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Dairy environment and seasons affect the microbiome of a traditional artisanal cheese

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ABSTRACT

Cheese microbiome is a complex community shaped by raw ingredients and by the production environment that significantly influences final product characteristics. While environmental microbiome can establish stable resident populations, their composition remains susceptible to seasonal shifts, hygienic practices and other external factors. In this study we investigate the interplay of these factors on the bacterial and fungal communities throughout the production of a full-fat semi cooked semi-hard cow's milk cheese produced in the Piedmont region, North-West of Italy, named *Maccagno*. Amplicon based sequencing was used to characterize bacterial and fungal diversity across environmental surfaces (contact and non-contact) and during the manufacturing and ripening of Maccagno cheeses over three seasons (autumn, winter and summer). Metabolomic profiling and texture analysis of the ripened cheeses allowed for direct correlation with microbial community shifts. The facility environment maintained a remarkably stable core microbiota, including *Staphylococcus*, *Streptococcus thermophilus*, *Lactococcus lactis*, *Debaryomyces*, *Penicillium* and *Cladosporium*. Among the monitored processing plant sampling sites, the metal stirring tool, milk inlet pipe and the ripening room ventilation system emerged as critical points for microbial transfer and persistence. During ripening, core microbial taxa including *Lc. lactis*, *S. thermophilus* and *Debaryomyces* were observed. Shotgun metagenomics was then performed on final cheeses and genome reconstruction highlighted that *Lc. lactis* genomes showed impressive seasonal genomic adaptability, particularly in autumn, where it contributed to favorable texture and flavor through proteolytic activity and production of aroma-associated metabolites like acetoin and linear ketons. Conversely, summer production exhibiting the highest prevalence of spoilage-associated microbes such as *Acinetobacter* and *Enterobacteriaceae*, mainly of facility origin that led to off-flavor profiles inconsistent with the typical Maccagno sensory identity. The fungal communities, mainly composed by *Debaryomyces* and *Penicillium*, also varied seasonally, influenced significantly by the ventilation system in the ripening room. Maccagno cheese quality is a direct reflection of these complex microbial dynamics. Seasonal variations in raw milk microbiome and microbial populations established in specific environmental niches significantly affected the final product's sensory and textural attributes. To this end, understanding seasonal influences and the role of resident environmental populations is crucial for optimizing production protocols, mitigating spoilage risks, and ensuring the consistent quality of traditional cheeses.

1. Introduction

Maccagno is a full-fat semi cooked semi-hard cow's milk cheese that is traditionally made in Piedmont (Northwest Italy). It is traditionally manufactured from raw cow's milk produced by the autochthonous cattle breed "Bruna Pezzata Rossa d'Oropa" that freely grazes at a minimum altitude of 900 m between May and October while during winter is fed with dry forages obtained from similar pastures. According to the technical data sheets for traditional agri-food products (PAT) of the Piedmont region, the cheesemaking of Maccagno cheese begins with the use of raw, whole milk from one or two milkings. Coagulation occurs at a temperature of 35–37 °C, with an acidity between 3.2 and 3.5°SH/50, using veal rennet and artisanal whey starter to achieve coagulation within 30–50 min. The curd is then broken at 48–50 °C in order to reach

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the size of a rice grain. The curd is then transferred into plastic perforated molds. Molded cheeses are then pressed and left to rest for 12–24 h. During this period, cheeses are manually turned upside down 4–8 times. The cheese is then salted in brine for 48 h, before ripening, which takes place in cellars at 85–90 % relative humidity and 8–12 °C for a minimum of 20 days up to 2 months. The cheese has a cylindrical shape with flat faces, weighing approximately 2 kg. The rind is thin, brown/grey in color, smooth and regular, becoming firmer and slightly wrinkled with longer maturation. Its flavor is sweet, reminiscent of milk cream and butter. The aroma is intense, especially in the summer product, with no sharp or pungent sensations. The texture is compact and elastic with a soft structure.

The sensory properties of cheeses result from complex microbiome interactions and compositions, which are susceptible to environmental changes. The microbiome composition and its modifications are influenced by several factors, including seasonality, the microbiome of raw milk and the production environment, the cheesemaking, the ripening temperature, the water activity and the potential contamination from operators (Ferrocino et al., 2022a).

Environmental microbiome in dairy industries can actively participate in cheese fermentation and ripening and contributes to the typical flavor and texture (De Filippis et al., 2024). In addition, shifts in raw milk and environmental microbiome according to season can have a significant impact not only on the sensory qualities, but also on the microbiological safety of the final products (Kable et al., 2016). A detailed analysis of the microbiome in both the production environment and along the cheesemaking is essential for characterizing the microbial profile of food products (Barcenilla et al., 2024). This approach not only helps define the authentic microbiological identity of traditional foods but also plays a critical role in identifying potential sources of contamination (Bokulich et al., 2015; Doyle et al., 2017; Zwirzitz et al., 2020), preventing sensory defects in the final product (Walsh et al., 2020), and guiding targeted interventions to shape the microbial community. Therefore, in-depth microbiome mapping is a foundational step in modern food microbiology, offering powerful insights for quality management (Ferrocino et al., 2022b). The aims of this study were to: i) investigate the microbiome of a cheesemaking plant that produces a traditional ripened cheese from raw cow milk over three seasons and ii) track the origins and dynamics of bacterial and fungal populations from the plant environment to the final products. Investigating the

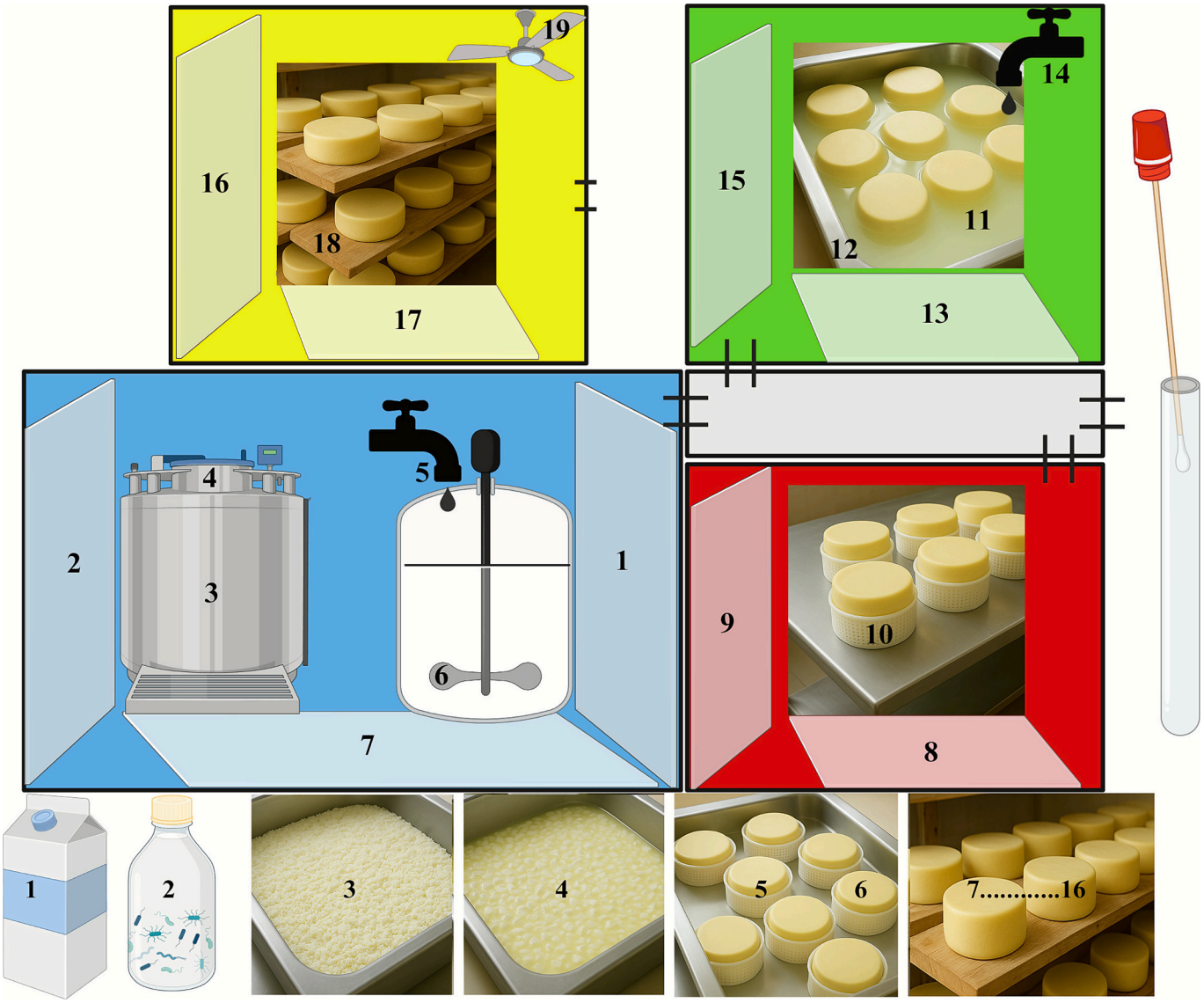

Fig. 1. The Fig. presents a floorplan of the dairy plant, with numbered locations indicating the environmental sampling points where swabs were collected. Additionally, the Fig. illustrates the numbered steps of the cheese making process, providing a comprehensive overview of both the facility layout and the cheese manufacturing workflow. The legend of the samples are reported in Supplementary Table 1.

microbiota, mycobiota, and metabolome was crucial for understanding how seasonal variations and the production environment impact the final quality of cheese. By discovering the route of microbial transmission, producers can implement targeted interventions to maintain desired sensory qualities and prevent off-flavors or textures. This study sheds light on how environmental microbes contribute to the unique characteristics of a traditional ripened cheese and how these interactions can be managed to maintain high product quality.

2. Materials and methods

2.1. Production plant and sample collection

All the samples for this study were taken from the same dairy plant, located in Biella, Piedmont region in northwest Italy. The plant included a processing area, a molding room, a brining room, and a ripening room. All these areas were connected by a corridor, with the exception of the ripening room which had a separate entrance (Fig. 1). The plant was visited three times, in October (autumn), January (winter) and July (summer). Environmental sampling was performed in the exact same locations within the facility during each visit and samples were collected before production started and a few hours after the cleaning and disinfection procedures. Along the three seasons, a total of 57 environmental samples (25 food contact surfaces and 32 non food contact surfaces) were considered (Fig. 1 and Supplementary Table 1). Additionally, 141 samples were collected throughout the production, covering the following stages: raw milk, whey, curd after heating, curd after cutting, cheese after brining and cheese at various stages of ripening (7, 15, 30, 45, and 60 days) (Fig. 1 and Supplementary Table 1).

Three independent samples were collected from each environmental site using a sterile swab:(Swab Rinse Kits Copan, VWR, Milan, Italy): 100 cm^2 or 10 cm^2 for metal stirring tool, milk inlet pipe, cheese mold and ventilation system ripening area. For brine and water, 10 mL were collected in a sterile tube. One hundred mL of raw milk and whey and 150 g of curd after heating and curd after cutting were collected in a sterile container. Three cheeses after brining and 3 cheeses from each ripening stage (7, 15, 30, 45, and 60 days) were sampled (Fig. 1 and Supplementary Table 1).

All samples were then transported under refrigeration to the laboratory and subjected to analysis within 2 h of collection.

Sampling on cheese loaves was carried out using a sterile punch that was inserted perpendicular to the center of the cheese and then rotated 360° to collect samples (Alessandria et al., 2016). The portion of cheese below 1 cm from the rind and the central portion were then used to separately analyze the rind and core of the cheese.

2.2. DNA extraction and bioinformatics analysis

For environmental swabs, the three independent swabs from each surface in each season were pooled to increase the microbial load recovered. The tip of each swab was carefully inserted into the 2 mL PowerBead Tube (Qiagen, Milan, Italy), together with 800 μL of solution CD1 (Qiagen). For brine, water, milk and whey, 2 mL were directly used.

For cheeses, 10 g were diluted into 45 mL of STE Buffer (100 mM NaCl, 10 mM Tris-Cl pH 8.0, 1 mM EDTA) in a stomacher bag and 2 mL were then used. DNA was extracted by using the DNeasy PowerSoil Pro Kits (Qiagen) according to the manufacturer's instructions.

