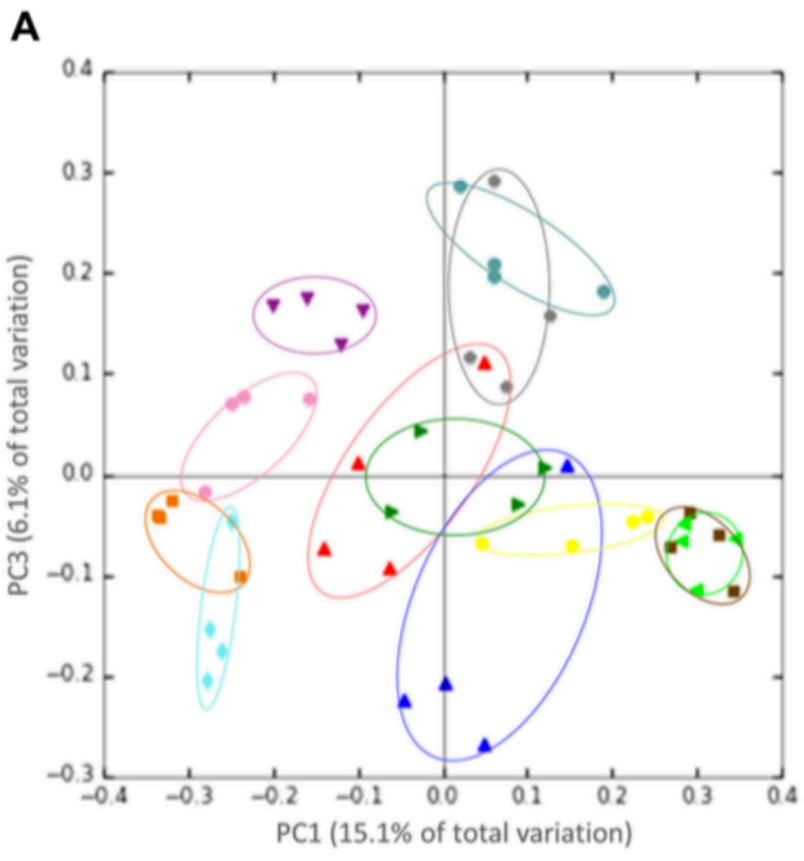
TABLE 1 | Significant differences in 16S rRNA gene abundance per gram of tissue between organically and conventionally managed apple tissues.

	Group1*	Group2*	Group1 mean	Group2 mean	*p*-Value
Organic tissues	Stem O	Peel O	7.91E+07 ± 6.99E+07	6.81E+04 ± 4.89E+04	0.001
	Peel O	Seeds O	6.81E+04 ± 4.89E+04	2.04E+08 ± 1.28E+08	0.002
	Fruit pulp O	Seeds O	2.51E+05 ± 2.80E+04	6.81E+04 ± 1.28E+08	0.004
Conventional tissues	Seeds C	Peel C	4.71E+07 ± 3.50E+07	2.18E+04 ± 1.12E+04	0.002
	Stem C	Peel C	2.28E+08 ± 6.16E+07	2.18E+04 ± 1.12E+04	0.001
	Stem C	Fruit pulp C	2.28E+08 ± 6.16E+07	6.96E+04 ± 1.76E+04	0.02

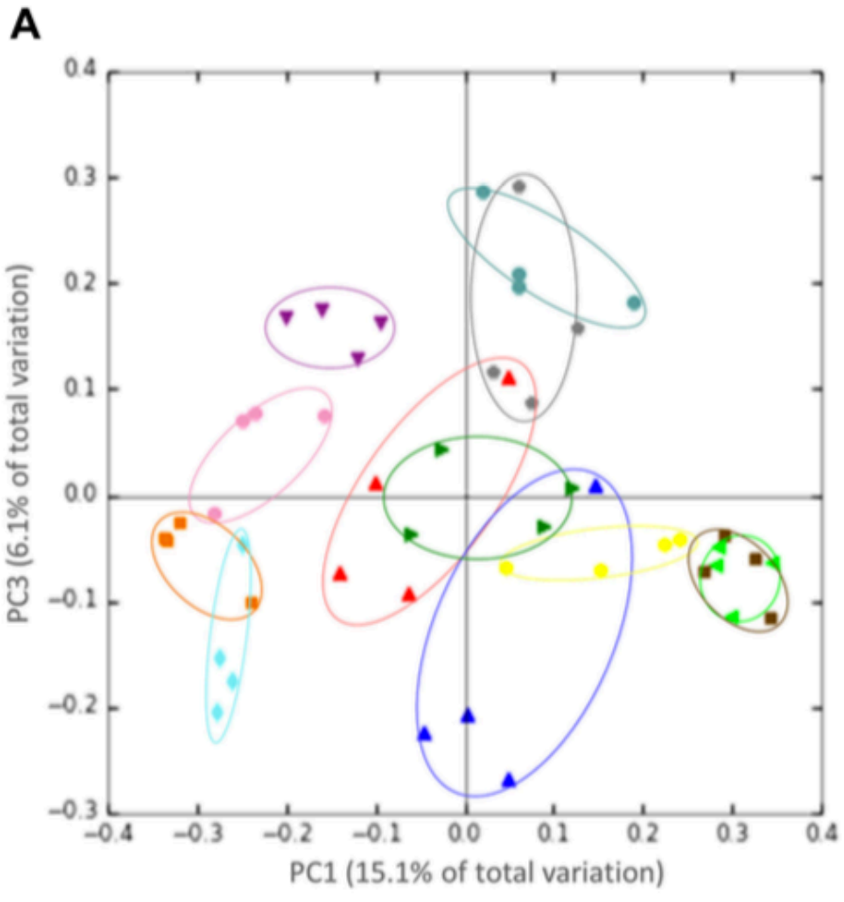
**O and C denote for organically and conventionally managed apples, respectively. Only significant differences in microbial abundance between apple tissues are listed.*

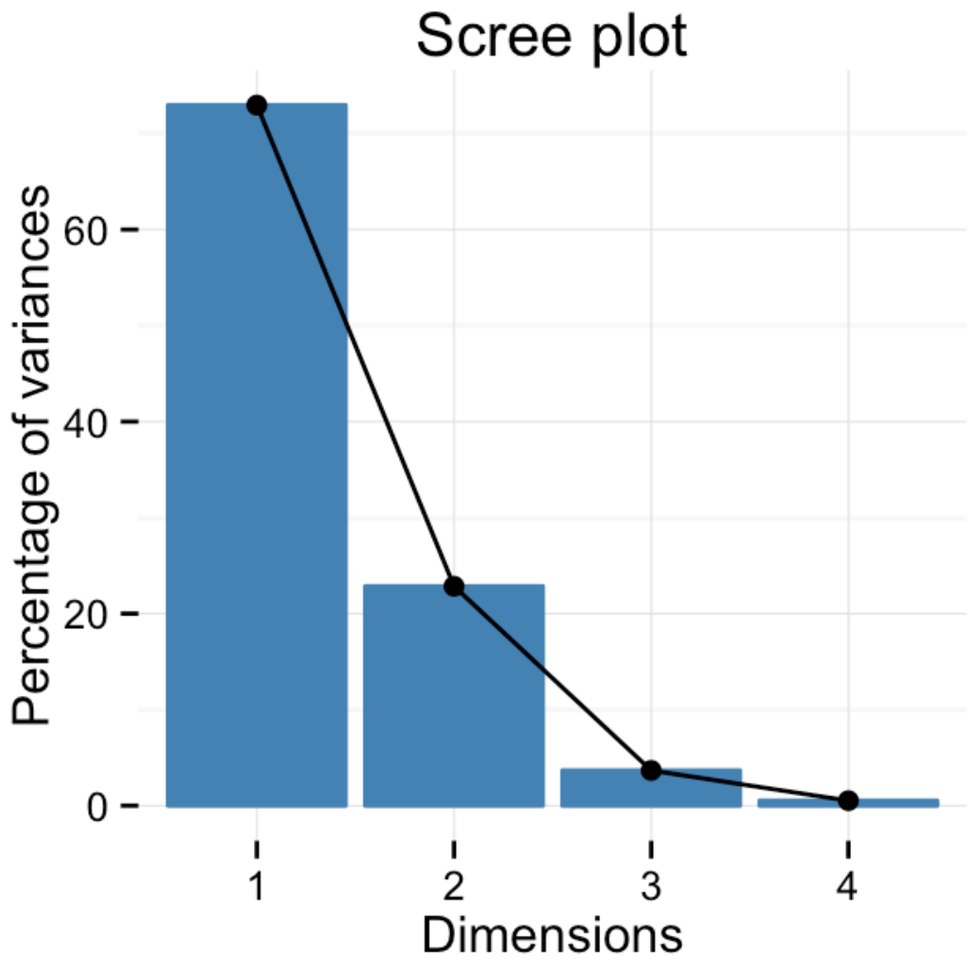
Principal Components


A
B
C
PC1 (15.1% of total variation)
PC3 (6.1% of total variation)
organic seeds
conventional seeds
organic
conventional
PC3 (16.0% of total variation)
PC3 (7.0% of total variation)

