The phosphorus cycle in forests revealed by ^{33}P and the stable isotope composition of oxygen of inorganic phosphate ($\delta^{18}O_{Pi}$)

Dissertation

der Mathematisch-Naturwissenschaftlichen Fakultät

der Eberhard Karls Universität Tübingen

zur Erlangung des Grades eines

Doktors der Naturwissenschaften

(Dr. rer. nat.)

vorgelegt von

M.Sc. Simon Hauenstein

aus Nürnberg

Tübingen

2024

Gedruckt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Eberhard Karls Universität Tübingen.

Tag der mündlichen Qualifikation:	16.12.2024
Dekan:	Prof. Dr. Thilo Stehle
1. Berichterstatter/-in:	Prof. Dr. Yvonne Oelmann
2. Berichterstatter/-in:	Prof. Dr. Emmanuel Frossard

Inhalt

The phosphorus cycle in forests revealed by ^{33}P and the stable isotope composition of oxygen of inorganic phosphate ($\delta^{18}O_{Pi}$)

Summary

The main objectives of my thesis were to assess the importance of biological processes controlling the P cycle in forest ecosystems along a gradient of decreasing soil biological activity and soil P availability using multi-isotopic approaches (^{33}P, $\delta^{18}O_{Pi}$, $\delta^{18}O_W$) and to investigate the importance of soil organic matter turnover on P cycling under environmental change. Particularly, the research aims were to answer whether (i) the fate of P in a tree sapling-soil system differs between nutrient-poor and nutrient-rich sites, (ii) what influence the organic layers have on the fate of P in a tree sapling-soil system at either site, (iii) if purification procedures from soil used to determine the stable isotope composition of oxygen (O) of inorganic phosphate ($\delta^{18}O_{Pi}$) are adequately transferable to measure xylem sap $\delta^{18}O_{Pi}$ and (iv) if the effect of site (BBR, LUE) i.e., a P rich versus a P poor forest ecosystem, and of fertilization (N, P, N+P) do alter Pi and Po concentrations as well as biologically cycled phosphate (inferred from the O isotope signature after adding an ^{18}O label) in xylem sap.

We could show that (i) the mobilization of P likely mediated by the microbial community followed by efficient uptake in organic layers under nutrient-poor conditions (LUE) supported tree sapling growth while at the same time reducing the leaching of P to underlying mineral soil horizons. In contrast, the presence of organic layers did not significantly influence P uptake by beech saplings under nutrient-rich conditions (BBR). (ii) via DAX 8 we were able to remove Po before measuring $\delta^{18}O_{Pi}$. (iii) We did not see fertilization effects reflected in xylem sap $\delta^{18}O_{Pi}$ either between the sites (BBR, LUE), nor the fertilization treatments (N;P; NP, Con). However, our investigations have shown that the ^{18}O labeling approach pinpoints a decoupling of water and P uptake at the P-rich site BBR representing an acquiring ecosystem. By contrast, trees relied on the organic layer for both water and P uptake at the P-poor site LUE representing a recycling ecosystem.

Such findings indicate the importance of the organic layers for P nutrition as well as water supply for beech trees at nutrient poor ecosystems. Since the distribution of most forest ecosystems in Europe is constrained to nutrient-poor soils, sustainable forest management should consider the formation and functioning of the forest floor. Foresters should consider that ecosystem resilience particularly at P-poor ecosystems might be strongly influenced by processes that deteriorate the organic matter and subsequently changes the forest floor structure and thereby its function as a place of water retention and nutrient retention from leaching to mineral soil layers. In this regard, ongoing N deposition and increasing soil temperatures especially in the forest floor due to climate change have the potential to considerably change forest floor turnover dynamics. My improved method of extracting xylem sap and subsequent silverphosphate purification for $\delta^{18}O_{Pi}$ analysis in xylem sap enables new fields of application and was applied successfully in two different in situ experiments.

Zusammenfassung

Das Hauptziel meiner Dissertation bestand darin, die Bedeutung biologischer Prozesse, die den P-Kreislauf in Waldökosystemen entlang eines Gradienten abnehmender biologischer Bodenaktivität und P-Verfügbarkeit im Boden steuern, mit Hilfe von Multi-Isotopenansätzen (^{33}P, $\delta^{18}O_{Pi}$, $\delta^{18}O_W$) zu bewerten und die Bedeutung des Umsatzes organischer Bodensubstanz für den P-Kreislauf unter veränderten Umweltbedingungen zu untersuchen. Die Forschungsziele waren insbesondere die Beantwortung der Frage, ob (i) sich die Nährstoffversorgung junger Buchen mit P zwischen nährstoffarmen und nährstoffreichen Standorten unterscheidet, (ii) welchen Einfluss die organische Auflage auf die Nährstoffversorgung junger Buchen mit P an beiden Standorten einnimmt, (iii) ob die zur Bestimmung der stabilen Isotopenzusammensetzung des Sauerstoffs (O) von anorganischem Phosphat ($\delta^{18}O_{Pi}$) verwendeten Reinigungsverfahren aus den Laborversuchen mit Boden, Pflanzen etc. auch angemessen auf die Messung von $\delta^{18}O_{Pi}$ im Xylemsaft von Buchen übertragbar sind und (iv) ob der Einfluss des Standorts (BBR, LUE), ein P-reiches gegenüber einem P-armen Waldökosystem, und die Düngung (N, P, N+P) die Konzentrationen von Pi und Po sowie von biologisch mobilisiertem Phosphat (abgeleitet aus der O-Isotopensignatur des Phosphats $\delta^{18}O_{Pi}$ nach Hinzufügen einer ^{18}O-Markierung) im Xylemsaft verändern.
Wir konnten zeigen, dass (i) die wahrscheinlich durch die mikrobielle Mobilisierung von P, gefolgt von einer effizienten Aufnahme innerhalb der organischen Auflage unter nährstoffarmen Bedingungen (LUE) das Wachstum der Jungbuchen gefördert und gleichzeitig die Auswaschung von P in tiefer liegende mineralische Bodenhorizonte reduziert wurde. Im Gegensatz dazu hatte das Vorhandensein einer organischen Auflage keinen signifikanten Einfluss auf die P-Aufnahme von Jungbuchen unter nährstoffreichen Bedingungen (BBR). (ii) Mittels DAX 8 konnte Po vor der Messung von $\delta^{18}O_{Pi}$ erfolgreich entfernt werden. (iii) Wir konnten weder zwischen den Standorten (BBR, LUE) noch zwischen den Düngungsvarianten (N;P; NP, Con) Auswirkungen auf die $\delta^{18}O_{Pi}$-Wert im Xylemsaft feststellen. Mittels Labelling durch erhöhte ^{18}O-Gehalte, konnten wir eine Entkopplung von Wasser- und P-Aufnahme am P-reichen Standort BBR aufzeigen. Im Gegensatz hierzu erfolgte am P-armen Standort LUE, sowohl die Wasser- als auch bei der P-Aufnahme hauptsächlich aus der organischen Auflage.

Dies zeigt deutlich die fundamentale Bedeutung der organischen Auflage für die Phosphor-, als auch die Wasserversorgung von Buchen in nährstoffarmen Ökosystemen. Da die meisten Waldökosysteme in Europa auf nährstoffarmen Böden beheimatet sind, sollte eine nachhaltige Waldbewirtschaftung die Erhaltung und Funktion der organischen Auflage berücksichtigen. Förster sollten bedenken, dass die Resilienz von Ökosystemen, insbesondere auf P-armen

Standorten, zentral durch Funktionen der organischen Auflage als Ort der Wasser- und Nährstoffrückhaltung geprägt ist. Der Schutz und die Pflege der organischen Auflage, also eine gute Humusbewirtschaftung der Wälder wird hier deutlich. In dieser Hinsicht haben die anhaltende N-Deposition und steigende Bodentemperaturen im Zuge des Klimawandels, insbesondere innerhalb der organischen Auflage das Potenzial, die Umsatzdynamik der organischen Substanz erheblich zu beschleunigen und somit zu einer Degradation der Humusauflage beizutragen. Des Weiteren möchte ich das Potential meiner angepassten Methode zur Extraktion von Xylemsaft und anschließender Silberphosphatfällung für die Analyse von $\delta^{18}O_{Pi}$ in Xylemsaft erwähnen. Hieraus erschließen sich eine Vielzahl neuer Anwendungsbereiche und die Methode wurde in zwei verschiedenen In-situ-Experimenten bereits erfolgreich angewandt.

Danksagung

Mein größter Dank gilt meiner Mentorin Prof. Dr. Yvonne Oelmann, die man im besten Sinne des Wortes Mentor einordnen muss. Mit dem nötigen Fingerspitzengefühl bewundere ich den Spagat aus fordern und fördern. Ich bin sehr dankbar für die gemeinsame Arbeitsweise, welche großen Anteil am Aufrechterhalten der eigenen Motivation hatte. Ihre kritische und motivierende Begutachtung meiner Arbeit und die daraus entstehenden Diskussionen haben maßgeblich zu dieser Dissertation beigetragen. Ich bin froh, das Glück gehabt zu haben Teil ihrer Arbeitsgruppe sein zu dürfen und auch möchte ich mich für das mir entgegengebrachte Vertrauen bedanken!

Prof. Dr. Emmanuel Frossard danke ich für seine Bereitschaft meine Doktorarbeit als Zweitgutachter zu betreuen und für viele hilfreiche Denkanstöße in einer Vielzahl von Treffen. Auch der direkte Austausch mit unseren Projektpartnern aus der Arbeitsgruppe von Herrn Prof. Dr. Frossard, Dr. Chiara Pistocchi, Dr. Maja Siegenthaler, Dr. Federica Tamburini und Dr. Éva Mészáros waren eine große Unterstützung. Ich blicke gerne auf die gemeinsamen Stunden in diversen Wäldern Deutschlands, sowie auf einer Vielzahl von Tagungen in ganz Europa zurück.

Auch Herrn Dr. Thomas Pütz aus dem IBG 3 des Forschungszentrums Jülich möchte ich als direktem Projektpartner großen Dank aussprechen für seine Unterstützung und Bereitstellung der Labormöglichkeiten für die Untersuchung radioaktiven Phosphors.

Allen Co-Autoren gilt mein Dank für das Lesen meiner Manuskripte und ihre hilfreichen Anmerkungen und Denkanstöße: Dr Harald Neidhardt, Micha Nebel, Dr. Thomas Pütz, Prof. Dr. Friederike Lang und Dr. Jaane Krüger.

Prof. Dr. Friederike Lang und Dr. Jaane Krüger gilt besonderer Danke für die vorbildliche Koordinierung und im speziellen natürlich auch für die Initiierung dieses Schwerpunktprogramms bei der Deutschen Forschungsgemeinschaft (SPP1685 – Forest Strategies for limited Phosphorus Resources) durch Prof. Dr. Friederike Lang. Ich bin sehr dankbar, Teil dieser großartigen Gemeinschaft gewesen zu sein und die vielen Blickwinkel aus verschiedenen Disziplinen auf das gleiche Thema waren unglaublich bereichernd.

Des Weiteren danke ich meinen Kollegen Dr. Harald Neidhardt, Dr. Arnim Kessler, Christiane Nagel, Norina Pagano, Wen Shao, Yao Li, Dr. Nina Hacker, Dr. Karla Dietrich, Dr. Elisabeth Sorkau, Dr. Eva Koller-France und Dr. Melanie Brunn für die sehr schöne und freundschaftliche Arbeitsatmosphäre.

Ich danke allen studentischen Hilfskräften, die mich tatkräftig unterstützt haben und denen auch ich als Mentor dienen durfte: Behrend Dellwisch, Christian Bödinger, Christiane Nagel, Conny Hüber, Daniel Greulich, Melanie Schäfer, Yolanda Rhode, Micha Nebel. Bei Herrn Daniel, Greulich und Herrn Micha Nebel möchte ich mich für deren tatkräftige Unterstützung bedanken. Die zur Erstellung von deren Abschlussarbeiten gewonnen Daten sind Teil meiner Veröffentlichungen.

Allen technischen Mitarbeitern in Tübingen und Jülich danke ich ebenfalls für ihre Unterstützung bei der Planung und Durchführung der Feld- und Laborarbeiten: Sabine Flaiz, Eleonora Streb, Martina Krause, Andrea Ecker, Philipp Meulendick, Dr. Stephan Köppchen, Dr. Diana Hofmann.

Mein Dank gilt der Deutschen Forschungsgemeinschaft DFG für die Finanzierung des Projektes (Oe516/6-2 in the priority program SPP 1685 ‘‘Ecosystem Nutrition: Forest Strategies for limited Phosphorus Resources’’).

Meiner Familie möchte ich danken, da wir nur zusammen diese Lebensphase erfolgreich meistern konnten. Wir sind mittlerweile zu viert und das familiäre Glück spendet viel innerliche Stärke, um die Herausforderungen einer solchen Arbeit bestehen zu können. Meiner Frau danke ich für ihr stetiges Verständnis und Ihre Unterstützung in jeder Lage.

Liste der Publikationen der Dissertation:

a) Akzeptierte Publikationen

Hauenstein, S., Neidhardt, H., Lang, F., Krüger, J., Hofmann, D., Pütz, T., Oelmann, Y. (2018). Organic layers favor phosphorus storage and uptake by young beech trees (*Fagus sylvatica* L.) at nutrient poor ecosystems. *Plant Soil* 432, 289–301. doi: 10.1007/s11104-018-3804-5

Hauenstein, S., Nebel, M., Oelamnn, Y. (2020). Impacts of fertilization on biologically cycled P in xylem sap of *Fagus sylvatica* L. revealed by means of the oxygen isotope ratio in phosphate. Frontiers: *Frontiers in Forests and Global Change (special issue: Forest soils)*

b) Noch nicht eingereichte Manuskripte

Hauenstein, S., Oelmann, Y. A novel oxygen stable isotope approach to study phosphate in xylem sap

Selbstständigkeitserklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst und keine anderen Hilfsmittel als die angegebenen verwendet habe.

Insbesondere versichere ich, dass ich alle wörtlichen und sinngemäßen Übernahmen aus fremden Werken als solche kenntlich gemacht habe.

_____ (Ort, Datum)

_____ (Unterschrift)

1.1 Introduction

Forest growth in temperate regions is generally limited by the availability of nitrogen (N) in soils. However, recent studies revealed a decrease in foliar phosphorus (P) concentrations in Central Europe for the last decades, especially on P poor soils indicating a shift towards P-(co)limitation. Continuously high deposition of atmospheric N and increased CO_2 concentrations due to climate change accelerate forest growth, consequently resulting in enhanced P demands by trees. In a conceptual approach Lang et al. (2016;2017) describe the adaption of forest ecosystems to low P supply as the development from an “acquiring system” e.g. on young, nutrient-rich or just P-rich soils, towards a “recycling system” e.g. on old, nutrient-depleted or just P-poor soils. This is the basis of the DFG priority program “SPP1685 - Ecosystem nutrition: Forest Strategies for limited Phosphorus Resources” by which this thesis is framed. The DFG priority program “SPP1685” follows a geosequence of decreasing availability of mineral bound P in the mineral topsoil horizons, i.e. a soil chronosequence on silicate material represented by five central study sites, Bad Brückenau (BBR), Mitterfels (MIT), Vessertal (VES), Conventwald (CON) and Lüss (LUE), respectively. Similar stand age (80-120a) and tree composition (European beech, Norway spruce) are given and C/P ratios of the forest floor range from 230 – 790. The five central sites are Level II monitoring sites (ICP Forests monitoring), which run since 15 years and are operated by federal forest institutions. Basic characteristics of soils and stands, tree growth analyses, climate data, and flux data of major cations and anions were collected.

Phosphorus (P) is an essential agent in a variety of vital processes like the build-up of DNA, RNA, and cell membranes, the energy transfer via free nucleotides and the carbon metabolism. Therefore, P is of paramount importance for plant growth and ecosystem performance (Jonard et al., 2015, Scheerer et al., 2018). Forest growth in temperate regions is generally limited by the availability of nitrogen (N) in soils (Aber 1992). However, recent studies revealed a decrease in foliar phosphorus (P) concentrations in Central Europe for the last 20 years, indicating a shift towards P-(co)limitation (Duquesnay et al. 2000; Ilg et al. 2009; Jonard et al. 2015). For example, a significant decline of 13% in foliar P concentrations was observed across Europe, most likely due to ongoing atmospheric N deposition and increased CO_2 concentrations. Consequently, forest productivity is increased, eventually resulting in a switch from N- to P-limitation (Jonard et al. 2015; Talkner et al. 2015). The drivers

for such a transition from nitrogen (N) to P limitation in European forest ecosystems are mainly associated with anthropogenic activities. In particular, continuously high deposition of atmospheric N and increased CO_2 concentrations due to climate change accelerate forest growth, consequently resulting in enhanced P demands by trees (Jonard et al., 2015; Talkner et al., 2015). Jonard et al. (2015) as well as Talkner et al. (2015) consider P as the upcoming limiting factor in nutrient poor forest ecosystems (Polglase et al., 1992; Clarholm, 1993). Therefore, mechanistic knowledge regarding the supply strategies of trees with P under different environmental conditions is required.

In order to understand these processes, it is important to distinguish between two sources of P for plant uptake, the geologic parent material and organic matter (OM). During pedogenesis the geologic parent material is important for the potential P supply to organisms in the first place, but with progressing pedogenesis biogeochemical weathering and erosion cause pronounced changes in the pools of bioavailable P (Walker and Syers, 1976; Wardle et al., 2004). With progressing pedogenesis, P released from primary minerals is either lost due to leaching or is fixed in the soil mainly through adsorption and incorporation into Al- and Fe-(hydr)oxides. Therefore, P supply from mineral sources is declining and shifted to deeper soil horizons during pedogenesis.

In the same regard in temperate forest ecosystems, the contribution of the organic layers to P nutrition of trees in addition to mineral P sources is increasing. This is because organic layers play a twofold role. On the one hand, organic matter and therefore nutrients including P gradually accumulate on the soil surface due to increasingly limited microbial decomposition with ongoing acidification. Additional effects contribute to the accumulation of OM on the forest floor: In dense matured forest ecosystems canopy closure intercept large proportions of rainfall, shading decreases surface temperatures and both effects subsequently lower microbial activities (Vogt et al., 1986). Furthermore, the bioavailability of nutrients like P, K, and Fe is reduced due to incorporation in organic molecules and thereby removed from active circulation, this in turn leads to a decreased forest productivity (Berg and McClaugherty, 2003; Fisher and Binkley, 2000; Kimmins, 1997). On the other hand, OM and humic metabolites serve as physicochemical adsorbents for P and other resources including water, reducing leaching through the soil column (Leuschner, 1998). Soil OM in general is considered to have positive effects on forest productivity, especially in coarse textured

soil (Grigal and Vance, 2000). Furthermore, the accumulation of OM in form of organic layers provides a new habitat for microbes, thus maintaining microbial activity and nutrient cycling in contrast to less favorable conditions in mineral topsoil layers (Spohn et al., 2013). Because trees preferentially acquire P from organic layers of the forest floor the cycle of P in the ecosystem is being tightened (Lang et al., 2017, Pistocchi et al., 2018). As a positive subsequent effect, leaching of P to deeper mineral soil horizons that are characterized by a higher P sorption capacity and larger retention times is minimized (Polglase et al., 1992). This is often reflected by a thick and dense rooting system in the forest floor (Brandtberg et al., 2004; Jackson et al., 1996) enabling an effective coupling of nutrient mobilization (for instance mediated by specific extra-cellular enzymes released by plants and microorganisms) and subsequent uptake (Wood et al., 1984).

If Phosphorus is released from the solid soil matrix into soil solution as a result of dissolution of P-containing minerals, or of mineralization of soil organic matter (SOM), is difficult to disentangle. A common method to trace the fate of nutrients in ecosystems are stable isotopes. In the case of P in ecosystems or even mesocosm studies this is restricted since there is only one stable isotope of P (^{31}P) and two radioactive isotopes ^{32}P (half-life of 14.3 days) and ^{33}P (half-life of 25.34 days) (Audi et al., 2003). Since the application of radioactivity under field conditions is constrained by safety issues, few authors extended their study beyond laboratory experiments. These few publications report on root uptake of forest ecosystems *in situ* (Brandtberg et al., 2004; McLaren et al., 2016). In a pot experiment, Jonard et al. (2009) found evidence that 99% of P taken up by pine trees in a forest plantation were mobilized from the forest floor. Furthermore, they calculated a P supply originating from organic layers of 93% and 95% for spruce and birch trees from results of Brandtberg et al. (2004).

We studied the fate of P by means of ^{33}P under nutrient-poor vs. nutrient-rich conditions associated by differences in characteristics of the organic layer.

Our overarching aims (**A1**) were i) to test if the fate of P in a tree sapling-soil system differs between nutrient-poor and nutrient-rich sites, and ii) to assess the influence of organic layers on the fate of P in a tree sapling-soil system with different P availability. As our investigated sites represent the initial and end members of the gradient described by Lang et al. (2017), we hypothesize that i) the majority of P is concentrated in organic compartments (organic layer and aboveground biomass) at the nutrient-poor site whereas more evenly distributed at the nutrient-rich site, and accordingly ii) the

presence of an organic layer is crucial to maintain tree sapling performance at the nutrient-poor site which is not the case for the nutrient-rich site.

Even though it is possible to label distinct soil fractions by radioactive ^{33}P or ^{32}P and therefore identify different P sources, the restrictions for the use of radioactive material even under laboratory conditions, is a strong limiting factor for the number of published studies on ^{33}P or ^{32}P. Recent studies used $\delta^{18}O_{Pi}$, the stable isotope ratio of ^{18}O to ^{16}O associated to P in Orthophosphate (PO_3), to distinguish biological P mobilization processes from other processes (Pistocchi et al., 2018; Hacker et al., 2019). This is possible because only biological processes involve an exchange between O atoms in phosphate and O atoms in ambient water (Blake et al., 2005), while no such exchange takes place during the dissolution of P-containing minerals and the desorption of $H_2PO_4^-/HPO_4^{2-}$ from charged surfaces (Liang and Blake, 2007).

$\delta^{18}O_{Pi}$ values have been used to reveal the release of phosphate by extracellular and intracellular enzymes (Liang & Blake, 2006, von Sperber et al., 2014 & 2017), the importance of microbial P cycling soil (Angert et al., 2011, Tamburini et al., 2012, Hacker et al., 2019, Pistocchi et al., 2018, Siegenthaler et al., 2022), and organic P pools in soil and plants (Pfahler et al., 2013 & 2017,Tamburini et al., 2018, Helfenstein et al., 2018). Yet, methods to identify different processes preceding P uptake into xylem sap and its soil sources are missing. Therefore, the ratio of stable isotopes of O in phosphate ($\delta^{18}O_{Pi}$) might represent a promising approach to disentangle the different sources of P in P poor ecosystems compared to P rich ecosystems. Consequently, the differentiation of these processes could also help inform foresters to improve P nutrition of forests by modifying either mineral-bond P or the composition/activity of the microbial community involved in SOM mineralization in soil.

