

# Microbial biogeography of the transnational fermented milk matsoni

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## Highlights

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Comprehensively profiled matsoni fermentation microbiota across Armenia and Georgia.

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Region of origin affects bacterial and fungal composition in matsoni.

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Milk type alters microbial composition of matsoni fermentations.

## Abstract

The fermented milk matsoni is a traditional, national food product of both Georgia and Armenia. Little is known about the effects of biogeography and [milk type](#) on the microbial biodiversity of matsoni or the fungal composition of matsoni fermentations. High-throughput marker-gene sequencing was used to survey the bacterial and fungal communities of matsoni from different milk types and regions throughout Armenia and Georgia. Results demonstrate that both production region and milk type influence matsoni microbiota, suggesting that the traditional production methods preserve the transfer of unique regional microbiota from batch to batch. Bacterial profiles were dominated by [Lactobacillus](#) and [Streptococcus](#) species. Yeast profiles varied dramatically, with [Kluyveromyces marxianus](#), *Candida famata*, *Saccharomyces cerevisiae*, *Lodderomyces elongisporus*, and *Kluyveromyces lactis* being the most important species distinguishing production regions and milk types. This survey will enable more detailed capture and characterization of specific microbiota detected within these fermentations.

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## Keywords

Fermentation

Microbial ecology

Next-generation sequencing

### 1. Introduction

Matsoni (*syn.* mazun, matsoon) is a traditional Caucasian fermented milk product largely used from ancient times. It is considered an analog of yogurt and prepared from milk of cow, sheep, goat, buffalo, or a mixture thereof. Matsoni is made from heated or [pasteurized milk](#), cooled to 35–42 °C, and inoculated with a portion (approximately 1% wt/wt) of finished matsoni, thereby maintaining an ongoing culture consortium of [lactic acid bacteria](#) and yeasts. It is fermented at 35–42 °C overnight and the finished product has a characteristic pleasant, cultured milk taste and aroma. It is a national food throughout the Caucasus, where it is widely considered to have beneficial health effects, particularly for intestinal disorders, and to increase longevity.

In contrast to yogurt, which is fermented

by [Lactobacillus delbrueckii subsp. bulgaricus](#) and [Streptococcus thermophilus](#), matsoni microbiota comprise more abundant and different microbial species. Essentially important is that the microbial composition of matsoni apparently differs from various Caucasian areas but is very stable and characteristic for the region of origin ([Afrikian, 2009](#), [Afrikian, 2012](#)). Several studies have described the microbiota of matsoni fermentations, variously reporting [Lactobacillus delbrueckii subsp. lactis](#), [L. delbrueckii subsp. bulgaricus](#), [L. acidophilus](#), [S. thermophilus](#), [Lactococcus lactis](#), [L. lactis subsp. cremoris](#), [Geotrichum candidum](#), [Saccharomyces](#), [Candida](#), and other species of yeasts ([Saroukhanian, 1960](#), [Yerzinkian, 1965](#), [Yerzinkian, 1971](#), [Ter-Ghazarian, 1993](#), [Afrikian, 2009](#), [Afrikian, 2012](#), [Quero et al., 2014](#), [Uchida et al., 2007](#), [Reddy et al., 1986](#), [Merabishvili and Chanishvili, 2001](#)). Other bacteria, including [Lactobacillus helveticus](#), [Lactobacillus paracasei](#), and [Leuconostoc lactis](#) are also involved in some fermentations ([Quero et al., 2014](#)). This inconsistent bacterial composition has led some to conclude that the bacterial diversity of matsoni fermentations may be influenced by region of production ([Uchida et al., 2007](#), [Afrikian, 2009](#), [Afrikian, 2012](#)). Such biogeographical patterns have been described previously in other [food fermentations](#) ([Bokulich and Mills, 2013a](#), [Bokulich et al., 2014b](#), [Yu et al., 2011](#)) and may explain regional differences in matsoni product qualities, but a comprehensive survey of regional matsoni fermentations has yet to be described. The

effect of [milk type](#) on microbial composition is similarly uncharacterized in matsoni or similar dairy products.

The goal of this study was to better characterize how production region and milk type shape the bacterial and fungal constitution of matsonis in Armenia and Georgia. We used high-throughput amplicon sequencing as a culture-independent surveillance tool to characterize regional and milk-derived patterns in the bacterial and yeast populations of matsoni. Results indicate that regional and substrate-driven conditions shape the bacterial and fungal consortia of matsoni fermentations, and indicate that matsonis are a diverse source of microbial cultures for dairy fermentations.

## 2. Methods

### 2.1. Sample collection

Samples were collected aseptically on-site at the farms or production facilities producing matsoni, or at local markets, and transported on ice to the laboratory for immediate processing. A total of 194 samples were collected from different sites within 18 distinct regions in Georgia, Armenia, and Nagorno Karabakh ([Fig. 1](#), [Table 1](#)). Matsoni fermentations typically last 24 h and all samples were collected from finished fermentations. DNA was extracted using the ZR-96 Fecal DNA MiniPrep Kit (Zymo Research, Irvine, CA), with bead beating in a FastPrep-24 bead beater (MP Bio, Solon, OH), and stored at  $-20\text{ }^{\circ}\text{C}$  until further processing.



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Fig. 1. Matsoni sample collection map. Each point represents the coordinates of a single sampling site within Armenia (red), Georgia (blue), or the Nagorno Karabakh region (green, bordered by dashed line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1. Description of matsoni samples collected.

