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Alpine archaeology and everyday life at high altitudes: from the excavation to the laboratory (Orgéres-La Thuile, AO, Italy)

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***Abstract* – The Orgères settlement (La Thuile-AO-1665m.) is located at the entrance of the Vallon des Chavannes, an alternative road to the Piccolo San Bernardo pass. The archaeological excavations have revealed a multi-layered context (1st-18th century AD) with buildings functional to the control of territorial management connected to the valley economy based on breeding, the production of wool, milk and derivatives, wood, hay, etc. On the basis of archaeozoological and isotopic analyses of animal teeth we know that Orgères was a permanent and non-seasonal settlement. In this work, DNA-based metabarcoding analyses were conducted on soil microbial communities and plant remains at different archaeological layers. The laboratory data made it possible to identify some important indicators in order to draw an initial picture of living conditions at high altitudes.**

Keywords: Alpine archeology; Metabarcoding analyses; Middle Ages; Roman age

I. INTRODUCTION

Historical-archaeological considerations - The archaeological investigations conducted in Orgères (Fig. 1) have returned important information on daily life at high altitudes [1]. The first data concern the housing structures, built with the *blockbau* technique (wooden bases elevated in wood), except for the stronghold, the headquarters of the lord who controlled the economy of the territory, built in local stone (essentially gray marble), well squared and with the use of the finest ophiolite inserted "at sight" in the jambs of the entrances and in the openings. All the entrances of the structures are located towards the East, i.e. on the side opposite the col des Chavannes, to protect them from the wind coming from the hill.

The settlement is located in a particularly favorable area, along an alternative road route to the Piccolo San Bernardo pass, occupying a flat and sunny plateau located near the Dora di Verney which guarantees water supply for both domestic and productive activities.

Two other factors make us understand the knowledge that in ancient times we had of the territory: the settlement was built between two avalanche cones protecting it from these catastrophic natural events, and on the southern side is naturally protected by a cliff [2]. From archival documents, dated to the early fourteenth century, we know that the care of the roads was entrusted to the *Marrones* who had the task of helping merchants, pilgrims, etc. to cross the Alps even in winter. The Orgères site was sedentary as demonstrated by the

archaeozoological analyses. As a matter of fact the isotopic analyses of teeth of newborn sheep and goats found in the stable, concrete archaeological evidence, have demonstrated that the animals were not subject to transhumance since the percentage of oxygen, nitrogen and strontium registered is constant [3-4]. A document dated 1305 which mentions an important market in Morgex, where the products of the mountain valleys were most likely sold, testifies that the economy of the valley provided for the control of the management of the meadows, breeding and production of milk and derivatives. Even the various areas of forging and processing testify to a self-sufficient economy and support for those who had to cross the Alps to reach Tarantasia and the market areas of northern Europe: a significant datum, in this regard, is the significant quantity of nails ice for shoeing animals [5]. In this work, these considerations related to daily life have been enriched by bio-molecular analyses carried out in order to characterize soil microbial communities and/or plant traces potentially informative on the past human activities in the site.

