

Longitudinal Study on the Milk Microbiota of Dairy Cows Diagnosed with *Staphylococcus aureus* Clinical Mastitis

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INTRODUCTION

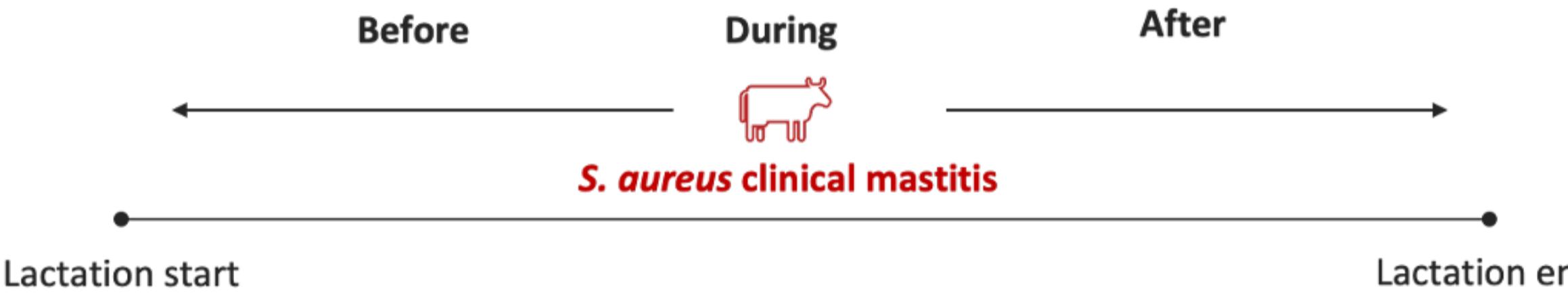


HYPOTHESIS

We hypothesize that the composition of the microbiota **may be predictive** of which quarters will eventually develop *S. aureus* clinical mastitis, and the presence of certain members of the microbiota **may have a more protective effect** against *S. aureus* colonization than others.

