

Background

Research on Alpine and sub-Alpine settlements remains limited due to the outdated perception of mountains as marginal areas. In reality, the Alps have long functioned as key corridors connecting the Mediterranean with Central and Northern Europe and as sources of essential raw materials. A multidisciplinary approach is therefore crucial to reassess human environment interactions, including forest exploitation, farming, mining, and mobility, which often leave subtle traces in soils.

In this project, archaeological sites in key areas of the Alpine arc were selected: one in the West (Orgères, La Thuile (AO), Italy) and two in the East (Monte Palazzo, San Tomio di Malo (VI), Italy; Trambileno, Valle dei Lombardi, (TN), Italy).

Methods

The archaeological sites were investigated using DNA-based metabarcoding on soil samples collected from different archaeological layers and surrounding areas. DNA was extracted using the DNeasy® PowerSoil® Pro Kit (QIAGEN). Targeted PCR amplification was performed on specific barcoding regions: the ITS2 region for fungal communities, the V3–V4 region of the 16S rRNA gene for bacteria, and the trnL (UAA) intron for plant identification.

Case studies

Orgères, La Thuile (AO), Italy 1.665 m a.s.l.

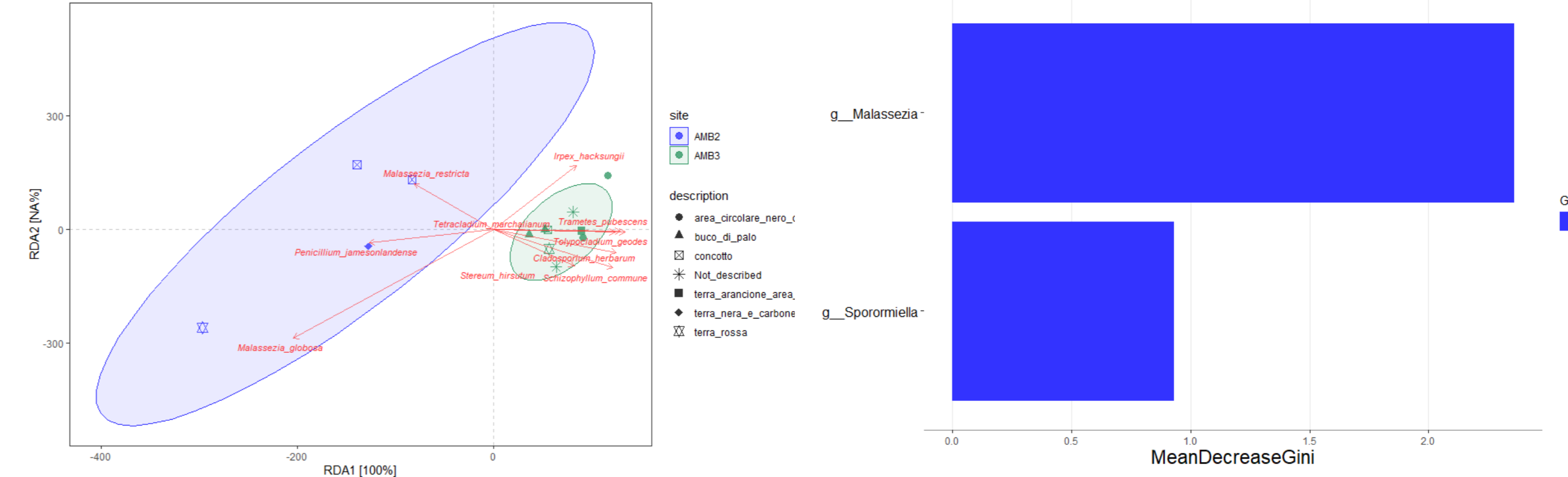


The integration of fungal, prokaryotic, and plant data enabled the functional interpretation of two adjacent environments: a likely stable (AMB2) and a barley storage/processing area (AMB3).

