

# BioKat – Biocatalysts in bioreactors: Functional microbial networks in semi-continuous operated biogas reactors

Katharina Willenbücher<sup>1\*</sup>, Marius Conrady<sup>2</sup>, Bianka Miltz<sup>1</sup>, Patrice Ramm<sup>2</sup>, Andreas Schlüter<sup>3</sup>, Dirk Benndorf<sup>4</sup>, Michael Klocke<sup>1\*\*</sup>

<sup>1</sup> Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Potsdam, Germany; <sup>2</sup> Institute of Agricultural and Urban Ecological Projects affiliated to Berlin Humboldt University (IASP), Berlin, Germany; <sup>3</sup> Bielefeld University, Center for Biotechnology (CeBiTec), Bielefeld, Germany; <sup>4</sup> Otto von Guericke University, Magdeburg, Germany

\*kwillenbuecher@atb-potsdam.de, \*\*mklocke@atb-potsdam.de

While reaching the start-up phase of an anaerobic digestion (AD) of silage resulting in methane-rich biogas, a functional microbiological community is formed. The aim here is to characterize the structure and functionality of this microbiome as well as its dynamics during the ongoing AD. Through a comprehensive analysis of the community at metagenome, metatranscriptome, and metaproteome levels and a chemical analysis, it becomes possible to identify microorganisms and/or genes as key drivers that provide information about the major factors in biogas processes. The focus of interest is the keystone species responsible for the primary hydrolysis of lignocellulosic substrates.

