Research proposal for application for beam time at LNLS

This document must consist of a maximum of two A4 pages (including references and figures) with a minimal font size of 10 pt. Proposals which do not respect these rules will be rejected. Delete the gray italic text to fill with the proposal information. Only PDF files format are accepted. It is compulsory to write the proposal in English.

PROPOSAL TITLE

In situ µ-XRF and µ-XANES shedding light on the mechanisms of phosphorus uptake by plants

1) Is this a continuation of a previous proposal?

No, it is not.

2) Scientific background.

In 2017/2018 Brazil is became the largest soybean producer and world exporter. This crop was responsible for nearly 12% of the Brazilian total exports in 2016. If one considers also meat exports (soybean is the main source of protein for livestock) this figure can reach up 20 %[1].

Most of the soybeans come from the *cerrado* region. There, soils show good physical features, but are extremely poor in available nutrients, especially phosphorus (P). *Cerrado* soils present large quantities of iron and aluminium oxides that have great affinity for phosphate ions, once adsorbed in soil particle the phosphate becomes hardly available to plants[2]. Attempts of farming without annual fertilization reduces crop yield, even though the total amount of P present in soil may be considered high[3]. In addition, soybean is highly dependent on P fertilizer.

In Brazilian *cerrado* soils, the P recovery, i.e. the ratio between the plant intake and the amount of P applied via fertilizer is around 50%[2]. Hence, half of the P remains in the soil, mostly unavailable to crops. As a result, P has been stored underground and every year the amount of P stock will increase. This stored P is commonly called legacy-P[2].

Like crude oil, P reserves are limited. Around 60% of all currently exploitable P to produce fertilizers is held by Morocco. Brazil is one the largest P consumers (3°), but its production is less than 50% of the own demand and the reserves are less than 2 % of global deposits[4]. As a commodity, essential to the production of other commodities, P shortages or merely pricing speculation may give risen to crises, threatening our economy and the global food supply.

Increasing the availability of the soil stored P is of strategic importance. It can: i) reduce famers dependence of fertilisers during peak prices; ii) decrease the quantity of phosphate fertilisers applied to crops, which can positively impact prices, the environment and extend life of the rock phosphate deposits.

The first step towards making the legacy-P available consists in: i) understanding the nature of the chemical bonding configuration between phosphate and soil; ii) understand how plants and to which extent desorb P from soil. Thus, we propose to employ the recently available and unique micro beam facilities at SXS beamline to perform *in situ* XRF mapping and P K edge XANES around the living roots and undisturbed soil of soybean plants.

3) Expected results.

The absorption of P occurs through a complex mechanism that is not completely understood yet. One strategy is to exudate organic acids that solubilize the P. This proposal will to shed light on the P solubilisation and transport taking place in the rhizosphere, the area (1-2 mm) affected by the roots. Specifically, the expected output of the measurements are illustrated in Figure 1(a) and stated below:

i) The high sensitivity of micro-XRF maps will allows us to draw rhizosphere P depletion surfaces as function of root age. Thus, we can better understand how roots drain the P in soil.

ii) Micro-XANES is paramount to this study as this will reveal how the P chemical species changes as function of the root distance. By doing so, we can better understand the solubilisation process. For example, we answer how phosphate is transported.

iii) Micro-XANES will also reveal how the P chemical species changes according to the time of contact with roots. This will be possible because the older roots will be in contact with the soil for longer periods than the younger ones.

4) Previous characterization.

Figure 1(a) illustrates the rhizobox developed to perform the study. Using this setup we are able to grow the plant and monitor the root development. The root face is covered by a 4 µm thick polypropylene film that allows the tender X-rays to escape. The soil used in this study comes from an experimental field cultivated with soybean and maize since 2008. Phosphate fertilizer has been added every year, which resulted in ten years of legacy-P accumulation.

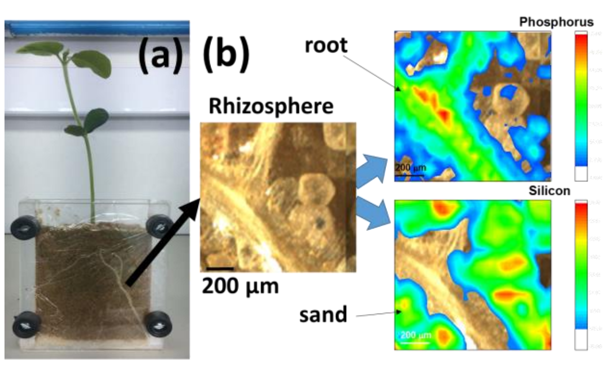

Fig1. (a) Rhizobox that allow recording in situ and in vivo micromaps; (b) chemical images of phosphorus and silicon.

Firstly, using ICP-OES, we determined the total content of P. The soil presents 971 mg P kg^{-}

^{1} soil. This concentration (ca. 0.1%) assures the feasibility of the XANES measurements at SXS beamline. Then we performed wet chemical P fractionation which indicated that despite of the high total P content, only 1.6% is readily available to the plants. The fractionation still showed that 3.8% is labile, i.e. plants can access at the expenses of root exudates. Nearly 24% is strongly adsorbed to the soil, whereas 70.6% is not any more accessible.

