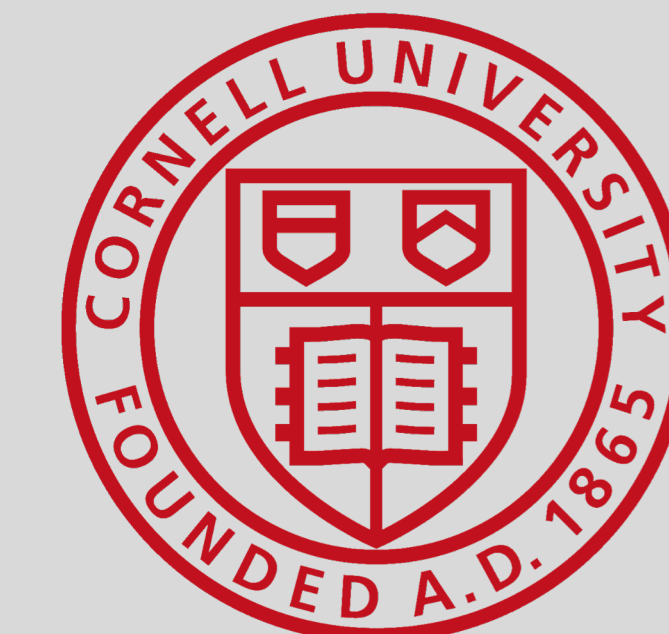


# Can biochar ameliorate triclosan impact on soil bacteria?

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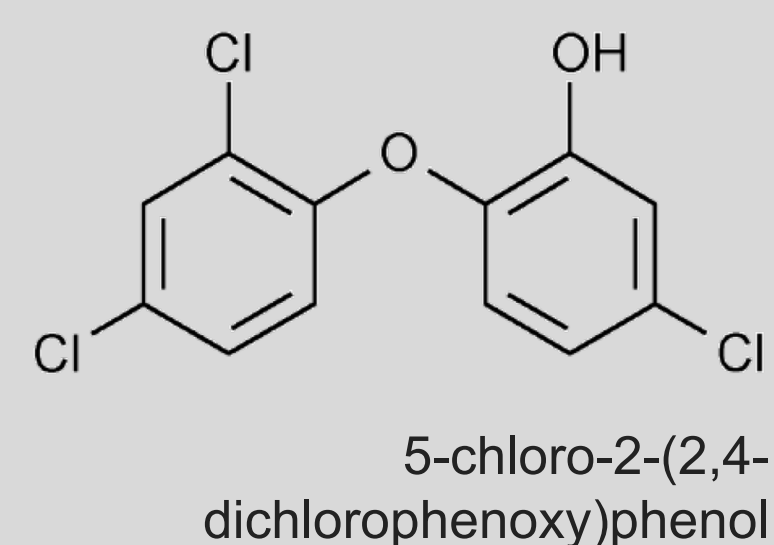
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## Background

