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*Facing Phosphorus Scarcity*

# Phosphorus in Soils and Plants





**Theme 1 – Phosphorus forms, availability and cycling in soils**



## **Theme 1 – Phosphorus forms, availability and cycling in soils**

### **Keynote presentation**

**Oxygen stable isotopes in phosphate: improving our understanding on the fate of phosphorus in the soil/plant system**Federica Tamburini <sup>1</sup>, Verena Pfahler <sup>1</sup>, Christian von Sperber <sup>1</sup>, Stefano M. Bernasconi <sup>2</sup>, Emmanuel Frossard <sup>1</sup>*1. Institute of Agricultural Sciences, ETH Zurich, Switzerland**2. Geological Institute, ETH Zurich, Switzerland*

The use of stable isotopes has brought a revolution in environmental studies, deepening our knowledge on the biogeochemical cycle of important nutrients, such as C, N, and S, and allowing to follow transfers from sources to sinks. Phosphorus, a key nutrient essential for life, has only one stable isotope, but it is often associated to oxygen, which has 3 stable isotopes. Since at ambient conditions only biological processes are able to affect the isotopic signature of oxygen in phosphate ( $\delta^{18}\text{O}-\text{PO}_4$ ), the scientific community has shown a strong interest in using this tracer, particularly to study the soil/plant system. The extraction and purification of phosphate from soil and plant material are not easy steps, because the final sample should be free of any O-containing compound other than phosphate. P is present in many soil pools, but to date only few have been investigated for  $\delta^{18}\text{O}-\text{PO}_4$ . Moreover, although we know the fractionation factors associated with several biological processes controlling P cycle, we are missing information on others, such as biological uptake, relevant phospho-enzymes, and synthesis of organic P-compounds. In terrestrial ecosystems,  $\delta^{18}\text{O}-\text{PO}_4$  was first used to trace P, but the relatively narrow window of  $\delta^{18}\text{O}-\text{PO}_4$  in P sources has shown the limits of this approach. Most importantly, biological cycling of P by microbes and plants can erase the source signal and the imprint of other processes. However, this last aspect has proven useful, because  $\delta^{18}\text{O}-\text{PO}_4$  seems to be an invaluable tool to assess the role of the microbial biomass in P cycling.



## **Theme 1 – Phosphorus forms, availability and cycling in soils**

### **Oral presentations**

## Contribution of anthropogenic phosphorus to agricultural soil fertility and food production

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Agricultural intensification over the last few decades has been accompanied by the extensive use of anthropogenic phosphorus (P) derived from mined phosphate rock. Given the increasing scarcity of P resources, accurate estimates of the reliance of agriculture on anthropogenic P are required. Here, we propose a modeling approach for assessing the contribution of anthropogenic P to agricultural soil fertility and food production. We performed computations at country level and France was chosen as a typical Western European country with intensive agriculture. Four soil P pools were identified based on their bioavailability (labile vs. stable) and origin (anthropogenic vs. natural). Pool evolution between 1948 and 2009 was estimated by combining international databases and a simple biogeochemical model. Mean simulated P pool sizes for 2009 (0–35 cm soil horizon) were 146, 616, 31 and 156 kgP/ha for natural stable, anthropogenic stable, natural labile and anthropogenic labile pools, respectively. We found that the simulated anthropogenic contribution of soil P sharply increased from 1948 to 1975 and remained almost constant over the last 35 years. The temporal evolution of this contribution is directly related to the integral of chemical fertilizer use over time, starting from 1948. In 2009, 68–91% of soil P involved in plant nutrition was anthropogenic. The contribution of anthropogenic P to food production was similar at 72–91%, which is greater than budget-based estimates (~50–60%) commonly reported in the literature. By focusing on soil fertility and food production, this study provides a quantitative estimation of human perturbations of the P cycle in agroecosystems.

## The fate of fertilizer phosphorus in pastures

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Phosphorus (P) fertilizers are essential for pasture production in the high rainfall zone of south-eastern Australia. However, the fate of fertilizer P in pasture systems remains unclear and is largely thought to accumulate in the topsoil layer in sparingly available forms. This study sought to understand the fate of fertilizer P in pastures using <sup>33</sup>P labeled single superphosphate applied (~ 10 kg P ha<sup>-1</sup>) to a subterranean clover sward at two sites (Naracoorte, South Australia; and Hall, Australian Capital Territory). Plants were analysed for uptake of <sup>33</sup>P and the 0 to 4 cm and 4 to 8 cm soil layers were fractionated and analyzed for <sup>33</sup>P in the total and inorganic P fractions (plus organic P by difference) of the Colwell (bicarbonate extraction), NaOH-EDTA and H<sub>2</sub>SO<sub>4</sub> (ignited and non-ignited) soil extracts. Pasture biomass recovered 40 % and 34 % of the fertilizer P applied at the Naracoorte and Hall field sites, respectively. A significant proportion of the remaining fertilizer P (~ 30 %) was recovered in the 0 to 4 cm layer within the NaOH-EDTA and ignition- H<sub>2</sub>SO<sub>4</sub> extracts. The Colwell soil P extractant recovered 20 % and 13 % of the fertilizer P in the 0 to 4 cm layer at the Naracoorte and Hall field sites, respectively. This suggests that only a small fraction of the fertilizer P is incorporated into sparingly available forms in the year of application. In all cases, the majority of fertilizer P in the soil was recovered as inorganic P forms in the 0 to 4 cm layer. Initial conclusions are that the accumulation of soil organic P typically found in fertilized pastures may involve slow processes rather than a rapid direct immobilization of fertilizer P into organic forms.

O103

### Soil organic phosphorus in critical and non-critical hydrological source areas

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Critical source areas (CSAs) are zones in the landscape where easily connected hydrology coincides with a phosphorus (P) sources in the soil. The P export risks in CSAs are hypothesised to be higher compared with non-critical source areas (Non-CSAs). Past research has often neglected forms of organic P, such as DNA and phospholipids which are among the most potentially biodegradable organic P compounds. The objectives of this study were i) to quantify the magnitude of organic P compounds in agricultural soils and determine whether these magnitudes differed significantly between CSAs and Non-CSAs; ii) determine the variation of P magnitude between and within individual fields. The study focussed on soils collected from the Morland sub-catchment of the River Eden catchment in Cumbria, England. Five CSA – Non-CSA pairs were identified using the SCIMAP (diffuse pollution risk mapping) modelling and field assessment. The results showed that there are significant differences in the concentrations of total P (TP), DNA-P, WETP (water extractable total P) and WEOP (water extractable organic P) between CSA and Non-CSA. Besides, the concentrations of all the P forms showed distribution variation between fields or even within the same field. DNA-P and PLD-P was presented considerable proportions of total P in soil, especially DNA-P which had a good correlation with TP. DNA-P in the ten areas accounted for a considerable proportion of soil TP (4.9 to 16.6%). Given the potential lability and bioavailability of DNA and phospholipids, our data demonstrate that these soil organic P could be a potential pool to support plant nutrition and a potential contributor to water pollution problems.

O104

### A $^{33}\text{P}$ tracing model for quantifying gross P transformation rates in soil

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Due to rapid sorption of phosphate released by mineralization processes, in most soils net organic P mineralization rates cannot be derived from changes in extractable inorganic P over time. Besides, a mechanistic understanding is only obtained if the individual gross P transformation rates are known. Available techniques for the quantification of gross P transformation rates rely on isotopic dilution principles. We developed a numerical  $^{33}\text{P}$  tracing model to quantify all relevant simultaneously occurring P transformation rates in soil. The tracing model combines a process-based numerical model with a parameter optimization routine to estimate gross P rates. Each rate can follow either zero-order, first-order or Michaelis-Menten kinetics. The model was able to simulate the observed dynamics in a previously published data set (Bünemann et al. 2012 SBB 51:84-95). Isotopic dilution due to microbial processes was partitioned differently from the conventional approach, and therefore gross organic P mineralization rates were lower than previously published. Net organic P mineralization rates tended towards zero, confirming the overriding dominance of microbial immobilization and re-mineralization processes in the studied grassland soil. Other published datasets are currently being reanalyzed using the modeling approach. The P tracing model presented here removes the need for the baseline of isotopic dilution due to physicochemical processes. It presents an important advancement over the conventional estimation of gross P transformations in isotopic dilution experiments and opens up new possibilities to study gross P transformation rates, also under non-steady-state conditions.

O105

### Colloidal phosphorus and its contribution to plant nutrition

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Bioavailability of P is related to its concentration and speciation in the soil solution. Andisols and Oxisols exhibit very low soil solution P concentration due to their high content of Al/Fe oxyhydroxides which strongly adsorb P. Free orthophosphate (Pi) is the P form taken up by plants; but it is not the only P species present in the soil solution. Mobile colloidal P (P associated with Al, Fe, range size from 1-1000 nm) constitutes an important fraction of water extractable P in these soils; however its availability is poorly understood. We hypothesized that colloidal P in soil-solutions of Andisols and Oxisols may contribute to the pool of plant available phosphorus and that this pool has been previously overlooked. The potential availability of colloidal P was assessed from <sup>33</sup>P labeled non-filtered and filtered (through 0.45-µm and 3-kDa membranes) soil-water extracts from two Andisols and an Oxisol. The results showed that a large fraction of this colloidal P, which constituted ~80% of the total P in solution, was isotopically exchangeable. The contribution of colloidal P to plant nutrition was evaluated by measuring P uptake of *Triticum aestivum* from labeled non-filtered and 3-kDa filtered (truly dissolved P) soil-water extracts of the same soils. In the Andisols, plant P uptake from the non-filtered solutions was up to 36-fold higher than the 3-kDa filtered solutions. In the Oxisols, no differences in the P uptake between both solutions were observed. Our results suggest that colloidal P of Andisols is not chemically inert and contributes to the plant P uptake. The different results obtained from the Andisols and Oxisols points out the dissimilar nature of the colloids of the soils.

O106

### Distribution and speciation of phosphorus in soils using µ-X-ray fluorescence and X-ray absorption near edge structure

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Gaining insights into both the distribution of phosphorus (P) in soils and the evolution of the fertilizer inputs is of great interest for a sustainable management of P in agriculture. Soil P is commonly studied with chemical methods on bulk powdered samples. Solid state RMN, an in situ technique, minimizes the chemical perturbations but the information retrieved is only partial. In the present study, X-ray fluorescence (XRF) and X-ray absorption near edge structure (XANES) spectroscopy were applied on thin sections of undisturbed soil mottles, using a micron size probe to provide spatial P-distribution, elemental co-localisation and identification of the P-bearing phases. Samples were picked in fields cultivated with different combinations of crop rotation and fertilizer inputs from the Morrow Plots (U. of Illinois, Urbana-Champaign, 140 years old). Analyses were conducted at the ID21 X-ray microscopy beamline at the ESRF in both unfocused (200 µm beam) and focused (0.3 x 0.6 µm<sup>2</sup>) modes. The µ-XRF maps evidenced the concentration of P in small hot spots (0.5-10 µm large) and a low diffuse P-background. A general Al-P correlation and punctual Fe-P correlations were observed. XANES on powdered bulk soils and on their fine fractions evidenced that P is mainly localized in the <2 µm size fraction, mainly adsorbed on organic matter. The variety of µ-XANES spectra shapes showed the presence of minor P species like apatite, polyphosphates, or phosphates adsorbed on goethite. Thus, µ-X-ray spectroscopy is a useful tool to investigate distribution and speciation of elements as P in soil samples, highlighting both major and minor phases.

O107

### Deep Ultraviolet Raman Microspectroscopy - Novel technique for the characterization of phosphorus in soil

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Phosphorus is of fundamental importance for virtually all living organisms including humans and is not substitutable. A sustainable phosphorus management would minimize the decreasing of phosphate resources. The availability for plants highly depends on the chemical state of phosphorus in soils which is difficult to analyze due to the limits of state of the art wet chemical and instrumental approaches. A novel direct analysis of organic and inorganic phosphates in soil was investigated by deep ultraviolet (DUV) Raman microspectroscopy. DUV Raman microspectroscopy has the advantage that no fluorescence interference particularly caused by organic matter in soils occurs. In addition, the sensitivity of organic phosphate bands increase because strong absorption bands lead to resonance enhancement of the Raman bands when excited in the deep ultraviolet. By this electronic resonance enhancement effect the intensity of the scattered light can be increased by a factor of 106 compared to non-resonance Raman excitation. Measurements were done without further sample preparation with a micro-Raman spectroscopy system with an Ar ion laser (244 nm). The chemical state of inorganic and organic phosphate phases were detected by mapping of the soil sample. DUV Raman microspectroscopy as a high resolution imaging technique also gives the opportunity to analyze local interactions between soil compounds. Furthermore, the chemical state of carbonates, sulfates and silicates in the soil were also determined. Thus, DUV Raman microspectroscopy could become the analytical key to improve the understanding of transformation mechanisms of mineral phases in soils.

O108

### Organic phosphorus characterisation by <sup>31</sup>P NMR spectroscopy and enzyme addition assays across a range of soil types

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Solution <sup>31</sup>P NMR spectroscopy is commonly used for the quantification of various soil organic P classes. Enzyme addition assays may be a cheaper, more accessible alternative, but a thorough comparison to results obtained by <sup>31</sup>P NMR is lacking. In this study, NaOH-EDTA extractable P of ten topsoil samples from four continents with varying total P (83 – 1560 mg kg<sup>-1</sup>), pH<sub>H2O</sub> (4.2 – 8.0) and land management (grassland or cropped land) was characterized using both methods. Four different classes of organic P were determined by enzyme additions (monoester-like P, DNA-like P, inositol phosphate-like P and enzyme-stable P) and related to the corresponding organic P classes as quantified by <sup>31</sup>P NMR spectroscopy. NaOH-EDTA extractable organic P ranged from 6 – 1115 mg P kg<sup>-1</sup> soil, of which 33 – 92 % was enzyme-labile. Inositol phosphate-like P was the largest organic P class in most soils (15 – 51%), followed by monoester-like P (10 – 47%) and DNA-like P (0 – 15%). A strong correlation was found between the four organic P classes characterized either by <sup>31</sup>P NMR spectroscopy or enzyme addition assays (R<sup>2</sup> 0.93 – 0.99). The correct interpretation of enzyme addition assays was further verified by <sup>31</sup>P NMR analysis of enzyme-treated extracts, and the molecular size of the enzyme-stable P was investigated using FPLC. Besides implications for the nature of soil organic P, our results suggest that enzyme addition assays are suitable for a quick, cheap and coarse determination of four soil organic P classes in NaOH-EDTA extracts.

O109

### Earthworms can increase plant growth through elevated phosphorus availability in their casts

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Earthworms are known to increase the availability of phosphorus (P) in their casts, but their effect on P uptake by plants has received little attention. We conducted a series of pot experiments to test whether earthworm activity increases P availability to grass under P-limited conditions. In a first experiment we tested the impact of three earthworm species (*Lumbricus rubellus*, *Aporrectodea caliginosa* and *Lumbricus terrestris*) on P availability. Dissolved P concentrations in water extracts from earthworm casts and bulk soil were analysed and compared. We observed a 500-fold increase in ortho-P ( $p < 0.001$ ) and organic P ( $p < 0.001$ ) in the water extracts of earthworm casts from all three species as compared to the bulk soil. In a second experiment we tested whether this resulted in increased plant growth and whether a pH increase in the earthworm casts was the main explanatory factor for the elevated ortho-P concentrations. *L. terrestris* significantly increased yield from 164 to 188 g dry matter  $m^{-2}$  ( $p = 0.044$ ) and P uptake from 0.21 to 0.27 g  $m^{-2}$  ( $p = 0.002$ ) in the absence of P fertilisation. The strong increase in pH of 2.5 units was, however, not the sole driving factor for increased P availability in the casts. Competition for binding sites between ortho-P and strongly elevated dissolved organic carbon concentrations in the casts is hypothesized to be the main mechanism explaining this P effect, but this remains to be confirmed with surface complexation modelling. In conclusion, earthworms can increase plant growth under P-limiting conditions. As such, stimulation of earthworm presence in agricultural fields may be a viable tool to increase production in P-limited systems.

O110

### Active and total microbial communities respond differently to P fertilization and pH in permanent grassland

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Soil properties, particularly pH and nutrient availability, shape the soil microbial community, with direct effects on biogeochemical processes such as organic P mineralization. Mineral P fertilization has been shown to affect gross organic P mineralization rates. However, the underlying microbial processes are not yet well understood. Therefore, we studied the effects of P fertilization and pH on archaeal, bacterial and fungal communities and phosphomonoesterase activities in a long-term grassland fertilization trial characterized by a natural pH gradient near Zurich (Switzerland). Both DNA and RNA were extracted, and the community composition examined by fingerprinting methods. In addition, we used 454-sequencing to examine the diversity of PhoD alkaline phosphatase as affected by P fertilization and pH. RNA-based profiles of archaeal, bacterial and fungal communities were distinct from DNA-based profiles. Soil pH greatly affected the structure of all communities at both RNA and DNA level. Interestingly, the structure of the active fungal community was mainly related to available P, whereas the one of the total community was affected by pH only. Acid and alkaline phosphomonoesterase activities were strongly related to the microbial communities at low and high pH, respectively. Transcripts of PhoD alkaline phosphatase corresponded to a subset of the genes in the community. Our results suggest that microbial groups respond differently to P availability and pH. These changes are reflected in the overall community structure, with effects on the organic P mineralizing activity.

O111

### Using $^{33}\text{P}$ to quantify phosphorus accumulation below-ground by canola and the contribution to following wheat

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Measures of phosphorus in coarse roots are an underestimate of P accumulation below-ground by crop species since they do not account for P in unrecovered fine roots. A  $^{33}\text{P}$  stem-wick feeding technique was used to label canola root systems 'in-situ' to provide quantitative measures of i) the amount of P accumulating below-ground in canola root systems, and ii) the contribution of that below-ground P to subsequently grown wheat. Canola (*Brassica napus*) was grown in sand or clay soil in sealed pots and 45 days after sowing was fed via the stem with  $^{33}\text{P}$ -labelled phosphoric acid. Pots were destructively sampled at peak biomass and maturity; another set had shoots removed at maturity and soil was left undisturbed. Three weeks later wheat was sown into the undisturbed pots and grown for five weeks. Mean recovery of fed  $^{33}\text{P}$  (459 kBq/plant) in the shoot plus fallen leaves/flowers, recovered roots and soil was similar at both growth stages (93% peak biomass and 92% maturity), and 7% was sorbed to the wick. More  $^{33}\text{P}$  was partitioned to canola roots (40-50%) than shoots (26-36%) regardless of soil type and plant age. Total below-ground phosphorus (BGP) accumulation was always greater (up to 30%) than P in recovered roots alone. The contribution of the BGP to P uptake of wheat was similar regardless of soil type with 6-7% of BGP recovered in wheat shoot plus coarse roots. The majority (92-94%) of the canola BGP remained in soil. Wick-feeding  $^{33}\text{P}$  via the stem enables improved quantitation of total P accumulation in root systems of break crop species and facilitates estimation of the contribution of break crop root system P to following wheat as well as to longer term P pools in soil.

O112

### Acid phosphatase activity in the rhizosphere: how a cereal and a legume create local hotspots

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Phosphorus is a poorly mobile, essential nutrient in terrestrial ecosystems. Phosphorus can be of greater local availability in the rhizosphere where greater biological activity can increase enzymatic activities and induce chemical changes such as to pH. However, many rhizosphere processes occur at the very fine-scale (millimetric) while sampling is difficult to do at less than the cm-scale. Using rhizoboxes with 2D access to the soil profile, we mapped and monitored acid phosphatase activity in situ in chickpea and durum wheat, sole and intercropped, as well as in bare soil rhizoboxes over 5 weeks, from small plants to post seed set for the legume. The soil originated from the till treatment of a long-term fertilizer trial that had not received phosphorus application over 40 years, and thus had low phosphorus availability. We also monitored pH at a very fine scale several times a week using novel non-invasive optical sensors (optodes) to understand fluctuations as well as development of pH shifts in the soil over time and with plant root passage. Greater acid phosphatase activity was frequently observed near roots, especially chickpea roots, some root apices, and root nodules. We also observed heterogeneity of hotspots of acid phosphatase activity within the soil and investigated the size and frequency of these hotspots using geostatistics. We combine these results with data on pH and inorganic phosphorus to try to understand how rhizosphere processes impact local phosphorus availability and how these processes may shift when different plant species interact with each other.




## **Theme 1 – Phosphorus forms, availability and cycling in soils**


### **Posters**

## P101

### Fine mapping of soil phosphatase activity as affected by endogeic earthworms, in presence or absence of plants

Esther Guillot <sup>1,2</sup>, Camille Cros <sup>1</sup>, Sarah Placella <sup>1</sup>, Josiane Abadie <sup>1</sup>, Gabrielle Daudin <sup>1</sup>, Claire Marsden <sup>3</sup>, Agnès Robin <sup>4</sup>, Jean Trap <sup>2</sup>, Philippe Hinsinger <sup>1</sup>

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
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Phosphorus is a key nutrient in crop production. A large fraction of soil P is however in mineral and organic forms, that are not directly and readily available for plants. Ecosystem engineers, like earthworms are deemed to modify the surrounding soil, both biologically and chemically. In the drilosphere, i.e. the soil volume that is influenced by earthworms, microbial activities have been often reported to be greater, resulting in enhanced enzymatic activities, thereby providing available nutrients for plants. Using rhizoboxes, we monitored acid phosphatase activity weekly over five weeks with endogeic earthworms (*Alolobophora chlorotica*) alone or with a chickpea / durum wheat intercrop. We employed a nitrocellulose membrane (16 x 30 cm), incubated for 3.5 hours against the soil, below the observation window, in order to sorb the acid phosphatase, and ultimately get a detailed map of its activity. The soil used had not received P-fertilizers for over 40 years, and was thus P-limiting for plants and microorganisms. The spatial distribution of hotspots of enzymatic activity was thereby measured at infra-millimetric scale, and assessed with geostatistics. Earthworms strongly enhanced the microbial activity in the bulk soil, without plant, with higher total areas of hotspots of acid phosphatase activity. In the presence of roots, earthworms seemed to concentrate the acid phosphatase activity in numerous little hotspots, which were generally co-located with earthworm casts.

## P102

### Fine mapping of soil phosphatase activity in the rhizosphere of intercropped chickpea and durum wheat

Camille Cros <sup>1</sup>, Esther Guillot <sup>1,2</sup>, Sarah Placella <sup>1</sup>, Josiane Abadie <sup>1</sup>, Gabrielle Daudin <sup>1</sup>, Claire Marsden <sup>3</sup>, Agnès Robin <sup>4</sup>, Jean Trap <sup>2</sup>, Philippe Hinsinger <sup>1</sup>

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Facilitation of phosphorus (P) has been reported to occur in cereal/legume intercropping systems, but the underlying processes are still unknown. Such positive interactions may occur as a result of an increase of P availability induced by the roots of one of the two intercropped species, for the benefit of the companion crop. Enhanced mineralization of organic P as a consequence of an increase in acid phosphatase activity in the rhizosphere of the intercropped species is one of the processes likely involved in such facilitation. Our hypothesis was: acid phosphatase activities are different when plants are growing alone or when they coexist. To test this hypothesis, we used rhizoboxes, in which we finely mapped and monitored acid phosphatase activity weekly over five weeks for three treatments: chickpea and durum wheat, grown alone or intercropped. We used a novel non-destructive method for spatialized measurements of acid phosphatase activity, based on nitrocellulose membranes (16 x 30 cm), which were incubated for 3.5 hours against the soil, in order to sorb the enzymes. The soil used had not received P-fertilizers over 40 years, and was thus P-limiting for plants and microorganisms. Chickpea was able to create more hotspots of acid phosphatase activity than durum wheat. Interestingly, the cereal-legume intercrop was exhibiting more hotspots than chickpea, and thus did not show an intermediate behaviour, as expected in the absence of interaction. This supports the occurrence of P facilitation. Our approach thus proved efficient to study the spatial distribution of enzymatic activities, at the microscale, in the rhizosphere.

P103

#### Using DGT (Diffusive Gradient in Thin films) as an indicator of phosphorus (P) availability in the Nordic countries

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Prediction of phosphorus (P) availability in soils is essential in order to secure efficient use of a limited natural resource in the future. Historically, a number of different extraction methods have been used to assess P availability. However, a growing awareness of the limitations associated to these methods has led to an increased attention to the DGT method (Diffusive Gradients in Thin films). Unlike common extractions, the DGT method is dynamic as the measurements are dependent on the re-supply of P from the solid phase in the soil – much like the actual uptake of P by plant roots. Much work has been done in Australia showing that the DGT method is superior at predicting P availability, and the technique is currently being implemented as a commercially available alternative to the common extractions. However, very little work has been done under temperate conditions for determining the possibility of using DGT as an advisory tool in agriculture outside Australia. Building on the experiences from Australia, we have conducted a number of both field and glasshouse trials in Denmark, Norway, Sweden, and Finland in order to compare official extraction techniques to the DGT method. Our initial results show that the DGT is superior for predicting plant available P compared to extraction techniques, but it has also showed important differences in P response between glasshouse experiments and field trials. These results will be presented at PSP5 together with an assessment of the possibilities of implementing the DGT as an official technique.

P104

#### The Hedley phosphorus fractions transformations of a soil incubated with different animal manures

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Animal manures, increasingly used as a source of nutrients to crops, enhance the potential for eutrophication. These residues vary in their organic and inorganic phosphorus (Po, Pi) composition depending, among others, on their origin. Thus, the improvement of manure recycling requires a better understanding of soil Pi and Po. Duck (Dk) and swine (Sw) manures were applied (85 mg P kg<sup>-1</sup>) to 2 lots of a sandy loam soil: S0 and S33, corresponding to a previous P enrichment with 0 and 33 mg P kg<sup>-1</sup> as KH<sub>2</sub>PO<sub>4</sub>. Soil manures mixtures and samples without added manure were incubated aerobically for 112 days. The Hedley P fractionation was performed at 7 and 112 incubation days. Pi was determined by cation/anion exchange resins (CAER), NaHCO<sub>3</sub>, NaOH, NaOHus and HCl, and Pt in the NaHCO<sub>3</sub>, NaOH, NaOHus fractions. Po was calculated as Pt-Pi. Results, expressed as % increases, reveal that manure P was mainly distributed by the CAER, NaHCO<sub>3</sub> and NaOH fractions (>50% in all the treatments). The previous P enrichment had no significant effect on percentage P distribution in all the fractions, except for the NaOHus-Pi, and S0 and S33 treatments followed similar trends. On the contrary, incubation time and manure type lead to significant differences for most Pi and Po fractions. In Dk manure, incubation time induced a redistribution of P increases, enhancing the CAER, NaHCO<sub>3</sub>-Pi and NaOH-Pi fractions, at the expense of decreases in the NaOH-Po. In Sw manure only the NaHCO<sub>3</sub>-Pi fraction presented a similar trend, with the NaOH-Pi fraction revealing no significant variations and CAER decreasing with time. Manure addition to soils promoted P dynamics, particularly of the labile Pi and moderately labile Po.

P105

### Phosphate recovery from iron phosphate rich sludge

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Ecological, geopolitical and economic concerns demand a more sustainable use of phosphate (P). During waste water treatment, huge amounts of P are eliminated by adding iron (Fe) to form insoluble iron phosphate compounds (FeP) that settle in the sludge. P recovery from FeP sludge, in a concentrated form, is not yet economical feasible. We envisage a biotechnological process, to recover P and Fe from FeP sludge. Our approach is based on natural P mobilising mechanisms well-known from soils and sediments. Key for P and Fe recovery is to understand the complex iron biogeochemistry that affects P binding and mobilization. Accordingly, our first experiments revealed distinct patterns of P release from different FeP upon sulphide addition. In the wastewater plant, FeP formed initially experience drastic changes in a variety of parameters (e.g. oxidizing and reducing conditions) that transform these compounds. The lack of information on these types of FeP makes it difficult to design experiments and develop thermodynamic models. Thus, the first step towards a recovery process is to characterize the FeP and Fe compounds that form at different stages of the wastewater treatment process. Methods will be developed to sample, separate and analyse sludge under anaerobic conditions. Characterisations of solid compounds include Mössbauer spectroscopy, SEM-EDX, XRD and TEM. Identifying FeP allows to find the stage where, thermodynamically and chemically, the most favourable iron phosphate compound exists for introducing a successful Fe and P recovery process. The developed methods bundle can be applied to other research fields as well. Up to now, despite intriguing differences, accurate characterizing of iron compounds and FeP has often been neglected.

P106

### Chemical nature of the residual phosphorus in Andisols

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Sequential fractionation has been widely used to study nature and dynamics of soil phosphorus (P), mainly using the 'Hedley' method. One of the main perceived limitations of this method is the assumption that unextractable fraction (residual fraction) is recalcitrant and composed of organic forms. The objective of this study was to quantify, to isolate and to characterize the residual fraction from three series of Andisols developed under grazed pasture. Our conceptual approach included the isolation of residual fraction after sequential Hedley fractionation from fertilized and unfertilized soils. We analyzed the chemical parameters and the contribution of organic P in the residual fraction. Moreover chemical composition of P was characterized by solution <sup>31</sup>P nuclear magnetic resonance spectroscopy (31P NMR). Our results showed that a significant proportion of the total P (45-63%) was present in the residual fraction, with most of it being extractable in NaOH-EDTA (61.2 – 114.0 %). Analysis of these extracts by solution <sup>31</sup>P NMR spectroscopy revealed that the residual fraction contained mainly inorganic forms of P (43.8 – 69.9%), with smaller amounts of inositol hexakisphosphate in four stereoisomeric forms (myo, neo, scyllo and d-chiro). Based on this first detailed analysis of residual P, we conclude that a large proportion of the total P in Andisols is found in the residual fraction, mainly as inorganic orthophosphates and as monoester orthophosphates.

P107

### Chemical nature of phosphorus in soils dominated by bracken and bluebell plants

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The role of plants in phosphorus (P) cycle in soil is of great importance and still not entirely understood.  $^{31}\text{P}$  NMR was used to assess phosphorus speciation, given as percentage of total P, in soil and plant samples collected in an area dominated by bracken (*Pteridium aquilinum*) and bluebells (*Hyacinthoides non-scripta*), in Snowdonia (UK). Samples were collected from April to September 2013 in order to cover the main lifecycle stages of these native plants. Total P in soil was between 1.5-2.2 g/kg, mainly sequestered as organic P (1.0-1.5 g/kg). Plant-available P ranged from 22 to 63 mg/kg.  $^{31}\text{P}$  NMR showed that soil samples contained between 60-78% of organic P; specifically monoesters (54-73%) comprising mainly phytate (28-42%) and Scyllo-inositol phosphate (13-16%); diesters (1-6%); phosphonates (1-2%); and phosphonolipid (1-4%); The inorganically-bound P (22-40%) occurred as orthophosphate (21-40%) and pyro/polyphosphate (0-5%). The total P concentration in soil increased after bluebell flowering with phytate as the main P-containing species. This is notable as phytate was not detected in the plant samples, with the exception of bluebell bulbs (up to 46%) which are contractile and bury themselves deeper into the soil during flowering. Phytate release from the bulb at this period is hypothesized. Also, the main P species found in this acidic soil (pH 3.98-4.67) support high levels of fungal activity because of phosphonates and pyrophosphate, and polyphosphate detected. P was bound predominantly in inorganic form in both bracken and bluebell samples (up to 85%). Also detected were monoesters (12-25%) and diesters (about 1%).

P108

### Effect of grassland management on amount and quality of organic P forms in a Cambisol

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Agricultural management such as installation of temporary grassland (ley period) influences soil organic matter as well as nutrient dynamics. Its impact on belowground coupling and decoupling biogeochemical cycling of elements and the resulting ecosystem services, such as C and nutrient storage is poorly known. In particular, we have little understanding about the changes of organic P compounds occurring belowground. Our previous work showed that SOM under ley grassland is characterized by contrasting molecular composition, when compared to soil under permanent cropping. Therefore, we hypothesized that grassland installation could also have an impact on the contribution and chemical composition of organic P. The aim of our study was to assess the response of P forms to installation of temporary grassland using a longterm monitoring site for environmental research in Western France. We studied the impact of 6 years grassland installation on organic P forms characterized by Headley fractionation. Our results showed that total P content of the 0-30 cm soil layer ranged between 226 and 231  $\mu\text{gP g}^{-1}$ , without difference between soils under permanent cropping and 6 years grassland. We observed a change in P forms between the two soils, as the ratio of organic P/inorganic P tended to be wider under 6 years grassland as compared to permanent cropping in agreement with our initial hypothesis. Headley fractionation showed increase of organic P of the humic fraction representing P associated with soil organic matter under grassland, while in both soils the residual, non-extractable fraction represented most P (36% of total). We conclude that further work is necessary to disentangle the relationship between organic P and organic C dynamics.

P109

### Soil properties affect the relationship between Olsen P and solution P

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The aim of this work was to characterize and rationalize the relationship between Olsen P and the concentration of P in the soil solution P –using the concentration of P in the 0.01 M CaCl<sub>2</sub> extract (1:10 soil:solution ratio; 30-min extraction) as a proxy for the latter. For that purpose, we collected and analysed 24 non-calcareous and 25 calcareous soils from the Mediterranean region of Spain. The relationship between Olsen P and CaCl<sub>2</sub>-P could be described by a Freundlich equation ( $y = ax^b$ ), whose b parameter adopted a mean value of  $0.53 \pm 0.16$ , and was slightly dependent on the contents of Fe oxides (in non-calcareous) and carbonates (in calcareous soils). The predicted Olsen P for a CaCl<sub>2</sub>-P critical value of 0.03 mg/L (typical for many field crops) ranged widely (2 – 39 mg/kg) and was positively influenced by the clay, carbonates and Fe oxides contents of the soils. Our results suggest that routinely determined soil properties can be combined with soil P test data to provide a better basis for the sustainable management of soil P.

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P110

### Long term effects of different soil management systems and winter cover crops on phosphorus forms in aggregates

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This study was carried out to evaluate the aggregation and the soil P forms in aggregates of an Oxisol under winter cover crops managed under no-tillage (NT) and conventional tillage system (CT) in a long term experiment. The experiment was established in 1986 with six winter treatments (blue lupine, hairy vetch, oat, radish, wheat and fallow) implanted in a Rhodic Hapludox in southern Brazil, managed under NT and CT. In September 2011, soil samples were collected in the 0-5 cm layer. In the dried soil samples were evaluated the total contents of C, N and P. The separation and the stability of the aggregates were determined by the modified method of Kemper & Chepil (1965), through wet sieving. After, the aggregate soil samples were subjected to the chemical fractionation of P proposed by Hedley. For all the winter treatments the soil managed under NT showed higher geometric mean diameter, weighted average diameter and index of aggregate stability, compared to CT. The larger aggregates showed higher content of labile organic P forms. The NT system favors the formation of larger aggregates and increases the contents of C, N and P in the soil. The rye and radish favor the formation of larger aggregates. The macroaggregates showed higher concentrations of labile organic P. The distribution of inorganic P forms in the aggregate distribution does not differ from the original soil.

P111

### Laboratory methods for estimating plant available P in manure and sludges

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Sewage sludge and manure contain considerable amount of phosphorus (P) that should be utilised in plant production. Plant available P is complicated to determine from biosolids that are prepared by various methodologies. In this study, we aimed to find a robust method for analysing plant available P from different organic fertilisers. We compared P availability from eight organic fertilisers prepared from sewage sludge and three organic fertilisers prepared from dairy manure. The methods used were water extraction 1:5 and 1:60, ammonium oxalate, ammonium citrate and ammonium acetate. We also made sequential Hedley fractionation. We compared extractions against P concentration determined by aqua regia and against plant P uptake in a pot experiment. Dairy manure P was easily soluble and 60-70% of P was extracted by water and ammonium acetate. Ammonium citrate and ammonium oxalate extracted 80-90% of manure P. Plant P uptake in pot experiment was high from added manure and thus supported the good availability of manure P. Iron precipitation was used for P removal in all tested sewage sludges and their Fe:P ratios were 0.2, 1.6 and 9.8. With the smallest Fe:P ratio, water and ammonium acetate extracted 10-15 % of P, whereas with higher Fe:P ratios their extractions was less than 5 % from total P. Ammonium citrate extracted efficiently 80-90% of total P. Hedley fractionation showed that most P (40-70%) in tested sludges was released by NaOH extraction. Plant availability of P from sewage sludges in the pot experiment was clearly better than expected by water extractions. In most sludges, P extracted by NaHCO<sub>3</sub> (Olsen-P) seemed to correspond well to P uptake.

P112

### Soil phosphorus forms in natural grassland affected by additions of soluble and natural phosphate

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Most natural grasslands in Southern Brazil grow in soils with low phosphorus (P) availability, but with high contents of total and organic P. The present study aimed to evaluate the changes in the soil P forms resulting from the application of phosphate sources over a growth cycle of the natural pastures. In three experiments under natural pasture P was applied in the form of Gafsa rock phosphate (GR), triple superphosphate (TS) and control, arranged in a randomized block with three replications. In the trials carried out under an Alfisol and an Entisol, were applied 100 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> in September 2010. In the natural pasture under an Ultisol, it was applied 180, 90, 100 and 100 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> at the years 1997, 1998, 2002 and 2010, respectively. Soil samples were collected (0-10 cm) throughout natural grasslands' growing season (0, 55, 116, 171 and 232 days after P application at Alfisol and Entisol and 0, 50, 83, 129, 159 and 186 days after in Ultisol). It was analyzed the levels of available P by anion exchange resin, P immobilized into the soil microbial biomass, P extracted by 0.1 mol L<sup>-1</sup> NaOH, total organic P and total P. The available P content increased rapidly with TS, but at the end of the evaluation period they were equivalent to the GR, which was similar to control. The application of P fertilizers in grasslands with low P availability increased the importance of the labile inorganic P forms to the plants, making them less dependent on the mineralization of organic P fractions. The organic fractions, including microbial P, are not good indicators of the bioavailability of P in natural pastures under Alfisols, Entisols and Ultisols in Southern Brazil.

P113

### Changes in soil P pools and phosphate enzyme activity following long term pig slurry application under no-tillage system

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Agricultural soils often receive annual applications of pig slurry (PS) for long periods in southern Brazil. The present study aimed to demonstrate the impact of the use of soil as a means of disposing PS on the forms of soil P accumulation and some biological and biochemical parameters of the soil P cycle after 19 successive applications of PS over 8 years under no-tillage (NT) system. The PS was applied at the rate of 0, 20, 40 and 80 m<sup>3</sup> ha<sup>-1</sup>. The results indicated that P added as PS preferentially accumulated in the inorganic fraction, with high saturation of the soil adsorption sites, showing high P availability and increasing the environmental risk of eutrophication of water bodies. The increase in the availability of inorganic P due to the application of PS provided higher crop yields and amount of organic waste deposited on the soil surface, leading to an increase in the levels of organic P, microbial P content and even in the acid phosphatase enzyme activity. This demonstrates that the addition of PS to the soil also affects biological and biochemical components in the soil P cycle. Moreover, it also shows that with the application of PS at higher rates, the soil turns into an environment that have greater P availability as well as high potential contribution of soil organic P forms to plant nutrition.

P114

### Impact of fertilizer integration with farmyard manure on crop yield and soil P pools in a long-term trial in Germany

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In this study a modified version of the Hedley sequential phosphorus fractionation procedure was used to determine the size and changes in soil P pools after 50 years of applying different schemes of plant nutrient management to a continuous cultivation trial (potato-rye-oats) in a sandy soil in Germany. The schemes of plant nutrient management correspond to depletion (no addition of P), maintenance (application of P either as mineral fertilizer or farmyard manure (FYM) alone) and increase (application of P fertilizer plus FYM). After 17 crop rotation variation in available P pool were related to changes in the readily available fraction (resin-P). Stability of the HCO<sub>3</sub> fraction indicates a rapid replacement of P consumed by the crops. Variations in moderately available Pool were related to changes in the Al/Fe-P attached to the organic matter (NaOH-Po) and the Ca-P (HCl-P) fractions. Application of FYM increases the Ca-P pool. Independently of the P fertilizer strategy used, no variation were observed in the non-available P pool (residual). In a total-P balance (input-output) analysis, the omission of P resulted in negative balance and reduction of the available P (P<sub>cal</sub>) while integration of FYM and NK fertilizers led to better total-P balances and maintained available P at the adequate level. Combined application of FYM and P resulted in high accumulation of total-P balance and increase the available P to high level. Application of FYM increased available P pool to adequate (C) level similar to the NPK fertilization (Balanced approach) without yield enhancement. Whereas, the integration of FYM with NK or NPK increased crop production and maintained/built up soil fertility.

P115

### Prediction of stock and fate of phosphorus forms according to soil classification

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Wallonia presents a high diversity of soils and the fate of P in the soil-plant systems can highly vary from one region to another. The fate of phosphorus depends upon its forms in the solid constituents of soils, which is seldom characterized. For example, total P determines the soil reserve of P but also the potential P content which can be lost to surface water by erosion but analysis of this parameter is time consuming and rarely performed. This study aims (i) to define functional groups of soils for a differentiated P management, (ii) to estimate total soil P by regression equations based on soil parameters, and (iii) to estimate the quality of these predictions. The study consists in a characterization of 12 parent materials in Wallonia, collected across different land uses. A classification of soils was defined by clustering analysis and 5 groups were defined according to P contents and forms. Using this information in regression improved the quality of predictions. The coefficients of determination vary from 0.83 to 0.99, in comparison to a coefficient of 0.77 for the global regression. Then, pedotransfer functions were validated with an independent external dataset of 55 soils. Estimation of the quality of the prediction of P content (mean error, standard deviation of prediction and root mean square error) was made with global and local regression models. In conclusion, using a soil classification allowed to improve P content assessment by specific regressions and to propose differentiated P management for each group of soils.

P116

### Predicting bioavailability of phosphorus in biochar: an assessment of analysis methods

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A new, human-driven flow of phosphorus has become dominant over natural cycling processes. In order to ensure food security for generations to come, phosphorus 'cycling' on an anthropogenic timescale must be realised. A sustainable solution to this phosphorus cycling imbalance lies in the utilisation of waste materials to both recover phosphate and return it to soil. Total phosphorus analyses do not indicate how readily phosphorus can be accessed by plants, so predicting the bioavailability of phosphorus captured in this process is important for the development of potential recycling materials. Biochar is a charcoal analogue produced at temperatures between 350 - 700°C under oxygen-limited conditions. It can be made from a wide variety of organic feedstock materials, including wastes, and is used for soil improvement. Many of the methods used to characterise biochars have been developed for the study of soils and plant tissues. Biochar is inherently different to these materials and so the prescribed methods may be unsuitable. Plant pot experiments have been used to assess the suitability of literature methods for the determination of short- (and long-) term bioavailability of phosphorus from a selection of biochars produced at the UK Biochar Research Centre. To date, total phosphorus concentration of these materials has been measured using the modified dry-ashing technique (analysed by ICP-OES) and XRF. Plant uptake and soil phosphorus concentrations will be assessed against these results and those of Hedley fractionation, 2% formic acid extraction and Mehlich 3 extraction to validate laboratory-based bioavailability assessments of biochar.

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### Phosphorus availability in an acidic Belgian Luvisol amended with biochar

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In recent years, application of biochar (pyrolyzed biomass produced under oxygen-limited conditions) to soil has been promoted as one of the tools that could be deployed to mitigate anthropogenically-induced increase in atmospheric CO<sub>2</sub> concentrations. Simultaneously, biochar has been considered a soil conditioner. However, the fate of phosphorus (P) in biochar amended soils is not fully elucidated so far. Here, using a 76-day incubation experiment, we investigated the effect of biochar from coffee husks (BC), woody material (BW) and miscanthus straws (BM) applied at two rates (1% and 3%; w/w) to an acidic Belgian Luvisol (pH 4.3) on the short-term availability of P. By increasing the available P concentration by 1.75 times relative to the control, the BW-3% treatment was by far the most efficient treatment to increase P availability. This was likely a result of the strong pH increase (+3.7 units) induced by this amendment since it is well known that P solubility highly increases with decreasing acidity. However, for the other treatments, the slight but significant pH increase (+0.3 to 0.9 units) could not explain the variation of available P concentrations. On the contrary, the P availability was significantly positively correlated to the rate of carbon mineralization. It was found that P was immobilized or released depending on the direction of the priming effect (negative or positive, respectively) induced by the biochar application. Our results suggest that short-term P availability in biochar amended soils is at least in part biologically controlled. Further investigations are nevertheless required to determine mechanisms and sources of priming effects.