METHODS

Longitudinal Cohort Study
S. aureus clinical mastitis via natural infections



RESULTS

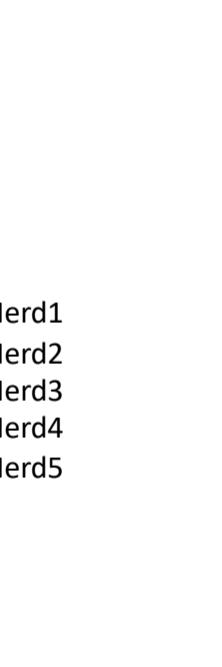
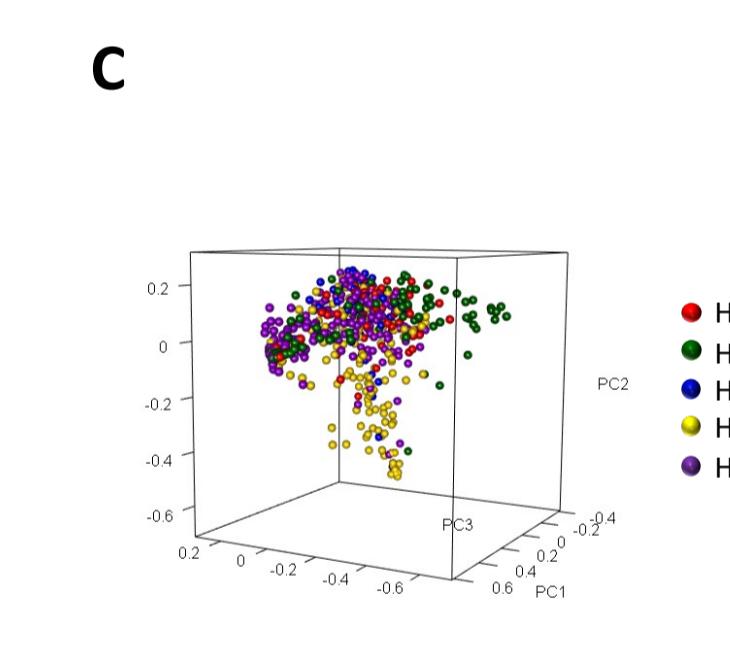
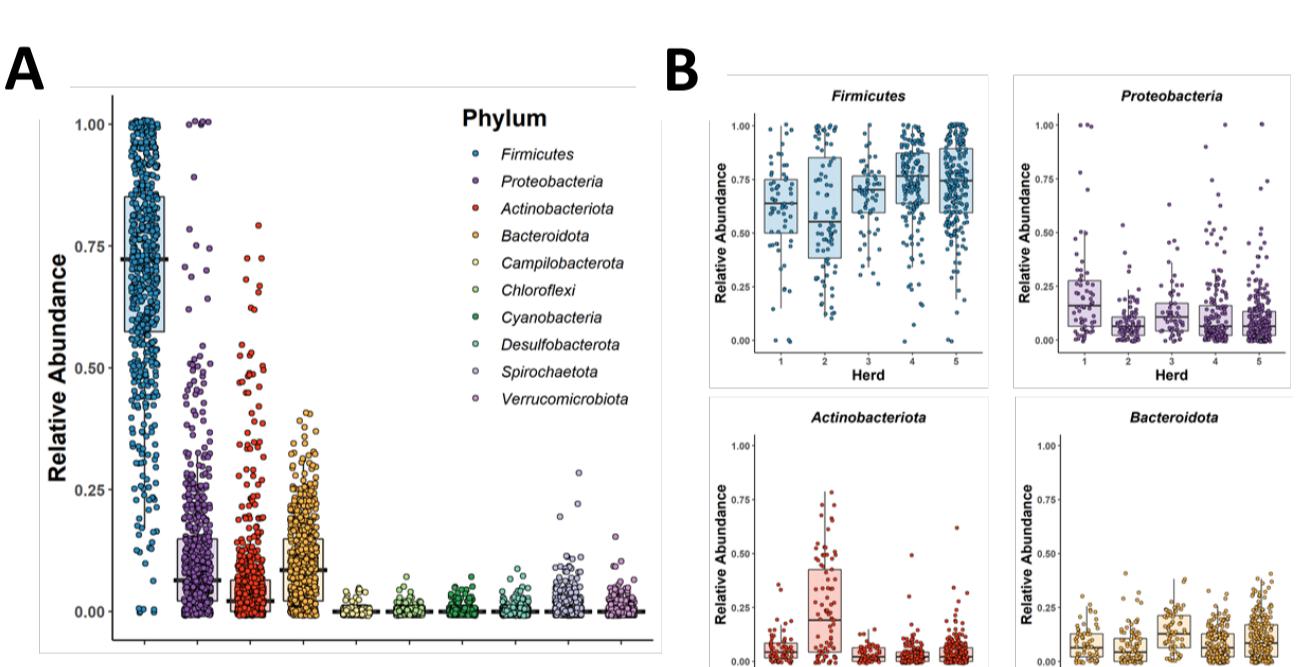


Figure 1. Relative abundance of the raw milk microbiota of five herds (1-5) and comparison of dissimilarity between herds and cows. (A) The relative abundance of each phylum in the milk samples showed four major phyla: Firmicute, Proteobacteria, Actinobacteriota, and Bacteroidota. (B) The relative abundance of the four major phyla varied in five herds. At the genus level, *Staphylococcus* was the most abundant genus in all five herds. The distribution of *Aerococcus* was high in Herd4 while two genera in Actinobacteriota and unclassified *Intrasporangiaceae* were mostly found in Herd2. (C) Beta-diversity varied significantly between herds ($F = 14.179$, $p < 0.001$) and between cows ($F = 7.4166$, $p < 0.001$). The level of dissimilarity between herds was higher than between cows.

