OP-161 [Bee Health]
Assessing pesticide impact on honeybee gut microbiota: a call for microbial diversity as an environmental risk assessment endpoint

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A recent roadmap for integrating environmental microbiota into risk assessments under the European Food Safety Authority (EFSA) remit has been published. The honeybee gut microbiota has emerged as a promising avenue to protect bees against stressors. Honeybees exhibit a stable core microbiota, and dysbiosis may serve as an indicator of adverse conditions.

We investigated the gut microbiota of newly emerged "Apis mellifera iberiensis" workers exposed to a single concentration of the insecticide flupyradifurone (FPF, 36 ppm). The control groups included pure syrup (negative control, NC) and syrup supplemented with 1% acetone (acetone control, AC). Laboratory trials followed official guidelines (OECD No. 245). The abdomen of each bee was separated from the thorax, and DNA extraction was performed individually. Full-length 16s rRNA amplicon metagenomic was sequenced through PacBio sequel II (HiFi/CCS mode).

The Shannon diversity index was used to analyze honeybee gut microbiota composition across experimental groups. Our results revealed a significant increase in bacterial community diversity (Shannon index, $P = 0.003$) after ten days of chronic exposure to FPF. This effect was more pronounced when compared to the AC group ($P = 0.003$) than to the NC group ($P = 0.03$). These findings demonstrate that FPF disrupts the honeybee gut microbiota.
This study represents the first characterization of honeybee gut microbiota strictly adhering to OECD guidelines without modifications or adaptations. Furthermore, we have provided new insights into pesticide risk assessment, highlighting an overlooked aspect of bee health assessment. We propose integrating this approach into pesticide risk assessments by using diversity indices as comparative parameters. Specifically, we advocate for the inclusion of honeybee gut microbiota dysbiosis as a sublethal effect in the initial screening phase of risk assessments (laboratory-based assays) and as a key parameter for evaluating pollinator health.

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MARIE CURIE ACTIONS

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WHY CONSIDER HONEYBEES?

Environmental microbiomes roadmap

2024

APPROVED: 30 Jan 2024
doi: 10.2903/sp.efsa.2024.EN-8602

Roadmap for the integration of environmental microbiomes in risk assessments under EFSA's remit

Environmental microbiomes roadmap

Appendix B – Case study: The microbiome of honey bees as an indicator of health and emerging risks

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journal homepage: www.elsevier.com/locate/scitotenv

Discussion

Bee gut microbiota as an emerging endpoint for the environmental risk assessment of pesticides

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Strategies to integrate microbiota as an endpoint into ERA