## Biogasreactor setup

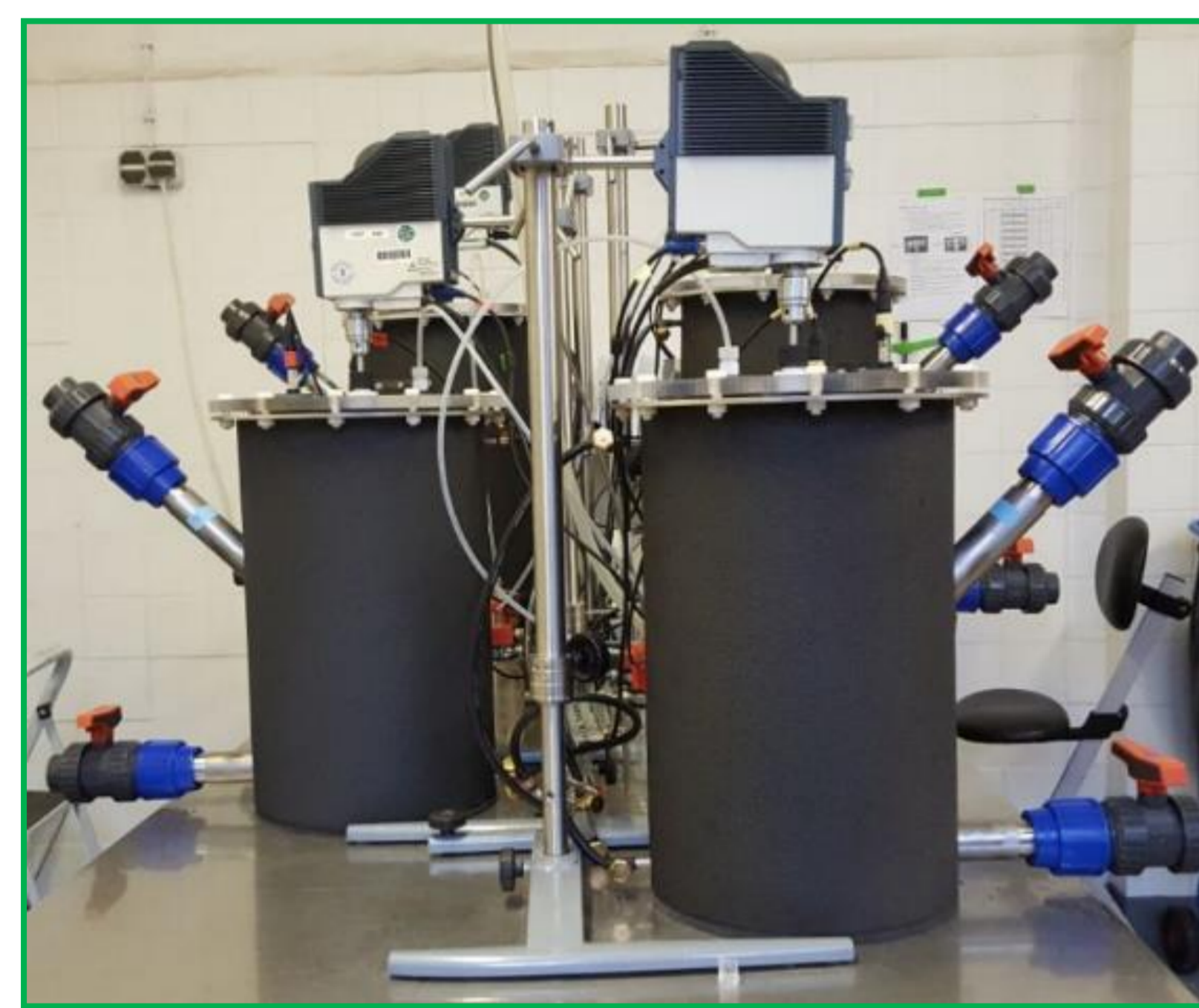
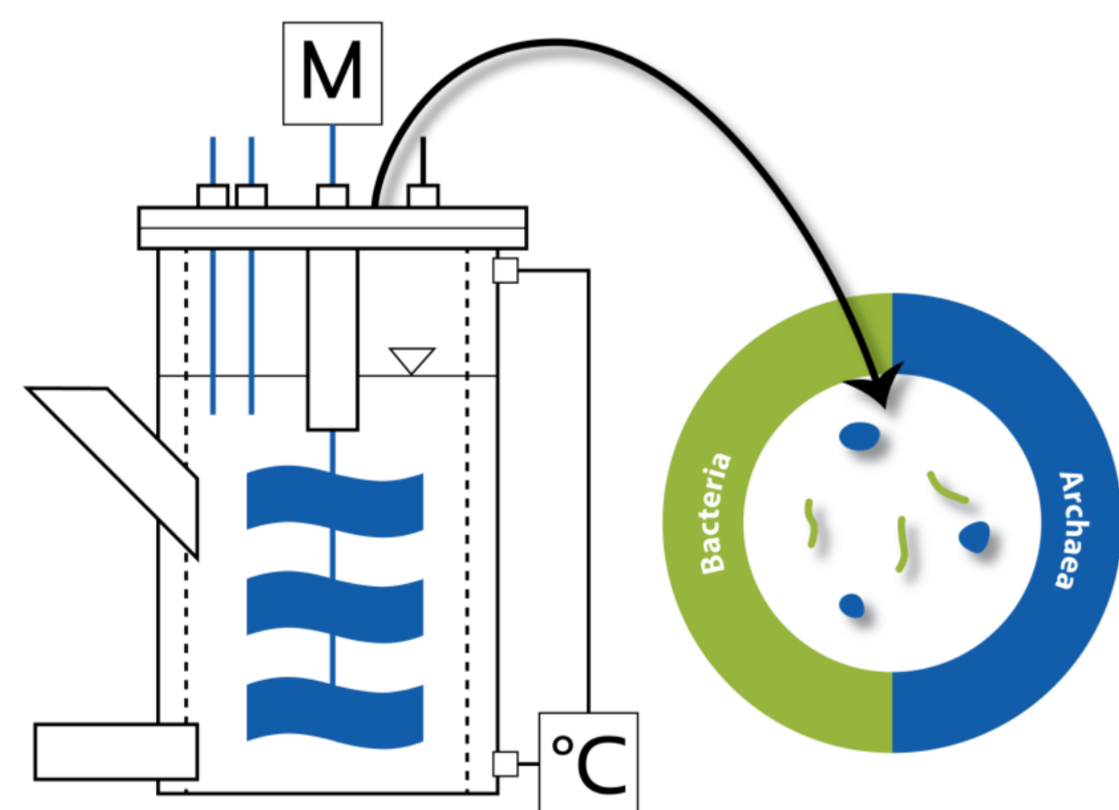


