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.

Swiss Gourmet / Arlet

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.

- What do you like about the article?
- Can you find shortcommings?
- Do you understand the sample design?
- Would you be able to reproduce the data analysis?
- Do you agree with the statistical tests applied?
- Can you download the raw data?
- What do you think is missing?

Swiss Gourmet / Arlet

⊕ biological replicates (n=4)

⊕ quantification with qPCR

⊕ simple design, clear question

⊕ some nice and appealing figures

⊕ conclusion are clearly formulated

Organic versus Conventional ?

Organically managed apples originated from an organic orchard, which follows the international “demeter” guidelines for organic farming1, 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.

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Both apple management groups (“organic” and “conventional”) were transported to laboratory immediately and processed under sterile conditions.

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)**.

Gene copy numbers of bacterial 16S rRNA per gram tissue of organic and conventional apples were measured by qPCR inquiry

Materials and Methods

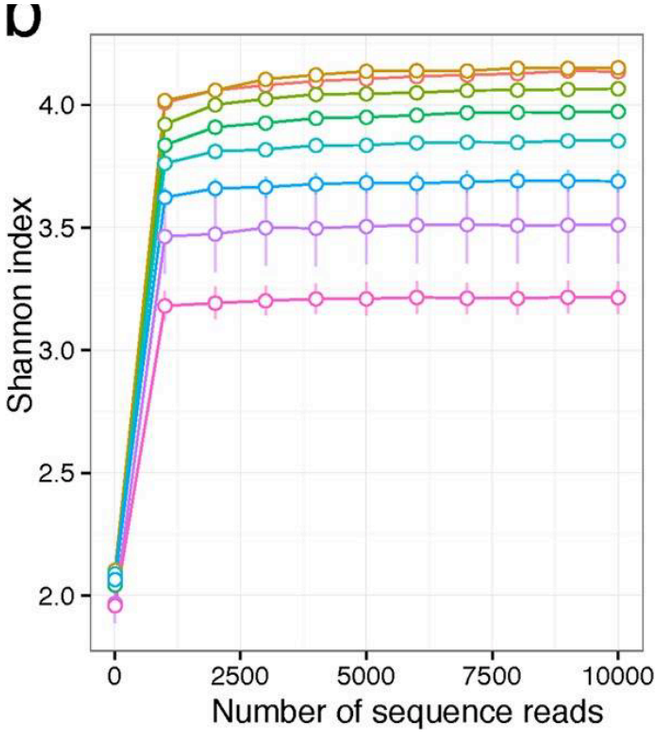
After taxonomy assignment, sequences assigned to host **mitochondria and chloroplasts were discarded**.

- How many OTUs were removed?

- Did you consider mt and chl realted for the quatifications?

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.



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).

Material and Methods

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Number of samples

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

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

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

Alpha diversity for organically (o) and conventional (c) grown 🍎 (tissues).

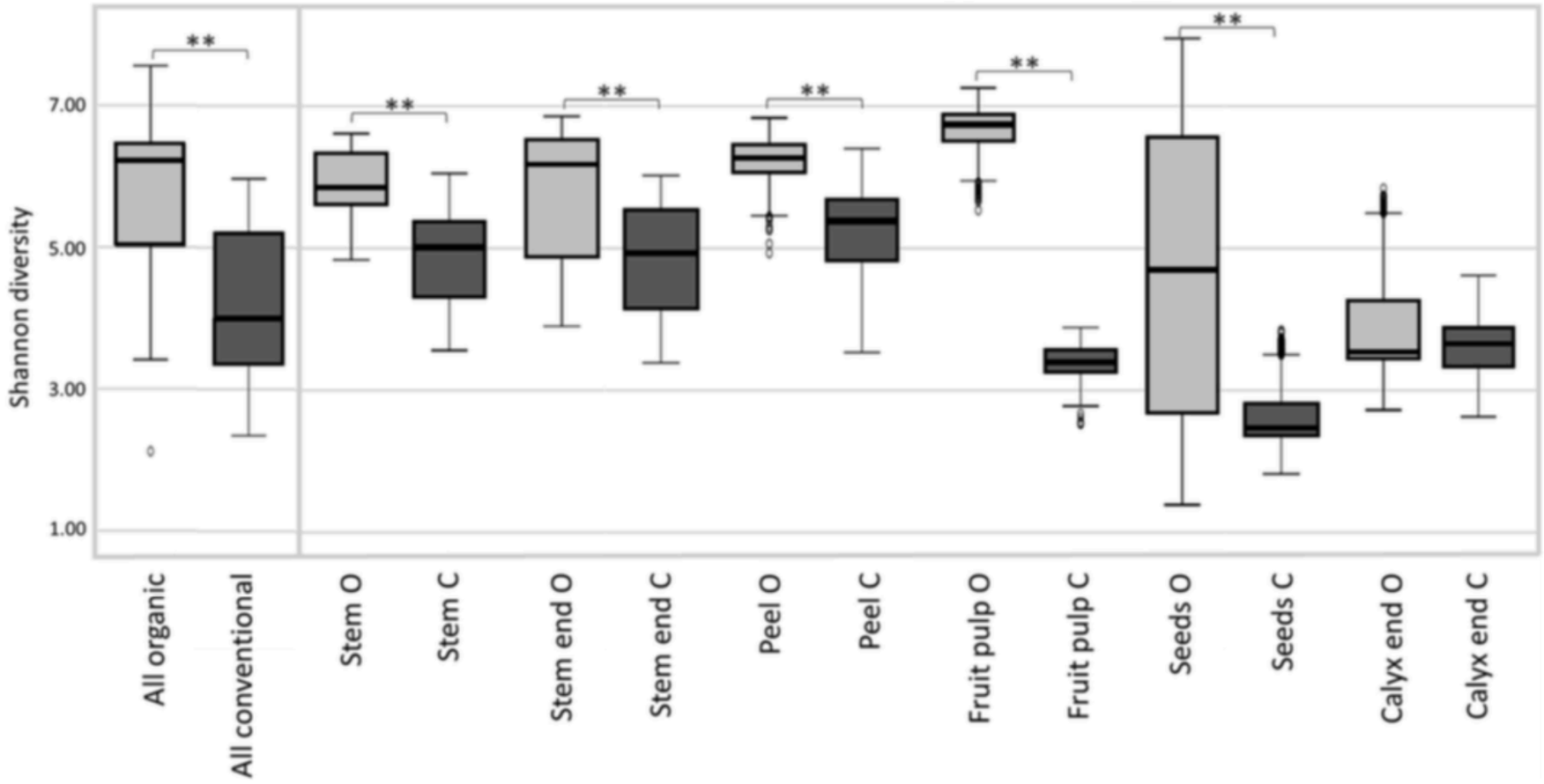


FIGURE 2 | Microbial diversity estimates of organically and conventionally managed apples and apple tissues. Suffixes O and C of carposphere tissue in the bottom legend, denote for organic and conventional management, respectively. Significant differences in Shannon diversity estimates of the apple management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