### What is triclosan?

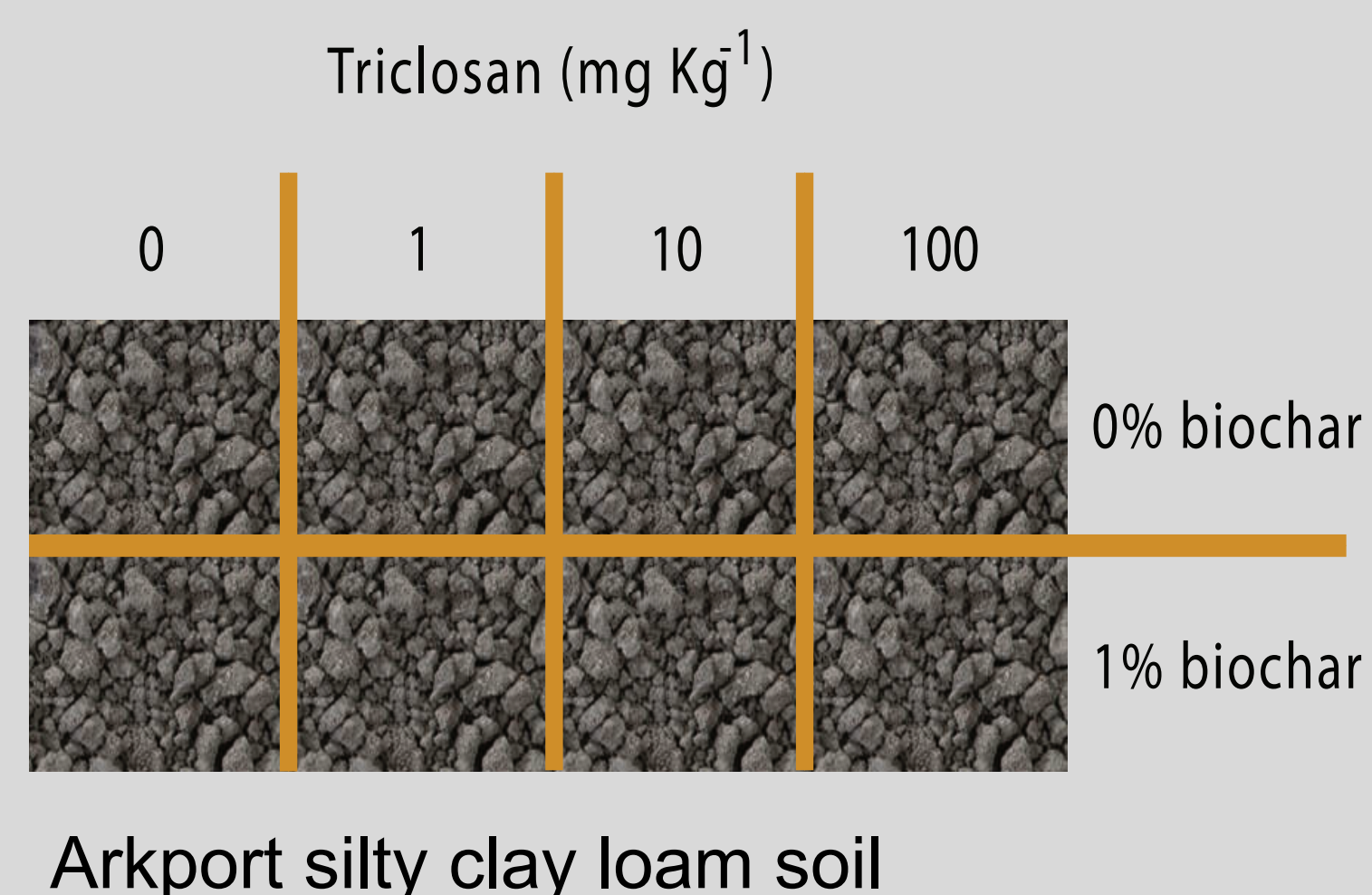
- Broad-spectrum antimicrobial
- Widely used in industrial, medical and personal care products
- Persists in biosolids (0.334-133 mg Kg<sup>-1</sup>)<sup>1</sup>
- Most of the triclosan remains in the surface of biosolids-amended soils<sup>2,3</sup>
- It can be transformed into more toxic metabolites<sup>4,5</sup>
- FDA removed triclosan from over-the-counter antibacterial hand and body washes



### Biochar modifies xenobiotics bioavailability

- Soil amendment<sup>6</sup>
- Enhance soil fertility<sup>7</sup>
- Carbon sequester and sorption of micropollutants<sup>8</sup>

## Methods



- Mineralization of triclosan
- Bacterial community analysis
  - V6 region of 16S rRNA genes via Illumina sequencing
  - Bacterial diversity
  - Bacterial community structure
  - Dynamic OTUs

