Phosphorus speciation across elevation and vegetation in soils of the subarctic tundra

A solution $^{31}$P NMR approach

Johannes Krohn
Abstract
In tundra, phosphorus (P) is an important macronutrient for plants and microorganisms. A major fraction of P exists as organic compounds in the topsoil which can be mineralized to bioavailable inorganic P. Since mineralization is positively related to temperature, climate warming is likely to increase P bioavailability but the extend of these changes may also depend on vegetation cover and soil properties. I assessed organic and inorganic P concentrations across an elevation and vegetation gradient in northern Sweden using one dimensional (1D) solution 31P nuclear magnetic resonance spectroscopy. I hypothesized that concentration of labile soil P will decrease with increasing elevation (decreasing temperature) and that soils with meadow vegetation will contain higher concentrations of labile P than heath soils. Concentration of labile P in the form of Resin-P and polyphosphates decreased with elevation whereas less labile orthophosphate monoesters increased. Across vegetation types, polyphosphates were more abundant in heath and meadow contained higher concentrations of monoesters. The inverse response of Resin-P and monoesters to elevation may be best explained by lowered organic P mineralization in colder climate. High concentrations of polyphosphates at the lowest elevation may indicate an increased presence of fungal communities associated with mountain birch forest. Heath seemed to be more dominated by fungal communities than meadow and higher concentration of monoesters in meadow indicated a higher soil sorption capacity. In a broader view, the results may suggest that a warmer climate increases mineralization of organic P in form of orthophosphate monoesters to more labile P forms. This effect might be enhanced by an upward movement of the tree line and might be more pronounced in heath than meadow soils due to a higher fungal activity.

Key words: Climate warming, arctic ecosystems, tundra, phosphorus, Solution 31P NMR
Terms and Abbreviations
Phosphorus: P
Nitrogen: N
Aluminum: Al
Iron: Fe
Calcium chloride: CaCl₂
Sodium hydroxide: NaOH
Ethylenediaminetetraacetic acid: EDTA
Methylphosphonic acid: MPA
Organic matter: OM
Loss on ignition: LOI
One dimensional: 1D
Nuclear magnetic resonance: NMR
Labile P: Combination of inorganic and organic P considered to be plant available
Resin-P: Labile P determined by an anion-exchange resins soil test
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1. Introduction

In the world’s tundra biome (Fig. 1), phosphorus (P) is an important macronutrient for plant growth which can be limiting or, together with nitrogen (N), co-limiting nutrient (Bowman et al., 1993; Bowman, 1994; Shaver and Chapin, 1995; Rien and Chapin, 2000; Seastedt and Vaccaro, 2001; Hobbie and Gough, 2002; Soudzilovskaia et al., 2005; Giesler et al., 2012). New P is mainly released by weathering from rocks, e.g. apatite, though the process is relatively slow in tundra systems due to the cold climate (Kitayama et al., 2000). A major pathway for new inputs of N to tundra soils is via fixation of atmospheric N by free-living cyanobacteria, lichen, moss associated blue green algae and legumes (Alexander and Schell, 1973; Liengen and Olsen 1997; Hobara et al., 2006; Rousk and Michelsen 2016).

Figure 1. Extension of worlds arctic and alpine tundra biome (yellowish) in the northern hemisphere (Data from World Wildlife Fund Terrestrial Ecoregions; Basemap from ESRI)

However, P weathering and N fixation are minor sources for biological utilization as compared to the mineralization of accumulated organic compounds in the humus soil and plant litter (Fig. 2). Consequently, P and N supply for plants and microorganisms is largely controlled by microbial (fungi and bacteria) mineralization of organic matter (OM) and plant litter to CO₂, H₂O and other inorganic compounds (Chapin et al., 1995; Weintraub, 2011). The mineralization rate is related to temperature and warming experiments showed that higher temperatures generally favor the availability of P for plants (Chapin et al., 1995; Jonasson et al., 1999; Schmidt et al., 2002). Moreover, studies from the subarctic tundra revealed a greater P availability for plants with decreasing elevation i.e. increasing temperature (Sundqvist et al., 2011a; Vincent et al., 2014).

Another important mechanism for P uptake by plants and microorganisms is the availability of N (Sundqvist et al., 2011b). Thus, a high N availability increases the demand of P which causes P limitation. N limitation, on the other hand, leads to an excess of bioavailable P unusable for plants and microorganisms. To this day, the influence of temperature on N:P stoichiometry in tundra soils is unclear. However, the study of Sundqvist et al. (2011b) in a subarctic tundra showed that the relative importance of N for P plant limitation increased with higher elevation (and thus decreasing temperature), though these findings only referred to N:P ratios in plant leaf and litter tissue. Further, N was found to be highly important for P limitation in boreal forest soils dependent on site-specific changes in hydrochemistry (Giesler et al., 2002).
Other than P limitation by high N availability, P can get limited for plants by microbial or non-biological (soil sorption processes) immobilization (Fig. 2; Jonasson and Chapin 1991; Nadelhoffer et al., 1991). Thus, a high proportion of plant available P released to the soil by mineralization is immobilized before plants utilization. In arctic tundra soils, P immobilization rates were reported be as high or higher than P mineralization rates (Chapin et al., 1988, Giblin et al., 1991, Nadelhoffer et al., 1991, 1992, Jonasson et al., 1993; Giblin et al., 1991; Hobbie and Chapin, 1996; Schmidt et al., 1999).

