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Determining the core bacterial and fungal genera in table olive fermentations

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Abstract

Table olives are among the most ancient and important fermented foods of the Mediterranean basin. Their production is still strongly related to traditional practices, and the lack of thermal treatments, the reliance on natural contamination and selective factors (NaCl, pH, occurrence of phenolics, etc.) determine the dynamics of the microbial community. Lactic acid bacteria (LAB) and yeasts have a pivotal role in table olive microbial communities, but several halophilic and alkalophilic microorganisms may also contribute, positively or negatively, to the quality and safety of this fermented vegetable.

We have use metataxonomic data extracted from the FoodMicrobionet database to provide quantitative insights on the structure of bacterial and fungal microbial communities of table olives and to identify core genera in different trade preparations.

Celerinatantimonas and *Lactiplantibacillus* were the most prevalent genera among bacteria, followed by several LAB, halophilic and alkalophilic lactic acid bacteria (HALAB) and Gram negatives, including non-halophilic species. Similarly, 3 fungal genera (*Pichia, Candida,* and *Wickerhamomyces*) were the most abundant and prevalent among fungi.

The distribution of both bacteria and fungi varied significantly in different olive varieties, among olives, brines and contact surfaces or materials, and at different production stages, and no clear grouping related to the combination of ripeness and trade preparation was found, although HALAB were characteristically abundant in Spanish style green olives.

Addition of starter cultures affected the composition and dynamics of microbial communities to a variable extent.

Keywords:

Table olives; core microbiota; fungal community; bacterial community; metataxonomic analysis.

Highlights

- Data from 16 metataxonomic studies on table olives, including 833 samples, were combined.
- Enhanced metadata facilitated the comparison among different studies.
- Composition of the microbiota of table olives was highly variable.
- *Celerinatantimonas* and *Lactiplantibacillus* dominated the core bacterial microbiota.
- *Pichia*, *Candida,* and *Wickermahomyces* were the most prevalent among fungi.

1. Introduction

Table olives are the result of the processing fruits of selected varieties of *Olea europea* L. *sativa* that have been treated to remove their natural bitterness and preserved by fermentation, heat treatment, drying or salting (IOOC, 2004). The preservation of olives in brines dates back to 7,000 BP (Langgut and Garfinkel, 2022). Nowadays, this fermented vegetable is still produced mostly in the Mediterranean Basin, but the production and export from the Americas is increasing. Thereby, world production for the crop year 2023/24 is estimated at 2,828,500 t, and is concentrated in Mediterranean countries such as Spain, Egypt, Turkey, Morocco, Italy, and Greece (source: https://www.internationaloliveoil.org/world-market-of-olive-oil-and-table-olives-data-from-december-2024/, last accessed March 2025).

While the level of innovation in industrial production may be high (Campus et al., 2018), table olive production for artisanal producers is still strongly related to traditional practices, and, as for many other vegetable fermentations (Gänzle, 2022; Hernández-Velázquez et al., 2024; Tamang et al., 2020), the lack of thermal treatments of raw material, the reliance on natural contamination and selective factors (NaCl, acidic pH, occurrence of phenolics, etc.) determine the dynamics of the microbial community and could affect the quality and safety of the final product (Anagnostopoulos and Tsaltas, 2022; Perpetuini et al., 2020; Vaccalluzzo et al., 2020). On the other hand, several chemical and physical treatments are used at the time of packaging to enhance stability of commercial products (Campus et al., 2018; Panagou et al., 2003).

IOOC recognizes 5 main trade preparation groups for table olives: i) treated olives (often referred to as Spanish style olives, treated by alkali, prior to fermentation in brine); ii) natural olives (Greek style olives, naturally fermented in brine with no alkali treatment); iii) dehydrated and/or shrivelled olives (which may or may not be treated in brines); iv) olives darkened by oxidation (California style; which may be fermented prior to oxidation treatment); and v) other specialties (which do not fall in previous categories) (https://www.internationaloliveoil.org/wp-content/uploads/2019/11/COI-OT-NC1-2004-Eng.pdf). Microorganisms play an important role in the production, safety and quality of all these products (Anagnostopoulos and Tsaltas, 2022; Vaccalluzzo et al., 2020).

Several PDO and PGI table olive varieties are recognized in the European Union (https://ec.europa.eu/agriculture/eambrosia/geographical-indications-register/) and their

microbiota may contribute to aspects related to identity and quality (Anagnostopoulos and Tsaltas, 2022). Additionally, the composition of the microbiota may be used to prevent frauds or identify the geographical origin of the product (Argyri et al., 2020; Kalogiouri et al., 2020; Kamilari et al., 2019).

Natural contamination from the olives, use of salt, spices, water, food contact surfaces and, in some cases, the addition of starter cultures, determine the assembly of the initial microbial community (Lanza, 2013; Penland et al., 2021), whose evolution is affected by a large number of factors, including initial pH, scarcity of nutrients (due to the repeated washing needed to remove alkali residuals in some varieties), high phenolic concentration, high salt concentration (brines with 6-12% NaCl are used, although there is a trend for reduction of salt concentration), temperature (which may be low in some areas at the beginning of fermentation), and anaerobiosis in large fermenters (Anagnostopoulos and Tsaltas, 2022; Benítez-Cabello et al., 2023; Blekas et al., 2002; Khalil et al., 2023; Perpetuini et al., 2018).

Due to the diversity of contamination sources, to the lack of heat treatments, and to the variety and variability of ecological factors influencing this fermentation process, the microbial communities of table olives are complex and dynamic.

As for many other food products, -omic approaches are increasingly being used to complement or even replace cultivation-based approaches (Anagnostopoulos and Tsaltas, 2022; Ferrocino et al., 2022; De Filippis et al., 2018; Tsoungos et al., 2023). Two main microbial groups, lactic acid bacteria (LAB) (Giavalisco et al., 2024; Portilha-Cunha et al., 2020) and yeasts (Arroyo-López et al., 2008, 2012), are recognized as having a pivotal role in table olive microbial communities, but several halophilic and alkalophilic microorganisms may also contribute, positively or negatively, to the quality and safety of table olives (de Castro et al., 2022; Lucena-Padrós and Ruiz-Barba, 2016; Posada-Izquierdo et al., 2021).

The microbiology of table olives has been reviewed multiple times, including recently (Arroyo-López et al., 2018; Anagnostopoulos and Tsaltas, 2022; Campus et al., 2018; Heperkan, 2013; Perpetuini et al., 2020; Portilha-Cunha et al., 2020; Tsoungos et al., 2023; Vaccalluzzo et al., 2020). These reviews provide qualitative data on the occurrence, dynamics and abundance of beneficial, functional, and spoilage microorganisms in table olive fermentation, but they usually lack quantitative insights nor provide re-usable data.

At the time of writing this article, there were 36 studies which investigated the microbiota of table olives using amplicon targeted approaches (Table 1). In most cases, studies focused on one or few olive varieties and trade preparations; while the most frequent approaches include targeting of regions the 16S rRNA gene or ITS region using the Illumina platform, the type of samples used (fruits, brines, or both; environmental samples) varies among studies. The quality of metadata is also highly variable, as are the experimental designs, although the comparison of started/unstarted fermentations or the evaluation of the effect of salt concentration is rather frequent. Qualitative lists of genera found in most studies have been presented in recent reviews (Tsoungos et al., 2023) but they are of little help in evaluating the variability in prevalence and abundance of the different species or in the identification of core microbial populations of fermented olives in general or of selected processes and no public repository with processed data on the microbiota of table olives is available. There is therefore a need for a reanalysis of published data in order to provide a comprehensive view of the structure and dynamics of the microbial communities of table olives and to foster access to this data by scientists and stakeholders.

Thereby, in this work our objective was to use the FoodMicrobionet database (Parente et al., 2016, 2022; Parente and Ricciardi, 2024) to provide quantitative insights on the structure of bacterial and fungal microbial communities of table olives and to identify core genera as a function of table olive elaboration. Even if an obvious shortcoming of our approach is the heterogeneity of data available (in terms of olive varieties, sampling strategies and experimental designs, gene targets and primers, sequencing platforms), we feel that the use of a standardized pipeline for raw sequence processing and an enhanced metadata structure will in part mitigate this problem, and provide transparent and reusable data and software to the scientific community. This in turn may allow a better understanding of the ecology of microorganisms which are important for the quality (including both beneficial and spoilage microorganisms) and safety of table olives and promote the development of novel microbiome-based starter cultures (Ferrocino et al., 2022; Gänzle et al., 2023).

