

Residual signature of sewage sludge in soil bacterial communities 15 years after application

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Soil microbial communities

- Vital in soil functionality, soil nutritive status and plant health
- Play a key role in soil nutrient cycling and OM decomposition
- Involved in soil GHGs fluxes
- Quickly and strongly respond to external changes → useful as bioindicators

Plant-Soil-Microbial interactions

Plant and soil properties affect soil microbial communities

Agroforestry systems are well studied and globally considered sustainable forms of land management, their impact on soil microbial biodiversity remains poorly understood.



Investigate the **residual effect of sewage sludge** addition (15 years after the last application) on the **soil bacterial communities** in an agroforestry system (*Pinus radiata* plantation in combination with pasture) in the NW of the Iberian Peninsula

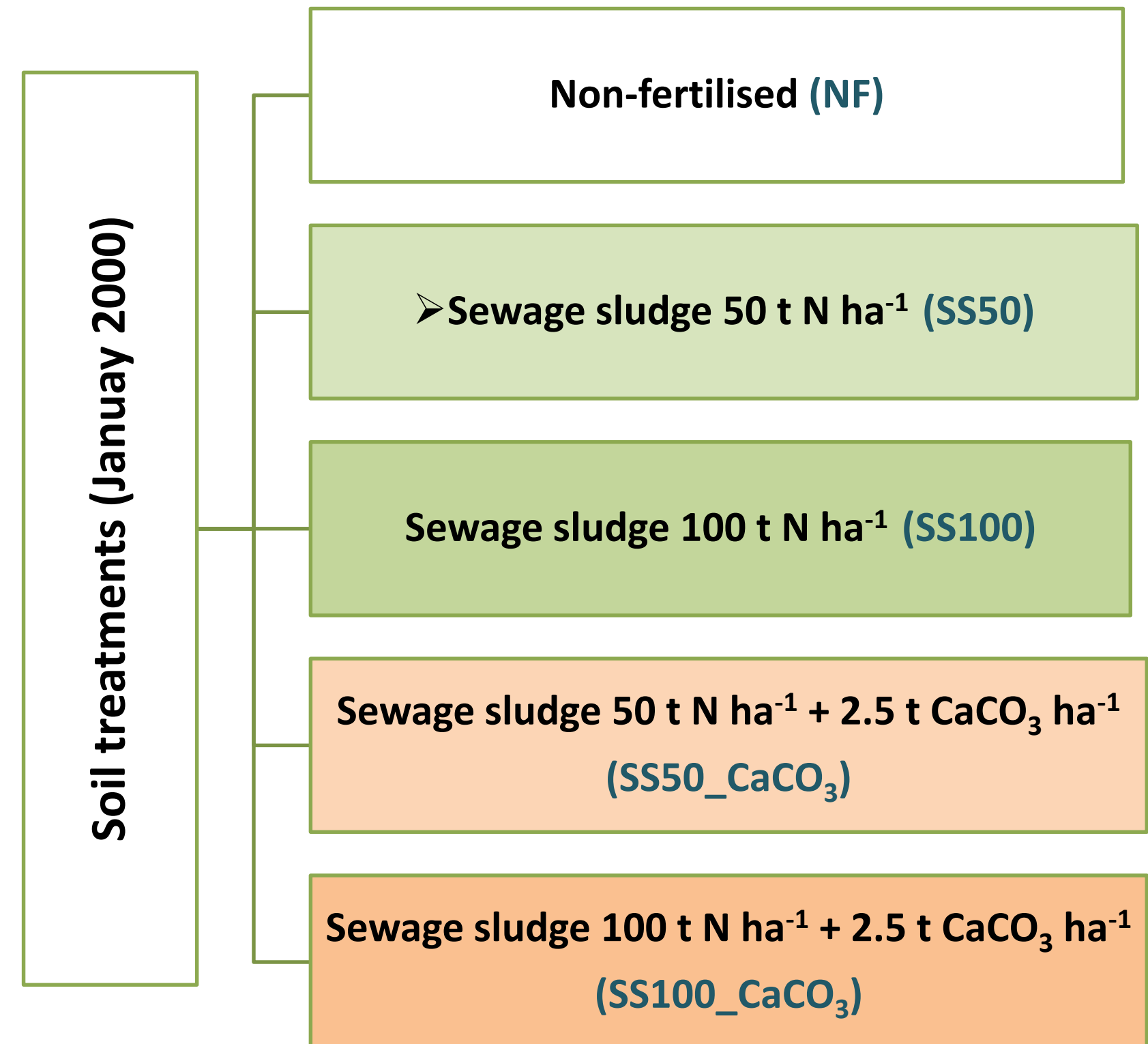
MATERIAL AND METHODS

EXPERIMENTAL DESIGN

- *Pinus radiata* D. Don plantation in the NW of the Iberian Peninsula 1,667 trees ha⁻¹ (distance 2 x 3 m) in October **1998**
- Four **soil fertilisation** treatments with sewage sludge in **2000 during 4 consecutive years**. Three replicate plots (12 x 8 m) per treatment

SOIL ANALYSIS

- **Soil sampling** (25 cm deep, 4 replicates per plot) in **October 2019**
- **Soil bacterial communities** → DNA extraction from soil and sequencing of hypervariable regions V2-4-8 and V3-6, 7-9 of the 16S rRNA gen using an Ion-Touch platform.
- Soil chemical properties: **pH, CEC, C, N, P** and mid-infrared spectral **signature of the soil OM**



