

# Understanding the micro-ecosystem of wild cacao in Colombia: the case study of the Cacao Colombia BIO expeditions

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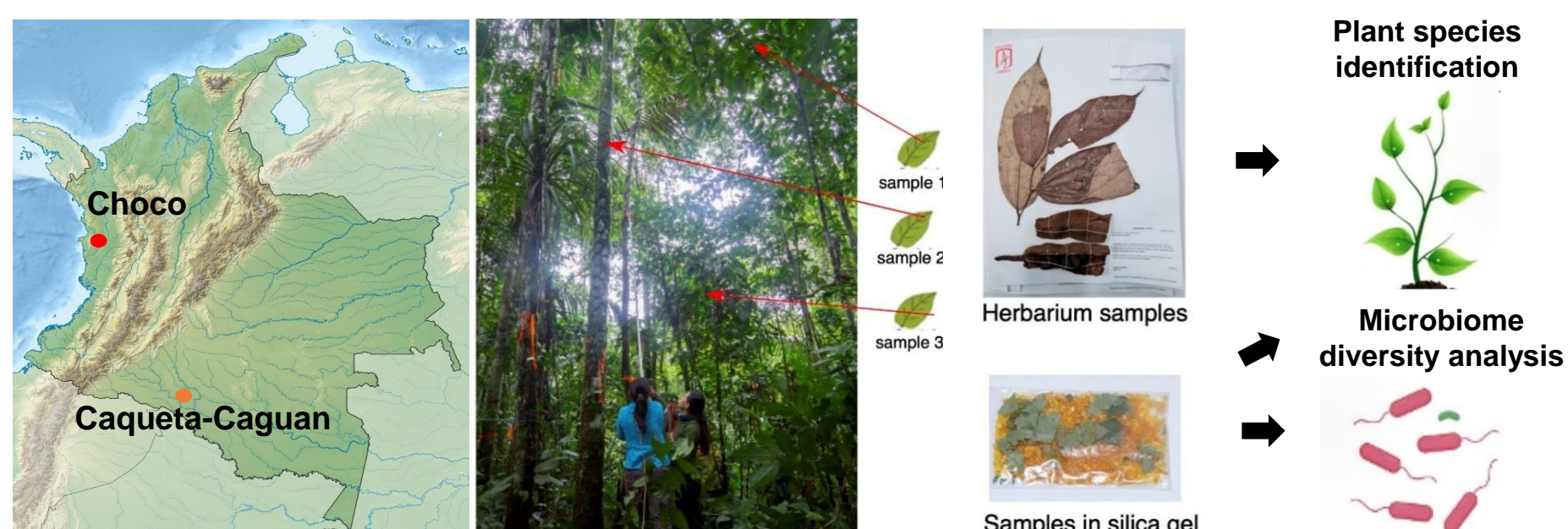
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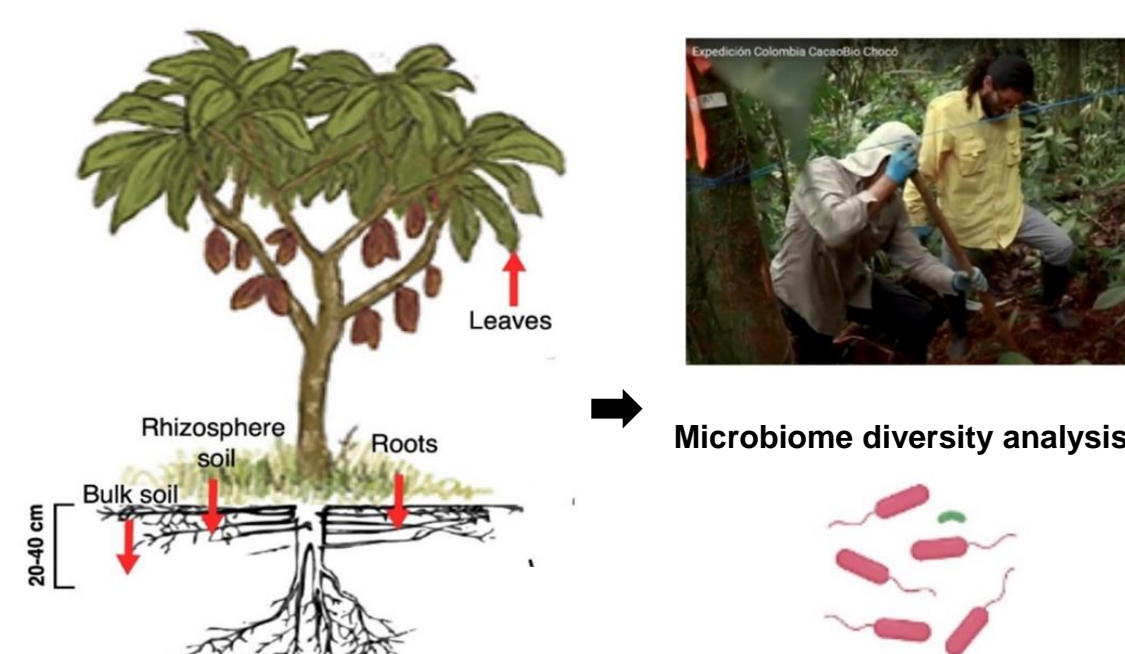
## INTRODUCTION

