**Title:** Artisanal and farmers bread-making practices differently shape fungal species community composition in French sourdoughs

**Running title:** Bread-making practices as a driver of yeast species community composition

**Authors**

Elisa Michel[†,°], Estelle Masson[‡], Sandrine Bubbendorf[‡], Léocadie Lapicque[‡], Thibault Nidelet[†], Diego Segond[†], Stéphane Guézenec[†], Thérèse Marlin[†], Hugo Devillers[†], Olivier Rué[¶], Bernard Onno[°], Judith Legrand[§*]*, Delphine Sicard[†] *and the participating bakers[#]

**Authors affiliation**

[†] SPO, Univ Montpellier, INRAE, Institut Agro, Montpellier, France

[°] Oniris, Laboratoire MicrobioTech, UMR GEPEA 6144, Rue de la Géraudière CS 82225, 44322 Nantes Cedex 3, France

[‡]Laboratoire de Psychologie : Cognition, Comportement, Communication – EA 1285, Université de Bretagne Occidentale, 20 rue Duquesne, CS 93837, F-29238 Brest 03, France

[§]Génétique Quantitative et Evolution-Le Moulon, Université Paris-Sud, INRAE, CNRS, AgroParisTech, Université Paris-Saclay, 91190 Gif-sur-Yvette, France

[¶]Université Paris-Saclay, INRAE, MaIAGE, 78350, Jouy-en-Josas, France

[¶]Université Paris-Saclay, INRAE, BioinfOmics, MIGALE bioinformatics facility, 78350, Jouy-en-Josas, France

*Judith Legrand and Delphine Sicard should be considered both last authors.

**Corresponding author**

Delphine Sicard, SPO, Univ Montpellier, INRAE, Institut Agro, Montpellier, France +33 624720501

delphine.sicard@inrae.fr

**Key words:** domestication, yeast, *Kazachstania*, *Saccharomyces cerevisiae*, fermentation

No competing interests

Financial supports: French National Research Agency (ANR-13-ALID-0005) and INRAE

**Abstract**

Preserving microbial diversity in food systems is one of the many challenges to be met to achieve food security and quality. Although industrialization led to the selection and spread of specific fermenting microbial strains, there are still ongoing artisanal processes that may allow the conservation of a wider diversity of microbial species. We examined whether the diversity of artisanal practices could lead to an increased level in fungal species diversity for bread making. We used an interdisciplinary participatory research approach including bakers, psycho-sociologists and microbiologists to analyze French bread-making practices and describe fungal communities in naturally fermented sourdough of 27 bakers and 12 farmer bakers. Bread-making practices were classified in two groups: the farmer-like practices group and the artisanal-like practices group. Surprisingly, the well-known bakery yeast, *Saccharomyces cerevisiae,* was dominant (i.e. with a relative abundance over 50%) in only 24% of sourdoughs while other yeast species of the closely related *Kazachstania* genus were dominant in 54% of sourdoughs. Bread-making practices were found to drive the distribution of yeast species. The most remarkable difference was the occurrence of *Kazachstania humilis* in sourdoughs made with artisanal-like practices and the occurrence of *Kazachstania bulderi* in sourdoughs made with farmer-like practices. Phenotyping of these two species in laboratory sourdough mimicking media revealed phenotypic divergence between sourdough and non-sourdough strains for *K. humilis* but not for *K. bulderi*. Overall, our results showed that preserving bread-making practices diversity allows the preservation of a higher taxonomic and phenotypic diversity in microbial communities.

# Introduction

Humans started to ferment food before the Neolithic using naturally fermenting microbial communities. In the 19th century, the industrialization and the increase of knowledge in microbiology resulted in changes in fermented food practices with the use of starters. This selection led to a reduction in species diversity and genetic diversity for fermented food processing and limited *in situ* conservation of microbial communities in industrialized systems [1–3]. Domestication of the yeast *Saccharomyces cerevisiae* for the production of beer, wine, cheese, leavened bread, that of the fungi *Penicillium roqueforti* or *Penicillium camemberti* for cheese production or that of the fungus *Aspergillus oryzae* for rice or soybean fermented products are well studied cases [1–10]. The recent renewed interest in artisanal practices that make use of naturally fermenting microbial communities could promote again the conservation of microbial diversity. However, the effect of artisanal practices on the distribution of microbial species remains poorly documented.

Among fermented foods, bread is still a symbol deeply engrained in the history, religious rites and medicine of several cultures. The origin of bread likely originated 14 000 years ago, suggesting that bread was made long before plant domestication [11]. Since the Neolithic, bread history is intimately combined with the evolution of cereals, bread-making associated tools and the advent of Mediterranean civilizations [12]. Leavened bread was traditionally made with flour, water and a fermenting agent, which was either a fermenting beverage or a fermenting dough, called sourdough. This sourdough was generally initiated from a mixture of flour and water, naturally colonized by lactic acid bacteria (LAB) and yeasts. Sourdough was then either maintained from one bread-making process to the other or initiated again and again, depending on the craftsman [13, 14]. In the 19th century, the use of yeast starters made of *S. cerevisiae,* often called « baker's yeast », spread as an alternative to sourdough. Nowadays, *S. cerevisiae* industrial starters are more frequently used than sourdoughs, although the latter are gaining interest. A recent study showed that industrial populations of *S. cerevisiae* have followed a different evolutionary path than sourdough populations of *S. cerevisiae* [10]. Both have been domesticated by humans and have improved their fermentation performance in a sourdough-mimicking

medium. However, industrial and sourdough strains of *S. cerevisiae* differ genetically and phenotypically, indicating that sourdough use contributes to the conservation of bread related *S. cerevisiae* lineages [10].

Beyond *S. cerevisiae*, sourdough can also host other yeast species. To date, more than 40 yeast species have been detected in sourdough [1, 15, 16]. The most frequently encountered species are *Wickerhamomyces anomalus* and *Kazachstania humilis*. Several other species in the genus *Kazachstania* (*Kazachstania barnettii*, *Kazachatania exigua*, *Kazachstania bulderi*, *Kazachstania unispora*) as well as several species in the polyphyletic genus Pichia have also been recurrently detected. The factors that determine the distribution of these species are still unknown. A recent large-scale study of 500 sourdoughs from four continents found no effect of geography or factors related to bread-making practices such as age of sourdough, storage location, feeding frequency, grain intake, house characteristics [17]. However, most of the sourdoughs in this study were made by private citizens who probably did not maintain the sourdough microbial community in the same way as a professional baker. To our knowledge, no studies have been conducted to date to investigate the effect of bakers' bread-making practices on sourdough yeast community composition.

In France, sourdough breads are made both by bakers and farmers who also grow and mill their own wheat. The number of farmers-bakers has increased in the 2000s with two motives: to grow wheat varieties meeting their needs and to assert their independence from industry [18]. Although farmer-bakers are less numerous than bakers, they participate in the renewed interest in local wheat varieties and artisanal know-how, which may contribute to the conservation of both socio-cultural diversity and microbial diversity.

Here, we used a participatory research approach involving psycho-sociologists, biologists, bio-statisticians, bakers and farmers-bakers to study whether and how bakers and farmers-bakers contribute to the preservation of socio-cultural and fungal species diversity in sourdough microbial community.

# Materials and methods

A total of 27 bakers and 12 farmer-bakers participated to the study. They were all making bread with organic flour except five. All of them sent sourdough to the lab for microbiological and metabolic analysis. Among them, 36 described their bread-making practices as well.

## A questionnaire survey, face-to-face interviews and focus-groups to collect bread-making practices

Data on bread-making practices were collected through a questionnaire survey, interviews and focus groups. The collected variables were related to *i)* the ingredients origin : wheat varieties types (ancient populations also called landraces / modern varieties), whether they produced flour from their own wheat, whether they had their own mill or use an external mill, water origin, *ii)* the sourdough recipe: its age, its hydration state, the origin of the chief sourdough (sample of dough or sourdough), the number of back-sloppings before bread making and per week, the temperature of water used for back-sloppings, *iii)* their bread-making practices: the number of bread makings per week, the percentage of sourdough, flour and salt in bread dough, the kneading methods, the total duration of fermentation and the addition of baker's yeast in dough.

## Sourdoughs samples, enumeration and strain isolation

Sourdoughs were collected before kneading and referred to as final sourdoughs (Table S1). On the day of collection, they were sent to the lab where yeast and bacteria were enumerated and isolated as in [19, 20], and sourdoughs stored at -20°C in sterile vials for non- culture based analysis. Ethics and rights associated with sourdough collection and strains isolation have been respected.

### Sourdough acidity and metabolic analyses

For each sourdough, three independent 1-g replicates were analyzed. pH and Total Titrable Acidity were measured as described in [20]. Organic acids, alcohol and sugars concentrations (expressed as g/kg of sourdough) were analyzed by liquid chromatography using an HPLC HP 1100 LC system (Agilent

technologies, Santa clara, CA, USA) equipped with a refractive index detector (RID Agilent G1382A) and a UV detector (Agilent G1314A). Two different columns were used, a Rezex ROA-organic acids column and a Rezex RPM-monosaccharide column (SDVB – Pb+2 8%, 300x7.8mm, Phenomenex, Torrance, CA, USA). The details of the experiments are described in supplementary information (Method S1).

**Yeast species identification**

The Internal transcribed spacer 1 (ITS1) ribosomal DNA of each 1216 yeast isolates was amplified by PCR from chromosomal DNA, either by using primers ITS1F and ITS2 [21, 22], or primers NSA3 and 58A2R [19, 22]. For isolates unidentified with the ITS1 region alone, DNA was extracted according to MasterPure Yeast DNA purification kit (Epicentre, Epibio). PCR reactions targeting partial genes, the D1D2 region of the large subunit of rRNA (LSU), a part of the RNA polymerase II large subunit encoding gene (*RPB1)*, a part of the RNA polymerase II encoding gene (*RPB2)*, a part of the actin encoding gene *Act1* and Transcriptional Elongation Factor TEF were performed. To discriminate three specific isolates, PCR on genes *GHD1*, *FSY1*, *URA3*, *DRC1*, *MET2* were performed [23–26] (Table S2). All PCR products were sent to be sequenced with Sanger sequencing (Eurofins, Germany). Species were identified using NCBI [27], YeastIP [28] and a personal database, which was constructed after *ITS1*, *RPB2*, LSU sequencing of all 33-yeast species reportedly found in sourdoughs in the literature [19].

**Sourdough DNA extraction, MiSeq sequencing, bioinformatics**

The ITS1 region was targeted with the PCR primers ITS1-F (5′- CTTGGTCATTTAGAGGAAGTAA - 3′) and ITS2 (5’-GCTGCGTTCTTCATCGATGC-3’).

The sequencing run was performed with MiSeq Reagent Kit v3. 2015 [20]. Sequences were analyzed through FROGS “Find Rapidly OTU with Galaxy Solution” [29] and home-made pipelines. Overlapped reads were merged with Flash [30] with a minimum overlap of 10 nucleotides, a maximum overlap of 300 nucleotides and a maximum mismatch density of 0.1. Primers were removed with Cutadapt [31] and data were cleaned with Sickle with quality-threshold and length-threshold equal to 20 [32]. Reads

were clustered with Swarm (d=3) [33] and chimeras deleted with VSEARCH [34]. Sequences were then filtered on minimum abundance of 0.005% of all sequences. From the OTU's abundance table and for each OTU, the taxonomic affiliation using UNITE Version 7.1, Release 2016-11-20 [35], YeastIP [28] and our own databases [19] was obtained by blasting OTUs representative sequences against each database.