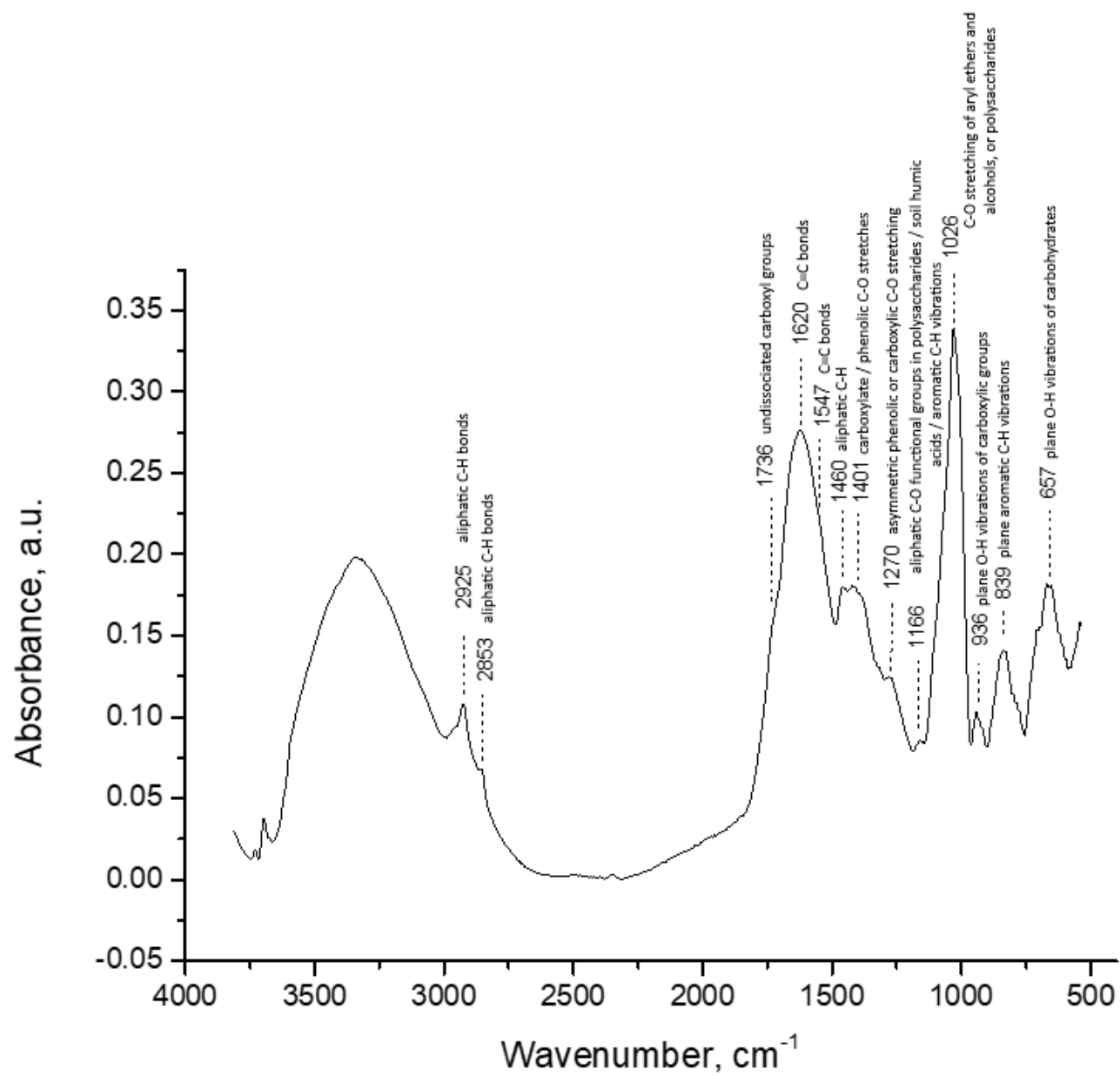
RESULTS: Soil chemical properties in NF and fertilised soils 15 years after sewage sludge application

Small changes in soil chemical properties 15 years after the last sewage sludge application

✓ Only slightly (but significant) higher soil pH and exchangeable Ca in the highest dose of SS together with CaCO₃

	pH_H2O	pH_KCl	Total_N	Total_P	Org_C	K(meq)	Na(meq)	Ca(meq)	Mg(meq)	Al(meq)	CEC
NF	4.75 ± 0.08 ab	4.05 ± 0.07 ab	0.25 ± 0.03	0.018 ± 0.002 ab	5.29 ± 0.91	0.21 ± 0.02 b	0.75 ± 0.09 b	1.11 ± 0.14 c	0.64 ± 0.14	5.37 ± 1.31	8.09 ± 1.53
SS_50	4.62 ± 0.22 b	3.85 ± 0.20 b	0.27 ± 0.07	0.018 ± 0.003 ab	5.59 ± 2.11	0.25 ± 0.03 a	0.83 ± 0.05 ab	1.76 ± 0.31 bc	0.89 ± 0.26	6.52 ± 1.93	10.25 ± 2.25
SS_100	4.70 ± 0.19 ab	3.96 ± 0.15 ab	0.28 ± 0.01	0.024 ± 0.008 a	5.66 ± 0.47	0.25 ± 0.02 a	0.89 ± 0.08 a	2.50 ± 1.17 abc	0.92 ± 0.18	6.21 ± 1.78	10.77 ± 1.84
SS_50_CaCO3	4.89 ± 0.11 ab	4.14 ± 0.09 a	0.25 ± 0.05	0.015 ± 0.001 b	4.21 ± 1.20	0.23 ± 0.01 ab	0.82 ± 0.08 ab	3.16 ± 0.88 ab	0.87 ± 0.24	4.60 ± 1.14	9.69 ± 1.43
SS_100_CaCO3	4.94 ± 0.17 a	4.11 ± 0.14 a	0.27 ± 0.02	0.019 ± 0.003 ab	5.75 ± 1.31	0.22 ± 0.01 ab	0.78 ± 0.10 ab	3.72 ± 1.61 a	0.98 ± 0.23	4.92 ± 0.89	10.62 ± 1.51