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140 **Table 1**. Metataxonomic studies on the microbial communities of table olives (in inverse
141 chronological order).
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Olive varieties	Olive trade preparation	Platform	Targeted region(s)	Description	Reference
Kalamata	Greek style olives, black	Oxford Nanopore	16S rRNA gene	Use of probiotic LAB starters in the production of Kalamata black olives	(Vougiouklaki et al., 2024)
Manzanilla	Spanish style olives, green	Illumina	ITS region	Impact of the addition of LAB starters on the fungal microbiota	(López-García et al., 2024)
Gordal, Manzanilla, Hojiblanca	Greek style olives, green	Illumina	16S rRNA gene, ITS region	Impact of wild yeast starters on the volatilome	(Ruiz-Barba et al., 2024)
Taggiasca	Greek style olives, turning color	Illumina	26S rRNA gene	Dynamics of yeast species during natural fermentation	(Traina et al., 2024)
Thassos	Naturally dry salted olives, black	Illumina	26S rRNA gene	Composition of yeast microbiota in dry salted olives	(Gounari et al., 2023)
Cypriot, Picual, Kalamata	Greek style, green or black	Illumina	16S rRNA gene, ITS region	Bacterial and fungal microbiota of three varieties, collected in different locations	(Kamilari et al., 2023)
Halkidikis, Thassou, Kalamon, Amfissis, and Konservolia	Greek style, Spanish style, dry salted	Illumina	16S rRNA gene, 18S rRNA gene	Composition of bacterial and yeast microbiota in market samples of dry olives	(Mougiou et al., 2023)
Manzanilla	Spanish style, green	Illumina	16S rRNA gene, ITS region	Fungal and bacterial microbiota in laboratory fermented olives inoculated with LAB and yeasts	(López-García et al., 2023)
Manzanilla	Spanish or Greek style, green	Illumina	16S rRNA gene, ITS region	Bacterial and yeast microbiota in uninoculated olive fermentation	(Ruiz-Barba et al., 2023a)
Gordal, Hojiblanca and Manzanilla	Greek style, green	Illumina	16S rRNA gene, ITS region	Bacterial and yeast microbiota in uninoculated natural green olive fermentation	(Ruiz-Barba et al., 2023b)
Manzanilla	Spanish style, green	Illumina	16S rRNA gene, ITS region	Optimization of a pretreatment technique for recovery of LAB and yeasts from biofilms	(López-García et al., 2022)

Manzanilla, Aloreña, Hojiblanca, Gordaln, Morona, Verdial, Empeltre, Empeltre Mallorquina, Kalamata, Conservolea, Galega, Picholine, Criolla, Lucques du Languedoc, Azapa, Arauco	Various	Illumina	ITS region	Fungal communities of packaged olives	(Benítez-Cabello et al., 2022)
Nyons	Greek style, black	Illumina	16S rRNA gene, ITS region	Effect of salt reduction and inoculation with a complex consortium or backslopping on microbiota dynamics	(Penland et al., 2022)
Hojiblanca	Spanish style, green	Illumina	16S rRNA gene	Effect of inoculation with LAB on the dynamics of bacterial microbiota	(Correa-Galeote et al., 2022)
Gordal, Hojiblanca and Manzanilla	Spanish style, green	Illumina	16S rRNA gene	Investigation of the role of *Celerinatantimonas* in gas pocket defect	(de Castro et al., 2022)
Ascolana tenera	Spanish and Greek style, green	Illumina	16S rRNA gene	Impact of starter addition on the microbiota in laboratory scale fermentation	(Maoloni et al., 2022)
Halkidiki, Conservolea	Spanish and Greek style, green	Illumina	ITS region	Fungal microbiota of green olives stored in two types of pouches	(Tzamourani et al., 2022)
Nyons	Greek style, black	Illumina	16S rRNA gene, ITS region	Source tracking of desirable and spoilage microorganisms	(Penland et al., 2021)
Aloréña de Malaga	Greek style, green, cracked	Illumina	16S rRNA gene, ITS region	Microbiota of traditionally packed olives during storage	(López-García et al., 2021)
Kalamata	Greek style, black	Illumina	16S rRNA gene, 18S rRNA gene	Evolution of microbiota during storage in modified atmosphere	(Michailidou et al., 2021)
Unknown/Unspecified	Greek style, green, cracked	Illumina	Unknown	Composition of the microbiota fungal and bacterial microbiota in brine of an unspecified Turkish variety	(Demirci et al., 2021)
Manzanilla	Spanish style, green	Illumina	16S rRNA gene	Diversity of the bacterial microbiota in spoiled industrial fermentations	(Arroyo-López et al., 2021)

Manzanilla	Spanish style, green	Illumina	16S rRNA gene	Impact of a LAB and yeast starter on bacterial communities	(Benítez-Cabello et al., 2020a)
Kalamata	Greek style, black	Illumina	16S rRNA gene, ITS region	Bacterial and fungal microbiota of olives and brines of industrially fermented Kalamata olives	(Kazou et al., 2020)
Nyons	Greek style, black	Illumina	16S rRNA gene, ITS region	Dynamics of fungal and bacterial microbiota during fermentation	(Penland et al., 2020)
Picual	Greek style, green	Illumina	16S rRNA gene	Dynamics of bacterial community in uninoculated and inoculated (LAB) fermentation	(Anagnostopoulos et al., 2020)
Konservolia and Halkidiki	Greek style, black and Spanish style, green	Ion Torrent (bacteria), Illumina (fungi)	16S rRNA gene, ITS region	Bacterial and fungal communities in samples from different regions	(Argyri et al., 2020)
Manzanilla, Aloreña, Hojiblanca, Gordal, Morona, Verdial, Empeltre, Empeltre Mallorquina, Kalamata, Conservolea, Galega, Picholine, Criolla, Lucques du Languedoc, Azapa, Arauco	Various	Illumina	16S rRNA gene	Bacterial communities of commercial packaged olives	(Benítez-Cabello et al., 2020b)
Manzanilla, Hojiblanca	California style	Illumina	16S rRNA gene	Bacterial communities at different stages of processing	(Medina et al., 2018)
Manzanilla, Gordal	Spanish style, green	Illumina	16S rRNA gene, ITS region	Bacterial and fungal microbiota of spoiled olives	(de Castro et al., 2018)
Nocellara Etnea	Greek style, green	Ion Torrent	16S rRNA gene	Bacterial microbiota dynamics in LAB started and spontaneous fermentation	(Randazzo et al., 2017)
Aloreña de Malaga	Greek style, green	Illumina	16S rRNA gene	Bacterial microbiota in cured or cracked olives, with or without heat shock	(Rodríguez-Gómez et al., 2017)
Nocellara del Bélice	Spanish style and Picholine, green	Illumina	16S rRNA gene	Effect of salt reduction and type of processing on bacterial microbiota	(Zinno et al., 2017)

Aloreña de Malaga	Greek style, green	F454	ITS region	Dynamics of fungal microbiota in brines and fruit	(Arroyo-López et al., 2016)
Aloreña de Malaga	Greek style, green	F454	16S rRNA gene	Dynamics of bacterial microbiota in brines and fruit	(Medina et al., 2016)
Nocellara Etnea	Spanish and Greek style, green	F454	16S rRNA and rRNA gene	Effect of type of processing on bacterial microbiota	(Cocolin et al., 2013a)

ITS internal transcribed spacer

2. Materials and methods

2.1. Database analysis

FoodMicrobionet v 5.01 (Parente and Ricciardi, 2024) includes 16 different metataxonomic studies (with a total of 833 samples) on table olives (Table 2) whose raw sequences are available from NCBI Short Read Archive (SRA). The sequences were processed with the scripts available in FoodMicrobionet repository (https://github.com/ep142/FoodMicrobionet) using computational approaches described elsewhere (Parente et al., 2022; Parente and Ricciardi, 2024).

Table 2. List of studies on metataxonomic table olives included in FoodMicrobionet 5.0.1 (Parente and Ricciardi, 2024) which were used in the present work.

study	target	region	Read length	bioproject	samples	reference
ST14	16S RNA gene and 16S RNA	V1-V3	323	PRJNA192807	20	(Cocolin et al., 2013b)
ST133	16S RNA gene	V3-V4	270	PRJNA600153	18	(Anagnostopoulos et al., 2020)
ST141	16S RNA gene	V3-V4	426	PRJEB3989	215	(Penland et al., 2020)
ST182	ITS region and 16S RNA gene	ITS1 and V3-V4	427	PRJEB43241	15	(López-García et al., 2021)
ST183	16S RNA gene	V3-V4	404	PRJNA688686	4	(Michailidou et al., 2021)
ST226	ITS region and 16S RNA gene	ITS1 and V3-V4	427	PRJNA878774	12	(Kamilari et al., 2023)
ST229	16S RNA gene	V3-V4	427	PRJNA811759	48	(Maoloni et al., 2022)

ST236	ITS region and 16S RNA gene	ITS1 and V3-V4	427	PRJEB61009	17	(Ruiz-Barba et al., 2023a)
ST239	ITS region	ITS2	374	PRJEB39898	208	(Penland et al., 2020)
ST240	16S RNA gene	V3-V4	425	PRJEB40675	47	(Penland et al., 2021)
ST246	ITS region	ITS1 + ITS2	339	PRJNA317749	28	(Arroyo-López et al., 2016)
ST247	ITS region	ITS2	422	PRJEB40674	47	(Penland et al., 2021)
ST248	ITS region	ITS1	268	PRJEB50357	69	(Benítez-Cabello et al., 2022)
ST251	16S RNA gene	V2-V3	275	PRJNA315418	28	(Medina et al., 2016)
ST252	16S RNA gene	V3-V4	330	PRJNA761998	8	(Vaccalluzzo et al., 2022)
ST253	ITS region and 16S RNA gene	ITS2 and V3-V4	322	PRJNA1034814	49	(Giavalisco et al., 2023)

ST253 is an unpublished study from our group, dealing with the effect of starter culture (*Lactiplantibacillus pentosus* O17) and timing of salt addition to the brine on the manufacture of black Itrana olives (Giavalisco et al., 2023). Sequences were deposited in NCBI SRA with accession number PRJNA1034814.

All studies were extracted from FoodMicrobionet using the extract_studies.R script available in the Metaolive repository (https://github.com/ep142/Metaolive) and saved as phyloseq objects (McMurdie and Holmes, 2013).

Until recently metadata models were not enforced for the deposit of sequence data in NCBI Short Read Archive (https://www.ncbi.nlm.nih.gov/biosample/docs/packages/): the metadata for studies deposited in SRA are therefore often incomplete and inconsistent and do not follow a common scheme and those available in FoodMicrobionet are not specific for table olives. We therefore assembled a complete set of metadata attributes (olive cultivar, olive variety, olive trade preparation, style, spoilage, starter addition, etc.), by using information available directly from metadata deposited in SRA or inferred from the papers describing the study, were added to the sample tables. The enriched metadata are available in our GitHub repository (https://github.com/ep142/Metaolive/tree/main/olive_FMBN).