**Phenotypic analysis of yeast strains**

Fermentation performance of the two most frequently encountered *Kazachstania* species were assessed as described in [10] for *S. cerevisiae*. Fifteen sourdough strains of *K. bulderi* and 16 sourdough strains of *K. humilis* were included in the analysis. *Kazachstania bulderi* strains were coming from sourdoughs B4, B12, B15, B17 B20, B21, while *K. humilis* strains were coming from sourdoughs B2, B5, B6, B7, B10, B17. From one to three strains per sourdough were analyzed in the experiment. In addition, four strains of *K. bulderi* (strain MUCL 38021 isolated from silage in Namur, Belgium, strain MUCL 54694 isolated from ensilage in Erezée, Belgium, strain NRRL Y-27205 and strain CLIB 604 isolated from maize silage in the Netherland) and three strains of *K. humilis* (strain CBS 7754 isolated from food dressing, strain CLIB 1323 isolated from bantu beer, and strain CBS 2664 isolated from alpechin) which were coming from non-sourdough habitats, were added as control to test the effect of habitat of origin. Each strain was phenotyped at least in triplicate leading to a total of 145 fermentations distributed over two blocks. Briefly, fermentations were carried out at 24 °C with constant magnetic stirring (300 rpm) during 24 h. $CO_2$ release was measured by weight loss every 40 min using an automated robotic system [36]. At the end of fermentation, population size and cell viability were determined by flow cytometer (C6 cytometer, Accuri, BD Biosciences) as described in [37].

**Data analyses**

To analyse bread-making practices, a multiple correspondence analysis (MCA) and hierarchical clustering (complete linkage clustering method) on principal components based on the first two axes of the MCA were performed using the FactoMineR R package [38].

To analyse fungal community, weighted Unifrac distances between sourdough communities were computed from a rooted phylogenetic tree based on the OTUs sequences using the R-packages Phyloseq and GUniFrac [39, 40]. Phylogenetic sequences were aligned with Clustalo and phylogenetic tree were built with the parsimony algorithm, with 100 replicates bootstraps, pairwise ktuple-distances with Seaview [41]. Different roots were tested (*Sporidiobolales sp.*, *Bullera globospora*, *Trichosporon asahii*, *Udeniomyces pyricola*). Tree architecture did not change with the root. It did not fit the expected phylogeny and, notably, some *Ascomycota* were misclassified among the *Basidiomycota*. However, the dominant sourdough species belonging to the *Saccharomycetaceae* family were clustered according to expected clades or subclades, except that *Kazachstania servazzi* and *Kazachstania unispora* were grouped in a clade closer to *Saccharomyces* species than to other *Kazachstania* species. Using the Unifrac distances matrix, we performed a Principal Coordinate Analysis (PCoA) and clustered sourdough communities using the first two axes of the PCoA, and the complete linkage clustering method (hclust R function). To check the sensitivity of our analysis to this misclassification, we performed the same analyses without the sourdoughs that had one misclassified species representing more than 10% of their reads, *i.e.* sourdoughs B20, B41, B42, and B44 and found the same clustering [40].

For each sourdough, the species richness, Chao1, Shannon and Simpson indexes were computed, and the Shannon and Simpson indices values were converted to the effective number of species per sourdough. This number was estimated from the Shannon diversity index as $exp^{Shannonindex}$ and from the Simpson diversity index as $\frac{1}{1-Simpsonindex}$ [42, 43]. For probability estimates, the exact 95% confidence intervals were computed using a binomial distribution.

To study the link between microbial species diversity and bakery practices, a univariate Permutational Multivariate Analysis (PERMANOVA) on the Unifrac distance matrix was performed for each bakery practice variable. We performed univariate analysis on the 30 bakers who had less than 8 missing values among the 29 bread-making practices variables and adjusted the p-value using FDR correction to account for multiple testing. In addition, independence exact Fisher tests between the variable providing fungal community PCoA groups and each of the bread-making practices variables were performed. Multiple testing was accounted for using the False Discovery Rate method [44].

The link between the baker practices group, the fungal community group or the yeast dominant species and the variation of each quantitative variable (microbial density, pH, TTA, metabolite concentration) was tested with the following mixed effect model: $Y_{ijk} = \mu + \alpha_i + B_j + \varepsilon_{ijk}$ with $\varepsilon_{ijk}$ ~N(0,$\sigma^2$), where $\alpha_i$ is the effect of the fungal community group $i$ modelled as a fixed effect and $B_j$is the effect of sourdough j modelled as a random effect and k represents the measurement replicates. For sourdough hydration rate, the variable was arcsin transformed but sourdough effect was not included in the model because no repetition was obtained from any sourdough. The model parameters were estimated using the lmertest R package [45]. To test the fixed effects, we used likelihood ratio tests. Multiple comparisons of means were performed using Tukey tests with the multcomp package. p-values were all adjusted for multiple testing with the FDR method. The geographical structuration was tested with a Mantel test on the Unifrac distances matrix and the geographical distances matrix computed with the package geosphere [46] and ade4 [47].

Finally, the phenotypic diversity of *K. bulderi* and *K. humilis* strains coming from sourdough and non-sourdough habitats were analyzed. Population size and mortality rate after 27h of fermentation measured by flow cytometer were used as proxy for absolute fitness. The cumulative $CO_2$ production curve was calculated and the kinetics of $CO_2$ production rate over time was estimated by successive linear smoothing over 5 points. Four fermentation parameters were then estimated: the maximum $CO_2$ release ($CO_2$max, in g/L) was estimated by the maximum of the cumulative $CO_2$ production curve, the fermentation latency-phase time (t1g, in h) was estimated by the time between inoculation and the

beginning of the fermentation calculated as 1g/L of $CO_2$ release, the maximum $CO_2$ production rate (Vmax in g/L/h) was estimated by the maximum of the $CO_2$ production rate kinetic and the time of the Vmax (tVmax in h) as the time between inoculation and the Vmax. Hence, the phenotype of each strain was characterized by six quantitative variables called "phenotype variables" below: its population size, its mortality rate and the four fermentation parameters. To determine whether the origin of the strain (sourdough or non-sourdough) had an impact on strain phenotype, each log-transformed quantitative variable was analyzed separately using a mixed linear model as described below. The experimental design was unbalanced between the two blocks with very few non-sourdough strains in one of the two blocks. Therefore, for each phenotype variable, we first estimated the block effect with a subset of 8 strains cultivated in both blocks using a linear model with two fixed effects : the strain and the block. Additive models were used as the interactions terms were not significantly different from zero after adjusting p-values with the Benjamini-Hochberg method. Second, each phenotype variable was corrected for the block effect and analyzed with the mixed effect model: $Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_{ij} + Z_k + \epsilon_{ijkl}$ where $Y_{ijkl}$ represents the log-transformed phenotype variable corrected for the block effect for the strain *k,* from species *i (i=1,2),* sampled in environment *j (j=1,2)*, observed for replicate *l*. $\mu$ represents the mean of the phenotype variable, $\alpha_i$ the additive effect of species *i*, $\beta_j$ the additive effect of environment *j,* and $\gamma_{ij}$ their interaction. $Z_k$ represents the gaussian random effect of strain *k* with $Z_k \sim N(0, \sigma_s^2)$ and $\epsilon_{ijkl}$ the gaussian residuals with $\epsilon_{ijkl} \sim N(0, \sigma^2)$. For each species *i*, the impact of the environment was quantified using the contrast $\Delta_i = \beta_S + \gamma_{i,S} - \beta_{NS} - \gamma_{i,NS}$ with NS standing for "non-sourdough" and "S" for '"sourdough" and tests ($H_0 = \Delta_i = 0, H_1 : \Delta_i \neq 0$) were performed and p-values were adjusted using the Benjamini-Hochberg method. As log-transformed data were analyzed, the exponential of this contrast can be interpreted as the ratio between the sourdough mean and the non-sourdough mean. Confidence intervals and tests were performed using the doBy R-package.

All statistics and plots (ggplot2 [48], leaflet package [49], with minor esthetical adjustment with Inkscape) have been done with R. Data and scripts are shared on Zenodo. http://doi.org/10.5281/zenodo.2600170

255

# 256 Results

## 257 Two groups of bread-making practices

258 A total of 39 French bakers producing natural sourdough bread and distributed all over France
259 participated to the study (Table S1). The bread-making practices of 35 of them were collected through
260 one or several methods: personal interviews (with 12 bakers), focus groups (3 groups), observation
261 during break-making workshops (2 workshops), and an online/phone survey (36 bakers). The general
262 process of sourdough bread making is presented in Figure 1. We analyzed 28 variables around this
263 general bread making process, describing variations of the practices at all steps of the bread making
264 process, from wheat grains to baked bread (Figure S1). Four bakers (B6, B10, B19, B20) who did not
265 provide enough information about their practices were excluded from the multivariate analysis.
266 According to a hierarchical clustering on principal components (HCPC), the 32 other bakers clustered
267 into two groups corresponding to two main types of bread-making practices (Figure 2). The first group,
268 hereafter termed “farmer-like” practices group, includes 6 bakers and 11 farmers-bakers using the
269 following practices: low bread production (<500 kg per week, 81% of the bakers of the “farmer-like”
270 group), use of ancient wheat populations (56%), manual kneading (63%), working at ambient
271 temperature (88%), long fermentation periods (more than 4 hours for 88%), and no use of commercial
272 baker’s yeast (88%). In addition, they tend to make their chief sourdough from dough after kneading
273 (75%). The second group, hereafter called “artisanal-like” practices group consists of 12 bakers and 4
274 farmer-bakers having more intensive practices, characterized by a large bread production (>500 kg per
275 week, 81%), mechanical kneading (100%), use of modern wheat varieties (63%), working at ambient
276 temperature (56%), using commercial yeast starters in addition to sourdough for bread making or using
277 commercial yeast starters for pastries and buns making (81%). In this second group, bakers tend to make
278 their chief sourdough from a final sourdough.

## Composition of sourdough fungal communities

Sourdough is a mix of flour and water naturally fermented by bacteria and yeasts. Sourdough yeast density ranged from 8.1 $10^4$ to 5.8 $10^8$ CFU per gram of sourdough, with a mean value of 2.9 $10^7$ CFU per gram, as commonly found in sourdoughs from all over the world [1, 16, 50, 51]. We isolated 20 to 40 yeast strains from each sourdough by picking colonies randomly and identified species using ITS sequence as well as other barcodes when needed (see M&M section). Among the 39 collected sourdoughs, one (sourdough B14) did not give any colony in the laboratory, suggesting that his sourdough microbiota was no longer alive. A total of 1216 strains were characterized from the other 38 sourdoughs. In addition, we developed an ITS1 meta-barcoding MiSeq sequencing method on sourdough (see sup M&M). After filtering 5,360,620 raw ITS1 sequences for quality, abundance (0.005%) and chimera, 3,542,801 sequences were further analyzed. Overall, the sequences clustered in 113 OTUs. The number of reads per sourdough ranged from 8421 to 194 557. Therefore, we carried out our analysis on the rarefied matrix. Among all OTUs, 64 were identified as non-yeast, including 10 assigned to the order *Triticodae* (especially to the species *Triticum aestivum*), 50 assigned to assigned to genera that include plant pathogen species together with saprobic ones, such as *Alternaria*, *Aspergillus* or *Fusarium*, *Gibberella*, while 4 OTUs remained unidentified. Among the 40 yeast or yeast-like OTUs, 96% of total reads were assigned to the phylum *Ascomycota*, 87.5% to the order *Saccharomycetales* and 85.7% to the family *Saccharomycetaceae*. Only 4% of the total reads were assigned to the phylum *Basidiomycota*. Overall, three OTUs assigned to the species *Kazachstania humilis*, *Kazachstania bulderi* and *Saccharomyces cerevisiae* represented 20.3%, 15.5% and 24.1% respectively of the total number of reads and 28.1%, 23.7% and 18.2% respectively of the number of reads identified as yeast species (Figure 3).

Both non-culture-based and culture-based methods allowed the identification of the same dominant species (defined as a species with an over 50% frequency) for all sourdoughs but five (B09, B20, B22, B25, B41) (Figure 3). In two cases, the discrepancy was explained by the detection of *Cladosporium* sp. at high frequency with metabarcoding while this species could not be isolated in the laboratory (Figure

3). In two other cases, it was explained by a high number of *S. cerevisiae* isolated in the laboratory compared to what was observed using metabarcoding sequencing. Finally, in the last case, the identification of *Pichia kudriavzevii* required additional sequencing as it shares an identical ITS with *Candida xylopsoci*. Because metabarcoding allows a deeper characterization of the fungal species diversity with few discrepancy cases, the distribution of fungal species diversity will be further described using metabarcoding data only. Previous analysis of the same sourdoughs revealed that *F. sanfranciscensis* was the dominant bacterial species in all analyzed sourdoughs but two, where the dominant species was either *L. curvatus* or *L. heilongjiangensis* [20, 52, 53]. Therefore, we decided to study the distribution pattern of microbial species in the fungal community only.