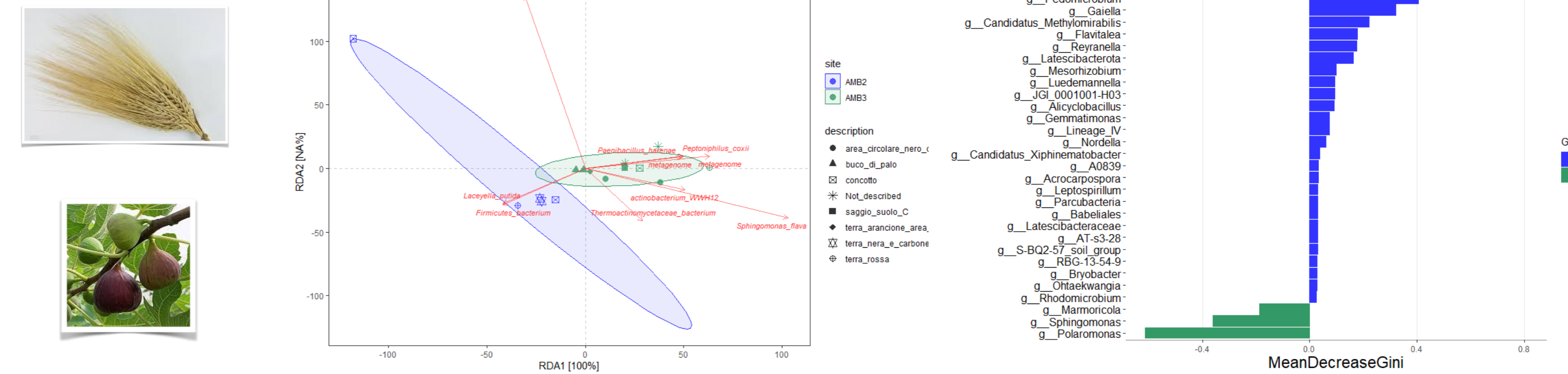
AMB2 is characterized by *Sporormiella*, indicative of herbivore activity, and *Malassezia*, associated with animal skin, supporting its use as a livestock area. In contrast, AMB3 shows plant-degrading taxa and the presence of *Hordeum vulgare* L. DNA, suggesting cereal storage or processing. Orgères often derives from orgere (barley), indicating a place where barley was cultivated or grew.

Plant DNA analysis also identified non-local species, such as *Ficus carica* L. (2nd–3rd century AD), pointing to cultural and economic exchanges.