The increasing human population, the loss of agricultural land due to climate change, erosion, lack of water, and the appearance of new pests and diseases have significantly altered crop biodiversity. The genetic diversity of a crop species such as *Theobroma cacao* L. is probably too narrow to respond to new adaptations required for the rapid environmental changes occurring in forested regions worldwide. To improve future crop resistance and productivity, it is necessary widening the gene pool of the cultivated species. Exploring new sources of genetic diversity in wild populations of the species and the crop wild relatives is necessary. *Theobroma cacao* has the highest levels of genetic diversity in the Upper Amazon areas from southern Perú to the Ecuadorian Amazon and the border areas between Colombia, Perú, and Brazil. In this region also occur many cacao wild relatives. The *Theobroma* genus contains 22 species, and its sister genus, *Herrania*, has around 18 species distributed in Amazonian and Pacific regions in the Neotropics. The rise of the Andes promoted the diversification of plant species separating the Chocó and Mesoamerican regions from the Amazonian and East of South America. To explore the ecosystem of Colombian wild populations of *T. cacao* and its wild relatives, two collecting expeditions in the Amazon region of the Caguan-Caqueta and the Pacific Central Choco region were conducted in 2018 and 2019. We conducted a multi-level and inter-disciplinary approach to study the macro and micro diversity (arthropods, flora and microbial communities) associated to cacao wild relatives in the wild to improve the understanding of chocolate native genetic resources. Here, we present the results on plant and microbiome analyses.