DNA was first quantified with a Qubit fluorometer using the Qubit high-sensitivity double-stranded DNA (dsDNA) quantification kit (Thermo, Milan Italy). A total of 100 ng of DNA was used to amplify the V3-V4 region of the 16S rRNA gene (Klindworth et al., 2013) for the microbiota and the D1-D2 region of the 26S rRNA gene for the mycobiota (Mota-Gutierrez et al., 2019). Amplicon sequencing (2X250bp) was performed by the Novogene company (Munich, Germany). Final cheese samples were then used for whole metagenomics shotgun (2X150bp) performed by the Novogene. QIIME2 software (v. 1.9) (Bolyen et al., 2019) was used to process the amplicon raw reads. Amplicon Sequence Variants (ASVs) were generated using the DADA2 algorithm (Callahan et al., 2016) and subsequently mapped against the Greengenes2 database for taxonomic assignment of bacteria or SILVA database for fungi. To ensure comparability across samples, ASV tables at the lowest taxonomic resolution were normalized to the lowest sequence count per sample. For the shotgun sequencing data, initial processing involved filtering out adapters, low-quality reads, and sequences shorter than 50 bp with adapter removal software (Schubert et al., 2016). Host reads were then excluded using Bowtie2 (v.2.4.4) (Langmead & Salzberg, 2012) against the *Bos taurus* genome in end-to-end sensitive mode. Cleaned, non-host reads were assembled into contigs using MetaSPAdes (v. 3.11.0) (Nurk et al., 2013). Metagenome-assembled genomes (MAGs) were constructed using MetaBat2 (Kang et al., 2019), with their quality assessed via CheckM (Parks et al., 2015). Only MAGs demonstrating completeness greater than 90 % and contamination less than 10 % were included in further analysis. Taxonomic assignment at the species-level genome bin (SGBs) pangenome calculations and gene annotation were performed with Tormes (Quijada et al., 2019).

2.3. Texture analysis and pH determination

Texture analysis was performed using a TAXT2i Texture Analyzer (Stable Micro System, Godalming, UK) equipped with a 25 kg load cell and a P/35 cylinder probe as already reported (Bertolino et al., 2011). Briefly, 3 cubes of 8 cm^3 were cut from the core of each cheese at the end of ripening and immediately analyzed. A TPA test was conducted by compressing the samples to 30 % of their original height using a test speed of 0.8 mm/s. A 5 s rest period was applied between the two compression cycles. For the acquisition of the force–time curve, a Texture Export Exceed software v2.54 (Stable Micro Systems, Godalming, UK) was used. The following parameters were measured in triplicates from the force–time curves: hardness (N), cohesiveness (adimensional), adhesiveness (mJ), gumminess (N), springiness (mm), chewiness (mJ) and resilience (adimensional). The pH values of cheese samples were determined with a pHmeter Model 300 (Hanna Instruments, Padova, Italy) equipped with a solid electrode HI2031 directly inserted in the food matrix.

2.4. VOCs determination

Volatile organic compounds analysis was conducted using solid-phase microextraction (SPME) according to Ruggirello et al. (2018). Briefly, 1.5 g of cheese sample at the end of ripening was dispersed in 1.5 g of 30 % NaCl solution and added 10 mL of internal standard solution (2-octanol) at 10 ppm and fitted with a PTFE silicone septum (Supelco, Bellefonte, PA, USA). Then, headspace solid phase micro extraction (HS-SPME) syringe needle, fitted with a Stable Flex 1 cm 50/30 mm divinylbenzene-carboxen polydimethylsiloxane (DVB-CAR-PDMS) fibre (Supelco, Bellefonte, PA, USA), was introduced through the septum. The microextraction procedure included a 15 min equilibration at 40 °C, 30 min of extraction at 40 °C, and a 2 min splitless injection at 260 °C. GC/qMS analysis was performed with a Shimadzu GC-2010 gas chromatograph equipped with a Shimadzu QP-2010 Plus quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan) and a DB-WAXETR capillary column (30 m × 0.25 mm, 0.25 μm film thickness, J&W Scientific Inc.,Folsom, CA, USA). The temperature program started at 40 °C for 5 min, increased at a rate of 10 °C min^{-1} to 80 °C, then at rate of 5 °C min^{-1} to 240 °C. The carrier gas (He) flow-rate was 1 mL min^{-1}. The injection port temperature was 260 °C; the ion source and the interface temperature were 240 °C. The detection was carried out by electron impact mass spectrometry in total ion current (TIC) mode, using an ionization energy of 70 eV. The mass acquisition range was m/z 33–300. Peak identification of each volatile metabolite was performed by comparison of the retention time and mass spectra of eluting

compounds to those of the pure standard. Semiquantification (μg/kg) was performed using the area relative to the internal standard. All measurements were conducted in triplicate.

2.5. Statistical analysis

The alpha diversity index (including Shannon and richness) was calculated using the *vegan* package of R (Dixon, 2003). Principal Component Analysis (PCA) was performed on ASV relative frequencies using the *ade4* package in R (Culhane et al., 2005) and ANOSIM statistical test was performed. To assess the statistical significance of differences between ASVs as a function of the season Wilcoxon rank-sum tests were performed. ANOVA and the Tukey's Honestly Significant Difference (HSD) post-hoc test was performed on texture parameters and metabolomic data. The machine-learning classification tool, SourceTracker2 (Knights et al., 2011), was used to assess the tracking of microbial communities from the environment to the final cheese product. Spearman's correlation between ASVs, texture parameters and metabolomic was performed through the *corr.test* package of R. Bar charts, box plots, PCA and pie/venn charts were generated using the *ggplot2* package whereas correlation network through the *ggraph* package of R.

3. Results

3.1. Alpha diversity and shift in microbiota and mycobiota composition

Alpha diversity calculation (based on Shannon and richness) of cheese throughout the production, showed a significant increase for both indexes, for microbiota (Fig. 2A and B, P ≤ ≤0.05) and mycobiota (Fig. 3A and B, $P \leq 0.05$) with the highest value in summer, followed by winter and autumn. No significant differences in alpha diversity between seasons were observed for the environmental swabs (Fig. 2A and B and 3A and B, $P > 0.05$).

PCA of microbiota composition showed a significant shift in the composition in cheese through ripening according to the seasons (Fig. 2C, P ≤ 0.05), while no clear clustering for the environmental swabs (Fig. 2D P > 0.05) was observed. On the other hand, for mycobiota, cheese during ripening collected in autumn and winter clustered together and were well separated from samples collected in summer (Fig. 3C, P ≤ 0.05), while no clear clustering for the environmental swabs was observed as a function of the season (Fig. 3D, P > 0.05).

3.2. Bacterial profiling of environmental swabs, milk, whey, and cheese throughout the cheese production chain

During autumn (Fig. 4), in the production environment,

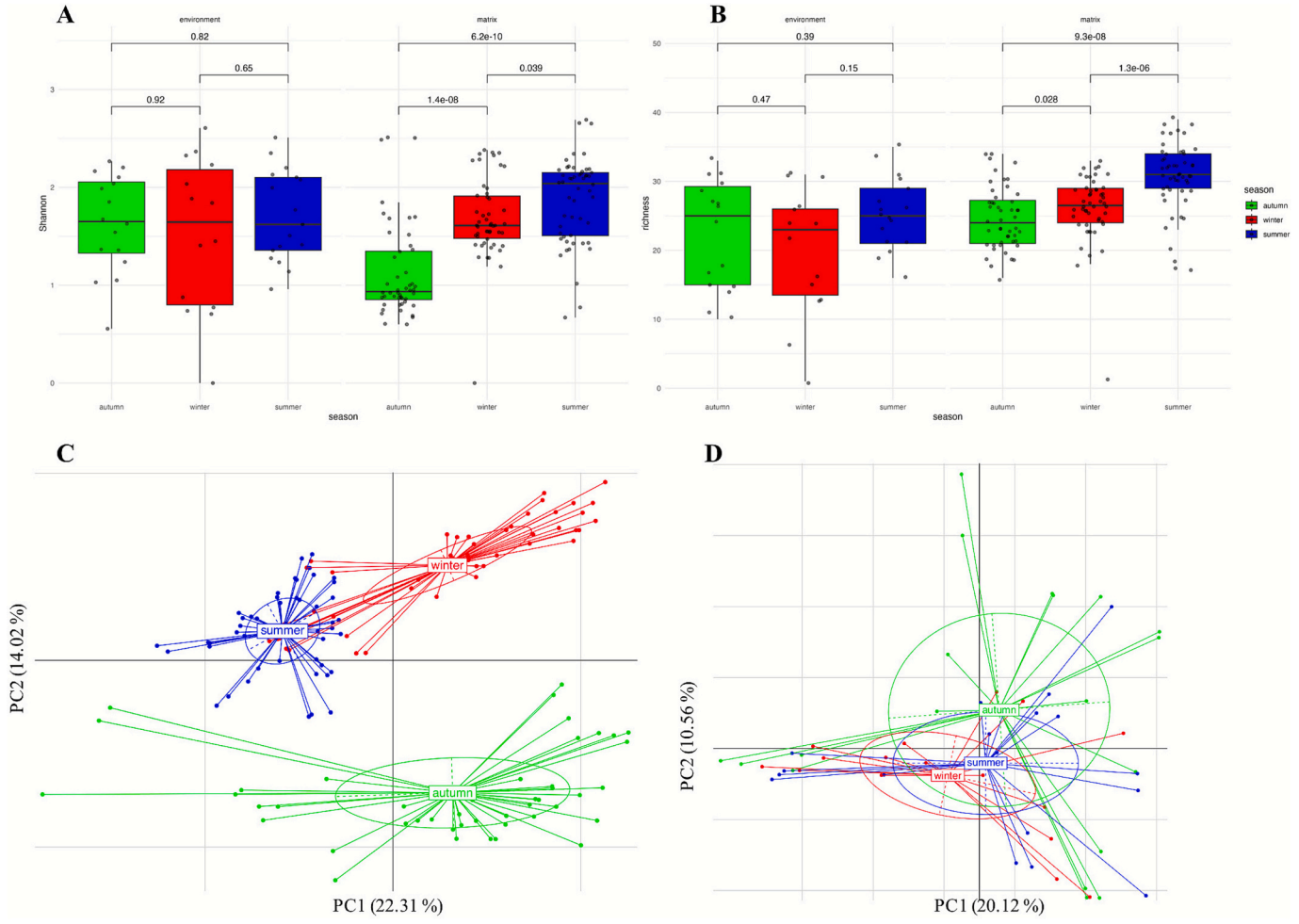

Fig. 2. Boxplots to describe the bacterial α-diversity measures (Shannon, plot A and richness, plot B) of microbiota of environmental or cheese related samples collected in autumn (green bars), winter (red bars) and summer (blue bars). Individual points and brackets represent the richness estimate and the theoretical standard error range, respectively. Plot C and D showed the Principal Component Analysis (PCA) based on ASVs relative frequency of cheese related samples (plot C) and environmental samples (plot D) collected in autumn (green), winter (red) and summer (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

