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Mapping of P and K nutrients using X-ray fluorescence: first insights on P exchanges in ectomycorrhizal roots



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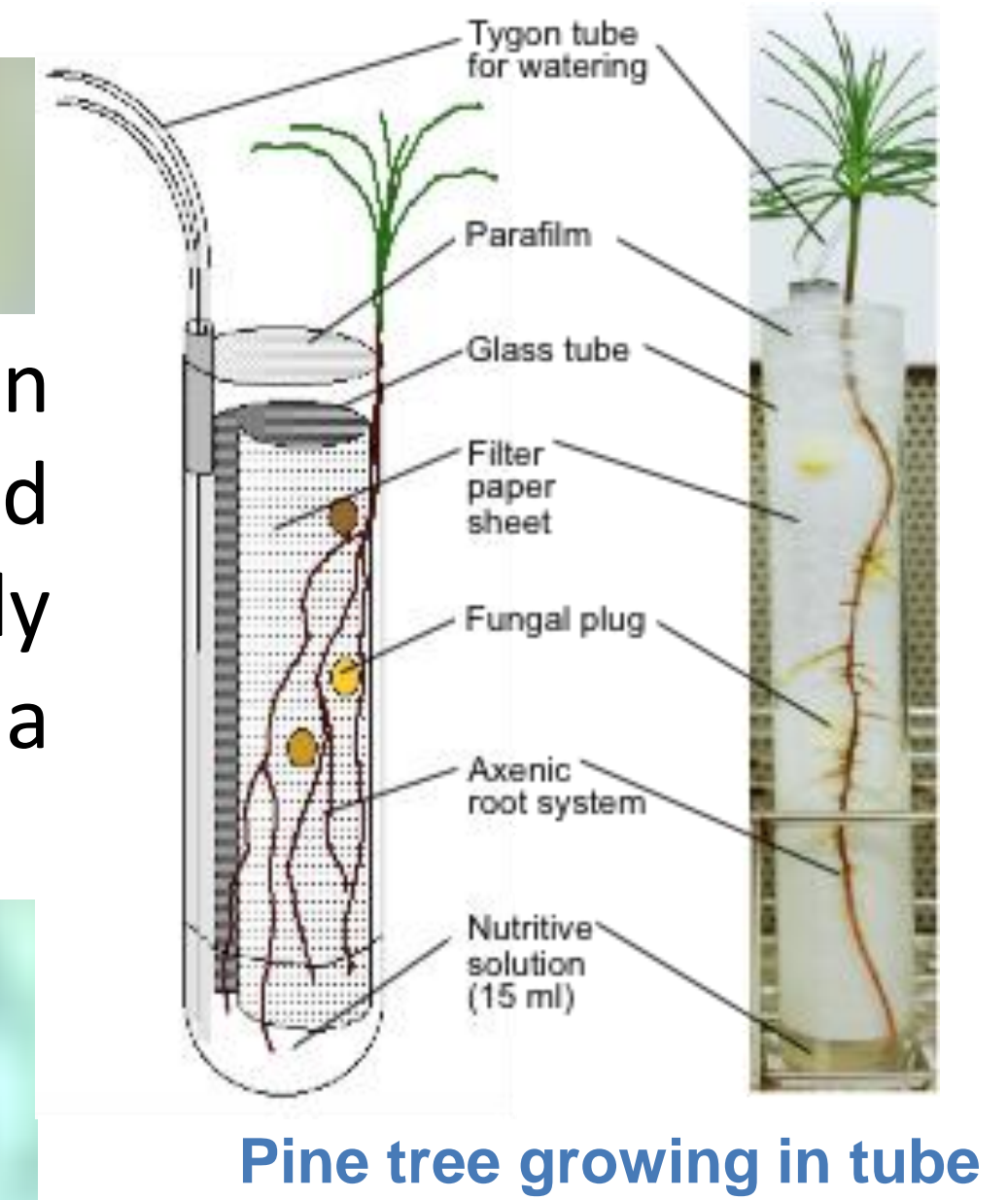