Quantitative Records of Diversity Estimates of Apple Microbiota

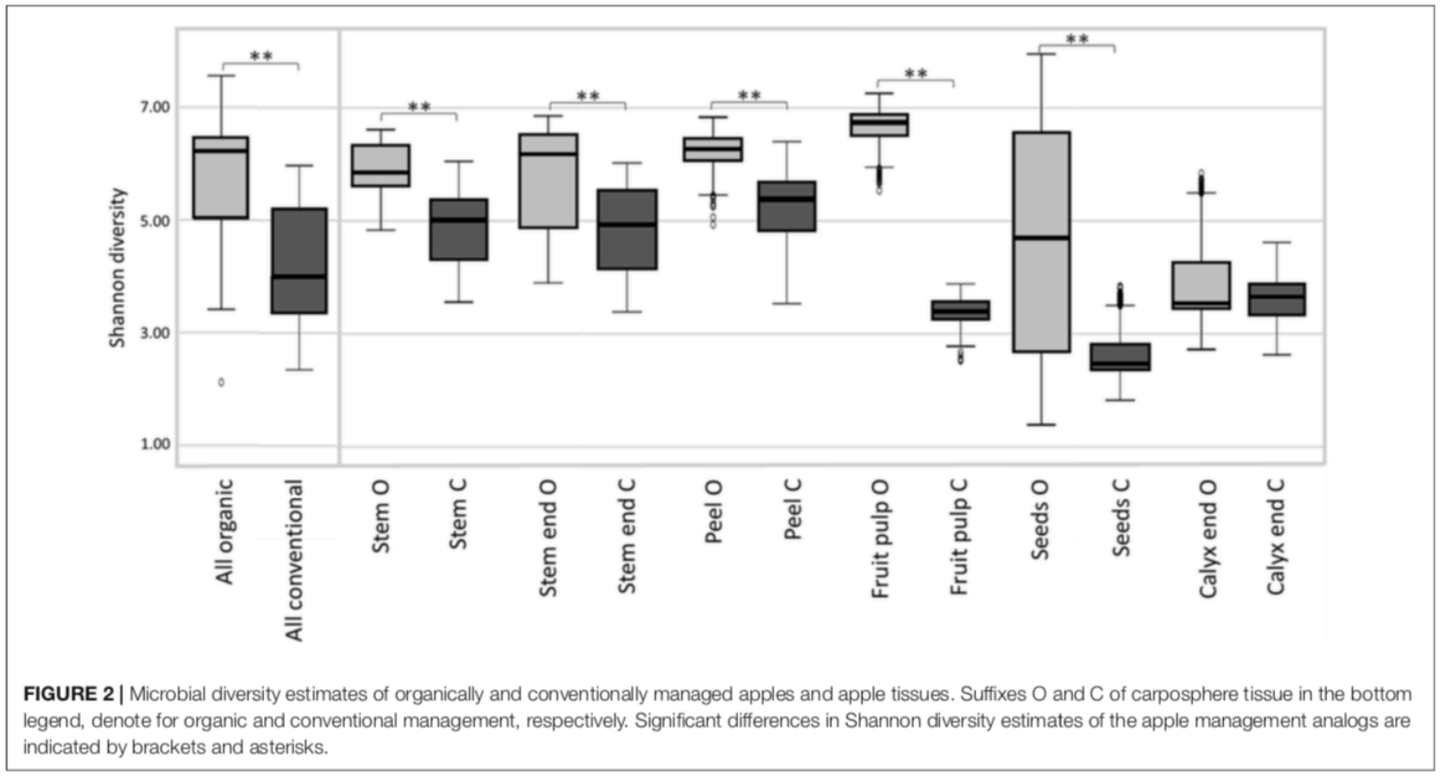


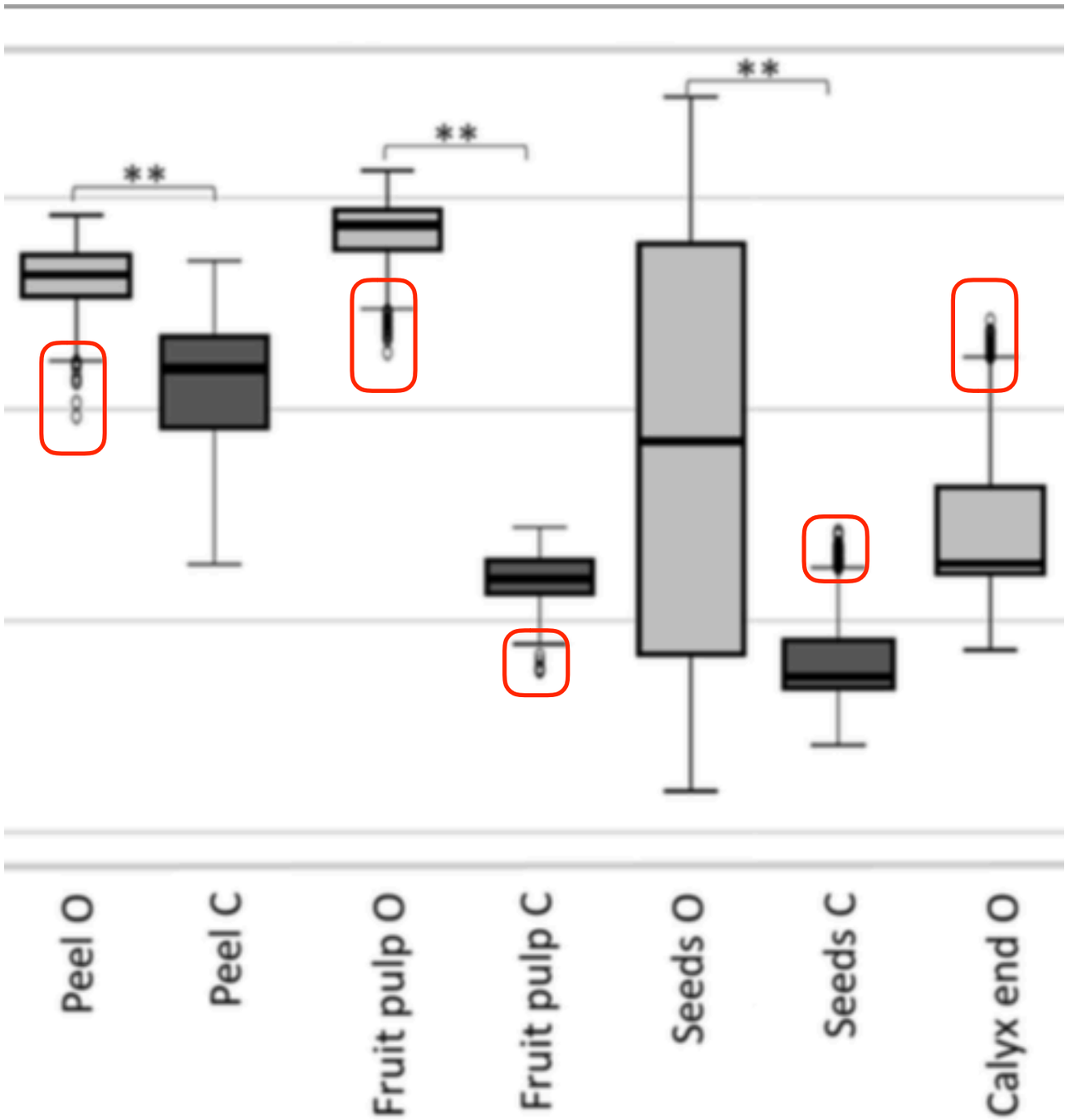
FIGURE 2 | Microbial diversity estimates of organically and conventionally managed apples and apple tissues. Suffixes O and C of carposphere tissue in the bottom legend, denote for organic and conventional management, respectively. Significant differences in Shannon diversity estimates of the apple management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

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.

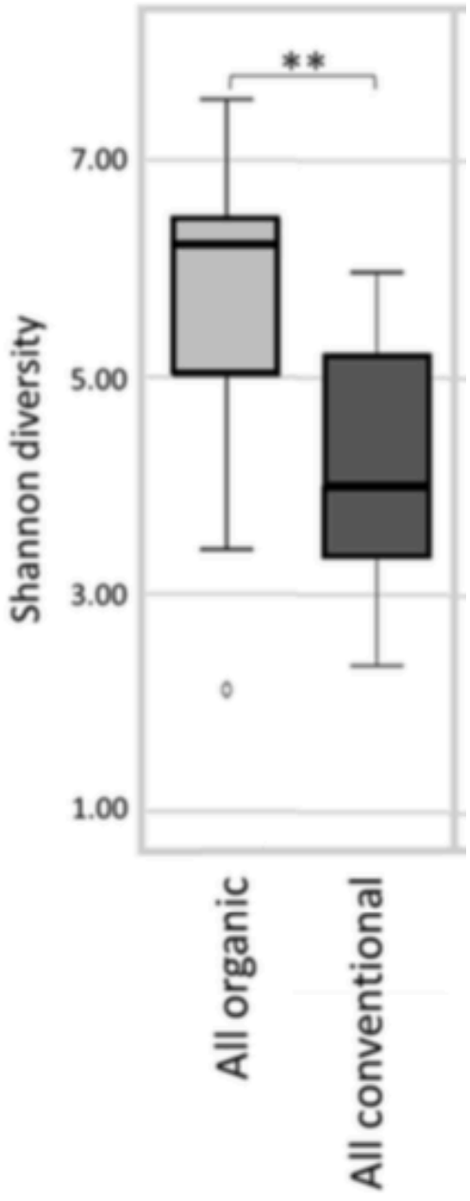
The Kruskal–Wallis test does NOT assume that the data are normally distributed; that is its big advantage. If you're using it to test whether the medians are different, it does assume that the observations in each group come from populations with **the same shape of distribution**, so if different groups 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.



How many samples per tissue were used?

Quantitative Records of Diversity Estimates of Apple Microbiota



- 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.