## References

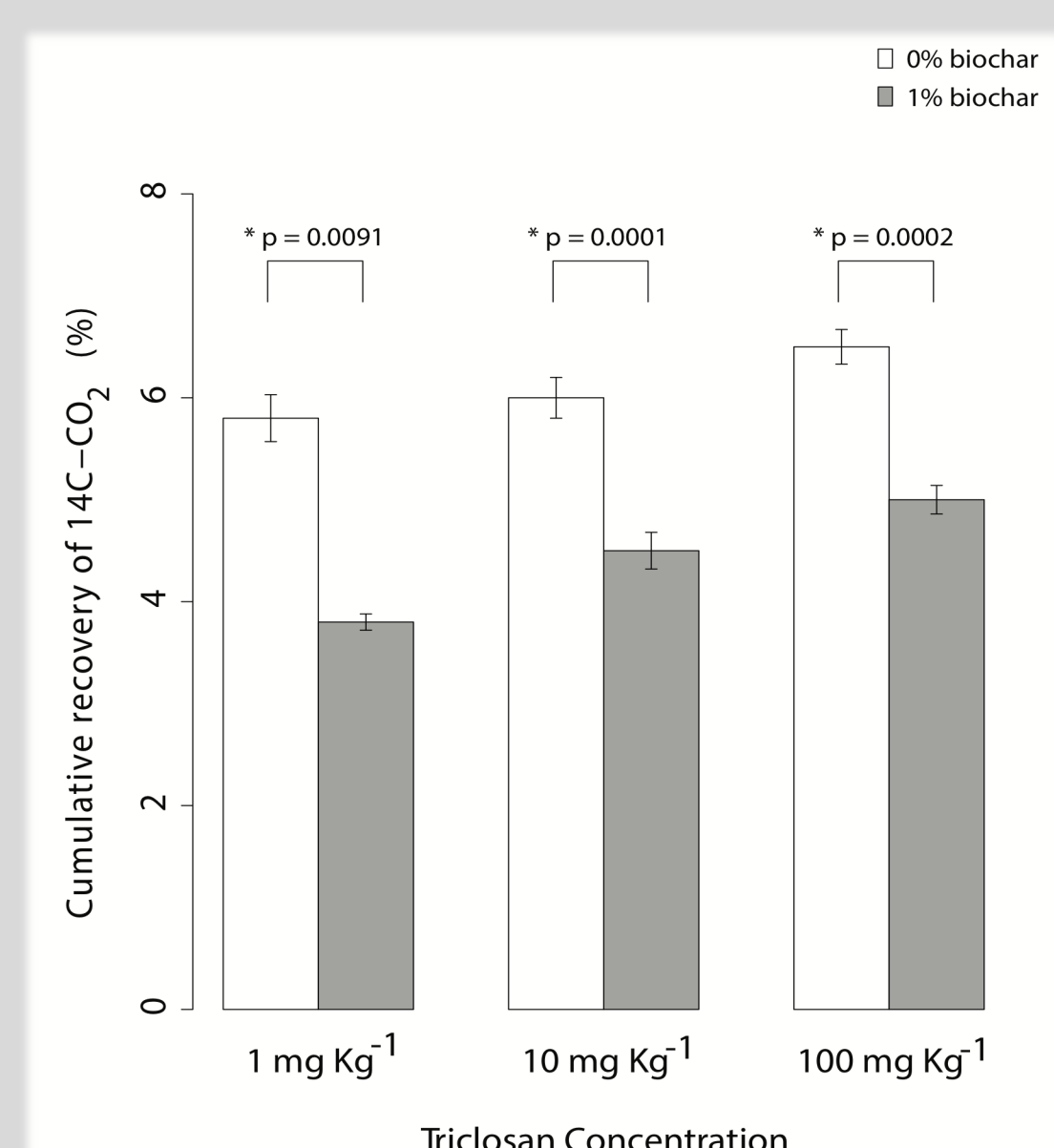
- U.S. Environmental Protection Agency (U.S. EPA). 2009. Targeted National Sewage Sludge Survey Statistical Analysis Report January 2009. EPA-822-R-08-018.
- Sabourin, L., Beck, A., Duenk, P.W., Kleywegt, S., Lapen, D.R., Li, H., et al. 2009. Runoff of pharmaceuticals and personal care products following application of dewatered municipal biosolids to an agricultural field. *Sci. Total Environ.* 407, 4596–4604.
- Xia, K., Hundal, L.S., Kumar, K., Armbrust, K., Cox, A.E., Granato, T.C., 2010. Triclocarban, triclosan, polybrominated diphenyl ethers, and 4-nonylphenol in biosolids and in soil receiving 33-year biosolids application. *Environ. Toxicol. Chem.* 29, 597–605.

- Latch, D.E., Packer, J.L., Stender, B.L., VanOverbeke, J., Arnold, W.A., McNeill, K., 2005. Aqueous photochemistry of triclosan: Formation of 2,4-dichlorophenol, 2,8-dichlorodibenzo-p-dioxin, and oligomerization products. *Environ. Toxicol. Chem.* 24, 517–525.
- Helbing, C.C., Van Aggelen, G., Veldhoen, N., 2011. Triclosan Affects Thyroid Hormone-Dependent Metamorphosis in Anurans. *Toxicol. Sci.* 119, 417–418.
- Jenkins, J.R., Viger, M., Arnold, E.C., Harris, Z.M., Ventura, M., Miglietta, F., et al., 2017. Biochar alters the soil microbiome and soil function: results of next-generation amplicon sequencing across Europe. *GCB Bioenergy* 9, 591–612.

