Organic Phosphorus 2013
Integration across Ecosystems

4–7 February 2013

Smithsonian Tropical Research Institute
Republic of Panama

Edited by:
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Welcome and Introduction

On behalf of the organizing committee, it is my pleasure to welcome you to the Smithsonian Tropical Research Institute in Panama for Organic Phosphorus 2013: Integration across Ecosystems.

Phosphorus is essential for life, but is commonly the element that limits productivity in both terrestrial and aquatic ecosystems. From tropical rain forests to the open oceans, organisms depend on organic phosphorus turnover for their phosphorus nutrition and have evolved complex adaptations that allow them to compete efficiently for this scarce resource. Yet organic phosphorus continues to be overlooked in biogeochemical and ecological research, and remains poorly understood in comparison to inorganic phosphorus. This limits our understanding of ecosystems and constrains our ability to develop sustainable agricultural practices and predict the response of the earth’s major biomes to global change.

Ten years ago, the first organic phosphorus workshop was held in the inspirational surroundings of Monté Verita – a hilltop retreat in the mountains of southern Switzerland. The aim was to unite a multidisciplinary group of scientists seeking to unravel the complexity of organic phosphorus in the environment. The meeting laid the foundation for a decade of research and resulted in collaborations and friendships that continue to this day.

Organic Phosphorus 2013 continues in the spirit of Monté Verita by combining state-of-the-art presentations at the Smithsonian headquarters in Panama City with workshop-style discussion and debate in the relaxed atmosphere of Barro Colorado Island, the most intensively studied tropical forest on the planet. Our aims are to review advances since the first meeting in Switzerland and define priorities for the next decade of research on organic phosphorus in the environment.

The workshop was made possible by the hard work of a number of people. We thank Audrey Smith and Ana Matilde Ruiz for planning and administration of the workshop, Oris Acevedo and her staff for support on Barro Colorado Island, Juan Perez for construction of the workshop website, and Dayana Agudo and Stephanie Bratkovics for practical assistance during the workshop. Adina Paytan provided financial support for US-based students to attend the workshop through a grant from the US National Science Foundation. Finally, the Smithsonian Tropical Research Institute generously provided the use of the Earl S. Tupper Research and Conference Center and granted access to Barro Colorado Island Research Station, and we thank the STRI Director Dr Eldredge Bermingham for his support.

We hope you enjoy your stay in Panama, and we look forward to what promises to be a fascinating and productive workshop.

Benjamin L. Turner

Smithsonian Tropical Research Institute, Republic of Panama
# Program at a Glance

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Detailed Program

Monday 4 February 2013

8:40–8:50: Ben Turner, Smithsonian Tropical Research Institute, Panama
Welcome and introduction

Session 1: Controls on the amounts and forms of soil organic phosphorus

8:50–9:10 David Nash, Victorian Department of Primary Industries, Australia
A meta-analysis of climate and soil effects on forms of soil organic phosphorus

9:10–9:30 Martin Blackwell, Rothamsted Research, UK
Isolating the influence of pH on the amounts and forms of soil organic phosphorus

9:30–9:50 Andrea Vincent, Swedish University of Agricultural Sciences
Speciation of soil organic phosphorus in tropical and boreal forests: effects of litterfall, metals, and time

9:50–10:10 Charlotte Jørgensen, University of Southern Denmark
Binding sites for inositol phosphates in soil and effects on their mobility

10:10–10:40 Coffee break

Session 2: Stability and bioavailability of organic phosphorus in soil

10:40–11:00 Luisella Celi, Università di Torino, Italy
Role of iron oxides in the fate of inositol phosphates in soil

11:00–11:20 Reiner Giesler, Umeå University, Sweden
Approaching near-surface processes in studying organic phosphorus

11:20–11:40 Tegan Darch, Rothamsted Research, UK
Using plant exudates and enzyme hydrolysis to determine plant available phosphorus in tropical soils

(session continues overleaf)
Courtney Giles, University of Vermont, USA

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

12:00–13:10  Lunch

Session 3:  Organic phosphorus in agroecosystems

Leo Condron, Lincoln University, New Zealand

Soil organic phosphorus mineralization in managed grassland and forest ecosystems

Tandra Fraser, University of Guelph, Canada

Soil phosphorus turnover in long-term organic and conventional management systems

Yuki Audette, University of Guelph, Canada

Effect of animal manure composts on soil phosphorus chemistry

William Roberts, Lancaster University, UK

Organic phosphorus retention in riparian vegetated buffer strip soils

14:30–16:00  Posters and coffee (see page 8 for a list of poster presentations)

Session 4:  Forms and dynamics of organic phosphorus in wetlands

Alexander Cheesman, Smithsonian Tropical Research Institute, Panama

Organic phosphorus forms and composition in palustrine wetland soils

Susan Newman, South Florida Water Management District, USA

Phosphorus transformations in emergent and submersed macrophyte dominated wetlands

William Cooper, Florida State University, USA

Linking phosphorus sequestration to carbon humification in wetland soils

17:00–18:00  Drinks
Tuesday 5 February 2013

8:40–8:50  Ben Turner, Smithsonian Tropical Research Institute
            *Introduction and BCI logistics*

**Session 5:**  **Microbes, genes, and organic phosphorus acquisition**

8:50–9:10  Else Bünnemann, ETH Zurich, Switzerland
            *The role of microbial processes in soil phosphorus cycling: approaches and outcomes*

9:10–9:30  Milko Jorquera, Universidad de La Frontera, Chile
            *Bacillus β-propeller phytase genes in pasture rhizosphere and volcanic soils from southern Chile*

9:30–9:50  Elise Morrison, University of Florida, USA
            *The composition and abundance of phosphorus-utilizing genes within Everglades microbial communities*

9:50–10:10 Curt Richardson, Duke University, USA
            *Differential nutrient limitation of soil microbes across ecosystems: Does phosphorus really matter?*

10:10–10:40  **Coffee**

**Session 6:**  **Phosphorus and tropical forest productivity**

10:40–11:00  Xiaojuan Yang, Oak Ridge National Laboratory, USA
            *Modeling phosphorus dynamics and phosphorus limitation on productivity in tropical forests*

11:00–11:20  Kasper Reitzel, University of Southern Denmark
            *Soil solution organic and condensed inorganic phosphorus in lowland tropical forests*

**Session 7:**  **Organic phosphorus in the marine environment**

11:20–11:40  Adina Paytan, University of California Santa Cruz, USA
            *Oxygen isotope of phosphate as an indicator of organic phosphorus utilization in the ocean*
11:40–12:00 Jason Chin, The Queen’s University of Belfast, UK  
*Phosphate insensitive aminoalkylphosphonate degradation in marine environments*

12:00–13:10 Lunch

**Session 8: Organic phosphorus in freshwater ecosystems**

13:10–13:30 Barbara Cade-Menun, Agriculture and Agri-Food Canada  
*Characterizing dissolved and particulate phosphorus forms in runoff from agricultural lands*

13:30–13:50 Julia Paraskova, Uppsala University, Sweden  
*Can phospholipids and nucleic acids shed light on organic phosphorus turnover in lake sediment?*

13:50–14:10 Philip Haygarth, Lancaster University, UK  
*Organic phosphorus compounds in riverbed sediment from source to estuary*

14:10–14:40 Coffee

**Session 9: Phosphorus in sewage and wastewater**

14:40–15:00 Will Brownlie, NERC Centre for Ecology and Hydrology, UK  
*The impact of human domestic behavior on phosphorus compounds in private sewage systems in the Loch Leven catchment (Scotland, UK)*

15:00–15:20 Darragh Hobbs, The Queen’s University of Belfast, UK  
*Can environmental stress be exploited for enhanced biological phosphorus removal?*

15:20–15:40 Bryan Spears, NERC Centre for Ecology and Hydrology, UK  
*Assessing the impact of a rural development policy on the phosphorus load from septic tanks to the Loch Leven catchment (Scotland, UK)*

16:00 Depart for Barro Colorado Island
Poster Presentations

Poster 1: Soil phosphorus forms and distribution determined by $^{31}$P NMR spectroscopy influenced by tillage practices
*Dalel Abdi, Université Laval, Canada*

Poster 2: Isolation of phytase-producing rhizobacteria from different natural ecosystems and agro-ecosystems of northern, central and southern Chile
*Jacquelin Acuña, Universidad de La Frontera, Chile*

Poster 3: Soil phosphorus fertility under organic nutrient management
*Yuki Audette, University of Guelph, Canada*

Poster 4: Delays, degrees and degradation: Evaluating the effects of experimental parameters on $^{31}$P NMR results
*Barbara Cade-Menun, Agriculture and Agri-Food Canada*

Poster 5: Improved peak identification in $^{31}$P NMR spectra with a standardized method and peak library
*Barbara Cade-Menun, Agriculture and Agri-Food Canada*

Poster 6: Improving bioavailability of phosphorus from cattle dung by using phosphatase immobilized on natural clay and nanoclay
*Marcela Calabi-Floody, Universidad de La Frontera, Chile*

Poster 7: A new solution $^{31}$P NMR extraction procedure for sediment samples with low phosphorus content
*Delphine Defforey, University of California Santa Cruz, USA*

Poster 8: Changes in soil organic phosphorus and their relationship with nutrient limitation in Chilean temperate forests
*Aurora Gaxiola, Institute of Ecology and Biodiversity and Pontificia Universidad Católica de Chile*

Poster 9: Characterization of particulate phosphorus in a hydrologic transect of the Lake Champlain Basin, Vermont, USA: phosphorus species analysis and enzyme lability
*Courtney Giles, University of Vermont, USA*
Poster 10: Phosphorus lability and speciation in two agricultural soils by sequential fractionation, solution $^{31}$P NMR, and phosphorus K-edge XANES spectroscopy
Jin Liu, Zhejiang University, China
(presented by Barbara Cade-Menun, Agriculture and Agri-Food Canada)

Poster 11: Distribution and hydrolyzability of organic phosphorus in aggregates of highly weathered tropical soils
M. Nesper, ETH Zurich, Switzerland
(presented by Else Bünemann, ETH Zurich, Switzerland)