Non-biological immobilization can be highly site-specific and related to multiple environmental factors such as topography (Giblin et al., 1991; Giesler et al., 1998), soil moisture, pH, depth of the organic horizon (Shaver et al., 1998; Lagerström et al., 2009) and the soil age (Hobbie and Gough, 2002; Vitousek, 2002). Studies reported an increased P sorption capacity in humus soils of a boreal forest and subarctic tundra which was related to high concentrations of aluminum (Al) and/or iron (Fe) (Giesler et al., 2002, 2004; Vincent et al., 2011). Thus, sorption processes including Al and Fe are highly dependent on the quantity of these elements and are favored in acidic soils. In calcareous soils P can precipitate with calcium by forming calcium-phosphates. However, the precipitated P is not ultimately plant unavailable. As plants remove P from the soil solution, the more calcium phosphate dissolve and solution P levels are partly replenished. In addition, P gets actively mobilized i.e. solved from precipitated forms to the soil solution by microorganisms (Richardson and Simpson, 2011) and plants root excretion of organic acids (Schachtman et al., 1998). Both P mobilization by root exudates and microbial processes are commonly known to enhance P availability for plants and microorganisms. In summary, sorption of P affect plant P availability and depend on the amount of surface sorption sites e.g. Al and Fe and soil pH.

As a second mechanism of P immobilization, the uptake of bioavailable P by microorganisms was reported to negatively influence plant P availability (Nadelhoffer et al., 1991). Despite microorganisms immobilize significant amounts of nutrients, studies from several tundra ecosystems, however, suggest that plant N and P uptake might be not affected by microbial immobilization in soils with an active root system (Fisk and Schmidt, 1996; Jonasson et al., 1993;...
The tundra is characterized by a mosaic of two main vegetation types - heath and meadow - dependent on the local topography, hydrology and snow cover (Fig. 2). Heath soils are characterized by low pH and N availability and slow-growing dwarf-shrubs, while meadow soils are characterized by high pH, high N availability and dominated by fast-growing herbaceous species (Björk et al., 2007; Eskelinen et al., 2009; Sundqvist et al., 2011b). Several studies found a relationship between P bioavailability and vegetation type. For instance, Giesler et al. (2012) and Vincent et al. (2014) found the distribution of P forms to be different across vegetation types with labile P (plant available P) concentrations being lower in meadow as compared to heath sites. This might be associated with decreased organic P mineralization and connected higher P sorption capacity in meadow sites. Interestingly, the study of Sundqvist et al. (2014) showed contrasting P dynamics across an elevation gradient for vegetation types with the temperature response of P mineralization and immobilization being stronger in heath than in meadow sites. Moreover, Vincent et al. (2014) found a significant interaction effect between elevation and the tundra vegetation type for multiple P forms. Since P mineralization and immobilization is related to temperature and vegetation, understanding turnover processes of organic P is critically important.

Organic P includes a large quantity of molecular forms with varying mobility, bioavailability and vulnerability to sorption (Condron et al., 2005; Vestergren et al., 2012). Before utilization by microorganisms or plants, most of the P forms require hydrolysis (Richardson and Simpson, 2011; Vincent et al., 2011). The main molecular forms of organic P are orthophosphate monoesters (e.g. inositol- and sugar orthophosphates), orthophosphate diesters (e.g. DNA and phospholipids) and phosphonates (Fig. S1). In the study of Turner et al. (2004) from several Fennoscandian subarctic mountain birch - tundra sites, these forms were reported to comprise 44-55%, 12-16% and 0-4% of the total P content in the surface soil, respectively. Proportion of inorganic P forms was relatively low with orthophosphates between 15-25%, pyrophosphate between 3-18% and polyphosphate between 0-15%, illustrating the dominance of organic P forms in organic tundra soils similarly to histosols such as peat soils. The composition of P forms is determined by several interconnected abiotic and biotic factors such as P utilization by microorganisms and plants, soil sorption processes and precipitation reactions (Celi and Barberis 2005). Although most soil P can be found as orthophosphate monoesters, soil organic P inputs are dominated by diesters (Magid et al., 1996), implying that plants and microorganisms highly control the composition of P forms. On the other hand, utilization of organic P by plants and microorganisms depends on organic P immobilization such as soil sorption processes which limits the organic P forms available for microbial degradation (Lung and Lim 2006). Thereby, the vulnerability to sorption to a certain soil compound, e.g. Al and Fe, increases with increasing number of P groups (Turner et al., 2002). Consequently, P forms which are weakly sorbed to soil constituents such as orthophosphate diesters are more available to plants and microorganisms (Celi and Barberis, 2005). In contrast, some orthophosphate monoesters are rapidly stabilized and less bioavailable (Vincent et al., 2011).