PCoA plots based on unweighted UniFraq distances

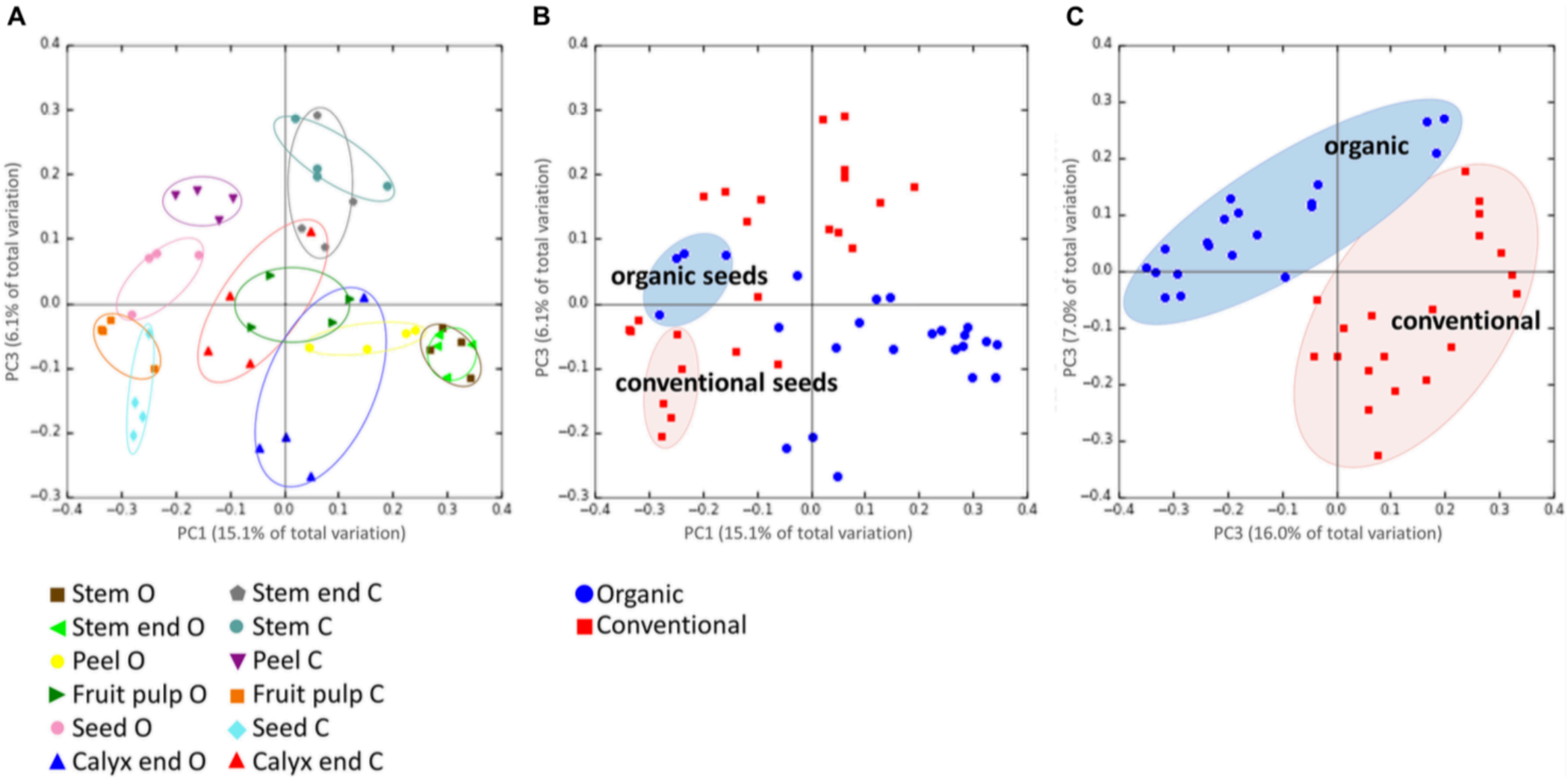
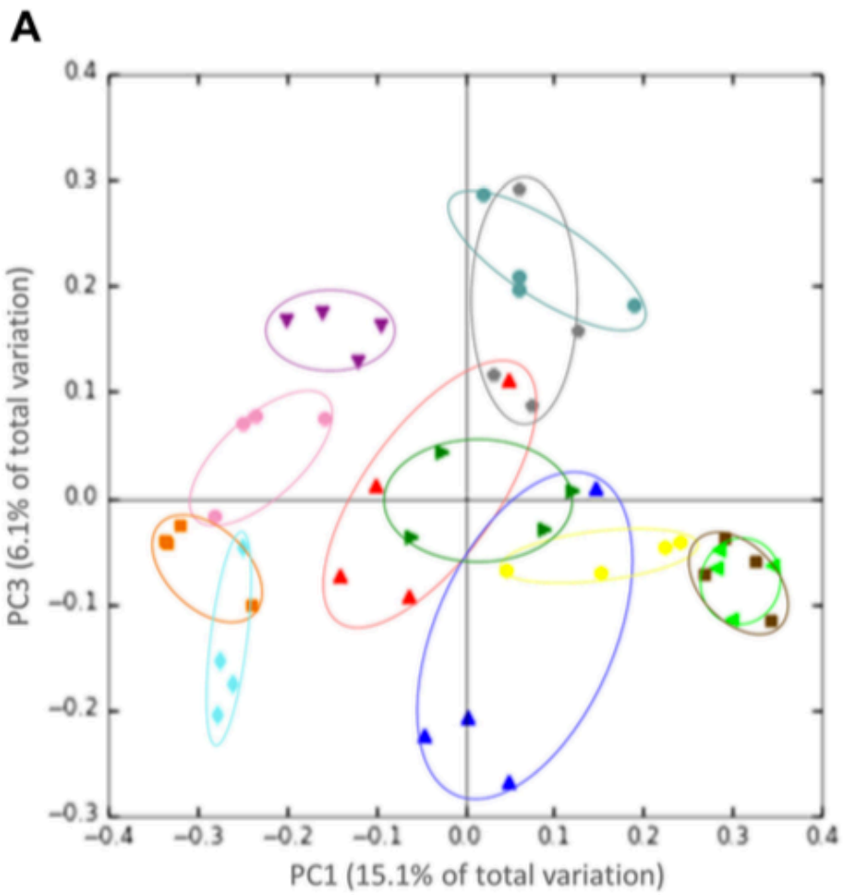
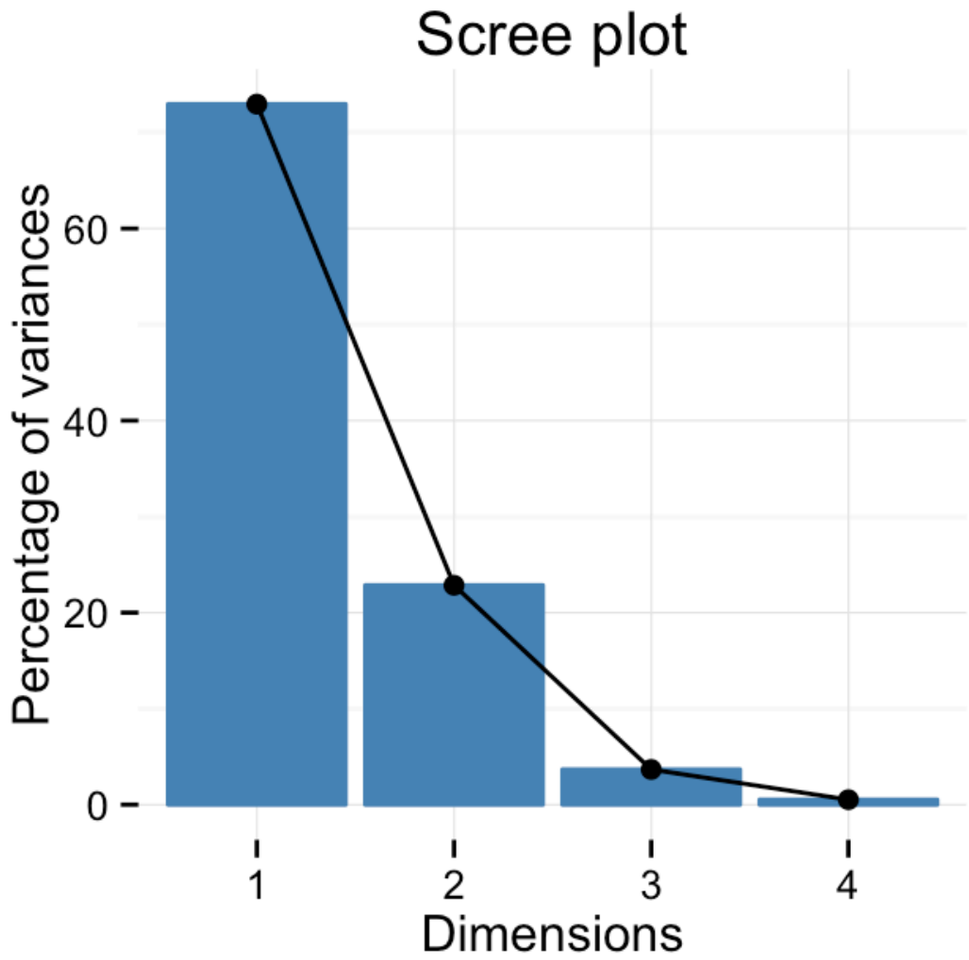
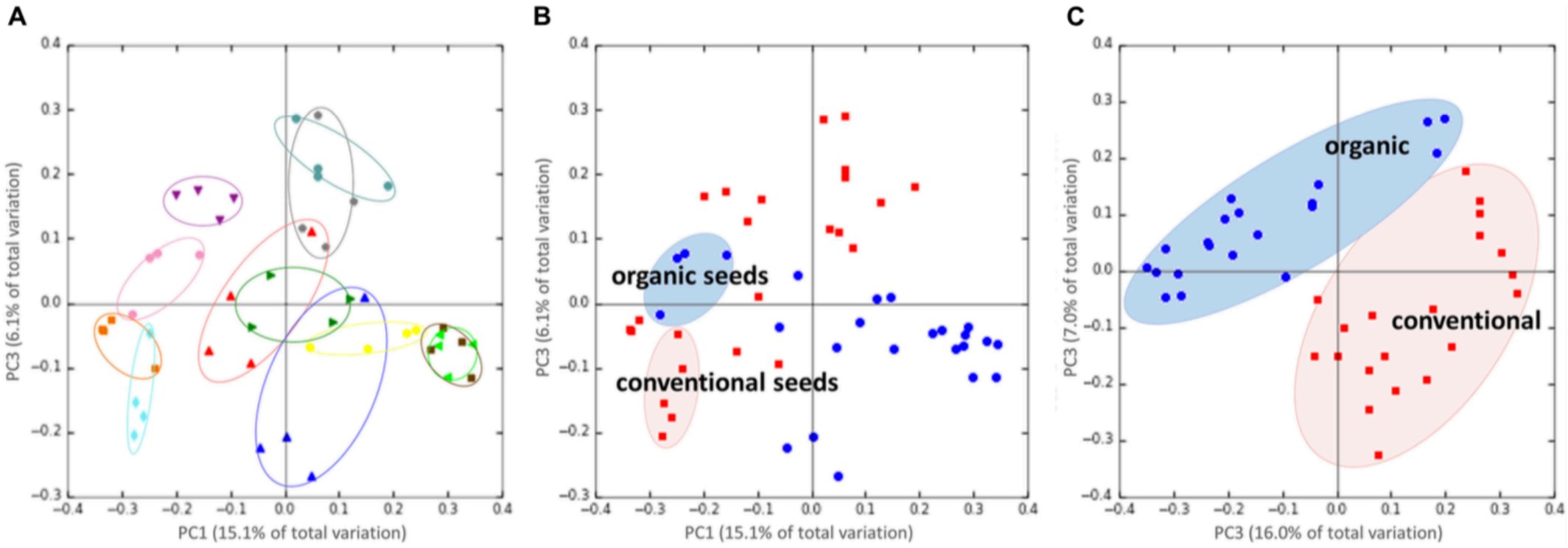


FIGURE 3 | Beta-diversity analysis on microbiota composition dependencies. Panel **(A)** shows the microbiota composition grouped by the tissue of the respective management group, where O and C in the bottom legend denote for organically and conventionally managed apples, respectively. Panel **(B)** 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.