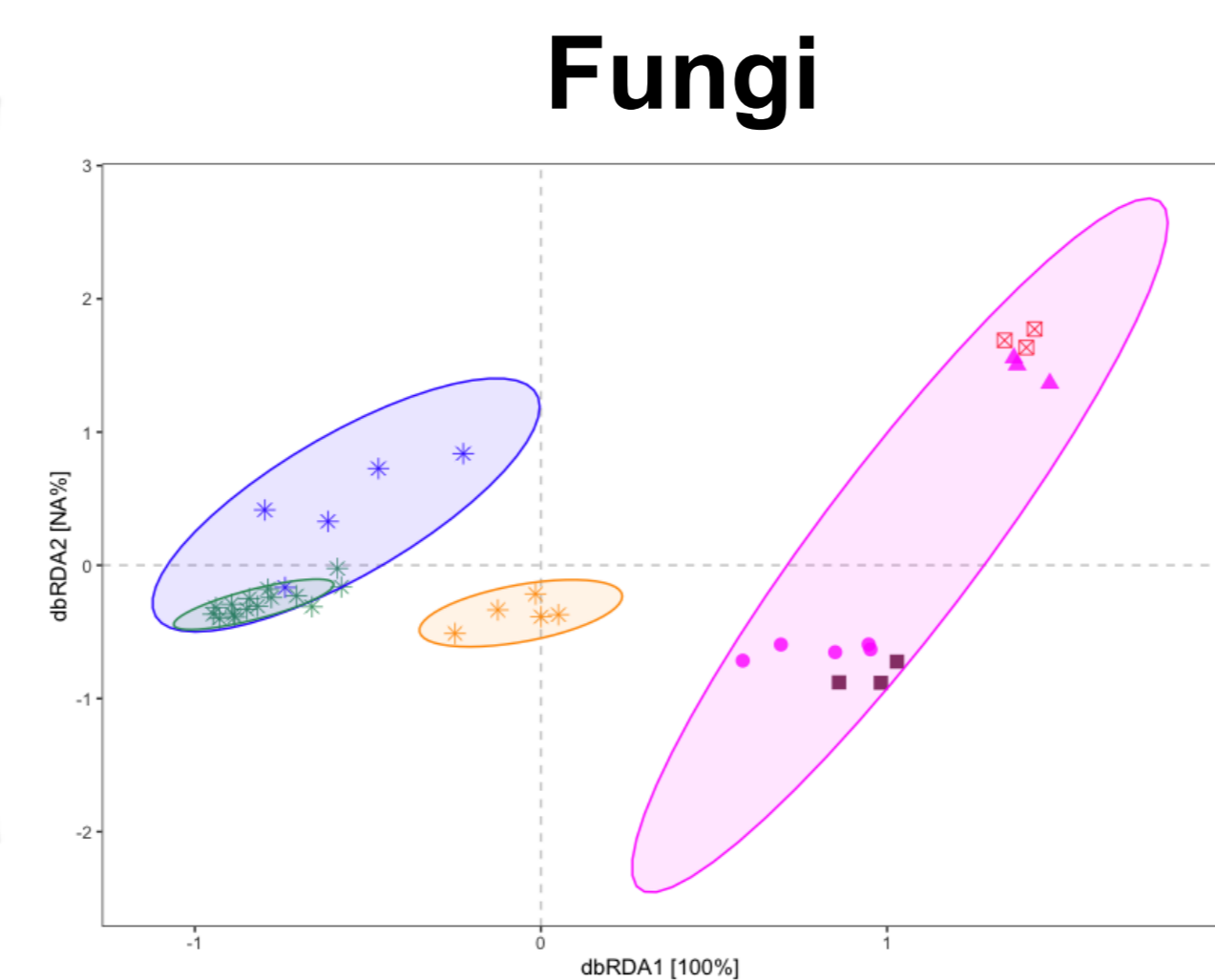
Fungi



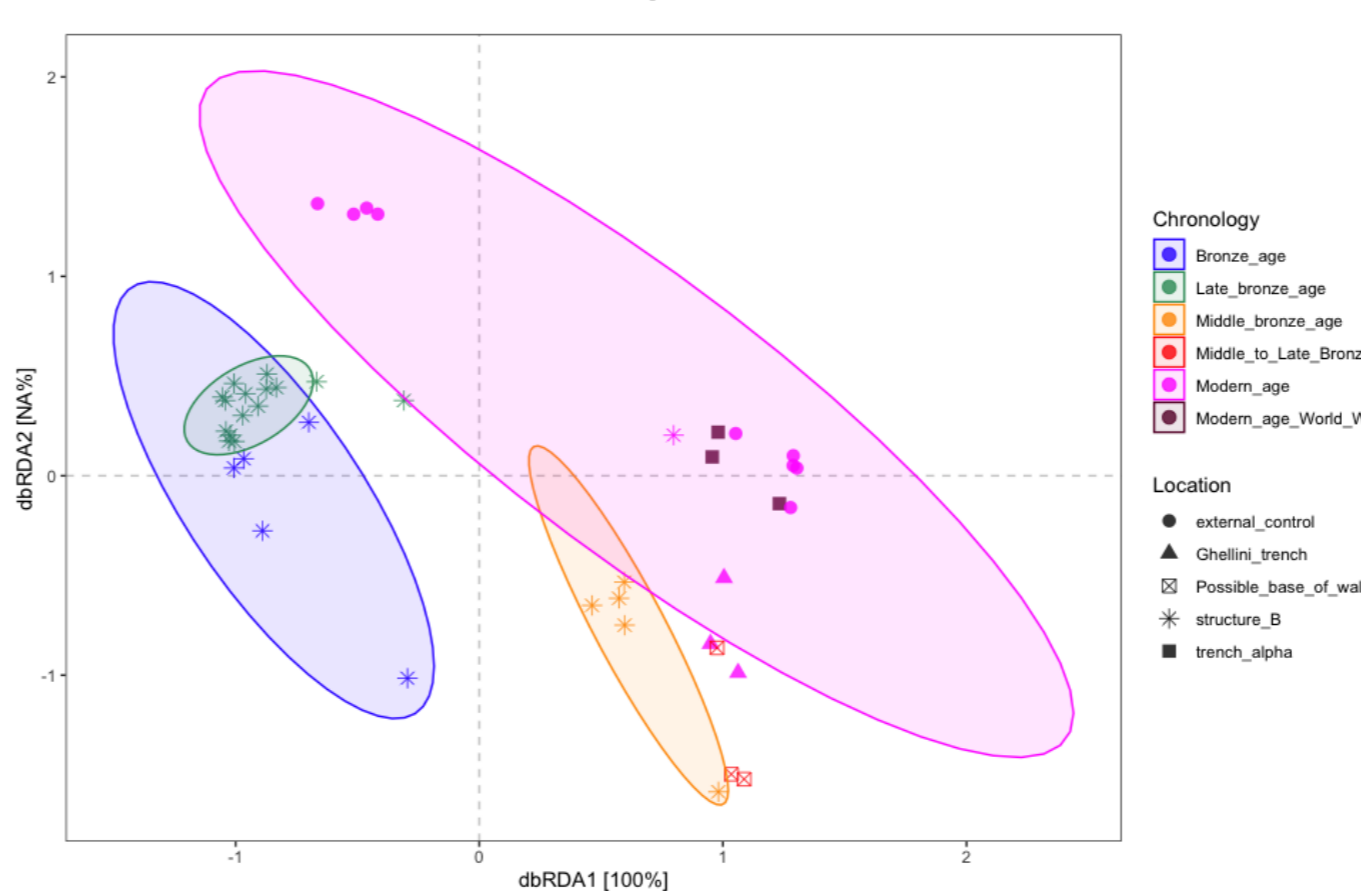
Prokaryotes



Monte Palazzo, San Tomio di Malo (VI), Italy 505 m a.s.l.