Poster 12: Linking soil partitioning, crop acquisition and compositions of novel phosphorus fertilizers to improve phosphorus resource management
Marc Stutter, The James Hutton Institute, UK
(presented by Martin Blackwell, Rothamsted Research, UK)

Poster 13: Contribution of recalcitrant phosphorus to phosphorus fractionation in an Andisol
Gabriela Velásquez, Universidad de La Frontera, Chile

Poster 14: Fate and transport of phosphorus compounds in the River Eden, UK
Ying Wang, Lancaster University, UK
Abstracts

Abstracts of oral presentations appear in chronological order, followed by abstracts of poster presentations in alphabetical order. All abstracts were edited for consistency of style, spelling, and grammar according to the American Society of Agronomy style guidelines. In some cases, abstracts were also edited to fit the one-page limit.
A meta-analysis of climate and soil effects on forms of soil organic phosphorus

David Nash\textsuperscript{1}, Murray Hannah\textsuperscript{1}, Richard McDowell\textsuperscript{2}

\textsuperscript{1}Future Farming Systems Research Division, Victorian Department of Primary Industries, Ellinbank, Australia
\textsuperscript{2}Land and Environment Group, AgResearch Limited, Invermay Agricultural Research Centre, Mosgiel, New Zealand

This paper presents a meta-analysis of soil organic phosphorus species in soils used for primary production (i.e. food and fiber). The meta-data used in the study included over 600 individual soil analyses from more than fifty different sites. Both relative and absolute concentrations of monoesters and diesters were considered in the study in addition to more specific compounds such as DNA. Random forests, an ensemble classifier consisting of many regression trees, were used to analyze the database for climatic (including Koppen-Geiger Classification), soil and land use characteristics.
Isolating the influence of pH on the amounts and forms of soil organic phosphorus

Martin S.A. Blackwell¹, Benjamin L. Turner²

¹Rothamsted Research, North Wyke, Okehampton, UK
²Smithsonian Tropical Research Institute, Republic of Panama

Soil pH influences the chemistry, dynamics, and biological availability of phosphorus, but few studies have isolated the effect of pH from other soil properties. We studied phosphorus chemistry in soils along the Hoosfield acid strip (Rothamsted, UK), where a pH gradient from 3.7 to 7.8 occurs in a single soil with little variation in total phosphorus (mean ± standard deviation 399 ± 27 mg P kg⁻¹). Soil pH was an important factor in determining the proportion of phosphomonoesters and phosphodiesters in the soil organic phosphorus, although total organic phosphorus concentrations were a relatively consistent proportion of the total soil phosphorus (36 ± 2%) irrespective of soil pH. In soils with pH < 4 organic phosphorus increased by ~ 20%, because inositol hexakisphosphate, DNA, and phosphonates were all present at highest concentrations. The increase in organic phosphorus in the most acidic soils was not related to organic carbon, because concentrations declined at pH < 4.0. Thus, the organic carbon to organic phosphorus ratio declined from ~ 70 in neutral soils to ~ 50 in strongly acidic soils. Readily-exchangeable inorganic orthophosphate extracted with anion-exchange membranes and a more stable inorganic pool extracted in NaOH–EDTA both increased markedly as soil pH declined. Inorganic orthophosphate concentrations were correlated strongly and negatively with amorphous manganese and positively with amorphous aluminum, suggesting that soil pH influences orthophosphate stabilization via metal oxides. We conclude that pH has a relatively minor influence on the total amounts of organic phosphorus in soil, but is an important factor in determining the forms of organic phosphorus present under strongly acidic conditions.
Speciation of soil organic phosphorus in tropical and boreal forests: effects of litterfall, metals, and time

Andrea G. Vincent¹, Benjamin L. Turner², E.V.J. Tanner³, J. Vesterågren⁴, P. Persson¹, G. Gröbner⁴, D.A. Wardle¹, J. Schleucher⁴, Reiner Giesler⁴

¹Swedish University of Agricultural Sciences, Umeå, Sweden
²Smithsonian Tropical Research Institute, Republic of Panama
³Department of Plant Sciences, Cambridge University, Cambridge, UK
⁴Umeå University, Umeå, Sweden

Understanding the factors that control the speciation of organic phosphorus is crucial to elucidate its biogeochemistry and role in ecosystem function. Here I present results from three studies in which solution ³¹P NMR spectroscopy was used to speciate organic phosphorus in soils from a long-term ecological experiment and two natural environmental gradients. In many tropical forests soils, most of the phosphorus is present in organic molecules or fixed onto metal oxides and considered stable and unavailable to plants. Three years of litter removal in a large-scale experiment in Central Panama reduced organic phosphorus in surface mineral soil by 23%, whereas three years of litter addition increased it by 16%. Organic phosphorus was dominated by monoesters and diesters, but no inositol phosphates were detected. The calculated turnover rate suggests that the upper 2 cm of the mineral soil contributes a fifth of the phosphorus needed to sustain above-ground tree growth, suggesting that organic phosphorus in surface soil is not stable and makes an important contribution to the nutrition of tropical forest plants. In a second study, changes in organic phosphorus speciation were characterized along a short but extreme phosphorus-sorption gradient caused by groundwater recharge and discharge in Northern Sweden. Boreal forests grow on podzols with thick humus horizons, but phosphorus dynamics are surprisingly similar because in both cases metal oxides can confer a high phosphorus fixation capacity. The composition of organic phosphorus changed sharply along the gradient: phosphate diesters were more abundant in sites with low metal oxide concentrations, whereas inositol phosphates dominated in sites with high metal oxide concentrations. As this gradient in metals encompasses the variation in soil properties in the wider Fennoscandian forest landscape, these findings provide insight into the factors controlling phosphorus speciation in the region. Finally, time is one of the main factors controlling the composition of soil phosphorus in general, but less is known about the effects of time and ecosystem development on the composition of organic phosphorus, and even less on the composition of organic phosphorus in young high latitude soils. I show preliminary results from a study in which we use ³¹P NMR and two dimensional, ¹H–³¹P correlation NMR to characterize changes in organic phosphorus across a 7800-year old chronosequence in northern Sweden, with a special emphasis on the monoester region.
Binding sites for inositol phosphates in soil and effects on their mobility

Charlotte Jørgensen¹, Benjamin L. Turner², Henning S. Jensen¹, Kasper Reitzel¹

¹University of Southern Denmark, Odense, Denmark
²Smithsonian Tropical Research Institute, Republic of Panama

Inositol hexakisphosphate (IP₆) is considered to be a relatively ubiquitous and recalcitrant form of organic phosphorus due to strong sorption and precipitation reactions in soil and sediments. In particular, amorphous metal oxides (i.e. extracted by oxalate) correlate with organic phosphorus and IP₆ in soils, suggesting that IP₆ is stabilized specifically by association with amorphous aluminum and iron. We examined the relationship between organic phosphorus and oxalate extractable aluminum and iron in soils known to contain IP₆ and in soils from tropical rainforests where IP₆ has not been detected. Soils were extracted with ammonium oxalate–oxalic acid adjusted to pH 3.0, the extracts were shaken with cation-exchange resin to remove paramagnetic ions, and pH was adjusted to 12–13. The remaining soil pellet was extracted with 0.25 M NaOH–0.05 M EDTA. Both extracts were freeze-dried and analyzed by solution ³¹P NMR spectroscopy. Only IP₆ and orthophosphate were detected in oxalate extracts, while other orthophosphate monoesters, diesters and pyrophosphate were detected only in the NaOH extracts. This clearly supports the evidence for binding of IP₆ in soils by amorphous aluminum and iron (hydr)oxides. A second study of sediments in an oligotrophic and slightly acidic Danish lake further supports the association of IP₆ to amorphous metal oxides. In this lake, IP₆ binds partly to humic matter via aluminum or iron bridges and partly to aluminum (hydr)oxides or fulvic acids. The IP₆ associated with humic matter was partly mobilized by a pH increase to 6–7, but not by a decline in redox potential. In conclusion, these studies demonstrate that IP₆ is bound to amorphous metals in soils and sediments, and we propose the use of oxalate extraction combined with ³¹P NMR spectroscopy as a relatively selective method for isolating and identifying IP₆ in the environment.
Role of iron oxides in the fate of inositol phosphates in soil

Luisella Celi, Maria Martin, Eleonora Bonifacio, Daniel Said-Pullicino, Elisabetta Barberis

Università di Torino, DISAFA-Chimica Agraria, Torino, Italy

The selective stabilization of inositol phosphates in soil is linked to their high affinity for iron oxide surfaces, which hampers their degradation and controls their fate in soil. This presentation will review the factors that control adsorption thermodynamics and kinetics of inositol phosphates on iron oxides differing in mineral properties, such as the degree of crystallinity, the proportion of reactive sites for adsorption on the total area, and the distances between contiguous hydroxyls. Computational studies show the optimal arrangement of the myo-inositol hexakisphosphate molecule on the mineral surface. Furthermore we will evaluate how the strength and extent of inositol phosphate binding to the minerals affect phytase activity and extractability in NaOH–EDTA.
Approaching near-surface processes in studying organic phosphorus

Reiner Giesler¹, Janice Kenney², Per Persson²

¹Climate Impacts Research Centre, Department of Ecology and Environmental Science, Umeå University, Abisko, Sweden
²Chemistry Department, Umeå University, Umeå, Sweden

Esters of phosphoric acid constitute a sizable fraction of the total phosphorus supply in the environment and thus play an important role in the global phosphorus cycle. Enzymatic hydrolysis of these esters to produce orthophosphate is often a required reaction preceding phosphorus uptake by plants and microorganisms. Generally, adsorption to environmental particles is assumed to limit this process. Here we show how spectroscopic techniques, such as Fourier Transform Infrared spectroscopy (FTIR), can be used as a tool to study surface interactions of sorbed organophosphates and enzymes or in bioassays. In an experimental study using glucose-1-phosphate sorbed to goethite (α-FeOOH), we found that the rate of acid phosphatase hydrolysis was of the same order of magnitude at the surface as in aqueous solution. The surface process releases carbon to the solution, whereas orthophosphate remains adsorbed on goethite. The hydrolysis reaction is strictly an interfacial process governed by the properties of the interface. Our results suggest that mineral surfaces with appropriate surface properties can be effective in concentrating substrates and enzymes, thereby creating micro-chemical environments of high enzymatic activity. The results also indicate that strongly adsorbed molecules in soils and aquatic environments may be subjected to biodegradation by extracellular enzymes. We are now using a similar approach to study the interface between live microorganisms growing at mineral surfaces with adsorbed organic phosphorus compounds. The FTIR technique allows us to detect temporal chemical changes at the nanomolar level at the interface between hyphae or bacteria and the mineral surface and we will present some preliminary results from this approach. Finally, our research team at Umeå University has been working recently to apply two dimensional $^1$H – $^{31}$P NMR to natural samples and we will report the latest results from these studies.
Using plant exudates and enzyme hydrolysis to determine plant available phosphorus in tropical soils