Determination of soil organic P forms across tundra vegetation types and its response to increasing temperature is crucial for the understanding of key ecosystem processes such as primary production. Recent studies showed that the bioavailability of P is influenced by numerous co-varying environmental factors including temperature, vegetation and soil properties (Sundqvist et al., 2011a, 2011b, 2014; Giesler et al., 2012; Vincent et al., 2014). However, little is known about the composition of organic P forms in tundra soils across elevation and vegetation gradients (i.e. changes in temperature, soil properties and microbial
processes) despite that organic P is the dominant form of P in tundra soils. It is therefore of high interest to determine organic P forms and processes that affect their mineralization for a better understanding of how environmental factors influence plant P bioavailability. A tool for the determination of organic P forms in high resolution is the one-dimensional (1D) solution $^{31}$P nuclear magnetic resonance spectroscopy (NMR) which has been successfully applied for molecular-level characterization of organic P in boreal forest (Vincent et al., 2011, 2013) and tundra soils (Turner et al., 2004; Yang et al., 2016). The main aim of this study is to characterize inorganic and organic P forms across vegetation and elevation on a molecular-level to test how abiotic (temperature) and biotic (vegetation) factors affect the distribution of major functional P forms.

I hypothesize that (i) with decreasing temperature (increasing elevation) non-labile organic P forms will become more abundant due to slower mineralization and that (ii) meadow will contain higher concentrations of labile P forms than heath due to better conditions for microbial mineralization of organic P.

The ultimate aim of this study is to broaden the knowledge about the response of tundra P forms to future global warming and its potential impact on net primary production of different tundra vegetation types.

2. Materials and Methods
2.1. Study sites and soil sampling
The study sites were located within a radius of 25 km around Abisko (Norrbotten, Sweden, Fig. 3). Three sites formed an elevation gradient (500, 750 and 1000 m) and both vegetation types (heath and meadow) to be present at each elevational level (Fig. 4). The sites at 500 m were located within the mountain birch forest whereas at 750 and 1000 m the tundra was treeless. The tree line was formed by Betula pubescens ssp. Czerepanovii at an elevation of 500-600 m. The dominant species in the tundra heath were related to the dwarf-birch Betula nana, lichens, bryophytes and ericaceous dwarf-shrubs. For tundra meadow, the main species were graminoids and forbs. See Sundqvist et al. (2011) for a detailed vegetation description. The bedrock type varied from phyllitic and mica hard schists towards the west of Abisko to a dominance of granite and feldspathic sandstone towards the east of Abisko. Accordingly, the studied humus soils underlie a varying calcareous influence being more pronounced in the western parts of the study area (Fig. 3). The mean air temperature at 388 m.a.s.l (Abisko Scientific Research station) for the sampling period (01.08. – 15.08.2017) was 11°C and cumulative precipitation for the same period was 37 mm. Assuming a temperature decrease of 0.5°C with an increasing elevation of 100 m, the mean air temperature for the sampling period at 500, 750 and 1000 m were 10.4, 9.2 and 7.9°C, respectively.

The soil sampling was conducted during the first half of August 2017 (Fig. 5). For each plot, five to thirteen randomly selected soil cores (8.3 – 10.2 cm diameter) were sampled down to 10 cm depth or, if the humus layer exceeded 10 cm, to the full humus depth. The humus depth varied strongly between and within the plots for both vegetation types with a minimum of 2 cm and a maximum of 23 cm. In average, the total humus depth at heath and meadow sites were $6.4 \pm 3.4$ (mean ± SD) and $4.1 \pm 2.0$ cm, respectively. The average total humus depths for the studied elevation were $6.0 \pm 3.9$, $4.7 \pm 2.7$ and $5.0 \pm 2.2$ cm starting at 500 m in ascending order. Between the soil cores, I maintained a minimal distance of 1 m to fully represent a plot. The soil cores of each plot were bulked together to obtain a total number of 24 composite samples, one for each elevation – vegetation combination. The composite samples were sealed in polyethylene bags, transported to the laboratory on the same day of sampling and immediately stored at 2°C (<12 h). For homogenization and separation of roots from the humus soil, samples were sieved (4 mm mesh size) within 24 h after sampling. The sieved humus soil was stored at 2°C.
Figure 3. Location of the study sites. Each of the four elevation gradients (500, 750 and 1000 m) is represented by three identical symbols (points, triangles, squares or hexagons).

Figure 4. Characteristic landscape of the tundra in northern Sweden. Photographs show the studied vegetation types (heath and meadow) across an elevation gradient (500, 750 and 1000 m). Photographs by J. Krohn.

