

# Organic Phosphorus Workshop 2016

## *Organic Phosphorus in the Environment: Solutions for Phosphorus Security*

#organicP2016

# Abstract Book



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## OP2016 Welcome Letter

Dear Colleagues,

Welcome to the Lake District. As I write this, it is Tuesday before the meeting and there is an excited anticipation in the air, as I know you will be starting to make plans to start your journey to northern England. It is going to be a great pleasure to welcome you and we are planning and hoping that you will have an inspiring time.

This meeting and this topic is deeply special to me, as it reflects a something close to the core of my intellectual interests and a personal journey. It started in the late 1980s when I was doing my PhD partly based with Tony (AF) Harrison as one of my supervisors, at the beautiful Merlewood Research Station on the edge of The Lake District. Although I was not primarily P focussed at this time, I became aware of the 'blue book' on 'Organic Phosphorus in Soils - A review of World Literature' published by Tony, and I am pleased to report that Tony will be Guest of Honour at our Dinner on Thursday. Thereafter I left The Lakes and Tony to work for 16 happy years at North Wyke (now part of Rothamsted), where my focus turned to studying phosphorus at the interface of land and rivers. This was the time, in the mid 1990s, when I became particularly fascinated to determine molybdate *unreactive* P and inositol P forms in soils and leachate waters. I became equally as intrigued as to why so many seemed to ignore this, in favour of inorganic phosphate?

Today I am back close to the Lake District at Lancaster University and phosphorus is perhaps more topical and prominent on the world stage than ever before, with talk of sustainability, managing resources for food production, as well as maintaining water quality, ecology and biodiversity. There are now quite a few international meetings on the element and only two weeks ago I was at the international Sustainable Phosphorus Summit in China (~500 delegates) and one of the main findings was that we need to make better use of the vast organic P stocks that exist in the world's soils. Is this the greatest challenge for our Workshop.....?

So it is my great pleasure to report that despite a busy world P calendar, we had to close registrants at 100 delegates for this week and we can now focus on an inspiring week together. Bringing you to the Lake District is special, it may not be the biggest or the best of the world's National Parks, but it does have a landscape that has inspired scholars before, like John Ruskin and William Wordsworth. Perhaps we can tap into their inspiration to connect this back to our phosphorus challenges? I hope so. So it remains for me to thank the team, especially those closest who have worked so hard, Daniel, Catherine, Tim and Courtney, plus the wider contributors too, and of course some of the seed funding that came from the UK BBSRC via the OPUS project team. Thanks to you all.

Oh – and those of you who use social media please use the hashtag #organicP2016 to keep things joined up.

Here's to an inspiring and enjoyable week!



Phil Haygarth, Chair of the Organising Team

## Keynote Speakers



**Prof. Leo Condon**

**Keynote Talk:** Historical perspective on the nature and dynamics of organic phosphorus in the environment



**Dr Barbara Cade-Menun**

**Keynote Talk:** Methods to characterize and quantify organic phosphorus in environmental samples: past successes and future directions



**Dr Marie Spohn**

**Keynote Talk:** How are organic carbon and phosphorus mineralization connected in the rhizosphere?



**Dr Mark Smits**

**Keynote Talk:** Plant-fungus interactions with apatite: ideas versus observations of mycorrhizal fungi-induced apatite weathering



**Dr Federica Tamburini**

**Keynote Talk:** Insights into the biological cycle of P (with a little help from oxygen isotopes).



**Dr Anna Rosling**

**Keynote Talk:** How mycorrhizal associations effects phosphorus cycling in deciduous forest soils.



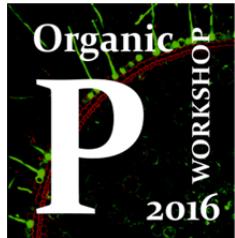
**Prof. Dr Erwin Klumpp**

**Keynote Talk:** Specification of nano-particulate phosphorus in terrestrial systems.



**Dr Ben Turner**

**Keynote Talk:** Organic phosphorus and the ecology of tropical forests.



## Abstracts: Oral Presentations

**Session 1: Organic phosphorus flows in the environment in context with other nutrient cycles: Integration across ecosystems**

## Observed C:N:P stoichiometry and P speciation as an indicator of P cycling across ecosystems: the chicken or the egg?

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*The environment not only determines the conditions under which life exists, but the organisms influence the conditions prevailing in the environment.—Alfred Redfield (1958).*

Although the marine microbes studied by Redfield conform to relatively consistent molar 106:16:1 C:N:P ratios soil microbes are known to have specialised strategies to exploit substrates of widely ranging ratios, with their own ratios following those of substrates<sup>1</sup>. Strong variation in aquatic microbial stoichiometry across freshwaters of varying nutrient compositions has also recently been shown<sup>2</sup>. In a catchment context the C:N:P ratios of the waterbody (dissolved substances, sediments) must result from (a) the mixing of different nutrient sources and (b) in-stream processing. A key question for understanding aquatic P cycling, in relation to major requirements for knowledge around waterbody resilience to pollution, is the degree of dominance of processes (a) and (b) in the observed nutrient concentrations. Under semi-natural conditions internal processing may dominate as biota draw-down key (often limiting) resources such as P. Conversely, under nutrient saturated conditions potentially this internal processing power is reduced and observed stoichiometry and concentrations of C, N, and especially, P become the product of physical mixing of P sources. This contribution asks ‘*What is the role of C:N:P stoichiometry in determining this resilience associated with within-ecosystem (e.g. soil, waterbody) processing ability?*’ and furthermore ‘*Can P speciation (e.g.  $P_i/P_o$  or  $^{31}P$  NMR speciation) improve process understanding above that knowledge provided by ratios of total C, or P?*’

The aim of this contribution is to use a combination of literature, own studies and knowledge from the audience to promote discussion and bring together insight into C:N:P stoichiometry and P speciation of soil, runoff, river water, sediment and biota to address nutrient limitations, catchment P processing and unravel whether observed ratios are either the influence on, or the result of ecosystem processing; the ‘chicken’ or the ‘egg’.

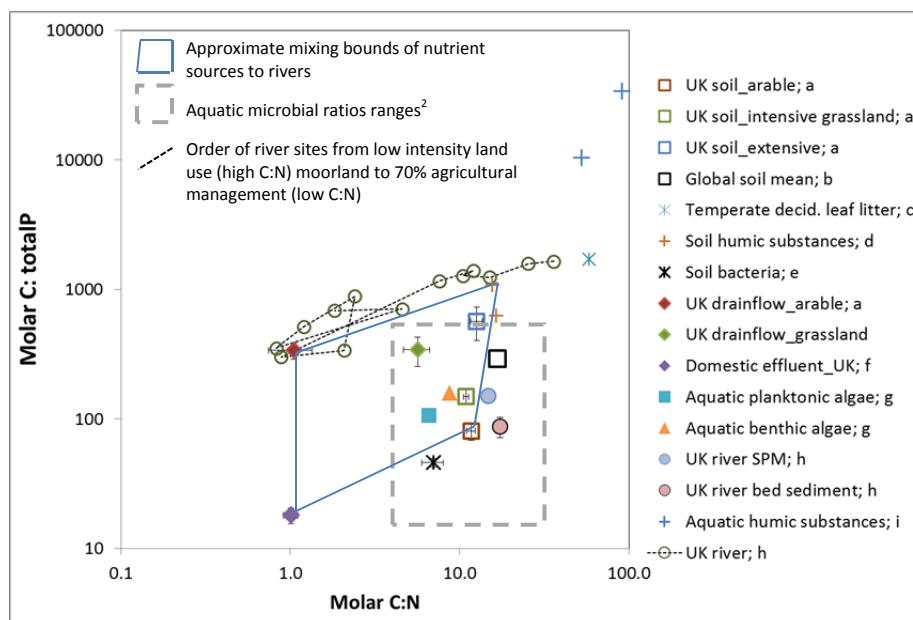


Fig. 1. Stoichiometry values across ecosystem components of the catchment continuum, including biota.

**References:** <sup>1</sup>Mooshammer et al. 2014. Front. Microbiol. 5, 22; <sup>2</sup>Godwin & Cotner, 2015. Front. Microbiol. 6, 159; a, this study; b, Xu et al. 2013; c, e, Cleveland & Liptzin, 2007; d,i, [www.humicsubstances.org](http://www.humicsubstances.org); f, Richards et al. 2015; g, Demars Pers.comm.; h, Stutter et al. 2008.

## Organic P in freshwaters: Rapid degradation and variable production have profound effects on stoichiometry

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Phosphorus (P) is a critical element to all life because it facilitates information and energy transfer within and among organisms. Its availability controls fluxes of carbon (C) in many soils, lakes and the global ocean, but surprisingly little is known about its bioavailability and sources. Although it is known that increasing dissolved P concentrations often translate to increased biomass at multiple trophic levels, many P compounds reside in a relatively inaccessible pool of which little is known. Dissolved organic P (DOP) compounds represent the bulk of the dissolved P in most aquatic systems, but where it originates, how rapidly it turns over and the extent to which it regulates production and decomposition processes is unclear. Ecological stoichiometry theory suggests that the availability of a limiting nutrient such as P should have strong effects on the lability of organic matter that is available for degradation. This role has been little explored in the past and will be examined in this talk.

In the oceans, DOC: DON: DOP ratios are much higher than the Redfield ratio and increase with depth<sup>1,2</sup>, presumably due to rapid degradation and re-incorporation of organic P compounds into biomass relative to organic N and C. Fewer systematic observations of these ratios have been made in terrestrial and freshwater ecosystems. We have found that extractable grassland soils DOC: DOP ratios are high (200-500) but not as high as the deep ocean. However, DOC: DOP ratios in freshwaters are extremely variable, greater than a factor of 10 in our surveys, but higher than either soils or marine ecosystems (values ca. 700-6,500), suggesting high DOC: DOP production ratios, rapid degradation and re-incorporation into biomass or both. Recent work in our group has shown that biomass stoichiometry is much more variable than currently assumed, with some C:P ratios approaching 10,000:1<sup>3</sup>. We will examine the factors affecting the production, lability and preferential degradation of organic P along the soil to ocean continuum and their significance to organic P in the environment.

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- [1] Clark, L L, E D Ingall, and R Benner. "Marine Phosphorus Is Selectively Remineralized." *Nature* 393 (1998): 426.
- [2] Ammerman, J W, R R Hood, D A Case, and J B Cotner. "Phosphorus Deficiency in the Atlantic: An Emerging Paradigm in Oceanography." *EOS* 84 (2003): 165, 170.
- [3] Godwin, Casey M, and James B Cotner. "Aquatic Heterotrophic Bacteria Have Highly Flexible Phosphorus Content and Biomass Stoichiometry." *The ISME Journal* 9 (2015): doi:10.1038/ismej.2015.34.

## The C:N:P:S stoichiometry of soil organic matter

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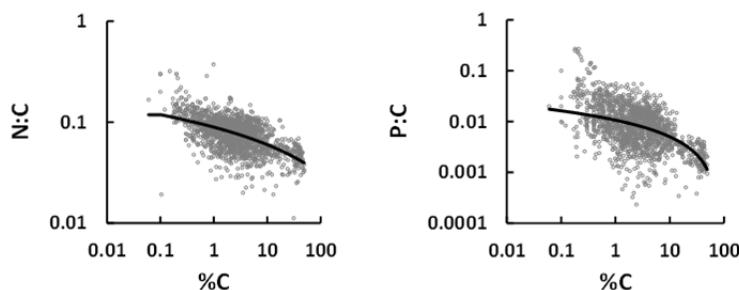
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The formation and turnover of soil organic matter (SOM) includes the biogeochemical processing of the macronutrient elements nitrogen (N), phosphorus (P) and sulphur (S), which alters their stoichiometric relationships to carbon (C) and to each other. We sought patterns among soil organic C, N, P and S in data for c. 2000 globally distributed soil samples, covering all soil horizons. For non-peat soils, strong negative correlations ( $p < 0.001$ ) were found between N:C, P:C and S:C ratios and % organic carbon (OC), showing that SOM of soils with low OC concentrations (high mineral matter) is enriched in N, P and S.

The results can be described approximately with a simple mixing model in which nutrient-poor SOM (NPSOM) has N:C, P:C and S:C ratios of 0.039, 0.0011 and 0.0054, while nutrient-rich SOM (NRSOM) has corresponding ratios of 0.12, 0.016 and 0.016, so that P is especially enriched in NRSOM compared to NPSOM. The following diagram shows the modelled trends for N:C and P:C superimposed on the data.



The trends hold across a range of ecosystems, for topsoils and subsoils, and across different soil classes. The major exception is that tropical soils tend to have low P:C ratios especially at low N:C.

We suggest that NRSOM comprises compounds that have been selected by their strong adsorptive properties towards mineral matter. The stoichiometric patterns established here offer a new quantitative framework for SOM classification and characterisation, and provide important constraints to dynamic soil and ecosystem models of carbon turnover and nutrient dynamics.

## The dynamics of the C/P<sub>org</sub> ratio of acid forest soils provide evidence for P limitation of SOM decomposing organisms

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The P limitation of soil organisms is still an open question. Acid soils bear a high potential for P limitation and high C/P<sub>org</sub> ratios are assumed to indicate P limitation of decomposing soil organisms. Thus, we hypothesize that in P-poor soils the C/P<sub>org</sub> ratio of soil organic matter (SOM) increases during decomposition of SOM, while the C/P<sub>org</sub> ratio decreases during decomposition of SOM at P-rich sites. To test our hypothesis we analyzed the C/P<sub>org</sub> ratio of the soil organic matter of five beech forest ecosystems on silicate rock with different P supply by the mineral soil (total P stock of the P poorest site: 164 g P m<sup>-2</sup>; P richest site: 900 g P m<sup>-2</sup>). Carbon/P<sub>org</sub> ratios were determined from soil samples and from SOM fractions of the soil samples with assumed different degree of SOM decomposition: 1. Samples from the Of and Oh horizon of the forest floor. 2. Samples from mineral soil horizons form the mineral topsoil horizons down to 1m below mineral soil surface. 3. The free light fraction of the soil organic matter and the mineral associated fraction. The free light fraction of SOM and the mineral associated fraction were derived as described by Golchin et al. [2]. The C<sub>org</sub> content of different soil samples and SOM fractions was determined in milled samples dried at 105°C using an elemental analyzer (Vario EL cube, Elementar, Germany), the P<sub>org</sub> content was calculated according to the method developed by Saunders and Williams [1].

The C/P<sub>org</sub> ratios of the forest floor samples increased with decreasing P content of the mineral soil. The largest C/P<sub>org</sub> value (972 g/g) was observed for the Oh horizon of the poorest site. The C/P<sub>org</sub> ratio decreased from Of samples (ca. 600) to Oh samples (ca. 300) of the forest floor. The P poorest site was an exception. Here the C/P<sub>org</sub> ratio increased from Of samples (500) to Oh samples (1000). The C/P<sub>org</sub> ratios decreased with increasing depth of the mineral soil. Yet, the gradient was steeper with decreasing P status. At the P richest sites the C/P<sub>org</sub> ratio decreased from 110 (topsoil) to 27 (1m soil depth), while at the P poorest sites it decreased from 982 (topsoil) to 31 (1m soil depth). All these results indicate a P enrichment of SOM during decomposition and do not provide evidence for P limitation (apart from the forest floor of the P poorest site). In contrast, results from SOM fractionation show different results. It is widely assumed that the degree of decomposition of mineral associated organic matter is higher than that of the free organic matter. Only the soil samples from the P richest site showed a lower C/P<sub>org</sub> ratio of the mineral associated SOM compared to the free organic matter. All the other sites showed much lower C/P<sub>org</sub> ratio of the free organic matter (ca. 200) than of mineral associated organic matter (ca. 2000). In contrast to the analyses of bulk SOM, the analysis of SOM fractions indicates P limitation of decomposing organisms at four of the five study sites. Phosphorus depletion during decomposition and continuous input of P rich root litter to the light SOM fraction might explain these results.

### References:

- [1] Saunders WMH, Williams EG. "Observations on the determination of total organic phosphorus in soils." *J. Soil Sci.*, 6, 254–67, 1955.
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## First application of the phosphorus-enabled version of ORCHIDEE: can we reproduce the deteriorating tree mineral nutrition in Europe?

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The effect of phosphorus availability on the land carbon balance remains largely unknown. The assessment of potential negative effects on the future land carbon balance is severely hampered due to large uncertainties in the availability of phosphorus<sup>1</sup>, the applied methodolgy<sup>2</sup> as well as due the interactions between the biogeochemical cycles of carbon, nitrogen and phosphorus<sup>3,4</sup>.

Here, we present the P-enabled land surface model ORCHIDEE-CNP which simulates the cycles of carbon, nitrogen, and phosphorus as well as their interactions as a function of climate and soil characteristics and lithology. We evaluate the performance of the model against a set of eddy-covariance sites. Further, we test if the model is able to simulate the observerd increase in the leaf nitrogen to phosphorus ratio of European tree species<sup>5</sup> with the aim to identify potential drivers behind the deteriorating tree mineral nutrition.

### References:

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- [2] Sun et al. Toward a consistent approach for diagnosing phosphorus limitations in natural terrestrial ecosystems by carbon cycle models (in revision)
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- [4] Brokvin & Goll. Land unlikely to become large carbon source. Nature geosciences. doi:10.1038/ngeo2598. 2015
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## Carbon, nitrogen and phosphorus net mineralization in organic layers of temperate forests: Stoichiometry and relations to litter quality

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Litter decomposition in the organic layer of forest ecosystems ends with the mineralization of carbon (C), nitrogen (N) and phosphorus (P). The rates of the mineralization processes can turn a forest into a C sink or source and determine its fertility, but despite their importance for ecosystem functioning, C, N and P net mineralization rates are seldom evaluated in combination. Hence, our objective was to assess the relationships between net mineralization rates and litter stoichiometry. We conducted an eight-week incubation experiment with Oi, Oe and Oa layer litter of six beech, one spruce and one pine site, in which we determined C, N and P net mineralization rates, litter organic C quality and C:N:P stoichiometry. Net N mineralization started at litter C:N ratios of 41 (Oi) or 28 (Oa) and N:P ratios of 40 (Oi) or 60 (Oa). Net P mineralization started at litter C:P ratios of 1400 (Oi) and N:P ratios of 41 (Oi). N and P net mineralization were positively correlated with each other ( $r = 0.68^{***}$  (Oi),  $r = 0.72^{***}$  (Oe),  $r = 0.31$  (Oa)) and were also positively related to litter N and P concentrations and negatively to N:P ratios. C mineralization was positively related to litter organic C quality and weakly positively correlated with net N and P mineralization. Beech and coniferous litter characteristics differed only in the Oi layers. The average C:N:P stoichiometry of net mineralization was 620:4:1 (beech, Oi), 15350:5:1 (coniferous, Oi), 1520:8:1 (Oe) and 2160:36:1 (Oa). On average, net mineralization C:N ratios were higher, and N:P ratios lower than litter C:N and N:P ratios. This difference contributes to the decrease of C:N ratios and increase of N:P ratios from the Oi to the Oa layers. In conclusion, the stoichiometry of net mineralization rates was closely related to litter element ratios.

## Dissolved phosphorus in forest soils

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Primary mineral sources of phosphorus (P) are becoming increasingly depleted during progressing ecosystem development and soil formation. Since inorganic P forms tend to be bound strongly to or within secondary minerals, they are hardly available to plants and assumed not to be leached from soil. What about organic forms of P? Since rarely studied, little is known on composition, mobility, and bioavailability of dissolved organic phosphorus (DOP). There are indications that dissolved organic P forms can be mobile in soils. However, they appear also easily hydrolysable by enzymes, which may limit their leaching from soil. This work addresses organic and inorganic P forms in soil water at forest sites along a P availability gradient. Soil solutions were sampled with lysimeters immediately beneath the organic layers overlying the mineral soils and with suction devices at different depths in the mineral soils. Solutions were analysed for dissolved organic carbon as well as for total and inorganic P; with the difference between total and inorganic P representing the organic P fraction. Solutions were also subjected to enzyme assays that allow for distinguishing hydrolysable monoester P, diester P, phytate P, and pyrophosphate. The enzyme assays were complemented by P speciation with liquid-state <sup>31</sup>P-NMR spectroscopy and tests for sorption of dissolved P forms.

Leaching of P from the organic forest floor layers decreased with decreasing P availability. The decrease was mainly due to the decrease in leached inorganic P; thus, the contribution of DOP increased with decreasing P availability. In the mineral soil, DOP was more mobile than inorganic P. Consequently, the contribution of DOP to total dissolved P leaching steadily increased with depth. Despite strong retention in the mineral soil, inorganic P occurred at small concentrations even in the deeper subsoils. Thus, inorganic P contributed to P leaching from soil and is not as immobile as often assumed.

The greater mobility of organic than of inorganic P was due to a combination of weaker sorptive retention and surprisingly poor bioavailability. Despite of consisting mainly of orthophosphate monoesters and diesters, a large fraction of the DOP leached from organic layers resisted enzymatic hydrolyses. The portion of P not hydrolysable by enzymes strongly increased with depth in the mineral soil. Sorption studies showed that organic compounds rich in P were far less strongly retained by interaction with reactive minerals than P-rich organic compounds and inorganic P forms.

In summary, leaching of P into the deeper subsoil and likely from the entire soil occurred at all studied forest sites. The findings imply that forest ecosystems loose dissolved P at small but steady quantities, irrespective the P availability or the vegetations' prevailing P nutrition strategy. This leaking could be one major cause for long-term P depletion of ecosystems and is mostly driven by the mobility of DOP in the mineral soil.