Tegan Darch¹,², Martin Blackwell¹, Jane Hawkins¹, Philip M. Haygarth², David Chadwick³, Benjamin L. Turner⁴

¹Rothamsted Research, North Wyke, Okehampton, UK
²Lancaster Environment Centre, Lancaster University, Lancaster, UK
³School of Environment, Natural Resources and Geography, Environment Centre Wales, Bangor University, Bangor, UK
⁴Smithsonian Tropical Research Institute, Republic of Panama

Organic phosphorus is abundant in tropical forest soils, but there is little information on its potential bioavailability. Plants can utilize soil organic phosphorus by exuding organic acids to solubilize phosphorus strongly bound to soil constituents. The solubilized organic phosphorus is then amenable to hydrolysis by extracellular phosphatase enzymes, releasing orthophosphate for uptake by roots. This raises the possibility that organic acid extracts could be used to estimate plant-available organic phosphorus in tropical soils. We used citric acid extraction and phosphatase hydrolysis to quantify biologically available soil organic phosphorus in five soils taken from the surface 10 cm of tropical forest plots in the Republic of Panama. We used 2 mM citrate to approximate rhizosphere organic anion concentrations; higher citrate concentrations appeared to cause dissolution of mineral surfaces and interfered with colorimetric detection of phosphate. Citric acid solubilized significantly more organic phosphorus than deionized water, but less than a conventional NaHCO₃ extraction, which is used in the Hedley fractionation procedure and assumed to represent ‘plant available’ organic phosphorus. In citric acid extracts, 71% of the extracted phosphorus was organic, of which 53% was hydrolysable by enzymes. Across the five soils, there was no significant difference between the quantities of enzyme hydrolysable organic phosphorus and reactive (inorganic) phosphorus, indicating that citrate-extractable organic phosphorus is a potentially important plant available pool. The proposed method provides information on potentially bioavailable organic phosphorus and is suitable for use in conjunction with organic phosphorus speciation methods such as phosphatase hydrolysis.
The role of plant and bacterial organic anion production in plant access to insoluble organic phosphorus

Courtney D. Giles¹, Gregory K. Druschel², Alan E. Richardson³, Jane E. Hill⁴

¹Experimental Program to Stimulate Competitive Research, University of Vermont, Burlington, Vermont, USA
²Department of Earth Sciences, Indiana University-Purdue University, Indianapolis, Indiana, USA
³CSIRO Plant Industry, Canberra, Australian Capital Territory, Australia
⁴School of Engineering, University of Vermont, Burlington, Vermont, USA

The inefficient use of phosphorus in agricultural systems has led to the global depletion of mineral phosphate supplies as well as the pollution of surface waters. By improving crop access to native soil phosphorus, external fertilizer requirements and agricultural nutrient losses could be minimized. myo-Inositol hexakisphosphate (IP₆) is a dominant organic phosphorus compound in soils and can be converted to plant-available orthophosphate through the action of plant or microbial phytases. In soils, IP₆ hydrolysis can be hampered by IP₆ precipitation and sorption to the solid phase, or by low solution phytase activity. Previous studies suggest that IP₆ bioavailability can be improved in the presence of organic anions (e.g., oxalate, citrate, gluconate) from plant or microbial sources. The influence of plant and bacterial organic anions on IP₆ solubility and bioavailability was assessed in tobacco (Nicotiana tabacum) grown on insoluble phosphorus sources (IP₆ and orthophosphate) which were (1) sorbed to goethite and (2) precipitated with calcium. Wild-type tobacco was transformed to over-express Peniophora lycii phytase and Multidrug-And-Toxic-Compound-Extrusion-type transporters (CIT), resulting in 33-fold higher phytase activity and 2.6-fold higher citrate in exudates, respectively. Consistent with exudate characteristics, IP₆ sorbed to goethite was four-fold more available to phytase plants, and orthophosphate sorbed to goethite was two-fold more available to the CIT plants relative to the wild type. In calcium phytate media, the phytase plant-line incorporated > 20-fold more shoot phosphorus than other plant-lines. The addition of an organic acid-producing Pseudomonas sp. to the calcium phytate growth system led to seven- and ten-fold more shoot phosphorus in wild type and CIT plants, respectively. Shoot P did not improve for inoculated phytase plants, likely due to direct hydrolysis of calcium phytate by exogenous plant phytases. A combination of phosphorus solubilization and hydrolysis services is therefore suggested as a suitable target for improving crop utilization of native soil phosphorus.
Soil organic phosphorus mineralization in managed grassland and forest ecosystems

Leo Condron, Ivan Chirino-Valle

Agriculture and Life Sciences, Lincoln University, Canterbury, New Zealand

It is acknowledged that the organic and microbial forms of phosphorus play an important role in determining the productivity and sustainability of managed grassland and forest ecosystems in a temperate environment such as New Zealand. However, quantifying the role and mechanisms of organic phosphorus mineralization in soil-plant systems remains a challenge. A variety of analytical techniques have been developed and applied to this task, including soil phosphorus fractionation, phosphatase enzyme assays and \(^{31}\)P NMR spectroscopy. This paper presents recent findings from three long-term field experiments (two grassland and one forest) that illustrate the deployment and relative success of different approaches to assessing and quantifying soil organic phosphorus mineralization. For example, results of analysis of soils obtained from a field trial that had been maintained under contrasting mowing regimen (no mowing, mowing with clippings retained, mowing with clippings removed) for 15 years showed that clippings retention enhanced short-term plant phosphorus availability by 50%. This was mainly attributed to increased biological activity, which was not reflected in the relative solubility of soil phosphorus. Furthermore, data from a grassland afforestation field trial, which included three contrasting tree species, revealed that changes in the nature and mineralization of soil organic phosphorus during the 10 years following forest establishment were driven mainly by processes associated with the cessation of grazing rather than the actions of tree roots and associated micro flora.
Soil phosphorus turnover in long-term organic and conventional management systems

Tandra Fraser¹, D. Lynch², M. Entz³, K. Dunfield¹

¹School of Environmental Science, University of Guelph, Ontario, Canada
²Faculty of Agriculture, Dalhousie University, Truro, Nova Scotia, Canada
³Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada

Low plant available phosphorus levels have been reported in organically managed soils across Canada. Manure can be a valuable source of phosphorus but large distances may prohibit regular application in the Great Plains region. Replacement of phosphorus in long-term organic systems is challenging since limited options are available under organic certification, making the turnover of organic phosphorus in these soils important. Soil samples were taken from the wheat phase of a long-term field experiment near Winnipeg, Canada, established in 1992. Soil phosphorus pools and turnover of organic phosphorus were measured in the wheat phase of a forage-grain rotation (flax-alfalfa-alfalfa-wheat) managed as organic no input, manure-amended organic (fall applied, 2002 and 2011 only), conventional, and restored native prairie systems. In spring 2011, Hedley phosphorus fractionation revealed significantly lower concentrations of both soil labile and moderately labile phosphorus fractions in the organically-managed treatments compared to the prairie and conventional systems, although plant phosphorus concentrations showed no difference (after one-time manure application). Alkaline phosphatase activity in July was highest in the no input organic and organic manure-amended treatments, with correspondingly low levels of plant available phosphorus. Samples collected in July 2012 from the same plots now in flax, following the second fall manure application, resulted in organic manure-amended alkaline phosphatase activity to be similar to the conventional and native prairie systems. The influence of long-term organic systems and periodic manure application on the abundance and diversity of bacteria harboring an alkaline phosphatase gene is currently being investigated to examine the relationship between alkaline phosphatase activity, plant phosphorus availability, and phosphorus uptake.
Effect of animal manure composites on soil phosphorus chemistry

Yuki Audette¹, L. Evans¹, I. P. O’Halloran¹, R.C. Martin², and R.P. Voroney¹

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In manures and composites, the distribution of organic and inorganic phosphorus forms varies widely depending on its source, animal physiology and diet, and the method of storage and preparation. Generally, these organic amendments contain large amounts of calcium, which increase soil pH and give buffering effects of added bicarbonate and organic acids. Recent studies show considerable shifts from aluminum- and iron- to calcium-phosphorus reaction products, and the transformation of the relatively soluble brushite into the more crystalline octacalcium phosphate in animal manure- and compost-applied soils. Organic phosphorus can constitute up to 60% of the total phosphorus in manures and composites, but the dominance of organic phosphorus decreases in excessively manure-applied soils. The mechanism of transformation of phosphorus forms in soils by application of animal manures and composites is not well understood. The objective of this study was to quantify the forms of phosphorus in animal compost and compost-amended soils, so as to understand the mechanism of their transformation in soils. The transformation of various phosphorus forms were studied in turkey litter compost and in an organically-managed calcareous soil amended with turkey litter compost by a sequential phosphorus fractionation method. Soil organic phosphorus pools were extracted using NaOH–EDTA method. The amount of phosphorus in each extract was measured and the organic P was characterized by solution $^{31}$P NMR spectroscopy. This study showed that turkey litter compost is > 90% inorganic phosphorus, with a specifically high concentration of octacalcium phosphate accounting for 69% of the total phosphorus. Application of turkey litter compost to soil increased the proportion of the total phosphorus as octacalcium phosphate from 17% to 32% by the end of the incubation study (126 days). A model of soil phosphorus mineral species predicts that octacalcium phosphate will dissolve in soils amended with turkey litter compost and become plant available when pH and total phosphorus in soil solution decrease.
Organic phosphorus retention in riparian vegetated buffer strip soils

