

# Soil microbial biodiversity and composition are not altered by thinning in a *Pinus nigra* forest

Antonio Rodríguez-García<sup>1</sup>, Rosario Pérez-Redondo<sup>2a</sup>, Eduardo Collado-Coloma<sup>3</sup>, Alberto Sola-Landa<sup>2a</sup>, Miriam Piqué-Nicolau<sup>3</sup>, Roberto Rubio-Gutiérrez<sup>2b</sup> and Javier de-Dios-García<sup>2b</sup>

<sup>1</sup> Área de Microbiología, Facultad de Ciencias Biológicas y Ambientales, Campus de Vegazana, Universidad de León, 24071, León, Spain (arodg@unileon.es).

<sup>2</sup> Castilla y León Wood & Forest Services Center (Cesefor), <sup>a</sup>Avda. Real 1, 24006, León, Spain. <sup>b</sup>Pol. Ind. Las Casas, Calle C, parcela 4, 42005, Soria, Spain.

<sup>3</sup> Forest Science and Technology Centre of Catalonia (CTFC), Ctra. de Sant Llorenç de Morunys, km 2, 25280 Solsona, Lleida, Spain.

## Objective

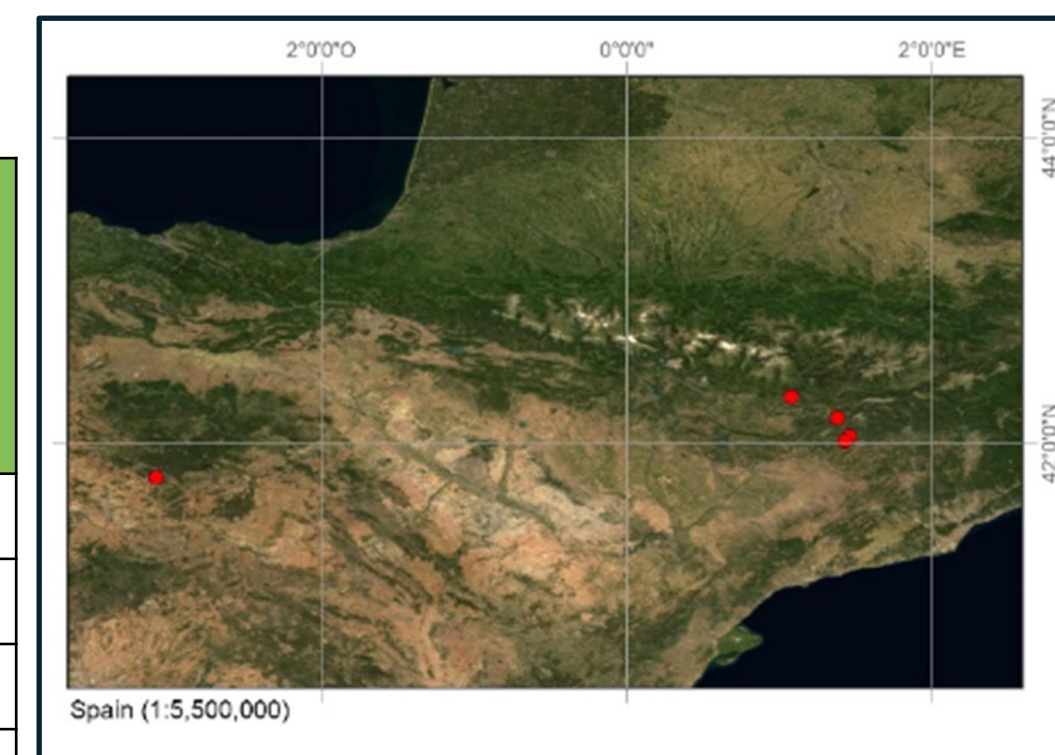
The objective of this study was to assess the impact of thinning on the diversity and composition of soil fungi and bacteria in *Pinus nigra* forests using metabarcoding profiling.



## Methods

Control and thinned plots, representing a variety of types, structures, and ages, were established across five sites in Soria and Catalonia, totaling 11 plots.