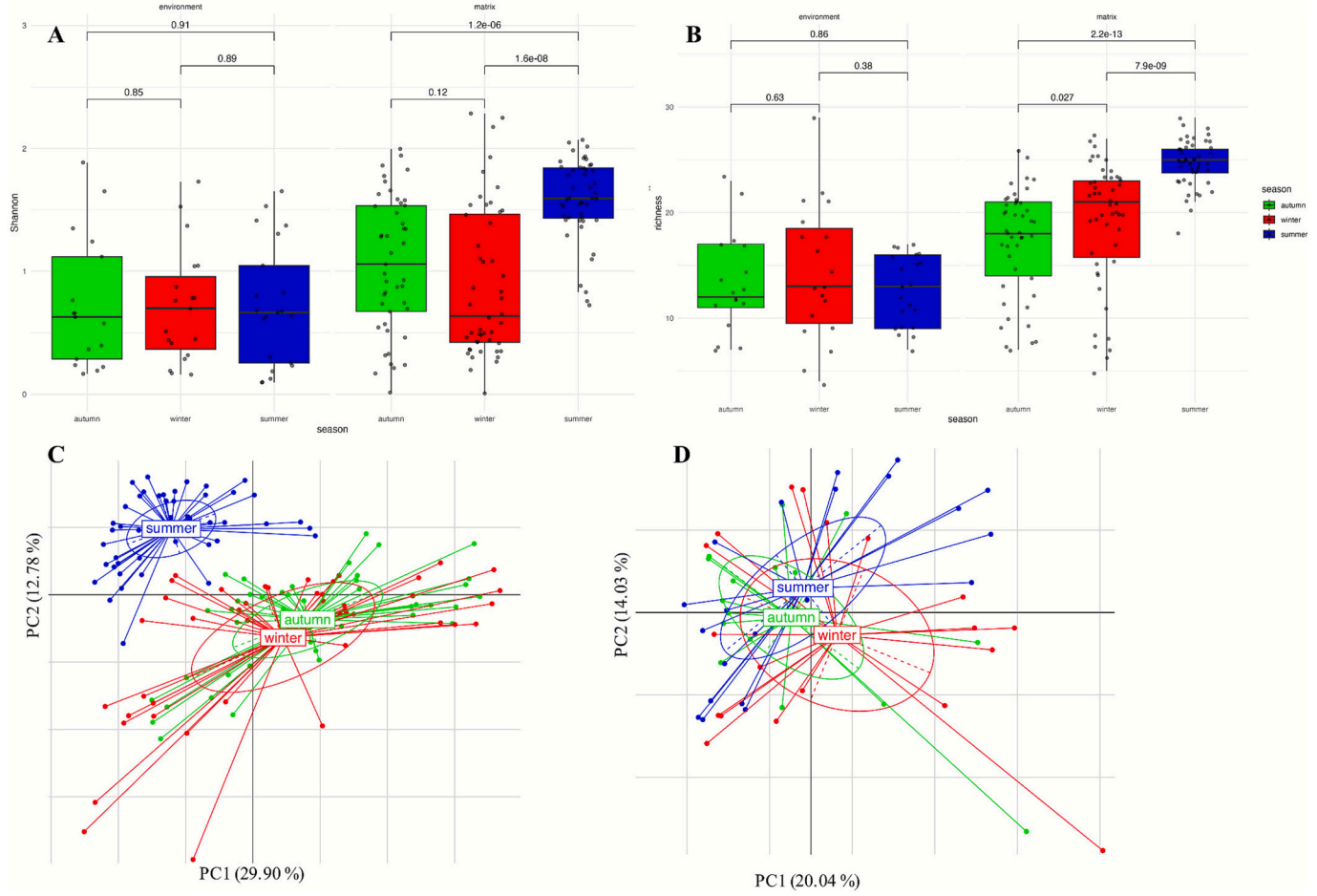

Fig. 3. Boxplots to describe the fungal α-diversity measures (Shannon, plot A and richness, plot B) of mycobiota of environmental or cheese related samples collected in autumn (green bars), winter (red bars) and summer (blue bars). Individual points and brackets represent the richness estimate and the theoretical standard error range, respectively. Plot C and D showed the Principal Component Analysis (PCA) based on ASVs relative frequency of cheese related samples (plot C) and environmental samples (plot D) collected in autumn (green), winter (red) and summer (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Staphylococcus (28 % of the relative frequency), *Methylobacterium adhaesivum* (14 %) and *Lactococcus lactis* (11 %) dominated the production room walls. Equipment such as heater vat and metal stirring tool were rich in *Lc. lactis* (44–50 %, respectively) and *Streptococcus thermophilus* (22–39 %). The floor in the molding room exhibited the highest levels of *Chryseobacterium* (24 %) and *Lc. lactis* (14 %). Brine and brine tanks were dominated by *Lactobacillus*, *S. thermophilus* and *Acinetobacter*. The floor and the wall in the brining room were rich in *Staphylococcus* (50 %) and *Lactobacillus* (56 %), respectively. The ripening room was dominated by *Brevibacterium* (up to 42 %), *Methylobacterium* (13 %) and *Serratia* (11 %). (Fig. 4). Raw milk was characterized by *Acinetobacter* (22 %), *Streptococcus parauberis* (12 %) *Lactococcus raffinolactis* (10 %) and in minor fraction from *Pseudomonas* and *Enterobacteriaceae*. The whey starter was dominated by *Lc. lactis* (52 %) and *S. thermophilus* (14 %). Cheese at the end of ripening displayed a simple microbiota characterized by *S. thermophilus* (68 % core, 63 % rind), and *Lc. lactis* (28 % core, 30 % rind).

In winter (Fig. 4), the production room was heavily colonized by *Lc. lactis* and *Staphylococcus*. Brine showed dominance by *Lactobacillus* (75 %) and *Pseudomonas fragi* (8 %), while the brine tank of *S. thermophilus* (33 %) and *Psychrobacter* (6 %). Ripening wooden boards and the ventilation system in the ripening area featured *Brevibacterium* (19 %), *Psychrobacter* (29 %), and *M. adhaesivum* (30 %). Milk inlet and metal stirring tool pipe were dominated by *Lc. lactis* (52 % and 54 %) and *Staphylococcus* (46 % and 45 %). Milk and whey starter displayed the predominance of L. *raffinolactis* (38 %), *Acinetobacter* (15 %), *P. fragi* (12 %), *Carnobacterium* (7 %) and *Leu. lactis* (9 %). Cheese maintained dominance by *S. thermophilus* (55 % core, 42 % rind), with highest levels of *Lacticaseibacillus zeae*, *Latilactobacillus sakei*, *Leu. mesenteroides*, *Leu. pseudomesenteroides*, and *Lactiplantibacillus plantarum*, particularly in the rind (Fig. 4).

In summer (Fig. 4), the production room walls showed high levels of *Lc. lactis* (52 %) and *Staphylococcus* (16 %). Milk inlet pipe and metal stirring tool were dominated by *S. thermophilus* (50 %), *Lc. lactis* (34 %), *Staphylococcus* (35 %), *M. caseolyticus* (15 %), *A. johnsonii* (8 %), *Acinetobacter* and (5 %), *Sphingomonas* (5 %). Heater vat was colonized by *Staphylococcus* (44 %), *Pseudomonas* and *M. caseolyticus* (15 %). Brine and brine tanks were dominated by *Lactobacillus* and *S. thermophilus.* The molding room floor displayed the dominance of *Pseudomonas* and *Staphylococcus*, while water carried *Brevibacterium* (46 %) and *Arthrobacter* (20 %). Ripening areas were dominated by *Psychrobacter* (53 %) and *Brevibacterium* (40 %) (Fig. 4). Summer milk exhibited a more diverse profile, with *Acinetobacter* (13 %), *Lc. lactis* (14 %), *L. raffinolactis* (10 %), *S. thermophilus* (9 %), *Enterobacteriaceae* (8 %), *Lc. garvieae* (7 %) and *M. caseolyticus* (4 %), along with a minor fraction composed by *Lc. garvieae*, *Citrobacter*, *Staphylococcus aureus*, *Enterococcus*, *Micrococcus*, *Brochothrix*, *Carnobacterium, A. guillouiae* and *Pseudomonas*.

Cheese was characterized by *S. thermophilus* (43 % core, 42 % rind), *Lc. lactis* (10 % core, 17 % rind) and a minor fraction of *Lc. zeae*, *Lactobacillus*, *Leu. mesenteroides*, *Acinetobacter*, *A. johnsonii*,

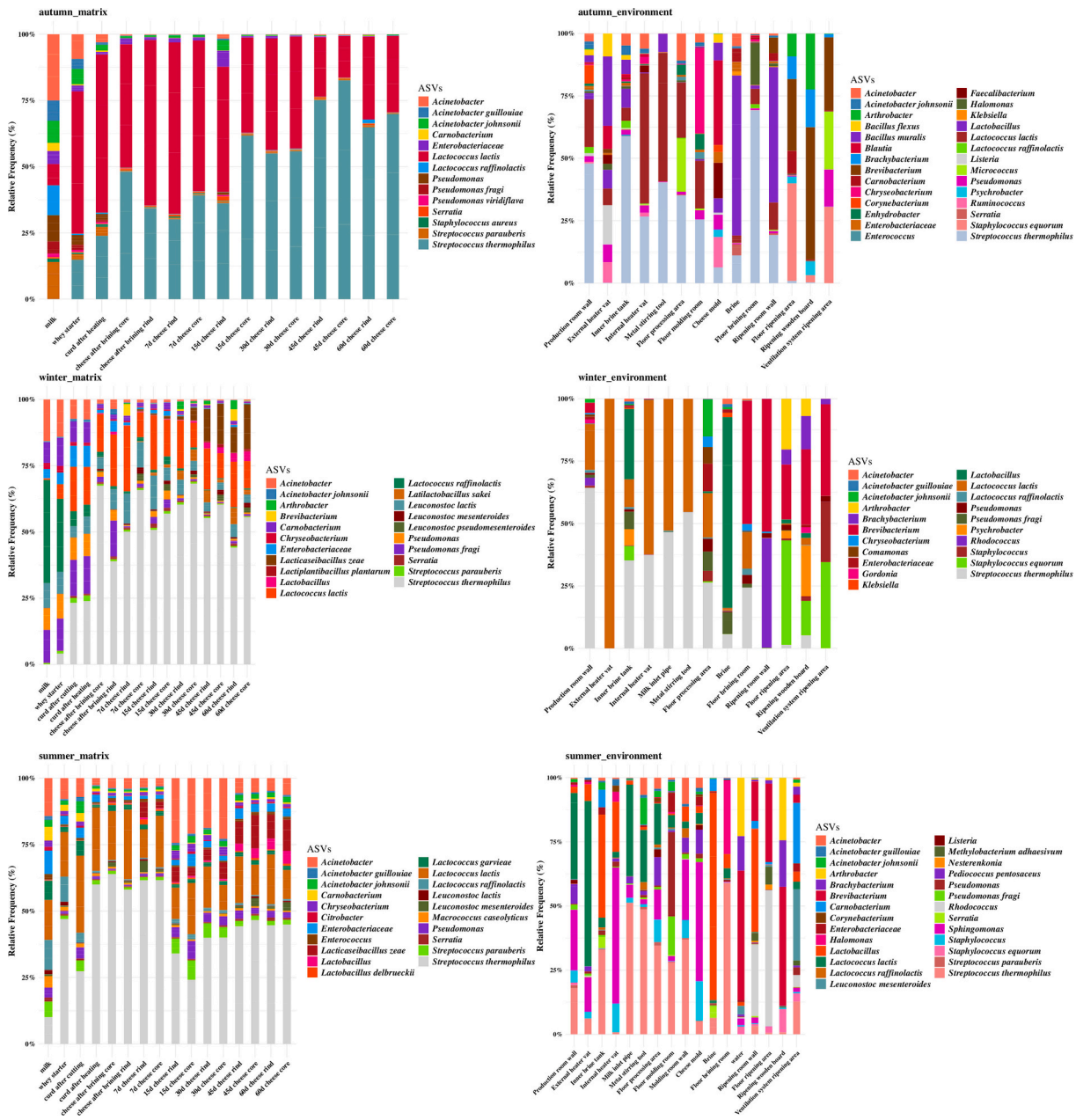

Fig. 4. Stacked bar plots show the relative frequency of bacterial ASVs (with a relative frequency > 2 % in at least 3 samples) across different sample types and seasons. Each bar represents a specific sampling point (environment or matrix), ordered according to the production workflow. Colors correspond to individual ASVs. The y-axis indicates proportional abundance expressed as relative frequency (%).

Enterobacteriaceae, *Serratia* and *Pseudomonas* (Fig. 4).

3.3. Fungal profiling of environmental swabs, milk, whey, and cheese throughout the cheese production facility

In autumn, *Candida* dominated the internal heater vat (49 %), while *Debaryomyces* prevailed on metal stirring tools (40.37 %), floor in the molding room (52 %), brine (95 %), and brine tank (91 %) (Fig. 5). *Penicillium*, *Cladosporium*, and *Fusarium* were also detected across surfaces, particularly in the molding and ripening areas. Ripening wooden boards were characterized by *Debaryomyces* (83 %), *Penicillium* (10 %) and *Galactomyces* (0.5 %). The ventilation system in the ripening area was dominated by *Debaryomyces* (64 %) and *Penicillium* (35 %). Autumn milk and whey were rich in *Galactomyces* (37 % and 26 %, respectively) and *Geotrichum* (26 % and 20 %), with notable presence of *Clavispora* and *Kluyveromyces*. In 60-day cheeses, *Debaryomyces* was abundant in the rind (95 %) and in the core (41 %), while *Kluyveromyces* dominated the core (43.48 %).