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Land-use types and management practices interact with phosphorus as it moves through the landscape shaping the forms of phosphorus that are eventually delivered to surface waters. Establishment of uncultivated riparian borders or ‘vegetated buffer strips’ are one such management practice and are becoming increasingly popular for the mitigation of phosphorus delivery from agricultural land, having been shown to reduce total phosphorus delivery. However, uncertainty remains about how buffer strips alter the delivery of dissolved forms of phosphorus. The aim of this study was to test the hypothesis that biological processes operating within buffer strip soils alter the forms of phosphorus delivered by increasing dissolved organic/molybdate unreactive phosphorus delivery. This was achieved by combining a literature review with a soil survey across multiple established buffer strip sites, a plot scale experiment measuring the quantity of phosphorus for ms in runoff, and a soil column leaching experiment. Results from the soil survey showed that buffer strip soils have significantly ($p < 0.05$) greater soluble organic phosphorus concentrations compared to adjacent arable fields, which was attributed to increased microbial turnover of phosphorus. Runoff plots with buffer strips also had significantly ($p < 0.05$) greater mean concentrations of dissolved organic phosphorus in runoff and therefore greater dissolved organic phosphorus loads compared to plots without buffer strips. Soil columns sown with two common riparian grass species had higher mean concentrations of dissolved organic phosphorus compared to columns with no plants. Plants and microorganisms provide a sink for phosphorus in riparian vegetated buffers strips, so the mechanisms employed to acquire this phosphorus and processes associated with its turnover may lead to the increased delivery of dissolved organic forms of phosphorus. This presents a threat to water quality as mineralization could occur during delivery or by aquatic algae.
Organic phosphorus forms and composition in palustrine wetland soils

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Organic phosphorus is abundant in wetland soils and is therefore critical in supporting productivity in these sensitive ecosystems. Modern spectrographic techniques like \textsuperscript{31}P NMR spectroscopy allow researchers to explore the functional nature of soil organic phosphorus, but there has been no comprehensive examination of organic phosphorus in freshwater wetlands. Here we present a survey of phosphorus forms determined by solution \textsuperscript{31}P NMR spectroscopy in 28 palustrine wetlands spanning a range of climatic, hydro-geomorphic and vegetation types. We demonstrate the broad range of functional forms in wetland soils (i.e. phosphomonoesters, phosphodiesters, phosphonates, pyrophosphate, and long-chain polyphosphates) and show that soils with similar concentrations of total organic phosphorus can contain markedly different phosphorus forms. Climate and vegetation appeared to play only a minor role in structuring the organic phosphorus composition of wetland soils, while pH and organic matter content were strong predictors. For example, phosphonates (up to 4\% of total phosphorus) were detected only in acidic soils, while polyphosphates (up to 15\% of total phosphorus) occurred in high organic matter soils irrespective of soil pH. Previous studies of high organic matter subtropical wetlands using \textsuperscript{31}P NMR highlighted an absence of higher-order inositol phosphates, yet we observed myo- and scylo-inositol hexakisphosphate in acidic mineral wetland soils (~ 50\% of the total phosphomonoesters pool). We conclude that the phosphorus composition of wetland soils can be predicted by soil biogeochemical properties (i.e. mineral content and pH) independently of climate and vegetation.
Phosphorus transformations in emergent and submersed macrophyte dominated wetlands

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Four decades of elevated phosphorus loads have converted several thousand hectares of Everglades marsh from a phosphorus-limited sawgrass (Cladium jamaicense Crantz) ridge and slough landscape, to one dominated by cattail (Typha domingensis Pers). We conducted a large-scale in situ study, the cattail habitat improvement study (CHIP), comprised of fifteen 6.25 ha plots, to assess our ability to rehabilitate cattail areas by creating submersed aquatic vegetation habitat. Dense cattail areas were treated using a combination of herbicides and fire to create replicated open areas in enriched and moderately enriched areas along a nutrient gradient in Water Conservation Area 2A in the northern Everglades. As hypothesized, the conversion to an algal and submersed aquatic vegetation dominated system resulted in a more oxygenated environment with more labile carbon sources, resulting in less carbon storage and changes in phosphorus storage and cycling. We documented the transformation of phosphorus as the ecosystem evolved using a combination of conventional phosphorus fractionation procedures and solution ³¹P NMR spectroscopy. Given the abundance of phosphomonoesters and diesters, we also assessed the potential for organic phosphorus cycling using phosphomonoesterase and phosphodiesterase activity. The phosphorus gradient in Water Conservation Area 2A was a model for the design of the Everglades Stormwater Treatment Areas, built to remove phosphorus before it enters the Everglades. A similar landscape evolution has been observed in many of these systems, with cattails near the inflow and submersed aquatic vegetation near the outflow. We compare the created and more natural systems to assess how phosphorus, primarily organic phosphorus, is cycled, to determine whether differences in these mechanisms may increase the phosphorus reduction capabilities of the Stormwater Treatment Areas.
Linking phosphorus sequestration to carbon humification in wetland soils

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We used hydrofluoric acid pretreatment to differentiate phosphorus incorporated into soil organic matter from phosphorus chelated to metals or sorbed to anionic sorption sites. A combination of solution ³¹P NMR and solid-state ¹³C NMR spectroscopy and liquid chromatography mass spectrometry (LC–MS) revealed differences among soils sharing similar physical properties but differing in the quality of soil carbon. Relatively little phosphorus was removed after hydrofluoric acid treatment in soils dominated by alkyl and aromatic groups (i.e. humified) compared to soils with abundant by O-alkyl groups. In contrast, organic phosphorus was the dominant form associated with humified soil carbon, although a surprisingly large amount of inorganic phosphorus was also sequestered. These differences support the hypothesis that phosphorus forms stable humic complexes in soils with humified organic matter consisting primarily of alkyl and aromatic functional groups, although the extent to which inorganic phosphorus behaves in this way remains unknown. Conversely, in soils dominated by carbohydrates, phosphorus appeared to be predominantly bound to free metals or small molecules. Inositol phosphates were specifically targeted in this work using ion chromatography coupled to high resolution time-of-flight mass spectrometry (TOF–MS). The behavior of inositol phosphate congeners containing between one and six phosphate groups could be determined at concentrations well below those observed by ³¹P NMR spectroscopy. Our data are consistent with previous results suggesting that phosphorus sequestration in wetlands is tightly linked to soil carbon humification, the process that converts labile biopolymers into stable geopolymers. Once sequestered in this way, release of phosphorus into overlying water would be minimized as long as the soil organic matter is stable.
The role of microbial processes in soil phosphorus cycling: approaches and outcomes

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Microbial processes in soil phosphorus cycling include immobilization of inorganic phosphorus and mineralization of organic phosphorus, which occur both under steady-state conditions at basal respiration and under dynamic conditions such as soil moisture fluctuations. We present a range of approaches to study these processes at different scales, from i) laboratory incubations using radioisotopes and enzyme additions, to ii) determination of microbial phosphorus fluxes and turnover during drying-rewetting events in the glasshouse and in the field, and finally iii) characterization of microbial phosphorus cycling in situ using stable oxygen isotopes in phosphate. We focus on two case studies from Switzerland: a phosphorus-limited permanent grassland and an alpine soil chronosequence after deglaciation. In the phosphorus-limited permanent grassland, microbial immobilization of inorganic phosphorus was extremely rapid and dominated basal gross mineralization (8 mg P kg\(^{-1}\) d\(^{-1}\)). The resulting low net organic phosphorus mineralization coincided with the absence of phosphorus release by phosphatase, phytase and nuclease added to soil water extracts, while 8 mg P kg\(^{-1}\) were hydrolyzable in a buffered suspension of the soil, the majority being DNA-like phosphorus. Drying-rewetting reduced microbial phosphorus by at least 50%, and maize planted into the rewetted soil benefitted from the resulting increase in available phosphorus. The smaller fluctuations in microbial and available phosphorus observed during the growing season in the field were partly governed by plant growth. With the microbial phosphorus stock being 2–3 times higher than plant phosphorus uptake, it could be both an important competitor for, and a supplier of, phosphorus. This important role of soil microorganisms in phosphorus cycling was supported by similar isotopic signatures of oxygen in available and microbial inorganic phosphorus, even in the youngest soils of the chronosequence. From these studies, we derive a conceptual model of microbial phosphorus cycling in soil–plant systems.
Bacillus β-propeller phytase genes in pasture rhizosphere and volcanic soils from southern Chile

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Phytate is one of the most abundant sources of organic phosphorus in Chilean volcanic soils (Andisols) and phytase-producing rhizobacteria have been proposed as potential biofertilizers to improve phosphorus uptake in plants. Although phytase-producing rhizobacteria are commonly isolated from the rhizosphere of pastures and cereal crops, little is known about their ecology in the rhizosphere and soils from southern Chile. Here, we report the detection, diversity and quantification of Bacillus β-propeller phytase genes in rhizosphere soils. The polymerase chain reaction (PCR) technique was used to identify Bacillus strains carrying β-propeller phytase genes, and then the β-propeller phytase genes were sequenced and used as molecular marker to assess by quantitative PCR the presence and persistence of a phytase-producing rhizobacteria, Bacillus MQH-19, after inoculation of soil microcosms and within rhizosphere. Our results revealed the occurrence of Bacillus and Paenibacillus carrying β-propeller phytase genes in pasture plants grown in Chilean Andisols and that the addition of phytate to nonsterile soils induced the expression of the Bacillus β-propeller phytase gene in the rhizosphere of ryegrass. This study demonstrated that phytase-producing rhizobacteria carrying β-propeller phytase genes are widely distributed in pastures from Chilean Andisols and show also that phytate addition to soils induced changes in the abundance and expression of Bacillus β-propeller phytase genes in the rhizosphere. This knowledge will help to identify management practices that can improve phosphorus availability and circumstances in which phytase-producing rhizobacteria inoculants might be useful for improving plant phosphorus nutrition in the field, particularly in plants grown in Andisols from southern Chile.
The composition and abundance of phosphorus-utilizing genes within Everglades microbial communities