	<b>Cow</b>	<b>Buffalo</b>	<b>Goat</b>	<b>Sheep</b>	<b>Total</b>
Armenia	21	22	46	32	121
Georgia	10	10	16	8	44
Nagorno Karabakh	4	11	14	0	29
Total	35	43	76	40	194

## 2.2. Marker-gene sequencing library construction

Amplification and sequencing was performed as described previously for bacterial ([Bokulich et al., 2012](#)) and yeast communities ([Bokulich and Mills, 2013b](#)). Briefly, the V4 domain of bacterial 16S rRNA genes was amplified using primers F515 (5'–*NNNNNNNN***GTGTGCCAGCMGCCGCGGTAA**–3') and R806 (5'–**GGACTACHVGGGTWTCTAAT**–3') ([Caporaso et al., 2011](#)), with the forward primer modified to contain a unique 8 nt barcode (italicized poly-N section of primer above) and 2 nt linker sequence (bold, underlined portion) at the 5' terminus. **PCR** reactions contained 5–100 ng DNA template, 1X GoTaq Green Master Mix (Promega, Madison, WI), 1 mM MgCl<sub>2</sub>, and 2 pmol of each primer. Reaction conditions consisted of an initial 94 °C for 3 min followed by 35 cycles of 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s, and a final extension of 72 °C for 10 min. Fungal internal transcribed spacer (ITS) 1 loci were amplified with primers BITS (5'–*NNNNNNNN***CTACCTGCGGARGGATCA**–3') and B58S3 (5'–GAGATCCRTTGYTRAAAGTT–3') ([Bokulich and Mills, 2013b](#)), with a unique 8 nt barcode and linker sequence incorporated in each forward primer. PCR reactions contained 5–100 ng DNA template, 1X GoTaq Green Master Mix (Promega, Madison, WI), 1 mM MgCl<sub>2</sub>, and 2 pmol of each primer. Reaction conditions consisted of an initial 95 °C for 2 min followed by 40 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s, and a final extension of 72 °C for 5 min. Amplicons were combined into two separate pooled samples (keeping bacterial and fungal amplicons separate) at roughly equal amplification intensity ratios, purified using the Qiaquick spin kit (Qiagen, Valencia, CA), and submitted to the UC Davis Genome Center DNA Technologies Core for Illumina paired-end library preparation, cluster generation, and 250-bp paired-end sequencing on an Illumina MiSeq sequencer (Illumina, San Diego, CA).

## 2.3. Data analysis

Raw fastq files were demultiplexed, quality-filtered, and analyzed using QIIME v.1.8.0 ([Caporaso et al., 2010b](#)). The 250-bp reads were truncated at any site of more than three sequential bases receiving a quality score <Q10, and any read containing ambiguous base calls or barcode/primer errors were discarded, as were reads with <75% (of total read length) consecutive high-quality base calls ([Bokulich et al., 2013b](#)). Reverse primer sequences were trimmed from the ends of ITS sequences following demultiplexing. Operational taxonomic units (OTUs) were clustered at 97% identity using the QIIME subsampled reference OTU-picking pipeline using UCLUST-ref ([Edgar, 2010](#)) against either the Greengenes 16S rRNA gene database (May 2013 release) ([McDonald et al., 2012](#)) or the UNITE fungal ITS database ([Abarenkov et al., 2010](#), [Koljalg et al., 2005](#)), modified as described previously ([Bokulich and Mills, 2013b](#)). OTUs were classified taxonomically against these same databases using RDP classifier ([Wang et al., 2007](#)). Any OTU comprising less than 0.01% of total sequences for each run were removed prior to further analysis ([Bokulich et al., 2013b](#)). Bacterial 16S rRNA gene sequences were aligned using PyNAST ([Caporaso et al., 2010a](#)) against a reference alignment of the Greengenes core set ([McDonald et al., 2012](#)). From this alignment, chimeric sequences were identified and removed using ChimeraSlayer ([Haas et al., 2011](#)) and a phylogenetic tree was generated from the filtered alignment using FastTree ([Price et al., 2010](#)). Sequences failing alignment or identified as chimera were removed prior to downstream analysis.

Beta-diversity (between-sample community dissimilarity) estimates were calculated within Phyloseq ([McMurdie and Holmes, 2013](#)) using weighted UniFrac ([Lozupone and Knight, 2005](#)) distance between samples for bacterial 16S rRNA reads (evenly sampled at 1500 sequences per sample) and Bray–Curtis dissimilarity for fungal ITS reads (evenly sampled at 100 reads per sample). Non-metric multidimensional scaling (NMDS) coordinates were computed from the resulting distance matrices to compress dimensionality into two-dimensional NMDS plots, enabling visualization of sample relationships. In order to determine whether regional factors (country, region, or district of production) or substrate factors (milk type or starter type) related to differences in phylogenetic or OTU diversity, permutational MANOVA ([Anderson, 2001](#)) with 999 permutations was used to test the null hypothesis that sample groups were not statistically significant. For all classifications rejecting this null hypothesis, a Kruskal–Wallis test was used to determine which taxa differed significantly (with Bonferroni error correction) between sample groups.

### 3. Results

Finished matsoni fermentation samples were collected from 194 locations spanning 18 distinct regions in Georgia, Armenia, and Nagorno Karabakh (Fig. 1, Table 1). Regional variation was assessed across three different geographic scales: between countries (including Nagorno Karabakh as a separate entity), between production regions, and between individual administrative districts. The effects of ingredients on microbial composition were also tested, comparing matsonis produced from different milks (cow, goat, buffalo, sheep) and different starter cultures (i.e., inoculated with matsoni made from one of these four milks). As we expected a high degree of interaction between regional factors and ingredients, the data were dissected to test individual factors in isolation within single geographic units and within individual milk types.

