



# Assessment of taxonomic and functional changes in the environmental symbiotic and free-living microbiome induced by Ag and Cu metals

M. Barbudo-Lunar<sup>1</sup>, C. Trombini<sup>2</sup>, C. Michán<sup>1</sup>, J. Alhama<sup>1</sup>, J. Blasco<sup>2</sup>

<sup>1</sup>Dpt. Biochemistry and Molecular Biology, ceiA3, Campus Rabanales, Universidad de Córdoba, 14071-Córdoba, Spain.

<sup>2</sup>Dpt. Ecology and Coastal Management, ICMAN-CSIC, Campus Rio San Pedro, 11510-Puerto Real, Cádiz, Spain.

Presenting/Corresponding Author: julian.blasco@csic.es (J. Blasco)

## INTRODUCTION

Despite the importance of coastal ecosystems, their ecological well-being is threatened by the increasing release of anthropogenic sources of pollution. Metals are present in the environment as a result of natural or anthropogenic inputs. Silver is well known for its toxicity to organisms. Although its levels are generally low, the associated risk in coastal ecosystems could be high due to sewage discharges. Copper is a widespread and well-known environmental contaminant. The development of pollutant exposure experiments in controlled microcosm systems makes it possible to simulate their effects in natural ecosystems. Due to their wide distribution and close contact with pollutants, microorganisms, both symbiotic and free-living in natural environments, could be good indicators of pollution.

## OBJECTIVES

- To identify the changes in the microbiome of aquatic sediments and in the digestive gland of *Scrobicularia plana* after exposure to metals.
- To identify biomarkers of response to environmental pollution caused by metals.

## MATERIAL AND METHODS

In this work, the changes in the microbial profile, both in sediments and in the digestive gland of the bivalve mollusc *Scrobicularia plana*, upon exposure to silver (Ag) or copper (Cu), or a mixture of both, at 1 µg/L for 10 days, were characterised in an aquatic microcosm system (Fig. 1). An unexposed control was also included. Three biological replicates were set per condition. The ZymoBIOMICS™ DNA Miniprep Kit was used to isolate gDNAs from both types of samples. 16S rDNA was sequenced and bacterial taxonomy was identified by database search (Ion Torrent, SCAI-UCO).

## RESULTS AND DISCUSSION

The phylum with the highest number of identifications in all samples was always Proteobacteria (Fig. 2). No significant changes at phylum level were observed. The *Desulfobactereaceae*, *Ectothiorhodospiraceae* and *Flavobacteriaceae* families were highly abundant in all samples (Fig. 3). After exposure to metals, there were several significant increases at the family level, highlighting *Xanthomonadaceae* in sediment samples after exposure to the Ag/Cu mixture with respect to individual exposure to Ag (Table 1), and *Chromatiaceae* in digestive gland samples after individual exposure to Cu with respect to unexposed samples (Table 2).