However, the presence of Po might hamper the application of this approach for xylem sap. Apart from P, xylem sap is a complex mixture of a variety of different constituents: N, K, Mg, Ca, S, B, Zn, Cu, sugars, phytohormones or polyvalent heavy metal cations complexed with organic acids, amino acids and peptides (White, 2012). This matrix including Po compounds might interfere with the analysis of $\delta^{18}O_P$ in xylem sap. The preparation of samples for $\delta^{18}O_P$ analysis includes several dissolution- and reprecipitation steps finally yielding silver phosphate. The purity of the precipitated silver phosphate is of decisive importance since small amounts of Po can bias $\delta^{18}O_P$ values (Tamburini *et al.*, 2010, Weiner *et al.*, 2011). Thus, the complete removal of Po accompanied by no effect on Pi in the sample matrix is essential. However, methods

commonly used to remove Po (strong oxidizing agents, high temperatures, strong basic conditions) may bias $\delta^{18}O_{Pi}$ values (Lécuyer, 2004). Tamburini *et al.* (2010) recommended the use of a DAX8 resin prior to the precipitation of silver phosphate for soil extracts that are colored mostly caused by dissolved organic matter (DOM). In soil, DOM is a complex mixture of mostly high-molecular-weight aromatic and aliphatic compounds (i.e. humic and fulvic acids), polysaccharides, and proteins (Schulze, 2005). Thus, the chemical composition of Po in xylem sap likely differs substantially from that in soil potentially requiring a modified pretreatment such as the removal of low-molecular-weight Po molecules before $\delta^{18}O_P$ analyses.

Our aims (**A2**) were to (i) assess Pi and Po concentrations in xylem sap, and to (ii) test the effect of a lipid removal agent (LRA) and DAX8 pretreatments on Pi and Po concentrations as well as on $\delta^{18}O_{Pi}$ values. For the latter, we used no pretreatment as the control and included the purification of Pi in xylem sap and standard P solutions (solely Pi and 1:1 or 1:2 mixture of Pi and Po) for O isotope analysis. We checked the potential influence of treatments on $\delta^{18}O_P$ values via ^{18}O-spiked and non-spiked chemicals used for sample preparation.

Being able to interpret $\delta^{18}O_{Pi}$ values in xylem sap, opens the possibility of investigating sources of phosphate in xylem sap. Since xylem sap of woody plant species can be related to the source of water use and thus, to root uptake of water and nutrients dissolved therein (Ehleringer and Dawson, 1992; Bauke et al., 2021). Particularly if the O isotope signature of ambient water is modified via isotope labeling by ^{18}O-enriched- or ^{18}O-depleted water, mobilization processes or distinct P sources can be identified (Hacker et al., 2019). Accordingly, an incorporation of O atoms from ^{18}O-enriched ambient water into phosphate by biological activity in soil should be visible in $\delta^{18}O_{Pi}$ values of xylem sap once phosphate has been taken up by trees. It is not yet known if the uptake of P by plants is associated with an isotopic fractionation, but it is probable as both plants and microorganisms take up P through transporters. (Blake et al., 2005)

Apart from these restrictions, the main P sources for plant nutrition are not only constrained by P availability at different sites, also the availability of other nutrients might strongly affect P uptake mechanisms and the relative importance of different P sources in soil. Jonard et al. (2015) as well as Talkner et al. (2015) consider P as the upcoming limiting factor in nutrient poor forest ecosystems which yet has to be approved by P fertilization trials (Polglase et al., 1992; Clarholm, 1993). The effect of

P fertilization likely depends on the type of nutrient acquisition in ecosystems. In forests on old, highly weathered and/or P-poor soils, trees rely on the mobilization from organically bond P in soil organic matter (Jonard et al. 2009). In case of such P-poor soils, P fertilization increased forest growth/productivity (Blevins et al., 2006; Trichet et al., 2009; Turner and Lambert, 2015). This effect was likely driven by soil processes. Mo et al. (2015) found that the addition of P to soils containing very little available P resulted in a significant 'priming' effect, which stimulated microbial activity and nutrient turnover of litter, whereas no effect of P addition was observed in soils containing sufficiently available P. This is in line with Bergkemper et al. (2016) and Siegenthaler et al. (2022) who described a shape of bacterial communities towards organic P acquisition on P-poor soils, whereas in P-rich soils inorganic P acquisition dominated. By contrast, several authors reported the downregulation of enzyme activity slowing down P mobilization from organic P after the addition of water soluble mineral P to soil irrespective of initial P availability in soil (DeForest et al., 2012; Marklein and Houlton, 2012; Shaw and DeForest, 2013). Whether these P fertilization effects on P availability in soil translate into tree P nutrition and in particular into tree P uptake via xylem sap has hardly been studied (Prietzel and Stetter, 2010).

Given the assumption that an P rich forest ecosystem relies much less on nutrient release from organic matter via biological activity than a P deficient ecosystem does (**A3**), different contributions of biologically cycled phosphate i.e., different extents of ^{18}O-enrichment, in xylem sap can be expected. Similarly, a stimulation of biological activity in soil by P fertilization should be reflected in a greater contribution of biologically cycled phosphate particularly so in a P poor forest ecosystem.

2 Material & Methods

Since the second manuscript in this thesis (**A2**) comprises a method development experiment, it does not seem reasonable to repeat the complete methodological part in this thesis. I have cited the methodological aspects needed to understand our progress in xylem sap extraction and the main outcomes of this experiments. Please refer to the manuscript in Appendix **A2** for the explicit method development scheme.

2.1 Study sites

In the frame of the priority program SPP 1685 'Ecosystem Nutrition, Forest Strategies for limited Phosphorus Resources' (Lang et al. 2016), two temperate forest sites were selected for the ^{33}P uptake experiment (**A1**) and the in situ fertilization - $^{18}O_{Pi}$ xylem sap experiment (**A3**). The site "Lüss" (LUE) is located in Lower Saxony, northwestern Germany in the Lüneburg Heath (52°83' N, 10°36' E), whereas the site "Bad Brückenau" (BBR) is located in Bavaria, southeastern Germany in a midrange mountain area of the Rhön (50°35' N, 9°92' E). The potential natural vegetation at the site BBR is Hordelymo-Fagetum (Lang et al., 2017. The forest stand comprises 99% *Fagus sylvatica* L. and 1% *Acer pseudoplatanus* L. Mean stand age for beech is 137 years with an average height of 26.8 m, a mean (breast height) diameter of 36.8 cm and a number of 335 tress per hectare. In Lüss, organic layers and soil types are classified as a mor-like Moder forest floor on hyperdystric folic Cambisols developed from Pleistocene sands. At the Bad Brückenau site, mull-like Moder forest floors on a dystric skeletic Cambisols developed on alkaline igneous rocks/metamorphites (Haußmann and Lux 1997). Further site characteristics are provided in Table 1.
The beech forest at the site JUE is located inside the research center Jülich (50.91°N, 6.42°E) in North Rhein Westphalia. It is a matured pure beech forest with a stand height of approximately 23m. The soil type is a gleyic cambisol (FAO). It is an intermediate site according to the P gradient from the main experimental sites BBR (high plant available P concentrations) and LUE (low plant available P concentrations).

Table 1 ***Field site characteristics of Lüss (LUE) and Bad Brückenau (BBR).***

(A2)

Chemical parameters are given for the A horizon (BBR: 0- -14 cm; LUE: A (0- -12 cm) according to our pot distinction (LUE=Ah; BBR=AE+E). MAT = mean annual temperature, MAP = mean annual precipitation, Pi = inorganic P, TP = total P; bioavailable Pi = PO_4-P citr., C = C-total (C-tot ≙ C_{org}) (Data from: Lang et al. 2017)

Study site	**BBR**	**LUE**
Site characteristics		
Altitude [m a.s.l.]	**809**	**115**
MAT [°C]	**5.8**	**8.0**
MAP [mm a^{-1}]	**1031**	**779**
Forest floor mass [kg m^{-2}]	**1.83**	**12.8**
TP forest floor [g m^{-2}]	**2.7**	**9.2**
Turnover rate of the forest floor [years-1]	**5**	**39**
pH (H_2O)	**4.0**	**3.7**
C [mg g^{-1}]	**136**	**46**
N [mg g^{-1}]	**7.5**	**1.8**
TP [mg kg^{-1}]	**3199**	**146**
Bioavailable Pi [mg kg^{-1}]	**43**	**53**
C:N	**18**	**25**

In order to gain an adequate amount of xylem sap for the novel oxygen stable isotope approach to study phosphate in xylem sap (**A2**), one beech tree was felled and xylem sap was extracted at the 10th of April 2018, located near the level 2 ICP forest-monitoring site Baltmannsweiler, southeast Germany.

2.2 Fertilization experiment

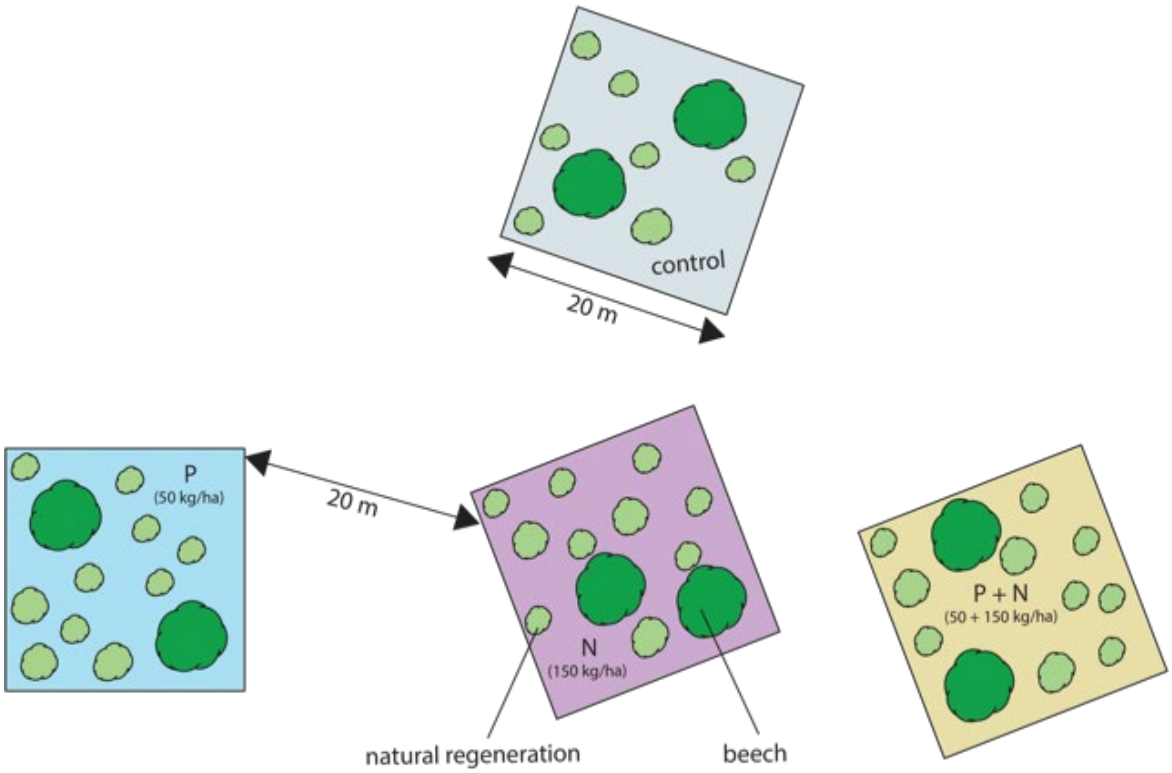


Figure 1: Sketch of the experimental design of the fertilizer treatments (done in triplicate). The amount of fertilizer applied is given in brackets in kg ha^{-1} $year^{-1}$. Nitrogen (N) and phosphorus (P) were applied as NH_4NO_3 and KH_2PO_4, respectively. Note that the control and N treatment received KCl to ease comparison with the P treatment which included the addition of K as well. Courtesy of Jaane Krüger.

At both sites (LUE-BBR), a full-factorial fertilization experiment was established in April-May 2016 by the SPP staff organized from Freiburg by Jaane Krüger. In 2017 the in situ fertilization - $^{18}O_{Pi}$ xylem sap experiment (**A3**) was conducted on these plots. The experiment comprised a control and three fertilization treatments: N addition (150 kg N ha^{-1} $year^{-1}$ as NH_4NO_3), P addition (50 kg N ha^{-1} $year^{-1}$ as KH_2PO_4), N+P addition (combination of N and P addition). Control and treatment plots (20 m x 20 m) were established in triplicates which were randomly distributed in the stands while keeping a minimum distance of 20 m between plots (Fig. 1). Each plot contained at least two individuals of adult beech trees (*Fagus sylvatica* L.) with a diameter at breast height of 46.8 ± SD 3.65 cm at the site BBR and 48.8 ± SD 5.81cm at the site LUE and an approximate age of 120 years.

The control and the N addition treatments received KCl to account for addition of K in the P-containing treatments. The addition of N was split into three parts (50 kg N ha^{-1}

each) in spring, summer, and autumn to account for the seasonal demand of trees and to reduce leaching losses. The addition of P took place once a year along with the N addition in spring. The fertilizer addition was carried out in dissolved form by means of garden sprayers. The experiment was conducted in Juli/August 2017. We acknowledge that by restricting the fertilization to N and P, we did exclude the potential limitation of forest growth by elements other than N and P which was speculated by (Jonard et al., 2012). We tried to reach a conservative estimate of maximum N input accounting for the next decade of atmospheric N deposition. Nevertheless, similar to many other forest fertilization experiments, our fertilization approach did not take into account direct interactions between the fertilizer and the canopy which would have occurred had we manipulated atmospheric deposition directly.

2.3 Sampling

In order to perform a ^{33}P-uptake experiment (**A1**) under controlled conditions, we established a greenhouse mesocosm experiment. Soil was collected from a flat surface position in LUE and BBR in between February and March 2014. Each horizon of the forest floor and mineral soil to a depth of 25 cm below ground was collected separately (horizon-specific characteristics summarized in Table S1. The order of soil horizons (IUSS 2015) was as follows, for LUE: Oi (3 cm) - Oe (4 cm) – Oa (1 cm) – AE (6 cm) - E(5 cm) – Bsh (9 cm+), and for BBR: Oi (3 cm) – Oe (3 cm) -Ah (11 cm) – BA (11 cm+). Each horizon was homogenized carefully before establishing the mesocosm experiment. The thickness of each layer in mesocosms resembled the average thickness in the field. We established two different mesocosm treatments: one including organic layers and one where the organic layers wert absent. Mesocosms without organic layers were covered by 0.5 cm thick synthetic fleece to mimic the presence of an organic layer and the associated reduction of evaporation.

Two-year old *Fagus sylvatica* L. saplings were purchased from a nursery in the Black Forest and planted into the pots. We allowed the plants to settle for 12 months before starting the ^{33}P-uptake experiment. During this period, mesocosms were watered twice a week with tap water. The ^{33}P-uptake experiment was conducted in July 2015. Two weeks before the start, tap water was added to the mesocosms to achieve 60% water holding capacity and maintained throughout the experimental course by daily watering. If water drained out of mesocosms, it was collected with a pipette and reapplied to the pot surface. Soil water tension was monitored by two tensiometers (Blumat digital) in each treatment, resulting in eight tensiometers (2 sites

x 2 treatments x 2 replicates). The mesocosms were then exposed to field conditions, but sheltered by a transparent Plexiglas enclosure to maintain soil moisture conditions. Every 48 hours after the start of the ^{33}P-uptake experiment, mesocosms were relocated randomly to minimize location effects below the enclosure. At the start of the ^{33}P-uptake experiment, we added 23 MBq ^{33}P-labeled orthophosphate 'carrier free' (>99.5% ^{33}P) as aliquots in 100 ml tap water per mesocosm, followed by additional 100 ml tap water to rinse the flask as well as force a rapid and homogenous infiltration. The amount of added P in this 100 ml labeling solution corresponds to 6.55 x 10^{-6} mmol P. We sampled mesocosms destructively at five time steps (0, 24, 48, 96, 192 hours). Therefore, in total we established 60 mesocosms (2 sites x 2 treatments x 5 time steps x 3 replicates). The short half-life time of ^{33}P of 25.34 days (Audi et al. 2003) constrained the experiment duration to 8 days (192 hours).

In order to trace the mobilization of P from organic horizons into xylem sap of beech trees we choose the endmembers of the investigated P gradient BBR (P-rich) and LUE (P-poor) and plots of the established fertilization trial from 2016 in the in situ fertilization $^{18}O_{Pi}$ xylem sap experiment (**A3**). The isotope labeling took place from 19.06.-21.06.2017 at the site BBR and 12.06-14.06.2017 at the site LUE. We used an area of 10.8 m^2 around each tree individual for the application of ^{18}O-enriched water. This area was formed by two circles with an identical center in the middle of each tree with radii of 0.4 m and 1.9 m. We prepared ^{18}O-enriched water by diluting tap water with ^{18}O-enriched water (>98 atom% ^{18}O, Hyox ^{18}O, rotem, Arava , Israel) aiming at $\delta^{18}O$ values of soil solution of around +40 ‰. Assuming thermodynamic equilibrium fractionation (Chang & Blake, 2015), $\delta^{18}O_{Pi}$ values of +61.7 ‰ would result. We applied the ^{18}O-enriched water using a syringe (100ml, Vektenxi, amazon.de) that was composed of a 12 cm long needle with a closed tip and five outlet holes (1 mm diameter). We injected the ^{18}O-enriched water into the Oe horizon and the injection depth was adapted to the site-specific forest floor structure in order to be centered in the Oe horizon. To assure homogeneous application of the label, the area was divided into eight subareas. 60 injections of 2.5 ml water were allocated randomly to each subarea. In total, 1.2 l of ^{18}O-enriched water was applied in the prescribed area around each tree individual and this equals 2.1% (BBR) and 1.7% (LUE) of the average soil water content in the organic layers. To check for resulting labeling of soil water, organic layer samples were taken and stored in gas-tight vials until isotope analysis.

2.4 Xylem sap extraction

During the ^{33}P uptake-experiment (**A1**), we determined the uptake of ^{33}P at different time intervals by extracting the xylem sap from the whole three-year-old beech trees by means of Scholander pressure bomb extraction (Soilmoisture 3000 Series Plant Water Status Console). First, trees saplings were harvested by cutting 1 cm above soil surface. Directly after harvesting, we removed 2 cm of bark and cambium to prevent phloem extraction and tightly pulled a PE hose (LDPE transparent hose, 200 mm long with 8 mm inner diameter) over the stem. The tree sapling was placed in the pressure chamber under approximately 15 bar as long as xylem sap was extractable (max. 5 minutes). The pressure was applied by compressed air. The amounts of extracted xylem sap (0.05-0.2 ml) were sufficient for ^{33}P measurement, but insufficient for further chemical analyses (determination of P concentrations and speciation).

Since a main hurdle of this thesis consisted of the extraction of adequate amounts of xylem sap for subsequent silverphosphate precipitation and $\delta^{18}O_{Pi}$ isotope analysis. A minimum amount of 0.8 mg Pi was required for each measurement. Therefore, the displacement method of Glavac et al. (1989) proved to be the most efficient method for this purpose. In order to establish a novel oxygen stable isotope approach to study phosphate in xylem sap (**A2**) we harvested an adult beech tree (*Fagus sylvatica* L.) near the ICP forest monitoring site Baltmannsweiler and cut the trunk into segments of 1.2 m length. Ten 1.2 m long trunk segments were needed to gain 13 l xylem sap which we considered sufficient for the needed number of samples for isotope analysis. For the extraction of xylem sap, 0.05 m of bark and cambium at the uppermost und lowermost part of each segment were removed to prevent contamination with phloem constituents. The trunk segments were rotated by 180° and thus arranged in a vertical position against the direction of growth. The lowermost end of the tree segment was thoroughly washed by distilled water. Subsequently, a rubber collar was fixed at the uppermost end of each trunk segment where bark and cambium had been removed and filled by approximately 1.5 l distilled water. A water-soluble fluorescent dye (Fluorescein sodium salt, Sigma Aldrich) was added to the water in excess of its coloring capacity (> 1 g l^{-1}). The water-dye mixture forced a downward flux of xylem sap and we collected xylem sap at the lowermost part of the trunk segments and replaced the collection container every ten minutes. During xylem sap extraction, the collecting container was externally cooled by ice and the xylem sap obtained was transferred to a collecting vessel stored in a mobile freezer (-20°C). The first 100 ml of

sample were discarded to exclude contaminating cell residues from cutting. Upon the first occurrence of the dye in the collection container, the sample was discarded as well and this marked the point in time where the first vascular bundles of the 1.2 m segments were filled by the fluorescent dye completely. The aliquots of xylem sap were combined to one composite sample which was transferred to the laboratory and stored at -80°C.

