Phosphorus availability and microbial respiration across biomes: from plantation forest to tundra

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Abstract
Phosphorus is the main limiting nutrient for plant growth in large areas of the world and the availability of phosphorus to plants and microbes can be strongly affected by soil properties. Even though the phosphorus cycle has been studied extensively, much remains unknown about the key processes governing phosphorus availability in different environments.

In this thesis the complex dynamics of soil phosphorus and its availability were studied by relating various phosphorus fractions and soil characteristics to microbial respiration kinetics. The soils used represent a range of aluminium, iron, carbon and total phosphorus content, and were located in four different biomes: subtropical forest, warm temperate forest, boreal forest and tundra.

The results showed that NaOH extractable phosphorus, a fraction previously considered to be available to plants only over long time scales, can be accessed by microbes in days or weeks. Microbial phosphorus availability was not related to aluminium or iron content in any of the studied systems, not even in highly weathered soils with high aluminium and iron content. This is in contrast with other studies of soils with high sorption capacity and shows the variability of factors that govern phosphorus availability in different environments.

In the boreal forest chronosequence, no difference could be seen with age in total phosphorus content or concentrations of occluded phosphorus forms. However, there were lower concentrations of labile phosphorus forms in older systems, which were correlated with a decrease in microbial respiration. This was most likely related to organic matter quality in the system, and not to geochemical factors.

Phosphorus availability was linked to differences in topography (water regime) and vegetation in the tundra ecosystems. The results suggest that the availability of phosphorus, both for microbes and plants, was lower on the meadow vegetation sites compared to the two types of heath vegetation.

Many factors are important for phosphorus availability in soils, but these results suggest that microbes can access less available phosphorus if not restricted by carbon, and this may be important in regard to forest management practices as well as effects of environmental change.

Keywords: phosphorus availability, microbial bioassay, soil respiration, microbial growth rate, Hedley fractionation, soil sorption, weathered soils, boreal forest, subarctic and tundra.
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Camilla Esberg
To everyone who put a smile on my face
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List of Papers

This thesis is based on the following papers, which are referred to by their Roman numerals in the text.

Paper I


Paper II


Paper III


Paper IV


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Introduction

Phosphorus is a large constituent in all living organisms, and it is essential in cellular structures (such as nucleic acids and phospholipids) and as an element in many processes inside living cells (for example, as an energy shuttle and ATP). It is therefore not surprising that phosphorus is seen as one of three primary macronutrients for plants (the other two being nitrogen and potassium). Phosphorus is naturally present in soil parent material and is gradually released through weathering. There is some phosphorus loss from the system, but it is mostly recycled locally among soil, plants, consumers and decomposers in a natural ecosystem (Buscot and Varma 2005).

In large areas of the world, phosphorus is considered to be a growth limiting nutrient, foremost in areas with old and highly weathered soils, such as in tropic and subtropic regions, and in calcareous soils with high pH. As a consequence, both agricultural land and forest plantations are being heavily fertilised with phosphorus to increase crop productivity. However, only between 10-20% of the added phosphorus is taken up by crop and pasture (McLaughlin et al. 1988, Holford 1997). This is a very inefficient use of a non-renewable resource, especially since the rock phosphate reserve is only predicted to last another 50-100 years (Cordell et al. 2009, and references therein).

It is evident that even though phosphorus in soil and the phosphorus cycle has been studied extensively over the years, there is a lack of knowledge concerning phosphorus dynamics in soil. Richardson (2001), for example, discusses how difficult it is to predict the effect of soil properties such as pH, total phosphorus and sorption capacity, on phosphorus mobilisation, even though these factors are known to affect phosphorus availability. This means that it is even harder to predict what is happening on a seasonal scale with different soil moisture and temperatures. Clearly we do not know enough about how interactions between biotic and abiotic factors affect phosphorus availability, or which soil processes dominate in a specific environment.

The orthophosphate released through weathering is easily adsorbed to soil particles, both mineral and organic. Phosphorus can be so tightly bound that it is unavailable to plants except over very long time periods (Cross and Schlesinger 1995). Phosphate can form insoluble complexes with elements such as aluminium and iron, or form non-soluble precipitates with calcium and magnesium. In old, highly weathered soils the sorption capacity for phosphorus is correlated to amorphous iron and aluminium oxides (Loganathan et al. 1987, Freese et al. 1992), since high concentrations of these compounds offer a large number of binding sites for phosphate. Its
high affinity to adsorb to soil particles and form complexes only leaves a small portion of free phosphate in the soil solution, and phosphorus can usually only be found in the order of micromolar in the soil solution while most other mineral nutrients occur in millimolars (Ozanne 1980).

