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# Comparison Between Full-Length 16S rRNA Metabarcoding and Whole Metagenome Sequencing Suggests the Use of Either Is Suitable for Large-Scale Microbiome Studies

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- 1 Comparison between full-length 16S metabarcoding and whole metagenome sequencing suggests the
- 2 use of either is suitable for large-scale microbiome studies.
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- 9 \*Corresponding author
- 10 Running title
- 11 Milk filters microbiome comparison by FL-16S and WMS

# 12 Keywords

- 13 full-length 16S rRNA; whole metagenome sequencing; bulk tank milk filters; microbiome; shotgun
- 14 metagenomic sequencing; metabarcoding
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#### 23 Abstract

24 Since the number of studies of the microbial communities related to food and food-associated 25 matrices almost completely reliant on Next Generation Sequencing techniques is rising, evaluations 26 of these high-throughput methods are critical. Currently, the two most used sequencing methods to 27 profile the microbiota of complex samples, including food and food-related matrices, are the 16S 28 rRNA metabarcoding and the whole metagenome sequencing, both of which are powerful tools for the monitoring of food-borne pathogens and the investigation of the microbiome. Herein, the 29 30 microbial profiles of 20 bulk tank milk filters from different dairy farms were investigated using both the full-length 16S rRNA metabarcoding, a third-generation sequencing method whose application in 31 32 food and food-related matrices is yet in its infancy, and the whole metagenome sequencing, in order to evaluate the correlation and the reliability of these two methods to explore the microbiome of 33 34 food-related matrices. Metabarcoding and metagenomic data were generated on a MinION platform (Oxford Nanopore Technologies, UK) and on a Illumina NovaSeq 6000 platform, respectively. Our 35 36 findings support the greater resolution of whole metagenome sequencing in terms of both increased 37 detection of bacterial taxa and enhanced detection of diversity; on the other hand, full-length 16S rRNA metabarcoding has proven to be a promising, less expensive and more practical tool to profile 38 39 most abundant taxa. The significant correlation of the two technologies both in terms of taxa diversity and richness, together with the similar profiles defined for both highly abundant taxa and core 40 microbiomes, including Acinetobacter, Bacillus and Escherichia genera, highlights the possible 41 42 application of both methods for different purposes.

The present study allowed the first comparison of full-length 16S rRNA sequencing and whole
metagenome sequencing to investigate the microbial composition of a food-related matrix, pointing
out the advantageous use of full-length 16S rRNA to identify dominant microorganisms and the

46 superior power of whole metagenome sequencing for the taxonomic detection of low abundant
47 microorganisms and to perform functional analysis of the microbial communities.

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## 49 **1**. Introduction

50 Recent developments in next-generation sequencing (NGS) technologies, together with the reduction in costs and the rise in efficiency, have led to an increase in the number of metabarcoding 51 52 and metagenomic investigations in different matrices and niches. Two main strategies can be used 53 for the analysis of microbial communities with NGS techniques: whole metagenome sequencing (WMS), also referred to as shotgun metagenomic sequencing, and high-throughput 16S rRNA 54 55 metabarcoding. Several studies reported on the bovine milk microbiota arising from its association with the quality and safety of dairy products (F. Addis et al., 2016; Rubiola et al., 2020) and more 56 often than not 16S rRNA metabarcoding was applied. The 16S rRNA gene is around 1,600 bp and 57 includes nine hypervariable loci (denoted V1-V9) (Bukin et al., 2019). The 16S rRNA metabarcoding 58 relies on a combination of amplification followed by sequencing of the 16S rRNA gene variable 59 60 regions, thereby allowing the taxonomic classification and determination of the relative abundance 61 of the bacterial component within a sample. This targeted approach is considered a robust and well-62 characterized method and has some advantages over shotgun metagenomic sequencing; indeed, it is less expensive than WMS and it does not require the same level of sequencing depth to obtain a 63 proper characterization of the microbiota. Besides, as it is based on a targeted amplification, this 64 65 technique is not affected by the presence of host (bovine) DNA which characterises milk and dairy 66 products, and data analysis does not require intensive computational power; a wide range of commonly used bioinformatics tools and pipelines for taxonomy and functional analysis are available 67 to facilitate reproducible and modular analysis of 16S rRNA sequencing data in free software 68 69 platforms, such as QIIME2 (Bolyen et al., 2019) and Mothur (Schloss et al., 2009). Nonetheless, some