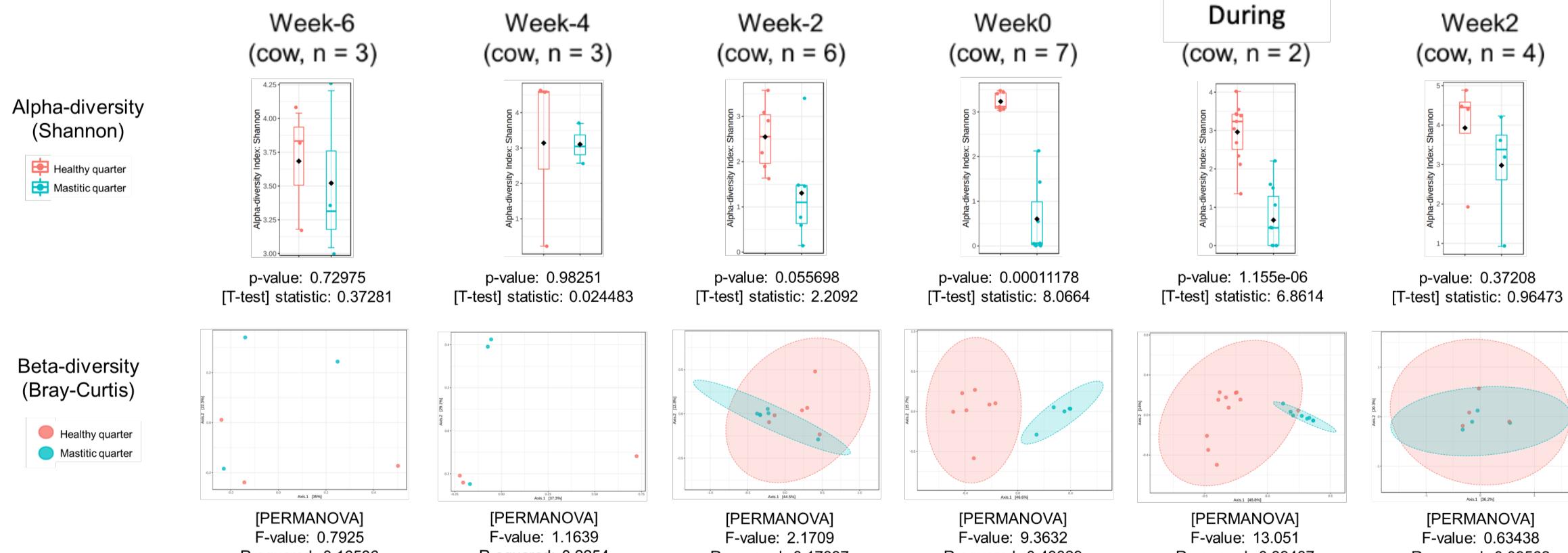


Figure 2. Microbial changes in alpha- and beta-diversity and biomarkers before, during, and after *S. aureus* clinical mastitis (CM) in Group I. Group I consisted of sick cows ($n = 7$) where *Staphylococcus* was detected in mastitic quarters at Week 0. Alpha-diversity in healthy quarters was significantly different from sick quarters at Week -2. Both alpha- and beta-diversity were significantly dissimilar between healthy and sick quarters at Week 0, which then remained as such while the mastitis continued for a few more weeks (Week0_during). Two weeks after the resolution of *S. aureus* CM (Week2), alpha- and beta-diversity were recovered similar to the healthy quarters.

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Bacteria found in bovine mammary glands can diagnose *S. aureus* clinical mastitis early and protect the mammary gland from mastitis pathogens.