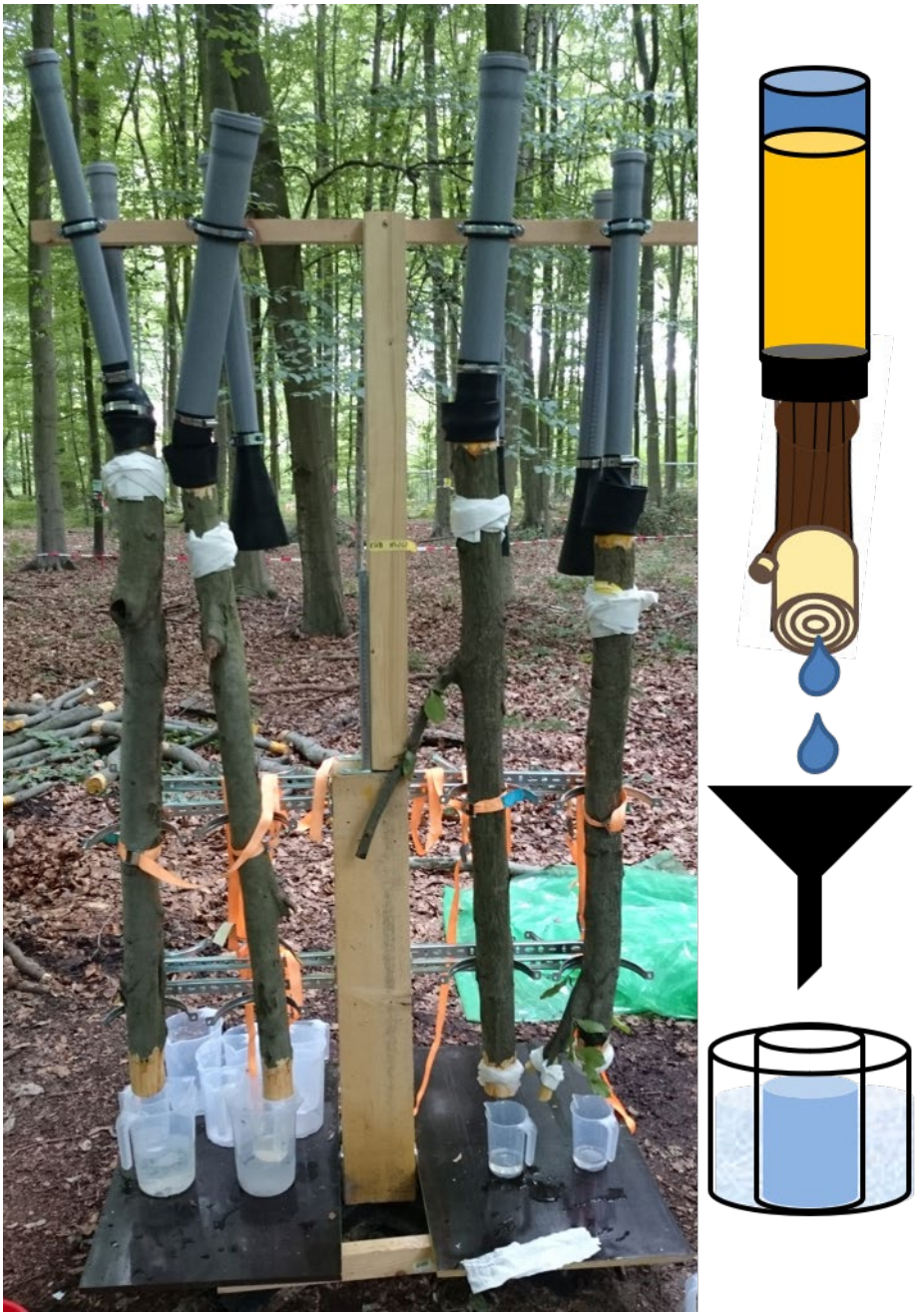


Figure 2 own picture: xylem sap displacement equipment/systematic

Xylem sap extraction in the in situ fertilization $^{18}O_{Pi}$ xylem sap experiment (**A3)** was conducted by Scholander pressure bomb extraction, since results from our experiment at the site BMW were not finished and “Scholander pressure bomb extraction” was successfully used to extract xylem sap in our first experiment (**A1**) from three-year-old beech saplings. In total, we sampled 48 trees (2 sites x 4 treatments x 2 time steps x 6 replicates). Two time steps were necessary to cover the potential duration until ^{18}O-enriched phosphate originating from incorporation of ^{18}O from the water label in soil were taken up by the trees. To follow the uptake of ^{18}O-enriched phosphate, we extracted xylem sap. To this end, branches (diameter of 0.8 - 2 cm) were harvested by tree climbers from the middle canopy. Directly after harvesting, we removed 2 cm of bark and cambium to prevent contamination with phloem constituents and tightly pulled a PE hose over the twigs. The twigs were placed in the Scholander pressure bomb (Soilmoisture 3000 Series Plant Water Status Console). The pressure was applied as N_2 gas (Grade 3). This procedure was repeated until a minimum amount of 30ml xylem sap was extracted. Directly after sampling the xylem sap was filtered through 1.2 µm PET syringe filters (CHROMAFIL Xtra PET-120/25, MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). The samples were stored in a portable fridge at 4°C. Aliquots were split for $\delta^{18}O_W$ (1 ml) and $\delta^{18}O_{Pi}$ analyses.

In total, we sampled 48 trees (4 treatments x 2 timesteps x 2 tracers x 3 replicates) for the Jülich (JUE) experiment 2018.

Two timesteps were necessary to cover the potential duration until ^{18}O-enriched phosphate originating from incorporation of ^{18}O from the water label in soil were taken up by the trees. To follow the uptake of ^{18}O-enriched phosphate, we extracted xylem sap. To this end, branches (diameter of 10-20cm) were harvested standing on an elevator platform from the middle treetop. Directly after harvesting, we removed 0.05m of bark and cambium on each side of the 1.2m long branch pieces to prevent contamination with phloem constituents. Xylem sap extraction was performed identically as described for the novel oxygen stable isotope approach to study phosphate in xylem sap (**A2**) and the most promising procedure identified to measure $\delta^{18}O_{Pi}$ in xylem sap was adapted.

Therefore, we processed xylem sap samples each day directly after returning from the field (<3h after extraction). Because some organic P compounds might be rapidly transformed into Pi in xylem sap, 100ml of DAX 8 Resin (Sigma Aldrich – Superlite DAX8 resin) was added to one liter of xylem sap and shaken for 2h. Subsequently we

purified Pi following the protocol of Weiner et al. (2011) and added anion exchange resin membranes (397cm^2 - VWR International GmbH, Bruchsal, Germany) to the xylem sap samples. After 16 h of shaking, the membranes were removed, rinsed with H_2O and eluated twice by 50ml 0.2M HNO_3. Eluates were again stored in a portable fridge at 4°C until preparation for isotope analysis in the laboratory. Subsequently the isotopic signature of oxygen associated to inorganic phosphate was measured by means of IRMS mass spectrometry as described in the chemical analysis chapter (2.5).

2.5 Chemical analyses

If not mentioned separately the following sample treatments were performed identically in each experiment. Tree sapling compartments (stems and twigs after xylem sap extraction combined with leaves) were dried at 60°C for 72 hours for determination of the biomass dry weight. Moist soil was sieved to pass a 2 mm mesh. Total dry soil mass per pot, as well as each horizons dry weight were determined. Plant (stem, twigs, leaves) and soil samples were digested for analyses of total P concentrations (TP): dried material was homogenized with a planetary ball mill, mixed with 65% HNO_3 and digested at 180°C (Loftfields Analytische Lösungen 6 AM; Heinrichs et al. (1986)).
Concentration of inorganic P (Pi) in extraction and digestion solutions was determined spectrophotometrically with a continuous flow analyzer (CFA, AA3, XY2, Seal-Analytic, Norderstedt, Germany) at λ = 660 nm, using the method of Murphy and Riley (1962). In soil extraction solutions, total dissolved P concentrations were measured by means of Inductively Coupled Plasma/Optical Emission Spectrometry (ICP-OES, PerkinElmer Optima 5300 DVGermany) at λ P 213.617. Organic P concentrations (Po) were calculated as the difference between total dissolved and Pi concentrations (Gurpal et al. 2006).
Phosphorus fractions in mineral soil were determined by a sequential P fractionation scheme after Hedley et al. (1982) modified by Kuo (1996). 'Labile P' was determined by the extraction of 0.5 g dry weight equivalent of fresh soil with 0.5 M $NaHCO_3$ (Roth, adjusted to pH 8.5) by shaking, centrifugation (ROTANTA 460 rs, Hettich Lab Technologies, Tuttlingen, Germany), decantation, and filtration through P-free filters (MN 619 G ¼, Macherey-Nagel GmbH & Co. KG, Düren, Germany). 'Moderately labile P' was extracted from the soil residuum by shaking with 0.1 M NaOH (Sigma-Aldrich) followed by the same procedure as outlined for the 'labile-P' fraction. In a third extraction step, the 'stable-P' fraction was determined by addition of 5 ml 37% HCl

(Sigma-Aldrich): samples were first heated in a water bath to 80°C, thereafter, shaken by hand followed by another addition of 5 ml 37% HCl. Samples were allowed to cool down before filtration.

We purified Pi following the protocol of Weiner et al. (2011) and added anion exchange resin membranes (VWR International GmbH, Bruchsal, Germany) to the xylem sap samples. After 16 h of shaking, the membranes were removed, rinsed with H_2O and eluated by HNO_3. Eluates were again stored in a portable fridge at 4°C until preparation for isotope analysis in the laboratory. In the laboratory, the eluate was used for precipitation of silver phosphate as described by Weiner et al. (2011). In brief, the mineral precipitation and dissolution of ammonium phospho-molybdate was followed by mineral precipitation and dissolution of magnesium ammonium phosphate. After removal of cations and proof of the absence of chloride, silver phosphate was precipitated. Because some organic P compounds might be rapidly transformed into Pi in the in situ fertilization $^{18}O_{Pi}$ xylem sap experiment (**A3)**, we processed xylem sap samples each day directly after returning from the field, whereas this was not necessary in prior experiments due to the nearby laboratories (**A1, A2**). Soil water for O isotope analysis was gathered by cryoextraction (Orlowski et al., 2013) (**A3**). $\delta^{18}O$ values of soil water extracts as well as of xylem sap water were measured by cavitiy ring down spectroscopy (PICARRO Inc., 480, Oakmead Parkway, Sunnvale, CA, 94085, US) The analysis of O isotope ratios of silver phosphate was carried out by means of a TC/EA (PYRO cube) coupled in continuous flow to an IRMS (IsoPrime100, both Elementar Analysensysteme; Hanau, Germany). Three triplicate subsamples (if enough silver phosphate was available) of each sample were weighed in silver capsules together with a small amount of glassy carbon powder to promote CO formation during combustion (Thermofisher scientific, Type 1, Kandel, Germany). The purity of the precipitated silver phosphate was ensured by the close match of standards and samples regarding the regression of O yield on analyte weight (Figure S2). Calibration and drift-corrections were accomplished by repeated measurements of two international benzoic acid standards, IAEA 601 and IAEA 602 ($\delta^{18}O$ =+23.3‰ and +71.4‰, respectively; distributed by the International Atomic Energy Agency, Vienna, Austria), and one internal Ag_3PO_4 standard ($\delta^{18}O$ = +10.2‰).

Radioactivity of ^{33}P (**A1**) was measured in all soil extracts ($NaHCO_3$-P, NaOH-P, HCl-P, HNO_3), as well as in HNO_3-digestion solutions of organic layer and plant material

after addition of Ultima Gold scintillation cocktail (Perkin Elmer, USA) by means of Liquid Scintillation Counting (TriCarb 3110 TR, Perkin Elmer, USA).

2.6 Calculations and statistical analyses

In the conducted ^{33}P mesocosm experiment (**A1**), aboveground P stocks were calculated as the sum of compartment-specific (stem, twigs, leaves) products of total aboveground tree sapling biomass and P concentrations. The activity of ^{33}P of each sample was first corrected for its radioactive decay (Audi et al. 2003). The specific ^{33}P activity (SA) was calculated as follows (Equ. 1; Di et al. 1997)

$$\mathrm{SA}\ [\mathrm{MBq}\ g^{-1}\ \mathrm{P}] = \frac{\text{Radioactivity of } {}^{33}P\ [\mathrm{MBq}\ g^{-1}\ \mathrm{DM}]}{{}^{31}P\ concentration\ [g\ P\ g^{-1}\ \mathrm{DM}]} \qquad \text{(Equation 1)}$$

Concentrations of ^{31}P correspond to Pi concentrations in soil P fractions and total dissolved P concentrations of P fractions in soil and plant compartments.

The ^{33}P recovery was calculated as the ratio between radioactivity initially applied to the sum of all radioactivity dispersed to soil and plant (compartments) at the end of the experiment (Equ. 2; Di et al. 1997)

$${}^{33}P\ recovery\ [\%] = \frac{\text{Radioactivity applied [MBq]}}{\sum Radioactivity\ soil\ [MBq] + \sum Radioactivity\ plant [\mathrm{MBq}]} \times 100 \qquad \text{(Equation 2)}$$

where *radioactivity soil* comprises all P fractions ($NaHCO_3$-extractable P, NaOH-extractable P, HCl-extractable P) including organic layers [if present], and *radioactivity plant* refers to radioactivity in aboveground tree sapling biomass (stem, twigs, leaves). We calculated the difference between treatments in SA of A horizons that is between the presence ($O_{present}$) and absence (O_{absent}) of organic layers (Equ. 3):

$$\Delta\ {}^{33}P\ [\mathrm{MBq}\ g^{-1}\mathrm{P}] = SA_{Opresent}\ [\mathrm{MBq}\ g^{-1}\mathrm{P}] - SA_{Oabsent}\ [\mathrm{MBq}\ g^{-1}\mathrm{P}] \qquad \text{(Equation 3)}$$

These differences were calculated for each P fraction in soil and for plant compartments.

Differences between sites were tested using a student's t test if no treatment was involved. For single time steps and the complete design, we used a repeated measures ANOVA with site as between-subject factor and treatment (presence/absence of organic layers) as within-subject factor. Furthermore, we tested whether calculated differences between treatments (Δ) significantly deviated from zero based on a t test against zero. For time series, a repeated measures ANOVA was conducted with site as between-subject factor and time step as within-subject factor. The data set did not

allow testing prerequisites for parametric tests. Therefore, we repeated all statistical analyses based on non-parametric tests (Mann-Whitney-U for site or treatment effects; Bredenkamp for interactions between site and treatment). Because a sample size of three results in a minimum level of significance of 0.1 in a Mann-Whitney-U test, we had to adjust the level of significance accordingly. Except for the difference in foliar P concentrations and the presence/absence effect of the organic layer on the NaOH fraction in Bad Brückenau, all significant results remained irrespective of whether we used parametric or non-parametric tests. As opposed to parametric tests it is not possible to account for interactions and repeated measures simultaneously in one non-parametric test. Consequently, an extensive number of non-parametric tests results increasing the probability of false positive results and therefore, we report on the outcome of parametric tests.

In our novel approach to measure the oxygen stable isotope composition in phosphate in xylem sap (**A2**), differences between treatments were tested by t-tests for dependent samples. Subsequently each treatment was tested this way against the prior treatment following the analytical concept of Figure 1, except for the AER supernatant, which was tested against the initial solution, the DAX8 supernatant or the LRA supernatant depending on which pretreatment before Po removal was used. If the prerequisite for statistical analyses was violated (non-normal distribution), Wilcoxon tests were performed instead.

In the in situ fertilization $^{18}O_{Pi}$ xylem sap experiment (**A3)**, general site effects were derived from a comparison of control plots between sites. In these cases, differences between sites were tested using a student's t test. For single time steps and the complete design (i.e. including all treatments), we used a repeated measures ANOVA with site as between-subject factor and treatment (fertilization) as within-subject factor. Furthermore, we tested whether calculated differences between treatments (Δ) significantly deviated from zero based on a t test against zero. For time series, a repeated measures ANOVA was conducted with site as between-subject factor and time step as within-subject factor. If the prerequisite for statistical analyses was violated (non-normal distribution), Wilcoxon tests were performed instead.

Statistical analysis was carried out by IBM SPSS Statistics 22 an all experiments.

3 Results and Discussion

3.1 Organic layers favor phosphorus storage and uptake by young beech trees (*Fagus sylvatica* L.) at nutrient poor ecosystems (A1)

The aim of my first experiment was to investigate the influence of P availability and the organic layers on P nutrition of beech saplings. This investigation is important because the accumulation of organic layers in forests is linked to decreasing nutrient availability. Organic layers might represent a source of phosphorus (P) nutrition of trees in forests. Our aims were i) to test if the fate of P in a tree sapling-soil system differs between nutrient-poor and nutrient-rich sites, and ii) to assess the influence of organic layers on the fate of P in a tree sapling-soil system at either site.

We conducted a ^{33}P labeling experiment of mesocosms of beech (*Fagus sylvatica L.*) saplings.

Recovery of ^{33}P in the organic layer was greater under nutrient-poor than under nutrient-rich conditions likely caused by the abundance of microorganisms and roots. Under nutrient-poor conditions, we found that the mobilization of P followed by efficient uptake promoted tree sapling growth if the organic layer was present. The presence of organic layers did not significantly influence P uptake by beech saplings under nutrient-rich conditions suggesting mechanisms of P mobilization in addition to organic matter mineralization.

Our results highlight the importance of organic layers for P nutrition of young beech trees growing on nutrient poor soils in temperate forest ecosystems. This crucial role of organic layers should be considered for sustainable forest management.

3.2 A novel oxygen stable isotope approach to study phosphate in xylem sap (A2)

Despite the pivotal role of the xylem sap in terms of nutrient transport to locations of metabolic demand, knowledge on phosphorus (P) and its origin in xylem sap is lacking. Since we are interested in deciphering the ecosystem adaptations of P acquisition in a P-rich versus a P-poor site, reliable $\delta^{18}O_{Pi}$ values in xylem sap would be a great step forward. As organic P compounds as well as other xylem sap components might alter the correct measurement of $\delta^{18}O_{Pi}$ values in xylem sap. Here we present a novel pretreatment approach and purification scheme of xylem sap before the regular stable isotope measurement of oxygen (O) associated to inorganic phosphate ($\delta^{18}O_{Pi}$) according to Tamburini et al. (2012). On average, we found inorganic P (Pi) and organic P (Po) concentrations of 0.04±0.01 and 0.53±0.01 mg P l^{-1} in xylem sap, respectively. If we concentrated Pi based on anion exchange membranes, we found an incorporation of ^{18}O from isotopically spiked chemicals. Therefore, interactions with resins and/or Po biased $\delta^{18}O_{Pi}$ measurements. Because phosphocholine was reported as the main organic P species in xylem sap, we used a lipid removal agent (LRA) preceding to the novel oxygen stable isotope approach to study phosphate in xylem sap. However, LRA did not remove Po quantitatively and even worse, concomitantly removed Pi.

Because the addition of DAX8 minimized pH shifts and inhibited enzymatically induced Po interferences, we recommend the use of DAX8 before O isotope analysis of phosphate in xylem sap. $\delta^{18}O_{Pi}$ values of xylem sap ranged between -2 and +15‰ indicating enzymatically mediated transformation reactions of Po to Pi. Therefore, the O isotope approach proved to be a promising tool to reveal plant-internal P cycling.

3.3 Impacts of fertilization on biologically cycled P in xylem sap of *Fagus sylvatica* (L.) revealed by means of the oxygen isotope ratio in phosphate (A3)

Since the main P sources in soil for plant nutrition are not only constrained by P availability at different sites, also the availability of other nutrients might strongly affect P uptake mechanisms and the relative importance of different P sources in soil. Studies on the effect of high atmospheric N deposition report inconsistent results on forest productivity and N cycling which might be related to P availability in soil and subsequently affect tree P nutrition. We wanted to test the effects of (i) site i.e., a P-poor versus a P-rich site and of (ii) fertilization (N, P, N+P) on inorganic P (Pi) and organic P (Po) concentrations as well as on biologically cycled phosphate (inferred from the O isotope signature after adding an ^{18}O-enriched label) in xylem sap. We measured Pi and Po concentrations and the O isotope signature in phosphate ($\delta^{18}O_{Pi}$) in xylem sap of beech (*Fagus sylvatica* L.) trees two and 14 days after addition of ^{18}O-enriched water to the organic layer in a full factorial fertilization experiment (control, +N, +P, +NP) at two sites differing in P availability.

Higher P concentrations in xylem sap at the P-rich than at the P-poor site originated from accelerated biological P cycling indicated by incorporation of ^{18}O from the isotope label into phosphate in xylem sap shortly after labeling. At this site, $\delta^{18}O_W$ values of xylem sap after label application remained close to background $\delta^{18}O_W$ values of soil solution.

We speculate that in contrast to P uptake, trees took up water from deeper (non ^{18}O-labeled) soil layers. At the P-poor site, the ^{18}O label was recovered both in xylem sap water and phosphate in xylem sap, the latter only after 14 days.

These results imply that trees relied on the organic layer for P acquisition and water uptake. However, biological processes associated with an incorporation of ^{18}O from the label were slower at the P-poor than at the P-rich site. P addition (P, NP) increased Pi concentrations in xylem sap at the P-rich site. Based on $\delta^{18}O_{Pi}$ values in xylem sap, the additional P originated both from the fertilizer and from accelerated biological P cycling in soil.

We conclude that P-poor sites likely suffer more from climate change in case of an increased frequency of droughts because as opposed to P-rich sites both water and nutrient uptake will be affected.

3.4 Variability in the stable isotope composition of oxygen of inorganic phosphate ($\delta_{18}O_{Pi}$) in xylem sap of *Fagus sylvatica* (L.) on inorganic and organic P

I was able to demonstrate the outstanding importance of organic layers for P nutrition on P-poor sites by means of of radioactive isotopes of phosphorus. Since we have approached a proper measurement scheme for the stable isotope composition of oxygen of inorganic phosphate ($\delta^{18}O_{Pi}$) in the xylem sap of trees (A2). We are now able to obtain data as a combination of the different experiments on both Pi and Po concentrations as well as $\delta^{18}O_{Pi}$ values in xylem sap from different sites as well as during different seasons. As raised in the introduction, we lack an in-depth knowledge of tree P nutrition. Furthermore, to the best of my knowledge, $\delta^{18}O_{Pi}$ values in xylem sap have not been measured yet. Here I summarize the comprehensive und still partially unpublished data combined from our various field experiments and discuss potential controls. These controls could be constrained by different methodological approaches, seasonal changes, or site effects.

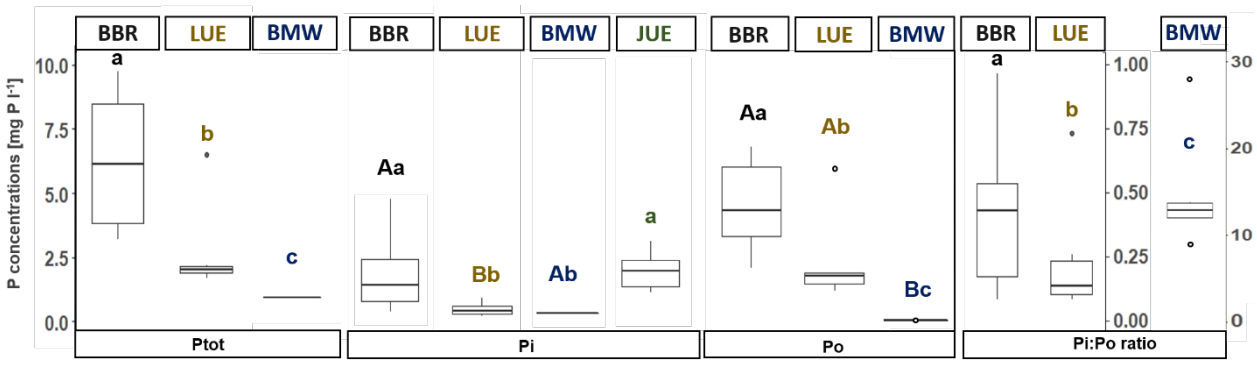


Figure 3: Total (Ptot), inorganic (Pi) and organic (Po) phosphorus concentrations (mg P l^{-1}), and Pi:Po ratios in xylem sap of trees (NPK control plots) from various experiments. Note that Pi:Po ratios refer to the second and third y axis. Lowercase letters indicate differences between sites whereas uppercase letters indicate significant differences between Pi and Po concentrations of a given site. Po at the site JUE was not measured. BBR = Bad Brückenau; LUE = Lüss; BMW = Baltmannsweiler; JUE = Jülich.