Graphical and statistical analysis of phyloseq objects was carried out using own R 4.3.2 (R core team, 2024) scripts, available in the GitHub repository. Taxonomic re-assignment was carried out using the assignTaxonomy() and addSpecies() functions of the dada2 package (Callahan et al., 2016), using Silva 138.1 (Quast et al., 2013) and UNITE (general release

04/04/2024) (Abarenkov et al., 2023) taxonomic reference databases. Statistical and graphical treatment of the results included:

1. sample filtering (by removing samples with < 1000 sequences)
2. primary taxa filtering: sequences to mitochondria, and chloroplasts were removed from bacterial datasets; sequences assigned to fungi were removed from bacterial datasets, and vice versa;
3. taxonomic agglomeration was performed at the genus level;
4. application of a very lenient prevalence (threshold for removal < 0.01) and abundance (threshold for removal < 0.005) filter (taxa were retained if they passed both thresholds) to avoid loss of rare taxa;
5. calculation of alpha diversity
6. production of a variety of boxplots and bar plots for alpha diversity
7. ordination using non-monotonic multidimensional scaling of the Bray-Curtis distance calculated on taxa abundance data

To this end, functions from packages phyloseq, vegan (Oksanen et al., 2022) and tidyverse (Wickham et al., 2019) were used.

Detailed metadata are available in our GitHub repository (https://github.com/ep142/Metaolive).

Of the 16 studies available, 8 included data for bacteria only, 4 for fungi only, and 4 for both bacteria and fungi. However, due to inconsistencies in the deposit of sequences in SRA (in several cases the same sample was deposited with two separate biosample accessions and/or data for bacteria and fungi were deposited with different bioproject or study accessions), the same samples might be present in two studies, one for bacteria and one for fungi (by convention in FoodMicrobionet a study must have a unique bioproject accession and a sample a unique biosample accession). The number of samples per study varied from 4 to 251, with a median value of 28.

3. Results and Discussion

214 *3.1 The bacterial microbiota of table olives*

215

216 Data on bacterial communities of table olives and their production environments were
217 obtained from 12 metataxonomic studies, whose number of samples was highly unbalanced
218 (Supplementary Table 1). The most common target and platform are the V3-V4 region of the
219 16S RNA gene and Illumina, respectively; data on 16S RNA were found in one study but were
220 removed from the analysis.
221 Samples were available for fruits and/or brine, or contact surface and raw materials
222 (Supplementary Table 2 and 3). After removing samples with less than 1,000 sequences, a
223 total of 411 samples were left for analysis (Supplementary Table 3).
224 The largest number of samples was available for Nyons (Penland et al., 2020, 2021) and
225 Itrana naturally fermented black olives (Giavalisco et al., 2023).
226 Overall, the data we assembled from SRA cover a reasonably large number of olive varieties
227 (12, see Supplementary Table 3) from 5 countries (France, Spain, Italy, Greece, and Cyprus;
228 Supplementary Figure 1), including alkali treated and natural olives making this the most
229 comprehensive study on the microbiota of table olives.
230 Alpha diversity indices were calculated before taxa filtering. Chao1 ranged from 2 to 100.
231 The variability of Chao1 as a function of olive variety, stage of fermentation (0 environmental
232 samples, 1 raw material, 2 samples during fermentation, samples at the end of
233 fermentation), ripeness, trade preparation, sample type is shown in Figure 1.
234

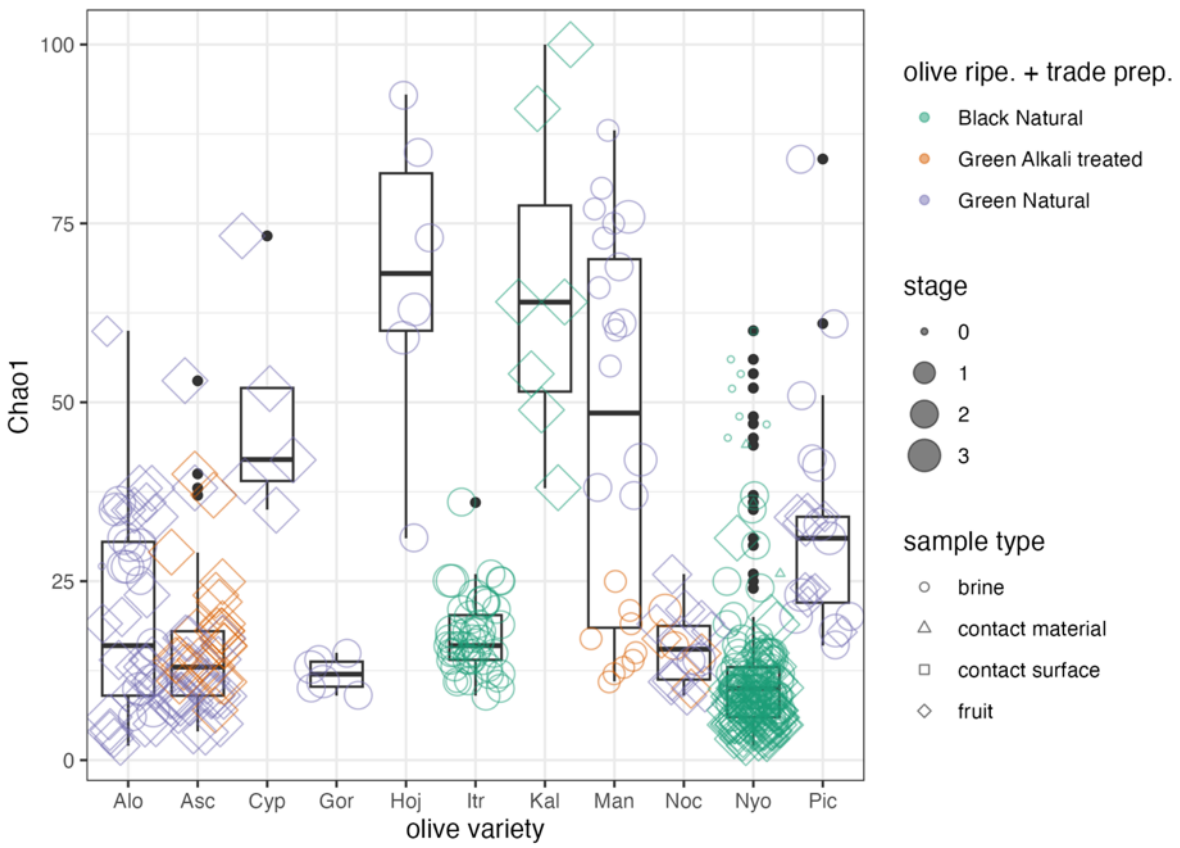


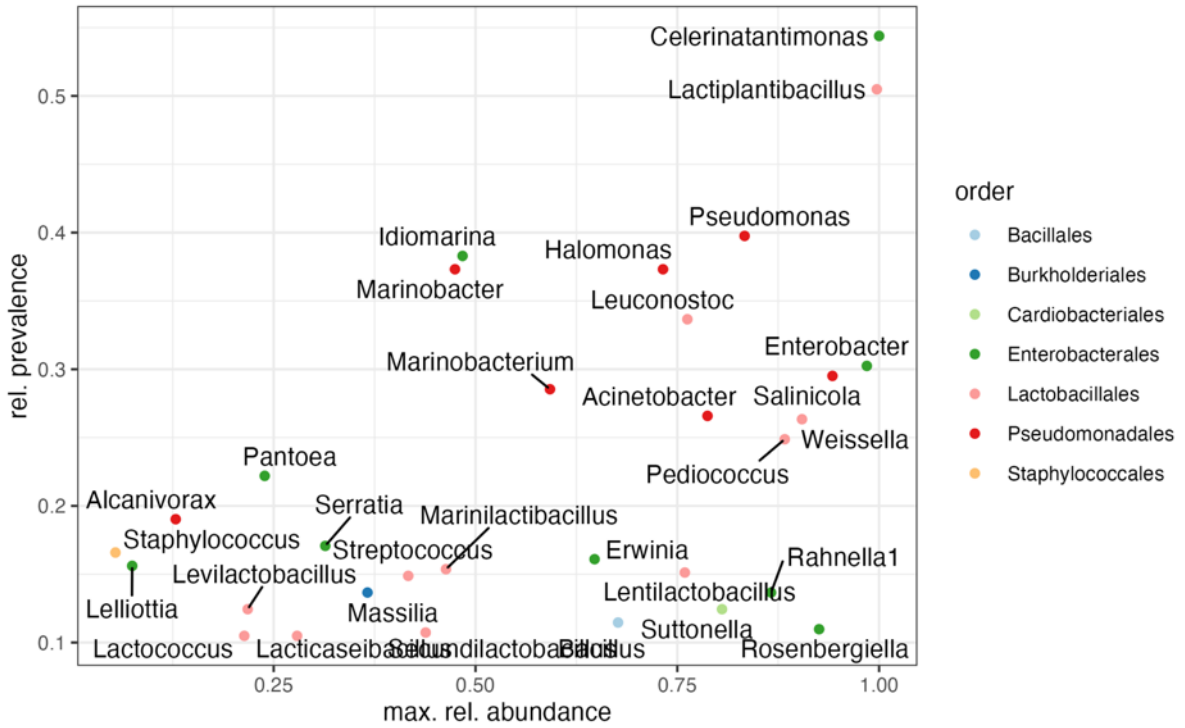
Figure 1. Distribution of Chao1 for bacterial communities from studies on table olives (Table 2) by olive variety, fermentation stage (1 raw materials, 2 samples during fermentation, 3 samples at the end of fermentation), olive ripeness and trade preparation, sample type.