70 limitations of this approach are recognised including, 16S rRNA metabarcoding does not provide 71 functional information about the genes encoded by those microbial communities being investigated 72 (Biegert et al., 2021) and it has a low taxonomic resolution, usually limited to family or genus level. Additionally, different reference databases (e.g. GreenGenes (DeSantis et al., 2006), SILVA (Quast et 73 al., 2013), the Ribosomal Database Project (Cole et al., 2014)) can influence the sample taxonomy 74 outcomes of the 16S rRNA metabarcoding (Abellan-Schneyder et al., 2021), which is furthermore 75 affected by a loss of diversity due to PCR bias (F. Addis et al., 2016). Indeed, different 16S rRNA 76 hypervariable regions exhibit differences in their ability to resolve taxa, and the choice of primer 77 designs used is crucial, as the amplification of some regions has been shown to exhibit a bias resulting 78 in over- or under-representation of specific taxa (Laudadio et al., 2018). Among commonly targeted 79 16S rRNA loci, the V3 – V4 and V4 – V5 are the most widely used and their different outcomes in 80 terms of bacterial taxa distribution and alpha diversity have been recognised in different matrices, 81 82 including biological and environmental samples (Cuccato et al., 2021; Rintala et al., 2017; Soriano-83 Lerma et al., 2020), as well food matrices, dairy products and fermented foods (Choi et al., 2020; Ferrocino et al., 2017; Liu et al., 2019; Macori and Cotter, 2018). As the short length of the targeted 84 85 16S rRNA loci represents one of the limitations for taxa identification below the family level, in recent years third-generation sequencing technologies facilitating long-read sequencing has been 86 87 developed, enabling full-length 16S (FL-16S) gene sequencing (Catozzi et al., 2020); although 88 platforms supporting these techniques, including Pacific Biosciences (PacBio) sequencers and Oxford 89 Nanopore Technologies (ONT) devices, generate read data with lower nucleotide accuracy when compared to the Illumina platforms, reading the FL-16S gene sequence can have better classification 90 91 resolution (Jeong et al., 2021), as confirmed in recent studies applying this sequencing technique on 92 mock communities and complex matrices such as wastewater samples (Numberger et al., 2019), 93 human faeces (Leggett et al., 2017; Matsuo et al., 2021) and water buffalo milk (Catozzi et al., 2020).

94 In contrast, shotgun metagenomic sequencing confers several advantages over 16S rRNA metabarcoding. First and foremost this strategy can provide functional information about the 95 96 investigated microorganisms; further, it provides an improved profile of the diversity of the sample 97 and can reach taxa resolution at the species level (Biegert et al., 2021). In this case, whole metagenomic DNA is first extracted, fragmented and then sequenced, independent of the 98 amplification of targeted genes (F. Addis et al., 2016). Thus, a large amount of data is generated to 99 100 be interrogated for features, including the taxonomic profile of the microbial community, its metabolic pathways and functions. Despite these advantages, some limitations are also recognised, 101 including the computational power required, tools and expertise necessary to properly analyse the 102 data generated; partial sequencing of those less represented microorganisms, whilst background 103 host DNA can be present in significant amounts, especially in host-derived samples including milk and 104 105 dairy products, requiring the use of different molecular and bioinformatic tools to mask these 106 features, such as pre-extraction methods applying commercially available kits or chemicals to lyse 107 mammals cells, and post-extraction methods enriching microbial DNA by selectively binding and removing CpG-methylated host DNA (Rubiola et al., 2020; Yap et al., 2020). Finally, the shotgun 108 109 metagenomic sequencing technique is usually more expensive when compared to 16S rRNA metabarcoding and requires a higher coverage (Catozzi et al., 2020). Comparison between WMS and 110 short-read 16S rRNA metabarcoding has been recently explored in different matrices, especially soil 111 112 and stool samples targeted to investigate the gut microbiome (Brumfield et al., 2020; Durazzi et al., 113 2021; Jovel et al., 2016; Laudadio et al., 2018; Shah et al., 2010; Tessler et al., 2017); indeed, the 114 extent to which these two sequencing technologies correlate with each other is a crucial assumption, which should be investigated in depth. However, food and food-related matrices have been poorly 115 investigated using both these sequencing techniques; further, the comparison between WMS and 116 FL-16S sequencing is still unexplored. In this context, several studies have suggested the use of milk 117