Marker-gene sequencing revealed a complex relationship between regional and substrate-driven (i.e., source milk and starter type) factors and the microbial consortia of matsoni fermentations. Country, region, and district of production all influenced microbial community composition across all samples, within individual countries and regions, and within individual milk types (Table 2). Source milk and starter type also impacted diversity across all countries, within individual regions, and within individual districts. However, these effects were not universally observed and some regions and milks displayed more homogeneous bacterial or fungal diversity than others. In particular, sheep milk fermentations and Georgian samples exhibited fewer patterns related to production region, milk type, and starter type.

Table 2. One-way permutational MANOVA comparisons between regions and substrates.

Comparison type		Subset	Comparison	Bacterial weighted UniFrac		Fungal Bray–Curtis		
				R	P	R	P	
Regional	All samples	All samples	Country	0.057	0.001***	0.084	0.001***	
			Region	0.296	0.001***	0.278	0.001***	
			District	0.690	0.001***	0.521	0.001***	
	Within country	Armenia	Region	0.279	0.001***	0.223	0.001***	
			District	0.768	0.001***	0.505	0.001***	
		Georgia	Region	0.186	0.275	0.338	0.006**	
			District	0.397	0.444	0.645	0.006**	
		Within region	Nagorno Karabakh	District	0.246	0.003*	0.125	0.11
			Kotayk	District	0.808	0.001***	0.350	0.001***
Tavush	District		0.382	0.017*	0.623	0.001***		
Within milk type	Buffalo	Country	0.147	0.001***	0.271	0.001***		
		Region	0.706	0.001***	0.696	0.001***		

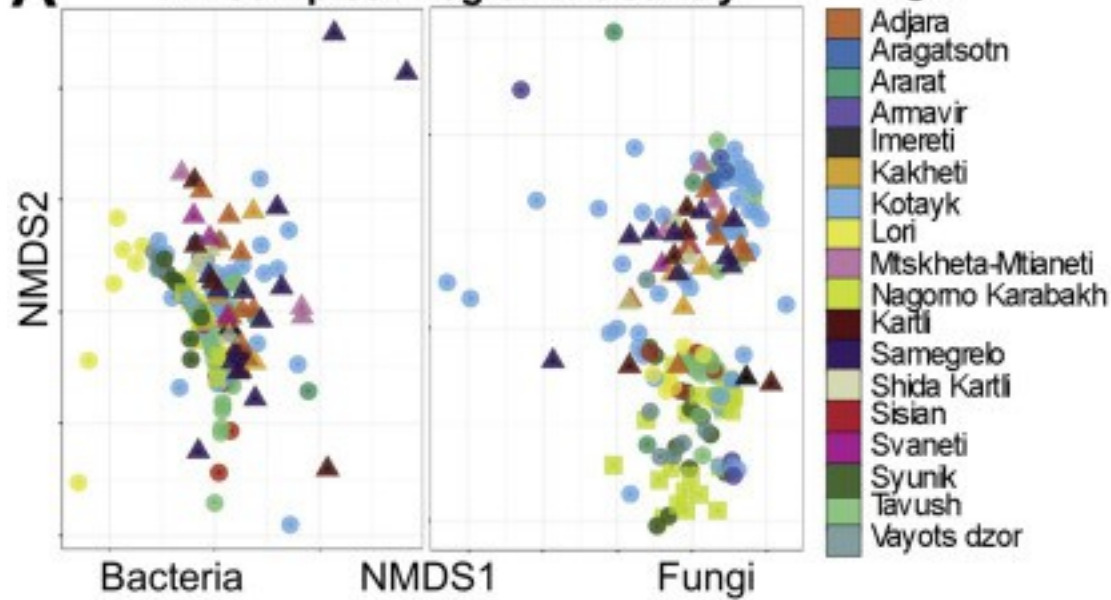
Comparison type		Subset	Comparison	Bacterial weighted UniFrac		Fungal Bray–Curtis		
				R	P	R	P	
Substrate	All samples	Cow	District	0.895	0.001***	0.847	0.001***	
			Country	0.172	0.003*	0.139	0.007**	
			Region	0.722	0.001***	0.387	0.007**	
		Goat	District	0.849	0.001***	0.636	0.009**	
			Country	0.098	0.002**	0.167	0.001***	
			Region	0.483	0.002**	0.392	0.001***	
		Sheep	District	0.596	0.01*	0.680	0.001***	
			Country	0.062	0.043*	0.043	0.118	
			Region	0.605	0.001***	0.378	0.205	
	Within country	Armenia	District	0.796	0.001***	0.795	0.001***	
			Milk	0.227	0.001***	0.050	0.001***	
		Georgia	Starter	0.360	0.001***	0.094	0.001***	
			Milk	0.328	0.001***	0.064	0.001***	
		Nagorno Karabakh	Starter	0.482	0.001***	0.145	0.001***	
			Milk	0.044	0.781	0.116	0.185	
		Within region	Kotayk	Starter	0.044	0.782	0.116	0.213
				Milk	0.193	0.022*	0.404	0.001***
			Tavush	Starter	0.241	0.001***	0.106	0.021*
				Milk	0.589	0.001***	0.143	0.002**
			Tavush	Starter	0.531	0.002**	0.171	0.001***
Milk	0.298			0.023*	0.175	0.152		
		Starter	0.371	0.001***	0.553	0.001***		

