## Differential abundance analyses of soil prokaryotes in two Amazon livestock-forestry systems with different algorithms

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## Abstract

Livestock-forestry integration systems (ILFS) have been proposed to promote soil health through several mechanisms, including increased biodiversity. Linear Discriminant Analysis (LDA) Effect Size (LEfSe), ANOVA-Like Differential Expression 2 (ALDEx2), Linear Models for Differential Abundance Analysis of Microbiome Compositional Data (LinDA), and Differential Gene Expression Analysis Based on the Negative Binomial Distribution (DESeq2) are methods for discovering biomarkers. They identify characteristics that consistently explain differences between microbial communities, using different statistical approaches. The objective of this study was to compare algorithms for the identification of differential microbial features in two ILFSs located in Porto Velho, Rondônia State, Brazil, Western Amazon. Soil samples were taken from the 0-10 cm layer from two ILFSs (at 0.00 and 5.25 m from the trees) and a native forest (NF) fragment (Am climate, Udox soil, clay texture), with five replicates, totaling 25 samples. Both ILFSs consisted of long-established pasture in which Samanea tubulosa (ILFS-ST) and Eucalyptus pellita (ILFS-EP) strips were planted. The soil DNA was extracted and 16S rRNA gene was sequenced. The sequences were grouped in OTUs and classified taxonomically (Silva 138.1 database). The relative abundance data were analyzed by the LEfSe, ALDEx2 kw, LinDA and DESeq2 methods using the microeco package for R, at the genus level. Of the 1120 genera present in the samples, LEfSe identified 42 as differentially abundant among areas, being 76% assigned to ILFS-ST. DESeq2 identified 189 genera, 39 coinciding with LEfSe. ALDEx2 and LinDA identified no significant feature. LEfSe presented the most practical result, but combining methods might be a way to compensate for biases and to obtain more consistent results.

Palavras-chave: Silvopastoral systems; Soil microbiome; Statistical methods.

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