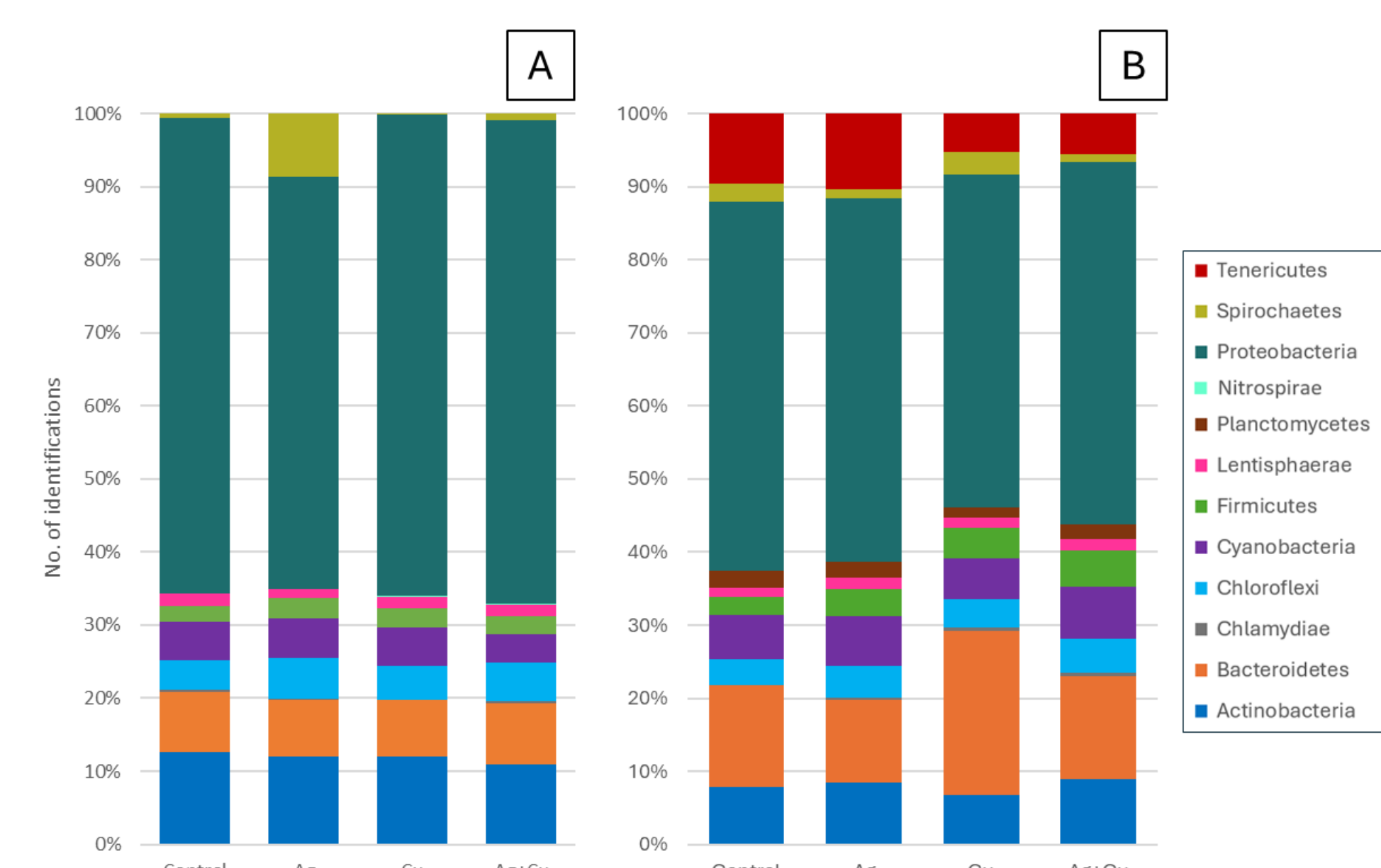


Fig 2. Taxonomic identification at the phylum level of microorganisms present in sediment (A) and digestive gland (B) samples exposed to metals. The number of identifications is represented as a percentage of 100%.