The complex interactions between regional factors and ingredients are apparent using Non-metric Multidimensional Scaling (NMDS) to visualize how these factors affected microbial community similarity at multiple geographical scales and within different types of matsoni (Fig. 2). Comparing all samples, matsonis from Georgia and Nagorno-Karabakh are separated by NMDS, but Armenian samples bridge both clusters; individual regions within each country are more highly differentiated (Fig. 2A). Regional variation is even clearer when comparing matsonis from individual milk types (Fig. 2B–D), highlighting production region as a salient factor driving the microbial composition of matsoni fermentations and suggesting that milk type also contributes. Milk type did not significantly alter microbial diversity across all samples (Table 2) and does not explain

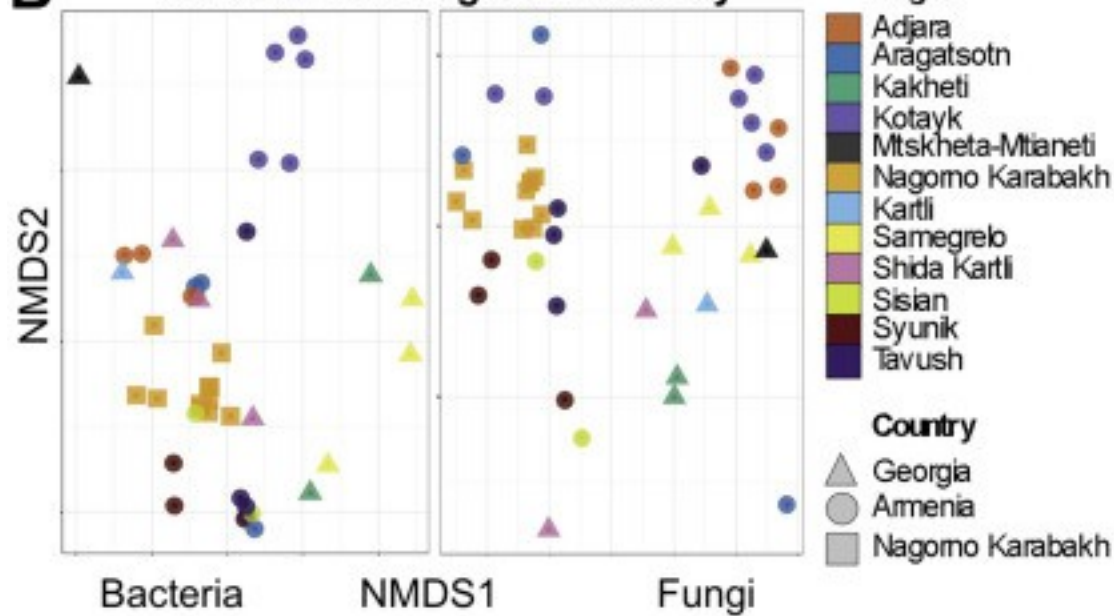
sample ordination by NMDS (data not shown). However, matsonis segregate by milk type within smaller geographic scales, e.g., within Armenia, Nagorno Karabakh, and individual production regions ([Fig. 2E–G](#)). In most of these cases, regional factors still explain sample ordination to a greater degree (e.g., Armenian samples cluster by production region), but sub-clusters based on milk type are apparent within many of these regional divisions ([Fig. 2E–G](#)). In some cases, starter culture type (i.e., inoculated with finished cow matsoni, goat matsoni, or matsoni made from another milk type) exerted a greater effect on microbial composition than milk type, such as in Tavush (a region of Armenia) where samples clearly cluster by starter type rather than milk type ([Fig. 2H](#)).