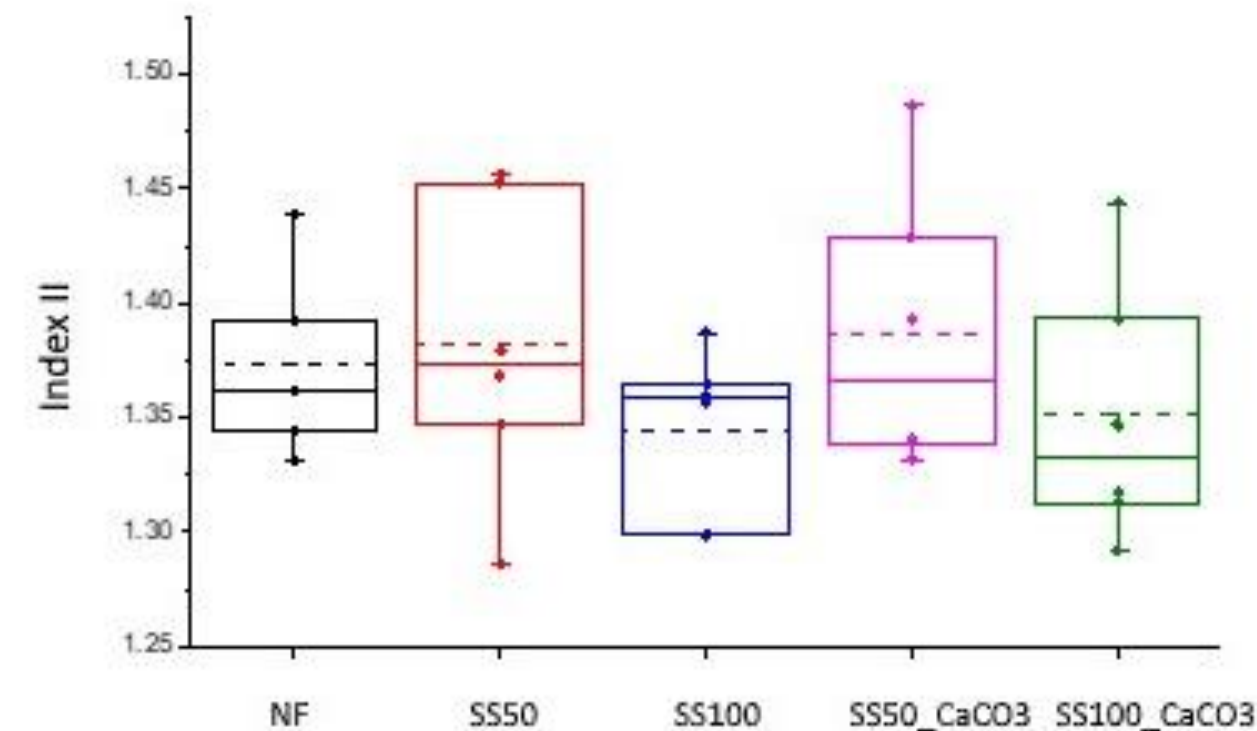
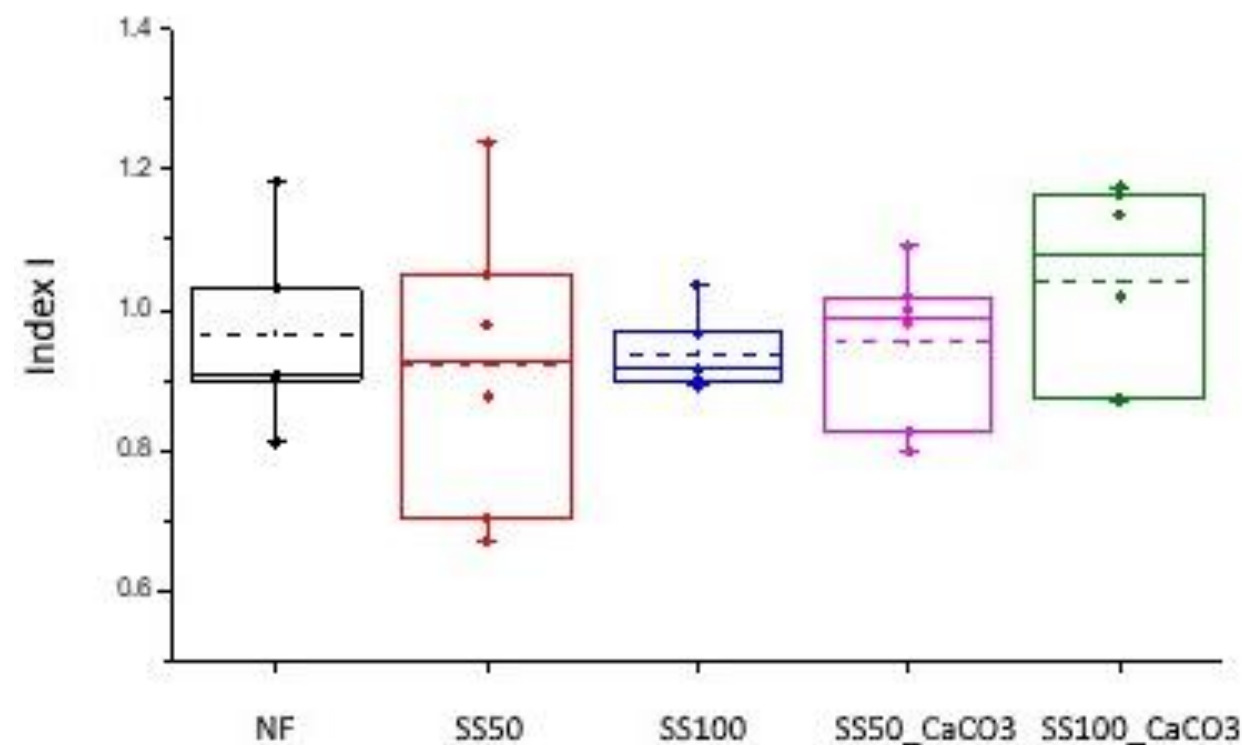
RESULTS: Mid-infrared spectral signature of the **soil OM compounds**