## Fungal species diversity within and between sourdoughs

All sourdoughs but two had a dominant yeast species with a relative abundance over 50% and many species with a lower relative abundance (Figure 3). Within sourdoughs, fungal species richness ranged from 10 to 33, with a 23.5 median (Table S3). The effective number of species per sourdough calculated from the Shannon diversity index ranged from 1 to 6.8 (Table S3), with 70% of sourdoughs having an index below 2 (Table S3). Between-sourdough species differences were analyzed using weighted Unifrac distances, computed from a phylogenetic tree built from the distances between OTUs using *Sporidiobolales species* as root (Figure S2). Unifrac distances computed with four differently rooted trees were highly positively correlated (Figure S3). Unifrac distances between sourdoughs ranged from 0.0005 and 0.71, with a median of 0.49 and a mean of 0.52. The clustering of sourdoughs according to their Unifrac distances is show Figure 5. There was no significant correlation between the Unifrac distances and geographical distances between sourdoughs (Mantel test, P=0.35).

We then analyzed specifically the distribution of yeast species as yeast, together with lactic acid bacteria, are the main functional player in a sourdough ecosystem and for bread quality. Over the 40 yeast species detected in the 38 sourdoughs, 12 had a relative abundance over 50% in at least one sourdough, four had a relative abundance between 20% and 50% and 24 had a relative abundance below 10%. All dominant species (relative abundance over 50%) were fermentative yeast species, except in

one sourdough that had a C*ladosporium* species. We found all the sourdough yeast genera (*Saccharomyces*, *Kazachstania*, *Pichia*, *Torulaspora* and *Hyphopichia)* commonly reported in the literature except the *Wickerhamomyces* genus that we did not detect in our samples [1, 15, 16].

## The baker's yeast species, *Saccharomyces cerevisiae* is not the most widespread yeast species in French organic sourdoughs

*Saccharomyces cerevisiae* was found in 53% of all sourdoughs (95% confidence intervals=36% - 69%) but was dominant (relative abundance over 50%) in only 24% (95% confidence intervals=11% - 40%) (Figure 3). In two cases, *S. cerevisiae* co-occurred with another yeast species at similar relative abundance. In the first case, *S. cerevisiae* was present at a relative abundance of 40% with *Candida sake* at a 41% relative abundance. In the second case, it was found at a relative abundance of 47% with *Pichia kudriavzevii* at a relative abundance of 52%. In all the other cases, *S. cerevisiae* had a relative abundance below 21% and was found with other dominant yeast species, such as *Kazachstania australis*, *Kazachstania humilis*, *Saccharomyces uvarum* or *Torulaspora delbrueckii.* This suggests that *S. cerevisiae* did not displace other species and can indeed be out-competed by other species in sourdoughs.

## Sourdough yeast species mostly belong to the *Kazachstania* genus

*Kazachstania* was the most represented yeast genus over all sourdoughs, when considering both the number of reads over all sourdoughs and the number of detected species. Indeed, this genus represented 57% of the total number of reads while *Saccharomyces* represented 26% of the total number of reads. In addition, eight species of the *Kazachstania* genus were found in sourdough, while the *Saccharomyces* genus was represented by two species (*S. uvarum* and *S. cerevisiae*) (Figure 3). The *Kazachstania* genus is one of the closest genetically related genus to *Saccharomyces* and contained Crabtree positive yeasts, able to ferment glucose even when oxygen is present if the amount of sugar is sufficient (Hagman & Piskur 2015). *Kazachstania* species dominated in 54% (95% confidence intervals=36%-69%) of sourdoughs while *Saccharomyces* species dominated in 27% only (95% confidence intervals=13%-43%). *Kazachstania humilis*, followed by *K. bulderi* were the most commonly dominant *Kazachstania*

356 species, and found in respectively 21% (95% confidence intervals=10%-37%) and 15% of sourdoughs
357 (95% confidence intervals=6%-31%) (Figure 3). A recently described *Kazachstania* species,
358 *Kazachstania bozae*, was also identified in five sourdoughs (4.5%-29%) and found dominant in three
359 (1.7%-22%) [64]. Strains of this species were closely related to a strain previously isolated from boza,
360 a Bulgarian fermented drink, as estimated with ITS and LSU (D1D2) barcodes (Source: NCBI,
361 GenBank: KC118125.1 and KX369579.1). In addition, *Kazachstania saulgeensis,* a recently described
362 species [65, 66], was dominant in one sourdough (0.07%-14%). *Kazachstania unispora* and
363 *Kazachstania servazzi* which had previously been detected in sourdough were also found [17, 17, 53,
364 57, 58, 63, 67–71]. Finally, some *Kazachstania* species were detected for the first time as dominant in
365 sourdoughs, whereas they had been previously found in other environments, like soil (*K. australis*),
366 sauerkraut (*K. barnettii*) [72–74]. None of the previous studies on sourdough have evidenced as many
367 *Kazachstania* species in sourdough.

368 **The composition of sourdough fungal communities was associated with differences in**
369 **bread-making practices**

370 We tested whether sourdough fungal community beta diversity could be explained by bread-making
371 practices. To do so, we performed univariate PERMANOVA analysis on the 30 bakers with less than 8
372 missing values for the 29 bread-making practices variables (Table S4). The univariate analysis revealed
373 that the weighted Unifrac distance is structured according to the use of commercial yeast in bakery
374 ($P<0.05$). It also varied significantly with sourdough age, chief sourdough origin (dough, sourdough or
375 both), the quantity of bread produced per week, the milling method (cylinder, millstone, Astrie, Tyrol),
376 the type of wheat variety (ancient, modern or a mix thereof) and the fermentation duration. However,
377 after FDR correction for taking into account multiple testing, none of these variables significantly
378 explained Unifrac distances.

379 In order to understand further the relationship between sourdough fungal community composition
380 and bread-making practices, we clustered sourdoughs according to their fungal community composition,
381 on the basis of the PCoA of their weighted Unifrac distances. Then, we tested the link between the

382 fungal community group and the bread making practice group (farmers/artisanal practices group) as well
383 as the link between the fungal community group and each of the different bread-making practices
384 (Figure 5). Sourdoughs were clustered into three fungal community groups. Group 1 clustered all
385 sourdoughs (but two) having *Kazachstania* species as dominant species (*K. humilis, K. barnettii, K.*
386 *bulderi, K. saulgeensis, K. bozae)*. Group 2 contained sourdoughs with *Saccharomyces sp.*, *K. servazzi*
387 or *K. unispora* as dominant species. Group 3 sourdoughs harbored *S. cerevisiae* together with other
388 species such as *Pichia kudriavzevii*, *Candida sake*, or a Dipodascaceae sp. Group 1 sourdoughs were
389 mostly made by bakers having farmer's bread-making practices while group 2 and 3 sourdoughs were
390 mostly made by bakers using artisanal practices (exact Fisher test, P=0.035). The fungal community
391 groups were significantly associated with two specific bread making practice variables: the quantity (in
392 kg) of bread made per week (Exact Fisher test, P=0.001) and the use of commercial yeast (Exact Fisher
393 test, P=0.05). All sourdoughs in group 2 but one were found in bakeries making between 500 kg and
394 1000 kg of bread per week, while groups 1 and 3 sourdoughs originated from bakeries producing very
395 different amounts of bread (ranging from amounts below 250 kg to over 1000 kg). In addition, group 1
396 sourdoughs were more frequently found in bakeries that do not use commercial yeast while group 2 and
397 3 were more frequently found in bakeries using the commercial yeast *S. cerevisiae* (Exact Fisher test,
398 P=0.01). Interestingly, group 1 sourdoughs harbored *S. cerevisiae* either at a relative abundancy below
399 1% or not at all, while all groups 2 and 3 sourdoughs had *S. cerevisiae* at a relative abundancy over 20%,
400 except in three cases where it was either absent or at a relative abundancy below 6%.

401 To test more specifically the link between bread-making practices and the distribution of
402 *Kazachstania* species, we analyzed more in depth group 1 sourdoughs. Within this group, 8 sourdoughs
403 had *K. humilis* as dominant species, 6 had *K. bulderi*, 3 had *K. bozae* and the remainder had still other
404 *Kazachstania* species. All sourdoughs made with artisanal practices carried *K. humilis* as dominant
405 species or, in one case, the *K. bozae*. By contrast, sourdoughs made with farmers' practices had as
406 dominant species *K. bulderi*, *K. australis*, *K. barnettii*, *K. saulgeensis* or *K. bozae* (exact Fisher test,
407 P=0.004).

## Fungal community composition was partly related to sourdough acidity, maltose concentration and hydration

The composition of fungal community may affect sourdough metabolic content (sugars, acids, alcohols) via fungal strains metabolite consumption and production. Inversely, the presence and concentration of different compounds (sugars, acids, alcohols) may affect differently the fitness depending on the strains and consequently be one of the drivers of fungal community composition. For example, lactic acid bacteria (LAB) are the main producers of acidity in sourdough, but yeasts also produce acetic acid and also indirectly affect acidity through positive or negative interaction with bacteria.

To investigate the relation between sourdough fungal communities and metabolic compounds, we quantified sourdough hydration, yeast density, bacteria density, sourdough pH, Total Titrable Acidity (TTA), sourdough concentration in seven sugars (maltose, glucose, fructose, raffinose, arabinose, mannose, xylose), four alcohols (glycerol, ethanol, mannitol, meso-erythtritol), six acids (lactate, acetate, glutarate, pyruvate, malate, succinate) and calculated the fermentative quotient (lactate over acetate ratio). For each variable, there was a wide range of variation (Table S5). The principal component analysis based on all variables showed no evidence of sourdough grouping (Figure S4). As expected in fermentation, yeast density was positively correlated to ethanol ($r=0.74$, $P<0.001$), glycerol ($r=0.67$, $P<0.001$), and acetate ($r=0.6$, $P<0.001$) concentration. However, it was not significantly correlated to sugar concentrations. This might be explained by the co-occurrence of bacteria which have their own metabolism and interact by competition and/or cross feeding with sourdough yeasts.

We then tested whether the variation of each quantitative variable was associated with the bread-making practices groups (farmer-like practices and artisanal practices). There was no significant effect of the bread making practice group except for sourdough hydration that was significantly higher in sourdoughs made using farmer-like practices ($F_{1,94}=11,69$, $P<0.001$). On average, sourdoughs made with

farmers-liker practices had 55% water while sourdoughs made with artisanal-like practices had in average 49% of water.

In addition, we tested whether variations in quantitative variables were associated with the fungal community groups (Table S5). Group 3 microbial community sourdoughs (defined by PCoA clustering on Unifrac distance, see below), which contains *S. cerevisiae* in co-dominance with a second yeast species (*Candida sake*, *Pichia kudriavzevii* or a *Dipodascus* species), had a significantly higher mean pH (mean $pH_{group3}$=4.2 against $pH_{group1}$=3.8, Tukey Contrasts, P<0.001), lower TTA (mean $TTA_{group3}$=7.7 against $TTA_{group1}$=17.1, Tukey Contrasts, P=0.002), and a higher maltose concentration (mean $Maltose_{group3}$=52.8 mg/gr of sourdough against $Maltose_{group1}$=24.1 mg/gr of sourdough, Tukey Contrasts P=0.002) than group1, having a *Kazachstania* dominant species. Compared to group 2 having in most cases *S. cerevisiae* as dominant species, it also had higher pH ($pH_{group2}$=3.9, Tukey Contrasts, P=0.003), and higher maltose concentration ($Maltose_{group2}$=23.7, Tukey contrast, P=0.003). These data may reflect a lower fermentative activity for group 3 fungal community having two co-dominant species, and/or a negative interaction effect of group3 fungal community on the activity of lactic acid bacteria (LAB), which are the main producers of sourdough acids. Previous studies on the bacteria content of the same sourdoughs showed that *L. sanfranciscensis* was most generally the dominant species, although *L. heilongjiangensis, L. curvatus* or *L. brevis* were also found as dominant species [20, 52, 53]. We found no significant correlation between LAB and yeast densities (r= -0.15, p= 0.45, Figure S4) but the link between fungal and bacteria community might be species and strains dependent. Additional studies on the interactions between fungal and bacterial communities need to be performed to better understand how they may drive sourdough acidity and sugar content.