Context and Objective of the study

It is well known that ectomycorrhizal (**ECM**) fungi improve the growth of their hosts by providing growth-limiting nutrients and particularly inorganic phosphate (**Pi**). However, the molecular players driving the Pi allocation from the fungus to the host are largely unknown thus far. The objective of this study was to understand the role of a candidate fungal P transporter gene for P efflux (*Hc-PT2*) in the ECM association between *Pinus pinaster* and the basidiomycete *Hebeloma cylindrosporum*. To fill this objective, we compared the P distribution in short roots of non mycorrhizal (**NM**) plants with those found in ECM roots associated with a fungal strain previously transformed with an over-expression vector, either containing no gene (**Ctrl**) or our candidate gene (**OE-PT2**).

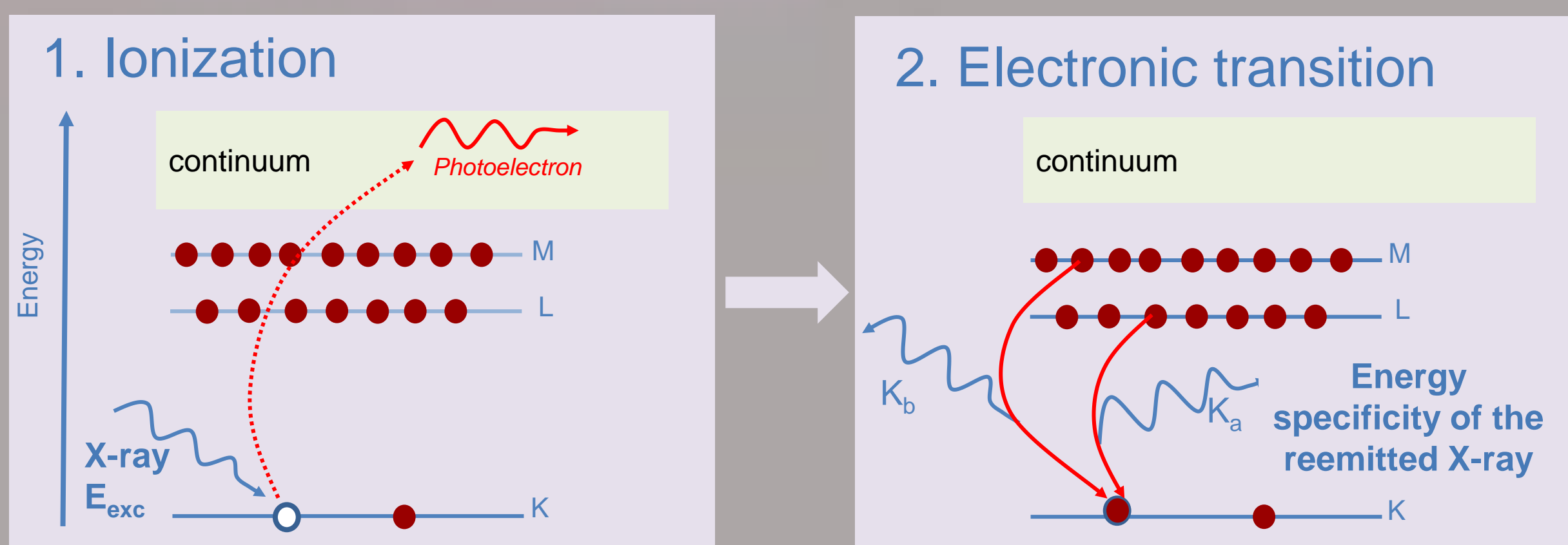
Plants growth and samples preparation

Plants were grown in tubes for 2 months, whether or not inoculated with Ctrl or OE-PT2 fungal strains. A nutritive solution containing nitrate (1 mM) and Pi (0.1 mM) was regularly added throughout the culture. Plants were extracted from the tubes and the root system rinsed in water. Short roots were cut under a stereomicroscope, embedded in a cryoprotectant and immediately plunged into isopentane cooled by liquid N₂ to fix the cells and their contents. Cryo-thin sections of 20 µm were prepared using a cryotome and analysed at SOLEIL synchrotron on the microfocused LUCIA beamline equipped with a N₂ cryostage.