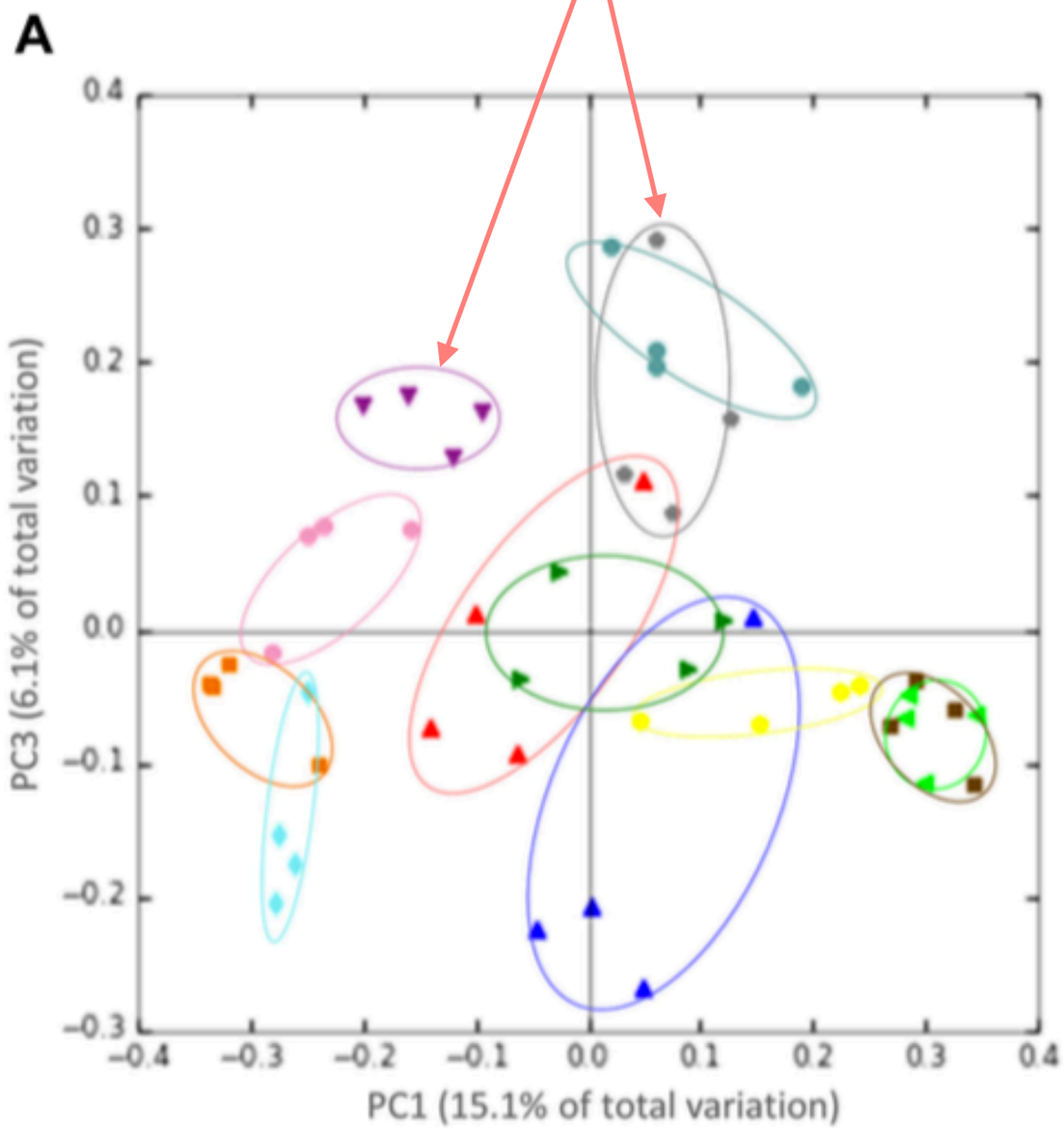


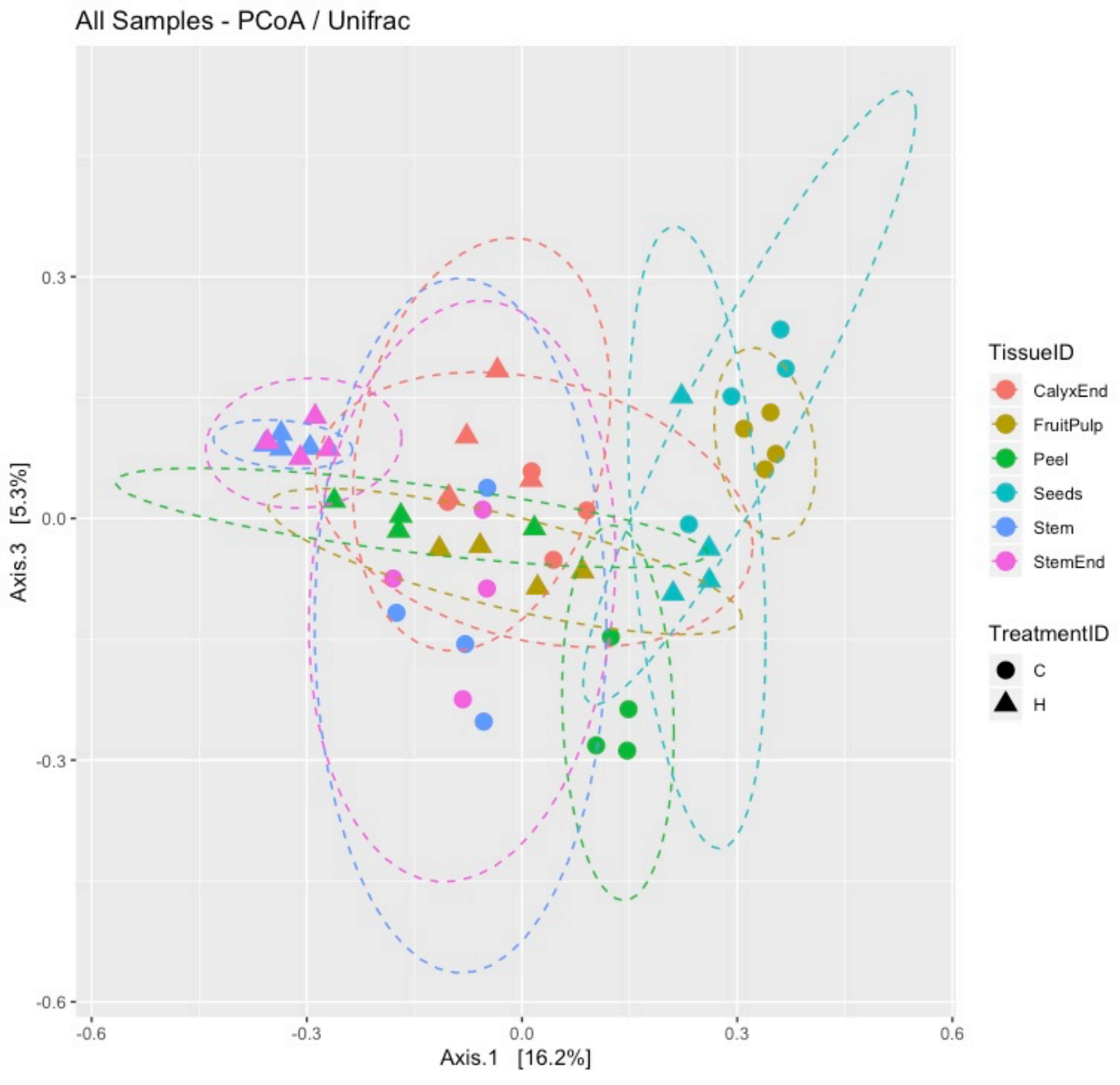

```
Explained Variance using PCoA with Unweighted Unifrac
=> PCA 1&2 = 29.0%
=> PCA 1&3 = 21.6%
=> PCA 1-3 = 34.3%

Explained Variance using PCoA with Weighted Unifrac
=> PCA 1&2 = 58.0%
=> PCA 1&3 = 48.7%
=> PCA 1-3 = 67.4%
```

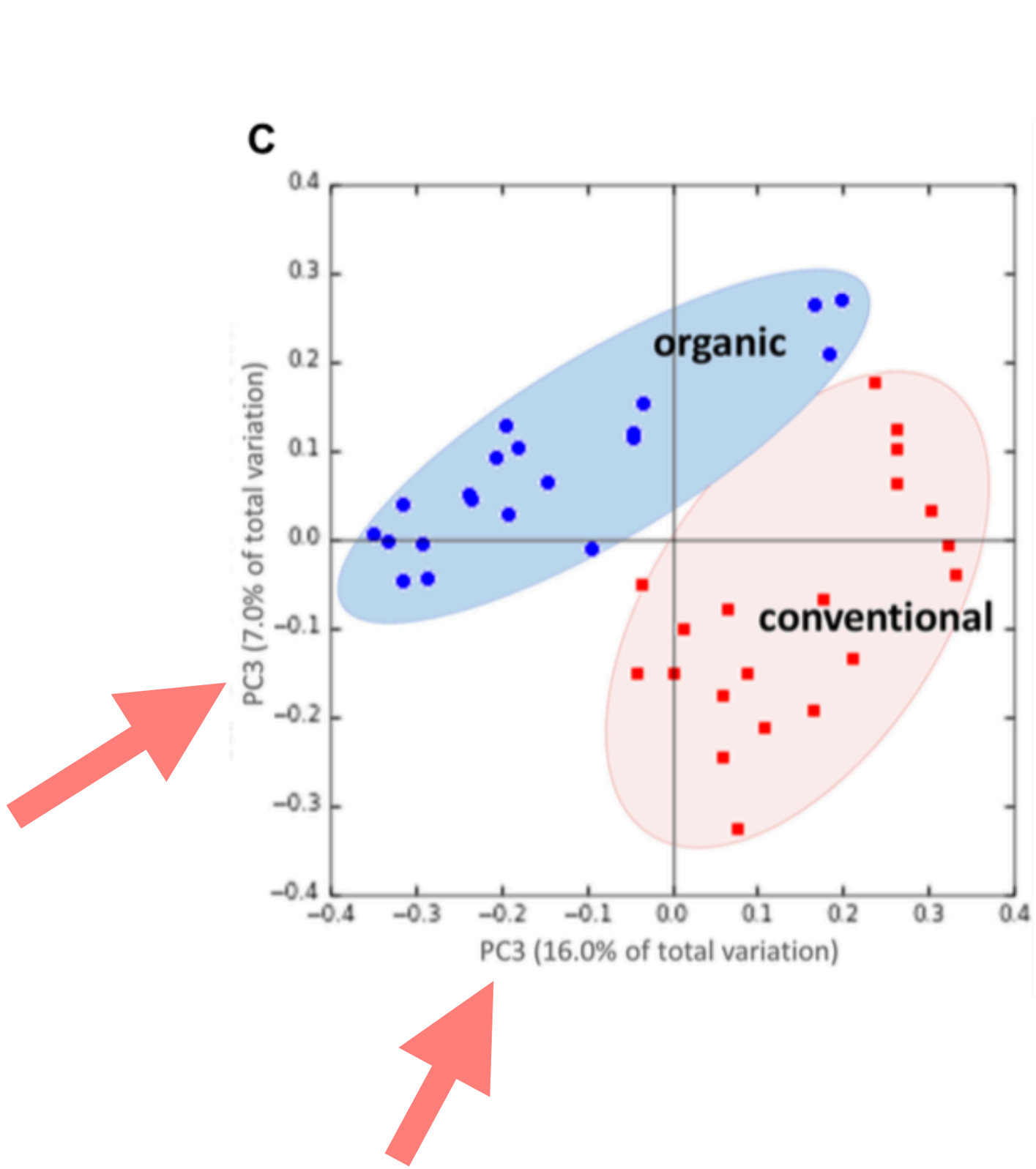

A
PC3 (6.1% of total variation)
PC1 (15.1% of total variation)
0.4
0.3
0.2
0.1
0.0
−0.1
−0.2
−0.3
−0.4
−0.3
−0.2
−0.1
0.0
0.1
0.2
0.3
0.4