Finally, we analyzed whether the variations of each quantitative variable was associated with the dominant yeast species. We only considered the 26 sourdoughs having either *S. cerevisiae* (9 sourdoughs), *K. humilis* (8 sourdoughs), *K. bulderi* (6 sourdoughs) or *K. bozae* (3 sourdoughs) as dominant species, since the other yeast species were found dominant only once. The differences in dominant species was not significantly associated to variation in sourdough sugar, acids or alcohol

concentration. However, on average, sourdoughs dominated by *K. bulderi* were more hydrated (63% water content in average) than sourdoughs dominated by *K. humilis*, *K. bozae*, and *S. cerevisiae*, having respectively 49%, 47%, 53 % water content in average ($P<0.001$ for the 3 Tukey Contrasts). *Kazachstania bulderi* was found to be dominant only in sourdoughs made using farmers' practices, a bread making practice group that was also found to be associated with more hydrated sourdoughs. Additional experiments should be carried out to test whether this species has indeed a better fitness in more hydrated sourdoughs or whether its presence in more hydrated sourdoughs is related to covariation with other farmer practices.

## Phenotypic signatures of domestication

A previous analysis on *S. cerevisiae* revealed that sourdough strains had higher average fitness and fermentation performance than strains from other environments in a sourdough-mimicking medium (Bigey et al. 2021). Here, we investigated whether evidence of a domestication syndrome could also be found in *K. humilis* and *K. bulderi*, the two *Kazachstania* species most commonly found in French sourdoughs. We tested whether fitness (log of population size and mortality at the end of fermentation) and fermentation performance (CO2max, Vmax, t1g, tVmax) differed between sourdough strains and strains from elsewhere.

A principal component analysis of 38 strains of *K. bulderi* and *K. humilis*, based on quantitative variation in the six phenotypic variables described below was carried out. The first two axis explained 80.5% of the variation and clearly separated strains by species (Figure 6). The *K. bulderi* strains were located at the right of the PCA and were characterized by high population size and low mortality at the end of fermentation, while the *K. humilis* strains were located at the left and were characterized by a rapid onset of fermentation (t1g), high maximum fermentation rate (Vmax), and a short time to reach Vmax (tVmax). Non-sourdough strains of *K. humilis* were located outside the cloud of sourdough strains while non-sourdough strains of *K. bulderi* were distributed within and outside the cloud of sourdough strains.

Statistical comparison of sourdough and non-sourdough strains of *K. bulderi* and *K. humilis* for each phenotypic variable revealed phenotypic divergence for *K. humilis* but not for *K. bulderi*. While the *K. bulderi* sourdough strains did not ferment significantly faster than the non-sourdough strains, the *K. humilis* sourdough strains showed significantly higher Vmax, lower t1g, and lower tVmax than the non-sourdough *K. humilis* strains (Figure 7, Table S6). On average, they started fermentation two hours before the others and reached Vmax three hours before the others. In addition, their Vmax were on average 34% higher than the others.

## Discussion

Sourdough microbial diversity has been intensively studied worldwide. Despite a cultural and historical interest on bread in France, French sourdough fungal diversity was only partly characterized before this study [19, 53, 71]. A recent large-scale (> 500 starters) study of sourdough microbial diversity revealed the fungal diversity that can be detected over the globe across home-made sourdoughs [17]. Comparison of these findings with the diversity found in France showed that all the yeast species detected at a relative abundance over 1% in this international collection of sourdoughs were detected in French sourdoughs except the species, *Wickerhanomyces anomalus*, *Pichia membranifaciens*, *Naumovozyma castellii*, and *Saccharomyces bayanus*. Inversely, French baker's sourdough harbored some yeast species that were never found elsewhere, such as *K. bozae*, *K. australis*, *K. saulgeensis* [65].

Beyond the baker yeast species, *S. cerevisiae*, the most represented yeast genus in French sourdough was *Kazachstania*. Eight *Kazachstania* species were found as dominant yeast species in at least one French sourdough. This includes *K. humilis*, *K. bulderi*, *K. barnettii*, *K. unispora*, *K. servazzii*, *K. bozae*, *K. australis*, *K. saulgeensis*. Three *Kazachstania* species (*K. exigua, K. lodderae, K. naganishi*) already reported in sourdough were not found in our collection of French sourdough. *Kazachstania lodderae* and *K. naganishi* are rarely found in sourdough. By contrast, *K. exigua* is a frequently cited sourdough species in the literature. It has been previously found in France, Finland [77], Italy [78, 79], Denmark [80], Ethiopia [81], USA [17]. It is also the first species to have been isolated from a sourdough (in San Francisco, [29]). However, the attempt to study its genetic variation has been hampered by the fact that

it probably originated from hybridization between unknown yeast species [82]. To date, the genus *Kazachstania* is composed of more than 40 species, of which 11 are present in the sourdough. It is possible that an adaptive radiation linked to the adaptation to different sourdoughs or to different anthropized niches has taken place, as it has been observed for example in cichlids during their adaptation to different lakes. Indeed, five of the *Kazachstania* species present in the sourdough have so far only been detected in human-related niches. These are *K. saulgeensis*, *K. barnettii*, *K. bozae*, *K. bulderi*, *K. humilis*. These species are genetically closer to each other than they are to *K. australis*, *K. servazzii* and *K. unispora* which have also been found in nature and are grouped in another part of the *Kazachstania* pylogenetic tree. Genomic analysis of these species would shed light on their evolution. So far, eight of the 11 *Kazachstania* species found in the sourdough have at least one genome assembly available in public databases, including *K. saulgeensis* and *K. barnettii* [44, 45], and the assemblies of *K. bulderi* and *K. humilis*, which were recently published in public databases (Bio project: PRJEB44438). The genomic and phenomic analysis of the large collection of *Kazachstania* strains obtained by our study, together with the world collection of *Kazachstania* strains, may shed light on the radiation and domestication processes of these species.

We found that yeast community composition was partly related with bread-making practices. Bread-making practices divergence also led to different phenotypic signatures. Strains of the *K. humilis* species, which was typical of sourdough made by artisanal-like practices, had increased fermentation rate while strains of *K. bulderi* which was typical of sourdough made by farmer-like bread-making practices had not. The species *K. humilis* has been found in many countries, viz. Austria, Canada, China, Denmark, Ethiopia, Finland, Germany, Greece, Italy, Morocco, the Netherland, Spain, UK, USA, and France [1, 15–17, 19, 53, 55–60]. It is also the most frequently encountered *Kazachstania* species in sourdoughs around the world. This species is therefore frequently found in bakeries, where short fermentations are often favored. This may explain why sourdough strains of *K. humilis* seem to have been selected for increased fermentation rate. Increased fermentation rate was also found in bakery strains of *S. cerevisiae* when compared to non-bakery strains.

In contrast, we did not find evidence of selection for improved fermentation performance in *K. bulderi*, which was the third most represented species in French sourdough. *Kazachstania bulderi* was found in bakeries with farmer-like practices. These bakeries often bake bread once or twice a week and store their sourdough for several days. They also often use long fermentation and thus may not have selected an increased fermentation rate. Farmer-bakers typically store their sourdough for several days during the week and therefore make a lower number of backslopping during the week. In addition, they make bread with longer fermentation times than artisanal bakers. It is therefore possible that they did not select to accelerate the speed of fermentation and instead let natural selection in the sourdough environment act alone. Alternatively, the lack of phenotypic divergence between sourdough and non-sourdough strains may reflect the limitation of our sampling. *Kazachstania bulderi* has been reported for the first time in anaerobic maize silage in the Netherlands and in fermented liquid feed for piglets [61, 62], more recently in French, Belgium and Spain sourdoughs [19, 53, 57, 63] but to our knowledge was never found in wild environment. Here, we compare sourdough strains with strains coming from ensilage and animal feed. It is unknown whether ensilage and animal feed strains are wild strains or feral strains that have escaped from other domesticated environments. This may explain why we did not detect any phenotypic divergence between sourdough and non-sourdough strains of *K. bulderi*. Other than fermentation phenotypes, there was no evidence of fitness differences between sourdough and non-sourdough strains of *K. humilis* and *K. bulderi* in sourdough mimicking media. Additional experiments in real dough should be performed to further test the effect of natural selection in this environment.

Other evolutionary process than selection could also explain the distribution of yeast species across sourdoughs. Interviews with the bakers working with sourdough hosting *K. bulderi* and *K. bozae* suggested the role of dispersion of these species in French sourdoughs. Indeed, these bakers have been connected over the years either through seed exchanges, sourdough mixing or gifts, bread making training in common or working in one another's bakery. Some yeast species have been found in the bakery house environment and baker's hands and may thus be dispersed through baker's tools or baker's travels [58, 67, 75, 76]. However, it is still unclear whether wheat seeds and flour are a source of sourdough yeasts.

To our knowledge, this is the first evidence of the influence of artisanal practices on taxonomic diversity in microbial communities. On the other hand, several studies have shown that making fermented products could lead to the selection of divergent phenotypes and genotypes. This is the case of sourdough and industrial populations of the baker's yeast *S. cerevisiae* that diverge among themselves and have a better fermentation performance than non-baker's strains. As for beer populations, sourdough populations have acquired a better capacity to assimilate maltose, linked at least in part to an increase in the number of copies of the genes involved in the assimilation of maltose. Several studies on wine populations of *S. cerevisiae*, *Torulaspora delbrueckii* et *Lachancea thermotolerans* also showed that wine populations are genetically differentiated from strains from other environments and present beneficial phenotypes in grape must and for wine quality. The analysis of filamentous cheese fungi *P. roqueforti* and *P. camemberti* populations also revealed cheese making genetically differentiated populations. Interestingly, different genetic groups associated with different cheese making practices were found. Strains of the blue cheese fungus, *P. roqueforti*, isolated from Roquefort cheese were more diverse and were genetically and phenotypically different than strains used to make other blue cheeses [83, 84, 85]. Two varieties of the white cheese making fungus, *P. camemberti*, with different phenotypic features, were associated with different kind of cheese (camembert and brie). All together these studies show that the diversity of practices used to make fermented products allows to maintain genetic, phenotypic and taxonomic diversity.

However, fungal domestication also involved important bottleneck. For example, the low level of genetic diversity found in blue-cheese P. roqueforti strains and P. camemberti strains revealed the risk of diversity erosion in fermented product making [6, 7, 84]. This risk is associated with the massive use of few industrial strains or the need to standardize products to meet the specifications of industrial production or AOP. Here, we show that despite the recurrent use of *S. cerevisiae* as industrial starter species in bakeries and homes, and the occurrence of this species in a wide range of habitats such as soil, trees, and humans [9, 54], this species does not appear to have overwhelmingly colonized French traditional sourdoughs (Figure 3, Figure 4). This result confirmed a recent analysis which revealed that *S. cerevisiae* sourdough strains have a different evolutionary history than industrial strains [10]. The

dynamic of microbial species colonization and invasion in food environment remain largely unknown. A recent study suggested that wild-type Penicillium can evolved in a few weeks into a domesticated form. Additional experiments at the level of microbial community will shed light on the dynamic of microbial community establishment in food production and on the ability of industrial strains to invade food microbial community.