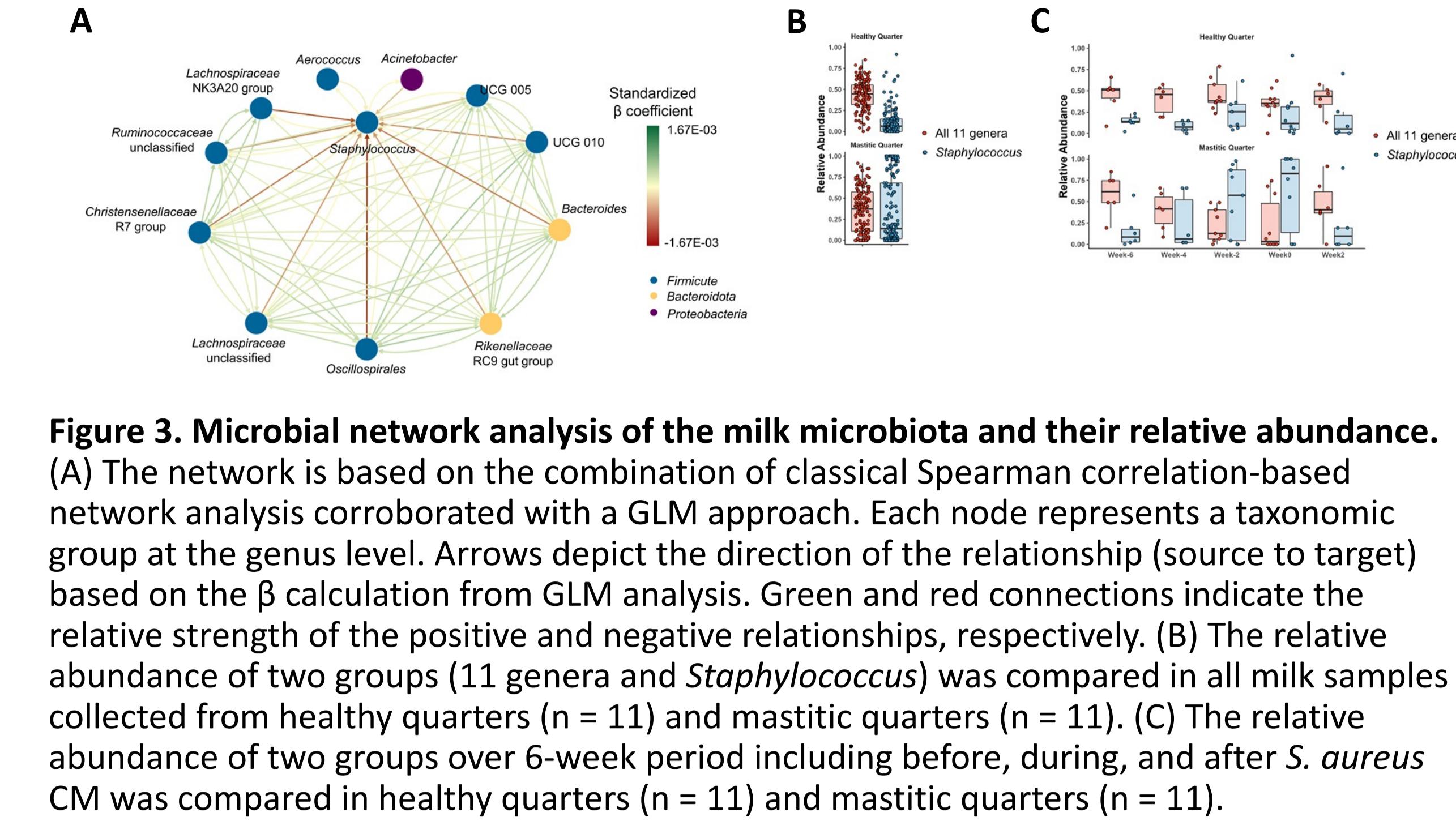
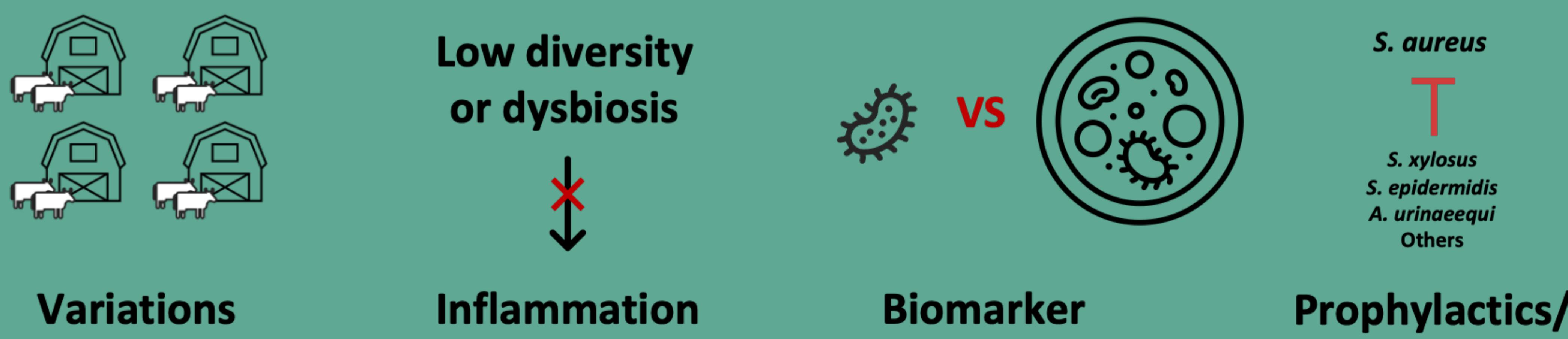