In winter (Fig. 5), *Debaryomyces* dominated most facility sites, especially brines (96 %), heater vat (78 %), and ripening boards (88 %). *Penicillium* showed high levels on the production room wall (77 %). Milk inlet pipe was dominated by *Debaryomyces* (18 %) and *Saccharomyces* (48 %), with a notable presence of *Penicillium* (9 %) and *Fusarium* (6 %). Metal stirring tools were also rich in *Debaryomyces* (84 %), *Saccharomyces* (2 %), *Alternaria* (0.6 %), and *Wickerhamiella* (2 %). Ripening boards also showed a strong dominance of *Debaryomyces* (88 %), *Penicillium* (5 %) and *Galactomyces* (2 %). The ventilation system in the ripening area was dominated by *Debaryomyces* (91 %) and *Starmerella* (1 %). Winter milk and whey were characterized by *Galactomyces* (milk: 55 %, whey: 43 %) and *Geotrichum* (Fig. 5). Cheese cores were primarily characterized by *Debaryomyces* (66 %) and *Kluyveromyces* (17 %), while rinds in *Debaryomyces* (90 %).

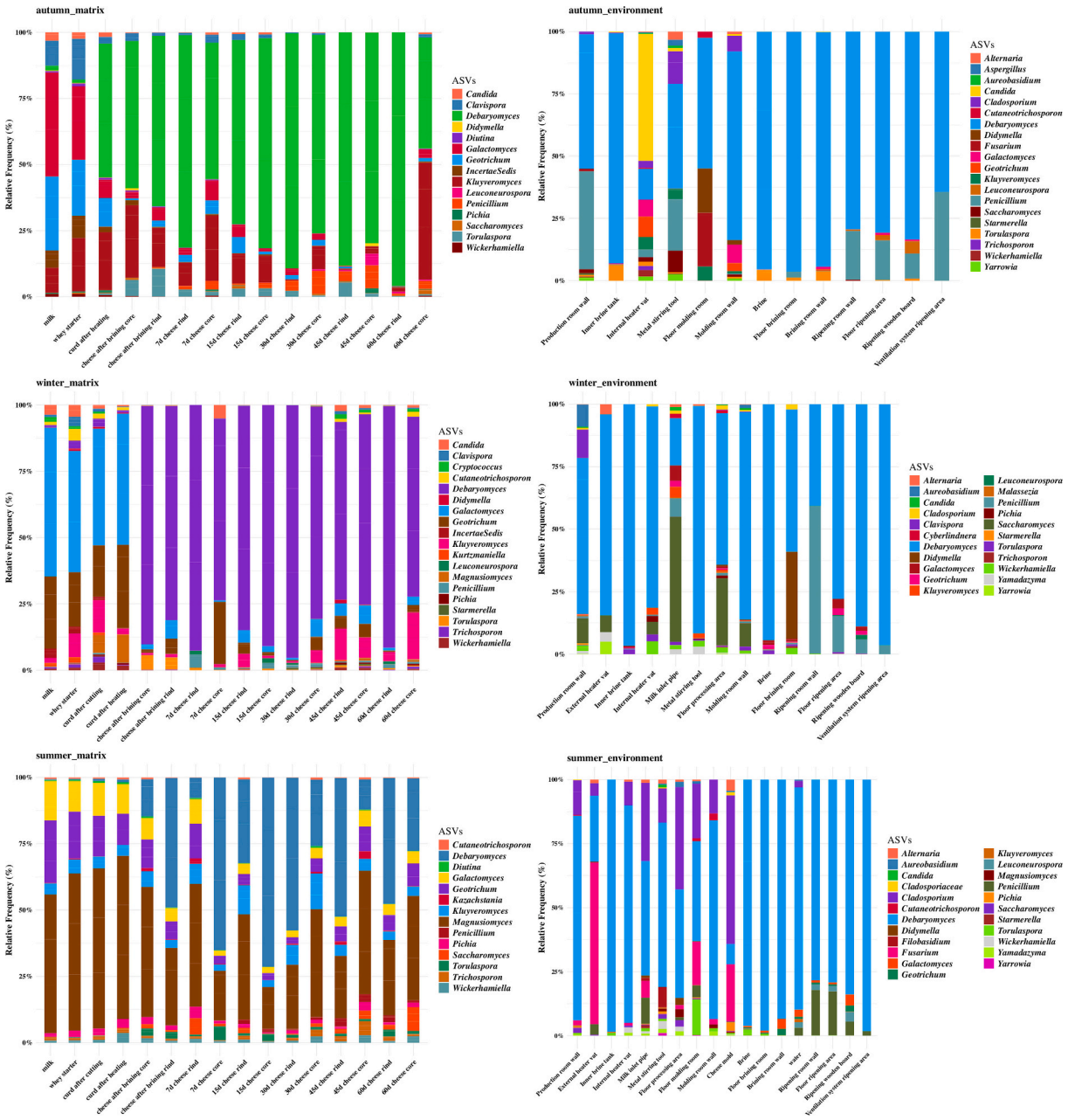

Fig. 5. Stacked bar plots show the relative frequency of fungal ASVs (with a relative frequency > 2 % in at least 3 samples) across different sample types and seasons. Each bar represents a specific sampling point (environment or matrix), ordered according to the production workflow. Colors correspond to individual ASVs. The y-axis indicates proportional abundance expressed as relative frequency (%).

In summer (Fig. 5), *Debaryomyces* remained dominant throughout the facility, with the highest value in the ventilation system in the ripening area (96 %), along with *Cladosporium* (37 % on molding room floors) as well as *Fusarium*, and *Penicillium* on floors and walls in all the facility. Milk inlet pipe was dominated by *Debaryomyces* (43 %), *Cladosporium* (29 %) and *Penicillium* (9 %). Metal stirring tools showed presence of *Debaryomyces* (63 %), *Cladosporium* (13 %), and *Alternaria* (1 %). Ripening boards were characterized by *Debaryomyces* (83 %), *Penicillium* (5 %), and *Galactomyces* (4 %). (Fig. 5). Summer milk and whey were characterized by *Magnusiomyces* (50 % and 56 % respectively), followed by *Geotrichum* and *Galactomyces*. Cheese cores were dominated by *Magnusiomyces* (38 %) and *Debaryomyces* (26 %) while rinds displayed *Debaryomyces* (46 %) but also notable contributions from *Geotrichum*, *Galactomyces*, and *Kluyveromyces*.

3.4. Seasonal variation in milk and final cheese microbiota and mycobiota composition

The microbiota and mycobiota composition of milk exhibited significant seasonal variation (ANOSIM $P \leq 0.01$). Specifically, milk collected in autumn showed a significant association with *A. guillouiae*, *A. johnsonii*, *Lc. lactis*, *Pseudomonas veronii*, *Pseudomonas viridiflava*, *Staphylococcus aureus*, *Clavispora* and *Kluyveromyces* (Supplementary Fig. 1, $P \leq 0.01$). In winter, the microbial community was associated with *Brochothrix*, *Carnobacterium*, *Lc. raffinolactis*, *Leuconostoc lactis*, *P. fragi*, *Candida*, *Galactomyces*, *Cryptococcus* and *Kurtzmaniella* ($P \leq 0.01$). Summer milk was characterized by *Citrobacter*, *Enterococcus*, *Lacticaseibacillus. zeae*, *Lb. delbrueckii*, *Lc. garvieae*, *Serratia*, *S. thermophilus*, *Kazachstania*, and *Pichia* (Supplementary Fig. 1, $P \leq 0.01$).

Final cheese microbiota displayed distinct significantly seasonal patterns. No significant differences in *Lc. lactis* and *S. thermophilus* were observed between summer and winter; however, both ASVs were significantly associated with autumn cheeses. (Supplementary Fig. 2, P $\leq$ 0.01). Winter was associated with *Ltb. sakei*, *Leu. Lactis* while summer with *Acinetobacter*, *A. guillouiae* and *A. johnsonii*, *Chryseobacterium*, *Citrobacter*, *Lactobacillus*, *Lb. delbrueckii* and *Pseudomonas.* No significant differences in *Enterobacteriaceae*, *Enterococcus*, *Geotrichum*, and *Lc. garvieae* were observed between autumn and winter, however those ASVs were mainly associated with summer cheeses (Supplementary Fig. 2, P $\leq$ 0.01).

3.5. Seasonal microbial dynamics and environmental contributions to cheese microbiome

The SourceTracker analysis revealed that both bacterial and fungal communities in 60-day cheese cores and rinds originate from multiple environmental reservoirs within the production facility (Fig. 6A and B). For bacteria, the stirring tool accounted for about 29 % of the bacteria in the core and 28 % on the rind, while the milk inlet pipe contributed 23 % and 22 %, respectively. Additional significant sources included the non-contact surfaces such as: wall near the heater (core: 5 %, rind: 4 %), wall of the production room (core: 5 %, rind: 4 %), suggesting indirect contamination through aerosols or splashing (Fig. 6A). In contrast, the fungal communities exhibited a more diverse and diffuse origin profile. Brine tank (core: 5 %, rind: 10 %), floor of the brine room (core: 5 %, rind: 12 %), and wall of the brine room (core: 5 %, rind: 10 %) were the main contributors to both cheese core and rind, especially for the latter (Fig. 6B). The ventilation system in the ripening room played a much greater role in fungal dispersion, contributing 4 % to the core and 6 % to the rind. The ripening boards (core: 4 %, rind: 7 %), walls of the ripening room (3–4 %), and molding and heater areas (e.g., wall near heater: 3 % core, 4 % rind) were also notable contributors, especially to the rind (Fig. 6B). Although equipment such as the metal stirring tool and milk inlet pipe were still relevant, their contributions were much lower for fungi (1–2 %) compared to bacteria. Considering the metal stirring tool as the main bacterial source and the ventilation system as the key fungal source we further evaluated the distribution of ASVs detected in these specific samples (Fig. 6C). Regarding bacteria on the metal stirring tool, the composition varied notably by season. In autumn and winter, the community was dominated by *Lc. lactis* and *S. thermophilus* while in summer we detected several spoilage microbes (*Acinetobacter*, *A. johnsonii, Pseudomonas, Enterobacteriaceae* and *Sphingomonas*) (Fig. 6C). For fungi, the analysis of the ventilation system in the ripening room revealed a clear seasonal pattern in the dominant taxa. *Debaryomyces* was the predominant genus across all seasons, representing 64 % of fungal abundance in autumn, increasing to 96 % in winter, and reaching 98 % in summer. Conversely, *Penicillium* was most abundant in autumn (35 %) but dropped markedly in winter **(**3 %**)** and summer (1 %) (Fig. 6D).

3.6. Seasonal impact on cheese texture, flavor, and microbial-metabolite relationships

The microbial seasonal profiles of cheese were distinct in texture as well as metabolites. In autumn, cheeses were characterized by significantly lower gumminess, chewability and elasticity (Supplementary Table 2, $P \leq 0.05$) with a prevalence of key aromatic compounds including 2-heptanone, acetoin and 8-nonen-2-one (P $\leq$ 0.05). Notably, several ASVs dominant in autumn and winter cheeses showed strong negative correlations with these compounds including: *Lc. zeae*, *Lp. plantarum*, *Ltb. sakei*, and *Leu. lactis.* Interestingly, *Lc. lactis*, another significant autumn ASV, showed a positive correlation with acetoin (Fig. 7, P $\leq$ 0.05, $R > 0.8$).