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### Soil and plant phosphorus status and crop yields after 40 years of mineral P fertilization

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Phosphorus (P) fertilizers are an essential input for economically sustainable crop production but they may represent a significant problem for water quality if managed incorrectly. Appropriate evaluation of soil P availability is a prerequisite for ensuring the productivity and long-term sustainable management of agroecosystems. A long-term trial at Agroscope-Changins in Switzerland has been conducted in a clay Gleyic Cambisol (FAO classification system) for 40 years to analyze the effect of P fertilization (0 to 60 kg P ha<sup>-1</sup> yr<sup>-1</sup>) on soil and plant P status, and crop yields. Soil analyses (0-20 and 20-50 cm depth) included total, organic, inorganic and available-P assessed by a process-based approach and different chemical extractions. Shoot biomass and plant P concentration of wheat in 2011 and corn in 2012 were measured approximately weekly during the growing season (7 sampling dates) and the P nutrition index [PNI:  $100 \times (P_{\text{measured}}/PC)$ ] was determined using a critical P concentration (PC) based on whole plant shoot biomass [ $PC = \alpha \times (DM)\beta$ ]. After 40 years of cultivation, cumulative P (applied minus exported P) ranged from -567 to +1554 kg P ha<sup>-1</sup>, respectively for 0 and 60 kg P ha<sup>-1</sup> yr<sup>-1</sup>. As a consequence, P-fertilization significantly affected, except organic P, all other measured soil P parameters for the 0-20 cm soil layer. Thus, the total P ranged from 0.78 to 1.14 g P (kg soil)<sup>-1</sup> while the concentration of phosphate ions in soil solution varied from 0.06 to 0.75 mg P l<sup>-1</sup>, respectively for 0 and 60 kg P ha<sup>-1</sup> yr<sup>-1</sup>. Phosphorus fertilization also significantly increased wheat and corn grain P concentration but did not affect grain yield. The PNI ranged at harvest from 97 to 166 % for wheat and 98 to 128 % for corn, showing that P was not crop-limiting even after 40 years of cultivation without P fertilization. This study highlights the interest of plant-based diagnostic methods alongside soil analyses for the optimization of P fertilization practices for croplands in Switzerland.

P119

### Phosphorus recyclable in agrifood residues

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Phosphorus (P) utilisation mainly in agrifood systems create a massive flow of P from edaphic deposits to waterways, leading to eutrophication and depletion of P reserves. Thus, recycling of mobilised P is necessary. Low and unpredictable recovery of P from organic fertilisers is considered an obstacle. Here, the determining factors for the recovery of P from agrifood residues were identified. The plant and soil responses to manure and sewage sludge with various P capture and hygienisation treatments were quantified in a pot experiment. The major factors in fertilisers determining P recyclability were organic substances. In addition, other sorptive agents, pH and conductivity played a role. Organic substances, especially those ones formed by composting, seemed to prevent sorption of P to forms unavailable to plants. Again, anaerobic digestion decomposed organic substances thus obviously enhancing sorption of P especially from manure. Altogether, P was more plant-available in manure than in soluble NPK compounds. Also, when sewage P was captured biologically or with a moderate dose of iron, or in struvite, P was more plant-available than in NPK. Plant-availability of P in sewage sludge was increased by hygienisation through acid and oxidizer treatment. We conclude that the recyclability of P in appropriately treated organic residues is at higher level than in soluble inorganic fertilisers. Organic substances in fertilisers play an essential role in maintaining P recyclability.

P120

### The phytate enigma: just how stable is it?

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Most soil P exists in stable inorganic or organic forms that cannot be accessed directly by plants. P fertilizer is commonly used in agro-ecosystems to circumvent plant P deficiency but is often highly inefficient and in some cases environmentally damaging. Considerable effort has been made to understand the forms and transformations of P in the soil environment. It has long been believed that myo-inositol hexakisphosphate (i.e. phytate) is an important and even dominant component of organic P stores. Phytate is primarily derived from seeds and is thought to accumulate due to its high reactivity toward soil constituents. However, recent research has questioned this view. Here we present a review of the evolution of our current understanding of organic P cycling in terrestrial ecosystems. Over 500 research articles from the late 1800s to current times were reviewed and the defining periods of organic P research were identified that led to the current model. Throughout, there has been an underlying assumption that soil organic P is derived from organic P compounds found in living organisms (predominantly phytate, phospholipids and nucleic acids). Phytate has usually been identified as the most likely candidate for stable soil organic P. However, in recent times the recognition of humic P (i.e. organic P in high molecular weight polymers) as an alternative dominant form of organic P in Australian soils suggests a radically different P model that involves the slow accumulation of non-living organic P forms and possibly the transformation of inorganic to organic P forms through abiotic processes. This model is more consistent with established models of organic C and N cycling.

P121

### Improved detection of organic P forms in pasture subsoils

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Organic phosphorus (P) is often enriched in the surface layer of pasture soils and its composition is relatively easy to determine using solution <sup>31</sup>P NMR spectroscopy following extraction with NaOH-EDTA. On the other hand, it has proven more difficult to determine the composition of organic P in subsoil layers because their lower organic P contents result in poor spectral sensitivity. This study sought to improve spectral sensitivity by employing a narrower (1:4) soil to solution ratio to concentrate the extractable organic P forms prior to solution <sup>31</sup>P NMR spectroscopy; resulting spectra were compared to those of the more commonly used 1:10 extracts. Soil was collected from seven sites and two depths (0 – 4 cm and 4 – 10 cm) from pastures located across the high rainfall zone of eastern Australia. The NaOH-EDTA extracts were analyzed for inorganic P using colorimetry, and total Al, Fe, Mg, Mn and P using ICP-OES. On average, 69 % and 50 % of the organic P determined by ignition-H<sub>2</sub>SO<sub>4</sub> was recovered in NaOH-EDTA extracts at 1:10 and 1:4 soil to solution ratios, respectively. There was a strong linear relationship between concentrations in 1:10 and 1:4 extracts for both P and extractable metals. Solution <sup>31</sup>P NMR spectra of 1:4 extracts were considerably better in terms of spectral sensitivity than those of the 1:10 extracts. This study demonstrates that in soils with low organic P content a NaOH-EDTA extraction using a 1:4 soil to solution ratio facilitates better characterization using solution <sup>31</sup>P NMR spectroscopy.

P122

### Phosphate-coated nanoparticles as mobile P carriers in soil: fate study

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In tropical soils, low P bioavailability is a major plant nutritional problem. Responses to fertilizer application are low and transient due to strong fixation of P by Fe- and Al-oxyhydroxides. Recently, it was shown that supplementing a low-P nutrient solution with phosphate-coated Al-nanoparticles (NPs) enhances P bioavailability to plants. The aim of this study was to assess if phosphate-coated NPs can also be used in tropical soils as a carrier of P. The mobility of P-coated NPs in soil and the P mobilisation potential of the NPs was tested with three different soils: sand (control) and a P fixing Ferralsol and Andosol. Four different types of Fe- or Al-based NPs were added to the soils in two set-ups: either NPs loaded with <sup>33</sup>PO<sub>4</sub> added to non-labelled soil or non-labelled NPs added to <sup>33</sup>PO<sub>4</sub> labelled soil. After 7 days incubation with soil, dissolved <sup>33</sup>P was measured in soil extracts (0.01M CaCl<sub>2</sub>, S:L 1/1 water and S:L 1/10 water) with liquid scintillation counting and Fe & Al with ICP-MS. Finally, advanced characterisation of the different types of NPs used, was performed with Asymmetric Flow Field Flow Fractionation (AF4)-ICP-MS and with single particle ICP-MS. In the 1/10 water extracts, <sup>33</sup>P solubility increased by the addition of <sup>33</sup>PO<sub>4</sub>-labelled NPs but this was generally not found when the ionic strength of the extracts was higher. There was no significant increase in the mobilisation of <sup>33</sup>P by the NPs with <sup>33</sup>PO<sub>4</sub> labelled soils. Other formulations of NPs are required with higher colloidal stability at the prevailing ionic strength in soils to mobilise PO<sub>4</sub> with NPs.

P123

### Phosphorus cycling in forest ecosystems as revealed by the isotopic composition of oxygen in phosphate

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The current view on the P cycle in forest ecosystems relies mostly on measurements and correlations of pools, and to a lower extent on measurement of fluxes. We have no direct insight into the processes P molecules go through at the ecosystem level, and into the relative importance of organic and mineral pools in sustaining P nutrition of trees. The analysis of oxygen isotopes associated to P ( $^{18}\text{O}$ ) is expected to bring this type of information. The German Priority Program SPP 1685 aims to test the overall hypothesis that the P-depletion of soils drives forest ecosystems from P acquiring systems (efficient mobilization of P from the mineral phase) to P recycling systems (highly efficient cycling of P). Our contribution to this project will consist in studying the relative importance of biological and geochemical processes in controlling the P cycle in temperate beech forest ecosystems in Germany. We will follow the fate of phosphate from litter fall to the uptake of P by forest plants via P release by decomposition of organic matter, and after release from P-containing minerals. We will use a multi-isotope approach (O in water, P and O in phosphate, C in organic matter) at natural abundance or under enriched conditions to address our research question. We will rely on measurements in the established forest ecosystems and on laboratory incubations of the organic layer or the mineral soil. After the first year, we expect to obtain a complete characterization of soil and plant P pools, including their  $^{18}\text{O}$  signature, in three study sites along a gradient of decreasing soil P availability.

P124

### Total and organic phosphorus as a basis of the regression model for mineral phosphorus prediction

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Regression models comparing organic, total phosphorus in the soil and AL extracted phosphorus are created to enable the prediction of mineral phosphorus without the soil analysis, based only on analytical data of one or more different soil properties. Samples were collected in the area of the eastern Croatian, and analysis were carried out on 94 samples and included the pH, organic matter content, organic and total phosphorus. Criteria for selection of samples were  $\text{pH}_{\text{KCl}}$  and organic matter content and all the samples were divided into five categories according to soil acidity and two categories according to organic matter content. The results of total and organic phosphorus in the soil obtained by analysis were used to predict the results for AL method. Models include the basic equation for calculating the concentration of the extracted phosphorus by particular method (AL) based on the amount of organic and total phosphorus regardless of other soil properties. Regression formula were described by equation  $Y = \text{intercept} + \text{OX1} + \text{TX2}$ . Correlation coefficient between AL phosphorus, organic and total phosphorus was  $r = 0.43^*$  ( $n = 94$ ), but dividing the samples into five acidity categories, correlation coefficient increased to  $r = 0.89^{**}$ . Likewise, the developed regression model was very responsive to the expansion of the input variables and inclusion of information about the organic matter content in the soil what increased model accuracy for 50 %. Although the positive relationship between organic phosphorus and organic matter in the soil has not been established, their interaction contributed to the improvement of the developed model.

P125

### Phosphorus fraction characteristics after P fertilization under aerated and flooded Ferralosols and Argosols

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Soil phosphorus (P) fractions and phosphatase activities are important for P nutrition in cultivated soils. Flooding plays important role in changing soil pH, Eh, which could influence soil phosphorus (P) status in soil. The lab study measured soil pH, Eh, sequential soil P fractions, activities of  $\beta$ -D-glucosidase, phosphomonoesterase and phosphodiesterase after 180 days of inorganic P (Pi) fertilizer addition under aerated and flooded Ferralosols and Argosols. Results showed that Pi addition had no effects on aerated soil pH but could significantly increase soil pH while decreased soil Eh under flooded conditions. Added Pi had no influence on activities of  $\beta$ -D-glucosidase and phosphodiesterase but could inhibit phosphomonoesterase activities significantly. Added Pi to Ferralosols mainly turned to be labile Po, then moderately labile P fractions and those effects could be weakened under flooding treatments; For Argosols, added Pi mainly turned to be recalcitrant P fractions and this trend could be strengthened by flooding with lower rate of Pi addition. The principal components analysis showed that Eh positively correlated with the three enzyme activities while soil pH negatively correlated with phosphatase activities; Results also showed that background concentration of total P influence concentrations of resin-P, moderately labile P and recalcitrant P fractions. We conclude that Pi added to Ferralosols are more reactive than that in Argosols; Flooding influence soil pH, Eh, soil enzyme activities and P fractions significantly, which reacts more on Argosols than Ferralosols.

P126

### Effects of soil Mg content on the adsorption isotherm constants and P solubility in agricultural soils of Wallonia

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The reserves of total P (P<sub>tot</sub>) in Walloon soils are high, 904 mgP/kg on average in the topsoil. It resulted from continuous and excessive fertilization and manure applications. However, only 9% of P<sub>tot</sub> on average (71.6 mgP/kg) is available in Walloon topsoil due to high soil P sorption capacity. Thus, as P-fertilizers are a finite resource, a better understanding of P behavior in soils is needed to adapt practices of fertilization. Recently, one study has shown that during the last decade, Mg content in soils of Wallonia increased between 10 to 35%. Hence, we investigate whether Mg content could influence the availability of P in agricultural soils. Results of a geochemical modelization with PHREEQC indicated that the hypothesis was justified. In that context, we studied soil-solution equilibrium of P in different agricultural soils of Wallonia (N=5) by batch experiments in order to define the influence of Mg content on the P adsorption isotherm. Our results show that the addition of Mg modifies the P adsorption isotherm in each soil studied. The more Mg added, the less P in soil solution. This suggests that addition of Mg can decrease the P availability in studied soils. However, further studies are needed to understand mechanisms and processes involved in the decrease of P availability and the impact of Mg fertilization on soil P availability in agricultural soils.

P127

### Pasture degradation affects forms and distribution of phosphorus in aggregates of tropical soils

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Degradation of tropical pastures on highly weathered soils is related to soil structure decline and loss of organic matter, which could also affect soil phosphorus (P) status. Our aim was to examine the effect of pasture degradation on contents and forms of P contained in aggregates and macroaggregate fractions (structural components). We conducted a study on nine farms in a deforested part of the Colombian Amazonia, each farm with degraded (DEG) and productive (PROD) *Brachiaria* spp. pastures. Topsoil samples were physically separated into aggregate size classes, and macroaggregates further separated into occluded fractions. Soils and structural components were analyzed for concentrations of total P, available P, NaOH-EDTA extractable organic and inorganic P, and enzyme labile P in NaOH-EDTA extracts. Degraded pasture soils had significantly fewer large macroaggregates (LM) and more microaggregates, both with significantly lower organic P (Porg) concentrations than the corresponding aggregates of PROD soils. At the same time total and extractable inorganic P did not differ between pasture types, indicating a shift from Porg to unextractable P upon degradation. Soils under PROD contained about 40% more Porg than DEG soils, mainly in the LM fraction. In bulk soil and most structural components, around 60% of Porg was enzyme labile. Lower contents of all enzyme labile forms as well as enzyme stable P in DEG than PROD indicate a reduction of all forms during pasture degradation and show the importance of physical protection of Porg. The LM fraction and in particular their occluded microaggregates were identified as important sites of Porg storage.

P128

### The effect of Acacias introduction in Eucalyptus plantations on phosphorus availability in Congo and Brazil

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Available phosphorus (P) is recognized as a major constraint for tropical forest productivity because in these areas deeply weathered soils dominate. This problem is critical since world P reserves are finite and thus induce an increase in fertilizer cost. In this context, improving exploration and exploitation of P soil resources by plants is essential to ensure forest sustainability. Introducing leguminous species in forest ecosystem or in cropping system is an ecological way to improve N nutrition, but we also hypothesized an improvement in P nutrition. In order to verify this hypothesis, P availability was determined in monospecific *Eucalyptus* and *Acacia mangium* plantations and in mixed-species stands at 1 site in Congo and 4 sites in Brazil. Exchangeable anionic membranes were incubated in situ in order to trap available P plus nitrate during one month at each site. In addition, results of P availability using incubation of membrane in situ were compared with available P extracted using membrane in laboratory. The behavior of P availability showed different pattern depending on the site. At some sites, soil P availability decreased by half under *Acacia* compared to soil under *Eucalyptus* while at other sites no statistical differences were found. Several factors may explain the decrease in soil P availability after introduction of *Acacia* (e.g. P requirement linked to symbiotic N fixation, P stocks in the biomass of the whole stands). The comparison of incubations in situ and P extractions in laboratory was discussed as incubation in situ integrated biological process (in particular root uptake and rhizosphere activity) contrary to classical extractions in laboratory.

P129

### Organically bound and microbial phosphorus in the topsoil of 20 European beech (*Fagus sylvatica* L.) stands in Germany

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Turnover of soil organic matter plays an important role in phosphorus (P) nutrition of forest ecosystems. Previous studies have shown that the litter layer is crucial for P nutrition of forest stands with moder humus form. In contrast, the mineral soil seemed to be more important than the litter layer for P nutrition of forest stands with mull humus form. From these findings the hypothesis was deduced that due to effective mixing of litter layer material into the mineral soil beech stands with mull humus form contain more organically bound P and more microbial P in the mineral soil than beech stands with moder humus form. Organically bound P and microbial P were determined on 20 beech plots in 0-5, 5-10, 10-30 and 30-50 cm soil depth. Plots with mull humus form did not differ in their contents and pools of organically bound P in all soil depths from plots with moder humus form. However, pools of microbial P in 0-5 and 5-10 cm soil depth were significantly larger on plots with mull humus form than on plots with moder humus form. In deeper soil layers (10-50 cm) microbial P did not differ between the two groups of plots. On all plots microbial P pools were high compared to annual litter fall P. The hypothesis was only partly confirmed by the results. Despite the missing intrusion of litter material into the mineral soil on plots with moder humus form, pools of organically bound P were similar on plots with mull and moder humus form. Probably, dissolved organic P was displaced from the litter layer to the mineral soil on plots with moder humus form. Though, biological activity in topsoils of plots with mull humus form was reflected in high microbial biomass P pools. Assumed that the turnover of microbial biomass is fast and microbial P is at least partially plant available, the large microbial P pools could play an important role in forest P nutrition.

P130

### High plant availability of P and low availability of Cd in ashes from combustion of straw and wood

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Combustion of biomass for energy production is expected to increase, and in Denmark large power plants are planned using dust combustion of biomass. Recycling of phosphorus (P) in the ash therefore becomes increasingly important, but the availability of P in bioashes has been reported low and variable. Furthermore the cadmium (Cd) content often limits recycling of ash on agricultural land. In this study we evaluate the availability of P and Cd in ashes from dust combustion of wood and straw pellets and from traditional combustion of wood chips. Increasing rates of four ashes and triple superphosphate (TSP) were applied to a P-depleted sandy soil in pots and to framed field plots on a loamy sand soil. The uptake of P and Cd was measured in spring barley, and in pots also in Ital. ryegrass. In the field plots the effects on available P in soil were evaluated as changes in resin and bicarbonate extractable P after barley harvest. Plant P uptake was proportional with total P application irrespective of P source indicating that P in ashes was approximately as available as P in TSP. The net increases in resin and bicarbonate extractable soil P were independent of the P source and were on average respectively 18% and 13% of the P input. Cd uptake in plants increased linearly with TSP application rate while Cd uptake remained low after application of wood-derived ashes despite of very high Cd application rates. We conclude that the ashes, independent of combustion type, showed high P and low Cd availability to plants.

P131

### Can deficit irrigation techniques be used to enhance phosphorus and water use efficiency and benefit crop yields?

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Soil drying and rewetting (DRW) affects the forms and availability of phosphorus (P). Water soluble P has been reported to increase 1.8- to 19-fold after air-drying with the majority of the increase (56-100%) attributable to organic P. Similarly, in two contrasting soil types DRW increased concentrations of total P and reactive P in leachate, likely due to enhanced P mineralisation and physiochemical processes causing detachment of soil colloids, with faster rewetting rates related to higher concentrations of P. How these dynamics affect crop P acquisition and growth remains unclear. Sensing soil drying stimulates root-to-shoot signalling of the phytohormone abscisic acid (ABA) which causes partial stomatal closure and reduces water use (transpiration). Water deficit caused a greater accumulation of ABA in plants subjected to P deficiency which increased stomatal closure, suggesting increased sensitivity. This effect was blocked by kinetin, suggesting an interactive effect of ABA and cytokinins. Further studies are needed to understand interactions between P availability and phytohormone signalling during DRW. The research aims to guide deficit irrigation techniques to increase P and water use efficiencies and crop yields. Presented here are the results of two experiments, the first investigating whether DRW increases phosphate uptake by plants and affects the compositions of xylem sap (ions via ICP-MS and ABA via radioimmunoassay). The second reports the soil water content and matric potential thresholds at which DRW increases P availability. Future experiments will investigate how different sources of P (organic and inorganic treatments) respond to DRW regimes.

P132

### Phosphorus for cereal production; comparing the uptake efficiency of different fertiliser application methods

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Phosphorus (P) is essential for all life and in recent decades there are increasing concerns around the security of future P supplies, increasing P fertiliser prices and P loss to the environment. Phosphorus fertiliser is required to maintain or improve the P status and productivity of many soils, however; sustainable approaches to P management are needed. Different soil types have varying capacity to attenuate P and consideration should be given to how P fertilisers are applied to soils. In cropping systems the placement of P close to the seed can offer significant advantages over more common place broadcast application methods. The aim of this study was to investigate the effects of P fertiliser application method on soil P availability, crop use efficiency of P, grain yield and quality across a range of soil types. Over the 2010 to 2014 cropping seasons, P fertiliser experiments using winter wheat and spring barley were conducted in Ireland. Phosphorus fertiliser application rates from 0 – 60 kg/ha in 10 kg increments as triple super-P were applied using 3 fertiliser application methods (surface broadcast; incorporation into the seedbed; and combine drilled with seed). Grain and straw yield response, P concentration, P uptake, P use efficiency and grain quality were determined. The total P, bio-available P and degree of P saturation in the soil was also determined. Spring barley grain yield was more responsive to P fertiliser additions than winter wheat. In general the apparent P use efficiency of surface broadcast P fertiliser was low (<20%). Placing P fertilisers close to or with the seed on low P fertility soils was the most efficient method of applying fertiliser P. There is potential to significantly increase P use efficiency in cereals production systems using more targeted P application methods.

P133

### Phosphorus fractions in a clay loam soil under natural and long-term (44 Years) managed agricultural eco-systems

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Long-term agricultural practices affect soil P status, and thus P bio-availability and vulnerability of losses. Previous studies mostly focused on short-term effects and limited to assessments within agricultural ecosystems. We evaluated the effects of long-term (44 years) cropping systems (continuous corn: CC, continuous bluegrass: CB, and crop rotation of corn-oats-alfalfa-alfalfa: CR) and fertilization on changes in soil P status as compared to that in natural forest ecosystem in eastern Canada. Soil P was fractionated into various inorganic (Pi) and organic (Po) forms in association with plant availability using a modified Hedley's sequential extraction procedure. Under natural forest, soil P was predominated by stable P, followed by moderately stable Pi (HCl-Pi) and moderately labile Po (NaOH-Po). Consistent cropping without fertilization reduced all forms of soil P relative to natural forest; i.e. labile P (NaHCO<sub>3</sub>-P) and moderately labile P were reduced by 60-76% and moderately stable and stable P were reduced by 30-39%. Consistent cropping with fertilization reduced all forms of Po, in the order of CC>CR>>CB, but increased labile and moderately Pi in the order of CB>>CC>CR. Moderately stable Pi increased with fertilization in CC and decreased in RC and CB. All forms of soil P are in a dynamic system, of which the transformation pathways may depend on the balance of soil P input vs. output. Cultivation of virgin soil causes losses of soil Po, which may not be remedied by fertilization.

P134

### Interactions between magnesium and phosphorus in silty soils of Wallonia and their uptake by ryegrass

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The reserves of total phosphorus (P) in Walloon soils are relatively high but only a small part of it is available for plant nutrition according to soil chemical tests. Furthermore, the general trend of evolution of available P in crop soils is a continuous decrease in Wallonia. Besides this, it has been noticed that available magnesium (Mg) content was increasing during the last 15 years. Risks of P immobilization are suspected. Indeed, geochemical modeling of soil solution has shown that an increase of Mg content could decrease the P content. In that context, the objective of our study is to evaluate whether the Mg content can affect P uptake by plants. The experimental protocol is based on a micro-culture in pots. The test-plant used is the ryegrass (*Lolium perenne* L.). The fertilizers selected are struvite and superphosphate. Struvite is used as the Mg source. It is a Mg-ammonium phosphate complex with high nutrient content precipitated from human urine. Superphosphate fertilizer is used as reference for comparison. Studied soil is a silty soil from the southern Belgium with a long agricultural past. Fertilizers were added to soil at following rates: 20, 40 and 80 kg P/ha. Plants were first grown in pure sand without addition of P. Ten days after the plant emergence, roots were brought into contact with the studied soil-fertilizer mixes during fifteen more days. At the end of the experimentation, analyses were performed on plant material (biomass, P content) and on soil (total P and Mg, available P and Mg, pH, phosphatases activity). We hypothesize to observe a decrease of the P availability and therefore, of the P uptake by plants, with an increase of Mg content.

P135

### Evaluation of the P availability of Gafsa phosphate rock in several soils using $^{32}\text{P}$ isotopic dilution method

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This study aims to test the effectiveness of Gafsa phosphate rock (GPR) use as phosphate fertilizer compared to water soluble fertilizer as triple super phosphate (TSP). An experiment was carried out to evaluate different soil chemical properties that control P solubility and mobility from GPR using the  $^{32}\text{P}$  isotopic kinetic method. Seven soils with different soil pH were mixed with GPR and TSP at three rates (0, 500, 2000 mg P/kg) corresponding to localized P fertilization usually applied in orchard. The treatments were replicated three times and incubated for one month in moist conditions. Changes in the amount of isotopically exchangeable P (E) and the amount of P transferred from the solid constituents to the soil solution after different periods (Pr) as well as changes in pH, Ca and P concentrations in soils suspensions were determined. The relative efficiency of the GPR to water soluble fertilizer in the seven soils was with an increase in soil acidity. For each soil, the relative efficiency varied differently for the two P applications rates of GPR. In fact, it was noticed that in two soils with different pH (5.5 and 6.2), the relative efficiency decreased with the high P rate of GPR. This result may be explained by a liming effect of GPR resulting in a high increase of pH and the calcium concentration in these soils limits the GPR dissolution and reduces mobility of soluble P in soil solution. These results contrast with the one obtained for a soil with pH (6.5) because of its high pH buffering capacity. Our results demonstrate the importance of considering the soil properties to evaluate the GPR efficiency and manage GPR fertilization.

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### Correlation of phosphorus leaf concentration with yield components and concentration of nutrients in leaves of pistachio

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To evaluate phosphorus concentration in the leaves of pistachio trees, and its relation with some yield components and leaf nutrients concentration, 48 uniform size trees were selected. The experiments were performed in 2010 (ON-year) and 2011 (OFF-year) in Rafsanjan region, Iran. Pistachios nuts were harvested during the commercial harvest period. Then, fruit set, nut weight (g), splitting rate (%) and blankness (%) were measured. However, for nutrient status evaluation, leaf samples were collected in mid-July from each tree, and some leaf nutrient concentrations were measured. Results indicated a positive correlation between nut weight and phosphorus leaf concentration while there was no significant correlation between phosphorus leaf concentration with fruit set, splitting rate and blankness. In this study, concentration of phosphorus in the leaves was greater by 25 % in ON than OFF-year. A negative correlation was obtained between the phosphorus leaf concentrations with zinc ( $r=-0.86$ ) and copper ( $r=-0.87$ ) concentration in leave of pistachio trees. The results of present study indicated that some yield components and leaf nutrient concentrations were influenced by phosphorus nutrition status of pistachio trees.

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#### Abiotic processes controlling the effects of citrate on phosphorus availability: study of a fertilized ferralsol

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Organic acids exuded by roots, especially citrate, can increase P availability in soils and thus facilitate P acquisition by plants. However, controlling processes are poorly known. In order to improve this understanding, we added different citrate concentrations (2, 10, 20, 50, 100, 250 and 500  $\mu$ M as trisodium citrate) to water extractions performed with a fertilized ferrallitic soil. We performed the experiments at T = 2°C in order to minimize the effects of biological activity. We measured dissolved inorganic P concentrations (available P) along with the concentrations of major solutes (Na, K, Ca, Al, Fe, Si and organic carbon) and pH. We also modeled aqueous speciation and determined mineral saturation indexes. Results showed that available P gradually increases with citrate concentration. Similar trends were observed with dissolved Al, Fe, Si, organic C, and Na. In contrast, pH decreased compared to control (i.e. 0 citrate) with addition of low citrate concentrations while it became more alkaline at higher citrate concentration. Dissolved K, Mg and Ca did not show monotonous variations as well. Dissolved K gradually decreased up to 250  $\mu$ M of citrate. Dissolved Ca slightly increased with low citrate concentrations, vanished at intermediate concentrations (50 and 100  $\mu$ M) and finally increased markedly at high citrate concentrations. Dissolved Mg exhibited intermediate variations. Multiple linear regression analysis showed that variations of P availability were significantly correlated with dissolved Si. This result along with these of the modeling suggested that available P was mainly controlled by dissolution of P-sorbing minerals, especially kaolinite.

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#### Factors controlling phytases produced by the fungi *Aspergillus niger* and *Debaryomyces castellii* in rhizosphere soils

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To face the phosphorus (P) scarcity, soil phytate degradation by exogenous phytase excreted by plants or through the soil trophic web is promising. However, numerous bottleneck factors limit phytase activity in roots vicinity. We aimed to prioritize these factors in order to propose best scenarios for efficient phytate degradation. In our study, we measured the orthophosphates ions (Pi) released by fungal phytase obtained from *Aspergillus niger* and *Debaryomyces castellii* in two simplified rhizosphere soils, with anionic resin membranes used as plant surrogate. A full-factorial design of experiments, with two levels per factor was carried out to rank effects of soil (Cambisol or Luvisol), enzyme, oxalate, glycine and pH. Each factor, replicated three times was chosen as representative of soil geochemistry modifications induced by root activities. For all experiments, the activity of the phytases was measured quantifying Pi adsorbed on anionic membranes. In Luvisol, the pH:oxalate interaction ranked first before oxalate and pH whereas in Cambisol, the pH ranked first before interaction oxalate:pH and oxalate. In both soils, a significant effect (p-value = 0.02) of the pH:oxalate interaction on the Pi liberation was noticed but any effect of the nature of enzymes and soil type. The Pi values were very important for initial pH (=7) and high oxalate concentration (15.3 mM). We concluded the geochemical effect of oxalate and pH on Pi liberation prevailed the biochemical effect on phytases. Thus, despite inherent complexity of soils, studies can focus on some few bottleneck factors as pH or oxalate liberation to optimize phytase activities.

P139

### Comparative uptake of phosphorus from organic and inorganic forms in Australian Mediterranean soils

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The phosphorus (P) status of Western Australian cropping soils often exceeds the critical values (CV) for optimal growth. Stored organic and inorganic P could supply several crops if fertilisers were withheld. Our aim was to determine the contribution of these pools of P to plant uptake in soils that exceed their CV. The light brown sandy loam (Birtle), brown sandy loam (Butcher), and dark brown sandy loam (McLeay) had P buffering indices of 29, 44 and 219 and sodium bicarbonate extractable P (Colwell P) concentrations of 65, 34 and 43 mg P/kg soil, respectively. These soils were placed in pots of ~5 kg soil and sown to wheat. Wheat was harvested at late anthesis in spring to measure P concentrations in shoot and root tissues. Rhodes grass followed by ryegrass were then grown in summer and winter respectively and harvested to measure P uptake. The plants were given basal nutrients except P. Soils were sampled from all pots after the final shoot and root harvest and were analysed separately. Soil P was extracted with NaOH-EDTA and analysed for total and inorganic P and organic P determined by difference. The total plant uptake of P expressed as a soil concentration was 62 in Birtle, 41 in Butcher and 61 mg P/kg soil in McLeay soils. The decrease in Colwell P was less than total plant uptake. It was 42 mg P/kg in Birtle, 14 in Butcher and 19 in McLeay soils. NaOH-EDTA extractable organic P contributed little to plant uptake. At final harvest, it increased slightly by 4.8 to 11 mg P/kg soil presumably due to residual roots left in soil. Plant uptake of stored P was from the inorganic pools, which made up between 71 to 91% of the initial total NaOH-EDTA extractable P.

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### Effects of P on *Vigna unguiculata* cv. 305 and *Stylosanthes hamata* cv. Verano in rubber plantation

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In order to assess the effects of P and N additions on nodulation and N<sub>2</sub> fixation *Vigna unguiculata* cv. 305 (cowpea) and *Stylosanthes hamata* cv. Verano were grown in fertilized and unfertilized associations with *hevea brasiliensis* in NE Thailand. One year later, *V. unguiculata* cv. 305 and *S. hamata* cv. Verano were studied for their adaptation to low P on the top of the toposequence. The results show that P addition not only increased above-ground biomass, but also nodulation and N<sub>2</sub> fixation of *V. unguiculata* and *S. hamata*. With P applications, N<sub>2</sub> fixation represented between 30% and 58% of total plant N. Without fertilization, the nodule dry weight for *V. unguiculata* increased strongly with the soil organic carbon, and was positively correlated with plant N content. We noticed that an increase of 1 mg in nodule dry weight produced an increase in total plant N of 1.29 mg plant<sup>-1</sup> in the control and of 1.48 mg plant<sup>-1</sup> in the PK treatment for *V. unguiculata* at flowering stage, and of 3.18 mg plant<sup>-1</sup> for *S. hamata* at 183 days after sowing. It is concluded that the correlation between total plant N and nodule dry weight seems thus a promising method to estimate N fixation in further researches on the effectiveness of N fixation in nodulated plants.

P141

### Soil and plant mechanisms affecting transformations of phosphorus from organic and inorganic amendments

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In Organically-managed farming systems animal manures and composts provide both plant available nutrients and organic matter to soils, resulting in benefits to the soil's biological, physical, and chemical properties. These amendments can directly affect plant availability of soil P by altering both the amounts and forms of P present. The study's objectives are to; (1) quantify the forms of P in turkey litter composts (TLC), (2) compare the kinetics of P transformation in soils amended with either TLC or with a synthetic P fertilizer, and (3) investigate the effects of amendments, plants, and time on soil P chemistry. The study found that labile organic P and calcium phosphates (Ca-Ps) are the major forms of P in TLC. An 18-week incubation study showed that synthetic P fertilizer increased the proportion of labile inorganic P, which then was gradually transformed into more stable forms. The TLC increased the proportions of labile inorganic and organic P, and Ca-Ps in soil. A 16-week greenhouse study with ryegrass showed significant plant growth in TLC-amended soil compared to synthetic fertilizer-amended soil. Ryegrass decreased the amounts of inorganic and organic labile P in fertilizer- and TLC-amended soils, where the latter was most likely caused by mineralization of organic P to plant available P. The contents of Ca-Ps also decreased in TLC-amended soil over time with ryegrass. Contributions of both organic P and Ca-Ps to plant available P need to be assessed for effective P management in organically amended soils.

P142

### Clay minerals and phosphate sorption in soils

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Solubilization of soil phosphate accumulated during the last decades in fertilized soils can be profitably used to decrease phosphate (PO<sub>4</sub>) fertilization while maintaining crop production. In this respect, it is important to clearly identify and rank the different soil constituents regarding their importance for the control of PO<sub>4</sub> sorption. However, the relative importance of clay minerals versus Fe oxide-hydroxides is still controversial. Indeed, some researchers only considered the influence of Fe oxide-hydroxides, while others also included clay minerals in their PO<sub>4</sub> sorption models. Through this communication our main objective is to present a set of evidences issued from the literature and also based on original data which support the significant influence of clay minerals on PO<sub>4</sub> sorption in soils. The first evidence is based on the adsorption capacity of minerals and on the mineralogical composition of soils. Second, we showed that the adsorption properties of clay minerals versus pH do not resemble to that of Fe oxide-hydroxides, thus precluding the consideration of Fe oxide-hydroxides as a surrogate for clay minerals. Last, preliminary results of synchrotron-based X-ray absorption spectroscopy at the phosphorus K-edge showed that PO<sub>4</sub> binding to clay minerals substantially involves structural Al-O sites and not only Fe-O sites. To conclude, both Fe oxide-hydroxides and clay minerals should be considered in PO<sub>4</sub> retention modelling in soils as they considerably influence sorption processes.

P143

#### Spatial variability of phosphorus concentration in leaves of pistachio trees in Rafsanjan area of Iran

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The aim of this study was to evaluate spatial variability of phosphorus concentration (August sample) in leaves of pistachio trees in Rafsanjan area, Iran. The experiment data after checking the status of their normality, they were analyzed geostatistically. The results showed that the fit model for phosphorus concentration of leaf (percentage) was Gaussian model. The nugget 0.101, range 2442 m and sill 0.39 were obtained. Ratio of nugget to sill is 0.25 that shows a strong spatial relation between the data. The ordinary kriging map of phosphorus concentration showed that most concentration of leaf phosphorus is in northern and central parts of studied area (about 21%) that the amount of it is nearly twice of southern and eastern parts. It seems that due to of placing the most of pistachio gardens in these regions and excessive use of phosphorus fertilizers, the amount of phosphorus in these regions was great. Also, according to this subject the critical limit of phosphorus in leaves of pistachio is % 0.14 and the optimal amount of it is 0.14 to 0.17, therefore, we can say that phosphorus concentration in leaves of trees in the studied region was great (% 0.19). The results of Pearson's correlation coefficient test showed that there was a positive correlation between the phosphorus concentration in pistachio leaf with the concentration of its in soil, but there was a significantly negative correlation between the phosphorus concentration in leaves with the zinc concentration.

P144

#### Phosphorus status in agricultural soils of Fribourg canton, Switzerland

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Phosphorus (P) is the second essential nutrient for plant growth but can become an ecological and economical concern in case of over-fertilization. Soil P dynamic is influenced by many parameters like soil physical-chemical properties and farming practices. A better understanding of the factors controlling its distribution is required to achieve best management of P in cropping systems. In Switzerland, the FRIBO network was launched in 1987 and consists of 250 sites covering a wide diversity of soils and three different land uses (cropland, grassland and mountain pasture) across the Fribourg canton. A spatial investigation of the different P forms (total, organic and available) for the FRIBO network led to the following main conclusions: (i) The P status in agricultural soils was significantly different among the three land uses encountered, with the highest mean values of available P found in croplands, from 2.12 (CO<sub>2</sub> saturated water extraction) to 81.3 mg kg<sup>-1</sup> (acetate ammonium + EDTA extraction); whereas total P was more abundant in permanent grasslands (1186 mg kg<sup>-1</sup>), followed by mountain pastures (1039 mg kg<sup>-1</sup>) and croplands (935 mg kg<sup>-1</sup>). (ii) Environmental variables such as altitude, slope, wetness index or plan curvature, derived from digital elevation model only explained a small part of the spatial variation of the different P forms (20 to 25%). Thus, the geostatistical analyses revealed that land use plays a significant role in soil P distribution. Improved predictions of the spatial distribution of P-related forms at landscape scales are needed and would require additional data points and variables such as parent material, soil types and terrain attributes.

P145

### Compost from anaerobic digestate: available phosphorus in soil and ryegrass utilization efficiency

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A compost (CSM) from the solid fraction of anaerobically digested maize, and one (CDB) from the residue of the dry-batch fermentation of household waste were compared in a soil incubation at 30 mg P kg<sup>-1</sup>, to test their capacity to supply available phosphorus (Olsen-P). A municipal solid waste compost (MSWC) and an inorganic P source [Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>·H<sub>2</sub>O] (P-chem) were added as reference in addition to a not-fertilized control (Ctrl). Moreover the plant P apparent recovery fraction (ARF) was assessed in pot on Italian ryegrass over 112 days, on the same products at the same rate. P sequential extraction of the organic products showed CSM had the greatest P content in the more available forms (H<sub>2</sub>O 29% > NaHCO<sub>3</sub> 27% > HCl 22% > NaOH 9%). Oppositely CDB showed the greatest P content in the less available fraction (HCl 43% > NaHCO<sub>3</sub> 9% > H<sub>2</sub>O 9% > NaOH 8%), being similar to MSWC (HCl 63% > NaOH 11% > NaHCO<sub>3</sub> 9% > H<sub>2</sub>O 4%). CSM showed the greatest Olsen-P at the beginning of soil incubation (28.5 mg kg<sup>-1</sup>), after an intense reduction (-50%), this treatment still showed 40% more than P-chem throughout the incubation. CDB and MSWC performed the worst (4.6 and 6.0 mg kg<sup>-1</sup> on average) very close to the unamended soil (Ctrl 3.0 mg kg<sup>-1</sup>). Pot test showed CSM had also the greatest utilization efficiency on ryegrass (ARF: 12.5%), then followed P-chem (5.6%) > MSWC (4.2%) > CDB (2.5%). Data from both soil incubation and plant pot test showed P availability is related to the inherent characteristics of organic samples. It appears therefore from sequential extraction that the presence of significant amount of Ca-P bounds reduced the P available for plant nutrition.

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### Phosphorus adsorption and desorption behavior on different biochars

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Biochar has been used as a soil conditioner that increases carbon sequestration and fertility, such as soil phosphorus availability. Hence the sorption characteristics of biochar need better understood. The objective of this study was to assess phosphorus-retention capacities via P adsorption and desorption by biochars from different pyrolysis processes and feed stock materials (coir dust and eucalyptus). Phosphorus adsorption and desorption behavior on different biochars was studied by fitting the equilibrium solution and sorbed/desorbed concentrations of P using Freundlich and Langmuir isotherms. Biochar produced from coir dust had very different chemical characteristics than eucalyptus. A well fitted Langmuir equation was obtained for the adsorption isotherm ( $R^2=0.994$ ), indicating a favorable adsorption in the studied range. Eucalyptus biochar had higher P sorption than coir dust biochar. Langmuir equation and Freundlich equation were well fitted the experimental isotherm of P desorption onto the biochar. Eucalyptus biochar had higher P desorption than coir dust biochar, but coir dust biochar had better appetency than eucalyptus biochar. Effects of different biochars on P adsorption were affected by different pH values. Coir dust biochar on P adsorption capacity increased as the pH decreases. Eucalyptus biochar on P adsorption capacity increased with the pH decreasing when pH < 6, P adsorption capacity increased with the pH increasing when pH > 6, the amount of adsorption was maximum at pH 6.0.

P147

### Effect of N derived from mineral fertilizer or leguminous species on P forms of grassland soil

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In order to reduce high water nitrate pollution and use of fossil carbon, agricultural systems need to limit nitrogen fertilizer use. In forages systems, legume-grass mixture may be an alternative solution. Indeed, legumes are able to fix atmospheric nitrogen by means of symbiosis with bacteria. Compared to soil treated with N fertilizer (150 kg.ha<sup>-1</sup>), these associations may have different effects on soil P in particular the abundance and distribution of its organic and inorganic forms. In this study, we investigated this effect using 4 forage associations including two graminaceous species (*Dactylis* and *Fescue*) receiving either mineral N fertilizer or being associated with alfalfa, a legume species for 5 years. We evaluated biomass yield, SOM storage and the contribution of extractable C, N, and P. Moreover, we used Hedley fractionation and plant available Olsen P to characterize the contribution of different P forms. Our data showed similar SOM content but higher biomass yields after 4 years on plots with alfalfa compared to mineral fertilized graminaceous species. We also noted reduction of total and inorganic P in soil under alfalfa/fescue mixture, whereas only inorganic P was reduced under *Dactylis*/alfalfa mixture. Plant available Olsen P was similar in all soils and organic P contribution accounted for 60% of total P, considering NaOH extractable and residual fractions as organic. We conclude that replacement of mineral N fertilizer by leguminous species increased biomass yields and showed some effect on the quantitative contribution of P forms.

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### N-fixing tree species introduced in Eucalyptus forest modify soil organic P and low molecular weight organic acid pools

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Eucalypts, fast-growing trees, are planted in nutrient-poor soils such as sandy savannas in Congo, and deplete highly soil macronutrients such as N and P. To increase N inputs, the N-fixing tree species acacia was intercropped in eucalypt plantations. Increasing N availability could also change organic P (Po) cycling via different litter decomposition rate and increased contents of low molecular weight organic acids (LMWOAs) released by microbes. To investigate the effects of acacia on P cycling, we used first high performance ion chromatography to quantify Po forms and LMWOAs in soil from different plantations: pure stand of acacia, of eucalyptus and the mixture of both species. We separated and identified six Po forms (glucose-6-P, AMP, ATP, phytate, fructose bis-P, uridine-diP) and four LMWOAs (malate, malonate, oxalate, citrate). Glucose-6-P and AMP were the dominant forms of Po in all stands, but their concentrations decreased in the mixture compared to the pure stands. Phytate was present in all soil samples and was much higher in eucalypt than in acacia stands, with intermediate values in the mixture. Oxalate and malate were the dominant LMWOAs, especially in eucalypt pure stands. Po mineralization was estimated in Olsen extract using two recombinant fungal enzymes, a phytase (from *Aspergillus niger*) and an acid phosphatase (from an ectomycorrhizal species). Both enzymes were able to release inorganic P from Po present in the soil extracts. To go further into the effect of LMWOAs on P bioavailability, two concentrations of organic acids were added to soil before running enzyme measurements. In conclusion, our results show that intercropping of acacia modified both organic P forms and LMWOAs concentrations in pure and mixed stands and presumably P cycling.