Prokaryotes

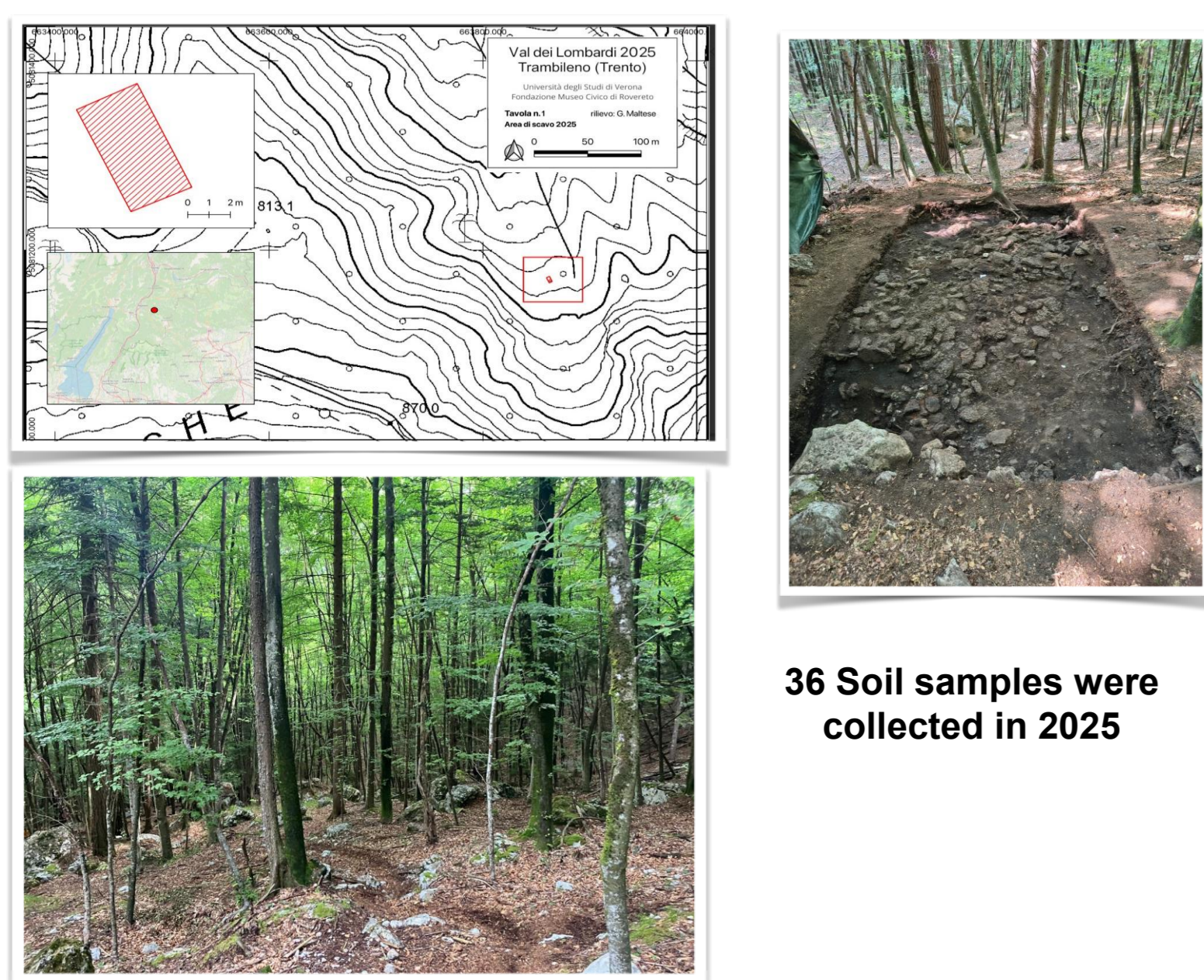


Structure B, less affected by modern disturbance, preserves plant DNA from medicinal species (*Galium verum* L., *Origanum vulgare* L., *Hypericum perforatum* L., *Cruciata laevipes*, Opiz), suggesting possible storage or processing of herbal plants.