Conversely, summer cheeses displayed a more intense and complex flavor development, marked by higher pH and hardness, and a richer presence of compounds like 2-butanone, ethyl alcohol, 2-butanol, isopentyl alcohol, 2,3-butanediol, methyl octanoate, methyl hexanoate, and various short- and medium-chain fatty acids such as acetic and butanoic and pentanoic acid (Supplementary Table 2, P $\leq$ 0.05). This complexity aligns with the richer summer microbiota, which included species like *Acinetobacter* (e.g., *A. guillouiae* and *A. johnsonii*), *Enterobacteriaceae*, *Enterococcus*, *Chryseobacterium*, *Citrobacter*, *Geotrichum*, *Lactobacillus*, *Lb. delbrueckii*, and *Lc. garvieae*. Many of these ASVs showed strong positive correlations with the characteristic summer metabolites. For instance, *A. guillouiae* was highly positively correlated with 2-butanone, ethyl alcohol, isopentyl alcohol, 2,3-butanediol, and methyl octanoate, as well as acetic, pentanoic, and hexanoic acids

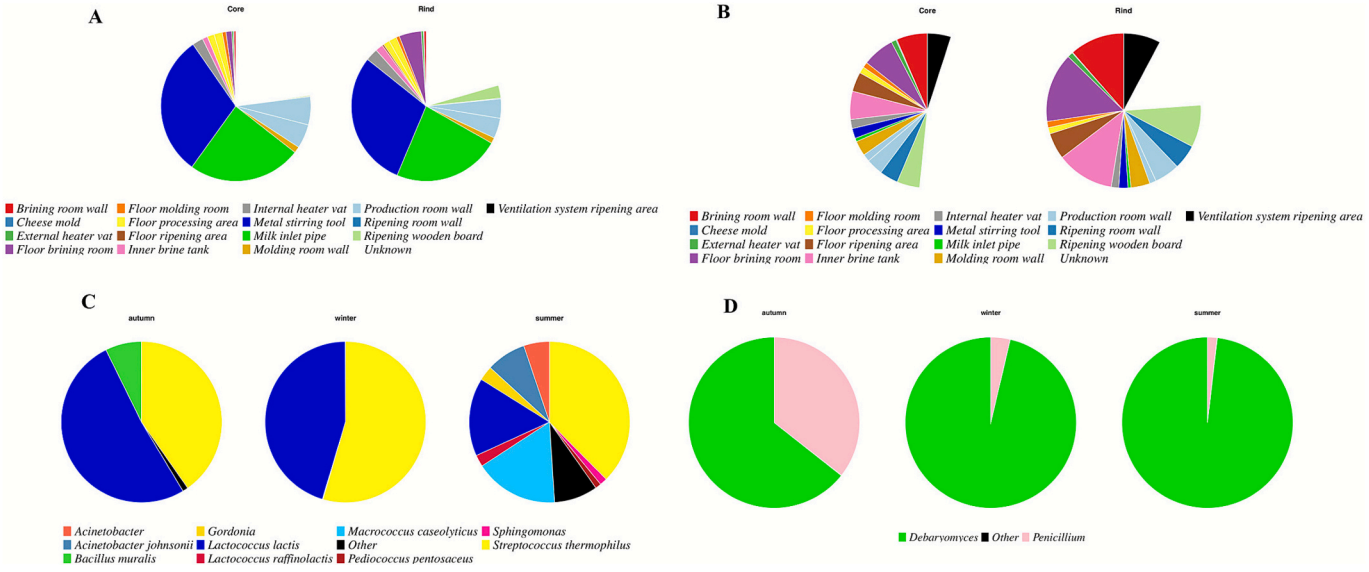

Fig. 6. Plots A and B are pie charts that illustrate the source bacterial (Plot A) and fungal (Plot B) composition in Maccagno cheese after 60 days of ripening. These compositions were determined using SourceTracker analysis. For both microbial domains, the data is presented for two distinct parts of the cheese: the Core and the Rind. Each colored segment within the pie charts represents a different bacterial or fungal source or environmental sample type, with its size proportional to its estimated relative contribution (percentage) to the cheese microbiota. Plot C displays the bacterial composition specifically found on the Metal Stirring Tool according to the season. Plot D shows the fungal composition of the Ventilation System in the Ripening Area according to the season.

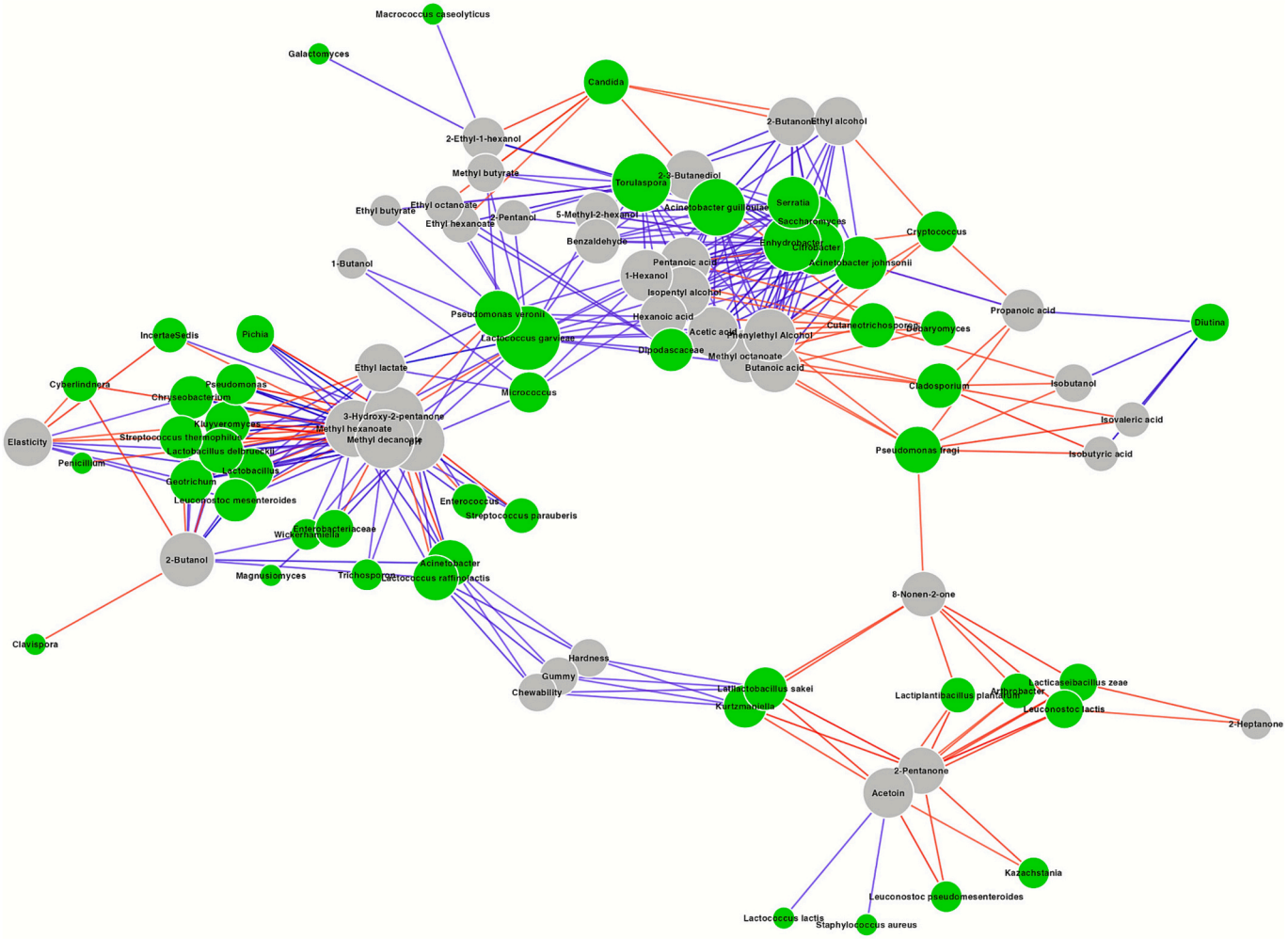

Fig. 7. Spearman correlation network of bacterial, fungal, metabolome and texture datas. This network illustrating the significant correlations between bacterial (green nodes), fungal (green nodes), metabolites (grey nodes) and texture data (grey nodes) of the ripened cheese ASVs (Amplicon Sequence Variants) found in Maccagno cheese. The size of each node is proportional to its degree, indicating the number of connections within the network. Edges (lines) connecting the nodes represent significant Spearman correlations ($P \leq 0.05$, $R > 0.8$). The color of the edges indicates the negative (red) or positive (blue) correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Fig. 7, $P \leq 0.05$, $R > 0.8$). Similarly, *A. johnsonii* correlated positively with acetic and butanoic acid. Other significant positive correlations include *Torulaspora* with 2-butanone, 2-ethyl-1-hexanol, and ethyl octanoate. Furthermore, pH was strongly positively correlated with *Pichia* and *Pseudomonas*. Conversely, certain microbial groups displayed consistent negative correlations with these summer-associated flavor compounds. *Candida* showed negative correlations with multiple alcohols and esters, including 2-butanone and 2-ethyl-1-hexanol (Fig. 7, $P \leq 0.05$, $R \leq -0.8$). *Cladosporium* was notably negatively correlated with various acids like isobutyric acid and isovaleric acid.

3.7. Unveiling seasonal genetic diversity of Lactococcus lactis strains

Across cheese samples at 60 days of ripening, *Lactococcus lactis* was the species-level genome bins (SGBs) mainly reconstructed in the dataset with 4 SGBs in autumn, 6 summer and winter (Fig. 8A). Few SGBs *Acinetobacter albensis*, *Brevibacterium aurantiacum*, *Ls. otakiensis*, *Ls. paracasei*, *Ltb. curvatus*, *Lc. lactis*, *Leu. mesenteroides*, *Leu.pseudomesenteroides* and *S. thermophilus* were also found in the dataset (Fig. 8A).

Lc. lactis SGBs largely segregate by season. Notably, the summer SGBs appear to be more numerous and form a cohesive cluster on one side of the tree, suggesting a shared evolutionary path or strong seasonal adaptation. The winter and autumn strains formed a smaller, more dispersed groupings within the overall tree, indicating a different pattern of genetic relatedness or diversity compared to the dominant summer cluster (Fig. 8B).

The pangenome analysis of *Lc. lactis* revealed distinct sets of unique genes associated with each cheese production season (Fig. 8C).

SGBs reconstructed in autumn showed 62 unique genes including multiple resistance mechanisms for heavy metals (arsenic, cadmium, copper) and antibiotics (tetracycline resistance ribosomal protection protein Tet(M), bacitracin export), transport systems genes for various compounds such as carnitine, dipeptides, iron, and taurine, genes involved in amino acid metabolism (Histidine ammonia-lyase, Urocanate hydratase), aminotransferases, lipid synthesis (Long-chain-fatty-acid–CoA ligase) lactate permease, bacteriocins genes and exotoxin genes.

In winter cheeses, *Lc. lactis* SGBs possessed 29 unique genes primarily related to carbohydrate metabolism, including enzymes for sugar utilization like 2,5-diketo-D-gluconic acid reductase B, 3-hexulose-6-phosphate synthase, beta-galactosidase GanA, sucrose-6-phosphate hydrolase.

In summer cheeses, the 11 unique gene repertoire of *Lc. lactis* SGBs was comparatively smaller but included genes crucial for core metabolic pathways like aconitate hydratase A (involved in the TCA cycle) and components for cell surface modification (Lipopolysaccharide 1,6-

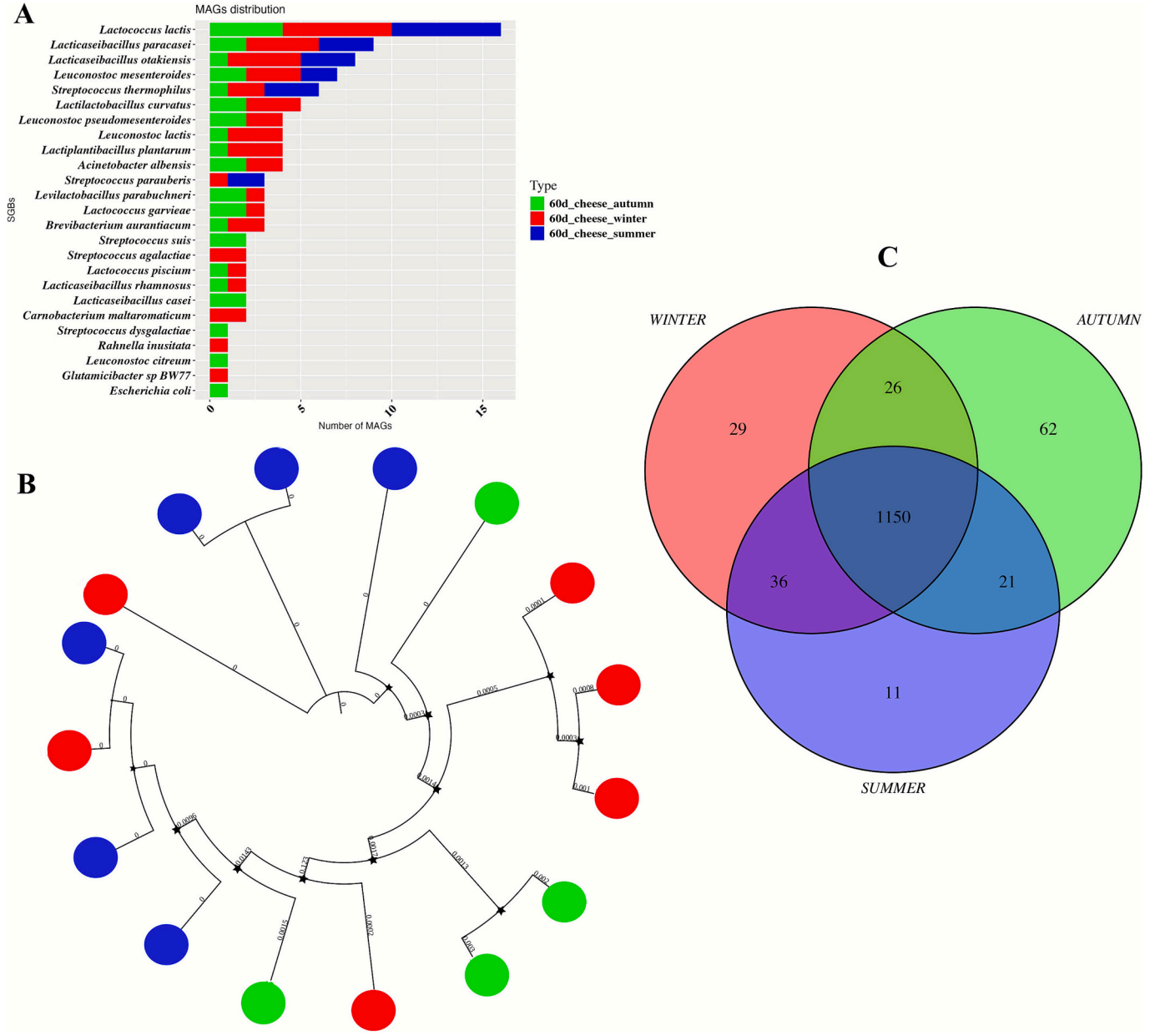

Fig. 8. Bar chart (plot A) showing the number of Metagenome-Assembled Genomes (MAGs) for the top 20 Species-level Genome Bins (SGBs) reconstructed in ripened cheese color code according to the season. (Plot B) Venn diagrams depicting the overlap between genes common or unique between the pangenome of *Lc. lactis*. (Plot C) Phylogenetic tree built on concatenated *Lc. lactis* SGBs.

