

Screening for glyphosate herbicide activity on an extensive collection of bacterial strains: an in vitro study

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Introduction

- Glyphosate is the most widely used herbicide in the world.
- It targets the enzyme **5-Enolpyruvylshikimate-3-phosphate Synthase (EPSPS)** in the shikimate pathway and disrupts the formation of aromatic amino acids and other secondary plant compounds (Figure 1) in plants, unicellular parasites, certain bacteria, and fungi [1].
- The presence of EPSPS in various microorganisms led to patenting the substance as a **broad-range antimicrobial** in 2010 (US Patent No. 7771736 B2) [2].
- In different ecosystems, such as the rhizosphere and microbiota, glyphosate could indirectly affect plant and animal health by **negatively affecting the composition, diversity, symbiotic relationships, and metabolic processes** of associated microbial communities [3–5].
- Glyphosate use in Africa has **increased dramatically and is unregulated** (overdosing and repeated applications) : From 12 million liters in 2008 to 20 million liters in 2012) and 85% of corn and soybean seeds are genetically modified [6].

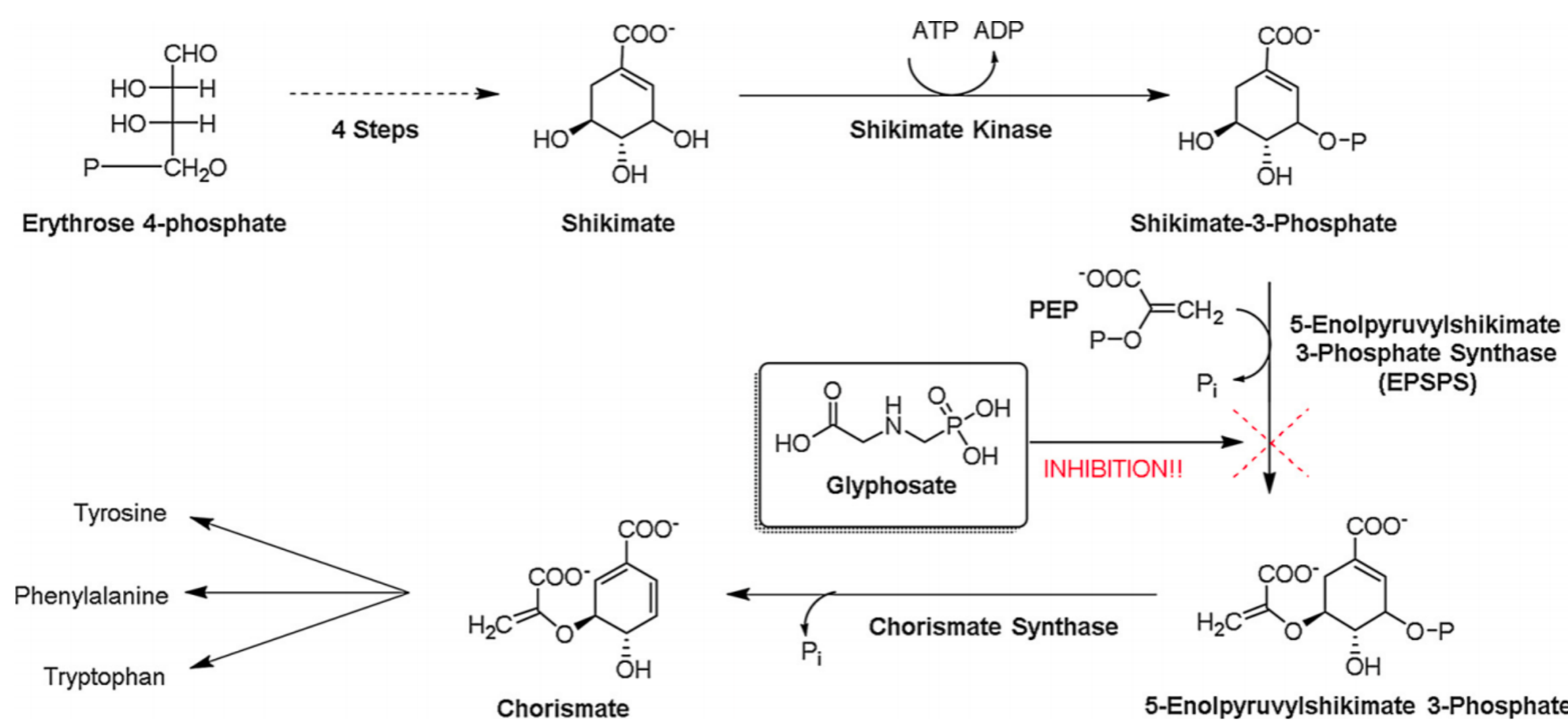


Figure 1. Mode of action of glyphosate [5]

