FOREST FACTS

4 ▪ 2013 ▪ Results from the Swedish University of Agricultural Sciences

40 år 1973-2013

Andrea G. Vincent ▪ Ulrik Ilstedt ▪ Johan Vestergren ▪ Reiner Giesler ▪ Per Persson ▪ Gerhard Gröbner ▪ Jürgen Schleucher

*Figure 1. Spruce (*Picea abies*) sapling in one of the study sites, located in the vicinity of Umeå, northern Sweden. Photo Andrea Vincent.*

# Phosphorus in forest soils

# – Disentangling the chemistry of an essential nutrient

- The boreal forest is important for carbon storage, biodiversity, and the Swedish economy. Therefore it is important to understand what mechanisms determine forest growth.
- Although nitrogen (N) is considered the most crucial nutrient for growth in the boreal forest, phosphorus (P) may be locally limiting.
- Less is known about the role of P because there are no easy methods to study its many organic and inorganic forms.
- In this research, advanced biochemical techniques were used to characterise organic P in forest soils to the molecular level.
- Forest soils were shown to contain a diversity of organic P molecules, most of them nucleotides from RNA and phospholipids from the cell membranes of organisms.
- Much of the P in boreal forest soils is in small, high-energy and high-nutrient organic molecules that in principle are easily degraded into mineral P by organisms. Hence, changes to these degradation rates could strongly influence P availability in boreal forests.

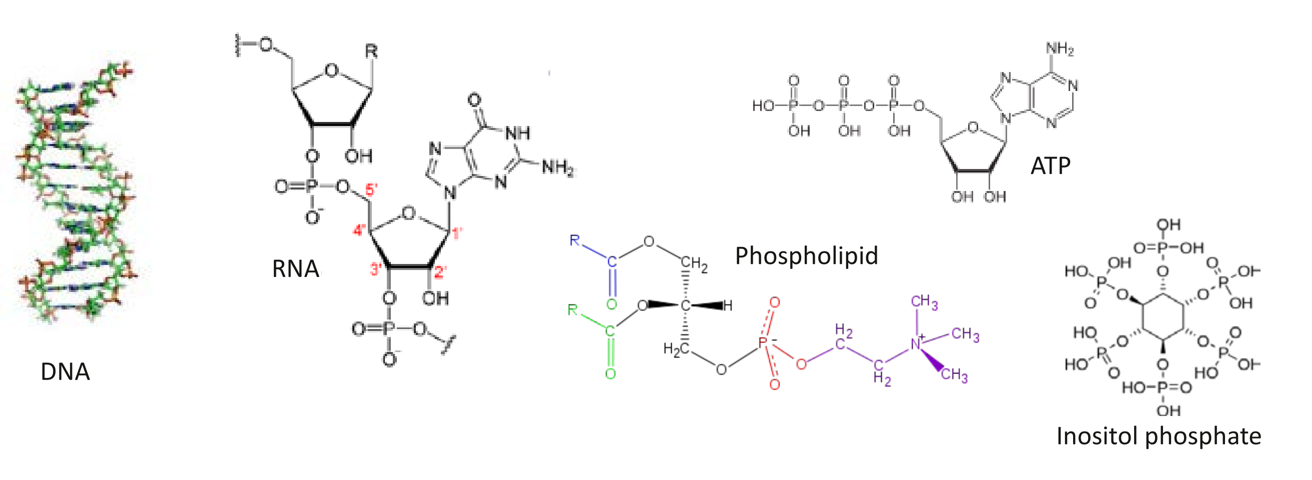



*Figure 2. Examples of organic P compounds commonly found in plants, microorganisms and animals.*

The boreal forest is the second largest biome in the world, holding 33 % of Earth's forest cover. Forests fulfil important ecosystem services such as the maintenance of biodiversity and carbon sequestration, and they are also crucial for the economy. As such, scientists have long been interested in understanding what factors influence the health and growth rate of forests. One of the most important factors are soil nutrients – a group of about 25 elements that plants and soil microbes need to grow and reproduce. Of these 25 elements, nitrogen (N) and phosphorus (P) are often the rate-limiting nutrients to forest growth worldwide. An increased availability of these elements, e.g. through fertilization, would yield the strongest growth response in trees. In the boreal forest, N is considered to be the most limiting nutrient to tree growth, and for a long time people have been investigating why and how N influences forest growth. Nevertheless, phosphorus is also an essential nutrient for life and tends to be scarce in the environment, but its role in boreal forests has been much less studied than that of nitrogen. This is partly because soil phosphorus is often bound to many different types of organic molecules requiring advanced techniques for their identification. Identifying the different forms of soil phosphorus is essential to know how it influences the growth of forests and obtain a more balanced picture of how boreal forests 'work'.