Plants have developed strategies to compensate for low phosphorus availability. They can adapt to the circumstances by either reducing growth rate, remobilising internal phosphorus, modifying phosphorus metabolism, or increasing the uptake by modifying root structure or function in order to increase the explored soil volume (Turner et al. 2005). Plants can also change the environment around the roots, the rhizosphere, by releasing exudates which either affect the availability of phosphorus or stimulate microbes by providing them with energy (carbon compounds) (Turner et al. 2005). However, most plants are not able to acquire enough phosphorus by themselves and have developed a symbiotic relationship with mycorrhizal fungi (Read and Perez-Moreno 2003).

The symbiosis between plants and microbes means that plants can gain access to more phosphorus in exchange for the carbon compounds needed by the fungi (Smith and Read 1997). However, there are also other soil microbes that can affect phosphorus availability. For example, there are free-living fungi that can excrete organic acids and iron siderophores that make insoluble forms of phosphate available and contribute to the weathering of soil minerals (Mehta et al. 1979, Sollins et al. 1981). Microbes also have a very important role as decomposers in ecosystem nutrient cycling and can decompose complex organic material and humus. For example, it has been shown that soil bacteria are important for the mineralisation of organic phosphorus (McLaughlin et al. 1988, Subba Rao 1997).

Most studies showing how ecosystems become increasingly phosphorus-limited with time are from tropical and temperate ecosystems (Walker and Syers 1976, Walker et al. 1983, Vitousek 2002, Wardle et al. 2004, Coomes et al. 2005, Allison et al. 2007). Initially (in primary succession), nitrogen is the main limiting nutrient in terrestrial ecosystems; as the nitrogen pool builds up as a result of nitrogen fixation, for example, the balance shifts towards co-limitation of nitrogen and phosphorus. In the very long-term absence of major disturbances, this stage is then followed by a phosphorus-limited system, since there is a gradual decrease in phosphorus availability. This decrease is a result of phosphorus being adsorbed into soil particles or otherwise protected from desorption or decomposition, both chemically and physically.

There is not much known, however, about whether this process of old ecosystems moving towards phosphorus limitation also occurs in boreal ecosystems. Even though nitrogen is considered to be the main limiting nutrient in the boreal forest, there is a gradual build-up of soil nitrogen with
time through biological nitrogen fixation and atmospheric deposition (Lagerström et al. 2007). This accumulation of nitrogen could potentially lead to a shift towards phosphorus limitation in boreal forests as well, although this scenario is not yet fully explored. Furthermore, phosphorus limitation has also been demonstrated in local areas in the boreal forest, such as groundwater discharge areas (Giesler et al. 2002, 2004). Groundwater in these areas has redistributed aluminium and iron from the mineral soil to the humus layer, which has increased the phosphorus sorption capacity of the soil and caused a plant phosphorus limitation.

The alpine ecosystem is another ecosystem that is primarily considered to be nitrogen limited (Körner 2003). However, as in tundra ecosystems it is likely that transport and redistribution of soil elements caused by water flow occur. Ping et al. (1998) found high aluminium concentrations at the foothills of arctic Alaska, and there are also studies that have found high calcium concentrations in subarctic meadow sites (Darmody et al. 2000, Campbell et al. 2001, Darmody et al. 2004). As mentioned above, both high aluminium and calcium concentrations affect phosphorus availability. Does this mean that we could detect the same indications on microbial and plant phosphorus limitation as Giesler et al. (2004) found in the boreal forest?

Phosphorus in soil has traditionally been studied using wet-chemical methods, which produces phosphorus pools defined according to each method, and not necessarily reflecting the reality as experienced by plants and microbes. The Hedley fractionation method is well known and has often been used to estimate plant phosphorus availability (Hedley et al. 1982, Tate et al. 1991, Gahoonia and Nielsen 1992, Schmidt et al. 1996); it is therefore interesting to consider the extent to which this method is comparable to microbial phosphorus availability.

Soil respiration measurement is a widely used tool for studying soil microbial processes. In a bioassay, environmental factors such as temperature, humidity and nutrient content can easily be controlled. The method used for measuring respiration in these studies was developed by Nordgren (1992) for boreal forest soils, but has also been used successfully in tropical (Ilstedt et al. 2003) and subtropical soils (Teklay et al. 2006, Gnankambary et al. 2008).

