

## Linking the Genetic Signature of Free-Living Soil Diazotrophs with Process Rates under Land Use Conversion in the Amazon Rainforest

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**Abstract :** The Amazon Rainforest is a global diversity hotspot and crucial carbon sink, but approximately 20% of its total extent has been deforested- primarily for the establishment of cattle pasture. Understanding the impact of this large-scale disturbance on soil microbial community composition and activity is crucial in understanding potentially consequential shifts in nutrient or greenhouse gas cycling, as well as adding to the body of knowledge concerning how these complex communities respond to human disturbance. In this study, surface soils (0-10cm) were collected from three forests and three 45-year-old pastures in Rondonia, Brazil (the Amazon state with the greatest rate of forest destruction) in order to determine the impact of forest conversion on microbial communities involved in nitrogen fixation. Soil chemical and physical parameters were paired with measurements of microbial activity and genetic profiles to determine how community composition and process rates relate to environmental conditions. Measuring both the natural abundance of  $^{15}\text{N}$  in total soil N, as well as incorporation of enriched  $^{15}\text{N}_2$  under incubation has revealed that conversion of primary forest to cattle pasture results in a significant increase in the rate of nitrogen fixation by free-living diazotrophs. Quantification of *nifH* gene copy numbers (an essential subunit encoding the nitrogenase enzyme) correspondingly reveals a significant increase of genes in pasture compared to forest soils. Additionally, genetic sequencing of both *nifH* genes and transcripts shows a significant increase in the diversity of the present and metabolically active diazotrophs within the soil community. Levels of both organic and inorganic nitrogen tend to be lower in pastures compared to forests, with ammonium rather than nitrate as the dominant inorganic form. However, no significant or consistent differences in total, extractable, permanganate-oxidizable, or loss-on-ignition carbon are present between the two land-use types. Forest conversion is associated with a 0.5- 1.0 unit pH increase, but concentrations of many biologically relevant nutrients such as phosphorus do not increase consistently. Increases in free-living diazotrophic community abundance and activity appear to be related to shifts in carbon to nitrogen pool ratios. Furthermore, there may be an important impact of transient, low molecular weight plant-root-derived organic carbon on free-living diazotroph communities not captured in this study. Preliminary analysis of nitrogenase gene variant composition using NovoSeq metagenomic sequencing indicates that conversion of forest to pasture may significantly enrich vanadium-based nitrogenases. This indication is complemented by a significant decrease in available soil molybdenum. Very little is known about the ecology of diazotrophs utilizing vanadium-based nitrogenases, so further analysis may reveal important environmental conditions favoring their abundance and diversity in soil systems. Taken together, the results of this study indicate a significant change in nitrogen cycling and diazotroph community composition with the conversion of the Amazon Rainforest. This may have important implications for the sustainability of cattle pastures once established since nitrogen is a crucial nutrient for forage grass productivity.

**Keywords :** free-living diazotrophs, land use change, metagenomic sequencing, nitrogen fixation

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