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Numerous studies have seen an increase in microbial phosphatase activity in response to phosphorus limitation. However, the genetics driving this enzymatic activity have not been investigated thoroughly, particularly in phosphorus-limited wetlands. This study represents a preliminary investigation of the composition and abundance of genes related to phosphorus utilization within the microbial community of Everglades Water Conservation Area 2A. From these data, we can draw conclusions about the phosphorus acquisition strategies of phosphorus-limited microbial communities. The genetic composition of the microbial community was analyzed using the functional microarray GeoChip 3.0 in surface sediments and periphyton along a phosphorus gradient. Microbial communities were found to contain genes coding for the production of phytase, phosphonatase (phn), exopolyphosphatase (ppx), and polyphosphate kinase (ppk). Genes for phytase production were found only within the fungal community of the low-phosphorus site, in both the sediment and periphyton. This suggests that alternative forms of phosphorus are utilized in phosphorus limited soils and highlights the important role that fungal communities play in the breakdown of compounds such as myo-inositol hexakisphosphate. Genes for phn were seen at all sites and within periphyton. Confirmatory polymerase chain reaction (PCR) amplification of phn confirmed that this gene was present at all sites, while real time PCR showed that only the most phosphorus impacted site expressed this gene at the time of sampling. Although ppx genes were detected, there were no great differences in relative concentrations between sites. These genes have many additional functions that confound interpretable trends, suggesting the need for further investigation. Genes for ppk were found in greatest abundance at the high phosphorus site, indicating that microbial communities will sequester inorganic phosphorus as polyphosphate when phosphorus limitation is alleviated. Overall, this study shows the complexity of the genetics driving microbial phosphorus cycling and the numerous ways it may be influenced by phosphorus limitation.
Differential nutrient limitation of soil microbes across ecosystems: Does phosphorus really matter?

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At global scales, the relative demand of soil microbes for carbon, nitrogen, and phosphorus are thought to occur in broadly fixed ratios, reflected by patterns in the elemental stoichiometry of both microbial biomass pools and soil enzyme activities. However, in ecosystem studies similar large-scale stoichiometric patterns may obscure considerable variability among habitats and stoichiometric variation is routinely observed in terrestrial and aquatic ecosystems among habitats, and among species of autotrophs and heterotrophs. Our analysis of a large range of ecosystem data sets indicated considerable differences in the C:N:P stoichiometry of terrestrial and wetland soils, especially with respect to the relative availability of phosphorus. To determine whether biological stoichiometry underlies patterns of soil microbial metabolism, we analyzed published data on microbial biomass carbon, nitrogen, and phosphorus pools in soils spanning the global range of climate, vegetation, and land use types. We compared element ratios in microbial biomass pools to the metabolic quotient $q_{\text{CO}_2}$ (respiration per unit biomass), where soil carbon mineralization was simultaneously measured in controlled incubations. Although microbial nutrient stoichiometry appears to follow somewhat constrained allometric relationships at the global scale, we found significant variation in the C:N:P ratios of soil microbes across land use and habitat types, and size-dependent scaling of microbial C:N and C:P (but not N:P) ratios. Our results showed a direct link between microbial stoichiometry and metabolic rates in soils, with a weak but significant relationship between microbial biomass C:P ratios and metabolic quotients ($q_{\text{CO}_2}$) across a wide range of global soils. Importantly, we found that while soil microbial biomass appeared constrained by soil nitrogen availability, microbial metabolic rates ($q_{\text{CO}_2}$) were most strongly associated with inorganic phosphorus availability. Our findings appear consistent with the model of cellular metabolism described by biological stoichiometry theory, in which nitrogen limits biomass, but rates of protein synthesis are limited by the high phosphorus demands of ribosomes.
Modeling phosphorus dynamics and phosphorus limitation on productivity in tropical forests

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One of the major uncertainties in the prediction of future carbon uptake and storage comes from the treatment of carbon-nutrient interactions in global carbon–climate models. Phosphorus, the most limiting nutrient in tropical regions, has yet to be incorporated into global carbon–climate models (except for CASA-CNP and JSBACH). Here we introduce phosphorus dynamics and carbon–nitrogen–phosphorus interactions into the CLM4-CN model and investigate the role of phosphorus cycling in controlling the productivity of tropical ecosystems. The newly developed CLM-CNP model includes all major biological and geochemical processes controlling phosphorus availability in soils and the interactions between carbon, nitrogen, and phosphorus cycles. We apply the CLM-CNP model at ten intensively studied RAINFOR sites in the Amazon watershed to evaluate the model performance against the observations and investigate how phosphorus status affects above ground productivity in tropical forests. We also investigate the relative importance of biochemical mineralization (enzymatic hydrolysis of organic phosphorus by phosphatase) and biological mineralization (release of phosphorus from organic from during oxidation of carbon) in maintaining soil available phosphorus in tropical forests. These site level simulations improve our understanding of the controls on soil available phosphorus and how soil phosphorus availability affects terrestrial carbon uptake. The evaluations against site-level observations increase confidence in applying the model more broadly to regional and global scales.
Soil solution organic and condensed inorganic phosphorus in lowland tropical forests

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Recently it was shown that transpiration rates correlate positively with P:C ratios in leaves from tropical trees, suggesting that phosphorus movement to the root zone occurs by transpiration-induced mass flow of soil solution containing dissolved phosphorus compounds. To investigate this, soil solution was isolated by centrifugation (10,000 g for 15 min) from 21 rain forest soils in Panama between April and July 2012. Dissolved reactive phosphate in the soil solution was determined by the malachite green method, with dissolved unreactive phosphorus, including organic and condensed inorganic phosphorus, determined by the same procedure following wet oxidation. Soil solutions were dominated by unreactive phosphorus (mean 90%, range 73–100%) with concentrations up to 470 µg P L⁻¹. The lability of the unreactive phosphorus was studied by adding four different hydrolytic enzymes to the soil solutions (phytase, nuclease, phosphomonoesterase, and pyrophosphatase). On average, 64% (range 8–100%) of the unreactive phosphorus was hydrolyzed during incubation for 24 hours at pH 5.5. A striking finding was that the soil solutions contained considerable concentrations of pyrophosphate, which was supported by solution ³¹P NMR spectroscopy of the soils. Native phosphatases, including both phosphomonoesterase and phosphodiesterase, were also active in soil solutions. Taken together, our results demonstrate that organic and condensed inorganic phosphorus compounds are abundant in soil solution and are susceptible to hydrolysis by phosphatase enzymes, supporting the hypothesis of transpiration-induced phosphorus acquisition by tropical trees.
Oxygen isotope of phosphate as an indicator of organic phosphorus utilization in the ocean

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Dissolved inorganic phosphorus concentrations in surface water of vast areas of the ocean are extremely low (< 10 nM) and phosphorus availability could limit primary productivity in these regions. We explore the use of oxygen isotopic signature of dissolved phosphate ($\delta^{18}O_{PO4}$) to investigate biogeochemical cycling of phosphorus in the Sargasso Sea, Atlantic Ocean and at the Hawaii Ocean Time series in the Pacific. In surface waters $\delta^{18}O_{PO4}$ values were lower than equilibrium by 3–6‰, indicative of dissolved organic phosphorus remineralization by extracellular enzymes. An isotope mass balance model using a variety of possible combinations of enzymatic pathways and substrates indicates that dissolved organic phosphorus remineralization in the euphotic zone can account for a large proportion of phosphorus utilized by phytoplankton (as much as 82%). Relatively short dissolved inorganic phosphorus turnover times (4–8 hours) and high expression of alkaline phosphatase (38–77% of the cells labeled) are consistent with extensive dissolved organic phosphorus utilization and low dissolved inorganic phosphate availability in the euphotic zone. In deep water, where dissolved organic phosphorus utilization rates are lower, $\delta^{18}O_{PO4}$ values approach isotopic equilibrium and dissolved inorganic phosphate turnover times are longer. Our data suggests that dissolved organic phosphorus in the euphotic zone of the Sargasso Sea may be appreciably remineralized and utilized by phytoplankton and bacteria to supplement cellular requirements. A substantial fraction of photosynthesis in this region is supported by dissolved organic phosphorus uptake.
Phosphate insensitive aminoalkylphosphonate degradation in marine environments

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Organophosphonates are compounds with direct carbon-phosphorus bonds, rather than the more common carbon-oxygen-phosphorus ester. Phosphonates tend to be more chemically, thermally and enzymatically stable than phosphates, and so microorganisms must possess dedicated phosphonate degradation pathways in order to use them as a nutrient source. To date multiple degradation pathways have been described which allow the metabolism of a wide range of phosphonates. Previously it was thought that these genes were repressed by environmental inorganic phosphate, and as such it was commonly accepted that phosphonates would only be degraded as a source of phosphorus in an inorganic phosphate depleted environment. However, some terrestrial isolates have been described which can use specific phosphonates as a sole carbon source regardless of the environmental phosphate levels. In the ocean, up to a third of the dissolved organic phosphorus pool can consist of phosphonates. Although the precise composition of these phosphonates is difficult to determine, 2-aminoethylphosphonate is known to be produced by a wide range of marine invertebrates and is believed to be the most common. Previous studies have identified 2-aminoethylphosphonate-degrading enzymes in the ocean under inorganic phosphate repressed regulation, and organophosphonates are widely accepted to be a bioavailable phosphorus source for marine microorganisms. However, using a range of coastal surface and deep ocean water samples we have obtained bacterial isolates capable of degrading 2-aminoethylphosphonate as the sole nitrogen source regardless of environmental inorganic phosphate levels. The degradation of 1mM 2-aminoethylphosphonate was able to support significant growth in these isolates even in the presence of 1 mM KH₂PO₄, and resulted in significant quantities of phosphate being released into the growth medium. Biochemical characterization of the isolates suggests that the enzymes involved are previously characterized 2-aminoethylphosphonate degradation proteins, which have not previously been shown to be phosphate-insensitive in marine environments. The isolation, growth and characterization of these isolates will be discussed.
Characterizing dissolved and particulate phosphorus forms in runoff from agricultural lands

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The loss of phosphorus from land to water is of concern because phosphorus is often the nutrient most limiting algal growth in freshwater systems. Agriculture is often blamed as the source for phosphorus loss from land to water, but little is known about the phosphorus forms and concentrations in runoff from agricultural land, or if differences exist among different cropping systems. Knowledge about the physical (dissolved or particulate) and chemical forms of phosphorus is essential to develop beneficial management practices to reduce phosphorus transfer, because physical and chemical phosphorus forms differ in their transfer mechanisms and their reactivity in the environment. To better understand phosphorus loss in runoff, I have characterized by $^{31}$P NMR spectroscopy the phosphorus forms in dissolved and particulate edge-of-field runoff samples from various agricultural practices. This technique is sensitive enough to show temporal differences in phosphorus forms within a single runoff event, allows the determination of flow-weighted concentrations of phosphorus forms, and has allowed the identification of a number of phosphorus forms, including agricultural chemicals such as glyphosate. In addition, the comparison of NMR spectra of phosphorus in runoff to spectra from source material in the field suggests that dissolved and particulate phosphorus in runoff may originate from different sources. This suggests that multiple management practices may be required to control phosphorus movement from a single field.
Can phospholipids and nucleic acids shed light on organic phosphorus turnover in lake sediment?