Furthermore, the combination of microbial respiration kinetics and operationally defined phosphorus pools can generate insight into the complex dynamics that determine phosphorus availability in the following four biomes: subtropical and warm temperate plantation forest on highly weathered soil, boreal forest and tundra.
Aims of the thesis

The main objectives in the projects included in this thesis were:

- To assess how microbial phosphorus availability relates to soil phosphorus pools in plantation forest, boreal and tundra soils;

- To investigate if surface sorption is important for the availability of phosphorus, using examples from highly weathered subtropical and warm temperate plantation forest soils;

- To study phosphorus dynamics in a boreal forest using a long-term chronosequence; and

- To test if plants and microbes in tundra soils are phosphorus limited.
Study areas

Three types of ecosystems were studied: plantation forest (subtropical and warm temperate), boreal forest and subarctic tundra.

Six forest plantation sites in South Africa were used for studying subtropical and warm temperate forest soils. Two of the sites are part of the Mozambique coastal plains and situated in Kwambonambi, where the soil consists of aeolian sand deposits. The climate is subtropical with a mean annual temperature of 21.7°C and a mean annual precipitation range from 1,084 to 1,223 mm. The two sites were plantations with *Eucalyptus grandis* and *Eucalyptus urophylla*. The other four sites are located in KwaZulu-Natal Midlands, where the parent materials were dolerite (two sites), mudstone (one site) or arenite (one site). The climate is cool to warm temperate with mean annual temperature and mean annual precipitation ranging from 14.8 to 17.0°C and 760 to 988 mm, respectively. Two of the four clay sites were forest plantations with *Eucalyptus* trees (*Eucalyptus grandis* or *Eucalyptus smithii*) and the other two were planted with *Pinus patula* or *Pinus taeda*. All six sites had very little field layer vegetation; at most, some scattered herbaceous plants and ferns were found.

The boreal forest sites were located on 30 forested islands in Lake Hornavan and Lake Uddjaure in northern Sweden, where mean annual precipitation is 750 mm, and mean temperature in July and January is +13°C and -14°C, respectively. The vegetation on the islands is dominated by the tree species *Pinus sylvestris*, *Picea abies* and *Betula pubescens*, and the dwarf shrubs *Vaccinium myrtillus*, *Vaccinium vitis-idaea* and *Empetrum hermaphroditum*. The ground layer is dominated by feather mosses. The islands were divided into three size classes with ten islands each: large (>1 ha), medium (0.1-1.0 ha) and small (<0.1 ha), with the average time since the last major fire of 585, 2,180 and 3,250 years, respectively (Wardle et al. 2003). The main disturbance factor on the islands is wildfire caused by lightning strike; the larger islands are struck more often than the small ones and are therefore exposed to fire with shorter intervals (Wardle et al. 1997, 2003).

The tundra sites are situated in northern Swedish Lapland, in the subarctic part of the Fennoscandian mountain range above the tree line in the alpine life zone. The climate is characterised as having short, cool summers and mild, snow-rich winters. The mean annual temperature ranges from -2 to -5°C, and mean annual precipitation is between 800 and 1,000 mm (Eriksson 1982, Molau and Larsson 2000, Jansson et al. 2008). Three sites were selected and at each site three types of vegetation community were studied. Each vegetation community has a different water regime. Dry heath is mainly supplied by precipitation and the vegetation is sparse and patchy,
dominated by cryptogams and creeping dwarf shrubs. The mesic heath community is found on more moist soils and dominated by a denser layer of dwarf shrubs. The vegetation is usually taller on mesic than on dry heath. Mesic meadow communities are usually found in the lower parts of slopes and receive water from higher parts of the landscape. The vegetation is dominated by herbaceous vegetation, intermingled by dwarf shrubs.

**Figure 1** Schematic graph of hourly measurements of soil microbial respiration rate (CO2 h⁻¹) before and after substrate addition. Basal respiration is the mean value of hourly measurements before substrate addition. Maximum respiration is assumed to occur when a substance limits further microbial growth. Substrate-induced respiration stands for the respiration rate directly following substrate addition. Lag time is defined as the time from substrate addition to when microbes start to grow exponentially. The exponential phase is expressed as growth rate, the slope of log transformed respiration rate versus time.
Methods

Soil samples from the subtropical and warm temperate forests were collected in 2004 as described in Papers I and II. The boreal forest soil samples and the samples from the tundra sites were collected in 2005 (described in Paper III) and 2006 (see Paper IV), respectively. At the tundra sites, plant samples were also collected in 2006 (Paper IV).

Microbial respiration was measured in a bioassay before and after substrate addition to all soil samples (Papers I to IV; Figure 1). The microbial kinetic parameters measured using the bioassay were basal respiration, growth rate, lag time, maximum respiration, time from substrate addition to maximum respiration, and accumulated respiration between substrate addition and maximum respiration. In addition to the microbial bioassay, total microbial biomass carbon, nitrogen and phosphorus were determined in untreated soil samples (Paper III).