## **Theme 2 – Phosphorus acquisition by plants and microorganisms**



## **Theme 2 – Phosphorus acquisition by plants and microorganisms**

### **Keynote presentations**

## K201

### Genetics and chemical genetics dissection of the *Arabidopsis* root growth response to low-phosphate

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The plant root system tends to preferentially colonize the upper horizons (top-soil foraging) where phosphorus accumulates. To better understand this morphological response we use a simplified system based on *Arabidopsis*. When grown on a low-phosphate (Pi) synthetic medium, the *Arabidopsis* primary root growth is rapidly inhibited whereas the laterals are induced, reminiscent to the top-soil foraging. For a long time, the induction of the laterals in low-Pi was assumed to be a consequence of the primary root growth inhibition. We have shown that, unexpectedly, these two responses can be uncoupled. In addition we show that LPR1 (a gene identified by analysing the *Arabidopsis* natural variation) has a role in both the primary root inhibition and the lateral roots stimulation by low-Pi. We used LPR1 as a tool to identify the tissue responsible for the primary root response to low-Pi in the root tip and to further demonstrate the uncoupling of the two responses. As part of a novel strategy to dissect the low-Pi signalling, we have screened a chemical library for small organic molecules that either mimic or suppress the expression of a low-Pi-specific molecular marker. We identified several such drugs. Interestingly, drugs that reduce the expression of several classical markers of the Pi-homeostasis, also suppress the root growth inhibition. Overall, our results indicate that the root architecture induced by low external Pi is triggered in some particular root cells, and is connected to Pi-homeostasis.

## K202

### Arbuscule development and phosphate acquisition through arbuscular mycorrhizal symbiosis

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In natural ecosystems, most vascular flowering plants live in symbiosis with arbuscular mycorrhizal (AM) fungi. These mutually beneficial associations develop in the roots, where the fungus obtains carbon from the plant and delivers phosphate to the roots. Development of the symbiosis is a complex process that requires the differentiation of both symbionts. The fungus grows into the root cortical cells where it undergoes terminal differentiation to form elaborately branched hyphae, called arbuscules. Differentiation of the fungal hyphae is coordinated with cellular differentiation of the root cortical cells which envelop the arbuscule in a new membrane called the periarbuscular membrane. Nutrient exchange occurs at the arbuscule/periarbuscular membrane interface and phosphate, released from the arbuscule, is transferred into the cortical cell by plant phosphate transporters in the periarbuscular membrane. Our research focuses on the molecular events that underlie development of the arbuscule/periarbuscular membrane and phosphate transport across this membrane interface. A combination of genomics coupled with reverse genetics has enabled the identification of several plant proteins required for arbuscule development, including DELLA proteins, which are transcriptional regulators of gibberellic acid signaling. A symbiosis-specific phosphate transporter MtPT4, plays a major role in the acquisition of phosphate delivered by the AM fungus and is also essential for maintenance of the symbiosis. Recent progress in understanding how these proteins regulate development and functioning of an AM symbiosis will be discussed.



## **Theme 2 – Phosphorus acquisition by plants and microorganisms**

### **Oral presentations**

O201

### Large rhizosheaths improve phosphorus acquisition and growth of wheat on P-deficient acid soils.

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Rhizosheaths provide root-soil contact and are considered to play an important role in nutrient acquisition. Large rhizosheaths are associated with long root hairs which are also the primary sites for phosphorus (P) uptake. The aims of this study were twofold; to determine the effect of a large rhizosheath on P acquisition and subsequent growth in P-limiting soils, and to investigate the genetic control of rhizosheath in two wheat populations. Near-isogenic wheat (*Triticum aestivum* L.) lines differing in rhizosheath size on acid soils were evaluated in two contrasting low pH, P-limiting soils containing high concentrations of available  $Al^{3+}$ . In separate experiments, generation means analysis was used to estimate gene effects for rhizosheath size measured on two wheat populations involving the same donor parent. Significant genotypic differences in rhizosheath size were apparent only when pH was low and coupled with high  $Al^{3+}$  concentrations. Under these conditions, a larger rhizosheath improved shoot biomass by about 20% when growth was limited by P availability. Generation means analysis for rhizosheath size revealed a largely additive-based genetic control with few major genes in both populations. This study demonstrated that improved phosphorus acquisition efficiency and growth of wheat in P-limiting conditions was conferred by large rhizosheaths. Genetic control of rhizosheath size in wheat grown on acid soils appears to be relatively simple allowing for genetic gains to be made through phenotypic selection providing population size is large enough to recover rhizosheath phenotype of the donor parent.

O202

### Assessment of root trait variation under phosphorus deficiency through Genome-Wide Association Analysis

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Phosphorus (P) is rapidly bound in soils and this 'fixation' makes P the least mobile nutrient. Consequently, P uptake depends on the size and spatial distribution of root systems, particularly in cereals. In rice, root biomass and surface area are typically good predictors of P uptake capacity and a major gene (OsPSTOL1) was recently identified as enhancing P uptake through more rapid root development. Presently no mechanisms is known in rice that would enhance P uptake efficiency per given unit root size (Root Efficiency, RE). Our objective was to screen rice genebank accessions for variation in RE and to identify genetic factors controlling this trait through Genome-Wide Association Studies (GWAS). A diverse panel of 200 rice accessions was phenotyped for P uptake and root traits in field and nutrient solution experiments. GWAS identified significant loci for root length plasticity on chromosome 5 and for lateral root number on chromosomes 1 and 2. Overall, P uptake in the field was correlated with root biomass ( $r=0.65$ ) but several 'outliers' achieved high P uptake with below-average root biomass and are therefore candidates to harbor specific root-efficiency traits. Loci associated with RE were mapped on chromosomes 1, 3 and 11. Genotypes were grouped as having high P uptake due to a) large root biomass or b) high RE and compared to standard and low P uptake types in order to identify causal attributes for high RE and uptake. Associations with mycorrhizae were negligible but root hair properties and root exudates differed between groups. How this variability within the rice gene pool can be harnessed to breed more P efficient varieties will be discussed.

### Breeding sorghum for P-limited soils in Western Africa: from field to gene level

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Sorghum (*Sorghum bicolor* L. Moench) productivity is severely impeded by low phosphorus (P) and aluminum (Al) toxic soils in sub-Saharan Africa and especially West Africa (WA). Improving productivity of this staple crop under these harsh conditions is crucial to improve food security and farmer's incomes in WA. This is the first study to examine the genetics underlying sorghum adaptation to phosphorus limitation in a wide range of WA growing conditions. A set of 187 diverse sorghum genotypes were grown in 29 -P and +P field experiments from 2006-2012 in three WA countries. Sorghum grain yield performance under -P and +P conditions was highly correlated ( $r=0.85^{***}$ ). Significant genotype-by-phosphorus interaction was detected but with small magnitude compared to the genotype variance component. Due to the slightly higher heritability estimates under -P conditions, direct selection under -P conditions proved to be feasible and more efficient than indirect selection under +P conditions. We observed a high genetic diversity within our panel for P uptake and P utilization traits. Using genome wide association mapping based on 220 934 SNPs we identified one genomic region on chromosome 3 that was highly associated to grain yield production. A major Al-tolerance gene in sorghum, SbMATE, was collocated in this region and SbMATE specific SNPs showed very high associations to grain yield production, especially under -P conditions. The results suggest that SbMATE has a pleiotropic role in providing tolerance to two of the most serious abiotic stresses for sorghum in WA, Al toxicity and P deficiency. Furthermore we identified homologs in sorghum for PSTOL1, a gene underlying a major P efficiency QTL in rice. SNPs within PSTOL1 homologs were significantly associated to P uptake and shoot biomass production. The identified SNPs can help accelerate breeding for increased sorghum productivity under unfavorable soil conditions and contribute to assuring food security in WA.

### Imaging of plant roots response to the placement of phosphate fertiliser using 4D X-ray tomography

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Plant root system architecture adapts to the prevailing soil environment and the distribution of nutrients. Many species respond to localised regions of high nutrient supply, such as those surrounding fertiliser granules, by proliferating or elongating root branches into these nutrient-rich areas. However, observation of this is limited to plant culture in idealised materials (e.g. hydrogels) with a structure-less homogenous mass, or rhizotrons which are spatially limited and provide only 2D images that are not fully quantitative. In this study, in vivo, time resolved, non-destructive, micro-focus X-ray CT imaging in 3D is used to visualise, quantify and assess interactions of the root system of living spring wheat plants with fertiliser in real soil during the life cycle of the plants. Two fertilisers (Triple superphosphate (TSP) & Crystal Green/Struvite (CG)) were used in 3 treatments to deliver 80 kg/ha of  $P_2O_5$  (CG only, TSP only & 50:50) to the plant. Weekly CT scans (60  $\mu$ m spatial resolution) of the plant roots were obtained over 12 weeks. This is the first time in situ root/soil/fertiliser interactions have been visualised in 3D from plant germination through to maturity. Preliminary results have shown that lateral roots tend to come within a few millimetres of the phosphorus (P) source. This would allow the root hairs to extend and access the diffused P in the soil surrounding the fertiliser. Quantitative analysis of root/fertiliser interaction, root volume and root architecture is ongoing. This is a promising method for visualizing root-fertiliser interactions in situ and would also be applicable to address other questions of root growth and architecture.

O205

### Phosphorus efflux from maize roots is highly localised to the root tip

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In addition to phosphorus influx, efflux is regarded an important component of P uptake by plant roots. Several studies demonstrated that generally both, apical as well as basal root parts contribute to P influx. However, data on the localization of efflux is hardly available in literature. In previous studies we observed high concentrations of P in closely confined regions around root tips of soil-grown *Brassica napus* L. and *Zea mays* L. plants. Supported by numerical simulation we hypothesized P efflux as a potential cause for these P hotspots. To clarify the nature of our observation, we labelled the shoot P of *Z. mays* plants grown in soil-filled rhizotrons by puncturing the coleoptile and placing a droplet containing  $^{33}\text{P}$  on the lesion. Phosphorus is phloem-mobile, therefore the radioactive spike is readily redistributed in the whole plant after labelling. Using a 2D sampling and imaging technique for P, we acquired images of the distribution of  $^{33}\text{P}$  release by the maize roots. After a sampling period of 48 h the efflux images showed that P release from maize roots is highly confined to the apical region of the root, with only negligible contribution of more basal root axes. Although the roots were actively growing during the sampling period and efflux localization at the mm scale is complicated, our data indicate that the route of P efflux is mainly via the apoplastic phloem-soil continuum at the initial, unsuberised phloem. We conclude that P influx and efflux are not co-localized in maize roots and discuss the consequences on root ion uptake and rhizosphere ecology.

O206

### Variation in mycorrhiza effects on P acquisition efficiency among inbred maize lines

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Maize (*Zea mays* L.) P acquisition and growth in low P soils is markedly improved by its symbiosis with arbuscular mycorrhizal (AM) fungi. Maize genotypes also differ in their tolerance to low P conditions and it has been suggested that AM may be used in plant breeding to further improve the P uptake efficiency of low P tolerant maize varieties. We investigated this hypothesis using six inbred maize lines and their wild ancestor teosinte (*Z. mays* ssp. *parviglumis*). All genotypes were grown at three P levels with and without inoculation with *Rhizophagus irregularis*. Genotype variation was analyzed in terms of growth and P uptake, using a linear model approach (Sawers et al., *Theor. Appl. Genet.*, 120: 1029–1039, 2010). The proportion of plant P uptake derived via the AM pathway was determined from the AM fungal uptake of  $^{33}\text{P}$  mixed into the soil of a root free hyphae compartment. Although most variation in tolerance to low soil P levels was related to early root growth and root hair formation, genotype variation was also observed in AM effects on P uptake and growth at low soil P levels. Interestingly, AM symbiosis was functionally important for P uptake even at the high and growth adequate P level. Here no positive AM effects were detected and some genotypes showed growth depressions when colonized by AM. We conclude that AM effects on P acquisition efficiency vary among inbred maize lines and that AM may therefore have a role in breeding of low-P tolerant maize varieties.

O207

### The phosphate transporter HcPT2, first candidate for phosphate efflux in ectomycorrhizal symbiosis?

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In forest ecosystems, the availability of phosphorus as inorganic P (Pi) limits tree growth. To overcome this deficiency, trees are associated with fungi through new organs called ectomycorrhizae (ECM). Despite the importance of phosphate nutrition, the mechanisms involved in the unidirectional transfer of P between the fungus and the plant are still unknown. Therefore, the identification of these mechanisms essential for symbiosis is a major issue. To understand better these mechanisms, we used a model association between the ECM basidiomycete *Hebeloma cylindrosporum* and the maritime Pine, *Pinus pinaster*. The fungal genome contains three transporters of Pi (HcPT1.1, HcPT1.2 and HcPT2) which have been already characterized as H<sup>+</sup>:Pi carriers. We hypothesized that one of these carriers could be responsible for both influx and efflux of Pi respectively from the soil to the fungus and from the fungus to the plant. Our first results suggest that HcPT2 would be the best candidate to fill both roles. Immunolocalization showed that the protein is located in (i) the extraradical hyphae and the fungal sheath involved in Pi uptake and (ii) the Hartig net, ie the interface between fungal and host cells. We also produced overexpressing and knock-down transgenic *H. cylindrosporum* strains to study specifically the role of HcPT2 in (i) the P efflux from the fungus measured in vitro and (ii) the P nutrition of the host plant grown at low and high P levels. Finally, if our hypothesis is confirmed, the main question will be to define how this transporter is regulated to provide both influx and efflux of phosphate in ECM symbiosis.

O208

### A purple acid phosphatase, GmPAP33, participates in phosphorus reutilization of arbuscular mycorrhizae in soybean

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Soybean is an important food and oil crop. Efficient phosphorus (P) acquisition and utilization could be considered as a critical strategy for improving soybean yield. Both purple acid phosphatases (PAPs) and arbuscular mycorrhizae (AM) play very important roles in enhancing soybean P efficiency. However, it is seldom reported that AM can specifically induce/enhance the expression of some plant purple acid phosphatase genes under low P conditions. In the previous study, we identified an AM-induced purple acid phosphatase gene from soybean, GmPAP33. In the present study, firstly, we cloned GmPAP33 and found that the enhancement of GmPAP33 expression by AM inoculation was independent in AM fungi species and soybean varieties. Subsequently, the promoter region of GmPAP33 was isolated, and cis-regulatory elements MYCS and P1BS involved in the transcriptional activation of AM-mediated genes were found in this region. Moreover, the double localization analysis with both GUS and AM infection structure staining showed that GmPAP33 promoter can drive GUS expression more intensively in the arbuscule-containing cells of the transgenic roots. Furthermore, the over-expression of GmPAP33 increased the yield and P efficiency in soybean. In summary, our results imply that GmPAP33 might be involved in P re-utilization in AM symbiosis and thereby improve P efficiency in soybean.

0209

### Hormonal networks involved in phosphate deficiency-induced cluster root formation of *Lupinus albus* L.

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In various plant species adapted to low fertility soils, formation of cluster roots (CRs) represents the most efficient plant strategy for root-induced mobilisation of sparingly soluble soil P sources. Massive local proliferation of short lateral roots and root hairs dramatically increases the root surface area, mediating the release of root exudates involved in mobilisation of sparingly soluble soil nutrients. This study addresses hormonal interactions involved in the regulation cluster root (CR) development of phosphate-(P)-deficient white lupin (*Lupinus albus* L.), in a combined approach of transcriptome sequencing, RT-qPCR based quantification of hormone-related gene expression, external application of hormones, hormone antagonists and hormone transport analysis. Shoot-to-root translocation of auxin remained unaffected by P limitation, while strong stimulatory effects of external sucrose on CR formation even in P-sufficient plants suggest that sucrose rather than auxins acts as major shoot-borne signal, triggering the induction of CR primordia. Ethylene may have functions as mediator of the sucrose signal, as indicated by moderately increased expression of genes involved in ethylene biosynthesis in the pre-emergent (PE) zone of CR formation, and by strong inhibitory effects of the ethylene antagonist CoCl<sub>2</sub> on CR formation induced by sucrose or P by limitation. As similarly reported in other plant species, moderately increased production of ethylene and also of brassinosteroids (BR) may induce biosynthesis and transport of root-borne auxins, indicated by increased expression of the respective genes in the PE zone of CR development. A role of BR in CR formation is further underlined by inhibitory effects of BR antagonists. The well-documented inhibition of root elongation by high doses of ethylene may be also involved in the inhibition of lateral root growth during CR maturation, indicated by a massive increase of gene expression involved in ethylene production.

0210

### Influence of soil fertility management on microbial phosphorus and nitrogen limitation in a lixisol

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Phosphorus (P) and nitrogen (N) availability are known to be the most limiting nutrients for plant growth in lixisols of semi-arid West Africa. Many studies have investigated the effects of mineral fertilizers and manure on soil physicochemical characteristics and crop productivity in these soils but little is known about the long-term effects of different types of fertilization on microorganisms. We investigated the long-term effects of mineral fertilizer (fm), manure and mineral fertilizer (fmo), and no fertilizer (control) on soil microbial biomass as well as microbial N and P limitation. Microbial carbon (C), N and P were measured on samples from a long-term field experiment (54 years) in the central-West of Burkina Faso. Subsequently, the effects of H<sub>2</sub>O, CN, CP, or CNP addition on microbial biomass were assessed by measuring soil respiration, and extracting microbial P and total PLFA at the peak of soil respiration. Microbial N was significantly higher in fmo than in fm and control, whereas microbial P was significantly higher in fmo and fm than in the control. In soil from all field treatments, CNP addition resulted in the highest peak of respiration. The response to CN or CP addition differed between field treatments, suggesting that at high levels of C availability, microbial activity was co-limited by N and P in the control and only N-limited in fmo and fm. Changes in microbial community patterns based on the relative abundances of PLFAs support the idea that soil microbes were N and P co-limited in the control, N limited in fmo and P limited in fm. Consequences for microbial processes in N and P cycling need to be further investigated.

O211

### The distribution of acid and alkaline phosphatase activity in the rhizosphere

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The spatial organization of the rhizosphere is little understood, despite its importance for terrestrial nutrient and phosphorus (P) cycling. We analyzed the distribution of acid and alkaline phosphatase activity in the rhizosphere of *Lupinus albus* L and *Hordeum vulgare* L. For this end, we used soil zymography – a novel in situ method for the analysis of the two-dimensional distribution of enzyme activity in soil. Both acid and alkaline phosphatase activity was up to 5.4-times larger in the rhizosphere of *L. albus* than in the bulk soil. While acid phosphatase activity (produced by roots and microorganisms) was closely associated with roots, alkaline phosphatase activity (produced only by microorganisms) was more widely distributed, leading to a 2.5-times larger area of high activity of alkaline than of acid phosphatase in soil. This finding indicates a spatial differentiation of plant and microbial extracellular P mineralization in the rhizosphere, which might alleviate a potential competition between plants and microorganisms for P. While alkaline phosphatase activity decreased strongly due to P fertilization, acid phosphatase activity was not affected by fertilization, suggesting that microorganisms were less adapted to low P availability than *L. albus*. Our studies indicate, first, a spatial differentiation of microbial and plant P mineralization in the rhizosphere, second it indicates *L. albus* is better adapted to low P availability than soil microorganisms in temperate soils. Finally, we conclude that soil zymography is a very promising method for studying the distribution of exoenzymes and organic P mineralization in the rhizosphere.

O212

### Coordination of root morphological and physiological adaptations to phosphorus deficiency for contrasting plant species

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Plants have evolved numerous adaptive mechanisms to cope with phosphorus (P) deficiency, which include modification of root morphology and enhancement of root physiological responses in terms of carboxylate exudation, proton release and phosphatase secretion. However, the coordinating relationship of root morphological and physiological adaptations to P deficiency for different plant species with contrasting root traits is not fully understood. Here, a comparative study was conducted to examine coordination mechanisms of root morphological and physiological adaptations for seven different plant species in P-deficient (no P applied) or P-sufficient (100  $\mu\text{g P g}^{-1}$  soil) acid or calcareous soils, including fibrous root-system maize (*Zea mays*), wheat (*Triticum aestivum*) and rape (*Brassica napus*), and taproot white lupin (*Lupinus albus*), soybean (*Glycine max*), faba bean (*Vicia faba*) and chickpea (*Cicer arietinum*). Root morphological traits (root biomass, root/shoot ratio, total root length, specific root length and total root surface area) and physiological traits (P utilization efficiency, P uptake efficiency, pH, acid phosphatase, malate and citrate exudation in the rhizosphere) were measured. The principal component analysis showed that white lupin and chickpea were classified into the species with strong root physiological adaptation to P deficiency. Soybean and fababean belonged to the morphological-physiological adaptation species. Maize, wheat and rape showed strong root morphological adaptation to P deficiency. The results were further confirmed by field experiments, suggesting a coordination of root morphological and physiological responses to cope with P deficiency for different plant species, which is important for manipulating root morphology and rhizosphere processes for a given plant species to enhance plant growth and P acquisition.

**Root functional traits and their plasticity drive grasslands' Fabaceae capacities to face phosphorus shortage**Florian Fort <sup>1</sup>, Pablo Cruz <sup>1</sup>, Olivier Catrice <sup>2</sup>, Ciprian Stroia <sup>3</sup>, Claire Jouany <sup>1</sup>*1. INRA, AGIR UMR 1248, 24 Chemin de Borde Rouge – Auzeville CS 52627, 31326 Castanet Tolosan cedex, France**2. INRA, LIPM UMR 2594/441, 24 Chemin de Borde Rouge – Auzeville CS 52627, 31326 Castanet Tolosan cedex, France**3. Banat University of Agricultural Sciences and Veterinary Medicine, Department of Biology and Plant Protection, Calea Aradului 119, 300645 Timisoara, Romania*

Understanding which strategies allow plants to cope with N and P shortages may lead to select species better able to valorize non-optimal growth conditions in agrosystems. This question is of particular importance for Fabaceae since they are able to free themselves from soil nitrogen supply, but are highly limited by P shortage. To test which root strategies allowed species to be more efficient under P shortage we grew, in a greenhouse, 13 grassland Fabaceae species under two levels of P availability. Ten root functional traits were measured, including cross-sectional area occupied by aerenchyma, mycorrhizal rate, root hair length, root phosphorus use efficiency (RPUE), root-surface phosphatase activity, and specific root length (SRL). Traits plasticity in response to P shortage was also evaluated. Results showed a negative relationship between mycorrhizal rates and biomass production in high and low P availability conditions. Long root hairs and high aerenchyma production are associated with high biomass production in, respectively high and low P availability conditions. We highlight that the increase of root-surface phosphatase activity and RPUE in response to P shortage were positively related to biomass production in this condition. Moreover, high SRL, the plasticity of SRL and root hair length in response to P stress limit the impact of this stress on species biomass production. Our results showed that grassland Fabaceae display a broad range of root functional strategies, which drive the different species performances in case of P shortage. Moreover, this study challenges the idea that arbuscular mycorrhizal fungi have always positive effect on plants' growth.



## **Theme 2 – Phosphorus acquisition by plants and microorganisms**

### **Posters**

P201

#### High yielding rice under low P application field: Pup1 breeding

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Pup1 (Phosphorus-uptake 1) is one of the major QTL identified in rice enhancing P-uptake in the field. Sequencing of the Pup1 locus in the tolerant donor Kasalath showed the presence of a novel protein kinase gene, named PHOSPHORUS STARVATION TOLERANCE 1 (OsPSTOL1). Currently developed IR64- and IR74-Pup1 seeds are being distributed and tested by collaborators in South Asia, Southeast Asia and Sub-Saharan African countries. Pup1 introgression lines into two Indonesian varieties, Situ Bandit and Batur (partial Pup1), have been developed by ICABIOGRAD and the selected Pup1 lines are ready to be tested in the large scale field experiment. Some IR64-Pup1 lines showed good tolerance to low P application in India. Molecular marker application for Pup1 is readily available for three top candidate genes of Pup1: OsPupK20-2, OsPupK29-1, and OsPupK46-2. PhilRice is developing P-efficient upland rice by Pup1 introgression into four famous varieties by marker-assisted backcrossing. In addition, another allele-specific marker for African rice of PSTOL1, Pup1b, was reported recently. Understanding various Pup1 allele types and their association with P-uptake and P-deficiency tolerance will contribute to the development of more P-efficient rice varieties. Currently, various multiple-trait pyramiding using Pup1 enhancing acceptability under direct seeding and drought conditions have been conducted in International Rice Research Institute. The developed lines are being validated in field screening conditions.

P202

#### Influence of root hairs on P deficiency in rice (*Oryza sativa*) varieties

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As phosphorus is a macronutrient for plant nutrition its acquisition from the soil is of high importance. By producing longer roots, more lateral roots, and root hairs a larger area of soil can be explored in order to take up more P. Here, we investigate several methods for understanding the impact of root hairs on the performance of rice varieties in response to P deficiency. Root hairs of field grown plants of several varieties were compared to those grown in nutrient solution or in a filter paper assembly, provided with sufficient or deficient levels of P. Roots grown in P deficient nutrient solution formed longer root hairs and initiated them earlier than roots grown in control solution. In contrast, roots grown in the filter paper experiment did not show significant differences in root hair development. With increasing distance from the root tip all tested varieties formed more and longer root hairs. Some genotypes like Sadri Tor Misri and DJ123 had consistently longer and denser root hairs in both artificial growth conditions, however, this was only partly confirmed in roots extracted from the field. In conclusion, the tested artificial methods are easy to control and perform, but do not completely represent the complex situation roots have to deal with in soil. On the other hand, field experiments are too time intense and difficult to reproduce for detailed comparison of natural variation and conditions. To establish a realistic, but controllable system for root hair investigation, soil-based pot experiments will be performed in the future.

## P203

### The P demand for growth of C3 and C4 plants at ambient and elevated atmospheric [CO<sub>2</sub>]

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Elevated [CO<sub>2</sub>] causes a carbon fertilizer response in C3 plants, while such response is less in C4 plants that have more efficient photosynthesis. We anticipate that increased growth of C3 plants at e[CO<sub>2</sub>] also increases the demand for phosphorus. This hypothesis was tested for the C3 species *Medicago truncatula*, *Brachypodium distachyon* and *Oryza sativa* and the C4 species *Zea mays*. Plants grew in a phytotron at 400 and 900 ppm [CO<sub>2</sub>] and at a range of soil P levels; possible interactive effects of colonization by AM fungi were also investigated. The C3 species grew better at e[CO<sub>2</sub>], but patterns differed and ranged from strong increases over the full soil P range in the two grasses *B. distachyon* and *O. sativa* to a soil P-dependent increase in the less P-efficient *M. truncatula*. Maximum growth in *B. distachyon* was reached at a lower soil P level at e[CO<sub>2</sub>] than at ambient [CO<sub>2</sub>], irrespective of mycorrhiza. In *Z. mays*, an unexpected carbon fertilizer effect at medium and high soil P was probably due to improved water use efficiency. Mycorrhizas increased growth of *M. truncatula* and *Z. mays*, had little influence on *B. distachyon* and decreased growth of *O. sativa*. However, growth responses to e[CO<sub>2</sub>] were only marginally influenced by mycorrhizas. Root length specific P uptake was similar at a[CO<sub>2</sub>] and e[CO<sub>2</sub>] but shoot P concentrations of C3 species were lower at e[CO<sub>2</sub>] than at ambient [CO<sub>2</sub>]. We conclude that the P use efficiency increases at e[CO<sub>2</sub>], that species with efficient P acquisition may require less P for optimal growth at e[CO<sub>2</sub>] than at ambient [CO<sub>2</sub>] and that e[CO<sub>2</sub>]-induced promotion of plant growth and P uptake is only little modulated by mycorrhizas.

## P204

### Multiscale Modeling for Water and Nutrient Uptake by Plant Roots

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Nutrient uptake by plant roots is a multiscale problem described by a single root and the entire root system scale simultaneously. This process requires a spatial resolution in the sub mm and hence is not feasible for simulations of the entire root system or soil profile. To address this problem, we present a numerical simulation model that uses a 1-D radially axisymmetric model to describe nutrient transport towards a single root segment. The network of connected cylindrical models was coupled to a 3-D regular grid that was used to solve the flow and transport equations at the root system scale. The coupling was done by matching the fluxes at the outer boundaries of the cylindrical domains of the radially axisymmetric model with the fluxes across the interfaces of the voxels of the 3-D grid that contain root segments and by matching the sink terms in these voxels with uptake by the root segments. Results include comparison of simulations of cumulative nutrient uptakes and nutrient depletion zone around the root zone, obtained at the single root and root system scale using the coupled (3D-1D) model, fine grid 3D model and the approximate analytical solution of Roose et al. (2001). Demonstrations using the coupled model have shown good agreement with the high resolution 3-D, which makes the coupling approach capable of accurately simulating a root system scale models with minimum computational cost. The coupling model also allows to account for the effect of water uptake and soil drying specially on arid lands on nutrient uptake and to account for spatial variations in root density and nutrient concentrations.

P205

***Bacillus subtilis* increases phosphorus uptake by plants from phosphate rock**

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Phosphorus mobilizing microorganisms can contribute to increase P uptake from sparingly soluble P sources, such as Ca phosphates present in soil or added as fertilizer. The aim of this work was to study the effect of *Bacillus subtilis* on P uptake by plants when rock phosphate (RP, mainly an apatite-type phosphate) is the P source present in the growing medium and how Fe oxides can interact with the potential effect of this microorganism. Experiment was performed on an artificial growing media (siliceous + calcareous sand; 3:1 ratio) with a complete randomized design with three factors: (i) inoculation/no inoculation with *B. subtilis*; (ii) P source (RP at 100 and 200 mg P kg<sup>-1</sup>, and KH<sub>2</sub>PO<sub>4</sub> at 100 mg P kg<sup>-1</sup>), and (iii) Fe oxides (ferrihydrite at 0 and 300 mg Fe kg<sup>-1</sup>). *B. subtilis* increased DM yield and P uptake by plants (greater accumulation in plant shoots and roots); this effect was independent of Fe oxides or P source. Fe oxides decreased P uptake by plants only when P was supplied as KH<sub>2</sub>PO<sub>4</sub>; this reveals that P adsorption on Fe oxides may negatively affect the efficiency of soluble P fertilizer. The effect of *B. subtilis* can be related to an acidification effect of the media. However, *B. subtilis* decreased the amount of organic anions in the media, as the likely result of their use as C source for the microorganism.

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P206

**Effect of modulation of phosphorus uptake and storage in plant by humic substances treatment**

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A number of researches on plant mechanism of adaptation to low phosphorus fertility has been studied in recent years, and their alterations as plant reaction to severe condition has been observed morphologically, physiologically and biochemically. Humic substances increase the solubility of P compounds and consequently make bioavailability available. Nevertheless, the information on the effect of humic substances on the P nutrition at the molecular level has been little known. The aim of our study is to observe the plant reaction to the interaction with the humic substance under the condition of low P content. Our research structure is based on observation of the phyto-hormonal effect of humic substance treatment under phosphate deficiency on plant growth.

P207

### Grassland Fabaceae grown under contrasted phosphorus supply induced changes in rhizospheric soil phosphatase activity

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Fabaceae performances in low fertility agro systems rely on their capacity to acquire phosphorus (P) since their growth is highly limited by P shortage. Recent work conducted on grassland's legumes demonstrated, that different roots strategies exist within fabaceae family resulting in a large range of responses to P stress. Our objective is to evaluate to what extent rhizospheric soil phosphatase activity is related to Fabaceae response to P limitation. In that purpose, we grew in a green house, 13 grassland Fabaceae species under two levels of P availability for more than 100 days. At harvest, rhizospheric soil were sampled then analysed for alkaline phospho-monoesterase activity together with bulk soil (control). Results show a significant effect of plant on species identity on rhizospheric phosphatase activity, *Anthyllis vulneraria* and *Vicia cracca* induce high phosphatase activity in their rhizospheric soil while *Securigera varia* and *Trifolium pratense* induce low phosphatase activity. There is also a significant and negative effect of P supply on phosphatase activity of rhizospheric soil. The activity measured for the bulk soil is always significantly lower than the one measured for the rhizospheric soil. It was interesting to notice that phosphatase activities measured for Fabaceae are not different from those obtained for Poaceae grown under similar conditions. We showed that Fabaceae species induce a strong increase of the phosphatase activity in their rhizospheric soil in comparison to bulk soil. However, further work is needed to understand the link between rhizospheric soil activity and P stress tolerance.

P208

### A simple technique for studying root hair development under varying levels of buffered P supply

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Root hairs contribute significantly to phosphorus (P) uptake and are particularly important when P supply is low and strongly diffusion-limited, which is the case for many depleted and P-fixing soils in the tropics. In view of the need to select and develop P-efficient crops, it may be of interest to explore genotypic variation in root hair development. However, the measurement of root hair development of soil-grown plants is difficult as root hairs are easily damaged while roots are washed out from soil. Growth pouches or hydroponics have been used to study root hair development but in these systems it is not possible to spatially buffer P supply. A method was developed to evaluate root hair growth under artificial conditions that closely mimic diffusion-limited P supply in soils. The method involves seedlings grown on an agar medium that contains P adsorbed on Al<sub>2</sub>O<sub>3</sub> nanoparticles at different levels of P intensity. The use of Al<sub>2</sub>O<sub>3</sub> nanoparticles as P buffer ensures clarity of the agar, allowing for in-situ measurements of root hair length and density. Results of a study on 8 soybean genotypes screened in both the agar medium and a pot experiment using a P-deficient soil at low and high P supply are presented. P intensity in the agar was buffered at 6 and 80 µM, while total P concentrations in the medium were 120 and 250 µM respectively. The P intensities corresponded to the P concentrations measured in the pore water of the amended soil samples used for the plant growth experiment. We conclude that the technique provides a simple way to study the plastic response of root hairs to P deficiency governed by localized P-sensing mechanisms.

P209

### Fertilization of phosphorus to the soil can increase iron chlorosis in sensitive plants

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The aim of this work was to clarify the influence of phosphorus (P) fertilization on iron (Fe) availability in chlorosis-prone plants grown on calcareous soils. A monocot plant (sorghum) and a dicot plant (lupin) having different Fe acquisition strategies were successively grown in a growth chamber on pots filled with 250 g of 24 different calcareous soils. Two treatments were applied to the soils before filling the pots, one with the intact soil (control) and one in which P was sprayed into the soil at a rate of 100 mg P kg<sup>-1</sup> soil. Leaf chlorophyll concentration (LCC) in lupin decreased with increasing available P/available Fe (Olsen P/ oxalate extractable Fe) ratio in the native soil but LCC in sorghum was unaffected by that ratio. In sorghum, LCC and dry weight were positively affected by P fertilization in the case of soils poor in available P (< 10 mg P kg<sup>-1</sup> soil), whereas the opposite effect was observed in the rest of soils (>10 mg P kg<sup>-1</sup> soil). In summary, high P fertilization increases Fe chlorosis symptoms depending on the plant and on the content in available Fe and P of the soil.

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P210

### Phosphate reduces iron bioavailability on model calcium carbonate-iron oxide systems

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Iron (Fe) deficiency or Fe chlorosis is an important nutritional problem for sensitive plant species grown on calcareous soils and is mainly related to the soil content on Fe oxides and carbonate. Interveinal yellowing of young leaves and reduced growth are the main symptoms of this deficiency. Phosphorus (P) reacts greatly with soil components, such as iron oxides (high affinity), clay and carbonates. Artificial substrate consisting on a mixture of Fe oxide (ferrihydrite)-coated sand (FOCS), calcium carbonate sand (CCS) and quartz sand in different proportions, to which P was sprayed homogeneously at several doses, were used to pot grown chickpea, lupin and peanut (preliminary experiment), and lupin and sorghum (factorial experiment). The objectives of the experiment were to test the hypothesis that fertilizing at high P rates ( $\approx 100$  mg P kg<sup>-1</sup>) reduces Fe bioavailability and to assess that this effect depends on plant Fe acquisition strategy. The FOCS content had a significant positive effect on leaf chlorophyll concentration (LCC, estimated from the SPAD value). Lupin LCC decreased with increasing CCS content in the substrate unlike the case of sorghum (factorial experiment). High P dose had a negative effect on LCC of plants grown on FOCS-poor substrates but this effect was almost negligible in FOCS-rich substrates. In conclusion, phosphorus decreases Fe oxide solubility because of the Fe oxides surfaces were covered by the added P reducing the Fe availability for plants.

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P211

### Exploiting root exudation of organic acids and phytases to enhance plant utilisation of soil phosphorus

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Here we introduce an organic P consortium who has recently been funded to test the hypothesis that: cropping systems selected to express favourable root exudate properties will facilitate sustainable agricultural production through improved access to soil organic phosphorus (Po). Specifically, we will use exemplar plant mixtures to investigate the ability of low molecular weight organic acid anions (LMWOAA) and phosphatases to access soil Po. Additionally, the interaction of Po, LMWOAA, and phosphatases with the soil solid phase will be investigated, through batch incubations and equilibrium modelling. A range of experiments are being conducted to identify potentially suitable strains of barley (*Hordeum vulgare* L.) and clover (*Trifolium subterraneum* L.) and will be followed by growth experiments incorporating different combinations of these plant lines to determine which combinations can most efficiently access soil Po, while minimizing losses of P via leaching. Experiments and techniques employed will include the screening of plant populations for LMWOAA and phosphatase exudation, using anion exchange resins and diffusive gradients in thin films (DGT), HPLC for root exudate analysis, enzyme hydrolysis and <sup>31</sup>P nuclear magnetic resonance analysis for identification of Po compounds and classes in soil extracts and soil pore water.

P212

### Effect of foliar application Urea and Putrecine on phosphorus content of pistachio leaves

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In order to study effects of foliar application Urea and Putrecine on concentrations of phosphorus in the leaves of pistachio trees in Rafsanjan area, Iran, a field experimental was carried out in factorial experiment, in completely randomized block design with three replications. Treatments were three levels of urea (0, 0.5 and 1%) and three levels of Putrecine (0, 0.5 and 0.7%). This study was done in May and June 2013. Our results showed that foliar application of urea (1%) significantly increased phosphorus concentration in pistachio leaves of Ohadi ( $p < 0.01$ ) and Kalaghoche ( $p < 0.05$ ) cultivars. The foliar application of Putrecine had no significant effects on phosphorus leaf concentration of pistachio trees. In both cultivars, interaction of Urea and Putrecine was significant ( $p < 0.01$ ). We concluded that the foliar application of Urea have positive effect on phosphorus content of pistachio leaves.

P213

#### How *Fagus sylvatica* L. deal with low P availability - investigation approach

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Tree root system as a whole interacts with soil, but in nutrient scarcity there are key areas involved in mobilisation and uptake. Those areas (microzones) which undergo investigation could be mycorrhizal root tips, elongation zone of young non-mycorrhizal root tips and tips of extraradical hyphae. The behaviour of the plant root may be a resultant of couple of factors including: inner P status of the plant, availability inorganic phosphate and microbial associations. Assessment of abilities of distinguished rhizosphere and hyphae zones can be studied using plants grown in rhizoboxes with transparent front plate allowing optical recognition of area of interest. In such set-up various 2D methods might be applied - phosphatase activity measurements by zymography, labile phosphorus mapping with DGT gels, measuring pH distribution with optodes, collection of soil solution using micro suction cups or filter papers. Plant nutrient status can be measured by phosphatase activity in cells, TCA extractions and NMR. Thanks to modern molecular biology methods also local microbial communities can be quantified and classified. Such strategy can give an answer to several questions about *Fagus sylvatica* strategy in soils of various phosphorus availability and microbial composition. Including what is the real reaction time of the tree to changing phosphorus availability and if microbial communities are inevitable for survival of allochthonous seedlings in new conditions.

P214

#### Characterization of two putative direct phosphate transporters in *Brachypodium distachyon*

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In low phosphorus (P) soil, the symbiotic relationship with arbuscular mycorrhizal (AM) fungi leads to increased uptake of P and increased growth. But in some cases AM fungi induce plant growth depressions. We test the hypothesis that the growth depressions can be explained by P limitation rather than the classical carbon drain theory. Our assumption is that an AM-induced functional impairment of direct Pi uptake at the root surface is not fully compensated by P uptake via the AM pathway. A transformation approach was used to manipulate the activity of the Pi transport pathways in the model grass *Brachypodium distachyon*. Transgenic over-expression and knock-down (RNAi) lines of two specific phosphate transporters (BdPT4 and BdPT8) have been generated and initial studies of a few of these transgenic lines show over-expression and down-regulation as expected. All of the transgenic lines are currently being analyzed to compare their non-mycorrhizal phenotype with expression levels of the targeted Pi transporter gene. Selected transgenic lines will be used to investigate the activity of the two transporters in Pi uptake by radioisotope uptake studies, also in mycorrhizal plants. The same transgenic lines will be used for more elaborate molecular studies focusing on the effect on the other Pi transporter genes. Besides, the subcellular localization of the transporter proteins is being investigated using GFP tagged lines. Eventually, this study shall clarify whether it is possible to improve the Pi uptake efficiency of crops by maintaining a high activity of the direct uptake pathway in mycorrhizal plants and thereby making the two pathways additive instead of complementary.

P215

### Real time imaging to analyze phosphate uptake and translocation in planta

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Imaging techniques based on reporter genes are broadly used in cell biology to analyze proteins. This has turned out to be very useful to investigate regulations of transporters however this does not provide much information on the activity of these molecules. The use of radioactive tracers has been widely developed to study plant mineral nutrition, providing useful general information on the kinetics of these mechanisms. Here we report a technique based on real time radioisotope imaging system (IRRIS), which provides access to ion movement in planta from whole plants to microscopic level. A microscope based system was able to image tracer in the plant tissue enlarged 40 times combined with chemiluminescent or fluorescence detection in the same plant. The behavior of <sup>33</sup>P was analyzed by a series of images, which were obtained every few minutes providing the opportunity to distinguish Pi uptake from Pi transport.

P216

### Enhancing fertilization efficiency by phosphate solubilising bacteria: A lab-scale screening study

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Asides the exploitation of waste-borne phosphorus an increase of phosphorus (P) fertilization efficiency is mandatory for the sustainable management of this non-renewable resource. Especially in calcareous soils, fertilizer P may be sequestered in sparingly soluble compounds (e.g. tricalcium phosphates, TCP) leading to reduced plant growth. Phosphate solubilising microorganisms (PSM) enhance the release of P by excretion of organic acids and/or complexing agents. This study aimed at the selection of PSM potentially suited for field inoculation. Plate and broth cultivation techniques were employed using a Pikovskaya medium with TCP as the inorganic P source. The diameter of halos was used as a proxy for the degree of P-mobilization from TCP by colony forming units on agar plates under different culture temperatures. As expected, larger halos developed under aerobic conditions and at the physiologic temperature optimum of the species. Phosphorus mobilisation was found to increase in the order *Rhizobium leguminosarum* < *Bacillus subtilis* < *Rhizobium meliloti* < *Pseudomonas fluorescens*. Based on these findings, the latter organism was selected for Pikovskaya-broth cultivation studies including the monitoring of microbial growth, pH development and phosphorus concentration in different fractions. Analyses comprised dissolved phosphate, residual TCP, and microbial as well as yeast extract-borne phosphorus remaining in the medium. Within 24 hrs the pH of the medium dropped from 7 to around 4.3 and remained fairly constant over one week. This was accompanied by a significant P-release. Overall, our findings suggest that *Pseudomonas fluorescens* may be suited for the inoculation of soils in order to mobilize soil-borne, but sparingly available P.

P217

### Phosphorus diffusion in a flooded P-deficient soil: a simulation study

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Transport of phosphate (P) from the soil to the root surface is mainly by diffusion. The quantity of P diffusing is mainly determined by its effective diffusion coefficient (De). This latter can be very low in high P-fixing soils. Upon flooding paddy soils, the De increases due to increased volumetric moisture content and reduced tortuosity and due to chemical factors mobilizing P, i.e. reductive dissolution of iron oxides. This study numerically evaluated the P concentration gradient as a function of time and distance under bulk diffusion in flooded P-fixing soil that is used as a typical soil for flooded rice in Madagascar. The model was set up to simulate self-diffusion of P between two adjoining blocks of soil, a <sup>33</sup>P labelled source and an unlabeled sink respectively. Input parameter data were obtained from an experiment on a flooded P-deficient soil using a tracer (<sup>33</sup>PO<sub>4</sub>-carrier) and from the literature. The De was calculated with the equation of Nye and Tinker (1977) the soil to soil pore water distribution coefficient (KD) of P in soil was equal to 4000 L/kg and De of 5.01 x 10<sup>-10</sup> cm/s. The concentration distance profile was calculated from Kaselowsky et al., (1989). Calculations showed that after a deployment time of 25 days, the <sup>33</sup>P concentration in the sink, relative to the source, was 0.5 at the interface depleting to 0.001 at 1.5 mm distance in the second soil block. The total <sup>33</sup>P flux that migrated out of the first 1 cm source into the sink was only 1.96 % of the total <sup>33</sup>P. In a corresponding non-flooded soil (lower volumetric moisture content, higher tortuosity and higher KD), that fraction decreased to 1.01 % suggesting why P deficiency in flooded soils is less pronounced than in aerated soils.