## Conclusion and perspectives

In conclusion, a great diversity of bread-making practices and fungal community composition was found in our sample of French sourdoughs. Surprisingly, the well-known baker's yeast *S. cerevisiae* was found dominant only in one fourth of the sampled sourdoughs. By contrast, several species of the neighboring genus *Kazachstania* were detected at high frequency, revealing a major role for this mostly unknown genus in the study of fungal domestication and in bread making. Therefore, our results highlight the necessity of maintaining socio-cultural diversity to maintain microbial diversity in food systems. These findings could not have been evidenced without the collaboration of bakers and scientists, showing the importance of participatory research projects to gain new insight into biodiversity preservation.

### Acknowledgements

This work has emanated from research conducted with the financial support of the French National Research Agency, ANR-13-ALID-0005 and INRAE. We thank all the bakers and farmers-bakers for constructing the project with us and for sharing their sourdoughs and knowledge. We thank Yoann Robert, Candice Aulard, Josette Bessière for lab assistance and Matthieu Barret for MiSeq sequencing. We also would like to thank Philippe Roussel for sharing his knowledge about French bread.

### References

1. Carbonetto B, Ramsayer J, Nidelet T, Legrand J, Sicard D. Bakery yeasts, a new model for studies in ecology and evolution. *Yeast* ; **0**.

2. Gibbons JG, Rinker DC. The genomics of microbial domestication in the fermented food environment. *Current Opinion in Genetics & Development* 2015; **35**: 1–8.

3. Steensels J, Gallone B, Voordeckers K, Verstrepen KJ. Domestication of Industrial Microbes. *Current Biology* 2019; **29**: R381–R393.

4. Gibbons JG, Salichos L, Slot JC, Rinker DC, McGary KL, King JG, et al. The evolutionary imprint of domestication on genome variation and function of the filamentous fungus Aspergillus oryzae. *Curr Biol* 2012; **22**: 1403–1409.

5. Watarai N, Yamamoto N, Sawada K, Yamada T. Evolution of Aspergillus oryzae before and after domestication inferred by large-scale comparative genomic analysis. *DNA Research* 2019; **26**: 465–472.

6. Ropars J, Didiot E, Rodríguez de la Vega RC, Bennetot B, Coton M, Poirier E, et al. Domestication of the Emblematic White Cheese-Making Fungus Penicillium camemberti and Its Diversification into Two Varieties. *Curr Biol* 2020; **30**: 4441-4453.e4.

7. Ropars J, López-Villavicencio M, Snirc A, Lacoste S, Giraud T. Blue cheese-making has shaped the population genetic structure of the mould Penicillium roqueforti. *PLOS ONE* 2017; **12**: e0171387.

8. Legras J-L, Galeote V, Bigey F, Camarasa C, Marsit S, Nidelet T, et al. Adaptation of *S. cerevisiae* to Fermented Food Environments Reveals Remarkable Genome Plasticity and the Footprints of Domestication. *Mol Biol Evol* 2018; **35**: 1712–1727.

9. Peter J, De Chiara M, Friedrich A, Yue J-X, Pflieger D, Bergström A, et al. Genome evolution across 1,011 *Saccharomyces* cerevisiae isolates. *Nature* 2018; **556**: 339–344.

10. Bigey F, Segond D, Friedrich A, Guezenec S, Bourgais A, Huyghe L, et al. Evidence for Two Main Domestication Trajectories in *Saccharomyces* cerevisiae Linked to Distinct Bread-Making Processes. *Current Biology* 2020; S0960982220316912.

11. Arranz-Otaegui A, Carretero LG, Ramsey MN, Fuller DQ, Richter T. Archaeobotanical

639 evidence reveals the origins of bread 14,400 years ago in northeastern Jordan. *PNAS* 2018; **115**: 7925–
640 7930.
641 12. Samuel D. Bread in archaeology. *Civilisations Revue internationale d'anthropologie et de*
642 *sciences humaines* 2002; 27–36.
643 13. Roussel P, Chiron H. Les pains français: évolution, qualité, production. 2005. Maé-Erti.
644 14. Decock P, Cappelle S. Bread technology and sourdough technology. *Trends in Food Science*
645 *& Technology* 2005; **16**: 113–120.
646 15. Van Kerrebroeck S, Maes D, De Vuyst L. Sourdoughs as a function of their species diversity
647 and process conditions, a meta-analysis. *Trends in Food Science & Technology* 2017; **68**: 152–159.
648 16. Arora K, Ameur H, Polo A, Di Cagno R, Rizzello CG, Gobbetti M. Thirty years of knowledge
649 on sourdough fermentation: A systematic review. *Trends in Food Science & Technology* 2021; **108**:
650 71–83.
651 17. Landis EA, Oliverio AM, McKenney EA, Nichols LM, Kfoury N, Biango-Daniels M, et al.
652 The diversity and function of sourdough starter microbiomes. *eLife* 2021; **10**: e61644.
653 18. Demeulenaere É, Rivière P, Hyacinthe A, Baltassat R, Baltazar S, Gascuel J-S, et al. La
654 sélection participative à l'épreuve du changement d'échelle. À propos d'une collaboration entre
655 paysans sélectionneurs et généticiens de terrain. *Natures Sciences Societes* 2017; **Vol. 25**: 336–346.
656 19. Urien C, Legrand J, Montalent P, Casaregola S, Sicard D. Fungal Species Diversity in French
657 Bread Sourdoughs Made of Organic Wheat Flour. *Front Microbiol* 2019; **10**.
658 20. Michel E, Monfort C, Deffrasnes M, Guezenec S, Lhomme E, Barret M, et al.
659 Characterization of relative abundance of lactic acid bacteria species in French organic sourdough by
660 cultural, qPCR and MiSeq high-throughput sequencing methods. *International Journal of Food*
661 *Microbiology* 2016; **239**: 35–43.
662 21. White TJ, Bruns T, Lee S, Taylor J. 38 - AMPLIFICATION AND DIRECT SEQUENCING
663 OF FUNGAL RIBOSOMAL RNA GENES FOR PHYLOGENETICS. In: Innis MA, Gelfand DH,
664 Sninsky JJ, White TJ (eds). *PCR Protocols*. 1990. Academic Press, San Diego, pp 315–322.
665 22. Martin KJ, Rygiewicz PT. Fungal-specific PCR primers developed for analysis of the ITS

region of environmental DNA extracts. *BMC Microbiology* 2005; **5**: 28.

23. Peris D, Sylvester K, Libkind D, Gonçalves P, Sampaio J, Alexander WG, et al. Population structure and reticulate evolution of *Saccharomyces* eubayanus and its lager-brewing hybrids. *Mol Ecol* 2014; **23**: 2031–2045.

24. Nguyen H-V, Gaillardin C. Evolutionary relationships between the former species *Saccharomyces* uvarum and the hybrids *Saccharomyces* bayanus and *Saccharomyces* pastorianus; reinstatement of *Saccharomyces* uvarum (Beijerinck) as a distinct species. *FEMS Yeast Res* 2005; **5**: 471–483.

25. Libkind D, Hittinger C, of the … V-E. Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. *Proceedings of the …* 2011.

26. Sampaio JP, Gonçalves P. Natural populations of *Saccharomyces* kudriavzevii in Portugal are associated with oak bark and are sympatric with *S. cerevisiae* and S. paradoxus. *Appl Environ Microbiol* 2008; **74**: 2144–2152.

27. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res* 2016; **44**: D7–D19.

28. Weiss S, Samson F, Navarro D, Casaregola S. YeastIP: a database for identification and phylogeny of Saccharomycotina yeasts. *FEMS Yeast Research* 2013; **13**: 117–125.

29. Escudié F, Auer L, Bernard M, Mariadassou M, Cauquil L, Vidal K, et al. FROGS: Find, Rapidly, OTUs with Galaxy Solution. *Bioinformatics* 2018; **34**: 1287–1294.

30. Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 2011; **27**: 2957–2963.

31. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 2011; **17**: 10–12.

32. Sickle 1.33 – Windowed Adaptive Trimming for Fastq files using Quality – My Biosoftware – Bioinformatics Softwares Blog.

33. Mahé F, Rognes T, Quince C, Vargas C de, Dunthorn M. Swarm: robust and fast clustering method for amplicon-based studies. *PeerJ* 2014; **2**: e593.

693 34. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool
694 for metagenomics. *PeerJ* 2016; **4**: e2584.
695 35. Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, et al. Towards a
696 unified paradigm for sequence-based identification of fungi. *Molecular Ecology* 2013; **22**: 5271–5277.
697 36. Bloem A, Rollero S, Seguinot P, Crépin L, Perez M, Picou C, et al. Workflow Based on the
698 Combination of Isotopic Tracer Experiments to Investigate Microbial Metabolism of Multiple Nutrient
699 Sources. *J Vis Exp* 2018.
700 37. Delobel P, Pradal M, Blondin B, Tesniere C. A 'fragile cell' sub-population revealed during
701 cytometric assessment of *Saccharomyces* cerevisiae viability in lipid-limited alcoholic fermentation.
702 *Lett Appl Microbiol* 2012; **55**: 338–344.
703 38. Lê S, Josse J, Husson F. FactoMineR: An R Package for Multivariate Analysis. *Journal of*
704 *Statistical Software* 2008; **25**: 1–18.
705 39. McMurdie PJ, Holmes S. phyloseq: An R Package for Reproducible Interactive Analysis and
706 Graphics of Microbiome Census Data. *PLOS ONE* 2013; **8**: e61217.
707 40. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial
708 communities. *Appl Environ Microbiol* 2005; **71**: 8228–8235.
709 41. Gouy M, Guindon S, Gascuel O. SeaView version 4: A multiplatform graphical user interface
710 for sequence alignment and phylogenetic tree building. *Mol Biol Evol* 2010; **27**: 221–224.
711 42. Chao A, Chiu C-H, Jost L. Phylogenetic diversity measures based on Hill numbers. *Philos*
712 *Trans R Soc Lond B Biol Sci* 2010; **365**: 3599–3609.
713 43. Jost L. Partitioning diversity into independent alpha and beta components. *Ecology* 2007; **88**:
714 2427–2439.
715 44. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful
716 Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological)*
717 1995; **57**: 289–300.
718 45. Kuznetsova A, Brockhoff PB, Christensen RHB. lmerTest Package: Tests in Linear Mixed
719 Effects Models. *Journal of Statistical Software* 2017; **82**: 1–26.

720 46. Hijmans RJ, Karney (GeographicLib) C, Williams E, Vennes C. geosphere: Spherical
721 Trigonometry. 2021.
722 47. Dray S, Dufour A-B. The ade4 Package: Implementing the Duality Diagram for Ecologists.
723 *Journal of Statistical Software* 2007; **22**: 1–20.
724 48. Pedersen HW Danielle Navarro, and Thomas Lin. Welcome | ggplot2.
725 49. Cheng J, Karambelkar B, Xie Y. Leaflet: Create interactive web maps with the
726 javascript'leaflet'library. *R package version* 2018; **2**.
727 50. Clément H, Prost C, Chiron H, Ducasse MB, Della Valle G, Courcoux P, et al. The effect of
728 organic wheat flour by-products on sourdough performances assessed by a multi-criteria approach.
729 *Food Res Int* 2018; **106**: 974–981.
730 51. De Vuyst L, Van Kerrebroeck S, Leroy F. Microbial Ecology and Process Technology of
731 Sourdough Fermentation. *Adv Appl Microbiol* 2017; **100**: 49–160.
732 52. Lhomme E, Orain S, Courcoux P, Onno B, Dousset X. The predominance of Lactobacillus
733 sanfranciscensis in French organic sourdoughs and its impact on related bread characteristics.
734 *International Journal of Food Microbiology* 2015; **213**: 40–48.
735 53. Lhomme E, Urien C, Legrand J, Dousset X, Onno B, Sicard D. Sourdough microbial
736 community dynamics: An analysis during French organic bread-making processes. *Food Microbiol*
737 2016; **53**: 41–50.
738 54. Goddard MR, Greig D. *Saccharomyces* cerevisiae: a nomadic yeast with no niche? *FEMS*
739 *Yeast Res* 2015; **15**: fov009.
740 55. Valmorri S, Tofalo R, Settanni L, Corsetti A, Suzzi G. Yeast microbiota associated with
741 spontaneous sourdough fermentations in the production of traditional wheat sourdough breads of the
742 Abruzzo region (Italy). *Antonie Van Leeuwenhoek* 2010; **97**: 119–129.
743 56. Wang X, Du H, Zhang Y, Xu Y. Environmental Microbiota Drives Microbial Succession and
744 Metabolic Profiles during Chinese Liquor Fermentation. *Appl Environ Microbiol* 2018; **84**: e02369-
745 17.
746 57. Chiva R, Celador-Lera L, Uña JA, Jiménez-López A, Espinosa-Alcantud M, Mateos-