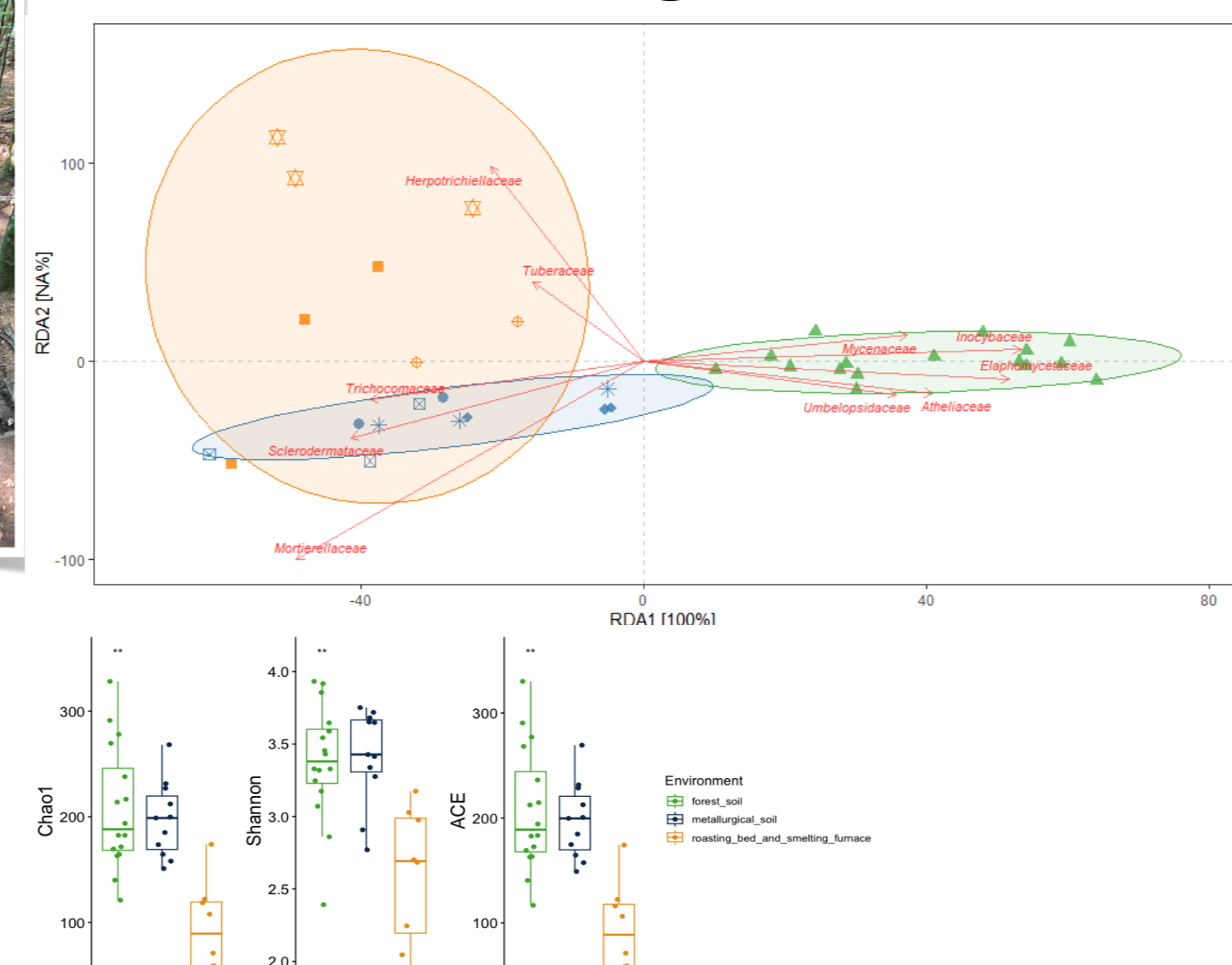


Monte Palazzo shows disturbed stratigraphy due to early 20th-century excavations and WWI trenches, complicating interpretation. Nevertheless, clear differences in microbial communities between archaeological layers and surrounding soils indicate effects of time, structures, and microenvironments. Trench deposits contain fungal DNA from human pathogens and bacteria tolerant to heavy metals, revealing both soldiers' sanitary conditions and the environmental impact of warfare. The presence of *Scedosporium dehoogii* suggests potential health risks, while genera such as *Zavarzinella* and *Anaeromyxobacter* point to metal contamination and chemically altered trench environments.