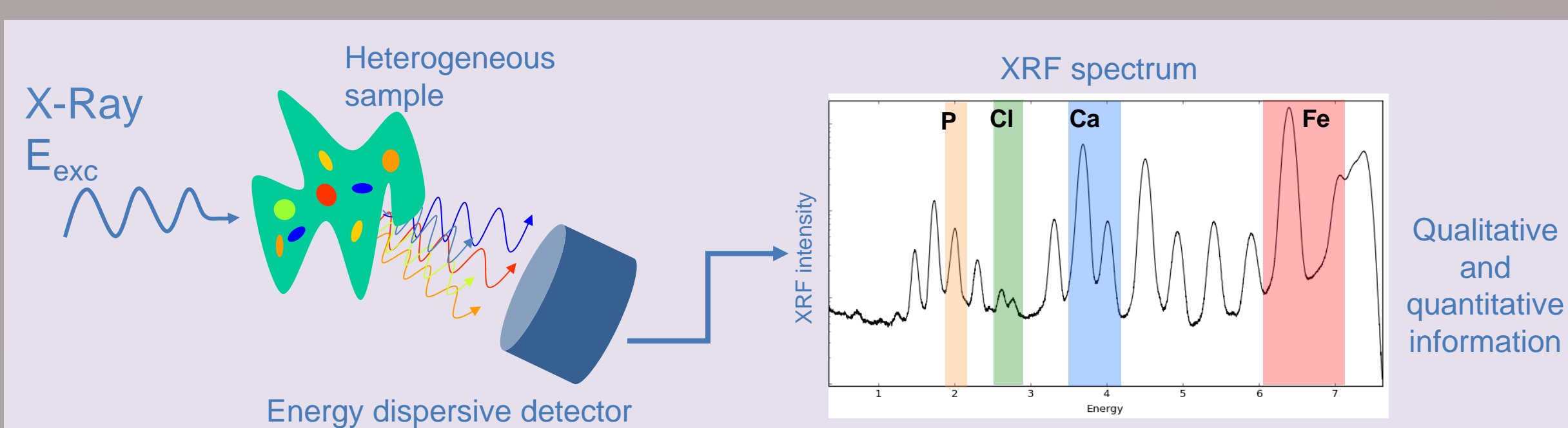


Elemental mapping using X-ray fluorescence (XRF) on LUCIA beamline

X-ray spectroscopy fluorescence principle



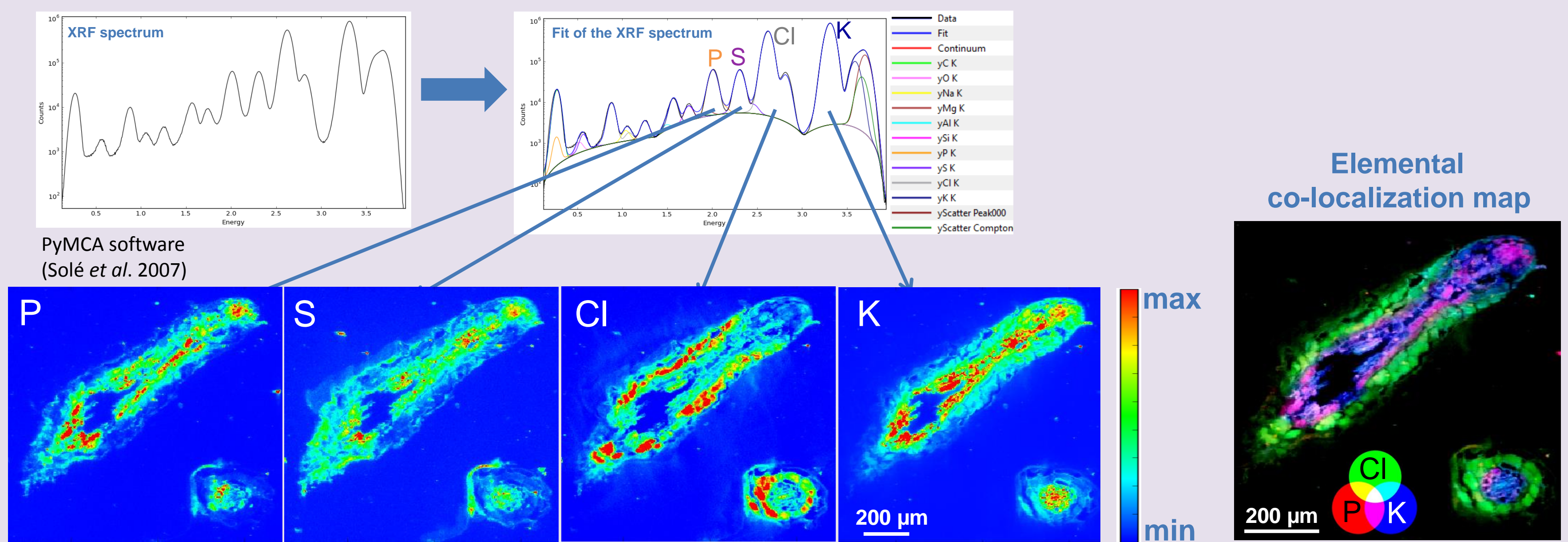
→ Elemental composition determination



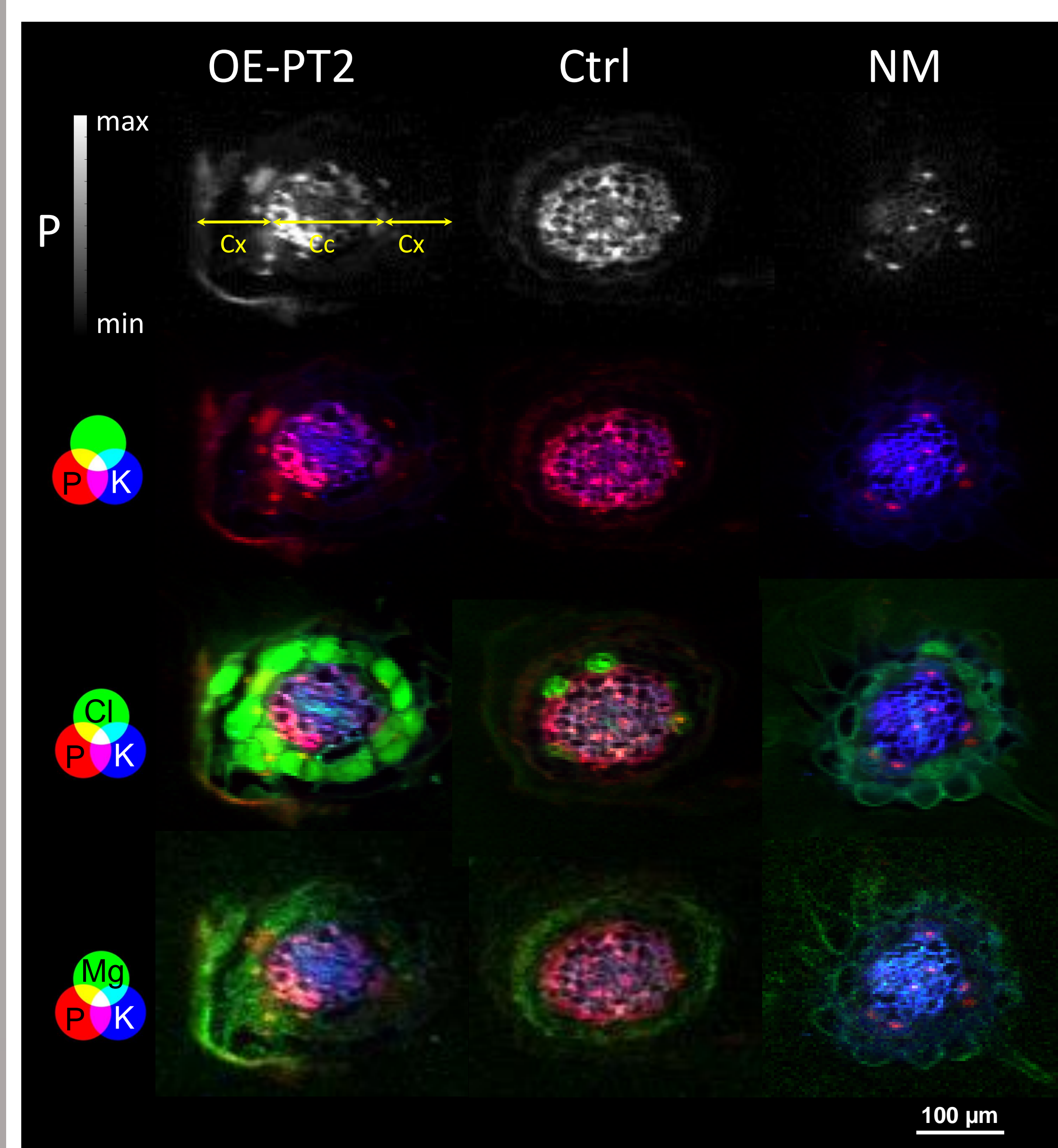
Elemental mapping

Samples are moved relative to the focused beam to scan the area of interest and an XRF spectrum is recorded in each pixel of the map.

XRF maps were recorded using a 3 x 3 µm² pixel size at 3.7 keV to collect signal of K, Cl, S, P, Si, Al and Mg.