https://doi.org/10.1016/j.scitotenv.2025.179977

Lack of standardization in sublethal effects

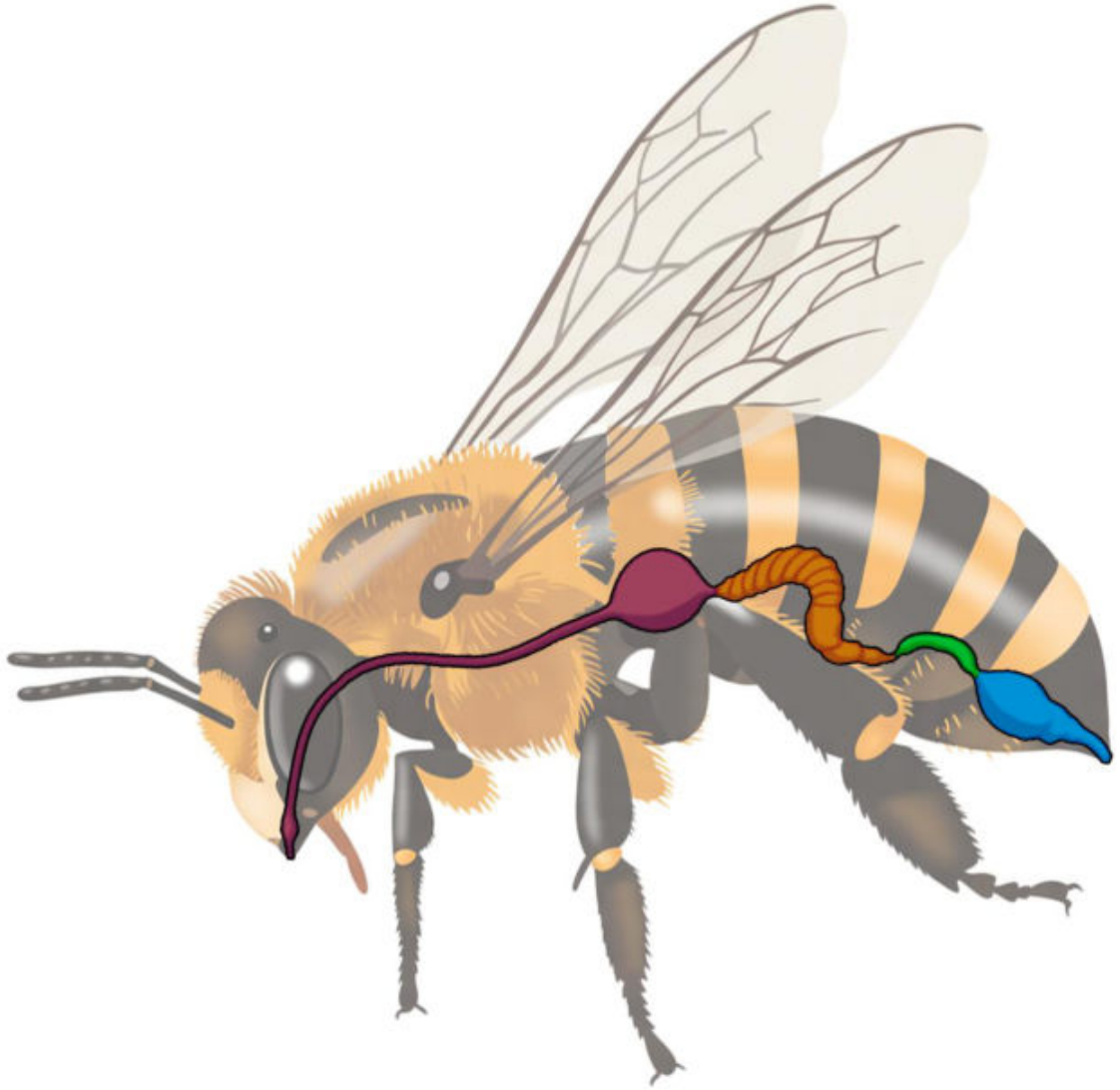
Ideal endpoint:

Potential for standardization – RING TESTS

Capability for use in a shorter timeframe

Ecological relevance

Extrapolating individual-level effects at colony-level



HONEY BEE GUT MICROBIOTA: AN EMERGING ENDPOINT

Rectum:
Dominated by *Lactobacillus*, *Bombilactobacillus* and *Bifidobacterium*.

Role in:

- **Nutrition**
- **Immune system**
- **Detoxification**

Rosa-Fontana et al., 2025. Science of the Total Environment, DOI: 10.1016/j.scitotenv.2025.179977

EXPOSURE BY ORAL INGESTION

Microbiota interacts with pesticides

DYSBIOSIS:

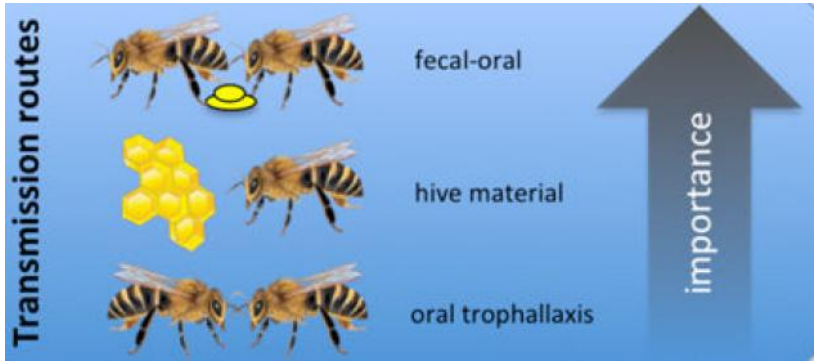
"A disruption or a displacement of the established community".