No changes in the decomposition or recalcitrance of the OM

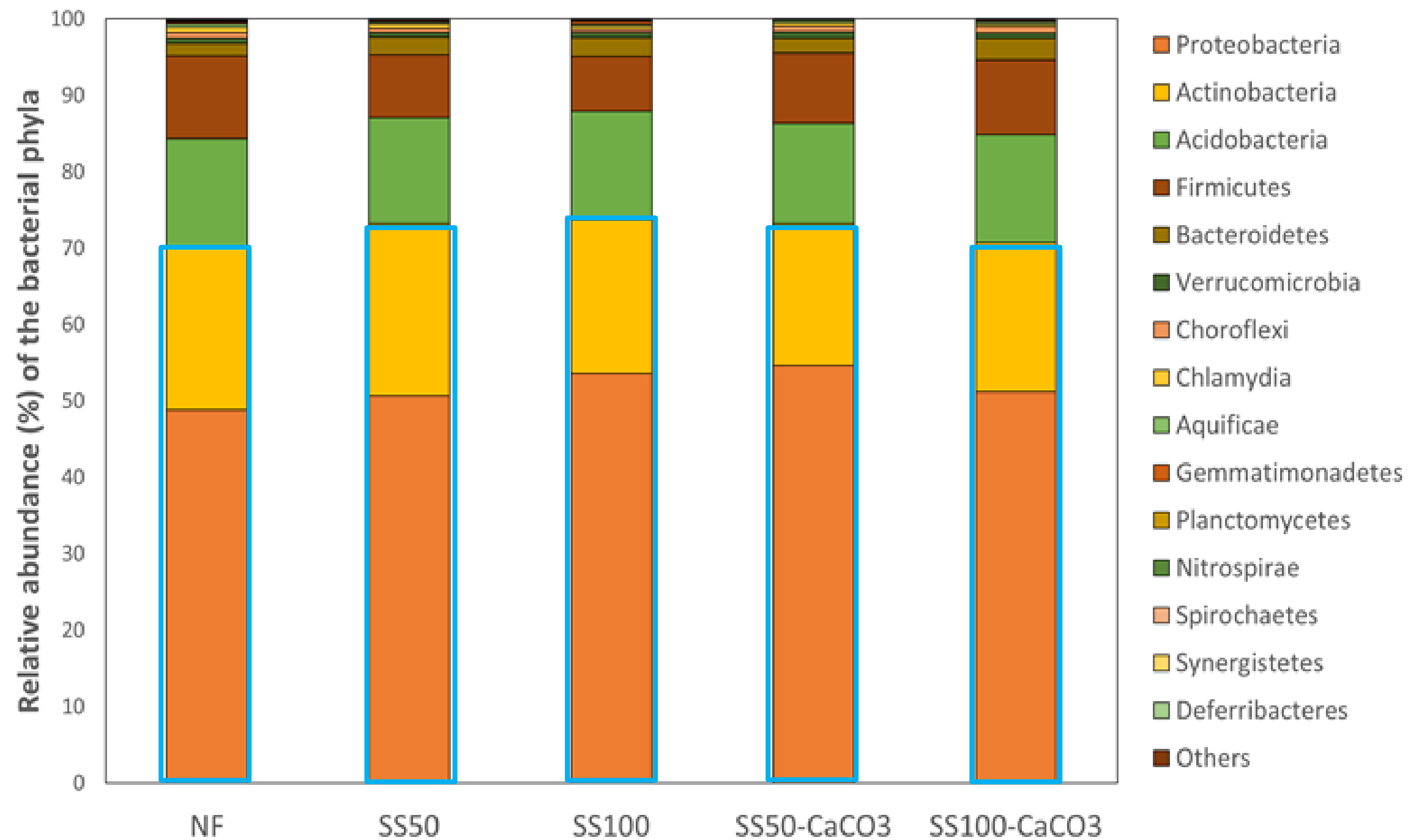
Index I (ratio between aromatic and aliphatic functional groups, which increases with increasing **degree of OM decomposition**)

Index II (ratio of C-containing to O-containing functional groups, which is an indication of the **recalcitrance of OM**)