## Phosphorus – essential for life

Phosphorus (P) is an essential ingredient for life and is found in molecules like DNA, RNA, and ATP, and phospholipids (Figure 2), which are the major constituents of cell membranes of organisms. All the P present on the surface of our planet originally comes from rocks, and cannot be fixed by the organisms from the atmosphere, which many so-called nitrogen fixers can do with N. With time, rocks break down releasing mineral P to the soil, where plants and soil microbes absorb it and use it to grow, metabolising it into organic P. When plants and microbes die, the P returns to the soil in organic forms, and gradually becomes the dominant form of soil P. This is a problem for plants, because they can only use organic P after enzymes produced by roots and microbes release the mineral phosphate from organic P molecules. An enzyme's ability to release mineral phosphate from an organic P molecule depends on the type and size of the molecule, and whether it sticks to minerals in the soil, as this will influence an enzymes' ability to release phosphate. Therefore, understanding how much of the soil P is available to plants requires the identification of the different organic P molecules in soil. Organic P occurs in different 'families', like the diesters and the monoesters. Diesters are abundant in the tissues of plants, animals, and microorganisms, for example DNA, RNA, phospholipids, and ATP. Monoesters like inositol phosphate are found in seeds (Figure 2).


*Figure 3. Glass tube containing an extract of a boreal forest humus soil (left) which is then inserted into the Nuclear Magnetic Resonance spectrometer (right) to obtain $^{31}$P NMR spectra of soils. Photo Andrea Vincent.*

## From brains to dirt – Nuclear Magnetic Resonance spectroscopy

The greatest advances in characterising organic P in soil have been achieved with Nuclear Magnetic Resonance (NMR) spectroscopy, a technology used in medicine, amongst other things, to visualize internal structures of the body like the brain. In the 1980's, soil scientists realised that they could use

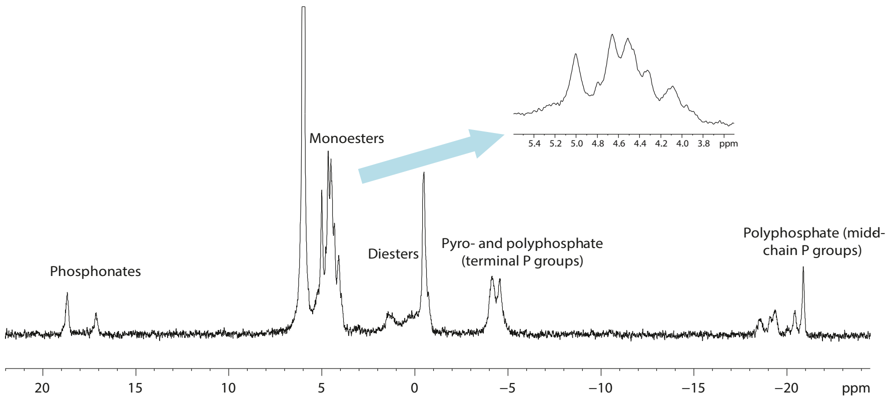



*Figure 4. Representative $^{31}$P NMR spectrum of a boreal forest soil, where each peak represents a different 'family' of phosphorus compounds. The inset is a zoomed-in section of the monoester region to show how broad it is.*

NMR to identify different molecules of organic P in soil. Soil samples are mixed with chemicals to bring the P out into a solution, and the solution put into a glass tube (Figure 3). The tube is then placed into a giant magnet (Figure 3), the central component of an NMR instrument.

A spectrum is produced (Figure 4), where each peak represents a different P compound, identifiable by its position on the horizontal axis. NMR studies of forest soils around the world show that they contain compounds belonging to the diester and monoester families, but that monoesters are by far dominant. While diesters are abundant in microbes, plants and animals, the origin and function of so much monoester P in soils is more uncertain, starting because their identification is especially difficult. As shown in the inset in Figure 4, the monoester region is composed of many overlapping peaks that look like a broad 'mountain'. It is more or less possible to tell what compounds some of the peaks are, but compounds are also 'buried' under the mountain and not visible. Soil organic P can amount to 90% of all the P in soil, and given that monoesters are the most abundant type of organic P, identifying them is crucial to understand if they are important for forest nutrition.