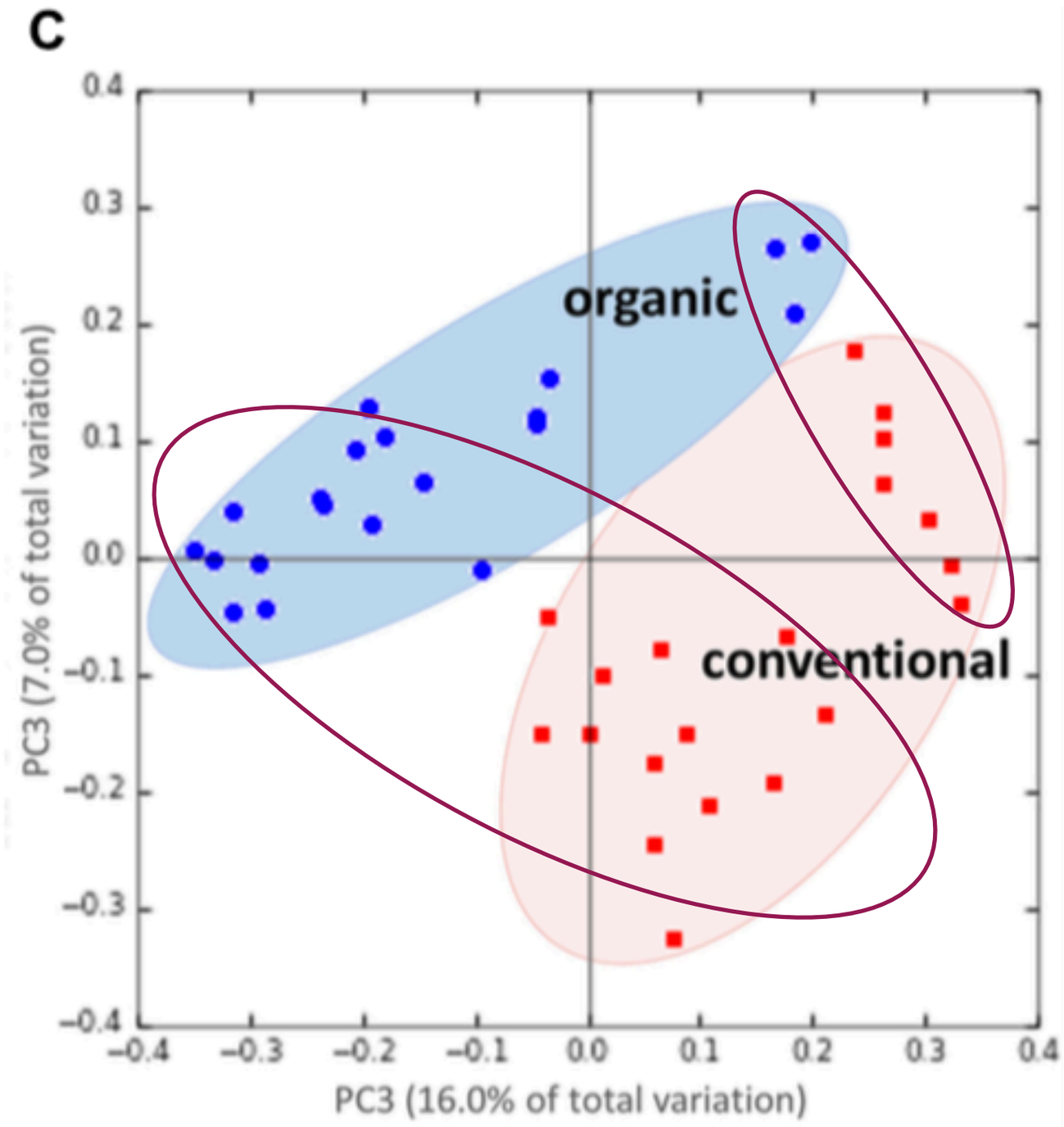


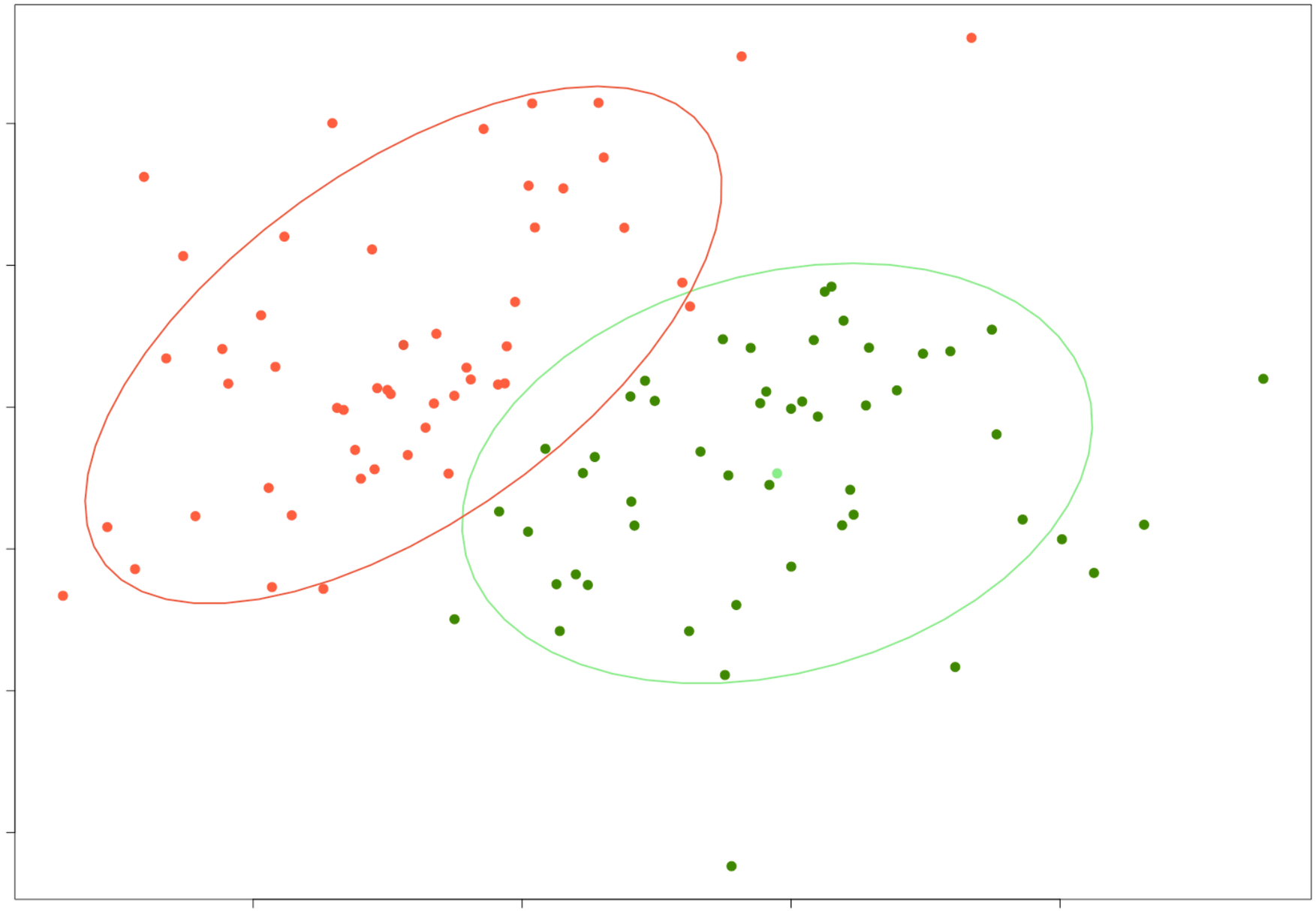
PC1 vs. PC3 →15.1% + 6.1%=21.2%

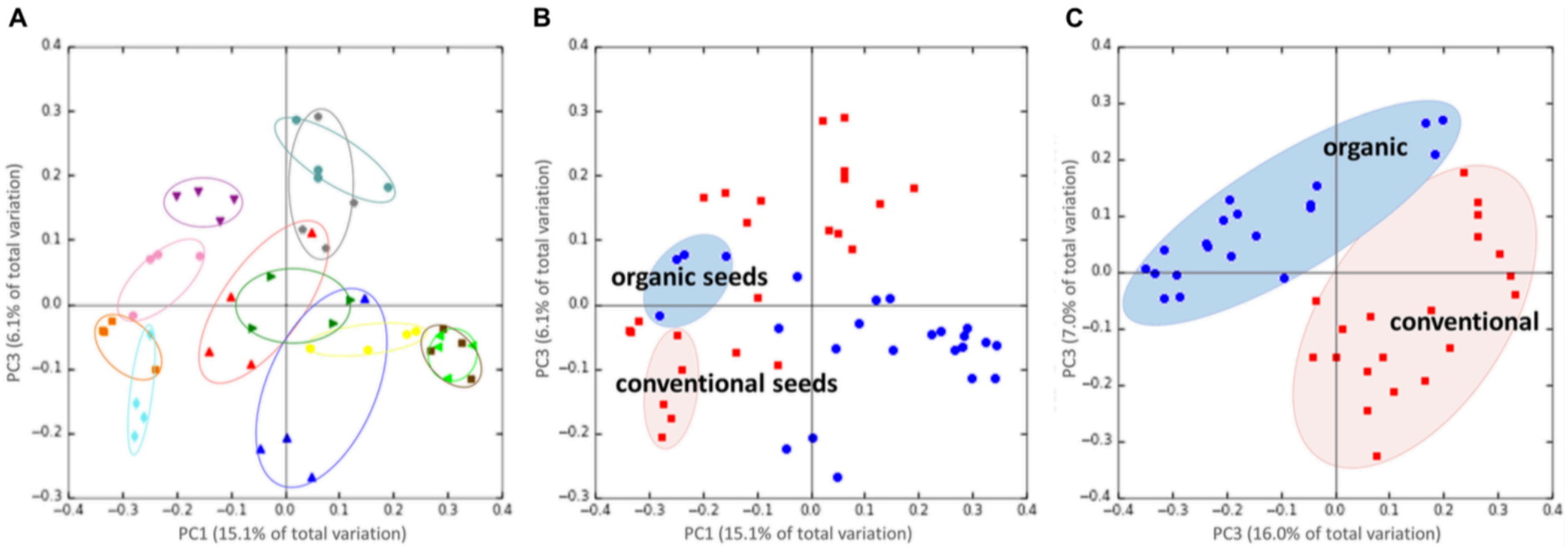


A & B → PC1 + PC2 → 15.1% + 6.1% = 21.2%

B → PC1 + PC3 → 16.0% + 7.0% = 23.0%


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





Highest beta diversity measures were observed when the replicates were grouped by the tissue of the respective management group (ANOSIM values: $R = 0.8$, $p = 0.001$; Figure 3A). Grouping samples by organic and conventional management revealed the ANOSIM values $R = 0.26$, $p = 0.001$(Figure 3B). Hence, we had a closer look on the management effect on each tissue separately, resulting in the ANOSIM values $R > 0.8$, $p < 0.05$ for all tissues, except seeds (ANOSIM values for seeds: $R = 0.4$, $p = 0.05$). The management practice therefore seems to have a profound impact on the microbiota composition of all tissues while the management effect on seed microbiota was lower. This observation was confirmed when seed samples were excluded from the dataset; ANOSIM values increased to $R = 0.45$ and $p = 0.001$ (Figure 3C).