filters as useful tools to investigate the microbiome of bulk tank milk and to identify the presence offoodborne pathogens (Murphy et al., 2005; Sonnier et al., 2018)

To fill the aforementioned knowledge gap, in the present study milk filters sampled in the context of a previous work aiming to evaluate the milk production environment resistome were reanalysed using both the FL-16S rRNA metabarcoding and WMS in order to compare the microbial community profiles and evaluate the reliability of these two methods to explore the microbial communities of food-related matrices.

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#### 127 2. Materials and methods

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#### 2.1 Farms selection, samples collection and DNA extraction

The samples were collected in May 2020 from the bulk tank of 10 dairy farms located in Piedmont, North-West Italy, with the support of ARAP (Associazione Regionale Allevatori del Piemonte). The sampling procedure included the use of disposable in-line milk filters that were taken from the bulk tank of each farm under aseptic conditions, then inserted in sterile plastic sampling bags (Whirl-Pack, NASCO) and transported in controlled temperature to the Laboratory of Food Inspection -Department of Veterinary Science, University of Turin - where DNA extraction was performed immediately. The sampling was repeated in May 2021, for a total of 20 milk filters.

Upon arrival at the laboratory, 10 g of each milk filter were added to 90 ml of sterile buffered
saline solution (Ringer's solution, Oxoid, Basingstoke, UK) in a sterile stomacher bag and
homogenized for 2 min at 230 rpm in a stomacher (Seward Stomacher Blender 400, London, UK).
Total DNA was then extracted from filter homogenates using the DNeasy Blood and Tissue Kit

140 (QIAGEN, Hilden, Germany), with minor adjustments. Samples were centrifuged for 10 min at 100 × g 141 to pellet and discard eukaryotic cells; milk serum was then centrifuged at 13,000 × g for 15 min at 142 4°C to pellet prokaryotic cells and pellets recovered resuspended in phosphate-buffered saline [PBS] (Oxoid Basingstoke, UK). Isolation of genomic DNA was then performed following the manufacturer's 143 protocol, including the recommended modification for Gram-positive bacteria (Schwenker et al., 144 2022); DNA was eluted in 50  $\mu$ l 10 mM Tris-HCl buffer (pH 8.5) and frozen at -20°C until analyzed. 145 Template DNA of each sample was quantified using a Qubit 2.0 Fluorometer (Life Technologies, 146 Carlsbad, CA, USA) with the Qubit double-stranded DNA (dsDNA) high-sensitivity assay kit. DNA 147 integrity and purity were verified by conventional 2% agarose gel electrophoresis and also using a 148 NanoDrop spectrophotometer (ThermoFisher Scientific, Belgium). Samples meeting quality criteria 149 were submitted for FL-16S rRNA metabarcoding and WMS. 150