Plot_ID	Type of silvicultural treatment	Last year of intervention	Thinning intensity (% Basal Area reduction)	Clearings
CL.01	None	-	-	No
CL.02	Thinning from below	2004	35-40 % BA	No
CA.01	Thinning from below	2016	21.5 % BA	Yes/Selective
CA.02	None	-	-	No
CA.03	Preparatory cutting	2016	32.5 % BA	Yes/Selective
CA.04	None	-	-	No
CA.05	Preparatory cutting (but similar to a thinning from below)	2016	29.4 % BA	No
CA.06	None	-	-	No
CA.09	None	-	-	No
CA.07	Mixed thinning (above, below)	2016	16.7 % BA	No
CA.08	Mixed thinning (above, below)	2016	23.2 % BA	No



## Soil sampling

- Circular plots (16 m Ø) randomly sampled.



- Eight soil cores (21 cm × Ø 5 cm) per plot.
- Cores combined in a plastic bag.



- Kept cold until processing in laboratory.

## Samples processing

- Processing of soil samples:
- Sieving to 2.5 mm
  - Manual homogenization
  - Aliquot partitioning and storage



## DNA extraction

- Mechanical disruption in Mikro-Dismembrator.
- DNeasy PowerSoil Pro kit (Qiagen).
- 3 technical replicates per plot (33 DNA samples).

## Amplicon sequencing

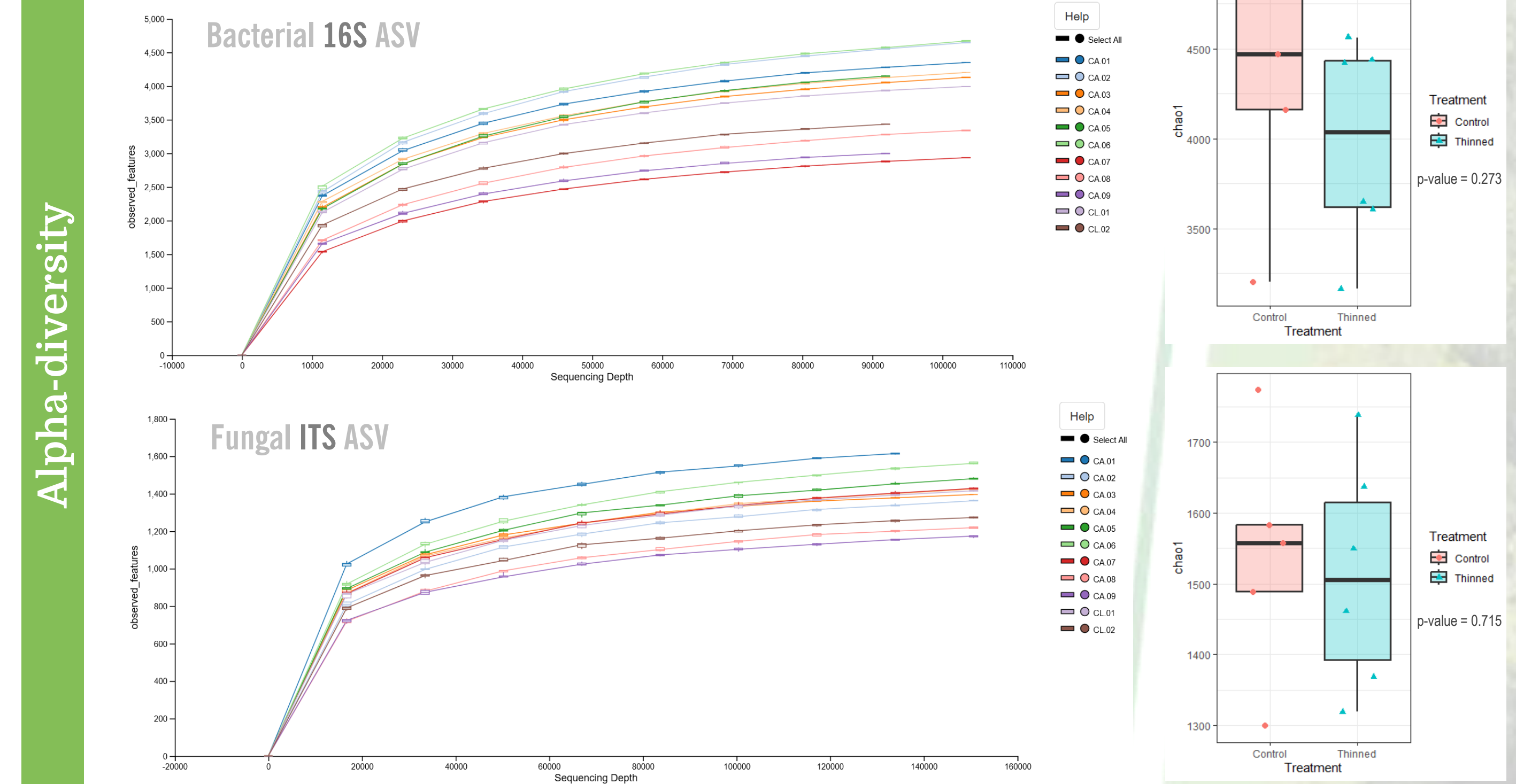
- PCR amplification 16S:
- PCR amplification ITS:
- Illumina PE 250 bp sequencing.