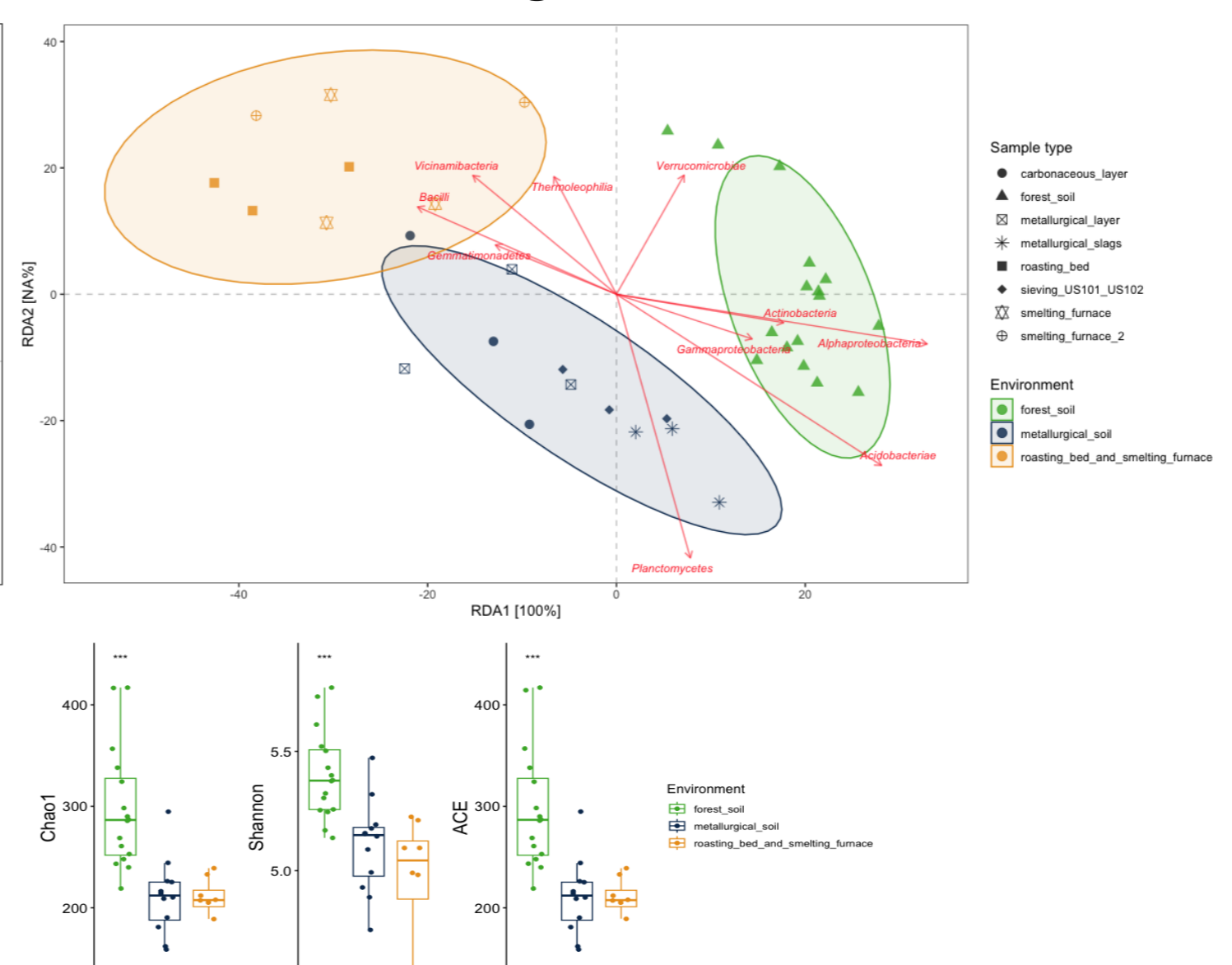
Trambileno, Valle dei Lombardi, (TN), Italy 525 m a.s.l.



Fungi



Prokaryotes



The site preserves a biological memory of metallurgical activities.

These activities not only altered microbial composition but caused a lasting ecological simplification (reduced alpha diversity), marking a clear signature of past human disturbance.

Microbial communities remain distinct today, indicating that Bronze Age activities produced long-term soil changes, that different activities were spatially organized rather than mixed, and that internal site zonation can be reconstructed even without visible structures.

Conclusions

Taken together, biomolecular analyses at Orgères, Monte Palazzo, and Val dei Lombardi show that environmental DNA, through the integrated study of microbial communities and plant genetic traces, not only represents a powerful tool for reconstructing past environments, land use, human and animal activities, agricultural practices, resource management, and cultural connections reflected by non-native species, but also serves as an important complement to traditional archaeological methods, enhancing the interpretation of past events.

Acknowledgements

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