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How much better is R = 0.8 ($p < 0.05$) for all tissues versus R = 0.4 ($p = 0.05$) without the seeds?

* Mantel test, anosim and permanova are multivariate statistical tests of significance. ANOSIM tests for significant difference between two or more classes of objects based on any (dis)similarity measure. permanova is a nonparametric method to conduct multivariate anova and test for differences between object classes. ANOSIM is based on ranks.

R vs. R^2

Figure 3A: ANOSIM values: $R = 0.8$, $p = 0.001$ → $R^2 = 0.8 \times 0.8 = 64\%$

Figure 3B: ANOSIM values: $R = 0.26$, $p = 0.001$ → $R^2 = 0.26 \times 0.26 = 6.8\%$

Figure 3C: ANOSIM values: $R = 0.45$, $p = 0.001$ → $R^2 = 0.45 \times 0.45 = 20.3\%$

ANOSIM values: $R = 0.8$, $p < 0.05$ → $R^2 = 0.8 \times 0.8 = 64\%$

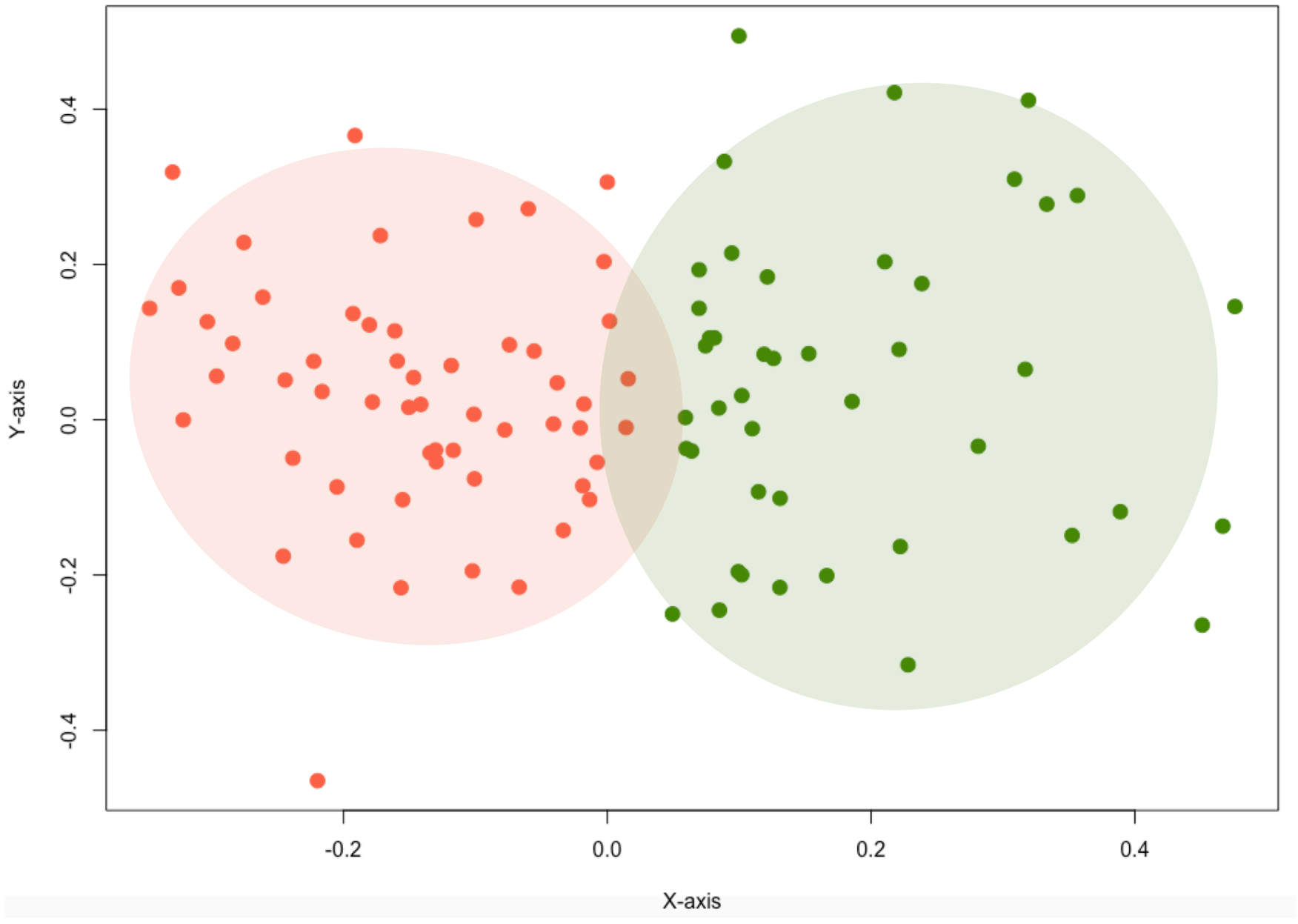
ANOSIM values: $R = 0.4$, $p = 0.05$ → $R^2 = 0.4 \times 0.4 = 16\%$