- Xu, N., Tan, G., Wang, H., Gai, X., 2016. Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. *Eur. J. Soil Biol.* 74, 1–8.
- Maurath, N., Orr, C., Ralebitso-Senior, T.K., 2015. Biochar adsorption properties and the impact on naphthalene as a model environmental contaminant and microbial community dynamics: A triangular perspective. In: Lucas Borja, M.E., editor. *Soil Management: Technological Systems, Practices and Ecological Implications*. Nova Science Publishers, p. 64-90

## Results

### 1. Mineralization of triclosan

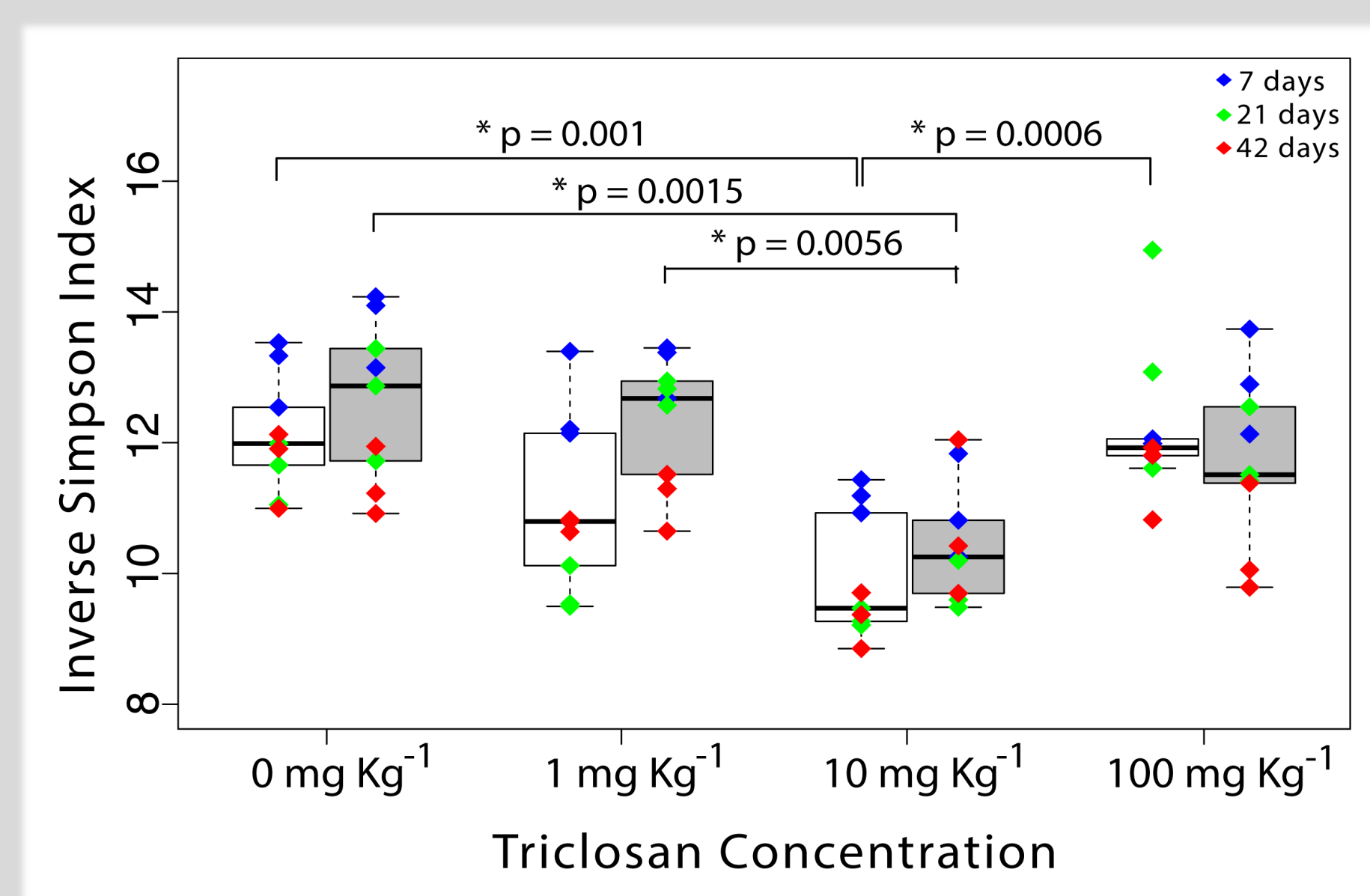


- Little triclosan was mineralized in soil samples
- Biochar decreased triclosan mineralization by 24-33%