## Past atmospheric phosphorus

*Helle Astrid Kjær*<sup>1</sup>, Remi Dallmayr<sup>2</sup>, Jacopo Gabrieli<sup>3</sup>, Kumiko Goto-Azuma<sup>2</sup>, Motohiro Hirabayashi<sup>2</sup>, Anders Svensson<sup>1</sup> and Paul Vallelonga<sup>1</sup>

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Phosphorus in the atmosphere is poorly studied and thus not much is known about atmospheric phosphorus and phosphate transport and deposition changes over time, though it is well known that phosphorus can be a source of long-range nutrient transport, e.g. Saharan dust transported to the tropical forests of Brazil.

Greenland ice cores provide archive of past climate and can inform on a variety of proxies, amongst these wind transported dust (originating in the Chinese deserts), forest fires, volcanic eruptions, black carbon and acid pollution in recent times with more.

Ice cores are well dated and can resolve sub-annual layers, while simultaneously reaching back up to 100,000 yrs. Thus polar ice cores offer a unique opportunity to study changes in the atmospheric transport of phosphorus on various timescales, from glacial-interglacial periods to recent anthropogenic influences. We have for the first time determined the atmospheric transport of phosphorus to the Arctic by means of ice core analysis [1,2].

We have determined total P,  $\text{PO}_4^{3-}$ , and dissolved reactive phosphorus (DRP) in two Greenland ice cores. One core (NEGIS) from North East Greenland covers the recent past and we have investigated changes in the atmospheric phosphorus deposition over the past 400 yrs. We find little change in absolute concentrations, but some changes related to volcanic eruptions and forest fire activity in the Anthropocene [1].

In glacial times it has been speculated that transport of phosphorus from exposed shelves would increase the ocean productivity by wash out. However whether the exposed shelf would also increase the atmospheric load to more remote places has not been investigated. Results from the NEEM ice core covering the past glacial show that glacial cold stadials had increased atmospheric total phosphorus mass loads of 70 times higher than in the past century, while DRP was only increased by a factor of 14 [2].

### References:

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## Phosphorus dynamics in a tropical forest soil restored after strip mining

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Bauxite mining is an ecotoxicologically benign form of surface mining that often occurs in forested lands on highly weathered oxisols. Approximately 500 ha of land may be cleared of forest, mined and subsequently restored annually at each mine, with leases often allowing for hundreds of square kilometres of mining and reforestation over many decades.

We carried out our study at a forested mine site in the remote Gove Peninsula on the north western fringe of the Gulf of Carpentaria in the Northern Territory of Australia. Here bauxite has been mined since the 1960's and for over quarter of a century the same forest restoration prescription was employed, allowing for informative chronosequence studies (space-for-time substitution) of ecosystem regeneration to be undertaken. The tall, biodiverse open-forest is mostly dominated by *Eucalyptus tetrodonta* and *Eucalyptus miniata* with underlying strata dominated by shrubs and perennial grasses in an almost flat landscape<sup>(1)</sup>. Soil handling practice was state-of-the-art direct-return system, where topsoil storage was unnecessary<sup>(2)</sup>.

We aimed to assess the changing P status of the restored soils from a 26 year chronosequence of sites. Specifically we assessed the distribution of P among fractions defined by the modified Hedley fractionation allowing us to understand how these fractions changed with restoration period and depth in the profile, and how they are distributed between organic and inorganic components. We considered the proportions P putatively available at short, intermediate and long-term scales.

Organic P dominated the labile and intermediate-term available fractions but the long-term available fraction was dominated by inorganic P. Concentrations of resin extractable P<sub>i</sub>, bicarbonate extractable P<sub>o</sub>, and HCl digested P<sub>i</sub> and P<sub>o</sub> fractions increased significantly with restoration age. For most fractions, clear depth trends were found with greatest values in the 0-10 cm layer and lesser values in those underlying.

The relative development of the various P fractions were strongly association with soil organic matter relationships. These, in turn, were regulated by the absolute amounts and stoichiometric balances of soil nutrients, notably N and P.

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## Phosphorus cycling within soil aggregate fractions: maize-pigeon pea as a case study

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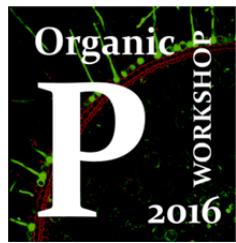
As worldwide mineral phosphorus (P) reserves continue to decline in conjunction with higher food production demands, there is a growing effort devoted to the development of innovative ways to more efficiently use P stored in the soil profile. In tropical soils, the organic P pool is of particular importance as a slow yet continuous source of P to plants. However, one of the first steps required to use this soil P effectively for crop production is to understand how P pools are stabilized and cycled within soil aggregates, as this is a key driver of soil nutrient storage and soil nutrient use capacity for plants. Compared to carbon (C) and nitrogen (N), this area remains distinctly under-studied, yet it is currently assumed that P dynamics, particularly organic P, closely follow those of C. The results of this study using highly weathered Lixisols of Malawi, in contrast, indicate that P dynamics differ distinctly from both C and N dynamics within aggregates, and hence, we suggest a new concept of P stabilization and turnover within soil aggregates. While C and N follow an “open” cycle, whereby C and N are mineralized during aggregate turnover and exit the soil system as gas and leachate, P is a relatively “closed” system, where most of the mineralized and desorbed P is lost from the plant-available pool via strong sorption to the unaggregated silt and clay-sized particles (<53 µm). This P is not returned to the soil solution as are C and N, and thus this strong P fixation process accounts for the notoriously low P-use efficiency of fertilizers as well as the large P sink of most agricultural soils. However, when comparing these P cycling patterns under two contrasting crop species, maize (*Zea mays*) and pigeon pea (*Cajanus cajan*), while the overall trend is the same, the degree and speed at which P is sorbed is strongly reduced under pigeon pea. The pigeon pea significantly increased soil aggregation and more strongly stabilized P pools, particularly organic P, within the occluded macroaggregate fractions, and thus slowed the rate of decomposition and sorption during aggregate turnover. We conclude that increasing soil aggregation can significantly reduce P mineralization and/or desorption from soil aggregate fractions and its subsequent sorption to silt and clay particles, thus reducing loss of plant-available P from cropping systems. Furthermore, significant improvements in aggregation and thus organic P storage in tropical soils can be attained by relatively simple changes in the management of crop species rotations.

**Keynote: Historical perspective on the nature and dynamics of organic phosphorus in the environment**

Leo M Condron<sup>1</sup>

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Organic forms of phosphorus play a vital role in determining the dynamics and bioavailability of phosphorus in soil, sediment and water. An extensive body of research carried out over the past 60 years has contributed to the elucidation of the nature and transformations of organic phosphorus in these diverse environments. The isolation, separation and analysis of organic phosphorus have proven to be significant challenges to unravelling organic phosphorus biogeochemistry. This presentation will provide an overview of progress that has been made to date on key aspects of organic phosphorus dynamics in different environments.



## **Abstracts: Oral Presentations**

### **Session 2: Methods of evaluating organic phosphorus stocks, concentration and speciation**

**Keynote: Methods to characterize and quantify OP in environmental samples: Past successes and future directions**

*Barbara J. Cade-Menun<sup>1</sup>*

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Although phosphorus (P) is an essential nutrient for all organisms and is a key regulator of productivity in terrestrial and aquatic ecosystem, knowledge about P biogeochemistry has lagged behind that of carbon and nitrogen due, in part, to technical limitations. Historically, research into agronomic and environmental aspects of P cycling has focused on inorganic P and P fertilizer needs. However, the important role of organic P in P cycling in many ecosystems has become more widely recognized in recent years. Research suggests that organic P forms may contribute significantly to the pool of plant-available P in some soils, while organic P transported in runoff may play a key role in the eutrophication of water bodies. In light of this, methods to accurately quantify P pools, characterize P species and track P dynamics are essential.

Methods for characterizing P, including organic P, have relied on simple extraction techniques, followed by colorimetric analysis. This includes fractionation techniques separating P into operationally defined pools of variable chemical specificity, depending on the method. However, in the last few decades there have been significant methodological advances, allowing the characterization of P forms with advanced tools such as <sup>31</sup>P nuclear magnetic resonance (P-NMR) and P K- and L<sub>2,3</sub>-edge X-ray absorption near-edge structure (PXANES) spectroscopy. These techniques have allowed the majority of P species in soil and environmental samples to be classified into general compound types, and often into specific P forms. However, no single technique is perfect, and a suite of methods is needed to fully understand P forms and their dynamics in most ecosystems. This presentation will review the techniques currently available for organic P research, discussing the strength and weakness of each method. It will also discuss areas where knowledge is still limited, and future research needs.

## A portfolio of enzymological and analytical tools for study of soil inositol hexakisphosphate transformations

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**Background:** Inositol (cyclohexanehexol) exists in 9 isomeric forms. Its phosphorylated derivatives can comprise a significant proportion of organic phosphorus in soils, but can be difficult to quantify because of complex formation with soil minerals. Among phosphorylated derivatives, *myo*-inositol hexakisphosphate, *scyllo*-inositol hexakisphosphate, *neo*-inositol hexakisphosphate and D-*chiro*-inositol hexakisphosphate were identified in soils by Cosgrove, Tate and coworkers (reviewed<sup>1</sup>). Remarkably little is known of the metabolic origins of *scyllo*-, D-*chiro*- or *neo*-inositol hexakisphosphates, though it seems plausible that they are products of ‘metabolism’, intracellular and/or extracellular, or geochemical transformation of the considerable inputs of *myo*-inositol hexakisphosphate from plant tissues and organs to soils. We noted that study of hexakisphosphate transformations would be aided by synthesis of <sup>32</sup> or <sup>33</sup>P-hexakisphosphates, by analytical tools for separation of isomers and separately by analytical tools capable of measuring and discriminating between different hexakisphosphates extracted from soils without resort to NMR.

**Methods:** Aided by description of the crystal structures of inositol phosphate kinases, we incubated *myo*-, *neo*- and D-*chiro*-inositol phosphate substrates with [ $\gamma$ -<sup>32</sup>P]ATP. Labeled and unlabeled inositol phosphates were resolved by assorted anion-exchange HPLC technologies. Additionally, *myo*-inositol hexakisphosphate, *scyllo*-inositol hexakisphosphate and D-*chiro*-inositol hexakisphosphate were incubated with a commercial E coli-derived phytase to identify products characteristic of degradation by dominant soil microbes of the HD class of Histidine Acid Phytases (HAPs).

**Results:** Strategies were established for radiolabeling of axial phosphates of *myo*-, D-*chiro*- and *neo*-inositol hexakisphosphate. Thus, syntheses of *myo*-inositol-(1,[<sup>32</sup>P]2,3,4,5,6)P<sub>6</sub>, *neo*-inositol-(1,[<sup>32</sup>P]2,3,4,[<sup>32</sup>P]5,6)P<sub>6</sub> and 1D-*chiro*-inositol-([<sup>32</sup>P]1,2,3,4,5,[<sup>32</sup>P]6)P<sub>6</sub> were achieved. Products were resolved by anion-exchange HPLC. Additional separations on other column technologies were achieved for *myo*-, *scyllo*- D-*chiro*- and *neo*-inositol hexakisphosphate and their degradation products. A method was established, on a third column technology, for simple conductimetric detection and quantification of inositol hexakisphosphate isomers in hydroxide-EDTA extracts of soil, the methods were applied to soils managed under fallow, grassland and fertilized grassland regimes<sup>2</sup>. Finally, total dephosphorylation of *myo*-, *scyllo*- D-*chiro*- and *neo*-inositol hexakisphosphate should yield *myo*-, *scyllo*- D-*chiro*- and *neo*-inositol. A pmol sensitive method for separation of these isomers of inositol was achieved with detection by pulsed amperometry.

**Conclusions:** These resources should allow facile description of interconversions of inositol hexakisphosphates in supplemented soils.

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## The chemical nature of soil organic phosphorus – evidence for complex phosphomonoesters in high molecular weight material

Timothy McLaren<sup>1,2</sup>, Ronald Smernik<sup>1</sup>, Richard Simpson<sup>3</sup>, Michael McLaughlin<sup>1,4</sup>, Therese McBeath<sup>4</sup>, Christopher Guppy<sup>5</sup> and Alan Richardson<sup>3</sup>

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Phosphorus (P) is an essential element of all living organisms and an innate constituent of soil organic matter. A large proportion of the total P in soil can exist in organic form. The major forms of organic P (Po) detected in living organisms (e.g. phospholipids, nucleic acids and inositol phosphates) are also those detected in soil. However, these typically account for <50% of the soil Po. The chemical nature of the majority of soil Po remains elusive, but has been generally been considered to be associated with high molecular weight (HMW) material.

Solution <sup>31</sup>P nuclear magnetic resonance (NMR) spectroscopy on alkaline extracts is the most widely used technique to determine the chemical nature of soil Po. However, there is some debate in the literature on how to interpret the orthophosphate and monoester region ( $\delta$  7.0 to 3.5 ppm) of the <sup>31</sup>P NMR spectra on soil extracts. We have hypothesized that this region contains a broad signal that underlies the sharp signals and, if not appropriately taken into account, will result in an overestimation of Po arising from sharp signals within this region (e.g. *myo*-inositol hexakisphosphate - *myo*-IHP). Our hypothesis is that the broad signal contains monoesters in HMW material, and that the chemical nature of this Po is distinct from that associated with the identified sharp signals.

Soil was sourced from five locations across the world, including: Australia, France, Germany, Sweden and the United States. Soil was extracted with alkali and then analyzed using solution <sup>31</sup>P NMR spectroscopy. Following NMR analysis, soil extracts were first fractionated into two molecular weight fractions using an ultrafiltration device with a 10 kDa molecular weight cut-off. The retentates (>10 kDa) and filtrates (<10 kDa) were then analyzed by NMR. The <10 kDa filtrates were then further fractionated into two molecular weight fractions using an ultrafiltration device with a 3 kDa molecular weight cut-off, and the retentates (<10 kDa and >3 kDa) and filtrates (<3 kDa) also analyzed by NMR.

There was large variation in the relative signal intensities within the orthophosphate and monoester region of <sup>31</sup>P NMR spectra between all soils. For example, the <sup>31</sup>P NMR spectra on soil extracts for France, Germany and Sweden exhibited higher signal intensities for *myo*-IHP compared to those of Australia and the United States. On average, 33% of the extractable Po was detected in the >10 kDa retentates, where the <sup>31</sup>P NMR spectra of the monoester region was dominated by a broad signal. In contrast, the <sup>31</sup>P NMR spectra of the <10 kDa filtrates were dominated by sharp signals, although there was still an underlying broad signal in this fraction. Further fractionation revealed much of the *myo*-IHP could be detected in the <10 kDa and >3 kDa retentates, whereas the <3 kDa filtrates contained both sharp and some broad signal. In conclusion, we demonstrate the existence of monoesters in HMW material that is dominated by a broad signal in <sup>31</sup>P NMR spectra. The presence and biological significance of monoesters associated with this fraction needs to be considered in relation to the cycling of soil Po in the environment.

## Revisiting organic phosphorus evaluation by the compositional analysis concept

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Phosphorus (P) forms determined by  $^{31}\text{P}$  nuclear magnetic resonance spectroscopy ( $^{31}\text{P}$ -NMR) are compositional data (i.e., parts of some whole bounded between 0 and the unit of measurement). Because compositional data are intrinsically related to each other within a closed pre-defined compositional space, a simple log transformation, variable by variable, or any other transformation of the compositional variables may produce statistically erroneous results. However, most studies analyze P forms as single components rather than parts of some whole such as total P (TP) or soil dry mass, leading systematically to methodological biases and conflicting interpretations. Compositional data analysis using centred log-ratio (*clr*) or isometric log-ratio (*ilr*) coordinates avoids such difficulties and preserves sub-compositional coherence in the analysis. The objective of this study was to compare classical and compositional methods for the statistical analysis of  $^{31}\text{P}$ -NMR P data expressed as proportions of TP or concentrations relative to soil dry mass. Two published datasets were used. Analyses of variance and regression analysis with soil pH were conducted on P species percentages scaled on TP or as untransformed concentrations scaled on a soil dry-weight basis as well as their ordinary log, centred log-ratios (*clr*) and isometric log-ratios (*ilr*). Contradictory F-statistics values and coefficients of correlation with soil pH were obtained for the untransformed and ordinary log transformed  $^{31}\text{P}$ -NMR P data expressed as proportions or concentrations. In contrast, statistical results were the same regardless of the measurement unit when P compound percentages were *clr*-transformed. Using orthogonal *ilr* coordinates,  $^{31}\text{P}$ -NMR P data were correlated to soil properties and to each other and synthesized into a multivariate distance without methodological bias. We conclude that the variance and regression analyses of molecular P species are scale-dependent and that the *clr*- and the *ilr*-transformations should be used to unbiasedly analyze the P fractions and avoid conflicting interpretations.

## Phytate degradation by different phosphatase enzymes: Contrasting kinetics, isomer compositions, decay rates, pathways, and isotope effects

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Phytate ( $IP_6$ ) is often the most common organic phosphorus compound in soils and sediments. Understanding the fate of inositol phosphate ( $IP_x$ ) isomers in the environment in terms of their composition and concentration and assessing their relative resistance (or preference) against degradation is essential to estimate their potential role on resupplying inorganic P ( $P_i$ ) and impacting water quality. Furthermore, distinction made among the suites  $IP_x$  compounds produced and degradation pathways generated allows potential identification of active phosphohydrolase enzymes in the environment. We sought to identify distinction among the suites  $IP_x$  compounds produced and degradation pathways generated by different enzymes. Particularly, we analyzed  $IP_6$  degradation by four different phosphohydrolase enzymes (phytase from wheat and *Aspergillus niger* and acid phosphatase from wheat germ and potato) with particular focus on degradation pathways, isomer kinetic decay rate, and isotope effect during degradation using a combination of HPIC, NMR, stable isotopes, and process-based modeling techniques. Our results show that all enzymes generate largely distinct sets of isomers and have both major and minor degradation pathways. The process-based model and Bayesian inverse modeling allowed to determine the decay kinetics parameters of phytate and the generated isomers and well captured the trend and magnitude of the measured concentrations for each  $IP_x$  isomer. Furthermore, oxygen isotope ratios ( $\delta^{18}O_P$ ) of released  $P_i$  enabled to identify isotopically identical phosphate moieties in phytate. We conclude that distinctly different fractionation factors, degradation pathways, and kinetic decay rate coefficients among enzymes studied could lead to potential discrimination of phytate sources and the presence of active enzymes in the environment.

## Application of high resolution mass spectrometry to high the characterisation of organic phosphorus in environmental matrices

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Cycling of phosphorus (P) in the environment plays a fundamental role in agricultural production and the health of freshwater ecosystems. However, the contribution of organic phosphorus ( $P_o$ ) species to biological processes, particularly eutrophication, is not fully understood. Characterisation of  $P_o$  in water quality testing is based on an operational definition<sup>1</sup>. This is sufficient for studying bulk chemistry processes, but provides limited insights into the role of organic phosphorus in the environment.

Attempts to characterise  $P_o$  in more detail have mostly focused on the use of nuclear magnetic resonance (NMR) spectroscopy<sup>2, 3</sup>. NMR can identify classes of  $P_o$  compounds, for example phosphate monoesters, or diesters, however, potentially many tens or hundreds of compounds can contribute to a given signal in an NMR spectrum, since individual molecular species of  $P_o$  cannot be resolved. In contrast, high resolution mass spectrometry (HRMS) has become a powerful tool in the analysis of complex biological samples and is being widely used in the field of metabolomics<sup>4</sup>. Thousands of compounds can be resolved simultaneously with the capability being extended still further by combination with liquid chromatography (LC) to provide enhanced characterisations and quantitative determinations.

This paper presents results to date from the development and application of electrospray-HRMS and LC/HRMS techniques to the determination of  $P_o$  from complex environmental matrices, including: agricultural manures, i.e. pig manure, cow manure, and poultry litter, wastewater effluents and river water extracts. We aim to demonstrate how molecular characterisation of  $P_o$  opens up the possibility of studying the transformation and transport processes involving  $P_o$  in the environment to better understand the role  $P_o$  plays in the P cycle, in particular the eutrophication of freshwater ecosystems.

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## Nanomaterials as a promising binding support to hydrolyse soil phytate

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Nanotechnology offers new avenues in terms of plant nutrition from soil phosphorus (P) legacy. Already used in medicine or industry, mesoporous nanomaterials are promising because they efficiently protect enzyme activities in adverse conditions by immobilization in their porous network<sup>1</sup>. Synthesis of nanomaterials can be fine-tuned in order to choose size-dependent and pore system qualities, appropriate surface-to-volume ratio respective to the aims of the enzyme application. In the present study, we hypothesized that mesoporous nanomaterials loaded with a phytase from *Aspergillus niger* can hydrolyse organic P from soil phytate.