The phosphorus pools were determined in all soil samples by using a wet-chemical extraction method (a modified version of the Hedley fractionation). The method extracts phosphorus in five steps. In the first two steps, the extractable phosphorus is assumed to be labile and readily available to plants and microbes: membrane and NaHCO$_3$ extractable phosphorus (Cross and Schlesinger 1995). The third step, NaOH extractable phosphorus, is assumed to be adsorbed more strongly to aluminium and iron complexes and available over an intermediate time scale (Cross and Schlesinger 1995). The HCl extractable phosphorus and the residual fraction are assumed to be unavailable to organisms, except perhaps over a very long time scale (Cross and Schlesinger 1995). Total soil C and N content were determined for all soil samples (Papers I to IV). Aluminium and iron content were analysed in Papers I, II and IV, and phosphorus sorption was determined in Paper I.

The plant leaf samples from the tundra sites were analysed for nitrogen and phosphorus content (Paper IV). Plant responses to the addition of nitrogen, phosphorus and the combination of the two nutrients were tested in a plant bioassay using *Solidago virgaurea* L (Paper IV).
Theory and interpretation of respiration curves

Soil respiration measurement is a very common method for estimating microbial activity in soils, by measuring either carbon dioxide production or oxygen consumption. It is an indirect rather than direct measurement of, for example, microbial biomass. In this chapter, a few of the terms used in respiration measurements are defined and explained.

Maintenance respiration, or when there is little to no microbial growth, is usually called basal respiration (Figure 1). During basal respiration, the release of carbon dioxide is relatively stable and can produce an estimate of the metabolically-active microbial biomass in soils (Anderson and Domsch 1978). However, the environment for microbes is not stable and is constantly changing. Easily available carbon and nutrient sources are made available in their surroundings, as root extracts or as litter. In a microbial bioassay, this is mimicked by substrate addition. Microbes have an instant respiration increase following the addition of glucose, defined as substrate-induced respiration (Figure 1; Anderson and Domsch 1978). Anderson and Domsch (1978) also showed that substrate-induced respiration could be used to estimate the microbial biomass in soil.

The initial respiration increase (substrate-induced respiration) is usually followed by an exponential increase in respiration rate (Figure 1). The time it takes between substrate addition to when the microbes begin to grow exponentially, or lag time (Figure 1), is an indication of how quickly the microbes can switch from catabolic (maintenance) to anabolic (growth) metabolism (Stotzky and Norman 1961).

Anderson and Domsch (1978) showed that the respiration rate after glucose amendment is proportional to microbial biomass; Marstorp and Witter (1999) found a similar relationship between respiration rates after glucose additions and microbial biomass estimated from soil dsDNA. Therefore, an exponential increase in growth rate after substrate additions could be interpreted as an increase in microbial biomass. In this thesis, microbial growth rate is defined as the slope of the log transformed respiration rate plotted against time (Nordgren et al. 1988, Giesler et al. 2004). Microbial growth rate in a phosphorus-limited system, like the one created in a bioassay by the addition of glucose and nitrogen in excess of microbial demand, depends on how quickly the microbes can take up phosphorus. The rate of microbial uptake is to a large extent controlled by the soil phosphorus release rate and the transport from substrate to
Figure 2 Respiration curves showing responses to glucose (C) and nutrient (phosphorus [P] and nitrogen [N]) additions in different configurations and for three different sites. The delta symbol indicates that the nutrient is added as a small amount, as opposed to in excess. Graphs a) and b) are respiration curves from Westfield, South Africa, from two depths, 0-10 cm and 50-60 cm. The samples were amended with C+N or C+N+∆P. Graphs c) and d) show respiration curves from 5 different treatments for a dry heath plot in Abisko, Sweden. The curve for C additions is shown in both graphs for comparison. Graphs e) and f) show respiration responses to four different addition combinations in soil samples from boreal forest in Arjeplog, Sweden.

Microbes. The soil phosphorus release rate, in turn, is dependent on the form that phosphorus takes in the soil; for example, if the phosphate is adsorbed to a soil particle surface, the bond has to be broken before it can be taken up by the microbe. A rate limiting factor may be the production of phosphorus solubilising compounds and/or enzymes needed to hydrolyse organic phosphorus. If phosphorus is present in easily available forms, the growth rate is higher (see, for example, respiration curves in Figures 2a, b, c and e). These curves show how growth rate is higher when an easily available
phosphorus form (phosphate) was added together with glucose and nitrogen, compared to only glucose and nitrogen addition. Demetz and Insam (1999) have also shown that growth rate is linearly proportional to the amount of added phosphorus. Using growth rate as indications of phosphorus availability does not offer an absolute value of the amount of available phosphorus. Rather, it gives a relative estimation of its accessibility. Lag time and the time it takes the microbes to reach maximum respiration rate after substrate addition are also related to phosphorus availability (Giesler et al. 2004), and can be used in a similar way as microbial growth rate.