Figure 3. Microbial network analysis of the milk microbiota and their relative abundance. (A) The network is based on the combination of classical Spearman correlation-based network analysis corroborated with a GLM approach. Each node represents a taxonomic group at the genus level. Arrows depict the direction of the relationship (source to target) based on the β calculation from GLM analysis. Green and red connections indicate the relative strength of the positive and negative relationships, respectively. (B) The relative abundance of two groups (11 genera and *Staphylococcus*) was compared in all milk samples collected from healthy quarters ($n = 11$) and mastitic quarters ($n = 11$). (C) The relative abundance of two groups over 6-week period including before, during, and after *S. aureus* CM was compared in healthy quarters ($n = 11$) and mastitic quarters ($n = 11$).

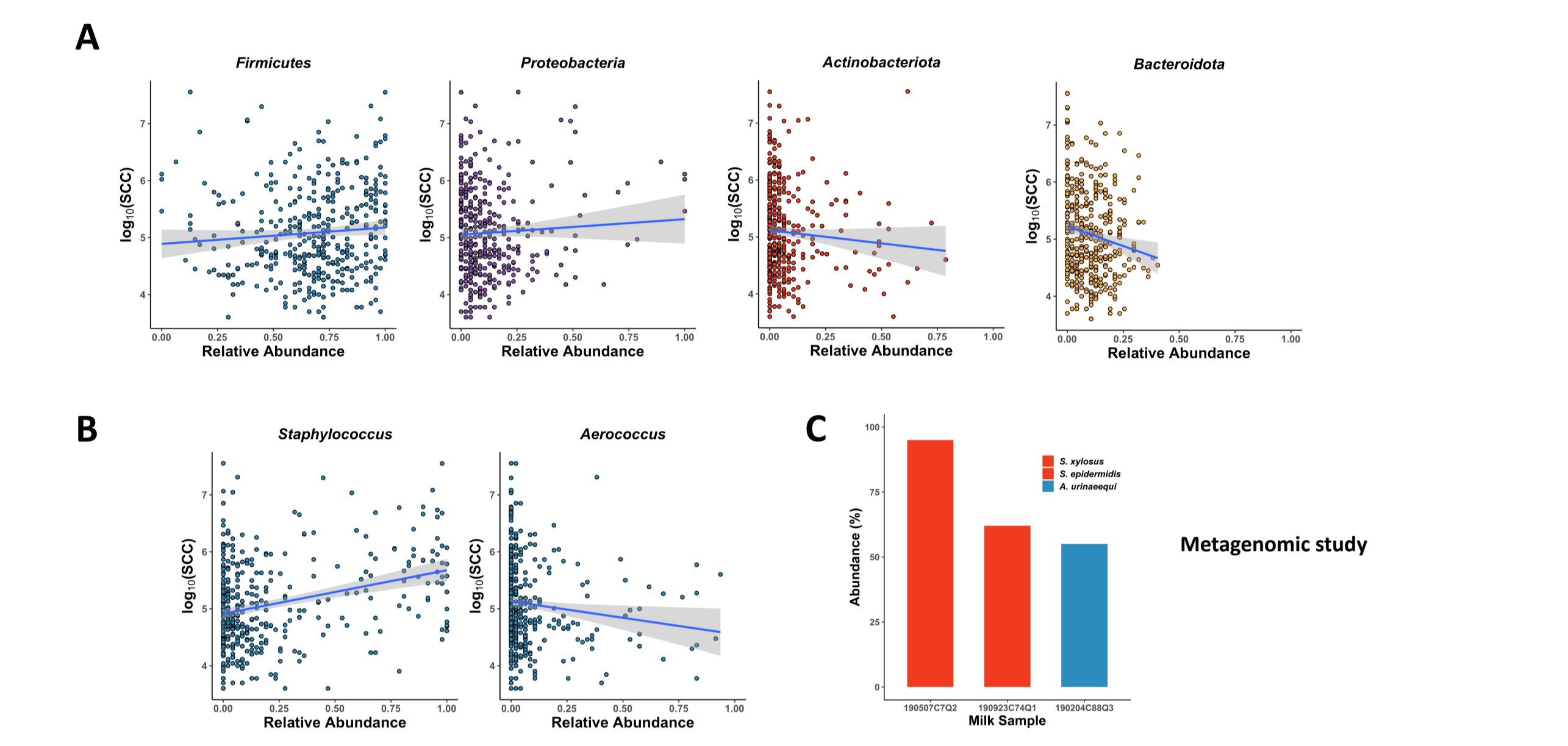


Figure 4. Relative abundance of milk microbiota. A broad range of SCC was detected in the milk samples ($n = 392$) analyzed in this study. (A) log₁₀ somatic cell counts (log₁₀(SCC)) for four major phyla by phyla prevalence showed no strong relationship between them. (B) *Staphylococcus* was associated with increased immune response. Only *Staphylococcus* and *Aerococcus* were highly predominant in milk samples with high SCC. (C) Low SCC was detected in three milk samples: 190507C7Q2 (59,000 cells/mL), 190923C7Q1 (143,000 cells/mL), and 190204C88Q3 (43,000 cells/mL). 16S rRNA amplicon sequencing (left) revealed a single genus highly predominant in the milk samples. Shotgun metagenomic sequencing (right) identified the bacterial species in each milk sample and revealed the abundance (%).