Thus, we first evaluated the efficiency of functionalization techniques of mesoporous nanomaterials (impregnation, grafting) by estimating their loading capacity and their catalytic activities. In this work we used two mesoporous materials, MCM-41 as a control because size pore is lower than median diameter of phytase, and Kit-6 as the putative nanomaterial. Both MCM 41 and Kit-6 were functionalized by impregnation and grafting. We characterized the materials by XRD, nitrogen volumetry and microscopy methods. We evaluated the loading of the enzyme by proteolytic treatment with trypsin. Afterwards, we evaluated catalytic activities of the functionalized nanomaterials by comparing it with the free enzyme for its pH (pH 3 to 8) and temperature (5-40°C) dependence, without plants. Finally, we estimated the efficiency of functionnalized mesoporous nanomaterials in presence of *Medicago truncatula* grown on sterile sand with phytate as the sole source of P.

Loading of enzyme in the Kit-6 is very efficient and was evaluated to more than 80% of initial amount. The trypsin enzyme exhibited a smaller diameter than nanomaterial pores allowing hydrolysis of phytase adsorbed onto but also inside the nanomaterial. After 30 min contact, a remaining phytase activity was evaluated to be one third of the initial activity suggesting that adsorbed phytase is concealed deep within the pores and then protected from proteolytic effects. Considering the normal range of pH and temperature encountered in agricultural soils in Europe, immobilization did not affect the optimal pH and temperature of the enzyme. However, the activity of functionalized nanomaterials became limited at the lower (pH 3.2) and upper (pH 8.7) ends of the pH range investigated in contrast with the free phytase behavior. After exposure at 55°C, the free enzyme was not stable and its activity dramatically dropped after 5 min while the immobilized enzyme remained mostly constant after 30 min (Kit-6). The amount of phosphorus accumulated in plant is about 10 µmoles per plant, 2.5 times higher than the control without Kit-6 nanomaterials. Statistical differences were noticed for both roots and shoots. If we consider the lowest quantity possibly absorbed, this accumulation in plants accounts for the net decrease in Pi concentration in the soil solution. Our results showed therefore that nanomaterials made it possible to preserve phytase activities and should be further explored to increase soil organic P recycling for sustainable agriculture.

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## Novel primers target group A and C bacterial non-specific acid phosphatase genes

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Molecular studies aiming to examine the community structure of microorganisms involved in environmental phosphorus cycling has been limited. To the best of our knowledge, the only molecular analysis of non-specific acid phosphatase (NSAP) genes determined near full-length gene sequences for specific bacterial isolates identified in relation to the bioremediation of uranium (VI)<sup>[1]</sup>. The lack of sequence conservation among NSAP classes have proved difficult for molecular studies aiming at the design of primers for the general quantification of NSAP gene targets. Thus the goal of the current study was to develop novel primers to target short segments of NSAP genes from three bacterial classes (Fig. 1).

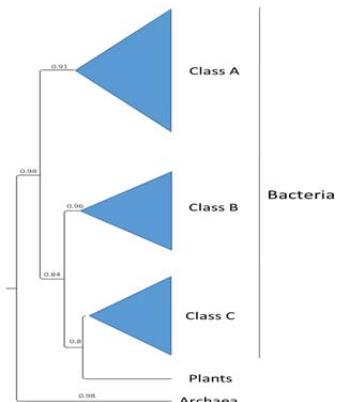
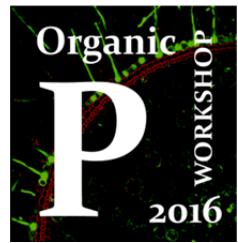


Fig. 1. Comparison of bacterial non-specific acid phosphatase protein sequences reveal three phylogenetic classes. Protein coding sequences were aligned and primers targeting active sites for each class (Class A acid phosphatase, Class B acid phosphatase and Class C acid phosphatase) were identified using CEMAsuite<sup>[2]</sup>. Archaeal phosphoesterases and plant (*Arabidopsis*) acid phosphatase sequences were included as outliers. Bootstrap values are provided.

Primer selection was supported by *in silico* hybridization results to target known sequences in the Genbank NCBI database, and tested for specificity through end-point PCR with genomic DNA. PCR products amplified from soil metagenomic DNA were sequenced, revealing unique sequences from Class A and C that grouped phylogenetically with known NSAP genes from their respective classes. No Class B NSAPs were identified from the Canadian soils tested. We are currently using primers to reveal NSAP gene abundance (DNA) and gene expression (RNA) in a 22-year old long-term ecology trial established at Lincoln University, New Zealand. In April 2016, soil (ca. 2g) was sampled and placed immediately into LifeGuard™ Soil Preservation Solution (MO BIO Laboratories, Inc. Carlsbad, CA) to stabilize nucleic acids *in situ* for transportation to the laboratory at the University of Guelph, Canada. Preliminary analysis revealed that soils contained over 7 log Class A NSAP genes per g dry soil, and that gene expression was confirmed through quantification of Class A NSAP transcripts. By developing these primers we hope to provide additional tools for researchers to study the bacterial-plant-rhizosphere interactions involved in the soil phosphorus cycle.

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## Abstracts: Oral Presentations

### Session 3: Biotic Interactions in organic phosphorus cycling - Plants

**Keynote: How are organic carbon and phosphorus mineralization connected in the rhizosphere?**

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The rhizosphere is an important hotpot of organic phosphorus (P) and organic carbon (C) mineralization in soil. Due to high inputs of organic C into soil in the form of root exudates, a large number of microorganisms are active in the rhizosphere. Both plants and microorganisms mineralize organic P in the rhizosphere and their relationship with respect to P acquisition can be mutualistic as well as competitive. In this talk, I will show recent results gained by isotope analysis and in situ enzyme measurements, giving new insights into the factors controlling organic C and P mineralization in the rhizosphere.

## Keynote: Plant-fungus interactions with apatite: ideas versus observations of mycorrhizal fungi-induced apatite weathering

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The mineral apatite is the primary source of P in most ecosystems. Mineral dissolution ('weathering') releases P (and Ca) into the soil solution. Although its dissolution rate is generally much higher than other minerals like quartz and feldspars, it still takes thousands of years to completely dissolve under normal soil conditions. As P is a major plant nutrient, and often limiting plant growth, it is not unlikely that during plant evolution mechanisms have been evolved to enhance the apatite dissolution rate. One proposed mechanism is via a mutualistic cooperation with ectomycorrhizal fungi. The plant delivers photosynthates to the fungus. The fungus directs that energy towards apatite grains in the soil, and, by the production of fungal weathering agents like protons, oxalate and citrate, enhances the dissolution of apatite. P is taken up efficiently, and returned to the host tree.

Several pot and microcosm experiments indeed demonstrate the ability of mycorrhizal fungi to (1) direct plant energy towards patches with apatite, (2) enhance the dissolution rate of apatite and (3) take up P from the apatite and deliver it to the plant. Also field data suggest a direct link between certain ectomycorrhizal trees and apatite. Finally, the simulation of this weathering mechanism in a global scale model environment supports the quantitative importance of direct mycorrhizal fungi-mineral interactions on total weathering rates<sup>1</sup>.

So far the idea of mycorrhizal weathering appears as a well established theory, but a critical review of above mentioned studies leaves only limited evidence, while (in spite of the modelling work<sup>2</sup>) mechanistic understanding is lacking. This is partly due to the experimental design more focused on plant nutrition than mineral weathering, and partly due the ignorance of (other) soil scale processes. For instance the weathering rate has been measured only in one laboratory study, and any detail on the dissolution chemistry is lacking in all studies. The latter is essential in order to reliably up-scale laboratory scale results to soil conditions.

Soil chemistry data only leave a minor role for other weathering agents than protons, unless these weathering agents stay within a micrometer range from the mineral surface. Indeed the modelling work mentioned above confirms the dominant role of protons in mineral dissolution. Their proposed important role for ectomycorrhizal fungi is based on the assumption that all proton exchange between plants and soil takes place at certain mineral surfaces. This totally ignores the well established knowledge that most protons are exuded in exchange for base cations which are dominantly recycled from organic matter and not from mineral surfaces.

Altogether evidence for mycorrhizal enhanced apatite weathering is weak. Chemical data suggest a dominant role for proton mediated weathering, which is driven by plant cation uptake and organic matter degradation. This is supported by recent field data<sup>2</sup>. This study also indicates a more direct (inhibiting) interaction between organic matter and mineral weathering.

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## Utilization of sparingly available P by the family Cyperaceae grown in low fertility soils in Western Japan.

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The flora in the poor-nutrient soils such as Regosol, broadly distributed in Western Japan, is highly diverse and frequently contains the family Cyperaceae. It is known that part of Cyperaceae forms a unique root structure so-called ‘dauciform roots’ (DR) under low P conditions. Morphologically, DR is consisted with dense numbers of long root hairs in short lateral roots<sup>1</sup>. It has been also shown that DR has a high ability to release root exudates such as carboxylate and phosphatase to mobilize the sparingly available P in soils<sup>2</sup>. The information of DR-forming plant species in Northern hemisphere is quite less than in Southern hemisphere, although the high diversity of the family Cyperaceae in Northern hemisphere. The aim of this study is to investigate the DR-forming species among Cyperaceae plants grown in Western Japan, and to analyze the contribution of DR in natural environments to mobilize P from sparingly available forms, such as organic P and sparingly soluble P.

Cyperaceae plants were sampled from wide range of Western Japan. DR was found from 13 of 20 species belonging to 6 of 7 genera (*Carex*, *Fimbristylis*, *Kyllinga*, *Rhynchospora*, *Schoenus*, and *Scirpus*) in Cyperaceae. Our confirmation of DR is the first discovery in genera *Fimbristylis*, *Kyllinga*, *Rhynchospora*, and *Scirpus*. Our result suggests that DR formation under low P availability is more general trait in the family Cyperaceae, and not specific in Southern hemisphere-species.

Since *Carex lenta* was found in several sites in our field, this plant was selected to investigate the contribution of DR to mobilize P from sparingly available forms. The density of DR in main roots and total P in soil were negatively correlated. DR formation was predominant in low P-availability soils (less than 3 mg-P/kg [Olsen-P]). Hedley’s sequential P fractionation revealed that the soluble organic P pool was high in the rhizosphere soil with a high number of DR than in bulk soil. Sparingly soluble inorganic P pools were less in the rhizosphere. We concluded that the DR in *C. lenta* increased by P deficiency and contributed to mobilize P from sparingly available forms.

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## A tale of two exudates: The role of citrate and phytase in unlocking the potential of soil organic phosphorus for plant nutrition

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The majority of soil phosphorus (P) occurs in sparingly soluble forms, which include organic moieties, with only a small proportion of the total soil P being directly available to plants. Plants respond to P deficiency through physiological and biochemical adaptations, which allow for the efficient exploration and extraction of P, for example, by root morphological changes or the production of organic anions and phosphatases. Recent conceptual models<sup>[1]</sup> and experimental work<sup>[2]</sup> suggest that the combination of citrate and phytase in the rhizosphere could expand the sources of P available to plants. We hypothesize that citrate and phytase exudation must co-occur in the rhizosphere to mobilize and mineralize organic P in the form of phytate for plant use. This hypothesis was explored using transgenic tobacco (*Nicotiana tabacum*) plant lines, whereby plants with contrasting exudation of citrate and phytase were grown alone or in combination to evaluate the effect of these traits on P acquisition and changes in soil P composition. Growth experiments in pots using a low-P soil with a proportionately high organic P content, showed that the exudation of citrate and phytase, either from the same tobacco plant or from plants with single traits grown in combination, resulted in a significant improvement in shoot P content and dry weight, albeit with restricted plant growth overall due to P limitation. This was associated with a depletion of citrate-extractable and phytase-labile soil P<sup>[2]</sup> but only occurred when the roots of intercropped citrate- and phytase-exuding plants were allowed to intermingle. Further work will evaluate the composition and function of microbiota in the rhizosphere of citrate- and phytase-exuding tobacco. Key functional traits such as bacterial phosphatase abundance and associated transformation of P will be assessed in rhizosphere soils (e.g., as measured by <sup>31</sup>P NMR), and 2D spatial mapping studies will be used to identify ‘hot-spots’ of soil microbial activity and P mobilization, which contribute to the utilization of organic P by plants.

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## Understanding soil phosphorus dynamics at increasing citric and oxalic acid doses

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The root exudation of organic acids has been postulated as a major plant and microbial mechanism for mobilizing soil phosphorus (P), nevertheless major gaps of knowledge still exists in understanding their behaviour and impact in rhizosphere P dynamics. We have used an integrated approach to study the mobility and resupply of inorganic P from soil solid phase as well as the microbial biomass P and respiration at increasing doses of citric and oxalic acid in 2 soils with contrasting agronomic P status. Citric or oxalic acids significantly increased soil solution P concentrations for doses over 1 mMol kg<sup>-1</sup>. However, low organic acid doses (0.5-1.5 mMol kg<sup>-1</sup>) were associated with a steep increase in microbial biomass P, which was not seen for doses over 2 mMol kg<sup>-1</sup>. In both soils, treatment with the tribasic citric acid led to a greater increase in soil solution P than the dibasic oxalic acid, likely due to the rapid degrading of oxalic acids in soils. Organic phosphorus was only significantly mobilized by citric or oxalic acids in the low P soil, possibly due to the lower lability of organic P and large concentration of inorganic P in the high P soil. After equilibration of soils with citric or oxalic acids, the adsorbed-to-solution distribution coefficient ( $K_d$ ) and desorption rate constants ( $K_{-1}$ ) decreased whereas an increase in the response time of solution P equilibration ( $T_c$ ) was observed. The extent of this effect was shown to be both soil and organic acid specific. Because the kinetics of P interchange between the soil matrix and the soil solution slowed with increasing organic acid doses, their net effect on P bioavailability is therefore expected to be much lower than the observed increase in solution concentration.

Our study suggests that low organic acid concentrations in soils (<1 mg kg<sup>-1</sup> soil) will have a limited direct effect on plant available P concentrations in soils, on the other hand, low doses of compounds such as citric and oxalic acid may have a strong effect on the microbial activity and the accumulation of microbial biomass P. These results support the hypothesis that low rates of exudation of citric and oxalic acid are not related to a P mobilization mechanism, but to a microbial stimulation in the rhizosphere, which may have many indirect implications on nutrient uptake by plants (positive or negative). Considering our case studies, only conditions of high organic acid exudation (e.g. proteoid roots of lupins) could be related to a direct P mobilizing mechanism.

## Can intercropping improve organic phosphorus use efficiency in agriculture?

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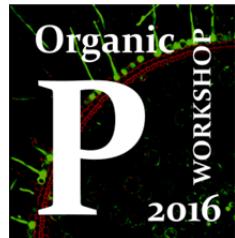
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Uncertainty over the future supply of rock phosphate means that we need to develop cropping systems that utilise phosphorus (P) more efficiently and rely less on phosphate fertilizer inputs. One method is to select crops that can access the ‘bank’ of soil P, which is commonly considered unavailable to plants. Plant exudation of carboxylates can solubilise P bound to soil, while phosphatase exudates can hydrolyse organic P, and both these responses can become upregulated under P deficient conditions. However, plants differ in their capacity to exude these compounds, and many modern crop breeds have been produced to respond to phosphate fertilizers, rather than utilising soil P. By intercropping plants with differing exudate properties, it may be possible to improve P use efficiency in crops.

After screening plants for citrate and phytase exudation, four barley cultivars and three legume species (*M. truncatula*, *O. sativus* and *T. subterraneum*) were selected for plant growth trials, on the basis that they represented a range of exudation properties. Plants were grown in a Scottish grassland soil (a freely drained podzol) selected for its low phosphate content (1.1 mg P kg<sup>-1</sup> phosphate in water extracts, 6.8 mg P kg<sup>-1</sup> Olsen P). The soil received either no phosphate addition, at a rate equivalent to 32 kg P ha<sup>-1</sup> to produce a P limited soil, or at a rate equivalent to 130 kg P ha<sup>-1</sup> to produce a P sufficient soil. At each P level, plants were grown as monocultures and as either barley-barley or barley-legume combinations for six weeks.

Intercropping of barley and legumes generally had a positive effect overall on the plant uptake of P. Barley benefitted most from being intercropped, with greater shoot P uptake at the intermediate and high P levels compared to the monoculture (range 7 to 124%). Conversely, at these P levels the legumes often had a lower shoot P than if they had been grown in a monoculture (range 59% decrease to 17% increase). In the no added P soils results were more variable (48% decrease to 73% increase in barley plants, 100% decrease to 167% increase in legumes), and this may be related to exudation properties under P stress. The barley-barley intercropping generally showed no significant differences compared to the monocultures. The similarity of the intercropped barley cultivars, for example in their root architecture, could explain this effect in that they were competing for similar niche spaces and resources in the soil.

Future work is required to determine whether the positive interactions seen for barley-legume intercropping translate to the field. However, indications are that intercropping shows promise as a method of better utilising poorly available soil organic phosphorus.



## Abstracts: Oral Presentations

### Session 4: Biotic Interactions in organic phosphorus cycling - Microbes

## Keynote: Insights into the biological cycle of P (with a little help from oxygen isotopes)

Federica Tamburini<sup>1</sup>, Chiara Pistocchi<sup>1</sup>, Éva Mészáros<sup>1</sup>, Emmanuel Frossard<sup>1</sup>

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Oxygen isotopes in phosphate ( $\delta^{18}\text{O-P}$ ) have become a useful tool in studying biological processes actively controlling the P cycle in the soil/plant system. Indeed, abiotic processes can change the isotopic signature of phosphate only in a limited measure. Two kind of enzymatic processes are responsible for changing the  $\delta^{18}\text{O-P}$  of phosphate pools in the soil. Over time, inorganic pyrophosphatase, an ubiquitous enzyme present in all living cells, promotes the complete exchange of the oxygen atoms of the phosphate with oxygen from the water<sup>1</sup>. This exchange favours an equilibrium fractionation, which is dependent on temperature. On the other hand, mono- and diesterases, such as acid<sup>2</sup> and alkaline phosphatase<sup>3</sup>, phytase<sup>4</sup> to cite few hydrolysing enzymes, are responsible for kinetic fractionation: the phosphate molecule is released from organic compounds with the incorporation of oxygen from the water plus a fractionation factor, which is related to the enzyme at work<sup>1</sup>.

Bearing the theory in mind, several studies have tried to understand the role of biological processes on the P cycle. Microbial biomass is key in controlling the available P pool in soils, as it was shown in several systems<sup>5</sup> and intracellular processes are able to erase the isotopic signature of the source phosphate. However, the importance of organic P compounds in replenishing the available phosphate pool in soils is still to be proven. The  $\delta^{18}\text{O-P}$  in pristine organic P compounds is an essential datum if one wants to determine the contribution of organic P hydrolysis, but it is still missing. There is no viable protocol to purify phosphate from organic compounds for oxygen isotopic analysis<sup>6</sup>.

In this talk, we will present the state of the art in oxygen isotope studies applied to P cycling in the soil/plant system together with an overview on the developments made in the analytical part concerning the determination of the  $\delta^{18}\text{O-P}$  of the organic compounds.

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## Keynote: How mycorrhizal association may effects phosphorus cycling in forest soils

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Phosphorus (P) is an essential nutrient for all living organisms. However, acquiring P from soil is challenging for plants and microorganisms alike because of the low availability and mobility of P in soil. Organisms that acquire P from soil have evolved different strategies to do so depending on the degree of P limitation and the form of P in soil. Many of these strategies involve forming symbiotic associations between plants and mycorrhizal fungi. In this presentation I want to explore how functional differences between dominant mycorrhizal types may affect soil P cycling. In the mycorrhizal association plants provide photosynthetically derived carbon to their root associated fungi and among other things obtain P in return. Except for conditions of extreme P limitation, most terrestrial plants obtain their P predominantly through such fungal pathways associating predominantly with either of the two main groups of mycorrhizal fungi arbuscular mycorrhizal (AM) or ectomycorrhizal (ECM). The two types are evolutionary, morphologically and functionally distinct and while both benefit plant growth they differentially affect the soil system that they are part of.

The mycorrhizal association relieve root symbiotic fungi from the carbon limitation experienced by most soil dwelling microorganisms. This allows them to efficiently forage for nutrients in soil. Fungal strategies for P uptake involve increasing P availability, by altering soil conditions and by exudation of organic weathering agents and enzymes, as well as increased acquisition efficiency. These mechanisms satisfy the P demand of the fungi and its associated plants. While both AM and ECM fungi increase acquisition by efficient soil colonization and high affinity P transporters, ECM fungi are far more efficient in increasing P availability, in particularity through enzymatic activity. Under P limiting growth conditions plants forming both AM and ECM are known to increase their carbon allocation to roots and associated fungi. Reciprocal reward in the carbon to P exchange at the plant fungal interface is thought to be an important mechanism in stabilizing the symbiotic interactions, in particular in AM associations. However, providing just enough P to plants to maintain a high carbon flow may be the most beneficial strategy for the fungi.

In forest ecosystems trees are often thought of as drivers of nutrient cycling. However mounting evidence supports the alternative view that soil microbial biomass has larger P demands and higher cycling rates than trees and thus driver P cycling in forest ecosystems. In a recent study from a temperate hardwood forest in southern Indiana, US, we compared stands dominated by trees forming predominantly either AM or ECM. We found the same annual average microbial biomass P in both stand types and that this pool was two – three times larger than the estimated annual P demand of the canopy. Fungal biomass was significantly higher in ECM dominated plots and fungal communities were different between AM and ECM plots. These differences were accompanied by significant differences in soil P pools, with higher availability of organic P in ECM dominated plots compared to AM plots with larger recalcitrant organic P pools. Interestingly organic P accumulated in both ECM and AM plots over the growing season, resulting in increasingly P-limited microbial biomass. These results demonstrates that while the size of the soil microbial biomass was the same between AM and ECM plots, its functional composition was linked to different phosphorus speciation in these plots and the carbon to nutritional balance of these communities drives seasonal P pool dynamics.