P218

### Interplay between direct and mycorrhizal P uptake in monocots

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Arbuscular mycorrhizal (AM) fungi can induce growth depression in e.g. grasses. We are testing the hypotheses that this growth depression could be caused by (1) fungal carbon drain and/or (2) phosphorus limitation. Two phosphate (Pi) uptake routes are present in AM plants: the mycorrhizal pathway and the direct pathway. A shift to the mycorrhizal pathway may occur at the expense of the direct pathway so that AM plants can become P limited if the decrease in the direct pathway is not fully compensated by mycorrhizal uptake. We are using *Brachypodium distachyon* and rice models to study growth depression by AM fungi. We are testing the hypotheses above by growing AM plants at elevated [CO<sub>2</sub>] to mitigate possible C limitations and by analyzing gene expression of phosphate transporter genes, combined with physiological radiotracer uptake studies, to distinguish between routes of Pi uptake. Localized mycorrhization in split-root plants is used to study whether the interplay between the two pathways is systemic. The transcriptomes of split-root rice plants are analysed to gain a deeper understanding of the molecular interplay between the direct and mycorrhizal pathways. Data from the different analyses will be presented and the pathway interplay discussed at the molecular and physiological levels.

P219

### Effects of mineral fertilizer and cropping system on endomycorrhizal fungi dynamic and upland rice nutrients acquisition

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Despite the expansion of the areas occupied by the rainfed rice, the production yield is still averagely low due to the lack of knowledge about the real needs of soil fertilization. The relationship between fertilizers, soil fertility and soil microorganisms, such as endomycorrhizal fungi, is not sufficiently exploited. This study conducted mainly in the field aimed to describe the effects of soil fertilization with triple superphosphate 20 kg/ha (TSP20) and the cropping system, monoculture of upland rice or coculture of upland rice with common bean, on the dynamic of endomycorrhizal fungi community (AM) associated with upland rice. Plant development was evaluated by measuring the amount of nitrogen and phosphorus in upland rice aerial part. Results showed that compared to control treatment, non-fertilized with TSP20, soil fertilization with TSP20 stimulated significantly the AM community by increasing up to 2.27 times the Most Probable Number of mycorrhizal propagules (MPN) which can infect a host plant and decreasing 1.5 times the number of soil AM spores. The same trends were also recorded with soil under the coculture bean-rice treatment compared to the monoculture treatment. Nitrogen and phosphorus amount in the upland rice aerial part were also significantly high for the treatment TSP20 and for the treatment combining the fertilization with TSP20 and the coculture bean-rice compared to control treatments. Then, soil fertilization with the TSP20 in cocultured bean-rice enhances the uptake of major nutrients by upland rice and stimulates also the AM community in soil.

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P220

### Root exudates affecting P phytoavailability in soils - biogeochemical mechanisms and experimental approaches

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Roots release a range of organic as well as inorganic molecules of which some have been implicated to play an important role in increasing plant P nutrition. Here we aim to summarize the biogeochemical mechanisms that can be triggered by roots to increase P solubility, with particular focus on the effectiveness of various root exudate compounds in contrasting soil types with differing P amounts and chemistries. Also, accurate sampling and determination of release rates of root exudates is a prerequisite for further mechanistic investigations that will lead to a better understanding of the processes and dynamics involved in P mobilisation. Different experimental approaches (soil vs hydroponic culture) to sample root exudates will be presented and compared, including a new, rhizobox-based approach that enables us to repeatedly collect unaltered root exudates from soil grown plants. Implications of the differences in results obtained by the different experimental approaches will be discussed.

P221

### Molecular characterization of phosphate-solubilizing rhizobia isolated from *V. faba* in Marrakech region field cultures

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Low soil phosphorus availability is among the major constraints for crops especially when they are depending on symbiotic nitrogen fixation. Rhizobial strains, beneficial N<sub>2</sub>-fixing symbiotic partners of legumes, were reported to solubilize both organic and inorganic complex phosphates. The current study is carried out to select rhizobial strains isolated from nodules of faba bean cultures in Marrakech-Haouz region in Morocco and to investigate their ability to solubilize the complex mineral P. Our results revealed that among 80 isolates of rhizobia strains, 20 are able to solubilize the mineral P forming halo around their colonies on TCP agar. We evaluated and compared the effect of 8 strains on growth and phosphorus uptake by two Moroccan varieties of faba bean (*Vicia faba* L.) plants (Aguadulce and Defes). The Greenhouse experiments showed contrasting effect of strains on nodulation rate and plant growth depending on the inoculated strain and the symbiotic combination. The molecular characterization of rhizobial strains was performed by PCR amplification and sequencing of 16s rRNA coding gene, rec A and nod D genes and also the pqq C gene encoding the pyrroloquinoline quinone synthase C applied in the phosphate-solubilization by bacteria. The majority of the rhizobial strains belonged to *Rhizobium leguminosarum*. Some strains were identified as *Ensifer meliloti* (formerly *Sinorhizobium meliloti*). The gene pqq C was detected in 9 rhizobia strains.

P222

### Phosphorus and nitrogen uptake of potato under water saving irrigation regimes

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Because of the worldwide shortage of fresh water, and depletion of non-renewable resources of phosphorus (P), concurrent maximization of nutrient use efficiency and saving water are demanded in crop systems. The objective of this study was to investigate whether potato production with high P and nitrogen (N) uptake can be obtained under the water saving irrigation regimes of: Partial Root-zone Drying (PRD) and Deficit Irrigation (DI). Potatoes were grown in split-root pots in a climate-controlled glasshouse. N and P were applied 153 and 37 kg/ha respectively. From four weeks after planting, the plants were treated with either full irrigation (FI), PRD or DI for two months. The result indicated that, compared with FI, both DI and PRD significantly improved water use efficiency (WUE) but decreased leaf area and biomass. Compared with FI, both PRD and DI saved 38% water and significantly improved tuber WUE with 35% but decreased tuber production with 15%. The total N and P uptake were same with DI and PRD and were significantly lower than FI. N uptake in shoot was significantly higher in PRD treatments than DI, while there was no difference in shoot P uptake. Physiological phosphorus use efficiency (PPUE) with FI and PRD were similar and both were significantly higher than DI. We conclude that, with limited freshwater resources, application of PRD irrigation could be a promising approach for saving water and when saving the same amount of water, PRD has advantages compared to DI in terms of improved shoot N uptake and PPUE. However, challenges remain as to maintaining P uptake under the decreased soil water regimes used in contemporary water saving irrigation strategies.

P223

### Cattle manure inoculation with a phytase producing *Bacillus*: organic P mineralization, bacterial community and P uptake

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The aim of this work was to evaluate the effect of manure inoculation with an alkaline  $\beta$ -propeller phytase producing bacterium (PBB) in the organic P mineralization, changes in bacterial community structure, inoculum survival and plant P uptake. Eleven strains selected from previous rhizosphere phosphobacteria screening studies were had their phytase activity briefly biochemically characterized. *Bacillus* sp. MQH-19 was the only strain with extracellular alkaline phytase activity, and was selected to be tested as an inoculant in neutral to alkaline wastes environments. The inoculation of manure with *Bacillus* sp. MQH-19 bacterium strain at under unbuffered (no pH control) and non-sterile conditions promoted an 8 and 13% increase in the NaHCO<sub>3</sub> and NaOH-EDTA extracts respectively. Changes in bacterial community structure due to PBB inoculation were studied using denaturing gradient gel electrophoresis analysis of 16S gene (16S-DGGE), which was PCR amplified from environmental genomic DNA extracts. Bacterial community structure was significantly affected by PPB inoculation up to 6 days after inoculation. Principal component analysis of 16S-DGGE relative band volumes showed that higher effect of inoculation on the bacteria community structure was at day 3. Inoculum persistence was evaluated using quantitative PCR of  $\beta$ -propeller gene harbored by *Bacillus* MQH-19. The relative abundance of  $\beta$ -propeller gene in respect to total 16S showed a rapid decrease pattern in time, caused by both bacterial biomass growth and absolute inoculum decrease. Curiously, the absolute increase of 16S gen copies during incubation was reduced by phytate addition to both inoculated and uninoculated samples. Although PPB inoculation resulted in a significant P hydrolysis in manures, the application of PPB inoculated manure to P deficient volcanic soils (Andisol) did not led to a significant response in wheat growth and P acquisition.

P224

### Improving upland rice production and soil nutrients availability by managing mycorrhizal propagules and cropping system

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Upland rice cultivation was developed in several regions of Madagascar to satisfy the food needs of the population. In this way, integrated leguminous plant within rice cropping system constitute one of adopted approaches. This study assessed the effects of the cropping system using upland rice and common bean in mono or mixed-culture and the origin of mycorrhizal inoculum on soil enzymatic activity and on the amount of nitrogen and phosphorus of upland rice aerial part under glasshouse conditions. In this experiment, plants of each treatment were inoculated with mycorrhizal propagules from roots of upland rice and/or common bean previously cultivated on soil amended with mixed phosphorus (Triple superphosphate) and manure fertilizer. Results showed that the mixed culture of upland rice and common bean increased significantly the amount of nitrogen and phosphorus in aerial part of rice up to 1.02 and 1.48 times respectively compared to those measured on monoculture plant. Soil phosphatase activity was also significantly increased by this treatment up to 1.7 times compared to those recorded on monoculture system. Concerning the origin of mycorrhizal inoculum, the bean root and the mixed bean-rice roots inoculum stimulated the soil phosphatase activity under upland rice soil and enhanced the amount of nitrogen and phosphorus in aerial part of this plant in monoculture. These results suggest that integrated common bean on upland rice crop system by establishing mixed culture or by inoculating with mixed roots of rice-bean improves soil nutrients availability and nitrogen and phosphorus uptake by rice plant.

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P225

### Effectiveness of citrate for mobilization of plant-available phosphorus in soil

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Organic anions are widely reported to enhance the availability of phosphate for plant uptake through mobilization of sparingly-soluble forms of P in soil. This is supported by observations that exudation from roots of organic anions, such as citrate, increases in response to P deficiency and that extraction of soils with citrate commonly increases soluble phosphate concentrations. Using a range of soils we found that increased mobilization of soil P occurred from both inorganic and organic forms, with a non-linear response of P release with increasing citrate concentration. Furthermore, extracted organic P exhibited high amenability to hydrolysis by phosphatase enzymes, and especially by phytase. To evaluate the role of root-released citrate in situ, near isogenic lines (NILs) of wheat were developed that differed in citrate efflux from the root tip. This trait was first identified in a Brazilian cultivar (cv Carazinho). Despite differences in citrate release, the NILs showed no consistent differences in biomass production or P uptake when grown in different soils and P treatments in both glasshouse and field experiments. A simple theoretical model, based on the expected mobilization of P in soil from the known citrate release, predicted that between 4 and 30% of the plants P requirement might be achieved through citrate efflux. The volume of soil into which the citrate diffuses was identified as the most important parameter that could explain the apparent lack of response by the plants.

P226

### Aluminum, phosphorus and mycorrhizal symbiosis interaction in wheat cultivars growing in volcanic soils

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Soil acidity is an impediment to agricultural production on a significant portion of arable land worldwide. The main features of these soils are the low levels of available phosphorus (P) and low amounts of exchangeable bases. However, the low productivity of acid soils is mainly due to nutrient limitation and the presence of high levels of aluminium (Al), which causes deleterious effects on plant physiology and growth. Therefore, the objective of this research is to understand under a field experiment the Al-P interactions and how arbuscular mycorrhizal (AM) symbiosis affect the growth and development of wheat when cropped in acidic volcanic soils. Two Al tolerant and P efficient wheat cultivar ('T1' and 'T2') and one cultivar Al sensitive and P no efficient were selected and a field experiments were carried out under two Al saturations (35 and 0.14 %) and three P levels (0, 100 and 200 ppm). The production of phosphatases (P-asa) and glomalin related soil protein (GRSP) was quantified. Also, AM root colonization and AM spores density were determined. Based in root architecture the results showed that 'T1' had high P efficiency by a longer root hair than the Al-sensitive cultivar. The high Al saturation and low P availability affected all factors studied whereas AM fungi colonization was not inhibited under this condition. Mycorrhizal propagules, P-asas and GRSP production were increased at high Al levels and low P. 'T1' and 'T2' had a high GRSP production (10.75 and 11.98 mg g<sup>-1</sup> respectively). In addition, Al-GRSP ranged from 5.6 to 8.3 % of total GRSP being higher in 'T1'. The mycorrhizal symbiosis could be giving an additional P efficiency in Al tolerant wheat cultivars through increased AM propagules, GRSP production and higher Al-GRSP and P-asas. We suggest that the presence of AM fungi populations adapted to these conditions is raising the adaptation of plants present in acidic soils oriented to annual crops.

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### Differences in root morphology of pasture legumes and their association with 'critical' soil phosphorus requirements

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Phosphorus (P) fertiliser is needed for high production by grass-legume pastures in southern Australia. The target for managing the available-P concentration of soil is determined by the high P requirements for growth of the pasture legume. Because the soils are moderate to highly P-sorbing, it is likely that reduced P fertiliser inputs will be achieved if productive legumes with lower P requirements were available. We examined the root morphology of subterranean clover (*Trifolium subterraneum*, a key pasture legume), 2 companion perennial grasses and 11 alternative pasture legumes after growth in soil for 3-4 weeks and found the legumes exhibited a 3.5-fold range in specific root length (SRL: 78-281 m/g DM), a 1.5-fold range in average root diameter (RD: 0.28-0.43 mm) and a 6.1-fold range in root hair length (RHL: 0.12-0.75 mm). Subterranean clover ranked among the legumes that had the lowest SRL (170 m/g), high RD (0.32 mm) and short RHL (0.23). The grasses had relatively high SRL (192-283 m/g), low to high RD (0.24-0.32 mm) and long RHL (0.86-1.0 mm). A grass and 5 legumes (including subterranean clover), selected to represent the range in root morphology traits, were then compared by growing them at 6 soil P levels (deficient to sufficient) for 6 weeks in a controlled-environment cabinet (900  $\mu\text{mol}$  quanta/m<sup>2</sup>/s; 25/15 °C day/night). The 'critical' external P requirement of the species (corresponding to 90% of maximum shoot yield) ranged from 6.6 (*Dactylis glomerata*) to 26.7 mg P applied/pot (*T. subterraneum*). RHL, specific volume of the root hair cylinder (RHC), and specific surface area of RHC of these species were correlated with their 'critical' P requirements.

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### Bacterial beta-propeller phytase transcripts are revealed in nodulated roots of *Phaseolus vulgaris* supplied with phytate

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Although soils are rich on phosphorus (P) compounds, their low availability is a wide spread abiotic constraint that causes plant yield instability. It is recognized that phytate is contributing significantly to soil organic phosphorus compounds. However, to be used by plants, the phytate must be hydrolyzed by specific phosphatase enzymes called phytases. The aim of this work was to determine if the *Bacillus subtilis* phytase (BPP) could make P available from phytate for the benefit of a nodulated legume. The P sensitive recombinant inbred line RIL147 of *Phaseolus vulgaris* was grown under hydroaerobic conditions with either 12.5  $\mu\text{M}$  phytate ( $\text{C}_6\text{H}_{18}\text{O}_{24}\text{P}_6$ ) or 75  $\mu\text{mol}$  Pi ( $\text{K}_2\text{HPO}_4$ ), and inoculated with *Rhizobium tropici* CIAT899 alone, or co-inoculated with both *B. subtilis* DSM 10 and CIAT899. The results indicated that the in situ RT-PCR of BPP genes displayed the most intense fluorescent BPP signal on root-tips. Some BPP signal was found inside the root cortex and the endorhizosphere of the root tip, suggesting endophytic bacteria expressing BPP. However, the co-inoculation with *B. subtilis* was associated with a decrease in plant P content, nodulation and the subsequent plant growth. Such a competitive effect of *B. subtilis* on P acquisition from phytate in symbiotic nitrogen fixation might be circumvented if the rate of inoculation were reasoned in order to avoid the inhibition of nodulation by excess *B. subtilis* proliferation. It is concluded that *B. subtilis* BPP gene is expressed in *P. vulgaris* rhizosphere.

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#### Phytate-mineralizing rhizobia from *Vicia faba* symbiosis in an agro-ecosystem of south of France

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Via its ability to fix atmospheric N<sub>2</sub>, *Vicia faba* - rhizobia symbiosis can contribute to N inputs into crops and soil. However, symbiotic nitrogen fixation can be limited by such abiotic factors as excess of nitrogen or deficiency in phosphorus, or biotic constraint as the ineffectivity of native rhizobia. Myo-inositol hexakisphosphate (phytate) constitutes the main organic P source of soils. Phytases, the only phosphatases able to hydrolyse phytate to inorganic phosphorus, can increase P bioavailability for plants growth and development. Widely distributed among bacteria, b-propeller phytase (BPP) has been largely studied particularly among *Bacillus* species. Histidine acid phosphatases (HAP) represent the most studied and diverse class of phytases found among bacteria, fungi and plants. In order to search for rhizobia with phytase activity, nodule isolates from multi-location observation in an agroecosystem of south of France were screened on phytate selective medium. Among 59 isolates from *V. faba* nodules, 26 were able to solubilize phytate among which six identified as *R. leguminosarum* *bv. viciae* under recA gene sequencing, confirmed their ability to mineralize phytate in liquid culture. Using BPP and HAP specific primers, rhizobial isolates genes were amplified and *V. faba* nodules transcripts were localized in situ. It is concluded that phytate-mineralizing rhizobia exist among *R. leguminosarum* *bv. viciae* spp..

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#### Impacts of rhizobacterial volatiles on the response of the model grass *Brachypodium distachyon* to phosphorus deficiency

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Beneficial biotic interactions in the rhizosphere are increasingly taken into account to achieve efficient and sustainable phosphorus use in agriculture. Plant growth-promoting rhizobacteria (PGPR) have shown their ability to promote plant growth and tolerance to abiotic stresses through multiple mechanisms. Among them, rhizobacterial volatile organic compounds have been involved in growth promotion effects, but they are still poorly documented in monocotyledonous plant species. Their possible effects on root architecture and activity are also essentially unknown, despite their relevance for P-use efficiency. In this context, our research project aims at studying the capacity of a model cereal plant, *Brachypodium distachyon* (L.) Beauv. Bd21, to face P deficiency when exposed to volatile compounds emitted by PGPR. The first step of this project consisted in characterizing Bd21 response to P deficiency. With this aim, Bd21 was cultivated in 2 mm sieved sand and watered with a modified Hoagland solution containing different P concentrations. After 30 days, plant biomass production and allocation, root architecture, total phosphorus content, as well as total and root-secreted acid phosphatase activities were measured. These results allowed us to define optimal P supply and mild to strong deficiency conditions for Bd21 growth, in order to assess PGPR volatile influence on plant response to P deficiency. The next step will use an ex-vitro co-cultivation system allowing volatile-mediated interactions and should help us to evaluate the ability of rhizobacterial volatiles to enhance plant tolerance to P deficiency.

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#### Root system characterization of two grasses functional types under different phosphorus levels

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Two grasses with high frequency and abundance in natural grasslands of the Pampa biome (*Paspalum notatum* and *Andropogon lateralis*) were collected and subsequently cultivated in hydroponics. Young plantlets were transferred to polyvinyl tubes (15 cm  $\Phi$  and 20 cm length) filled with undisturbed soil collected on a natural pasture. For each species, 14 repetitions were done, in which half of them received 60 mg P kg<sup>-1</sup> of soil. The pots were daily irrigated with distilled water. After 25 days of growth, plants were harvest and split into shoot and roots. The root systems were scanned and analyzed with the aid of WinRHIZO software. These species were pre classified in different plant functional types considering its leaf physiological attributes and morphology (leaf dry matter content and specific leaf area). However, those grasses exhibit traits of plasticity to grazing pressure, and could be clustered in the same group. Both species showed increase in root length, root project area and surface area with P supply. However, in *P. notatum* the increase on these characteristics was higher than in *A. lateralis*. Other interesting feature is related to the average of root diameter, which either decreased in *P. notatum* or increased in *A. lateralis* with P supply. This difference was a consequence of higher number of new roots, with fine diameter in *P. notatum* as compared to *A. lateralis*. These data suggest that *P. notatum* plants have high acquisitive capability and consequently with high responsiveness to mineral nutrition. On the other hand, *A. lateralis* roots have high mineral resources storage capability (resources conservation) but show reduced growth response to P supply.

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#### Functional characterization of OsPT13, a rice phosphate transporter required for arbuscular mycorrhizal symbiosis

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Phosphate (Pi) acquisition of crops via arbuscular mycorrhizal (AM) symbiosis gains increasing importance due to limited high-grade rock Pi reserves and demand for environmentally sustainable agriculture. We found that 70% of the overall Pi acquired by rice is delivered via the symbiotic route. Besides, mutations in either PT11 or PT13, two symbiosis specific rice Pi transporters, all affected fungal colonization and arbuscule formation demonstrating that both genes are essential for AM symbiosis. However, for symbiotic Pi uptake, only PT11 is necessary and sufficient. The PT11 lineage of proteins is present in mono and dicotyledons whereas PT13, while found across the Poaceae, is absent from dicotyledons. Further investigations showed that the expression of PT13 is up-regulated by an elevated Pi status of rice roots. The PT13 promoter activity in arbusculated or innermost cortex cells suggests a function of PT13 might be associated with the high Pi concentration of these cells. These data indicated that PT13 might be responsible for the Pi distribution under high Pi condition. To further characterize PT13 function we are currently investigating the subcellular localization and physiological consequences of over-expression of PT13 in *Arabidopsis*. PT13 is localized to the plasma membrane in *Arabidopsis*. Moreover, PT13 overexpression lines showed reduced growth, less shoot Pi concentration and up-regulation of phosphate starvation response genes compared to wild type, suggesting that PT13 overexpression in the plasma membrane affected Pi uptake. The possible function of PT13 as a Pi exporter is under the investigation.

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#### Quantitative prediction of plant P uptake by infinite sink extraction

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Besides batch extraction methods, infinite sink extraction techniques like Fe-oxide papers, anion resin methods and diffusive gradients in thin films are applied to estimate soil phosphorus availability to plants. These methods selectively sample the reversibly adsorbed P fraction of soils by the continuous removal of pore water P, which induces P desorption from soil surfaces. To date, the application of these methods for bioavailability estimation is confined to the use as P indices. In this work we investigated the possibility to use infinite sink extraction to quantitatively predict the P uptake of plants from soil. We used a modified infinite sink extraction protocol to ensure that there were no contributions to the amount of extracted P from microbial degradation of organic P sources and no P solubilisation by reductive dissolution of sorption sites (Fe (oxy)hydroxides). The extracted P quantities were compared to the P uptake of *Zea mays* L. in a pot experiment and to the amount extracted by some standard soil P tests (CAL P, Colwell P,  $\text{Ca}(\text{NO}_3)_2$  P, cDGT P). Plant P uptake was correlated with the P amounts extracted by all extraction techniques. The correlation with P extracted was highest, however, in the infinite sink approach ( $P_{\text{max}}$ ). More importantly, the absolute amounts of P taken up by plant roots quantitatively matched  $P_{\text{max}}$  to a high degree, with the deviation of  $P_{\text{plant}}$  to  $P_{\text{max}}$  being  $\leq 30\%$ . We conclude that plant P uptake may quantitatively be assessed by suitable infinite sink methods. Although this approach may be too tedious for routine use it can be employed as a research tool and as a benchmark test for calibrating routine soil P tests.

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#### Nitrate supply controls phosphorus availability and microbial properties in the rhizosphere of intercropped species

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Positive interactions between cereals and  $\text{N}_2$ -fixing legumes have been recently evidenced for phosphorus (P) but the underlying rhizosphere mechanisms are still unknown. We assumed that rhizosphere acidification induced by the  $\text{N}_2$ -fixing activity of the intercropped legume in interaction with changes in microbial activity is one of the mechanisms enhancing P availability in neutral soils. To further investigate those mechanisms we applied an artificial gradient of nitrate supply since nitrogen nutrition is supposed to drive changes of rhizosphere pH. Durum wheat and chickpea were grown in a mini-rhizobox device as intercrops and sole crops on a luvisol with neutral pH. We applied 3 levels of nitrate supply to obtain a broad range of rhizosphere pH. Phosphorus availability, pH, and abundance of selected microbial groups were investigated in the rhizosphere and bulk soil. Plant biomass, P and N concentration in shoots and roots were measured. Phosphorus availability was enhanced when durum wheat and chickpea were intercropped. A negative relation between P availability and values of pH in the rhizosphere was obtained for chickpea grown alone, explaining the observed substantial increase of available P when no nitrate was added. Values in intercrop were distributed in a zone where root-induced changes of pH did not influence P availability. Abundance and relative abundance of BPP (beta-propeller phytase gene), microbial biomass and CNP stoichiometry were modified in the rhizosphere of intercropped species. Those results suggested that nitrate supply controls the fate of phosphorus and microbial properties in the rhizosphere of intercropped durum wheat and chickpea.

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### Enhancement of phosphate acquisition and delivery to plants using the cyanobacterium *Nostoc punctiforme*

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Microorganisms are integral to the soil phosphorus cycle and as such play an important role in mediating the availability of P to plants. Understanding the microbial contribution to plant P nutrition and the opportunities for manipulating specific microorganisms to enhance P availability in soil is therefore of considerable interest. We propose that the cyanobacterium *Nostoc punctiforme* plays a significant role in enhancing plant growth by enhancing phosphate availability. Further to this, its robust nature and competence to form symbiosis with a broad range of hosts, which potentially includes wheat and canola as well as rice, makes it a potentially more attractive option for commercial development over other bacterial species. The role *N. punctiforme* potentially plays in enhancing phosphate bioavailability to its host during symbiosis are possibly through the two following pathways: (i) phosphate is transferred through *N. punctiforme* into the host, (ii) phosphate is chelated from the silicates in soil where it is bound by extracellularly excreted bioactive enzymes. To determine the mechanisms facilitating phosphate trafficking by *N. punctiforme* to its hosts, we have produced mutants that are deficient of an imperative phosphate transport system component (PstB-) and mutants over-expressing the PstB system component, potentially resulting in respective phosphate deficient and over-accumulating phenotypes. The genetically manipulated *N. punctiforme* phenotypes are to be investigated for their capacity to alter cellular phosphate levels in *Geosiphon pyriformis*.

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### Metabolite profiling of shoot extracts, root extracts, and root exudates of rice cultivars under phosphorus deficiency

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The low P tolerance of plants is mainly determined by the ability of P acquisition from soil and the efficiency of utilization of P absorbed from soil. Organic acids and phosphatase are known as P mobilizing metabolites among various kinds of metabolites in plants. The roles of other metabolites in low P tolerance are as yet not clarified. We applied a metabolite profiling technique to investigate shoot extract, root extract and root exudates of low P tolerant and sensitive rice cultivars under P deficiency. *Oryza sativa* (cv. Koshihikari and Akamai) were grown in a culture solution at P concentration of 0 (P0) and 8 (P8) mg P L<sup>-1</sup> for 5 and 10 days after transplanting. Shoot and root extracts and root exudates were obtained and their metabolites were determined by capillary electrophoresis/time-of-flight mass spectrometry (CE-TOF MS). Cultivar Akamai had higher low P tolerance than cultivar Koshihikari. One hundred and fifty-one, 155, and 136 metabolites were identified in the shoot extract, root extract, and root exudates, respectively. The concentrations of several metabolites were increased, decreased or unchanged at P0. Akamai decreased concentration of more metabolites in shoot extract than those in Koshihikari. Concentration of nucleotide and sugar phosphate changed more at P0. Degree of change was higher in Akamai than in Koshihikari. These results suggest that ability of degradation of metabolites especially P-related metabolites is different between low P tolerant and low P sensitive rice cultivars.

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### Diversity of arbuscular mycorrhizal fungi and P-solubilising bacteria in rubber tree rhizospheres in Thailand

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Rubber tree (*Hevea brasiliensis*) is a crop of major importance for smallholders in Southern Asia because it produces latex, a substantial source of income for farmers. Rubber tree can grow on very poor soils (sandy soils, low fertility, subject to erosion and leaching of applied fertilizers), which are unsuitable for other commonly cultivated cash crops. It covers 2.7 million ha across Thailand, but its establishment in areas with very poor soils, especially in Northeast Thailand, represents a major potential for increased production. The important roles of rhizosphere microbial communities in supporting soil fertility and plant nutrition has been widely recognized. In particular, root-interacting P-solubilizing bacteria (PSB) and arbuscular mycorrhizal fungi (AMF) contribute to plant P nutrition by increasing mineral nutrient availability and by enhancing plant nutrient uptake. The diversity of AMF in roots and of PSB in rhizosphere soil was assessed along a chronosequence of rubber tree plantations (3, 6 and 16 year-old) and compared to cassava fields. AMF diversity was assessed by 454 sequencing of SSU 18S rDNA. PSB strains were characterized after culturing on selective media. AMF communities in cassava roots were twice as rich as in rubber tree samples. AMF diversity was not affected by the age of rubber trees, but was related to the soil P content. The improved understanding of the diversity of root- or rhizosphere-associated microbes will contribute to the development of alternative sustainable practices to improve and sustain soil fertility.

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### The role of plant and bacterial organic anion production in plant access to insoluble organic phosphorus

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The inefficient use of phosphorus (P) in agricultural systems has led to the global depletion of mineral phosphate supplies and surface water pollution. External fertilizer requirements and nutrient loss could be minimized by improving crop access to native soil P. Phytate (IHP) is the dominant organic P compound in most soils and is converted to plant-available orthophosphate (Pi) by plant or microbial phytases. The bioavailability of insoluble IHP may be improved in the presence of low molecular weight organic anions (OAs) from plant or microbial sources. The influence of plant and bacterial OAs on the solubility and bioavailability of IHP was assessed in tobacco (*Nicotiana tabacum*) grown on insoluble P sources (IHP, Pi), which were either sorbed to goethite (Gt) or precipitated with calcium (Ca). Wild-type (WT) tobacco was transformed to over-express *Peniophora lycii* phytase (PHY) and Multidrug-And-Toxic-Compound-Extrusion-type transporters (CIT), leading to increased phytase activity (33-fold) and citrate exudation (2.6-fold), respectively. Consistent with exudate characteristics, PHY plants accessed 4-fold more P from Gt-IHP, whereas CIT plants incorporated 2-fold more shoot P relative to the WT. The PHY plant-line incorporated >20-fold more shoot P compared to other plants grown on Ca-IHP. Transgenic plants inoculated with *Pseudomonas* sp. incorporated >7-fold more P from Ca-IHP, suggesting a synergistic effect of plant exudates and microbial products on plant acquisition of insoluble organic P. A combined strategy of P solubilization and hydrolysis is therefore suggested as a suitable target for improving crop utilization of native soil P.

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### Understanding the genetic control of rhizosheath formation and impacts of multiple stress on phosphorus acquisition

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There is urgent need for simple rapid screens of root traits that improve acquisition of nutrients and water. Temperate cereals produce rhizosheaths, a trait first noted on desert species over 100 years ago and thought to be limited to grass species of the Poales order. Here we screen association genetics populations of barley for rhizosheath weight and derive QTL and candidate genes. We assess whether rhizosheath weight was correlated with plant performance and phosphorus (P) uptake under combined drought and P deficiency. Rhizosheath weight was investigated in relation to root hair length, and under both laboratory and field conditions. We go on to perform a phylogenetic study of angiosperm species to assess whether this trait is isolated to the Poales. Our data demonstrate that rhizosheath weight was correlated with P uptake under dry conditions and that the differences in rhizosheath weight between genotypes were maintained in the field. Rhizosheath weight also varied significantly within barley populations, was correlated with root hair length, and was associated with a genetic locus (QTL) on chromosome 2H. Putative candidate genes were identified. We demonstrate that a number of other angiosperm orders have rhizosheaths, which include orders containing domesticated crop species. Rhizosheath weight is easy and rapid to measure and associated with relatively high heritability. Breeding cereal genotypes for beneficial rhizosheath characteristics is achievable and the same is possible for other crop species. Understanding of this trait could contribute to agricultural sustainability in nutrient and water-stressed environments.

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### Chemical imaging of phosphorus dynamics in the rhizosphere

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Phosphorus (P) is one of the most limiting nutrients to plant growth and crop yield. To increase P uptake, plants can actively solubilise P by releasing e.g. protons or organic anions. Innovative approaches for 2D chemical imaging of rhizosphere processes can be used to investigate the complex interactions of plant roots and soil. Diffusive gradients in thin films (DGT) combined with laser ablation – inductively coupled plasma mass spectrometry (LA-ICP-MS) allow for the 2D visualisation of the distribution of cationic and anionic solute species in the rhizosphere. Furthermore, pH-sensitive planar optodes can map the rhizosphere pH. The aim of this work was the development and testing of a combined setup of DGT-LA-ICP-MS and pH-sensitive planar optodes to investigate biogeochemical processes controlling P solubility in the rhizosphere. This was achieved by simultaneous imaging of P, Al, Ca, Fe and pH to assess if P solubilisation is related to the dissolution of P sorption sites (Fe- and Al-(oxy)-hydroxides) and of soil P minerals such as Ca-, Fe-, Al-phosphates. To this end, a plant experiment using *Triticum aestivum* L., *Fagopyrum esculentum* L., and *Lupinus albus* L. on calcareous and non-calcareous soils was conducted. We show the applicability of simultaneous 2D chemical imaging of soluble Ca-, Fe-, and Al together with P using DGT-LA-ICP-MS as well as 2D mapping of plant induced pH-changes based on planar optodes. We conclude that this combined imaging approach offers great potential for investigating P depletion around roots, mineral dissolution due to root activities as well as plant induced pH-changes at sub-mm resolution.

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### A conceptual model of root hair ideotypes for future agricultural environments

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Phosphorus (P) commonly limits crop yield and is frequently applied as fertilizer derived from rock phosphate, a diminishing resource. Plants have evolved a number of mechanisms to improve P-acquisition, including the proliferation of root hairs. Through an understanding of these mechanisms it is possible to improve the P-efficiency of crops. Observations of interactions between root hair traits and P-acquisition made from experimentation utilising a genetically nearly identical mutant population of barley (*Hordeum vulgare* L.), along with conclusions taken from the literature have been synthesised to produce a conceptual model of root hair ideotypes. Understanding the impacts of root hair presence and length on P-limited yield and interactions with Arbuscular Mycorrhizal fungi allow us to postulate beneficial modifications to root hair traits and their implications for plant yield. Possible cost/benefit relationships of such traits are described, recommending potential ideotypes encompassing important traits associated with root hairs and P-acquisition, which represent targets for research and breeding programmes in future crop plants. Using the model we have identified and focused on the potential to utilise low cost roots in improving phosphorus use efficiency. Screening has begun for roots with increased longevity, larger cells, reduced cell file number or aerenchyma which all have potential to improve P-acquisition at reduced cost to the plant. Given finite resources and an increasing global population, crop plants with such traits have the potential to play an important role in improving crop varieties and the long term sustainability of agriculture.

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### Changes in wheat root morphology under different phosphorus fertiliser supply rates

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Wheat is one of the most important food crops in the world, thus improving its phosphorus utilisation efficiency is an important aspect of the drive towards sustainable intensification, and requires better understanding of the root response to soil phosphorus supply. To overcome low phosphorus availability many plants have developed adaptive mechanisms for maintaining phosphorus homeostasis. One of the main morphological mechanisms involves increased root growth or enhanced root hair production, leading to increased root-soil contact which is an important factor controlling the uptake of phosphorus in soils. Soil organic phosphorus can also be utilized following hydrolysis by phosphatase enzymes. The responses of wheat (*Triticum aestivum* L.) root traits, phosphatase activity and phosphorus forms to different levels of phosphorus fertiliser supply in two contrasting soil types from the UK were investigated by growing wheat for 3 weeks in rhizotrons containing soils fertilised with either 0, 10, 25, 50, 100, 200 mg P kg<sup>-1</sup> dry soil. Significant differences in root morphological responses occurred in the two soil types, but generally greater root length density was observed at low P fertilizer rates, and greater root hair length and density occurred at high rates, with thresholds for significant changes varying between 25 and 50 mg P kg<sup>-1</sup> treatments. In conclusion, wheat roots respond morphologically to phosphorus fertilizer rates, and thresholds for responses vary in different soils.

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#### Effect of copper application on P concentration of pistachio seedlings in some calcareous soils of Iran

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Pistachio is one of the most important commercial trees grown in Iran, Turkey and the USA. Pistachio is considered a potential crop for many arid and semi-arid regions. In pistachio orchards, micronutrients uptake due to high pH, high amount of calcium carbonate and soil and water salinity is a serious problem. Therefore, pistachio trees are encountered with deficit micronutrients such as copper. This study was conducted to study the effect of soil copper application on phosphorus concentration of pistachio root, stem and leaf. The experimental design was a  $3 \times 12$  factorial experiment in a completely randomized design with three replications in greenhouse conditions. Treatments were 3 levels of copper (0, 5, and 10 mg Cu kg<sup>-1</sup> soil as CuSO<sub>4</sub>) and 12 soils with different physical and chemical properties. Pistachio seedlings were harvested 6 months after planting and P concentration was determined in root, stem and leaf. The results indicated that copper application had a significant effect on leaf and stem P concentration. The highest stem and leaf P content was observed at level of 10 mg Cu kg<sup>-1</sup> soil. Soil copper application had no significant effect on root P concentration. Also, P concentration of leaf, stem and root in various soils was different. For example, the highest leaf P content was observed in sandy soils and the lowest leaf P concentration was observed in clay soils.

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#### Effect of residual copper on P concentration of pistachio seedlings in some calcareous soils of Iran

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Pistachio is one of the most important commercial trees grown in Iran, Turkey and the USA. Pistachio is considered a potential crop for many arid and semi-arid regions. Soil in pistachio growing area is characterized by high pH, high amount of calcium carbonate and low organic matter. In these soils, deficiency of micronutrients can become severe. At present, in many pistachio orchards of Iran, it's common to use excessive chemical fertilizers each year. Therefore, it is necessary to perform a comprehensive study about residual effects of chemical fertilizers in different soils. This research was carried out with the aim of study the effects of residual copper on phosphorus concentration of pistachio seedlings in soils with different physical and chemical properties. In the first year, 12 soil types with different physical and chemical characteristics were collected from pistachio orchards in Rafsanjan region. Three copper levels (0, 5 and 10 mg kg<sup>-1</sup> soil as CuSO<sub>4</sub>) were added and pistachio seedlings were grown for six months. The experiment was conducted on a completely randomized design with three replications. After removal of roots, residual soil of previous cultivation was passed through a two millimeters sieve. After seed germination, eight seeds were planted in each pot at a depth of 3 cm, pots were irrigated with distilled water and soil moisture was reached to the field capacity. About a month after cultivation the number of plants per pot was reduced to four. At the end of the growth period (30 weeks after planting) pistachio seedlings were harvested and P concentration was determined in root, stem and leaf. The results indicated that residual copper had a significant effect on leaf and stem P concentration. The highest stem and leaf P concentration was observed at level of 10 mg Cu kg<sup>-1</sup> soil. Soil copper application had no significant effect on root P concentration. Also, P concentration of leaf, stem and root in various soils was different.

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#### Effect of foliar application of calcium and zinc on phosphorus content of pistachio buds and leaves

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Deficiency of some macro and micronutrients are widespread throughout all pistachio growing areas of Iran. In areas where pistachio is planted, salinity and high pH of calcareous soils limit the availability and uptake of elements. Soil application is most common method to supply essential nutrients to plants, however, higher plants can also absorb mineral nutrients when applied as foliar sprays in appropriate concentrations. This study was conducted to investigate the effects of foliar application of zinc and calcium on phosphorus concentration of pistachio buds and leaves. The experimental design was a 4\*3 factorial experiment in a randomized complete block with three replications. Each replication consists of three pistachio trees cv Ahmadi Aghayi. Treatments were four levels of calcium (0, 1, 2, and 3 percent as calcium chelate) and three levels of zinc (0, 5, and 7 percent as zinc sulfate). The results indicated that foliar application of Ca and Zn had a significant effect on pistachio P concentration of leaves and buds. Calcium foliar spray significantly decreased P concentration of buds and leaves whereas, foliar application of zinc significantly increased P concentration of leaves and buds.

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#### A Pi starvation responsive gene, GmMDH12, mediating malate synthesis and facilitating nodule growth in soybean

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Most legumes could develop a symbiotic organ, nodule, with rhizobia to fix atmospheric nitrogen (N). It has been documented that low phosphorus (P) availability severely limits nodule growth and N fixation, but its underlying mechanisms still remains unclear. Recently, we identified 44 phosphate (Pi) starvation responsive proteins from soybean nodules through proteomic analysis. Among them, a malate dehydrogenase, GmMDH12, was dramatically enhanced by Pi starvation in nodules. Since malate is the main carbon source for growth of rhizobia in nodules, malate synthesis might directly affect nodule growth and thereby N fixation. We further cloned GmMDH12 gene and found that the expression of GmMDH12 were also elevated by P deficiency in nodules. GUS staining of soybean transgenic hairy roots with GmMDH12 promoter fused GUS, revealed that GmMDH12 mainly expressed in root tips, vascular tissues of roots and nodules. Biochemical analysis of recombinant GmMDH12 demonstrated that it favored to catalyze the reduction of oxaloacetate to malate in vitro. Furthermore, GmMDH12 overexpression increased malate concentration in both roots and nodules, and subsequently increased nodule number and nodule weight of the transgenic composite soybean plants, especially when supplied with sufficient P. Taken together, we conclude that GmMDH12 might function in modifying malate synthesis in both roots and nodules at different P supply conditions, which is critical for developing adaptive changes through carbon metabolic pathway to respond Pi starvation in nodules.

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### Elevated CO<sub>2</sub> favors microbial immobilization of phosphorus in the rhizosphere of crop plants

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Increasing atmospheric CO<sub>2</sub> concentrations are expected to increase the demand for phosphorus (P) by crops, because elevated CO<sub>2</sub> (eCO<sub>2</sub>) enhances plant growth and biomass production. Understanding the mechanisms by which plants access soil P pools under eCO<sub>2</sub> is important for developing P management strategies to cope with global climate change. The aim of this study was to investigate how eCO<sub>2</sub> affects P transformation in the rhizosphere. Chickpea (*Cicer arietinum* L.) and wheat (*Triticum aestivum* L.) plants were grown in rhizo-boxes containing Vertosol soils with two contrasting P fertilizer histories, and exposed to ambient (380 ppm) or eCO<sub>2</sub> (700 ppm) for 6 weeks. Wheat plants were labelled with <sup>13</sup>CO<sub>2</sub> to quantify the flow of plant carbon in the plant-soil continuum. Elevated CO<sub>2</sub> enhanced the accumulation of organic P in the rhizosphere of wheat and chickpea grown in soils with high P status. Elevated CO<sub>2</sub> also significantly increased microbial P in the rhizosphere. The two crop species however did not differ in their ability to access soil P pools irrespective of CO<sub>2</sub> treatment. Elevated CO<sub>2</sub> increased <sup>13</sup>C enrichment in the rhizosphere of wheat and the number of rDNA copies from the <sup>13</sup>C-DNA fraction. Furthermore, the microbial biomass C and the respiration rate in the rhizosphere also increased, along with the organic P, under eCO<sub>2</sub>. These results suggest that eCO<sub>2</sub> increases photosynthetic C efflux from roots, which in turn stimulates microbial activity and leads to P immobilization in the rhizosphere

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### The interaction of AM fungi with phosphorus resource determine plant composition and productivity in desert grassland

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Plant diversity and species richness is essential to ecosystem stability and productivity. Previous studies suggested arbuscular mycorrhizal fungi (AM fungi) can determine plant community structure, but the mechanism that AM fungi influence plant community composition is still not well understood. Using a microcosm system, we demonstrate that AM fungi and phosphorus resource composition can alter plant community composition. In the absence of AM fungi, the plant diversity and species richness decreased with increasing P resource diversity. When P resource from one increased to four, plant diversity and species richness declined 29% and 37% respectively. Compared to without AM fungi treatment, AM fungi highly increased plant species diversity and richness. In the presence of AM fungi, the mean plant diversity declined less than 2% when P resource from one increased to four, while the species richness increased 13%. Furthermore, the significant differences were observed among different AM fungi treatment under the same P treatment. The results indicate that the plant community was influenced by AM fungi and P resources. Our results suggest that the interactions of soil P resource-AM fungi-Plant play a vital role in determining plant community composition and productivity.