On average, we found Pi concentrations of 1.38 ± standard deviation (SD) 1.07 mg P l^{-1} and Po concentrations of 2.26 ± SD 2.25 mg P l^{-1}. In general, the variability of P concentrations in xylem was high at the sites LUE and BBR and low at the site BMW (coefficient of variation [CV] 41% in BBR, 63% in LUE and 1% in BMW). The mean Pi:Po ratio was 0.33 ± SE 0.08 (LUE and BBR; JUE only Pi values are available; BMW is treated separately due to its very low Po values). We found significantly different site effects in Ptot and Po concentrations in the decreasing order BBR, LUE, BMW (Fig. 3).

The ranges of 0.1 to 4.8 mg Pi l^{-1} and 0.02 to 6.7 mg Po l^{-1} in xylem sap found in our studies are comparable to concentrations reported in the literature, 0.5 to 70 mg Pi l^{-1} and 0.5 to 80 mg Po l^{-1}, respectively (Bollard, 1960, Saur et al., 1995, Prima-Putra & Botton, 1998, Netzer et al., 2017). Most of our P concentrations in xylem sap are at the lower range of reported P concentrations in literature. For Pi we found lower values as reported in literature at the site LUE and for Po we found lower values as reported in literature at the site BMW, 0.13 mg Pi l^{-1} and 0.02 mg Po l^{-1}, respectively. (Dambrine et al., 1995, Peuke & Rennenberg, 2004, Netzer et al., 2017).

While literature about organic P concentrations in xylem sap is rare it is also biased if Po is even a component of xylem sap. For example, Schachtmann et al. (1998) describe Pi transport in xylem sap solely as Pi, whereas Maizel et al. (1956), Gout et al. (1990) and Netzer et al. (2017) also describe Po as a component in xylem sap.

It is worth to mention that three out of four sites, where xylem sap of beech trees was extracted contained significant amounts of organic P. Since Po in xylem sap is a critical component in our approach to determine the stable isotope composition of oxygen (O) of inorganic phosphate ($\delta^{18}O_{Pi}$), due to its coprecipitation during silverphosphate precipitation (**A2**). NMR studies might help to identify Po species in xylem sap, thus helping to verify findings from Tolbert et al. (1960) and giving a better understanding of how to purify inorganic P in xylem sap. The very few scientific publications that addressed the issue of Po composition in xylem sap describe the majority as organic esters in the form of phosphochline (PC) and phosphoethanoloamin (<1%) (Maizel et al., 1956, Martin & Toblert, 1983). Since I do not assume cholineester synthesis to take place in roots after Pi uptake, I much prefer the idea of PC being the most prominent organic P form transferred from storage organs via xylem to places of metabolic demand. Gout et al. (1990) demonstrate that PC was first hydrolyzed outside the cell

and that choline thus formed entered the cytosolic compartment where choline kinase reconverted it to PC.

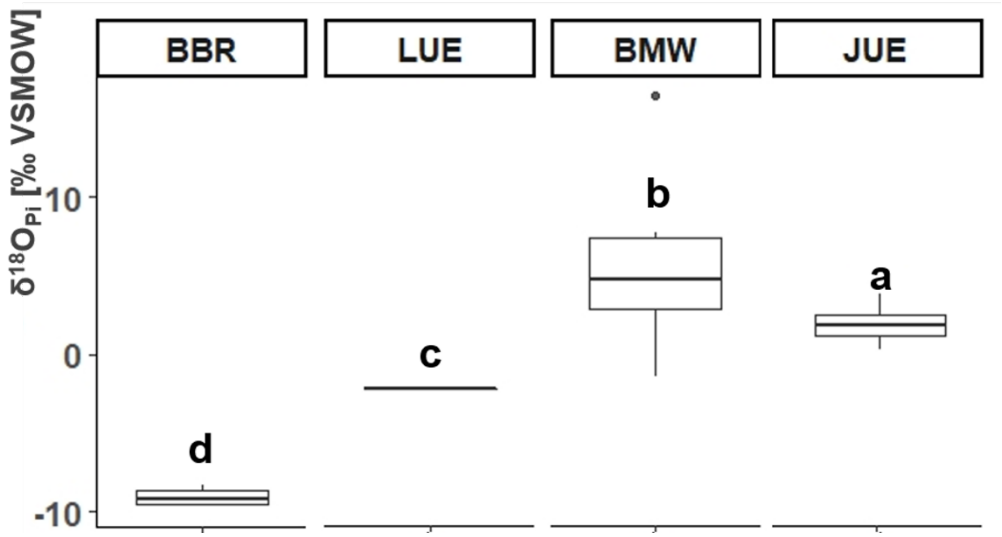


Figure 4 Oxygen isotope signatures ($\delta^{18}O_{Pi}$) of inorganic phosphorus in xylem sap (NPK control plots) from different experiments. Lowercase letters indicate significant differences between sites. BBR = Bad Brückenau; LUE = Lüss; BMW = Baltmannsweiler; JUE = Jülich.

During the course of each experiment, we also collected data about the natural abundance of $\delta^{18}O_{Pi}$ in xylem sap. The site JUE has a mean $\delta^{18}O_{Pi}$ background value of 2.3 ± SD 1,1‰ and the site BMW 5.8‰ ± SD 5.5‰. Both are in the range of values to be ever reported in literature (Sperber et al., 2023). Whereas xylem sap samples from the site LUE have $\delta^{18}O_{Pi}$ background values of -2.2 ± SD 0.2‰ and -9.1 ± SD 0.6‰ at the site BBR. (Fig. 4)

We are the first to measure $\delta^{18}O_{Pi}$ in xylem sap with the intention to trace mobilized P from organic layers into xylem sap of beech trees. To date, only ten studies reported on the analysis of $\delta^{18}O_{Pi}$ from plant material are available, of which non was conducted in plant xylem sap (Larsen et al. 1989, Young et al., 2009; Tamburini et al. 2012; Pfahler et al. 2013/2017; Scheerer et al. 2018, Qin et al, 2018, Bauke et al. 2021,). These results range from 2.5 to 52‰ (Sperber et al., 2023).

All $\delta^{18}O_{Pi}$ values from plant materials in these studies represent extractions from whole plant samples using different extractants (TCA; HNO_3; H_2O). Therefore, comparability to directly extracted xylem sap is limited, due to extracted phloem and cytosol.

The site JUE has a mean $\delta^{18}O_{Pi}$ background value of 13.3 ± SD 1‰ in soil solution, while we found a $\delta^{18}O_{Pi}$ signal of 2.3 ± SD 1,1‰ in xylem sap. Bauke et al. (2021) describe the TCA extractable $\delta^{18}O_P$ signal in root tissue to be comparable to the $\delta^{18}O_P$ soil signal. A certain proportion of the $\delta^{18}O_{Pi}$ signal in xylem sap therefore refers to P derived from soil solution, while there are some interfering aspects. The interpretation of this difference is a multifactorial issue. A distinct proportion of Pi in xylem sap might originate from P storage organs, which are also located in the roots (Netzer et al., 2017). Phloem/xylem exchange is also described in some papers (Van Bel, 1990, White & Ding, 2023). Pfahler et al. (2017) and Qin et al. (2018) describe the influence of arbuscular mycorrhizal fungi as an $\delta^{18}O_{Pi}$ altering agent during plant P uptake. Furthermore, there is no data available if fractionation during root uptake might also alter the $\delta^{18}O_{Pi}$ signal. Additionally enzymatic activity in xylem sap might influence the resulting $\delta^{18}O_{Pi}$ signal. Therefor $\delta^{18}O_{Pi}$ has a great potential for understanding soil plant P uptake and its involved mechanisms.

The extraction method used for xylem sap extraction at the sites BMW and JUE was xylem sap displacement according to the method of Glavac et al. (1989) and at the sites BBR and LUE the method mostly used in literature - Scholander pressure bomb extraction was used. I compared P concentrations from the same tree compartments (branches from the site BMW) extracted either by Scholander pressure bomb extraction or xylem sap displacement, which resulted in the same P concentrations in inorganic P and organic P (data not shown). Therefore, I would also exclude dilution by the displacement solution (ddH_2O) during the displacement technique. Even though I could not exclude different results for $\delta^{18}O_{Pi}$ in these samples, since I didn't measure it in these exact samples. By regression of CO peak areas on analyte weight of Ag_3PO_4 standards and samples as a measure of the O yield we have checked for measurement impurities (**A3-S2**). Furthermore, I would not assume biotic/enzymatic turnover, since the samples were frozen (-20°C) immediately after extraction. In a student's laboratory course, we examined the stability of Pi and Po in xylem sap. We could show that xylem sap samples at 22°C are stable (<5% loss of Pi) for about 3h and multiple (5-times) freezing/thawing cycles had no effect either on Pi or on Po concentrations (data not shown). Since the extracted volume by xylem sap displacement technique (Glavac et al. 1989) is sufficient for xylem sap extraction of more than one liter per sample and therefore P_{tot} is usually greater than 1 mg P per sample, this technique should be the choice for further $\delta^{18}O_{Pi}$ investigations in xylem sap. As the successful precipitation of

silver phosphate requires a minimum quantity of 0.8 mg P. Whereas the collection of 1 mg P per sample is quite laborious and challenging by the use of scholander pressure bomb extraction, especially on P poor sites. Remarkably we found no Po contamination of in situ measured $\delta^{18}O_{Pi}$ in xylem sap (**A3**), due to the given plausibility in mass spectrometry measurements. By determing the oxygen yield (Tamburini et al. 2010; Jaisi & Blake 2014) both Ag_3PO_4 purity and plausibility of samples <200µg was checked (**A3-S2**). Therefore, our new approach to measure $\delta^{18}O_{Pi}$ in xylem sap (**A2**) was operational in our given experimental design (**A3**). Since the $\delta^{18}O_{Pi}$ results in xylem sap extracted by scholander pressure bomb extraction at the sites LUE and BBR have very negative values (LUE -2.2 ± SD 0.2‰ and BBR -9.1 ± SD 0.6‰) compared to those extracted by xylem sap displacement technique (JUE 2.3 ± SD 1.1‰ and BMW 5.8 ± SD 5.5‰), I cannot exclude an altering influence of the different methods. The distinct different pressures during xylem sap extraction between the methods might have altered $\delta^{18}O_{Pi}$ values, despite the plausibility in mass spectrometry measurements. Zuecco et al. (2022) describe altered $\delta^{18}O_w$ signals by different extraction methods especially when scholander pressure bomb extraction was used.

Since P concentrations, as well as Pi:Po ratios differ due to seasonal changes (Netzer et al. 2017) and across tree compartments (Dambrine et al. 1992, Bauke et al. 2021) it is difficult to disentangle explicit explanations for our very low total P concentrations at the site Baltmannsweiler due to the lack of seasonal and tree compartment specific data. Since only Pi concentrations at the site LUE during that specific period of extraction (beginning of august) were below concentrations ever shown in literature, while the lowest Po concentration in the same samples was 1.16 mg P l^{-1}, I account tree internal P cycling/remobilization to be dominant at that specific moment of sampling.

For the very low Po concentrations at the site BMW I most reasonably account sampling period to be decisive. We sampled xylem sap in early spring (10th of April) before budbreak (April/May), when xylem pressure was increasing several weeks before its seasonal peak at the end of April (Cochard et al., 2001). High xylem pressure is a result of potential-driven water uptake and thus, likely diluted concentrations of xylem constituents in our study. This contrasts with findings in literature (Netzer et al. 2017), where highest Pi and Po values are described during budbreak, due to increased remobilization of stored P from plant organs. Since I found no P data in

literature for the time several weeks before budburst when xylem pressure begins to increase, I assume tree internal P remobilization to start later, shortly before budburst. Even these low Po concentrations in xylem sap are favorable for our novel oxygen stable isotope approach to study phosphate (**A2**) due to the exclusion of a possible interfering contaminant. Our results indicate the activity of enzymes in xylem sap to be critical in our approach to determine the stable isotope composition of oxygen (O) of inorganic phosphate ($\delta^{18}O_{Pi}$).

Therefore, the timing of the measurement can have a decisive influence on the results. Since Netzer et al. (2017) also describe changing Pi and Po concentrations in xylem sap during the several seasonal changes, for example during the beginning of senescence shortly before the end of the vegetation period, it is very important to account seasonal tree internal P cycling for Pi, Po and $\delta^{18}O_{Pi}$ measurements in xylem sap. Since the experiments at the sites BBR, LUE (**A1**) and JUE (**A3**) took place during the mid of the vegetation period, I would not assume seasonal changes to be decisive for altered results. Furthermore, $\delta^{18}O_{Pi}$ from the site BMW (5.8 ± SD 5.5‰) is intermediate compared to the other sites, indicating that the different seasonal sampling periods may not have altered $\delta^{18}O_{Pi}$ in this case. Even though the high variation between the results are a very strong limiting factor for accurate interpretation of seasonal effects.

Since the experimental design of the SPP follows a P gradient in parental P availability to disentangle forest adaptions to P limitation, the impact of site on our results should be fundamental. The low Pi concentrations in xylem sap at the site LUE most likely result from the very low plant available Pi concentrations in soil solution, in line to the sites P limitation for tree growth. In our results **(A2, A3)** the comparison of ICP-OES data for total P (P_{tot}) and the UV-VIS/CFA data for inorganic P in xylem sap are significantly different at the sites BBR, LUE **(A3)** and JUE, indicating a distinct proportion of Po in our xylem sap samples at these sites. At the site BMW P_{tot} and Pi results were not significantly different and calculated Po concentrations indicate negligible proportions of Po in these samples. Despite the very limited knowledge about the Po composition of xylem in literature (Maizel et al., 1956, Martin & Tolbert, 1983) our results also corroborate the presence of phospholipids in xylem. The resulting Pi:Po ratio of 15 ± 6 (SD) in xylem sap of beech at the site BMW is also highly above Pi:Po ratios found in literature. Netzer et al. (2017) describe a maximum Pi:Po

ratio of 2 in xylem sap of beech trees. Despite that Pi:Po ratios of xylem sap are quite rare in literature.

Natural abundance $\delta^{18}O_{Pi}$ values in xylem sap from our investigated sites do not reflect comprehensible results supporting the investigated P gradient and its inherent forest ecosystem adaptions.

4 Error discussion

In the following section my aim is to discuss data uncertainties. Later in the general conclusions section I additionally want to conclusively discuss our results and give advice for upcoming experiments to tackle these uncertainties.

In general, mesocosm experiments **(A1)** do not necessarily reflect the conditions in natural ecosystems. However, the possibility to keep environmental conditions as constant as possible, as well as to minimize the complexity of a natural system, results of mesocosm experiments help to understand fundamental processes especially when adapting new methods to disentangle distinct pathways of a single nutrient like P in a complex ecosystem like a forest. In a further step, using results from mesocosm experiment studies are helpful to verify if identical patterns do occur in natural ecosystems **(A3).** Thus, simplifications of complex natural ecosystem in experimental mesocosm studies are important to disentangle the mechanisms underlying the adaptions of a forest ecosystem to cope with different plant available P concentrations (**A1, A3**).

The detection limit of the continuous flow analyser (CFA, AutoAnalyzer3, SEAL Analytical, Norderstedt, Germany) and the spectrophotometer (UV VIS, Specord 200, Analytic Jena AG, Jena, Germany at λ = 660 nm) for measuring Pi with the phosphomolybdate blue method after Murphy and Riley (1962) was 0.014 mg L^{-1}(CFA) and 0.02 mg L^{-1}(UV VIS). For measuring P_{tot} by means of an inductively coupled plasma optical emission spectroscopy (ICP OES, DV 5300, Perkin Elmer, Waltham, Massachusetts, USA) the detection limit was 0.05 mg L^{-1}. Since the mean Pi concentration in xylem sap of beech tree from the site LUE (**A3**) is 0.39 ± SD 0.23 mg P l^{-1} the detection limit should not be critical, whereas the mean Po concentration in xylem sap from beech trees of the site BMW (**A2**) is 0.04 ± SD 0.011 mg P l^{-1} the detection limit is critical.

In situ experiments always have uncertainties like differing microclimate, or local disturbances, therefore a small degree of heterogeneity is inherent in such experiments. The in situ ^{18}O labelling experiment explained in **A3** was conducted in July/August 2017. Due to financial limitation, it was not possible to label the whole forest floor (organic layers) above the estimated area of rhizosphere distribution around each tree. Additionally, we had to exclude the possibility of an uptake of the tracer by roots from a second monitored tree in our 20x20m fertilized plots. Since the

availability of adult beech trees in the fertilized plots was limited (4-6 individuals) and 2 were already in use of other experiments we had to guarantee 5m minimal distance between the ^{18}O labelled areas around each tree. Therefore, we chose to label each tree as describes in **A3** inside a radius of 1.9m circular around each tree. Since the estimated radius of the rhizosphere around each tree individual is more than 1.9m the applied ^{18}O label found in xylem sap represents only a proportion of water and P uptake from the organic layers.

In the course of this work, laboratory methods were used which are still new (**A2**), not frequently used (**A3**) and/or had to be adapted to the given conditions (**A1, A3**).

Using multi label experiments in the case of P is an upcoming idea to target the limited use of radioactive ^{32}P or ^{33}P (half-life 14.3 d for ^{32}P and 25.3 d for ^{33}P), since P has only one stable isotopologue (^{31}P) it is not possible to use ratios of stable isotopologues like ^{18}O to ^{16}O in water samples (**A1**). As described in the introduction oxygen in phosphate is an ideal tracer for biologically mediated P and in combination with a radioactive P tracer it becomes possible to distinguish biologically mediated inorganic P from inorganic P mobilized by weathering (Jaisi et al. 2011, Joshi et al. 2016) or to identify distinct locations of P mobilization (**A1, A3**). Since it is not possible to directly compare radioactive P tracers to the stable isotope composition of oxygen (O) of inorganic phosphate ($\delta^{18}O_{Pi}$), due to the interdependence of an ^{18}O incorporation in inorganic phosphate in relation to microbial/enzymatic activity. Therefore, both methods in combination can give complimentary results but it is not possible to directly link mass budgets of radioactive ^{32}P or ^{33}P uptake to $\delta^{18}O_{Pi}$ uptake kinetics in the plant (Helfenstein et al., 2018; Siegenthaler et al., 2020; Pistocchi et al. 2020).

As described in **A3** small amounts of Ag_3PO_4 precipitate (<200µg, ~30% of the samples) include a certain degree of uncertainty and must be checked for plausibility in relation to samples >200µg Ag_3PO_4 precipitate. Furthermore, the contamination of Ag_3PO_4 by organic matter is a problem when solubilized during P extraction leading to an incorrect interpretation of isotope measurements (Jaisi & Blake 2014). Thus, it is suggested to determine the oxygen yield (Tamburini et al. 2010; Jaisi & Blake 2014). By correlating the weight of Ag_3PO_4 samples and the CO beam area of the mass spectrometer it is possible to estimate Ag_3PO_4 purity (Weiner et al. 2011). If this ratio ranges in the same order for samples and commercially produced standards it is likely that the samples are not polluted by organic matter. Since both Ag_3PO_4 purity and plausibility of samples <200µg was checked (**A3**), I do not assume organic matter

contamination of the samples. This does not explain the different $\delta^{18}O_{Pi}$ values in each experiment.

The main limitation of the NPK fertilization experiments is that only short-term effects can be analyzed, since the fertilization trial was established in April/May 2016 and our experiment (**A3**) started in late summer July/August 2017 two week after the last of three N applications, while P was applied only once at the beginning in 2016.

5 General conclusions

In our first experiment **(A1)** we intended to test if the fate of P in a tree sapling-soil system differs between nutrient-poor and nutrient-rich sites and to assess the influence of organic layers on the fate of P in a tree sapling-soil system at either site.
We could show that the mobilization of P likely mediated by the microbial community followed by efficient uptake in organic layers under nutrient-poor conditions (LUE) supported tree sapling growth while at the same time reducing the leaching of P to underlying mineral soil horizons. In contrast, the presence of organic layers did not significantly influence P uptake by beech saplings under nutrient-rich conditions (BBR). This suggests that mechanisms of mobilization do not only encompass OM mineralization but also dissolution of mineral-bond P as well as desorption of P from charged surfaces. However, our results do not match with suspected increased leaching losses likely due to the high sorption capacity of the nutrient-rich site BBR. In conclusion, the importance of the organic layer for tree nutrition increases with decreasing P availability. We were able to demonstrate the importance of the organic layers for tree nutrition at P poor sites. Furthermore, the forest floor serves the ecosystem trait as a place of tight nutrient cycling as well as water uptake and retention.
In our second and third experiment **(A2,A3)** we intended to test if purification procedures from soil used to determine the stable isotope composition of oxygen (O) of inorganic phosphate ($\delta^{18}O_{Pi}$) adequately transferable to measure xylem sap $\delta^{18}O_{Pi}$.
The oxygen isotope signature of phosphate can be analysed reliably **(A3)**. *Higher P concentrations in xylem sap at the P-rich than at the P-poor site originated from accelerated biological P cycling indicated by incorporation of ^{18}O from the isotope label into phosphate in xylem sap shortly after labelling. At this site, $\delta^{18}O_W$ values of xylem sap after label application remained close to background $\delta^{18}O_W$ values of soil solution. We speculate that in contrast to P uptake, trees took up water from deeper (non-^{18}O-labelled) soil layers. At the P-poor site, the ^{18}O label was recovered both in xylem sap water and phosphate in xylem sap, the latter only after 14 days. These results imply that trees relied on the organic layer for P acquisition and water uptake. However, biological processes associated with an incorporation of ^{18}O from the label were slower at the P-poor than at the P-rich site.*

However, there are restrictions in that, interferences of Po on $\delta^{18}O_{Pi}$ values that were likely linked to the activity of extracellular enzymes are observed **(A2)**. *Since our experiment was not planned to discover and explain such interferences, the assessed explanatory variables are insufficient to fully explain Po contamination. More research is needed and the assessment of phosphatase enzymes in plants and of their isotope fractionation factors could guide a conceptual model of plant-internal P cycling in the future.*

In our third experiment **(A3)** we intended to test the effect of site (BBR,LUE), a P rich vs a P poor forest ecosystem (a), and of (b) fertilization (N, P, N+P) on Pi and Po concentrations as well as on biologically cycled phosphate (inferred from the O isotope signature after adding an ^{18}O label) in xylem sap.
Higher P concentrations in xylem sap at the P-rich than at the P-poor site originated from accelerated biological P cycling indicated by incorporation of ^{18}O from the isotope label into phosphate in xylem sap shortly after labeling. At this site, $\delta^{18}O_W$ values of xylem sap after label application remained close to background $\delta^{18}O_W$ values of soil solution. (b) We did not see fertilization effects reflected in xylem sap $\delta^{18}O_{Pi}$ either between on neither site. However, our investigations have shown that the ^{18}O labeling approach pinpoints a decoupling of water and P uptake at the P-rich site BBR representing an acquiring ecosystem. By contrast, trees relied on the organic layer for both water and P uptake at the P-poor site LUE (recycling ecosystem).