## Phosphate-insensitive organophosphonate cycling by marine bacteria

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Phosphorus is often the limiting nutrient for planktonic growth in marine systems<sup>1</sup>, and as such marine microbes have developed many strategies for coping with phosphorus starvation. In particular, the hydrolysis of organophosphates is a common occurrence<sup>2</sup>. However, an additional pool of organic phosphorus is the organophosphonates, reduced phosphorus compounds which are commonly synthesised by diverse marine organisms<sup>3</sup>, make up ~25% of the marine high molecular weight dissolved organic phosphorus pool<sup>4</sup> and are characterised by a direct carbon-phosphorus bond which requires a dedicated C-P bond cleavage enzyme to hydrolyse<sup>3</sup>. Traditionally these enzymes have been shown to be under the control of the phosphorus-starvation induced *pho* regulon, a set of genes for coping with phosphate starvation and which is repressed by environmental phosphate<sup>5</sup>. This prevents these phosphonates from being degraded as anything other than a phosphorus source by phosphorus starved microbes. In areas of the oceans which are not phosphorus starved these phosphonates are considered to be recalcitrant, and presumed to lead to the compound being trapped in ocean sediments and removed from biological nutrient cycles.

Here we show that some of the enzymes which hydrolyse the most common phosphonate in the ocean, 2-aminoethylphosphonate (2AEP), can be under alternative forms of gene control which allow bacteria to digest 2AEP regardless of phosphorus starvation. This allows these microbes to degrade 2AEP for the carbon and/or nitrogen in the molecule, returning the phosphorus and other nutrients to actively cycled biological nutrient pools. This research is paralleled by studies which have suggested that organic phosphate degradation in the oceans is also not always controlled by phosphate starvation, and so active organic phosphorus cycling in the oceans may occur well beyond the traditionally considered phosphorus-starved zones and may be important for returning nitrogen and carbon to marine biological nutrient cycles.

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## Effect of phosphorus addition on the total bacterial communities and alkaline phosphomonoesterase-harboring bacterial populations in the rhizosphere of ryegrass

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Rhizobacterial communities may play a crucial role in phosphorus (P) nutrition of plants [1]. P is an essential macronutrient and is frequently abundant in soils; however, bioavailability of P may be limited in soil for a variety of reasons [2]. Several studies have shown that addition of P modulated the bacterial composition in rhizospheres [3]. Microorganisms can access and recycle P by reducing pH via production of organic acids and releasing enzymes such as phosphomonoesterases, which hydrolyze the orthophosphate group from organic compounds [4]. However, our knowledge of how P fertilization modulates rhizobacterial communities in crops and pastures is still poor. Here we investigated the effect of P addition (phosphate [PHO] and phytate [PHY]) on total bacterial communities and alkaline phosphomonoesterases (APase) gene-harboring bacterial populations in the rhizosphere microsites (root tip [RT] and mature zone [MZ]) of *Lolium perenne*. Sizes and diversities of bacterial communities were studied by 454-pyrosequencing of 16S rRNA genes, denaturing gradient gel electrophoresis (DGGE) and quantitative PCR (qPCR). Our results suggested that P addition induces significant changes in the rhizobacterial community composition. Despite that pyrosequence analysis showed that members of the *Proteobacteria*, *Actinobacteria*, *Chloroflexi* and *Acidobacteria* were the dominant phyla in all sampled rhizosphere microsites, differences in the relative abundances of some bacterial genera were detected (e.g. *Arthrobacter* and *Acidothermus*). Our results also revealed a higher richness in rhizosphere microsites of plants supplied with PHY compared with PHO. With respect to APase-harboring bacterial populations, DGGE (*phoD* gene) showed significant differences between rhizospheres supplied with PHO, PHY and controls. qPCR (16S rRNA genes, *phoD* and *phoX*) showed significantly higher abundances of bacteria and APase genes in RT than in MZ microsites. This study contributes to our understanding of the effect P fertilization on rhizobacterial community compositions of pastures grown in Chilean Andisols.

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## Carbon:phosphorus ratio drive bacterium mediated phytate mineralization and competition for phosphorus among plant-AM fungi and bacteria association

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We focus on the three-partner relationship among plants, arbuscular mycorrhizal (AM) fungi and hyphosphere bacteria to ask if the interaction between AM fungi and bacteria can pay back the resource (phosphorus here) to host plants by consuming the plants derived carbon (C). Microcosm experiments which separate the plant roots and mycorrhizal hyphae and bacteria were conducted to demonstrate the direct effects of root or hyphal exudates on the growth and activity of phosphorus solubilizing bacteria in organic phosphorus (P) mineralization and the direct effects of bacteria on the growth and activity of the AM fungus in P uptake which were transferred to plants. The root or AM fungal hyphae was both supplied with or without 100 mg P kg<sup>-1</sup> phytate and inoculated with or without P solubilizing bacteria (PSB). 5 mg P kg<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub> was added or not to root or hyphae to reduce C:P ratio in rhizosphere or hyphosphere soil. The plant was also labelled with <sup>13</sup>CO<sub>2</sub> and <sup>13</sup>C -DNA stable isotope probing was used to track the C flow through plant root to hyphae and to hyphosphere bacteria community. Results showed that (1) PSB competed for P that was mineralized from phytate with plants and AM fungal hyphae in low available P soil. Reducing of C:P ratio by adding small amount of inorganic P in the rhizosphere or hyphosphere promoted phytate mineralization and shifted the PSB effects from negative to positive. (2) AM fungus released substantial C to the environment, triggering bacterial growth and stimulating their activity on organic P mineralization and turnover. (3) *Pseudomonas alcaligenes* which has been previously identified to mineralize phytate was labeled with <sup>13</sup>C derived from maize photosynthates via extraradical mycelium of *R.irregularis*, indicating the PSB strain used hyphae exudates. Our results suggest that AM fungi and bacteria share photosynthates of plant, and as reciprocation, the AM fungi-bacteria interaction repays the plant with P by jointly mobilizing soil organic P.

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## Soil *phoD* and *phoX* alkaline phosphatase gene composition responds to multiple environmental factors

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Alkaline phosphatases such as PhoD and PhoX are key players in organic phosphorus cycling in soil. We identified the key organisms harboring the *phoD* and *phoX* gene in soil and explored the relationships between environmental factors and the *phoD*- and *phoX*-harboring community structures across three land-uses located in arid to temperate climates on two continents using 454-sequencing. *phoD* was investigated using the primers published by Ragot et al. [1] and new primers were designed to study *phoX* in soil.

*phoD* was found in 1 archaeal, 13 bacterial and 2 fungal phyla, and *phoX* in 1 archaeal and 16 bacterial phyla. Dominant *phoD*-harboring phyla were *Actinobacteria*, *Cyanobacteria*, *Deinococcus-Thermus*, *Firmicutes*, *Gemmatimonadetes*, *Planctomycetes* and *Proteobacteria*, while abundant *phoX*-harboring phyla were *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Planctomycetes*, *Proteobacteria* and *Verrucomicrobia*. The *phoD*-harboring community was always composed of the same dominant phyla, suggesting that the *phoD*-harboring community composition is rather stable across various environmental factors. In contrast, dominant *phoX*-harboring phyla varied significantly between soils. Additionally, our results suggest that concentrations of soil organic phosphorus followed by climate, soil group, land-use and other soil nutrient concentrations are common environmental drivers shaping biogeographical patterns of the *phoD*- and *phoX*-harboring community structure and composition. The *phoX*-harboring community structure was also affected by pH.

Our results showed that key *phoD*- and *phoX*-harboring microorganisms occur partly in different phyla. Additionally, our study shows that the composition of *phoD* and *phoX* is mostly governed by the same environmental drivers. This is the first study identifying the dominant *phoD*- and *phoX*-harboring phyla in soil.

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## Utilisation of soil organic phosphorus in response to long-term phosphorus depletion

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Most studies that have attempted to assess and quantify the relative bioavailability and utilisation of soil phosphorus have involved short-term glasshouse or field experiments [1]. In 1994 a field trial was established at Lincoln University in New Zealand to investigate and quantify the long-term effects of grassland biomass management regimen on plant and soil properties and processes. The trial included undisturbed plots (unmown) together with treatments that either retained biomass (mown 4-5 times annually with clippings returned) or removed biomass (mown 4-5 times annually with clippings removed) [2, 3]. The biomass retained and removed regimen represented sustainable and depletive systems, respectively. In 2015 above ground plant biomass and topsoil (0-2.5cm) samples were taken to determine the cumulative impact of 21 years of biomass and phosphorus removal on the nature and relative bioavailability of phosphorus. Results presented in the table below showed that as expected long-term biomass removal significantly reduced plant phosphorus uptake after 21 years, together with significant depletion of topsoil total phosphorus (13%). Phosphorus fractionation analysis revealed that continued biomass removal resulted in substantial and significant decreases in bicarbonate (48%) and hydroxide extractable (24%) soil inorganic phosphorus pools. On the other hand, while bicarbonate extractable soil organic phosphorus was significantly depleted by biomass removal (22%), the much larger hydroxide extractable organic phosphorus pool remained unchanged. The findings of this study demonstrated that limited mineralization of soil organic phosphorus occurred in response to long-term biomass and phosphorus removal, and highlight the need to investigate biological means of improving the utilization of organic phosphorus in soil-plant systems.

	Biomass Retained	Biomass Removed
<b>plant biomass P (g/m<sup>2</sup>)</b>	0.59	0.22*
<b>total soil P (mg/kg)</b>	946	823*
<b>bicarbonate soil inorganic P (mg/kg)</b>	75	39*
<b>hydroxide soil inorganic P (mg/kg)</b>	189	143*
<b>bicarbonate soil organic P (mg/kg)</b>	63	48*
<b>hydroxide soil organic P (mg/kg)</b>	302	287 <sup>ns</sup>

<sup>ns</sup> = no significant difference between biomass treatments  
\* = significant difference between biomass treatments ( $p < 0.05$ )

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## Phosphate solubilising bacteria: Utilizing soil organic and inorganic phosphate resource for sustainable agriculture

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Only about 5-30% of phosphorous (P) fertilizer is utilized by plants. The remainder complexes with metal ions and accumulates as unusable deposits. Soils can accumulate P at 5-15 kg ha<sup>-1</sup> yr<sup>-1</sup> and many agricultural soils have sufficient P deposits to last 100 years. These deposits are mainly inorganic P salts that are converted to organic forms due to biological activities.

Rhizosphere bacteria can make both organic and inorganic P reserves available by phosphate solubilisation (PS). Bacteria are known to release organic acids, phytases and phosphatases to release bound P for plant uptake. Many studies have screened phosphate solubilising bacteria (PSB) *in vitro*, but most studies screen bacteria using inorganic forms of P only and very few studies have been proven *in planta*. This means there are currently no large scale bacterial commercial products for PS and potential bacterial inoculants mostly target inorganic P only. The objective of this study was to optimize the screening process to target both, organic and inorganic P deposits and study the effectivity of PSB on legume growth.

A total of 1440 nodule inhabiting bacteria were collected from a long term P fertilizer trial site with white (*Trifolium repens*) and subterranean (*T. subterraneum*) clovers were used as bait plants. Isolates were screened for *in vitro* PS using two inorganic P sources, tri-calcium phosphate (TCP) and hydroxyapatite (HA) and calcium phytate (PA) as an organic P source. Proportion of positive isolates found with PA (16.3%) was much higher than inorganic TCP (6.4%) and HA (4.2%). PA also showed bigger ( $p<0.05$ ) halo sizes on plates. The same isolates constantly solubilized all 3 media types ( $p<0.001$ ), which suggests that either the same mechanism is responsible for solubilisation of organic and inorganic forms or both mechanisms widely coexists.

Among PSB, *Rhizobium spp.*, *Enterobacter spp.* and *Pseudomonas spp.* were commonly found. Of these, only 11 isolates (*R. leguminosarum* bv. *trifolii*), were able to fix nitrogen (N). When white clover plants were supplemented with minimum N and HA as the sole P source, all 11 isolates increased plant growth in comparison with the uninoculated control. The most effective isolates produced a 73% increase in dry weight compared with our standard commercial inoculant *R. leguminosarum* bv. *trifolii* TA1. It seems likely that increase in plant growth was the result of bacteria solubilising P, which was then available as an energy source to increase the amount of nitrogen fixation.

Targeting both organic and inorganic P deposits in soil may give access to a larger P reserve. A steady release of P by bacteria could be used to reduce P fertilizer requirements and indirectly address the problems associated with P runoff and eutrophication.

## Release of acid phosphatase from extraradical hyphae of arbuscular mycorrhizal fungus *Rhizophagus clarus* under low P condition

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Arbuscular mycorrhizal (AM) fungi enhance uptake of available phosphorus (P) from soil. The mechanism underlying this P uptake enhanced by AM fungi is the increase in surface area for absorption of available P. Little is known about utilization of unavailable P by AM fungi. We investigated whether extraradical hyphae of AM fungi exude ACP and whether ACP activity responds to phosphorus condition. Sterilized Andosol was packed in pots that were separated into the mycorrhizal and hyphal compartments with a nylon net of 30 µm pore size. Seeds of *Allium fistulosum* L. were inoculated or uninoculated with the AM fungus *Rhizophagus clarus* in P fertilized soil (0 and 0.5 g P<sub>2</sub>O<sub>5</sub> g<sup>-1</sup>). Soil solution was collected by using mullite ceramic tubes. Hyphal extracts were extracted from extraradical hyphae grown on sand culture and *in vitro* monoxenic culture, respectively. Root exudate of *A. fistulosum* was collected from hydroponic culture. The soil solution, hyphal extracts, root extract and root exudates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis. *L. usitatissimum* inoculated with *R. clarus* was grown on solid minimal media with three (3 and 30 µM) P levels. The extraradical hyphae and hyphal exudates were collected and subjected to analysis of ACP activity by using *p*-nitrophenylphosphate. P concentration, P content and dry weight of shoot were higher in the inoculated treatment than in the uninoculated treatment. Activity staining of the gel revealed that ACP activity at 187 kDa was observed in the soil solution in the inoculation treatment, and in the hyphal extract collected from sand culture and *in vitro* monoxenic culture, but neither in the root exudate of non-mycorrhizal plant grown in the hydroponic culture nor in the root extracts irrespective of mycorrhizal status. ACP activity of extraradical hyphae and hyphal exudates were higher in 3 µM treatment than 30 µM treatment. These findings suggest that the fungus releases ACP from extraradical hyphae into the hyphosphere under low P condition.

## Effect of microbial bioeffectors inoculation and fertilizers application on soil organic phosphorus as evaluated by $^{31}\text{P}$ -NMR spectroscopy

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Soil organic phosphorus (P) compounds are an important fraction of total soil P, and include numerous molecular species with varying bioavailability and mobility through ecosystems. Identification of organic P species is important to understand their origin and turnover in soils, and ultimately, ascertain their effects on soil fertility. Soil microorganisms are key components of soil fertility and may contribute to the P nutrition of plants through the mobilization of inorganic soil P or the decomposition of soil organic matter and subsequently increase P availability through mineralization. At present, the use of plant growth-promoting microorganisms (PGPM) is becoming more popular as an additive to chemical or organic fertilizers for improving crop yield in an integrated plant nutrient management system. The type of P fertilizer used can affect the activity of applied PGPM, but the complementary effect of different P fertilizers and PGPM application on soil organic P is scarcely known.

Therefore, we investigated the effects of the application of different commercial PGPM strains (no inoculation, *Trichoderma harzianum* T 22, *Pseudomonas* sp., and *Bacillus amyloliquefaciens*) alone or in combination with different P fertilizers (no P fertilization, triple superphosphate, rock phosphate, composted cow manure and composted horse manure) on soil organic P forms as characterized by solution  $^{31}\text{P}$ -NMR spectroscopy (1) in a greenhouse pot experiment under maize cultivation, using a clay-loam soil mixed with quartz sand (2:1).

Results showed that for all treatments, orthophosphate (Ortho-P) was the dominant soil P form. Organic P accounted only for about 20% of the total extracted P. The most abundant fraction of the organic P in soils was orthophosphate monoesters (Mono-P), whereas the percentage of orthophosphate diesters (Di-P) was less than 20%. A small amount of polyphosphates and pyrophosphate was also detected. The Principal Component Analysis (PCA) score-plots for P fractions of soils amended with different P fertilizers and microbial inocula showed that both factors affected soil P composition. P fertilizers application brought to a significant increase in Ortho-P, Mono-P and Di-P content as compared to unfertilized soil. However, triple superphosphate increased Ortho-P more than the other P fertilizer treatments. A significant enhancement in organic P content was observed when both composts were applied to the soil. All bioeffectors increased Di-P content (including the DNA contribution) as compared to non-inoculated soil, but the largest increase in organic P content was found with application of *Pseudomonas* sp., in particular, under compost fertilization. This study indicates that inoculation with bioeffectors in combination with organic fertilization is an efficient way of increasing the soil organic P levels in soil.

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## Soil microbial phosphorus dynamics are affected by cover crops and minimum tillage

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Phosphorus (P) constitutes an essential, but non-renewable resource restricting plant growth in many environments. The soil microbial community controls the cycling of organic P. However, little is known about the mineralization of organic P and its availability to microbes. In order to reduce unnecessary fertilizer inputs and associated environmental pollution, cover crops and reduced tillage are both used in conservation agriculture providing multiple benefits for the agroecosystem. The present project studies the influence of conservation agriculture on soil microbial community and associated P dynamics in a field experiment at the agricultural station Tachenhausen (SW Germany) on a loamy clay. Samples were taken in 0-5 and 5-20 cm depth on five occasions over 15 months.

We elucidated the relationship between P limitation for microbial growth, assessed via a respiration microcompensation system with substrate additions, the soil microbial biomass P content, determined by hexanol fumigation-extraction and the structure of the microbial community using lipid biomarkers, as well as the activity of extracellular enzymes using MUB-labeled substrates. Additionally, we determined the hydrolyzability of soil organic P with the Enzyme Addition Assay (EAA) based on the excess addition of enzymes to alkaline extracts of soil samples<sup>1</sup>.

We detected an influence of the agricultural management on the composition of the soil microbial community and the vertical distribution of saprotrophic and mycorrhizal fungi in depth. Cover crops increased the activity of P-cycling enzymes, which was possibly the reason for trends towards a shift in the availability of different substrates of the organic P pool in the soil. Some of the effects of cover crops were still detectable after more than one year.

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## Priming phosphorus effect in volcanic soils

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The food production in the world is alarmingly dependent of phosphate fertilizer. Phosphorus (P) is a finite resource and continued inputs are required to maintain the productivity of agroecosystems under sustainable scenario. The question is: how to obtain a better P recycling from wastes applied into the soil? Volcanic ash derived soils, represents between 50-60% of the total arable land area of southern Chile. In these soils, high inorganic phosphorus retention and, in turn, low P availability, and high aluminum (Al) soluble concentrations (at low pH) are the most limiting factors in food production. The total P is in range between 1200 mg kg<sup>-1</sup> to 3000 mg kg<sup>-1</sup> or more, organic P from 40 to 60 % and the recalcitrant fraction after sequential extraction procedure range from 45 to 63 % in this soils. Our preliminary result by <sup>31</sup>P NMR analysis revealed that most (48-72%) of the residual P was present as inorganic orthophosphate and pyrophosphate, although organic P monoesters accounted for 25-46% of total P [1]. Preliminary, studies using dairy cattle dung suggest that we can improve P availability using organic P sources within the soil because of microorganism. [2; 3; 4]. Thus, we hypothesize that the “Priming P effect” is one of the most important biotechnological tool for P remobilizing in soil. The aim of this work was to test the “Priming P effect” in soil by using cow manure, glucose and urea. We utilized soil incubation studies at 25 °C degree at field moisture. The status of P was determined by the sequential Hedley extraction procedure. The results indicated an increment of available P. Soil plus manure plus glucose and urea produced an increment in P bicarbonate (organic and inorganic) of 179 mg kg<sup>-1</sup> respect to control until 60 days. We found an increment of 43 mg kg<sup>-1</sup> of inorganic P extracted in bicarbonate after 60 days. This is a very high content in our soils. We found in complementary studies that Al extracted in oxalate were correlated positively with labile organic P in soil, specifically with both NaHCO<sub>3</sub> and NaOH fractions extracted. This observation was also reinforced by P-NMR analysis that showed higher monoester P in soils with higher oxalate Al. These results showed that P priming process is producing available P in volcanic soils and we can use this effect as P starter in cropping production system.

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## Land-use influences phytate mineralization capacity and phosphatase microdiversity in soils

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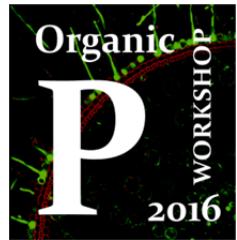
**Background:** Cycling of phosphorus (P) – largely controlled by microbial activity - can exert significant influence upon soil fertility and productivity. Inositol hexakisphosphates (phytates, IP<sub>6</sub>) may form a significant proportion of organic P (P<sub>o</sub>) in soils. Microbial release of P from IP<sub>6</sub> is achieved by phytases. Of these, histidine acid phytase (HAPhy), protein tyrosine phosphatase-like cysteine phytase (CPhy) and β-propeller phytase (βPPhy) are active in prokaryotes. Lower-order products of IP<sub>6</sub> dephosphorylation may also be hydrolyzed by phosphomonoesterases such as PhoA, PhoD and PhoX. In common with many organic compounds in soil, P<sub>o</sub> may become stabilized by incorporation into macro- and micro-aggregates within relatively short timescales. The resulting physical separation between microorganisms and P<sub>o</sub> may require the activity of extracellular enzymes for access.