### The modern breeding selecting of maize is not always against arbuscular mycorrhizal association from 1950s to 2000s

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Arbuscular mycorrhizal fungi have been considered as parasitic fungi for plants in high P soil condition. Breeding under high soil fertility selecting pressure may reduce mycorrhizal responsiveness (MR). However, few evidences show the role and to what extent that the mycorrhizal pathway (MP) contribute to plant P uptake in high P soil. The MR and the contribution of MP to P uptake among old and new Chinese maize genotypes (released from 1950s to 2008) in low (4 mg/kg), optimal (8 mg/kg) and high (50 mg/kg) Olsen-P soils were compared to understand the constitution of maize P uptake efficiency. The results showed: (1) Old and newly released cultivars differed in MR under low and high P soils. New variety had higher MR than the old in the high soil. (2) Principal component (PC) analysis showed that the first PC comprised morphological and physiological traits of maize roots, and the second PC comprised mycorrhizal traits. The opposite was the case, however, in high-P soil. (3) The MP contribution in all cultivars was the highest in growth-optimal soil. There were no significant differences between the old and new cultivars in low or optimal P soil, while it was higher in new variety than the old variety in high P soil. The changes of the genes encoding the P transporters of DP were consistent with MP at transcriptional levels in the new or old cultivars in both the high and the low P soils. It is concluded that the P uptake efficiency of DP was affected by MP in maize. The modern breeding selecting of maize does not always be against AM association from 1950s to 2000s and MP play positive roles in promoting the growth of some maize genotypes in high P soil.

### Native arbuscular mycorrhizal fungi enhanced phosphorus capture and regulated ions uptake of crops in salinity soils

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Previous study has proved that salt stress on crops could be alleviated by AM fungi and crops inoculated with AM fungi had higher biomass. However, the results published in function of AM fungi do not appear to be convincing as most of them were finished in greenhouse or laboratory. It is relatively difficult to quantitative AM fungal function in natural conditions because of limited method. To determine function of native AM fungi, we used 2 common crops in salinity soils seeded in one modified core systems, constructed by polyvinylchloride (PVC) tube and 30  $\mu$ m nylon mesh. No difference was found in growth of beet (non-mycorrhizal plant) indicated that core system with 30  $\mu$ m nylon mesh could provide a relative reliable control for quantitative AM fungal function. Mycorrhizal colonization of cotton and maize in rotated cores (relative control, crops without AM fungi) was significantly lower than that in static cores (crops with AM fungi) in all salinity soils. Crops with AM fungi grew better than that without AM fungi with significantly promoted biomass and P uptake in all 3 experiments. In Exp 1 and 2, AM fungi colonization advanced cotton ability to capture P by promoted root length and proline concentration and photosynthetic capacity by expanded leaf area. Salt ions in crops with AM fungi were regulated by selective uptake more K<sup>+</sup> and less Na<sup>+</sup> to keep higher K<sup>+</sup>/Na<sup>+</sup> ratio. Our results suggest that native AM fungi could promote crops growth by enhancing P capture and regulating ions uptake in salinity soil with stronger salt tolerance.

### Challenges and opportunities on the use of biofertilizers: Examples from Senegal and Kenya

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Not only phosphorus (P) bioavailable in soil is very low but phosphate fertilizing efficiency is also low. Consequently, annual world P demand increases predicting phosphorus stock end in the coming 125 years. In addition to that, the high cost of chemical fertilizers obliges most Sub Sahara African smallholder farmers to do not use fertilizers which ultimately results in poor yields. In this paper, we present opportunities and challenges of using bio fertilizers as sustainable way of alleviating soil P deficiency effects in Kenya and Senegal. In Kenya where soil P deficiency has been identified as the biggest challenge of crop productivity increases, we share results on the use of commercialized arbuscular mycorrhizal inoculants to replenish soil P. While in Senegal known having huge quantities of P rock deposit and important quantities of feed stock material that can be charred (biochar), we present results on the capacities of biochar to improve P availability for plant cultivated in sandy soil. Results from both countries show that current expectations on the use of bio fertilizers are numerous and justified. However challenges on sustainable agriculture through the use of the called bio fertilizers especially mycorrhizal inoculants and biochar are still ahead.

### Do nitrogen-fixing plants show higher root phosphatase activity on phosphorus-poor soils?

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Symbiotic dinitrogen (N<sub>2</sub>) fixation in N<sub>2</sub>-fixing plants may enhance plant performance on N-poor soils, but may not be favoured on phosphorus- (P) poor soils, due to its high P costs. Yet surprisingly, N<sub>2</sub>-fixing species are abundant in ecosystems with N-rich soils such as lowland tropical rainforests, where P is likely to limit plant growth. A prominent hypothesis seeking to explain this paradox is that N<sub>2</sub>-fixing plants have a greater ability to acquire organic P through higher root phosphatase activity. However, evidence to support this hypothesis remains limited. We measured extracellular root phosphomonoesterase (PME) activity from 18 species of N<sub>2</sub>-fixing (including legumes and non-legume *Allocauarina* spp.) and non-N<sub>2</sub>-fixing species along a soil age gradient in Western Australia that shows a ~40-fold decline in total soil [P] from the youngest to the oldest soils, leading to some of the most P-impooverished soils found in any terrestrial ecosystem. In support of the hypothesis, we found that N<sub>2</sub>-fixing legumes had higher PME activity than co-occurring non-legumes on all sites, and that the difference in PME activity between legumes and non-legumes increased with declining soil [P]. However, PME activities of N<sub>2</sub>-fixing *Allocauarina* spp. (which form associations with *Frankia*) were consistently low across all soils, which do not support the hypothesis. We conclude that the high root phosphatase activity of legumes on P-poor soils is likely a phylogenetically conserved trait that is not necessarily linked to their N<sub>2</sub>-fixing ability.

P253

### Hyphosphere interactions between an AM fungus and a PSB promote phytate mineralization

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Both arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) are involved in phosphorus (P) mobilization and turnover but the influence of their interaction on organic P mineralization in the root free soil (hyphosphere) have been little studied. We investigated the interactive effects of an AM fungus (*Rhizophagus irregularis*, RI) and/or PSB (*Pseudomonas alcaligenes*, PA) on phytate mineralization and subsequent transfer to the host plant (*Medicago sativa*) using a two-compartment microcosm with a central 30  $\mu\text{m}$  nylon mesh barrier. The root growth compartment containing 5 mg inorganic P (Pi,  $\text{KH}_2\text{PO}_4$ )  $\text{kg}^{-1}$  soil was inoculated with RI or uninoculated and the AM fungal hyphal soil containing 75 mg organic P (Po, Na-phytate) plus 0 or 5 mg Pi  $\text{kg}^{-1}$  soil was inoculated with RA or uninoculated. Sole inoculation with RI increased shoot P content compared with the uninoculated treatment and dual inoculation with both RI and PA did not increase shoot P compared with sole RI inoculation. Sole PA inoculation significantly increased microbial biomass P (MBP). Compared with sole PA inoculation soil MBP increased under zero-Pi addition but decreased under 5 mg Pi  $\text{kg}^{-1}$  soil addition in the dual inoculation RI/PA treatment. The uninoculated microcosms had the lowest acid phosphatase activity and the highest phytate-P remaining in the soil. Inoculation with PA led to higher acid phosphatase activity and lower phytate-P than did RI. Dual RI/PA inoculation had the highest acid phosphatase activity and the lowest phytate-P remaining in the soil. Addition of 5 mg Pi  $\text{kg}^{-1}$  soil to the hyphal compartment decreased phytate-P remaining in the RI and/or PA treatments. The phytate-P remaining in the soil was negatively correlated with soil acid phosphatase activity or MBP in the presence of RI but there was no correlation between shoot P and soil phytate-P. In conclusion, our results indicate that the mineralization of soil phytate was promoted by the interaction between the AM fungus and its hyphosphere PSB.

P254

### Comparison of plant growth and root-released organic acids of major Norwegian crops under phosphorus deficiency

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Phosphorus (P) is an essential macronutrient for plant growth and development. The world population is expected to reach an estimated 9.2 billion by 2050, which means food production globally has to increase by 70% in order to feed the world. P deficiency is one of the major limitations to crop productivity globally. Root exudates play an important role in releasing soil P and increasing P availability to plants. Here, we compared the effects of P supply on plant growth and organic acids (OAs) secretion from the roots of rape, potato, wheat and barley (two dicots and two monocots), which are important crops in Norway, using hydroponic culture. Similarly with other reports, our results showed that P starvation caused different extents of phenotypical alterations like biomass reduction, anthocyanin accumulation, and increased root length and root/shoot ratio in all of the four crops. The results also showed that P deficiency induced exudates of malic acid, citric acid and succinic acid, but with some differences among different crops. The total amount of OAs released by plant roots is rape>barley>wheat>potato. Moreover, our preliminary results indicated that P deficiency induced a pronounced boron uptake in dicots (rape and potato), particularly in plant shoots. Interestingly, in our system, over-accumulation of boron by adding more exogenous  $\text{H}_3\text{BO}_3$  inhibited P deficiency induced OAs significantly in rape. All these results suggest that different plant species have different physiological responses to P deficiency. Those results are also of importance for understanding genetic diversity and adaptation of crops to P deficiency, and for effective utilization of P in future agriculture. Our ongoing study on physiology and molecular analyses will provide more information about the relationship between P uptake and organic acids produced by plant roots.

P255

Arbuscular mycorrhiza and P uptake of cucumber (*Cucumis sativus* cv. Nahid) under different levels of sodium bicarbonate

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To study the effect of arbuscular mycorrhiza (*Glomus mosseae*) on P uptake of greenhouse cucumber (*Cucumis sativus* cv. Nahid) under bicarbonate stress originated from irrigation water, a completely randomized design was adopted in an experiment with four levels of bicarbonate in irrigation water (0, 400, 800 and 1200 mg/L), two levels of mycorrhiza (inoculated and un-inoculated plants) with five replications. Results showed that at highest level of bicarbonate, leaf, stem and root P concentration were decreased by 56, 31 and 28% respectively. Mycorrhizal treatment improved P status of cucumber plants considerably. In brief, inoculated plants had 43% more P in comparison with control.

P256

From A to B: mechanisms in A (*Aspergillus*) to phosphate release for B (Barley)

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Phosphorus is an essential nutrient for plant growth and development, and deficiencies in soil phosphorus limit agricultural crop yields worldwide. Rock phosphate, the basic constituent of many inorganic phosphorus fertilizers, is an increasingly expensive resource with a low efficiency of use. Naturally-occurring fungal interactions with such sparingly-soluble phosphorus sources could enhance the release of soluble phosphate into the soil nutrient cycle and promote plant growth. This research aims to characterize the mechanisms involved in fungal phosphate solubilization, and examine the abilities of the fungus *Aspergillus niger* to enhance plant phosphorus uptake and growth under phosphorus-deficient conditions and in the presence of inorganic phosphorus sources, including rock phosphate. The results presented will include fungal growth and solubilization of phosphorus sources, phosphate mineral transformations and the underlying mechanisms, as well as the influence of such activities on growth and uptake of phosphates by plants. Our results varyingly show that soil fungi are able to solubilize sparingly-soluble phosphate minerals, including those in rock phosphate, and that this ability can be beneficial to plant acquisition of phosphorus. Future agricultural practices may benefit from greater understanding of the interactions between plants, fungi and phosphorus-containing minerals.



### **Theme 3 – Phosphorus utilization and signaling in plants**



### **Theme 3 – Phosphorus utilization and signaling in plants**

#### **Keynote presentations**

K301

### Enhancing internal phosphorus use efficiency in crops: concepts and approaches

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Enhancing phosphorus (P) uptake efficiency of crops alone will lead to higher rates of soil P mining in low-input agriculture. Improvements in internal P use efficiency are therefore required, but research to date has not led to any practical advances. What traits are actually desirable? What approaches can we use to identify and exploit useful traits? We have recently revisited the definitions of internal P use efficiency to investigate which criteria may be useful in a crop breeding context. While the ultimate goal is to achieve higher crop yields per unit of P acquired by plants, simply measuring grain yield per unit of P uptake has not led to any specific improvements in internal P use efficiency. We propose that two separate traits may be desirable: firstly, the capacity of plants to produce large amounts of vegetative biomass per unit of P acquired (high vegetative PUE); and secondly, a reduction in P translocation to grain during reproductive growth phases (a low grain P trait). From a conceptual viewpoint, these traits go hand-in-hand and would not only reduce the amount of P required by crops to produce maximum yields, but would reduce the export of P from fields in harvested grains. These concepts, and approaches to capture these traits for use in breeding, are discussed.

K302

### Regulation of phosphate homeostasis: microRNA-mediated surveillance of phosphate transport

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Phosphorus is acquired and translocated by phosphate (Pi) transporters in plants. Regulation of these transporters plays a central role in the maintenance of whole-plant Pi homeostasis especially when Pi availability in the soil is changed. Recently, we revealed the interplay of two Pi starvation-induced microRNAs, miR399 and miR827, in regulating Pi transport activities. In *Arabidopsis*, miR399 and miR827 direct the cleavage of PHO2 and NLA transcripts, respectively. PHO2 encodes a ubiquitin E2 conjugase associated with endomembranes while NLA encodes a RING-type ubiquitin E3 ligase localized in plasma membranes. Loss-of-function of PHO2 or NLA results in excess Pi accumulation. PHO2 modulates the activity of xylem loading of Pi via mediating the protein degradation of PHO1. Moreover, PHO2 and NLA regulate the degradation of PHT1 Pi transporters at different cellular compartments. PHO2 regulates the abundance of PHT1s in the secretory pathway destined for plasma membranes whereas NLA controls the abundance of PHT1s in plasma membranes by endocytosis. Analyses of *nla pho2* mutants suggest that NLA and PHO2 function cooperatively to control Pi acquisition. Upon Pi deprivation, upregulation of miR399 and miR827 suppresses the expression of PHO2 and NLA, respectively, thereby alleviating the negative regulation of PHO1 and PHT1s and reinforcing the activities of Pi translocation and uptake. Intriguingly, miR399 can move systemically from shoots to roots, serving as a signal to coordinate the Pi demand in shoots and Pi supply in roots. Our studies demonstrate that miRNAs modulate the functioning of the membrane-associated ubiquitin machinery to regulate Pi homeostasis.



### **Theme 3 – Phosphorus utilization and signaling in plants**

#### **Oral presentations**

O301

**A rice cis-natural antisense RNA acts as a translational enhancer for its cognate mRNA and contributes to Pi homeostasis**

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Cis-natural antisense transcripts (cis-NATs) are widespread in plants and often associated with down-regulation of their associated sense genes. We found that a cis-NAT positively regulates the level of a protein critical for phosphate homeostasis in rice. OsPHO1;2, a gene involved in phosphate loading into the xylem, and its associated cis-NATPHO1;2 are both controlled from promoters active in the vascular cylinder of roots and leaves. While the OsPHO1;2 promoter is unresponsive to the plant phosphate status, the cis-NATPHO1;2 promoter is strongly up-regulated under phosphate deficiency. Expression of both cis-NATPHO1;2 and the OsPHO1;2 protein increased in phosphate-deficient plants, while OsPHO1;2 mRNA level remained stable. Down-regulation of cis-NATPHO1;2 expression by RNA interference resulted in a decrease in OsPHO1;2 protein, impaired the transfer of phosphate from root to shoot and decreased seed yield. Constitutive over-expression of NATPHO1;2 in trans led to a strong increase of OsPHO1;2 protein, even under phosphate-sufficient conditions. Under all conditions, no changes occurred in the level of expression, sequence, or nuclear export of OsPHO1;2 mRNA. However, expression of cis-NATPHO1;2 was associated with a shift of both OsPHO1;2 and cis-NATPHO1;2 towards the polysomes. These findings reveal an unexpected role for cis-NATPHO1;2 in promoting OsPHO1;2 translation and affecting phosphate homeostasis and plant fitness.

O302

**Methylomes of plants starved for Pi reveals dynamic DNA methylation changes at Pi starvation responsive genes**

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Cytosine DNA methylation is a covalent modification of the genome involved in regulating the expression of coding and non-coding genomic elements. Here, we demonstrate that phosphate (Pi) starvation in rice leads to diverse dynamic changes in DNA methylation. To do so, 36 DNA methylomes from rice seedlings grown under medium- and long-term Pi starvation and Pi re-supply were profiled using single-base resolution whole-genome bisulfite sequencing, identifying more than 200 conserved differentially methylated regions (DMRs) in roots in response to long-term Pi starvation. Most of these DMRs displayed hyper-methylation in response to Pi starvation and occurred within transposable elements (TEs). In contrast, most of the hypo-methylated phosphate starvation induced DMRs rarely overlapped with TEs and were preferentially intragenic. In addition, half of the DMRs were associated with genes being significantly induced upon long-term Pi starvation, including most of the key factors involved in Pi homeostasis. Interestingly, similar analyses performed in Arabidopsis roots revealed only two phosphate starvation induced DMRs, at known regulators of Pi homeostasis. Altogether our data demonstrates that an abiotic stress, namely Pi starvation, induced dynamic and widespread changes in DNA methylation, to an extent which is species specific, to potentially regulate the expression of specific repeat elements proximal to highly induced genes, as well as to control correct gene expression in response to Pi stress. This finding paves the way for the development of new strategies to develop crops with increased nutrient use efficiency.

O303

### Multiple loci identified in a Genome-Wide Association Studies of internal phosphorus utilization efficiency in rice

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The realization that phosphorus (P) is a non-renewable, finite and increasingly costly resource, has renewed interest in breeding more P-efficient rice varieties. Internal P efficiency (PUE) is a principal concern because there has been no progress in breeding for high PUE so far as most resources have been directed toward enhancing P acquisition. In this study our objective is to assess the genotypic variation present for PUE within the rice gene pool and to map loci controlling PUE through Genome-Wide Association Studies (GWAS). A diverse set of 292 rice accessions were grown in nutrient solutions, assuring each genotype had access to an equal and low (0.8 mg) amount of P. PUE was then estimated as biomass produced per P taken up. GWAS using a 44K SNP data set identified most influential loci for PUE on chromosomes 1, 4, 11, and 12. Highest PUE (2.54 g biomass mg<sup>-1</sup> P) was detected in a group of accessions having a rare haplotype on chromosome 11. This group belonged to the aus subspecies of rice. A second influential and rare haplotype on chromosome 1 further increased PUE in a group of indica accessions. A unique SNP pattern surrounded three candidate genes at this locus, possibly suggesting that they were introgressed from a distant relative and maintained through positive selection in the group of high-PUE accessions. Expression profiling of these candidates identified differential expression patterns for one of the genes and its putative role in P redistribution during P deficiency stress will be discussed.

O304

### *Arabidopsis* HPS10/ALS3 interacts with AtSTAR1 to serve as a signaling hub for responses to P deficiency and Al toxicity

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In most acid soils, phosphorus (P) deficiency and aluminum (Al) toxicity are the two most important constraints to plant growth and crop productivity. In the past, most studies on plant responses to P deficiency and Al toxicity were conducted separately. In fact, it has long been observed that P and Al interact in affecting plant growth and metabolism. Some evidence indicates that such interactions might result from the sharing of signalling components involved in plant response to both of these nutritional stresses, but the molecular identity of such components is unknown. By analysing the *Arabidopsis* mutant hps10 (hypersensitive to Pi starvation10), we show that HPS10 encodes the previously characterized ALS3 (ALUMINUM SENSITIVE 3). HPS10 is an ABC (ATP-Binding Cassette) transporter-like protein that contains a transporter domain but lacks an ATP-binding domain. In roots, HPS10 is specifically expressed in the vascular tissues; under P deficiency, however, its expression domain extends to all cell layers of roots. HPS10-GFP fusion proteins are localized in both the plasma membrane and cytoplasm. AtSTAR1 is also a transporter-like protein that contains an ATP-binding domain, but lacks a transporter domain, and is localized in the cytoplasm. Interestingly, using luciferase complementation image and BiFC assays, we found that HPS10 interacts with AtSTAR1 in tonoplasts. The knockout mutant of atstar1 displays the same phenotype as hps10 in response to P deficiency and Al toxicity. Based on these results, we propose that HPS10/ALS3 and AtSTAR1 form a functional protein complex that serves as a signaling hub to coordinate plant responses to P deficiency and Al toxicity.

O305

### The ESCRT-III-associated protein AtALIX mediates high affinity phosphate transporter trafficking in *Arabidopsis*

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High affinity phosphate transporters (PHT1) allow phosphate uptake from the soil and the extracellular space into plant cells. PHT1 levels at the plasma membrane are tightly regulated by the endomembrane system that eliminates excess PHT1 proteins under phosphate sufficient conditions by sorting them into endosomes to the lytic vacuole. Prior to the release of their cargoes into the vacuolar lumen, sorting endosomes mature into multivesicular bodies (MVB) through the action of ENDOSOMAL COMPLEX REQUIRED FOR TRANSPORT-III (ESCRT-III) protein complexes. Here, we describe AtALIX a cytosolic protein that associates to MVB by interacting with ESCRT-III subunit SNF7 and enables protein cargo trafficking to the vacuole. Thus, by using a partial loss of function mutant, Atalix-1, we show that AtALIX facilitates vacuolar degradation of PHT1;1 and brassinosteroid receptor BRI1. In line with broad target specificity, Atalix-1 mutants displayed a pleiotropic phenotype, including reduced plant growth and vacuole malformations, whereas null mutants were lethal, indicating that AtALIX controls diverse processes in plants being essential for their life. AtALIX versions containing the Atalix-1 mutation showed reduced ability to interact with SNF7, providing a potential molecular basis for impaired cargo trafficking in Atalix-1 mutants. Defects in Pi transporter trafficking and in vacuole morphology, where phosphate is mainly stored, very likely affect phosphate uptake and distribution within Atalix-1 mutant cells, leading to distorted phosphate homeostasis and altered phosphate starvation responses.

O306

### Systems biology of plant phosphate and sulphate metabolism

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Optimal nutrient supply to plants and its utilisation is a prerequisite for optimal plant performance. However, not only the supply of nutrients but a balanced supply of these and all other nutrients is paramount. However, given that we hardly understand the effects and control of nutrient uptake and use of individual nutrients at molecular and physiological levels our knowledge with respect to the interplay between multiple nutrients is quite limited. Here we provide a study on the interplay between sulfate and phosphate employing systems biology tools as transcriptomics and metabolomics. The aim of this study is to catalogue communalities and differences between both nutrient deprivations. We try to employ the concept of modular responses that we deduce from research on sulfate metabolism on comparing P and S starvation. We further used these analytical tools on a mycorrhization model system of *Medicago truncatula* interaction with *Rhizophagus irregularis* (formerly *Glomus intraradices*) asking for the uptake of sulphate occurring by mycorrhiza when symbiosis has been established under mild P starvation. We could show that indeed sulphate is supplied in vivo to the plant. Thus, altering nutrient use efficiency by breeding or transgenic approaches has always to be seen in the context of its effects on other nutrients as the response systems are not strictly separated from one another.

O307

### Effect of the phosphorus status on the isotopic composition of oxygen bound to phosphorus in *Glycine max*

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The use of the isotopic composition of oxygen bound to phosphorus ( $\delta^{18}\text{O}\text{-PO}_4$ ) to study phosphorus (P) cycling in the soil-plant system has increased in recent years. But detailed studies about the effect of the P status of plants on the  $\delta^{18}\text{O}\text{-PO}_4$  are missing. By combining the analysis of the  $\delta^{18}\text{O}\text{-PO}_4$  with the radioisotope  $^{33}\text{P}$  we investigated the effect of the P status on P translocation and on the  $\delta^{18}\text{O}\text{-PO}_4$  of inorganic phosphate extracted from different plant parts. Soybean (*Glycine max*) was grown in hydroponic cultures first with ample P supply. During this period, the plants were labelled with  $^{33}\text{P}$  for eight days. In order to obtain plants with different P status, part of the plants were further grown with an ample amount of P supplied (+P), while the other plants were grown in a P-free nutrient solution (-P) for ten days. The plant material was extracted with 0.3 M trichloroacetic acid (TCA) to obtain inorganic phosphate (TCA P). The residue was extracted with 10 M nitric acid ( $\text{HNO}_3$ ) targeting P remaining in the plant material after the extraction with TCA (residual P). The  $^{33}\text{P}$  data show an enhanced translocation of P from old leaves and roots in the -P plants compared to the +P plants. This was reflected in the  $\delta^{18}\text{O}\text{-PO}_4$  of TCA P in the old leaves, as the  $\delta^{18}\text{O}\text{-PO}_4$  of TCA P was significantly lower in the -P plants compared with the +P plants, but not in the roots. This was attributed to an enhanced mobilisation of P from the residual P in the old leaves compared to the roots of the -P plants. Finally, our study suggests that  $^{33}\text{P}$  and  $\delta^{18}\text{O}\text{-PO}_4$  are complementary approaches, but cannot replace each other.

O308

### Root cap cells play a key role in phosphate nutrition

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Mineral ion uptake by plants represents one of the initial food chain inputs, and a crucial factor that controls yield. Cellular and molecular mechanisms driving ion absorption remain poorly characterised and often involve broad multigenic families and overlapping functions. In the model plant *Arabidopsis*, the expression of several high affinity phosphate (Pi) transporters (PHT1 family) is highly regulated depending on the phosphate status of the plant and restricted to specific cell layers of the root. By specific complementation of *phf1-1* mutant (strongly altered in phosphate uptake regulation), using GAL4-enhancer trap strategy, we investigated the role of several root cell layers. Coupling this technique with high-resolution real time  $^{33}\text{P}$ -imaging, we were able to differentiate Pi uptake and Pi translocation in the plant and more specifically in the root tip. We showed that the root cap accounts for a significant amount of the whole plant phosphate uptake (around 20%). Such Pi absorption is efficient for shoot biomass production and repression of Pi-starvation-induced genes. This work reveals that the root cap surrounding and protecting the root stem cell niche (meristem) at the root tip harbors an unexpected crucial role for Pi nutrition, extending the role of this tissue from that previously described in environmental perception (gravity, light, moisture).

**Organ-specific phosphorus-allocation patterns and transcript profiles linked to P efficiency in wheat**Tariq Aziz <sup>1,2</sup>, Patrick M. Finnegan <sup>1</sup>, Hans Lambers <sup>1</sup>, Ricarda Jost <sup>1</sup>*1. School of Plant Biology, Faculty of Science, The University of Western Australia, Crawley (Perth), WA 6009, Australia**2. Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad-38040, Pakistan*

Molecular mechanisms underlying genotypic variations in phosphorus (P) efficiency have been studied mostly in model plants, but rarely in wheat. Physiological and molecular adjustments under P deficiency in two wheat cultivars (cultivars Chinese 80-55 and Machete) were studied in a hydroponics experiment. The plants were grown with adequate P (0.2 mM P) for 15 days and later on were divided into two groups, receiving i) no P and ii) 0.2 mM P. We demonstrate that the greater P efficiency of Chinese 80-55 compared to Machete involved maintaining higher inorganic phosphate (Pi) concentrations in all organs upon Pi withdrawal in combination with higher Pi-acquisition rates when Pi is present. These findings correlated with differential organ-specific expression of Pi transporters TaPHT1;2, TaPHT1;5, TaPHT1;8, TaPHT2;1 and H<sup>+</sup>-ATPase TaHa1. Observed transcript level changes suggest that maintaining higher de novo phospholipid biosynthetic and anabolic activities in Pi-limited elongating basal leaf sections could be another crucial adaptation in Chinese 80-55 for maintaining growth upon Pi withdrawal. We surmise that these activities are supported through enhanced breakdown of starch in Chinese 80-55 stems suggested by higher TaGPho1 transcript levels which contrasts the stronger transcript-level suppression of genes involved in glycolysis, and transcriptional and ribosomal activities in Chinese 80-55 fine roots. Our work shows that major differences exist in the way the two contrasting cultivars allocated Pi and organic P compounds between source and sink tissues and how their metabolism acclimated to changes in Pi availability.



### **Theme 3 – Phosphorus utilization and signaling in plants**

#### **Posters**

P301

### Importance of phosphorus (P) remobilization during grain filling in rice plant regarding the P regime

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Phosphorus (P) is a key nutrient for plants and deficiencies of P in cropping soils frequently limit plant growth. Given the increasing need for P fertilizer to meet the global food demand, and the finite nature of world rock phosphate reserves, security of P supply will become a priority in the next decades, particularly for staple crops such as rice. At maturity, P is principally found in the rice grains as a consequence of P loading into the grain, a process starting at grain filling (immediately after anthesis) with a rapid phase of P accumulation between 6-15 days after anthesis (DAA). However, the loading mechanisms involved remain unclear, particularly the contribution of exogenous uptake from roots post-anthesis vs remobilisation of P from vegetative tissues. Using a  $^{33}\text{P}$  isotope tracer, we studied the uptake, distribution and redistribution of P during grain filling in rice plants (cv. IR64) that differed in P status. Plants were grown in hydroponic system from sowing to maturity and  $^{33}\text{P}$  was spiked in the solution at 9 DAA. The distribution of  $^{33}\text{P}$  among organs (root, stem, leaves, flag leaf, rachis, husk, grain) was determined at 3h, 24h and 120h after spiking. The ultimate aim is to understand the physiology of P loading into grains in order to reduce the P concentration of cereal grains and improve the P efficiency of cropping systems. The results are therefore discussed in terms of importance of vegetative P remobilisation vs post-anthesis P uptake of exogenous P.

P302

### Remobilization of seed phosphorus reserves and their role in attaining phosphorus autotrophy in maize seedlings

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Remobilisation and utilization of seed P resources are crucial for achieving high yield potential in crops. The remobilization of stored maize seed phosphorus (P), and its allocation towards growing seedlings is critical for P-autotrophy during early ontogeny. We aimed to (i) evaluate the time frame of the origin of P utilized by maize seedlings including the heterotrophic, transitional and autotrophic phases, and (ii) compare P and carbon (C) dynamics in both seed and seedling compartments during the same phases. Using isotopic labelling ( $^{32}\text{P}$ ), we identified different P fluxes (P-heterotrophy, P-transition and P-autotrophy) and determined the proportion of P fluxes from heterotrophic seed P and external P uptake within seedling compartments during 23 days of early ontogeny. Phosphorus heterotrophic growth phase lasted from the first to the fourth day after sowing when seedlings were entirely made up of heterotrophic P originating from remobilized seed P pool. In our experimental conditions, the P-transitional phase, when growing seedlings were supported by both heterotrophic and autotrophic P, lasted from the 5<sup>th</sup> to the 15<sup>th</sup> day after sowing. Thereafter, seed P reserves were exhausted and seedlings depended entirely on external P uptake, indicating the beginning of P-autotrophic stage. Our results show that seed P reserve can sustain seedling early growth for up two weeks, but where external P availability are not limiting, seedlings switch to external resources within few days of germination. Further researches are needed to identify regulatory processes governing the transition from P and C heterotrophic to autotrophic growth in seedlings.

P303

### Non-coding RNAs in the adaptation of root growth to phosphate starvation

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Phosphorus (P) is an essential mineral for plant growth but its availability in soil is often limited. Molecular and genetic approaches have revealed several regulatory elements controlling many responses triggered in plants to cope with P starvation, notably root architecture. Large differences in root architecture are present in *Arabidopsis* accessions grown in P-depleted conditions. In a previous QTL analysis we had shown that the primary root tip is essential for the control of root growth when P is scarcely available. In addition, we have observed recently that the primary root growth repression by low-Pi is rapid and independent of the level of Pi accumulation inside the root. Non-coding RNAs are emerging actors in the adaptation of plants to environmental constraints. In order to decipher the role of non-coding RNA in the natural variation of the root response to low-P conditions, we are using deep sequencing (RNAseq) of RNA extracted from the root tip of different *Arabidopsis* accessions grown in low and high P. We will present our results about the induced and repressed mRNAs, long-non-coding RNAs and small RNAs. This study should allow us to identify new elements controlling root growth in response to P and to address the impact of non-coding RNAs in adaptation to environmental constraints and in the evolution of gene regulation.

P304

### Genetic regulation of phosphorus homeostasis during grain filling in rice

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Phosphorus (P) is an essential plant nutrient because of its role in nucleic acids, phospholipids, ATP and other phosphor-esters. Ultimately, 70-80% of total plant P is mobilised to seeds during reproductive development, which results in the removal of large quantities of P from fields in harvested seed and drives the need for continual P fertiliser inputs. At present, we have a poor understanding of the plant gene networks involved in P mobilisation into seeds as most research on P has focused on improving P uptake or understanding the gene networks involved in the response to P deprivation. We studied the physiological behaviour of P movement during grain filling and have found that grain P content reached a maximum 15 days after anthesis (DAA). We therefore used high-throughput RNA-seq to investigate transcript expression in the P source/sink tissue (i.e. flag leaf/grain) during the rapid phase of P accumulation (6-15 DAA). Differences in gene transcript levels and implications for P transport to developing grains will be discussed.

P305

### Synthesis and characterization of caged-phosphates for the controlled release of phosphate in living cells

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The light-induced release of a biomolecule from a caged precursor is a non-invasive method to generate a concentration jump in a time- and space-controlled manner that has found useful applications mainly in cellular biology and neurobiology. Here we report the development of two photolabile precursors of inorganic phosphate (Pi): 2-(4'-((di(tris-ethoxy(methoxy))amino)-4-nitro-[1,1'-biphenyl]-3yl) propan-1-ol (EANBP) and 7-(Dialkylamino)coumarin-4-yl)methyl (DEACM) caged-Pi. In vitro, the EANBP-Pi showed high photolysis efficiency at 405 nm with 95% release of free phosphate and a 0.28 quantum yield. Two acetoxymethyl bioactivatable protection groups were added to the caged-Pis. The resulting phosphoric triesters were able to diffuse through the cellular membranes of plant cells. Once inside the cell, the cleavage of these biocleavable motifs by intracellular esterases allows intracellular accumulation of EANBP-Pi. Therefore, membrane permeant bis(AM)-EANBP-Pi represents a very attractive tool to trigger Pi fluctuations in living cells including (but not limited to) plant cells. In particular, the properties of EANBP-Pi make it superior to DEACM-Pi for this application. Current efforts are focused on the in vivo application of these new compounds.

P306

### Phosphorus use efficiency in *Hakea prostrata*: Role of other nutrients

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Phosphorus (P), as inorganic phosphate (Pi, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>), is an essential plant macronutrient. Therefore, soil that does not contain large amounts of available Pi, or is not amended with Pi fertiliser, will limit plant growth, function and ultimately yield. To achieve higher agricultural yields, Pi fertiliser is being applied in intensive farming in every cropping season. However, excessive application increases farming costs and pollutes our environment. Phosphorus is a finite and dwindling natural resource, which requires our best stewardship. Phosphorus-efficient crop varieties may alleviate this quandary. Highly P-efficient Australian plants may guide us towards developing more P-efficient crop varieties by informing new selection strategies for breeding programs. *Hakea prostrata* naturally grows on extremely P-impooverished soils whilst maintaining high photosynthetic rates. Our research aims to further understand this native plant's unique metabolic adaptations to severe P limitation. In a world-first study, we are determining key metabolite and transcript responses of *H. prostrata* to changes in the availability of three major plant nutrients, namely P, nitrogen and sulfur. These findings will reveal new mechanisms underlying the adaption of this species to extremely low levels of soil P. Progress will be reported on the nutrient-assimilation patterns, transcript responses for key transporter genes, and their cross-talk in limiting and replete nutrient regimes.

P307

### Overexpression of a *Bacillus subtilis* phytase in *Arabidopsis thaliana* enhances its ability to mobilize P from phytate

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The low availability of phosphorus (P) is a major constraint to the growth and development of vegetable crops worldwide. In fact, 50-80% of total P in agricultural soils exists as organic P where phytic acid is the most dominant component, that is not available to plants unless hydrolyzed by specific enzymes called phytases. We are particularly interested to study a microbial phytase PHY US417 of *Bacillus subtilis* strain which was isolated in the Laboratory of Enzymes and Metabolites of Prokaryotes (LEMP) of the Centre of Biotechnology of Sfax (CBS). We plan to make use of this enzyme in the engineering of a Pi-enriched plant product for animal feeding, to meet the needs of the animal in this nutrient. On the other hand, we aim to develop biotechnological approaches to increase via this phytase, the ability of plants to mobilize soil P to improve crop production, hence reducing the use of phosphate fertilizers, which over time can cause environmental pollution problems especially eutrophication. We have demonstrated in this work that the PHY US417 phytase added to the medium has the ability to stimulate the growth of *Arabidopsis thaliana* plants in vitro under P-limited conditions. In addition, we engineered transgenic *Arabidopsis* plants overexpressing this phytase. The enzymatic tests revealed variable levels of phytase activity at pH 7.5 in these lines. Finally, physiological analyzes showed that overexpression of PHY US417 seems to improve (albeit modestly) growing plants submitted to P deficiency. All together, these results show that PHY US417 is active in *Arabidopsis* and can be exploited in other crops such as cereals.

P308

### Rice phosphate transporter OsPT4 functions on Pi-uptake and embryo development irrespective of Pi-status

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Phosphate is taken up by the roots, and then can be translocated from the roots to the leaves and seeds by the phosphate transporters. In this work, the functions of OsPT1;4 (OsPT4), one of the thirteen members of the Pht1 family in rice, was investigated. High expressions of the gene were detected in vegetative organs with the peak in roots, and in reproductive organs with the peak in embryos irrespective of Pi-statuses.  $\beta$ -Glucuronidase and green fluorescent protein reporter gene expression driven by OsPT4 promoter confirmed the expression pattern of this gene. OsPT4 was able to complement a yeast Pi-uptake mutant and increase Pi accumulation of *Xenopus laevis* oocytes when supplied with Pi at the external solution, indicating that OsPT4 is a phosphate transporter. Transgenic plants of OsPT4- overexpression, RNA-interference knockdown and Tos 17 insertion contained significantly higher or lower P concentration, respectively, compared to that of wild type control, in roots and shoots under both Pi-sufficient and -deficient conditions. These responses of the transgenic plants to Pi-utilization were further confirmed by the assay of <sup>33</sup>P uptake rate and transportation. Moreover, OsPT4 overexpression or suppression resulted in an increase or decrease of phosphate content in the untilled rice hulls, rice hulls and embryos. The *ospt4* mutants revealed a novel role of phosphate in embryo development. The seed-setting rates in *ospt4* mutants were significantly reduced, and interestingly, there was a positive correlation between the reduction of the seed-setting rate and knockdown or knockout of mutants, suggesting that the OsPT4 is required for the embryo development of rice. Finally, we showed that OsPT4 expression was strongly enhanced by mutation of Phosphate Over-accumulator (OsPHO2), but not by Phosphate Starvation Response 2 (OsPHR2), indicating that OsPT4 is involved in OsPHO2-regulated Pi pathway. Altogether, our data suggest that OsPT4, a phosphate transporter on the plasma membrane, plays predominately a role in phosphate uptake in the roots and the embryo development.

P309

### Characterization of OsSIZ1 reveals the involvement in the regulation of phosphate responses in rice

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Previous research has demonstrated that AtSIZ1 participates in Pi-starvation signaling by targeting the central transcription factor, PHR1. In this work, OsSIZ1 and its involvement in the regulation of phosphate responses was investigated. OsSIZ1 was abundantly and constitutively expressed during the whole-plant growth stage. Mutation of OsSIZ1 resulted in significant increase of P concentration in both roots and shoots independent of Pi-supplied conditions at seedling stage, and imbalanced Pi distribution at lateral growth stage. Overexpression of OsSIZ1 also caused enhanced P concentration, which depends on the transcripts of OsSIZ1 and showed different increase of P concentration in shoots and roots compared to *ossiz1* mutants. Quantitative RT-PCR analysis revealed an attenuated response to Pi starvation in OsSIZ1 mutants by suppressing Pi-starvation induced genes, such as OsIPS1, OsmiR399a, OsSPX1, OsSPX2 and phosphate transporters (OsPT2, OsPT3, OsPT4 and OsPT6). The expression of OsPHR2 and OsMYB2P-1 were down- or up-regulated, respectively, in *ossiz1* mutants under Pi-sufficient condition, and tended to similar to the wild-type by Pi deprivation. Taken together, the data demonstrate that OsSIZ1 has multiple effects on Pi homeostasis and regulation of gene clusters express that are involved in Pi uptake and translocation.

P310

### OsPHT2;1, one of rice Pht2 family, affects phosphate utilization and yield per plant

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A number of Pi transporters belonging to the Pht2 family have been functionally reported in several plant species, whereas functions of OsPHT2;1 (*Oryza sativa* L.) have not been characterized yet. In this study, we identified that OsPHT2;1 was strongly expressed in leaves and was up-regulated in Pi deficiency condition. In addition, OsPHT2;1 was strongly regulated by light. Localization of a OsPHT2;1-green fluorescent protein(GFP) fusion protein indicates that it is present in the chloroplast envelope. OsPHT2;1 was able to complement the yeast Pi-uptake mutant. Transgenic plants of OsPHT2;1 overexpression lines resulted in higher soluble Pi amounts and biomass of plants under sufficient and deficient Pi conditions. The OsPHT2;1-Ox(overexpression) lines exhibited higher total P contents and field yield compared with WT(wild type). These results indicated that OsPHT2;1 is a key member of Pht2 family and is critical for Pi translocation and utilization efficiency in rice.

P311

### Fine characterization of OsPHO2 knockout mutants reveals its key role in Pi utilization in rice

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Previous research using forward genetics approaches demonstrated that OsPHO2 regulates multiple phosphate-starvation responses in rice. In this work, we finely characterized two independent OsPHO2 knockout rice mutants under Pi-sufficient conditions. The *ospho2* mutants exhibited defects in growth and reproductive development in the whole growing period. The cells in the elongation zone of *ospho2* seedling roots were much shorter than those of the wild type. The P concentration in the blades of *ospho2* mutants was approximately five-fold that of wild-type plants, whereas it was only slightly higher in the sheaths, culms, spikelets and seeds. Furthermore, Pi levels in the *ospho2* mutants were highest in the oldest leaf and lowest in the youngest leaf, whereas there was no significant difference in the corresponding leaves of wild-type plants. These results suggest that *ospho2* phenotype results from a partial defect in Pi translocation and remobilization in the shoot of rice. This study thus provides evidence that OsPHO2, which function downstream of OsPHF1, modulates Pi utilization by regulating the expression of Pht1 transporters in rice.

P312

### Learning from the extremists - the phosphorus-efficient southwest Australian native *Hakea prostrata*

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Proteaceae in Australia's southwest are well adapted to the extremely low phosphorus (P) content of the highly weathered soils, e.g. in the Bassendean dunes and Peron slopes of the Swan Coastal Plain. They have evolved phosphate (Pi)-mining capabilities to access sparingly available P resources, producing dense clusters of short tertiary lateral rootlets, so called proteoid or cluster roots. These increase the surface area of the root to boost local organic acid, nuclease and phosphatase secretion to liberate Pi from organic molecules or soil particles. Proteaceae species also efficiently remobilise P from senescing organs. Since these adaptive traits are very costly they will require extensive reprogramming of primary metabolism. We have chosen harsh hakea (*Hakea prostrata* R.Br.) as a model to study acclimation responses to varying Pi supply on a physiological and molecular level. Metabolite profiling revealed relatively high levels of essential P metabolites such as phospholipids in growing tissues, which may help to sustain the formation of cluster roots and new leaves. Moreover, harsh hakea appears to adjust rates of N assimilation and protein synthesis during organ development and in response to external Pi availability, most likely by tightly controlling nitrate uptake. These and other metabolic adjustments will be discussed in the context of this species' extraordinary P efficiency and opportunities for translational research into creating smarter crop plants.

P313

### Interacting signalling pathway co-regulates the phosphate and zinc loading into root xylem in *Arabidopsis*

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Inorganic phosphate (Pi) and Zinc (Zn) are two essential elements for plant growth. Control of tissue Pi and Zn content is of major importance for normal plant growth and development. Zn deficiency typically leads to Pi overaccumulation in shoots and inversely, attesting thus the presence of complex interactions link the regulation of the homeostasis of these two nutrients. Yet despite their primary importance, the molecular bases of these interactions remain poorly understood. Our recent research work (Khan et al., 2014) has placed the co-regulation of these two elements at one of limiting step in Pi and Zn distribution within plants — the loading of Pi and Zn into root xylem. In *Arabidopsis*, this process mainly involves members of the Phosphate 1 (PHO1 and PHO1;H1) and the heavy metal ATPases protein (HMA2 and HMA4) families for Pi and Zn respectively. The recent progress on our understanding of the molecular mechanisms regulating the Pi and Zn loading into root xylem individually through these specific genes will be presented, including the first molecular evidences for their signalling crosstalk at this particular step of their transport in plant with the emerging role for PHO1;H3. Such progress is important to enable biotechnological and agronomic strategies aimed at enhancing Pi uptake and transfer to the aerial part of the plants by modifying the Zn deficiency signalling pathway.