Figure 5. Soil sampling at 750 m elevation from a heath soil (left) with a soil core sampler (middle) and an achieved soil sample at 1000 m (right). Photographs by R. Giesler (left) and J. Krohn (middle, right).
2.2. Determination of soil properties

The moisture content was determined gravimetrically by drying the soil at 105°C for 24 h. Loss on ignition (LOI) was determined on the dried samples by ignition in a muffle furnace (5 h, 550 °C). The pH was measured in a soil-water suspension with the ratio 1:20 (50 ml deionized water and fresh soil equivalent to 2 g d.w.) in 150 ml polypropylene bottles. After the first measurement, 5 ml of 0.11 M calcium chloride (CaCl2) was added to the distilled water suspension and the pH was measured again with a CaCl2 concentration of 0.01 M. Prior to each pH measurement, the bottles were shaken on a shaking table (2 h, 210 rpm) and left for sedimentation (1 h). For all pH measurements a Mettler-Toledo MP220 pH meter and a Mettler-Toledo Inlab Science glass electrode was used.

2.3. One dimensional $^{31}$P NMR

Soil extraction

I used 1D $^{31}$P NMR to quantify the concentration of organic P forms extractable by a sodium hydroxide (NaOH) and ethylenediaminetetraacetic acid (EDTA) such as inorganic orthophosphate, orthophosphate diesters and monoesters, phosphonates, pyrophosphate, and polyphosphates (Fig. S1). For this purpose, P was extracted for two replicates of each sample by shaking fresh soil equivalent to 5 g d.w. in 100 ml of a solution containing 0.25 M NaOH and 0.05 M EDTA for 4 h at room temperature (20°C). Extracts were immediately centrifuged at 12,000 rpm for 30 min in 250 ml centrifuge bottles and the supernatant (90 ml) was transferred to 250 ml polypropylene bottles and frozen at -80°C. Frozen samples were lyophilized over several days and stored in sealed bottles for further analysis.

Spectra acquisition

Samples for 1D $^{31}$P NMR were prepared from lyophilized NaOH-EDTA extracts; the lyophilization yielded 2.7±0.2 g of material. About 120 mg of this material were re-dissolved in 600 µL 50 mM Na2S in D2O, vortexed for 2 min, left to precipitate at room temperature for 15 h, and then centrifuged for 30 min at 10,000 rpm. The sulfide treatment was done to achieve linewidths narrow enough for 2D 1H, $^{31}$P NMR as described in Vestergren et al. (2012). A portion of the supernatant, 400 µL, was transferred into a 5 mm NMR tube for 1D $^{31}$P NMR. Additions of 30 µL of 2.8 M NaOH in D2O and 50 µL of 10 mM methylphosphonic acid (MPA, CH3O3P, Sigma-Aldrich product number 289868) in D2O were made directly into the NMR tube. The MPA served as P references for the quantification of individual compounds, and each 50 µL spike contained 0.5 µmol P. Spectra were obtained using a Bruker DRX 600 MHz spectrometer (Bruker, Germany) equipped with a CP-BBO-H/F cryoprobe tuned to 242.9 MHz for $^{31}$P. NaOH-EDTA extracts were analyzed using a 19 µs pulse (90°), a recycle time of 16 s including an acquisition time of 0.986 s, and broadband proton decoupling. Depending on sample, 440-720 scans were acquired, each run lasting approximately 3 h. Spectra were processed with a line broadening of 15 Hz, and chemical shifts of signals were determined in parts per million (ppm) using the signal of MPA at 20.5 ppm as internal standard. Identification of the main P classes was based on chemical shifts in the literature (Makarov et al., 2002; Turner et al., 2003, 2008; Vestergren et al., 2012, Table 1). Peaks were first ‘picked’ using an automatic peak fitting procedure, and peaks not ‘picked up’ by the program but clearly visible, were manually defined using the NMR software TopSpin 2.0 (Bruker, Germany). Signal areas were calculated by manual integration of spectral regions and of the MPA signal. Concentrations of P compounds were calculated using the known concentration of the MPA spike and are presented based on air dried soil (µg P g⁻¹ d.w. soil). All spectral processing was done using the program TopSpin 2.0.
Table 1. Reported chemical shifts of NaOH-EDTA extractable main phosphorus forms detected by solution $^{31}$P NMR