747 Horganero E, et al. Yeast Biodiversity in Fermented Doughs and Raw Cereal Matrices and the Study
748 of Technological Traits of Selected Strains Isolated in Spain. *Microorganisms* 2020; **9**: 47.
749 58. Reese AT, Madden AA, Joossens M, Lacaze G, Dunn RR. Influences of Ingredients and
750 Bakers on the Bacteria and Fungi in Sourdough Starters and Bread. *mSphere* 2020; **5**.
751 59. Syrokou MK, Themeli C, Paramithiotis S, Mataragas M, Bosnea L, Argyri AA, et al.
752 Microbial Ecology of Greek Wheat Sourdoughs, Identified by a Culture-Dependent and a Culture-
753 Independent Approach. *Foods* 2020; **9**: 1603.
754 60. Sugihara TF, Kline L, Miller MW. Microorganisms of the San Francisco sour dough bread
755 process. I. Yeasts responsible for the leavening action. *Appl Microbiol* 1971; **21**: 456–458.
756 61. Middelhoven WJ, Kurtzman CP, Vaughan-Martini A. *Saccharomyces* bulderi sp. nov., a yeast
757 that ferments gluconolactone. *Antonie Van Leeuwenhoek* 2000; **77**: 223–228.
758 62. Gori K, Bjørklund MK, Canibe N, Pedersen AØ, Jespersen L. Occurrence and identification
759 of yeast species in fermented liquid feed for piglets. *Microb Ecol* 2011; **61**: 146–153.
760 63. Comasio A, Verce M, Van Kerrebroeck S, De Vuyst L. Diverse Microbial Composition of
761 Sourdoughs From Different Origins. *Front Microbiol* 2020; **11**: 1212.
762 64. Gouliamova D, Dimitrov R. *KAZACHSTANIA* CHRYSOLINAE AND *KAZACHSTANIA*
763 BOZAE TWO NEW YEAST SPECIES OF THE GENUS *KAZACHSTANIA*. TRANSFER OF FOUR
764 *KAZACHSTANIA* SPECIES TO GRIGOROVIA GEN. NOV. AS NEW COMBINATIONS. 2020.
765 65. Jacques N, Sarilar V, Urien C, Lopes MR, Morais CG, Uetanabaro APT, et al. Three novel
766 ascomycetous yeast species of the *Kazachstania* clade, *Kazachstania* saulgeensis sp nov.,
767 *Kazachstania* serrabonitensis sp nov and *Kazachstania* australis sp nov Reassignment of Candida
768 humilis to *Kazachstania* humilis f.a. comb. nov and Candida pseudohumilis to *Kazachstania*
769 pseudohumilis f.a. comb. nov. *International Journal of Systematic and Evolutionary Microbiology*
770 2016; **66**: 5192--5200.
771 66. Sarilar V, Sterck L, Matsumoto S, Jacques N, Neuvéglise C, Tinsley CR, et al. Genome
772 sequence of the type strain CLIB 1764T (=CBS 14374T) of the yeast species *Kazachstania*
773 saulgeensis isolated from French organic sourdough. *Genomics Data* 2017; **13**: 41–43.

774 67. Minervini F, Lattanzi A, De Angelis M, Di Cagno R, Gobbetti M. Influence of Artisan
775 Bakery- or Laboratory-Propagated Sourdoughs on the Diversity of Lactic Acid Bacterium and Yeast
776 Microbiotas. *Appl Environ Microbiol* 2012; **78**: 5328–5340.
777 68. Salovaara H, Savolainen J. Yeast type isolated from Finnish sour rye dough starters. *Acta*
778 *Alimentaria Polonica* 1984; **10**.
779 69. Decimo M, Quattrini M, Ricci G, Fortina MG, Brasca M, Silvetti T, et al. Evaluation of
780 microbial consortia and chemical changes in spontaneous maize bran fermentation. *AMB Express*
781 2017; **7**: 205.
782 70. Fraberger V, Unger C, Kummer C, Domig KJ. Insights into microbial diversity of traditional
783 Austrian sourdough. *LWT* 2020; **127**: 109358.
784 71. Lhomme E, Lattanzi A, Dousset X, Minervini F, De Angelis M, Lacaze G, et al. Lactic acid
785 bacterium and yeast microbiotas of sixteen French traditional sourdoughs. *Int J Food Microbiol* 2015;
786 **215**: 161–170.
787 72. Jacques N, Sarilar V, Urien C, Lopes MR, Morais CG, Uetanabaro APT, et al. Three novel
788 ascomycetous yeast species of the *Kazachstania* clade, *Kazachstania* saulgeensis sp. nov.,
789 *Kazachstania*serrabonitensis sp. nov. and *Kazachstania* australis sp. nov. Reassignment of Candida
790 humilis to *Kazachstania* humilis fa comb. nov. and Candida pseudohumilis to *Kazachstania*
791 pseudohumilis fa comb. nov. *International journal of systematic and evolutionary microbiology* 2016;
792 **66**: 5192–5200.
793 73. Kurtzman CP. Phylogenetic circumscription of *Saccharomyces*, Kluyveromyces and other
794 members of the Saccharomycetaceae, and the proposal of the new genera Lachancea, Nakaseomyces,
795 Naumovia, Vanderwaltozyma and Zygotorulaspora. *FEMS yeast research* 2003; **4**: 233–245.
796 74. Boynton PJ, Greig D. The ecology and evolution of non-domesticated *Saccharomyces* species.
797 *Yeast* 2014; **31**: 449–462.
798 75. Minervini F, Lattanzi A, De Angelis M, Celano G, Gobbetti M. House microbiotas as sources
799 of lactic acid bacteria and yeasts in traditional Italian sourdoughs. *Food Microbiology* 2015; **52**: 66–76
800 76. Minervini F, Lattanzi A, Dinardo FR, De Angelis M, Gobbetti M. Wheat endophytic

lactobacilli drive the microbial and biochemical features of sourdoughs. *Food Microbiol* 2018; **70**: 162–171.

77. Infantes M, Schmidt JL. Characterisation of the yeast flora of natural sourdoughs located in various French areas. Sciences des Aliments (France) [Internet]. 1992, available on: https://agris.fao.org/agris-search/search.do?recordID=FR9204966search/search.do?recordID=FR9204966

78. Pulvirenti A, Solieri L, Gullo M, Vero LD, Giudici P. Occurrence and dominance of yeast species in sourdough. *Letters in Applied Microbiology* 2004; **38**: 113-7.

79. Minervini F, Lattanzi A, De Angelis M, Di Cagno R, Gobbetti M. Influence of Artisan Bakery- or Laboratory-Propagated Sourdoughs on the Diversity of Lactic Acid Bacterium and Yeast Microbiotas. *Appl Environ Microbiol* 2012; **78**: 5328-40.

80. Nuobariene L, Hansen ÅS, Arneborg N. Isolation and identification of phytase-active yeasts from sourdoughs. LWT - *Food Science and Technology* 2012; **48**:190-6.

81. Desiye A, Abegaz K. Isolation, characterization and identification of lactic acid bacteria and yeast involved in fermentation of Teff (EragrostisTef) Batter. 2013; /paper/Isolation%2C-characterization-and-identification-of-Desiye Abegaz/3373ba786cbed5f07d2c6330a5362ea56198adc2

82. Bon E, Neuvéglise C, Lépingle A, Wincker P, Artiguenave F, Gaillardin C, et al. Genomic Exploration of the Hemiascomycetous Yeasts: 6. *Saccharomyces* exiguus. *FEBS Letters* 2000; **487**: 42-6.

83. Cheeseman K, Ropars J, Renault P, Dupont J, Gouzy J, Branca A, et al. Multiple recent horizontal transfers of a large genomic region in cheese making fungi. *Nat Commun* 2014; **5**: 2876.

84. Dumas E, Feurtey A, Rodríguez de la Vega RC, Le Prieur S, Snirc A, Coton M, et al. Independent domestication events in the blue-cheese fungus Penicillium roqueforti. *Molecular Ecology* 2020; **29**: 2639-60.

85. Ropars J, Rodríguez de la Vega RC, López-Villavicencio M, Gouzy J, Sallet E, Dumas É, et al. Adaptive Horizontal Gene Transfers between Multiple Cheese-Associated Fungi. *Current Biology* 2015; **25**: 2562-9.

## Figure Legends

**Figure 1.** The sourdough bread making process. Sourdough is a mix of flour and water naturally fermented by bacteria and yeasts. It is initiated by mixing flour, water and occasionally other ingredients. It is then “fed” by regularly adding flour and water, a process termed back-slopping. Once considered mature by bakers based on their acidity, flavour and bubbling activity, the sourdough is called "chief", or “mother” sourdough, and can then be used for bread making. The bread making process starts from this “chief sourdough”, or from a piece of dough or sourdough sampled from the preceding bread making process, or initiated from a mix of flour and water naturally colonized by yeasts and LAB following several back-sloppings. Once or several times, the chief sourdough is refreshed by adding flour and water to constitute the final sourdough, which is used for bread making. This final sourdough is mixed with flour, water, and other ingredients (salt, seeds, yeasts starters, etc.) during kneading to constitute the dough. After kneading, primary fermentation occurs during the first rising. The dough is then divided and shaped. The pieces of dough are then left to rise during a second fermentation and finally oven-baked.

**Figure 2.** Multiple Correspondence Analysis (MCA) based on 28 categorical variables describing bread-making practices.

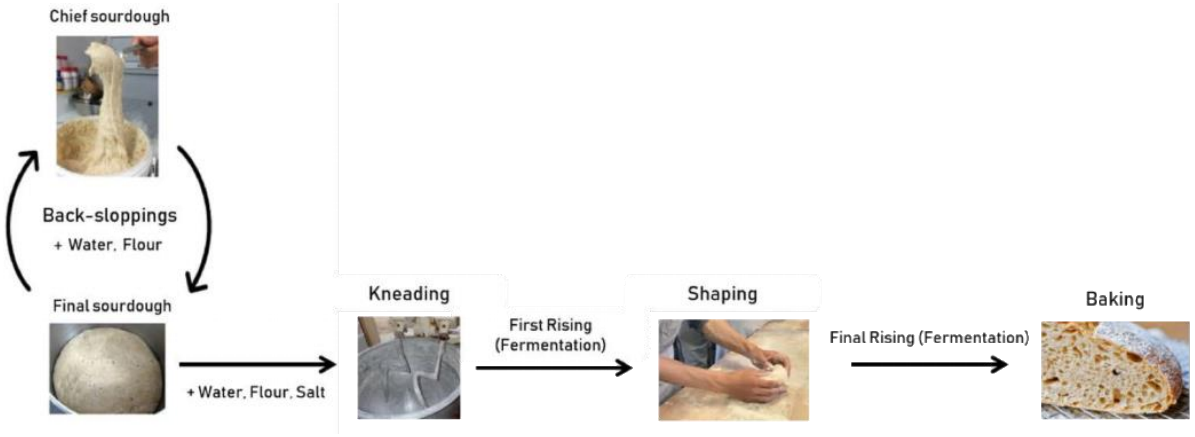
A) Representation of bakers. Each point represents a bakery. The purple area on the left brings together baker with “artisanal” practices and the light blue area on the right the bakers with “farmer” practices. The dot’s colors indicate the PCoA cluster of the sourdough fungal community (see Figure 5). Black dots for group 1, empty dot for group 2, grey for group 3. The fungal community of the sourdough of baker 14 was not studied. B) Representation of the 20 first categories that contributed the most to the MCA axes. The category, which corresponds to a class of a variable, is written next to the triangle. C) Distribution of each variable for each bread-making practices group. Only variables that mostly explained differences between bread making practice groups are shown: use of commercial yeast, kneading method, chief origin, kg of bread production per week, number of bread making per week,