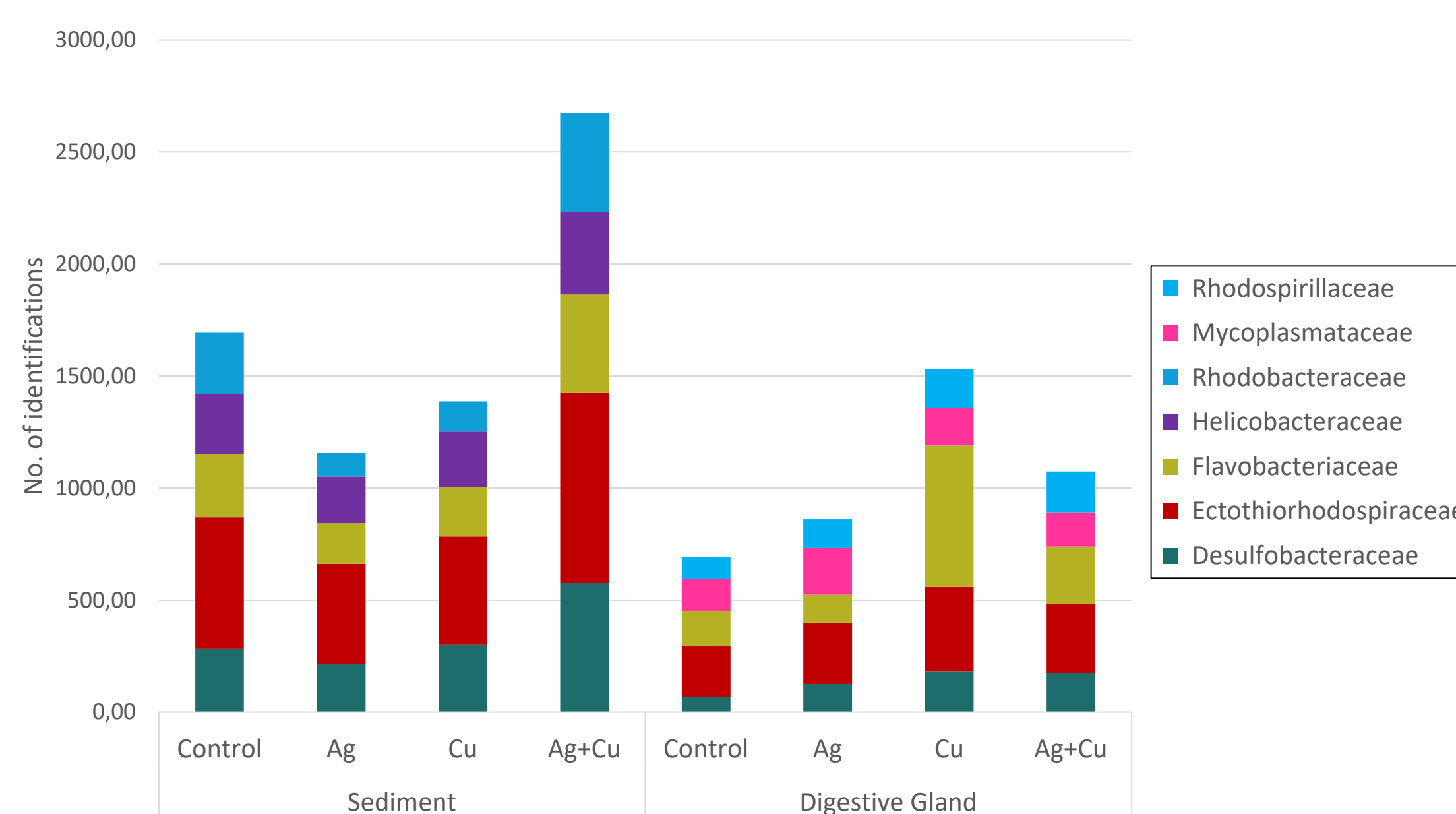


Fig 3. Number of identifications at the family level of microorganisms present in sediment and digestive gland samples exposed to metals. Only the 5 most represented families for each type of sample are represented.

Table 1. Statistically significant changes at the family level (\*\*,  $p < 0.01$ ; \*,  $p < 0.05$ ) in sediment samples.

Families	Sediment	
	Ag+Cu vs. Ag	
Hyphomonadaceae	*	
Pelobacteraceae	*	
Xanthomonadaceae	**	

Table 2. Statistically significant changes at the family level (\*\*,  $p < 0.01$ ; \*,  $p < 0.05$ ) in digestive gland samples.