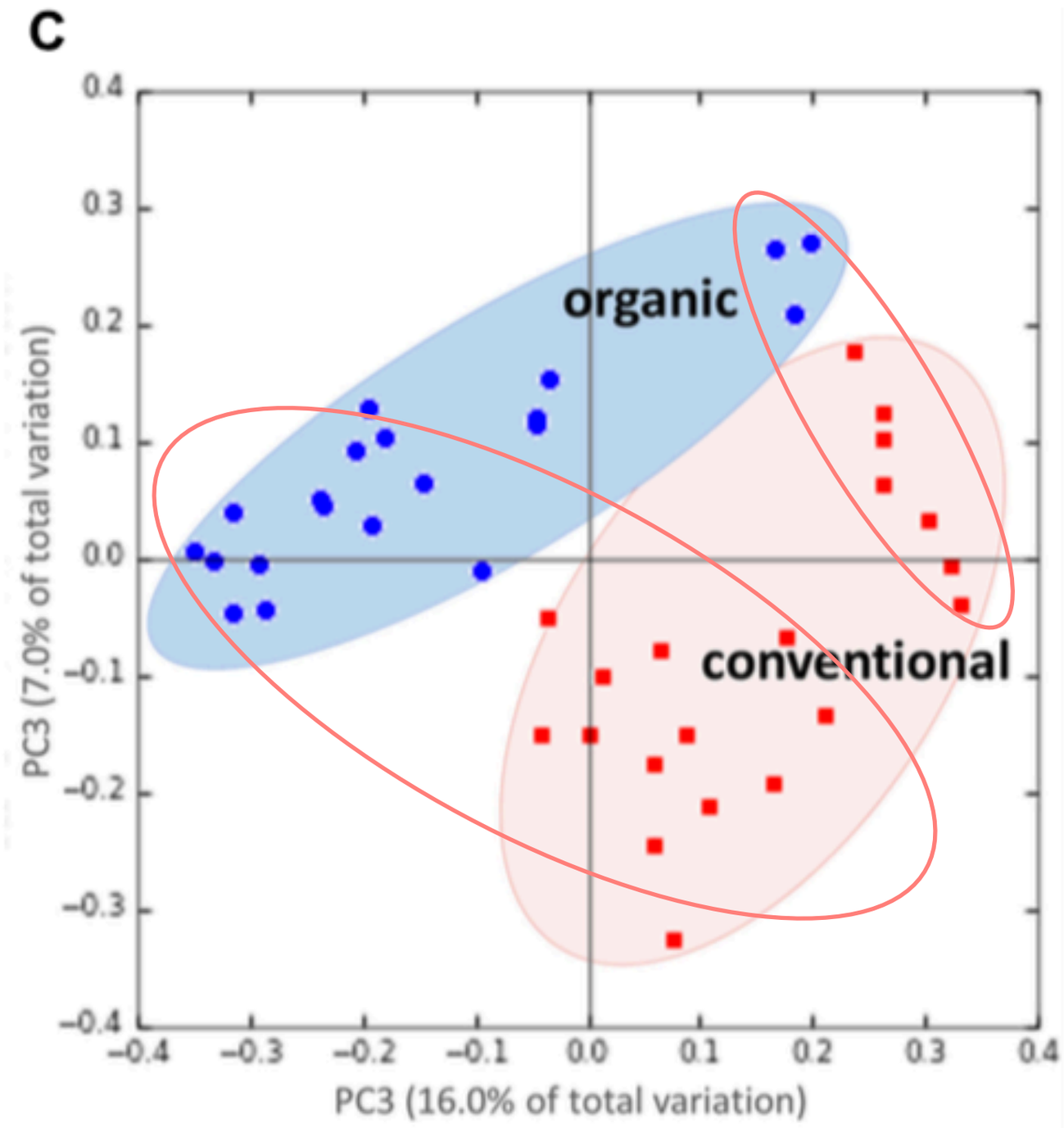

Scree plot
Percentage of variances
60
40
20
0
1
2
3
4
Dimensions

Photoshop?

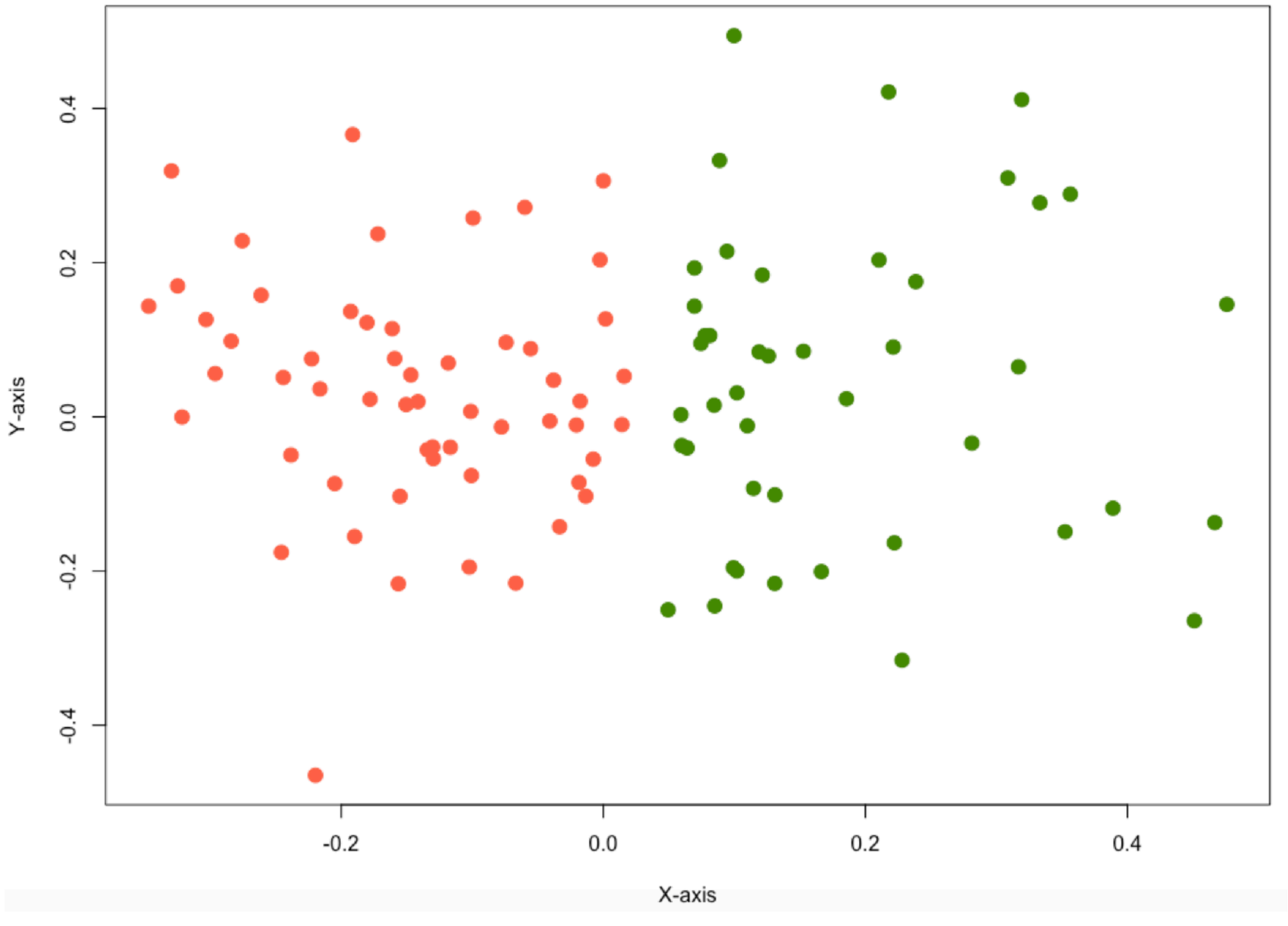

A
0.4
0.3
0.2
0.1
0.0
−0.1
−0.2
−0.3
PC3 (6.1% of total variation)
−0.4
−0.3
−0.2
−0.1
0.0
0.1
0.2
0.3
0.4
PC1 (15.1% of total variation)


All Samples - PCoA / Unifrac
0.3
0.0
-0.3
-0.6
Axis.3 [5.3%]
-0.6
-0.3
0.0
0.3
0.6
Axis.1 [16.2%]
TissueID
CalyxEnd
FruitPulp
Peel
Seeds
Stem
StemEnd
TreatmentID
C
H