### 151 2.2 DNA sequencing

152 Purified DNA was submitted to both FL-16S gene sequencing and WMS. Library preparation for FL-16S was carried out starting from 10 ng of purified DNA from each sample using the 16S Barcoding 153 154 Kit 1–12 (SQK-RAB204, ONT, UK), following the manufacturer's instruction which includes the generation of FL-16S rRNA genes amplicons using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') 155 and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') starting with 10 µl input DNA (10 ng), 1 µl 16S 156 Barcode, at 10 µM, 25 µl LongAmp Taq 2X master mix (NEB, UK). The amplification was conducted 157 using the following cycling conditions: initial denaturation 1 min at 95 °C (1 cycle); denaturation 20 158 159 secs at 95 °C (25 cycles); annealing 30 secs at 55 °C (25 cycles); extension 2 mins at 65 °C (25 cycles); 160 final extension 5 mins at 65 °C (1 cycle). The samples were processed following the manufacturer's instruction with no modifications. Pooled libraries were then sequenced on a MinION platform (ONT, 161 162 UK) using Flongle (FLO-FLG001) flow cells (ONT, UK) for 24 h.

WMS DNA library preparation was carried out according to the NEBNext Ultra II DNA Library Prep Kit
for Illumina (New England Biolabs, Ipswich, MA); four PCR cycles were used to amplify the library.
Libraries quality and fragment lengths were determined using the Agilent Bioanalyzer 2100 and the
High-Sensitivity DNA kit (Agilent Technologies, Santa Clara, CA, USA).

The samples were sequenced on the Illumina NovaSeq 6000 platform using an S2 flow cell (Illumina,
San Diego, USA) with a 150-cycles paired-end (PE) chemistry, generating 50 million PE reads for each
sample.

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## 2.3 Bioinformatic and statistical analyses

FL-16S base-calling was performed using Guppy (version 5.0.15) and Flye (version 2.9) was used as*de novo* assembler.

The Fastp tool (Chen et al., 2018) was used to remove reads shorter than 1,000 bp and those reads retained thereafter filtered on a minimum average read quality score of 9, according to the recommendations from Nygaard et al (Nygaard et al., 2020). Processed sequencing data quality was assessed with MultiQC v1.11 (Ewels et al., 2016). Taxonomic classification was performed using Kraken2 v2.1.2 (Wood et al., 2019) and Bracken v2.5.0 (Lu et al., 2017) (threshold=10) with the NCBI NT database.

180 Raw reads generated by WMS were quality assessed using FastQC v.0.11.9 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) and MultiQC v1.11 with default 181 parameters. Raw reads were quality-trimmed using Trimmomatic version 0.39 (leading, 3; trailing, 3; 182 slidingwindow, 4:20; minlen, 36), removing low-quality regions, adaptor sequences and sequencing 183 primers. After the quality filtering step, clean reads were aligned using Bowtie2 v.2.4.4 against the 184 185 Bos taurus ARS-UCD1.2 bovine reference genome (NCBI Genome ID: 82), to remove host DNA sequences. Taxonomic classification of host-filtered reads was carried out using Kraken2 (Wood et
al., 2019); the package Bracken (Lu et al., 2017) was then used on Kraken reports to re-estimate
species abundance (threshold=10). Microbial taxonomic assignments of both amplicon and shotgun
metagenomic sequence data were performed using the NCBINT database.

Relative abundance tables for all samples were merged and imported into MicrobiomeAnalyst (Chong et al., 2020) for statistical and diversity analysis. Data from both WMS and FL-16S were analysed using alpha diversity metrics to assess the divergence of the microbial communities within each filter sample. Shannon Diversity (Mouillot and Leprêtre, 1999) and Simpson Diversity indexes were calculated from the observed operational taxonomic unit (OTU) counts for FL-16S and WMS data after centered log-ratio (clr) normalization. Rarefaction curves were generated to assess the saturation of samples analyzed using the WMS and FL-16S sequencing.