## A new method to indentify mystery organic P compounds

To solve this problem a different set of NMR tools were needed. In biochemistry, there is a type of NMR which 'sees' not just phosphorus (one-dimensional, or 1-D NMR), but also the hydrogen atoms attached to that phosphorus (2-dimensional, or 2-D NMR), and so it can easily tell apart overlapping P peaks, provided they have different hydrogens. The problem was that 2-D NMR does not work when samples have a lot of iron, and soils have lots of it. A logical solution would be to remove iron from the soil before the 2-D NMR analyses, but how to do that without altering the P itself? An unexpectedly simple yet successful solution was found in that sulphide ions like to bind to iron, forming an insoluble iron sulphide solid that can be removed by centrifugation. The resulting iron-free sample was perfectly suitable for 2-D NMR. The first time ever 2-dimensional NMR spectrum of a forest soil is shown in Figure 5. The spectrum is like a topographic map, where each of the 'blobs', or crosspeaks represents a different monoester. Just as in a topo map, the crosspeaks with many lines are the 'tallest mountains', i.e the most abundant monoesters.

Crosspeaks joined by horizontal lines (Figure 5) have similar numbers on the vertical (P) axis, but they come up in totally different positions on the horizontal (H) dimension. As such, the extra hydrogen dimension in 2-D NMR is crucial to tell apart compounds that look the same in 1-D NMR.

## Forest soils are full of RNA and phospholipids

Unexpectedly, 2-D NMR revealed that much of the P detected in forest soils was in the form of the nucleotides which are the building blocks of RNA, or ribonucleic acid (Figure 5). RNA is used by organisms for many different things including protein synthesis, and as a rule, only organisms that are active and growing produce RNA. But why is there so much RNA in soils? RNA is a molecule that breaks down quickly when located outside of living organisms, meaning that the RNA detected was probably inside living microbes that were killed and split open during the NMR analysis. The 2-D NMR also revealed the

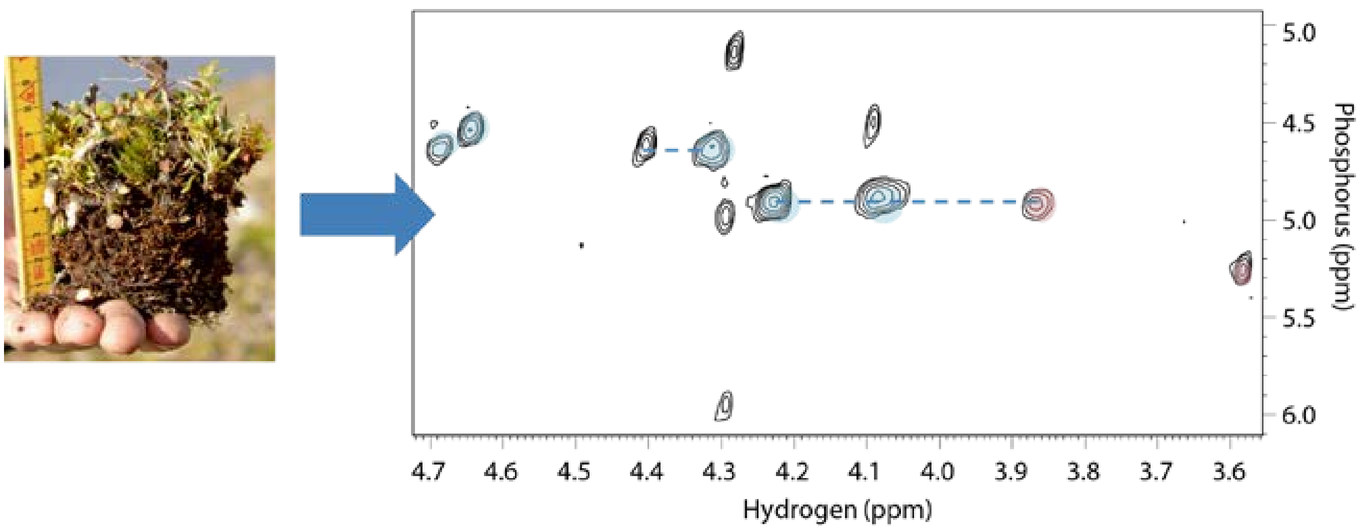



*Figure 5. Boreal forest humus soil (left) and representative two-dimensional $^{1}$H, $^{31}$P NMR spectrum of a boreal forest soil. Each crosspeak represents a different phosphorus compound. Blue indicates nucleotides from RNA and pink indicates phospholipids. Photo Reiner Giesler.*