<table>
<thead>
<tr>
<th>Phosphorus class</th>
<th>Chemical shift (ppm)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphonates</td>
<td>19 to 20.5</td>
<td>Turner et al. (2004)</td>
</tr>
<tr>
<td>Inorganic orthophosphate</td>
<td>5.7 to 6.2</td>
<td>Turner (2008)</td>
</tr>
<tr>
<td>Orthophosphate monoesters</td>
<td>3 to 6</td>
<td>Turner et al. (2004)</td>
</tr>
<tr>
<td>DNA</td>
<td>-0.3 to -0.5</td>
<td>Vestergren et al. (2012), Turner et al. (2004)</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>0.6 to 1.75</td>
<td>Makarov et al. (2002), Turner et al. (2003a)</td>
</tr>
<tr>
<td>Pyrophosphate</td>
<td>-4.4</td>
<td>Turner et al. (2003a, 2004)</td>
</tr>
<tr>
<td>Polyphosphate end groups</td>
<td>-4</td>
<td>Turner et al. (2003a)</td>
</tr>
<tr>
<td>Mid-chain polyphosphate</td>
<td>-17 to -21</td>
<td>Turner et al. (2003a)</td>
</tr>
</tbody>
</table>

2.4. Anion-exchange resins
In addition to the 1D $^{31}$P NMR an anion-exchange resins soil test was performed to determine the amount of P “easy” available for plants and microorganisms. I placed soil equivalent to 2 g d.w. from each sample together with 180 ml deionized water and an anion exchange membrane (4.5 x 8.5 cm$^2$) into 250 ml polypropylene bottles and shake them for 18 h at room temperature on a shaking table at 10000 rpm. The membrane was then removed and rinsed with deionized water to flush of remaining soil remains. After, the membrane was transferred to 150 ml polypropylene bottles where it was elute with 40 ml of a 0.5 M NaCl and deionized water solution and shaken for 1 h (10000 rpm). The membrane was removed and the eluate was frozen at -20°C until analysis with a flow injection analyzer (FIAstar$^\text{TM}$ 5000 Analyzer, FOSS Analytical AB, Höganäs, Sweden).

2.5. Statistical analysis
The data was checked for normality (Shapiro-Wilk test) and homogeneity (Levene’s test), and variables were treated as independent for vegetation types across elevational levels. The differences in concentration of P forms and Resin-P between vegetation types and elevation were evaluated with two-way ANOVA and Tukey’s HSD test. The significance of differences was determined at 95% confidence level (p < 0.05). Linear regression was used to test the relationships between soil properties, Resin-P and phosphorus forms. All analyses were performed using R Statistical Software Version 3.2.3 (www.r-project.org).
3. Results

3.1. Anion-exchange resins and soil properties

Resin-P concentration did not differ between vegetation types but significantly decreased with increasing elevation (Fig. 6). Soil pH was significantly lower (p<0.001) in heath than in meadow with an average difference for pH (H$_2$O) of 1.2 and pH (CaCl$_2$) of 0.9 (Table 2). Total OM content was significantly higher (p=0.002) in heath and soils at 500 m elevation contained a higher proportion of total OM than soils at 750 and 1000 m (Table 2).

Table 2. Soil properties and total concentration (μg P g d.w.$^{-1}$) of NaOH-EDTA extractable total phosphorus (n=4, mean ± SD) across vegetation types (meadow and heath) and elevation (500, 750 and 1000 m) by solution $^{31}$P NMR spectroscopy

<table>
<thead>
<tr>
<th>Vegetation</th>
<th>Elevation</th>
<th>pH (H$_2$O)</th>
<th>pH (CaCl$_2$)</th>
<th>Moisture content, % f.w.</th>
<th>LOI, %</th>
<th>NaOH-EDTA total P, μg P g$^{-1}$ d.w.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meadow</td>
<td>500 m</td>
<td>6.3 ± 0.5</td>
<td>5.7 ± 0.6</td>
<td>75 ± 5</td>
<td>68 ± 10</td>
<td>344 ± 68</td>
</tr>
<tr>
<td></td>
<td>750 m</td>
<td>5.8 ± 0.4</td>
<td>4.9 ± 0.4</td>
<td>63 ± 7</td>
<td>49 ± 26</td>
<td>443 ± 105</td>
</tr>
<tr>
<td></td>
<td>1000 m</td>
<td>5.9 ± 0.5</td>
<td>5.0 ± 0.7</td>
<td>64 ± 9</td>
<td>46 ± 18</td>
<td>443 ± 110</td>
</tr>
<tr>
<td>Heath</td>
<td>500 m</td>
<td>4.5 ± 0.2</td>
<td>3.5 ± 0.3</td>
<td>71 ± 3</td>
<td>85 ± 6</td>
<td>284 ± 102</td>
</tr>
<tr>
<td></td>
<td>750 m</td>
<td>5.0 ± 0.6</td>
<td>3.8 ± 0.7</td>
<td>75 ± 5</td>
<td>76 ± 12</td>
<td>277 ± 32</td>
</tr>
<tr>
<td></td>
<td>1000 m</td>
<td>4.9 ± 0.1</td>
<td>3.9 ± 0.2</td>
<td>74 ± 5</td>
<td>73 ± 15</td>
<td>411 ± 56</td>
</tr>
</tbody>
</table>

Figure 6. Average Resin-P concentration (n=4) across vegetation (meadow and heath) and elevation (500, 750 and 1000 m). Overall significant difference (Tukey’s HSD, p < 0.05) across elevation is represented by different uppercase letters. Error bars correspond to 95% confidence intervals.