854 percent of water in dough, number of back-sloppings before making bread, water origin, sourdough age
855 and flour percentage in dough. The categories of each of these variables are indicated on the right.
856
857 **Figure 3.** Yeast species diversity was analyzed for 38 out of the 39 sourdoughs with both cultural and
858 metabarcoding methods. Left: species were identified by traditional microbial isolation and
859 identification using ITS sequencing. Right: species were identified using ITS1 metabarcoding. The
860 three most frequently-encountered species are shown in contrasting colors surrounded by black (blue:
861 *Saccharomyces cerevisiae*, red: *Kazachstania bulderi*, yellow: *Kazachstania humilis*). The bread-
862 making practice of the baker who supplied the sourdough is indicated on the left ("artisanal" in purple,
863 "farmer" in light blue).
864
865 **Figure 4.** Distribution of yeast species diversity across French sourdoughs. Each bar represents the yeast
866 species diversity of one sourdough and is placed on the map where the baker is located. Sourdoughs
867 from "farmer" practice are surrounded in light blue and sourdoughs from "artisanal" practice are
868 surrounded by purple.
869
870 **Figure 5.** Representation of sourdough depending on the weighted Unifrac distances between
871 microbial communities. A) shows the clustering of sourdoughs according to their Unifrac distances on
872 a tree (left) or on a PCoA (right). Sourdough fungal community can be clustered in three groups
873 according to their weighted Unifrac distances. B to E) shows on the left: a tree constructed from the
874 Unifrac distance matrix, in the center: sourdoughs represented on the first 2 axes of the PCoA and on
875 the right: the distribution of modalities of a variable for each group of sourdoughs (number of each
876 group on the x-axis). B) shows the distribution of bread-making practices among fungal community
877 groups, C) shows the distribution of the dominant or most frequent species among fungal community
878 (K: *Kazachstania*, C: Candida, T: Torulaspora, P: Pichia), D) shows the use of commercial
879 *Saccharomyces cerevisiae* starter and E) shows the quantity of bread per week.
880

881 **Figure 6**. Principal component analysis of 37 *K. humilis* and *K. bulderi* strains based on the
882 quantitative variation of maximum CO2 production (CO2max), fermentation latency phase (t1g),
883 maximum CO2 production rate (Vmax), time to reach the maximum production rate (tVmax), log of
884 population size and mortality at the end of the fermentation. The correlations between variables are
885 presented on the left while the figure on the right shows the projection of strains on the first two axes
886 representing 70.64% of the variation. The strains are colored according to their habitat of origin
887 (sourdough/non-sourdough). Their species is indicated by symbol.
888
889 **Figure 7**. Ratio between the sourdough strains mean and the non-sourdough strains mean values of
890 each quantitative variable measuring fermentation performance: maximum CO2 production
891 (CO2max), fermentation latency phase (t1g), maximum CO2 production rate (Vmax), time to reach
892 the maximum production rate (tVmax), log of population size. Confidence intervals are indicated by
893 bars.

894

895 Figure 1

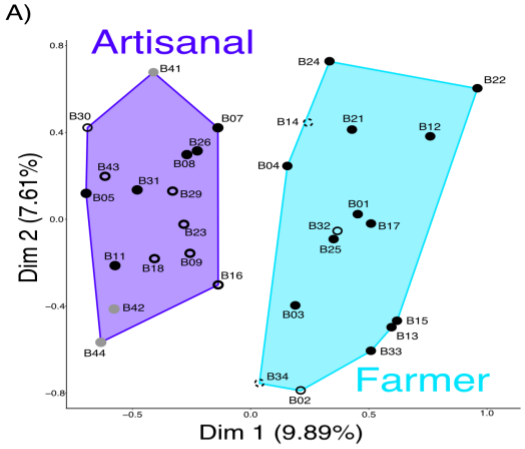



896

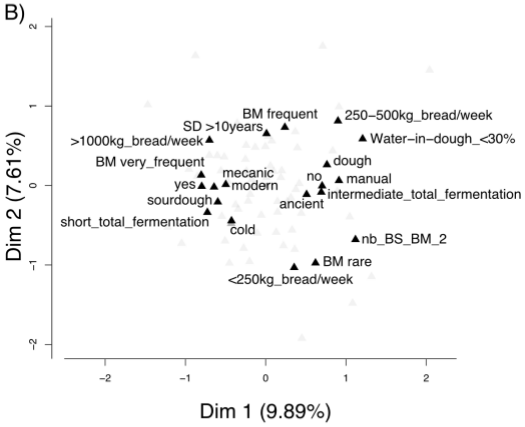
897

898 Figure 2

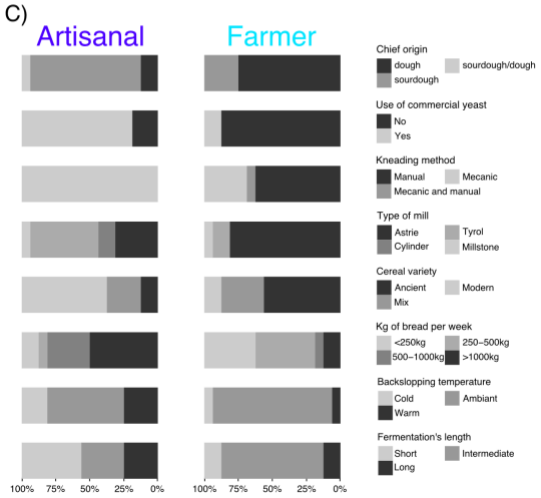
A)


Artisanal
Farmer
Dim 1 (9.89%)
Dim 2 (7.61%)
B41
B30
B07
B26
B08
B43
B31
B29
B05
B23
B11
B18
B09
B16
B42
B44
B24
B22
B14
B21
B12
B04
B01
B32
B17
B25
B03
B15
B13
B33
B34
B02

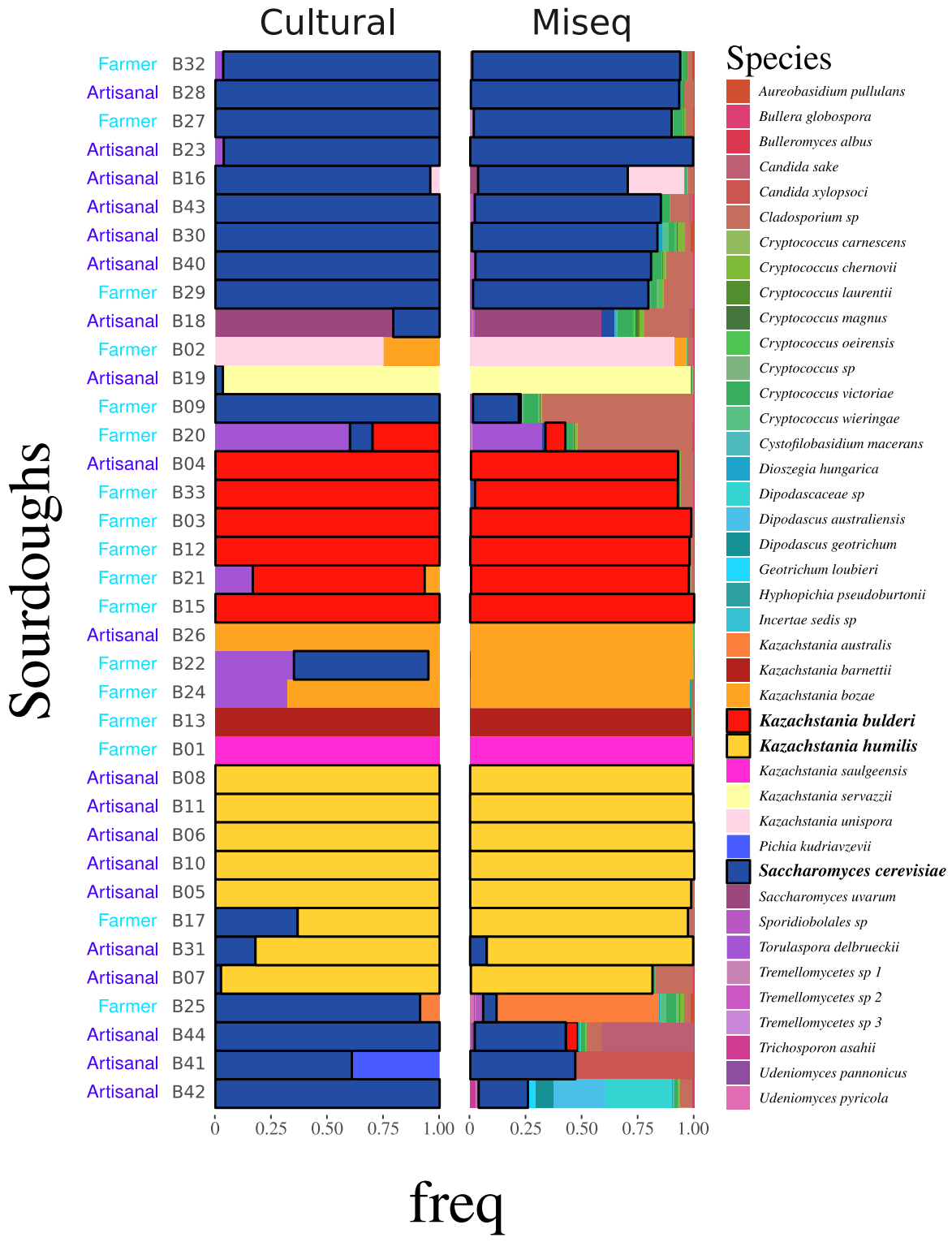
B)


BM frequent
250–500kg_bread/week
SD >10years
Water–in–dough_<30%
>1000kg_bread/week
BM very_frequent
dough
mecanic
no
manual
yes
modern
intermediate_total_fermentation
sourdough
ancient
short_total_fermentation
cold
nb_BS_BM_2
BM rare
<250kg_bread/week
Dim 1 (9.89%)
Dim 2 (7.61%)

C)