RESULTS: Composition of the soil bacterial community at the phylum level

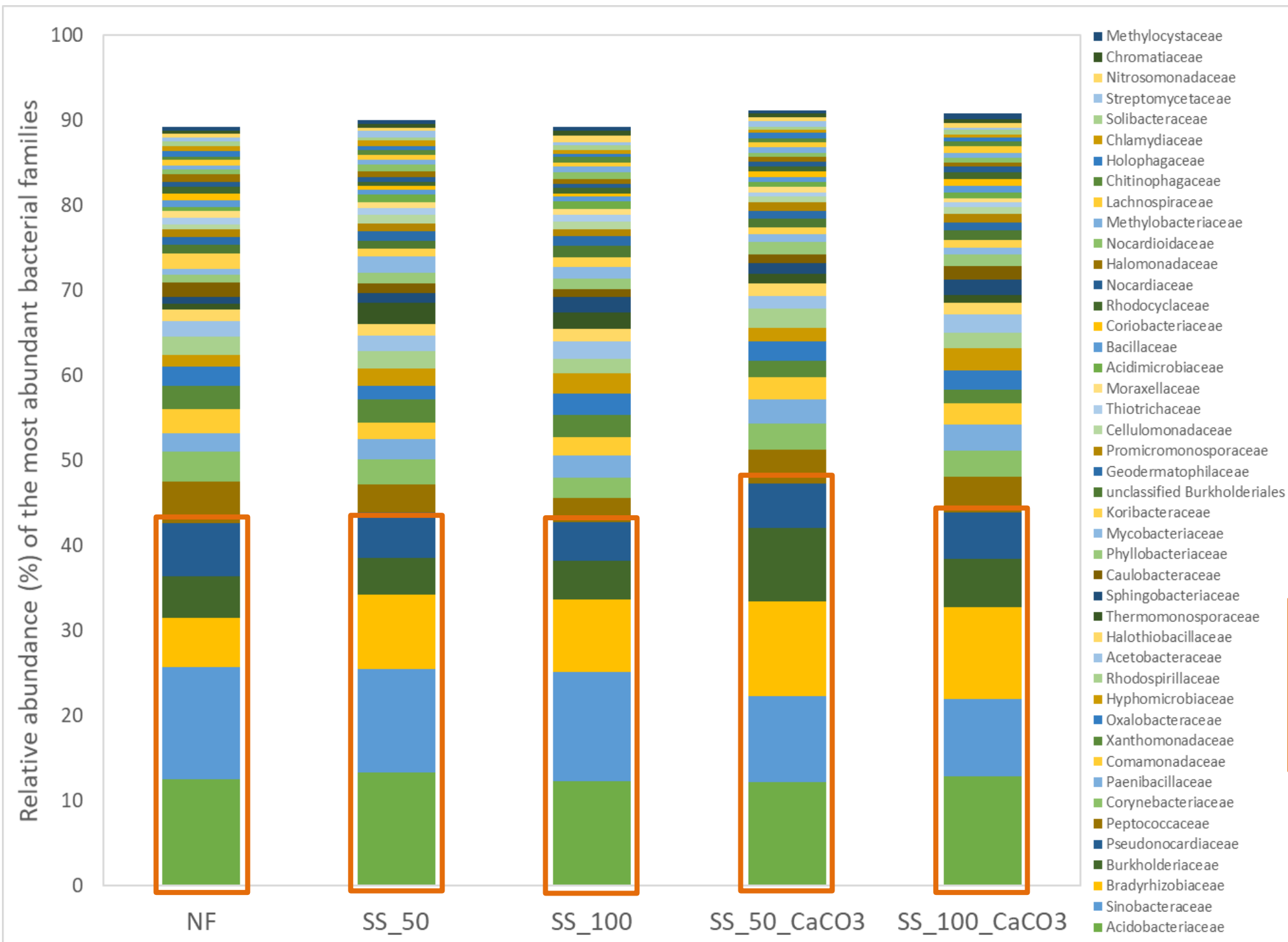
No differences at the **phylum** level among soil treatments



Bacteria community dominated by:

- **Proteobacteria (from 48.8% to 54.6%)**
- **Actinobacteria (from 18.6% to 22.4%)**

RESULTS: Composition of the soil bacterial community at the **family** level (only the most abundant families > 0.45%)

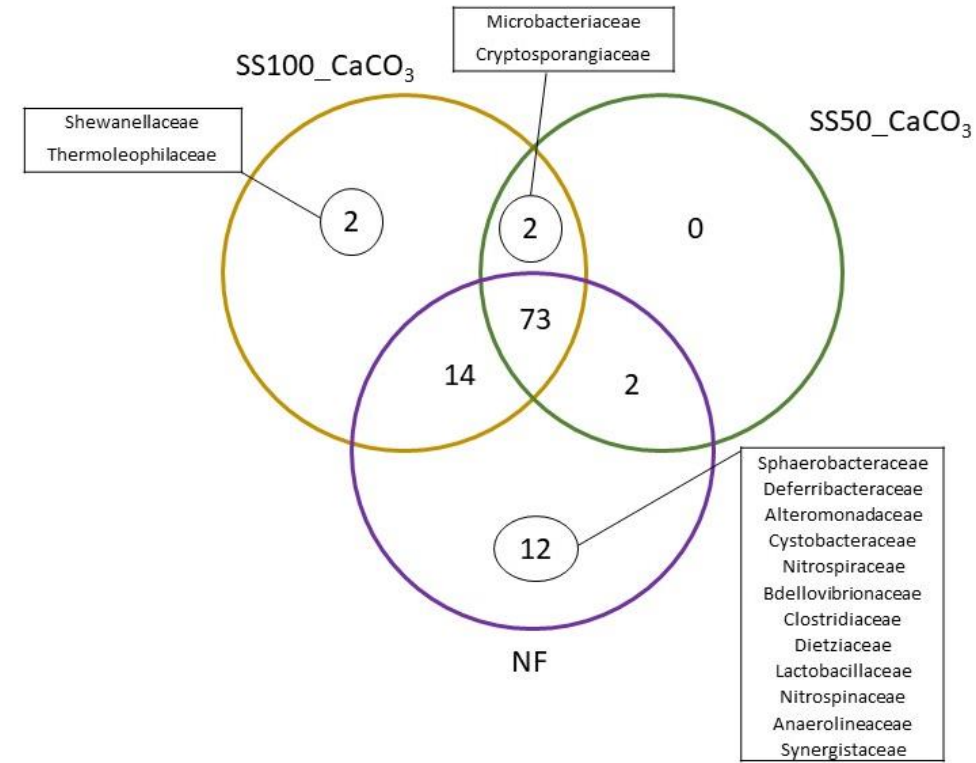
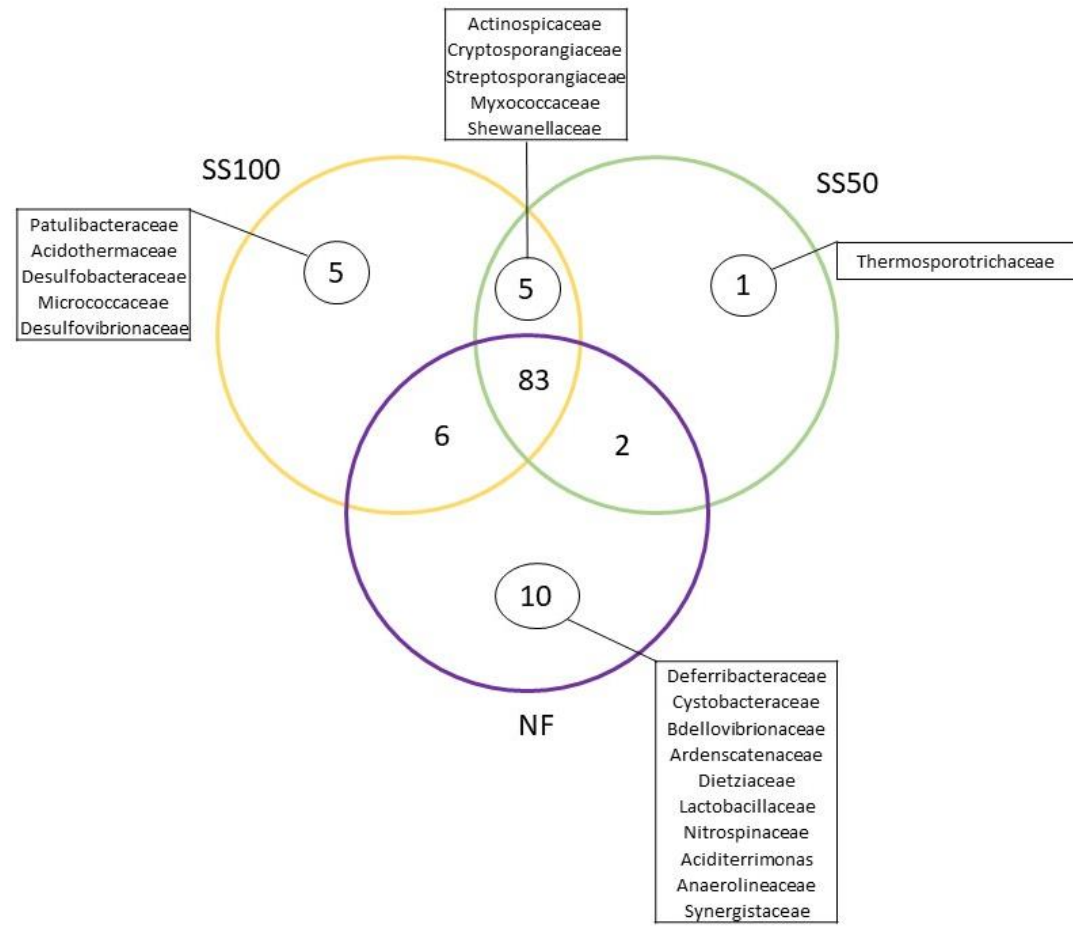