After the exponential growth phase, the respiration rate levels off and microbial respiration reaches its maximum respiration rate (Figure 1). Maximum respiration is assumed to occur when growth is limited by a substrate (Stotzky and Norman 1961). By adding glucose and nitrogen in excess of microbial demand, phosphorus becomes the growth-limiting nutrient (Nordgren 1992).
Results and discussion

Comparison between respiration kinetics and phosphorus pools

The old, weathered plantation forest soils, the boreal forest soils and the tundra soils all show very different distribution of phosphorus fractions. The easily available phosphorus fraction was only found in very low concentrations in the plantation forest soils, and for a couple of the clay soils the membrane extractable phosphorus was not even detectable in the top layer (Paper II). In these soils, a large part of soil phosphorus was found in forms considered to be unavailable to plants, except over long time scales. For example, these clay soils had a large percentage of total phosphorus in NaOH-extractable inorganic phosphorus, especially if compared to what was seen in the boreal and the tundra soils (Papers II, III and IV). Among these three ecosystems, the boreal forest soils were the ones with the largest percentage of total phosphorus as easily available phosphorus fractions. Membrane-extractable phosphorus was also the main predictor of various parameters from respiration measurements, such as time from substrate addition to maximum respiration in the boreal forest soils (Paper III). In the tundra soils, which also had more easily available phosphorus fractions compared to the plantation forest soils, we saw a positive relationship between microbial growth rate (after glucose and nitrogen in excess) and NaHCO₃-extractable organic phosphorus (Paper IV). The exponential growth rate following addition of glucose and nitrogen in excess is one of the more interesting parameters retrieved from respiration kinetics, as it has been suggested to be related to phosphorus availability (Demetz and Insam 1999, Giesler et al. 2004).

However, our results from the plantation forest clay soils (Papers I and II) clearly indicate that the microbes are not only able to use easily available phosphorus, they are also able to use parts of the NaOH-extractable phosphorus, especially the inorganic fraction (Papers I and II). This is evident since inorganic NaOH-extractable phosphorus is an important predictor for microbial growth rate (Figure 3; Paper I). In the sandy soils from the plantation forests, negative relationships were found between NaOH-extractable phosphorus and lag time, as well as time to reach maximum respiration rate after substrate addition. Lag time is the time it takes for the microbes to change from catabolic to anabolic metabolism (i.e., the time it takes for the microbes to start growing). The negative relationship therefore suggests that the concentration of this fraction affects microbial metabolism. This is further supported by the correlation between the estimated amount of microbially available phosphorus and NaOH-extractable phosphorus (Paper II). Although the relationships are
correlative, and not absolute values, the trends clearly indicate the importance of this fraction.

The NaOH inorganic phosphorus fraction is considered to be surface bound to aluminium and iron, and there are studies showing that microbes can use sorbed phosphorus (Shang et al. 1996, He and Zhu 1998). The NaOH-extractable phosphorus fraction has been considered to be available only over long time scales for plants (Cross and Schlesinger 1995), but these findings indicate that the fraction might be available within a short time frame (days to weeks) and more labile than has been previously considered. The same indications can be seen in a study by Ilstedt et al. (2003), where the estimation of the amount of microbially available phosphorus was correlated to the NaOH-extractable phosphorus. It has also been shown that NaOH-extractable phosphorus was depleted in the rhizosphere of Pine roots (Chen et al. 2002), and it is clear that this adsorbed phosphorus fraction can be used by microbes when they have access to an easily degradable carbon source.

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**Figure 3** Microbial growth rate plotted against NaOH-extractable inorganic phosphorus (P). A relationship was only found between the two variables for the clay soils from plantation forests. Plantation forest is represented by black triangles, boreal forest by gray diamonds, and tundra by open squares.
In Paper II, microbial respiration kinetics was used to estimate microbiologically-available phosphorus across a forest productivity gradient. This is an indirect method to estimate available phosphorus based on respiration measurements, but the results clearly showed that the microbiologically-available phosphorus pool was larger than the plant available phosphorus pool, defined by a chemical extraction method (Paper II). As previously mentioned, the amount of available phosphorus was related to NaOH-extractable phosphorus, a fraction that may be available to microbes when not carbon or nitrogen limited.