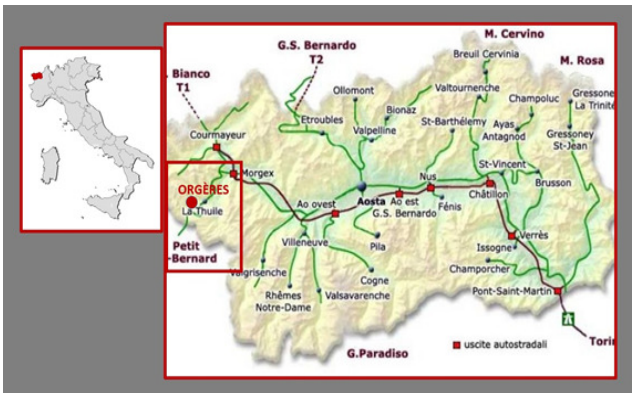

Fig. 1. Location of the archaeological excavation of Orgères

Analysis - The object of the molecular analyses was the environmental DNA (eDNA), that is the set of DNA molecules of various origins (i.e fungi, bacteria, archaea, plants, etc.), present in a non-biological matrix such as soil. The analysis of eDNA, through high-throughput DNA sequencing methods, namely metabarcoding, allows to assess the biodiversity present in a given substrate. The diversity and structure of microbial communities are driven by abiotic and biotic factors, of which they reflect present-day conditions [6-9].The survey of microbial communities present in archaeological sites has revealed the possibility of using them as indicators of past human activities [10, 11 with refs. therein]. As a matter of fact, microbial communities may indicate, after centuries or even thousands of years, the presence of fireplaces, animals, food waste disposal, grain pits, or can be informative on the building history of a construction [11-15]. Moreover, the study of past vegetation can provide information about the past climatic conditions or highlight the existence of particular commercial exchanges. On this basis we have conducted, at the Orgères archaeological site, a biomolecular analysis with the aim of characterizing the microbial (fungi and prokaryotes) and plant communities associated with the different archaeological layers.

II. MATERIALS AND METHODS

Soil samples were collected during an archaeological excavation campaign in July 2021 from 5 archaeological layers (AL), 4 from the ground level (GL) of the archeological site and 3 from the surrounding pasture (PA).

Total soil DNA has been extracted in triplicate by means of the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany), following the manufacturer's protocols.The quality and quantity of DNA samples were assessed by spectrophotometry (ND-1000 Spectrophotometer NanoDropH; Thermo Scientific). Each sample was amplified in triplicate. For prokaryotic community the 16S rDNA region was amplified using the primer set 515fB [16] and 806rB [17], for fungi the ITS2 region, with primer fITS9 [18] and ITS4 [19] and for plant community the tnrL genes by means of primer tnrLc and tnrLh [20]. PCR products were then sent to IGA technologies (Udine, Italy) for Illumina MiSeq sequencing.

Forward and reverse reads were analyzed by means of the bioinformatics platform QIIME2 (Quantitative Insights Into Microbial Ecology 2) version 2019.7 [21]. The taxonomic ssignment of retrieved fungal communities was obtained by means of a classifier built using the release of UNITE Community (2019): UNITE QIIME release for Fungi version 10.05.2021 [22]. The trophism of the fungal community was assessed by means of FUNGuild [23]. For prokaryotic community the taxonomic assignment was achieved using, as reference database, the SILVA Release 138.1 [24], functions were evaluated by means of FAPROTAX: Functional Annotation of Prokaryotic Taxa Version: 1.0 [25]. For the plant community the taxonomical assignment was achieved by doing a BLAST analysis against the NCBI nucleotide database (https://blast.ncbi.nlm.nih.gov). The three generated datasets (fungi, prokaryotes and plants), including OTU table, taxonomy table, phylogenetic tree from Qiime2, and metadata, were then imported into Rstudio [26] and used to create 3 phyloseq objects by means of the R package qiime2R [27]. The R packages phyloseq version 1.36.0 [28], ggplot2 version 3.1.0 [29] and vegan version 2.5-4 [30] were employed for data analyses.

Plant vegetation surrounding the archeological site was surveyed in July 2021 as a comparison with respect to metabarcoding data. Plants sampled in the area surrounding the archaeological site were identified on a morphological basis and named according to "Flora d'Italia" [31].

III. RESULTS

After bioinformatic analysis, 163,125 and 46,703 high-quality sequences were retained for fungi and prokaryotes respectively.

Concerning fungal taxonomy, Ascomycota were the dominant phylum in all soil samples (archeological layers: AL; pasture: PA and ground level :GL), Basidiomycota were particularly abundant in AL and PA (16%). Mortirellomycota accounts for the 9, 10 and 6% in AL, PA and GL respectively, Chytridiomycota was especially abundant in GL (Fig. 2 A).

Regarding prokaryotes, at kingdom-level, Bacteria was the most abundant through all the sites (AL 98%; PA 99%, GL 99.5%): At phylum level, the most represented is Proteobacteria (AL 32%; PA 30%, GL 29%) followed by Acidobacteriota (AL 18%; PA 10%, GL 19%), Bacteroidota (AL 8%, PA 13%, GL 20%), Verrucomicrobiota (AL 2%; PA 17%, GL 12%), Firmicutes (AL 4%; PA 10%, GL 2%) and Actinobacteriota (AL 5%; PA 12%, GL 4%) (Fig. 2 B).