3.2. P forms composition by one dimensional $^{31}$P NMR

The acquired one dimensional $^{31}$P NMR spectra of NaOH-EDTA extracts treated with Na$_2$S showed signals at high resolution (Fig. 7). Organic P forms (orthophosphate monoesters, diesters, phosphonates) dominated in all soils with an average contribution of 75% as compared to inorganic P forms (orthophosphate, pyrophosphate and polyphosphates; Table 3). Polyphosphates were classified inorganic due to missing chemical shifts in the spectral region between -9 and -10 ppm (Turner et al., 2003a). Among all P forms, orthophosphate monoesters dominated and were followed by inorganic orthophosphate, diesters (here DNA and phospholipids), polyphosphates, pyrophosphate and phosphonates in descending order (Table 3).
Figure 7. Solution 31P NMR spectra (in ppm) of NaOH-EDTA extracts treated with Na$_2$S from heath at 500 m elevation (A), meadow at 1000 m (B) and 500 m (C). Line broadening: 15 Hz; Number of scans: 440 (C), 620 (A) and 720 (B).
Table 3. Phosphorus forms proportion (%; n=4, mean ± SD) across vegetation types (meadow and heath) and elevation (500, 750 and 1000 m) detectable by solution \(^3\)P NMR spectroscopy

<table>
<thead>
<tr>
<th>Vegetation</th>
<th>Elevation</th>
<th>Phosphonates</th>
<th>Inorganic Orthophosphate</th>
<th>Orthophosphate monoesters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meadow</td>
<td>500 m</td>
<td>1.2 ± 0.3</td>
<td>23.6 ± 1.5</td>
<td>52.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>750 m</td>
<td>1.2 ± 0.1</td>
<td>19.1 ± 0.8</td>
<td>65.1 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>1000 m</td>
<td>1.1 ± 0.3</td>
<td>16.2 ± 1.3</td>
<td>70.1 ± 1.3</td>
</tr>
<tr>
<td>Heath</td>
<td>500 m</td>
<td>2.0 ± 0.5</td>
<td>25.0 ± 0.8</td>
<td>39.5 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>750 m</td>
<td>1.8 ± 0.2</td>
<td>15.5 ± 1.2</td>
<td>57.4 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>1000 m</td>
<td>1.6 ± 0.2</td>
<td>14.9 ± 0.9</td>
<td>57.0 ± 2.2</td>
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<table>
<thead>
<tr>
<th>Vegetation</th>
<th>Elevation</th>
<th>DNA, Phospholipids</th>
<th>Pyrophosphate</th>
<th>Polyphosphates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meadow</td>
<td>500 m</td>
<td>14.6 ± 0.6</td>
<td>4.2 ± 0.5</td>
<td>3.7 ± 1.3</td>
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<tr>
<td></td>
<td>750 m</td>
<td>9.8 ± 0.4</td>
<td>4.3 ± 0.3</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>1000 m</td>
<td>8.4 ± 0.5</td>
<td>3.5 ± 0.5</td>
<td>0.3 ± 0.1</td>
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<tr>
<td>Heath</td>
<td>500 m</td>
<td>14.9 ± 0.7</td>
<td>5.0 ± 0.9</td>
<td>13.7 ± 1.7</td>
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<tr>
<td></td>
<td>750 m</td>
<td>15.3 ± 0.8</td>
<td>4.5 ± 0.5</td>
<td>5.4 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>1000 m</td>
<td>18.2 ± 0.8</td>
<td>3.2 ± 0.4</td>
<td>5.1 ± 0.8</td>
</tr>
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</table>
**Phosphorus forms across vegetation and elevation**

Concentration of NaOH-extractable total P significantly differed across vegetation types (p=0.027) being higher in meadow and showed a tendency to increase with elevation (p = 0.062; Table 2). Orthophosphate monoesters concentration significantly increased with elevation by twofold for heath and 1.5 fold for meadow whereas concentration in heath was significantly lower than in meadow (Fig. 8; Table 4). Inorganic orthophosphate showed similarly to monoesters a significant lower concentration in heath but no effect to elevation. Interestingly, phosphate diesters concentration significantly increased from 500 and 750 m to 1000 m which was only observed for heath. Thus, vegetation and elevation significantly interacted for diesters. Moreover, diesters concentration tend to be higher in heath than in meadow (p=0.068). Both phosphonates and pyrophosphate showed no response to changes in vegetation and elevation. Polyphosphates concentration was significantly different across vegetation types and elevation. Interestingly, polyphosphates concentration substantially decreased from 500 m to 750 and 1000 m by sevenfold for meadow and twofold for heath.