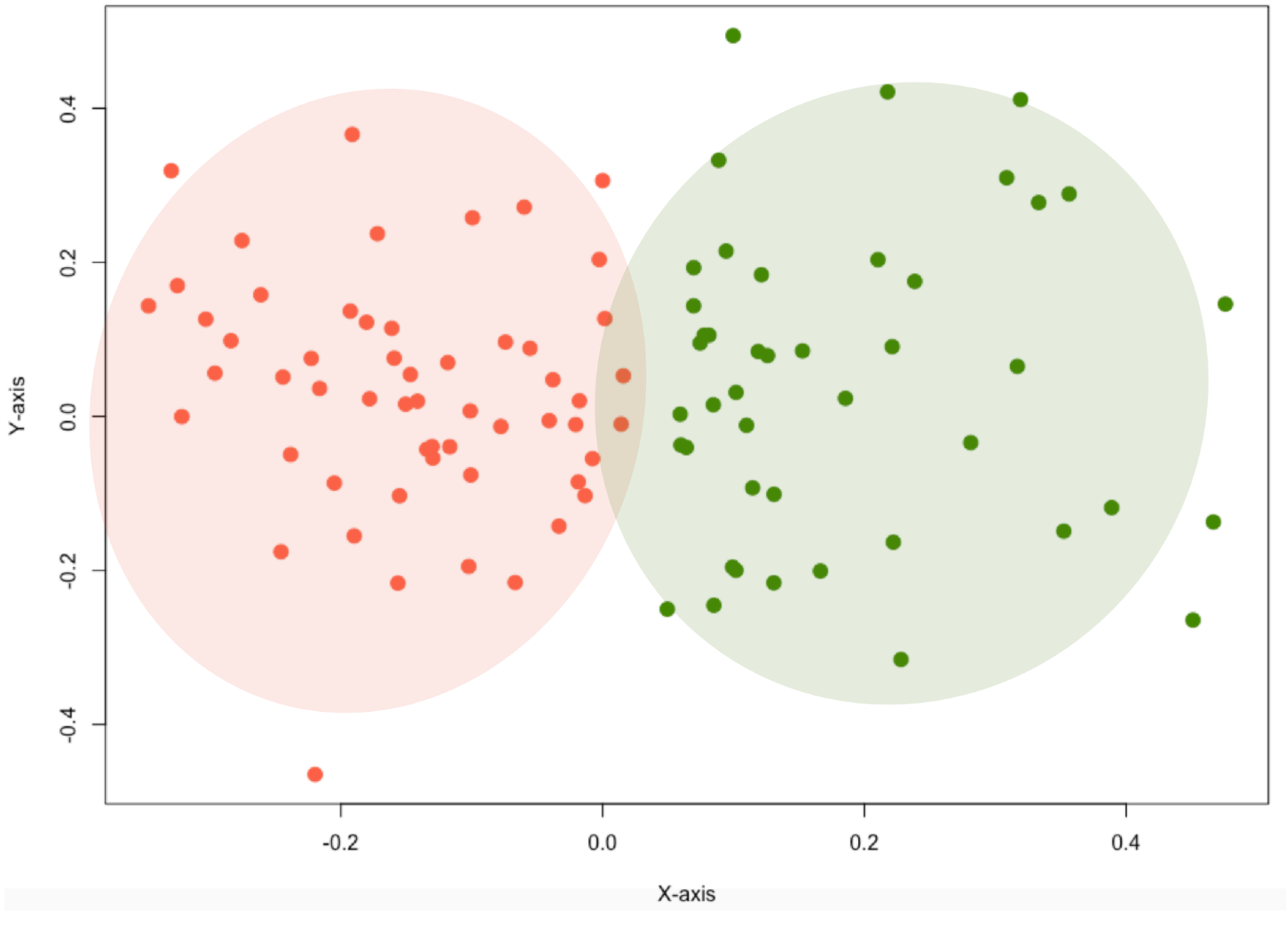

C
organic
conventional
PC3 (7.0% of total variation)
PC3 (16.0% of total variation)
0.4
0.3
0.2
0.1
0.0
−0.1
−0.2
−0.3
−0.4
−0.4
−0.3
−0.2
−0.1
0.0
0.1
0.2
0.3
0.4


C
organic
conventional
PC3 (7.0% of total variation)
PC3 (16.0% of total variation)
0.4
0.3
0.2
0.1
0.0
−0.1
−0.2
−0.3
−0.4
−0.4
−0.3
−0.2
−0.1
0.0
0.1
0.2
0.3
0.4

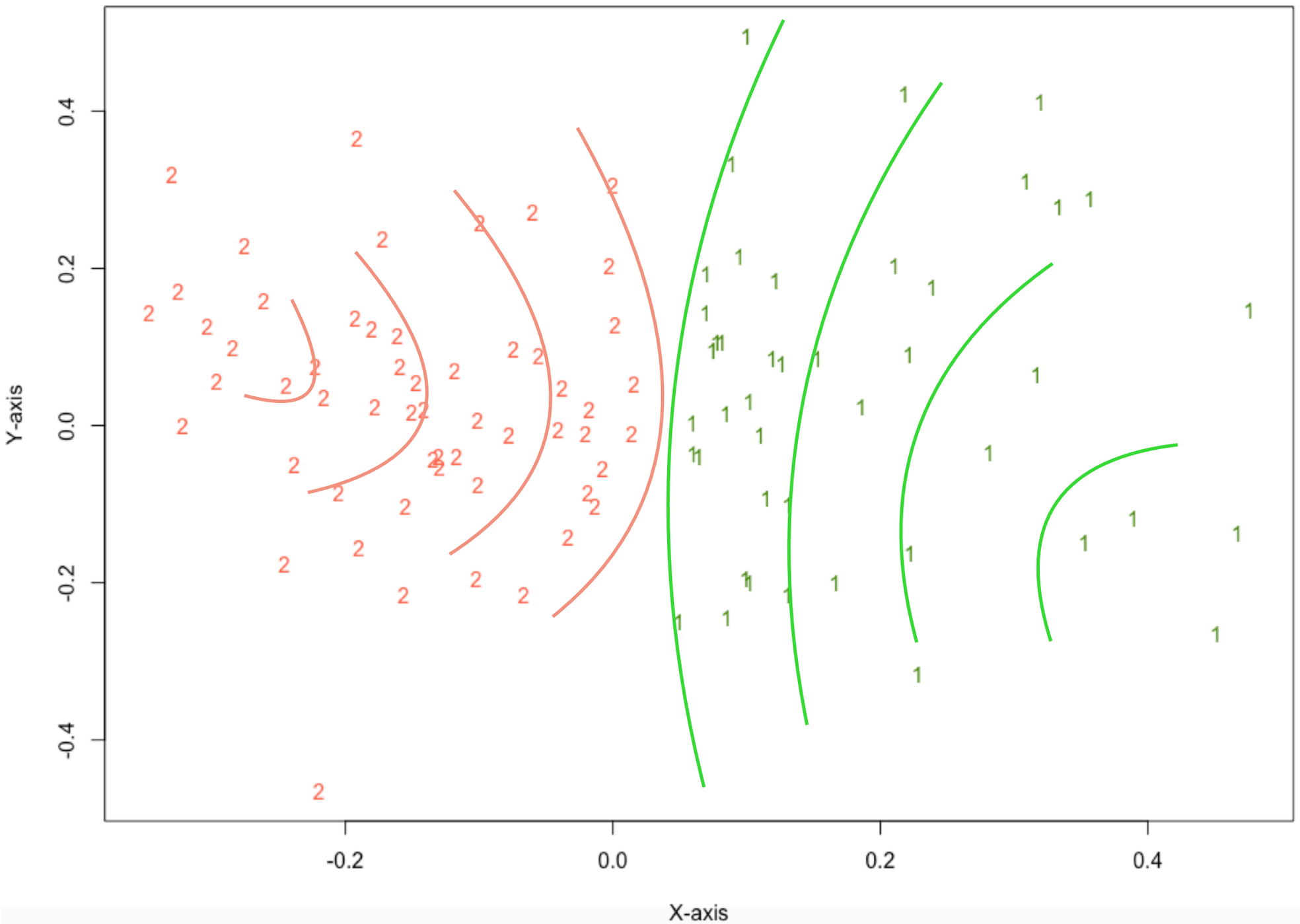
Do you see the two clusters?



Do you see the two clusters?



Do you see the two clusters now?



Just because you like to see a diffrence does not mean there is one.

Photoshop is not a scientific application!