P314

### Forms of phosphorus in tissue of cover crops grown in an Oxisol under no-tillage and conventional tillage

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The assessment of the ability of cover crops in absorb and accumulate P can help to identify species that promotes P cycling reducing then the use of fertilizers in the subsequent crops. This study was carried out to evaluate the amount and forms of P accumulated in shoots of cover crops managed under no-tillage (NT) and conventional tillage system (CT). The experiment was established in 1986 with six winter cover crops treatments (blue lupine, hairy vetch, oat, radish, wheat and fallow) implanted in a Rhodic Hapludox in southern Brazil, managed under NT and CT. In September 2011, shoot samples were collected in the six cover crops at approximately the flowering stage. In the dried shoots samples were evaluated the total contents of C, N and P, and the P forms in the tissue by P the method of Miyachi & Tamiya (1961) adapted from Schmidt & Thannhauser (1945). The forms and amounts of P in the tissue of cover crops were different among the species, but in all of them the soluble inorganic P was the main form of P storage. Moreover, the plants cultivated under NT showed an increase in the soluble P forms that was promoted by the higher contents of soil available P.

P315

### The auxin influx carrier AUX1 plays a critical role in roots during adaptive responses to phosphorus limitation

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Phosphorus (P) is an essential macronutrient for plant growth that accumulates in topsoil. Plant roots are reported to employ a variety of mechanisms to forage for such P reserves such as reducing their gravitropic response. We initially attempted to improve P foraging in rice by isolating mutants disrupting root gravitropism. Mutants defective for the auxin influx carrier gene *OsAUX1* were isolated and observed to disrupt root gravitropism on agar plates. X-ray based CT imaging later revealed that *Osaux1* mutants also exhibited a clear agravitropic root phenotype, causing their roots to accumulate in the topsoil. Despite the change in root distribution in soil, this genetic modification did not improve P uptake compared to wild-type. Further studies revealed that mutating *OsAUX1* had also disrupted root hair (RH) elongation, another important adaptive response to P limitation. Like rice, *Arabidopsis aux1* mutants were also defective for RH elongation under P limitation, suggesting this represented a highly conserved AUX1-dependent root response in angiosperms. Targeted expression studies revealed that AUX1 functioned to mobilise auxin from the root apex to elongation zone cells to promote root hair elongation under P limitation. Our study reveals that auxin and AUX1 play key roles mediating this important P adaptive response.

P316

### Relationships nitrogen and phosphorus and the nutritional indices in maize hybrids submitted to nitrogen levels.

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Improving knowledge about nutrient behavior and function into within the plant allows to can increase the performance in the effective management of P supply. The objective was to study the effect of nitrogen (N) doses in the interaction nitrogen:phosphorus (N:P) and in the efficiency of absorption and transport of P in maize hybrids (*Zea mays* L.) in the V4 stage grown in nutrient solution. The study was done in FZEA/USP/Brazil, in a hydroponic system with Hoagland and Arnon (1950) nutritive solution in greenhouse. The treatments were four doses of N: 1, 3, 15 and 30 mM of N and three maize hybrids: DKB390 PrórR2 (H1), Pioneer 30F35 (H2) and Syngenta Status (H3), with 4 (four) repetitions. At stage V4 were performed: a) concentration of N and P; b) N:P interactions were calculated in shoots and roots and c) efficiency of absorption and transport of P (Turkey test at 5%). A quadratic increase was observed in N concentration in shoots and roots along the increase in N concentration in solution in all hybrids. The P concentration increased in shoots and decreased in roots with the increment of N in solution, and fitted to a linear model to H1 and H2, and a quadratic model to H3. The N:P ratio in the shoots and roots increased due to the increase in N concentration in plants, ranging from 4:1 to 15:1 in shoots and 0.7:1 to 3.4:1 in the roots. The lowest efficiency of absorption and transport of P occurred with the application of lower doses of N for hybrids. The increased N in solution promotes increase in concentration of P in shoots, in N:P ratio in shoots and roots and on the efficiency of absorption and transport of P in maize.

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### Morphological root differences between two rice cultivars in response to phosphorus availability

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Soil phosphorus (P) availability is a relevant constraint limiting crop productivity worldwide. However, little is known about the characterization of the adaptative changes of the root system under field conditions. This study aimed to evaluate the root system of two rice cultivars, BR-IRGA 409 and IRGA 425, grown under two P levels (control - 27 mg P dm<sup>-3</sup>, and addition of 600 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>). Polyvinyl tubes (30 cm of diameter and 60 cm of length) with two open ends (being the bottom covered with a plastic net) and with small lateral orifices, were arranged in a rice field. The tubes were filled with polystyrene granules in order to provide root support, but allowing the soil solution flux. Once collected, the roots were scanned and analyzed with the aid of WinRHIZO software. Under natural P level, BR-IRGA 409 cultivar had lower root length, and number of tips and forks per plant, as compared to IRGA 425 cultivar. Conversely, with high P level the length and number of forks did not differed between the cultivars, however BR-IRGA 409 showed higher number of root tips as compared to IRGA 425. Apparently, IRGA 425 invested the photoassimilates in root biomass under low P levels, while BR-IRGA 409 showed increment in root architecture under high P availability. Moreover, a higher grain yield in IRGA 425 as compared to BR-IRGA 409 was observed. It is possible to say that the natural soil P level was close to the appropriate level for the rice culture, but on the other hand, the differences in P use efficiency of the cultivars probably determinate the response in root production. Thus, IRGA 425 showed a more efficient pattern of P acquisition under low P.

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### *Arabidopsis* inositol pentakisphosphate 2-kinase AtIPK1 modulates phosphate homeostasis via transcriptional regulation

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Inositol hexakisphosphate (IP6 or phytate) not only provides a phosphorus reservoir in plant seeds, but itself and the intermediates in its synthesis also play important roles in regulating diverse developmental and physiological processes. Disruption of the *Arabidopsis* inositol pentakisphosphate 2-kinase AtIPK1 was previously shown to reduce IP6 content and cause elevated Pi accumulation in plants. Here we investigated the molecular mechanism underlying misregulation of Pi homeostasis in *atipk1* mutants, and found an additional role of AtIPK1 in sustaining growth and development as null mutants cease shortly after germination. Using an incomplete loss-of-function *atipk1* allele, *atipk1-1*, we demonstrated AtIPK1 plays a role in maintaining Pi homeostasis through upregulation of a subset of Pi starvation-responsive genes involved in Pi uptake, allocation and remobilization. Notably, the transcriptional activation of a number of Pi starvation-responsive (PSR) genes in *atipk1-1* mutants was associated with reduction of histone H2A.Z occupation. Genetic studies indicate that transcriptional activation of PSR genes in *atipk1* mutants involve regulators in addition to the central PHR1(-like) factors. Reduction of vegetative IP6 level may not be a sufficient factor for causing misregulation of Pi homeostasis in *atipk1* mutants, as mutations in two *myo*-inositol-1-phosphate synthase coding genes, AtMIPS1 or AtMIPS2, which exhibited comparable reduction of IP6 content in the vegetative tissues, did not phenocopy *atipk1-1* mutants. This study revealed a regulatory mechanism of Pi homeostasis mediated by the inositol polyphosphate biosynthesis pathway at the transcriptional level.

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### Contribution of phosphatases to adaptation of rhizobial symbiosis with legumes to phosphorus deficiency

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To understand the mechanisms used by legumes to improve their phosphorus use efficiency (PUE) for symbiotic nitrogen fixation under P-deficiency, an in situ RT-PCR methodology was used to localize and quantify the transcripts of candidate phosphatases genes in nodules of two common bean RILs 115 and 147 in hydro-aeroponic culture under deficient versus sufficient P supply. Our findings have revealed that expression of phytase and fructose 1,6 bisphosphatase (FBPase) genes and activities of the corresponding enzymes were positively correlated with increases both of the rhizobial symbiosis efficiency in use of P for N<sub>2</sub> fixation and nodule O<sub>2</sub> permeability. Under P-deficiency, this positive correlation was more significant for the P-efficient RIL115 than for the P-inefficient RIL147. It is concluded that these phosphatases play a role in adaptation to P-deficiency and are likely involved in nodule respiration linked to symbiotic nitrogen fixation.

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### Comparison of P recycling abilities among rice cultivars showed an importance of lipid remodeling

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It has been shown that rice plant has a higher ability to recycle internal P, although the mechanism was not well clarified. The P-recycling ability could be considered as a target of breeding for P efficient cultivars. This study aimed to analyze the P-recycling ability based on differences among Japanese cultivars of rice. To compare the allocation of P, 9 cultivars were cultured for 21 days in nutrient solution containing P in 0 (–P) or 64 µM (+P) and harvested after separation into roots and upper and lower leaves. It was suggested that P-recycling ability was highly varied among cultivars. Akamai showed the lowest ration of P allocation in lower leaves and the highest ratio in upper leaves, suggesting that P concentration in young leaves was maintained by a higher P-recycling ability. In contrast, P content of upper leaves of Koshihikari was lowest among analyzed cultivars. A substitution of phospholipids by glycolipids is known as a P-recycling strategy. Thus, the composition of membrane lipids of leaves was analyzed by 2D-separation on TLC. A lesser decrease of phospholipid in upper leaves of –P plants was found in Akamai, although the rate of phospholipid in lower leaves were decreased around half. In Koshihikari, the alteration of phospholipid distribution was similar level between upper and lower leaves of –P plants. These facts suggest that the lipid remodeling of membrane, at least in part, can explain differences of P-recycling ability among rice cultivars. The result of microarray analysis supported the differences of lipid remodeling between Akamai and Koshihikari under –P conditions.

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### Deciphering the role of PHO1 in phosphate homeostasis in leaves and seeds

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PHO1 is expressed primarily in the root vascular cylinder and acts as a phosphate (Pi) exporter, important for the loading of Pi into the root xylem. The PHO1 family in *Arabidopsis* consists of 11 members, and of those only the closest homologue of PHO1, named PHO1;H1, contributes to Pi loading into the root xylem. While the PHO1 transcript is only weakly increased in roots of Pi-deficient plants, expression of PHO1;H1 is strongly up-regulated in both the leaves and roots of Pi-deficient plants. Beyond the vascular cylinder, PHO1 is also expressed in guard cells, and previous experiments have shown that it contributes to the response of stomata to abscisic acid. Analysis of transgenic lines expressing PHO1-GFP and PHO1-GUS lines expressed under the PHO1 promoter revealed a wider expression pattern of PHO1 than expected, including PHO1 expression in the shoot vascular cylinder and in developing seeds. The goal of this work is to examine the role of both PHO1 and PHO1;H1 expression on Pi homeostasis in tissues other than the roots, and in particular in leaves and developing seeds. Detailed expression pattern of both PHO1 and PHO1;1 in various tissues will be reported, in particular in developing seeds. Furthermore, we are characterizing the phenotypes of *pho1* and *pho1;h1* single and double mutants using a combination of hypocotyl and inflorescence grafting to WT roots, analysing Pi content and Pi transport dynamics in plants grown under various conditions.

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### P supply effect on photosynthetic performance and antioxidant responses in ryegrass cultivars grown under Mn excess

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We previously found that Mn-excess cause photosynthetic impairments, mainly in the Mn-sensitive cultivar of ryegrass. Also, our findings indicated that despite the increase of Mn in ryegrass tissues at increasing P supply, plant-growth inhibition caused by Mn-excess was decreased. We evaluated the P nutrition effect on photosynthetic performance of perennial ryegrass cultivars (Nui and Kingston) treated with increasing Mn under hydroponic solution. Two P treatments [100  $\mu$ M (control) and 400  $\mu$ M (high P); supplied as  $K_2HPO_4$ ] in combination with two Mn concentrations [2.4  $\mu$ M (control) and 350  $\mu$ M (excess Mn), supplied as  $MnCl_2$ ] at pH 4.8 during 10 days were performed. Results showed that despite P supply increased Mn tissue concentrations compared to control, P additions significantly reduced the detrimental effect of Mn excess on photochemical parameters ( $\Phi$ PSII and ETR), mainly in Nui (Mn-sensitive). We also found that high P additions increased the superoxide dismutase (SOD) activity in both cultivars, mainly in Kingston (Mn-tolerant), decreasing the oxidative stress induced by Mn excess. We observed that shoot phenolic (TPH) content was slightly increased in response to Mn excess, mainly in the Mn-tolerant cultivar. Under high P, these Mn-induced TPH increments decreased may be due to its low Mn-stress level in plants. These results confirm that P alleviates Mn toxicity in perennial ryegrass reducing the negative effect of Mn on photosynthesis and oxidative stress. Moreover, it is postulated that the greatest absorption of Mn in plants exposed to high P suggests the possible inactivation of Mn through complexation P-Mn.

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### Regulation of primary root growth by trihelix transcription factors in response to environmental limitations

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Under limiting Pi conditions, plants trigger local and systemic signaling networks causing dramatic changes in gene expression and metabolism to adjust plant growth, Pi uptake and mobilization of internal Pi reserves. This response is collectively called phosphate starvation response (PSR). Despite the identification of key factors involved in local Pi sensing and systemic signaling, molecular mechanisms that transmit signals from the local sites of Pi sensing at the root apex into systemic growth responses are unknown. We recently isolated two trihelix transcription factors with redundant functions to control the PSR. Both transcription factors are expressed in root tips, and their corresponding loss-of-function mutant continuously exhibit PSR-like phenotypes under sufficient Pi supply. Using genome-wide chromatin-immuno-precipitation studies and genetic approaches, we reveal that these trihelix factors transcriptionally modulate the balance of distinct hormone signaling pathways. Furthermore, phenotypic analysis of mutants corresponding to putative trihelix factor target genes identified an additional factor, which is required to promote the PSR under Pi starvation. Thus, our results provide a series of molecular and genetic evidence that trihelix factors are key transcriptional switches to adjust root development in response to the environmental stimulus Pi via direct regulation of hormone signaling pathways.

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### Evaluation of genotypic variation for phosphate deficiency tolerance in Sri Lankan rice varieties

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Rice (*Oryza sativa*) is one of the world most important crops and phosphorus (P) is an essential macronutrient to plant growth. Plant roots acquire P as phosphate (Pi), primarily in the form of  $H_2PO_4^-$  from the soil solution. Phosphorus deficiency severely limit rice yields and is a major constrain for rice production throughout the world. Phosphorus becomes one of the most limiting and least available macronutrient as it form insoluble complexes with inorganic ions in soil. The aim of the study was to evaluate P deficiency tolerant cultivars for marker assisted breeding. In this study selected thirty traditional and improved rice varieties were screened under field and greenhouse conditions. Growth medium was soil and the design was Completely Randomized Design in green house and Randomized Complete Block Design in field with four replicates. Nutrients such as Nitrogen, Potassium and Zinc were added in recommended level and compare growth at two phosphate levels as P0 (less than 0.05 kg/ha) and P30 (30 kg/ha). Plant growth parameters such as plant height, number of tillers were taken, plant dry weight, shoot and soil P concentration were measured and P uptake was calculated. Tolerant cultivars performed well in deficient soil with high P uptake and less P retain in soil. Variation for P uptake was in the range of 6.6 to 22.8 mg P/plant. Traditional varieties were superior to modern varieties. Varieties H4, Marss, Murungakayan, Kaluheenati, Pokkali were classified as highly tolerant and Bg 357, Bg 352 were intolerant. It can combine stress tolerant traditional varieties as donor of genes to modern varieties with high harvesting index to improve grain yield under P deficiency.

P325

### Effects of phosphorus fertilization on phytic acid in seeds of two maize hybrids

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Phytic acid, the primary storage form of phosphorus (P) in cereal grains, is indigestible to non-ruminant livestock due to the lack of phytase, and the excretion would result in serious P pollution, thus reducing the phytic acid P content in maize seed has a potential to decrease its risk. To explore the impact of variety and P fertilization on the contents of phytic acid P during seed filling, a field experiment with 2 hybrids (Jinhai 5 and Xundan 20) and three P levels (0, 120, 240 kg/ha  $P_2O_5$ ) was conducted. The seed samples were collected at 10, 14, 20, 25, 28, 34, 38, 44, 50, and 57 days after silking (DAS). Results showed that the phytic acid P of seed could not be detected until 14 DAS for all treatments. During the period of 14 to 57 DAS, the change of phytic acid P concentrations could be divided into 4 stages for all treatments, which were (1) rapid increase from 14 to 28 DAS, (2) stable from 28 to 38 DAS, (3) rapid increase again from 38 to 50 DAS, and (4) stable from 50 to 57 DAS. However, there was no such distinguishable stages in the accumulation of seed phytic acid P, which was increased steadily from 14 to 50 DAS. At the later of filling stage, the concentration and accumulation of phytic acid P were increased by P application, and they were higher in Jinhai 5 than that in Xundan 20. In conclusion, the concentration and accumulation of phytic acid P in maize seed could be decreased by selecting maize variety and reducing P fertilization.

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### Local and systemic regulation of the root-associated acid phosphatase activity induced by Pi starvation

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The induction and secretion of acid phosphatases (APases) is a universal response of plants to phosphate (Pi) starvation. In *Arabidopsis*, Pi starvation-mediated induction of root-associated AtPAP10 (*Arabidopsis* purple acid phosphatase 10) activity is tightly regulated at multiple levels; however, the roles of local and systemic signalling involved in this response remain largely unknown. Using split-root and other assays, we show that a decrease in local, external Pi availability is sufficient to induce AtPAP10 transcription in roots; however, the magnitude of the induction is affected by the Pi status in the whole plant. In addition, the triggering of AtPAP10 transcription through local signaling depends on the presence of sucrose, a systemic signal from shoots. Once the AtPAP10 mRNAs are synthesized in roots, subsequent accumulation and secretion of AtPAP10 proteins is mainly controlled by local signaling. After secretion, the activity of AtPAP10 on the root surface is further stabilized by external, low Pi levels. Sucrose and ethylene have been previously demonstrated to be two positive regulators of root-associated AtPAP10 activity. In this work, we provide evidence that under Pi deficiency, ethylene is primarily involved in the control of AtPAP10 protein secretion but not in the control of AtPAP10 transcription or protein accumulation. We also show that the effect of ethylene on the induction of root-associated APase activity depends on sucrose but the effect of sucrose does not depend on ethylene. These results provide insights into the distinct roles of local and systemic signaling in the regulation of plant responses to Pi starvation.

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### Phosphorus distribution in maize under phosphorus application and tillage systems on Ferric Acrisol in Ghana

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Phosphorus deficiency in soils is a major problem confronting crop production in both tropical and temperate climates. In sub-Saharan Africa, the deficiency has caused decline in crop yield over the years. There is dearth of relevant information on phosphorus distribution in plants, its use efficiency and implications on crop yield under different soil management system which is a prerequisite for sustainability of cropping systems. Field experiments were carried out at the Awomaso Agricultural Research Station of the Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana located within the semi-deciduous forest zone and Ejura, Council for Scientific and Industrial Research (CSIR) within the Forest-Savannah Transition zone of Ghana. The study aimed at evaluating phosphorus distribution in maize plants following phosphorus application under tillage systems and the implication on crop yield. The field experiment at both locations were split plots arranged in randomized complete block design with three replications. The main plot factors consisted of two tillage systems namely, conventional tillage (CT) and no-tillage (NT) systems whereas the sub-plot factors consisted of four levels of phosphorus fertilizer: 0, 30, 60 and 90 kg P<sub>2</sub>O<sub>5</sub> per ha. Phosphorus distribution in different parts of the plants (leaves and shoot) was determined at the end of juvenile stage (V6), peak of vegetative growth (VT) and also at physiological maturity, R6 (ear, shoot and leaves). Indices of phosphorus use efficiency such as agronomic efficiency, apparent phosphorus recovery (APR), phosphorus utilization efficiency (PUE) and partial factor productivity (PFP) were determined. Results indicated significant differences ( $p < 0.05$ ) in the four levels of the phosphorus with regard to the nutrient distribution in the various plant parts at both locations of study. PUE and APR were significantly higher under application of 60 kg/ha P<sub>2</sub>O<sub>5</sub> at the two locations. Higher phosphorus concentrations were found in the leaves at the peak of vegetative growth than at physiological maturity especially under the NT system. The results of the study generally indicated higher phosphorus use efficiencies under lower rate of P application (60 kg/ha P<sub>2</sub>O<sub>5</sub>) than observed under the higher rate (90 kg/ha) under the tillage systems. Though higher grain yield was generally obtained under application of 90 kg/ha P<sub>2</sub>O<sub>5</sub>, results were not significantly different from that under 60 kg/ha P<sub>2</sub>O<sub>5</sub>.

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### Phenotyping for drought and low phosphorus tolerance in tropical legume crops

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Water deficit and low soil fertility are the major environmental constraints to crop yield in the semi-arid regions of West and Central Africa where accessibility and affordability of fertilizer is an issue. Moreover, phosphorus is important for crop production but is inherently low in most of tropical soils. However, it is hypothesized that phosphorus improves root growth and number of nodules, which could result in increased nitrogen and water uptake, leaf expansion rate, canopy development, radiation interception, grain yield, and seed quality. Therefore, knowledge of drought tolerance and nitrogen fixation mechanisms induced by phosphorus may contribute to improve the management practices for the farmers land. Further, selection of plant genotypes that produce good yield under low-P soil or those with high-P response efficiency can be a low-input approach to solving this problem. Clearly, an integrated approach combining plant traits improvement and optimum land management are needed to revitalize the crops performance under climate change and degraded land conditions. In this presentation, we would like to describe some methodologies (dry-down, infrared-thermography, lysimetric system) and present some results from our work on the phenotyping of plants for water-use, nitrogen fixation and yield under progressive drying soil. Also, we will show how these techniques are well suitable and relevant for agronomical and physiological assessment of the effects of phosphorus on plants nutrition, growth, yield, and products quality. In prospects, we will be investigating on phosphorus efficiency in agriculture and its ability to improve the cropping systems and their productivity in the semi-arid regions.

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### What is the optimal phosphorus supply for growth of faba bean inoculated with rhizobia

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In order to study the influence of phosphorus fertilization on nodulation and growth of faba bean, the Alfia genotype was inoculated with *Rhizobium leguminosarum* bv *viciae* and grown in hydroaeroponics under glasshouse with eight phosphorus supplies: 5, 15, 30, 50, 75, 125, 175 and 225  $\mu\text{mol week}^{-1} \text{ plant}^{-1}$ . The results indicate that phosphorus supply in combination with rhizobia inoculation increased nodules and biomass production until a maximum P supply of 125  $\mu\text{mol P week}^{-1} \text{ plant}^{-1}$  for nodulation and  $\text{N}_2$ -dependent growth, above which P toxicity was observed. It is concluded that faba bean crop should be grown preferably with 125  $\mu\text{mol P week}^{-1} \text{ plant}^{-1}$  along with rhizobia inoculation.

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### Phosphate/zinc interaction analysis in two lettuce varieties reveals contrasting effects on biomass and Pi transport

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Inorganic phosphate (Pi) and Zinc (Zn) are two essential nutrients for normal plant growth. An interaction between these two elements has been observed in many crop plants. However, despite its agronomic importance, the biological significance and genetic basis of this interaction remain largely unknown. Present work aimed at examining the Pi/Zn interaction in two lettuce (*Lactuca sativa*) varieties, namely "Paris Island Cos" and "Korlaat". The effects of variation in Pi and Zn supply were assessed on biomass and photosynthesis for each lettuce variety. Paris Island Cos displayed a better growth and photosynthesis compared to Korlaat under all the conditions tested. A correlation analysis was performed to determine the interconnectivity between Pi and Zn intracellular contents in these lettuce varieties. Paris Island Cos showed a strong negative correlation between the accumulation levels of Pi and Zn in shoot and roots. However, no relation was observed for Korlaat. These results are in line with the observed decreases in the dynamic of Pi transport in Paris Island Cos following the increase of Zn concentration in the medium, but not in Korlaat plants. Taken together, these results revealed a contrasting behavior between the two lettuce varieties in term of the co-regulation of Pi and Zn homeostasis, and provided evidences in favor of a genetic basis for the interconnection between these two elements. Identification of key genes involved in this interaction will certainly facilitate the task of lettuce researchers and breeders in the future to improve Pi and Zn nutrition while reducing fertilizers use.



**Theme 4 – Ecosystem dynamics and environmental impacts of phosphorus**



## **Theme 4 – Ecosystem dynamics and environmental impacts of phosphorus**

### **Keynote presentations**

K400

## The IMBALANCE-P Lecture

### Effects of phosphorus limitations on Life, Earth system and Society

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P is an earthbound and finite element and the prospect of constrained access to mineable P resources has already triggered geopolitical disputes. In contrast to P, availabilities of carbon (C) and nitrogen (N) to ecosystems are rapidly increasing in most areas of the globe. The resulting imminent change in the stoichiometry of available elements will have no equivalent in the Earth's history and will bear profound, yet, unknown consequences for life, the Earth System and human society. The ongoing shifts in C:N:P balances in ecosystems will necessarily affect the structure, function and diversity of the Earth system. P-market crises might put pressure on the global food system and create environmental ripple effects ranging from expansion of agricultural land to P-price-induced changes in land management exacerbating the stoichiometric resource imbalance. Yet, the impacts of this unprecedented human disturbance of elemental stoichiometry remain a research enigma. The IMBALANCE-P-team, that gathers four groups in the fields of ecosystem diversity and ecology, biogeochemistry, Earth System modelling, and global agricultural and resource economics, will address this Earth System management challenge by providing improved understanding and quantitative foresight needed to formulate a range of policy options that will contain the risks and mitigate the consequences of stoichiometric imbalances. IMBALANCE-P will integrate Europe's integrated assessment and Earth system models, calibrated using ecosystem nutrient limitation data obtained from field experiments. The project will establish an international process of science-based P-diplomacy.

K401

## Landscape exports of P and their effects on aquatic ecosystems

Val H. Smith

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Human-induced disturbances to the landscape result in the mobilization of plant nutrients, especially phosphorus (P) and nitrogen (N), to local receiving waters. This anthropogenic export of nutrients has caused the nutrient over-enrichment, or eutrophication, of rivers, lakes, and coastal waters worldwide. Eutrophication is typically accompanied by nuisance algal growth, taste and odor problems in drinking water supplies, fish kills, and other undesirable symptoms of eutrophication. This presentation will focus on the effects of excess phosphorus loading on the frequency, intensity, and toxicity of Harmful Algal Blooms (HABs), as well as disruptions of aquatic ecosystem structure and function. For example, dominance by nuisance bloom-forming cyanobacteria (blue-green algae) in freshwater lakes increases steadily with phosphorus enrichment. Furthermore, recent evidence suggests that the toxicity of HABs depends upon the relative supplies of N and P in the water column. This presentation also will focus upon recent controversies concerning the feasibility and desirability of nutrient control as a tool for objective eutrophication management. In particular, mounting evidence suggests that restriction of both N and P inputs must occur in order to achieve reliable eutrophication control. Finally, this presentation will focus briefly on strategies and tools that can be implemented in the landscape to help minimize the quantities of N and P that are exported to local surface waters.



## **Theme 4 – Ecosystem dynamics and environmental impacts of phosphorus**

### **Oral presentations**

O401

### Phosphorus a pivotal element in plant-soil system stoichiometry: equilibrium and imbalances

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Phosphorus is a crucial element for all living organisms because of its role in the DNA structure, protein synthesis and energy transfer. In addition to its crucial role in all organisms and its frequent low availability, P role is strongly interdependent of the availability and internal cycle of other nutrients. The relationships among the ratios of the main nutrients such as C:N:P ratios of organisms and the environment (water and soil) and their relationships with ecosystem processes and structure are the subject of the ecological stoichiometric studies. Ecological stoichiometric studies have reached advances in aquatic ecosystems (mainly in lakes) but there is a lack of clear results in terrestrial ecosystems. Most studies have been focused on leaves and on the N:P ratio, relating it mainly to plant growth and/or to consequences of growth and production on ecosystem traits. But N and P can be allocated to more organs than leaves and to more functions than growth in terrestrial plants. Moreover, other elements have been proven to be important in terrestrial ecosystems and to indirectly affect N and P status and use, and yet they have been frequently neglected in the ecological stoichiometric studies in terrestrial ecosystems. We have conducted a set of new studies taking into account more plant organs than leaves, more functions than growth and more elements than only N and P. To do this, we have applied metabolomic and phylogenetic analyses coupled to elemental analyses in the study of plant responses to abiotic and biotic changes taking into account overall plant organs. The results have shown that plant elemental concentrations and stoichiometry changes are related to plant responses to environmental changes through the different shifts in the various plant functions (growth, anti-stress, storage and so on). Different plant functions use different elements asymmetrically, being P concentration and stoichiometry outstanding in these processes. Moreover, and together with this plant capacity to shift its elemental stoichiometric composition in response to environmental changes, we have also observed that each plant species has a specific elemental composition related to its long-term evolutionary process under determined and singular environmental conditions. The phylogenetic distances among species explain a significant part of the elemental composition differences among species. We are working with the general hypothesis that coupling of stoichiometric studies with studies of overall plant function in different environmental conditions can improve the knowledge of ecological processes and the underlying mechanisms

O402

### Do trophic relationships in soil enhance plant P nutrition? Phytate mineralization as a case study

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Phosphorus (P) strongly limits plant productivity as plants can only absorb free inorganic orthophosphate (Pi) at very low concentrations in soil solution. However, soil contains high levels of poorly available P, especially phytate, considered as the most abundant plant-unavailable organic P source. Here, we investigated a new strategy based on rhizosphere trophic relationships to mobilize P from phytate. We hypothesized that the interactions between plant (*Pinus pinaster*), phytase-producing bacteria (*Bacillus subtilis*), mycorrhizal fungi (*Hebeloma cylindrosporum*), representing the more widespread strategy to improve plant P acquisition, and bacterial grazer nematodes (*Rhabditis* sp.) may improve plant P acquisition from phytate and thus P sustainability from soil organic P. We grew seedlings in microcosms containing soil with Pi or phytate, with or without the above-mentioned organisms for 2.5 months. With Pi, no significant differences were observed among inoculation or mycorrhizal treatments. In contrast, with phytate, nematode grazing was required for non-mycorrhizal plants to acquire P into their shoots. In mycorrhizal plants, bacteria alone improved net P accumulation and nematode grazing enhanced this positive effect. Soil microbial P contents and in situ probing of bacterial phytase gene expression are currently under study to understand better the mechanisms underlying the strong positive effects of nematode grazing on plant P nutrition. Our first results indicate that the use of trophic relationships should be considered as a sustainable strategy for plant P nutrition to enhance organic P cycling and P availability in the rhizosphere.

O403

### Linking P and C cycling in the decomposer system of an Amazonian rainforest

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Coastal tropical rainforests of the Amazon grow on some of the oldest and most nutrient impoverished soils on Earth. Phosphorus (P) availability is particularly low, increasing the competition among organisms for this key element. Accordingly, we found that microbial decomposer communities from a tropical forest of French Guiana sequester leaf litter P at higher rates than leaf litter N with clear shifts in microbial biomass C:N:P stoichiometry. Apparent P limitation of microorganisms was tested with a two-year fertilization experiment in the field where we added the key elements C, N, and P in all possible combinations. We measured a 47% higher biomass and substantially increased activity of soil microbial communities that grew in P-fertilized plots compared to plots without P fertilization. These responses were amplified with a simultaneous C fertilization suggesting P and C co-limitation of soil microorganisms. Stimulated microbial activity under P fertilization led to strongly reduced soil organic C concentrations after two years. Supplementary laboratory incubations of soil samples from our experimental site confirmed higher respiratory C loss and higher dissolved organic carbon (DOC) leaching from P fertilized soils. Our data suggest that microbial soil communities in the studied tropical forest respond strongly to increased P availability leading to a substantial breakdown of soil organic matter (SOM). We conclude that P availability has a strong control on SOM dynamics in the studied tropical rainforests. Tropical forest soils may shift from C sinks to sources much more rapidly than previously thought.

O404

### The bioavailability of colloidal P to freshwater algae

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The eutrophication of freshwaters is a major environmental concern in developed countries and is often attributed to excessive P fertilizer application. However, the eutrophication risk depends strongly on P bioavailability, which in turn depends on P speciation. Colloidal P species, e.g. P associated with colloidal Fe and Al oxyhydroxides, are included in routine colorimetric measurements of the available P fraction as "molybdate reactive P", but the availability of this colloidal P fraction remains questionable. The aim of this study was to address the bioavailability of colloidal P in a well-defined model system. Growth and P uptake by a freshwater green alga (*Raphidocelis subcapitata*) were measured in synthetic solutions with or without colloidal Fe oxyhydroxides. Short term (1 hour) uptake experiments using radiotracers show that algal P uptake decreases with increasing colloidal P fraction. The P uptake rate is related to the free orthophosphate fraction (quantified by 10 kDa ultrafiltration), i.e. colloidal P does not contribute to the actual P uptake. Growth experiments on the longer term (up to 14 days) under P-limited conditions reveal that colloidal P contributes partially, but not completely, to algal growth. This is likely a result of desorption when free orthophosphate is taken up and becomes depleted. This potential P bioavailability correlates to the "labile P pool", which is quantified by dialysis of the test solution against ferrihydrite as infinite P sink. It is concluded that colloidal P is only partially bioavailable, and that the eutrophication risk in freshwaters may be overestimated if P is measured as "molybdate reactive P".



## **Theme 4 – Ecosystem dynamics and environmental impacts of phosphorus**

### **Posters**

P401

### Dynamics of soil carbon, nitrogen, and phosphorus and microbial community composition after land-use abandonment

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Since many forests and grasslands have previously been cultivated it is important to understand long-term effects of former arable land use on these ecosystems. We studied the impact of former agricultural land use on soil total organic C (TOC), total nitrogen (TN), phosphorus (P) fractions, and microbial community structure over 200 years in two chronosequences of abandoned vineyards, differing in aspect and slope. We found that more TOC accumulated in soils of south- than in southwest-exposed sites, which can be attributed to the drier microclimate that decreases organic matter decomposition. The concentration of fertilizer-derived bioavailable P diminished during the first 50 years after land-use abandonment, leading to low P availability at the later stages of the succession. The TOP concentration first increased during the first 40 years after abandonment, and decreased in the later stages of the secondary succession. The ratio of TOC-to-total organic P (TOC/TOP) increased strongly during secondary succession, which likely can be attributed to a P limitation of the vegetation in the later stages of succession. The ratio of arbuscular mycorrhizal fungi-to-bacteria (AMF/bacteria) increased strongly during the first decade after land-use abandonment, indicating a quick recovery of AMF. Along with an increase in the abundance of trees, the AMF/bacteria ratio slowly decreased again. In conclusion, we found that even though the bioavailable P concentration decreased quickly after land-use abandonment, other parameters such as the vegetation, the soil TOC/TOP ratio and the AMF/bacteria ratio were still in transition after over 150 years of secondary succession.

P402

### Belowground interactions between a cereal and a legume along a P gradient: a test of the Stress Gradient Hypothesis

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According to the stress gradient hypothesis, poor resource availability should favor facilitation interactions while high resource availability should lead to strong competition between two plants relying on the same resources. However, there are still some discrepancies about the shape of the relation between the stress level of the resource availability, and the nature and strength of the interaction between the interacting plants. Here, we present the results of a glasshouse experiment in which a cereal (wheat) and a legume (white lupin) are grown together or separately along a phosphorus gradient. As white lupin, by exuding carboxylates, protons and phosphatases, can increase P availability in soils containing sparingly available P, we make the hypothesis that the cereal could benefit from this increase as long as P availability is low enough. Moreover, by taking up the bioavailable P, wheat may maintain the level of soil P sufficiently low to stimulate the mechanisms responsible for the facilitation effect from white lupin. We thus investigated the relationships between the P supply level and the nature and strength of belowground interactions between wheat and white lupin, in order to determine the conditions leading to transgressive overyielding (i.e. when the amount a species yields when grown with another species, is higher compared to that when grown alone). Designing more performant agroecosystems is of particular interest in the global context of food insecurity, increasing cost/decreasing efficiency of fertilizers, and better using ecological processes that operate in natural ecosystems is worth an option.

## P403

### Microbial biomass and catabolic activities in the rhizosphere as impacted by P fertilization, a field study

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In the context of the EURoot project, a large maize phenotyping field experiment was conducted in order to assess root architecture and functional traits amongst a panel of 23 maize genotypes across a gradient of P fertility (long term P fertilizer trial of INRA Auzeville near Toulouse SW France). Four P levels were tested, from P0 (treatment without P fertilizer since 1969) to P4 (treatment with rates of P fertilizer application between 3- and 4-fold the offtake by crops). Rhizosphere (soil adhering to roots) and bulk soil were destructively sampled at the 6-8 leaves stage when P deficiency was clearly visible in many genotypes in the P0 treatment. We measured the substrate-induced respiration with the MicroResp<sup>TM</sup> technique (3 substrates and a proxy of microbial biomass) and basal respiration of the 350 soil samples from the topsoils of the two extreme P treatments (P0 and P4). Our first data analysis shows a positive P effect on microbial biomass and a significant interaction of phosphorus/type of soil effect (bulk/rhizosphere). No maize genotype effect was evident from the analysis. In general, P fertilization (P4 versus P0) seemed to have increased the differences in microbial parameters between rhizosphere and bulk soils.

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## P404

### Arbuscular mycorrhizal colonization in maize genotypes grown under contrasted P-regimes in a long-term field experiment.

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Most terrestrial flowering plants have the ability to establish symbiotic associations with arbuscular mycorrhizal (AM) fungi. The fungus improves the uptake of water and mineral nutrients in the host plant, mainly phosphorus and nitrogen, in exchange of photoassimilates. AM symbiosis not only promotes plant growth through facilitation of nutrient uptake, but it might also protect the plant from abiotic and/or biotic stresses. The benefits received by the AM symbiosis vary depending on the host genotype and the AM fungus species. In nature, as well as in crop fields, different AM fungi can be present in the soil or the plant, and affected by P availability or plant genotype. This has been little documented in field-grown crops however. In this study, we examined AM colonization in roots of a panel of maize genotypes grown in soils with contrasted P levels. We examined the relative level of colonization by 5 different AM fungi. Field experiment was carried out at the long-term P fertilizer trial of INRA-Auzeville (SW France). The field consisted in 4 P levels replicated in 4 blocks, ranging from P-deprived soils (P0, soil that had not been fertilized for 45 years), to soils that have been under excess P fertilization (P4, 4-fold the P offtake by crops). A total of 23 maize genotypes (selected for their contrasting root traits) grown in the two extreme P regimes (P0 and P4) were assessed. The level of root colonization was examined by microscopy observations of trypan-blue stained roots. AM colonization was further quantified by qPCR. Differences in susceptibility to AM colonization among the different maize genotypes grown under different P levels will be presented.

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P405

### Phosphorus fractions in discharge from artificially drained lowland catchments

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Tile-drainage systems have been identified as a major pathway for phosphorus (P) from agricultural land to surface water bodies including the Baltic Sea. We were aiming at assessing the importance of various dissolved and particulate P-forms ( $\text{PO}_4$ , DOP, POP, adsorbed  $\text{PO}_4$ ), for P leaching in order to derive mitigation strategies to improve the protection of the Baltic Sea against anthropogenic nutrient loads. Flow-driven water samples were collected at three spatial scales (tile-drain=5ha; drainage ditch=180ha; brook=1600ha) in a Northern German moraine landscape over the 2013/14 discharge period. In general, total P (TP) losses ranged between 60 and 120 g ha<sup>-1</sup> over 4 months of discharge with increasing concentrations at larger scales. The TP temporal dynamic in the brook was discharge-driven with greater concentrations at increasing discharge while the ratio of particulate P (PP) to TP tended to decrease upon rising discharge intensity. However, the overall contribution of the PP fraction to total losses was 54 % (median value, range: 12-93%). The dissolved organic P fraction (DOP) accounted for 11% (range 1-37%) of TP. A raster electron microscope analysis revealed that particulate P is basically bound to iron and calcium. The overall importance of the PP fraction to total P losses suggests that end-of-pipe measures such as drainage ponds or filter ditches in which particles are filtered or allowed to settle will substantially reduce P loading to surface waters.

P406

### Leibniz ScienceCampus Rostock Phosphorus Research – an interdisciplinary network for a more sustainable P management

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Agriculture and the entire economy are challenged in the future by the limited phosphorus (P) availability. Despite depletion of P-resources, undesired amounts of P are still 'wasted' causing environmental problems, especially in aquatic systems such as the Baltic Sea. Because of the central importance of P in a variety of production and environmental systems, an interdisciplinary research approach is necessary. Therefore, five Leibniz Institutes and the University of Rostock have created a network to intensify collaboration and research around this essential element and its sustainable management. Cooperation partners are the Leibniz-Institute for Baltic Sea Research Warnemünde (IOW), Leibniz Institute for Catalysis (LIKAT Rostock), Leibniz-Institute for Farm Animal Biology (FBN Dummerstorf), Leibniz Institute for Plasma Science and Technology (INP Greifswald), Leibniz Institute of Plant Genetics and Crop Plant Research (IPK branch North/Groß Lüsewitz), and the University of Rostock (with 4 faculties). About 40 research groups with more than 70 scientists with relevant expertise are active in the ScienceCampus. Research foci are 'P-cycles and fluxes in the environment', 'sufficiency and efficiency of P use, P-recovery', 'P as an element in and out of catalytic processes'. Additionally, the development of advanced analytical methods in P-research has been defined as cross-sectional task for serving and stimulating the research clusters. More than 20 thematically assigned projects covered by various disciplines are ongoing and new projects are emerging continuously.

P407

### Long and short time effects of phosphates sources on forage mass of natural grasslands in Southern Brazil

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The purpose of this study was to evaluate the contribution of different species into forage mass and total herbage mass in natural grasslands as related to either recent application of different phosphate sources or with 13 years history fertilization management. The treatments were applications of P from Gafsa rock phosphate and triple superphosphate and a control treatment, organized according to a randomized blocks design, with three replications. The experiments to evaluate the effect of recent additions were carried out in Candiota-RS, in which around 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was added in Sep 2010. In Santa Maria-RS was carried out the long historical fertilization experiment, where additions of 180, 90, 100 and 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> were done in 1997, 1998, 2002, and Aug 2010, respectively, which represents 470 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. The grassland was mowed on spring-summer season after the last P fertilization and responses were evaluated by the total forage mass. In experiments of Candiota, total forage mass was similar for the different phosphate sources (2608 kg ha<sup>-1</sup>). In Santa Maria, total forage mass with Gafsa rock phosphate application was similar to the control (2922 kg ha<sup>-1</sup>) and soluble phosphate application had increased the contribution of ryegrass and *Eustachys uliginosa*, however, the contribution of *Paspalum notatum* had decreased, resulting in higher total forage mass (4061 kg ha<sup>-1</sup>). Recent application of phosphate fertilizers did not affect total forage mass and contribution of different species, showing that the effect of phosphate fertilization is not immediate.

P408

### Distribution and estimated release of sediment phosphorus in a mixed land use catchment from Southern Brazil

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Phosphorus (P) and suspended sediment (SS) mobility from runoff and their subsequent delivery to freshwater reservoirs is of most concern in catchment scale soil and water quality management. The aims of this study were to characterize SS phosphorus forms; total P (TP), inorganic P (IP), total organic P (TOP) and bioavailable P (BAP) and monitor the spatial variation of the SS P content among the different management systems. The study area Guapore catchment is located between latitudes 29.2 S and 28.2 S and longitude 52.4 W and 51.8 W in Southern Brazil with basin area of 2000 km<sup>2</sup>. A total of 11 monitoring points with different land uses including city effluent, conventional tillage (CT) and no-tillage (NT) systems were selected. The SS were collected by using time-integrated suspended sediment samplers from March 2011 to October, 2013. The results indicated that mean soil TP for cultivated land was 442.5 mg kg<sup>-1</sup>. The SS exhibits high mean TP 2825.6 mg kg<sup>-1</sup> in the CT in December months in both years. In contrast, the TOP was maximum 584.86 mg kg<sup>-1</sup> in SS from non-treated city effluent. Generally, across the different land uses, the TP content of SS showed 4-7 fold increase when compared to respective soil TP. The highest IP was in NT system and average across the monitoring points IP showed as much as 95 to 99% of the TP. The BAP content of SS ranges from 18.7 to 84 mg kg<sup>-1</sup>. The study explains seasonal P export from different management systems and emphasize that moderation of the P fluxes from sediments requires considerable reductions of the phosphorus load from land based sources.

P409

### Soil properties affect relation between soil phosphorus and plant species diversity of extreme tropical habitats

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High concentrations of nitrogen (N) and phosphorus (P) can decrease species richness of plant communities due to negative effects of higher biomass and more intense competition for light. However this relation is unclear in habitats with severe drought or high metal concentrations in soils. In these extreme habitats, only tolerant species (metals, drought) with low requirements for water, N and P and no competitor for the light, would be selected. The hypothesis is that species richness in the extreme habitats is slightly affected by N and P. The present study was conducted in tropical grasslands of South Central Africa associated with Cu/Co natural rich soils. The aim was to examine the relation between available phosphorus in these soils and plant species diversity among habitats with high range of P [1.7–499 mg.kg<sup>-1</sup>], Cu [29–10136 mg.kg<sup>-1</sup>] and Co [2–927 mg.kg<sup>-1</sup>] concentrations in soils. For this purpose, the properties of 200 vegetation records and associated soil samples were determined. Results suggested that P does not contribute to variation of plant species diversity, unlike Cu, Co and other edaphic factors influencing metal availability (i.e. pH, C, Ca, and Mn content). It can be thus inferred that selection pressure may be more strongly influenced by metal phytotoxicity than by high variation of available P concentrations in soil in tropical grasslands. However, this conclusion should be confirmed by P limitation experimentation along Cu/Co gradient where both the plant diversity (species and functional diversity) and productivity would be measured.