- ### MOST ABUNDANT FAMILIES
- **Acidobacteriaceae** (between 12.1% 13.3%)
 - **Sinobacteraceae** (from 9.0% to 13.2%) ↑ **NF**
 - **Bradyrhizobiaceae** (from 5.8% to 10.9%) **NF < SS < SS_CaCO3**
 - **Burkholderiaceae** (from 4.3% to 8.6%)
 - **Pseudonocardiaceae** (from 4.5% to 6.3%) ↑ **NF**

• **Role in N₂ fixing, C and N cycling, decomposition of complex organic substrates, phosphate solubilisation and siderophore production**

• **Methylocystaceae → CH₄ oxidisers → positively correlated with Soil Ca**

RESULTS: Overlap of the bacterial communities from the different soil treatments

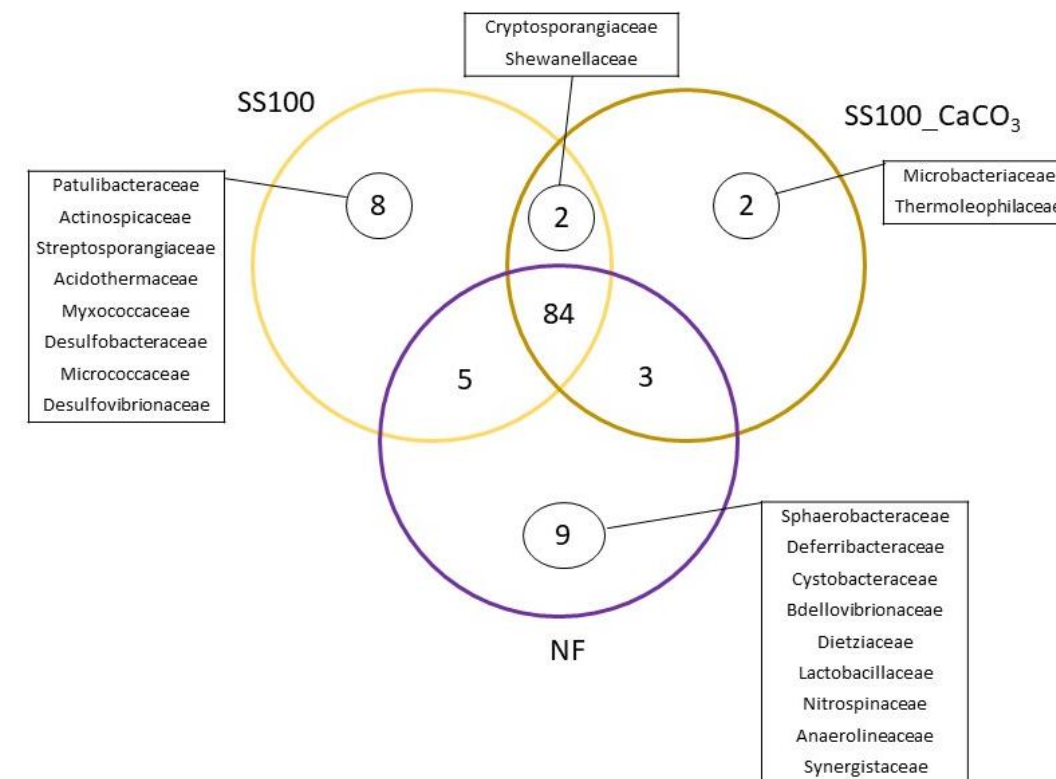
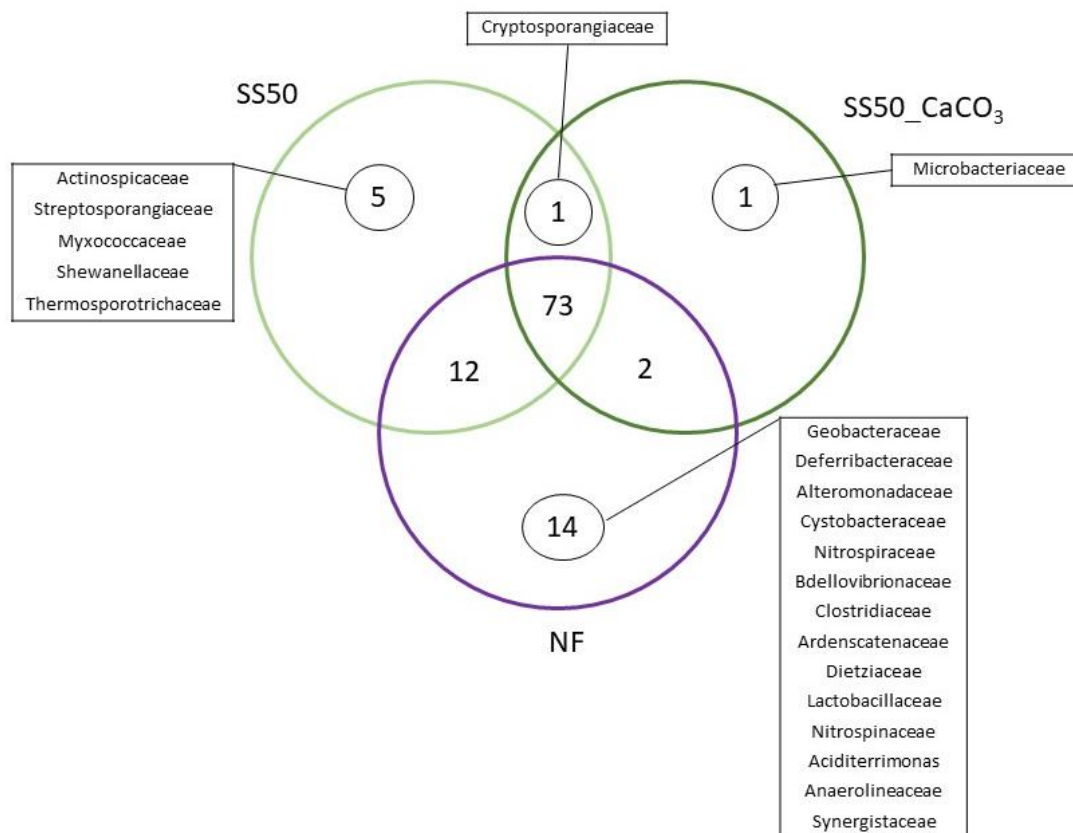