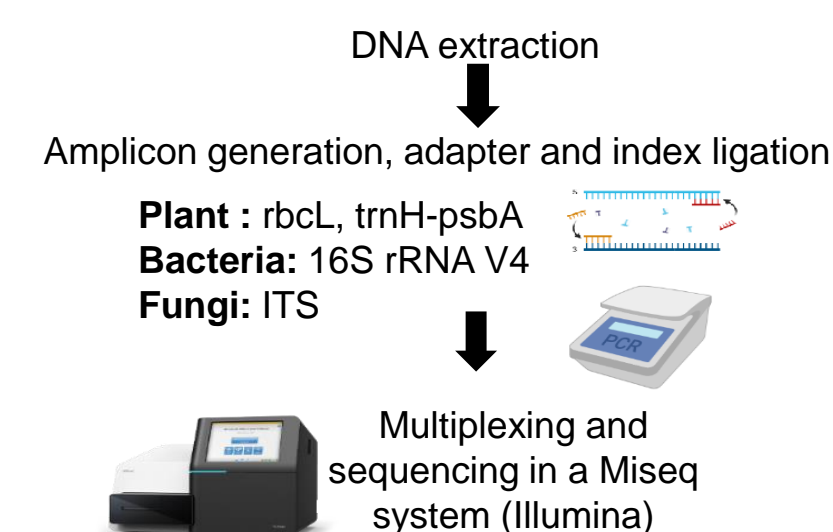
## MATERIAL AND METHODS



**Fig. 1.** Sampling in plots of 25 m of diameter of tree species around a *Theobroma* species. Specimens were mounted, identified and conserved in the Universidad de los Andes herbarium. Samples in silica gel were used to extract DNA to identify the plant species and study foliar endophytes



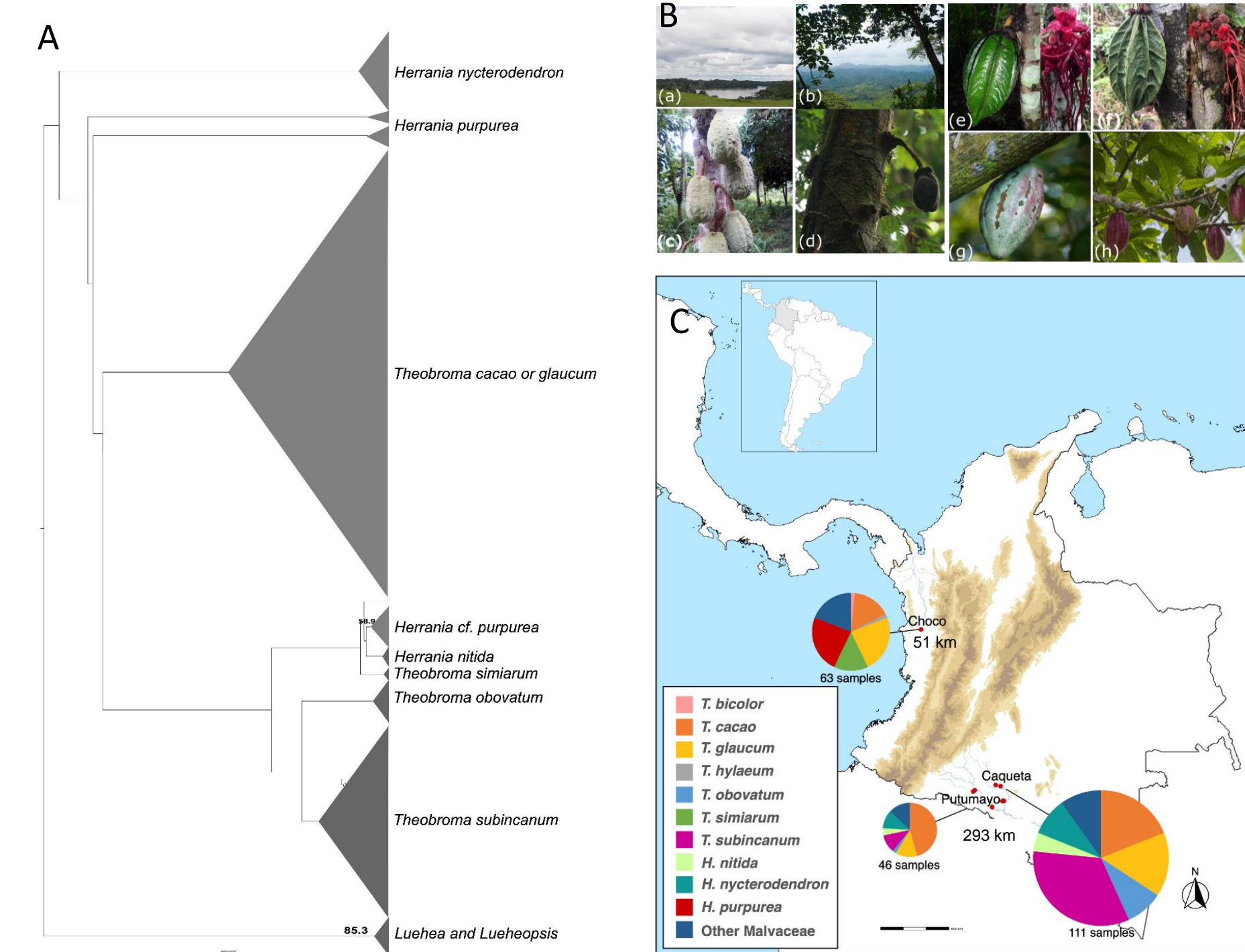
**Fig. 2.** Sampling of *Theobroma* trees to study the associated microbiota. Leaves, trunk, root, rhizosphere soil and bulk soil were sampled.