Core Microbiota

GDC Genetic Diversity Centre Zurich

Core Taxa

core = taxa occurring in 75% of all replicates

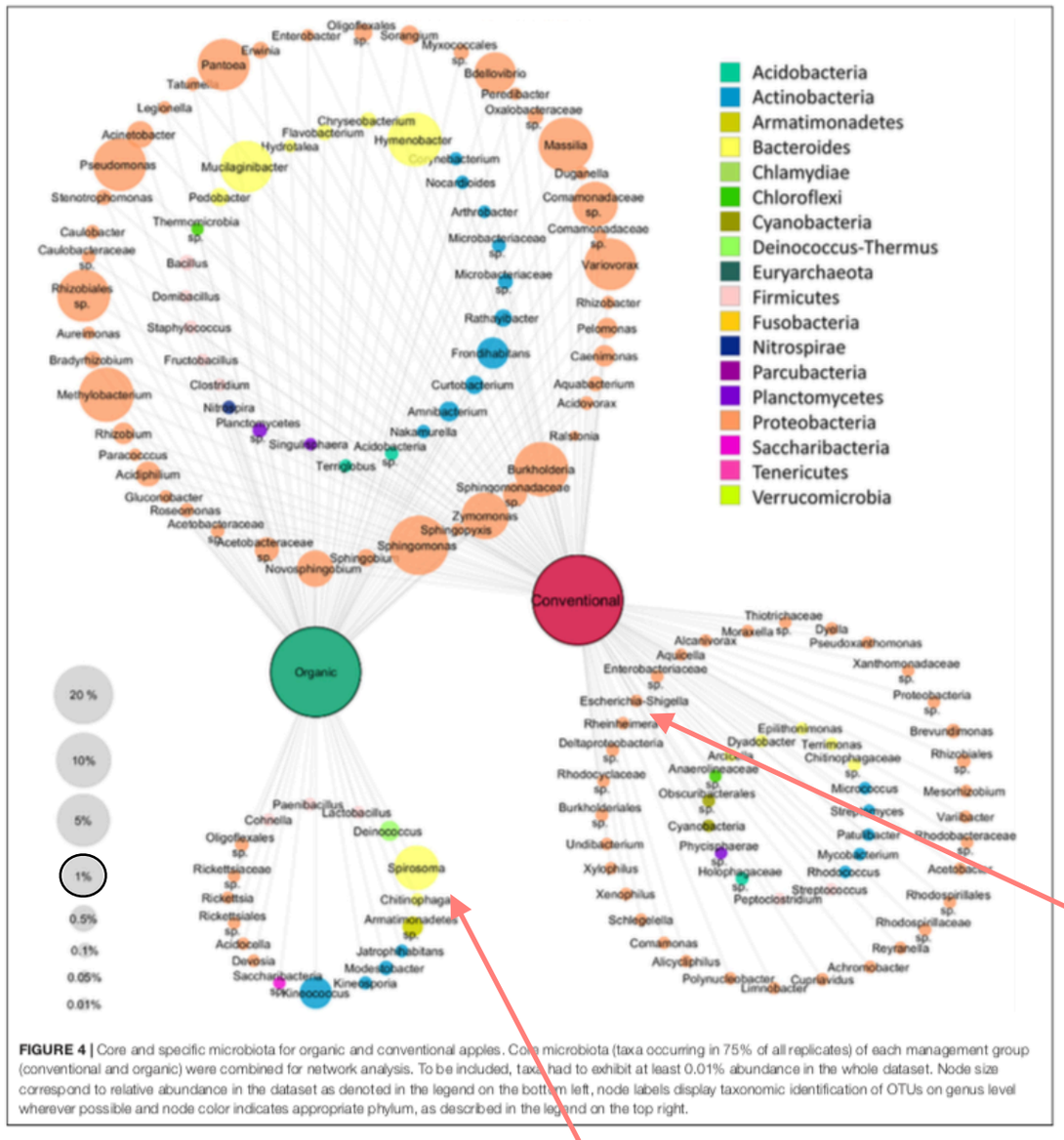
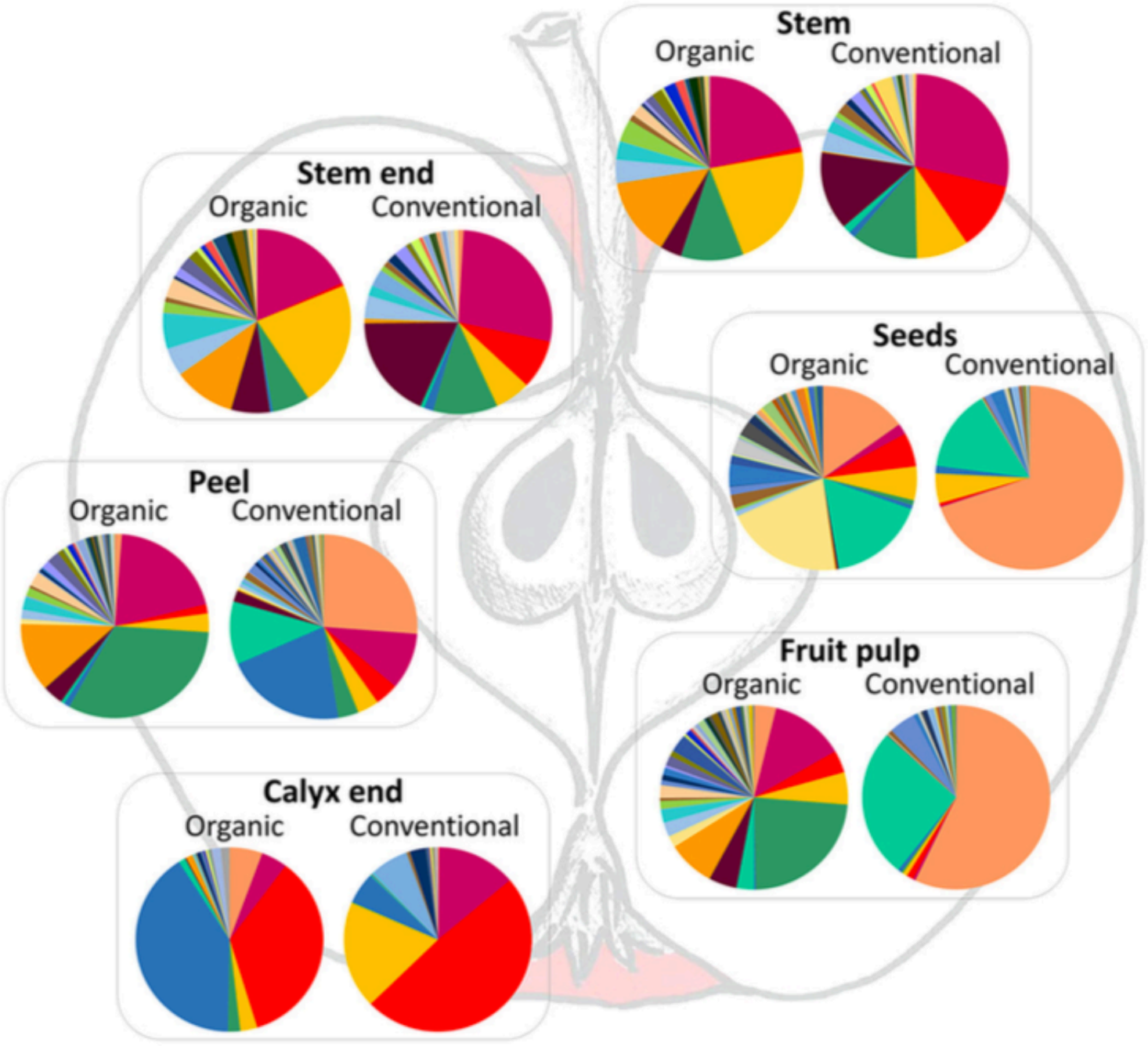


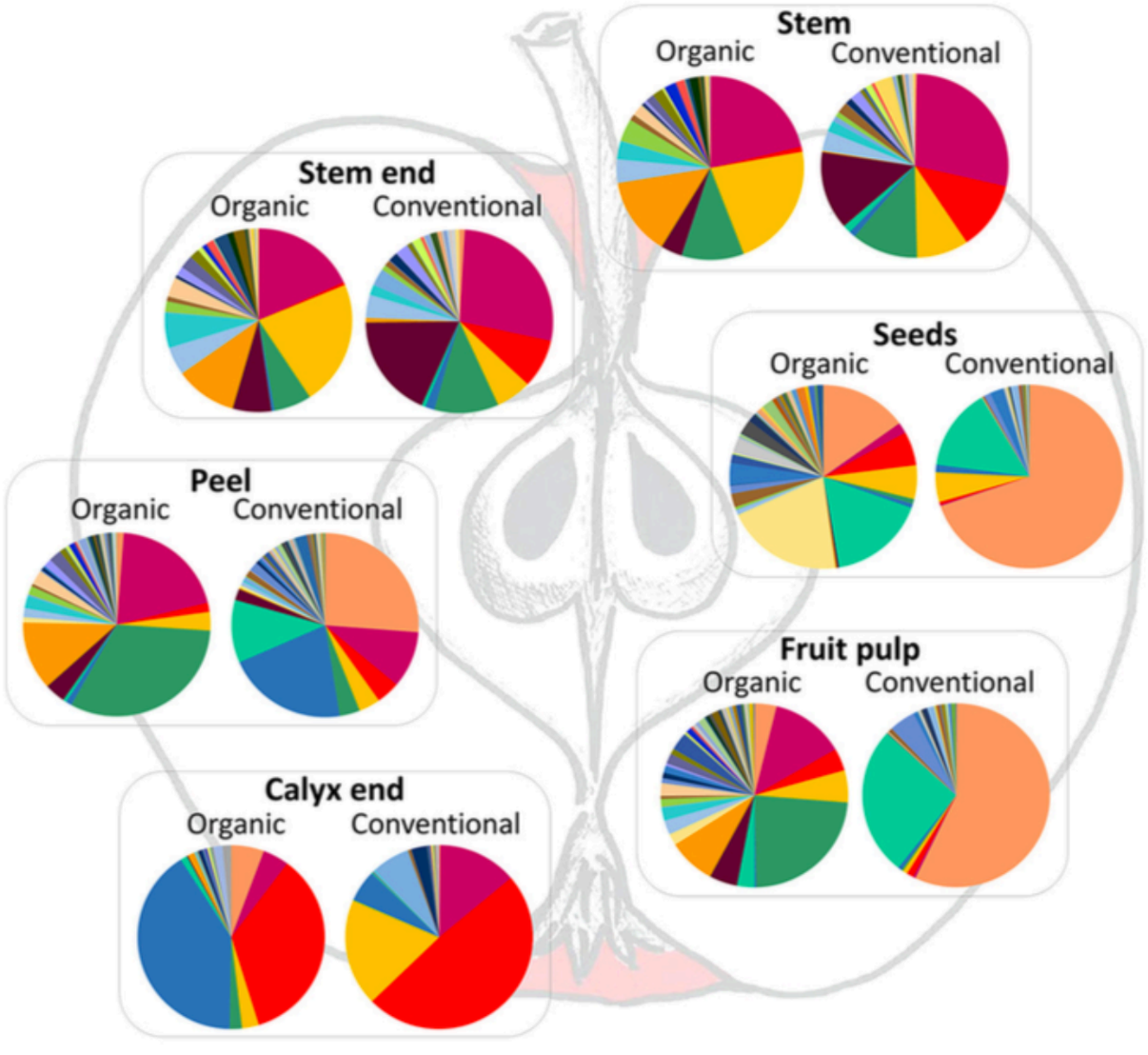
FIGURE 4 | Core and specific microbiota for organic and conventional apples. Core microbiota (taxa occurring in 75% of all replicates) of each management group (conventional and organic) were combined for network analysis. To be included, taxa had to exhibit at least 0.01% abundance in the whole dataset. Node size correspond to relative abundance in the dataset as denoted in the legend on the bottom left, node labels display taxonomic identification of OTUs on genus level wherever possible and node color indicates appropriate phylum, as described in the legend on the top right.