![Image of bar graphs](image.png)

**Figure 8.** Average concentration (n=4) of NaOH-EDTA extractable phosphorus forms across meadow (A, C) and heath vegetation (B, D) and elevation (500, 750 and 1000 m). Overall significant difference (Two-way ANOVA, p < 0.05) across vegetation and elevation is represented by different lowercase letters and by an asterisk, respectively. Error bars correspond to 95% confidence intervals.
**Relationship between phosphorus forms**

For heath (but not for meadow), there was a positive relationship between diesters (DNA, phospholipids) and orthophosphate monoesters (Fig. 9 A, B), and Resin-P was positively related to polyphosphates (Fig. 9 C, D). Resin-P and inorganic orthophosphate were positively related for both vegetation types (Fig. 9 E, F).

![Graphs showing relationships](image)

Figure 9. Linear relationship between orthophosphate monoesters and diesters (DNA, phospholipids; A, B), Resin-P and polyphosphates (C, D), Resin-P and inorganic orthophosphate (E, F) for meadow and heath vegetation.
Table 4. Comparison (Two-way ANOVA) of Resin-P and the main NaOH-EDTA extractable phosphorus forms across vegetation types (meadow and heath) and elevation (500, 750 and 1000 m).

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<th></th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
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<td></td>
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<td></td>
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<td>22763</td>
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<td></td>
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<td>Vegetation</td>
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<td>2536.9</td>
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<td></td>
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<td>91.74</td>
<td>45.87</td>
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4. Discussion

The obtained results revealed that mineralization of organic P in form of orthophosphate monoesters was affected by temperature in the hypothesized manner. Resin-P was likely connected to temperature but high polyphosphates concentration at 500 m seemed to be also related to Resin-P. However, and not consistent with my hypothesis, vegetation did not affect Resin-P but instead monoesters concentration. Possible causes for these findings are discussed below.

4.1. Elevation

I hypothesized that the abundance of non-labile organic P forms will increase with decreasing temperature (increasing elevation) which was confirmed by an increasing concentration of orthophosphate monoesters. Monoesters are organic P forms considered to be resistant against mineralization. Thus, a low microbial activity in higher elevation likely slows mineralization and favors stabilization of organic P in form of monoesters. With decreasing elevation P stabilization by monoesters tend to decrease and instead, monoesters may become an important P source for microorganisms. This agrees with the increasing Resin-P concentration with decreasing elevation which was also found by Vincent et al. (2014) in soils from the same study area. Thus, labile P may partly originate from the mineralization of monoesters and this process may be more pronounced at higher temperature. However, other mechanisms might be also relevant for the relatively higher abundance of Resin-P in lower elevation. I suggest three possible mechanisms why Resin-P concentrations may be negatively related to elevation. First, the decreasing pool of orthophosphate monoesters resulted in an increased sorption of labile P to elements such as Al and Fe. This reduces P uptake by plants and microorganisms which results in relatively higher labile P concentration in the soil. Second, the microbial need for carbon causes mineralization of organic P forms without utilizing the P products (Spohn and Kuzyakov, 2013). Thus, labile P accumulates as a side product of the microbial metabolism. Third, an excess of labile P in lower elevation might result from an increased N limitation. This agrees with the study of Sundqvist et al. (2011b) about N:P ratios in plant leaf and litter tissue which reported a lower importance of N for P plant limitation with decreasing elevation. Moreover, the higher abundance of polyphosphates with decreasing elevation can be an indicator for nutrient imbalance (Ghonsikar and Miller, 1973). Polyphosphates are related to mycorrhizal communities which accumulate long-chained polyphosphate for storage purposes (Read, 1991; Makarov et al., 2002, 2005). The increase in polyphosphates at 500 m elevation therefore suggest that not temperature but the presence of mountain birch forest increased the abundance of mycorrhizal communities. Birch trees are known to have a strong symbiosis with ectomycorrhiza which represents an important nutrient absorbing organ and is involved in the mobilization of organic resources (Read, 1991). Thus, ectomycorrhiza is essential for birch (Betula pendula) to have access to organic N since N mineralization rates of many forest litters is slow (Abuzinadah and Read, 1989). The high presence of polyphosphates associated with ectomycorrhiza and relatively high labile P concentration therefore indicate N limitation at the sites with mountain birch forest.