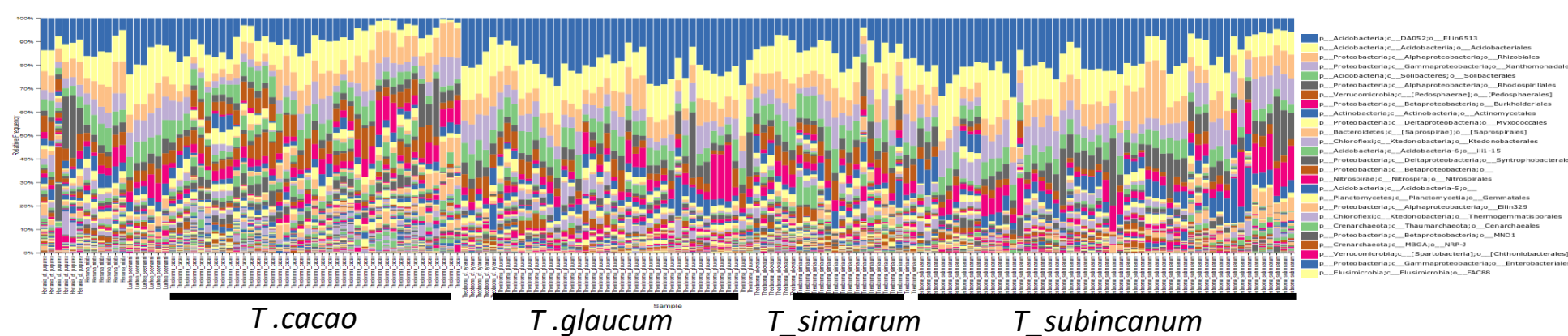
**Fig. 3.** Protocol used to generate sequences from four different regions in one sequencing lane of Miseq Reads are demultiplexed and used for diversity, phylogenetic and taxonomic analyses using Blastn, PhyML and Qiime 2 microbiome