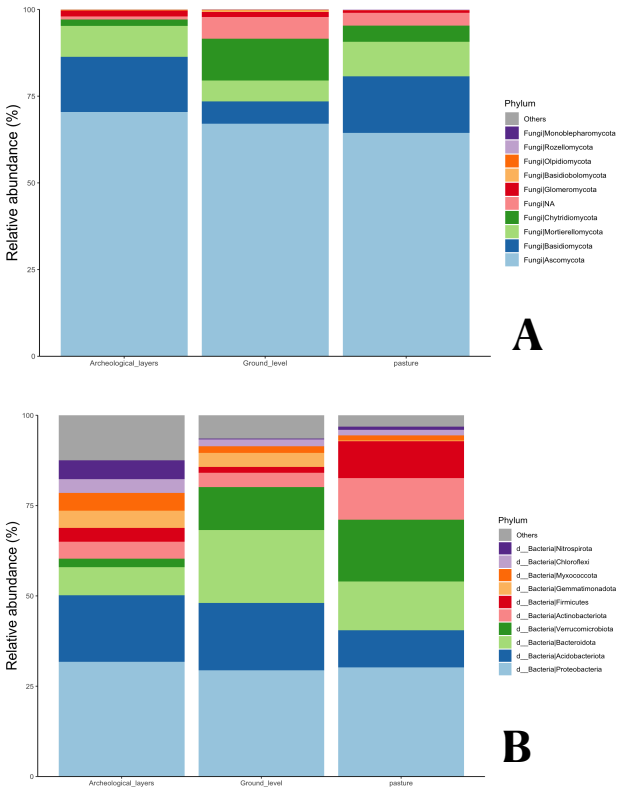

*Figure 2. **A** Taxonomic distribution (based on ITS2 target), at phylum level, of fungal communities retrieved from the sampled sites. **B**: Taxonomic distribution (based on 16S target), at phylum level, of prokaryotic communities retrieved from the sampled sites.*

In terms of trophic diversity, the recovered fungal community shows a statistically significant abundance of animal pathogens in the AL dated as modern age (Chi Square test $p \leq 0,05$). Functional annotation of prokaryotes also reveals that the AL dated as modern age is characterized by the statistically significant presence of human and animal pathogens (Chi Square test $p \leq 0,05$).

The analysis of the plant community associated with AL has revealed the presence of DNA belonging to species not present today in the area surrounding the site and, for the past climatic conditions, most likely not present even in past ages. More in detail, we found DNA sequences belonging to *Taxus baccata* L. in an AL dated VIII-X century AD, and DNA sequences belonging to *Ficus carica* L. in an AL dated I-III century AD.

IV. DISCUSSION

The results of the molecular analyses have shown that the microbial communities (fungi and prokaryotes) associated with the archaeological site differ from those present in the surrounding pasture. As a matter of fact only few operational taxonomical units (OTUs) are shared by the three analyzed environment, and as a consequence, the three microbial communities retrieved in AL, GL, and PA differ in terms of taxonomical and trophic(fungi) or functional (prokaryotes) profile.

Moreover if we consider only the archeological site, the sampled AL are characterized by the presence of different microbial communities compared to the microbial communities present on GL of the site. Our results demonstrate that past human activities have left traces on the soil microbiota, in agreement with reports on other sites [11-14].

Our data have highlighted a statistically significant abundance of DNA sequences belonging to fungi and bacteria pathogens of animals, and to bacterial pathogens of humans in the more modern archaeological layers of the Orgères site. In particular, the retrieve of DNA sequences attributable to bacteria of human pneumonia in the early medieval phases (VIII-X AD) is very probable considering the climatic conditions in the winter periods and could contribute to explain why the site was abandoned in modern age.

Finally, the molecular analysis of the vegetation associated with the AL made it possible to identify traces of plant species not present in the environment surrounding the site. In particular, the presence, in the Roman phases, of traces of dried figs testifies to a strong eating habit connected to Mediterranean contexts; the *T. baccata* (VIII-X century AD), which does not grow at these altitudes, was useful for making bows or tools. Such results can therefore give us an idea about the territory connections and/or commercial activities carried out at the Orgères site.

For these reasons, it is our opinion that the molecular analyses conducted in an archaeological site are a significant complement to traditional archaeological methods and that and can be informative for the interpretation of past events.