Our findings indicate the importance of the organic layers for P nutrition as well as water supply for beech trees at nutrient poor ecosystems. In general, the hypothesized adaption of a forest ecosystem along pedogenesis and accompanied nutrient depletion and acidification from an “acquiring system” on P rich soils towards a “recycling system” on P poor soils (Lang et al. 2016), is ideally demonstrated by our contrasting sites BBR and LUE (**A1, A3**). Both soil Plant available P and xylem sap Pi concentrations in LUE were very low indicating P limitation at that site (Fig.1). Corroborated by results from ICP forest monitoring that indicate a transformation from N- to P-limitation, or at least to NP colimitation, in beech trees especially on nutrient poor soils (Jonard et al. 2015, Talkner et al. 2015), I was able to identify a fundamental agent in the biogeochemical cycling of P at nutrient poor soils named the organic layers / forest floor (synonym). In conclusion, the importance of the organic layers for tree

nutrition at the site LUE proofed to be fundamental. In general, the importance of the organic layer for tree nutrition increases with decreasing P availability. This is corroborated by findings of Pistocchi et al. (2018) and Siegenthaler et al. (2021). Since the distribution of most forest ecosystems in Europe is constrained to nutrient-poor soils, sustainable forest management should consider the formation and functioning of the forest floor (**A1**).

Therefore, foresters should consider that ecosystem resilience particularly at P-poor ecosystems might be strongly influenced by processes that deteriorate the organic matter and subsequently changes the forest floor structure and thereby its function as a place of water retention and nutrient retention from leaching to mineral soil layers. In this regard, ongoing N deposition and increasing soil temperatures especially in the forest floor due to climate change have the potential to considerably change forest floor turnover dynamics. I was able to identify the importance of the forest floor for beech nutrition in mesocosm-, as well as in situ experiments (**A1,A3**). I have shown that enzymatic activity to be critical in our approach to determine the stable isotope composition of oxygen (O) of inorganic phosphate ($\delta^{18}O_{Pi}$) in xylem sap (**A2**). Nevertheless, the oxygen isotope signature of phosphate can be analyzed reliably as shown in our last experiments (**A3 and the Jülich experiment**) due to the use of DAX8 prior to silverphosphate precipitation. Therefore, I will give some ideas for upcoming experiments.

6 Approaches for future experiments

Since the amount of inorganic P (>0.8 mg) in xylem sap is decisive for sufficient Ag_3PO_4 precipitation we found xylem sap displacement technique according to the method of Glavac et al. (1989) to be the most promising.

We are not able to link our results to findings in literature, because we are the first to measure $\delta^{18}O_{Pi}$ in xylem sap. Therefore, more experiments on $\delta^{18}O_{Pi}$ in xylem sap are needed to verify our results.

Furthermore, the assessment of phosphatase enzymes in plant xylem sap and of their isotope fractionation factors could guide a conceptual model of plant-internal P cycling in the future. Furthermore, plant-internal P cycling strongly depends on season and thus, requires temporally resolved experiments. Since I was able to trace ^{18}O from applied water into xylem sap of beech trees, hydroponic experiments might help to identify a potential fractionation factor during root P_i uptake, and/or to differentiate the influence of arbuscular mycorrhizal fungi on $\delta^{18}O_{Pi}$. By labelling distinct plant organs by different ^{18}O tracers it might now become possible to trace tree internal P turnover mechanisms by analyzing $\delta^{18}O_{Pi}$ in xylem sap. Tamburini et al. (2018) describe a new method to analyze the isotopic composition of oxygen associated with organic phosphorus in soil and plant material. Adapting this method to xylem sap might give multiple opportunities for future experiments.

Von Sperber et al. (2023) critically describe the state of art and actual limitations and uncertainties of $\delta^{18}O_P$ in environmental science. Since there are some new principal analytical approaches like Raman spectroscopy, triple oxygen isotope analysis and high resolution mass spectroscopy the field seems to be levelled for further profound insights on $\delta^{18}O_P$.

7 References

Aber JD. (1992). Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. *Trends in Ecology & Evolution* 7:220–224. doi:10.1016/0169-5347(92)90048-G

Angert A, Weiner T, Mazeh S, Tamburini F, Frossard E, Bernasconi SM, Sternberg M. (2011). Seasonal variability of soil phosphate stable oxygen isotopes in rainfall manipulation experiments. *Geochimica et Cosmochimica Acta* 75: 4216–4227.

Audi G, Wapstra AH, Thibault C. (2003). The Ame2003 atomic mass evaluation. *Nuclear Physics* 729: 337–676. doi:10.1016/j.nuclphysa.2003.11.003

Augusto L, Achat DL, Jonard M, Vidal D, Ringeval B. (2017). Soil parent material-A major driver of plant nutrient limitations in terrestrial ecosystems. *Global change biology* 23: 3808–3824. doi:10.1111/gcb.13691

Bauke, S.L., Schnepf, A., von Sperber, C., Orlowski, N., Lewandowski, H., Selzner, T., Tamburini, F. and Amelung, W. (2021). Tracing uptake and translocation of phosphorus in wheat using oxygen isotopes and mathematical modelling. *New Phytol* 230. 1883-1895. https://doi.org/10.1111/nph.17307

Berg B, McClaugherty C. (2003). Plant litter: decomposition, humus formation, carbon sequestration, Springer Verlag, Heidelberg, Germany

Bergkemper F, Scholer A, Engel M, Lang F, Kruger J, Schloter M, Schulz S. (2016). Phosphorus depletion in forest soils shapes bacterial communities towards phosphorus recycling systems. *Environmental microbiology* 18: 1988–2000. doi:10.1111/1462-2920.13188

Blake RE, O'Neil JR, Surkov AV. (2005). Biogeochemical cycling of phosphorus Insights from oxygen isotope effects of phosphoenzymes. *American Journal of Science* 305: 596–620.

Blevins LL, Prescott CE, and van Niejenhuis A. (2006). The roles of nitrogen and phosphorus in increasing productivity of western hemlock and western redcedar plantations on northern Vancouver Island. *Forest Ecology and Management* 234: 116–122. doi: 10.1016/j.foreco.2006.06.029

Bollard EG. (1960). Transport in the Xylem. *Plant Physiology* 11: 141–166

Brandtberg P-O, Bengtsson J, Lundkvist H. (2004). Distributions of the capacity to take up nutrients by Betula spp. and Picea abies in mixed stands. *Forest Ecology and Management* 198:193–208. doi:10.1016/j.foreco.2004.04.012

Cochard H, Lemoine D, Améglio T, Granier A. (2001). Mechanisms of xylem recovery from winter embolism in *Fagus sylvatica*. *Tree Physiology* 21: 27–33.

Dambrine E, Martin F, Carisey N, Granier A, Hällgren J-E, Bishop K. (1995). Xylem sap composition. A tool for investigating mineral uptake and cycling in adult spruce. *Plant and Soil* 168: 233–241.

DeForest JL, Smemo, KA, Burke DJ, Elliott HL, and Becker JC. (2012). Soil microbial responses to elevated phosphorus and pH in acidic temperate deciduous forests. *Biogeochemistry* 109, 189–202. doi: 10.1007/s10533-011-9619-6

Di HJ, Condron LM, Frossard E. (1997). Isotope techniques to study phosphorus cycling in agricultural and forest soils. A review. *Biology and Fertility of Soils* 24: 1–12. doi:10.1007/BF01420213

Duquesnay A, Dupouey JL. (2000). Spatial and temporal variability of foliar mineral concentration in beech (*Fagus sylvatica*) stands in Northeastern France. *Tree Physiology 20* 1: 13–22

Fisher RF, Binkley D. (2000). Ecology and Management of Forest Soils. Wiley, New York.

Finzi AC. (2009). Decades of atmospheric deposition have not resulted in widespread phosphorus limitation or saturation of tree demand for nitrogen in southern New England. *Biogeochemistry* 92: 217–229. doi: 10.1007/s10533-009-9286-z

Glavac V, Koenies H, Jochheim H, Ebben U. (1989). Mineralstoffe im Xylemsaft der Buche und ihre jahreszeitlichen Konzentrationsveränderungen entlang der Stammhöhe. *Angewandte Botanik* 63: 471-486.

Gout E, Bligny R, Roby C, Douce R. (1990). Transport of phosphocholine in higher plant cells: ^{31}P nuclear magnetic resonance studies. *Proceedings of the National Academy of Sciences of the United States of America* 87: 4280–4283.

Grigal DF, Vance ED. (2000). Influence of soil organic matter on forest productivity. *New Zealand Journal of Forestry Science* :169–205

Hacker N, Wilcke W, Oelmann Y. (2019). The oxygen isotope composition of bioavailable phosphate in soil reflects the oxygen isotope composition in soil water

driven by plant diversity effects on evaporation. *Geochimica et Cosmochimica Acta* 248: 387-399

Hauenstein S, Neidhardt H, Lang F, Krüger J, Hofmann D, Pütz T. (2018). Organic layers favor phosphorus storage and uptake by young beech trees (*Fagus sylvatica* L.) at nutrient poor ecosystems. *Plant Soil* 432: 289–301. doi: 10.1007/s11104-018-3804-5

Hedley MJ, Stewart JWB. (1982). Method to measure microbial phosphate in soils. *Soil Biology and Biochemistry* :377–385

Helfenstein J, Tamburini F, Sperber C von, Massey MS, Pistocchi C, Chadwick OA, Vitousek PM, Kretzschmar R, Frossard E. (2018). Combining spectroscopic and isotopic techniques gives a dynamic view of phosphorus cycling in soil. *Nature communications* 9: 3226.

Ilg C, Foeckler F, Deichner O, Henle K. (2009). Extreme flood events favor floodplain mollusc diversity. *Hydrobiologia* 621, 63–73

Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED. (1996). A global analysis of root distributions for terrestrial biomes. *Oecologia* 108: 389–411. doi:10.1007/BF00333714

Janssens IA, Dieleman W, Luyssaert S, Subke JA, Reichstein M, Ceulemans R. (2010). Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geosci* 3: 315–322. doi: 10.1038/ngeo844

Jonard M, Augusto L, Morel C, Achat DL, Saur E. (2009). Forest floor contribution to phosphorus nutrition. Experimental data. *Annals of Forest Science* 66: 510. doi:10.1051/forest/2009039

Jonard M, Furst A, Verstraeten A, Thimonier A, Timmermann V, Potocic N, Waldner P, Benham S, Hansen K, Merila P, Ponette Q, de la Cruz, Ana C, Roskams P, Nicolas M, Croise L, Ingerslev M, Matteucci G, Decinti B, Bascietto M, Rautio P. (2015). Tree mineral nutrition is deteriorating in Europe. *Global change biology* 21: 418–430. doi:10.1111/gcb.12657

Joshi SR, Li X, Jaisi DP. (2016). Transformation of Phosphorus Pools in an Agricultural Soil. An Application of Oxygen-18 Labeling in Phosphate. *Soil Science Society of America Journal* 80: 69-78.

Kimmins JP. (1997). Forest ecology a foundation for sustainable development. Prentice-Hall, Inc., Upper Saddle River, New Jersey. 596 p

Lang F, Bauhus J, Frossard E, George E, Kaiser K, Kaupenjohann M, Krüger J, Matzner E, Polle A, Prietzel J, Rennenberg H, Wellbrock N .(2016). Phosphorus in forest ecosystems. New insights from an ecosystem nutrition perspective*. Journal of Plant Nutrition and Soil Science* 179: 129–135. doi:10.1002/jpln.201500541

Lang F, Krüger J, Amelung W, Willbold S, Frossard E, Bünemann EK, Bauhus J, Nitschke R, Kandeler E, Marhan S, Schulz S, Bergkemper F, Schloter M, Luster J, Guggisberg F, Kaiser K, Mikutta R, Guggenberger G, Polle A, Pena R, Prietzel J, Rodionov A, Talkner U, Meesenburg H, Wilpert K von, Hölscher A, Dietrich HP, Chmara I. (2017). Soil phosphorus supply controls P nutrition strategies of beech forest ecosystems in Central Europe. *Biogeochemistry* 127: 255. doi:10.1007/s10533-017-0375-0

Larsen S, Middelboe V, Saaby Johansen H. (1989). The fate of O-18 labeled phosphate in soil plant-systems. *Plant and Soil* 117: 143–145.

Lécuyer C. (2004). Oxygen Isotope Analysis of Phosphate (Chapter 22). *Handbook of stable isotope analytical techniques* 1: 482-496.

Leuschner C. (1998). Water extraction by tree fine roots in the forest floor of a temperate Fagus-Quercus forest. *Annals of Forest Science*: 141–157

Liang Y, Blake RE. (2006). Oxygen isotope composition of phosphate in organic compounds: Isotope effects of extraction methods. *Organic Geochemistry* 37: 1263–1277.

Liang Y, Blake RE. (2007). Oxygen isotope fractionation between apatite and aqueous-phase phosphate: 20–45 °C. *Chemical Geology* 238;: 121–133. doi: 10.1016/j.chemgeo.2006.11.004

Lovett GM, Arthur MA, Weathers KC, Fitzhugh RD, Templer PH. (2013). Nitrogen Addition Increases Carbon Storage in Soils, But Not in Trees, in an Eastern U.S. Deciduous Forest. *Ecosystems* 16: 980–1001. doi: 10.1007/s10021-013-9662-3

Magill AH, Aber JD, Currie WS, Nadelhoffer KJ, Martin ME, McDowell WH. (2004). Ecosystem response to 15 years of chronic nitrogen additions at the Harvard

Forest LTER, Massachusetts, USA. *Forest Ecology and Management* 196: 7–28. doi: 10.1016/j.foreco.2004.03.033

Maizel JV, Benson AA, Tolbert NE. (1956). Identification of Phosphorylcholine as an important constituent of plant saps. *Plant Physiology* 31: 408–409.

Marklein AR, Houlton BZ. (2012). Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytol* 193: 696–704. doi: 10.1111/j.1469-8137.2011.03967.x

Martin BA, Tolbert NE. (1983). Factors Which Affect the Amount of Inorganic Phosphate, Phosphorylcholine, and Phosphorylethanolamine in Xylem Exudate of Tomato Plants. *Plant Physiology* 73: 464–470.

McLaren TI, McLaughlin MJ, McBeath TM, Simpson RJ, Smernik RJ, Guppy CN, Richardson AE. (2016). *Plant Soil* 401: 23-38. https://doi.org/10.1007/s11104-015-2610-6

Mo Q, Zou B, Li Y, Chen Y, Zhang W, Mao R. (2015). Response of plant nutrient stoichiometry to fertilization varied with plant tissues in a tropical forest. *Scientific reports* 5: 14605. doi: 10.1038/srep14605

Murphy J, Riley JP. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* 27: 31–36. doi: 10.1016/S0003-2670(00)88444-5

Nadelhoffer KJ, Colman BP, Currie WS, Magill A, Aber JD. (2004). Decadal-scale fates of tracers added to oak and pine stands under ambient and elevated N inputs at the Harvard Forest (USA). *Forest Ecology and Management* 196: 89–107. doi: 10.1016/j.foreco.2004.03.014

Netzer F, Schmid C, Herschbach C, Rennenberg H. (2017). Phosphorus-nutrition of European beech (*Fagus sylvatica* L.) during annual growth depends on tree age and P-availability in the soil. *Environmental and Experimental Botany* 137: 194–207.

Odum EP. (2014). “The strategy of Ecosystem development,” in *The Ecological Design and Planning Reader*, ed. F. O. Ndubisi (Washington, DC: Island Press/Center for Resource Economics), 203–216.

Pfahler V, Dürr-Auster T, Tamburini F, Bernasconi SM, Frossard E. (2013). ^{18}O enrichment in phosphorus pools extracted from soybean leaves. *New Phytologist* 197: 186–193.

Pfahler V, Tamburini,F, Bernasconi SM, Frossard E. (2017). A dual isotopic approach using radioactive phosphorus and the isotopic composition of oxygen associated to phosphorus to understand plant reaction to a change in P nutrition. *Plant Methods* 13: 75.

Pistocchi C, Mészáros É, Tamburini F, Frossard E, Bünemann, EK. (2018). Biological processes dominate phosphorus dynamics under low phosphorus availability in organic horizons of temperate forest soils. *Soil Biology and Biochemistry* 126: 64–75. doi: 10.1016/j.soilbio.2018.08.013

Polglase PJ, Attiwill PM, Adams MA. (1992). Nitrogen and phosphorus cycling in relation to stand age of Eucalyptus regnans F. Muell*. Plant and Soil* 142:177–185. doi:10.1007/BF00010964.

Pregitzer KS, Burton AJ, Zak DR, Talhelm AF. (2007). Simulated chronic nitrogen deposition increases carbon storage in Northern Temperate forests. *Global change biology* 0, 071121035853002-??? doi: 10.1111/j.1365-2486.2007.01465.x

Prietzel J, Stetter, U. (2010). Long-term trends of phosphorus nutrition and topsoil phosphorus stocks in unfertilized and fertilized Scots pine (Pinus sylvestris) stands at two sites in Southern Germany. *Forest Ecology and Management* 259: 1141–1150. doi: 10.1016/j.foreco.2009.12.030

Prima-Putra D, Botton B. (1998). Organic and inorganic compounds of xylem exudates from five woody plants at the stage of bud breaking. *Journal of Plant Physiology* 153: 670–676.

Qin Y, Duan G, Zhao Z, Tian H, Solaiman ZM. (2018) ^{18}O-labeled phosphate applied to soil appears in the shoots of maize after uptake by roots but not after uptake by an arbuscular mycorrhizal fungus. *Mycorrhiza* 28(8): 787-793. doi: 10.1007/s00572-018-0849-5. Epub 2018 Jun 27. PMID: 29951862.

Saur E, Brechet C, Lambrot C, Masson P. (1995). Micronutrient composition of xylem sap and needles as a result of P-fertilization in maritime pine. *Trees* 10: 52–54.

Schachtmann DP, Reid RJ, Ayling SM. (1998). Phosphorus Uptake by Plants: From Soil to Cell. *Plant Physiol.* 116: 447–453.

Scheerer U, Netzer F, Bauer AF, Herschbach C. (2018). Measurements of ^{18}O-Pi uptake indicate fast metabolism of phosphate in tree roots. *Plant biology* 21: 565-570.

Schulze WC. (2005). Protein analysis in dissolved organic matter: What proteins from organic debris, soil leachate and surface water can tell us – a perspective. *Biogeosciences, European Geosciences Union* 2: 75–86.

Shaw AN, and DeForest J. L. (2013). The cycling of readily available phosphorus in response to elevated phosphate in acidic temperate deciduous forests. *Applied Soil Ecology* 63, 88–93. doi: 10.1016/j.apsoil.2012.09.008

Siegenthaler, MB, Ramoneda, J, Frossard, E, Mészáros, E. (2022). Microbial community responses to phosphorus and nitrogen inputs in the organic soil horizons of two contrasting temperate beech forests. *Applied Soil Ecology* 172: doi.org/10.1016/j.apsoil.2021.104357.

Sperber C von, Kries H, Tamburini F, Bernasconi SM, Frossard E. (2014). The effect of phosphomonoesterases on the oxygen isotope composition of phosphate. *Geochimica et Cosmochimica Acta* 125: 519–527.

Sperber C von, Lewandowski H, Tamburini F, Bernasconi SM, Amelung W, Frossard E. (2017). Kinetics of enzyme-catalysed oxygen isotope exchange between phosphate and water revealed by Raman spectroscopy. *Journal of Raman Spectroscopy* 48: 368–373.

Sperber, C. von, Pistocchi, C., Weiler, M., & Tamburini, F. (2023). Oxygen isotope ratios of phosphates in the soil-plant system: Limitations and future developments. *European Journal of Soil Science*: 74(6): e13434. https://doi.org/10.1111/ejss.13434

Spohn M, Ermak A, Kuzyakov Y. (2013). Microbial gross organic phosphorus mineralization can be stimulated by root exudates – A ^{33}P isotopic dilution study. *Soil Biology and Biochemistry* 65: 254–263. doi:10.1016/j.soilbio.2013.05.028

Talkner U, Meiwes KJ, Potočić N, Seletković I, Cools N, Vos B de, Rautio P. (2015). Phosphorus nutrition of beech (*Fagus sylvatica* L.) is decreasing in Europe. *Annals of Forest Science* 72: 919–928. doi:10.1007/s13595-015-0459-8

Tamburini F, Bernasconi SM, Angert A, Weiner T, Frossard E. (2010). A method for the analysis of the $\delta^{18}O$ of inorganic phosphate extracted from soils with HCl. *European Journal of Soil Science* 61: 1025–1032.

Tamburini F, Pfahler V, Bünemann EK, Guelland K, Bernasconi SM, Frossard E. (2012). Oxygen Isotopes Unravel the Role of Microorganisms in Phosphate Cycling in Soils. *Environmental Science & Technology* 46: 5956–5962.

Tamburini F, Pistocchi C, Helfenstein J, Frossard E. (2018). A method to analyse the isotopic composition of oxygen associated with organic phosphorus in soil and plant material. *European Journal of Soil Science* 69: 816–826.

Trichet P, Bakker MR, Augusto L, Alazard P, Merzeau D, Saur E. (2009). Fifty Years of Fertilization Experiments on Pinus pinaster in Southwest France: The Importance of Phosphorus as a Fertilizer. *Forest Science* :390–402.