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The fate of organic phosphorus in lake sediments is elusive due to our limited understanding of the turnover rate of various phosphorus compounds in aquatic and terrestrial systems. Orthophosphate diesters, including nucleic acids and phospholipids, represent a potentially degradable phosphorus pool that could support future primary production and eutrophication. In the presented study, a novel non-destructive method for the extraction and quantification of phosphorus bound to lipids and nucleic acids is applied to the investigation of lake sediments. Nucleic acids were extracted directly by lysis, combining shaking with glass beads, sodium dodecyl sulfate, lysozyme and proteinase K, purified with a phenol/chloroform mixture and further cleaned through ultrafiltration. Phospholipids were extracted with a single-phase buffer solution (chloroform/methanol/sodium citrate), which was subsequently split into two phases and separated. Phosphate in the nucleic acid material was released through digestion with potassium persulfate and in the phospholipid material through dry ashing, and final phosphorus concentration was determined. Sediment samples from two Swedish lakes were investigated using this approach. Sediment cores were collected at Lake Erken, a mesotrophic lake, and Lake Ånn, an oligotrophic lake, both in steady state regarding phosphorus input. Sediment profiles down to 30 cm were studied. Preliminary results reveal trends in the degradation rate of phospholipids in the top layer. We hope these findings contribute to increasing our understanding of the possible sinks and sources of these forms of phosphorus on a catchment scale by exploring the loss of nucleic acids and phospholipids over time (i.e. with increased sediment depth).
Organic phosphorus compounds in riverbed sediment from source to estuary

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We tested the hypothesis that phosphorus compounds varied with catchment area in the riverbed sediment of the River Taw, southwest England. The River Taw basin is approximately 1000 km² and runs from its source at Taw Marsh on Dartmoor (~ 600 m above sea level) to the estuary at Barnstable in the Bristol Channel, ultimately contributing to the Irish Sea and the Atlantic Ocean. Six sampling locations representing catchment areas from 1 to 862 km² were used to collect riverbed sediment and samples from the water column. Sediment < 63 µm was freeze-dried, extracted in NaOH–EDTA, and phosphorus in the extracts was characterized by solution ³¹P NMR spectroscopy and colorimetry. When expressed as a proportion of the total extractable phosphorus, organic phosphorus declined from 46% upstream to 23% downstream. Inositol hexakisphosphate, including the myo and scylo isomers, increased from 22% of the organic phosphorus in the headwaters to 36% at the estuary, while DNA decreased in the same direction, from 20% of the organic phosphorus in the headwaters to < 10% downstream. We conclude that the composition of the sediment organic phosphorus reflects the increasing influence of agricultural land management as the river travels from its headwaters to the estuary.
The impact of human domestic behavior on phosphorus compounds in private sewage systems in the Loch Leven catchment (Scotland, UK)

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Human domestic behavior influences the forms and transport of phosphorus to aquatic ecosystems. Private sewage systems provide an opportunity to assess these links in a replicated manner. This paper combines an assessment of human domestic behavior with an assessment of phosphorus compounds in sewage sampled from private sewage systems in the Loch Leven catchment (Scotland, UK), using \textsuperscript{31}P NMR spectroscopy. Human domestic behavior of users of private sewage systems within the catchment was assessed using questionnaires focusing on domestic habits and maintenance of the private sewage systems (n=155), and a locus of control personality test (n=21). Of the users surveyed, 17\% had never ‘de-sludged’ their private sewage system and 33\% had ‘de-sludged’ their system within the last 12 months. The ‘impact on the environment’ of dishwasher or washing machine detergents influenced the purchasing choice of 34\% and 45\% of users, respectively, but 65\% felt that available information was insufficient to help them change domestic habits to improve their private sewage system performance. Solution \textsuperscript{31}P NMR spectroscopy was conducted on PAX-18 extracted material from a subset of eight private sewage systems, selected to represent a replicated distribution of behaviors and designs. Orthophosphate (60–80\% of total extracted phosphorus), phosphonates (0–3\%) phosphate monoesters (13–24\%), pyrophosphates (2–3\%), phospholipids (2–9\%), and deoxyribonucleic acid (2–3\%) were all detected. Variations in phosphorus composition will be related to human domestic behavior and personality type across the subset of private sewage systems and the implications for phosphorus load control to the Loch Leven catchment will be discussed.
Can environmental stress be exploited for enhanced biological phosphorus removal?

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Eutrophication is the single greatest threat to fresh water quality in Europe. It is a direct result of elevated nutrient levels such as nitrogen and phosphorus in the water body. The over-reliance on phosphorus based fertilizers and the inconsistency of current wastewater treatment technologies are the main contributing factors to the problem. With global rock phosphorus reserves on a rapid decline, the demand for high quality agricultural produce increasing with global population and a rising awareness of environmental issues, there has never been a more pressing need for new and improved technologies for the removal, recovery and recycling of key nutrients. Certain microorganisms have an innate ability to accumulate large amounts of phosphorus, much greater than those required for normal metabolic activity and store it as polyphosphate, a biopolymer made up of tens or hundreds of orthophosphates linked together by high energy phosphoanhydride bonds. These are known as polyphosphate accumulating organisms. Commonly found in activated sludge, polyphosphate accumulating organisms provide the perfect test subjects for a novel approach to nutrient removal and recovery from wastewater. Using Acinetobacter calcoaceticus and Pseudomonas putida as model organisms, as well as the surrogate activated sludge community Polytox, we investigated whether it was possible to enhance their ability to accumulate phosphorus through the exploitation of an environmental stress. Starting out in batch culture and then scaling up using a 2 L state-of-the-art bioreactor, it was found that after altering specific environmental conditions over a range of times some cultures showed increases of up to 300% in phosphate removal from a defined medium as well as increases in intracellular polyphosphate levels. These findings offer an invaluable insight into the potential for this novel biotechnological process to be implemented at a full scale wastewater treatment plant as well as opening the door to its potential as an economical and efficient technology for the generation of secondary phosphorus.
Assessing the impact of a rural development policy on the phosphorus load from septic tanks to the Loch Leven catchment (Scotland, UK)

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Loch Leven, a shallow eutrophic lake in the lowlands of Scotland, UK, is a good example of a water body that supplies a wide range of ecosystem services across local, national and international scales. Over the past 150 years, this loch has been subjected to a range of management initiatives that have sought to improve the provision of specific services by the lake, including human waste management and the provision of biodiversity. This paper assesses the implementation of a novel piece of environmental policy designed to control eutrophication in Loch Leven through the management of organic phosphorus loading from private sewage systems. This policy states that prospective developers proposing sewage treatment via private sewage systems within the catchment, must (1) estimate the phosphorus load from their proposed system, and (2) propose and implement mitigation measures to reduce the phosphorus load within the catchment by 125\% of the estimated phosphorus load associated with the development. To assess the efficacy of this policy, we measured the range of total and soluble reactive phosphorus concentrations in seven private sewage systems over a period of 4 months and used these data to predict phosphorus loads from private sewage systems at the catchment scale, over a 90 year projected population increase. Total and soluble reactive phosphorus concentrations in private sewage systems ranged from 1.99 mg P L\textsuperscript{-1} to 15.35 mg P L\textsuperscript{-1} and from 0.32 mg L\textsuperscript{-1} to 11.10 mg L\textsuperscript{-1}, respectively. It was estimated that 1668 people were served by private sewage systems in 2010 and that this number would increase to 5333 by 2100. This population increase represents an increase in total phosphorus load to the catchment from a current load (2010) of about 1.01 t P yr\textsuperscript{-1} to 3.22 t P yr\textsuperscript{-1} in 2100. The annual total phosphorus load to Loch Leven from the catchment in 2005 was 7.69 t P yr\textsuperscript{-1} (0.6 g P m\textsuperscript{2} yr\textsuperscript{-1}). We present these results in the context of ecological responses in Loch Leven, both with and without implementation of the rural development policy.
Abstracts of Poster Presentations
Soil phosphorus forms and distribution determined by $^{31}$P NMR spectroscopy influenced by tillage practices

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A better understanding of organic phosphorus transformations is needed to improve management of phosphorus fertilization and tillage. Conservation tillage practices have become increasingly common in recent years to reduce soil erosion, improve water conservation, and increase soil organic matter. However, the effects on soil properties, especially phosphorus forms, amounts and distribution in the soil profile remain widely unknown. This study was conducted to assess the long term effects of tillage practices (no till and moldboard plowing to 20 cm depth) on phosphorus composition and distribution in the soil profile. The long term corn-soybean rotation experiment was established in 1992 on a deep clay loam soil of the St-Blaise series (Dark Grey Gleysol) in Acadie, Canada. We used 18 air-dried soil samples collected from three depths (0-5, 5-10, and 10-20 cm) and analyzed using solution $^{31}$P NMR spectroscopy. Results showed differences in phosphorus forms between the two tillage systems. For instance, the amounts of orthophosphate monoesters were higher under no-till than moldboard plowing in the three depths. In contrast, the reverse was true for the phosphonates, orthophosphate diesters and polyphosphates. In addition, phytate is more abundant under moldboard plowing, in particular in the upper 5 cm, suggesting that tilling led to the mixing of phytate from crop residues into the soil.
Isolation of phytase-producing rhizobacteria from different natural ecosystems and agro-ecosystems of northern, central and southern Chile