No microbiota approach for RAs

MAIN CHALLENGES

- Simple and constant X natural fluctuations.
- Defining acceptable fluctuation thresholds.
- Establishment of a ‘safe zone’ for ecotoxicological assays.

GUT MICROBIOTA IN YOUNG BEES



Newly emerged bees: few or 0 bacteria

Microbiota established

Day 0

Day 4

Bees (max. 2 days old)

LAB ASSAYS???

OECD 245:
Chronic Oral Toxicity Test

Kwong, W. K., & Moran, N. A. (2016). Gut microbial communities of social bees. *Nature reviews microbiology*, *14*(6), 374-384.

GUT MICROBIOTA IN YOUNG BEES

Newly emerged bees: few or 0 bacteria

Microbiota established

Day 0 — Day 4

Other studies:

Homogeneisation of microbiota

FORAGER GUT MIXED WITH FOOD

Variations by external factors

FLUCTUATIONS

GUT MICROBIOTA IN YOUNG BEES

Newly emerged bees:
few or 0 bacteria

Microbiota
established

Day 0 — Day 4

Our study:

Bees (max.
2 days old)

OECD 245:
Chronic Oral Toxicity Test
(10-Day Feeding)

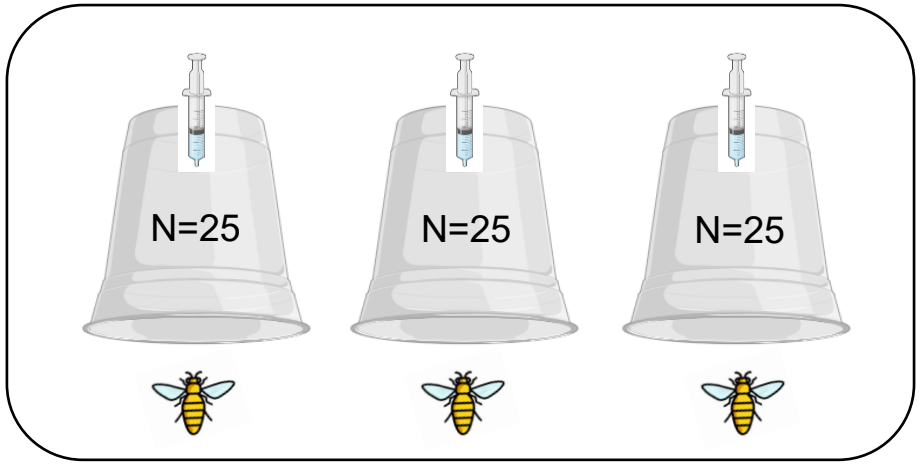
NO HOMOGENEISATION

Variations by external factors

FLUCTUATIONS

BIOASSAYS

Apis mellifera iberiensis



X

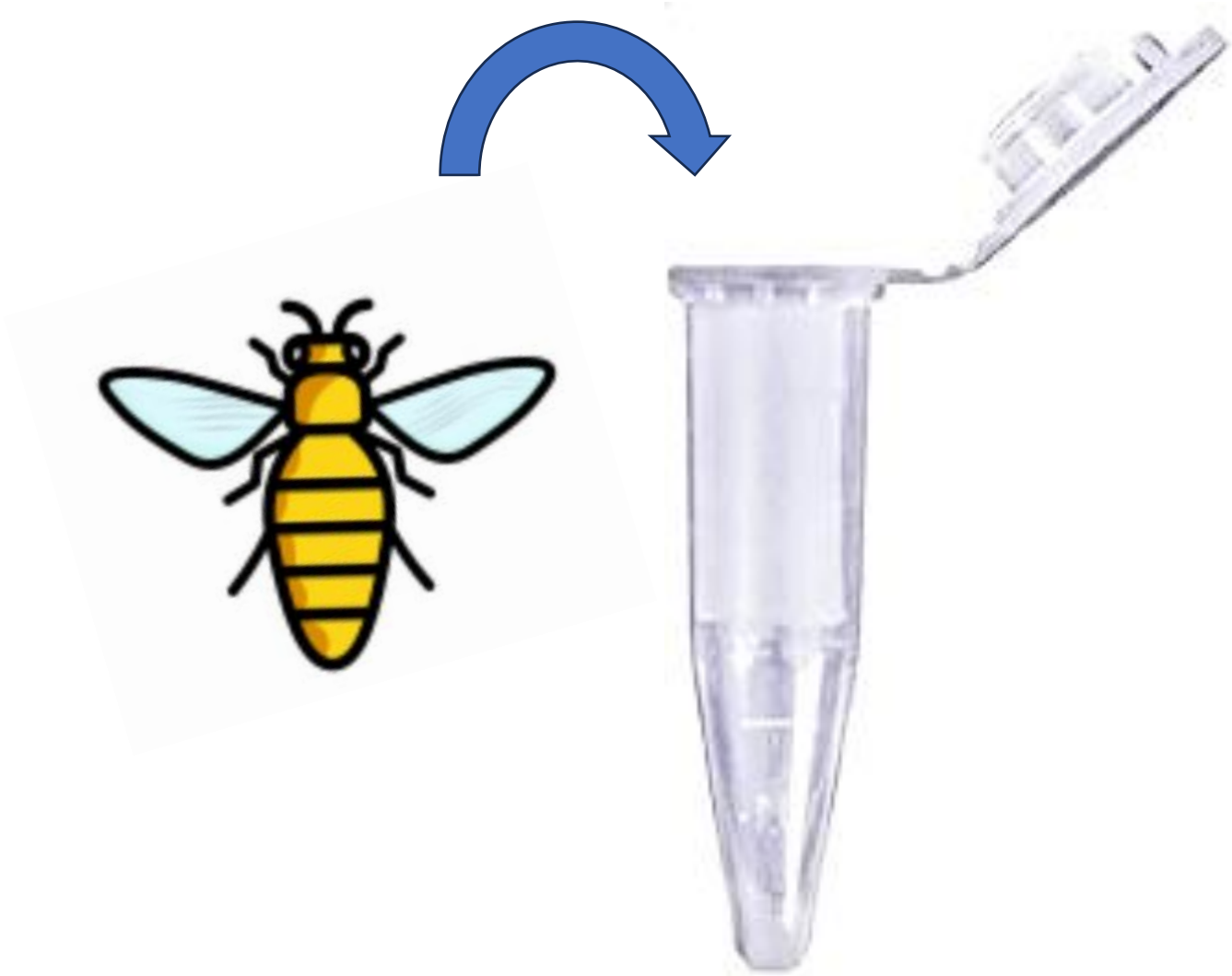
- **FLUPYRADIFURONE (FPF): 36 PPM**
- **NEGATIVE CONTROL (NC): PURE SYRUP**
- **ACETONE CONTROL (AC)**
- **POSITIVE CONTROL: Dimethoate (1 PPM)**

https://www.latiendadelapicultor.com/blog/razas-de-abejas/

http://coronaapicultores.blogspot.com/2012/11/especiesy-subespecies-de-abejas-reino.html