**Figure 1.** Cumulative recovery of <sup>14</sup>C-CO<sub>2</sub> from soil samples exposed for 42 days to different concentrations of <sup>14</sup>C-radiolabeled triclosan. \* indicates statistically significant differences (t-test, p<0.01)

### 2. Bacterial diversity

- Lower alpha diversity in soil samples exposed to 10 mg Kg<sup>-1</sup> both in absence and presence of biochar



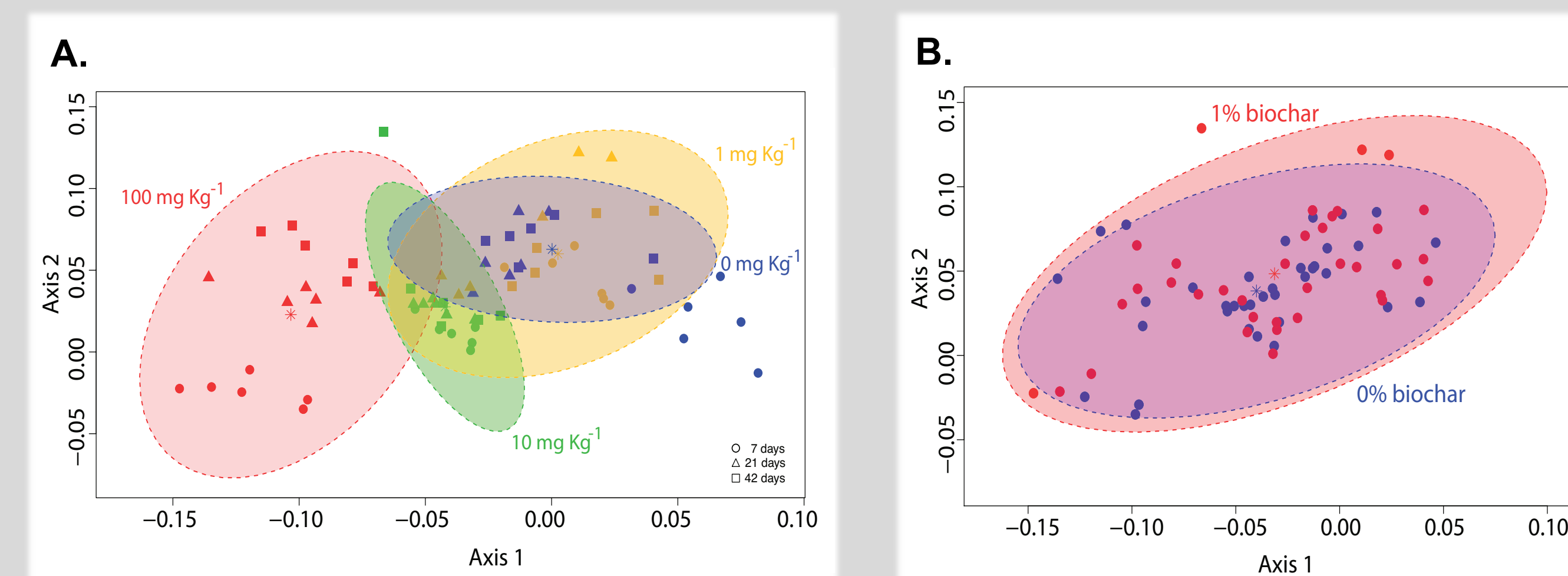
**Figure 2.** Bacterial alpha diversities per triclosan exposure measured by the Inverse Simpson Index in absence (white box) and presence (grey box) of biochar. \* indicates statistically significant differences (Tukey HSD, p<0.01)

## Conclusions

- Even though biochar appeared to reduce triclosan bioavailability, biochar could not overcome the effect of triclosan on the soil bacterial communities
- Biochar was found to more than double the level of *Bacteroidetes* in the control (0 mg Kg<sup>-1</sup>) and 1 mg Kg<sup>-1</sup> of triclosan, but did not have positive effect at higher concentrations of triclosan