Δ 48% expalined by the seeds

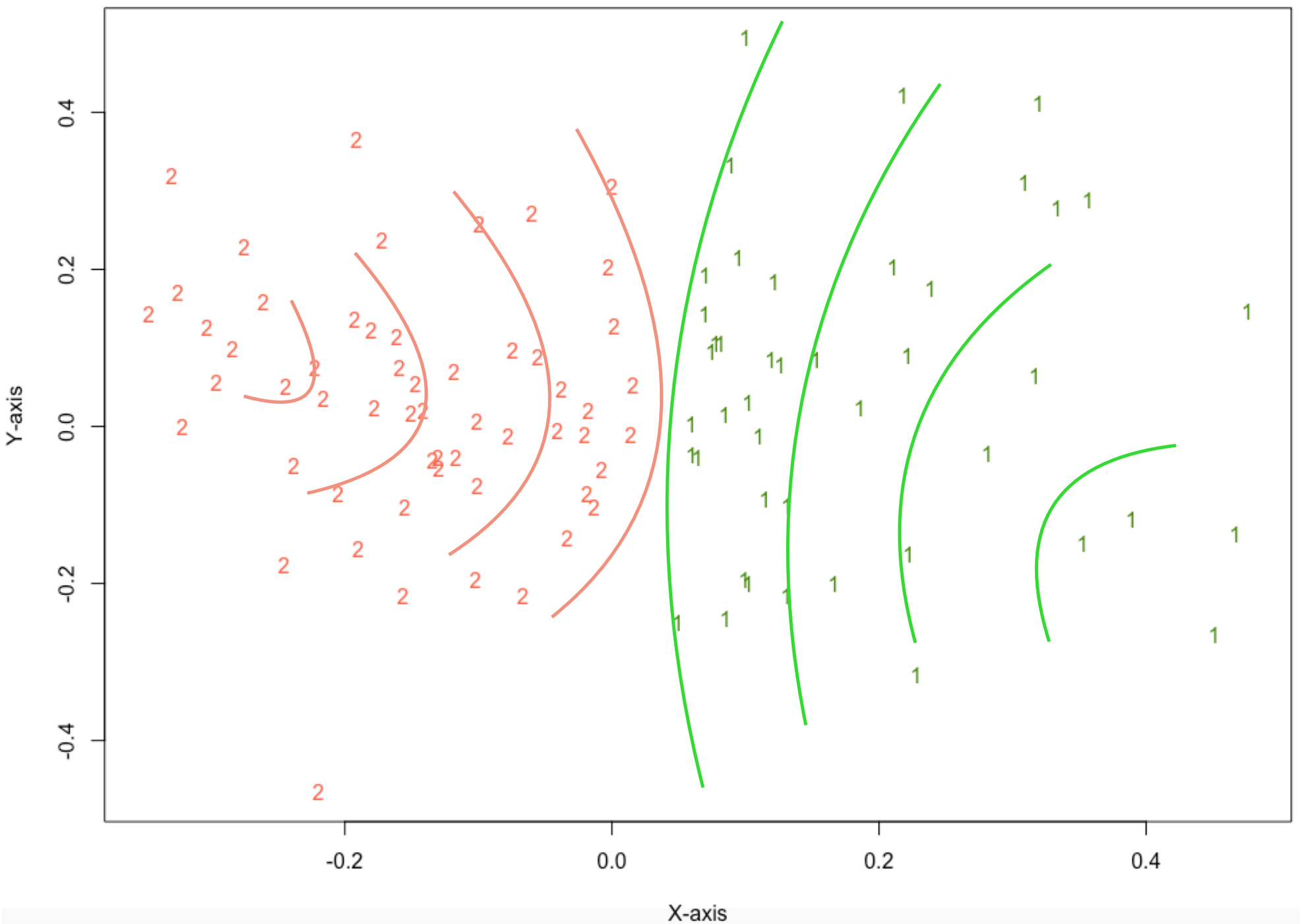
$R^2 = 0.86$ ($p < 0.05$) - Interpretation: Good! The relationship between the two variables explains 86% of the variation in the data siginifcantly.

$R^2 = 0.01$ ($p < 0.001$) - Interpretation: why should I care if it is significant or not since it only explains 1% of the total variance! What about the remaining 99%.

Do you see two clusters?



Do you see two clusters now?



Taxonomic network for **core** microbiota

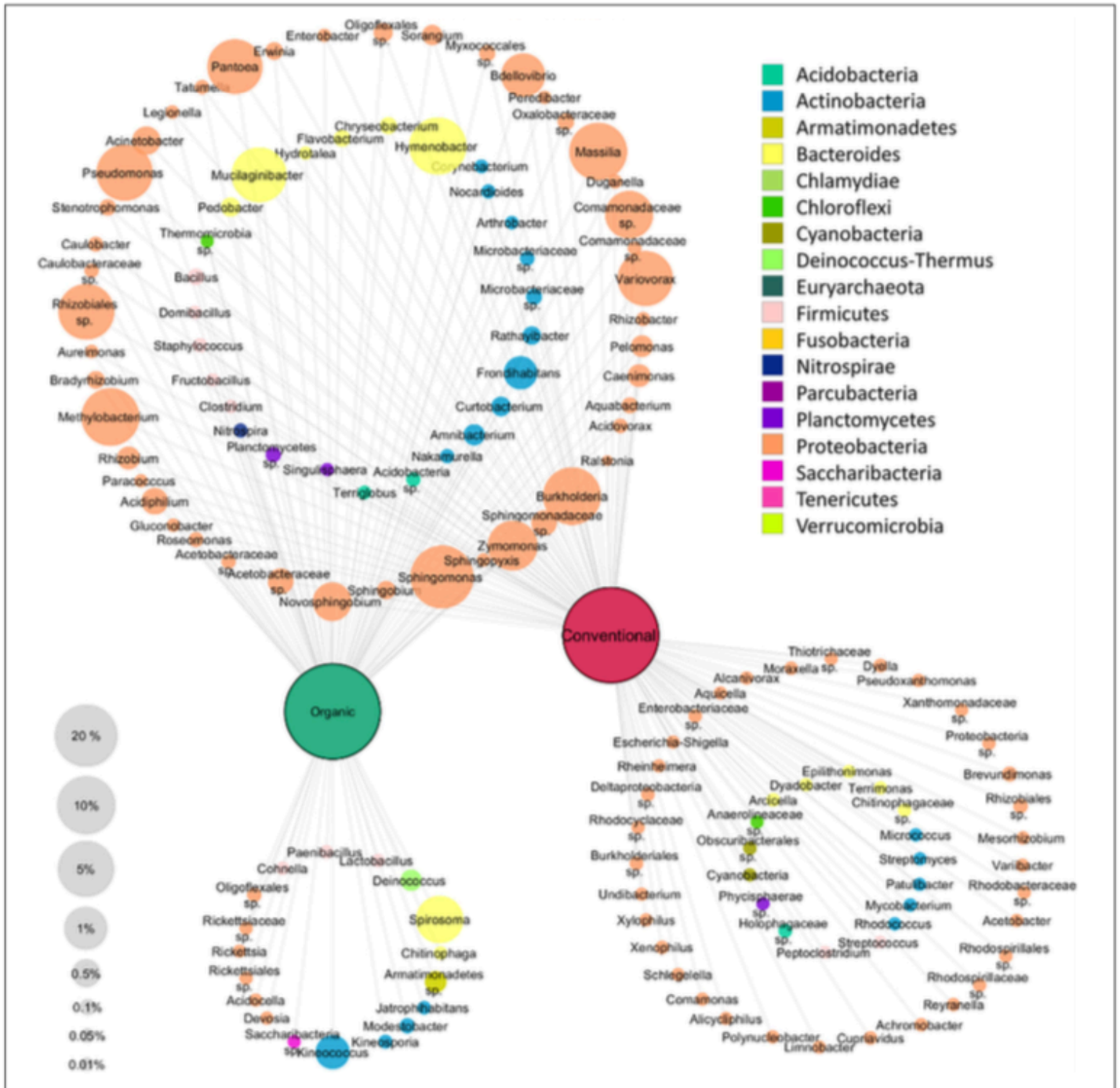
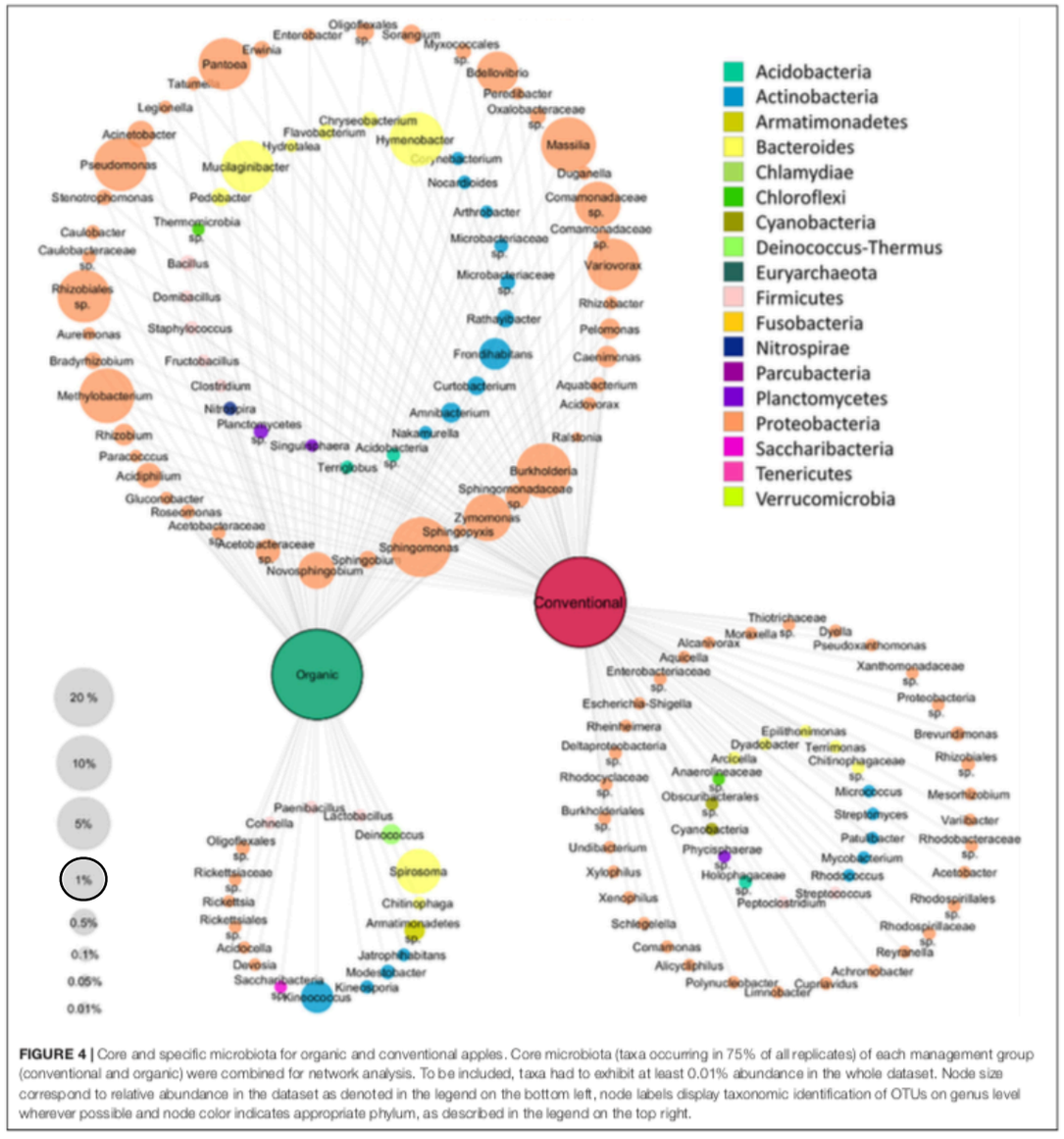


FIGURE 4 | Core and specific microbiota for organic and conventional apples. Core microbiota (taxa occurring in 75% of all replicates) of each management group (conventional and organic) were combined for network analysis. To be included, taxa had to exhibit at least 0.01% abundance in the whole dataset. 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.