galactosyltransferase, Putative glycosyltransferase EpsF), genes related to oxidative stress resistance (such as Manganese catalase and an organic hydroperoxide resistance transcriptional regulator).

4. Discussion

Cheese microbiome harbors a complex microbial composition mainly originating from raw materials, but is also determined by the environmental microbiome. The latter includes both bacteria and fungi that can become resident once they find an appropriate niche. However, their composition can shift due to changes in seasons, atmospheric conditions, cleaning procedures, or even by other external factors (e.g. operators, machinery, aereosol and so on) (De Filippis et al., 2021). In our study we found that milk microbiota and mycobiota were mainly affected by the season with the highest alpha diversity and microbial shift especially in summer. As already reported, type of feeding, temperature and atmospheric conditions during breeding and milking can enrich the microbiota during the warm season (Kable et al., 2016). Regarding environmental samples for both microbial domains we did not find a significant difference in the alpha diversity as a function of the season. This indicated that the facility environment contains a quite stable residential microbiome. As already reported, some microbes can be present temporarily or accidentally, while others can form residential populations in the production environments despite the use of controlled hygienic routines (Møretrø & Langsrud, 2017).

4.1. Core and season-specific bacterial communities in Maccagno cheese production environment

The microbial communities within the Maccagno cheese production environment exhibited both stable and season-specific bacterial compositions. *Staphylococcus*, *S. thermophilus* and *Lc. Lactis,* commonly

originating from raw milk and cheese (Quigley et al., 2013), consistently dominated the core environmental microbiota found on various contact and non-contact surfaces in Maccagno cheese production.Our data highlighted the significant contribution of metal stirring tools and milk inlet pipe as primary sources of *S. thermophilus* and *Lc. lactis*, underlining the role of equipment in shaping the final cheese microbiome (Johnson et al., 2021). *Staphylococcus*, often originating from humans and milk, is a stable component of equipment surfaces (Bokulich et al., 2014), floors (Einson et al., 2018), and ripening rooms (Calasso et al., 2016) due to its acid sensitivity and salt and psychrotolerance (Quijada et al., 2018). *S. thermophilus* is another ubiquitous core environmental microbe, frequently present on dairy equipment (Stellato et al., 2015), food contact (Settanni et al., 2012) and non-food contact surfaces (Schön et al., 2016), and consistently dominant in brine due to its salt tolerance (Sun & D'Amico, 2021). *Lc. lactis* is the most widespread and frequently dominant bacterium in wood vats, cheese hoops and air samples (Montel et al., 2014; Sun & D'Amico, 2021). The core environmental microbiota in Maccagno facilities also included salt-tolerant *Lactobacillus* especially in brine and *Brevibacterium*, which consistently characterizes ripening rooms and areas (Schornsteiner et al., 2014). *Psychrobacter*, a common dairy environmental microbe (Stellato et al., 2015), and *Methylobacterium* (including *M. adhaesivum*) also appear as core members of the environment and of the late ripening cheeses. The latter is recognized as a contaminant in various products and processes, originating from soil, dust, and water systems (Gallego et al., 2006).

Beyond these core members, specific seasonal associations were observed. During colder seasons, the environment was associated with spoilage psychrotrophic taxa such as *Chryseobacterium* on the floor of the molding room, *Serratia* in ripening rooms during autumn, *Pseudomonas fragi* in brine and *Psychrobacter* in ripening environments during winter (Aldrete-Tapia et al., 2014; Zwirzitz et al., 2021, 2020). Consistent with SourceTracker analyses, summer highlighted the presence of cheese-associated *Macrococcus caseolyticus* (Aldrete-Tapia et al., 2014) on metal stirring tools and heater vat surfaces, and *Arthrobacter* in water samples (Chaillou et al., 2015), alongside *Enterobacteriaceae* and *Citrobacter*.

Similar to the environment, the microbial profiles of raw milk and cheese reveal a stable presence of *Lc. raffinolactis*, *Lc. lactis*, *S. thermophilus*, and *Acinetobacter* across seasons in both raw ingredients and throughout the ripening. *Lc. raffinolactis* may play a synergistic role by utilizing metabolic byproducts of *Lc. lactis*, potentially aiding acid production, as other studies have indicated (Quigley et al., 2013).

Conversely, during summer, several spoilage-associated microbes from the metal stirring tool were linked to raw milk and subsequently to the final products, including *Acinetobacter*, *Enterobacteriaceae*, *Pseudomonas*, *Citrobacter*, *S. aureus*, *Enterococcus*, *Micrococcus*, *Brochothrix*, and *A. guillouiae*. The presence of *Enterococcus* and *Acinetobacter* can indicate unsanitary conditions in dairy manufacturing, sometimes leading to non-compliance with good manufacturing practices (Johnson et al., 2021; Martin et al., 2016). Our analysis confirmed their presence in the metal stirring tool probably due to their ability to form biofilm in dairy manufacturing environments (Zou & Liu, 2018). It was observed that they can be transferred to food during the production, directly (from dairy equipment) or indirectly (from non foodcontact surfaces) (Møretrø & Langsrud, 2017).

4.2. Core and season-specific fungal communities in Maccagno cheese production environment

The environmental analysis reveals a consistent and dominant core fungal community across the facility including *Debaryomyces*, *Penicillium* and *Cladosporium*. *Debaryomyces* is a cornerstone of production facilities mycobiota (Monnet et al., 2015; Schön et al., 2016) and here we found it as expected (Vermote et al., 2018) mainly in brine and brine tank due to its alotholerance (Estrada et al., 2023). *Debaryomyces* was observed as the predominant yeast also in non food contact surfaces along the three seasons and our results showed that the ventilation system in the ripening room was one of the main sources of this yeast for the final cheeses. Among the core mycobiota of the environment, we observed the predominance of *Penicillium* detected on contact surfaces, especially in molding and ripening areas and on ripening boards, indicating its widespread presence in dust, air (Visagie et al., 2014) and on surfaces where cheese is handled or aged (Anelli et al., 2024). *Cladosporium* and *Fusarium* were also present on non food contact surfaces, particularly on floors and walls, suggesting a link to general environmental aerial contamination as observed in food facilities (Abdo et al., 2020; Garnier et al., 2017). Raw milk and whey showed *Geotrichum* and *Galactomyces* as a core mycobiota commonly found in dairy products and milk (Grygier et al., 2020; Quigley et al., 2011). Cold season enriched the milk mycobiota with dairy associated yeasts including *Kluyveromyces*, *Candida*, *Kurtzmaniella* and *Clavispora* (Bintsis, 2021). The latter has already been associated with cheeses collected in colder seasons (Anelli et al., 2024). Summer milk was characterized by *Magnusiomyces*, found in a wide variety of sugar-rich habitats (Dudhat et al., 2022) and cheeses (Cardinali et al., 2021, 2024). The fungal communities in the 60-day ripened cheeses demonstrate the influence of the milk mycobiota on the final cheeses. The consistent presence of *Debaryomyces*, *Geotrichum* and *Galactomyces* in both core and rind across seasons underscores its importance in cheese ripening as already reported (Kothe et al., 2022; Merchán et al., 2025). *Debaryomyces* is the most dominant taxa during ripening of the red-brown defective cheese since it is responsible for the deacidification activity (Guzzon et al., 2017) and it can accelerate surface alkalinization and enhance cheese flavor (Lessard et al., 2012). However, the seasonal shifts in other dominant genera like *Kluyveromyces* (autumn/winter) and *Magnusiomyces* (summer) suggest that ripening dynamics are seasonally influenced, potentially impacting the final sensory characteristics of the cheese. The SourceTracker analysis highlighted that the ventilation system in the ripening room plays a large role in fungal dispersion, directly impacting the cheese. The analysis of the ripening room ventilation system further revealed the seasonal dominance of *Debaryomyces*, which steadily increases from autumn to winter and summer. Conversely, *Penicillium* was most abundant in autumn but significantly declined in winter and summer.

4.3. The interplay of Lactococcus lactis genomics and seasonal conditions in shaping Maccagno cheese characteristics

The pangenome analysis of *Lc. lactis* revealed distinct sets of unique genes associated with each cheese production season, reflecting potential adaptations to varying environmental conditions and metabolic demands. *Lc. lactis* SGBs in autumn display the most abundant unique genes compared to the other seasons. We observed several resistances to heavy metals and antibiotics that suggest a possible resistance mechanism to disinfectants or competition with other microorganisms. Genes for resistance to disinfectants and heavy metals are often found together with other mobile elements such as antimicrobial resistance genes (Zhu et al., 2019) and appears to be one of the longest-known environmental adaptations (Stanborough et al., 2017). In addition, resistance to heavy metals can be a matter of concern because of possible co-selection of antibiotic resistance (Belloso Daza et al., 2022). *Lc. lactis* is actively involved in the metabolism of aromatic amino acids, including phenylalanine, tyrosine, and tryptophan. Through this metabolic activity, *Lc. lactis* can produce a variety of aromatic compounds essential for developing a unique aroma and flavor (Yang et al., 2025). Aminotransferases are present in the genome of *Lc. lactis* and could also contribute to VOCs production (Pangallo et al., 2019; Ruggirello et al., 2018). The presence of lactate permease, bacteriocins genes and exotoxin also hints at competitive advantages within the microbial community. In summer we then observed that *Lc. lactis* SGBs display the presence of a CRISPR system endoribonuclease Csm6, suggesting an active defense mechanism against bacteriophages, which could be more prevalent in summer conditions. This seasonal genetic partitioning of *Lc.*

lactis underscores its remarkable adaptability and the specific selective pressures exerted in different cheese production seasons (Millen et al., 2012). The metabolomic profile of cheeses was strongly influenced by the microbial succession. In autumn we observed the highest concentration of acetoin, which has a buttermilk, fruity, and ethereal odor (Cardinali et al., 2024) and linear ketones associated with fruity and floral notes, with a positive effect on cheese flavor (Di Cagno et al., 2011; Ruiz et al., 2023). From the correlation network we identified that those metabolites were associated with the highest presence of *Lc. lactis* in autumn cheeses. The ability of *Lc. lactis* to produce these particular flavor compounds was already reported (Ruggirello et al., 2018; Terzić-Vidojević et al., 2020). The pangenome analysis of *Lc. lactis* SGBs in autumn showed the presence of several transport systems genes for aminoacid underscoring a highly proteolytic activity as already reported for this species (Broadbent et al., 2011; Cavanagh et al., 2015). As a consequence, we observed that the texture of the cheese in this season was softer and less gummy compared to the other seasons, as expected in this type of cheese. On the other hand in summer, cheeses display the predominance of alcohols, ketons and SCFAs all correlated with several food processing associated spoilage microbes including *A. guillouiae*, *A. johnsonii*, *Enterobacteriaceae*, *Enterococcus* (Alessandria et al., 2016; Calasso et al., 2016; Stellato et al., 2015). In particular the highest concentration of SCFAs in this season contribute to a piquant taste (Gatti et al., 2014). Maccagno cheese is commonly characterized by a very intense flavor with no sensations of pungency or spiciness, indicating that summer cheeses did not reflect the expected organoleptic flavor.