## Bioinformatics

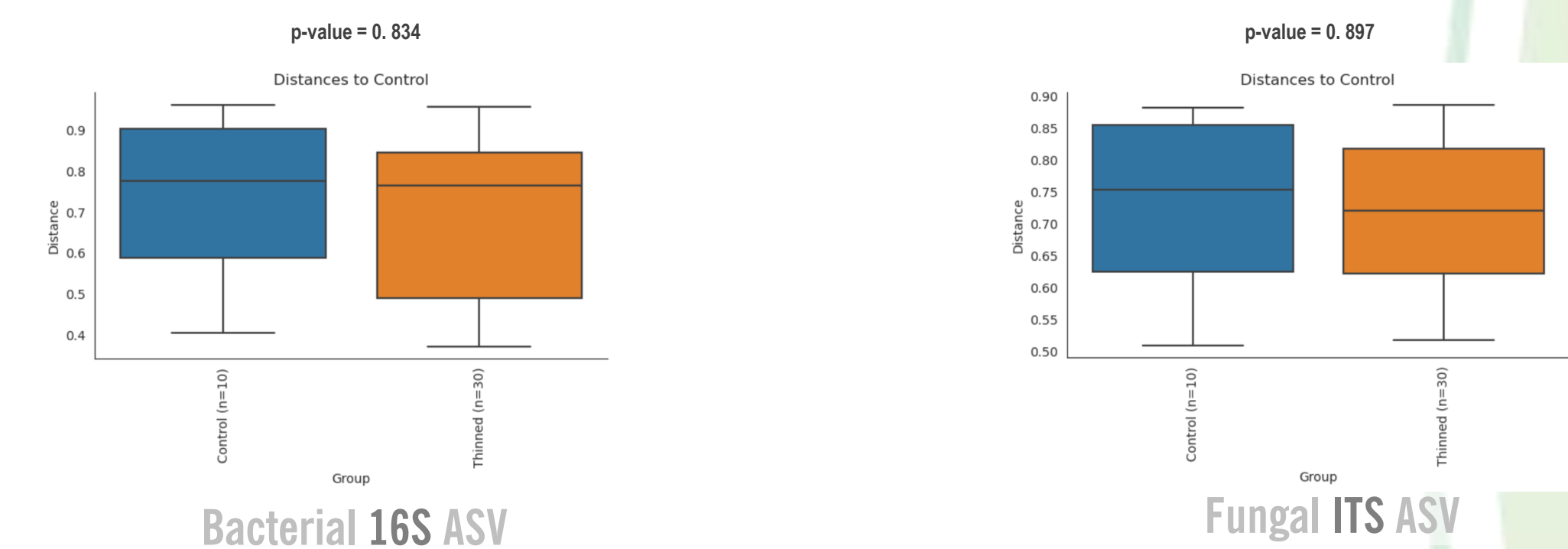
- BBDuk for primers removal
- dada2 for denoising, merging and dechimerization, yielding ASV tables.
- QIIME2 for classification with Unite (ITS) and Silva (16S), diversity (also *microbiome*) and differential abundance analysis (ANCOM-BC).