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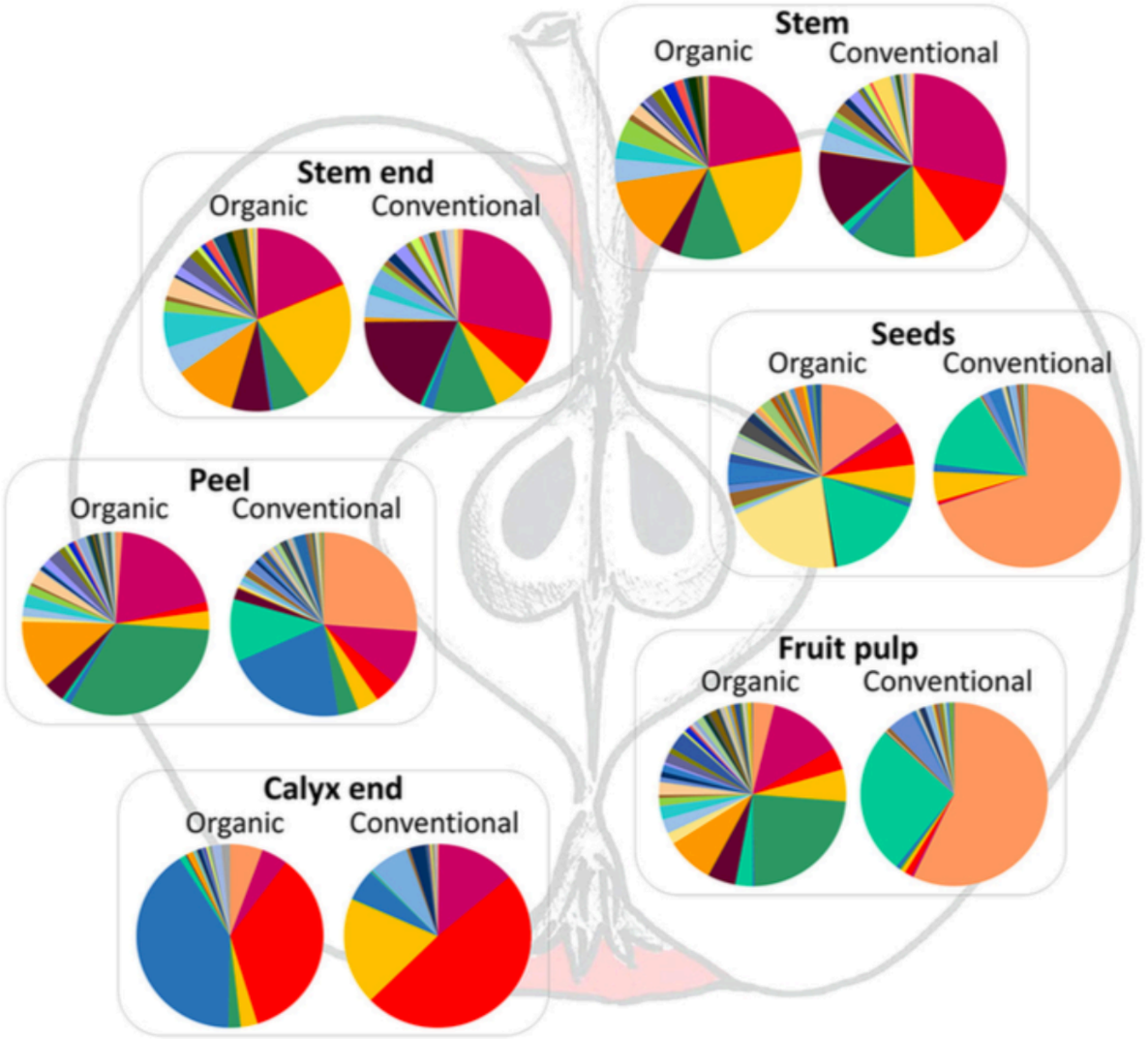
How much and what tissue?

$$50 \times 0.01\% = 0.5\%$$

$$13 \times 0.01\% + 2 \times 0.1\% + 1 \times 0.5\% + 1 \times 1\% = 1.83\%$$

- Spirosoma
- Kineococcus



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**.

Where is the core sample?
Did you get confused with the term "core microbiota"?

Relative abundance for the order Enterobacterials

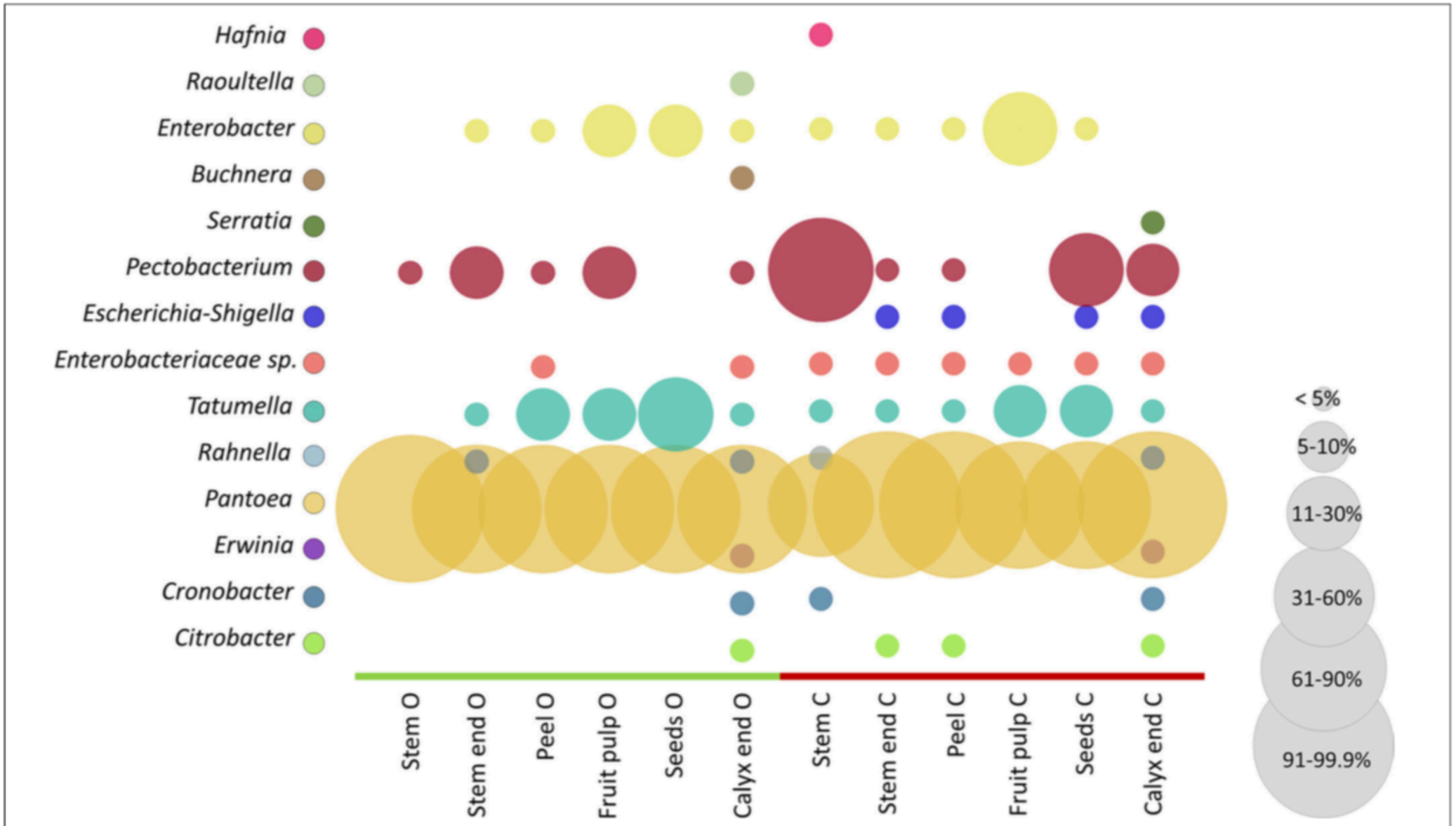
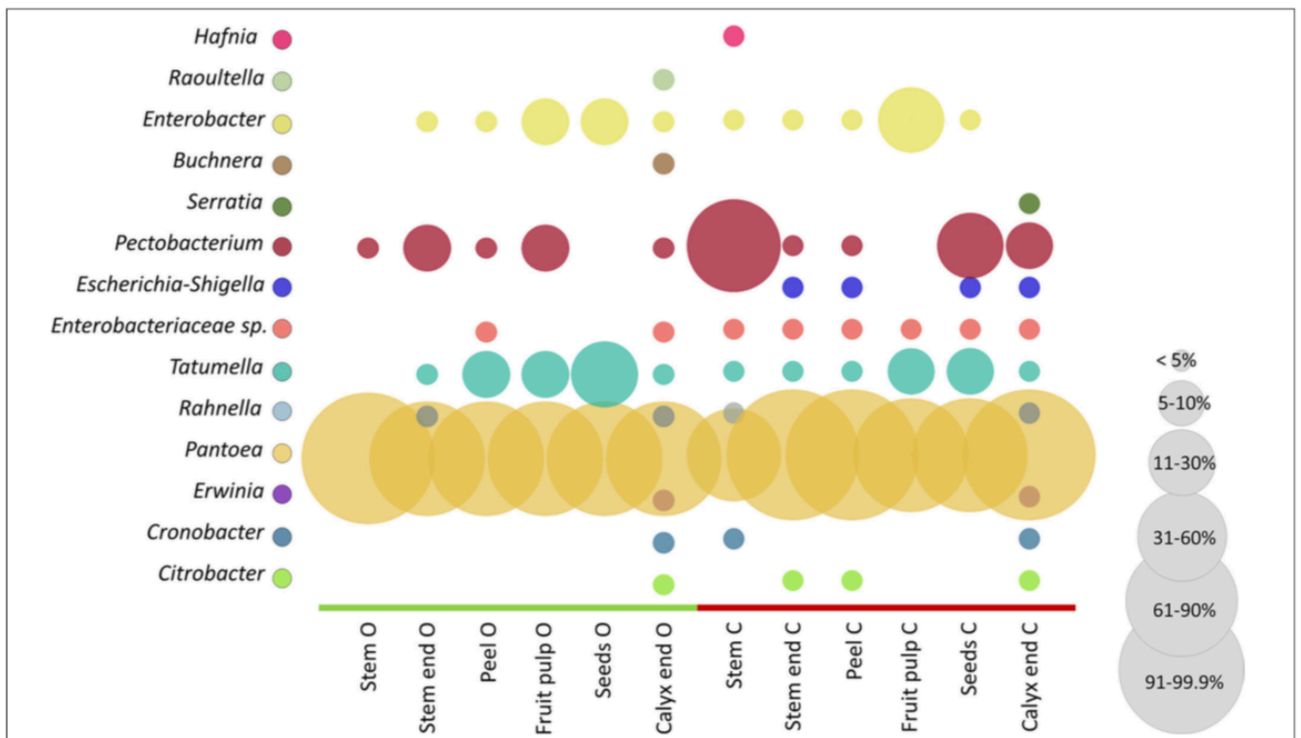