Turner J, and Lambert MJ. (2015). Long-term growth responses to phosphatic fertilisers in a Pinus radiata plantation. *Australian Forestry* 78: 207–218. doi: 10.1080/00049158.2015.1071679

Van Bel, AJE (1990) Xylem-Phloem Exchange Via the Rays: The Undervalued Route of Transport, *Journal of Experimental Botany* 41; 631–644: https://doi.org/10.1093/jxb/41.6.631

Veresoglou SD, Penuelas J, Fischer R, Rautio P, Sardans J, Merilä P. (2014). Exploring continental-scale stand health – N:P ratio relationships for European forests. *New Phytologist* 202(2): 422–430. doi: 10.1111/nph.12665

Vitousek P. (1982). Nutrient cycling and nutrient use efficiency. *Am.Nat.* 119: 553-572

Vogt KA, Grier CC, Vogt DJ. (1986). Production, Turnover, and Nutrient Dynamics of Above- and Belowground Detritus of World Forests. *Advances in Ecological Research* 15: 303-377. https://doi.org/10.1016/S0065-2504(08)60122-1

Waldrop MP, and Firestone MK. (2004). Altered utilization patterns of young and old soil C by microorganisms caused by temperature shifts and N additions. *Biogeochemistry* 67: 235–248. doi: 10.1023/B:BIOG.0000015321.51462.41

Walker TW, Syers JK (1976) Fate of phosphorus during pedogenesis. *Geoderma* 15: 1-19.

Wardle DA, Bardgett RD, Klironomos JN, Setälä H, Van Der Putten WH, Wall DH. (2004). Ecological linkages between aboveground and belowground biota. *Science* 304(5677): 1629-1633

Weiner T, Mazeh S, Tamburini F, Frossard E, Bernasconi SM, Chiti T, Angert A. (2011). A method for analyzing the $\delta^{18}O$ of resin-extractable soil inorganic phosphate. *Rapid Communications in Mass Spectrometry* 25: 624–628.

White, PJ. (2012). Long-distance Transport in the Xylem and Phloem (Chapter 3). In: *Marschner's Mineral Nutrition of Higher Plants* 3: 49–70.

White, PJ. Ding, G. (2023) Long-distance transport in the xylem and phloem (Chapter 3) This chapter is a revision of the third edition chapter by P.J. White, pp. 49–70. *Marschner's Mineral Nutrition of Plants (Fourth Edition)* 73-104: DOI: https://doi.org/10.1016/B978-0-12-384905-2.00003-0.

Wood T, Bormann FH, Voigt GK. (1984). Phosphorus cycling in a northern hardwood forest: Biological and chemical control. *Science* 223: 391-393

Zuecco G, Amin A, Frentress J, Engel M, Marchina C,Anfodillo T, Borga M, CarraroV, Scandellari F, Tagliavini M, Zanotelli D, Comiti F, Penna D. (2022) A comparative study of plant water extraction methods for isotopic analyses: Scholander-type pressure chamber vs. cryogenic vacuum distillation. *Hydrol. Earth Syst. Sci* 26: 3673–3689. https://doi.org/10.5194/hess-26-3673-2022

Appendix

A1 Organic layers favor phosphorus storage and uptake by young beech trees (*Fagus sylvatica* L.) at nutrient poor ecosystems

Abstract

Aims: The accumulation of organic layers in forests is linked to decreasing nutrient availability. Organic layers might represent a source of phosphorus (P) nutrition of trees in forests. Our aims were i) to test if the fate of P in a tree sapling-soil system differs between nutrient-poor and nutrient-rich sites, and ii) to assess the influence of organic layers on the fate of P in a tree sapling-soil system at either site.

Methods: We conducted a ^{33}P labeling experiment of mesocosms of beech (*Fagus sylvatica*) saplings.

Results: Recovery of ^{33}P in the organic layer was greater under nutrient-poor than under nutrient-rich conditions likely caused by the abundance of microorganisms and roots. Under nutrient-poor conditions, we found that the mobilization of P followed by efficient uptake promoted tree sapling growth if the organic layer was present. The presence of organic layers did not significantly influence P uptake by beech saplings under nutrient-rich conditions suggesting mechanisms of P mobilization in addition to organic matter mineralization.

Conclusions: Our results highlight the importance of organic layers for P nutrition of young beech trees growing on nutrient poor soils in temperate forest ecosystems. The role of organic layers should be considered for sustainable forest management.

1 Introduction

Forest growth in temperate regions is generally limited by the availability of nitrogen (N) in soils (Aber 1992). However, recent studies revealed a decrease in foliar phosphorus (P) concentrations in Central Europe for the last 20 years, indicating a shift towards P-(co)limitation (Duquesnay et al. 2000; Ilg et al. 2009; Jonard et al. 2015). For example, a significant decline of 13% in foliar P concentrations was observed across Europe, most likely due to ongoing atmospheric N deposition and increased CO_2 concentrations. Consequently, forest productivity is increased, eventually resulting in a switch from N- to P-limitation (Jonard et al. 2015; Talkner et al. 2015). Therefore, mechanistic knowledge regarding the supply strategies of trees with P under different environmental conditions is required.

The geologic parent material is important for the potential P supply to organisms in the first place, but with progressing pedogenesis biogeochemical weathering and erosion cause pronounced changes in the pools of bioavailable P (Walker and Syers 1976; Wardle et al. 2004). Apatite group minerals represent the primary source of P, with concentrations spanning from 120 mg kg^{-1} (ultramafic rocks) to >3,000 mg kg^{-1} (alkali rocks) (Augusto et al. 2017). With progressing pedogenesis, P is relased from primary minerals and is either lost due to leaching, or is fixed in the soil mainly through adsorption and incorporation into Al- and Fe-(hydr)oxides. Augusto et al. (2017) consider the parent material as the principal component controlling P availability in soils, beside the progression of pedogenesis, or climatic controls.

This view is supported by Porder and Ramachandran (2012), who showed that parent material serves as principal control of bioavailable P in soil.

In temperate forest ecosystems, organic layers might contribute to P nutrition of trees in addition to mineral P sources. This is because organic layers play a twofold role. On the one hand, organic matter (OM) and therefore nutrients including P gradually accumulate on the soil surface due to increasingly limited microbial decomposition with ongoing acidification. Additional effects contribute to the accumulation of OM on the forest floor: In dense matured forest ecosystems canopy closure intercept large proportions of rainfall, shading decreases surface temperatures and both effects subsequently lower microbial activities (Vogt et al., 1986). Furthermore, the bioavailability of nutrients like P, K, and Fe is reduced due to incorporation in organic molecules and thereby removed from active circulation, this in turn leads to a decreased forest productivity (Berg and McClaugherty 2003; Fisher and Binkley 2000; Kimmins 1997). On the other hand, OM and humic metabolites serve as physicochemical adsorbents for P and other resources including water, reducing leaching through the soil column (Leuschner 1998). Soil OM in general is considered to have positive effects on forest productivity, especially in coarse textured soil (Grigal and Vance 2000). Furthermore, the accumulation of OM in form of organic layers provides a new habitat for microbes, thus maintaining microbial activity and nutrient cycling in contrast to less favorable conditions in mineral topsoil layers (Spohn et al. 2013). Because trees preferentially acquire P from organic layers of the forest floor the cycle of P in the ecosystem is being tightened. As a positive subsequent effect, leaching of P to deeper mineral soil horizons that are characterized by a higher P sorption capacity and larger retention times is minimized (Polglase et al. 1992). This is often reflected by a thick and dense rooting system in the forest floor (Brandtberg et al. 2004; Jackson et al. 1996) enabling an effective coupling of nutrient mobilization (for instance

mediated by specific extra-cellular enzymes released by plants and microorganisms) and subsequent uptake (Wood et al. 1984). We expect a prominent role of organic layers in temperate forests where P availability is low, forcing trees to mobilize P from organic layers (Lang et al. 2016).

However, the fate of nutrients commonly studied using stable isotopes is difficult to follow for P in ecosystems or even mesocosm studies since there is only one stable isotope of P (^{31}P). In contrast, two radioactive isotopes ^{32}P (half-life of 14.3 days) and ^{33}P (half-life of 25.34 days) (Audi et al. 2003) can be utilized to follow the fate of P in the short-term (Di et al. 1997). Since the application of radioactivity under field conditions is constrained by safety issues, few authors extended their study beyond laboratory experiments. These few publications report on root uptake of forest ecosystems *in situ* (Brandtberg et al. 2004; McLaren et al. 2016). In a pot experiment, Jonard et al. (2009) found evidence that 99% of P taken up by pine trees in a forest plantation were mobilized from the forest floor. Furthermore, they calculated a P supply originating from organic layers of 93% and 95% for spruce and birch trees from results of Brandtberg et al. (2004). However, these calculations relied on assumptions regarding the fate of ^{33}P in soil that yet remain to be tested. The authors assumed constant radioactivity (specific activity) in soil across time and thus, did not account for the successive temporal shift of radioactivity from soil to tree seedlings by P uptake. This can be resolved by setting up a budget of ^{33}P in the tree-soil system as a whole over time.

We studied the fate of P under nutrient-poor vs. nutrient-rich conditions associated by differences in characteristics of the organic layer. In this context, Lang et al. (2016) describe a gradual shift from a ‘recycling’ temperate forest ecosystem on nutrient-poor soils towards an ‘acquiring’ forest ecosystem on nutrient-rich soils. Under nutrient-poor conditions, nutrients originate from OM mineralization with a tight P cycling whereas under nutrient-rich

conditions, nutrients and P in particular are mobilized by mineral dissolution associated with a gradual decline in bioavailable P stocks. According to this conceptual model, a temperate forest ecosystem maintains its ecosystem biomass and even a lower, but in relation to the nutrient availability high productivity, if growing under nutrient-poor conditions. Lang et al. (2017) follow a geosequence with a strong gradient in P stocks and bioavailable P pools, in relation to the aboveground woody biomass.

Our overarching aims were i) to test if the fate of P in a tree sapling-soil system differs between nutrient-poor and nutrient-rich sites, and ii) to assess the influence of organic layers on the fate of P in a tree sapling-soil system with different P availability. As our investigated sites represent the initial and end members of the gradient described by Lang et al. (2017), we hypothesize that i) the majority of P is concentrated in organic compartments (organic layer and aboveground biomass) at the nutrient-poor site whereas more evenly distributed at the nutrient-rich site, and accordingly ii) the presence of an organic layer is crucial to maintain tree sapling performance at the nutrient-poor site which is not the case for the nutrient-rich site.

2 Methods

2.1 Study sites

In the frame of the priority program SPP 1685 'Ecosystem Nutrition, Forest Strategies for limited Phosphorus Resources' (Lang et al. 2016), two temperate forest sites were selected for the purpose of this study. The site "Lüss" is located in Lower Saxony, northwestern Germany in the Lüneburg Heath (52°83' N, 10°36' E), whereas the site "Bad Brückenau" is located in Bavaria, southeastern Germany in a midrange mountain area of the Rhön (50°35' N, 9°92' E). In Lüss, organic layers and soil types are classified as a mor-like Moder forest floor

on hyperdystric folic Cambisols developed from Pleistocene sands. At the Bad Brückenau site, mull-like Moder forest floors on a dystric skeletic Cambisols developed on alkaline igneous rocks/metamorphites (Haußmann and Lux 1997). Further site characteristics are provided in Table A1-1.

Table A2-1 **Field site characteristics of Lüss (LUE) and Bad Brückenau (BBR).**
Chemical parameters are given for the A horizon (BBR: 0- -14 cm; LUE: A (0- -12 cm) according to our pot distinction (LUE=Ah; BBR=AE+E). MAT = mean annual temperature, MAP = mean annual precipitation, Pi = inorganic P, TP = total P; bioavailable Pi = PO_4-P citr., C = C-total (C-tot ≙ C_{org}) (Data from: Lang et al. 2017)

Study site	**BBR**	**LUE**
Site characteristics		
Altitude [m a.s.l.]	**809**	**115**
MAT [°C]	**5.8**	**8.0**
MAP [mm a^{-1}]	**1031**	**779**
Forest floor mass [kg m^{-2}]	**1.83**	**12.8**
TP forest floor [g m^{-2}]	**2.7**	**9.2**
Turnover rate of the forest floor [$years^{-1}$]	**5**	**39**
pH (H_2O)	**4.0**	**3.7**
C [mg g^{-1}]	**136**	**46**
N [mg g^{-1}]	**7.5**	**1.8**
TP [mg kg^{-1}]	**3199**	**146**
Bioavailable Pi [mg kg^{-1}]	**43**	**53**
C:N	**18**	**25**

2.2 Greenhouse experiment

In order to perform a ^{33}P-uptake experiment under controlled conditions, we established a greenhouse mesocosm experiment. Soil was collected from a flat surface position at each of the two forest sites in February to March 2014. Each horizon of the forest floor and mineral soil to a depth of 25 cm below ground was collected separately (horizon-specific characteristics summarized in Table S1. The order of soil horizons (IUSS 2015) was as follows, for Lüss: Oi (3 cm) - Oe (4 cm) – Oa (1 cm) – AE (6 cm) - E(5 cm) – Bsh (9 cm+), and for BBR: Oi (3 cm) – Oe (3 cm) -Ah (11 cm) – BA (11 cm+). Each horizon was homogenized carefully before establishing the mesocosm experiment. The thickness of each layer in mesocosms resembled the average thickness in the field. We established two different mesocosm treatments: one including organic layers and one where the organic layer was absent. Mesocosms without organic layers were covered by 0.5 cm thick synthetic fleece to mimic the presence of an organic layer and the associated reduction of evaporation.

Two-year old *Fagus sylvatica* L. saplings were purchased from a nursery in the Black Forest and planted into the pots. We allowed the plants to settle for 12 months before starting the ^{33}P-uptake experiment. During this period, mesocosms were watered twice a week with tap water. The ^{33}P-uptake experiment was conducted in July 2015. Two weeks before the start, tap water was added to the mesocosms to achieve 60% water holding capacity and maintained throughout the experimental course by daily watering. If water drained out of mesocosms, it was collected with a pipette and reapplied to the pot surface. Soil water tension was monitored by two tensiometers (Blumat digital) in each treatment, resulting in eight

tensiometers (2 sites x 2 treatments x 2 replicates). The mesocosms were then exposed to field conditions, but sheltered by a transparent Plexiglas enclosure to maintain soil moisture conditions. Every 48 hours after the start of the ^{33}P-uptake experiment, mesocosms were relocated randomly to minimize location effects below the enclosure. At the start of the ^{33}P-uptake experiment, we added 23 MBq ^{33}P-labeled orthophosphate 'carrier free' (>99.5% ^{33}P) as aliquots in 100 ml tap water per mesocosm, followed by additional 100 ml tap water to rinse the flask as well as force a rapid and homogenous infiltration. The amount of added P in this 100 ml labeling solution corresponds to 6.55 x 10^{-6} mmol P. We sampled mesocosms destructively at five time steps (0, 24, 48, 96, 192 hours). Therefore, in total we established 60 mesocosms (2 sites x 2 treatments x 5 time steps x 3 replicates). The short half-life time of ^{33}P of 25.34 days (Audi et al. 2003) constrained the experiment duration to 8 days (192 hours).

2.3 Xylem sap extraction and destructive harvest

During the experiment, we determined the uptake of ^{33}P at different time intervals by extracting the xylem sap from the whole three-year-old beech trees by means of Scholander pressure bomb extraction (Soilmoisture 3000 Series Plant Water Status Console). First, trees saplings were harvested by cutting 1 cm above soil surface. Directly after harvesting, we removed 2 cm of bark and cambium to prevent phloem extraction and tightly pulled a PE hose (LDPE transparent hose, 200 mm long with 8 mm inner diameter) over the stem. The tree sapling was placed in the pressure chamber under approximately 15 bar as long as xylem sap was extractable (max. 5 minutes). The pressure was applied by compressed air. The amounts of extracted xylem sap (0.05-0.2 ml) were sufficient for ^{33}P measurement, but insufficient for further chemical analyses (determination of P concentrations and speciation).

The destructive harvest comprised collection of tree sapling and soil material. Tree sapling compartments (stems and twigs after xylem sap extraction combined with leaves) were dried at 60°C for 72 hours for determination of the biomass dry weight. Organic layer horizons were pooled (O) and the mineral soil was separated into A (Ah) and B (below Ah) horizons. Moist soil was sieved to pass a 2 mm mesh. Total dry soil mass per pot, as well each horizons dry weight was determined.

2.4 Chemical analyses

Phosphorus fractions in mineral soil were determined by a sequential P fractionation scheme after Hedley et al. (1982) modified by Kuo (1996). 'Labile P' was determined by the extraction of 0.5 g dry weight equivalent of fresh soil with 0.5 M $NaHCO_3$ (Roth, adjusted to pH 8.5) by shaking, centrifugation (ROTANTA 460 rs, Hettich Lab Technologies, Tuttlingen, Germany), decantation, and filtration through P-free filters (MN 619 G ¼, Macherey-Nagel GmbH & Co. KG, Düren, Germany). 'Moderately labile P' was extracted from the soil residuum by shaking with 0.1 M NaOH (Sigma-Aldrich) followed by the same procedure as outlined for the 'labile-P' fraction. In a third extraction step, the 'stable-P' fraction was determined by addition of 5 ml 37% HCl (Sigma-Aldrich): samples were first heated in a water bath to 80°C, thereafter, shaken by hand followed by another addition of 5 ml 37% HCl. Samples were allowed to cool down before filtration. In the following, P fractions are termed according to the extraction solutions: $NaHCO_3$-P, NaOH-P, and HCl-P.

Plant (stem, twigs, leaves) and soil samples were digested for analyses of total P concentrations (TP): dried material was homogenized with a planetary ball mill, mixed with 65% HNO_3 and digested at 180°C (Loftfields Analytische Lösungen 6 AM; Heinrichs et al. (1986)).

Concentration of inorganic P (Pi) in extraction and digestion solutions was determined spectrophotometrically with a continuous flow analyzer (CFA, AA3, XY2, Seal-Analytic, Norderstedt, Germany) at λ = 660 nm, using the method of Murphy and Riley (1962). In soil extraction solutions, total dissolved P concentrations were measured by means of Inductively Coupled Plasma/Optical Emission Spectrometry (ICP-OES, PerkinElmer Optima 5300 DVGermany) at λ P 213.617. Organic P concentrations (Po) were calculated as the difference between total dissolved and Pi concentrations (Gurpal et al. 2006). Radioactivity of ^{33}P was measured in all soil extracts ($NaHCO_3$-P, NaOH-P, HCl-P, HNO_3), as well as in HNO_3-digestion solutions of organic layer and plant material after addition of Ultima Gold scintillation cocktail (Perkin Elmer, USA) by means of Liquid Scintillation Counting (TriCarb 3110 TR, Perkin Elmer, USA).

2.5 Calculations and statistical analyses

Aboveground P stocks were calculated as the sum of compartment-specific (stem, twigs, leaves) products of total aboveground tree sapling biomass and P concentrations. The activity of ^{33}P of each sample was first corrected for its radioactive decay (Audi et al. 2003). The specific ^{33}P activity (SA) was calculated as follows (Equ. 1; Di et al. 1997)

$$\mathrm{SA}\ [\mathrm{MBq}\ g^{-1}\ \mathrm{P}] = \frac{\text{Radioactivity of } {}^{33}P\ [\mathrm{MBq}\ g^{-1}\ \mathrm{DM}]}{{}^{31}P\ concentration\ [g\ P\ g^{-1}\ \mathrm{DM}]} \qquad \text{(Equation 1)}$$

Concentrations of ^{31}P correspond to Pi concentrations in soil P fractions and total dissolved P concentrations of P fractions in soil and plant compartments.

The ^{33}P recovery was calculated as the ratio between radioactivity initially applied to the sum of all radioactivity dispersed to soil and plant (compartments) at the end of the experiment (Equ. 2; Di et al. 1997)

$$^{33}P\ recovery\ [\%] = \frac{\text{Radioactivity applied [MBq]}}{\sum Radioactivity\ soil\ [MBq] + \sum Radioactivity\ plant [\text{MBq}]} \times 100 \qquad \text{(Equation 2)}$$

where *radioactivity soil* comprises all P fractions ($NaHCO_3$-extractable P, NaOH-extractable P, HCl-extractable P) including organic layers [if present], and *radioactivity plant* refers to radioactivity in aboveground tree sapling biomass (stem, twigs, leaves).

We calculated the difference between treatments in SA of A horizons that is between the presence ($O_{present}$) and absence (O_{absent}) of organic layers (Equ. 3):

$$\Delta\ ^{33}P\ [\text{MBq}\ g^{-1}\text{P}] = SA_{Opresent}\ [\text{MBq}\ g^{-1}\text{P}] - SA_{Oabsent}\ [\text{MBq}\ g^{-1}\text{P}] \qquad \text{(Equation 3)}$$

These differences were calculated for each P fraction in soil and for plant compartments.

Differences between sites were tested using a student's t test if no treatment was involved. For single time steps and the complete design, we used a repeated measures ANOVA with site as between-subject factor and treatment (presence/absence of organic layers) as within-subject factor. Furthermore, we tested whether calculated differences between treatments (Δ) significantly deviated from zero based on a t test against zero. For time series, a repeated measures ANOVA was conducted with site as between-subject factor and time step as within-subject factor. The data set did not allow testing prerequisites for parametric tests. Therefore, we repeated all statistical analyses based on non-parametric tests (Mann-Whitney-U for site or treatment effects; Bredenkamp for interactions between site and treatment). Because a sample size of three results in a minimum level of significance of 0.1 in a Mann-Whitney-U test, we had to adjust the level of significance accordingly. Except for the difference in foliar P concentrations and the presence/absence effect of the organic layer on the NaOH fraction in Bad Brückenau, all significant results remained irrespective of whether we used parametric or non-parametric tests. As opposed to parametric tests it is not possible to account for interactions and repeated measures simultaneously in one non-parametric test.

Consequently, an extensive number of non-parametric tests results increasing the probability of false positive results and therefore, we report on the outcome of parametric tests. Statistical analysis was carried out by IBM SPSS statistics 22.