Due to the differences in methods and experimental designs, only very general conclusions can be drawn:

- there is no evidence of a relationship between number of sequences per sample and Chao1.
- olive variety, degree of ripening and process are almost always confounded, therefore comparisons need to be done within the same variety.
- for the few studies for which the same olive variety was processed according to different trade preparations (usually Green Natural and Green Spanish style) the diversity is not consistently higher for either type.
- for the few studies for which inoculated and inoculated olives were compared, we found no significant (Kolmogorov-Smirnov two sample test) evidence of lower diversity in inoculated fermentations; median Chao1 was even higher in some cases for inoculated fermentations (see Supplementary Figure 2).

253 • there is no clear evidence of fermentation stage affecting the diversity (see Figure 1),
254 i.e. diversity does not apparently decrease with increasing fermentation time due to
255 the selection of a less diverse community adapted, for example, to high salt / high
256 polyphenol / low nutrient conditions, although in some environmental samples (see
257 Nyons olives) diversity is much higher than in olives or brines. The reasons for this
258 are not clear. One reason is that, although one study (Cocolin et al., 2013a) included
259 both 16S RNA (which may depict the active fraction within the community) and the
260 16S RNA gene (which may depict the structure of both the active and inactive parts
261 of the fermentation), the number of samples was too low to make any conclusion, it
262 is impossible to decide which portion of the microbial community as determined by
263 amplicon targeted sequencing was active. This problem is common to many other
264 fermentations and there is no consensus on the best approach to be used (Emerson
265 et al., 2017; Erkus et al., 2013, 2016; Yap et al., 2021) to solve it.
266 After this stage taxonomic filtering and agglomeration was applied. As a result, the number
267 of taxa was reduced from 929 (pre-taxonomic agglomeration) to 157, although the
268 proportion of sequences retained was 99.5%. This reveals that the diversity of bacterial
269 genera in table olives is much larger than what can be hypothesised from a single study.
270 A prevalence and abundance plot is shown in Supplementary Figure 3. Bacterial genera in
271 table olives and their production environments belonged to 24 phyla, but the majority
272 belonged to *Bacillota, Pseudomonadota, Actinobacteriota*, and *Bacteroidota*. The proportion
273 of sequences removed by filtering varied significantly among samples: this was mostly due
274 to the poor quality of taxonomic assignment for some samples (data not shown). However,
275 75% of the samples retained more than 86% of the original sequences after filtering.
276 We realize that the very unequal sample number per study may have significantly affected
277 the results of filtering and the calculation of average prevalence and abundance: the
278 majority of samples were in fact Nyons olives and their production environments (Penland
279 et al., 2020, 2021), and Itrana olives (Giavalisco et al., 2023) (see Table 2). Given the nature
280 of our composite dataset, this is inevitable, and subsampling to an even number of samples
281 would greatly reduce the size of the data set and hide part of the observed diversity. Since
282 this analysis is, in its nature, repeatable, this problem will be mitigated as the number and
283 variety of samples for table olives in FoodMicrobionet increases. In fact, using the scripts

284 provided in the METAolive GitHub repository (https://github.com/ep142/Metaolive), adding
285 new data as they become available is straightforward.
286 Prevalence and abundance for different genera varied within different combinations of olive
287 ripeness and trade preparation (data not shown), but a set of 31 bacterial genera whose
288 relative abundance exceeded 0.01 in at least one sample and whose prevalence was >0.1 in
289 at least one olive group (Green Alkali treated, Green Natural, and Black Natural) may be
290 representative of the dominating core microbiota in table olives. These data are summarized
291 in Figure 2, and shown in detail in Supplementary Table 4 where minimum and mean relative
292 abundance are also shown.
293



294
295 **Figure 2.** Relative prevalence and maximum relative abundance of the dominating bacterial
296 genera in the Olive FMBN data set.
297

298 Core bacterial genera belong to two main groups: *Lactobacillales*, including Halophilic and
299 Alkalophilic Lactic Acid Bacteria (HALAB), and Gram-negative and Gram-positive halophiles
300 (*Enterobacterales*, *Pseudomonadales*, *Burkholderiales*, *Staphylococcales*).
301 Their distribution varied significantly in different olive varieties, among olives, brines and
302 contact surfaces or materials, and at different production stages (raw materials, olives during
303 fermentation, products at the end of fermentation). A bar plot showing the average
304 composition of bacterial communities for olives and brines of different varieties and

305 different trade preparations and at different stages of production is shown in Supplementary
306 Figure 4. The 31 most abundant and dominant genera covered more than 50% of the
307 bacterial communities for most, but not all varieties. Notable exceptions were Kalamata,
308 Manzanilla, Aloreña, Nocellara Etnea, Hojiblanca, and Picual in which some microorganisms
309 which were not prevalent in other varieties occurred at relatively high abundance. In
310 Kalamata olives from Cyprus a large number of sequences was assigned to species of the
311 genera *Streptococcus* and *Lactobacillus* (Kamilari et al., 2023), which are more frequently
312 found in dairy products rather than in table olives. In Kalamata olives from Greece stored
313 under modified atmosphere (Michailidou et al., 2021) a high proportion of Alpha- and
314 Gammaproteobacteria was found at time 0, followed by an increase in the proportion of
315 *Lentilactobacillus parafarraginis,* which was the most abundant species from 6 months
316 onward. Further studies on this widespread variety, whose sequences were not available at
317 the time FoodMicrobionet 5.0 was assembled, have shown that the composition of the
318 microbiota may differ between brines and olives, and among products from different
319 geographic areas firms (Kazou et al., 2020). *Celerinatantimonas, Lactiplantibacillus,*
320 *Propionibacterium, Staphylococcus, Sediminibacterium, Leuconostoc, Sphingomonas,* and
321 *Enterobacter* were found among the most abundant core genera by Kazou et al. (2020).
322 In Green Spanish style Manzanilla olives, a high proportion of *Alkalibacterium* was found.
323 This genus was found in these olives in several studies (Arroyo-López et al., 2021; Benítez-
324 Cabello et al., 2019; Ruiz-Barba et al., 2023a). In Nocellara Etnea green alkali treated olives
325 (Cocolin et al., 2013a) members of the genus *Kosakonia, Citrobacter,* and more occasionally
326 *Pantoea* and *Cobetia*, were also abundant (rel. abundance >0.10). In Aloreña de Malaga
327 green natural olives some Actinobacteria (*Cutibacterium, Modestobacter*,
328 *Pseudokineococcus*) were occasionally found with relative abundances >0.2 (López-García et
329 al., 2021; Medina et al., 2018). In Picual olives from Cyprus (Anagnostopoulos et al., 2020;
330 Kamilari et al., 2023), in addition to core genera (*Lactiplantibacillus*, *Secundilactobacillus,*
331 *Pediococcus* were all relatively abundant), *Sediminibacterium,* a relatively prevalent (0.13
332 average prevalence) genus, was abundant (>0.3 rel. abundance in some samples, compared
333 to 0.006 in the whole data set).
334 In general, we found that the composition of microbial communities detected by our
335 pipelines using raw sequences were in good agreement with those of the original studies.
336 Differences due to pipelines and taxonomic reference databases can be expected (Bokulich

et al., 2020; Pollock et al., 2018) and DADA2 has systematically performed well in benchmarking studies (Callahan et al., 2016). The use of SILVA v138.2 allowed assignment of Amplicon Sequence Variants belonging to family *Lactobacillaceae* to the most recent taxonomic units within this family (Zheng et al., 2023) thus updating the taxonomic assignment available in older studies.

The lack of clear differences among different groups defined by combinations of ripeness and olive trade preparation is confirmed by the non-monotonic Multidimensional Scaling ordination of the Bray-Curtis distance matrix shown in Supplementary Figure 5. Rather, groups of samples within the same study (see samples for Nocellara Etnea, Itrana, Ascolana Tenera and Nyons olives for studies ST14, (Cocolin et al., 2013b); ST253, (Giavalisco et al., 2023); ST239-240, (Penland et al., 2020, 2021); and ST229, (Maoloni et al., 2022) are generally close among them. Non-monotonic Multidimensinal Scaling is an ordination technique which seeks to represent proximities in a multidimensional space in a space with typically 2 or 3 dimensions (Borg et al., 2018) while keeping the dissimilarities/distances in the new space as close to the original as possible. Therefore, sample points which lie close together in the MDS map have a similar bacterial community structure. The configuration shown in Supplementary Figure 5 confirms the results obtained from alpha-diversity analysis (see Supplementary Figure 4): selective factors which may be associated to olive ripeness (sugar concentration, polyphenol concentration, etc.) or trade preparation (alkali treatment followed by fermentation in Spanish style olives compared to fermentation alone in naturally fermented olives) are apparently not powerful enough to result in clearly separated microbial communities or to overcome confounding factors related to individual producers (differences in initial contamination, fermentation conditions, including salt concentration, temperature and atmosphere composition, etc.). Additionally, studies with several producers for the same variety are very rare and more data are needed to clarify this issue.