**Objective:** To identify the effects of land-use management upon the activity and functional diversity of P-related genes in soil microbiomes.

**Methods:** Soils were collected from permanent grassland, arable and bare fallow plots of the Highfield Ley-Arable long-term experiment at Rothamsted Research. Extensive physical and biological data has already been reported for these soils. Total P in bicarbonate (P<sub>Olsen</sub>) and alkaline EDTA (PEDTA) extracts was assessed with ICP-OES: orthophosphate and IP<sub>6</sub> stereoisomers in PEDTA extracts were separated and determined using suppressed ion conductivity-HPLC. Functional phenotypic and assembly-free metagenomic approaches were used to investigate abundance and microdiversity of phosphorus cycling genes within soils. Bioinformatics was used to predict the subcellular localization of each of the target enzymes.

**Results:** Microbial communities in bare fallow soil showed a marked capacity to mineralize IP<sub>6</sub> compared to communities from arable or grassland soils. Bare fallow soil contained the least P<sub>Olsen</sub> and a relatively high IP<sub>6</sub> component. Analysis of shotgun metagenomes indicated *phoA*, *phoD* and *phoX*, and HAPhy and CPhy genes were most abundant in grassland soil which contained the greatest amount of P<sub>Olsen</sub>. In contrast, βPPhy genes were most abundant in bare fallow soil. *PhoD* was the dominant phosphatase in all soils. The dominant phytase gene was land-use dependent: CPhy was dominant in grassland soil, βPPhy dominant in bare fallow soil. Phylogenetic analysis of metagenome sequences indicated the phenotypic shift observed in the capacity to mineralize phytate in bare fallow soil was accompanied by a shift in *phoD*, *phoX* and βPPhy genes coding for extracellular enzymes.

**Conclusions:** A clear effect of land-use upon the capacity of soil microbes to utilize IP<sub>6</sub> as a source of P was observed. In response to limited P<sub>Olsen</sub> and relatively high IP<sub>6</sub> in bare fallow soil, the microbiome exhibits a phenotypic shift towards an increased capacity to exploit IP<sub>6</sub> and an increase in the abundance of βPPhy, *phoD* and *phoX* gene sequences with homology to extracellular enzymes. Environmental genes associated with P-cycling are poorly represented in sequence collections. In the case of βPPhy, well characterized enzymes such as those of *Bacillus* spp. do not appear to be well represented in the soils described in this study.



## Abstracts: Oral Presentations

### Session 5: Organic phosphorus in soil and waters: Stocks, flows and impact of scale

## Keynote: Specification of nano-particulate phosphorus in terrestrial systems

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It is recently recognized that natural nanoparticles ( $d=1\text{ nm}-100\text{ nm}$ ) representing the finest size fraction of colloids ( $d=1\text{ nm}-1\text{ }\mu\text{m}$ ) play a crucial role in nutrient acquisition and cycling processes in aquatic and terrestrial ecosystems. Especially, the high specific surface area and reactivity of natural nanoparticles and colloids (NPC) render them predominant carriers of nutrients like P in ecosystems. Recent works have shown that NPC can bind the majority of P present in soil solutions [Jiang et al., 2015, Missong et al., 2016] and stream waters [Gottselig et al., 2014], and that they can even support P uptake of plants from solution [Montalvo et al., 2015]. To identify, quantify and characterize P-carrying NPC and colloids in water and soil, the field flow fractionation (FFF) technique coupled to ICP-MS and an organic carbon detector was developed (Nischwitz et al., 2016). The characterisation of water dispersible NPC and of NPC from different soil leachates (drainage water and soil column effluent) and stream waters showed comprehensively that NPC-P was associated to various NPC-types such as  $\text{C}_{\text{org}}$ , Fe-/Al-OOH and phyllosilicates as well as their aggregates. The  $^{31}\text{P}$ -NMR investigations with forest soils revealed a strong enrichment of  $\text{P}_{\text{org}}$ , especially labile- $\text{P}_{\text{org}}$  forms in NPC in comparison to bulk soil. Our most recent German grassland soil study indicated that the proportions of organic phosphorus (mainly orthophosphate monoesters) in NPC P fraction significantly decreased within a soil wetness transect (i.e. from Cambisol to Stagnosol).

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## Legacy and organic P dynamics: phosphorus flows from agricultural muck soils to stream and lake sediments

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One of the most difficult aspects of assessing agricultural P best management practices (BMPs) is the lag experienced between the initiation of land management changes and the ecosystem response to reduced P loads. This delay, lasting for up to a decade, has been attributed to the internal loading of P in sediments and is referred to as legacy P.

The P load entering Lake Simcoe, Ontario, Canada has been estimated at 77 tonne P year<sup>-1</sup>, and while efforts to reduce the total amount of P entering the lake have been successful, water quality remains poor<sup>[1]</sup>. Of the 21 subwatersheds which drain into Lake Simcoe, the West Holland subwatershed contributed an average of 14.5 tonne P year<sup>-1</sup>, the most for any one subwatershed<sup>[2]</sup>. Within the West Holland sub-watershed lies the Holland Marsh, Ontario's most productive vegetable farming region. This unique landscape was a former wetland area that has been drained to form a polder where water levels are carefully controlled by a series of dykes, canals, and pumping stations.

The goal of this project is to identify the effects of P originating from the agricultural muck soils of the Holland Marsh on legacy P forms in the stream sediments of the West Holland River and the lake sediments of Lake Simcoe. Soil and sediment core samples were collected in November 2015. Forms of P in muck soils as well as surface (0–2 cm) and deep (11–15 cm) sediments were analyzed by sequential fractionation and solution <sup>31</sup>P NMR spectroscopy techniques. Organic P accounted for a higher proportion of total P in surface sediment (~17 to 35%) than that in deep sediment (~14 to 27%) at all study sites. Orthophosphate diesters were mainly concentrated in surface sediments compared to that in deep sediments. Substantial changes in the abundance of inorganic and organic P between muck soils, stream sediments, and lake sediments were observed. The proportion of organic P was lowest in the muck soils (~17%) and highest in the lake sediments (~49%). Forms of legacy P within the aquatic environment were different from those found in the agricultural soils. Bioavailable forms of inorganic P entering water bodies are immobilized or taken up by phytoplankton or bacteria upon entering the water column and then returned to sediments as biomass decays over time. Relatively little is known about legacy P in aquatic environments and understanding organic P dynamics in soils, sediments, and water is important for establishing effective BMPs for restricted legacy P accumulation in these disparate ecosystems. This ongoing research will continue to examine the temporal, spatial, and vertical distribution of P to better understand transformations between specific P forms that affect aquatic environments.

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## Grass yield response to fertiliser P applications in organic matter-rich soils

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Current phosphorus (P) recommendations for grassland in Ireland are well established for mineral but not for high organic matter soils<sup>1</sup>. Poor P retention capacity due to competitive sorption reactions between labile phosphate anions and organic acids in organic matter-rich soils could have implications for sustainable production on these soils<sup>2</sup>. Furthermore, the chemical nature of P fractions in organic-rich soils and their ability to supply P for pasture is not well understood<sup>3</sup>. The aim of this research is to compare and evaluate grass yield response to fertiliser P under controlled conditions across a range of mineral, peaty mineral and peat soils in Ireland. A growth chamber study was established on low P status soils with organic matter ranging from 8 to 76%. Fertiliser P rates from 0 to 145 kg/ha were applied and dry matter yield of perennial ryegrass measured after a 4 month period. Results indicate that grass yield responses differ between mineral and organic matter-rich soils at similar fertiliser P rates under controlled conditions. For example, at low fertiliser P rates (0-40 kg P ha<sup>-1</sup>), grass yields were significantly ( $p < 0.05$ ) higher in mineral compared to organic matter-rich soils, which is indicative of the limited ability of the latter to mobilise soluble P from moderately labile and stable P fractions . In this work we describe the distribution of inorganic and organic P fractions in mineral and organic matter-rich soils and combine these data with yield responses following P treatments. This research will enhance our knowledge of P uptake in high organic matter soils and establish criteria for sustainable P management for grassland on these soils.

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## Effects of citrate on the solubilisation of soil phosphorus

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Phosphorus in soil exists in a number of different organic and inorganic phases. The solubilisation of P from these phases into soil solution involves a number of different processes. Here we consider those processes influenced by the efflux of citrate from plant roots with particular focus on the release of organic P. Citric acid can act in a number of different ways to release P from soil. One is acidification, which directly affects the solubility of solid phases such as iron and aluminium (oxy)hydrides (containing sorbed P) or discrete P containing minerals. Additional mechanisms include (i) competitive adsorption of citrate on mineral surfaces, which can displace both inorganic P and some organic P compounds (e.g. inositol hexaphosphate [IHP]) (ii) increased solubilisation of soil organic matter (SOM) caused by removal of Fe, Al and Ca via complexation with citrate and (iii) increased solubility of Ca, Fe, Al IHP compounds. Here we report on three experiments involving the addition of citrate to an agriculturally productive soil (Olsen P 76 mg kg<sup>-1</sup>, 17 g C kg<sup>-1</sup>, pH<sub>water</sub> 6.2) known to contain IHP. The objective was to understand the contribution of the aforementioned processes to the release of P into soil solution and compliment related work including the effect of citric acid doses on P sorption/desorption in soil as measured by diffusive gradient in thin films (DGT) and the effect of citrate exudation on plant P nutrition.

*Experiment 1:* We studied the dynamics of soil P release using anion exchange resin sheets in 3.5 g:75 mL water extractions at two temperatures (25 and 4 °C). After 12 h equilibration the resin sheet was replaced with a fresh sheet a further 3 times. Phosphorus was eluted from the resin sheets with 0.25 M H<sub>2</sub>SO<sub>4</sub> and analysed for molybdate reactive P (MRP) and total P by ICP spectroscopy. The resin extracted approximately equivalent P to an Olsen extraction, but in all resin extractions MRP was the dominant fraction and minimal organic P was collected on the resin. The resin extraction carried out at 25°C released 76% more P than at 4 °C, indicative of biological decomposition or increased diffusion.

*Experiment 2:* To study the effects of citrate on the pH of bulk soil, we added 75 ml of 10, 1 or 0.1 mM citrate (adjusted with NaOH to the soil pH of 6.2) to 3.5 g soil in beakers. The mixtures were stirred and the pH recorded over 3 days. The pH increased in a non-linear fashion and for the 10 mM citrate treatment, increased from the initial value of 6.2 to 8.3; possibly indicating citrate decomposition or proton sequestration. There were smaller pH increases in the soil for the other citrate concentrations. Hence, depending on the form of citrate release into the rhizosphere, it may increase rather than decrease soil pH.

*Experiment 3:* We also report on ongoing experiments investigating (i) the effect of citrate on the solubility of major cations and on soil organic matter, and (ii) the after-effect on the water solubility of MRP, TDP and SOM from the residual soil with water and buffer solutions at increasing pH. It is hypothesised that removal of Fe, Al and Ca from solid phase soil organic matter will allow increased solubility of SOM, thus increasing the solubility of organic P compounds.

## A structural equation model analysis of phosphorus transformations in global unfertilized and uncultivated soils

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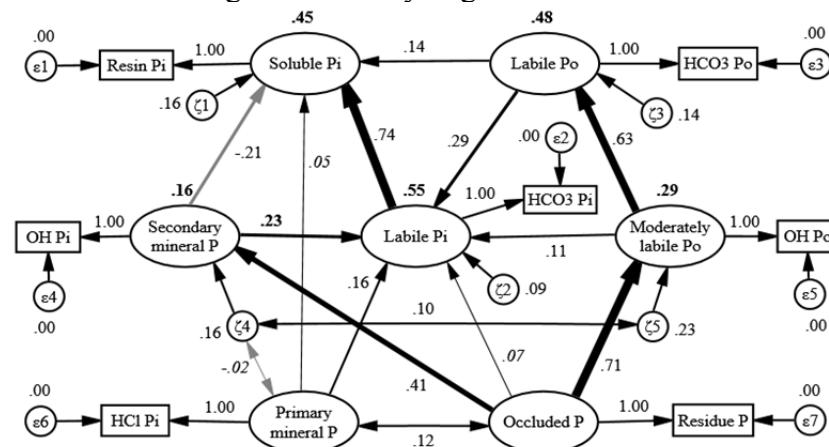
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Understanding the soil phosphorus (P) cycle is a prerequisite to predict how environmental changes may influence soil P dynamics and bioavailability. We compiled a database of P fractions (626 values) sequentially extracted by the procedures of Hedley *et al.* (1982) and Tiessen and Moir (1993) in global unfertilized and uncultivated soils, and applied structural equation modeling to select appropriate model(s) describing soil P transformations. The selected model (Fig. 1) revealed that soluble inorganic P ( $P_i$ , a readily available P pool) was positively directly influenced by labile  $P_i$ , labile organic P ( $P_o$ ) and primary mineral P and negatively directly influenced by secondary mineral P; soluble  $P_i$  was not directly influenced by moderately labile  $P_o$  or occluded P. In terms of overall effect, soluble  $P_i$  was most strongly influenced by labile  $P_i$ , secondarily by the organic P pools, followed by occluded P and primary mineral P, with minor total influence from secondary mineral P. Labile  $P_i$  was directly linked to all other soil P pools and was more strongly linked to labile  $P_o$  and primary mineral P than soluble  $P_i$ . Our study highlights the important roles of labile  $P_i$  in mediating P transformations and determining P availability in global soils.



**Fig. 1.** A structural equation model of P transformations in global soils. All data were  $\log_{10}$  transformed. The numbers on arrows are unstandardized path coefficients with values that did not differ significantly ( $P \geq 0.05$ ) from zero shown in italics. Width of the arrows indicates the strength of the paths. Arrows with positive and negative path coefficients are shown in black and grey colours, respectively. Numbers close to endogenous variables are the R-squared values (numbers in bold). Error variables ( $\varepsilon 1-\varepsilon 7$ ,  $\zeta 1-\zeta 4$ ) are standardized values. Overall fit of the model:  $\chi^2 = 6.51$ ,  $df = 6$ ,  $P = 0.369$ , RMSEA = 0.015.

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## Extreme events and grassland ecosystem function: The impact of flooding on P mobilization

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Improved grasslands make up an important part of UK agriculture and in addition to providing forage for livestock, grasslands provide additional and important ecosystem services including the regulation of water quantity and quality. In the context of global climate change the most recent IPCC report predicts greater uncertainty in weather patterns and an increased incidence of extreme weather events, such as heat waves, drought, heavy rains and storms<sup>1</sup>. It has been predicted that these episodic extreme events pose a bigger threat to European agroecosystems than progressive climate change<sup>2</sup>. Despite the large risks posed, our understanding of how extreme events will impact on plant and soil functioning and the downstream benefits/impacts remains poor. One vital ecosystem service provided by grasslands is the regulation of nutrient cycling to facilitate plant production and control the release of nutrients, including phosphorus (P) runoff.

To investigate the impact of extreme climatic events on grassland ecosystems we have set-up a plot-scale field trial on an improved lowland Welsh sheep grazed pasture. The trial design consists of 20 field plots 3 m by 3 m which will be subjected to five different treatment (stress) regimes with four replicates as follows; (i) control – natural climatic conditions, (ii) spring flood, (iii) summer drought, (iv) spring flood + summer drought and (v) summer flood.

This presentation will focus on the results of the spring flood initiated in April 2016. The flood plots were flooded to a depth of 30 cm with water from the adjacent river. The flood will be maintained for 8 weeks and the grass sward will be completely submerged throughout. Large pulses of P release have been observed following inundation on dry soils<sup>3</sup> as a result of both abiotic desorption, dissolution and biotic release related to the soil microbial pool. In this presentation we will report the changes in the flood and soil water chemistry over the course of the 8 weeks under flooding and the following month of recovery. Analyses will include dissolved reactive P (DRP) and dissolved organic P (DOP), Fe(II)/Fe(III) and DOC concentrations. In addition, we will report the water extractable P concentrations (both inorganic and organic forms), and the microbial community structure (from PLFA analysis) from soil cores taken immediately before and after the flood event and at the end of the month's recovery period.

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## Characterizing phosphorus mineralogy, chemistry, and speciation in Utah Lake sediments

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With a new phosphorus (P) total maximum daily load (TMDL) in preparation for Utah Lake, more work is needed to understand P cycling in surface water and sediments. Utah Lake is a unique freshwater body that is naturally shallow, turbid, eutrophic, and alkaline with high dissolved oxygen levels. Calcium carbonate precipitation is the primary form of sedimentation, with sedimentation rates of approximately 5 cm per 100 years. Co-precipitation of minor amounts of apatite (calcium phosphate), silicate minerals, and iron hydroxides may be an important sink of P in Utah Lake. The mineral-bound P in bottom sediments are likely an efficient removal mechanism of P in Utah Lake. As such, there may be an almost unlimited pool of legacy P trapped in Utah Lake sediments that far overwhelms the amount of P inflows from tributary streams. Thus, reductions in P loads from tributary streams to Utah Lake, as proposed by new state DWQ regulations, potentially will not result in improved water quality in Utah Lake. The purpose of this study is to identify the sinks and subsequent mobility of P in Utah Lake.

## Evaluation of soil organic phosphorus fractions in extensive and intensive Irish grasslands

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Irish grasslands differ largely in their land-use intensity, depending on several management factors including fertilization regimes, dairy or meat production and grazing periods<sup>1</sup>. In this study we evaluated the organic phosphorus (OP) content and its soil fractions in extensive and intensive grassland soils. We hypothesize that soil phosphorus (P) biological cycling will be distinctive between these grassland management systems. Intensive farming will provide an optimal soil nutrient status for plant production, however in extensive sites, the system will be driven to seek P in the soil reserves, releasing it from stable inorganic and organic P forms.

Soil OP was evaluated in 12 grassland sites, characterize as intensive (6) and extensive (6) according to their soil P index value, fertilizer (chemical and organic) application per year and grazing livestock (sheep or dairy). Microbial biomass and soil properties of the topsoil were investigated through chemical and biological measurements. P fractions were characterized applying sequential fractionation procedure<sup>2</sup> and the following soil OP fractions were defined: labile OP ( $H_2O$ -OP and  $NaHCO_3$ -OP); stable OP ( $NaOH$ -OP,  $NaOHsn$ -OP, HCl-OP) and residual-P.

Higher inputs of P in intensive sites increase the inorganic P content of the soil, significantly increased Morgan's P and total soil P ( $p<0.05$ ). Soil available carbon (C) and nitrogen (N), soil biomass and concentration of soil OP were not significantly affected by soil management. Residual P fraction showed an increase ( $p<0.05$ ) in its concentration in intensive sites. The percentage of soil OP and soil OP fractions was affected ( $p<0.05$ ) by grassland regimes and labile soil OP % was positively correlated with soil available C and microbial N/P ratio, indicating that labile OP forms are potentially used as nutrient sources. In extensive sites, no correlation was found between inorganic and organic P fractions, with exception of a positive correlation ( $p<0.05$ ) between organic labile and stable fractions. In intensive sites, strong correlations ( $r=0.90$ ,  $p<0.05$ ) were observed between inorganic and organic fractions. These results reveal that OP retains a distinct role as a source/sink of soil P between extensive and intensive grasslands. P biological cycle in extensive grasslands appear to have a crucial role in plant production, opposite to P cycle in intensive regimes, more prone to be driven by abiotic factors. Nevertheless, lack of correlation between soil biomass pool and soil OP indicate a need for further investigation regarding OP mineralization in distinctive grassland soils, targeting potential distinct profiles in microbial communities and P-cycling functional genes.

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## A new framework for lake ecological change due to phosphorus enrichment

Katrina A. Macintosh<sup>1</sup>, Brian Rippey<sup>2</sup>, Elena Forasacco<sup>3</sup>, Yvonne McElarney<sup>4</sup>, Louise Vaughan<sup>4</sup> and Kevin Gallagher<sup>4</sup>

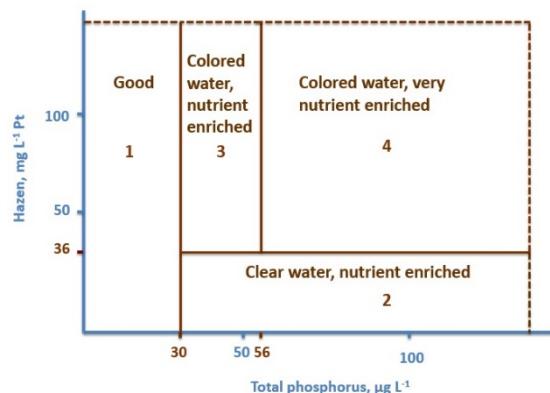
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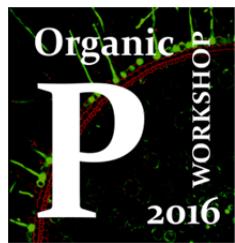
Fifty lakes, selected on nutrient status and catchment land use, were used to develop a new framework to describe lake ecological change due to enrichment. Lake physicochemical properties and species abundance of phytoplankton, macrophytes, zooplankton and fish were investigated. Regression tree analysis identified four lake groups, determined by nutrient concentration (total phosphorus, TP, ug/L) and water colour (Hazen, mg/L Pt): TP<30; TP>29 and Hazen<36; TP between 30 and 56 and Hazen>35; TP>56 and Hazen>35<sup>1</sup>.