5. Conclusions

Based on our findings, the physico-chemical and microbiological characteristics of the analyzed cheese are strongly influenced by both raw milk and the environmental microbiome, which interact in a seasonally dynamic way.

This study highlighted the crucial role of equipment like metal stirring tools and pipes in the dissemination of key bacteria and showed how the ripening room ventilation system is a critical reservoir for fungal dispersal. *Lc. lactis*, *S. thermophilus*, and *Debaryomyces* were identified as the core microbes. *Lc. lactis* not only dominated the bacterial community but also displayed season-specific genomic adaptations, especially in autumn, associated with desired flavor compounds and softer textures structure. Conversely, summer cheeses showed the highest microbial diversity in raw milk, including several spoilage-related taxa like *Acinetobacter* and *Enterobacteriaceae*, leading to off-flavors and deviations from Maccagno's expected organoleptic profile according to the technical data sheets for traditional agri-food products. Regarding fungi *Debaryomyces* and *Penicillium* were largely dispersed from the ripening room air system. Overall, this study underscores the importance of monitoring seasonally responsive environments and equipment to preserve product quality. It also highlights the need for targeted interventions during summer to mitigate spoilage risks and ensure that Maccagno cheese retains its distinct sensory and texture characteristics. In this study, a culture-independent method was essential to provide an unbiased, comprehensive view of microbial transfer dynamics, as culturomics makes it difficult to accurately trace contamination sources. Understanding the complex interactions between the environment, milk and cheese microbiome, and the seasonal variations is fundamental for controlling and optimizing Maccagno cheese production, ensuring consistent sensory quality.

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CRediT authorship contribution statement

Ilario Ferrocino: Conceptualization, Data curation, Formal analysis, Methodology, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Federica Biolcati:** Formal analysis. **Manuela Giordano:** Formal analysis, Methodology. **Marta Bertolino:** Formal analysis, Methodology. **Giuseppe Zeppa:** Methodology, Resources, Supervision, Writing – review & editing. **Luca Cocolin:** Conceptualization, Funding acquisition, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The metataxonomic (16S rRNA and 26S rRNA genes) and metagenome sequences are available at the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under the BioProject accession numbers PRJNA1297600 and PRJNA1297951, respectively.

References

Abdo, H., Catacchio, C. R., Ventura, M., D'Addabbo, P., Alexandre, H., Guilloux-Bénatier, M., & Rousseaux, S. (2020). The establishment of a fungal consortium in a new winery. *Scientific Reports, 10*(1). https://doi.org/10.1038/s41598-020-64819-2

Aldrete-Tapia, A., Escobar-Ramírez, M. C., Tamplin, M. L., & Hernández-Iturriaga, M. (2014). High-throughput sequencing of microbial communities in Poro cheese, an artisanal Mexican cheese. *Food Microbiology, 44*, 136–141. https://doi.org/10.1016/j.fm.2014.05.022

Alessandria, V., Ferrocino, I., De Filippis, F., Fontana, M., Rantsiou, K., Ercolini, D., & Cocolin, L. (2016). Microbiota of an Italian grana like cheese during manufacture and ripening unraveled by 16S rRNA-based approaches. *Applied and Environmental Microbiology, 82*(13), 3988–3995. https://doi.org/10.1128/AEM.00999-16

Anelli, P., Dall'Asta, C., Cozzi, G., Epifani, F., Carella, D., Scarpetta, D., … Susca, A. (2024). Analysis of composition and molecular characterization of mycobiota occurring on surface of cheese ripened in Dossena's mine. *Food Microbiology, 123*, Article 104587. https://doi.org/10.1016/j.fm.2024.104587

Barcenilla, C., Cobo-Díaz, J. F., Puente, A., Valentino, V., De Filippis, F., Ercolini, D., … Álvarez-Ordóñez, A. (2024). In-depth characterization of food and environmental microbiomes across different meat processing plants. *Microbiome, 20*. https://doi.org/10.1186/s40168-024-01856-3

Belloso Daza, M. V., Milani, G., Cortimiglia, C., Pietta, E., Bassi, D., & Cocconcelli, P. S. (2022). Genomic insights of *Enterococcus faecium* UC7251, a multi-drug resistant strain from ready-to-eat food, highlight the risk of antimicrobial resistance in the food chain. *Frontiers in Microbiology, 13*, 1–15. https://doi.org/10.3389/fmicb.2022.894241

Bertolino, M., Dolci, P., Giordano, M., Rolle, L., & Zeppa, G. (2011). Evolution of chemico-physical characteristics during manufacture and ripening of Castelmagno PDO cheese in wintertime. *Food Chemistry, 129*(3), 1001–1011. https://doi.org/10.1016/j.foodchem.2011.05.060

Bintsis, T. (2021). Yeasts in different types of cheese. *AIMS Microbiology, 7*(4), 447–470. https://doi.org/10.3934/MICROBIOL.2021027

Bokulich, N. A., Bergsveinson, J., Ziola, B., & Mills, D. A. (2015). Mapping microbial ecosystems and spoilage-gene flow in breweries highlights patterns of contamination and resistance. *ELife, 2015*(4), Article e04634. https://doi.org/10.7554/eLife.04634

Bokulich, N. A., Ohta, M., Lee, M., & Mills, D. A. (2014). Indigenous bacteria and fungi drive traditional kimoto sake fermentations. *Applied and Environmental Microbiology, 80*(17), 5522–5529. https://doi.org/10.1128/AEM.00663-14

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology, 37*(8), 852–857. https://doi.org/10.1038/s41587-019-0209-9

Broadbent, J. R., Cai, H., Larsen, R. L., Hughes, J. E., Welker, D. L., De Carvalho, V. G., … Steele, J. L. (2011). Genetic diversity in proteolytic enzymes and amino acid

metabolism among *Lactobacillus helveticus* strains. *Journal of Dairy Science, 94*(9), 4313–4328. https://doi.org/10.3168/jds.2010-4068

Calasso, M., Ercolini, D., Mancini, L., Stellato, G., Minervini, F., Di Cagno, R., & Gobbetti, M. (2016). Relationships among house, rind and core microbiotas during manufacture of traditional Italian cheeses at the same dairy plant. *Food Microbiology, 54*(11), 115–126. https://doi.org/10.1016/j.fm.2015.10.008

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods, 13*(7), 581. https://doi.org/10.1038/nmeth.3869

Cardinali, F., Ferrocino, I., Milanović, V., Belleggia, L., Corvaglia, M. R., Garofalo, C., … Aquilanti, L. (2021). Microbial communities and volatile profile of queijo de Azeitão PDO cheese, a traditional Mediterranean thistle-curdled cheese from Portugal. *Food Research International, 147*(06), Article 110537. https://doi.org/10.1016/j.foodres.2021.110537

Cardinali, F., Rampanti, G., Paderni, G., Milanović, V., Ferrocino, I., Reale, A., … Garofalo, C. (2024). A comprehensive study on the autochthonous microbiota, volatilome, physico-chemical, and morpho-textural features of Montenegrin Njeguški cheese. *Food Research International, 197*(09), Article 114484. https://doi.org/10.1016/j.foodres.2024.115169

Cavanagh, D., Fitzgerald, G. F., & McAuliffe, O. (2015). From field to fermentation: The origins of *Lactococcus lactis* and its domestication to the dairy environment. *Food Microbiology, 47*, 45–61. https://doi.org/10.1016/j.fm.2014.11.001

Chaillou, S., Chaulot-Talmon, A., Caekebeke, H., Cardinal, M., Christieans, S., Denis, C., & Champomier-Vergès, M.-C. C. (2015). Origin and ecological selection of core and food-specific bacterial communities associated with meat and seafood spoilage. *The ISME Journal, 9*(5), 1105–1118. https://doi.org/10.1038/ismej.2014.202

Culhane, A. C., Thioulouse, J., Perrière, G., & Higgins, D. G. (2005). MADE4: An R package for multivariate analysis of gene expression data. *Bioinformatics (Oxford, England), 21*(11), 2789–2790. https://doi.org/10.1093/bioinformatics/bti394

De Filippis, F., Valentino, V., Alvarez-Ordóñez, A., Cotter, P. D., & Ercolini, D. (2021). Environmental microbiome mapping as a strategy to improve quality and safety in the food industry. *Current Opinion in Food Science, 38*, 168–176. https://doi.org/10.1016/j.cofs.2020.11.012

De Filippis, F., Valentino, V., Yap, M., Cabrera-Rubio, R., Barcenilla, C., Carlino, N., … Ercolini, D. (2024). Microbiome mapping in dairy industry reveals new species and genes for probiotic and bioprotective activities. *npj Biofilms and Microbiomes, 10*(1). https://doi.org/10.1038/s41522-024-00541-5

Di Cagno, R., De Pasquale, I., De Angelis, M., Buchin, S., Calasso, M., Fox, P. F., & Gobbetti, M. (2011). Manufacture of Italian Caciotta-type cheeses with adjuncts and attenuated adjuncts of selected non-starter lactobacilli. *International Dairy Journal, 21*(4), 254–260. https://doi.org/10.1016/j.idairyj.2010.12.007

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science, 14*, 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

Doyle, C. J., O'Toole, P. W., & Cotter, P. D. (2017). Metagenome-based surveillance and diagnostic approaches to studying the microbial ecology of food production and processing environments. *Environmental Microbiology, 19*(11), 4382–4391. https://doi.org/10.1111/1462-2920.13859

Dudhat, J., Sakpuntoon, V., Angchuan, J., Kaewwichian, R., & Srisuk, N. (2022). Magnusiomyces siamensis sp. nov., a yeast-like fungus isolated from food waste. *International Journal of Systematic and Evolutionary Microbiology, 72*(6), 1–8. https://doi.org/10.1099/ijsem.0.005435

Einson, J. E., Rani, A., You, X., Rodriguez, A. A., Randell, C. L., Barnaba, T., … Sela, D. A. (2018). A vegetable fermentation facility hosts distinct microbiomes reflecting the production environment. *Applied and Environmental Microbiology, 84*(22), e01618–e01680. https://doi.org/10.1128/AEM.01680-18

Estrada, M., Navarrete, C., Møller, S., Procentese, A., & Martínez, J. L. (2023). Utilization of salt-rich by-products from the dairy industry as feedstock for recombinant protein production by *Debaryomyces hansenii*. *Microbial Biotechnology, 16*(2), 404–417. https://doi.org/10.1111/1751-7915.14179

Ferrocino, I., Rantsiou, K., & Cocolin, L. (2022a). Investigating dairy microbiome: An opportunity to ensure quality, safety and typicity. *Current Opinion in Biotechnology, 73*, 164–170. https://doi.org/10.1016/j.copbio.2021.08.009

Ferrocino, I., Rantsiou, K., & Cocolin, L. (2022b). Microbiome and -omics application in food industry. International Journal of Food Microbiology, 377(5), article 109781. Doi: https://doi.org/10.1016/j.ijfoodmicro.2022.109781.