## Regional Variation

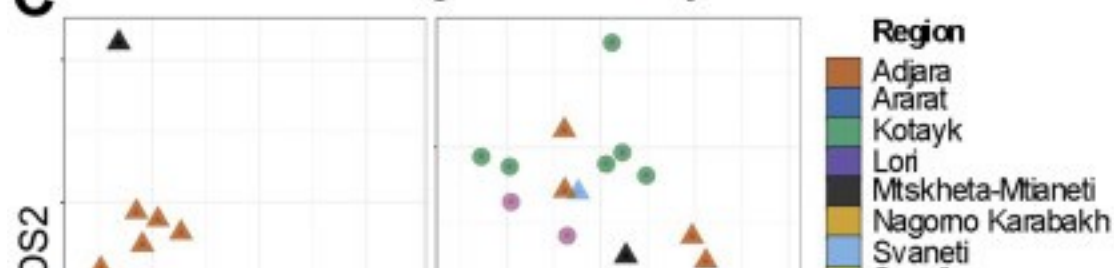
**A** All Samples: Region x Country



**B** Buffalo Milk: Region x Country

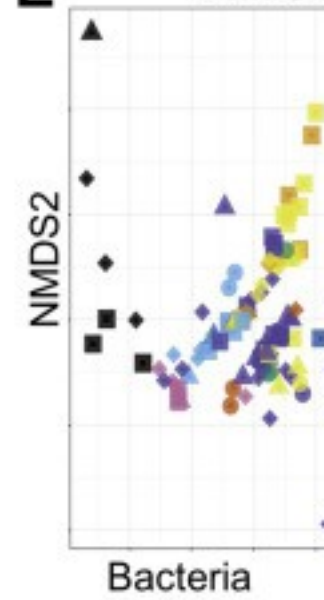


**C** Cow Milk: Region x Country

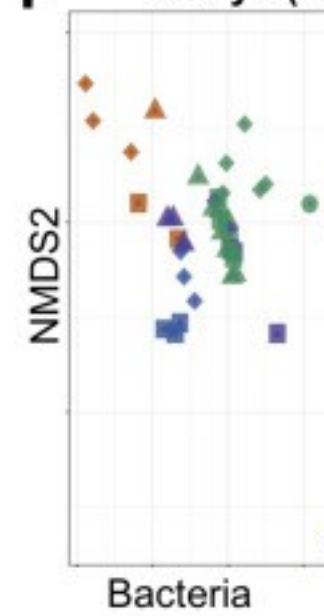


## Sub

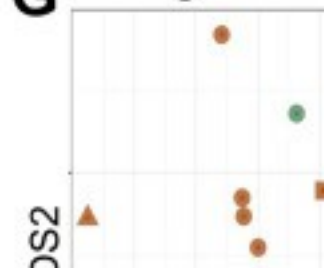
**E** Armen



**F** Kotayk (A



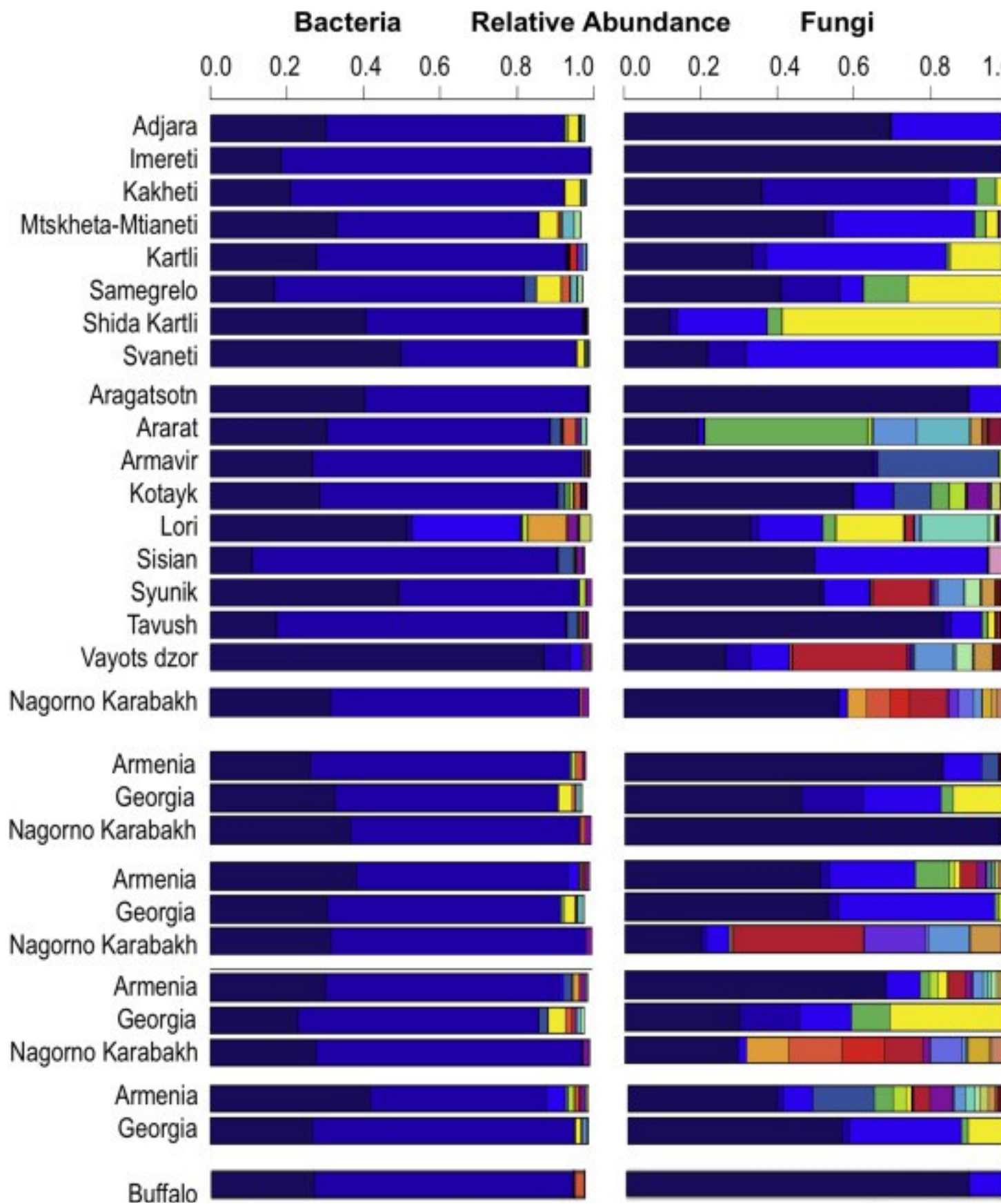
**G** Nagorno K



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Fig. 2. Matsoni [microbial communities](#) vary by region, starter, and [milk type](#). Non-metric multidimensional scaling plots of bacterial and fungal communities across different sample subsets. Each pair of panels depicts non-metric multidimensional scaling plots of bacterial (left panels) and fungal communities (right panels) within sample subsets. The title above each twin panel indicates the sample subset, followed by the metadata categories used to determine the color and shape of each point, respectively, in the following format: (subset): (color category) × (shape category). Individual samples are represented by a single point, and the proximity between points indicates the degree of similarity between those samples. In the plots comparing production regions (left-hand side of figure), regional variation is observed as clusters of samples from different regions (colors); the separation of these clusters indicates that the microbial communities within the samples identify this region (or regions) from other, disparate clusters. In the plots comparing substrates (milk and starter types) within sub-regions (right-hand side of the figure), samples cluster by milk type, starter type, or sub-clusters explained by milk type are apparent within the regional clusters (e.g., the Armenia and Kotayk plots).