Fig. 1: Two 18 L biogas reactors

### 4 biogas reactors – A, B, C & D

Semi-continuously stirred 18 L biogas reactors (CSTRs)  
Daily fed with a mixture of maize (90%) and grass (10%) silages  
Mesophilic AD (38 °C)  
Organic loading rate (OLR): 3.5 g VS L<sup>-1</sup> d<sup>-1</sup>

## Methods



### Physico-chemical process parameters

pH, temperature, FOS/TAC, biogas composition (CH<sub>4</sub>, CO<sub>2</sub>, O<sub>2</sub>, H<sub>2</sub>S), biogas production rate

### TRFLP analysis (terminal restriction fragment length polymorphism)

Pre-characterization of structure and development of microbial communities

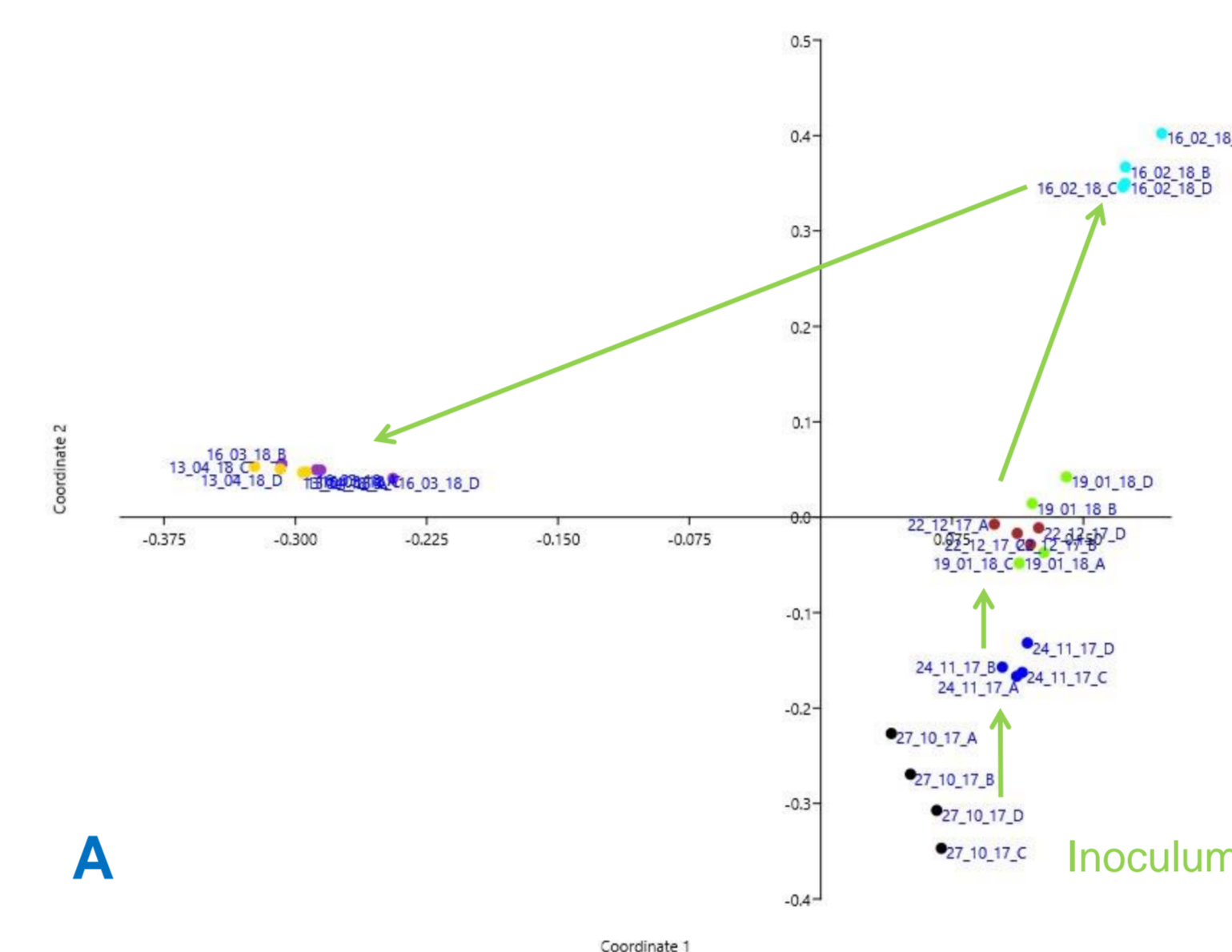
### Integrated –omics

16S rRNA gene amplicon & metagenome NGS  
Determining the genetic potential for AD biomass degradation and biomethanisation

Metatranscriptome & metaproteome analysis  
Real-time tracking of gene activities through the biogas process

