

**TITLE:** PREDICTION OF NITROGEN METABOLISM IN THE PHYLLOSHERE, RHIZOSPHERE AND LITTER OF THE AMAZON RAINFOREST USING METAGENOME RECONSTRUCTION

**AUTHORS:** DELBAJE, E.; BARRERA, S. E.; MOREIRA, J. C. F.; SILVA, B. M.; SOUZA A. A.; LAMBAIS, M. R.

**INSTITUTION:** ESCOLA SUPERIOR DE AGRICULTURA “LUIZ DE QUEIROZ” – UNIVERSIDADE DE SÃO PAULO (AV. PÁDUA DIAS, 11, CEP 13418-900, PIRACICABA – SP, BRAZIL)

**ABSTRACT:** The Amazon rainforest is one of the most biodiversity-rich tropical ecosystems with global ecological implications. However, the knowledge of the structures and functions of the microbial communities in this biome is limited. One of the key functions of microorganisms in tropical forests is nitrogen cycling. It has been shown that biological nitrogen fixation (BNF) may be a key activity in tropical forests and that free-living diazotrophs may be major contributors. In this study, we characterized the microbial communities associated with the phyllosphere, litter and rhizosphere of six tree species through sequencing of the V4 region of the 16S rRNA gene, using the Illumina MiSeq sequencing platform. A total of 17180612 sequences were processed using the pipeline of the Brazilian Microbiome Project. Sequences were clustered into Operational Taxonomic Units (OTUs) for a total of 9645 for phyllosphere, 6796 for rhizosphere, and 5688 for litter. The OTUs were affiliated to taxonomic groups using the Greengenes database as a reference. Metagenome reconstruction was performed using PICRUSt, which predicts functional genes based on the 16S rRNA gene taxonomic affiliation and available taxonomically related genomes. Our data show that *nifH* gene, a proxy for BNF, was enriched in the phyllosphere, as compared to litter and rhizosphere. The differences in abundance of diazotrophs, based on the abundance of *nifH* genes, showed that the effect of tree species is less significant than the effect of microhabitats, suggesting that microhabitats are determinant factors in the assembling of free-living diazotroph communities. This study provides valuable resources to generate new hypothesis to investigate the roles of different diazotroph taxa in the nitrogen cycle in the Amazon forest.

**Keywords:** Amazon; Diazotrophs; Metabolism prediction; PICRUSt; Nitrogen cycle

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