3 Results

3.1 Site effect on the partitioning of P in mesocosms

Phosphorus fractions in soil of the two sites differed significantly (Fig. A1-1a, Table A1-2). The sum of P concentrations in all P fractions of the mineral soil horizons (A and B) were significantly lower in Lüss than in Bad Brückenau ($p \leq 0.001$; Fig. A1-1a). Remarkably, bioavailable P concentrations ($NaHCO_3$-P) in the mineral soil horizons of Bad Brückenau were about 8.4 times greater than in mineral soil horizons of Lüss. In contrast, P concentrations of organic layers were similar ($p = 0.51$) for both sites, 0.64 in Lüss and 0.70 g P kg^{-1} in Bad Brückenau. The aboveground biomass of beech tree saplings one year after start of the mesocosm experiment (before the application of the ^{33}P tracer) did not differ significantly between the two sites (Fig. A1-1b). However, there was a significant site effect regarding foliar P concentrations with significantly lower concentrations for the tree saplings grown in soil of Lüss as compared to Bad Brückenau (Fig. A1-1c). As a result, aboveground P stocks (sum of P stocks in stem, twigs, and leaves) were significantly different between sites (between-subject effect $p \leq 0.01$;, 61.9 ± 8.9 mg P per pot (SE) in Lüss and 188 ± 19 mg P (SE) per pot in Bad Brückenau at the last time point of harvest).

Table A3-2 **Phosphorus fractions in soil [mg P kgDM^{-1}] before ^{33}P application.**
Means are given in bold letters followed by the standard error. Pi = inorganic P, Po = organic P. LUE = Lüss; BBR = Bad Brückenau. Statistically significant ($p \leq 0.05$) differences between sites are written in bold letters (Pi+Po). Horizon depths: BBR: A (0- -11), B (-11 - -22); LUE: A (0- -11), B (-11 - -20)

Study site		BBR		LUE		Site effect	
Horizon		A	B	A	B	A	B
$NaHCO_3$	Pi	49 ± 7	17 ± 0.3	4 ± 2	2 ± 0.2	F = 214	F = 773
	Po	160 ± 7	54 ± 1	11 ± 4	8 ± 1.5	**p ≤ 0.001**	**p ≤ 0.001**
NaOH	Pi	278 ± 4	359 ± 35	19 ± 3	18 ± 2.5	F = 716	F = 1770
	Po	771 ± 18	918 ± 62	34 ± 6	28 ± 3.3	**p ≤ 0.001**	**p ≤ 0.001**
HCl	Pi	413 ± 11	250 ± 34	39 ± 11	27 ± 1.0	F = 236	F = 101
	Po	121 ± 4	27 ± 11	5 ± 1	2.6 ± 0.7	**p ≤ 0.001**	**p ≤ 0.001**

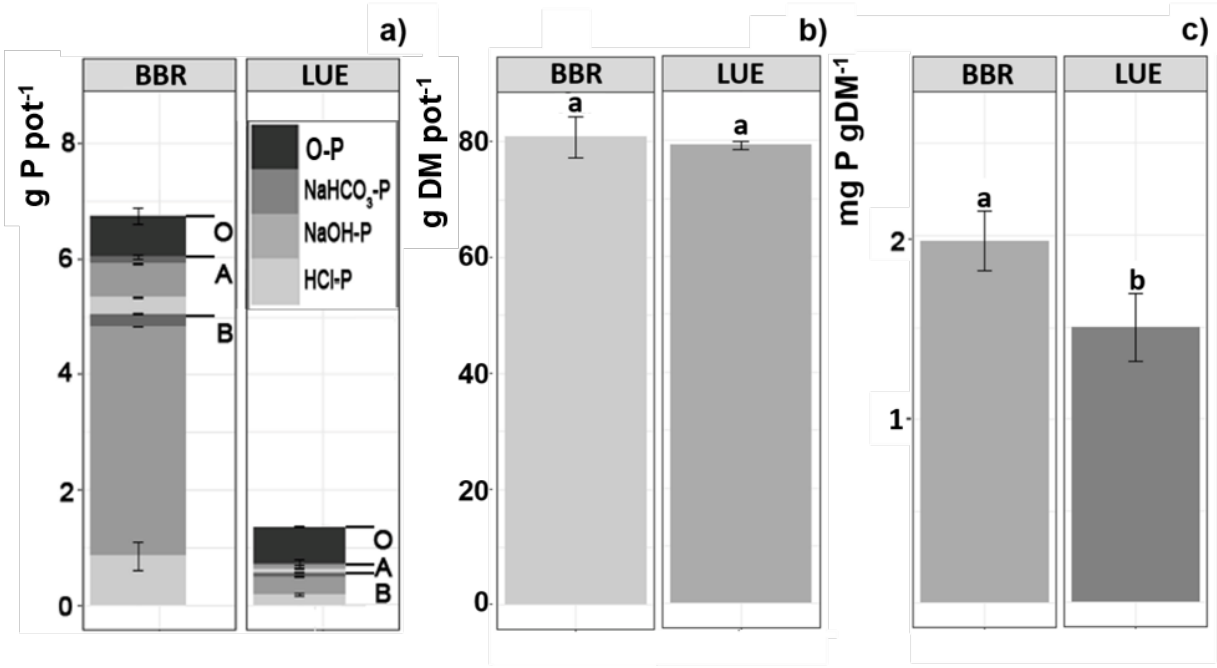


Figure A1-1
Phosphorus fractions in soil (g P per pot) (a), aboveground biomass (stem+twigs+leaves; g DM per pot) (b) and foliar P concentrations [mg g^{-1} dry matter (DM)] of 3 year old beech saplings at time step 0 (c). Mineral soil horizons A and B: Sequentially extracted plant-available P fractions (sum of inorganic and organic) according to Hedley et al. (1982). Organic layers (O-P): P determined by HNO_3 digestion. Different letters above bars indicate significant differences – to ease readability, these are not displayed in Subfigure 1a, but instead provided in the text. Error bars refer to the standard error of three replicates. BBR = Bad Brückenau; LUE = Lüss.

3.2 Site effect on the fate of applied 33P

At the end of the ^{33}P uptake experiment (after 192 h), specific ^{33}P activities in all soil horizons were three to seven times higher in Lüss than in Bad Brückenau ($0.04 > p > 0.0001$; Fig. A1-2a). The most pronounced difference was the 7-fold specific ^{33}P activity of the NaOH fraction in the B horizon of Lüss as compared to Bad Brückenau. Furthermore, the ^{33}P specific activity in organic layers of Lüss was 4.6 times higher than in Bad Brückenau. Similarly, Lüss was characterized by significantly higher specific ^{33}P activities in plant tissue (stem, twigs, leaves) than Bad Brückenau ($0.027 > p > 0.0001$; Fig. A1-2b). In detail, specific ^{33}P activities of stem, twigs, and leaves were nine, nine, and five times higher in Lüss than in Bad Brückenau.

The recovery of ^{33}P applied was similar between Bad Brückenau and Lüss for organic layers, the $NaHCO_3$-P, and the HCl-P fraction, but differed for the NaOH-P fraction (Fig. A1-3). In contrast, more of the radioactivity applied was retained by the biomass fraction in Lüss than in Bad Brückenau ($p = 0.008$; Fig. A1-3).

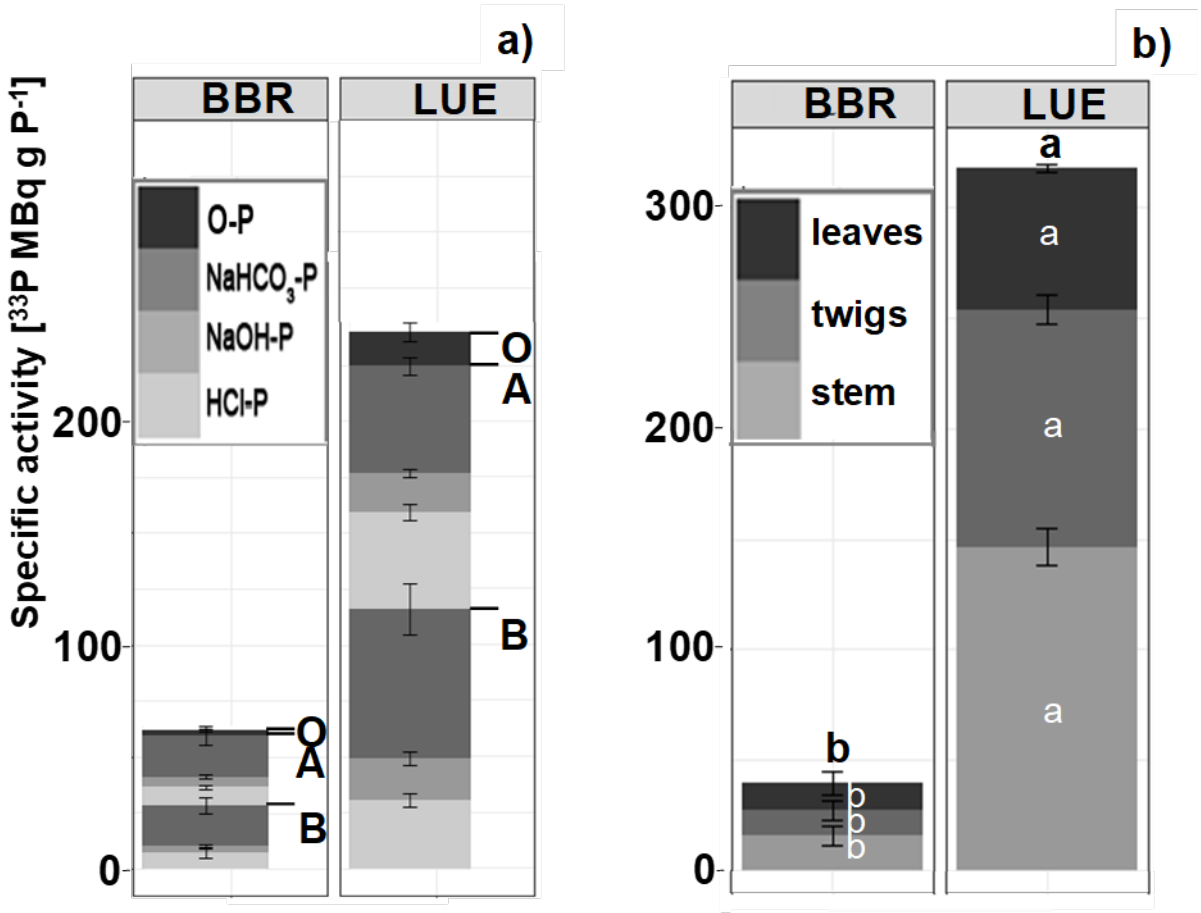


Figure A1-2 **Specific activity [^{33}P MBq g P^{-1}] in P fractions (Pi+Po) according to Hedley et al. (1982)** (a) and in aboveground biomass (b) 192h after application. Error bars refer to the standard error of three replicates. Different letters above bars indicate significant differences – to ease readability, these are not displayed in Subfigure 2a, but instead provided in the text. BBR = Bad Brückenau; LUE = Lüss; Organic layers (O-P): P determined by HNO_3 digestion.

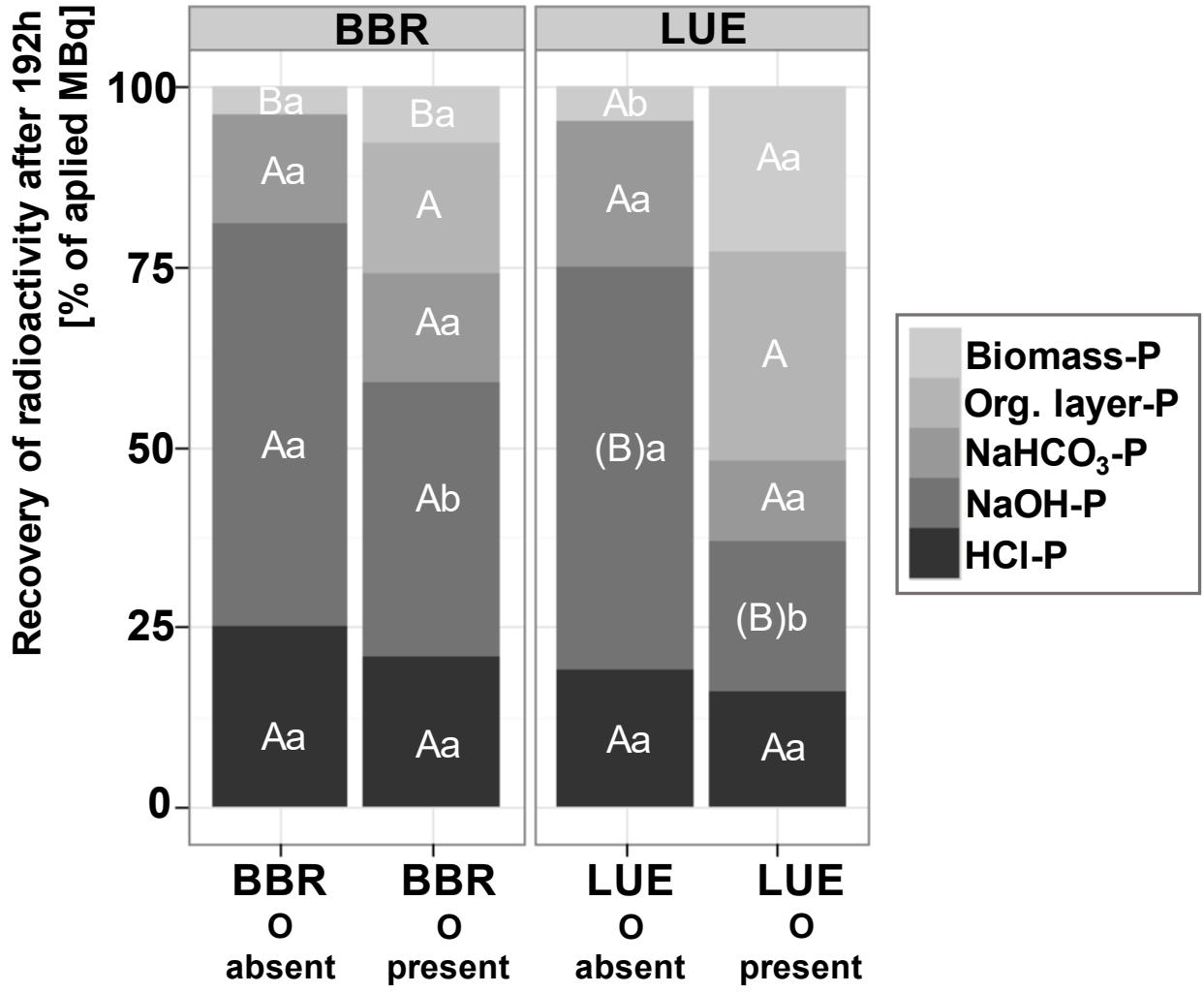


Figure A1-3 **Distribution of ^{33}P in tree saplings and soil 192 h after application.**
Different uppercase letters indicate significant differences between sites (extended by parenthesis = $p < 0.1$), whereas different lowercase letters refer to significant differences between treatments.Recovery rates according to the total applied ^{33}P: BBR: 90%; BBRO: 100%; LUE: 139%; LUEO: 81%. O = Organic layers; BBR = Bad Brückenau; LUE = Lüss; Organic layers & biomass P = P determined by HNO_3 digestion.

3.3 Effect of the presence/absence of organic layers on the fate of ^{33}P

Considering the specific ^{33}P activity of the A horizon in P fractions, the difference (Δ) between the presence and absence of organic layers after the 1-yr settlement phase and the ^{33}P uptake experiment period was site-dependent (between-subject effect: $p = 0.009$; Fig. A1-4). Differences in the specific ^{33}P activity of the A horizon differed significantly from zero throughout the experiment for all P fractions in soil in Lüss with only three exceptions (Fig. A1-

4). Contrarily, for Bad Brückenau, differences in the specific ^{33}P activity of the P fractions deviated significantly from zero only occasionally (NaOH fraction: 0 h, 48 h, and 96 h; $NaHCO_3$ and HCl fraction: 48 h; Fig. A1-4). These patterns did not change during the experiment (within-subject contrasts > 0.05 for all horizons). However, significant differences between the presence and absence of organic layers after 192 h were hardly identifiable due to a high variability in specific ^{33}P activities.

The absence of organic layers significantly limited the growth of the beech tree saplings at the site Lüss ($p \leq 0.001$) during the 1 year settlement phase and the subsequent ^{33}P uptake experiment (Lüss: 79 g dm^{-1} – 56 g dm^{-1} ≙ -30%; Bad Brückenau: 81 g dm^{-1} – 72 g dm^{-1} ≙ -11% (incl. the org. layer/without the org. layer; n=3; time point 0). At the end of the experiment, the reduction of aboveground biomass as related to the treatment (presence/absence of organic layers) reached -18% ± 4% (Lüss) -7% ± 16% (Bad Brückenau).

In the presence of organic layers, radioactivity in the xylem sap of the beech tree saplings cultivated in Lüss soil increased on average by a factor of 11.9 between 48 and 192 hours after the ^{33}P-tracer application (Bad Brückenau: 3.5-fold increase, only; Fig A1-5.). The difference between the presence and the absence of organic layers in specific ^{33}P activity in plant tissue was site specific (between-subject effect $p < 0.006$; Fig. A1-6) and deviated significantly from zero in Lüss at four out of five time steps (Fig. A1-6). In addition, ^{33}P accumulation in plant tissue over time followed a linear trend (linear within-subject contrast $0.027 > p > 0.001$; significant interaction of within- and between-subject effects $p < 0.008$). In contrast, the presence/absence of organic layers had no effect on the specific ^{33}P activity, resulting in a Δ specific ^{33}P activity close to zero for Bad Brückenau (Fig. A1-6).

If mesocosms were considered as a whole (soil + tree sapling), we found a significantly lower recovery of the applied radioactivity in the NaOH fraction in Lüss and in Bad Brückenau

if the organic layer was present than in pots without the organic layer (within subject effect p = 0.008; Fig. A1-3). At the same time, more radioactivity was recovered in aboveground biomass in the presence of organic layers than without organic layers in Lüss (within subject effect p = 0.004; Fig. A1-3) which was not the case for Bad Brückenau (significant interaction of within- and between-subject effects p = 0.05).

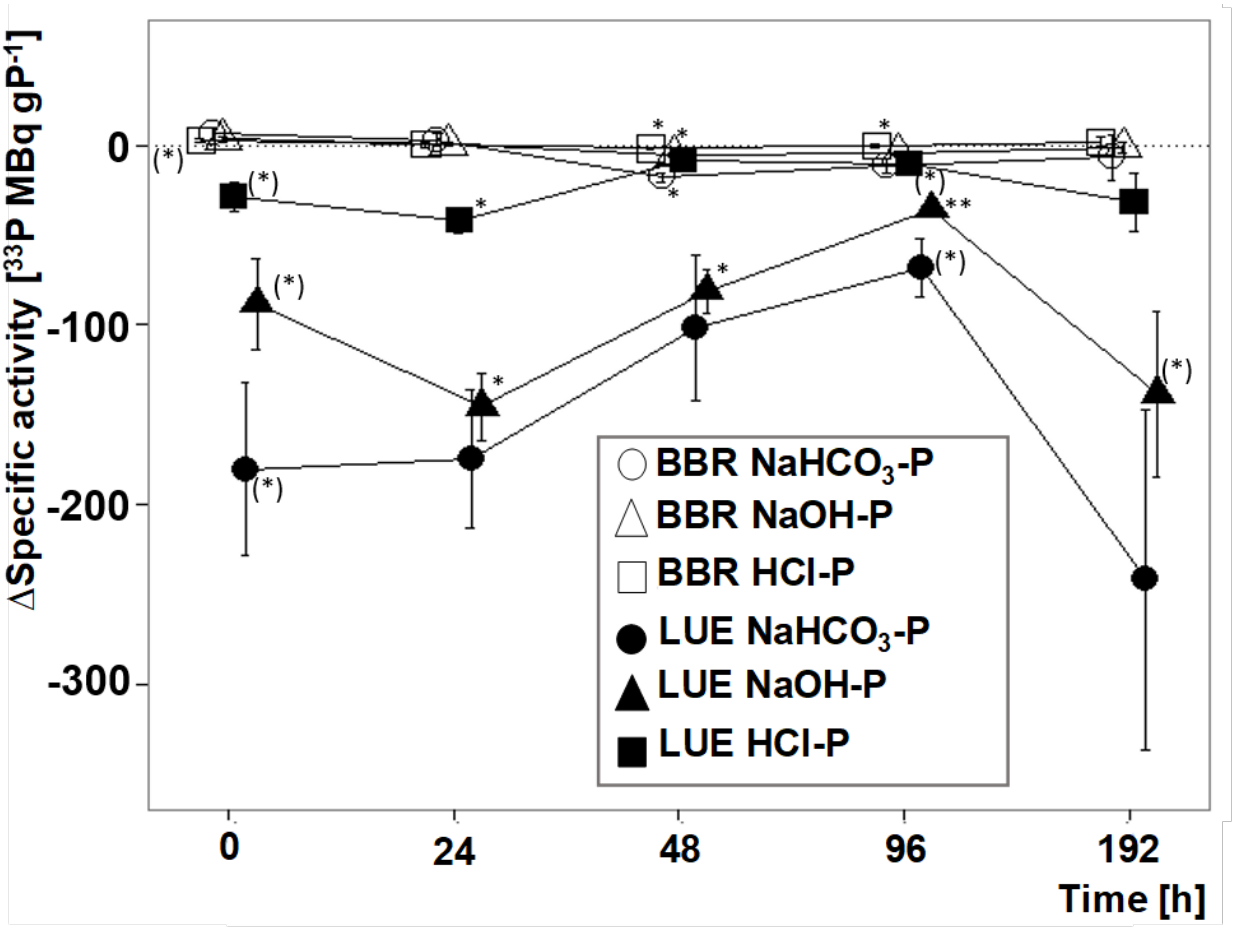


Figure A1-4 **Differences in specific activity [Δ ^{33}P MBq g^{-1} P] in P fractions in the A horizon between the treatments (presence – absence of organic layers).**
Error bars refer to the standard error of three replicates. Asterisks next to symbols (right: LUE, left: BBR) represent differences significantly deviating from zero with * p < 0.05, **, p < 0.01, and (*) p < 0.1. BBR = Bad Brückenau; LUE = Lüss.