Nº of Unique Families

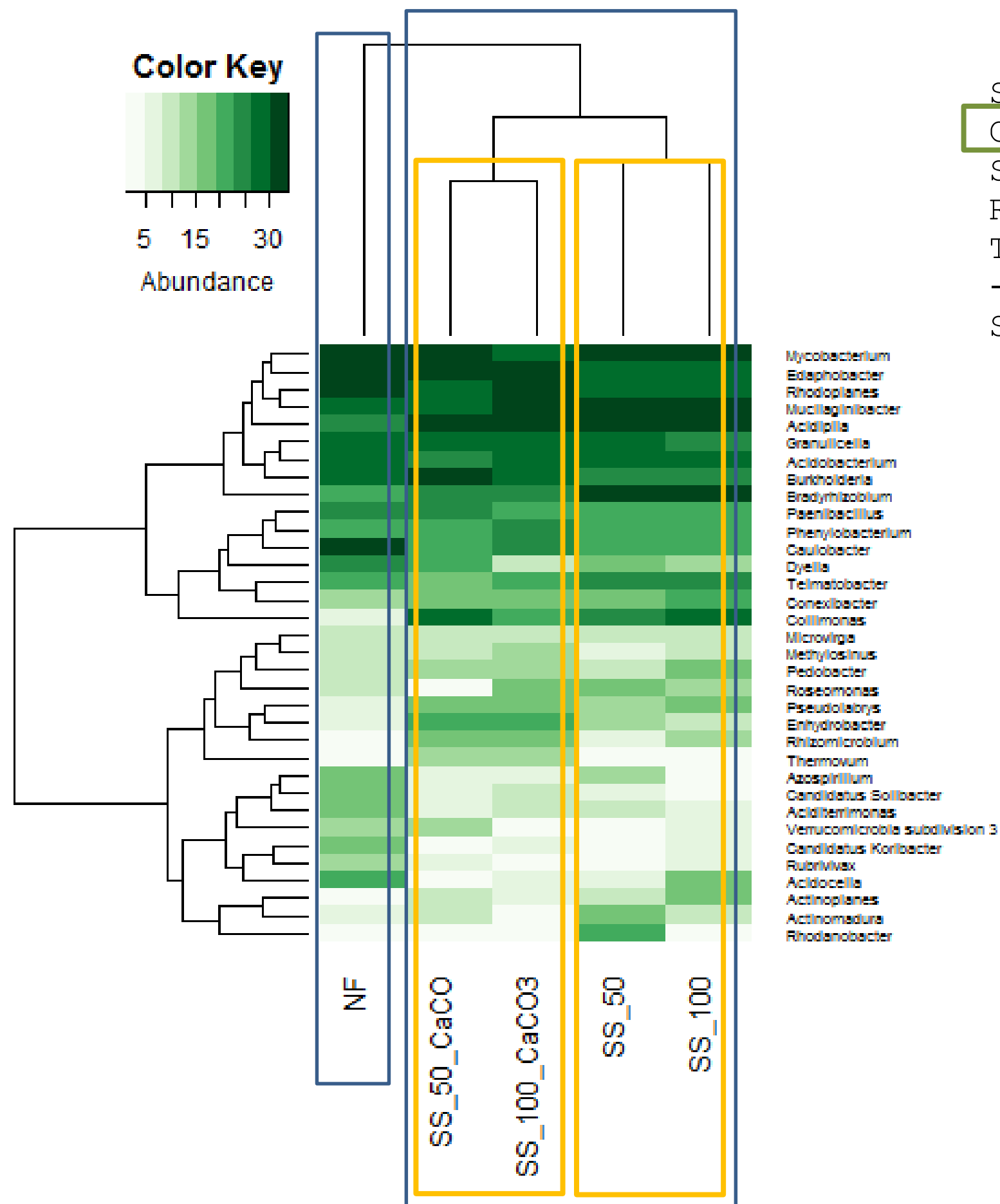
Fertilisation decreased the number of soil specific bacteria: **Non fertilised > treated soils**