## Results

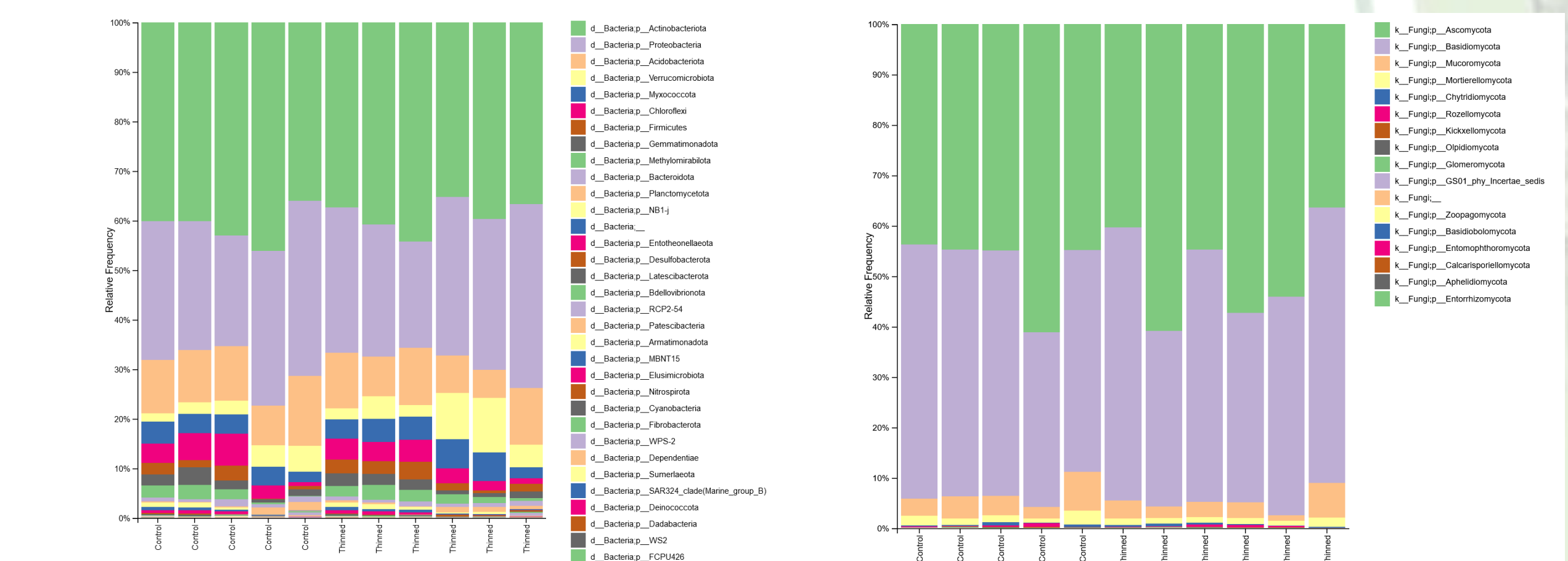
### Rarefaction plots and Kruskal-Wallis tests



