STSM report

- Learning how to evaluate DNA sequence of rumen microbes –

General info

Grantee:	Stefanie Engelke
STSM Reference Number:	COST-STSM-ECOST-STSM-FA1302-060217-081890
Home institution:	Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany
Host Institution:	National Institute for Agriculture Research (INRA), Saint-Genès-Champanelle, France
Grant Period:	2017-02-06 to 2017-03-05
Purpose of STSM:	Learning how to evaluate DNA sequence of rumen microbes

1. Purpose of the STSM

Methane emission from dairy cows is a fermentation product from the rumen metabolism and contributes to global warming. My PhD project is dealing with the effects of feeding different diets (corn silage- or grass silage-based with and without linseed) on the level of methane output of dairy cows and the aim is to validate an indirect marker to quantify the individual methane emission based on milk fatty acids. Both, milk fatty acid composition and methane production depend on rumen metabolism, which is shaped by the microbial population. DNA from rumen microbial samples of my PhD-project were analyzed for bacterial and archaea gene sequence and the aim is to integrate the microbial information in the interpretation of my project results. My purpose is to learn to evaluate the sequencing results and extend my knowledge about ruminal microbes from the experts at the research team DINAMIC - Digestion, Nutrition, Aliments, Métabolisme et Microbiote at UMRH (Herbivores Research Unit).

2. Work carried out

When I arrived at UMRH, the DNA from the rumen microbial samples of my PhD-project have already been subjected to next-generation sequencing (NGS; Illumina®). The first goal was to understand the imperative necessity processing chain of the generated raw data by NGS to prepare them for the statistical analysis or create illustrations. This includes learning how NGS is working and what contains an output file. After a literature study, I started to work on the processing chain using the software “mothur”. The software provides a tutorial for standard operation procedure to process 16S rRNA gene sequences that are generated by Illumina's MiSeq platform using paired end reads. This tutorial includes working on quality filtering, OTU picking, taxonomic assignment, phylogenetic reconstruction, diversity analyses and visualizations and is used for overlapped reads. In addition, the research team of DINAMIC created a standardized workflow for DNA analysis of bacteria, archaea,

protozoa and fungi using the Galaxy® workflow management system to facilitate the processing and repeatability of the enormous raw data set. The DNA from the rumen microbial samples of my PhD-project was analysed using the Galaxy® system. Afterwards I "played" with my real data and simulated parts of the Galaxy® workflow in mothur. Subsequently, I used the prepared data to create first graphs in different feeding groups. In addition, I learned more about different software programs to perform microbiome analysis from generated raw DNA data (QIIME™, IM-TORNADO, PIPITS). In the end of this internship, I could say that I considerably extended my knowledge about DNA sequencing and microbial communities. Furthermore, I could visit a cattle farm of INRA and see there the GreenFeed system and also preparations for future SF6 measurements.

3. Main results

The main results are presented in the figures below and show the relative abundance of bacteria phyla (Figure 1) and archaea genera (Figure 2) as well as principal coordination analysis of bacterial (Figure 3) and archaeal dissimilarity matrix (Figure 4) for each animal in different feeding groups. Figure 1 shows an increase of *Bacteroidetes* in feeding groups with linseed supplementation and with grass silage compared to feeding groups without linseed and corn silage. Figure 2 shows a decrease of *Methanobrevibacter* and *Methanomassilliicoccaceae, Group 12* and an increase of *Methanomassilliicoccaceae, Group 8* in grass silage diets compared to the corn silage diets. Compared with this, the principle coordination analysis of dissimilarity matrix indicate no clear differences in the feeding groups.