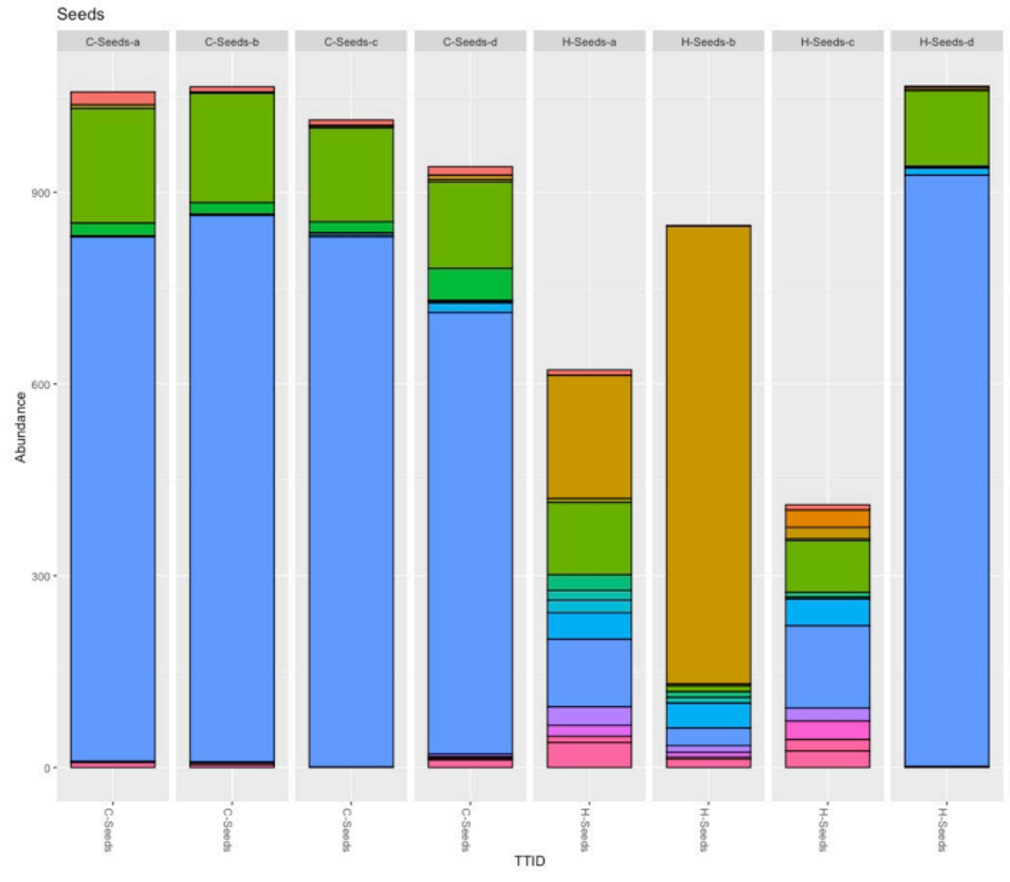
Mixed tissue, why?

Escherichia-Shigella (0.01%)

Spirosoma (1%-5%)


Stem
Organic
Conventional
Stem end
Organic
Conventional
Seeds
Organic
Conventional
Peel
Organic
Conventional
Fruit pulp
Organic
Conventional
Calyx end
Organic
Conventional


Stem
Organic
Conventional
Stem end
Organic
Conventional
Seeds
Organic
Conventional
Peel
Organic
Conventional
Fruit pulp
Organic
Conventional
Calyx end
Organic
Conventional


Seeds
C-Seeds-a
C-Seeds-b
C-Seeds-c
C-Seeds-d
H-Seeds-a
H-Seeds-b
H-Seeds-c
H-Seeds-d
900
600
300
0
Abundance
C-Seeds
C-Seeds
C-Seeds
C-Seeds
H-Seeds
H-Seeds
H-Seeds
H-Seeds
TTID

Pooling replicates is not a good strategy to reduce variance!

Food-Borne Pathogens

Results

The taxonomic assignment of OTUs revealed 44 different phyla, 325 orders and 1,755 genera. Among bacterial phyla, Proteobacteria highly dominated with 80%, followed by Bacteroidetes (9%), Actinobacteria (5%), and Firmicutes (3%). Burkholderiales were highly abundant concerning bacterial orders (31% abundance), followed by Sphingomonadales (14%), Rhizobiales (12%), Pseudomonadales (11%), **Enterobacteriales (7%)** and Cytophagales (5%); Micrococcales, Sphingobacteriales, Bacillales, Rhodospirillales, and Flavobacteriales, in ascending order, represented between 5 and 1% of total OTUs. OTUs assigned to the genus Ralstonia were most frequent with 13%, while Sphingomonas (12%), Pseudomonas (11%), Massilia (7%), Methylobacterium (7%), Burkholderia (5%), Pantoea (5%), and Hymenobacter (5%) were furthermore high abundant.

Burkholderiales were highly abundant concerning bacterial **orders** (31% abundance), followed by Sphingomonadales (14%), Rhizobiales (12%), Pseudomonadales (11%), **Enterobacteriales (7%)** and Cytophagales (5%); Micrococcales, Sphingobacteriales, Bacillales, Rhodospirillales, and Flavobacteriales, in ascending order, represented between 5 and 1% of total OTUs.

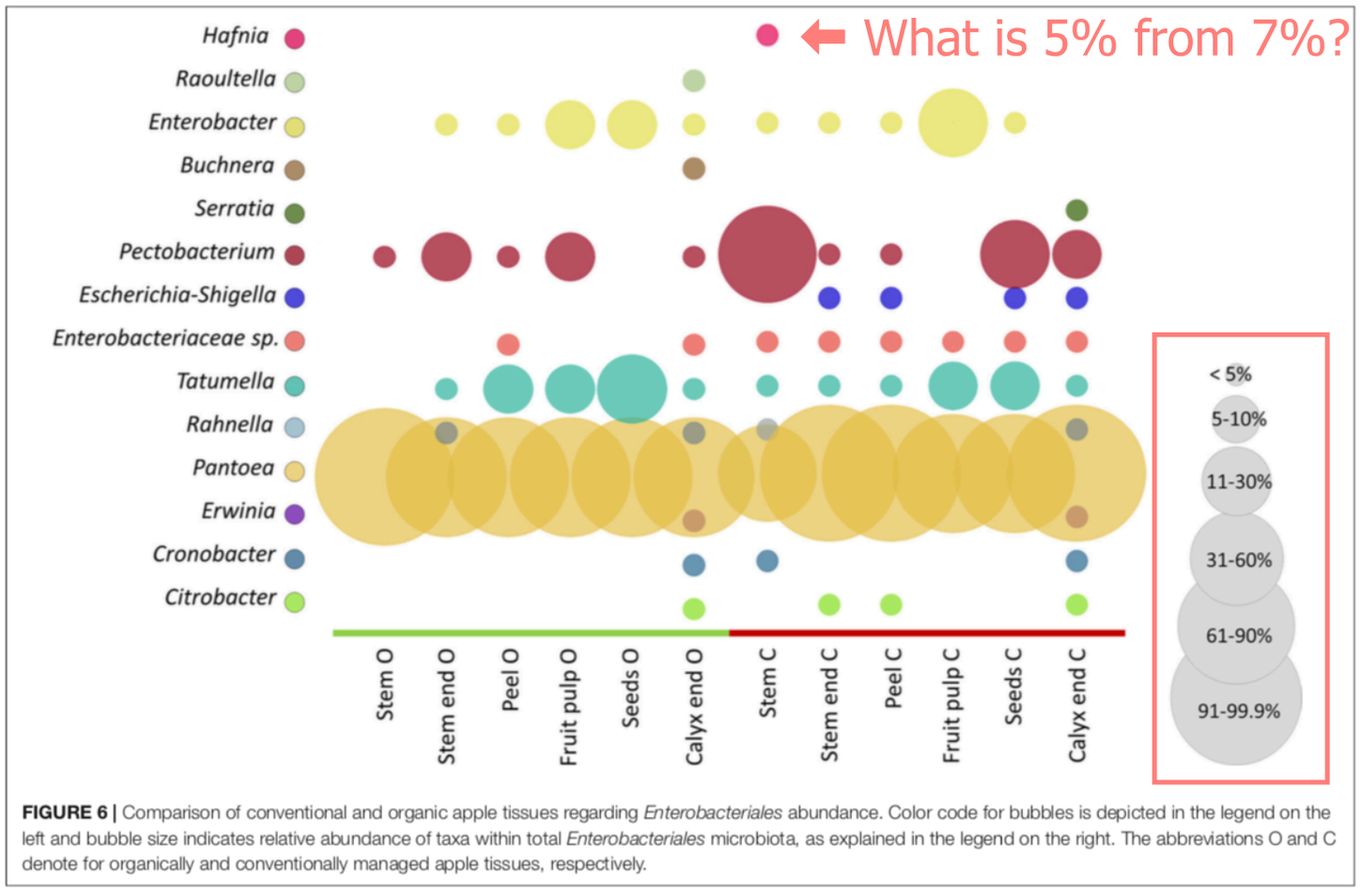


FIGURE 6 | Comparison of conventional and organic apple tissues regarding *Enterobacteriales* abundance. Color code for bubbles is depicted in the legend on the left and bubble size indicates relative abundance of taxa within total *Enterobacteriales* microbiota, as explained in the legend on the right. The abbreviations O and C denote for organically and conventionally managed apple tissues, respectively.

What is 5% from 7%?

Do not get fooled by figures!

The order Enterobacteriales was one of the signature taxa of conventional apples as well; among them, we would like to highlight the almost ubiquitous occurrence **of OTUs assigned to Escherichia-Shigella** in the tissues of conventional apples (although low abundant) and their absence in organically managed apples.

Accurate differentiation of *Escherichia coli* and *Shigella* serogroups: challenges and strategies

N. K. Devanga Ragupathi, D. P. Muthuirulandi Sethuvel, F. Y. Inbanathan and B. Veeraraghavan

Department of Clinical Microbiology, Christian Medical College, Vellore, India

The differentiation of *E. coli* and *Shigella spp.* could not be achieved using 16S rRNA gene sequences as a result of the narrow (<1%) divergence between EHEC, EIEC and *Shigella spp.* Jenkins et al. [14] concur with this finding; their 16S rRNA gene comparison could not distinguish between E. coli and Shigella spp. as a result of >99% sequence identity. **We therefore deem this approach to be unacceptable to differentiate certain inter- and intraspecies identity.**

Jenkins et al. (2012) Detection and identification of bacteria in clinical samples by 16S rRNA gene sequencing: comparison of two different approaches in clinical practice. J Med Microbiol. 61:483–488.