197 In order to perform a comparative statistical analysis of FL-16S and WMS data, each sample value 198 from each dataset was paired with its corresponding value for the same sample in the other dataset. The pairwise Spearman's correlation test was applied to investigate the amount of agreement 199 200 between the two datasets, including alpha diversity measures, richness (observed OTUs) and indexes 201 of Shannon's and Simpson's diversity. The composition of the core microbiome was assessed at genus 202 and family levels for FL-16S and WMS datasets using 50% and 1% cut-off values for occurrence and relative abundance of the OTUs, respectively (Neu et al., 2021); the abundance of shared OTUs was 203 visualized using heatmaps and Venn diagrams. 204

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207 **3.** Results

Shotgun metagenomic sequencing yielded 1.06 billion reads, with an average of 53.1 million reads per sample (range 44.8-76.8 million); out of 1.06 billion reads, a total of 6.2 million were identified at the bacterial and archaeal phyla level. FL-16S sequencing resulted in 166.928 reads, with an average of 8.346 reads per sample (range 2.759-30.168).

212 The number of observed OTUs gained from WMS was found to be higher in comparison with FL-16S sequencing datasets at each taxonomic level. In particular, at the family level, the number of families 213 214 detected by WMS was significantly greater compared to the number of families detected by FL-16S 215 sequencing (p-value <0.001, T-test), ranging from 7 to 37 OTUs per sample for FL-16S (mean 24.9) and from 227 to 301 OTUs per sample for WMS (mean 278,9); similarly, at the genus level, the 216 217 number of annotated genera observed by WMS was significantly greater compared to the number 218 of genera detected by FL-16S sequencing (p-value <0.001, T-test), ranging from 12 to 64 OTUs per 219 sample for 16S sequencing (mean 41,2) and from 614 to 850 OTUs per sample for WMS (mean 779.7). The number of genera and families identified in each sample by the two sequencing techniques are 220 221 reported as boxplots in **Figure 1**. Alpha diversity patterns were calculated at the family and genus 222 level using Shannon's and Simpson's indexes (Figure 1). At the family level, across samples analysed by WMS, both Shannon index alpha diversity and Simpson index alpha diversity were significantly 223 224 greater than alpha diversity values of samples analysed by FL-16S sequencing (difference between 225 means = 1,138 ± 0,1926,95% CI 0,7483-1,528, p-value < 0.0001; difference between means = 0,1324 ± 0,04367, 95% CI 0,04396 to 0,2208, p-value < 0.005, T-test). Similarly, at the genus level, across 226 227 samples analysed by WMS, both Shannon index alpha diversity and Simpson index alpha diversity 228 were significantly greater than alpha diversity values of samples analysed by FL-16S sequencing (difference between means = 1,238 ± 0,2132, 95% CI 0,8069-1,670, p-value <0.0001; difference 229 between means = 0,07861 ±0,03808,95% CI 0,001535-0,1557, p-value < 0.05, T-test). Thus, both the 230 observed Shannon index alpha diversity values and the Simpson index alpha diversity values were 231

greater for samples analysed by WMS compared to samples analysed by FL-16S sequencing at each
taxonomic level. Rarefaction curves showed that almost all samples reached the asymptote or started
to plateau despite the different technique applied (Supplementary File S1).

The top 10 most abundant genera profiled across the 20 samples by FL-16S sequencing and WMS corresponded to *Acinetobacter, Lactococcus, Escherichia, Streptococcus, Staphylococcus, Bacillus, Corynebacterium, Pseudomonas, Lactobacillus* and *Clostridium* (Figure 2). Most of the highly abundant genera detected per farm were detected by both FL-16S and WMS; however, different relative abundances were observed, mainly due to the overall lower number of OTUs annotated by full-length sequencing, consistently with the results of richness and diversity indexes.

All the diversity and richness measures, including observed OTUs, Shannon and Simpson diversity
indexes, were tightly correlated between FL-16S sequencing and WMS, at both the family (Observed
OTUs Spearman R = 0.6, p-value = 0.005; Shannon Spearman R = 0.75, p-value = 0.0002; Simpson
Spearman R = 0.6, p-value = 0.006) and genus level (Observed OTUs Spearman R = 0.68, p-value =
0.0008; Shannon Spearman R = 0.66, p-value = 0.001; Simpson Spearman R = 0.52, p-value = 0.01)
(Figure 3).