Figure 1(b) also shows a preliminary rhizosphere map using a sandy soil. This map was recorded in a laboratory benchtop micro probe X-ray fluorescence spectrometer. The image highlights the high content of P in the root whereas in the rhizosphere mainly silicon is observed. The main drawback of this type of equipment regards the lower sensitivity compared to the one offered by SXS beamline. Additionally, it is not possible to perform chemical speciation.

5) Experimental method.

Figure 2(a) illustrates the setup that we plan to assemble at the beamline. We propose to use the 20 μm X-ray beam produced by the polycapillary optics at SXS beamline to record and P Kα XRF maps and P K edge XANES in the rhizosphere of the plants. The measurements will be carried out using the Si(111) monochromator and XRF detector. The 3 mm working distance allows the measurements be carried out under air atmosphere.

The study will monitor three root regions: i) region a: root 5 weeks old; ii) region b: root 3 weeks old and iii) region c: root 3 days old. The goal here is capturing how the organic compounds exudate by the roots modify the soil environment and, therefore, the P concentration profile and its chemical environment. In each of these regions we will record a 1 x 1 mm^2 P XRF map using the monochromatic excitation beam. Then, in each region we will record 3-5 micro XANES spectra (-100 up to 250 eV relatively to the edge) according to the P concentration gradient. The experiment will be performed in triplicate. To complete the data gathering the rhizoboxes will be brought to our laboratory and the same regions will be mapped again revealing the spatial distribution of Ca and Fe. Additionally we will record XANES for the following pelletized reference compounds: $P_{adsorbed}$-Al_2O_3, $P_{adsorbed}$-αFe_2O_3, $P_{adsorbed}$-α-FeOOH, $FePO_4.2H_2O$, KH_2PO_4, $Ca_4H(PO_4)_3.2.5H_2O$; $CaHPO_4$, $CaHPO_4.2H_2O$, $Ca_5(PO_4)_3OH$, $AlPO_4.2H_2O$, DNA, phytic acid and ATP. Figure 2(b) outlines the overall measurement strategy.

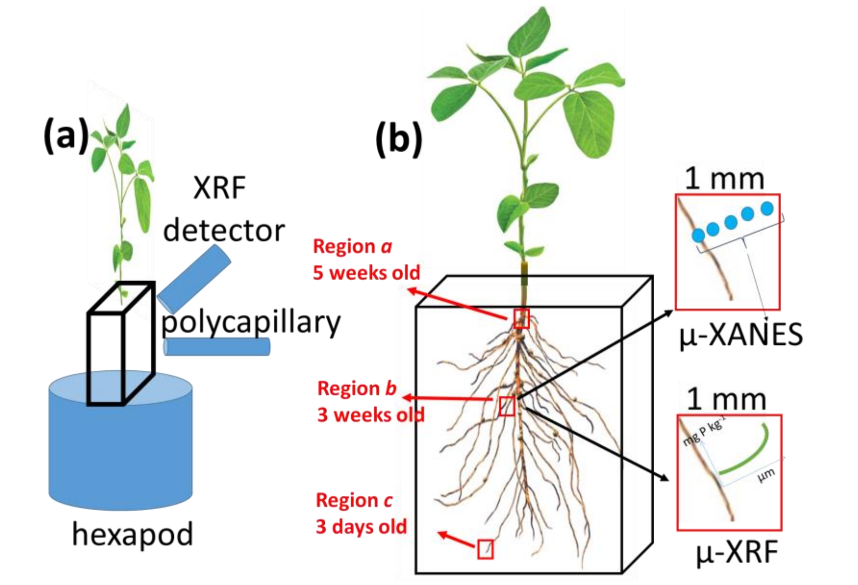

Fig2. (a) Rhizobox assemble at the beamline; (b) Selected regions for mapping and XANES measurements.

6) Beam time requested justification.

We expect measuring maximum 5 XANES per rhizosphere x 5 scans to improve statistics (15 minutes each) x 3 regions. This sums up ca. 24 hour per rhizobox. The experiment must be replicated, thus the chemical speciation will require three days. Considering the time necessary to record P exploratory XRF maps, XANES for reference compounds and setting up the experiments, another 24 hours are required. Altogether we request 4 days of beamtime, i.e. 12 shifts.

7) References.

1) Atlas of Economic Complexity, Center for International Development of Harvard University. Consulted in 2018.

2) Werner et. al. Standard Protocol and Quality Assessment of Soil Phosphorus Speciation by P K-Edge XANES Spectroscopy Env. Sci. Technol. 2015, 49, 10521-10528.

3) Roy, E. D. et al. The P cost of agricultural intensification in the tropics. Nature Plants, 2016, 2, 16043.

4) Vaccari, D. A., Phosphorous: A Looming Crisis. Scientific American 2009, 300, (6), 5.