Clinical Microbiology: Open Access

Delmas et al., Clin Microbiol 2015, 4:2
DOI: 10.4172/2327-5073.1000195

Commentary Open Access

Escherichia coli: The Good, the Bad and the Ugly

Julien Delmas*, **Guillaume Dalmasso and Richard Bonnet**

Microbes, Intestine, Inflammation and Host Susceptibility, INSERM U1071, INRA USC2018, Université Clermont Auvergne, Clermont-Ferrand, France

***Corresponding author:** Julien Delmas, Microbes, Intestine, Inflammation and Host Susceptibility, INSERM U1071, INRA USC2018, Université Clermont Auvergne, Clermont-Ferrand, France, Tel: +334731779; E-mail; jdelmas@chu-clermontferrand.fr

Received date: March 11, 2015, **Accepted date:** April 21, 2015, **Published date:** Aptil 28, 2015

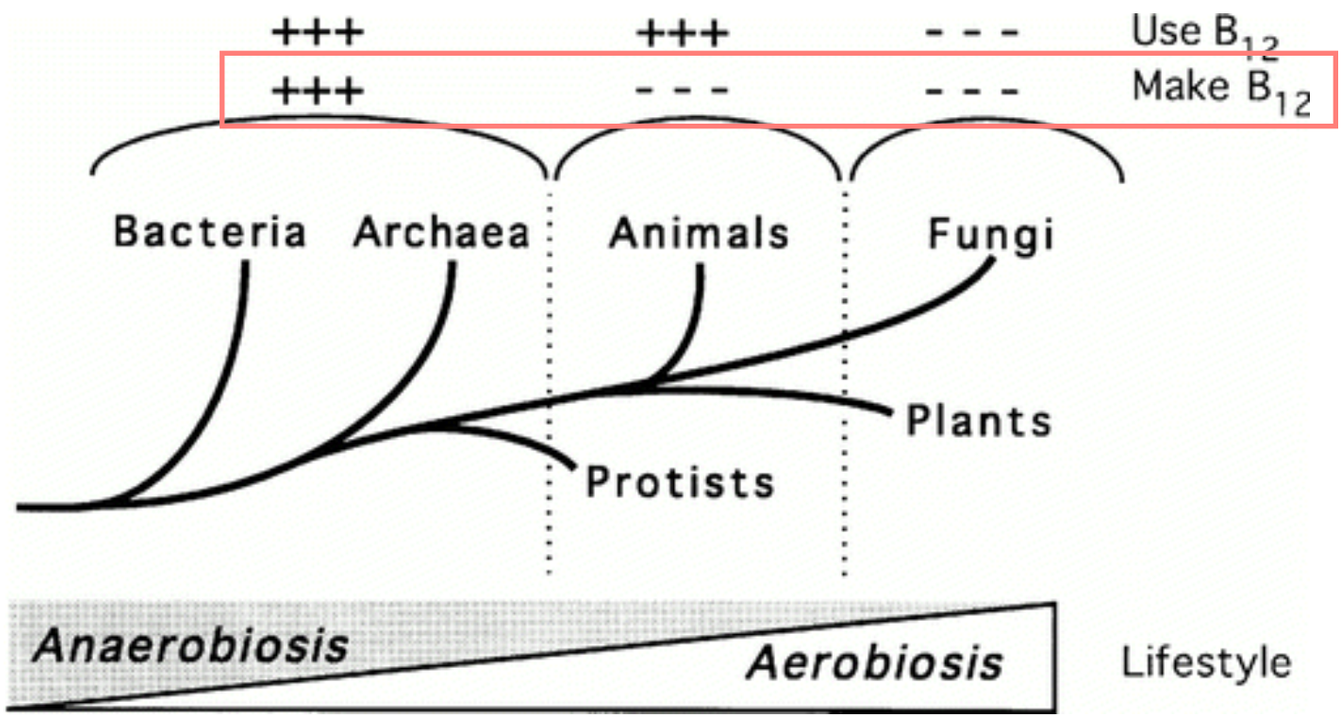
Abstract

The species *Escherichia coli* comprises non-pathogenic commensal strains that form part of the normal flora of humans and virulent strains responsible for acute infections inside and outside the intestine. In addition to these pathotypes, various strains of *E. coli* are suspected of promoting the development or exacerbation of chronic diseases of the intestine such as Crohn's disease and colorectal cancer.

The species ***Escherichia coli*** comprises non-pathogenic commensal strains that from part of the **normal flora** of humans and virulent strains responsible for acute infections inside and outside the intestine.

Understand your limits!

If you've been eating an apple a day to keep the doctor away but haven't been consuming the **core**, you are likely missing out on some of the **most beneficially nutritious parts of the apple**.



These results suggest that the selection pressure to maintain **B12 synthesis varies with the lifestyle** of the organism. E. coli seems to fill a niche that does not require full de novo B12 synthesis, perhaps one in which B12 (or Cbi) is prevalent, and ethanolamine (but not propanediol) is an important carbon source. For **Salmonella spp.**, the ability to synthesize B12 must be strongly selected; its main use may be to degrade propanediol under anaerobic conditions in the presence of a suitable alternative electron acceptor.

Roth J, Lawrence J, Bobik T. COBALAMIN (COENZYME B12): Synthesis and Biological Significance. Annual Review of Microbiology. 1996;50:137–81. pmid:8905078.

Cobalamin biosynthetic pathway in microbes

Microorganisms	De novo synthesis pathway	Salvage pathway	References
Aerobes			
Pseudomonas dentrificans	Yes	Yes	[3]
Rhodobacter capusulatus	Yes	Yes	[3]
Rhodobacter sphaeroides	Yes	Yes	[3]
Sinorhizobium meliloti	Yes	Yes	[3]
Anaerobes			
Salmonella typhimurium	Yes	Yes	[4]
Bacillus megaterium	Yes	*	[5]
Propionibacterium shermanii	Yes	*	[5]
Escherichia coli	No	Yes	[4]
Thermotoga sp. RQ2	No	No	[6]
Thermotoga maritima MSB8	No	No	[6]
Thermotoga neapolitana	No	No	[6]
Thermotoga petrophila	No	No	[6]
Thermotoga naphthophila	No	No	[6]
Thermotoga thermarum	No	Yes	[6]
Thermotoga lettingae	No	Yes	[6]
Fervidobacterium nodosum	No	Yes	[6]
Thermosipho melanesiensis	Yes	Yes	[6]
Thermosipho africanus	Yes	Yes	[6]
Kosmotoga olearia	No	Yes	[6]
Mesotoga prima	No	No	[6]
Petrotoga mobilis	No	No	[6]

Unidentified pathways are marked with "*"

In this review, we provide a comprehensive understanding of advances in the microbial production of **vitamin B12**, with a particular focus on establishing a heterologous host for the vitamin B12 production, as well as on strategies and tools that have been applied to increase microbial cobalamin production. Several worthy strategies employed for other products are also included.

Fang, H., Kang, J., & Zhang, D. (2017). Microbial production of vitamin B12: a review and future perspectives. Microbial cell factories, 16(1), 15.

Apple seeds contain amygdalin, a substance that releases **cyanide** into the blood stream when chewed and digested. However, **apple seeds** in small amounts do not contain enough **cyanide** to cause harm. However, it is better to spit out **seeds** to avoid any potential issues.

Think bevore you give advices! Seriously!!

Controversially, **Lactobacillus**, which is frequently used within probiotics (Derrien and van Hylckama Vlieg, 2015), was one of the **core taxa** of organic apples.

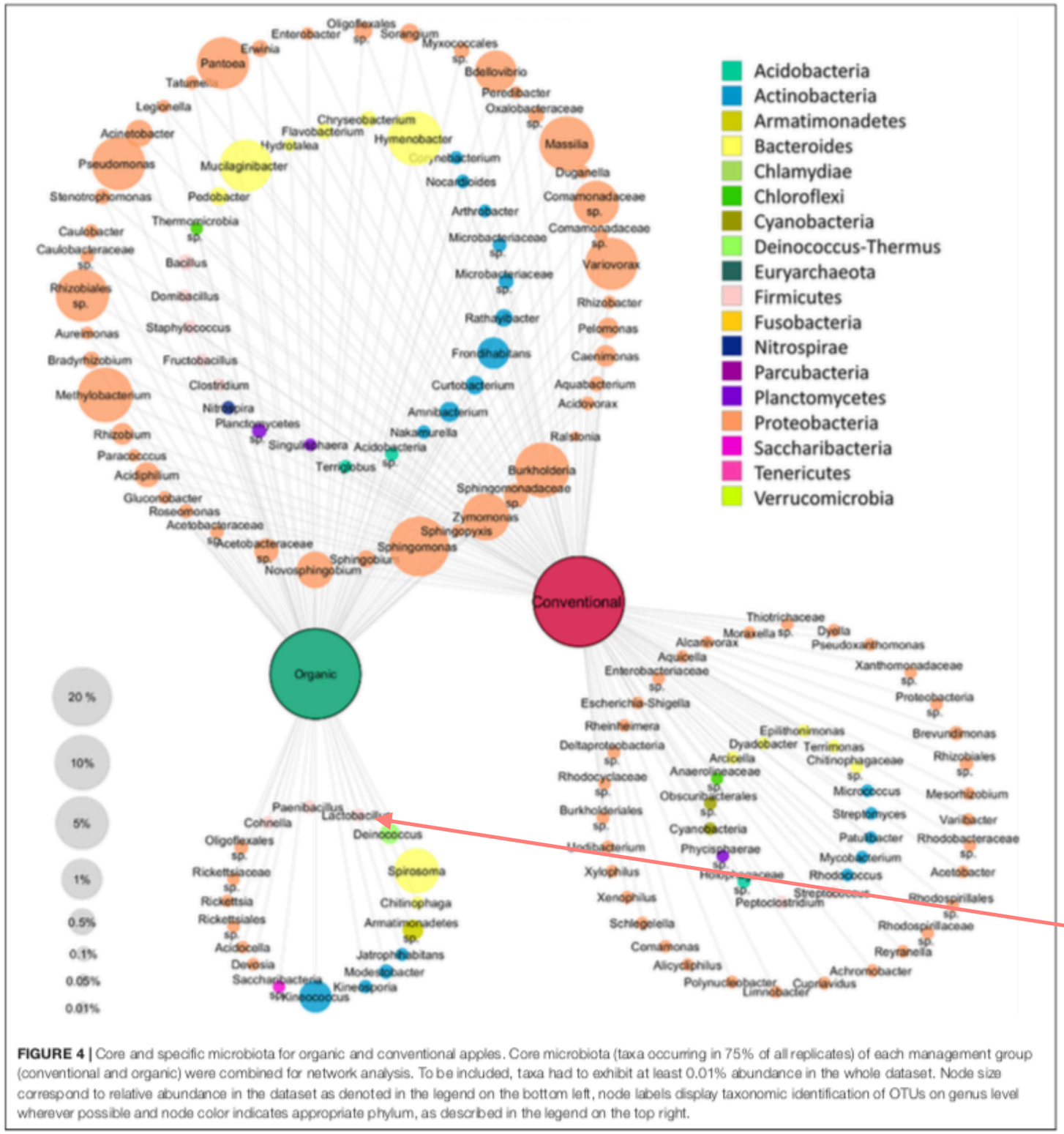


FIGURE 4 | Core and specific microbiota for organic and conventional apples. Core microbiota (taxa occurring in 75% of all replicates) of each management group (conventional and organic) were combined for network analysis. To be included, taxa had to exhibit at least 0.01% abundance in the whole dataset. Node size correspond to relative abundance in the dataset as denoted in the legend on the bottom left, node labels display taxonomic identification of OTUs on genus level wherever possible and node color indicates appropriate phylum, as described in the legend on the top right.