4.2. Vegetation

My second hypothesis was that meadow will contain higher concentrations of labile P forms than heath, which was rejected due to similar Resin-P concentrations for both vegetation types. Contrary, the higher concentration of monoesters in meadow suggest that stabilizing processes are more important than mineralization for P form composition across vegetation types. Another study from the same study area reported similar higher organic P concentrations in meadow than heath (Vincent et al., 2014). The study also found a higher sorption index in meadow than heath soils which was related to higher Al and Fe concentrations. This may indicate that Al and Fe were related to a higher monoesters stabilization in meadow. Such relationship between Al, Fe and monoesters was already reported in boreal forest soils (Vincent et al. 2011) and is likely to apply for the tundra. However, the study of Giesler et al. (2012) from the same study area found no different sorption index between vegetation types and only
higher oxalate extractable Fe concentration in meadow. This indicates that sorption processes are highly site-specific and differ at relatively small spatial scales. Interestingly, the positive relationship between monoesters and diesters in heath may indicate a slower P turnover rate than in meadow. Diesters are favored as a microbial food source against monoester. A slow degradation of diesters might therefore lead to the accumulation of diesters similarly to monoesters which partly consist of diesters degradation products. In meadow, diester may be mineralized relatively quickly compared to monoesters. Such mechanism may explain the lacking relationship between these two P forms in meadow. Moreover, diesters concentration tend to be lower in meadow than in heath which may be additional evidence for higher turnover rates in meadow.

The higher polyphosphates concentration in heath indicate a higher presence of mycorrhizal communities which are favored against bacteria in a low pH environment (Björk et al., 2007). This agrees with the study of Sundqvist et al. (2011a) from the same study area which found a higher fungal presence in heath. Heath vegetation tend to have a symbiosis with ericoid- and ectomycorrhizae whereas for meadow vegetation arbuscular or no mycorrhizae are more common (Björk et al., 2007). Ericoid- and ectomycorrhizae are more efficient in mineralizing organic compounds than arbuscular mycorrhizae. Therefore, P turnover processes may be more driven by mycorrhzal mineralization of organic P in heath than in meadow. Monoesters, which were significantly lower in heath, are likely to be the organic P form which is affected the most by mycorrhizal mineralization in heath. The positive relationship between polyphosphate and labile P in form of Resin-P in heath may partly reflect the dependency of organic P mineralization and mycorrhizal processes. In contrast, meadow was reported to be more dominated by bacterial communities (Sundqvist et al., 2011a) which explains the lacking relationship between polyphosphates and Resin-P.

4.3. Interaction between vegetation and elevation
The significant interaction between vegetation and elevation for diesters may be best explained by a different microbial community between vegetation types. Thus, from the same study area it was reported that fungal dominance increased with elevation and that heath soils had a higher fungal biomass as compared to meadow (Sundqvist et al., 2011a). However, this does not explain the significant higher diesters concentration in heath soils at 1000 m as compared to 500 and 750 m elevation (consistent across all study sites) which was not observe for meadow. Thus, other unidentified mechanism(s) likely affected diesters concentration. The overall highest polyphosphates concentration in heath soils at 500 m indicated especially favorable conditions for mycorrhizal communities (ericoid- and/or ectomycorrhiza). Thus, the combination of mountain birch forest and a field layer dominated by heath vegetation may result in a mycorrhizal “hotspot”.

4.4. Future implications
Vincent et al. (2014) stressed the importance of determining organic P forms in high resolution in the tundra landscape to reveal mechanisms of P turnover. My study addressed this concern and gained knowledge about the dependency of the main P forms to vegetation and temperature. However, it is of relevance to investigate P forms composition in even more detail to reveal key molecular forms driving P turnover processes. For instance, orthophosphate monoesters differ in their vulnerability to mineralization (Condron et al., 2005). Thus, myo-inositol hexakisphosphate, a specific orthophosphate monoester, was found to be the key molecule for P stabilization processes in boreal forest soils (Vincent et al., 2011). The same study reported Al and Fe to be closely related to myo-inositol hexakisphosphate. This illustrates the importance of accounting for soil sorption. Determination of N and enzymatic analysis will help to identify P limitation. Lastly, I suggest the application of two dimensional -31P NMR to achieve a distinct identification of molecular P forms.
4.5 Conclusions

This study characterized inorganic and organic P forms across tundra vegetation types – heath and meadow – and across an elevation gradient – 500, 750 and 1000 m. P forms were found in a high diversity including forms which are considered as “easy” degradable such as diesters and phosphonates. Less labile orthophosphate monoesters were the most common organic P form. Both elevation (and thus temperature) and vegetation influenced monoesters towards higher concentrations in a colder environment and in meadow soils. Contrary, Resin-P showed decreasing concentrations with increasing elevation (decreasing temperature) which may be related to a decreasing mineralization of monoesters. Polyphosphates were negatively related to an increasing elevation and were more abundant in heath vegetation. This was likely connected to a high abundance of mycorrhizal communities in soils of mountain birch forests and heath vegetation. Mountain birch forests with a field layer dominated by heath vegetation may therefore be a “hotspot” for mycorrhizal mineralization of organic P. In a broader view, the results may suggest that a warmer climate increases mineralization of organic P in form of orthophosphate monoesters to more labile P forms. This effect might be enhanced by an upward movement of the tree line and might be more pronounced in heath than meadow soils due to a higher fungal activity.

Acknowledgement

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References


Figure S1. Organic phosphorus forms with generic and example structures and information on the relative lability and prevalence in soil (adapted from Darch et al., 2014) from George et al. (2017; in press)