Across regions and milk types, marker-gene sequencing detected similar bacterial compositions. Most samples were dominated by [Lactobacillus](#) and [Streptococcus](#) species (>80% combined relative abundance) with minor populations of other bacteria ([Fig. 3](#)). [Lactococcus](#) and [Enterococcus](#) species were prevalent secondary populations observed in many samples. [Pseudomonas](#), Rummeliibacillus, [Enterobacteriaceae](#), *Erwinia*, *Wautersiella*, and [Yersinia](#) were also frequently observed.



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Fig. 3. Microbial composition of matsoni fermentations. Bar plots indicate the average relative abundances of bacteria (left) and fungi (right) in matsoni fermentations from different production regions, [milk types](#), and starter types within the Armenian region of Tavush. Only taxa detected at 1% or more average abundance in at least one sample subset are shown. The length of each bar represents the average relative abundance of that taxon in the designated sample subset; empty space represents the sum of other species detected at less than 1% average abundance in all sample subsets.

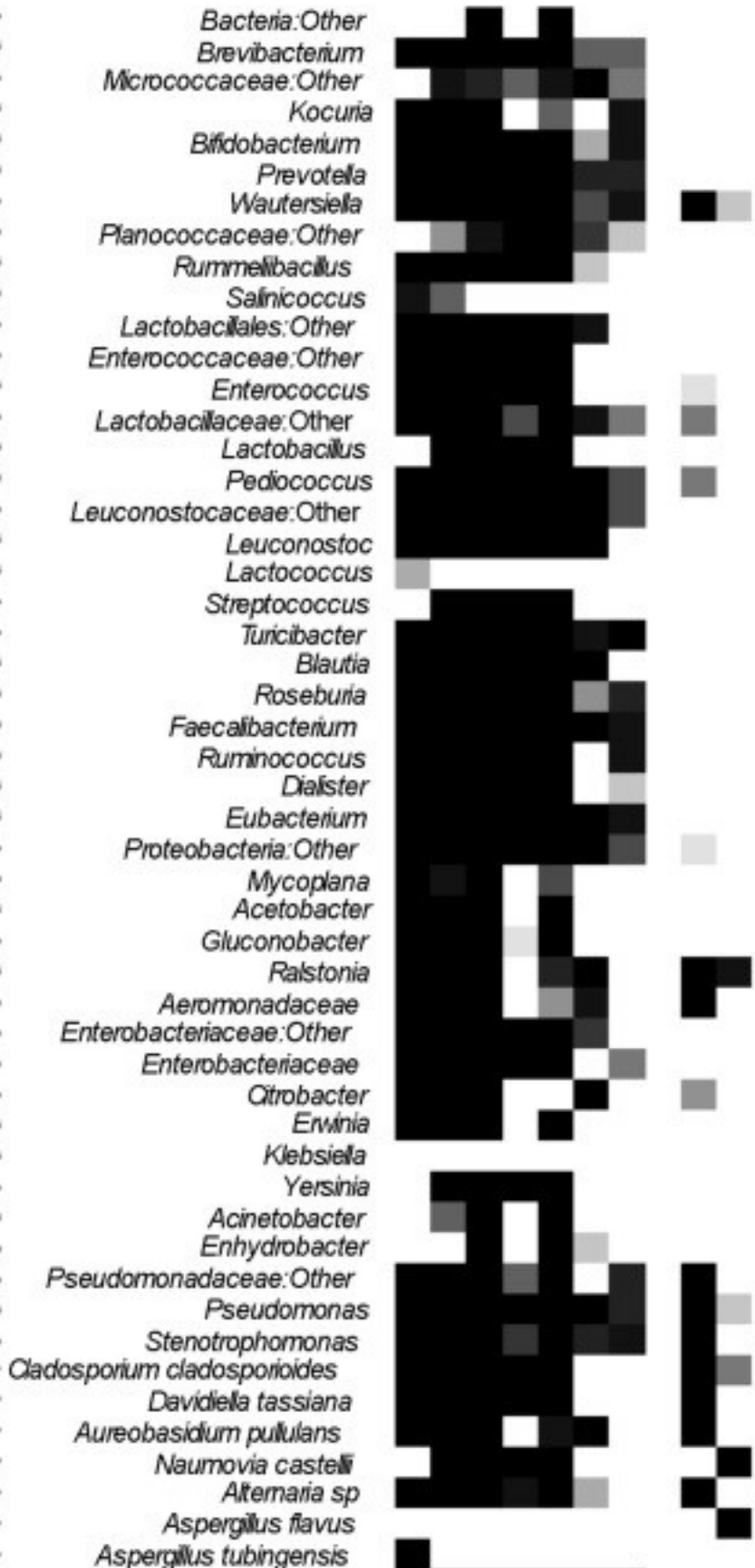
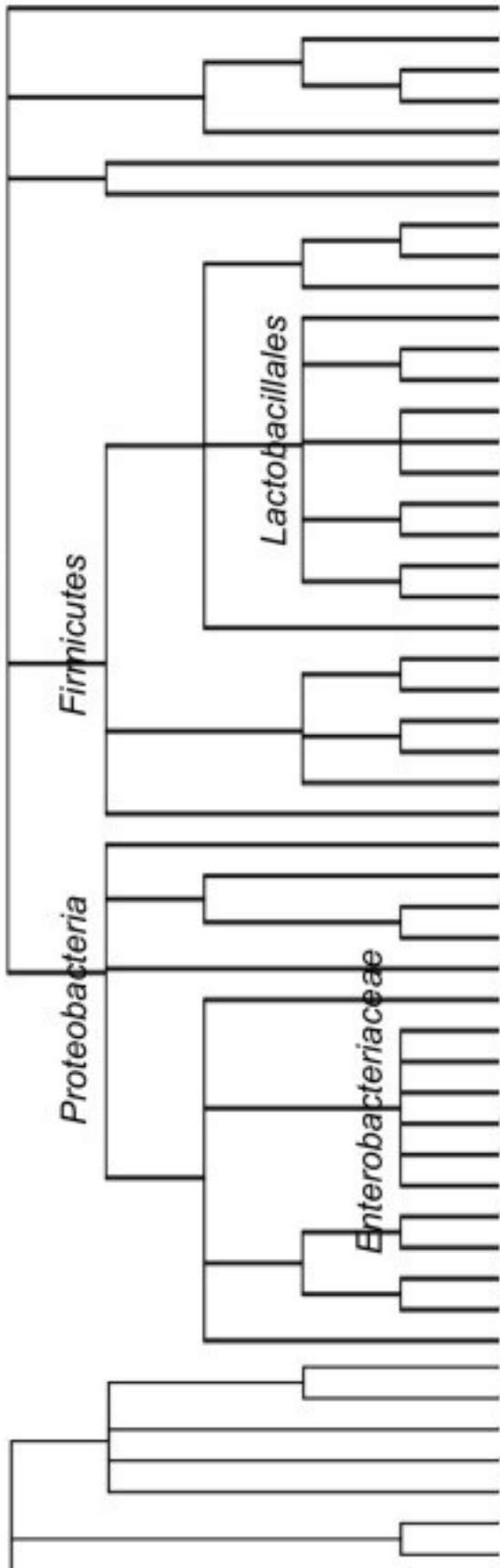
NK = Nagorno Karabakh.

