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## Bacterial communities in decomposing litter from cacao and shade trees in a long-term agroforestry trial

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### Abstract

Compared to monocultures, cacao cultivated within agroforestry systems could benefit from enhanced nutrient cycling - potentially reducing the need for fertiliser. Nutrient cycling encompasses litter decomposition processes, which are ultimately carried out by microorganisms. As part of the long-term SysCom trial in Alto Beni (Bolivia), we aimed to investigate how different cacao cropping systems affect the main bacterial groups present in decomposing leaf litter from cacao and shade trees. We placed litterbags with two different mesh sizes (0.2 and 2 mm), containing either cacao leaves or mixtures of cacao and shade tree leaves under different cacao cropping systems ranging from conventional monoculture to successional agroforestry. At 4, 8, and 12 months after laying, we collected the litterbags for total DNA extraction. We amplified and obtained the sequences of the V3-V4 region of the 16S ARN gene. Raw sequences were analysed using the dada2 pipeline to obtain amplicon sequence variants (ASVs). Downstream analyses were performed using the phyloseq and DESeq2 packages in RStudio. The 50 most abundant ASVs accounted for only around 30 % of the total abundance, highlighting the high diversity within the samples. No significant effect of decomposition time, cropping system, leaf material or mesh size for  $\alpha$ -diversity (Shannon, Inverse Simpson and Faith indices) was observed.  $\beta$ -diversity, evaluated by UNIFRAC distances, was strongly influenced by decomposition time, but not by the other predictors. The five most abundant families were Streptomycetaceae, Xanthobacteraceae, Sphingomonadaceae, Rhizobiaceae, and Chitinophagaceae. Differential abundance analysis showed that some families were affected by the cultivation system (Promicronosporaceae and Bifidobacteriaceae at 4 months after laying; Sphingomonadaceae at 8 and 12 months after laying). At 8 months after laying, Sphingomonadaceae, Rhizobiaceae, Weeksellaceae and others were significantly more abundant in the larger mesh litterbags. These probably facilitated the entry of mesofauna, small roots and soil particles. No effect of the leaf material (cacao vs leaf mixtures) on the differential abundance of bacterial families was observed. Our results show that the time of decomposition and the litterbag mesh size, had a stronger influence on the composition of the bacterial communities than the cropping systems.

**Keywords:** Agroforestry, microbiome, nutrient cycling, Streptomycetaceae

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