

TITLE: ABUNDANCE OF AMMONIA-OXIDIZING MICROORGANISMS IN THE FLOODPLAIN OF THE TOCANTINS RIVER

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ABSTRACT:

Ammonia oxidation is the first and rate-limiting step of nitrification and until recently, autotrophic ammonia-oxidizing bacteria were thought to be the major contributors to ammonia oxidation in soils, with all representatives belonging to a monophyletic group within *Betaproteobacteria*. However, recent studies revealed the existence of ammonia-oxidizing *Archaea* (AOA). In this study we analyzed the abundance of ammonia-oxidizing *Archaea* and *Bacteria* inhabiting different soil layers under agroforestry and primary forest in the floodplain of the Tocantins River during the flooded and non-flooded periods. Total RNA was isolated from 24 soil samples (two soil layers × two sampling sites × two seasonal periods × three sampling points) using RNeasy PowerSoil Total RNA Kit in duplicate for each soil sample and stored at -80°C until further use. Complementary DNA (cDNA) was synthesized using a QuantiNova Reverse Transcription Kit, and amplicon libraries were prepared using the cDNA as template in the amplification reactions and specific archaeal and bacterial primers for the 16S rRNA genes. Equal concentrations of libraries were loaded independently on MiSeq Reagent Kit v3 sequencing and sequenced in a MiSeq Personal Sequencing System. All 16S rRNA gene sequence reads were processed and analyzed using QIIME v.1.9.1. Statistical analyses were carried out using the R packages vegan 2.5–7 and ggplot2 3.3.5. The AOA groups consisted of two acidophilic groups within the Thaumarchaeota phylum belonging to Nitrososphaeraceae and Nitrosotaleaceae families, while AOB consisted mostly of uncultured members of the Nitrosomonadaceae family. As expected, AOA outnumbered AOB in all samples and represented 97,5% to 77% of the ammonia-oxidizing community, with highest abundance during in forest sites and in the non-flood period. Variance partition of the factors revealed that the soil chemical properties allied with land use and depth had the highest impacts in AOA (10%) and AOB (11%). Permanova analysis of the soil chemical properties revealed that soil pH was the only significant factor driving AOB variance, whereas for AOA, dissolved organic carbon, sulfate, total carbon, total nitrogen, and ammonia concentrations were the most significant. These results indicate the singularity of the Amazon soil microbial community, where microorganisms are subjected to greater environmental filtering, resulting in an evolutionary niche specialization for ammonia-oxidation in acidic soils.

Keywords: Ammonia oxidation, Ammonia-oxidizing Archaea and Bacteria, Amplicon sequencing, Microbial ecology

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