Information about how much phosphorus is potentially available on a site can have implications for land management practices and could enable the implementation of alternative management strategies. Apparently, there is a large inherent phosphorus pool that can be utilised by microbes under the right circumstances, and studies show how microbial phosphorus solubilising activity increases plant growth (Kucey et al. 1989, Rodriguez and Frago 1999, Whitelaw 2000). Ehlers et al. (2010) also showed that microbes that received additions of an energy source and nitrogen would use the inherent phosphorus pool. When crops or forests are harvested, carbon and nitrogen are removed from the system; the removal of these two elements can therefore also decrease phosphorus availability. The question for the future is how to put this knowledge into practice. There are two main ways currently in practice to increase phosphorus plant uptake by manipulation of microbes: by managing the existing microbial population or by inoculating specific microbes (Richardson 2001). For the management practise to be successful, it is important to understand the diversity and ecology of the microbial population (Richardson 2001), as well as which phosphorus pools they can access and how much phosphorus is potentially available in the soil.

**Soil parameters and phosphorus sorption**
The soils in these studies are not only found in different climates, they also have very different soil characteristics. The clay soils from the plantation forest have the characteristics of old, highly weathered soils with a high phosphorus adsorption capacity (Papers I and II). The sandy soils from the same region are slightly different with lower carbon, aluminium and iron content (Papers I and II). These soils were chosen to represent a range of aluminium and iron concentrations in order to examine the importance of surface sorption for phosphorus availability. High concentrations of aluminium and iron means that there is a large number of sorption sites both for inorganic and organic phosphorus compounds, which has a high affinity for these two elements (Anderson and Arlidge 1962, Anderson et al. 1974, Celi et al. 2003).
The strong sorption effect of aluminium and iron in the plantation forest clay soils could be seen as confirmation that the phosphorus sorption index was positively related to aluminium and iron concentrations (Paper I). Total phosphorus and various phosphorus fractions concentrations showed the same pattern (Paper I). One of these phosphorus fractions was, unsurprisingly the NaOH-extractable phosphorus pool, which is assumed to be adsorbed to aluminium and iron complexes (Cross and Schlesinger 1995). High levels of this phosphorus fraction indicate a high surface sorption (Giesler et al. 2004). Giesler et al. (2004) also found aluminium and iron being a strong predictor of microbial growth rate, suggesting that high concentrations of aluminium and iron have a negative impact on phosphorus availability. In the sandy plantation forest soils, which were low in aluminium and iron content, oxalate-extractable iron was mainly related to phosphorus extracted by NaOH (the organic fraction) (Paper I). It is worth mentioning that NaOH-extractable organic phosphorus is the dominant fraction in these sandy soils, constituting about 50% of total phosphorus (Paper I).

In the tundra ecosystem, we expected the phosphorus availability to be lower in the meadow sites than on the heath sites, as a result of aluminium and iron being transported to the meadow sites via groundwater. The meadow sites would therefore have higher concentrations of aluminium and iron compared to the heath sites. This theory was supported by lower concentrations of two easily available phosphorus fractions: membrane-extractable phosphorus and the organic fraction extractable with NaHCO$_3$ in the meadow soils (Paper IV). However, although we did find a relationship between extractable aluminium and NaOH-extractable phosphorus, a fraction associated with phosphorus sorption (Cross and Schlesinger 1995, Giesler et al. 2002), our data suggests that phosphorus sorption is not an important factor separating meadow from heath. In fact, the NaOH-extractable phosphorus fraction was higher in mesic heath than in meadow, despite higher oxalate-extractable iron concentration in meadow sites (Paper IV). It is therefore possible that factors besides aluminium and iron content are controlling phosphorus availability on meadow sites. One explanation for this could be higher calcium content on the meadow sites than on the heath sites. The HCl-extractable phosphorus fraction was much higher on the meadow sites compared to our heath sites (Paper IV); this is most likely related to calcium phosphates. The meadow sites have higher pH than the heath sites (Paper IV), which has been linked to high calcium saturation in this and other tundra ecosystems (Darmody et al. 2004, Virtanen et al. 2006). None of the meadow sites are on calcareous bedrock, but it is present in the area. It is therefore not unlikely that the sites receive calcium with the groundwater, which can then form secondary apatite in the humus layer. As high calcium concentrations and high pH usually mean low phosphorus
availability (Tunesi et al. 1999), this process is important when discussing phosphorus availability and dynamics in meadow communities, and should be studied further.

None of the studies presented in this thesis showed any indication of phosphorus availability for microbes being affected by the soil’s sorption capacity. Even though there are many positive correlations between aluminium and iron content in the soil and various phosphorus fractions (Papers I, II and IV), there are no relationships found between microbial parameters and sorption capacity of the soils.