Liming decreased the number of specific bacteria: **Sewage Sludge > Sewage Sludge+CaCO₃**

Predominance and colonisation of some groups which are able to successfully use the SS-derived nutrients



RESULTS: Similarity analysis of the soil bacterial communities



	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)					
SS dose	2	0.19135	0.095674	1.7447	0.11117	0.060 .					
Carbonate	1	0.15431	0.154310	2.8140	0.08965	0.012 *					
SS dose:Carbonate	1	0.05944	0.059439	1.0839	0.03453	0.328					
Residuals	24	1.31610	0.054837		0.76464						
Total	28	1.72119			1.00000						

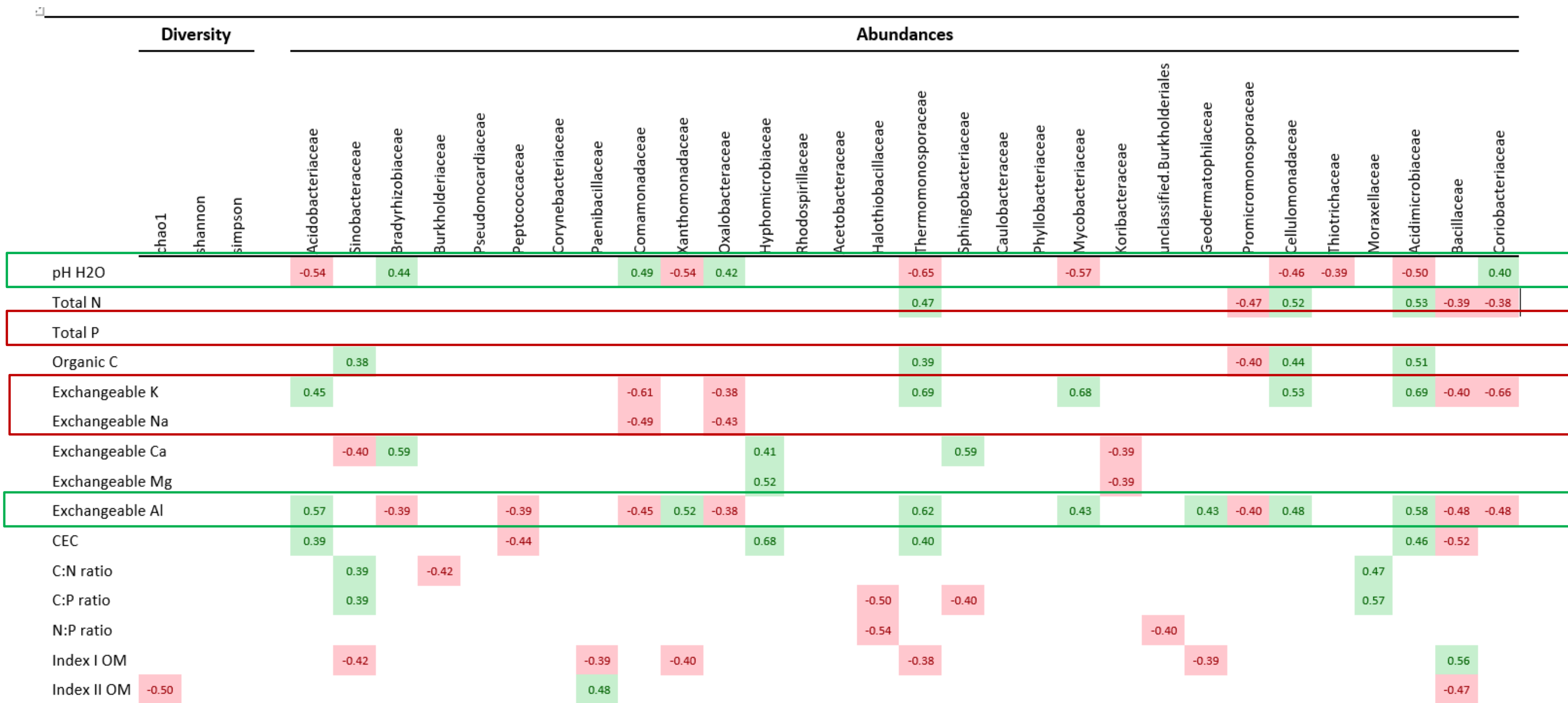
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STRUCTURE OF SOIL BACTERIAL COMMUNITIES

- NF soils clustered separately from all other soil treatments
- Soils including only SS or SS combined with CaCO₃ were also clustered separately
- The bacterial communities were significantly influenced by the addition of CaCO₃ together with the sewage sludge (Permanova analysis)

RESULTS: Correlation soil properties and bacterial abundances and diversity

- Soil pH (most negative) and exchangeable Al (negative and positive) showed the highest number of correlations
- Total P, exchangeable K and exchangeable Na showed the lowest number of correlations
- No correlations with soil diversity indices



CONCLUSIONS

- Addition of sewage sludge and CaCO_3 to soils changed the structure and composition of soil bacterial communities for more than 15 years due to:
 - a lasting change in the original soil bacterial communities
 - to maintained changes in soil properties by nutrient cycling
- Sewage sludge application was more pronounced in the soil bacterial communities than in soil chemical properties
- Changes in soil bacterial communities should also be assessed regularly to ensure the long-term sustainability of the management system

Thank you for your attention!!

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