## RESULTS

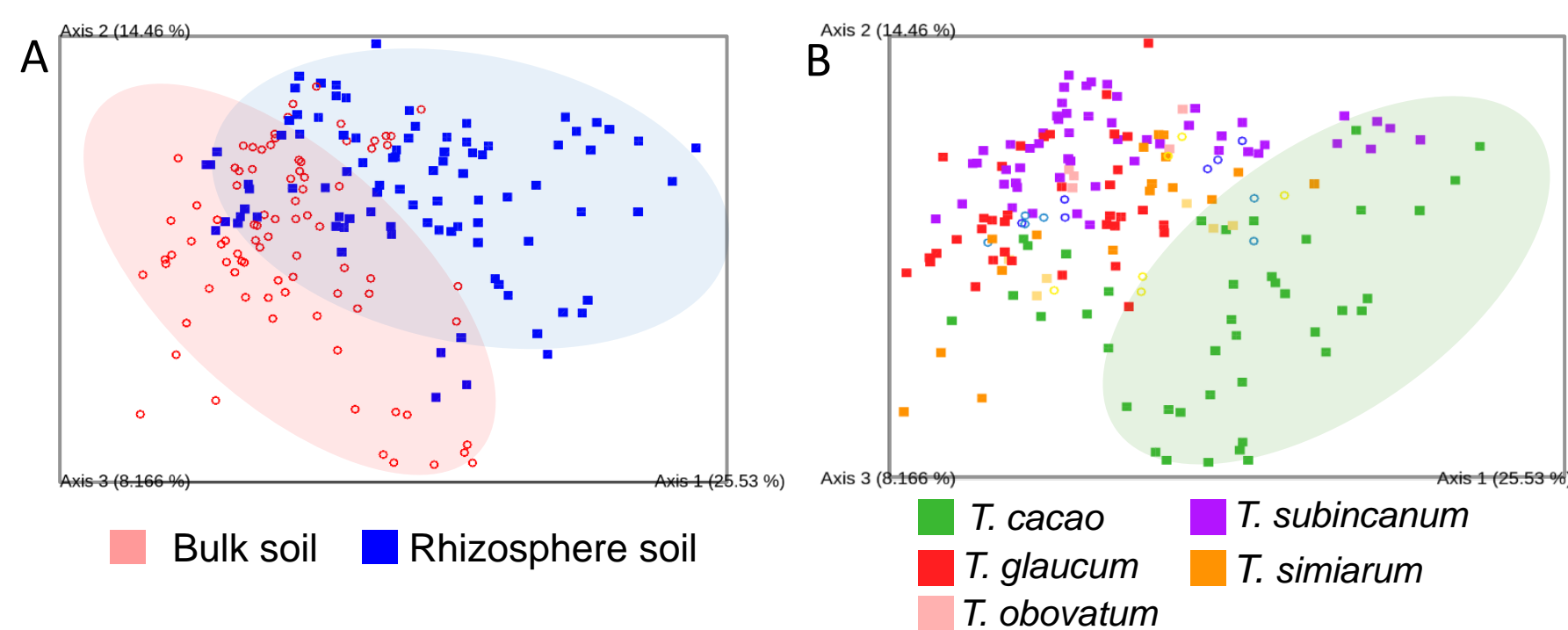
We collected 540 plant samples from which 220 belonged to Malvaceae species. Taking in account the traveled distance, the Malvaceae species richness in the Choco region is 3.4 higher than in Caqueta and Putumayo regions.



**Fig. 4.** A. Maximum likelihood phylogeny of Malvaceae species based on the *rbcL* region. B. Pictures of *Theobroma* and *Herrania* species in the Caguan-Caqueta Region C. *Theobroma*, *Herrania* and Malvaceae samples collected in the three Colombian regions, Caqueta, Putumayo, and Choco.



**Fig. 5.** Stacked bar plot showing bacteria diversity in bulk and rhizosphere soils of different *Theobroma* species



**Fig. 6.** Analysis of Principal Coordinates Analysis using weighted UNIFRAC for the surrounding soil and rhizospheric soil samples. The relationship between type of sample, that is, rhizospheric or surrounding soil (Panel A), and *Theobroma* species from which the sample was taken (Panel B).

In our study, we observed multiple factors associated with the bacterial and archaeal communities' structure in the root and surrounding soil. The most significant clustering pattern is the effect of the root of each species, exerting a strong selection of the accompanying microorganisms, and these differences appear to be species specific. We observed that *Theobroma cacao* plants, in the two evaluated territories, separated by the Andes and more than 500 km away, recruited more similar microbial communities than other species that share the same geographic location.

## CONCLUSIONS

CacaoBIO expeditions allowed to sample cacao wild relatives and their microbiomes associated and to study how in natural and less disturbed environments, the association of microbes and plants occurs. Traits not found in cultivated species due to local adaptation to unique environmental and climatic conditions are necessary to offer to the farmers and producers cacao materials that respond to the environment and climate change.

## REFERENCES

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## ACKNOWLEDGMENTS

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