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Plants associate with a wide variety of bacteria. Those associated with roots (rhizobacteria) and with the ability to provide some benefit to them are known as plant growth promoting rhizobacteria. Currently, rhizobacteria with the ability to produce phytase (myo-inositol hexakisphosphate phosphohydrolase) are being isolated and studied as plant growth promoting rhizobacteria for agricultural crops. However, only a small proportion of bacteria (<1%) taxa can be grown in culture. We used various culture media supplemented with vitamins (B5, H, Bx, B3 B6 and b1) and different sources of phosphorus (phytate), carbon (glucose, galactose) and nitrogen to isolate a diverse collection of rhizobacteria. Isolation was obtained from different plants from both natural ecosystems (Atacama Desert, Conguillio National Park, and Patagonia) and agroecosystems (crops of fruit, cereals and pastures) located throughout Chile. Our results showed that the use of different culture media allowed the isolation of a wide variety of bacterial phenotypes, including those with motility, ability to form biofilms, pigmentation and production of endospores. In addition, rhizobacteria with phytase activity were isolated at all sites sampled. This study showed that Chilean ecosystems contains a wide rhizobacterial diversity and highlight our limited knowledge of their ecology, interaction with plants, and their potential as plant growth promoting rhizobacteria based on the production of phytase.
Soil phosphorus fertility under organic nutrient management

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Organic nutrient management, involving applications of animal manures and composts, provides both organic matter and plant available nutrients to soils. These amendments affect the chemistry of soil phosphorus, altering both the amount and distribution of the various phosphorus fractions. It has been reported that most of the phosphorus in manures and composts is inorganic phosphorus. A substantial proportion of the organic phosphorus fraction is inositol phosphate, which reacts strongly in soils to form stable phytate complexes. In manures and composts, the amount and distribution of organic and inorganic phosphorus forms varies widely depending on its source, animal physiology and diet, bedding material, and the method of storage and preparation. The objectives of this study were to quantify the forms of phosphorus in animal composts, and to measure the kinetics of their transformation in soils. The transformations of various phosphorus forms during an 18-week incubation were analyzed in an organically-managed calcareous soil amended with either turkey litter compost or water soluble inorganic phosphorus (KH₂PO₄). A sequential phosphorus fractionation method was used to assess these transformations. Soil organic phosphorus pools were extracted in NaOH–EDTA and organic phosphorus was characterized by solution ⁳¹P NMR spectroscopy. The study showed that the majority of phosphorus in turkey litter compost and soils amended with turkey litter compost was inorganic phosphorus. The organic phosphorus pools were not significantly different among treatments. Amendment with KH₂PO₄ affected brushite (labile phosphorus) most, while the effect of turkey litter compost amendment was on the octacalcium pool. A soil phosphorus mineral species model predicted that octacalcium phosphate would become plant available in soils amended with turkey litter compost when total phosphorus in soil solution decreases. Octacalcium phosphate might play an important role in phosphorus fertility in organically-managed soils.
Delays, degrees and degradation: Evaluating the effects of experimental parameters on $^{31}$P NMR results

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The technique of $^{31}$P NMR spectroscopy has done more to advance the knowledge of organic phosphorus forms in environmental samples than any other method. However, to optimize the information gained from $^{31}$P NMR experiments and minimize the introduction of artifacts such as from sample degradation, it is essential that the correct parameters are used during each NMR experiment. In recent years, as more research groups use $^{31}$P NMR, we have observed the use of a wide range of experimental parameters, particularly with respect to delay time, pulse angle and experiment length. While the effects of delay time have been studied recently, there have been no published studies to date examining the effects of pulse angle or experiment length on $^{31}$P NMR spectra of environmental samples. As such, we devised a series of experiments to test experimental conditions on a range of sample types. Our experiments show that using a short pulse angle will significantly decrease the signal-to-noise of an experiment, significantly increasing the number of scans required to achieve the same signal-to-noise as would be achieved from using a 90-degree pulse. Our results also show that sample degradation can occur over time. As such, experiments using short pulse angles require longer experiments, in turn experiencing more sample degradation. This raises questions about the phosphorus form distributions determined from $^{31}$P NMR studies that have used short pulses and long experiment times.
Improved peak identification in $^{31}\text{P}$ NMR spectra with a standardized method and peak library

Barbara J. Cade-Menun

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The technique of $^{31}\text{P}$ NMR spectroscopy has done more to advance the knowledge of organic phosphorus forms in environmental samples than any other method. Early $^{31}\text{P}$ NMR work limited identification to peaks that were clearly separated, such as orthophosphate and pyrophosphate, grouping the remaining peaks into broad categories such as orthophosphate monoesters and orthophosphate diesters. Advances in $^{31}\text{P}$ NMR methodology for environmental samples now produce clearer spectra, providing the potential to identify more peaks in the monoester and diester regions. However, there is at present no standard method for peak identification, and no standardized library of chemical shifts of phosphorus forms. Various research groups have conducted spiking experiments and have developed their own peak libraries. However, because the chemical shifts of phosphorus forms are affected by sample conditions such as pH and salt concentration, it can be difficult to use the work of one lab group to identify phosphorus forms in samples analyzed under different conditions. In order to improve and standardize peak identification, I have developed a library of more than 40 phosphorus compounds, analyzed by $^{31}\text{P}$ NMR individually and grouped with nearby peaks. These were analyzed under standard conditions that could be easily replicated by any research group, to directly compare chemical shifts in their samples to those in the library. I also propose a standardized set of spiking experiments that should be used for all $^{31}\text{P}$ NMR experiments.
Improving bioavailability of phosphorus from cattle dung by using phosphatase immobilized on natural clay and nanoclay

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The high phosphorus retention in Chilean Andisols is one of the most important limiting factors in agricultural systems. Organic phosphorus hydrolysis by phosphatase enzymes could improve the plant nutrient uptake in such soils. Studies have reported that the use of nanomaterials as immobilization support improves enzymatic stability and catalytic activity against other materials. In soils derived from volcanic ash such as Andisols, is possible to find different structures of nanoparticles like aluminosilicate with nano-ball (allophane) and nano-tube (imogolite) morphology within clay fraction. Here, we evaluated the clay- or nanoclay-acid phosphatase complexes for improving phosphorus mineralization from degraded cattle dung. We implemented an immobilization mechanism of acid phosphatase using natural clays (allophane and montmorillonite) and nanoclays as support materials. We also evaluated the mineralization of organic phosphorus in decomposed cattle dung with clay- and nanoclay-phosphatase complexes by incubation studies. The nanoclays were extracted from an Andisol and montmorillonite, and clays and nanoclays were characterized by a variety of microscopy techniques. We found that the support materials stabilized acid phosphatase by encapsulation and that immobilization on allophane or montmorillonite materials improved both the specific activity (4 to 48%) and the maximum velocity of the reaction (28 to 38%) of acid phosphatase. Moreover, the enzyme performed better when immobilized on clay and nanoclay from Andisol than on montmorillonite materials. Phosphorus mineralization in cattle dung was regulated by water-soluble phosphorus present in the dung and phosphorus re-adsorption on allophane materials. However, we were able to detect a potential capacity of acid phosphatase immobilized on allophane nanoclays as the best alternative for phosphorus mineralization. Further research with initially low water-soluble phosphorus containing organic materials is required to quantify the phosphorus mineralization potential and bioavailability of phosphorus from dung.
A new solution $^{31}$P NMR extraction procedure for sediment samples with low phosphorus content

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Solution $^{31}$P NMR spectroscopy has been successfully used in a number of environmental studies to determine the nature of organic phosphorus compounds in soils, sediments and particulate organic matter. However, this tool cannot be used efficiently on samples with high inorganic to organic matter ratio, since the orthophosphate peak ($\delta = 6$ ppm, pH $> 12$) appears to represent 100% of phosphorus in the samples and the signal to noise ratio of organic phosphorus species is poor. The aim of this study is to develop a method to concentrate organic phosphorus species in sediment extracts while removing orthophosphate in order to increase the signal of organic phosphorus molecules in $^{31}$P NMR spectra. Sediments spiked with pure phosphorus compounds will be used to test the method. The method will then be applied to open ocean sediments collected during the Integrated Ocean Drilling Program expedition 336 on the flanks of the Mid-Atlantic Ridge in an effort to characterize organic phosphorus forms in deep-sea sediments and evaluate their bioavailability. Understanding phosphorus sources and cycling in deep-sea sediments is critical, since a large fraction of Earth’s prokaryotes thrives in this environment and little is known about their metabolism. It is hypothesized that in such sediments where phosphorus is scarce, phosphorus forms fueling microbial life are mainly in mineral phases. However, the role of the organic phosphorus pool in this setting is poorly understood and could be of great importance should it represent an important phosphorus pool fueling the deep biosphere. In addition, this method could also be applied to soil samples containing high inorganic to organic phosphorus.
Changes in soil organic phosphorus and their relationship with nutrient limitation in Chilean temperate forests

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Soils derived from volcanic ash are abundant and widespread in southern Chile, and together with glacial and sedimentary soils support biologically diverse temperate forests. Soils in southern Chile are rich in organic matter, have pH-dependent cation exchange capacity, and vary in texture from fine sands, silt loams to silty clays. Similarly, studies have reported high capacity to fix phosphorus in these soils. However, in highly weathered soils a high proportion of phosphorus is in the humic fraction, which can be higher than the fraction associated with aluminum or iron compounds. Soil organic phosphorus can occur in a broad spectrum of compounds that differ in their suitability for plant use. In this study we evaluated changes in soil phosphorus fractions in soils of southern Chile of different geologic origin, texture and with different plant species composition. We tested whether differences in soil nitrogen, phosphorus, and other plant nutrients along this soil gradient were in agreement with previous studies. We found a positive correlation between organic phosphorus and organic carbon, although this correlation was weaker in relation to previous studies. We also found negligible concentrations of total carbon and phosphorus in surface soils in young soils of volcanic origin, where tree species with contrasting leaf traits dominate. We did not detect a correlation between foliar phosphorus and total soil phosphorus, which suggests that leaf and soil nutrient contents are decoupled. However, this pattern changed when we analyzed different soil phosphorus fractions and contrasted these with foliar phosphorus and N:P ratios. Finally, foliar N:P ratios differed between functional groups (evergreen and deciduous trees), and among study sites; however, the general trend was to find values > 12.5, suggesting that several plant species in these temperate ecosystems can be phosphorus limited.
Characterization of particulate phosphorus in a hydrologic transect of the Lake Champlain Basin, Vermont, USA: phosphorus species analysis and enzyme lability