Addition of starter cultures affects the composition of the microbiota, and, as a consequence, the sample position in the ordination to a variable extent: differences are larger in some study (ST253; Itrana olives) than in others (ST229, Ascolana Tenera, (Maoloni et al., 2022); ST133, Picual, (Anagnostopoulos et al., 2020) possibly reflecting different abilities of the starters in colonizing fruit and brines and in affecting directly and indirectly other members of the microbiota. Maoloni et al. (2022) used a four-species LAB starter which only caused small changes in the composition of the microbiota, while a commercial

Lactipl. plantarum starter did dominate the microbial community of cracked and, to a lesser extent, whole Picual olives, especially at the species level. The effect was also indirect: Anagnostopoulos et al. (2020) found that *Lactipl. plantarum* addition apparently promoted the growth of *Marinilactobacillus.* We were unable to replicate this finding but we did confirm that *Pediococcus* abundance was lower in started fermentations. In our work (Giavalisco et al., 2023) on Itrana olives, the addition of a *Lactipl. pentosus* starter clearly reduced the relative abundance of *Leuconostoc* and *Pseudomonadota* compared to unstarted fermentations, at least at later fermentation stages.

Spoilage does also often cause notable changes in the composition of the microbiota, as shown in the ordination of Supplementary Figure 6. Spoiled samples of Nocellara Etnea and Nyons olives clearly stand apart from unspoiled samples in the same varieties/studies.

The variability in the relative abundance and distribution of LAB and HALAB in table olives is shown in Figure 3. In this and in the other boxplots samples in which a given genus was absent are shown at the bottom of the graph. The significance of 0s in metataxonomic data has always been an issue: they could reflect lack of sensitivity (due to shallow sampling) or real absence (Lutz et al., 2022). Due to differences in sequencing depth in our dataset (we did not to apply any rarefaction; McMurdie and Holmes, 2014) we decided not to replace 0s with dummy values. As a consequence, the quantiles reflect the abundance of a given genus solely in the sample in which it was found, while its prevalence is shown in Supplementary Table 4.

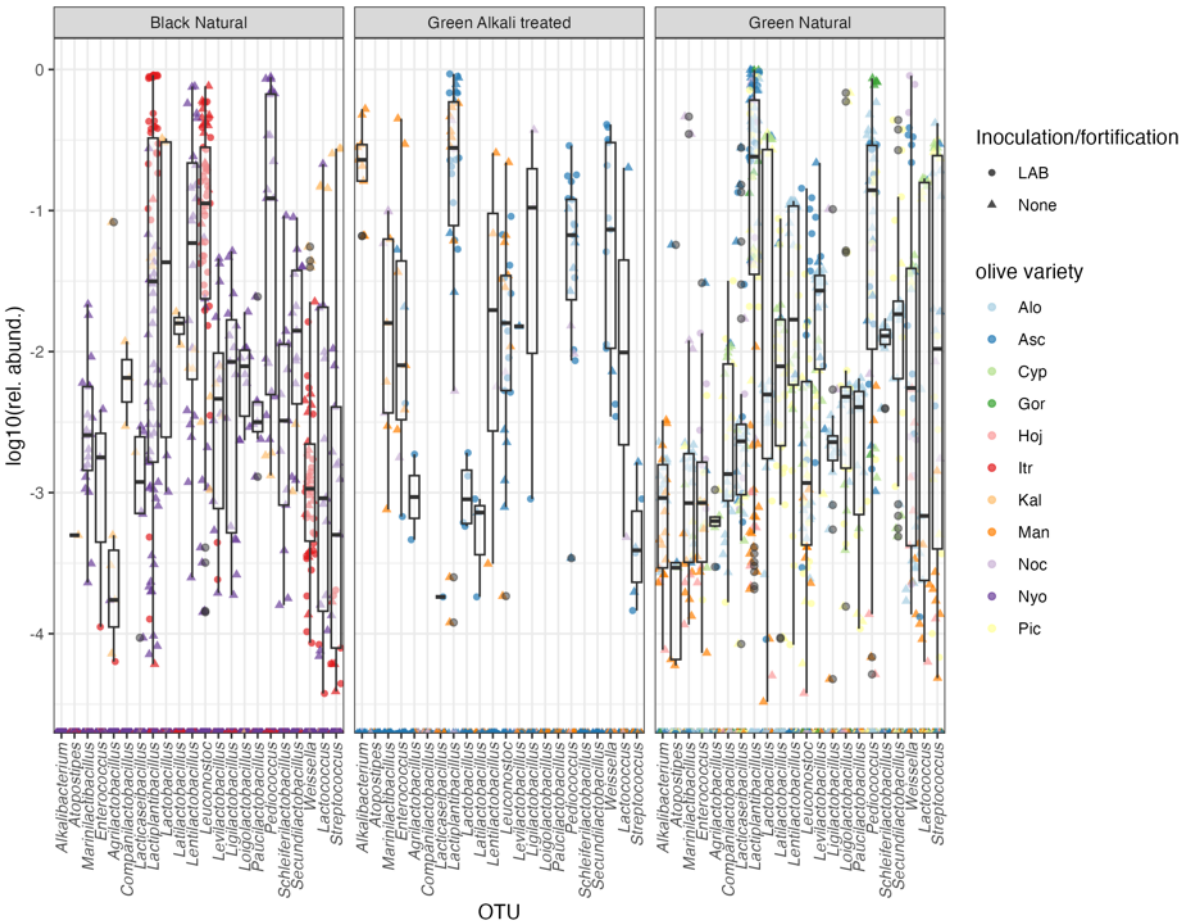


Figure 3. Distribution of the relative abundance of Lactic Acid Bacteria (including Halophilic and Alkalophilic LAB) in table olives. See Supplementary Table 3 for the abbreviations of table olives varieties

Homofermentative genera, with *Lactiplantibacillus* as the most prevalent and abundant genus, followed by members of the genera *Lacticaseibacillus, Pediococcus* and *Latilactobacillus,* are frequently found by amplicon targeted metagenomics in several varieties of olives (Portilha-Cunha et al., 2020; Tsoungos et al., 2023), and they are sometimes used as starters (Giavalisco et al., 2024). Other minor homofermentative genera (*Schleiferilactobacillus*, *Loigolactobacillus*) are far less prevalent but can sometimes be subdominant. Some heterofermentative genera (*Leuconostoc*, *Lentilactobacillus, Levilactobacillus, Ligilactobacillus, Secundilactobacillus, Weissella)* frequently occur at relative abundances >0.01 and can occasionally be abundant in table olives: their role is unclear (Portilha-Cunha et al., 2020; Tsoungos et al., 2023), and *Lentilactobacillus* can be involved in spoilage *(Lent. parafarraginis* and *Lent. buchneri* have been found in brines of spoiled Nyons olives; Penland et al., 2021) or produce biogenic amines. Enterococci, streptococci and lactococci are occasionally found as abundant members of microbiota in

some table olive samples, but it is unclear if some of them are contaminating sequences. While *Lactococcus lactis* is in fact commonly isolated from vegetable sources (Cavanagh et al., 2015), *Streptococcus thermophilus* has low salt tolerance and is pre-eminently associated to dairy products (Iyer et al., 2010). Other genera (homofermentative *Atopostipes*, *Companilactobacillus*; heterofermentative *Paucilactobacillus*) are usually below 0.01. HALAB (*Alkalibacterium*, *Marinilactobacillus*, but also *Halolactobacillus*) are characteristically abundant in green, alkali treated olives (Lucena-Padrós and Ruiz-Barba, 2019, 2016; Portilha-Cunha et al., 2020). *Alkalibacterium* have been found to be abundant in spoiled olive samples (Arroyo-López et al., 2021)

The distribution of the abundance of other halophilic genera is shown in Figure 4.