V. REFERENCES

1. C.M. Lebole, G. Di Gangi, L'archeologia racconta: lo scavo dell'insediamento alpino di Orgères, La Thuile, in D. Elia (ed.) Chiedi alla terra. Scavi e ricerche archeologiche del Dipartimento di Studi Storici dell'Università di Torino, Torino 2020, pp. 223-237.
2. G. Di Gangi, C.M. Lebole, Archeologia ed indagini in un territorio alpino, Atti della Conferenza Nazionale di geomatica ed informazione geografica, ASITA, Genova 20-24 giugno 2022, Genova 2022, pp. 173-184.
3. C.M. Lebole, C. Mascarello, G. Di Gangi, Archaeology and archaeozoology: the alpine settlement of Orgères (La Thuile-Aosta, Italy), in Metrology for Archaeology and Cultural Heritage. IEEE International Conference, Cassino 22-24 ottobre 2018, Cassino 2018, pp. 61-65.
4. G. Di Gangi, C.M. Lebole, G. Sartorio, La complessità dell'archeologia alpina: il sito di Orgères (La Thuile-AO) tra storia e territorio, in ISCUM (ed.) Tiziano Mannoni. Attualità e sviluppi di metodi e idee, Atti del Convegno Nazionale, Genova 14-15 ottobre 2021, Firenze 2021, voll-1-2, vol. 2, pp. 455-462.
5. G. Di Gangi, C.M. Lebole, Lo scavo di Orgères (La Thuile-AO). Un insediamento alpino tra ricerca ed archeologia pubblica, Atti dell'VIII Congresso Nazionale di Archeologia Medievale, Matera 12-15 settembre 2018, Firenze 2018, voll. 1-3, vol. 2, pp. 11-15.
6. W. Gams. The analysis of communities of saprophytic microfungi with special reference to soil fungi. In: Winterhoff, W. (Ed.), Fungi in Vegetation Science. Handbook of Vegetation Science, vol. 19. Springer, Dordrecht, Netherlands, 1992, pp. 183–223. https://doi.org/10.1007/978-94-011-2414-0_7.
7. J.S. Kim, D.E. Crowley, A. Buerkert. Bacterial communities from soil sediments of a mountain oasis in northern Oman. Catena, 2010, 82, 102–111. https://doi.org/ 10.1016/j.catena.2010.05.007.
8. L. Tedersoo, M. Bahram, S. Po˜lme, U. Koljalg, N.S. Yorou, R. Wijesundera, L. Villarreal Ruiz, L et al. and K. Abarenkov. Fungal biogeography. Global diversity and geography of soil fungi. Science, 2014, 346, 1256688. https://doi.org/10.1126/science.1256688.
9. D.C. Schlatter, K. Kahl, B. Carlson, D.R. Huggins, T. Paulitz. Fungal community composition and diversity vary with soil depth and landscape position in a no-till wheat-based cropping system. FEMS Microbiol. Ecol. 2018, 94, fiy098. https://doi.org/10.1093/femsec/fiy098.
10. T.S. Demkina, T.E. Khomutova, N.N. Kashirskaya, I.V. Stretovich, V.A. Demkin. Microbiological investigations of paleosols of archeological monuments in the steppe zone. Eurasian Soil Sci., 2010, 43, 194–201.
11. S. Peters, A.V. Borisov, S. Reinhold, D.S. Korobov, H. Thiemeyer. Microbial characteristics of soils depending on the human impact on archaeological sites in the Northern Caucasus. Quat. Int., 2014, 324, 162–171. https://doi.org/10.1016/j.quaint.2013.11.020.
12. A. Ivanova, O. Marfenina. Soil fungal communities as bioindicators of ancient human impacts in medieval settlements in different geographic regions of Russia and southwestern Kazakhstan. Quat. Int., 2015, 365, 212–222. https://doi.org/10.1016/j.quaint.2014.10.016.
13. E.V. Chernysheva, D. Korobov, A.V. Borisov. Thermophilic microorganisms in arable land around medieval archaeological sites in Northern Caucasus, Russia: novel evidence of past manuring practices. Geoarchaeology, 2017, 32, 494–501.
14. A.V. Borisov, T.S. Demkina, N.N. Kashirskaya, T.E. Khomutova, E.V. Chernysheva. Changes in the past soil-forming conditions and human activity in soil biological memory: microbial and enzyme components. Eurasian Soil Sci., 2021, 54 (7), 1078–1088. https://doi.org/10.1134/S1064229321070024.
15. S. Voyron, C. Tonon, L. Guglielmone, L. Celi, C. Comina, H. Ikeda, N. Matsumoto, D. Petrella, J. Ryan, K. Sato, A. Seike, I. Varriale, I. Yamashita, S.E. Favero-Longo, E. Bonifacio. Diversity and structure of soil fungal communities unveil the building history of a burial mound of ancient Japan (Tobiotsuka Kofun, Okayama Prefecture),Journal of Archaeological Science, 2022, Volume 146, 105656, ISSN 0305-4403, https://doi.org/10.1016/j.jas.2022.105656.
16. A. E. Parada, D.M. Needham & J.A. Fuhrman. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology, 2016, 18(5), 1403–1414. http://doi.org/10.1111/1462-2920.13023.
17. A. Apprill, S. McNally, R. Parsons, & L. Weber. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquatic Microbial Ecology, 2015, 75(2), 129–137. http://doi.org/10.3354/ame01753.
18. K. Ihrmark, I.T.M. Bödeker, K. Cruz-Martinez, H. Friberg, A. Kubartova, J. Schenck, Y. Strid, J. Stenlid, M. Brandström-Durling, K.E. Clemmensen, et al. New Primers to Amplify the Fungal ITS2 Region - Evaluation by 454-Sequencing of Artificial and Natural Communities. FEMS Microbiol Ecol, 2012, 82, 666–677, doi:10.1111/j.1574-6941.2012.01437.x.
19. T. White, T. Bruns, S. Lee, J. Taylor, M. Innis, D. Gelfand, J. Sninsky, J. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In PCR Protocols: a Guide to Methods and Applications, 1990, Vol. 31, pp. 315–322.
20. P. Taberlet, L. Gielly, G. Pautou, et al.. Universal primers for amplification of three non-coding

