How farming practices change the soil microbial communities involved in nutrient cycling on tropical ecosystems

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

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Summary
Soil contains about 25% of all the Earth's biodiversity, and microorganisms living in it contribute to a number of life-supporting functions performed by soils, such as maintaining soil fertility for the production of food and fiber for human consumption. However, together with climate change, the increasing intensification of agriculture jeopardizes the functionality of soils, contributing to their degradation and consequent loss of agricultural productivity. Several studies point to a generalized loss of soil biodiversity with the intensification of agriculture, which can have consequences in terms of its fertility, since microorganisms are responsible for recycling soil nutrients. Biogeochemical cycles control the availability of nutrients that are absorbed by plants and depend on the activity of specific communities of soil microorganisms that transform the various forms of nutrients among themselves, determining soil fertility and agricultural production. The biogeochemical processes that regulate carbon (C) and phosphorus (P) cycles are still poorly understood, mainly due to the lack of adequate microbial indicators. Recent studies have identified several marker genes that are good indicators of the biogeochemical processes associated with the C and P cycle. These genes encode proteins that catalyze the transformations between the various forms of C and P in the soil and are called “functional genes”. Several of these genes have been used as molecular markers to quantify the abundance of microbial communities involved in the various stages of biogeochemical cycles, which in turn constitutes an indicator of the main transformations that are operating on that ecosystem. Thus, this master's project aims to assess how the abundance of functional microbial groups related to the C and P cycle changes when natural ecosystems (native forest) are converted to agricultural use (cashew and legumes). The analyzed soils come from a tropical region (Guinea-Bissau) which will allow an increase in knowledge about the C and P cycles in this region of the world that is still so little studied. As main techniques, molecular biology techniques will be used, such as DNA extraction from soil, agarose gel electrophoresis, PCR, cloning, DNA sequencing, real-time PCR, determination of enzymatic activities, among others. The work will benefit from an ongoing project with the 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 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, legumes) 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 "functional genes" by quantitative real-time PCR

The extracted DNA will be used to quantify the abundance of microorganism communities involved in the degradation of aromatic C compounds (pcaH gene), methane assimilation (pmoA gene) and mineralization of organic P compounds (phoD gene), using the real-time PCR technology with SYBR Green and specific primers (described in the literature). The total bacterial and fungal community will be quantified using the 16S rRNA and ITS gene, respectively. The calibration curves for determining the number of DNA copies of each gene will be obtained from the cloning of the C and P functional genes in plasmids containing a known number of copies of the respective gene.

3 - Enzymatic activities

To complement the quantification analysis of the C and P functional genes, the activity of enzymes involved in the transformations between the various forms of C and P in the soil, such as β-glucosidase, dehydrogenase, phosphatase and phosphomonosterase, will be determined in the samples of each soil type (native forest, cashew, legumes). The methods used will be based on spectrophotometry.

3 - Statistical analysis

The results of the quantification of 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 C and P cycle.

Student 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.