Yeast populations exhibited greater variation between samples

([Fig. 3](#)). *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* displayed the greatest average abundance across all samples. Some samples were clearly dominated by other yeasts, often distinguishing individual regions and milk types. For example, *Lodderomyces elongisporus* dominated a subset of Armenian sheep's milk matsonis; *Kluyveromyces lactis*, *Candida zeylanoides*, and *Candida famata* were prominent in Georgian goat matsonis; *K. lactis* and *Naumovia castellii* in some Armenian goat matsonis; *Pichia kudriavzevii* in some Armenian sheep matsonis; and *Candida parapsilopsis*, *Candida albicans*, and *Pichia guilliermondii* in various goat matsonis from Nagorno Karabakh.

Many of these microbial populations were differentially abundant between milk types and especially between regional zones, becoming defining features of certain local matsonis ([Fig. 4](#)). Most *Firmicutes* (including *Lactobacillales*), *Actinobacteria*, *Enterobacteriaceae*, and other bacterial clades were differentially abundant between countries, regions, and districts at multiple geographic scales and in different milk types. [Buffalo and goat milks](#) exhibited a particularly large number of regionally defining taxa, whereas regional cow and sheep matsonis had fewer defining features.

Bacteria



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Fig. 4. Microbial populations exhibit regional and substrate-driven patterns of abundance. Heat map indicates which bacteria and fungi are differentially abundant between production regions and substrate types across various sample subsets, according to Kruskal–Wallis tests. Microbes are ordered along rows according to taxonomic affiliation (as indicated by the taxonomic dendrogram). Category comparisons between regions and substrates are ordered by column, as are the sample subsets in which these comparisons were made, as indicated below. Bonferroni-corrected Kruskal–Wallis  $P$  values for each organism  $\times$  comparison are indicated at the intersections between each row and column. Only organisms with at least one significant interaction are shown.

Milk and starter types had fewer differential taxa compared to regions, but several trends were still observed. Species of *Lactococcus*, *Klebsiella*, *Salinicoccus*, *Planococcaceae*, *Acinetobacter*, *Enhydrobacter*, and *Micrococcaceae* were consistently differential between milk types at multiple geographic scales. Broad swathes of *Lactobacillales* and [bacilli](#), *Gluconobacter*, *Ralstonia*, *Enterobacteriaceae*, *Enhydrobacter*, *Pseudomonas*, and [Bifidobacterium](#) species defined starter types at different geographic scales. Between starters in Tavush district, where starter cultures were the primary factor driving microbial composition, various [Clostridia](#), *Turicibacter*, *Brevibacterium*, *Bifidobacterium*, *Enterobacteriaceae*, [Prevotella](#), and *Wautersiella* were the most defining bacterial taxa. Many of these taxa were detected at <1% relative abundance, and influence on flavor development during active fermentation is unlikely. Even fewer yeast and other fungal species were differentially abundant between milk and starter types. *K. marxianus*, *K. lactis*, *Issatchenkia* spp., *Alternaria* spp., *Davidiella tassiana*, and *Cladosporium cladosporioides* differentiated milk types in Nagorno Karabakh and *C. parapsilopsis* differentiated milk types in Kotayk, but no species were differentially abundant between larger geographic areas. *C. cladosporioides*, *N. castellii*, *P. kudriavzevii*, *K. marxianus*, *S. cerevisiae*, and *Hanseniaspora uvarum* differentiated starter types variously across all regions as well as at smaller scales. In Tavush, *Botryotinia* spp., *L. elongisporus*, and *Zygoascus hellenicus* defined starter types.