Biological communities demonstrated strong and consistent evidence that Group 1 (low nutrient concentration; good quality) lakes were ecologically distinct from all the other lakes, while those in colored water nutrient enriched lakes showed gradual ecological change with increasing nutrient concentration. Communities in clear water nutrient enriched lakes were similar to those in colored water moderately enriched lakes, but different from very enriched lakes<sup>1</sup>. The new framework consists of an ecologically distinct, low nutrient concentration regime that, when disturbed, changes gradually with increasing nutrient concentration, with alternatives for clear and colored water: a solution to the imprecision of interrelationships between biological properties through the use of conditions or regimes. Recommendations are that the biological and chemical characteristics of Group 1 lakes are taken to represent '*Good Ecological Status*' and that impacted lakes are assigned to Group 2, 3 or 4 in order to establish the starting point of a trajectory of management to achieve Good Status. Distinction between clear and coloured water nutrient enriched lakes, should be researched further, as there may be implications for remediation or timescales for improvement.

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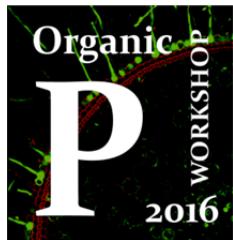


## Abstracts: Oral Presentations

### **Session 6: Global challenges for organic phosphorus research**

**Keynote: Organic phosphorus and the ecology of tropical forests**Benjamin L. Turner<sup>1</sup><sup>1</sup>Smithsonian Tropical Research Institute, Republic of Panama

Organic phosphorus is a dynamic and quantitatively important pool of bioavailable phosphorus in tropical soils that shapes the above and below ground ecology in a number of ways. Tree species distributions are strongly influenced by phosphorus availability, which reflects in part a trade-off between growth rates and investment in phosphorus acquisition via mycorrhizal symbionts and root phosphatase enzymes. There is evidence for widespread species-specific phosphorus limitation of tropical trees, yet some of the fastest growing species occur on the lowest phosphorus soils, presumably because they efficiently acquire phosphorus from organic compounds. There is some evidence that plants can use different forms of organic phosphorus, suggesting the potential for phosphorus partitioning, even though this does not appear to occur for nitrogen. However, trees that can fix nitrogen express particularly high root phosphatase activities, offering an explanation for their abundance in otherwise nitrogen-rich forests. Organic phosphorus accounts for almost all the soluble phosphorus in soil solution and probably supplies a considerable fraction of the phosphorus taken up by plants via transpiration-driven mass flow of water to the root surface. This has implications for phosphorus acquisition in the future, given that forest-wide transpiration will be reduced by increasing atmospheric carbon dioxide concentrations. Much of the soil organic phosphorus is contained within live microbial biomass, which contains about twice as much phosphorus as the vegetation. Phytase genes are abundant in the microbial community, which perhaps explains why tropical forest soils rarely contain inositol phosphates despite a high capacity for phosphorus fixation. Soil phosphatase activities increase exponentially below 2 mg P kg<sup>-1</sup> resin-extractable phosphate, which coincides with a marked shift in tree growth rates and community composition. This increasing biological investment in organic phosphorus acquisition indicates a critical threshold for phosphorus limitation above and below ground. Taken together, these findings demonstrate the fundamental importance of organic phosphorus cycling as a driver of the world's most productive and diverse biome.



## Abstracts: Poster Presentations

**Session 1: Organic phosphorus flows in the environment in context with other nutrient cycles: Integration across ecosystems**

## Eco-enzymatic stoichiometry and enzymatic vectors reveal differential C, N, P dynamics in decaying litter along a land-use gradient

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To evaluate carbon (C), nitrogen (N), and phosphorus (P) dynamics during the decomposition process, we investigated the temporal variability of extracellular enzymatic activities (EEA) associated with C, N, and P acquisition in microbial communities from different land uses. We hypothesized that EEA ratios would reveal different primary resource requirements with respect to microbial demand, depending on soil properties, litter type and the relative proportion of bacteria:fungi in the microbial community. To test this hypothesis, we implemented an experiment using four litters (*Triticum aestivum*, *Fagus sylvatica*, *Festuca arundinacea* and *Robinia pseudoacacia*) in four soils (cropland, plantation, prairie and forest) located in close proximity to one another on the same parent material. Analyses of EEA showed that overall N requirement increased relative to P during litter decay, but C requirement increased more rapidly than either N or P in most ecosystems. Soil type was the main factor controlling N vs. P limitation whereas litter type was the primary driver of C vs. nutrients limitation. Shifts in EEA were related to changes in metabolic quotient (C respired per unit biomass) but there was no evidence that the relative proportion of fungi:bacteria drove changes in EEA. We concluded that the use of EEA as a proxy of microbial resource demand improved our understanding of temporal shifts in resource requirements to microbial communities, their associated respiration efficiency and dynamics of C and nutrients among different ecosystems.

## Phosphorus cycling along a subalpine proglacial soil chronosequence in the Italian Alps

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Glacier retreat is one of the most visible effects of climate change in mountain ecosystems. The establishment of vegetation on recently deglaciated surface initiates gradients in many soil properties, but vegetation succession pathways strongly influence pedogenetic evolution. In undisturbed areas there is a trend towards the formation of podzols, while Cambisols develop where forest vegetation succession is inhibited, leading to soils differing in element mobility, leaching rates and biological communities. To evaluate the relationships between plant community succession and soil development as a function of moderate disturbances, we studied the biogeochemical cycling of phosphorus (P) in a proglacial area in the Western Italian Alps. Sites colonised by *Larix decidua* and *Rhododendron ferrugineum* were compared to areas affected by grazing of the same age of surface deglaciation. Phosphorus speciation in genetic horizons of soil profiles was determined by sequential fractionation and solution <sup>31</sup>P NMR spectroscopy. The first few years of ecosystem development under larch forest involves a marked decline in pH and accumulation of organic matter. Phosphorus transformations were shaped by geochemical processes in the layers explored by roots, with a rapid release of inorganic P from primary minerals and surface accumulation of organic P to concentrations similar to those found in the climax soil. Adsorbed and/or precipitated P increased less rapidly, although the distribution of P organic forms indicated that eluviation was already active after 90 years. In the grazed chronosequence, herbaceous species reduced geochemical P transformation. However, following P release from primary minerals, an active and dynamic biological cycle developed in the shallow surface horizon, as suggested by the accumulation of phosphodiesters and monoesters, the latter represented predominantly by microbially derived *scyllo*-inositol phosphates. These results highlight that natural succession of larch in a proglacial landscape enhance the rate of P transformations, while inhibition of forest vegetation slows pedogenesis by limiting biogeochemical cycles and organic matter accumulation in the surface horizons.

## Nitrogen fertilization influences on the acidification of P rich calcareous soil: Consequences on soil organic phosphorus transformation

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High phosphorus (P) accumulated in arable field has the potential risk to accelerate eutrophication. The labile and moderately labile organic P (Po) pools, accounting for 5-52% of total P in the soils of some long-term experiments, highlighted the role of Po to plant or the potential of P transporting to water bodies [1]. Nitrogen (N) fertilization possibly affects Po transformation and mobility with soil properties changing, i.e. soil acidification, which may dominantly contribute to Po transformation in fertile calcareous soil. This hypothesis was addressed on the laboratory incubation experiment with the incubating conditions of 25°C temperature and soil moisture content of 60% water holding capacity for 20 days. Three treatments (T1, control; T2, application of urea at rate of 300 kg N ha<sup>-1</sup>; T3, addition of H<sup>+</sup> equivalent to the H<sup>+</sup> produced by completely nitrification of urea using 1.0 mol L<sup>-1</sup> HCl) were treated in two soils (low P soil from cereal field: Olsen-P 20 mg kg<sup>-1</sup>, pH 8.4; rich P soil from greenhouse vegetable field: Olsen-P 505 mg kg<sup>-1</sup>, pH 7.5). The results showed that after 20 days incubation, urea application significantly increased contents of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N, with pH decrease 0.62 and 0.17 units in low and rich P soils, respectively, however, it had no significant effects on total P, and Olsen-P, except for slight increase in Po contents in the two soils by 3.7-7.2% compared to control treatment. Urea application inhibited the activity of alkaline phosphatase (ALP) by 31% in high P soil after incubation. Similar effects on soil P contents, pH change and ALP activity were investigated in the treatment with H<sup>+</sup> addition (T2 treatment), including increased Po by 12.3%, decreased pH by 0.27 and ALP activity by 71% in rich P soil, respectively, compared to control treatment after incubation. The preliminary results suggested that urea application affected Po transformation through restraining enzyme hydrolysis and soil acidification influenced by the nitrification of applied ammonium nitrogen in rich P calcareous soil. However, more complex mechanisms need to be explored and related items (i.e. Po forms, microbial P etc.) to Po transformation should be determined.

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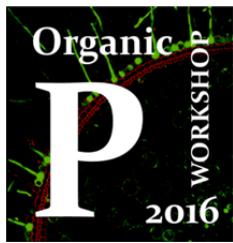
## Soil Organic Phosphorus in Cacao Agroforests in the Atlantic Forest Region, Brazil

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In tropical regions there is little knowledge about the phosphorus (P) species of agroforests soils. Our hypothesis was that the species' diversity and concentrations of P in Yellow Argissol (Ultisol) could be influenced by different types of cacao agroforestry systems (*Theobroma cacao* L.) with low input of phosphate fertilizer. We assessed the selected sites: (1) open cacao-cabruca, 40 years; (2) close cacao-cabruca, 40 years; (3) cacao+erythrina, 35 years; (4) cacao+rubber tree mixed, 3 years; (5) cacao+rubber tree mixed, 40 years; (6) cacao+rubber tree intercropping, 4 years after replacing a natural forest; (7) cacao+rubber tree intercropping, 4 years after replacing an unfertilized pasture; (8) unfertilized pasture and (9) natural forest. P soil (0-10 cm) was extracted with a solution of 0.25 M NaOH + 50 mM Na<sub>2</sub>EDTA in conjunction with <sup>31</sup>P nuclear magnetic resonance spectroscopy operating with proton decoupling at 202.446 MHz with a 5mm probe, using a 6.0  $\mu$ s pulse (30°), an acquisition time of 0.4 s, an delay time of 0.5 s operating at 21 °C. Approximately 30,000 scans were acquired for each sample. All of the sites exhibited high soil acidity (4.0–4.8). Mean soil organic carbon (SOC) ranged from 3.1 to 3.9%, total N from 0.2 to 0.5%, and total soil P (H<sub>2</sub>O<sub>4</sub>+H<sub>2</sub>O<sub>2</sub> digestion) from 500 to 1,300 mg kg<sup>-1</sup>. The soil organic P ranged from 24 to 96 mg P kg<sup>-1</sup>, with the predominance of phosphate monoesters (P-monoester) ranged from 14 to 70 mg kg<sup>-1</sup> (60-94% of organic P), while phosphate diester (P-diester), in the DNA form, exhibited lower contribution to the organic pool, ranging from 4 to 22 mg kg<sup>-1</sup> (6-40% of organic P). The P-diester was correlated strongly and positively with SOC and total N, highlighting the close association with the dynamics of soil organic matter. The stabilization of the soil P-diester was closely and positively associated with the clay content. Phosphonates and organic polyphosphates were not detected. The otho-phosphate exhibited predominance in concentrations at all sites ranging from 107.9 to 1,085 mg kg<sup>-1</sup> (92-98% of inorganic P), while pyrophosphate exhibited lower contribution to the inorganic pool. However, highest concentrations of pyrophosphate were found in cacao agroforests (mean 18.8 mg kg<sup>-1</sup>) compared to unfertilized pasture and natural forest (mean 7.2 mg kg<sup>-1</sup>). The high levels of C:organic P ratio (348-1304) and N:organic P ratio (41-107) indicated the predominance of immobilization of P in soils. The cacao agroforests exhibited the highest concentrations of soil organic P (mean 60.9 mg kg<sup>-1</sup>) and inorganic P (mean 381.1 mg kg<sup>-1</sup>) compared to unfertilized pasture and natural forest (means 53.4 mg kg<sup>-1</sup> of organic P and 154.4 mg kg<sup>-1</sup> of inorganic P). The principal component analysis revealed high dissimilarity between the cacao sites and forest and pasture and also between the cacao sites themselves. In conclusion, concentrations of P species were strongly affected by soil properties and the different cacao agroforests with low input of phosphate fertilizer.



## **Abstracts: Poster Presentations**

### **Session 2: Methods of evaluating organic phosphorus stocks, concentration and speciation**

## Modeling $^{31}\text{P}$ -NMR P forms in Canadian grasslands using compositional analysis with balances

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Phosphorus (P) fertilization has been shown to influence soil P forms. The preferred technique to characterize soil P forms is  $^{31}\text{P}$  nuclear magnetic resonance (NMR) spectroscopy and compositional data analysis using balances is appropriate for modeling the interactive P forms [1]. Our objective was to model changes in soil  $^{31}\text{P}$ -NMR P forms in response to mineral P fertilization at two grassland sites in Québec, Canada, using compositional analysis with balances. Four P rates (0, 10, 20, and 40 kg P ha<sup>-1</sup>), replicated four times, were applied each year since 2010 to a previously established timothy (*Phleum pratense* L.) sward at two sites, one located at Lévis on a Kamouraska clay and the other at Normandin on a Labarre clay loam. However, only three replications and three P rates (0, 20, and 40 kg P ha<sup>-1</sup>) were considered in this study. Soil samples to a 10-cm depth were collected in fall 2013 and were analysed for different parameters including available P as extracted by Mehlich-3 solution (PM3, the recommended method for soil test P in Québec) and P forms as extracted with solution  $^{31}\text{P}$ -NMR. We used isometric log-ratio coordinates [2] to compute balances between  $^{31}\text{P}$ -NMR P forms. At the two sites, PM3 was greater with the highest rate of P fertilization (31.2 and 76.0 mg kg<sup>-1</sup> at Lévis and Normandin, respectively) than without P (13.1 mg kg<sup>-1</sup> and 16.8 mg kg<sup>-1</sup>). Total inorganic P increased over total organic P as P fertilizer was added, and inorganic orthophosphate accumulated relative to pyrophosphate and polyphosphate suggesting an increase of P availability but also an increased risk for P loss in runoff. Phosphonate slightly increased over the other organic P forms with 20 kg P ha<sup>-1</sup> at Lévis and with 20 and 40 kg P ha<sup>-1</sup> at Normandin. The P fertilization also increased the monoesters relative to the diesters with 20 kg P ha<sup>-1</sup> at Lévis and with 20 and 40 kg P ha<sup>-1</sup> at Normandin. Modeling changes in P forms using balances improved understanding of P dynamics in grasslands that could result in improved P management strategies.

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## Comparison of phytate and other organic P forms in Mehlich-3 and Alkaline-EDTA matrices by NMR, ICP and mass spectrometry.

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The favored method of organic P identification over the last few decades has been <sup>31</sup>P NMR. While this technique has the distinct advantage of speciating the organic P fraction, it has a relatively poor detection threshold (0.05 mg/ml), which typically limits <sup>31</sup>P NMR to qualitative or confirmative applications<sup>[1]</sup>. Additionally, ICP and colorimetry have been used in tandem as an indirect determination of organic-P by way of subtracting the colorimetric inorganic fraction from the determination of total P by ICP. Amongst these methods, there are also a number of chromatographic techniques that have been reported, however typical detectors (UV or conductivity) have been relatively unsuccessful at quantifying phytate. More recently, the increasing availability of high-resolution, accurate mass spectrometers has made the selective quantitation (single µg/l or lower) of many organic P species possible. Together, these techniques give a reliable overview of what properties are contributing to organic P processes in soils.

Several extracts (Mehlich-3, NaOH-EDTA, and Mehlich-3 followed by NaOH-EDTA) were chosen to test on a variety of soils from various areas, which have a range of total P concentrations and different land uses. Soils from the Delmarva Peninsula in Maryland, Northeastern Arkansas, Saskatchewan, and Prince Edward Island were analyzed for phytate and lower inositol phosphates.

Beyond the question of phytate concentration, the goal was to look at how pH and different cations (Al with respect to Alum treatment, and Ca with respect to general soils) stabilize phytate, its stereoisomers and the lower inositol phosphates in soil. We will show the distribution of phytate isomers (syclo, neo, chiro) found naturally in soil and attempt to correlate these with a stabilizing factor such as cations. In addition to this, we also investigated how reliably the lower inositol phosphates were extracted with respect to the same factors found with phytate and its isomers.

While different extracts show different P forms (especially different levels of phytate), our results also show that organic P is not extracted from these different soils equally. This suggests that care needs to be used when interpreting extractions analyzed by ICP.

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## Stable oxygen isotope as a tracer ( $\delta^{18}\text{O-PO}_4$ ) for polyphosphate in fungi

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It is challenging to identify the source of phosphorus (P) from soil in plants, since P can be taken up as phosphate (Pi) from available P in soil solution, mineral P, as well as organic matter. Soil microorganisms play an important role in P cycling with fungi taking a particularly prominent role because of high biomass, P storage, and combined usage of different nutrient pools. Pi immobilized by fungi represents a potentially important P pool for plants in agricultural and natural ecosystems. Particularly under high Pi concentrations fungi accumulate P in the storage compound, polyphosphate [1]. Hence, studying fungal polyphosphate by means of the stable oxygen isotope ( $\delta^{18}\text{O-PO}_4$ ) could reveal sources of P for plant nutrition. This is possible because P is always bound to oxygen atoms in Pi, and only biological processes can modify the signature of  $^{18}\text{O}$  in phosphate [2]. Here we evaluated whether oxygen isotopes in Pi provide a viable tracer for the polyphosphate pool in fungi and verified whether it retains the  $\delta^{18}\text{O-PO}_4$  signature of its source pool. For a first assessment, we used baker yeast (*Saccharomyces cerevisiae*) as a model, since it is known to accumulate large quantities of phosphate. We could verify that, indeed, the majority of accumulated P is polyphosphate, using three different chemical analytical assays. Acid hydrolysis of fungal biomass produced under low and high Pi conditions revealed differences in the signature of released  $^{18}\text{O-PO}_4$ , further indicating that we can reliably quantify changes in fungal polyphosphate pools. We will also assess the  $\delta^{18}\text{O}$  of metabolic water in the studied fungi to check if it is distinct from ambient water as reported previously [3]. This additional information will help in better understanding isotopic fractionation during polyphosphate synthesis and Pi remobilization. Next, we plan to follow polyphosphate accumulation in saprophytic and mycorrhizal fungi, using measurements of the  $\delta^{18}\text{O-PO}_4$  signature in accumulated P to determine whether there is isotopic fractionation during uptake and polyphosphate synthesis.

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## Land uses induced phosphorus transformation in agricultural soils using sequential fractionation, solution P-NMR and P K-edge XANES spectroscopy

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Agricultural sustainability of pasture and crop lands greatly depends on the finite P resource that is largely preserved in soils. However, soil P transformation and cycling under pasture and crop land usage remain largely unknown. In this study, four agricultural fields containing adjacent sites for each land use type (native and tame grasslands, annual crop lands and roadside soils) were selected and multiple techniques, including sequential fractionation (SF), solution <sup>31</sup>P nuclear magnetic resonance (P-NMR) and P K-edge X-ray absorption near-edge structure (XANES) spectroscopy, were applied to investigate soil inorganic P ( $P_i$ ) and organic P ( $P_o$ ) transformation mechanisms induced by these land uses. Compared to the roadside soils without fertilization and intensive vegetation, there were significant increases in the proportions of  $P_o$  and orthophosphate monoester (Mono) in grasslands by P-NMR analysis. Consistently, increasing trends of NaOH-extracted  $P_o$  by SF and inositol hexakisphosphate (IHP) by P-NMR and XANES analysis were observed in the grasslands relative to the roadside soils. In contrast, lower Mono proportions occurred in croplands under fertilization where acid phosphatase was less active as compared to grasslands. Furthermore, P-NMR analysis firstly indicated that neo-IHP, thought to be of microbe origin, significantly increased in the native grassland than the annual crop lands, which suggested the more important role of microbes on P transformation and cycling under native grass cultivation. Additionally, P K-edge XANES analysis revealed the proportion of tricalcium phosphate (TCP) significantly decreased in the two grasslands and was relatively drawn down in annual crop lands compared to the roadside soils, implying the stronger impact of grass-induced mobilization of TCP in the grasslands than the annual crop lands. This study demonstrated the distinguished impact of grass and annual crop cultivation on soil P transformation, and provides the first direct evidence of changes in the microbe-related  $P_o$  species in native grasslands, thus giving valuable clues for further investigating microbe-mediated  $P_o$  degradation in agricultural soils and probably facilitating sustaining agricultural productivity by using  $P_o$  as an alternative resource for finite  $P_i$  fertilizer.

## Extracting the $\text{PO}_4\text{-}\delta^{18}\text{O}$ record from carbonate speleothems – methods development and potential applications.