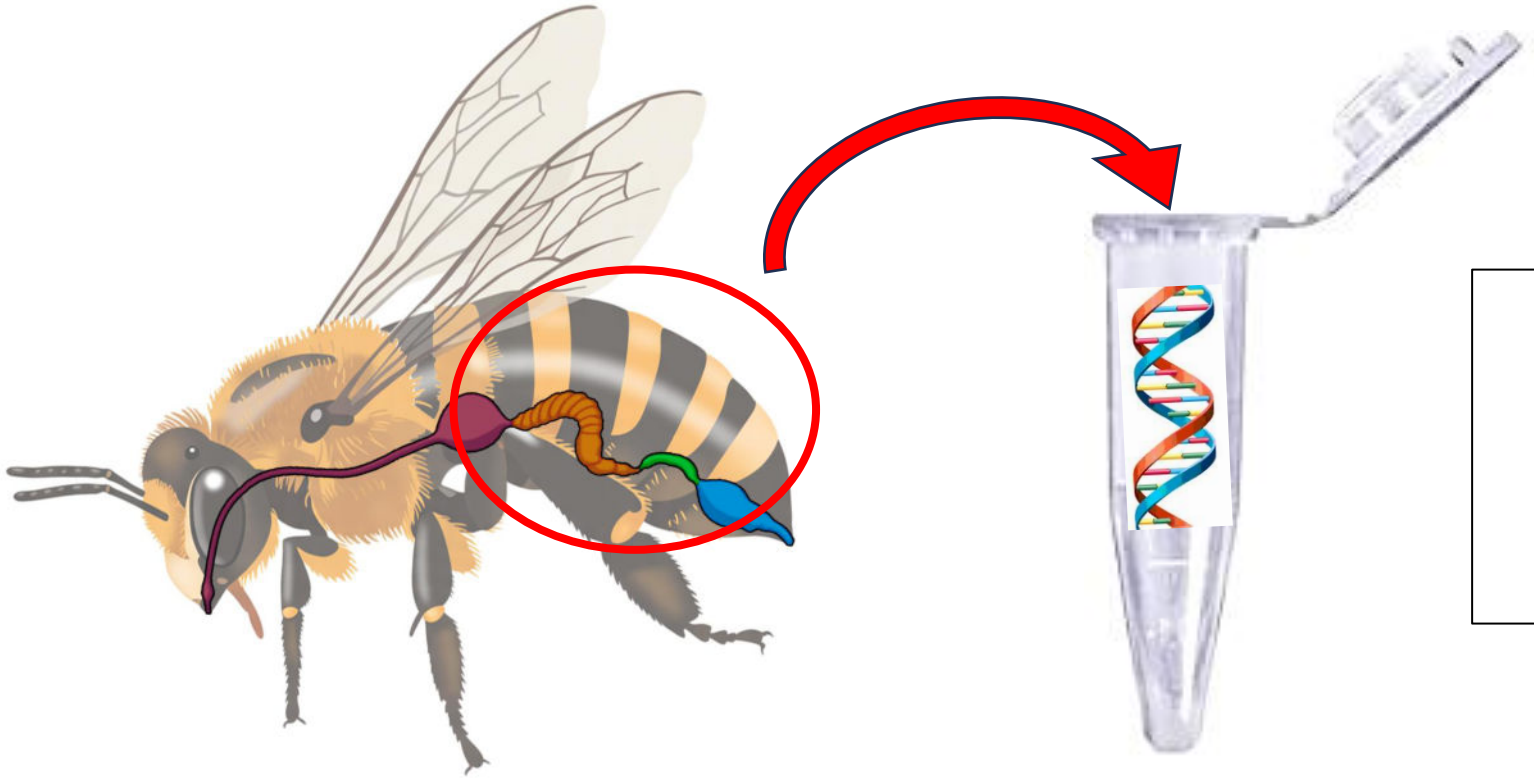
LIVE BEES AT DAY 10

- INDIVIDUALLY FROZEN AT -80°C UNTIL MOLECULAR ANALYSIS



SEQUENCING

- Abdomen separated from the thorax.
- DNA extraction performed individually.