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

– Knowledge for sustainable development

## Phosphorus in forest soils – Disentangling the chemistry of an essential nutrient

presence of lots of phospholipids, which are present in the cell membranes of organisms and are also released into soils when microbes die.

### Implications and future directions

This research shows that a large proportion of P in forest soils is probably located inside the bodies of active, living microbes. It also shows that these compounds have molecular structures which suggest they are easily degraded and thus that the plant-available mineral phosphate in them can be easily released. One implication of this finding is that any factors that speed up the rate of degradation of organic P molecules have the potential to greatly increase P availability in boreal forest soils. This might be the case for example if the activity of soil microorganisms were to increase in the future, as is expected with climate warming. Having obtained a better better picture of the different compounds that make up organic P in soils, the next step is to track their movement across the soil-plant system, in order to continue building knowledge of how and when forest plants and microbes use organic P to grow.


### Acknowledgements

This research was financed by the Swedish research councils VR and FORMAS, together with CMF and the Wallenberg and Kempe foundations.


### Read more


Vestergren, J., Vincent, A. G., Jansson, M., Persson, P., Ilstedt, U., Gröbner, G., Giesler, R. & Schleucher, J. 2012. High resolution characterization of organic phosphorus in soil extracts using two-dimensional $^{1}$H–$^{31}$P NMR correlation spectroscopy. Environmental Science & Technology 46:3950–3956.

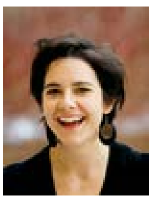
Vincent, A. G., Schleucher, J., Gröbner, G., Vestergren, V., Persson, P., Jansson, V. & Giesler, R. 2012. Changes in organic phosphorus composition in boreal forest humus soils: the role of iron and aluminium. Biogeochemistry 108:485–499.



### Keywords

Phosphorus, forests, soil, nutrients, nuclear magnetic resonance (NMR) spectroscopy.


### Authors




**ANDREA VINCENT**
Postdoctoral Researcher,
Department of Forest Ecology and Management,
SLU, SE-901 83 Umeå
+46-70-670 35 75
Andrea.Vincent@slu.se



**ULRIK ILSTEDT**
Associate Professor,
Department of Forest Ecology and Management,
SLU, SE-901 83 Umeå
+46-90-786 83 90
Ulrik.Ilstedt@slu.se



**REINER GIESLER**
Professor,
Department of Ecology and Environmental Sciences,
Umeå University
SE-901 87 Umeå
+46-980-400 13
Reiner.Giesler@emg.umu.se



**JOHAN VESTERGRAN**
PhD student,
Department of Chemistry,
Umeå University
SE-901 87 Umeå
+46-90-786 59 73
Johan.Vestergren@chem.umu.se



**PER PERSSON**
Professor,
Department of Chemistry,
Umeå University
SE-901 87 Umeå
+46-90-786 55 73
Per.Persson@chem.umu.se



**GERHARD GRÖBNER**
Professor,
Department of Chemistry,
Umeå University
SE-901 87 Umeå
+46-90-786 63 46
Gerhard.Grobner@chem.umu.se



**JÜRGEN SCHLEUCHER**
Professor,
Department of Medical Biochemistry and Biophysics
Umeå University
SE-901 87 Umeå
+46-90-786 53 88
Jurgen.Schleucher@chem.umu.se



FAKTA SKOG – Rön från Sveriges lantbruksuniversitet. ISSN: 1400-7789. Produktion: SLU, Fakulteten för skogsvetenskap 2013. Ansvarig utgivare: Peter.Hogberg@slu.se. Redaktör: Goran.Sjoberg@slu.se. Illustratör: Fredrik Saarkoppel, Kobolt Media AB. Tryck: Danagård LiTHO, Linköping. Prenumeration: www.slu.se/faktaskog.