Figure 1

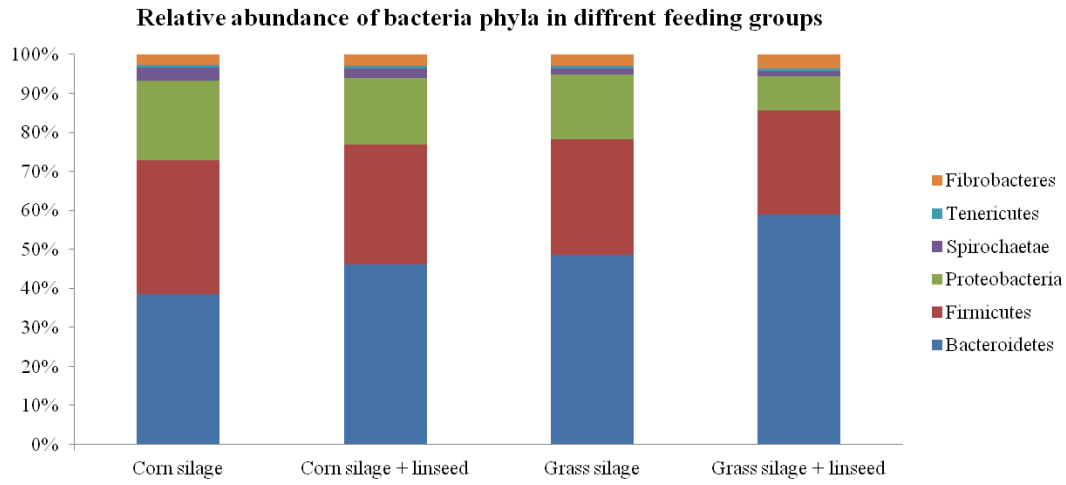


Figure 2

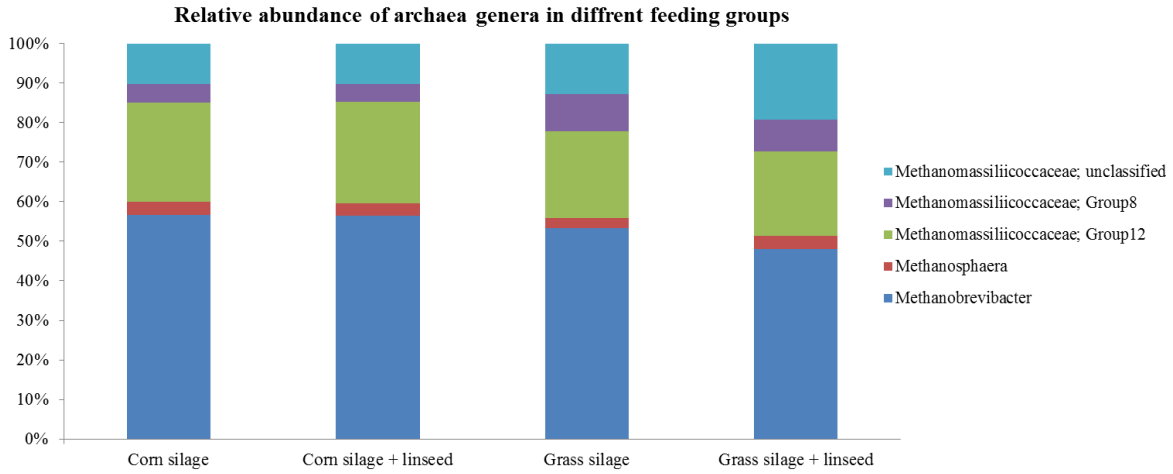


Figure 3

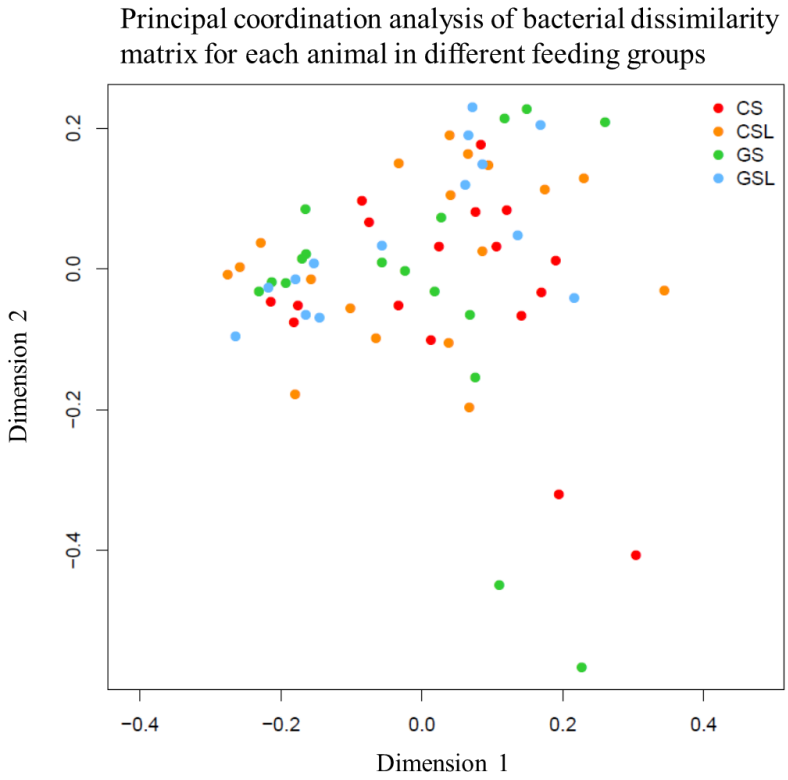
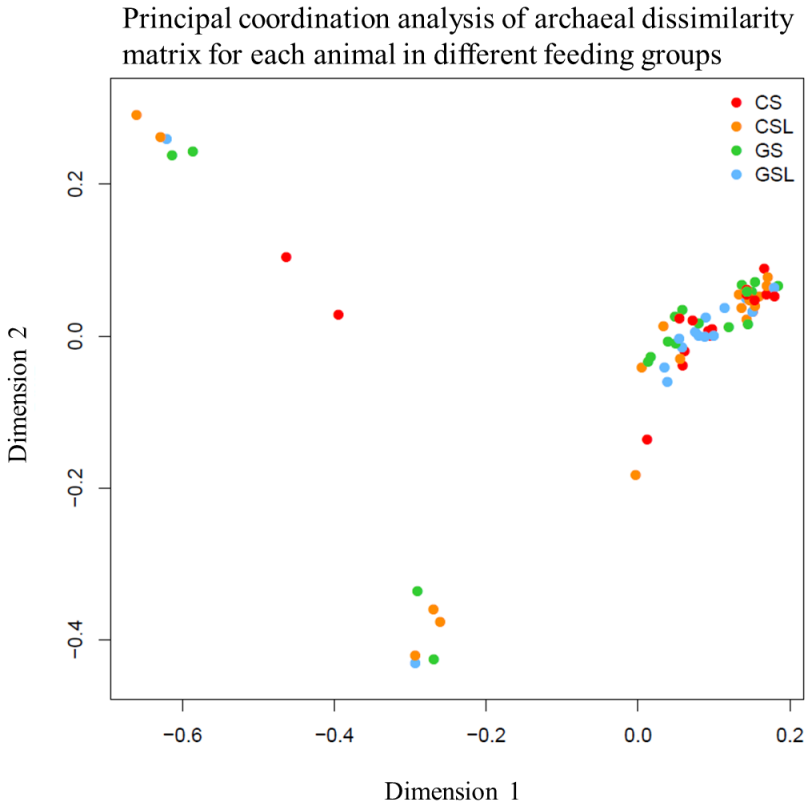


Figure 4



4. Benefits from the STSM to the METHAGENE network

Our contributions to METHAGENE are data on milk fatty acid profile as an indicator of methane production and its relationship with methane emission. The generated microbial DNA data will help to explain the variability of methane emission and milk fatty acids across different feeding conditions and may also contribute to (in)validate the prediction equations already published in the scientific literature to predict methane emission from milk fatty acid.

5. Future collaboration with the host institution

It was the first time that I worked with DNA sequence analysis. Further analysis of the obtained data during the STSM will be carried out together.

6. **Foreseen publications resulting from the STSM**

A collaborative publication manuscript with UMRH colleagues is planned and will deal with the relationship between methane emission, milk fatty acid composition and the bacteria and archaea community.

7. Confirmation by the host institution of the successful execution of the STSM

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Destinataire :

METHAGENE COST ACTION

Coordination bureau

Theix, 3rd March 2017

Objet : *Short-Term Scientific Mission*

To whom it may concern,

This is to certify that we received Stefanie Engelke from the Institute of Nutritional Physiology 'Oskar Kellner', Leibniz Institute for Farm Animal Biology (FBN) in Dummerstorf, Germany in our laboratory at the Institute National de la Recherche Agronomique (INRA) Centre Clermont-Ferrand – Theix as a guest visitor from February 6th to March 3, 2017.

The object of the visit, under the auspices of the Methagene Cost action - Short Term Scientific Mission, was to analyse high throughout put sequencing data to characterize rumen microbial communities.

Yours sincerely,

Milka Popova

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