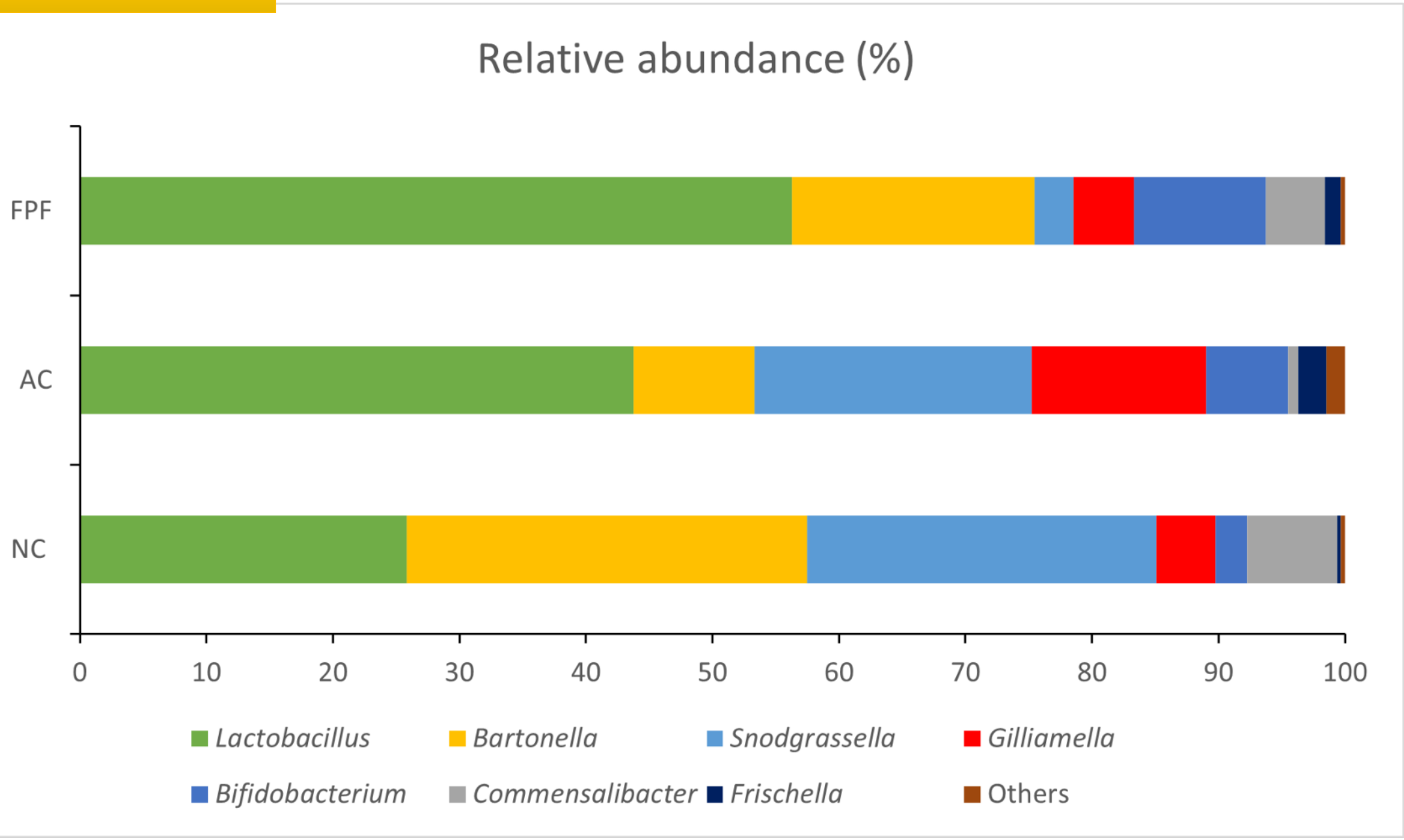
Parameters assessed:

- Relative abundance
- Bacterial diversity

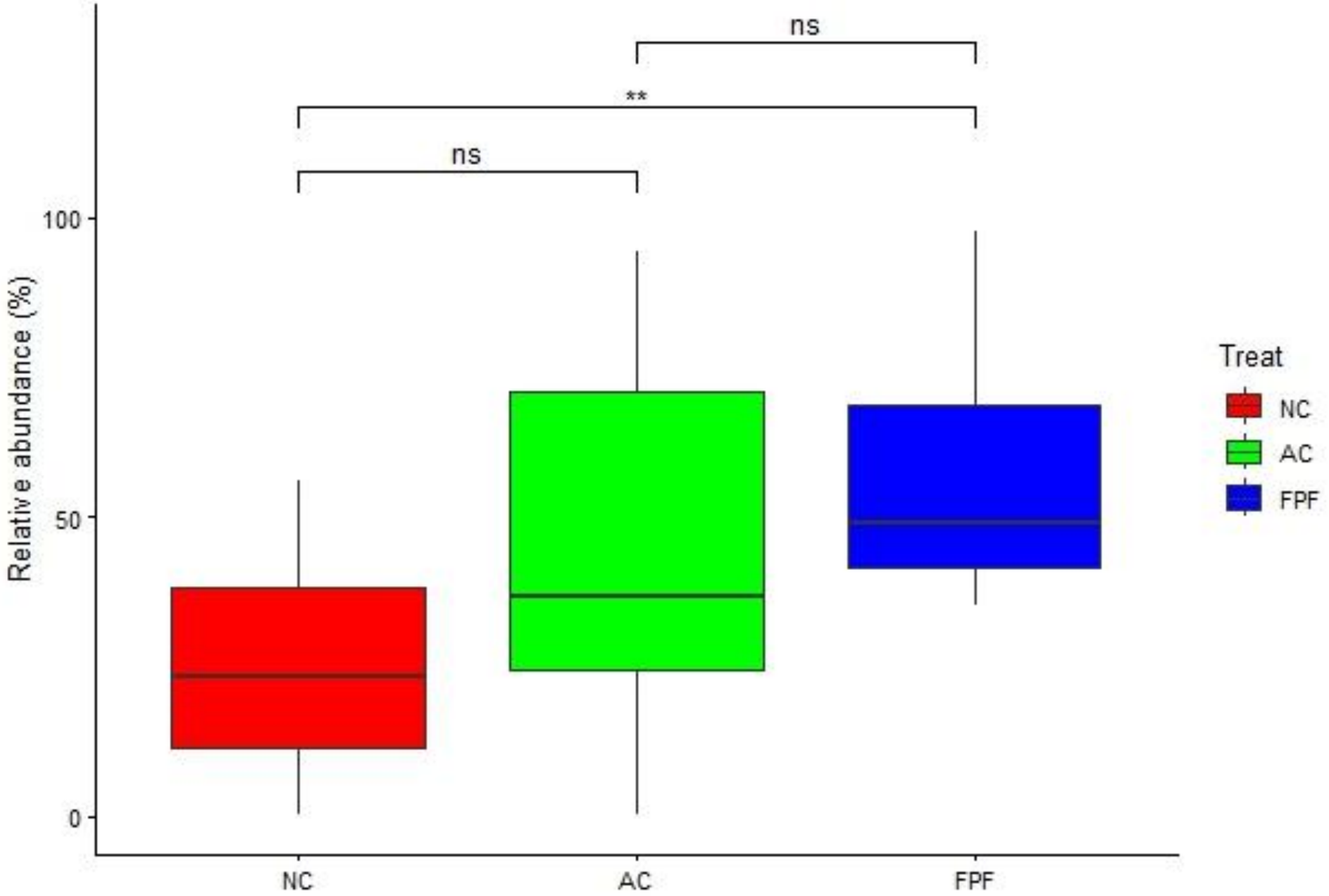
- Full-length 16s rRNA amplicon metagenomic was sequenced through **PacBio sequel II** (HiFi/CCS mode).