Microbes have a phosphorus solubilising capacity (Whitelaw et al. 1999) and experiments have shown that they can use phosphate sorbed to amorphous aluminium oxides and minerals such as goethite (He and Zhu 1998). However, the production of phosphorus solubilising compounds involves a carbon/energy cost for the microbes, and soil microbes are usually carbon-limited (Galardo and Schlesinger 1990, Wardle 1992). In this microbial assay, the microbes are supplied with glucose in excess and by using glucose as an energy source have enough carbon to produce the needed products to acquire enough phosphorus—even if it is tightly bound to soil minerals or in organic matter. In nature, the energy to produce organic acids or phosphorus solubilising enzymes can come from fresh litter input and plant exudates. There have been experiments with girdling studies that show that the direct flow of photosynthates from tree roots have large impacts on microbes and their activity (Högberg and Högberg 2002, Scott-Denton et al. 2006, Giesler et al. 2007).

Is phosphorus availability changing with time?
The chronosequence in Paper III is situated in the boreal forest and is formed by a series of islands, which are divided into three size classes. The large islands exemplify the youngest ecosystems, and the medium-sized islands are where biomass production is at its peak. The ecosystems on the small islands are in retrogression, where biomass production, microbial production and soil respiration are decreasing (Wardle et al. 2004).

We expected to see a larger amount of the more stable phosphorus forms on the smaller islands compared to the medium-sized and large islands. However, the increased amount of stable forms over time seen in other systems in retrogression was not noticeable in this system. None of the different stable phosphorus forms (such as residual and NaOH-extractable phosphorus) differed between the island size classes (Paper III). This was somewhat surprising since other processes on the small islands indicate that the system is in retrogression (i.e., production, decomposition and nutrient fluxes) (Wardle et al. 1997, 2003). This suggests that geochemical factors are not important factors driving the system towards retrogression, although it
has been for temperate and subtropical ecosystems in earlier studies (Walker and Syers 1976, Crews et al. 1995, Turner et al. 2007). It is more likely that the retrogressive development in this boreal system has to do with organic matter. There is a dominance of biological sinks or sources of phosphorus in the humus layer compared to geochemical sinks; this is also in agreement with findings by Wood et al. (1984).

Even though we did not find significant differences between island size classes in the stable phosphorus fractions with the method we used, there might still be qualitative differences in the organic fractions. The lower phosphorus availability on the small islands, as suggested by the lower microbial growth rate in the bioassay (Paper III), could be related to the organic phosphorus being less available. It has previously been shown that the small islands have a lower litter quality compared to the other islands (Wardle et al. 1997, 2003, 2004). The recycling of organic phosphorus compounds is a biologically driven process and involves enzymatic degradation (Attiwill and Adams 1993), a process which, in turn, is dependent on the quality of the organic compounds.

The results show a lower concentration of readily available phosphorus fractions on the small islands compared to the medium-sized islands (Paper III). These results are consistent with earlier findings, where the biologically-available phosphorus is decreasing during retrogression (Walker and Syers 1976, Crews et al. 1995, Turner et al. 2007). The boreal system studied in Paper III is somewhat different compared to other well-studied chronosequences in the literature. The humus layer in the boreal forest gradually becomes deeper if the system is not disturbed (Wardle et al. 2003), and it is not unlikely that the organic phosphorus is bound in more stable forms with time in deeper humus layers, which were not sampled. Thus, the quality of the litter on the small islands may have a negative effect both on the microbial growth rate and the concentration of labile phosphorus; consequently, it could be a major driver of retrogressive ecosystem development.

**Phosphorus limited systems?**

We found indications of decreased availability of phosphorus in the studied boreal forest chronosequence with age, but there were no signs of the soil microbes of the ecosystem in retrogression being limited by phosphorus. When the soils were amended with glucose and nitrogen in excess, the respiration curves always had a higher maximum respiration rate than soil samples amended with glucose and phosphorus. This suggests that there is a higher amount of available phosphorus compared to nitrogen in relation to the specific needs of the microbes in the experiment (see the example of a site in Figures 2e and f). These findings were also supported by the nitrogen-to-phosphorus ratio, which indicated that the nitrogen was the main limiting
nutrient on all the islands. Even though there are areas in the boreal forest where microbes seem to be phosphorus-limited (Giesler et al. 2002, 2004), this phenomenon appears to be site-related rather than age-related.

The respiration kinetics from the three vegetation types on the tundra also responded more strongly to the addition of glucose and nitrogen than to glucose and phosphorus (see the example of a dry heath in Figures 2c and d). These results suggest that microbes have a sufficient supply of energy and are more limited by nitrogen than phosphorus availability. However, the results also suggest that phosphorus is less available in the meadow sites than on the heath sites. For example, microbial growth rate is significantly lower in meadow sites compared to dry heath sites, and it took the microbes longer to reach their maximum respiration rate in meadow sites compared to heath sites (Paper IV). Giesler et al. (2004) found the same patterns when humus soils from nitrogen and phosphorus limited sites were compared.