247 The presence of a core microbiome common to the sampled milk filters was confirmed in both 248 samples analysed by FL-16S sequencing and WMS. Out of 361 families detected across all samples, thirteen families were found in the core microbiome associated with milk filters analysed by FL-16S 249 sequencing and thirteen families were found in the core microbiome associated with milk filters 250 251 analysed by WMS; four of them were shared between the two core microbiomes, namely *Moraxellaceae, Enterobacteriaceae, Bacillaceae* and *Streptococcaceae*. Consistently, of the 1,078 252 253 genera identified across all samples, thirteen were found in the core microbiome associated with milk filters analysed by FL-16S sequencing, namely Acinetobacter, Escherichia, Staphylococcus, 254 Lactococcus, Bacillus, Streptococcus, Aerococcus, Clostridioides, Lactobacillus, Clostridium, 255

Oscillibacter and Paeniclostridium, eight were found in the core microbiome associated with milk
 filters analysed by WMS, namely Acinetobacter, Corynebacterium, Bifidobacterium, Actinoalloteichus,
 Pseudomonas, Bradyrhizobium, Escherichia and Bacillus, and three were shared between the two
 core microbiomes, that is Acinetobacter, Escherichia and Bacillus (Figure 4).

260 **4**. Discussion

The two most used sequencing methods to profile the microbiota of complex samples, including food 261 and food-related matrices, are the 16S metabarcoding and shotgun metagenomic sequencing. Both 262 these NGS techniques offer different advantages over culture-based methods; the 16S 263 metabarcoding has been used more frequently mainly due to its low cost, low computational power 264 265 requirements and standardized analysis methods, WMS is becoming more attractive for in-depth studies of microbial populations due to the large amount of information provided by this untargeted 266 sequencing technique, which facilitates study of the functional profile of complex microbiomes. 267 Recently, comparisons between high-throughput 16S rRNA sequencing and WMS have been 268 performed in selected matrices, including gut, soil and water samples (Brumfield et al., 2020; Ranjan 269 270 et al., 2016; Tessler et al., 2017). However food and food-related matrices are poorly investigated for 271 several reasons including, the large amount of host DNA that characterizes these samples might greatly interfere with different sequencing techniques; those comparative studies performed have 272 been based on selected hypervariable loci within the 16S rRNA gene, while the FL-16S sequencing 273 has proved to allow a less biased study of different microbial ecosystems (Catozzi et al., 2020). This 274 275 study reports on the comparison of FL-16S and WMS to investigate the microbial population of bulk 276 tank milk filters, both of which are powerful tools for the monitoring of food-borne pathogens and the investigation of the microbiome of bulk tank milk. 277

Although 16S metabarcoding is a promising, less expensive and more practical tool to investigate themicrobiome when compared to WMS, in the present study it allowed the identification of only most

abundant microorganisms in the biological samples investigated. Consistently, some previous studies 280 281 highlighted a significant amount of agreement between 16S metabarcoding and WMS methods at a 282 higher order of taxa, with a high degree of correlation found between 16S and WMS (Biegert et al., 283 2021; Vogtmann et al., 2016). Our findings support the greater resolution of WMS in terms of both 284 increased detection of bacterial taxa and enhanced detection of diversity; the superior richness in 285 the profiles of microbes obtained and their diversity must also be weighted with the already known advantages related to the possibility of investigating the function of predicted genes. Our results are 286 287 in accordance with studies analysing human faecal (Ranjan et al., 2016) and soil (Brumfield et al., 2020) microbiomes, which, despite investigating targeted hypervariable regions of the 16S rRNA 288 289 gene, revealed a greater diversity of microorganisms through the use of WMS. In this context, it must 290 be stated that the actual composition of the microbiome of analysed milk filter samples was 291 unknown; thereby, our approach, while enabling us to draw some conclusions on sensitivity, does 292 not enable the evaluation of the specificity of each sequencing technique. This issue goes beyond the 293 aims of the present study and can be addressed using simulated NGS data.