	C				H			
	CalyxEnd				CalyxEnd			
Burkholderia-Paraburkholderia	0.1	0.0	0.4	0.4	0.2	0.6	0.4	1.8
Lactobacillus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Legionella	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Saccharibacteria	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Deinococcus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	FruitPulp				FruitPulp			
Burkholderia-Paraburkholderia	20.2	22.0	26.0	20.2	1.8	2.7	2.1	0.8
Lactobacillus	0.1	0.0	0.2	0.0	0.2	0.1	0.1	0.0
Legionella	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Saccharibacteria	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Deinococcus-Thermus	0.0	0.0	0.0	0.0	0.5	0.6	0.1	1.7
	Peel				Peel			
Burkholderia-Paraburkholderia	9.2	6.4	10.3	7.3	0.2	0.1	0.3	1.3
Lactobacillus	0.1	4.7	0.1	0.3	0.0	0.0	0.0	0.2
Legionella	1.9	1.6	3.5	0.1	0.1	0.1	0.0	0.0
Saccharibacteria	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.0
Deinococcus-Thermus	0.0	0.0	0.0	0.0	0.4	0.1	0.2	0.1
	Seed				Seeds			
Burkholderia-Paraburkholderia	15.7	12.3	14.2	11.8	7.7	1.2	7.4	11.7
Lactobacillus	0.0	0.0	0.0	0.0	0.4	0.4	0.0	0.0
Legionella	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
Saccharibacteria	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Deinococcus-Thermus	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0
	Stem				Stem			
Burkholderia-Paraburkholderia	0.2	8.1	1.3	0.2	0.1	0.0	0.0	0.0
Lactobacillus	0.5	0.0	0.0	0.1	0.1	0.0	0.0	0.0
Legionella	0.5	0.0	0.0	0.1	0.0	0.2	0.0	0.0
Saccharibacteria	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.0
Deinococcus-Thermus	0.0	0.0	0.0	0.0	0.3	0.2	0.1	1.0

Want to eat some Lactobacillus?

Eat a Joghurt or some Sauerkraut!

World Health Organization

According to the study, which was published this month in the journal Frontiers of Microbiology, a single apple contains about **100 million bacterial cells — but if you toss out the core, you're only consuming about 10 million of these precious cells.**

What is the recommended minimum daily intake of bacteria cells?

Missing bacteria? Here are a few good sources:

- bus handels
- bathtub rubber ducks
- shopping carts


JOURNAL

Beall's List - Potential, possible, or probable predatory scholarly open-access publishers

Frontiers' peer review process is flawed. It is stacked in favor of accepting as many papers as possible in order to generate more revenue for the company. Frontiers is included on my list, and I recommend against publishing in its journals, which are rather expensive to publish in anyway.

Is frontiers in microbiology a predatory Journal?

The **Frontiers journals** use **open peer review**, where the names of reviewers of accepted articles are made public. As of 2017, 24 of their journals had impact factors. ... Some journals, such as Frontiers in Human Neuroscience or **Frontiers in Microbiology** are considered megajournals on their own.

OPEN ACCESS

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For Better Science

BY LEONID SCHNEIDER, ON RESEARCH INTEGRITY, BIOMEDICAL ETHICS AND ACADEMIC PUBLISHING

Beall-listed Frontiers empire strikes back

BY LEONID SCHNEIDER
SEPTEMBER 14, 2016

COMMENTS 65

Willy
December 1, 2018

I think that both views have their own right to co-exist. I published articles in Frotniers and agree that their review process is sloppy. I also published articles with Wiley and Elsevier and experienced their review process as biased, unfair, and not constructive.

I reviewed for Frontiers and admit that my criticism of trash manuscripts was dismissed. However, it was possible to withdraw from the reviewing process so not to be stained by junk papers. I reviewed for Elsevier, Wiley, AAAS, NPG and others. Elsevier surely has the highest trash fraction among the manuscripts. However, they are happy to reject such manuscripts. Still, Elsevier is steering towards junk status as well as their reviewing process is flawed by nepotism. Plus, I consider Elsevier predatory as they bully university into a loan-shark style subscription model (https://www.the-scientist.com/news-opinion/universities-in-germany-and-sweden-lose-access-to-elsevier-journals-64522).

I agree with CVAK in that Frontiers gives room to unconventional authors and ideas – and I applaud them for this. They also waive fees if one can't afford them as they have done for me.

The bottom line is that publishers are just the executive branch of science. They scientific system with its focus on quantity enables all sorts of spam, regardless of the publishers. Just remember the Mozart effect (https://www.nature.com/articles/365611a0) or the memory of water (https://www.nature.com/articles/333816a0). Still, this is part of scientific discovery.

PUBPEER
The online Journal club

#1 Elisabeth M Bik

The main conclusions of this paper, which was funded by the Austrian Sparkling Science Research Program, are the following:

- Apples contain lots of bacteria; about 100 million per apple
- The different tissues within an apple (fruit pulp, seeds, stem) vary dramatically in microbial amounts and composition
- Lots of microbial differences also were found between organically managed and conventionally managed apples. In particular Lactobacillus was found in organic apples, and Escherichia and Erwinia were found in conventional apples.
- Eating organic apples is better for your health than conventional apples

This is a fun exploratory study, but the experimental design is not strong enough to support the third and fourth conclusions mentioned above.

What I really want to know

Is it possible to predict the treatment (conventional or organic) based on the bacterial community signature?

What OTUs are responsible for the discovered differences?

Re-Take on the matter:

https://www.gdc-docs.ethz.ch/Varia/Wassermann2019/site/

What does it really matter?

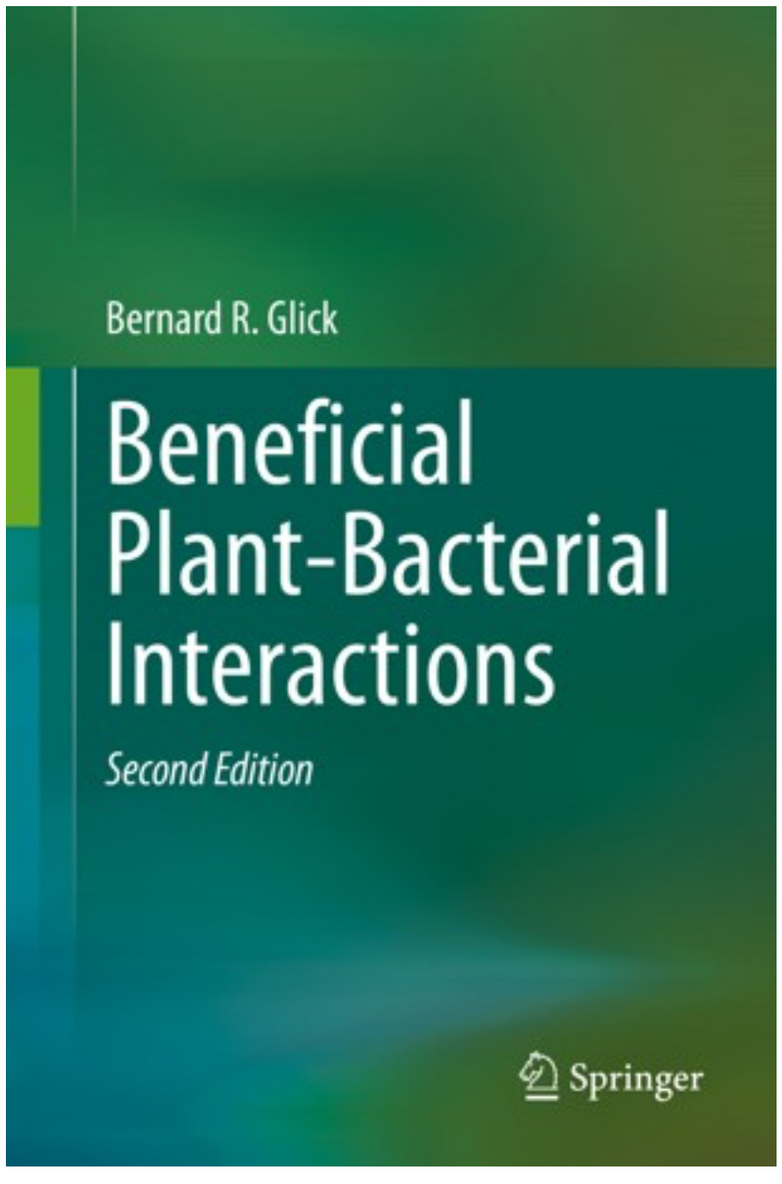

Table 2.1
Abundance of bacteria found in various apple fruit tissues from either organically or conventionally grown apples

Apple tissue	Organic, 16S rRNA gene copies per g apple tissue	Conventional, 16S rRNA gene copies per g apple tissue
Stem	8×10^7	2×10^8
Stem end	3×10^6	3×10^5
Peel	8×10^4	1.5×10^4
Fruit pulp	3×10^5	8×10^4
Seeds	1×10^8	3×10^7
Calyx end	5×10^6	4×10^6