RESULTS

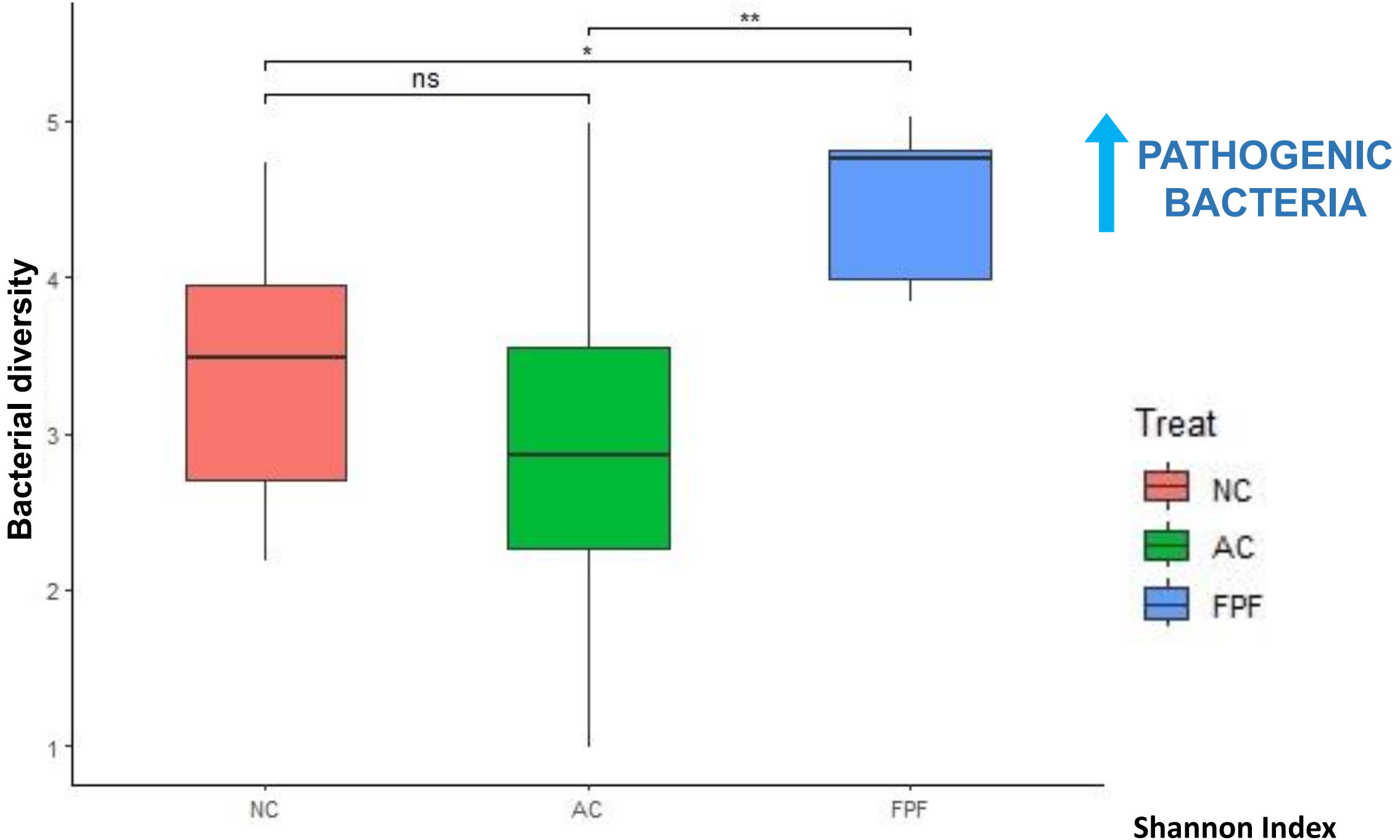
TOP BACTERIAL GENERA



Lactobacillus



Dunn test; p. adjust: **Bonferroni**


**
*
ns
Bacterial diversity
5
4
3
2
1
NC
AC
FPF
PATHOGENIC BACTERIA
Treat
NC
AC
FPF
Shannon Index

CONCLUSIONS

- First approach to standardising the honeybee gut microbiota.
- No homogenisation of the gut microbiota (OECD standards).
- Relative abundance + Bacterial diversity:

  Reliable indicators of **DYSBIOSIS**

- Dysbiosis: sublethal effect in ERA of pesticides.

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MARIE CURIE ACTIONS

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