Objectives

Our study aimed to :

- (1) assess the impact of glyphosate use on **bacterial biodiversity** and
- (2) screen different Gram-negative bacteria (GNB) of treated and untreated areas origin for **susceptibility to glyphosate**

Materials and Methods

- In November-December 2020, **175 samples** were collected in the Kédougou region of Senegal from the soil (n=58), termites (n=62), and chimpanzee stools (n=54) from glyphosate-treated agricultural fields, untreated fields, and forests (Figure 3).
- Samples were grown on MacConkey agar and bacterial identification was performed by MALDI-TOF [7].
- The glyphosate susceptibility testing was performed by liquid microdilution method according to Wiegand et al. [1].
- The statistical analyses were carried out with the software R version 4.2.2. The Kruskal-Wallis test was used to highlight significant difference.

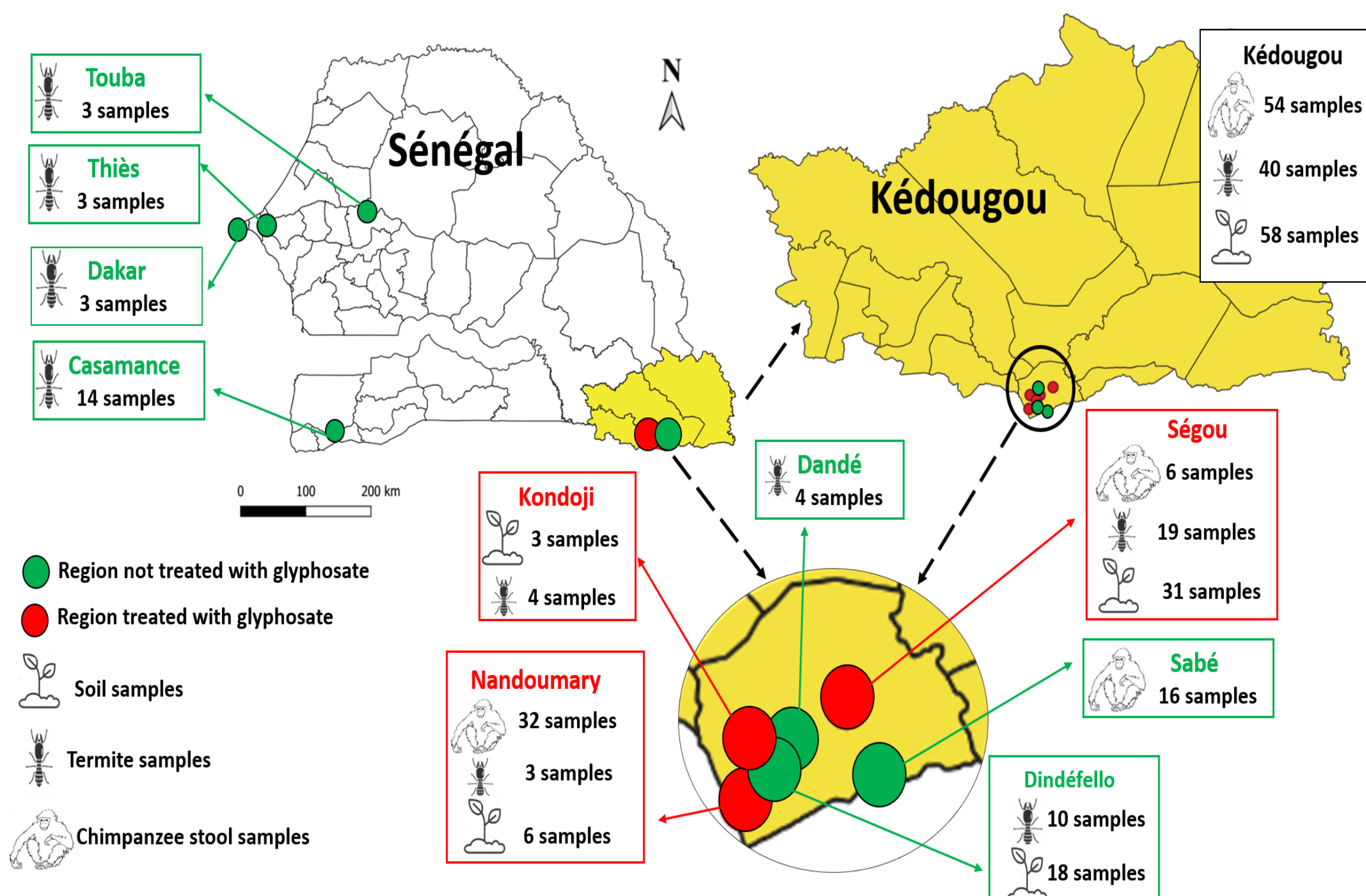


Figure 2. Map of Senegal showing collection sites, 175 Samples were collected in November-December 2020

Main Results

- The greatest number of strains (n=175) was isolated from Chimpanzee stools, followed by soil samples (n=115). *Enterobacteriaceae* constituted the majority of isolates (91%) and (70%) respectively (Figure 4 A and C). A small number of isolates were recovered from termite digestive contents (N=22) (Figure 4 B).
- Vast biodiversity at the species level was observed in the strains isolated from glyphosate-treated areas.
- Non-fermentative GNB species (surrounded in red in figure 4 A and C) were isolated predominantly from the glyphosate-treated zones [8].