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Freshwater ecosystem management could be improved by identifying sources of bioavailable phosphorus within impacted watersheds. In the Lake Champlain Basin, an estimated eighty percent of the total external phosphorus load is transported in association with sediments from developed and agricultural land-use regions. However, due to low phosphorus concentrations in surface waters, the identity and relative lability of phosphorus compounds associated with stream particulates is not well established. As a biologically vulnerable phosphorus fraction, enzyme-labile phosphorus is proposed as a surrogate for estimating bioavailable organic phosphorus in riverine samples. Six locations were chosen along a hydrologic transect of the Missisquoi sub-basin and sampled for particulate phosphorus during base-flow and post-storm events (August-September 2012). Stream particulates were collected by filtration (4–30 L; 0.2 \( \mu \)m) and extracted with NaOH–EDTA for analysis by solution \(^{31}\)P NMR spectroscopy and enzyme hydrolysis assays. Phosphatases (acid phosphatases from potato and wheat germ; nuclease P-1 from \textit{Penicillium citrinum}) were added to extracts singly or in combination to target simple monoester phosphorus, phytate-like-phosphorus, and nucleic-acid-like-phosphorus classes of enzyme labile particulate phosphorus. Established enzyme hydrolysis methods and associated colorimetric protocols have been modified to account for low phosphorus concentrations in particulate extracts, and are currently being optimized for application to these riverine samples.
Phosphorus lability and speciation in two agricultural soils by sequential fractionation, solution $^{31}$P NMR, and phosphorus K-edge XANES spectroscopy

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Environmentally sound phosphorus management without soil phosphorus deficiencies requires a full understanding of phosphorus speciation and lability, as these determine phosphorus bioavailability and mobility. With the stable economical development and fast urbanization in China, there has been an increasing trend of land use change from conventional crops to cash crops, like nursery stock and bamboo, since the end of the last century. We used sequential fractionation solution, $^{31}$P NMR spectroscopy and phosphorus K-edge X-ray absorption near-edge structure (XANES) spectroscopy to probe phosphorus speciation in two agricultural soils covered with nursery stock and bamboo. The largest phosphorus pool in soils was NaOH-extractable phosphorus. In bamboo soils with higher phosphorus fertilization, NaHCO$_3$-extractable phosphorus was the second dominant fraction, while residual phosphorus was the second largest pool in the nursery stock soil with little phosphorus fertilization. Orthophosphate monoesters, more likely to be sorbed to soil than diesters, were the major organic phosphorus compounds in these two soils. myo-Inositol hexakisphosphate and DNA were the prevailing organic phosphorus forms in the orthophosphate monoesters and diesters, respectively. The XANES spectroscopy indicated that iron associated phosphorus and dibasic calcium phosphate were the dominant inorganic phosphorus forms in both soils. The combination of techniques qualitatively determined phosphorus species in soils and offered quantitative results both on labile organic phosphorus and total phosphorus with a good agreement (standard deviations $< 10\%$), which has been little reported. These results demonstrate that using combined analysis provides comprehensive and accurate information on soil phosphorus speciation, essential for effective phosphorus management in agricultural lands.
Distribution and hydrolyzability of organic phosphorus in aggregates of highly weathered tropical soils

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Highly weathered pasture soils of the deforested areas of tropical South America usually have low available phosphorus contents. Because inorganic phosphorus is strongly sorbed, organic phosphorus could play a major role in supplying plant available phosphorus in these low fertility soils. Availability of organic phosphorus requires that the organic phosphorus compounds are accessible to hydrolyzing enzymes. Soil aggregation is important for soil organic matter turnover and could therefore affect the distribution and hydrolyzability of organic phosphorus in soil aggregates of highly weathered pasture soils, which has not yet been studied. We conducted a case study on nine farms in Colombian Amazonia, each farm with degraded and productive pastures dominated by Brachiaria spp. Our objectives were to determine i) the aggregate distribution in degraded and productive pastures, and ii) the concentration of organic phosphorus and its potential to be hydrolyzed as affected by aggregate size and pasture type. Results showed that degraded pasture soils contained significantly fewer large macro-aggregates (> 2000 µm) and more micro-aggregates (53–250 µm), both with significantly lower organic phosphorus concentrations than productive pasture soils, while total phosphorus did not differ between pasture types. On average, 65% of total organic phosphorus was enzyme labile, with no differences between pasture types or aggregate size classes. Application of different enzymes to specify organic phosphorus forms showed that the proportion of DNA-like phosphorus to total enzyme labile phosphorus was highest in the silt and clay fraction (< 53 µm) and lowest in the coarse particulate organic matter fraction. The proportion of phytate-like phosphorus was highest in the small macro-aggregates in which simple-monoester-like phosphorus was lowest. While similar proportions of enzyme labile phosphorus in all aggregates suggest similar potential organic phosphorus availability, the occurrence of different organic phosphorus forms points to different processes of organic phosphorus synthesis and stabilization in aggregates of different size.
Linking soil partitioning, crop acquisition and compositions of novel phosphorus fertilizers to improve phosphorus resource management

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Phosphorus is a crucial resource in agricultural production. Excessive consumption of mineral fertilizers has caused soil phosphorus to accumulate in forms currently inaccessible to crops. A recent review of literature suggests considerable global reserves of orthophosphate monoesters exist in topsoil, yet such compounds require specific crop strategies to make them accessible. We undertook \textsuperscript{31}P NMR analysis of 32 UK soils to evaluate forms of phosphorus stabilized under arable, grassland and semi-natural management, including adjacent crop and set-aside areas. Residual phosphorus has accumulated by becoming ‘fixed’ by sorption to mineral phases and/or converted to complexed forms. We examined phosphorus partitioning in relation to soil properties. Soil organic matter quantity and quality was a key parameter in the phosphorus mineralization/immobilization balance. The agricultural phosphorus input, largely from rock phosphate sources, is channeled through consumers into waste streams that currently flow as pollution into rivers and seas. Two vital steps in improving phosphorus resource use efficiency are therefore (1) the better utilization of residual and applied phosphorus by crops to drive down excessive soil fertilizer inputs and reduce soil phosphorus, and (2) the return of phosphorus in ‘wastes’ back to soils. We need to understand how phosphorus partitions in soils and the strategies that certain plants have to improve access to complexed phosphorus. Together this will improve efficient use of residual soil phosphorus, future applied inorganic fertilizers, and reclaimed phosphorus materials. We discuss how soil-plant strategies with organic acids and enzymes may unlock improved crop acquisition of complexed soil phosphorus reserves. In addition, we explore the composition of new recycled phosphorus fertilizer materials, how these may require similar tactics to make them accessible to crops and implications for the accompanying organic matter inputs to soils.
Contribution of recalcitrant phosphorus to phosphorus fractionation in an Andisol

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In Chile, Andisols are of great importance because these soils support livestock and cereal production. The high phosphorus fixation capacity of Andisols is a limiting factor for crop nutrition. Thus most crops and grasslands require periodic phosphorus-based fertilizer addition. However, > 90% of phosphorus applied to Andisols is not taken by crops and accumulates in the topsoil. A large proportion of the total phosphorus in Andisols from southern Chile is present in organic form. In Chilean Andisols a significant correlation between carbon and organic phosphorus suggests a high affinity between phosphorus and soil organic matter and we hypothesized that organic phosphorus might be stabilized in soil by similar mechanisms as carbon. The aim of this work was to study the contribution of chemical recalcitrant organic phosphorus to stabilized phosphorus in Andisols. Our conceptual approach included the fractionation of two different fertilized and unfertilized Andisols from two different depths using the Headley fractionation method for organic phosphorus. Two Andisols in the Piedras Negras and Pemehue series were chosen. For each soil we analyzed two treatments (fertilized and unfertilized) and two depths (0-20 cm and 20-40 cm). Phosphorus fractionation showed that > 40% of the phosphorus in all soils occurred in the residual faction and was therefore chemically recalcitrant. A significant correlation between pyrophosphate extractable aluminum and iron and total phosphorus \( r = 0.511, p < 0.05 \) was observed in the residual fraction, suggesting a relation between aluminum and iron present in humus complexes with phosphorus in the chemically recalcitrant fraction. We conclude that phosphorus in fertilized as well as unfertilized Andisols is chemically recalcitrant, which may be due to its strong association with humus complexes.
Fate and transport of phosphorus compounds in the River Eden, UK

Ying Wang, Phil Haygarth, Ben Surridge

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Generally, phosphorus in the environment can be divided into two different forms: inorganic and organic phosphorus. Inorganic phosphorus has been very well documented, while knowledge of organic phosphorus still remains uncertain, despite its potentially vital role in soils, waters and other matrices. To find ways of reducing phosphorus transfers to surface waters, it is essential to understand the forms, transport and transformations of phosphorus within catchment systems, including the soils, water and biotic materials that they are comprised of. This abstract describes an approach to determine the different organic phosphorus compounds in a catchment context (focused on the River Eden in North West England). The ultimate aim is a budget of organic phosphorus forms across the catchment system, with a view to making improved recommendations for sustainable phosphorus management. The lack of direct and effective organic phosphorus determination methods is a vital limitation to the organic phosphorus understandings. High performance liquid chromatography (HPLC) has the potential to separate organic phosphorus compounds in water or soil extracts. We shall describe our development of the HPLC approach for separating organic phosphorus compounds. The main focus is on the amounts and forms of phytic acid present in the streams and soils of the Eden catchment, with transformations along the transport pathways also considered.
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