## 4. Discussion

Matsoni is an interesting case study for food biogeography, as fermentation is still performed by inoculating milk with portions of previous fermentations instead of using defined starter cultures. Hence, locally unique cultures likely establish and persist, potentially leading to the regional character of this transnational product. Matsoni

fermentation microbiota exhibit a large degree of biodiversity, influenced by both geography and [milk type](#). The basic techniques for producing matsoni are highly similar in all areas but the combination of local ingredients, local techniques, and microbial biogeography inherent across these regions distinguish individual source locations. These findings explain the noted inconsistencies between earlier surveys of matsoni bacteria ([Uchida et al., 2007](#)). Matsoni fermentations are not conducted by one standardized culture, but instead display a large degree of heterogeneity, linked to the source milk and production region. Several core taxa dominated most matsoni fermentations — *Lactobacillus* spp., [Streptococcus](#) spp., and *K. marxianus* — but other taxa are present and distinguish matsonis of unique provenance. The bacterial profiles, consisting predominantly of a mixture of *Lactobacillus* and *Streptococcus* species, are similar to other yogurt-type fermentations, though the minor bacterial taxa (e.g., [Enterococcus](#), [Pseudomonas](#), [Enterobacteriaceae](#)) are not typical. Another factor that distinguishes matsonis from other yogurts is that the fermentations contain large populations of yeasts. *K. marxianus* was the most common, dominant yeast observed, followed by *S. cerevisiae* and *C. famata*. *K. lactis* was dominant in some Georgian samples — particularly from Shida Kartli — while *L. elongisporus*, *C. albicans*, and *C. zeylanoides* were prevalent in matsonis from various regions of Armenia. Yeasts, including species of [Kluyveromyces](#), *Saccharomyces*, and *Candida*, can significantly alter the sensory profiles of dairy products through the production of alcohols, organic acids, CO<sub>2</sub>, and proteolytic and lipolytic activities, distinguishing yeast-fermented products from other thermophilic dairy fermentations ([Roostita and Fleet, 1996](#), [Fleet, 1990](#), [Wouters et al., 2002](#)). These behaviors may explain many of the unique characteristics of regional matsonis, including effervescence and fruity and yeasty flavors. However, more work must be done to examine the role of these cultures in flavor production during matsoni fermentation and storage. The biodiversity of matsoni contributes to the rich variety of flavors and textures typical of different matsonis ([Quero et al., 2014](#)), and may be a valuable source to mine for [dairy starter cultures](#) ([Wouters et al., 2002](#)).

Milk variety also shapes matsoni microbiota, though the effect is most apparent within defined geographical scales (e.g., within a single production region). It is unclear whether these microbiota are influenced by milk chemistry, animal-associated microbiota, or other factors, and to what degree these effects reflect site-specific differences. Although some villages purvey matsonis from different milk types, these different animals may be raised on different farms, leading to differences in both local microbial inputs, as well as differences in milk handling. Milks from different animal

species or breeds can contain widely different contents of fat, protein, [lactose](#) and other sugars, [oligosaccharides](#), and other [macronutrients](#), as well as [lactoferrin](#), [immunoglobulin](#), and other antimicrobial compounds ([Jensen, 1995](#), [Peterson et al., 2013](#)). [Milk composition](#) could thus influence fermentation microbiota in matsoni or other dairy fermentations. Further studies are needed to assess what role milk chemistry may play in driving fermentation microbiota. More work is needed to understand several aspects of microbial activity in matsoni production. Seasonal variation in ingredients can influence the microbiota of dairy fermentations ([Wullschleger et al., 2013](#)), potentially explaining variation between batches of matsoni ([Quero et al., 2014](#)). Repeated sampling of matsoni fermentations at multiple individual sites will clarify the effect of seasonal variation on matsoni microbiota. The equipment and production environment in which matsonis are made could be important vectors for microbial transmission between batches, similar to other foods ([Bokulich and Mills, 2013a](#), [Bokulich et al., 2014a](#), [Bokulich et al., 2013a](#), [De Filippis et al., 2013](#)), Studying the production environment in matsoni production sites will be important to understand how processing areas contribute to the biogeographical diversity of matsoni fermentations. Finally, the influence of the microbiota on matsoni [sensory properties](#) is poorly described, particularly for low-abundance microbiota and yeasts. Thus, it is currently unknown whether regional biogeography of matsoni microbiota is actually related to the regional flavor characteristics of different matsoni fermentations. Even the dominant yeasts in these matsoni fermentations have undetermined roles that deserve better characterization for their utility in these thermophilic dairy fermentations.

This study describes the first complete culture-independent survey of matsoni bacteria, yeasts, and other fungi in a range of milk types throughout Armenia and Georgia. Results demonstrate that matsoni microbiota are shaped by region of production and milk type, indicating that the traditional method of serial inoculation preserves the transfer of unique regional microbiota from batch to batch. This survey will enable more detailed capture and characterization of specific microbiota detected within these fermentations.

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