294 The present investigation of the milk filters' core microbiome through the application of both techniques has allowed the definition of a group of bacterial genera common to all the selected 295 296 samples; in particular, while different sequencing methods defined different core microbiomes, 297 Acinetobacter, Bacillus and Escherichia genera were shared between the FL-16S and the WMS cores. Although the microbiota profiles of distinct bulk tank milk filters were different, the presence of a 298 299 well-defined core microbiome, characterized by both the sequencing technique applied, highlights 300 the possibility to integrate multiple techniques to confirm the consistency of the achieved outcomes. The overall high occurrence and relative abundance of members of the Moraxellaceae, 301 302 Enterococcaceae, Bacillaceae and Streptococcaceae families in milk filters are consistent with the 303 profiled core microbiome of recent studies focusing on raw bovine milk collected in tankers (Kable et al., 2016; McHugh et al., 2020), thereby highlighting the deep correlation of microbial communities
 of bulk tank milk and microbial communities of in-line milk filters; most of the taxa belonging to the
 core microbiomes profiled by FL-16S and WMS are known to be associated with dairy processing
 environments.

This study set out the use of different high-throughput molecular methods to provide an in-depth 308 309 description of the microbiota of a food processing environment using milk filters as promising tools; 310 however certain limitations must be considered. This research was performed using a small number 311 of samples, although this was sufficient to identify significant differences between the compared 312 methods. Furthermore, a comparison including the most commonly used hypervariable regions of 313 the 16S rRNA gene (e.g. the variable V3 and V4 regions), together with the FL-16S and the WMS approach could provide further data to choose the more suitable method for different scientific 314 315 purposes. To our knowledge, this is the first study aiming to compare the use of FL-16S and WMS to investigate the microbial composition of a food-related matrix. Although, as anticipated, the 316 317 resolution power of WMS has proved to be greater than that provided by 16S sequencing, the 318 significant correlation of the two technologies both in terms of taxa diversity and richness, together with the similar profiles defined for both highly abundant taxa and core microbiomes, highlights the 319 320 possible application of both methods for different purposes. Thus, our findings suggest that the use 321 of FL-16S to perform large-scale microbiome studies can provide rapid and valuable data at a fraction 322 of the cost of WMS, which, on the other hand, is an incomparable tool to perform in-depth studies 323 of the microbiome, including low abundance taxa and functional profiles.

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#### 335 Authorship contribution

- 336 Selene Rubiola: Writing original draft; formal analysis; investigation; conceptualization. Guerrino
- 337 Macori: Writing-review & editing; resources; validation; conceptualization. Tiziana Civera: Writing-
- review & editing; funding acquisition. Séamus Fanning: Writing review & editing; resources. Molly
- 339 Mitchell: Writing review & editing; formal analysis. Francesco Chiesa: Writing review & editing;
- 340 visualization; supervision; conceptualization.

### 341 Conflict of Interest

- 342 The authors declare that the research was conducted in the absence of any commercial or financial
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#### 484 Figure legends

Figure 1. Boxplots showing the number of OTUs, the Shannon and Simpson alpha-diversity indexes
observed at the family and genus level. All indexes showed a statistically significant difference
between the mean measures observed in samples analysed by FL-16S and WMS.

Figure 2. The top-10 most abundant genera identified across the 20 samples analysed after centeredlog-ratio normalization; genera with a lower relative abundance are binned into "others" category.
Samples are organized by farm and year of sampling.

Figure 3. Correlation between WMS and FL-16S in terms of diversity at family and genus level. Each data point represents a single sample. Consensus between both sequencing methods in terms of alpha diversity was calculated by Spearman's correlation. The slope of the correlation is represented by the grey, continuous line, while the 95% confidence interval is represented by the area delimited by the grey dotted lines. The data derived from FL-16S sequencing correlates well with the diversity assessment values derived from WMS for diversity.

497 Figure 4. Core heatmaps and Venn diagrams showing bacterial families and genera detected in more
498 than 50% of samples with more than 1% of relative abundance. Four OTUs at both family and genus
499 levels were detected in all samples by FL-16S sequencing and WMS, thereby representing the shared
500 core microbiome.

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