Lambic beers are produced through the spontaneous microbial inoculation of wort, which initiates a long-lasting fermentation and maturation process that is carried out in wooden barrels and results in a noncarbonated sour-tasting beer. Lambic beers are traditionally produced in Belgium in the proximity of the Senne river valley. The unique and complex flavor of these beers originates from the metabolic activities of various yeasts, lactic acid bacteria (LAB), and acetic acid bacteria (AAB).

Despite the increasing attention for acidic beers worldwide, scientific data on these beers were rather limited. The present study contributed to a more objective management of future lambic brews by assessing different lambic beer production processes with a multiphasic approach, encompassing various microbiological and metabolomic analysis methods. It turned out that the AAB, which were not examined extensively in previous studies, were present during two major phases of the lambic beer production processes, which could be explained by differences in their adaptation towards carbohydrate- or ethanol-rich and acidic environments. Generally, the AAB were more prevalent and displayed a higher metabolic activity at the liquid/air interface of the wooden barrels. Further, it was revealed that changing physicochemical parameters and substrate and metabolite compositions of the fermenting wort and maturing beer caused several transitions between the occurring microbial species and installed the typical four-phase fermentation and maturation process. The wooden barrels used in the lambic beer productions were partly responsible for the restricted microbial diversity and limited batch-to-batch variability between different lambic brews by acting as an additional microbial inoculation source, besides the environmental air during the coolship step. Moreover, they seemed to play an important role during the fermentation and maturation processes by establishing optimal heat transfer and microoxyxygenation conditions. A shotgun metagenomic analysis confirmed the functional potential of the microorganisms present during lambic beer productions, with some of these properties directly impacting the flavor profile of lambic beers.