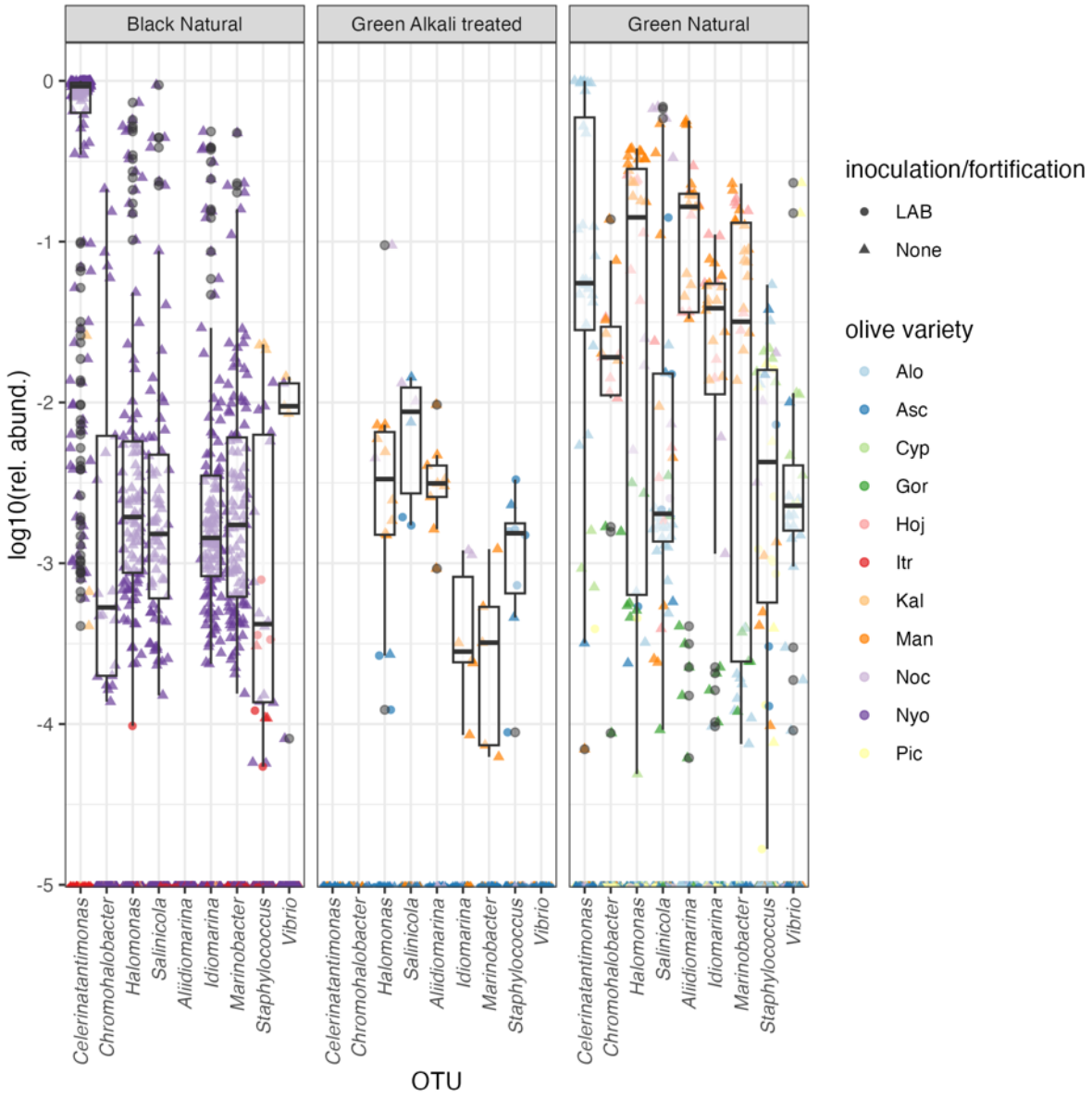


Figure 4. Distribution of the relative abundance of halophilic genera (other than HALAB) in table olives.

A number of halophilic *Pseudomonadota* are found in table olives, sometimes with high abundance and prevalence. The most notable genus is *Celerinatantimonas* which has been found to be abundant in Nyons black olives (Penland et al., 2021, 2020) where they are not associated with spoilage, and is also often found at relatively high abundances in green naturally fermented olives (Benítez-Cabello et al., 2020b; López-García et al., 2021; Medina et al., 2016; Rodríguez-Gómez et al., 2017). *Celerinatantimonas diazotrophica* has been specifically associated with the spoilage of Manzanilla and Gordal olives (de Castro et al., 2022; Ruiz-Barba et al., 2022). Most of the other species are associated with salt and brines. *Vibrio* have been isolated from table olives (Lucena-Padrós et al., 2015) but several *Vibrio* species are apparently unable to grow in olive fermentation brines, possibly because of inhibition by phenolic compounds (Posada-Izquierdo et al., 2021). However, they are found among the dominant members of the microbiota of Hojiblanca green Spanish style olives and might be favored by alkaline conditions with high salt; their presence in brine is not desirable (Correa-Galeote et al., 2022).

Other halophilic *Pseudomonadota* (*Alidiiomarina*, *Halomonas*, *Idiomarina*, *Marinomonas*, and *Salinicola*) are also abundant in green natural olives and in some black natural olives (Nyons olives), but less so in green alkali treated olives (Cocolin et al., 2013a; Randazzo et al., 2017; Ruiz-Barba et al., 2023a, 2023b). Fresh brines are a likely source of some of these species (Penland et al., 2021).

Because of low pH, relatively high NaCl and presence of polyphenols, table olives are a safe food (with the possible exception of olives darkened by oxidation, which need to be submitted to a botulinum cook (Campus et al., 2018)) and, to our knowledge, they have never been implicated in a food borne outbreak. Short reads used in amplicon targeted metagenomics seldom allow the resolution of closely related species and, in our study, we choose to agglomerate data at the genus level. A boxplot showing the distribution of the abundance of some genera which include human pathogens is shown in Supplementary Figure 7. Sequences assigned to *Brucella* and *Campylobacter* were only occasionally found and only at very low levels (relative abundance <0.001). On the other hand, sequences assigned to the genera *Salmonella* and *Yersinia* were found in Itrana, Ascolana Tenera,

Aloreña and Hojiblanca, sometimes with relative abundances >0.01. *Listeria* was never found. The significance of these findings is unclear. First of all, there is no way of ascertaining if these sequences come from live microorganisms. Secondly, almost none of the studies in our dataset included information which could have allowed the removal of contaminating sequences (blanks, DNA measurements, mock communities) using, for example, package decontam (Davis et al., 2018). The samples of Ascolana tenera were produced with addition of herbs and spices (Maoloni et al., 2022), which may be a source of undesirable microorganisms, but the potential source of potential pathogens in other samples is unclear.

3.2 The fungal microbiota of table olives

Data on fungal communities for table olives are less available in public sequence repositories compared to those on bacteria. This is rather surprising, considering the importance of yeast in table olive quality and spoilage (Arroyo-López et al., 2012; Benítez-Cabello et al., 2020a; Ciafardini and Zullo, 2019; Tsoungos et al., 2023; Tufariello et al., 2019). They are available in FoodMicrobionet for 8 studies (Supplementary Table 5) from 6 countries (Cyprus, France, Greece, Italy, Perú, and Spain; Supplementary Figure 8) with a total of 453 samples (including fruits, brine, fruit+brine, raw materials and contact surfaces). Although in most cases samples were collected before or during the fermentation, in a single study packaged commercial samples from a large variety of sources was used (Benítez-Cabello et al., 2020b).

The number of samples per study was highly unbalanced (12-208, median 37.5). As for bacterial communities, most were contributed by two studies, one on black natural Nyons olives (Penland et al., 2020, 2021) and one on Itrana olives (Giavalisco et al., 2023). Samples were available for fruits and/or brine, or contact surface and raw materials. After removing samples with less than 1,000 sequences, 390 samples were left for analysis (Supplementary Table 6), which makes this the largest and more diverse combined dataset on the fungal microbiota of olives.

As for bacterial microbiota, alpha diversity indices were calculated before taxa filtering. Chao1 ranged from 2 to 100. The variability of Chao1 as a function of olive variety, stage of fermentation, ripeness, trade preparation, sample type is shown in Figure 5.

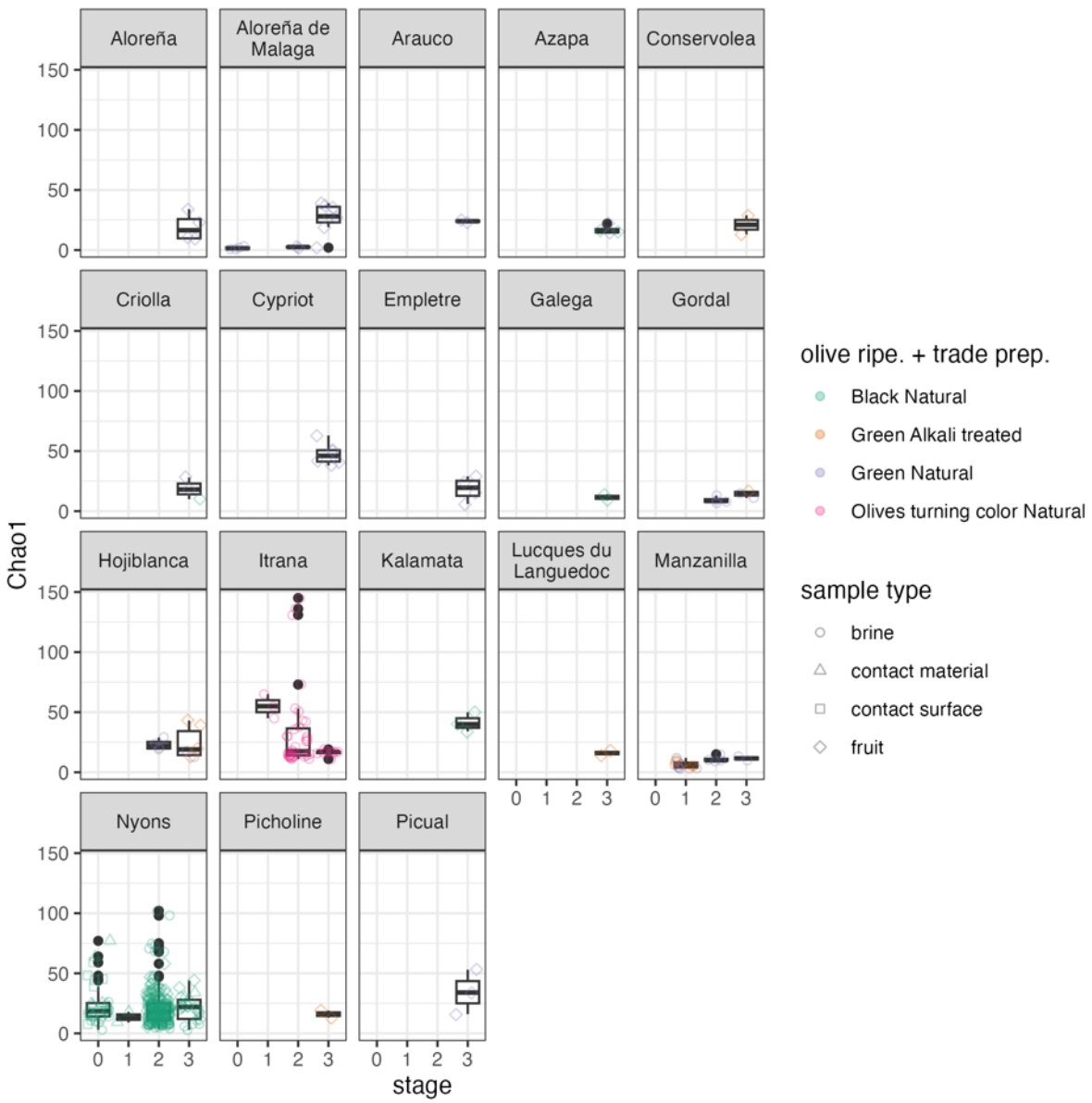


Figure 5. Distribution of Chao1 for fungal communities from studies on table olives (Table 2) by olive variety, fermentation stage (1 raw materials, 2 samples during fermentation, 3 samples at the end of fermentation, 0 other, including environmental samples), olive ripeness and trade preparation, sample type.