Gallego, V., García, M. T., & Ventosa, A. (2006). *Methylobacterium adhaesivum* sp. nov., a methylotrophic bacterium isolated from drinking water. *International Journal of Systematic and Evolutionary Microbiology, 56*(2), 339–342. https://doi.org/10.1099/ijs.0.63966-0

Garnier, L., Valence, F., Pawtowski, A., Auhustsinava-Galerne, L., Frotté, N., Baroncelli, R., & Mounier, J. (2017). Diversity of spoilage fungi associated with various French dairy products. *International Journal of Food Microbiology, 241*, 191–197. https://doi.org/10.1016/j.ijfoodmicro.2016.10.026

Gatti, M., Bottari, B., Lazzi, C., Neviani, E., & Mucchetti, G. (2014). Invited review: Microbial evolution in raw-milk, long-ripened cheeses produced using undefined natural whey starters. *Journal of Dairy Science, 97*(2), 573–591. https://doi.org/10.3168/jds.2013-7187

Grygier, A., Myszka, K., Juzwa, W., Białas, W., & Rudzińska, M. (2020). *Galactomyces geotrichum* mold isolated from a traditional fried cottage cheese produced omega-3 fatty acids. *International Journal of Food Microbiology, 319*(6), 0–7. https://doi.org/10.1016/j.ijfoodmicro.2019.108503

Guzzon, R., Carafa, I., Tuohy, K., Cervantes, G., Vernetti, L., Barmaz, A., … Franciosi, E. (2017). Exploring the microbiota of the red-brown defect in smear-ripened cheese by 454-pyrosequencing and its prevention using different cleaning systems. *Food Microbiology, 62*(1107), 160–168. https://doi.org/10.1016/j.fm.2016.10.018

Johnson, J., Curtin, C., & Waite-Cusic, J. (2021). The cheese production facility microbiome exhibits temporal and spatial variability. *Frontiers in Microbiology, 12*(3), 1–13. https://doi.org/10.3389/fmicb.2021.644828

Kable, M. E., Srisengfa, Y., Laird, M., Zaragoza, J., McLeod, J., Heidenreich, J., & Marco, M. L. (2016). The core and seasonal microbiota of raw bovine milk in tanker trucks and the impact of transfer to a milk processing facility. *MBio, 7*(4), 1–13. https://doi.org/10.1128/mBio.00836-16

Kang, D., Li, F., Kirton, E. S., Thomas, A., Egan, R. S., An, H., & Wang, Z. (2019). MetaBAT 2: An adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. *PeerJ Preprints*. , Article 27522v1. https://doi.org/10.7287/peerj.preprints.27522v1 |

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research, 41*(1), Article e1. https://doi.org/10.1093/nar/gks808

Knights, D., Kuczynski, J., Charlson, E. S., Zaneveld, J., Mozer, M. C., Collman, R. G., … Kelley, S. T. (2011). Bayesian community-wide culture-independent microbial source tracking. *Nature Methods, 8*(9), 4–15. https://doi.org/10.1038/nmeth.1650.Bayesian

Kothe, C. I., Mohellibi, N., & Renault, P. (2022). Revealing the microbial heritage of traditional Brazilian cheeses through metagenomics. *Food Research International, 157*(2), Article 111265. https://doi.org/10.1016/j.foodres.2022.111265

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with bowtie 2. *Nature Methods, 9*(4), 357–359. https://doi.org/10.1038/nmeth.1923

Lessard, M. H., Bélanger, G., St-Gelais, D., & Labrie, S. (2012). The composition of camembert cheese-ripening cultures modulates both mycelial growth and appearance. *Applied and Environmental Microbiology, 78*(6), 1813–1819. https://doi.org/10.1128/AEM.06645-11

Martin, N. H., Trmcic, A., Hsieh, T. H., Boor, K. J., & Wiedmann, M. (2016). The evolving role of coliforms as indicators of unhygienic processing conditions in dairy foods. *Frontiers in Microbiology, 7*(9), 1–8. https://doi.org/10.3389/fmicb.2016.01549

Merchán, A. V., Román, Á. C., Ruiz-Moyano, S., Vázquez-Hernández, M., Cabañas, C. M., & Benito, M. J. (2025). Mycobiota composition through the ripening of artisanal soft cheeses 'torta del Casar' and 'queso de la Serena' monitored by high-throughput sequencing. *Applied Food Research, 5*(1). https://doi.org/10.1016/j.afres.2025.100711

Millen, A. M., Horvath, P., Boyaval, P., & Romero, D. A. (2012). Mobile CRISPR/Cas-mediated bacteriophage resistance in *Lactococcus lactis*. *PLoS One, 7*(12). https://doi.org/10.1371/journal.pone.0051663

Monnet, C., Landaud, S., Bonnarme, P., & Swennen, D. (2015). Growth and adaptation of microorganisms on the cheese surface. *FEMS Microbiology Letters, 362*(1), 1–9. https://doi.org/10.1093/femsle/fnu025

Montel, M.-C., Buchin, S., Mallet, A., Delbes-Paus, C., Vuitton, D. A., Desmasures, N., & Berthier, F. (2014). Traditional cheeses: Rich and diverse microbiota with associated benefits. *International Journal of Food Microbiology, 177*, 136–154. https://doi.org/10.1016/j.ijfoodmicro.2014.02.019

Møretrø, T., & Langsrud, S. (2017). Residential bacteria on surfaces in the food industry and their implications for food safety and quality. *Comprehensive Reviews in Food Science and Food Safety, 16*(5), 1022–1041. https://doi.org/10.1111/1541-4337.12283

Mota-Gutierrez, J., Ferrocino, I., Rantsiou, K., & Cocolin, L. (2019). Metataxonomic comparison between internal transcribed spacer and 26S ribosomal large subunit (LSU) rDNA gene. *International Journal of Food Microbiology, 290*(10), 132–140. https://doi.org/10.1016/j.ijfoodmicro.2018.10.010

Nurk, S., Bankevich, A., Antipov, D., Gurevich, A. A., Korobeynikov, A., Lapidus, A., & Pevzner, P. A. (2013). Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. *Journal of Computational Biology, 20*(10), 714–737. https://doi.org/10.1089/cmb.2013.0084

Pangallo, D., Kraková, L., Puškárová, A., Šoltys, K., Bučková, M., Koreňová, J., & Kuchta, T. (2019). Transcription activity of lactic acid bacterial proteolysis-related genes during cheese maturation. *Food Microbiology, 82*(3), 416–425. https://doi.org/10.1016/j.fm.2019.03.015

Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P., & Tyson, G. W. (2015). CheckM: Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Research, 25*(7), 1043–1055. https://doi.org/10.1101/gr.186072.114

Quigley, L., O'Sullivan, O., Beresford, T. P., Ross, R. P., Fitzgerald, G. F., & Cotter, P. D. (2011). Molecular approaches to analysing the microbial composition of raw milk and raw milk cheese. *International Journal of Food Microbiology, 150*(2–3), 81–94. https://doi.org/10.1016/j.ijfoodmicro.2011.08.001

Quigley, L., O'Sullivan, O., Stanton, C., Beresford, T. P., Ross, R. P., Fitzgerald, G. F., & Cotter, P. D. (2013). The complex microbiota of raw milk. *FEMS Microbiology Reviews, 37*(5), 664–698. https://doi.org/10.1111/1574-6976.12030

Quijada, N. M., Mann, E., Wagner, M., Rodríguez-Lázaro, D., Hernández, M., & Schmitz-Esser, S. (2018). Autochthonous facility-specific microbiota dominates washed-rind Austrian hard cheese surfaces and its production environment. *International Journal of Food Microbiology, 267*(12), 54–61. https://doi.org/10.1016/j.ijfoodmicro.2017.12.025

Quijada, N. M., Rodríguez-Lázaro, D., Eiros, J. M., & Hernández, M. (2019). TORMES: An automated pipeline for whole bacterial genome analysis. *Bioinformatics, 35*(21), 4207–4212. https://doi.org/10.1093/bioinformatics/btz220

Ruggirello, M., Giordano, M., Bertolino, M., Ferrocino, I., Cocolin, L., & Dolci, P. (2018). Study of *Lactococcus lactis* during advanced ripening stages of model cheeses characterized by GC-MS. *Food Microbiology, 74*, 132–142. https://doi.org/10.1016/j.fm.2018.03.012

Ruiz, M. J., Salatti-Dorado, J. A., Cardador, M. J., Frizzo, L., Jordano, R., Arce, L., & Medina, L. M. (2023). Relationship between volatile organic compounds and microorganisms isolated from raw sheep milk cheeses determined by sanger sequencing and GC–IMS. *Foods, 12*(2). https://doi.org/10.3390/foods12020372

Schön, K., Schornsteiner, E., Dzieciol, M., Wagner, M., Müller, M., & Schmitz-Esser, S. (2016). Microbial communities in dairy processing environment floor-drains are dominated by product-associated bacteria and yeasts. *Food Control, 70*, 210–215. https://doi.org/10.1016/j.foodcont.2016.05.057

Schornsteiner, E., Mann, E., Bereuter, O., Wagner, M., & Schmitz-Esser, S. (2014). Cultivation-independent analysis of microbial communities on Austrian raw milk hard cheese rinds. *International Journal of Food Microbiology, 180*, 88–97. https://doi.org/10.1016/j.ijfoodmicro.2014.04.010

Schubert, M., Lindgreen, S., & Orlando, L. (2016). AdapterRemoval v2: Rapid adapter trimming, identification, and read merging. *BMC Research Notes, 9*(1), 1–7. https://doi.org/10.1186/s13104-016-1900-2

Settanni, L., Di Grigoli, A., Tornambé, G., Bellina, V., Francesca, N., Moschetti, G., & Bonanno, A. (2012). Persistence of wild *Streptococcus thermophilus* strains on wooden vat and during the manufacture of a traditional Caciocavallo type cheese. *International Journal of Food Microbiology, 155*(1–2), 73–81. https://doi.org/10.1016/j.ijfoodmicro.2012.01.022

Stanborough, T., Fegan, N., Powell, S. M., Tamplin, M., & C. P.. (2017). Insight into the genome of *Brochothrix thermosphacta*, a problematic meat spoilage bacterium. *83*(5), 1–20.

Stellato, G., De Filippis, F., La Storia, A., & Ercolini, D. (2015). Coexistence of lactic acid bacteria and potential spoilage microbiota in a dairy processing environment. *Applied and Environmental Microbiology, 81*(22), 7893–7904. https://doi.org/10.1128/AEM.02294-15

Sun, L., & D'Amico, D. J. (2021). Composition, succession, and source tracking of microbial communities throughout the traditional production of a farmstead cheese. *MSystems, 6*(5), e00821–e00830. https://doi.org/10.1128/msystems.00830-21

Terzić-Vidojević, A., Veljović, K., Tolinački, M., Živković, M., Lukić, J., Lozo, J., … Golić, N. (2020). Diversity of non-starter lactic acid bacteria in autochthonous dairy products from western Balkan countries - technological and probiotic properties. *Food Research International, 136*(June). https://doi.org/10.1016/j.foodres.2020.109494

Vermote, L., Verce, M., De Vuyst, L., & Weckx, S. (2018). Amplicon and shotgun metagenomic sequencing indicates that microbial ecosystems present in cheese brines reflect environmental inoculation during the cheese production process. *International Dairy Journal, 87*, 44–53. https://doi.org/10.1016/j.idairyj.2018.07.010

Visagie, C. M., Hirooka, Y., Tanney, J. B., Whitfield, E., Mwange, K., Meijer, M., … Samson, R. A. (2014). *Aspergillus*, *penicillium* and *Talaromyces* isolated from house dust samples collected around the world. *Studies in Mycology, 78*(1), 63–139. https://doi.org/10.1016/j.simyco.2014.07.002

Walsh, A. M., Macori, G., Kilcawley, K. N., & Cotter, P. D. (2020). Meta-analysis of cheese microbiomes highlights contributions to multiple aspects of quality. *Nature Food, 1*(8), 500–510. https://doi.org/10.1038/s43016-020-0129-3

Yang, C., Borjihan, Q., Liu, H., Kwok, L. Y., Wang, D., & Chen, Y. (2025). Terroir-driven flavor, microbiome, and metabolome signatures of Jiaoke: A multi-omics exploration of traditional fermented dairy from Inner Mongolia. *Food Bioscience, 66*. https://doi.org/10.1016/j.fbio.2025.105922

Zhu, Y. G., Zhao, Y., Zhu, D., Gillings, M., Penuelas, J., Ok, Y. S., … Banwart, S. (2019). Soil biota, antimicrobial resistance and planetary health. *Environment International, 131*, Article 105059. https://doi.org/10.1016/j.envint.2019.105059

Zou, M., & Liu, D. (2018). A systematic characterization of the distribution, biofilm-forming potential and the resistance of the biofilms to the CIP processes of the bacteria in a milk powder processing factory. *Food Research International, 113*, 316–326. https://doi.org/10.1016/j.foodres.2018.07.020

Zwirzitz, B., Wetzels, S. U., Dixon, E. D., Fleischmann, S., Selberherr, E., Thalguter, S., … Stessl, B. (2021). Co-occurrence of *Listeria* spp. and spoilage associated microbiota during meat processing due to cross-contamination events. *Frontiers in Microbiology, 12*. https://doi.org/10.3389/fmicb.2021.632935

Zwirzitz, B., Wetzels, S. U., Dixon, E. D., Stessl, B., Zaiser, A., Rabanser, I., … Selberherr, E. (2020). The sources and transmission routes of microbial populations throughout a meat processing facility. *npj Biofilms and Microbiomes, 6*(1), 1–12. https://doi.org/10.1038/s41522-020-0136-z