The data retrieved from studying the plants at these three tundra vegetation types show a more complex picture of the system. By analysing nutrient content in the leaves of a few species, we could get an idea of the nutrient status for the plants, since the nitrogen to phosphorus ratio can be used to study a possible nutrient limitation for plants (Güsewell 2004). The nitrogen-to-phosphorus ratio in the plants on the meadow sites were higher than in the plant material from the heath sites, and the ratio was above what is considered to be the case in nitrogen-limited ecosystems (Tessier and Raynal 2003, Güsewell 2004). The ratio for *Vaccinium uligonosum* was, on average, 16.4 (Paper IV), which is similar to what has been found for the same species at phosphorus-poor sites in a subalpine area (Gerdol 2005). The high ratio is most likely a result of lower phosphorus availability (lower growth rate and less labile phosphorus, Paper IV) and higher nitrogen mineralisation rates found on meadow sites compared to heath sites (Björk et al. 2007). The plant bioassay with *Solidago virgaurea* suggests a co-limitation of nitrogen and phosphorus, a situation which is most evident for the meadow soil (Paper IV). Thus, even though nitrogen seems to be the main limiting nutrient, phosphorus availability might also be restricted, at least in the meadow sites.

As mentioned above, microbes might be able to access soil phosphorus that is considered to be unavailable to plants in the short term (Cross and Schlesinger 1995). How microbial activity can increase plant uptake of phosphorus has also been discussed (Smith and Read 1997, Whitelaw 2000, Chen et al. 2004, George et al. 2006), but microbes can also act as a phosphorus sink (Stewart and Tiessen 1987, McLaughlin et al. 1988, Magid et al. 1996, Oberson et al. 2001). Around 35% of total soil phosphorus can be stored in the microbial biomass in arctic-alpine fellfields (Jonasson et al. 1995). Therefore, competition can be strong between microbes and plants, and high microbial phosphorus demand may influence the partitioning
between microbial and plant phosphorus uptake. It has been shown that microbes are effective in competing with plants for nutrients (Schimel and Chapin 1996). The ability of the microbial biomass to store phosphorus has large temporal variations, and stress can lead to cell death and subsequent release of phosphorus to the soil solution (Turner and Heygarth 2001), making it available for plant uptake (Bardgett 2005). Studies have shown that free nitrogen concentration is highest in the spring, as microbial biomass is decreasing, and when plant uptake is the greatest (Lipson et al. 1999, Jaeger et al. 1999). Lipson et al. (1999) also report an increase of microbial biomass in the autumn as a result of plant senescence, but argue that there is ongoing competition for nutrients throughout the growing season. This can also be the case for phosphorus, particularly in our meadow sites, as our results indicate lower phosphorus availability and the plants show increased phosphorus limitation compared to the heath sites.
Conclusion

The membrane and NaHCO$_3$-extractable phosphorus are the two most readily available phosphorus fractions in the method used in this thesis. Our results suggest that the NaOH-extractable inorganic phosphorus fraction, a fraction considered to be available only over long time frames (Cross and Schlesinger 1995), is related to microbial growth and is at least partly available to microbes within a relatively short time frame (days to weeks). It is argued here that this fraction is more labile than previously expected—not only under laboratory conditions but also in nature, as soil microbes can receive large amounts of labile carbon from, for example, tree roots.

Many factors are important for phosphorus availability in soil. However, different phosphorus controlling factors are more or less important in different soil types. In these three studied systems, microbial phosphorus availability was unrelated to the soil sorption capacity in both high and low sorption capacity systems. Furthermore, in the tundra meadow site in this study, phosphorus availability is more likely affected by high calcium content, a compound known to largely affect phosphorus availability. In many chronosequence studies, geochemical processes have been of importance for decreasing phosphorus availability in the retrogressive stage. However, in this boreal chronosequence it is more likely that the decreasing soil organic matter quality is the reason for decreasing phosphorus availability in the retrogressive stage.

Which are the key processes that influence phosphorus availability in different systems? A few different scenarios are presented above, but until we know the answer to this question we cannot make predictions of phosphorus dynamics in an ecosystem. Using a microbial bioassay to estimate phosphorus availability can be a good complement to studies of soil phosphorus and its availability to organisms. It has already produced a large amount of interesting data, and could be even more useful if developed further.

Soil phosphorus has been studied extensively, especially in soils where phosphorus strongly limits productivity. Phosphorus dynamics in arctic and alpine soils have not been subject to the same attention. However, since high latitude tundra ecosystems are assumed to be especially vulnerable to climate change (ACIA 2005), the lack of knowledge about phosphorus dynamics in these systems is a growing problem.
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