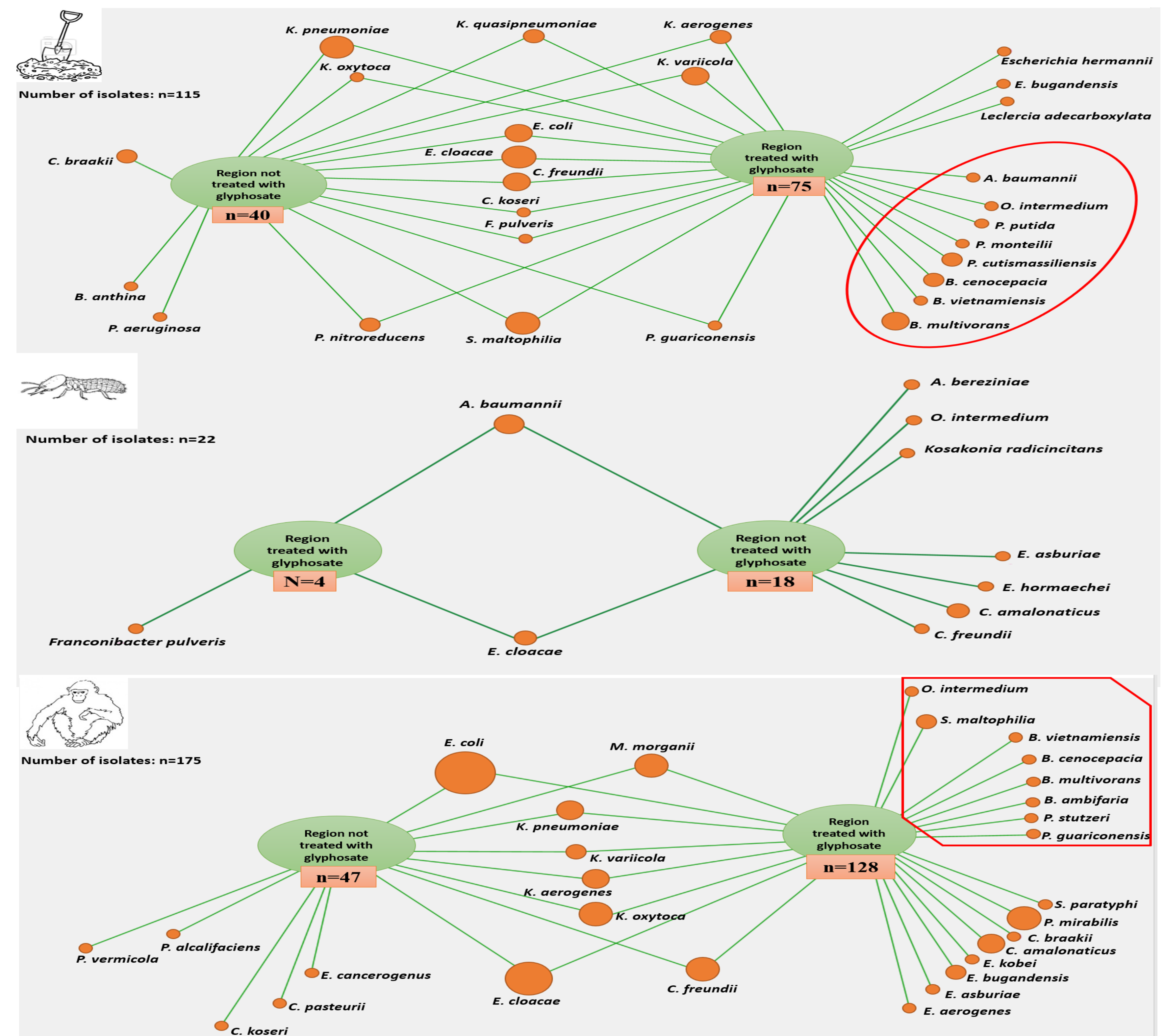


Figure 3. Cytoscape vizualisation of the unique and common species isolated from soil (Figure A), termite (Figure B) and, chimpanzee stool (Figure C) samples in glyphosate treated and untreated regions (Cytoscape v3.7.2. software).