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Speleothem carbonate is routinely used for palaeoclimate reconstruction, offering high resolution, accurately dated, multi proxy archives of environmental and climatic change. In recent years our understanding of karst hydrology and speleothem growth dynamics have improved dramatically, aiding the interpretation of a range of new isotopic and trace element proxies preserved within stalagmite records<sup>1</sup>. Phosphorus is one of the trace elements which have the greatest potential to inform us of past climate and environmental evolution. Currently, work on speleothem P focuses on changes in P concentration as a proxy for vegetation decay and rainfall intensity<sup>2</sup>. However, our understanding of P sources and incorporation into the carbonate crystal lattice is still in its infancy<sup>1–3</sup>. With the aim of unravelling some of the mystery behind speleothem P we have started to develop a method by which we extract and analyse phosphate oxygen isotopes ( $\text{PO}_4\text{-}\delta^{18}\text{O}$ ). Our work builds upon an established method<sup>4</sup> for the precipitation of  $\text{Ag}_3\text{PO}_4$  from organic rich soil waters. Due to the low concentrations of P in speleothem carbonate (frequently <100 ppm) and the finite amount of carbonate material available, we are optimising the existing method to work with small volumes of carbonate material (200 to 500 mg). This technique will be able to address the following key areas: 1) In  $\text{PO}_4$  rich environments,  $\delta^{18}\text{O}_{\text{PO}_4}$  can inform how the source of  $\text{PO}_4$  may have changed over time related to climate and environmental upheavals 2) in  $\text{PO}_4$  limited systems,  $\delta^{18}\text{O}_{\text{PO}_4}$  may be used to distinguish between biological and inorganic mechanisms of phosphate incorporation into speleothem carbonate, and 3) where incorporation into speleothem carbonate is inorganic, temperature dependent equilibration with  $\text{H}_2\text{O}$  in the overlying soil zone may bring us a step closer to an accurate speleothem palaeothermometer.

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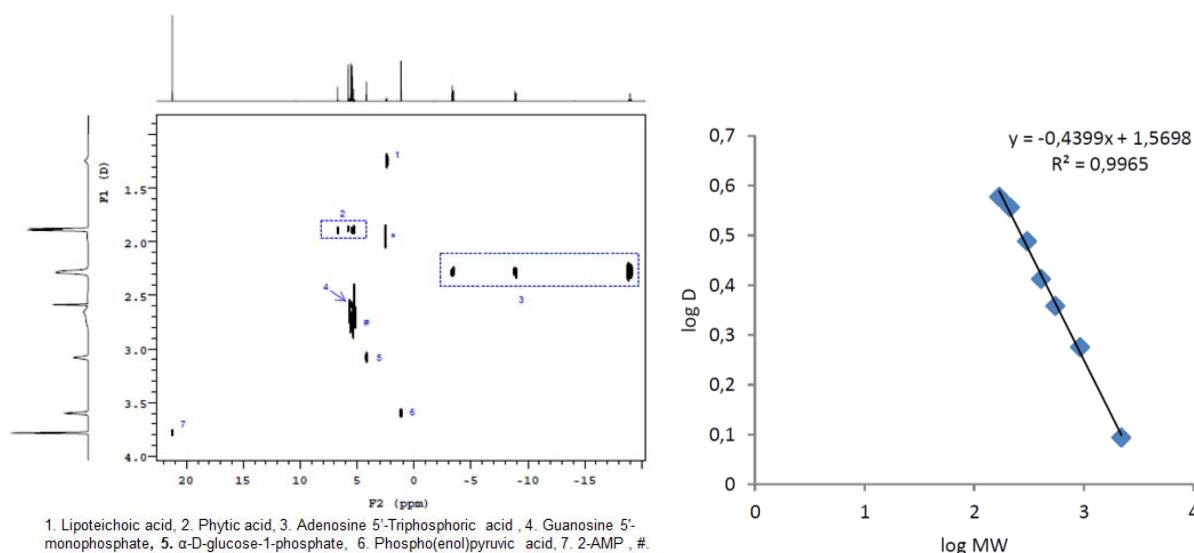
## Application of $^{31}\text{P}$ DOSY in the characterization of phosphorus compounds in soil extracts

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$^{31}\text{P}$  NMR has been widely applied to characterize organic phosphorus compounds in soil samples. However, confident peak assignment is greatly hampered by the dependence of peak position of individual phosphorus compound on different matrices<sup>[1]</sup>. A new approach named diffusion ordered nuclear magnetic spectroscopy (DOSY) allows virtual separation of different components based on their translational diffusion coefficient (D)<sup>[2]</sup>. Its utility was firstly illustrated in this study with the application to a mixture of 8 model P compounds that most commonly exist in soil (see spectrum below). The D versus molecular weight (MW) fitting result demonstrated an ideal correlation ( $r>0.9982$ ) in aqueous solution with high dielectric constant.



1. Lipoteichoic acid, 2. Phytic acid, 3. Adenosine 5'-Triphosphoric acid, 4. Guanosine 5'-monophosphate, 5.  $\alpha$ -D-glucose-1-phosphate, 6. Phospho(enol)pyruvic acid, 7. 2-AMP, #. degradation products of RNA, \*. Impurity or degradation product of Lipoteichoic acid.

DOSY was then applied to soil extracts that have been treated to remove paramagnetic ions with sodium sulfide followed by high-speed centrifugation (215000g, 45min) at 4°C to get high-resolution spectra. The fitting result of the soil extracts ( $r>0.9845$ ) was also satisfactory. In general, the additional information from the D domain in DOSY spectrum helps identify unknown peaks and prevent wrong peak assignment.

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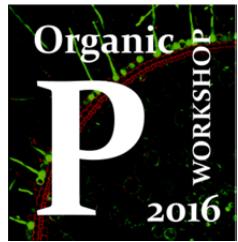
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## Holistic approach for understanding bioavailability and biogeochemical processes of phosphorus in soils using DGT

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Although it is more than 20 years since Diffusive Gradients in Thin-films (DGT) was developed, the strengths and applications of this unique technique for assessing dynamic characteristics of metals and nutrients (mainly P) are only now being really appreciated. During in situ deployment, DGT perturbs the solution equilibria and therefore provides information on chemical speciation, including the rates of complex dissociation. A well-developed theory is now established for extracting equilibrium and kinetic information from a suite of DGT measurements. When DGT is deployed in soils and sediments, it provides thermodynamic and kinetic information on the partition of phosphorus between solution and solid phase. The understanding of the interactions of DGT in complex solution and in soils and sediments has been greatly aided by the development of dynamic models which have allowed visualisation of the processes occurring in both the device and the surrounding media. As DGT automatically collects information in 2 dimensions, if analysis is undertaken in 2-D, a visual image of the solute fluxes is obtained. This feature has been used to uncover a previously unrealised solute structure in sediments, which occurs on a small scale in 2-D, due to the microniche activity of micro-organisms. Highly localised biogeochemical processes of phosphorus, occurring at the sub-mm scale at the rhizosphere, have been revealed using this approach. When plants take up phosphorus, it is removed from the surrounding medium. Similarly, DGT actively removes phosphorus from its deployment medium, and so if the rates of uptake are similar, DGT mimics P uptake by plants. In fact, DGT measures the phosphorus which is potentially bioavailable. Rather than measuring P in a single compartment, it measures the P which is relevant to plant uptake. By using DGT alongside traditional, single compartment measurements, it can provide additional information of direct relevance to the ambient biota.



## Abstracts: Poster Presentations

### Session 3: Biotic Interactions in organic phosphorus cycling – Plants

## Availability of phosphorus in soil cultivated with ruzigrass during soybean offseason

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**Introduction:** Crop rotation with some plant species with great capacity in phosphorus (P) uptake and also enhance the solubility of less labile P forms, can increase P cycling [1], and may result in improvement of P availability for the next crops. Ruzigrass [*Urochloa ruziziensis* (R. Germ. & C.M. Evrard)] is a well-adapted species to low soil P availability, and has been preferentially adopted in crop rotation during soybean off-season in Brazil. The objective of this work was to verify the possibility of increasing the yield of soybean in rotation with ruzigrass in the off-season compared to no crop rotation, according to soil P level.

**Material and Methods:** A field experiment in no-till has been performed in Botucatu, SP since 2001. The experiment has a randomized block design with four replicates. The treatments were soil fertilized with 0, 13, and 26 kg ha<sup>-1</sup> P as TSP applied to soybean seed furrows; and soil where ruzigrass was grown or soil kept fallow during soybean off-season. Soil sampling was conducted in November of 2015, after ruzigrass desiccation and before soybean sowing, at depths 0.00-0.05 and 0.05-0.10 m. Soil analysis was performed and the available P (Resin-P) was extracted with pearl resin [2]. The results were subjected to ANOVA, and compared by Tukey's test ( $p < 0.05$ ).

**Results and discussion:** Resin-P level was greater after ruzigrass cultivation than fallow when the soil was fertilized with 26 kg ha<sup>-1</sup> P at 0-0.05 and 0.05-0.10 m of soil depth. However, the yield of soybean was lower after ruzigrass (3609 kg ha<sup>-1</sup>) than after fallow (4008 kg ha<sup>-1</sup>), at the rate of 26 kg ha<sup>-1</sup> P. Once soil organic matter was higher and other nutrients were not reduced after ruzigrass than fallow, we believe, that ascertainment of organic P in soil may be fundamental to elucidating P bioavailability and the sustainability of crop rotation with ruzigrass. The decrease in soybean yield grown in rotation with ruzigrass may be function of the accumulation of soil organic P forms that are less available. Among all soil P compounds, phytate is frequently found as a mainly organic P form, accounting more than 80% of total organic P [3], and has the lowest bioavailability and the strongest affinity to soil particles.

**Conclusion:** Soil P availability is not correctly estimated with the routine soil test (resin-P) in soil cultivated with ruzigrass during soybean off-season. Despite the level of Resin-P increase after ruzigrass crop, it is not possible to get advantages of the rotation with this species over the soybean yield.

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## Tree species influences on phosphatase activity and microbial community composition

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Tropical forests cycle more CO<sub>2</sub> and water than any other terrestrial biome. The Next Generation Ecosystem Experiments–Tropics project is centered on minimizing ecosystem model uncertainties by using modeling and data to inform and understand processes that affect tropical forest CO<sub>2</sub> flux<sup>1</sup>. Phosphorus (P) limitation is one such factor that can constrain tropical ecosystem productivity because any available organic P compounds must be mineralized to inorganic P before plants and microbes can use them. This highlights the importance of both root function (phosphatase activity) and microbial communities in understanding how biological mineralization of P aids in supporting tropical forest growth and development<sup>2</sup>.

Three forested sites in Puerto Rico: Rio Icacos, El Verde Ridge, and El Verde Valley were chosen to investigate the effect of local phosphorus availability on phosphatase activity of four common species: *Cecropia schreberiana*, *Prestoea montana*, *Cyrilla racemiflora*, *Dacryodes excela*, and *Manilkara bidentata*. Root phosphatase activity is comprised of phosphomonoesterase (PME) and phosphodiesterase (PDE), both of which act on compounds to provide inorganic P. The two El Verde sites, on volcanic parent material contain higher amounts of available P than the Rio Icacos site on quartz-diorite. Fine root clusters collected from three trees of each species were assayed for PME and PDE activity. Results from a two-way repeated measures MANOVA indicate the importance of tree species in determining enzyme activity (P value <0.001) and that PME was produced in significantly higher amounts than PDE.

DNA extracted from the rhizosphere and endosphere of fine root clusters within El Verde Ridge and Valley sites were used for 16S rRNA gene profiling. Microbial community composition, assessed through QIIME, showed few visible differences in relative abundance between *Dacryodes excela* and *Prestoea montana*, indicating that microsite factors or high heterogeneity within species confound results. Taken together, these results suggest that tree species plays a much stronger role in determining phosphatase activity than in microbial community composition. This suggests that there are tree species differences in response to P limitation, which may influence distribution and ecosystem function under changing climates. Furthermore, the lack of similarity between microbial communities of the same species hints at an added layer of local-based acclimation to P availability.

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## Effect of citrate on *Aspergillus niger* phytase adsorption and catalytic activity in soil

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Phytase enzymes from bacteria, fungus and plant root exudates are known to hydrolyse organic phosphorus ( $P_o$ ) to bioavailable inorganic orthophosphate in soil. Exploiting such biochemical functions in agricultural systems, offers the potential for alternative sustainable phosphorus sources. Phytase adsorption to soil particles and phytate metal complexation has been shown to inhibit phytate dephosphorylation. Organic acid anions such as citrate increase phytase catalytic efficiency towards complexed forms of phytate, but the mechanisms are poorly understood. The aim of this work was to evaluate *Aspergillus niger* phytase inactivation and changes in its catalytic properties upon addition to soil, as well as the effect of citrate on phytase adsorption and activity towards free, precipitated and adsorbed phytate. *A. niger* phytase showed a relatively low absorption affinity for the Cambisol test soil. Phytase activity reduced by 37.3 % on adsorption. Citrate had no effect on the rate or total amount of phytase adsorption or residual activity thereafter. Free phytases and phytases in soil solution, showed optimum activity (> 80 %) at pH 4.5-5.5. Activity decreased slightly for immobilised enzymes compared to enzymes which were free or in soil solution > pH 5 and < 4 in the pH dependency curve. A decrease in activity was seen only at ionic strengths ( $NaCl$ ) > 0.6 M in immobilised and free enzymes, while activity from enzymes in soil solution reduced in all tested ionic strengths. Citrate significantly increased phytase activity towards phytate adsorbed to soil when the phytases were free ( $p \leq 0.003$ ) but not in other treatments (Na, Al and Ca-phytate). These results suggest that the effect of citrate on soil phytate dephosphorylation is associated with the availability of the substrate rather than its effect on the enzyme *per se*. The ionic strength and pH of soil solution has been shown to impact on phytase activity, suggesting that salinity, quality of irrigation water, wetting/drying cycles and fertilisation will have discrete impacts on the activity of phytases once released in soil and thus the ability to make  $P_o$  available for uptake by plants and microbes. The optimum acidic pH of *A. niger* also brings into question its suitability for application in many agricultural soils.

## Interactions of citrate and phytase exudates in the rhizosphere of tobacco (*Nicotiana tabacum*): Plant growth, microbial community structure, phosphatase gene abundance, and phosphorus species composition

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In order to sustainably intensify agricultural production, alternative and supplemental forms of phosphorus (P) are needed to replace or reduce the demand for inorganic phosphate fertilizers. Insoluble and organic forms of P are abundant in many soils, but are sparingly available to plants. The interaction of organic anions (OAs) and phosphatase enzymes has been shown to improve the availability of organic P under controlled experimental conditions in soils<sup>[1]</sup> or using synthetic mineral preparations<sup>[2]</sup>. The benefits of this interaction however have been less tractable in non-sterile plant-soil systems. Rhizosphere microorganisms may facilitate the acquisition of P by plants, for example, through the production of OAs, phosphatases, or phytohormones. In contrast, microbes may also limit the ability of plants to utilize soil P through processes such as P sequestration, degradation of root carboxylates, or through the reduction of key microbial functions such as phosphatase production. Transgenic tobacco plants (*Nicotiana tabacum*) that differentially express citrate and phytase either solely or in combination, were grown in a P limited soil to investigate the influence of citrate and phytase exudation (and the combination of these traits) on plant growth and P acquisition. In addition, we investigated the diversity of microorganisms associated with the root-soil interface (16S amplicon sequencing, fungal:bacterial abundance), the abundance of phosphatase (PhoC, PhoD) and beta-propeller phytase (BPP) genes, and the composition of soil P (determine by <sup>31</sup>P NMR) in the rhizosphere. Consistent with our previous study<sup>[1]</sup>, the growth of citrate- and phytase-exuding plants in intercropped combinations resulted in a greater accumulation of biomass and shoot P in comparison to individual plant-lines or plants lacking both traits, albeit with restricted plant growth due to P limitation. Preliminary analyses of soils indicated a reduction in BPP gene abundance in the rhizosphere of single-trait phytase-exuding plant lines relative to the other plant treatments. The poor growth of the unpaired phytase-exuding plant line may therefore be explained by a decrease in the production of phosphatases by soil microbiota in the presence of heterologous plant phytases. Information on microbial community structure and P species composition will be used to identify key mechanisms in the rhizosphere which could limit or enhance the effects of plant exudation on soil P utilization. These results suggest that the biological and biochemical components of the rhizosphere and their interactions could be managed to optimize the cycling of soil P for plant use.

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## Bioavailability of organic phosphorus across a riverine nutrient gradient

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A whole range of organic phosphorus compounds are present in our freshwaters as a result of export of organic matter from catchments and *in situ* decompositional processes. The increased loading of organic matter to freshwaters is a cause of concern, since organic nutrients may contribute to the enrichment of rivers and lakes and the resulting eutrophication problem of nuisance algal growth. However, the role of organic rather than inorganic phosphorus in promoting algal productivity has received little attention to date and few studies have considered the relative bioavailability of different organic phosphorus compounds across landscapes with differing productivity. This study presents some preliminary organic phosphorus bioassay data for phytoplankton and periphyton from two rivers in the UK, whose catchments differ geologically and in the intensity of agricultural land use. Three sites within each catchment were chosen to represent an environmental gradient of differing organic and inorganic nutrient concentrations. Periphyton were collected using artificial substrates deployed at monthly intervals at each site and phytoplankton were collected in water samples. In the laboratory, the periphyton and phytoplankton were separately exposed to five different phosphorus compounds and after two weeks, the biomass was measured as chlorophyll *a* pigment to assess bioavailability. Initial results from this work will inform on the role of organic, as well as inorganic, phosphorus as a driver of primary productivity across a gradient in river nutrient conditions.

## The effect of application of organic acids produced by citrus waste on the mobilization of inorganic and organic phosphorus present in cattle manure and its potential use as a biofertilizer in pastures growing in volcanic acid soils

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In recent times, the scarcity of phosphorus (P) has gained greater attention in the world due to the increase in the application of phosphorus fertilizers. These fertilizers are made from phosphate rock, a finite, non-renewable resource.

In addition, we know that the major characteristics of Chilean volcanic soils are the high adsorption capacity of P with a concomitant low P availability to plants; this situation is a problem due to that the Chilean Andisols are of great importance in the economy of Southern Chile supporting the bulk of agricultural production.

Cattle manure is one of the most widely used organic sources of plant phosphorus for crop production and its application to agricultural land has been viewed as an excellent way to recycle nutrients. Furthermore, P in cattle manure is present in inorganic (orthophosphate and pyrophosphate) and organic forms (phospholipids, DNA, phosphonates and phytate), which differ in their availability [1]

Organic phosphorus (Po) mineralization plays a key role in soil P cycling and may be involved in P availability to plants. Little is known about the capacity of organic acids in solubilizing Po in soil, but the low molecular weight organic acids may also induce Po release into soil solution although the mechanism for Po release is not well explained. These low-molecular mass organic acids occur widely in soils; they are secreted from plant roots and produced through the decomposition of plant residues. On the other hand, the organic acids are possible to find on citrus fruits. Citrus fruits are classed as acid fruits, because their soluble solids are composed mainly of organic acids and sugars. The acidity of citrus juices is due primarily to their content of citric and malic acids. Citric acid accounts for the largest portion of the organic acids in citrus fruits, and may make up as high as 60 percent of the total soluble constituents. The ability of low molecular weight organic acids to release inorganic anions, such as P, has been reported and has been attributed to desorption of inorganic anions and solubilization of phosphate compounds.

Now, we are doing incubation with cattle manure and citrus fruit residues to evaluate the mobilization of inorganic and organic phosphorus present in cattle manure and its potential use as a biofertilizer in pastures growing in volcanic acid soils.

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## Plant mobilization of soil P – what can the $\delta^{18}\text{O}_\text{P}$ tell us?

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Exudation of low molecular weight organic acids (LMWOA) by plant roots is suggested to increase the availability of scarcely available inorganic P, e.g. inorganic P adsorbed by oxides and hydroxides<sup>1</sup>. LMWOAs can also decrease the adsorption of organic P and thus make organic P available for enzymatic hydrolysis<sup>2</sup>. Due to the complex nature of the soil and the influence of other processes which increase the availability of P like acidification of the rhizosphere by the exudation of protons, the degree to which LMWOAs can increase the availability of P in soils is still under discussion<sup>3</sup>.

We will use the oxygen stable isotopes associated to P ( $\delta^{18}\text{O}_\text{P}$ ) in order to investigate the effect of LMWOAs on P availability in soils. Studies using the  $\delta^{18}\text{O}_\text{P}$  have focused mainly on the effect of phosphoenzymes on the  $\delta^{18}\text{O}_\text{P}$  of inorganic P and organic P, but the effects of other plant strategies to acquire P have been neglected. If LMWOAs affect the  $\delta^{18}\text{O}_\text{P}$ , then the use of the  $\delta^{18}\text{O}_\text{P}$  could possibly help to understand better the role of LMWOAs in increasing P availability in soils.

We will conduct three experiments. The first experiment will investigate the  $\delta^{18}\text{O}_\text{P}$  of different organic P compounds, including organic P extracted from soils and pure organic P compounds like phospholipids. The second experiment will be carried out using hydroponic systems in a controlled environment. Different inorganic and organic P compounds will be supplied in the nutrient solution. Plants which are known to exude phosphatase enzymes and LMWOAs from their roots will be grown in the nutrient solution for 3 weeks, and the  $\delta^{18}\text{O}_\text{P}$  of P fractions within the solution will be characterized after 0, 10 and 21 days. The three experiments will be carried out in pot trials in a glasshouse. The same plants used in the hydroponic experiment will be grown for six weeks in the three contrasting soil types. Soil samples will be taken and analyzed at the beginning of the experiment before planting and after 14 and 36 days replicates will be destructively sampled and analyzed.