FIGURE 6 | Comparison of conventional and organic apple tissues regarding *Enterobacteriales* abundance. Color code for bubbles is depicted in the legend on the left and bubble size indicates relative abundance of taxa within total *Enterobacteriales* microbiota, as explained in the legend on the right.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



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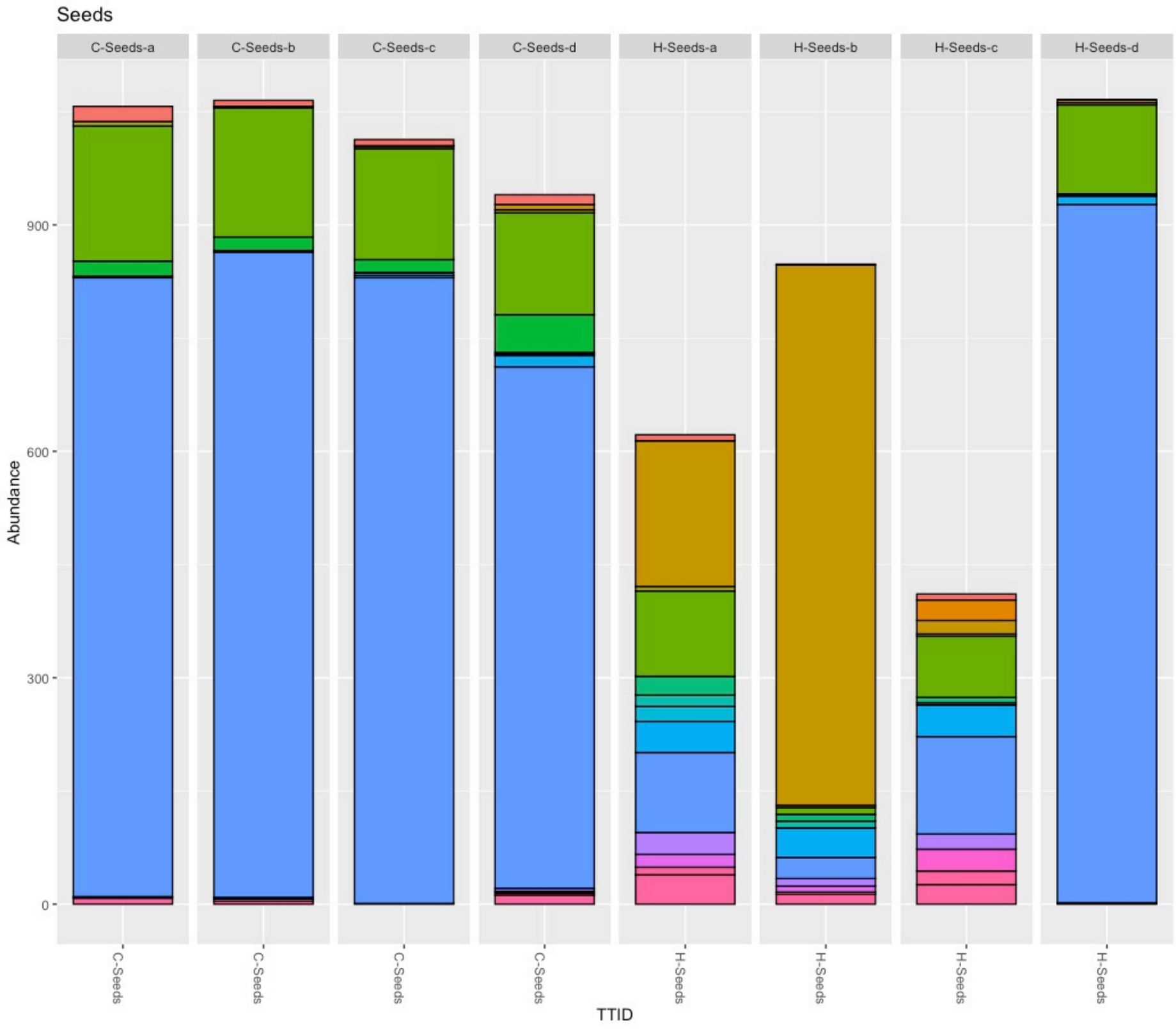
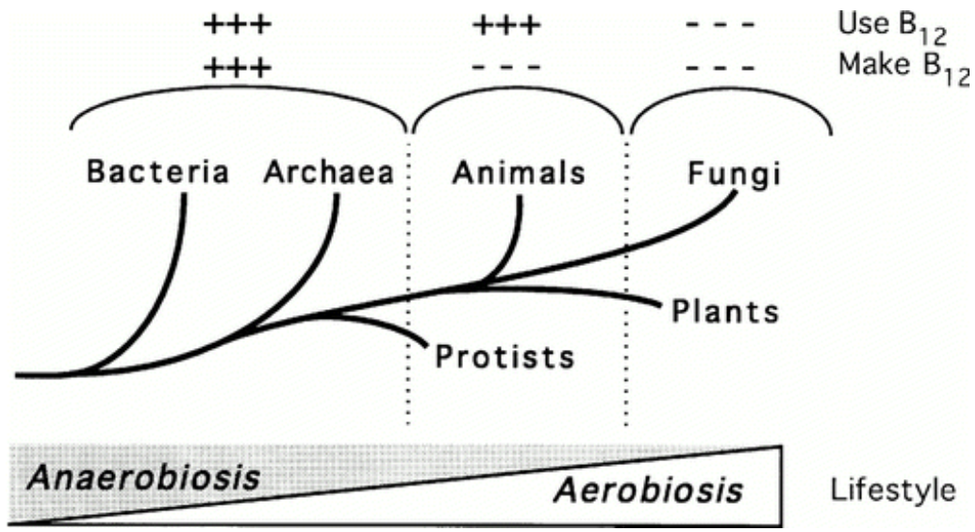
Burkholderiales were highly abundant concerning bacterial **orders** (31% abundance), followed by Sphingomonadales (14%), Rhizobiales (12%), Pseudomonadales (11%), **Enterobacteriales (7%)** and Cytophagales (5%); Micrococcales, Sphingobacteriales, Bacillales, Rhodospirillales, and Flavobacteriales,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


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
Abundance
900
600
300
0
C-Seeds
C-Seeds
C-Seeds
C-Seeds
H-Seeds
H-Seeds
H-Seeds
H-Seeds
TTID

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 "*"

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**.

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.

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.

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.

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

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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.

In-silico PCR

825 Escherichia

57 Shigella

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

0.01%

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.**

Was is the recommended minimum daily intake of bacteria cells?

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?