### 3. Bacterial community structure

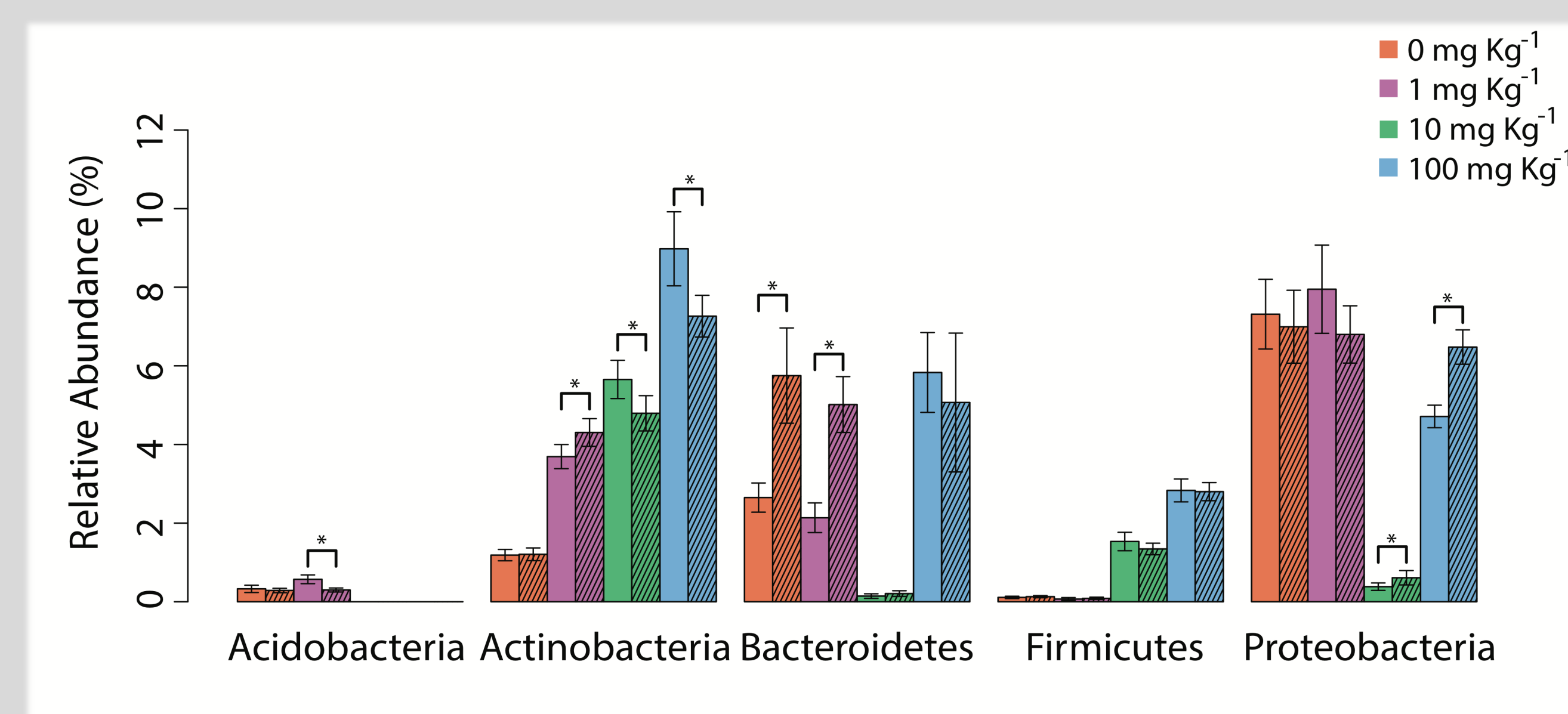
- Bacterial communities clustered by triclosan concentration (AMOVA, p<0.01), but not by presence or absence of biochar (AMOVA, p=0.678)



**Figure 3.** NMDS analysis of the bacterial community structures using  $\theta$ YC distances according to A. triclosan exposure, and B. presence of biochar. The 2 axes represent 95% of the variance. The lowest stress is 0.104 with and R-squared value of 0.97.

### 4. Bacteria taxa impacted by biochar

- Non-parametric Metastats analysis showed few OTUs impacted by biochar in a triclosan-concentration dependent manner (Control: 32 OTUs, 1mgKg<sup>-1</sup>: 43 OTUs, 10mgKg<sup>-1</sup>: 18 OTUs, 100mgKg<sup>-1</sup>: 32 OTUs)



**Figure 4.** Relative abundance (%) of significantly impacted OTUs (NPM analysis, p<0.05) at phyla level with (□) and without (▨) biochar for each triclosan concentration. \* indicates significant difference between the relative abundances with and without biochar (Wilcoxon test, p<0.05)

- Proteobacteria* was the only phylum positively affected by biochar at higher concentrations of triclosan (10 and 100 mg Kg<sup>-1</sup>)
- Though known triclosan degraders were not significantly affected by biochar, other xenobiotic degraders such as *Propionivibrio* and *Variovorax* were positively affected by biochar