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## Organic phosphorus fate in grassland Andisols with a poultry manure application history

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It is known that in the future the global phosphate rock mineral reserve will be depleted, so it will be necessary to find alternative sources to provide P to plants. Poultry meat production and the generation of its residues have increased. Poultry manure application to soils is considered as an agricultural management for the re-utilization of this material and also an important input source of nutrients for plant uptake. Although poultry manure has a high total P content (2%), the fate of the P when added to soil is not clear. With the aim to investigate the influence of continuous poultry manure addition in phosphorus soil mobilization, soil samples were taken at 5 different sites (Carilafquén, Copihual, San Ignacio, Santa Teresa and Huifquenco) where poultry manure applications (2 – 3 Ton ha<sup>-1</sup>) were performed. Sites consist in pasture systems development, sampling two paddocks of each site and its respective control which consisted of soils without application of poultry manure. Olsen P and total P content were higher in soils with poultry manure applications than the control. However, in the San Ignacio soils the values of Olsen P and total P content were lower in pasture soils with poultry manure than in the control soil. The phosphorus mobilization in the labile soil fraction ( $\text{NaHCO}_3$ ) showed a higher inorganic and organic phosphorus content in paddocks with poultry manure applications compared to the control soil. The total P content increased significantly for all soils analyzed in comparison to the control soil. The sparingly labile soil fraction ( $\text{NaOH}$ ) showed a higher inorganic phosphorus content in only 4 sampling sites with poultry manure applications. The organic P content increased in both paddocks with poultry manure in Huifquenco soils. In San Ignacio only in one paddock an increased was observed. While for Carilafquen, Copihual and Santa Teresa soils, the controls values were above the values obtained for paddocks with poultry manure applications. The total P contents were higher for Copihual, Santa Teresa and Huifquenco soils. San Ignacio soils remained with lower values than the control and in Carilafquen soil no differences in total phosphorus content were obtained. The application of poultry manure provide a high inorganic phosphorus content in the available soil fraction for plant uptake. Greenhouse and field assays will be necessary to determine the optimal dose and the pasture capacity to uptake this P source.

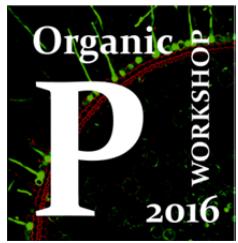
## Phosphorus uptake efficiency by wheat cultivars contrasting in aluminum tolerance growing in an aluminum-rich Andisol

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Phosphorus deficiency and aluminum (Al) phytotoxicity are major limitations for crop yield in acid soils. To ameliorate such limitations, agricultural management includes the application of lime, phosphorus (P) fertilizers and the use of Al-tolerant plant genotypes. While the mechanisms of Al tolerance and P efficiency may be closely related through strategies that decrease the toxicity of the Al<sup>3+</sup> ion and increase P availability in soils, the effects of soils with high Al saturation on P acquisition by wheat has scarcely been studied under field conditions in spite of the extended area used to produce this cereal worldwide. The aim of this work was to study Al-P interactions on wheat genotypes of contrasting Al tolerance when growing under field conditions in an Andisol with high Al saturation (32%) and low pH (5.0). A field plot trial experiment was performed using two Al tolerant (TCRB14 and TINB14) and one Al-sensitive (STKI14) winter wheat genotypes with the application of 0, 44 and 88 kg P ha<sup>-1</sup>. At the end of tillering and after physiological maturity (90 and 210 days after sowing), plants were harvested and yield, P and Al concentrations in shoots and roots were measured. Soil acid phosphatase, root arbuscular mycorrhizal (AM) colonization, AM spore number and glomalin were also determined. Shoot and root production and P uptake were higher in Al tolerant genotypes compared with the sensitive genotype. In addition, root mycorrhizal colonization and soil acid phosphatase activity were also higher in tolerant genotypes. In contrast, Al concentration in shoots and roots was higher in the sensitive genotype with a concomitant decrease in P concentration. Grain yield of Al-tolerant genotypes was also higher than that of the Al-sensitive genotype with and without P fertilizer. Overall, the Al tolerant genotypes were more effective at P acquisition from soil as well as from P fertilizer added, suggesting that plant traits such as Al tolerance, P efficiency, and mycorrhizal colonization potential are co-operating in overcoming adverse acid soil conditions.

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## Abstracts: Poster Presentations

### Session 4: Biotic Interactions in organic phosphorus cycling – Microbes

## Release of acid phosphatase from extraradical hyphae of arbuscular mycorrhizal fungus *Rhizophagus clarus* under low P condition

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Arbuscular mycorrhizal (AM) fungi enhance uptake of available phosphorus (P) from soil. The mechanism underlying this P uptake enhanced by AM fungi is the increase in surface area for absorption of available P. Little is known about utilization of unavailable P by AM fungi. We investigated whether extraradical hyphae of AM fungi exude ACP and whether ACP activity responds to phosphorus condition. Sterilized Andosol was packed in pots that were separated into the mycorrhizal and hyphal compartments with a nylon net of 30 µm pore size. Seeds of *Allium fistulosum* L. were inoculated or uninoculated with the AM fungus *Rhizophagus clarus* in P fertilized soil (0 and 0.5 g P<sub>2</sub>O<sub>5</sub> g<sup>-1</sup>). Soil solution was collected by using mullite ceramic tubes. Hyphal extracts were extracted from extraradical hyphae grown on sand culture and *in vitro* monoxenic culture, respectively. Root exudate of *A. fistulosum* was collected from hydroponic culture. The soil solution, hyphal extracts, root extract and root exudates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis. *L. usitatissimum* inoculated with *R. clarus* was grown on solid minimal media with three (3 and 30 µM) P levels. The extraradical hyphae and hyphal exudates were collected and subjected to analysis of ACP activity by using *p*-nitrophenylphosphate. P concentration, P content and dry weight of shoot were higher in the inoculated treatment than in the uninoculated treatment. Activity staining of the gel revealed that ACP activity at 187 kDa was observed in the soil solution in the inoculation treatment, and in the hyphal extract collected from sand culture and *in vitro* monoxenic culture, but neither in the root exudate of non-mycorrhizal plant grown in the hydroponic culture nor in the root extracts irrespective of mycorrhizal status. ACP activity of extraradical hyphae and hyphal exudates were higher in 3 µM treatment than 30 µM treatment. These findings suggest that the fungus releases ACP from extraradical hyphae into the hyphosphere under low P condition.

## Bacterial alkaline phosphatase in the rhizospheres of plants grown in Chilean extreme environments

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Bacterial alkaline phosphomonoesterase (APase) are relevant for organic phosphorus (Po) recycling in many soils. The ability of rhizosphere bacteria to mobilize insoluble P forms has been attributed to their capacity to: i) reduce pH by the exudation of organic acids and protons [1]; and ii) produce P-hydrolyzing enzymes [2]. Alkaline phosphomonoesterases (APase) are exclusively associated to metabolism of bacteria, fungi and earthworms [3], while acid phosphomonoesterase can be synthesized and released by both plants and soil microorganisms [2]. These enzymes are responsible for hydrolyzing Po to inorganic phosphate under P limiting conditions, particularly in alkaline soils [4, 5]. However, the abundance and diversity of bacterial APase in the rhizospheres of native plants have are poorly known, particularly in extreme environments. In this study, we studied the composition of total and APase-harboring bacterial communities, abundances of selected APase genes (*phoD* and *phoX*) and APase activities in rhizosphere soils from native plants grown in extreme environments of northern (Atacama Desert), central (Andes volcano [Quetrupillan and Mamuil Malal] and hot-spring [Liquiñe]) and southern-polar (Patagonia and Antarctic) regions of Chile. Differences in the composition of bacterial communities in the rhizosphere soils were revealed by denaturing gradient gel electrophoresis (DGGE) and quantitative PCR (qPCR) of 16S rRNA, *phoD* and *phoX* genes. In general, the significant lowest bacterial diversities, APase gene abundances and APase activities were observed in rhizosphere soils from Atacama Desert, whereas the highest values were observed in rhizosphere soils of Patagonia. In addition, APase gene abundances were positively correlated among them and with APase activity of rhizosphere soils, but negatively correlated with P availability in rhizosphere soils. Although bacterial APases were observed in all studied rhizosphere soils, their relevance to soil Po recycling in soils of extreme environments remains unclear and further studies are needed.

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## Aluminum-tolerant bacteria improve the phosphorous content in ryegrass grown in an Andisol amended with dung manure

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Plant crops highly depend on sufficient phosphorous (P) and nitrogen (N) uptake. In Chilean, acidic Andisols, crop production often is limited by a combination of the low concentrations of available P and significant levels of toxic aluminum (Al)<sup>1</sup>. In this study we aimed to isolate Al-tolerant plant growth promoting bacteria from the rhizosphere and the endosphere of ryegrass originating from acidic Chilean Andisols in order to constitute a bacterial consortium able to alleviate the Al<sup>3+</sup> toxicity and to support plant growth in Andisol<sup>2</sup>. A total of 5 strains, i.e. *Klebsiella* sp. RC3, *Stenotrophomonas* sp. RC5, *Klebsiella* sp. RCJ4, *Serratia* sp. RCJ6 and *Enterobacter* sp. RJAL6, were selected based on their capacity to tolerate high Al concentration (10 mM) and to exhibit multiple plant growth promoting traits under two P source (P solubilization, indol acetic acid production, 1-aminocyclopropane-1-carboxylate deaminase activity, organic acids and siderophore production). Our results showed that selected bacteria alleviated Al stress forming intracellular Al<sup>3+</sup>-siderophore complexes. The plant growth promoting potential of the consortium was confirmed in an assay with ryegrass plants. Enriched with dung manure the consortium was able to promote plant growth and the phosphatase activity in rhizosphere soils. Increased phosphatase activity thereby coincided with elevated P contents in shoot of the plants. Our results suggest that a combined amendment native Al-tolerant bacteria and organic fertilizers (e.g., dung manure) are suitable to reduce the Al toxicity and to promote the plant growth in Andisols of southern Chile.

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## Soil P bioavailability in a heathland restoration study on the Isle of Purbeck, UK

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The Isle of Purbeck is a multifunctional landscape with competing land uses including arable farming, livestock grazing, and recreational activities. Soil resources in the region have been under pressure from persistent physical and chemical manipulation. Historically, heathlands dominated the landscape but the expansion of agricultural lands and urban areas has resulted in a dramatic decline in lowland heaths. Initiatives such as the UK Biodiversity Action Plan [1] attempt to protect remaining areas and initiate heathland restoration projects on some agricultural lands.

Heathlands are typically characterised by low P and N availability but agricultural improvements involved large-scale application of amendments to increase soil pH, along with fertilisation to increase the productivity of the land. Subsequent restoration to heathland often involves chemical manipulations to lower the soil pH and re-establish heathland vegetation, thereby affecting the availability of nutrients and biochemical transformations in the soil. To examine the effectiveness of acidification on heathland restoration, an experiment was initiated in 1999 on National Trust land with applications of elemental sulphur or ferrous sulphate [2].

As part of the RECARE project (Preventing and Remediation Degradation of Soils in Europe through Land Care) funded by the European Commission FP7 Program, we are assessing soil degradation and the long-term effects of chemical and physical manipulations to the soil on the Isle of Purbeck. The heathland restoration experiment provides the opportunity to look at long-term effects of artificial acidification on P biochemistry across a range of soil pH and in relation to above- and belowground biodiversity. Preliminary results collected by Martinez et al. (unpublished data) in autumn 2014 suggest that treatment with elemental sulphur effectively decreased the soil pH to levels comparable to adjacent heathland. Available P (0-5 cm) had increased to 30.7 and 14.0 mg kg<sup>-1</sup> in the ferrous sulphate and elemental sulphur treatments compared to 8.9 and 3.3 mg kg<sup>-1</sup> for the control plot and target heathland. The microbial biomass of the acidified plots was significantly lower than the heathland targets and although there was no difference in microbial biomass P, there was a shift towards bacterial communities as demonstrated by phospholipid fatty acid analysis.

The potential bioavailability of P is being assessed by sequential chemical extractions to determine operationally defined inorganic and organic P pools. The impact of acidification treatments on soil P, along with changes in enzyme activities as an indicator biological functions, will be discussed in reference to the long-term reestablishment of heathland.

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## Phosphorus through the eye of the needle: On the mostly neglected role of soil biodiversity and biomass in affecting soil transformations?

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Soil P transformations involve complex physical, chemical, and biological reactions. Phosphorus is an essential nutrient to all life and many organisms have evolved with mechanisms to obtain P from less available sources, for example, excretion of organic acids and extracellular enzymes. In addition, orthophosphate tied up in microbial biomass will be released only when the cell is lysed, a process accelerated through grazing by soil fauna or protozoa. Through such mechanisms, the actions of soil biota and interactions among various components largely determine the quantity and turnover of organic P in soil.

Here we used a meta-analysis to develop a global perspective of the relationship between soil biodiversity and the transformation and fate of P. The primary objective of this synthesis was to understand how variations in P availability affect soil biota [abundance/function/ richness] and ultimately how these changes influence biologically mediated P transformations. Classically, the role of soil microbial biomass was described by Jenkinson [1] as the “eye of the needle” through which all key biogeochemical processes flow. Our hypothesis is that the soil organisms at multiple trophic levels, and thus the biomass, play the critical role reflecting Jenkinson’s “eye of the needle”.

A web of science search was conducted using search terms to include publications on soil and phosphorus and biodiversity. Soil organisms were grouped based on body size (i.e. microbes, microfauna, mesofauna, macrofauna) and further divided by trophic category where information was available. Since various methods are used to quantify soil phosphorus this creates difficulty in comparisons across studies, so only studies that reported a treatment and a control were included in the analysis. Likewise, it is possible to include different measures of soil biodiversity by considering the treatment effect relative to the control to determine if an overall change in soil P is correlated with changes in soil biota.

Although the initial goal of this synthesis was to include information on the functional outcome of soil biota on P transformations, there are few studies reporting such results and information was included where possible. Through reflection on past studies, we aim to gain a better understanding of the circumstances and to what extent soil biodiversity impacts P cycling and thereby identify priorities for future research.

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## Screening for phytase-producing bacteria from hydrothermal environments

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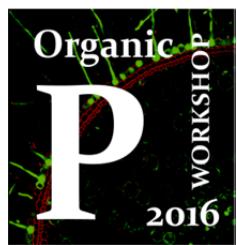
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Phytases are ecologically important enzymes involved in the organic phosphorus cycling in nature. These enzymes are widely used as feed additives to improve mineral bioavailability in animal diets [1]. For their biotechnological application, thermal tolerance is, among others, a desired property of phytases. However, so far thermal tolerance has scarcely been included into phytase-screening programs. The objective of this study was isolate and characterize thermo-tolerant bacterial phytases from Chilean hydrothermal environments (Liquiñe hot-spring [39°44'S; 71°51'W] and El Tatio geyser [22°19'S, 68°0'W]). Screening of 69 thermo-tolerant (60°C) culturable bacteria was carried out and the strains *Bacillus* sp. 9B and *Geobacillus* sp. 15 were selected and identified (16S rRNA gene) as producers for intra- and extracellular phytases. The results also indicate that both strains are able to synthesize more than one individual phytase. The characterization of intracellular phytase revealed that *Bacillus* sp. 9B produces phytase that shows an optimum pH at 5.0. This result suggests a novel property for phytases from genus *Bacillus* which are mostly characterized by β-propeller phytases with optimal pH in the range of 6.5 to 8.0. An optimum at pH ~5.0 is known for other phytase classes such as histidine acid phosphatase and purple acid phosphatase [2]. The strain *Geobacillus* sp. 15 also produces phytase that shows optimum pH at 5.0, but with an unusual residual activity at low pH (12% and 30% at pH 1.0 and 4.0, respectively). Most microbial phytases are inactive at low pH, except *Aspergillus niger* and *Escherichia coli* phytase [3,4]. The characterization of an acidic thermo-tolerant phytase produced by *Geobacillus stearothermophilus* from hot-spring has recently been reported [5]. The temperature optimum for phytate dephosphorylation was determined to be 60°C for the phytase from *Bacillus* sp. 9B and 50°C for the *Geobacillus* sp. 15 phytase. Interestingly, the phytase from *Geobacillus* sp. 15 shows a residual activity of 46% after incubation at 90°C for 20 min. Our study demonstrates that Chilean hydrothermal environments represent an unexplored source of novel thermo-tolerant bacterial phytases with more favorable properties for biotechnological applications, for example in food processing or as feed additives.

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## Abstracts: Poster Presentations

### Session 5: Organic phosphorus in soil and waters: Stocks, flows and impact of scale

## Translocation of P-carrying natural nanoparticles and colloids in forest soil mesocosm effluents

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Soil nanoparticles ( $d < 100\text{nm}$ ) and colloids ( $d < 1\mu\text{m}$ ) exert a decisive control on the mobilisation of strongly sorbing compounds such as phosphorus (P), due to their surface charge and high specific surface area. We investigated the nanoparticles and colloids (NPC) present in forest soil leachates examining their role in the transfer of P in soils. Mesocosm experiments with three German forest soils (upper 20 cm) were conducted. The mesocosms were planted with young beech trees and three different treatments were imposed: i) Ambient: irrigation with artificial rain, ii) Acidification: irrigated after treatment with ammonium-nitrate and iii) Liming: irrigation after application of carbonate. Soil leachates from three soil depths (below the organic layer, the upper mineral soil, and mineral soil) were collected. The leachates were analysed concerning their vertically translocated NPC-associated P content.

The field flow fractionation (FFF) technique coupled online to UV- and DLS- detectors and inductively coupled plasma mass spectrometry (ICP-MS) or to an organic carbon detector (OCD) enabled a size resolved characterization and quantification of the nanoparticulate and colloidal fractions and their elemental composition (P, C<sub>org</sub>, Fe, A, Si, Ca, Mn). To visualise and better characterise the particles present in the leachates, transmission electron microscopy with energy-dispersive x-ray spectroscopy (TEM-EDX) measurements were performed.

The leachates from the various soil depths showed distinct compositions pointing interactions of translocated NPC with the soil matrix. The translocated particles exhibited sizes up to 350 nm with up to 90% (on average ~30 %) of the leached P being associated with these NPC. The acidification treatment enhanced the mobilisation of NPC especially NPC carrying P and C<sub>org</sub>. The higher NPC-P output was probably linked to the higher NPC-C<sub>org</sub> output indicating that a higher percentage of NPC-P<sub>org</sub> was mobilised by acidification. Furthermore, the liming enhanced the NPC mobilisation of both P and calcium. Our qualitative and quantitative analysis of the soil leachates showed that NPC-P is a crucial vector controlling the P fluxes in forest ecosystems and could be a significant, but as yet still poorly quantified P loss factor.

## Examination of long-term soil development and phosphorus dynamics in a hypermaritime chronosequence, Calvert Island, British Columbia, Canada

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Long-term soil chronosequences are used to understand ecosystem succession, nutrient cycling and soil development through space-time substitution. This approach has been central to studies of ecosystem retrogression - the pronounced decline in primary productivity that accompanies soil aging in diverse environments<sup>1,2</sup>. Ecosystem retrogression has large implications on soil phosphorus (P) forms, plant diversity, litter quality, and plant nutrient acquisition strategies<sup>1,3,4,5,6</sup>. However, ecosystem retrogression cannot be examined unless the time span of the chronosequence is great enough to show the prominent biomass declines with age<sup>1</sup>. In this study, a newly identified chronosequence on Calvert Island, British Columbia, Canada spanning 11,000 years will be used to examine soil development and P transformations with age. This is the oldest, documented chronosequence on the British Columbia coast, and has the potential to be characterized as “retrogressed”. This chronosequence is located in a humid, temperate environment, and represents a series of coastal sand dunes. Visual examination of these dunes indicates progressive soil development and podzolization with increasing age, and previous analysis illustrates low levels of phosphorus on the oldest site. We will present P data from samples collected in spring 2016, including: total P, organic P and Mehlich P results. It is anticipated that total P and available P will significantly decline along the chronosequence; whereas, total organic P will increase to a maximum, and then decline on the oldest site. Further research on this chronosequence will include analysis of P using P-NMR on the organic horizons and enriched mineral horizons, as well as plant foliage and litter.

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## Nutrient losses and reductions: a comparison of physiographic regions and conservation practice in North Carolina

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Agricultural nonpoint source pollution often represents 50% of the loads to water resources (estuaries and lakes) in North Carolina (NC) [1]. Water resources in NC are co-limited so it is essential to reduce both sources of nutrients in the watersheds and river basins, such as the Neuse and Tar-Pam River Basin and Jordan and Falls Lakes, which are nutrient impaired. Four-paired watersheds have been monitored in the Jordan Lake watershed (piedmont region) for eight years and three-paired watersheds have been monitored in the Neuse River Basin (coastal plain watershed) for two years. These watersheds represent different physiographic regions within the state of NC. Pre-conservation treatment flow and nutrient concentration data have been collected in all watersheds; post-conservation treatment data flow and nutrient concentration in the Jordan Lake watersheds to determine effectiveness of conservation practices. Land use information and rainfall have also been collected. Conservation practices to exclude organic cattle waste from a stream in a pasture reduced both nitrogen and phosphorus export via stream discharge, whereas nutrient management had no effect on nutrient loads in runoff from cropland. Despite lower concentrations of nutrients in the Neuse River Basin watersheds, total loads were about the same between physiographic regions.

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