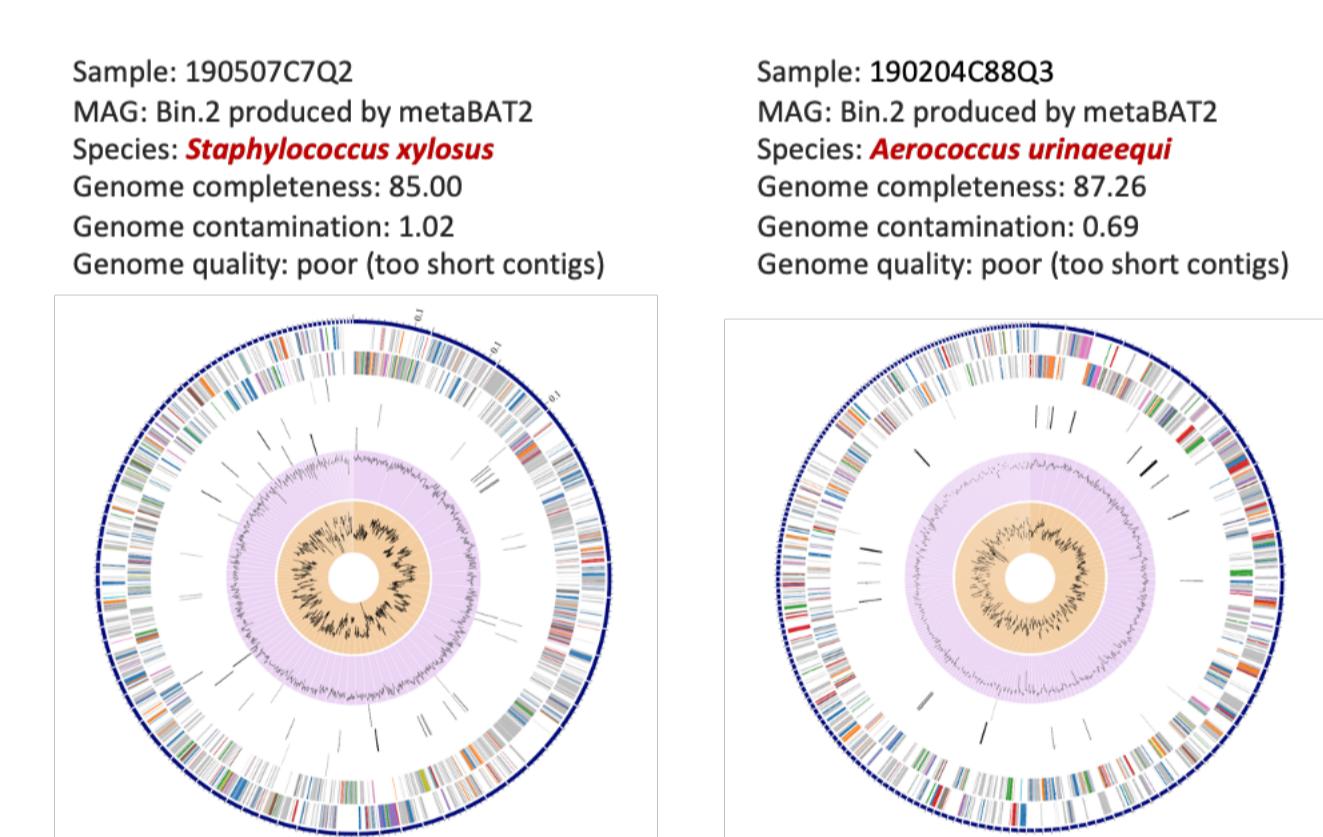
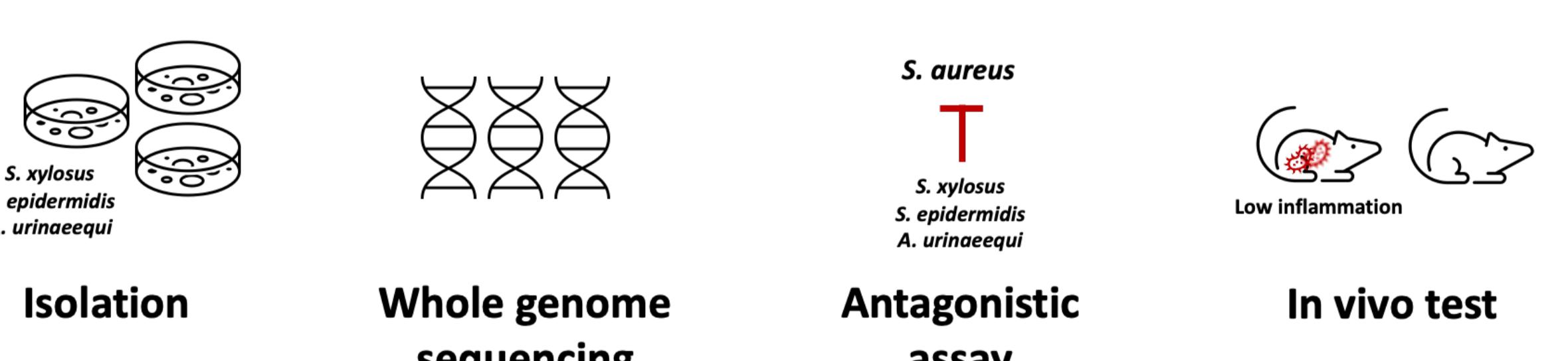


Figure 5. Metagenome assembled genome (MAG) of *Staphylococcus xylosus* and *Aerococcus urinasequei*. *S. xylosus* MGA included five gene clusters associated with secondary metabolites while *A. urinasequei* MGA showed no predicted gene cluster of secondary metabolite. However, the trace of lycopene biosynthesis gene cluster was found before final binning.

We have shown non-aureus *Staphylococci* and *A. urinasequei* inhibited *S. aureus* quorum-sensing and growth, respectively.
DOI <https://doi.org/10.1186/s12866-021-02265-4>

FUTURE WORKS



SIGNIFICANCE