For black natural olives of the Itrana variety (Giavalisco et al., 2023) there was a clear trend for decrease of diversity from raw olives to the end of fermentation (Supplementary Figure 9), but this is almost entirely due to the higher diversity in raw, uninoculated olives and before day 25, while no significant differences were found between started and uninoculated brines (data not shown).

However, there is no evidence of a similar pattern for the few other studies for which time-course data are available (ST182, ST236, ST239), even when duration of fermentation was

very long (ST239). Therefore, with data currently available we cannot conclude that fungal diversity in table olives will always decrease over time.

Taxonomic filtering and agglomeration at the genus level was performed as described for bacteria. Even if our pipeline allowed taxonomic assignment at the species level, given the differences in targets used in this study and the potential biases in taxonomic assignment related to different regions of ITS used for metataxonomic analysis (Hakimzadeh et al., 2023; Kauserud, 2023; Rué et al., 2023) the use of genus level assignment may be more prudent when comparing several studies. As a result of taxonomic filtering and agglomeration, the number of taxa was reduced from 535 to 140, and the proportion of sequences retained was 99.5%. A prevalence and abundance plot is shown in Supplementary Figure 10. Most of the genera passing the filters belonged to the phyla Ascomycota and Basidiomycota.

Prevalence and abundance for different genera varied within different combinations of olive ripeness and trade preparation (this is evident from Supplementary Figures 11-14), but a set of 31 fungi genera whose relative abundance exceeded 0.01 in at least one sample and whose prevalence was >0.1 in at least one olive group (Green Alkali treated, Green Natural, and Black natural) may be representative of the dominating core fungal microbiota in table olives (Figure 6, Supplementary Table 7).

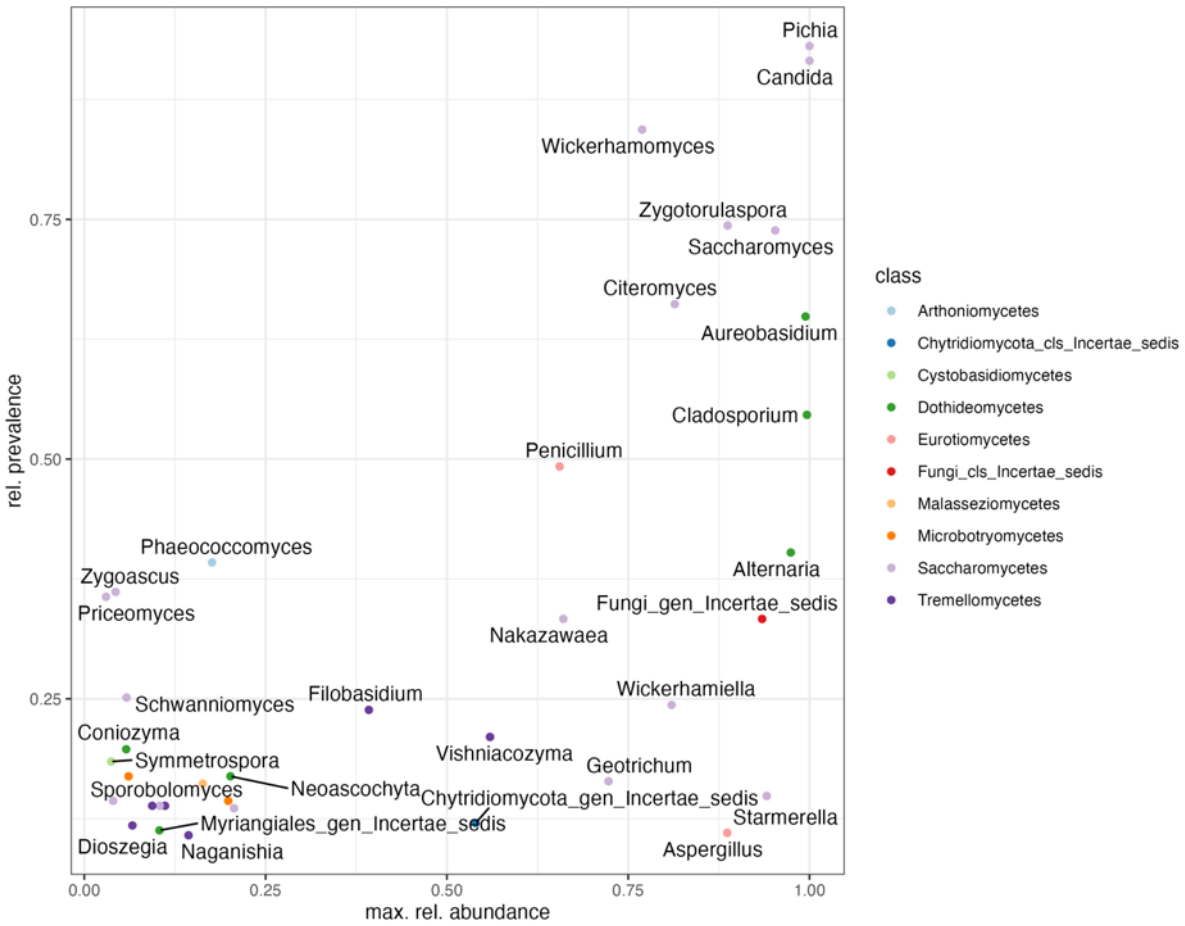


Figure 6. Relative prevalence and max relative abundance of the dominating fungal in the Olive FMBN data set

Given the high number of olive varieties it is impossible to show them in a single plot. The distribution of the core fungal genera is shown in Figure 7, while data on individual varieties are shown in the Supplementary Figures 11-13.

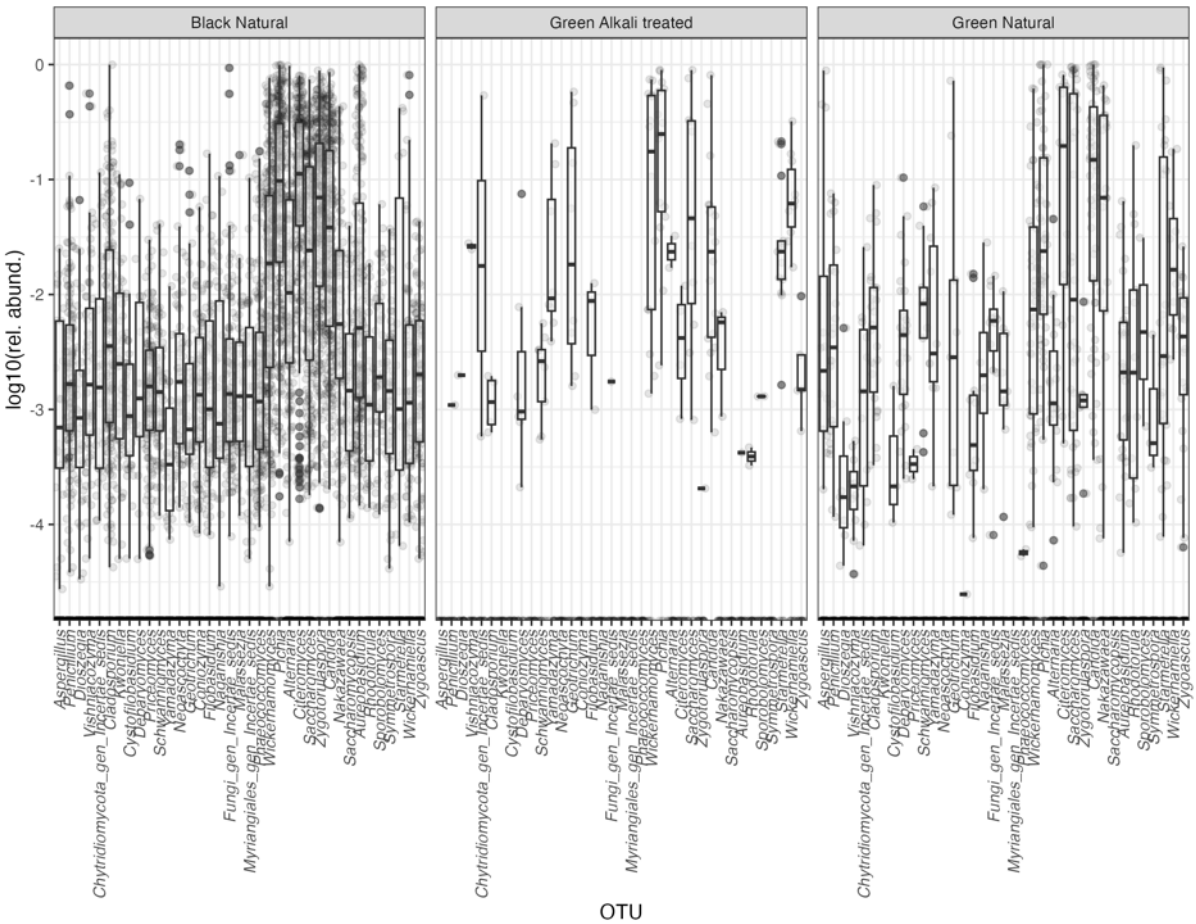


Figure 7. Distribution of the dominating fungal genera in the different types of table olive processing.

The distribution of abundance of fungi in table olives and their brines and food production environments was highly variable among olive groups and varieties (Supplementary Figure 14). The largest diversity was found in black natural olives, in which all core genera were found (although only *Pichia* and *Cyteromyces* had a median relative abundance >0.1). The variety of core genera found in green natural and green alkali treated olives was lower, and the most abundant genera were different (*Pichia* and *Wickerhamomyces* in green alkali treated olives, and *Pichia*, *Cyteromyces,* and *Candida* in green natural olives).