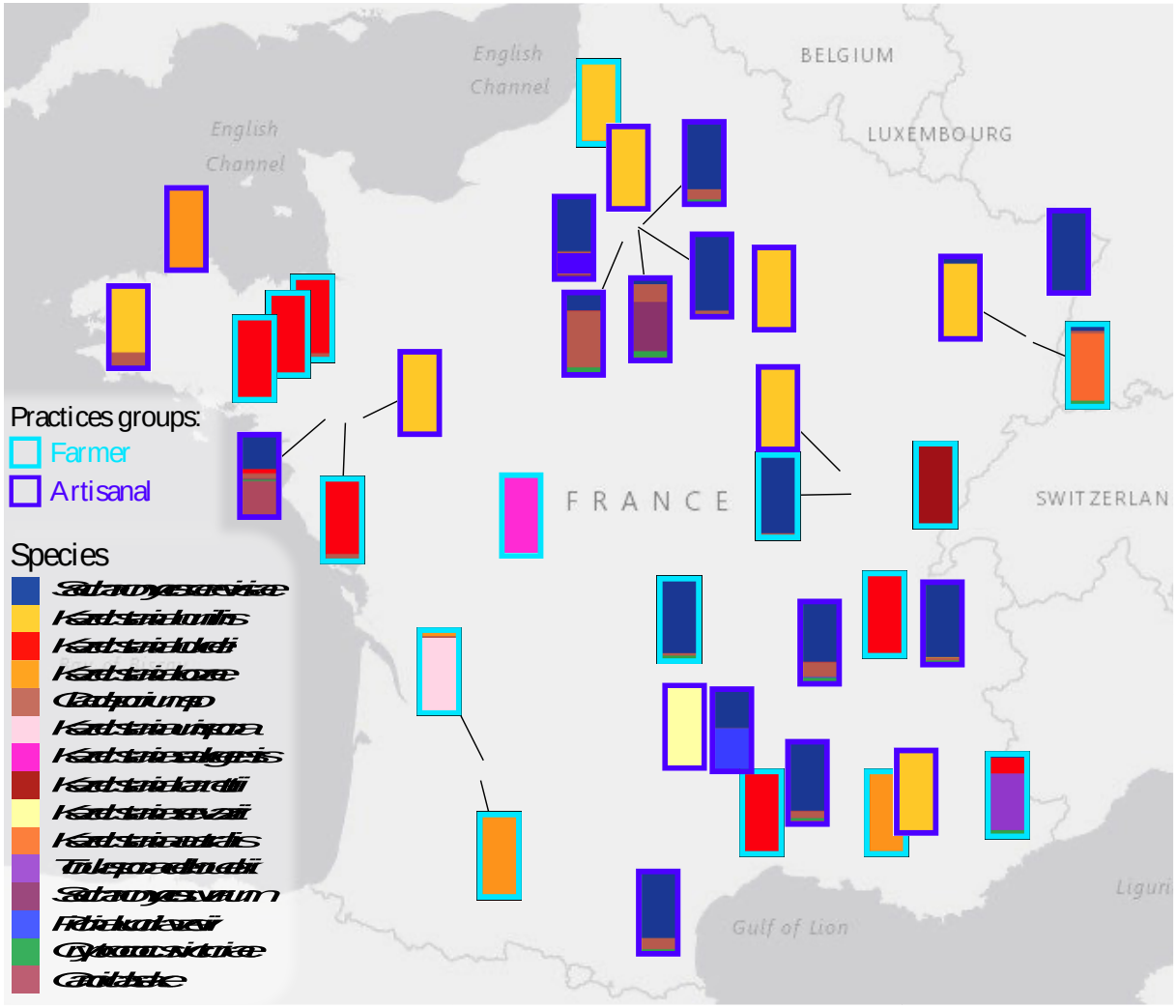

Artisanal
Farmer
Chief origin
dough
sourdough/dough
sourdough
Use of commercial yeast
No
Yes
Kneading method
Manual
Mecanic
Mecanic and manual
Type of mill
Astrie
Tyrol
Cylinder
Millstone
Cereal variety
Ancient
Modern
Mix
Kg of bread per week
<250kg
250–500kg
500–1000kg
>1000kg
Backslopping temperature
Cold
Ambiant
Warm
Fermentation's length
Short
Intermediate
Long
100%
75%
50%
25%
0%

901 Figure 3

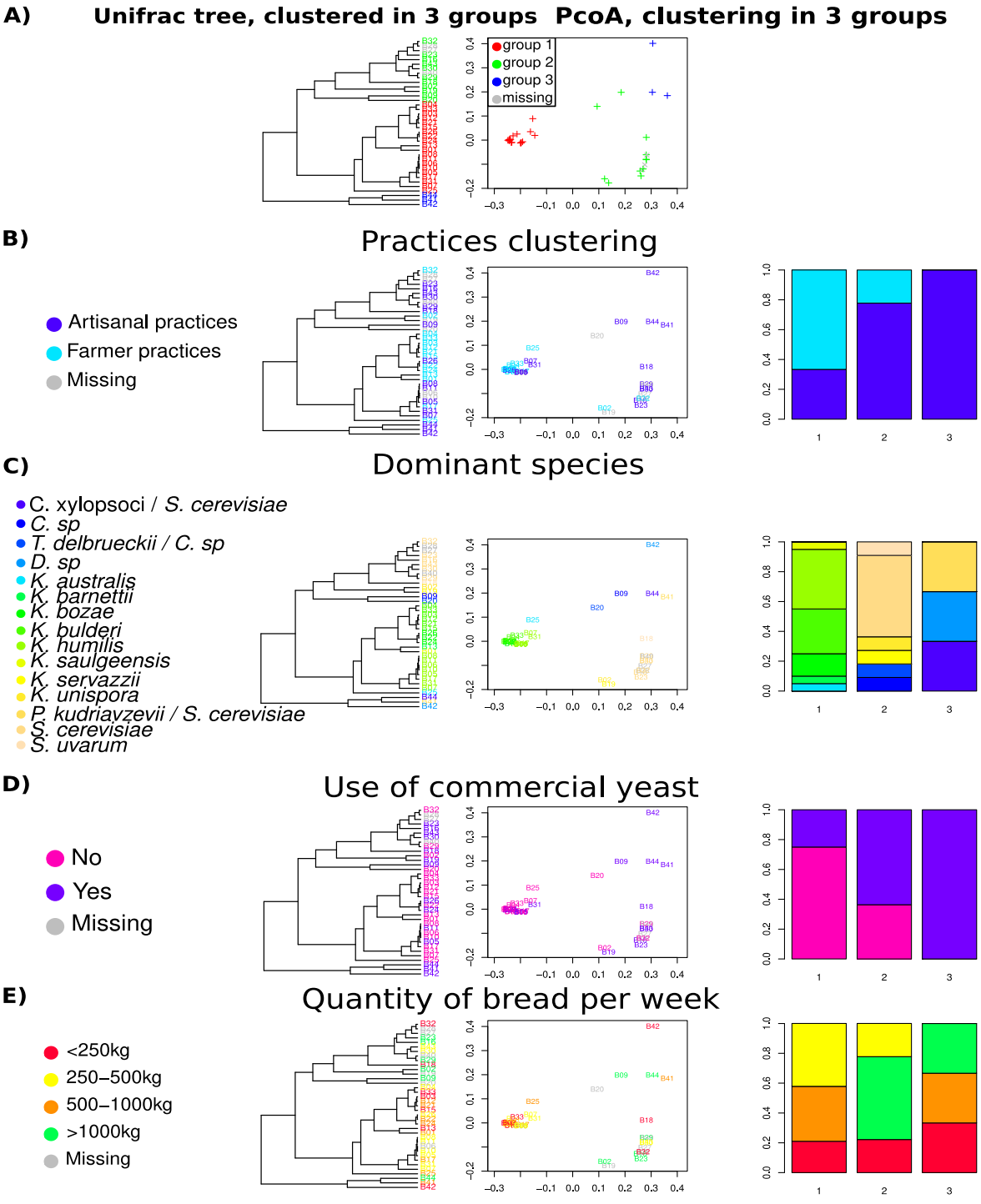



902
903

Figure 4

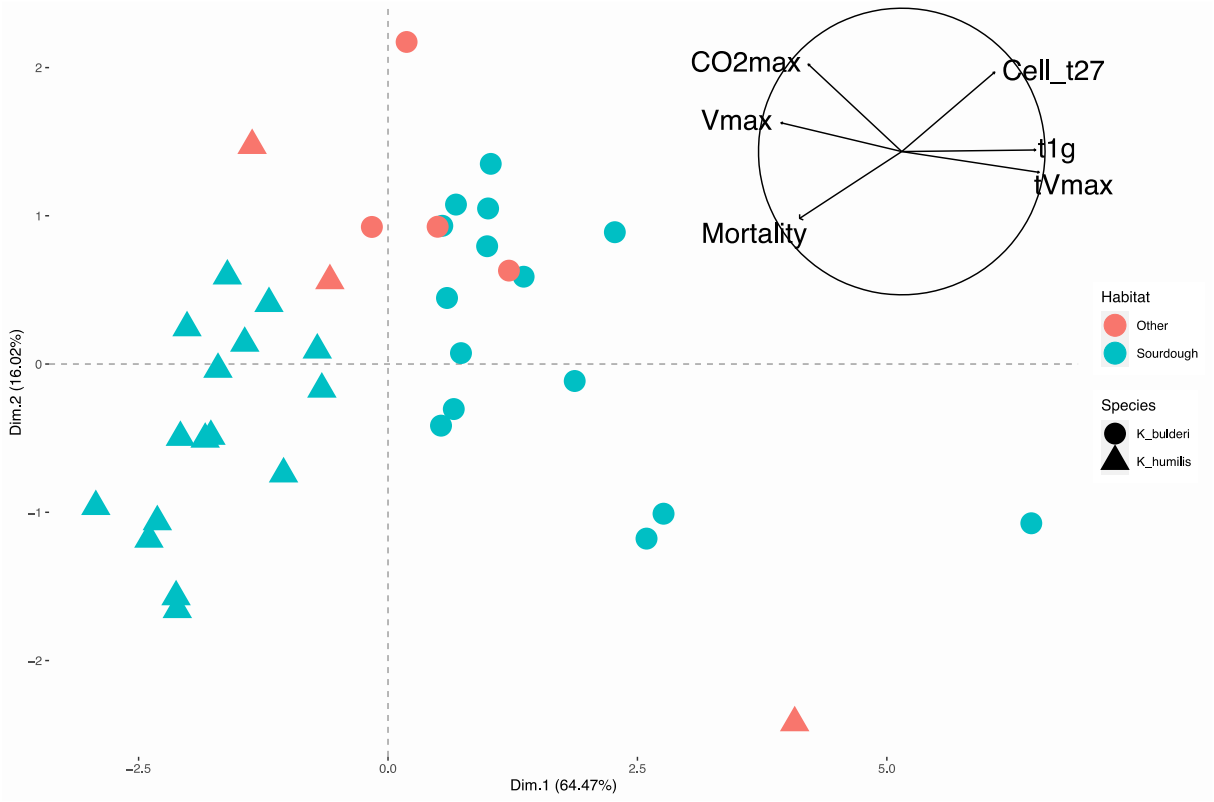

English Channel
English Channel
BELGIUM
LUXEMBOURG
FRANCE
SWITZERLAN
Practices groups:
Farmer
Artisanal
Species
Gulf of Lion

908 Figure 5
909
910




911

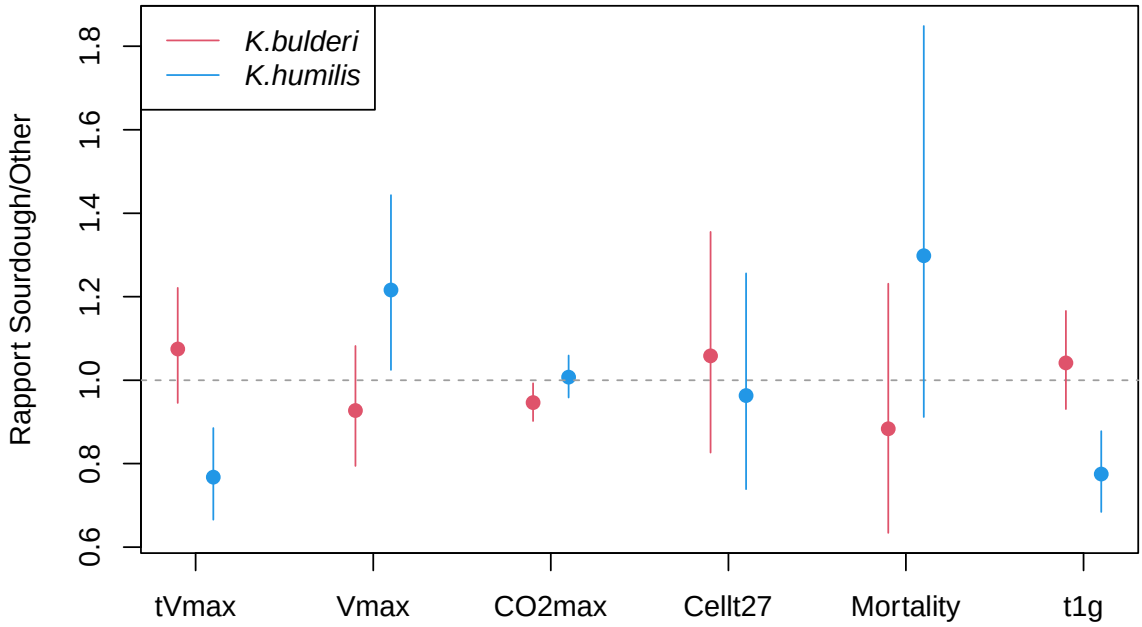
912 Figure 6
913




914

915 Figure 7

916




917