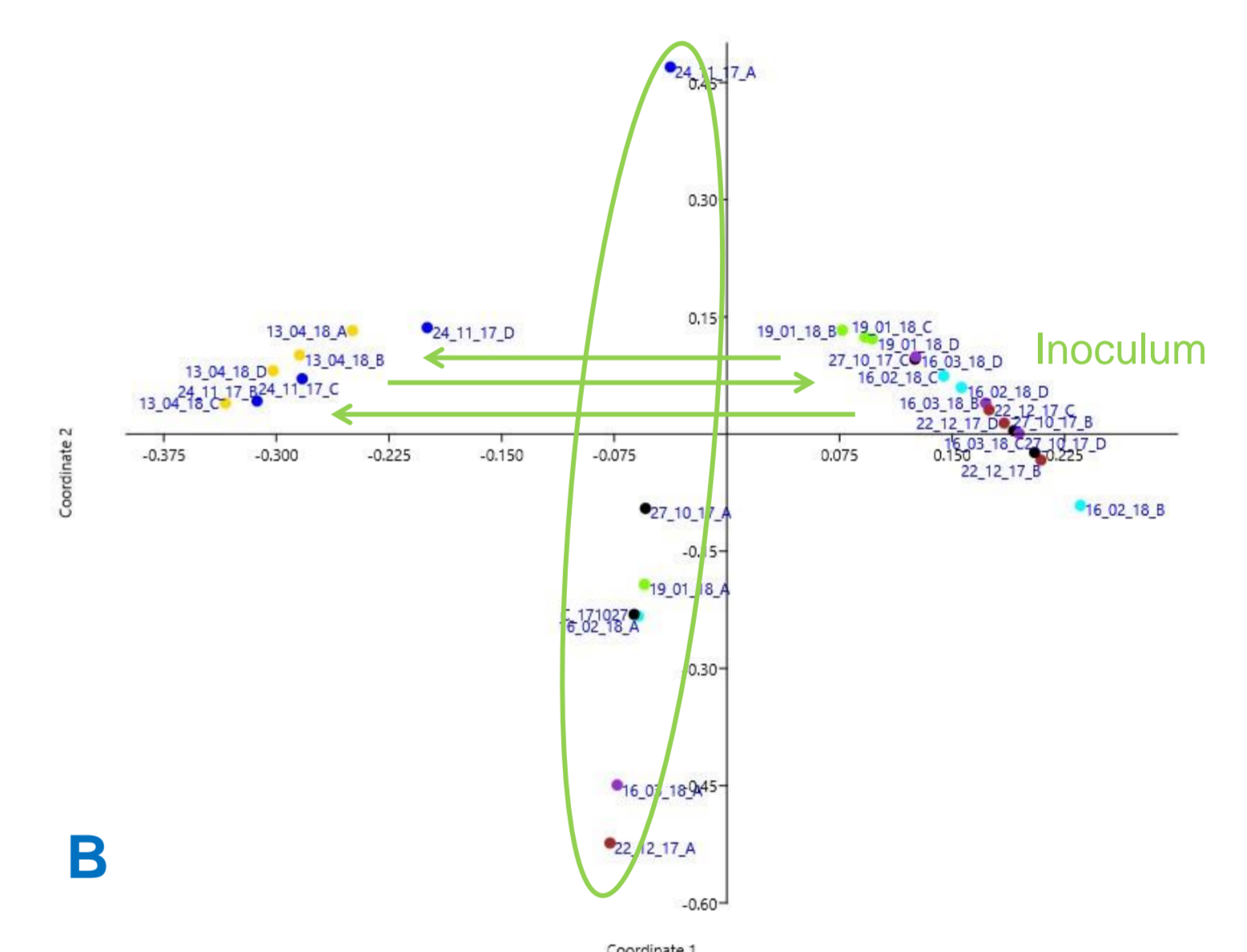
## First results

### Bacteria community

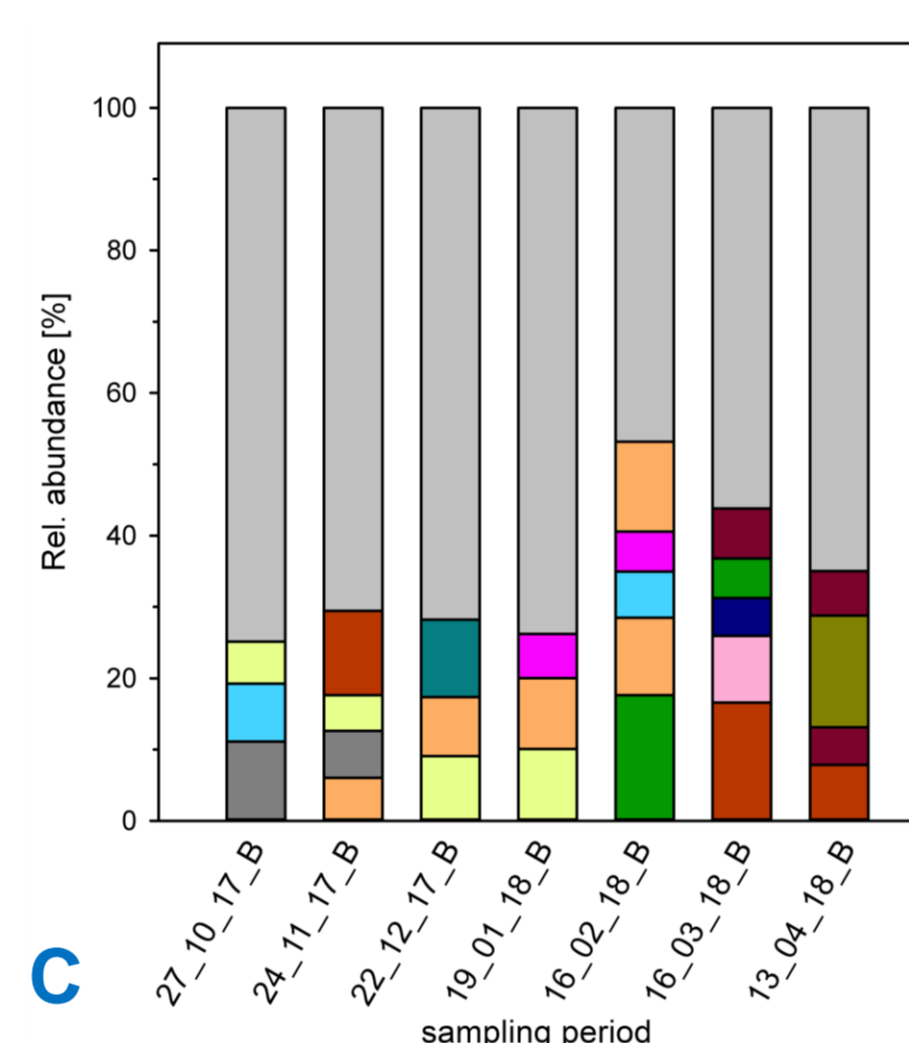


A

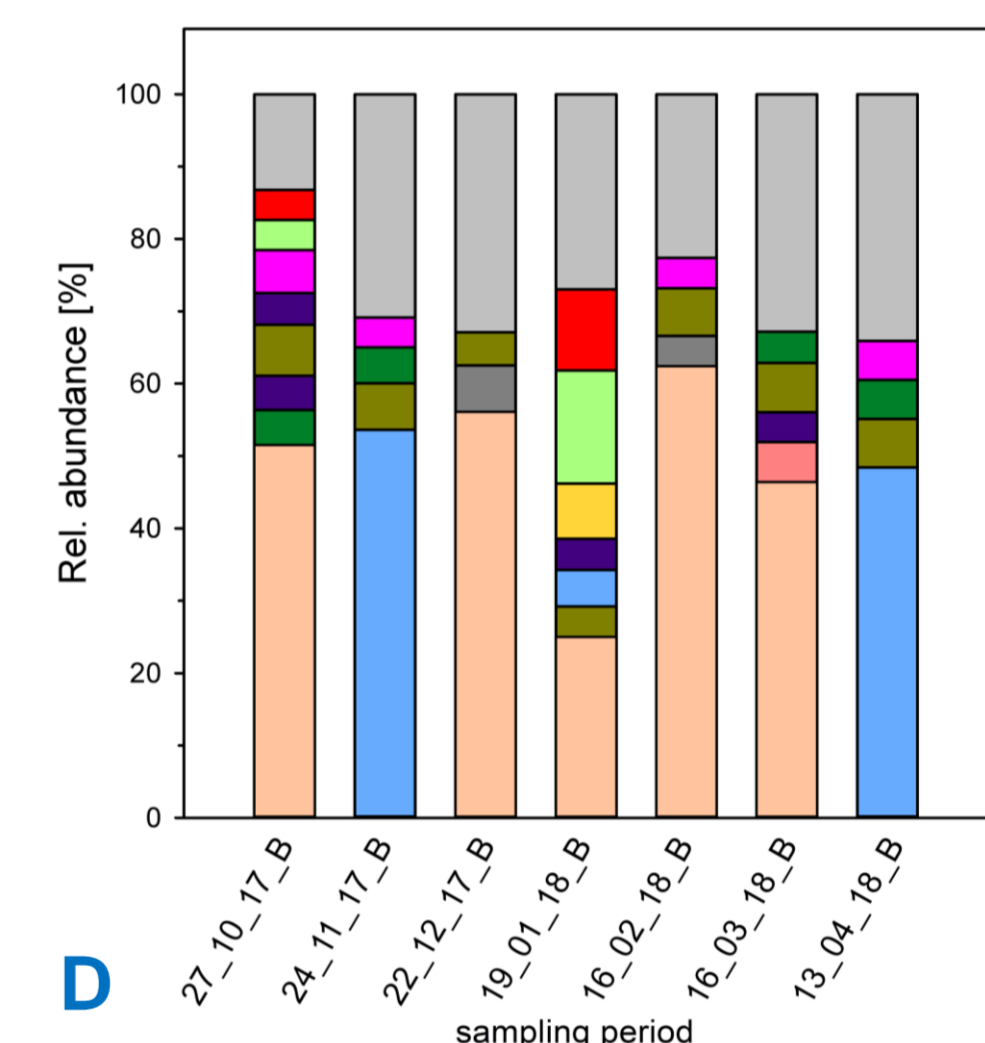
### Archaea community



B

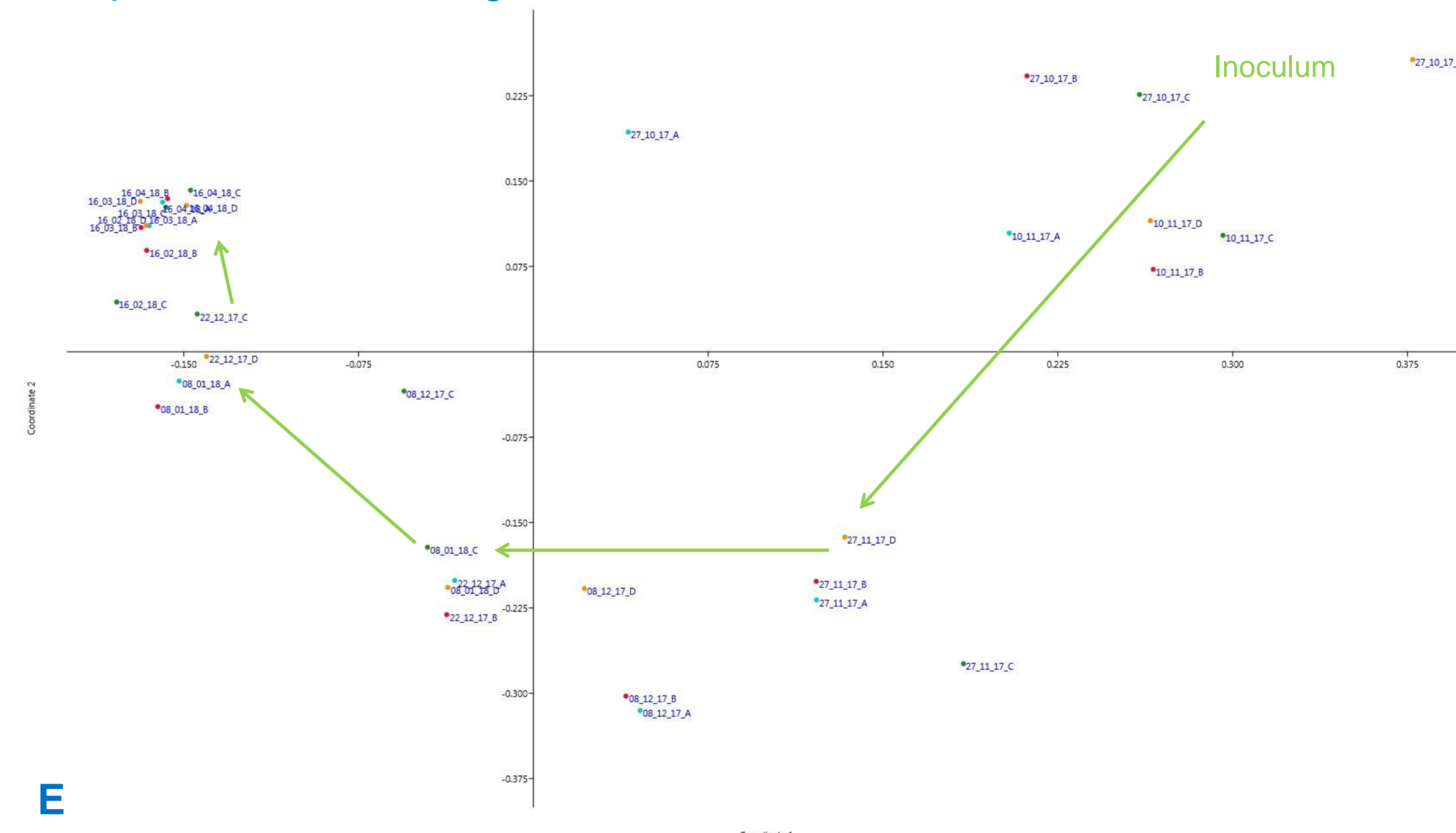


C



D

### Metaproteome functioning



E

Fig. 2: Principal Coordinates Analysis (PcoA) of the *Bacteria* Community (A) and the *Archaea* Community (B) reaching a steady state phase. Based on medians of identified TRFs (with at least six technical replica). PcoA of metaproteome-profiles (E) reaching a Steady State phase is based on identified metaproteins (only metaproteins with a total of more than 10 spectra in all samples). The green arrows visualize the successive change of the microbial community during fermentation. TRFLP profiles of the archaeal community (D) and bacterial community (C) in reactor B during the first 7 months are shown (others, <4%) .

The PcoA analysis of the TRFLP and metaproteome profiles for the bacterial communities shows noticeable changes during the first five months of reactor operation. The steady state phase was reached at month 6 to month 7. The PcoA for the archaeal community reveals a more homogeneous group than the bacterial community. In addition, the PcoA of the archaeal community in reactor A suggests a different development over time than in reactors B, C and D.

Further analysis, such as metagenome, 16S rRNA gene amplicon, metatranscriptome NGS as well as 16S rRNA gene full-length sequencing will complement these first results to identify the determined TRFs.