

## Microbial communities in agricultural soil fertilized with animal manure in Northern Italy.

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Soil is one of the largest reservoir of microbial diversity. Land application of chicken and swine manure is a common agricultural practice worldwide, including Italy. Some studies suggest that livestock manure used as fertilizer in agriculture may cause changes of microbial communities. However, little is known about the impact of manure fertilization on soil microbiomes and potential consequences on the natural environment.

This study aimed at investigating the possible influence of fertilization with manure from intensive swine and chicken farms on agricultural soil microbiome.

Sixty samples of manure and soil (before manure application and 30 days after) were collected from ten chicken farms and ten swine farms. The microbiome was determined by NGS sequencing employing a multiplexing 16S rRNA gene amplicon sequencing approach based on two-step PCR amplifications with tagged primers. Libraries were pooled and sequenced with Miseq Illumina 2X300 Pair Ends. Raw sequence data were analyzed with QIIME 2, while Silva database and Calypso software were employed for taxonomical and statistical analyses, respectively.

Microbial diversity in each sample ( $\alpha$ -diversity) was evaluated: at phylum level, *Firmicutes* and *Bacteroidetes* dominated manure microbiome, while *Proteobacteria* were the most abundant in soil samples. At order level, *Clostridiales*, *Bacteroidales* and *Bacillales* were the most abundant in manure samples, while in soil samples the most abundant order was *Chitinophagales*. At family level, bacterial families such as *Lachnospiraceae*, *Ruminococcaceae* and *Rikenellaceae* dominated the manure microbiome, whereas *Chitinophagaceae* were most abundant in soil samples. Manure and soil samples (before and after fertilization) clustered at phylum, order and family levels within two distinct groups. The assessment of the microbial diversity between samples ( $\beta$ -diversity) confirmed the uniqueness of microbial communities of manure and soil suggesting that manure application may not significantly alter soil microbiome. However, some differences were observed: *Enterococcaceae* and *Lactobacillaceae* were more abundant in manure and soil samples after fertilization than in soil samples before manure application. Similarly, *Burkholderiaceae*, *Sphingobacteriaceae*, *Flavobacteriaceae* and in particular *Bacillaceae* were more abundant in chicken manure and some soil samples after fertilization than before fertilization.

This represents one of the first study evaluating the effects of swine and chicken manure fertilization on the agricultural soil microbiome in Italy. Future studies aiming to assess the presence of antimicrobial residues and resistance genes in these samples would be useful to obtain key information regarding the impact of antimicrobial use in intensive animal farming on soil microbial communities.