Families	Digestive gland	
	Cu vs. Control	
Chromatiaceae	**	

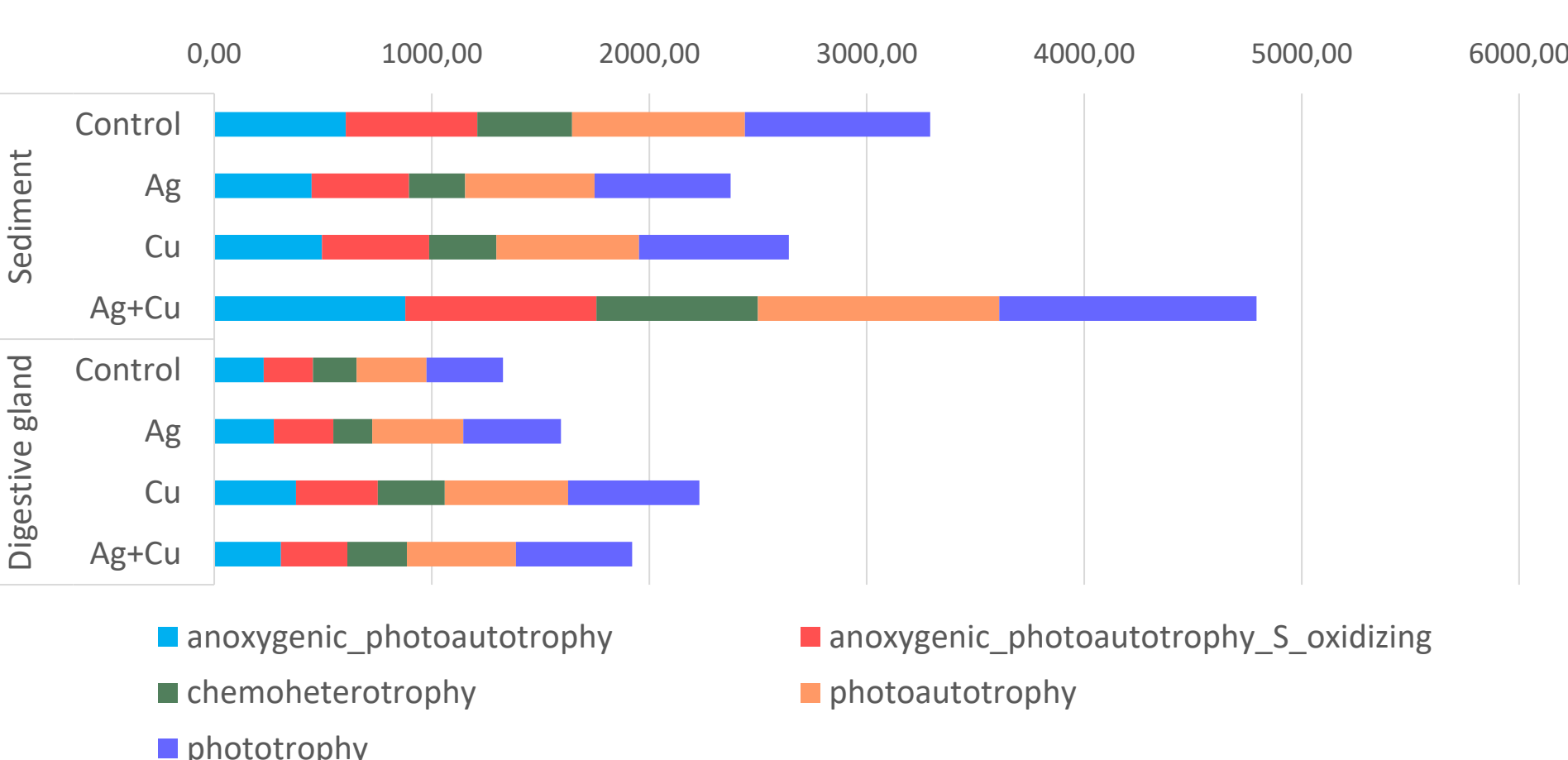


Fig 4. FAPROTAX analysis of the biological functions of the microbiome of sediment and digestive gland samples upon metals exposure. The 5 most abundant functions (higher number of microbial identifications associated to biological functions) for each type of sample are represented.

Table 3. Statistically significant changes at the functional level (\*\*,  $p < 0.01$ ; \*,  $p < 0.05$ ) in sediment and digestive gland samples

Functions	Sediment		Digestive gland	
	Ag+Cu vs. Ag		Cu vs. Control	
aromatic_compound_degradation	*		*	
aromatic_hydrocarbon_degradation	*		*	
hydrocarbon_degradation	*		*	

## CONCLUSIONS

- The exposure to Ag+Cu caused a significant increase in several families when compared to Ag.
- The exposure to Cu caused a significant increase of the *Chromatiaceae* family when compared to the control.
- Cu plays an important role in the microbiome structure.
- More significant changes at the family level were observed in sediment when compared to digestive gland samples.
- The presence of these metals did not affect the microbial diversity.
- Changes at the taxonomic and functional levels of the microbiome, symbiotic and free-living, have shown great potential as an environmental monitoring tool in coastal aquatic ecosystems.

**Acknowledgments:** MICINN (Projects PID2019-110049RB-I00 and PID2022-139807OB-I00). Fundings to BIO187 and RNM306 groups (PAIDI, UCO/CSIC). Research Support Services (SCAI) of the University of Córdoba. M. Barbudo-Lunar is recipient of a predoctoral contract of the University of Cordoba ("Plan Propio").