Elemental distribution of P, K, Cl and Mg in pine tree roots

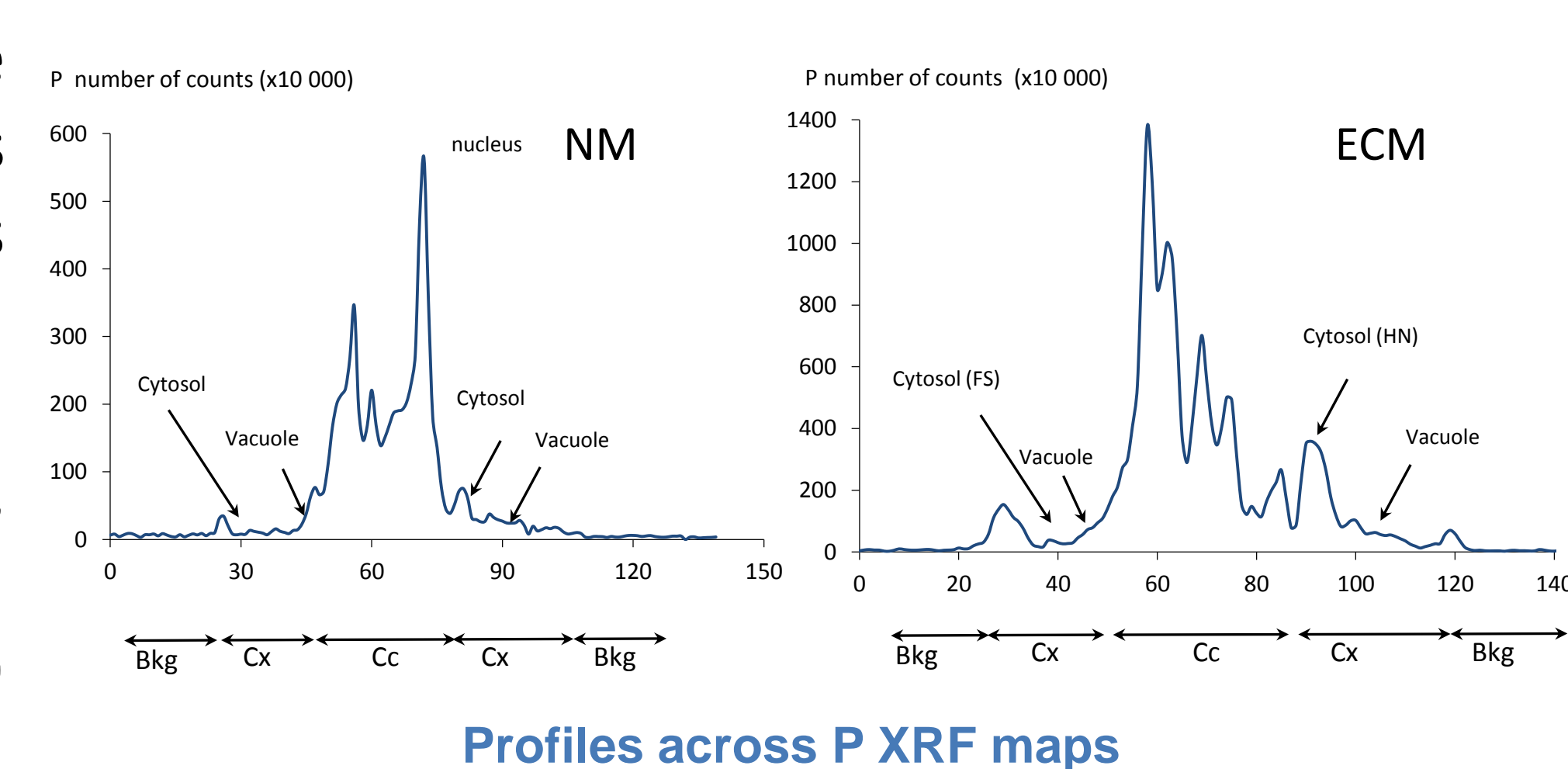


P XRF and co-localisation XRF maps

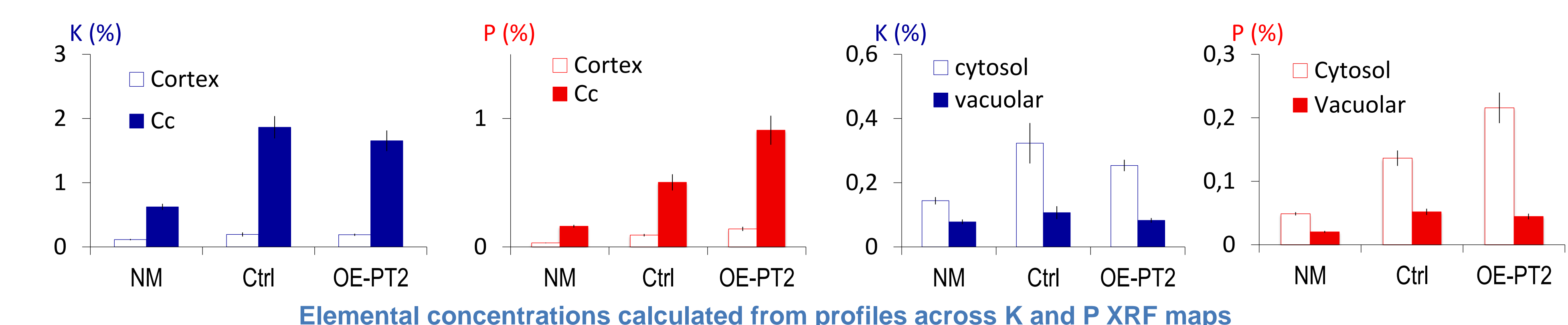
To compare the levels of P and K along the different growth conditions, P and K XRF values of the pixels were extracted along six profiles using ImageJ.

Several locations can be distinguished as a function of the pixel value: background (Bkg), cortex (Cx), central cylinder (Cc) and cortical cytosol and vacuoles in non mycorrhizal (NM) root, accordingly to the pixel values.

Interestingly, in mycorrhizal roots (ECM), it was also possible to distinguish the external fungal sheath (FS) and also the fungal cells belonging to the Hartig net (HN).



Profiles across P XRF maps



Elemental concentrations calculated from profiles across K and P XRF maps

Using a standard (NIST bovine liver), K and P % were calculated in different cells / compartments. The presence of the fungus increased always K and P contents of root cells compared to NM roots. Remarkably, OE-PT2 increased specifically the P contents in the central cylinder and in the cytosol of the cortical cells of ECM roots. These results support a role of *Hc-PT2* in the unloading machinery of P from the fungal cells in the Hartig net, enabling a higher P content in the xylem towards the shoots.

Conclusion

Mapping the elements on root sections can enable us to understand better the biological mechanisms sustaining the functioning of the symbiosis. These first characterizations of ECM using synchrotron XRF evidenced, in particular, the role of *HcPT2* in increasing P concentration in the xylem.