Assessment of the impact of agriculture on soil microbial communities involved in the nitrogen cycle in tropical ecosystems

Place of work:
FCUL, Ed C2, Lab. 2.4.37 (Plant Functional Genomics Lab)

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Soil microorganisms contribute to the maintenance of fertility, since they transform dead organic matter into nutrients capable of being absorbed by plants, thus being crucial for the production of food for human consumption. However, several studies point to a generalized loss of soil biodiversity with the intensification of agriculture and climate change, which can have consequences in terms of its fertility and, consequently, agricultural productivity.
Nitrogen (N) is a vital nutrient for plant growth. The nitrogen cycle depends on the activity of specific communities of soil microorganisms that transform the various forms of N among themselves, taking up atmospheric N into the soil (e.g., fixing bacteria) and transforming the N present in dead organic matter into nitrogenous nutrients capable of being taken up by plants (e.g., nitrifying bacteria). Recent studies have identified several genes that can be used as molecular markers to quantify the abundance of these microbial communities (N functional genes), which in turn constitutes an indicator of the main N cycle transformations that are operating in a given ecosystem (e.g., fixation, mineralization, nitrification). This project aims to evaluate how the abundance of functional microbial groups related to the N cycle changes when natural ecosystems (native forest) are converted to agricultural use (cashew and rice fields), in soils of tropical regions, in Guinea-Bissau.
As main techniques, molecular biology will be used, such as DNA extraction from soil, agarose gel electrophoresis, PCR, cloning, DNA sequencing, real-time PCR, enzyme activity, among others. The work will benefit from an ongoing project with Guinean Institutions financed by the FAO (Food and Agriculture Organization of the United Nations) and which aims to study the changes in soil biodiversity that are induced when natural tropical ecosystems are converted to agriculture.

Tasks
1 - Soil DNA extraction
Using a specific commercial kit, microbial genomic DNA from each type of soil (native forest, cashew, rice field) will be extracted and its concentration (NanoDrop) and quality (agarose gel electrophoresis, PCR) will be evaluated. At least 5 replicates of each DNA will be extracted and evaluated.
2 – Quantification of N functional genes by quantitative real-time PCR

The extracted DNA will be used to quantify the abundance of microorganism communities involved in N fixation (nifH gene), nitrification (AOA and AOB genes) and denitrification (nirK, nirS and nosZ1), using real-time PCR technology with SYBR Green and specific primers (described in the literature). The total bacterial community will be quantified using the 16S rRNA gene. The calibration curves for determining the number of DNA copies of each gene will be obtained from the cloning of N functional genes in plasmids containing a known number of copies of the respective gene.

3 - Enzymatic activities

To complement the quantification analysis of the N functional genes, the activity of enzymes involved in the transformations between the various forms of N in the soil, such as urease and nitrate reductase, will be determined in samples of each soil type (native forest, cashew, rice paddy). The methods used will be based on spectrophotometry.

3 - Statistical analysis

The results of the quantification of N functional genes and enzymatic activities in the different soil types will be analyzed using statistical methods (eg analysis of variance; ANOVA) to determine the effect of soil conversion on the communities of microorganisms involved in the N cycle.

Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship (BII), being the selection criterium the academic merit of the candidates.