P410

### Freshwater phosphate limits are based on a poorly standardized molybdate reactive P method

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Phosphate is becoming a main water quality determining factor in regions with intensive animal husbandry. The freshwater limits in Flanders are based on phosphate determined by the molybdate reactive P (MRP) method and the certified laboratories have to adhere to ISO protocols (ISO 15681-2:2003 or ISO 15923-1:2013). Surprisingly, filtration is not specified in these guidelines and sample preparation procedures range from unfiltered to 0.45 µm filtration in these certified laboratories. It is well established that MRP includes both free orthophosphate and phosphate associated with colloidal material such as Fe and Al oxyhydroxides while organic P is only weakly included. The aim of this study was to compare analytical results among certified laboratories and to identify filtration effects on the analytical results. In a round-robin test, ten waters were collected from streams in Flanders and sent to certified laboratories. In addition, the same waters were tested in our laboratory assessing effects of filtration (paper filter, 0.45 µm, 0.1 µm) and dialysis (12-14 kDa) on MRP. The MRP concentrations in the water samples decreased gradually by filtration over progressively smaller membrane pore sizes and after dialysis. Filtration over a 0.45 µm membrane filter reduced MRP concentrations to 74-84% of MRP in unfiltered waters with low Fe (<0.5 mg F/L) and to 6-33 % of MRP in unfiltered waters with high Fe (>2.5 mg Fe/L). In the round-robin test, the coefficient of variation of MRP among certified laboratories ranged from 4-71%. We postulate that this variation can be reduced by more stringent laboratory guidelines.

P411

### Physiological responses of *Hakea laurina* (Proteaceae) to low-P and high-P conditions

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Proteaceae species are generally known as low-P tolerant and high-P sensitive plants, although the strategies and mechanisms are still undetailed. The aim of this study is to investigate the physiological responses of *Hakea laurina* to low-P and high-P conditions and interactions between other elements and P status. *Hakea laurina* was hydroponically cultivated in nutrient solution containing 0, 6.4 (Low-P) and 64  $\mu\text{M}$  P (High-P). The 0, 14, 27, and 43 days after treatment (DAT), seedlings were used for measuring plant total fresh weight, shoot and primary root length, and number of leaves and cluster roots. All plants were harvested at 43 DAT after separation into roots and upper, middle and lower leaves, and used for elementary analysis for P, N, K, Ca, Mg, Fe, Mn, B, Cu and Zn. *Hakea* plants grew well and formed many cluster roots in Low-P condition, whereas the growth, especially root, was inhibited under High-P condition. This suggests that *Hakea* prefers to grow under low-P condition and shows P sensitivity under high-P condition like other Proteaceae species. P concentration of leaves at 43 DAT was 0.4-0.7 and 8.6-13.5 mg-P/g DW under Low-P and High-P condition, respectively. Concentration of Ca, Fe, Mn and Zn in upper leaves were significantly lower in High-P, while K concentration was opposite. In addition, B concentration of root was increased depending on P concentration. Considering the fact of huge exudation of carboxylate from cluster roots together, cluster roots formed under low P condition could support uptake of other elements also. The growth inhibition in High-P might be caused by alteration of other element distribution accompanied with P status.

P412

### Effects of myo-inositol hexakisphosphate on Zn(II) sorption on Aluminum Oxide

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Myo-inositol hexakisphosphate (IHP), the most abundant organic phosphate (OP) in most soils, strongly interacts with aluminum (Al) oxides and metal ions. In this study, the effects of IHP on Zn (II) sorption on  $\gamma\text{-Al}_2\text{O}_3$  ( $\gamma$ -alumina) were investigated using a batch technique, powder X-ray diffraction (XRD), in situ attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), and  $^{31}\text{P}$  and  $^{27}\text{Al}$  solid-state nuclear magnetic resonance spectroscopy (NMR). The XRD analysis indicates that without the post-sorption of IHP on  $\gamma\text{-Al}_2\text{O}_3$ , the formation of Zn-Al layered double hydroxide (LDH) precipitates occurs at Zn (II) concentration  $\geq 0.72$  mM, and with the post-sorption of IHP, Zn-Al LDH precipitates are formed at Zn (II) concentration  $\geq 1.50$  mM. Therefore, the post-sorption of IHP restrains the formation of LDH. The ATR-FTIR and solid-state NMR spectra further suggest that after the post-sorption of IHP,  $\gamma\text{-Al}_2\text{O}_3$ -IHP-Zn ternary surface complexes are formed with the addition of low concentration of Zn(II) ( $< 0.72$  mM), Zn-IHP precipitates are developed with the addition of moderate concentration of Zn(II) (0.72-1.50 mM), and Zn-IHP precipitates and LDH are formed when the Zn(II) concentration is higher ( $> 1.50$  mM). These results demonstrate that the simultaneous presence of IHP and divalent metals significantly affects the solubility and speciation of these compounds in environmental settings.

P413

### Phosphorus leaching risk assessment with manure fertilizer application in South China

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To investigate the phosphorus (P) leaching risk caused by applying manure fertilizer, six different manure fertilizers, namely, 0 (CK), 3000 (F200), 4500 (F300), 7500 (F500), 10500 (F700), and 15000 kg ha<sup>-1</sup> (F1000), were applied to the surface soil (0-20 cm) prior to vegetable planting. The maximum Olsen P decreased with increasing amounts of the manure fertilizer. Total P did not change with treatments below 7500, but decreased in the higher manure treatments. Water dissolved P (WDP) was highest in the upper (0 - 20 cm soil) layer, intermediate in the middle (20 - 40 cm soil) layer, and lowest in the lower (40 - 60 cm soil) layer. The 7500, 10500, and 15000 treatments increased the WDPs in both the middle and lower soil layers and enhanced the degree of P saturation in the lower layer. This indicates that vertical leachate movement of P accumulation in the middle soil layer may be underestimated and the P leaching risk is enhanced using manure fertilizer levels greater than 7500 kg ha<sup>-1</sup>.

P414

### Phosphorus characterization in manures excreted by pigs at three different growth stages

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In order to identify comprehensively the P components and concentrations contained in manure excreted at different pig grown periods, we characterized the manures excreted during different pig growth periods with the different feeds, using a sequential procedure and a simplified two-step procedure (NaHCO<sub>3</sub>/NaOH+EDTA). The results indicated that P concentration in sow feed was 7.3 g kg<sup>-1</sup>, which was greater than that in piglet and growing-finishing pig with 6.9 and 4.9 g kg<sup>-1</sup>, respectively. The total P concentration in manures determined by the sequential fractionation procedure were 14.4 g P kg<sup>-1</sup> in piglet manure, 13.5 g P kg<sup>-1</sup> in growing-finishing pig manure and 31.3 g P kg<sup>-1</sup> in sow manure, respectively. The percent of cumulative P to the total P extracted in piglet manure was 31, 17, 46, 6 and 0 % associated with extractants H<sub>2</sub>O, NaHCO<sub>3</sub>, NaOH, HCl and residues, respectively. Corresponding P fractions for the growing-finishing pig manure and sow manure, H<sub>2</sub>O extracted 59 and 22% (as H<sub>2</sub>O-P); NaHCO<sub>3</sub>-P, 24 and 26%; NaOH-P, 10 and 9%; HCl-P, 8 and 43%; and residue-P, 0 and 0% of the total P, respectively. Total P extracted by the NaHCO<sub>3</sub>/EDTA were 22.6, 17.7 and 32.5 g P kg<sup>-1</sup> in piglet, growing-finishing pig manure and sow manure, respectively. Majority of P extracted by NaOH/EDTA from sow manure was organic forms. In general, both of the procedures could analyze the composition of manure P with the various diets, which will ultimately contribute to the optimal management of manure P and animal diet.

**Eco-enzymatic stoichiometry in a land-use gradient**

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Ecological stoichiometric theory uses C to nutrient ratios to predict nutrient immobilization versus mineralization and decomposers growth efficiency. At a global scale, eco-enzymatic ratios were shown to provide a functional measure of the threshold at which control of community metabolism shifts from nutrient to carbon limitation. However, at a finer scale the controls of nutrient availability on microbial activity in contrasted ecosystems are still poorly understood. To explore a large range of potential microbial nutrient limitation in relation to enzyme allocation, we performed a microcosm experiment using contrasted soils from a land-use gradient (grassland, forest, culture, and plantation). Overall, C:N:P enzymatic ratios were relatively well related isometrically across all ecosystems. However, significant variations were found between different soils within the gradient. A deviation from the line 1:1 for the N:P ratio was observed in the soils of forest and grassland. In these natural ecosystems, the relative proportion of fungi was more important than bacteria indicating that the fungal biomass may be more limited by N than the bacterial biomass. On the other hand, bacteria were more abundant at lowest C:P and N:P enzymatic ratios, suggesting that fast-growing organisms required more P to maintain their metabolism. We concluded that eco-enzymatic stoichiometry is a useful indicator of nutrient or energy limitation of soil microorganisms and that some specific groups of microorganisms may be limited by different resources according to the differential limitation hypothesis.



**Theme 5 – Sustainable phosphorus use in agroecosystems**



## **Theme 5 – Sustainable phosphorus use in agroecosystems**

### **Keynote presentations**

K501

### Overcoming phosphorus deficiency in agricultural systems of sub-Saharan Africa: recent advances and limitations.

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Phosphorus deficiency is widespread in sub-Saharan Africa (SSA) due to continuous P depletion without replenishment and the prevalence of P-fixing soils. Phosphorus replenishment, particularly under small-scale farming systems, remains a challenge for it is mainly fertilizer dependent. While soluble P fertilizers are the obvious way to overcome P deficiency, the dependency on fertilizer imports by most SSA countries and the poor infrastructure make the cost of P fertilizers prohibitive for smallholder farmers. Recent initiatives and approaches for boosting agricultural production in SSA (e.g. the African Green Revolution, the Integrated Soil Fertility Management (ISFM)) are focussing on increasing fertilizer use and efficiency by small-scale farmers, including the use of local resources. Phosphate rock deposits are widespread in SSA, but various challenges, associated with their low reactivity, the lack of infrastructure and at times high Cd-concentrations, have limited their utilization. Numerous studies have developed strategies for improving the availability and efficiency of soil P through the use of organic resources, and the identification and development of P-efficient germplasm towards higher P-efficiency in crop rotation. This paper summarizes recent advances to combat P deficiencies in SSA. We focus on the use of P-fertilizers, the contribution of organic resources and P-efficient germplasm, and the role of ISFM. One major constraint hampering progress in understanding the processes driving P availability in tropical soils is the limited power of analytical methods when used in P-fixing soils. In this paper a particular attention is given to progress and limitations in developing methods towards understanding soil P processes.

K502

### Sustainable phosphorus use in agroecosystems: a story of global imbalance and resource recycling

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Phosphorus (P) plays a key role in many agroecosystems both as a limiting nutrient of crop production and as a factor controlling eutrophication in freshwater ecosystems. The context of increasing global rock phosphate scarcity requires more efficient farming systems that both promote P resource recycling and limit P losses to aquatic systems. This keynote will provide a global picture of P management in agroecosystems: we will point out the current imbalanced distribution of P application to soils at global scale and will stress the key role of past accumulated P in many agricultural soils in allowing for crop productivity for future decades. We will also address issues related to P resource recycling within and to agroecosystems: using an industrial ecology perspective, we will examine how crop and livestock integration, agricultural specialisation, and trade act as drivers of P flows at different scales ranging from small territories to global scales. Finally, we will discuss to what extent P recycling from urban sources (e.g., as compost or sludge) can contribute to meeting P demand in agroecosystems.



## **Theme 5 – Sustainable phosphorus use in agroecosystems**

### **Oral presentations**

O501

### Modelling the optimal phosphate fertiliser and soil management strategy for crops

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The readily available global rock phosphate (P) reserves are set to run out within the next 50-130 years, causing soils to have a reduced P concentration, which will affect plant P uptake. Careful use of this finite resource in agriculture systems is clearly warranted. We therefore present a model which searches for optimal fertiliser and soil management strategies for crop production while maintaining a sustainable plant P uptake. In this paper we present the results for wheat; however the model is adaptable for other types of crops, subject to root structure data being available. The model describes the development of the phosphate and water profiles within the soil space. Current cultivation techniques such as ploughing and a reduced till gradient are simulated along with fertiliser options to feed the topsoil or below the seed. By trying to minimise the amount of fertiliser used and achieving a sustainable level of P uptake we create a multi-objective problem, which is solved using optimisation algorithms. We find that a well-mixed soil (ploughing) is critical for optimal P uptake and provides the best environment for the root system. The combination of modelling and experimental data provides useful predictions for site-specific locations.

O502

### Reducing grain P concentration in rice through genetic improvement – an option for sustainable P management?

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The sustainable use of P fertilizers can be enhanced by reducing the amount of P exported from the fields. A Global Rice Science Partnership project was established to identify and develop rice genotypes with a lower concentration of P in the grains. In order to anticipate on potential negative effects of low grain P concentration on seedling vigor, management options to alleviate such an effect are tested. Field trials were carried out in Benin, Burkina Faso, Nigeria, the Gambia and Tanzania to explore genotypic variation in grain P concentration and genotype x environment interaction effects on grain P concentration. Further, field and greenhouse experiments were established to assess the effect of reduced grain P concentration on rice vigor at the early growth stage and yield. The use of a commercial seed P coating for alleviating potential negative effects of reduced grain P concentration on plant vigor was evaluated under upland conditions, while the application of fertilizers to the nursery bed was evaluated in an irrigated lowland trial. Results from the multi-location field trials indicated that the main effect of environment accounted for the largest part of the variation in grain P concentration. The effect of reduced grain P concentration on rice growth was not consistent across trials, and found to be genotype-, environment- and growth stage-specific. The preliminary results suggest that grain P concentration may be reduced without a significant threat to plant vigor. The implications of our results for sustainable use of P in rice-based systems through genetic improvement are discussed.

O503

### Trends of phosphorus use efficiency in the food chain of China

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Phosphorus (P) inputs by fertilizer and feed additives have greatly contributed to the increased food production in China during the last decades, but have also contributed to increased P losses to water systems. The aim of this study was to quantify the historical changes of P use efficiency and losses in the food chain in China between 1980 and 2005, and to explore possible trends for the period 2005 – 2030, using the NUFER (NUtrient flows in Food chains, Environment and Resources use) model. The results show that the P use efficiency in the food chain decreased from 19% to 7% and P losses to waters increased from 1 kg P/ha to 21 kg P/ha in the period 1980 to 2005. Scenario analyses suggest that P fertilizer consumption and losses will increase by 25% and 71% respectively in the period 2005 to 2030 in the business as usual scenario. Implementation of a package of integrated nutrient management measures (including balance P fertilization and improved manure management), changes in human diets, and increased imports of animal food and/or feed, are effective management options for increasing P use efficiency in the food chain. Our analyses indicate that the P use efficiency in the food chain may increase again to 13-15% and P losses may decrease by a factor of 3 to 5 compared with business as usual. We conclude that combination of diet change and integrated nutrient management has the highest values of P use efficiency in the food chain, and improved manure management is the most effective simple strategy for decreasing P losses.

O504

### Is P acquisition facilitated by intercropping of durum wheat and grain legumes in field conditions?

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Intercropping systems consist in growing two or more species simultaneously in the same field for at least part of their growth cycle. In situations of limiting nutrient availability, acquisition of resources in intercrops is generally improved compared to sole crops, as a result of positive interactions between the intercropped plant species, i.e. either niche complementarity or facilitation. Evidence has recently accumulated that intercropped cereals and grain legumes promote the use of soil P resources, though few reports derived from field trials. We investigated whether (i) intercropping of durum wheat with pea or faba bean does improve soil P acquisition; (ii) facilitative interactions can be detected, and (iii) soil P availability does influence such effects. We conducted two 1-year experiments in a long term P fertilizer field trial with a gradient of Olsen-P soil content from <5 to 30 mg/kg (Auzeville, SW France; 2009 and 2011). Aboveground parameters such as growth, productivity, nutritional status (N and P), fixed N ratios and grain quality were regularly monitored on intercrops and sole crops. Relative total biomass was always higher in intercrops vs. sole crops as illustrated by LER>1, except in the lower P soil content, where faba bean was outcompeted by wheat in the later stages. P export was increased in intercrops vs. sole crops at the legume flowering stage, while it decreased afterwards to similar amounts mainly due to faba bean suppression. Our data suggest positive plant-plant interactions (niche complementarity/facilitation) at earlier growth stages of wheat-legume intercrops that transiently resulted in a more efficient use of soil P resources but then receded until harvest.

O505

### Soil phosphorus and N<sub>2</sub> fixation of leguminous trees: consequences for rates and transfer in agroforestry systems

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In order to reduce excess fertilizer use, plant-mediated nutrient supply through N<sub>2</sub>-fixation, transfer of fixed N and mobilization of soil P may be important processes for the nutrient economy of low-input tree-based intercropping systems. *Acacia senegal*, an important leguminous tree in semi-arid environments, has shown promise as a multipurpose species, including gum production and soil fertility replenishment linked with N<sub>2</sub>-fixation capabilities. Our research objectives were to investigate the effect of tree age and site phosphorus conditions on (1) tree N<sub>2</sub>-fixation determined by <sup>15</sup>N natural abundance methodology and (2) N transfer processes determined by a direct <sup>15</sup>N tree leaf feeding technique. In natural populations (under field conditions), the rates of N derived from atmosphere (Nd<sub>fa</sub>) increased with increasing soil P availability from 0 to 39% Nd<sub>fa</sub> and slightly decreased with tree age in relation with increasing soil N mineralization. However, under controlled and non-limiting N conditions, Nd<sub>fa</sub> values were higher than in field conditions while increasing P supply stimulated growth and mineral N uptake without affecting the amount of Nd<sub>fa</sub>. Given high soil P conditions, we show that under controlled conditions, N transfer from *A. senegal* to an associated crop, wheat, amounted to upwards of 14% of total wheat N. Conceptually, our data indicates that i) controlled conditions may not reflect estimated values of Nd<sub>fa</sub> in the field, ii) a trade off point exists between soil P influence on N<sub>2</sub>-fixation and soil N acquisition and iii) facilitation for N transfer from the legume tree to the crop is more effective when crop N uptake was stimulated as occurred under high P conditions.

O506

### Future trends in soil cadmium concentration under current cadmium fluxes to European agricultural soils

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The gradual increase of soil cadmium concentrations in European soils during the 20<sup>th</sup> century has prompted environmental legislation to limit soil cadmium (Cd) accumulation. Mass balances (input – output) reflecting the period 1980-1995 predicted larger Cd inputs via phosphate (P) fertilizers and atmospheric deposition than outputs via crop uptake and leaching. This study updates the Cd mass balance for the agricultural top soils of EU-27+Norway (EU-27+1). Over the past 15 years, the use of P fertilizers in the EU-27+1 has decreased by 40 %. The current mean atmospheric deposition of Cd in EU is 0.35 g Cd ha<sup>-1</sup> y<sup>-1</sup>, this is strikingly smaller than values used in the previous EU mass balances (□3 g Cd ha<sup>-1</sup> y<sup>-1</sup>). Leaching of Cd was estimated with most recent data of soil solution Cd concentrations in 151 soils, which cover the range of European soil properties. No significant time trends were found in the data of net applications of Cd via manure, compost, sludge and lime, all being small sources of Cd at a large scale. Modelling of the future long-term changes in soil Cd concentrations in agricultural top soils under cereal or potato culture predicts soil Cd concentrations to decrease by 15 % over the next 100 years in an EU average scenario. This Cd balance has reverted from the general positive balances estimated 10 or more years ago. Country specific analysis with specific input/output data, including fertiliser Cd concentrations, climate, P rates and soil pH, reveal significant differences in trends within EU.

O507

### The potential of agricultural sector waste streams for phosphorus recovery on global scale

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Phosphorus is non-renewable resource of crucial importance for food production and life. Different characteristics concerning the limited supply of mineral phosphorus, increasing demand, substantial phosphorus losses, and negative effects of phosphorus dissipation on the aquatic environment have led to increasing concerns about current management of phosphorus. Recent scientific debates concluded that changes in current phosphorus management required actions in two directions: reducing phosphorus losses due to fertilizer application and phosphorus recycling from agricultural waste streams. This analysis uses mathematical programming to quantify the potential of waste streams from agricultural sector for phosphorus recovery under different hypothetical situations concerning price changes for mined mineral phosphorus, phosphorus externalities internalization and agricultural waste stream regulation. We extend an existing agricultural sector model by integrating a new agricultural waste recycling module which links recycled phosphorous supply from agricultural waste streams to phosphorus demand from crop production. We find that the livestock waste streams have fairly large potential for phosphorus recovery. The introduction to recycled phosphorus, phosphorus externality internalizations and, a continuation of the observed strong price trend may substantially reduce the use of mined mineral phosphorus fertilizers and increase the area supplied with recycled phosphorous sources. Our results do indicate a little physical scarcity of recycled phosphorous sources. The overall impact of higher cost of mineral phosphorous, phosphorus and agricultural wastes externalities regulations, and the supply of recycled phosphorus has little impact on aggregate crop and livestock production, trade, and prices, however the negative effects of mineral phosphorus and agricultural sector waste streams to the environment substantially decrease. Substitution of the mineral phosphorus with recycled phosphorus increases farmers' production cost, but their income effects does not change substantially because of market price adjustments and associated welfare shifts from producers to consumers.

O508

### Wet-chemically recovered sewage sludge ash P: high plant availability despite low water solubility

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Global policy on sustainable P use continues to evolve. In Switzerland, the authorities of the Canton of Zurich recently implemented a P recovery action plan for sewage sludge. Within this framework, a wet-chemical process to recover P from sewage sludge ashes was developed. This study aimed at characterizing the P species in the resulting recovery product and at assessing its availability to plants. The P speciation was obtained by solid-state <sup>31</sup>P NMR and sequential chemical extraction. The plant availability was studied in a growth trial under controlled conditions using <sup>33</sup>P isotope dilution techniques. The factorial design of the trial included three soils (pH 6.5, 7.6 and 7.8), four plants (Ital. ryegrass, rapeseed, soybean, alfalfa), and three fertilizer treatments (recovery product, no P, water-soluble control fertilizer). The spectroscopic analysis of the recovery product allowed distinguishing three molecular environments of P: sorbed P (43%), uncondensed amorphous Al-P (40%), and amorphous Ca-P (17%). Only 3% of the P was soluble in water, but additional 31% was dissolved in the presence of an anion sink. For the majority of soil-plant combinations, the recovery product was as effective in supplying P to plants as the control fertilizer, resulting in an overall relative effectiveness of 85%. This indicates that the weakly associated and amorphous P in the recovery product is highly reactive, even though it is not soluble in water. The wet-chemically recovered product can therefore effectively substitute rock P based, water-soluble fertilizers and the novel recovery process presents a promising technology to recycle P from sewage sludge ashes.

O509

### Bone char as renewable P-fertilizer with cadmium-immobilization by-effect

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Expected shortage of mineable phosphorus (P) and the contamination of P fertilizer with cadmium (Cd) and uranium (U) stimulated the search for alternative P sources to produce P fertilizer from renewable resources. Bone char (BC) was tested for its potential as clean and renewable P fertilizer with cadmium (Cd) immobilization capability. Incubation experiments in the laboratory and pot experiments in greenhouses were carried out with Cd-contaminated soils in which the P and Cd solubility and uptake by plants were tested. For most soils, BC increased the concentration of labile P immediately after application, reaching a maximum after 34 days. The P release was partially similar to highly soluble triple-superphosphate (TSP), and the Cd immobilization resulting from BC dissolution exceeded that of TSP by a factor of 1.4 to 2.7. Two multiple equations were established that enable the prediction of the P dissolution from BC and the subsequent Cd immobilization in acidic soils from the initial soil pH, soil P sorption capacity, and released P. Synchrotron-based P- and Cd-speciation gave evidence for the precipitation of insoluble Cd-phosphates following BC dissolution in the Cd-contaminated soil. In the subsequent pot experiments, BC was less effective than the highly soluble commercial P fertilizers in enhancing the dry matter yield of plants in a P deficient soil. However, in a soil sufficiently supplied with available P the plant yields in BC treatments exceeded those in the mineral P treatments. The Cd concentration in plants was slightly reduced with fertilizer application, being more pronounced in the BC treatments compared to TSP and diammonium phosphate. Furthermore, we introduced surface modifications at the BC in order to improve P-solubility without losing the Cd-immobilization effect. The success of these surface modifications is shown in incubation studies and by various extraction methods. Interestingly, the enhanced P solubility due to surface modification was not accompanied by an equivalent Cd mobilization. In conclusion, it appears possible to provide an alternative clean P fertilizer of moderate solubility with Cd immobilizing by-effects.

O510

### Designer riparian buffers – using plant phylogeny to close the arable agricultural phosphorus (P) cycle

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Phosphorus (P) loss from agricultural fields is the primary source of diffuse P pollution of watercourses, causing detrimental environmental consequences such as eutrophication. Establishment of vegetated riparian buffers is one tool in a suite of mitigation options aimed at reducing the contribution of P loss from agricultural land to surface water P loads. It is currently unclear how the vegetation in buffers affects the dynamics of dissolved forms of P and there are likely to be differences in the ability of plant species to accumulate P in biomass. To investigate this we performed a phylogenetic study of the ability of plant species common to riparian buffers to take up P supplied at a range of concentrations. We screened tens of species of plants and were able to establish phylogenetic clades of species that had particular P uptake abilities. We then went on to investigate the leaching of P from columns packed with soils of differing organic matter contents and sown with extreme examples of grass species taken from the phylogenetic study. These studies showed that the plants ability to accumulate P in tissue reduced the amount of P leached. Plants increased carbon mobility in the soil and this impacted rhizosphere traits which had impacts on the availability of organic P. Current studies are looking at the utility of green manure taken from riparian buffers to act as a P source to agricultural plants, with the aim of closing the arable P cycle. Designed buffers, taking into account the physiological characteristics of plants, could be used to reduce the amount of P leaching to watercourses and provide an alternative fertilizer source for arable cropping systems.

O511

### How to optimize the use of phosphate resources by producing alternative totally acidulated phosphate fertilizers

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Global phosphorus (P) cycle (GPC) points out substantial losses of P from mining to consumption by humans and it is necessary to increase efficiency as to lower such losses. Totally acidulated phosphate fertilizers (TAPF) are generally required to have high water solubility and such type of fertilizers are obtained either by using high premium grade phosphate rock (PR) or by purifying lower grade rocks at high cost and energy demand. High grade PRs are becoming scarce and the industry will have to rely more frequently in lower grade quality rocks. Researchers studied if it is always necessary that TAPF have high water solubility. Through adequate characterization of the fertilizers' water insoluble P fraction and also the agronomic evaluation of several fertilizers, it was found that many water insoluble compounds may still be good sources of P to plants. Some results show that fertilizers with as low as 43% water solubility in the available P fraction can be as good as fertilizers containing basically only water soluble P compounds. It was also found that some of the water insoluble P compounds may be very good sources of P in specific soil conditions, like flooded soils for rice. The possibility of producing alternative TAPF with lower water solubility can lead to a more adequate use of a finite resource like PR in many parts of the world, which can help to increase the P efficiency in the GPC. Also, results show that P fertilizers with lower water solubility may help to minimize environmental impacts of P in water resources. There is no scientific reason for wasting part of a so valuable resource like PR targeting only the production of TAPF with high water solubility.

O512

### Population of native PSM increased by Minjingu PR application and positive impact on crop yields in a Kenyan Ferralsol

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Soil microbes such as plant growth promoting rhizobacteria play significant roles in the solubilization of inorganic P, mineralization of organic P and in improving plant P uptake. It is known that phosphate solubilizing microorganisms (PSM) populations largely vary depending on the ecosystems, the cropping systems or the soil management. A total of 150 isolates of PSM were isolated from 13 soils coming from different agricultural provinces in Kenya. Strains belonging to *Bacillus megaterium*, *Bacillus* sp. and *Arthrobacter* sp. were the most abundant and well distributed strains. However, although PSM strains were well present in the different soils, only 5% of the strains were effectively solubilizing P in vitro. The capacity of Minjingu Phosphate Rock (PR) to enhance the populations of native PSM under 3 cereal-legume rotation systems was assessed over two (2) consecutive years. Triple Super Phosphate (TSP) was used as a positive control. In comparison to the negative control, application of Minjingu PR increased the fungal diversity and phosphate solubilizing bacteria (PSB) population by 67-90% while high rates of TSP significantly reduced bacterial diversity and populations of PSB by 46-69%. In addition, Minjingu PR resulted in both crop and legume yields increase (+41-104% compared to the control), which were similar to those obtained with TSP application. Cropping systems incorporating sparingly soluble P sources such as Minjingu PR into soils can stimulate the populations of native PSB and agronomic productivity. The combination of Minjingu PR - PSB may represent a promising way of minimizing the utilization of mineral P fertilizers.

**Seedling and adult plant phosphorus uptake and utilization in West and Central African pearl millet inbred lines**

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Since Sub-Saharan Africa has the least fertilizer use in the world, plant available P in the soil (Bray-1) is always below the critical level of 7 mg P kg<sup>-1</sup> soil in the Sahel region. Pearl millet production on the acid sandy soils is severely limited by the low P in addition to erratic rainfall. We sought to examine the genetic variability for P uptake and utilization efficiency in West and Central African inbred lines both at early growth stage in pot experiments as well as their subsequent field performance, to determine the relationships among the measured traits and grain yield and to speculate on the extent to which results from pot experiments can be extrapolated to performance under field conditions. About 180 inbred lines were evaluated in two pot experiments for six weeks in addition to one field experiment (160 inbred lines) in the same location, under high P and low P. In addition to measured traits, P-concentration in shoots, stover and grain were measured. Grain yield under low P was evaluated in four countries. We observed genetic variation for P uptake and utilization in both seedling and mature plants. P utilization increased under low P. Most traits were correlated among themselves and to grain yield though field measured traits were more correlated to grain yield than pot traits. We conclude that the inbred lines panel can be used in breeding for phosphorus efficiency and that both pot and field measured traits could be used as secondary traits though results from a pot experiment ought to be extrapolated cautiously to field performance.



## **Theme 5 – Sustainable phosphorus use in agroecosystems**

### **Posters**

P501

### Grain yield of narrow leafed lupin increased by phosphorus and *Bradyrhizobium* inoculation in Kenya

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Use of legumes to supplement cattle diets and improve soil nitrogen (N) levels has continued to receive attention. Several studies have reported increased yield and biological N fixation upon inoculation and phosphorus (P) applications but limited information on lupin exist in low fertility soils of Kenya. The effects of P fertilizer and *Bradyrhizobium* inoculation on nodulation and yield of lupins were investigated in three sites. The trial was laid in a split plot design with two varieties (broad leafed Var. Kiev mutant and narrow leafed Var 28137) as main plot and soil fertility strategies (control, rhizobia (Biofix®), triple super phosphate (TSP) and TSP + Biofix®) as sub plots replicated four times. The site without history of lupin production had positive response to nodulation in the narrow leafed but not in the broad leafed –variety. Inoculated treatments had between 78 – 100% effective nodules in narrow leafed compared to between 33 to 47% in broad leafed variety. Phosphorus addition showed a significant increase ( $p=0.02$ ) in narrow leafed lupin but no response in the broad leafed variety ( $p=0.28$ ). Grain yields from both varieties were 100% higher in the cooler areas compared to the warmer zones, showing that the cooler zones would be more suitable for lupin production. Broad leafed lupin was found to be more efficient in P acquisition from the soil and nodulated highly even in absence of P compared to the narrow leafed lupin. For farmers with low resource endowment, broad leafed variety would be a cheaper alternative.

P502

### Long-term P dynamic in the Ferralsol of Malagasy highland

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Improvement of soil fertility increases the crop production in order to alleviate poverty in Africa. The Malagasy highland, known as an available land for smallholders, is marked by high P sorption owing to the higher iron content in soils and limiting strongly plant productivity. Long term field experiment was carried out in Antananarivo in order to study the fertilizer management practice for the improvement of soil P in Ferralsol of Madagascar. The mineral P fertilizer input as triple super phosphate (TSP) applied as 0, 5, 10, 20, and 50 kg P ha<sup>-1</sup> was tested annually on different crops (Maize, Bambara groundnut, and Upland rice) during 4 consecutive years from 2006 to 2009. The grain yield was determined and the soil available P as phosphorus concentration in soil solution (Cp), resin P and Olsen P was assessed in order to analyze the annual and cumulative P budget. Grain yields were significantly increased with P levels and the highest yield was reached at 20 kg P ha<sup>-1</sup> in particular in 2008 and 2009. Fertilizer input was followed by significant increase of soil P status in particular for P Olsen. These crop responses by P application may suggest P as the main limiting factor in the Malagasy highland Ferralsol. Cumulative effect of fertilizer input over the year was highlighted in terms of rice yield and P Olsen. Level of Cp remains low (<0.1 mg P l<sup>-1</sup>) suggesting the high P-fixing by soil constituents. High correlation between cumulated P budget and soil available P in 2009 showed that chemical extraction method of soil P especially Olsen method is a best indicator of P plant availability under cultivated agrosystem in Malagasy highland.

P503

### Earthworms modify soil phosphorus and plant interactions in a Mediterranean legume-cereal intercrop

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Intercropping of legumes and cereals appears as an alternative agricultural practice to decrease the use of chemical fertilizers while maintaining high yields. A better understanding of the biotic and abiotic factors determining interactions between plants is required. Our study aims to analyse the effect of earthworms on the legume-cereal interactions with a focus on the modifications induced by earthworms on the forms of soil phosphorus (P). In a glasshouse experiment we investigated the effect of an endogeic earthworm (*Allolobophora chlorotica*) on the plant biomass and on N and P acquisition by durum wheat (*Triticum turgidum durum* L.) and chickpea (*Cicer arietinum* L.) either grown alone or intercropped. The modifications of the different organic and inorganic P forms in the bulk soil were measured. There was no overyielding of the intercrop in the absence of earthworms. Earthworms had a strong influence on biomass and resource allocation between roots and shoots whereas no modification was observed in terms of total biomass production and P acquisition. Earthworms changed the interaction between the intercropped species mainly by reducing the competition for nutrients. Facilitation (positive plant-plant interactions) was only observed for the root biomass and P acquisition in the presence of earthworms. Earthworms decreased the amount of organic P extracted with NaOH, while they increased the water soluble inorganic P content. In this experiment, earthworms could be seen as "troubleshooter" in plant-plant interaction as they reduced the competition between the intercropped species. Our study brings new insights into how earthworms affect plant growth and the P cycle.

P504

### P availability in two different ecosystems: cropping (Kenya) and mixed acacia and eucalyptus plantations (Congo)

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P availability is one of main soil fertility constraints to crop production and forest productivity in many high fixing P and weathered tropical soils. Two alternative practices were tested (i) Mineral and organic-manure P were added to increase microbial P biomass in low and high fixing Kenyan soils; (ii) Acacia mangium was introduced in eucalypt plantations growing on poor nutrient Congolese soils to improve P availability and N soil status. We determined microbial P biomass by fumigation-extraction and P availability using a modified Hedley P fractionation method at 16 weeks of incubation in the cropping soil. Total N and available P were quantified under acacia (100A), eucalypt (100E) and mixed-species stands (50A:50E); N and P were determined in aboveground litters and in the leaves, bark and wood at the end of rotation (7 years). In the high P fixing (0-0.1m) soil, addition of mineral P and manure increased microbial P biomass (x4.8 after 2 weeks to x15.2 after 16 weeks), and modified the P Hedley fractions partition. In the (0-0.5m) soil layer, the introduction of acacias in eucalypt plantations increased N concentrations (N> 0.06% in 50A:50E and N<0.05% in 100E) and decreased P concentration (6.94 mg P kg<sup>-1</sup> soil in the 50A:50E against 8.07 (100A) and 8.46 (100E)). Leaf N was lower in 50A:50E than 100A for acacia, and higher than in 100E for eucalypt. Leaf P was similar for acacia but higher for eucalypt in 50A:50E than in 100E. Addition of inorganic P and manure increased P microbial biomass in the high P fixing soil as alternative practice, while in the inverse, the association of acacia in the eucalypt plantations decreased P availability in the 50A:50E stand.

P505

### Natural reactive rock phosphate efficiency when mixed with soluble phosphate for soybean cropped under no-tillage

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An increased efficiency of reactive rock phosphates (RRP) when mixed with soluble phosphates such as triple superphosphate (TSP) has been observed in short term experiments, under conventional tillage. However, there are no studies on no-tilled areas considering the residual effect of the phosphates. The aim of this study was to examine soybean [*Glycine max* (L.) Merr.] response to mixtures of Arad reactive rock phosphate with triple superphosphate under no-tillage for three years. TSP or RRP were broadcasted or not on soil surface at a rate of 35 kg ha<sup>-1</sup> of P in the first and third year, over a winter crop cultivated before soybean. Mixtures containing 0, 20, 40, 60, 80 and 100 % of RRP were then applied to soybean seed furrows at 80 kg ha<sup>-1</sup>, for three years. There was no interaction of broadcast and seed furrow applied P on soybean yields. Soybean responded to broadcast triple superphosphate in the first year. The availability of phosphorus increased linearly with the proportion of TSP on the mixture in the third year, and soybean response was linear for two years, and in the third year the mixture of 40% RRP resulted in similar yields as TSP. The efficiency of reactive rock phosphate when mixed with a soluble P source applied to seed furrows did not increase, even with a high proportion of triple superphosphate. The mixture of reactive rock phosphate with a soluble phosphate is not effective for soybean under no-till.

P506

### Phosphorus uptake by rice from organic fertilizers applied to a Ferralsol assessed by isotope technique

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Organic fertilizers such as legume residues and animal manure are important to sustain the fertility of highly weathered tropical soils. However, little information is available on the phosphorus (P) uptake by crops from organic fertilizers when applied to strongly P sorbing soils. The crop P uptake from fertilizers can be assessed using direct labeling technique (DLT) and indirect labeling technique (ILT) (<sup>33</sup>P) in a glasshouse study. This study was conducted (i) to compare DLT and ILT to assess the recovery of P added with *Stylosanthes guianensis* (stylo) residues or mineral fertilizer, (ii) to assess the uptake of P added with stylo residues and manure to upland rice in a strongly P sorbing soil and (iii) to study the recovery of fertilizer P from amended soils by anion exchange resin extraction. We applied DLT and ILT in a pot study with four treatments (control 0P, stylo residues, mineral water soluble P and manure) in a Ferralsol with *Oryza sativa* (Nerica 4) and in parallel an incubation experiment. Comparable results were observed by using DLT and ILT for stylo residues and mineral P treatment in the pot study. It suggests that ILT can be applied to the manure treatment for which it was not possible to use DLT. Irrespective of the fertilizer added more than 60% of P in the rice shoots derived from the fertilizer, suggesting a good availability of P added with each fertilizer. Similar result as for the rice shoot was observed in the resin extractable P pool in the incubation study. Proportions of P derived from fertilizer in the plant and in the resin extract were similar, suggesting that resin extractable P is a good indicator for plant available P in this type of soil.

## P507

### Life Cycle Assessment (LCA) of phosphorus on AVP1 transgenic lettuce

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Human P usage is very inefficient. For example, about 80% of P mined for fertilizer, but only 1.5% of that is consumed by humans and 46% is lost from soil erosion and runoff, causing eutrophication. One means of improving agricultural P efficiency involves genetic engineering strategies to increase crop use of soil P so that results in reducing P fertilization rate. AVP1 transgenic romaine lettuce (*Lactuca sativa* cv. conquistador) had been engineered to improve dry tolerance and resistance, as well as nutrient use efficiency. The future scenarios for growing AVP1 transgenic crops are to reduce fertilization rate but not compromise yield. In this study, I used Attributional LCA, with kg nutrient/ha/yr as functional unit, to evaluate the environmental impacts by comparing wild type (WT) and AVP1 lettuce in California and Arizona (system boundary), as these two states produce 98% of lettuce over the U.S.A. I assumed that the biological effects from AVP1 gene were equal in head, leaf, and romaine lettuce. I also assumed that WT and AVP1 lettuce consume that same amount of water, while AVP1 lettuce requires more water in a shorter growth period to reach to equivalent yield as WT. Results showed that applying AVP1 lettuce would conserve 78% N and 55% P fertilizers applying to grow lettuce. It would also reduce 20 kgN/ha/yr emitted as ammonia to the air, 5.7 kgN/ha/yr as NO and N<sub>2</sub>O, 117 kgN/ha/yr as NO<sub>3</sub> being leached out from the soil, 0.065 kgP/ha/yr into groundwater, and 0.23 kgP/ha/yr into surface water. Applying AVP1 transgenic crops has large impact on reducing eutrophication potential and should be considered in future farm practices.

## P508

### Effect of phosphorus and soil on nodulation and production at flowering stage of different small faba bean varieties

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In order to evaluate faba bean nodulation and production at flowering stage under different phosphorus and soil conditions, two trials were conducted during 2013-2014 under field conditions in two different locations: experimental station (L1) and farmer's field (L2). Three Moroccan faba bean varieties (V) (*Vicia faba* minor) Alfia 5, Alfia 17 and Alfia 21 were combined with four phosphorus (P) rates (0, 20, 80 and 160 kg P<sub>2</sub>O<sub>5</sub>/ha). The experimental design was a split plot with four replicates where P was affected to the main plot and variety to the sub plot. At flowering stage, a sample of 0,5 linear meter of plants (shoots and roots) by sub-plot was harvested. Results show that in both locations P, V and P\*V have significant effects on above ground dry biomass. For nodulation; in L1, P have a significant effect on nodule number and biomass while, V and V\*P have no significant effect. The rate of 160 kg P<sub>2</sub>O<sub>5</sub>/ha gave the best nodules number and biomass per plant. In L2, nodule number per plant was significantly affected by the interaction V\*P, while the nodule biomass was significantly affected by V where Alfia 17 and Alfia 21 gave the best nodule biomass per plant. For above ground dry biomass (AB): the models developed are respectively for L1 and L2: ABL1 =  $-0,81 \cdot P^2 + 4,11 \cdot P + 11,46$  ( $R^2 = 0,61$ ) and (ABL2) =  $-11,25 \cdot P^2 + 64,17 \cdot P + 61,83$  ( $R^2 = 0,62$ ). Under L1 conditions the best production was performed by treatments Alfia 5 and Alfia 17 with 20 kg P<sub>2</sub>O<sub>5</sub>/ha. While under L2 conditions, the best production was performed by treatments Alfia 17 and Alfia 21 with 80 kg P<sub>2</sub>O<sub>5</sub>/ha.

### Pig manure solids and derived char or ash as P fertilizer: Effect of slurry acidification and separation technology

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Acidification, solid-liquid-separation and thermal treatment, i.e. pyrolysis or combustion, are options for improved management of the large volumes of pig slurry in areas of intensive livestock production. As the separated solid fraction can be used as phosphorus (P) fertilizer, the change of easily available P, affected by the (combined) factors of acidification, separation technology and thermal processing was studied. Acidified and non-acidified slurry was separated with different technologies: screw press (SCR), decanting centrifuge (DEC) and drainage after chemical pretreatment (CHE). After drying, pyrolysis (400°C or 600°C) or combustion (625°C), the materials were applied to two Danish soils and application of triple superphosphate (TSP) was included as a reference treatment. P availability during incubation (12 weeks) was determined with the diffusive gradients in thin films (DGT) technique. The initial availability of P in DEC- and CHE-Solids was high and within the same range as TSP in both soils. After 6 weeks of soil incubation, the higher P availability from the dried solids-, compared with derived char- and TSP-amended soils, were not significant anymore. Acidification did not significantly affect P availability of the solids over time, but it reduced the P availability from char and ash after processing at 600°C in most cases. Pig manure, separated by advanced technologies (DEC & CHE), can be used as effective P fertilizer with a higher initial P availability than derived char or ash, which however could serve as slow-release P fertilizers. Further studies on P speciation in manure-derived materials and mechanisms governing P dynamics in soils are needed.