regions of chloroplast DNA. Plant Mol. Biol., 1991, 17, 1105–1109. https://doi.org/10.1007/BF00037152.
21. E. Bolyen, J.R. Rideout, M.R. Dillon, N.A. Bokulich, C.C. Abnet, G.A. Al-Ghalith, H. Alexander, E.J. Alm, M. Arumugam, F. Asnicar, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol., 2019, 37, 852–857.
22. K. Abarenkov, A. Zirk, T. Piirmann, R. Pöhönen; F. Ivanov, R.H. Nilsson, U. Kõljalg. UNITE QIIME release for Fungi, Version 10.05.2021. UNITE Community. https://doi.org/10.15156/BIO/1264708.
23. N.H. Nguyen, Z. Song, S.T. Bates, S. Branco, L. Tedersoo, J. Menke, J.S. Schilling, P.G. Kennedy. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol., 2016, 20, 241–248.
24. S. Michael, M. S. Robeson II, D.R. O'Rourke, B.D. Kaehler, M. Ziemski, M.R. Dillon, J.T. Foster, N.A. Bokulich. RESCRIPt: Reproducible sequence taxonomy reference database management. PLoS Comput. Biol., 2021, 17, e1009581.
25. S. Louca, L.W. Parfrey, M. Doebeli. Decoupling function and taxonomy in the global ocean microbiome. Science, 2016, 353, 1272–1277.
26. RStudio Team. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA. 10/01/2020. Available online: http: //www.rstudio.com/ (accessed on 3 June 2022).
27. J.E. Bisanz. qiime2R: Importing QIIME2 Artifacts and Associated Data into R Sessions. 2018, Available online: https://github. com/jbisanz/qiime2R (accessed on 3 June 2022).
28. P.J. McMurdie, S. Holmes. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE, 2013, 8, e61217.
29. H. Wickham.. ggplot2: Elegant Graphics for Data Analysis; Springer: New York, NY, USA, 2016.
30. J. Oksanen, F.G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P.R. Minchin, R.B. O'Hara, G.L. Simpson, P. Solymos, et al. Vegan: Community Ecology Package. R Package Version 2.5-4. 2019. Available online: https://CRAN.R-project.org/package=vegan (accessed on 3 June 2022).
31. S.Pignatti, R. Guarino & M. La Rosa. Flora d'Italia, 2a Edizione. Eda- gricole - Edizioni Agricole di New Business Media, Bologna, 2017-2019.