- Enterobacteria strains had higher MIC values for glyphosate (probably adaptation or tolerance).
- Non fermenting Gram-negative bacilli were more sensitive to glyphosate than enterobacteria
- No statically significant differences were detected between the MIC values of strains isolated from the treated and untreated regions (test Kruskal-Wallis $P > 0,05$)

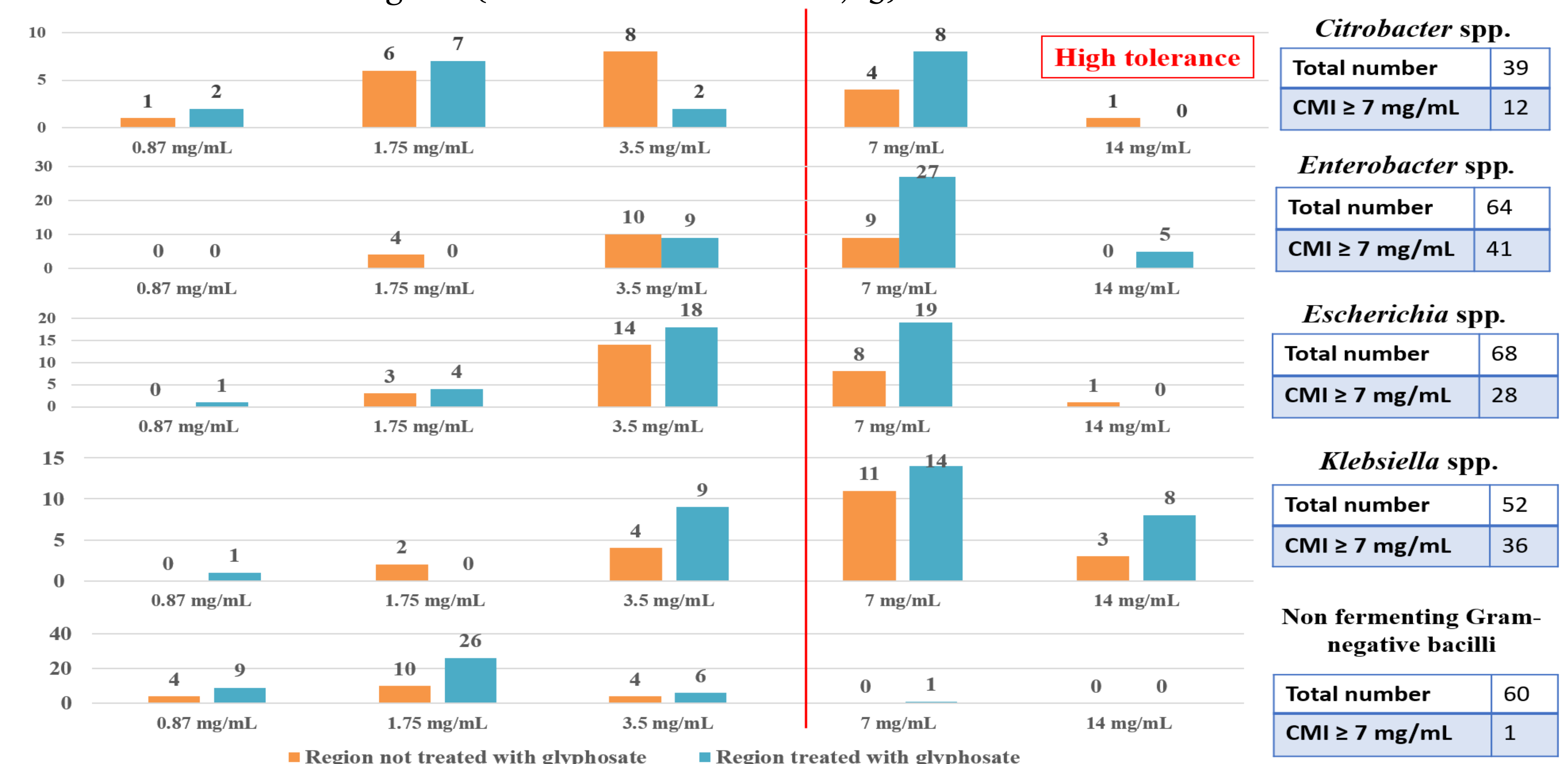


Figure 4. The distribution of glyphosate MICs in the main bacterial groups isolated from treated and untreated regions.

Conclusion

- Glyphosate can affect bacterial biodiversity in exposed ecosystems.
- The MIC values of glyphosate are not statically different between strains originating from glyphosate-treated and untreated areas. Still, they are different between bacterial groups, especially Enterobacteriaceae and Non-fermentative Gram-negative bacteria.
- More extensive sampling and determination of glyphosate residues in the samples must confirm these preliminary results.

References

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