### Foliar application of Zn improves shoot-grain zinc concentrations of winter wheat decreased by high available P in soil

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High soil available phosphorus (P) was considered as one of main factors which caused zinc (Zn) deficiency in crops, further impacted human health. The field experiments were conducted to investigate the effect of different applied P levels on soil available P, shoot, grain and straw Zn concentrations and contents and their relationships during two crop growing seasons. The applied phosphorus levels were 0 (control), 25, 50, 100, 200 and 400 kg P ha<sup>-1</sup>. And the foliar application of 0.4% ZnSO<sub>4</sub>·7H<sub>2</sub>O was used to study whether this approach could improve the grain Zn status. Compared with control, P application increased soil Olsen-P concentration from 0.1 to 5.7 folds in 2012 and 0.7-8.6 folds in 2013, respectively, and decreased shoot Zn concentration 8.1%-55.8% in 2012 and 18.2%-66.0% in 2013, respectively. Shoot Zn content was increased while the applied P was lower than 25 kg P ha<sup>-1</sup>, after that it was decreased with the applied P level increasing. The similar results were observed for grain Zn concentrations and contents. There was a significant negative correlation between soil Olsen-P and grain Zn concentration or shoot Zn concentration. However, the foliar application of Zn could significantly improve the grain Zn concentration even if the higher P was applied. In conclusion, increasing soil Olsen-P concentration reduced shoot and grain Zn concentrations and contents. Favourable yields and grain Zn concentrations were got at 50 kg P ha<sup>-1</sup>. Foliar application of Zn could improve poor grain Zn nutrition caused by higher P application.

P511

### Development of slow and controlled release P fertiliser using layered double hydroxides

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In calcareous soils, P fertilization efficiencies are low due to fast and irreversible P precipitation to Ca-phosphates. Slow and controlled release fertilizers can be proposed to supply plants with P in response to uptake of root signals. Here, layered double hydroxides (LDHs) are tested. The LDHs are layered inorganic anion exchangers consisting of layered divalent (e.g.  $Mg^{2+}$ ) and trivalent (e.g.  $Al^{3+}$ ) hydroxides and intercalated anions such as  $HPO_4^{2-}$ . The  $HPO_4^{2-}$  ions can be exchanged with  $CO_3^{2-}$  (from respiration) or with organic anions in rhizosphere, thereby avoiding immediate precipitation. The aim of this study is to synthesise P exchanged LDHs and determine their P desorption kinetics in response to  $CO_3^{2-}$ . Using cheap and readily available Mg, Ca, Fe and Al nitrate salts, LDHs were successfully synthesised and their layered structure was confirmed by X-ray diffraction (XRD). The nitrate LDHs were successfully loaded with  $PO_4$  solutions, yielding an uptake of up to 6 wt% P, confirming the theoretical anion exchange capacity of the LDHs. Desorption kinetics of P from P exchanged LDHs were examined in a carbonate solution. XRD-analysis confirmed the uptake and removal of P in the interlayer of the material after adsorption and desorption, which proves P is bound electrostatically and reversible in the LDHs. It can be concluded that LDHs can be a promising new class of P fertilizers to overcome current P fertilization problems in lime soils.

P512

### Changes in phosphorus use efficiency and losses in livestock production in China between 1980 and 2010

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Livestock production in China is in a rapid transition. An increasing production occurs now in land-less industrial systems instead of the traditional land-based systems. These changes have major impacts also on the nutrient use efficiency and losses in livestock production. Here, we report on the changes in phosphorus (P) use efficiency and losses in livestock production between 1980 and 2010. We used data statistics and the NUFER model to examine the effects of structural changes in livestock production on P use efficiency and losses. Our results indicate that the P use efficiency increased from about 6% in 1980 to about 17% in 2010 at herd level. However, P use efficiency at the whole system level, which include feed production and manure management, decreased by 50% during this period. The inputs of P fertilizer and P in feed additives increased strongly during this period. Manure management changed dramatically during this period. In the early 1980s most of the manure was still applied on own farm land. By 2010, a significant fraction of solid fraction manure was exported to vegetable and fruit producing farms while the liquid fraction was discharged on landfilled. The grassland-based livestock production system showed much higher P use efficiency than the land-less systems at system level in 2010. Total P losses increased by a factor of 5, from 475 Gg in 1980 to 2350 Gg in 2010. Largest P losses occurred in beef production (48%) and pig production (32%) production in 1980. Thirty years later, pig production (36%) was the largest contributor, followed by beef (29%) and poultry production (17%). Manure discharge was the main P loss pathway in both 1980 (80%) and 2010 (93%). In conclusion, structural changes in livestock production had diverse effects on P use efficiency. Total P losses increased dramatically.

P513

### Role of cow dung application on soil properties and phosphorus pool in an acid chilean soil

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Chilean Andisols promotes the phosphorous (P) immobilization decreasing its availability to plants. In a grazed pasture soil, P transformations and availability to plants are influenced by plant uptake and the return of P through animal excreta, mainly in dung. In this study, we determine the effect of applying fresh cow dung on phosphorus pools and other soil properties (nitrate, ammonium and pH) in an acid Chilean Andisol. Properties were monitored for 6 months in soil columns, on the top of which cow dung was applied. Soil pH, and phosphate concentration increased in 0-10 cm fraction at 30 days. After, pH decreased from 5.4 to 4.9 at the same depth. P-NaHCO<sub>3</sub> fraction increased (69 to 75 mg kg<sup>-1</sup>) in 60 day. Then, this fraction decreased (10 mg kg<sup>-1</sup>) due to microbial activity and pH change. From the 120 to 180 days the P-NaHCO<sub>3</sub> fraction recovered its original level. P-NaOH fraction decrease according to soil depth; this was correlated to the sonicated P-NaOH fraction increase (bivariate correlation=-0.295 p<0.01). P-residual increased to 100 mg kg<sup>-1</sup> at 40-60 cm depth soil. P-HCl fraction decreased significantly (p<0.01) at all depths from 10 to 5 mg kg<sup>-1</sup>. Only organic P-form was found (5-10 mg ml<sup>-1</sup>); in the leaching solution. <sup>31</sup>P-RMN indicated that this is an orthophosphate diester. Results showed phosphorus diffusion and fixation from 0-10 cm toward 30-60 cm, because of the less organic matter content. A mathematical model for describing the P dynamic is proposed. Its parameters were calculated using the experimental data. No significant differences were observed between experimental and calculated values of P-labil fraction, P-NaOH, sonicated P-NaOH. However, the model was not able to simulate the behavior of P-HCl and P-residual fractions and the difference between experimental and calculated values were significant.

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P514

### Phosphorus and liming effects on forage production, floristic composition and soil properties in the Campos biome

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Studies of responses of native and introduced grassland species to lime and phosphorus (P) applications could contribute to improved understanding of the potential production of South American natural grasslands. In order to determine the effect of applying different P sources and liming on forage production, diversity and floristic composition, and on soil chemical properties, a small-plot experiment was conducted over 12 years in a natural grassland oversown with *Lolium multiflorum* and *Trifolium vesiculosum* in the Campos biome of southern Brazil. In treatments with soluble phosphate application, dry matter (DM) yield in November 2008, after 164 days of winter and early spring growth, increased from 2.3 to 3.2 t ha<sup>-1</sup>. Differences in DM yield in March 2009, at 111 days of growth during late spring and early summer, were not significant. The DM yield in April 2010, after 419 days of growth, increased from 7.7 to 9.2 t ha<sup>-1</sup> in the treatments with P, regardless of the source. Increased forage yield during the slow growth period in winter was only possible with the introduction of winter-growing species (*L. multiflorum* and *T. vesiculosum*) and soluble phosphate application. Assessment of annual forage yield showed the effectiveness of Gafsa rock phosphate was equivalent to soluble phosphates in the long term. Soluble phosphates and liming modified the botanical composition of natural Campos grassland in the long term, but floristic diversity was not altered.

P515

### Which P inputs are compatible with a sustainable agriculture at short and long-term?

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During the past 20 years, there has been a constant reduction in mineral fertilizer use due to price increases and environmental concerns. These changes can lead to a decrease in soil P content, which is already observed in some regions in Wallonia. Some new issues are now emerging. Is current cropping systems compatible with yield maintenance? Do organic fertilizers have a similar effect than mineral fertilizers? To answer to these questions, a short-term experiment in controlled conditions and 2 long-term experimental plots were studied. The short-term experiment permitted to study the kinetics of P after an input and differences between fertilizer types, whereas the long-term experiments studied 3 levels of P and K input and different organic compounds. Although an evolution of P content was observed, no difference of yield was found before about 20 years. However, after 47 years, available P levels were considered as low in zero P-input plots and attention must now be focused on these parcels. Zero P-input caused a mean yield decrease of 7%, while a double input increased yield by 2% in comparison to plots with input corresponding to crop export. Thus the zero P-input option is rarely economically profitable in the long-term and providing double the amount of P removed is never financially sustainable. Finally, no difference of P content was observed between organic and mineral fertilizers, except for manure which engendered a higher P content. In conclusion, organic and inorganic fertilizers had a relatively similar effect and overlooking P fertilizer is possible in the short-term but P content has to be followed at the long-term, although yield loss was limited.

P516

### Study of Togo's carbonate-apatite for direct application

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Togo is a phosphate producer country. Besides the fluorapatite ore currently mined, it has a reserve of yet untapped carbonate-apatite rock estimated to 2GT. However, local agriculture does not benefit much from the mining of these phosphate rocks. Togo's phosphate is exported to United States, France, South Africa, to be processed into fertilizer which, finally is imported at very expensive price to farmers. The aim of this work is to investigate using these phosphate rocks for direct application. After removing calcite by triammonium citrate, phosphorus and calcium were determined by ICP-AES; the fluorine by fluorine ion-selective electrode and standard addition method; the carbonate by volumetry. Average molar ratio  $\text{CO}_3^{2-}/\text{PO}_4^{3-}$ ,  $\text{CaO}/\text{P}_2\text{O}_5$  and  $\text{F}/\text{P}_2\text{O}_5$  which give meaningful information about francolite's degree substitution are respectively 0,23 and 0,12. The range of values of these ratios which are respectively 0 to 0,3; 1,318 to 1,621; 0,089 to 0,148 shows that Togo carbonate-apatite must be considered as moderate to strongly substituted francolite. Theoretically, this composition suggests a chemical reactivity that allows direct application. The crystallographic parameter  $a$  estimated at 9,338 Å can be considered as relatively low comparatively to extreme values reported elsewhere for francolite which are 9,33 and 9,36. This value of crystallographic parameter  $a$  is also responsible for the relatively high value of the Index of Absolute Solubility (IAS) of this carbonate-apatite. The suitability of the carbonate-apatite rock from Togo for direct application will be tested by a series of chemical test and by plant cultivation experiments in phytotron.

P517

### Phosphorus use efficiency of potato - the influence of maturity type

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Phosphorus fertilizer recommendations for potatoes and vegetables are often high. Large amounts of fertilizers, in combination with light soils and irrigation, may increase the risk of phosphorus losses by runoff or leakage. The objective of the present work was to compare phosphorus use efficiency for six potato varieties varying in maturity type. During 2011 and 2012, field experiments were conducted on soils containing low levels of available phosphorus. Phosphorus fertilizer was added in the amounts of 0, 30 and 60 kg phosphorus per ha. In 2011, soil heterogeneity was large and tuber yield was increased by phosphorus addition as a mean over all varieties only. In 2012, an improved tuber yield was observed when phosphorus was added for most varieties. Phosphorus fertilization improved yields mainly by increasing the weight of the larger tuber fractions. Phosphorus use efficiency, calculated as tuber dry weight per unit of fertilizer phosphorus added, was reduced by fertilizer addition and was generally higher for late than for early or intermediate varieties. As tuber phosphorus uptake was correlated with tuber weight, tuber phosphorus uptake efficiency (i.e. tuber phosphorus content per unit of phosphorus added) and phosphorus use efficiency were closely related. This study confirms that there are large differences in phosphorus use efficiency between late and early potato varieties and shows that phosphorus fertilization should be adapted to maturity type. Late varieties might be the better choice for locations where the risk of phosphorus losses by runoff or leakage is high.

P518

### Nodular diagnosis of contrasting genotypes for rhizospheric phosphorus in *Phaseolus vulgaris*

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Legumes have the capacity to fix large amounts of atmospheric N<sub>2</sub> into the biosphere through their symbiosis with soil rhizobia. However this legume contribution to the N bio-geochemical cycle varies with the nodulated-root rhizospheric environment, in particular phosphorus availability. In order to assess the environmental constraints that might limit this symbiosis, a nodular diagnosis was performed in field-sites chosen with farmers of the Mediterranean basin, with common bean as a model grain-legume, and a major source of plant proteins for world human nutrition. The engineering of the legume symbiosis is addressed by participatory assessment of bean recombinant inbred lines contrasting for their efficiency in use of phosphorus for symbiotic nitrogen fixation. With this methodology, a large spatial and temporal variation in nodulation and in the efficiency in use of the rhizobial symbiosis for plant growth, with a positive effect of the later on soil P-bioavailability. Soil P availability was a major limiting factor of the rhizobial symbiosis. It is concluded that by increasing the phosphorus use efficiency for symbiotic nitrogen fixation, a virtuous cycle of fertility is activated within legume rhizosphere, which can contribute to the sustainability of agricultures through the use of appropriate legumes and cultural systems.

P519

### Phosphorus in the soil-plant system in long-term field experiment under organic and inorganic soil fertilizing

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Since 1989 the fertilization of agricultural soils with phosphorus in the Czech Republic decreased several times (currently 5 kg P ha<sup>-1</sup> year<sup>-1</sup>). The basic phosphorus reserve in soils continues to decrease due to continual phosphorus uptake by crops. Also the animal production in Czech Republic decreased and the production of organic fertilizers is lower. The 30 year field experiment with inorganic and organic fertilizing at sites with different climatic and soil conditions showed that absence of phosphorus fertilizing decreased available phosphorus fractions in soils. According to tests of optimal phosphorus concentrations in soils, the long-term deficiency of phosphorus fertilizing led to insufficient P concentrations available for crops. The type of fertilization affected phosphorus balance in soils. The nitrogen fertilization increased crop yields, consequently also the P uptake by plants. The negative phosphorus balance in N fertilized soils was observed particularly in more fertile chernozem due to greater phosphorus remove from soils caused by higher yields. The organic treatments maintain better an adequate phosphorus balance in the soil-plant system. Therefore N fertilizing without adequate phosphorus supply leads to even faster decrease of phosphorus reserve in soils. The field trial showed that the adequate and balanced phosphorus supply in intensively used productive areas should improve the phosphorus reserve and adequate available P concentrations in soils. The regional soil and climatic characteristics and yield potential should be taken in consideration.

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P520

### Sand to soil, best practice for soil amendments and sustainable land mangement in Australia

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It is evident that we must become better managers of waste and resources. The use of both organic and inorganic waste materials as soil amendments is one way of closing the loop when it comes to nutrients. Western Australia's sandy soils are characterised by low natural fertility, poor water-holding capacity, poor nutrient retention and a tendency to become water-repellent. The greater demands on these soils to sustain today consumers' needs require regular inputs of nutrients and high quality water. In most cases, the required nutrients are applied in the form of water-soluble chemical fertilisers. This practice is not environmentally sustainable and has been identified as one of the major contributors to algal pollution problems in the Swan and Canning River systems in Western Australia. Addition of soil amendments to sandy soils can provide significant benefits in terms of improving turf quality and minimising environmental impacts. Added benefits in terms of diversion of wastes from landfill can be delivered if waste materials can be successfully used as soil amendments. Both organic and inorganic waste materials were evaluated in this project. Organic materials included composted products produced from wastes including green waste, municipal waste, 'biosolids' and animal wastes. Inorganic materials included both natural soils and residues from industrial processes including energy, general and mineral extraction. The main objectives of this project were to: (i) demonstrate that application of composted organic wastes to sandy soil provides benefits for turf in terms of plant health and nutrition, water use efficiency and soil fertility, (ii) demonstrate that application of inorganic materials including industrial residues can result improved nutrient and water retention by soil, (iii) demonstrate that applications of blends of organic and inorganic wastes to sandy soil can result in sustainable turf production, (iv) evaluate a novel technique for rejuvenation for existing turf by applying soil amendment blends without major disruption to the turf, (v) demonstrate that use of organic and inorganic soil amendments based on waste materials can divert a significant amount of material from disposal to landfill waste facilities.

P521

### Phosphorus (P) applied with sewage sludge - distribution on soil P fractions and effect on P adsorption characteristics

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There is a growing awareness of the limitations in global mineral P reserves, increasing the need to create efficient P cycles in food production. Although a considerable amount of P is returned to Norwegian agriculture with treated sewage sludge, this mainly supplements P in mineral fertilizers instead of substituting it. This implies a potential environmental risk as it increases the P concentration in the soil. The aim of our study was to characterize P in different types of treated sewage sludge and explore the fate of P in uncalcareous silt loam applied with sludge by analysing different soil P fractions and soil P adsorption characteristics. Hedley P fractionation procedure was performed on both sludge and the soil applied with sludge. P adsorption characteristics in soil were measured both as the sum of oxalate extractable Fe and Al and by measuring adsorption of added P. The results from Hedley P fractionation of different sludges showed that P in Fe/Al precipitated sludges, which were stabilized by anaerobic digestion, was mainly found as P strongly bound to Fe and Al. Concentrations of labile P was low. Liming of Fe/Al precipitated sludge with either CaO or Ca(OH)<sub>2</sub> resulted in a shift to Ca-bound P and the concentration of labile P seemed to increase. In soil it was found a significant increase in P strongly bound to Fe and Al after application of limed or unlimed sludge. The limed sludge resulted also in a significant increase in the most available P fraction. Application of sludge with a high concentration of Fe +Al increased the concentration of oxalate extractable Fe +Al in soil, but P adsorption studies revealed that binding of added P did not increase after sludge application.

P522

### Farming practices with limiting nutrients for legumes cereals intercropping in the Sudano-Sahelian region of Burkina

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The legume/cereal intercropping in the Sudano-sahelian agro-systems generally achieve low yields. This is due to low rainfall and declining soil fertility, especially N and P. In order to evaluate the role of farmers' management on the nutrient balance in their fields, a survey was performed with 60 smallholders in the northern region of Burkina following semi-structured exchanges associated to direct observations and laboratory analysis. The results showed that farmers use composts or manure produced at their farm to fertilize about 60% of their total cultivated area of about 3ha. Chemical NPK fertilizers are sometimes added at low dose, of about 6-3-5 kg.ha<sup>-1</sup> yr<sup>-1</sup> of N - P - K. The average grain yield at harvest is 0.4-0.6 t.ha<sup>-1</sup> for the cowpea legume and 0.5-0.7 t.ha<sup>-1</sup> for the millet or sorghum. 85% of smallholders export the crops residues for feeding their livestock. It is concluded that the nutrient balance associated with these cropping systems are often negative. Soil amendment with Burkina rock phosphate should be tested as a low cost alternative to increase the legume biological N fixation and soil P balance.

P523

### Maize root growth and P uptake dependency on spatial distribution of sewage sludge, sewage sludge ash and TSP

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Sewage sludge represents one of the largest pools of phosphorus (P) in urban waste streams. Thus, knowledge about effects of sludge treatment and sludge application methods on plant P availability is important to ensure an effective recycling of P from waste. In this study we aimed to test the effect of incineration of sewage sludge on plant P availability and effects of a near seed placement compared to a homogenous application to soil. Furthermore, we wanted to quantify if increased root proliferation can be detected around a localized P source and whether this can be related to the P uptake from the source. Anaerobically digested, dewatered sewage sludge (SS) and ashes derived from subsequent incineration (ASH) were tested against triple superphosphate (TSP) in a rhizobox setup where shoot and root growth of maize was followed for 30 days. The three P sources were either mixed (M) homogeneously into the soil or placed (P) localized in a spot in a distance of 7 cm from the seed. Also a treatment receiving no P (CON) was included. The fraction of plant P derived from the P source was determined through an indirect labeling of the soil with <sup>33</sup>P combined with an additional experiment to determine the contribution of P from the seed. Preliminary results indicate that the localized placement had positive effects for TSP, negative effects for ASH, and no effect for SS. Dry matter yields ranked in the order TSP-P > ASH-M = SS-M = SS-P = TSP-M > ASH-P = CON. Increased root proliferation around the localized P source seemed present at highest degree for TSP, at a lesser degree for SS and not at all for ASH. In conclusion, placement effects depended on the P source properties.

P524

### Does phosphorus mobilised by break crops benefit subsequent wheat?

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The challenge for increasing phosphorus (P) use efficiency is to design farming systems that can access non-labile P reserves and improve sustainable use of the soil resource. Glasshouse studies have shown certain break crop species (some oilseeds and legumes) can mobilise P in soil, but there is less evidence regarding the extent this occurs in the field. This research aimed to identify P mobilisation by break crops and assess if this was linked to a P benefit for the following cereal crop. Plant samples were analysed for P content and root zone soil samples (0-10cm) for resin P, microbial P and mineral N. Break crop residue management treatments included mature stubble retention and green/brown manuring. Mobilisation of P by break crops (oilseeds, legumes and rye) was apparent between sowing and peak vegetative growth, as indicated by resin P plus crop P content at peak vegetative sampling exceeding pre-sowing resin P plus added P fertiliser and increased microbial P at one site. Input of mobilised P as break crop mature stubbles or green/brown manures did not result in increased soil P availability at sowing of the following wheat. Wheat following the break crops generally produced greater dry matter and grain yields, and accumulated more P compared to wheat grown continuously. P added in residues from break crops correlated poorly with P content and grain yield of wheat, whereas there was a positive relationship between soil mineral N at sowing and wheat P uptake. Since greater P uptake by wheat could not be directly linked with P mobilisation or break crop residue P inputs, further work is required to identify specific edaphic and plant interactions involved.

### Relationships between change in total P stock of the plough layer and P balance after 15 years of composts applications

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Understanding soil phosphorus (P) changes under continuous cropping over decades is an important agronomic and environmental issue. The objective of this study was to examine changes in total P stock of the plough soil layer of a cultivated ecosystem in relation to P balance. The long-term field experiment Qualiagro (INRA – Veolia Environnement) was established at Feucherolles, France, in 1998. Five treatments were designed and replicated in four blocks. Farmyard manure (FYM) and three types of composts (Municipal Solid Waste (MSW), Biowastes (BIOW) and Green Waste + Sewage Sludges (GWS)) were applied every two years at a rate of 4 t C ha<sup>-1</sup>. A control received no organic amendment. Changes of total P stock in the plough layer (0-28 cm) were compared to cumulated soil P balance since 1998, calculated as the difference between cumulated P input, as supplied through organic amendment and at seedling, and cumulated P output, by harvest of grains and crop residues if removed. Total soil P content and bulk soil density were determined at five soil sampling dates, crop yield and P content were measured annually. Cumulated P input for the 1998-2013 period ranged from 340 to 1695 kg P ha<sup>-1</sup> for MSW and GWS respectively. Cumulated P export by crops was influenced by treatments. Soil bulk density increased less in amended soils than in control. A linear relationship between total soil P stock variation and soil P balance was found, close to the 1:1 line. These results confirm that P outputs through erosion, runoff and leaching were negligible. Contribution of subsoil to crop nutrition was estimated to be close to 0.5-1 kg P ha<sup>-1</sup>.

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### RecoPhos P38: Upgrading sewage sludge ash to triple superphosphate

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Deteriorating phosphate rock qualities along with an increasing awareness of its limited static lifetime have triggered the exploitation of phosphate from secondary raw materials. In recent years, efforts have been made to recover high quality phosphate from the ash of thermally treated sewage sludge. Although some of the technologies have already been implemented on a larger scale their maturity and cost-effectiveness have been questioned. In this study we evaluated a novel technique for the full-scale treatment of sewage sludge ash. The proprietary process aims at the production of a phosphate fertilizer which may either be readily used for agricultural purposes or further customised to yield a polynutrient soil amendment. Ash from three municipal sewage sludge incineration plants used as input material in an approved full-scale application conducted in accord with the regional waste management authorities. Alike the industrial production of triple superphosphate the RecoPhos process involves digesting the ash with phosphoric acid to transform sparingly soluble minerals into readily available monocalcium phosphate. Results showed that the RecoPhos product complies with the German fertiliser ordinance regarding the contents of metals, metalloids and perfluorinated surfactants. As to the levels and fractionation of P in the RecoPhos product conforms to the standards of triple superphosphate according to the European Commission Regulation (EC) No 2076/2004. Based on these findings the product was officially discharged from the waste legislation. In 2012 1,000 metric tonnes of fertiliser per month were manufactured at a marketable price.

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### Soil aggregation and phosphorus cycling in maize-pigeon pea intercropping systems of Malawi

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Maize is the key staple crop in Malawi and supports the majority of livelihoods in the country. However, continuous maize cropping and low nutrient inputs have caused yields to decline, keeping smallholders in poverty. One potential low-cost method to increase phosphorus availability is maize-pigeon pea (PP) intercropping. Studies show that PP, a protein-rich legume, has a unique ability to access soil P pools less available to maize. Still, little is known about how this is done, what type of P is accessed, and if the P accessed by PP is transferable to maize. The goal of this project was thus to compare crop yields and aggregate-associated P pools between intercropped and sole maize to elucidate the mechanisms involved in P transfer between PP and maize. This study consisted of on-farm field trials in Central Malawi comparing sole maize and maize-PP intercropping systems. Soils were separated into aggregate fractions using a wet-sieving fractionation method. Each aggregate fraction was then assessed for total P (wet digestion with H<sub>2</sub>SO<sub>4</sub>), organic P (NaOH-EDTA extractable), microbial biomass P (hexanol-labile), and plant-available (resin) P. Our preliminary tests revealed that because of the presence of Fe-rich concretions (~5% of bulk soil by weight) there is a large variation within each plot. Total P concentrations in these concretions are 1.5 times greater than those in the bulk soil and aggregate fractions. They could potentially have a large effect on P dynamics (sorption, desorption, hydrolysis of organic P, etc.) within the cropping systems and must be studied in more detail to determine their overall effect on grain yield in this area.

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### Biosolids as a source of phosphorus in Australian agriculture

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The agricultural land application of biosolids (stabilised solid organic residuals from treated sewage sludge) as a source of nutrients for crop production is considered a long-term sustainable management option throughout many regions in Australia. Approximately 70% of the 330,000 tonne dry solids produced nationally are land applied per annum. The phosphorus (P) content of biosolids varies and is dependent on the wastewater treatment process, with mean total P values of 1.3-3.9% reported throughout Australia. It is estimated that approximately 6,000 tonne of P is returned to the soil annually in Australia from the land application of biosolids. Hence the recycling of P from biosolids is a useful substitute for inorganic fertiliser P and prevents the loss of P from the food chain. This paper presents findings for P availability in four biosolids products (dewatered mesophilic anaerobically digested, lime-amended, pelletised and alum sludge) relative to inorganic P fertiliser as investigated by combinations of field crop, laboratory and pot experiments in south-western Australia over several years. The risk of off-site movement of biosolids-P compared with inorganic fertiliser P at typical application rates was further examined on several soil types to assess the environmental implications of this practice in Australia. The effectiveness of biosolids-P compared to inorganic-P as a source of fertiliser in field experiments as measured by soil available P, uptake of P by shoots and crop yield was dependent on many factors over time including the soil conditions and biosolids type. Biosolids produced from wastewater treatment processes that used aluminium treatment to remove excess P had lower phytoavailability than those without chemical addition and pose additional challenges for land application as a source of P.

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### New pasture plant options to reduce P-input costs of grazing systems

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The cost of phosphorus (P) fertiliser is presently increasing at approximately twice the rate of inflation in Australia. P is also a significant proportion of the input costs for grazing farms. Consequently, there is growing interest in whether grazing systems that require less P fertiliser can be developed to reduce input costs. This objective requires pasture plants with lower critical P requirements for growth and the ability to sustain existing production levels/stocking rates. Critical P is defined, in this case, as the soil test P or fertiliser rate that corresponds to 90% of maximum shoot growth rate in spring. In the study described here, the response of *Trifolium subterraneum* (an annual legume used commonly in southern Australia) to application of fertiliser P, was compared with four alternative annual pasture legumes being assessed as part of a larger trial. Six levels of soil P fertility were achieved by applying triple superphosphate (21% P) to a P-deficient soil at Burrinjuck near Yass, New South Wales. The results indicate that the critical P levels for these species differed: *T. subterraneum* had a relatively high critical P level. However, *Ornithopus compressus*, *O. sativus* (pasture legume types) and *T. purpureum* and *T. incarnatum* (forage legume types) had lower critical P levels than *T. subterraneum*. Of the two pasture legumes types with a lower critical P level, only *O. sativus* produced an equivalent amount of dry matter at its critical P level to that of *T. subterraneum*.

P530

### Better P<sub>2</sub>O<sub>5</sub> fertilizer efficiency of intercropping than monocropping systems in different tropical soils

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In the 21<sup>st</sup> century, the growing demand for food and energy, concomitant with a scarcity of natural resources, incentives to produce more and sustainably. The best way to achieve these goals is to increase yields per unit area by the intensification of agriculture to generate a land reserve. The current trend is towards the rise in prices of phosphate fertilizers, that is a resource increasingly coveted. In addition, the proliferation of the use of phosphate fertilizers, reflects a net decrease of efficiency of the latter. This study was conducted to verify the role of intercropping on the efficiency of phosphate. Phosphate fertilizer trials were conducted in six sites and on seven soils in West Cameroon on Maize-Colocasia-Xanthosoma mixed crops. The response to increasing doses of 400Uha<sup>-1</sup> in monocrops and 1120Uha<sup>-1</sup> in intercrops, is 80% linear in monocrops and 90% quadratic in intercrops. The optimal average doses are 160Uha<sup>-1</sup> for sole corn, and 100Uha<sup>-1</sup> for sole tubers, whereas they are of 70 (median = 78), 77.5 (median = 177.5), and 82 Uha<sup>-1</sup> (median = 100) in intercrops, respectively the maize, colocasia and xanthosoma. The results show an average LER of 1.47 and median of 1.41 (CV = 49%) for the intercrop. To calculate the efficiency of the P<sub>2</sub>O<sub>5</sub> Equivalent Ratio of the intercrop, 40% of the optimal dose was assigned to maize and 30% for each tuber. In this case the P<sub>2</sub>O<sub>5</sub> Efficiency Equivalent Ratio is 6 average and 5 median. This improved fertilizer efficiency in intercrops may be due to free EcoSystemic Services that must be identified.

P531

### Growth of Australian native pasture species (genus *Rytidosperma*) in response to application of phosphorus fertiliser

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*Rytidosperma* species (wallaby grass) are native perennial grasses found in temperate grasslands of southern Australia. They are considered to be well adapted to low phosphorus (P) soils and reports vary as to whether they can respond and/or persist when P fertiliser is applied to increase production. However, the responses of *Rytidosperma* species to management are often considered collectively because the species cannot be distinguished using vegetative traits. This makes grassland botanical composition difficult to assess. This study examined the growth of nine *Rytidosperma* species, *Lolium perenne* and *Bromus hordeaceus* (introduced species) to six levels of applied P (ranging from deficient to sufficient) in a glasshouse under microswath conditions. Maximum shoot yield between the highest (*R. duttonianum*) and lowest (*R. erianthum*) yielding *Rytidosperma* species varied almost 2-fold. Two *Rytidosperma* species, *R. richardsonii* and *R. duttonianum*, yielded as well as *L. perenne* at high and low P supply. However, a number of *Rytidosperma* species were slow-growing and relatively unresponsive to P. Species that had a high maximum yield tended to also grow well at low P, except for *B. hordeaceus*, which did not thrive at low P, despite having a high maximum yield when fertilised. The productive *Rytidosperma* species had lower leaf area ratios, lower specific leaf areas and higher leaf dry matter contents than the introduced species. The study demonstrated that growth responses and P-requirements of the *Rytidosperma* species differ markedly. It is essential to know which species are present when managing a *Rytidosperma* grassland and making P-fertiliser decisions.

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### Increasing the diversity of leguminous plant improves soil functionalities and wheat growth in a P-deficient soil

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A study was conducted in glasshouse conditions to assess the influences of the leguminous plant diversity on the soil microbial functions and their consequences on the wheat growth. Three legume species were targeted: faba bean, alfalfa and pea. All the combinations of one, two or three species were performed in pots filled with a P deficient soil collected from a field located near Marrakech (Morocco). After 3 months culture, the plants were harvested and the shoot and root parts were dried, weighed and analyzed for their N and P contents. The soil catabolic functions were measured using the SIR (Substrate Induced Respiration) method. The mycorrhizal soil infectivity was assessed according to Kisa et al. (2007) and the arbuscular mycorrhizal (AM) diversity was estimated by PCR/Sequencing. Fluorescent Pseudomonads (FP), known to have PGPR capacities have been enumerated, identified (PCR/sequencing) and characterized for their inorganic phosphate solubilizing activities and for their effect on wheat growth. The results showed that an increase of legume diversity involved: (i) significant differences between the microbial functions within the treatments, (ii) changes in the abundance and diversity of the AM communities, (iii) an increase of the FP abundance, most of them solubilizing inorganic phosphate and promoting the wheat growth. These results suggest that the management of the legume plant cover diversity can optimize the positive impact of legume on the agrosystem productivity resulting from an increase in soil microbial functions, soil microbial diversity, AM symbiosis efficiency in sustainable agricultural practices (crop rotation, intercropping systems, etc).

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### Phosphorus balance and utilization efficiency of lactating cows in dairy operations with different sizes in China

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Critical demand for the amount and quality of milk is a key factor driving the transition of China's dairy production from small holders to intensive operations. It is believed that such changes in dairy operation sizes would very likely change the amount of feed phosphorus (P) input and milk production. In order to explore the relationship of P utilization efficiency and the size of dairy operations and clarify the P balance, a survey across twenty four collective dairy operations (CDOs) and sixteen intensive dairy operations (IDOs) in Hebei province was carried out in this study. The average milk production of surveyed CDOs and IDOs was 4958 and 6587 kg·cow<sup>-1</sup>·yr<sup>-1</sup>, respectively. Although the feed P inputs of the investigated dairy operations were characterized with large variation, the averaged annual P input in CDOs (20.2 kg·cow<sup>-1</sup>·yr<sup>-1</sup>) was lower than that in IDOs (28.0 kg·cow<sup>-1</sup>·yr<sup>-1</sup>); On the basis of 1 ton milk production, however, the P input in CDOs (4.0 kg) was similar to the IDOs (4.2 kg). Among the CDOs and IDOs, the annual utilization efficiency (APUE) and lactation utilization efficiency (LPUE) of feed P in IDOs were 24.3% and 27.4%, respectively, which were slightly higher than the APUE (23.0%) and LPUE (24.8%) of CDOs. In conclusion, in comparison with collective dairy operations, intensive dairy operations were characterized with higher and steadier milk production and greater P use efficiency.

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### Phosphorus fertilisation of Malagasy agronomic systems in the context of climate variability and change

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Farmers in Madagascar are among the most vulnerable groups in the World. Malagasy agronomic systems are characterised by low to very low soil fertility resulting to food insecurity. The availability of phosphorus (P) is a key issue of climate change mitigation of agronomic systems, as P is the first limiting factor of agricultural productivity. In fact, combination of organic and inorganic phosphorus fertilisers allows overcoming P deficiency in rain fed rice and, at the same time, permit coping with the negative impacts of climate variability when farmers have to modify their conventional farming system and practices, such as the use of triple superphosphate or the delay in crop installation. The production of rain fed rice can be improved from 1t to 3t per ha using mixed organic and inorganic sources of phosphorus. A transformational change is needed for an effective support from the decision makers either in training than in financial and technical supports. The participatory action research is the way for scaling up research results in the field.

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### Predicting phosphorus fertilisation effects of waste products by chemical extraction

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With rock phosphate becoming an increasingly precious resource, there has been growing interest in the use of waste products as alternative P fertilisers in agriculture. Their fertilisation effects are little known and strongly variable. Therefore, the aim of the present study was to evaluate 10 chemical extraction methods in terms of their ability to predict readily-available P and late-season P fertilisation effects of various waste products (two biomass ashes, meat bone meal, fish sludge, catering waste and two products of food waste-based digestate) at two soil pH levels (pH 5.5 and 6.9). Extractable P was compared with plant-available P as studied in a pot experiment with ryegrass (*Lolium multiflorum* var. *italicum*) and a nutrient-deficient sand-peat mixture as experimental soil. Our results showed that at soil pH 5.5, P extraction with 0.005 M CaCl<sub>2</sub> and simultaneous adsorption to iron-oxide impregnated filter paper predicted relative P efficiency best at harvest 1 (Rpred<sup>2</sup>= 0.57), and the strong desorption agent neutral ammonium citrate predicted relative P efficiency best as the sum of harvests 2, 3 and 4 (Rpred<sup>2</sup>= 0.57). At soil pH 6.9, NaHCO<sub>3</sub> adjusted to pH 8.5 was the only extraction method that significantly correlated with relative P efficiency (Rpred<sup>2</sup>= 0.54, sum of all harvests) because all other extraction methods overestimated P fertilisation effects of apatite-rich waste products. We concluded that the choice of extraction method will dependent on pH in the soil to be fertilised and on whether readily-available P or late-season P fertilisation effects are to be predicted.

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### An initiative of sustainable phosphorus use in P- rich mining zone of lower Himalaya region of Pakistan

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Phosphorus from inorganic sources such as tricalcium P (rock phosphate), though constitutes the major proportion in soils of lower Himalaya regions of Pakistan, is mainly unavailable to plants. The need of available P in this zone, is deteriorating the natural ecosystem of the area via environmental pollution through mining and artificial P fertilizers. Despite the well-known effects of rhizosphere trophic relationships on N mineralization, almost no work has yet been done on P mineralization. We, therefore, hypothesized that the interactions between phosphorus solubilizing bacteria and bacterial grazer nematodes are able to improve plant available P use from abundantly present P source. We tested the hypothesis by isolating P solubilizing bacterial strains and growing them with Pine seedlings in sand containing TCP as sole P source. The plants were grown alone or with a P solubilizing bacterial strain along with bacterial-feeder nematodes. The bacteria and the nematodes were isolated from same region of rich unavailable P zone of lower Himalaya. Interestingly the grazing of bacteria by nematodes enhanced plant P accumulation. Although plants increased the density of P solubilizing bacteria, these bacteria alone did not improve plant P nutrition as in the case of nematode predation. Our results open an alternative route for better utilization of poorly available inorganic P to plants. In future we plan to introduce a combined inoculum (biofertilizer) of P solubilizing bacteria and their predator nematodes for the use of small farmers of lower Himalaya region of Pakistan which will ultimately help to lead the region using their P reserves in a sustainable way.

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### Conventional and recovered P-fertilizer: A Monte Carlo approach to the exceedance of heavy metal limits in soils

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RecoPhos P38 is a recovered P-fertilizer derived from high-quality municipal sewage sludge ash. The commercially competitive product complies with the German Fertilizer Ordinance and was officially dismissed from the waste legislation framework. Compared to conventional Triple superphosphate the product exhibits lower levels of Cd but is higher in Cu. Since the application of P-fertilizers may lead to an accumulation of heavy metals in agricultural soils, this study aimed at delineating the timeframe for the exceedance of precautionary limits set out by the German Soil Protection Act. Using Monte Carlo simulations, continued maintenance fertilization with Triple superphosphate was compared to equivalent applications of RecoPhos P38 for a series of relevant field crops. Model inputs were based on literature data and own analytical results regarding (i) Cd and Cu in RecoPhos P38 and conventional Triple superphosphate, (ii) Cd and Cu background levels in German agricultural soils, and (iii) crop-specific element withdrawal rates. Results show that exceedance of the precautionary limits is likely to occur a timeframe of hundreds to thousands of years. Legal values are exceeded faster for Cd derived from the conventional fertilizer than for Cu stemming from RecoPhos P38. For sugar beet and maize withdrawal rates for Cu are higher than RecoPhos-borne Cu loads leading to zero accumulation in the soil. Overall, soil protection issues seem more affected by the application of conventional Triple super-phosphate than by use of RecoPhos P38. Monte Carlo simulations offer the benefit of including the distribution of parameter values into the soil accumulation model rather than restricting it to best-case/worst-case scenarios. This allows for a probabilistic interpretation of the time-to-exceedance of regulatory standards.

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### Mobilization of soil soluble phosphorus by low molecular weight organic acids in a neutral soil of China

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Low molecular weight organic acids (LMWOAs) are known to be effective in enhancing the mobilization of inorganic P, thus increasing plant-available P in soils. But little is known about their effect on organic P, especially in agricultural soil. The aim of this study was to investigate the role of three common organic acids (oxalic, citric and malic acids) and their mixture (ratio, 1:1:1) in the mobilization of soil soluble inorganic phosphorus (SIP) and soluble organic phosphorus (SOP) in a neutral agricultural soil of China. Surface layer (0-20cm) of the soil was collected from Hailun (47°26'N, 126°38'E) Experimental Stations of Ecology, Chinese Academy of Sciences. The test soil was the major type of agricultural soil in its local area. Soil samples were air dried and ground to pass through a 2-mm sieve before use. Triplicate 30ml of 1 mmol l<sup>-1</sup> of oxalic, citric, or malic acid, or their mixture (ratio, 1:1:1) were added to 3 g soils in 50-ml centrifuge tubes. The LMWOAs concentration was 10 mmol kg<sup>-1</sup> soil. The suspensions were shaken for 24 h at 25±1°C. Inorganic P concentration of the extracts was determined by the malachite-green method using UV-721 Spectrophotometer at 610 nm. Total P contents (10 ml of extracts) were quantified by digesting in autoclave (103.4 K Pa, 121 °C) for 60 min with 0.6 g K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> and 10 ml 0.9 mol l<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>. Concentrations of soluble organic P were calculated as the difference between total P and inorganic P. All the three organic acids and their mixture at 10 mmol kg<sup>-1</sup> soil enhanced the mobilization of SIP and SOP of the neutral soil as compared with water, but no evident combined effects among the three organic acids were observed. A comparison of impacts of LMWOAs types indicated that the amount of SIP and SOP decreased in the order of oxalic acid > mixture > citric acid > malic acid. The ability of LMWOAs to mobilize SIP and SOP was linked to the shift in pH values and their ability to complex or precipitate with cations (e.g., Fe, Al and Ca) bound to P. LMWOAs at 10 mmol kg<sup>-1</sup> soil can enhance the mobilization of SIP and SOP in the neutral soil of China. Although the amount of P mobilized by organic acids is small, it may play an important role in plant growth due to its labile nature.

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### Importance of subsoil phosphorus in wheat response on Mediterranean sandy soils

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Sandy soils are common in the Mediterranean region of Western Australia (WA). Phosphorus leached down these soils remains partly available and affects crop response. Subsoil P is however not taken into account in fertiliser recommendation. The aims of this work were to determine the difference in yield predictions based on 0-10 cm and 0-30 cm samples and to assess the importance of subsoil P. Yield response functions valid for WA were used to predict wheat yields. Soil P status of 184 focus paddocks across WA was assessed using bicarbonate-extractable P (Colwell P) and P buffering index (PBI) for the 0-10, 10-20, 20-30 cm soil layers. In 2010-13, 71% of all sites had annual average Colwell P >25 mg P/kg and PBI < 80. These soils exceeded their PBI-dependent critical values for Colwell P. The frequency of Colwell P > 25 mg P/kg in 0-10 cm was higher in the southern region (93%) than the northern (63%) region characterized by sandier soils with lower PBI values. Most sites (91%) had average Colwell P for 2012-13 >4 mg/kg in the 10-30 cm layer. The frequency of Colwell P > 4 mg/kg in the 20-30 cm soil layers was higher in the northern (70%) than the southern region (38%). The lower frequency of subsoil P >4 mg/kg and common exceedance of CV in the south resulted in similar yield predictions using 0-10 and 0-30 cm data; both approaches identified 5 responsive sites out of 50. In the northern region with annual rainfall >400 mm, up to 33% predicted yield difference occurred. There, 19 of 30 sites were predicted to be responsive using 0-10 cm depth compared with 9 of 30 sites using 0-30 cm depth suggesting that subsoil P is more important in these northern sands.

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### The potential of dehydrated food wastes as phosphatic fertiliser

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Organic waste is a large and growing environmental, social and economic cost. Around half of the waste produced in Australia each year is organic waste, of which the vast majority goes to landfill. In Victoria it is estimated that around half of all purchased food is thrown away each year. For every kg of food waste that is sent to landfill, it is estimated that 1.5 - 3.8 kg of CO<sub>2</sub>-e greenhouse gas emission is produced. In response the Victorian State Government is encouraging the development of market-based approaches to divert organic waste from landfill. To move toward sustainable food systems we need to identify ways of closing leaks in our food chains. A key element to affect this closure is the return of the nutrients in food waste to the soil. Phosphorus is a non-renewable nutrient and the use of food waste as a soil conditioner may help to alleviate a predicted future scarcity of this element. A typical organic food waste is around 3 % N, 0.2 % P and 0.8 % K on a dry weight basis. The University of Melbourne student union, along with a number of urban restaurants is attempting to process food waste on-site using commercial dehydrator units. Advantages of this process include: diversion of the material from landfill; reduced transport costs; closing the food production loop and; greater awareness of food production and waste avoidance. However as the value of this resource for land application is largely unknown, most of the treated food waste is sent to landfill. This paper examines the chemical and physical attributes of dehydrated food waste from a number of sources and its potential as phosphatic fertilizer.