For the varieties for which samples at different time points were available (Itrana, Nyons, Hojiblanca, Manzanilla) a clear evolution of the composition of the microbiota over time is evident. The abundance of *Pichia* tended to increase over time for Nyons olives. Although this genus was also among the most abundant for the other three varieties at the end of fermentation, it was not the most abundant (*Candida* dominated in Itrana olives and *Saccharomyces* in Hojiblanca and Manzanilla).

541 The most abundant and prevalent fungal genera included members of the genus *Candida*
542 (species level taxonomic assignment included *C. boidinii*, the species with the highest
543 average relative abundance and prevalence*, C. diddensiae,* and *C. spencermartinsiae*), *Pichia*
544 (with the two closely related species *P. membranifaciens* and *P. manshurica*) and
545 *Wickerhamomyces* (*W. anomalus*). These species have been regularly found in culture
546 dependent and independent studies on the fungal communities of olives, both during
547 fermentation and in commercial packages (Anagnostopoulos and Tsaltas, 2022; Arroyo-
548 López et al., 2012; Tsoungos et al., 2023). Members of the genus *Saccharomyces* (*S.*
549 *cerevisiae*) are also prevalent and may be abundant in some varieties (Nyons, Penland et al.,
550 2021, 2020; Itrana, Giavalisco et al., 2023). They have been frequently reported as
551 dominating and subdominating members of the fungal microbiota of table olives (Arroyo-
552 López et al., 2016; Kamilari et al., 2023; Kazou et al., 2020; Ruiz-Barba et al., 2023a, 2023b;
553 Tsoungos et al., 2023; Tzamourani et al., 2022).
554 *Cyteromyces* (*Cyt. nyonsensis*) was found in both black and green natural olives with
555 relatively high abundance (see below). It was first described in Nyons olives (Casaregola et
556 al., 2013), but is also found in Taggiasca olives (Traina et al., 2024) and in black dry salted
557 olives (Gounari et al., 2023).
558 Other yeast genera which have been commonly associated to table olives and olive brines
559 (Arroyo-López et al., 2012; Tsoungos et al., 2023) like *Dekkera, Nakazawea, Zygotorulaspora,*
560 *Wickerhamiella, Starmerella, Schwanniomyces*, are much less abundant and prevalent.
561 Other like *Dioszegia*, *Kwoniella* and *Vishniacozyma* are found occasionally and at lower
562 abundances and may be associated to the phylloplane rather than to the fermentation
563 (Ferluga et al., 2024; Wang et al., 2008; Zhu et al., 2021) (see also the Omnicrobe database
564 https://omnicrobe.migale.inrae.fr/index).
565 Yeast-like fungi (*Aureobasidium*) and molds (*Penicillium, Cladosporium* and *Aspergillus*) may
566 have the same origin, although they are also associated with spoilage (Arroyo-López et al.,
567 2016; Sánchez et al., 2022).
568 Overall, these figures confirm that there is very little in terms of common patterns of the
569 composition of fungal microbiota among different combinations of olive ripeness and olive
570 trade preparation. Although some genera (*Pichia, Candida, Wickerhamomyces* and
571 *Saccharomyces*) tend to be more abundant and prevalent than others in almost all varieties,
572 their abundance can be highly variable among different varieties in the same group and, for

573 the few cases in which data are available, large changes are observed between the earliest
574 stages of fermentation (raw olives, olives at the beginning of fermentation) and intermediate
575 stages. The latter tend to be similar to late stages. In addition, in the few cases in which data
576 for both fruits and brine are available, differences existed between the two niches.
577 This is confirmed by the ordination plots shown in Supplementary Figures 15 and 16. For
578 individual studies, a clear separation of samples can be found, and this can be related to
579 factors for a given study. For example, evolution over time is an important factor for Itrana,
580 Nyons, Hojiblanca and Manzanilla, see above; differences among unfermented olive fruits,
581 contact surfaces and materialas and fermented brines or olives for Nyons; spoilage for Nyons
582 olives.

583 **4. Conclusions**

584 In this paper, we have used in a transparent and reproducible manner, public metataxonomic
585 data sets on the composition and dynamics of microbial communities in table olives to
586 obtain a comprehensive view of the bacterial and fungal core microbiota of table olives.
587 Even if the microbiota of table olives has been thoroughly reviewed in recent years
588 (Anagnostopoulos and Tsaltas, 2022; Campus et al., 2018; Corsetti et al., 2012; Perpetuini et
589 al., 2020; Portilha-Cunha et al., 2020; Tsoungos et al., 2023), the quantitative insights
590 provided in this paper significantly advance our knowledge of the main microbiota of
591 different trade preparations of table olives.
592 On the other hand, the data we have collected point to some limitations of the experimental
593 approaches used so far and future research needs.
594 Table olive fermentation is, in most cases, an artisanal process. This may explain the
595 variability of the composition of the microbiota even within the same olive variety, which
596 may be controlled, in part, by modern technologies for decontamination of the fruits,
597 improved control of the fermentation process and starter addition (Campus et al., 2018).
598 Part of the variability observed in our study may be due to the diversity of type of samples
599 (brines, whole olives, homogenized olives, combinations of olives and brines), to differences
600 in DNA extraction and purification procedures and other differences in wet laboratory
601 approaches and sequencing platform (here, a common analysis pipeline was used for the
602 analysis of raw sequences). It is well known that the microbiota of the surface of the olives
603 and of the brines may differ (López-García et al., 2022) and it would be highly desirable if the

604 scientific community working on table olives would agree on common protocols and
605 sampling approaches.
606 Moreover, most of the metataxonomic studies available in the literature (Table 1) are small
607 and narrowly focused. We feel that larger and more structured studies are needed to
608 identify more reliably the microbiota associated with the fermentation of individual varieties
609 and to relate its composition and dynamics to contamination and dispersal and to selective
610 forces operating during the fermentation.
611 More and more diverse data would also be needed for the development of machine-
612 learning approaches for the identification of specific subsets of the microbiota which are
613 reliably associated to specific varieties or to distinguish between PDO and non-PDO
614 processes, or to identify the microbial spoilage associations (Arroyo-López et al., 2021;
615 Montaño et al., 1992; Penland et al., 2021).
616 Use of LAB and yeast starters does indeed improve the quality of table olives, as pointed out
617 in several recent reviews (Anagnostopoulos and Tsaltas, 2022; Campus et al., 2018;
618 Giavalisco et al., 2024). The starter cultures tested vary in their composition (Giavalisco et al.
619 2024): some are very simple (a single strain of a single species) and their development has
620 been empirical. The rational design of complex, microbiome-based starters which mimic the
621 composition and microbial interactions in the original microbial community requires both
622 the knowledge of the composition and dynamics of microbial communities and the ability to
623 track the survival and growth of individual strains during fermentation (Ferrocino et al.,
624 2022; Gänzle et al., 2023). Our study does provide a more comprehensive view of the
625 potentially beneficial genera which dominate different olive fermentations. On the other
626 hand, amplicon targeted approaches have a limited ability to evaluate the impact of starter
627 culture addition (see Supplementary Figures 5 and 15), because of limitations in taxonomic
628 resolution. When cultivation based, strain typing approaches are used (Martorana et al.,
629 2015) population dynamics are evident. Long read, 3rd generation sequencing approaches
630 can greatly improve the taxonomic resolution of metataxonomic studies (Srinivas et al.,
631 2025). One recent, small study, has been published on the use of Oxford Nanopore
632 Technology for the study of the microbiota of Kalamata black olives (Vougiouklaki et al.,
633 2024). More data are needed to understand if the cost/benefit ratio of using this approach is
634 favourable.

Evaluation of the microbial diversity of table olive fermentations is extremely important to understand strain level dynamics and the role of selection and drift in shaping, over time, the metagenome of table olive fermentations. Amplicon targeted approaches do not have enough resolution for this purpose. The number of shotgun metagenomic studies on food microbiota has been rapidly increasing and large databases are being created to allow the exploitation of this wealth of information (Carlino et al., 2024). To our knowledge, a single study reporting shotgun metagenomic data on table olives exists (Soto-Giron et al., 2021) and more data are clearly needed. Finally, comparative metagenomic studies on the main actors of table olive fermentations, such as *Lactipl. pentosus* (Page et al., 2023) would be extremely useful in analysing the patterns of domestication of these species and understanding the relationships, if any, between selective patterns associated with specific olive fermentation processes and the dominance of individual strains.

Another major issue is the detection of active fractions of the microbial community. To our knowledge, a single study used both rRNA and the 16S RNA gene as a target (Cocolin et al., 2013b), and concluded that the composition of the bacterial communities detected with both methods was similar, with the exception of one fruit sample. It is impossible to say if this is the case for all fermentations and it is well known in other food fermentations that the structure of active and inactive microbial communities may differ (Erkus et al., 2016, 2013).

Even with all these limitations in mind we feel that our work provides a significant contribution to the understanding of table olive microbiology. Our repository is publicly available (https://doi.org/10.5281/zenodo.13729346), as are the data and software used to generate it. Our data, software and analyses are therefore reproducible and reusable, and we feel they conform to the principles of FAIR (Findable, Accessible, Interoperable, Reproducible) paradigm of open science (Wilkinson, 2016). The availability of large and well annotated repositories is key to the development of machine-learning approaches for pattern recognition (Abdil et al., 2025; Kumar et al, 2024) and may contribute, as more data become available, to knowledge and applications in the table olives production.

Acknowledgements.

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