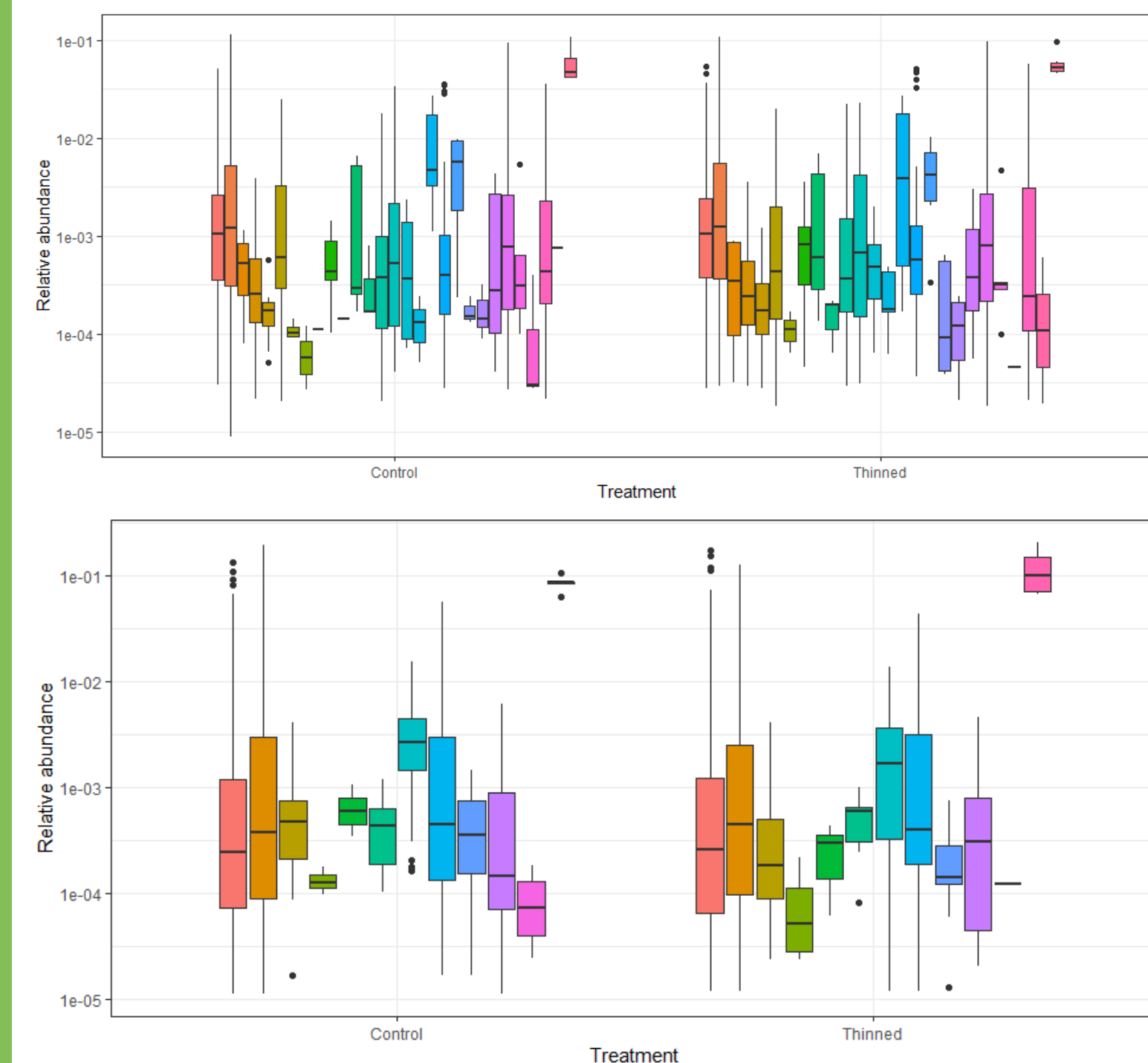
### PERMANOVA (Bray-Curtis)



## Composition & differential abundance



### Genus-level agglomerated abundances:



### ANCOM-BC

- Significance: q-value < 0.1
- Bacteria: just one species & one family enriched by thinning
- Fungi: no significant abundance differences at species, genus, family or order levels; only three fungal ASV showing differential abundance.

## Conclusion

Thinning did not significantly affect the diversity and composition of soil microbiota.

## Acknowledgements

This work was funded by the H2020 project ONEforest. We thank Carmen Alonso, Josefina Merino and Bernabé Martín from Cesefor and Haozhen Su from Novogene for the support provided to carry out this study.



This project has received funding from the European Union's Horizon 2020 research and innovation programme under the grant agreement N° 101000406.