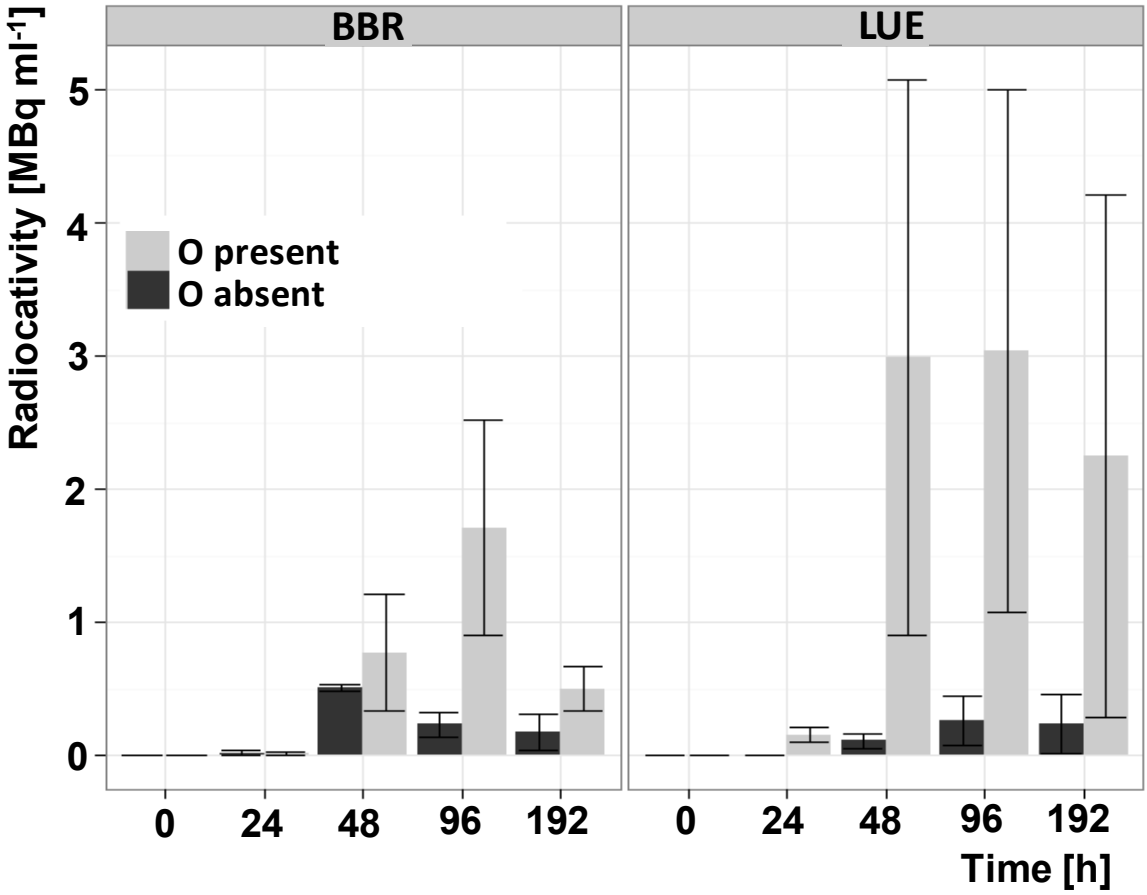


Figure A1-5 **Radioactivity in xylem sap after Scholander pressure bomb extraction at the different time steps of harvest.**

Error bars refer to the standard error of three replicates. BBR= Bad Brückenau; LUE= Lüss.

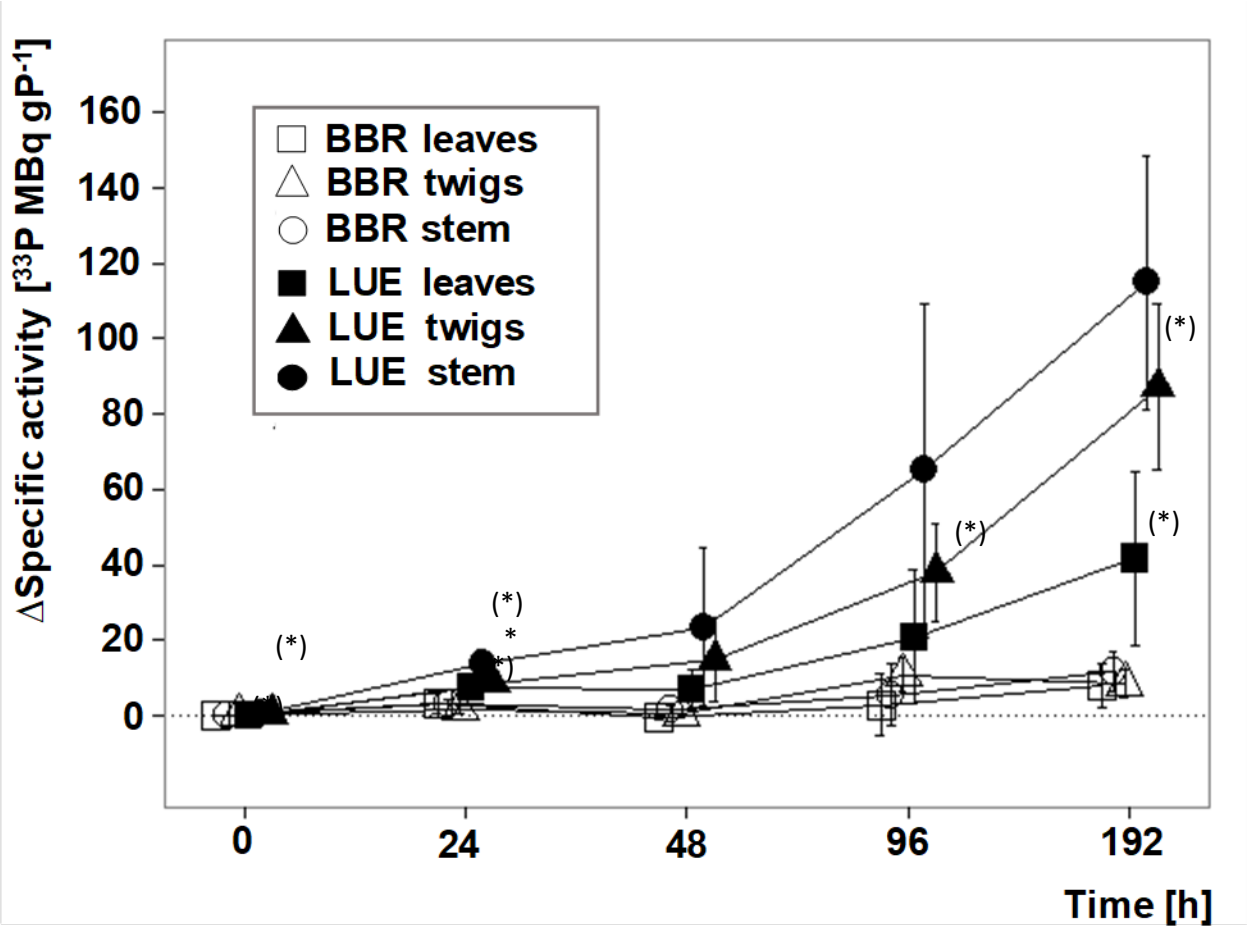


Figure A1-6 **Differences in specific activity [Δ ^{33}P MBq g^{-1} P] in plant tissue between the treatments (presence – absence of organic layers).**
Error bars refer to the standard error of three replicates. Asterisks next to symbols (right: LUE only) represent differences significantly deviating from zero with * $p < 0.05$ and (*) $p < 0.1$. BBR = Bad Brückenau; LUE = Lüss.

4 Discussion

4.1 Site effect on the fate of ^{33}P

Labeling of soil P pools was stronger for Lüss compared to Bad Brückenau as indicated by higher specific ^{33}P activities in all P fractions in soil. In general, the higher specific ^{33}P activities for Lüss resulted from overall lower P concentrations (Fig. A1-1), which served as denominator in the calculation of the specific activities (SA) (Equ. 1). In the organic layer and in the NaOH fraction of Lüss, this difference in the specific ^{33}P activity was especially pronounced (Fig. A1-2a). The exceptionally high labeling of organic layers of Lüss is surprising at a first glance, as organic layers of forest ecosystems are generally considered as very poor sorbents for P (Achat et al. 2009; Jonard et al. 2009; Kang et al. 2009). The increased specific ^{33}P activity in organic layers could be related to the retention/uptake of ^{33}P by microorganisms. Recently, several authors described the importance of microbes for P cycling in temperate forest ecosystems (Achat et al. 2013; Bergkemper et al. 2016; Hofmann et al. 2016). However, these previous studies on Pmic in forest soils excluded organic layers, although it represents a crucial component of microbial biomass (Chen et al. 2003) and thus likely of Pmic of nutrient-poor forest ecosystems. This is corroborated by Saggar et al. (1998) who reported Pmic concentrations of 212 ± 18 mg P kg^{-1} corresponding to 66% of total P in organic layers of a *Pinus radiata* forest on a P-limited Ultisol in New Zealand. Another explanation could be related to plant P uptake by roots within organic layers. Although we did not specifically measure radioactivity and P concentrations in roots, root length density in organic layers of Lüss was 23 times higher than in Bad Brückenau under field conditions (Fig. A1-S1). In combination with a 7-fold higher forest floor mass under field conditions, this results in an approximately 3-fold increased root density in Lüss (m roots g dm^{-1} forest floor). Therefore, sampling and subsequent acid digestion of organic layers material might comprise a

proportion of roots but also microorganisms that took up the ^{33}P tracer. Finally, the physical retention of ^{33}P in the soil solution through water retention caused by different hydraulic conductivities as indicated by different characteristics of organic layers might play a role. Similar forest floor water dynamics have been described by Marin et al. (2000) for tropical organic layers. At the site Lüss, a thick holorganic layer (Mor-like Moder) of 8 cm including an Oa-horizon (1 cm) has accumulated. The forest floor mass at Lüss was 3-fold higher than in Bad Brückenau in our pot experiment, resulting in a pronounced waterholding capacity. Therefore, retention times of infiltrating water as well as of mobilized nutrients seem to be longer at the site Lüss likely increasing the accumulation of the radioactive label in organic layers as compared to Bad Brückenau.

In addition to the organic layer, the site-specific difference between ^{33}P activities was high in the NaOH-P fraction of the mineral soil horizons (Fig. A1-2a). Phosphorus concentrations in the NaOH-P fraction of the A and B horizons of Lüss were 20 and 27 times lower, respectively, than those in the mineral horizons of Bad Brückenau (Table A1-2). Therefore, this fraction was particularly highly labeled as compared to the other P fractions. However, considering the NaOH-P fraction pool sizes, a lower percentage of ^{33}P was recovered in the NaOH-P fraction of Lüss than in Bad Brückenau (Fig. A1-3). The above-mentioned increased recovery of ^{33}P in organic layers serves as an explanation for the reduction of the proportion of the radioactive label that reached the mineral soil of Lüss as compared to Bad Brückenau.

The fate of ^{33}P in soil was linked to the performance of tree saplings (Fig. A1-1, Fig. A1-3). Focusing first on tree sapling performance, the aboveground biomass of beech tree saplings did not differ significantly between the two sites (Fig. A1-1b) although bioavailable P concentrations ($NaHCO_3$-extracable P) in mineral soil layers differed significantly (Fig. A1-1a).

This is surprising because a link between productivity and nutrient availability in soil is commonly assumed. For example, Minotta and Pinzauti (1996) found that the growth of beech seedlings was favored by nutrient-rich soils. On the one hand, the discrepancy might be explained by the fact that the initial nutrition of the tree saplings during seedling establishment and growth in the nursery was near-optimum. Therefore, during the one-year settlement phase and the following tracer application tree saplings in Lüss might have compensated for potential nutrient deficiencies by retranslocation (Salifu and Trimmer 2000) thereby increasing their nutrient use efficiency (the productivity a given species can achieve based on one unit nutrient taken up; Vitousek 1982). This would obscure site effects on productivity. Apparently, tree saplings increased their nutrient use efficiency in Lüss as compared to Bad Brückenau as indicated by significantly lower foliar P concentrations (Fig. A1-1c). In other words, tree saplings grown in Lüss soil produced more foliar biomass per unit of P than it was the case for Bad Brückenau saplings. Such plasticity has been described by numerous NUE studies, recently reviewed by Baligar and Fageria (2015).

We accounted for these site-specific foliar P concentrations in the calculation of specific ^{33}P activities (Equ. 1). Differences in specific activity of ^{33}P in the tree sapling - soil system were even more pronounced for plant tissue than for soil (Fig. A1-2b). Similar to differences in the specific ^{33}P activity in soil, lower foliar P concentrations for the plants grown in Lüss soil as compared to Bad Brückenau can serve as one explanation (Fig. A1-1c). However, the following results corroborate that this was not the only cause. 192 hours after tracer application, the ^{33}P label has accumulated in excess (leaves 5-fold) in plant tissue compared to the specific activity in 'bioavailable' P fractions in soil ($NaHCO_3$-P 3-fold; Fig. A1-2a). Consequently, a greater portion of the ^{33}P label was transferred from soil into plants over the course of the experiment also indicated by an increasing radioactivity in the xylem sap (Fig. A1-3; Fig. A1-5).

Thus, the stronger specific labeling of P fractions in soil resulted in a pronounced transfer of P from soil to plants. This resulted in a higher recovery of radioactivity in aboveground biomass of plants grown in Lüss soil than in Bad Brückenau soil. Taken together, we could verify our first hypothesis namely that the majority of P is recovered in the organic compartments at the nutrient-poor site.

4.2 Effect of the presence/absence of organic layers on the fate of ^{33}P

As outlined in Chapter 4.1, the organic layer is characterized by a considerable P retention capacity and increases water and nutrient retention times. Therefore, the presence of organic layers resulted in increased retention and thus, decreased recovery of ^{33}P in the NaOH fraction in soil at both sites. If we had relied on batch experiments of mineral soil (as a basis for ^{33}P pool dilution calculations done by Jonard et al. 2009), we would have neglected the pathway of the ^{33}P tracer through the plant-soil system. By calculating ^{33}P recovery, we could show that this pathway differs depending on the presence/absence of the organic layer and thus, is crucial to judge on the importance of organic layers for P nutrition. A closer look on the direct comparison of specific ^{33}P activities between the presence and absence of the organic layer revealed that the retention effect of the organic layer was most pronounced in Lüss and affected all P fractions in soil similarly. That is, throughout the experimental course, consistently we observed significantly negative differences in specific ^{33}P activities in the A horizon between treatments in Lüss irrespective of P fractions but rarely so in Bad Brückenau (Fig. A1-4). Therefore, the importance of the presence of an organic layer depends on site characteristics related to P cycling. Increased recovery of the radioactive label in the organic layer resulting in decreased recovery in mineral soil if the organic layer is present is in line with the concept proposed by Lang et al. (2016) of 'recycling' forest ecosystems associated minimal

P leaching losses at nutrient-poor sites and 'acquiring' forest ecosystems potentially linked to P leaching losses at nutrient-rich sites (Lang et al. 2017). However, in the latter case leaching losses might be low as indicated by non-significant differences in ^{33}P recovery of the most mobile fraction ($NaHCO_3$-P) between the treatments caused by the high sorption capacity in mineral soil of the nutrient-rich study site (Lang et al. 2017). For the nutrient-poor site corresponding to a 'recycling' forest ecosystem, our results provide evidence for the crucial role of the organic layer in minimizing P losses.

At the same time, more ^{33}P was recovered in plant biomass if organic layers were present as compared to the treatment without, indicating a pronounced nutrition effect of organic layers for Lüss, which was not the case for Bad Brückenau (Fig. A1-3). Missing effects of the presence of the organic layer on aboveground biomass, P concentrations in plant tissue, and ^{33}P recovery in Bad Brückenau indicate that roots can mobilize P from other layers in soil suggesting that mechanisms of mobilization do not only encompass OM mineralization but also dissolution of mineral-bond P as well as desorption of P from charged surfaces. In contrast, at Lüss, organic layers further acted as an important source for nutrients, as indicated by a dense rooting system (Fig. A1-S1) and for microbial activity as observed at the field site (Hofmann et al. 2016). Accordingly, tree saplings in Lüss benefitted from increased nutrient availability if organic layers were present. As a result and in line with several studies (Carey et al. 1982; Hallsby 1994; Pare and Bernier 1989), trees increased foliar P concentrations and produced more aboveground biomass than trees in pots without the organic layer. This is further in agreement with Jonard et al. (2006), who documented an increase in the foliar N, P, and K concentrations associated with accumulating forest organic layers. These studies infer the role of the organic layer whereas Jonard et al. (2009) relied on ^{33}P and calculated that < 95% of P taken up by *Pinus pinaster* seedlings derived from the

organic layer. Their calculations did not account for treatment-specific differences in the fate of ^{33}P in soil and thus, likely overestimated the contribution of the organic layer to tree nutrition. Nevertheless, their results derived from a site with even lower P stocks than in Lüss corroborate our main finding: the importance of the organic layer for tree nutrition increases with decreasing soil P stocks. Recovery of ^{33}P in aboveground biomass was 5-fold comparing treatments (presence and absence of the organic layer) at Lüss and therefore, our study proves that organic layers are an essential component in guaranteeing tree P nutrition at nutrient-poor sites. Again, our results corroborate the concept of Lang et al. (2016) in terms of tight P cycling in 'recycling' forest ecosystems and are in line with findings of Marin et al. (2000). The organic layer contributes to minimizing P losses (see previous paragraph) as well as increasing aboveground P uptake by tree saplings. Altogether our results confirm our second hypothesis on the crucial role of the organic layer in nutrient-poor forests.

5 Conclusions

Commonly, the accumulation of organic layers is regarded as an indication of low nutrient availability in temperate forests ignoring the role of organic layers itself as a potential source of nutrients such as P. Tree sapling-soil systems under nutrient-poor and nutrient-rich conditions seem to be well adapted to the mobilization of nutrient resources. Many aspects pinpoint at a complex recycling system guaranteeing nutrition mainly by mineralization of OM in the forest floor under nutrient-poor conditions. At the same time, a dense fine-root system in organic layers at nutrient-poor sites fosters an efficient uptake of mineralized P. We could show that the mobilization of P likely mediated by the microbial community followed by efficient uptake in organic layers under nutrient-poor conditions (Lüss) supported tree sapling growth while at the same time reducing the leaching of P to underlying mineral soil horizons.

In contrast, the presence of organic layers did not significantly influence P uptake by beech saplings under nutrient-rich conditions (Bad Brückenau). This suggests that mechanisms of mobilization do not only encompass OM mineralization but also dissolution of mineral-bond P as well as desorption of P from charged surfaces. However, our results do not match with suspected increased leaching losses likely due to the high sorption capacity of the nutrient-rich site Bad Brückenau. In conclusion, the importance of the organic layer for tree nutrition increases with decreasing P availability. Since the distribution of most forest ecosystems in Europe is constrained to nutrient-poor soils, sustainable forest management should consider the formation and functioning of the forest floor.

6 References

Aber JD (1992) Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. Trends in Ecology & Evolution 7:220–224. doi:10.1016/0169-5347(92)90048-G

Achat DL, Bakker MR, Morel C (2009) Process-based assessment of phosphorus availability in a low phosphorus sorbing forest soil using isotopic dilution methods. Soil Science Society of America Journal 73(6): 2131-2142

Achat DL, Bakker MR, Augusto L, Derrien D, Gallegos N, Lashchinskiy N, Zeller B (2013) Phosphorus status of soils from contrasting forested ecosystems in southwestern Siberia: effects of microbiological and physicochemical properties. Biogeosciences 10(2): 733-752

Audi G, Wapstra AH, Thibault C (2003) The Ame2003 atomic mass evaluation. Nuclear Physics 729:337–676. doi:10.1016/j.nuclphysa.2003.11.003

Augusto L, Achat DL, Jonard M, Vidal D, Ringeval B (2017) Soil parent material-A major driver of plant nutrient limitations in terrestrial ecosystems. Global change biology 23:3808–3824. doi:10.1111/gcb.13691

Baligar VC, Fageria NK (2015) Nutrient Use Efficiency in Plants: An Overview. Rakshit A, Singh H B, Sen A (eds) Nutrient Use Efficiency from Basics to Advances. Springer, New Delhi

Berg B, McClaugherty C (2003) Plant litter: decomposition, humus formation, carbon sequestration, Springer Verlag, Heidelberg, Germany

Bergkemper F, Scholer A, Engel M, Lang F, Kruger J, Schloter M, Schulz S (2016) Phosphorus depletion in forest soils shapes bacterial communities towards phosphorus recycling systems. Environmental microbiology 18:1988–2000. doi:10.1111/1462-2920.13188

Brandtberg P-O, Bengtsson J, Lundkvist H (2004) Distributions of the capacity to take up nutrients by Betula spp. and Picea abies in mixed stands. Forest Ecology and Management 198:193–208. doi:10.1016/j.foreco.2004.04.012

Carey ML, Hunter IR, Andrew I (1982) Pinus radiata forest floors: Factors affecting organic matter and nutrient dynamics. New Zealand Journal of Forestry Science:36–48

Chen CR, Condron LM, Davis MR, Sherlock RR (2003) Seasonal changes in soil phosphorus and associated microbial properties under adjacent grassland and forest in New Zealand. For. Ecol. Manage. 177(1-3), 539-557

Di HJ, Condron LM, Frossard E (1997) Isotope techniques to study phosphorus cycling in agricultural and forest soils. A review. Biology and Fertility of Soils 24:1–12. doi:10.1007/BF01420213

Duquesnay A, Dupouey JL (2000) Spatial and temporal variability of foliar mineral concentration in beech (Fagus sylvatica) stands in Northeastern France. Tree Physiology 20 (1), 13–22

Fisher RF, Binkley D, (2000) Ecology and Management of Forest Soils. Wiley, New York.

Grigal DF, Vance ED (2000) Influence of soil organic matter on forest productivity. New Zealand Journal of Forestry Science:169–205

Toor SG, Hunger SJ, Peak DJ, Sims T, Sparks DL (2006) Advances in the Characterization of Phosphorus in Organic Wastes. Environmental and Agronomic Applications. Acta Scientiarum. Agronomy 89:1–72. doi:10.1016/S0065-2113(05)89001-7

Haußmann T, Lux W (1997) Dauerbeobachtungsflaechen zur Umweltkontrolle im Wald: Level II. BMELF. Erste Ergebnisse, Bonn, p. 148

Hallsby G (1994) The influence of different forest organic matter on the growth of one-year old planted Norway spruce seedlings in a greenhouse experiment. New Forests:43–60

Heinrichs H, Brumsack HJ, Loftfield N, König N (1986) Verbessertes Druckaufschlußsystem für biologische und anorganische Materialien. Zeitschrift für Pflanzenernährung und Bodenkunde 1986:350–353

Hedley MJ, Stewart JWB (1982) Method to measure microbial phosphate in soils. Soil Biology and Biochemistry:377–385

Hofmann K, Heuck C, Spohn M (2016) Phosphorus resorption by young beech trees and soil phosphatase activity as dependent on phosphorus availability. Oecologia 181:369–379. doi:10.1007/s00442-016-3581-x

Ilg C, Foeckler F, Deichner O, Henle K (2009) Extreme flood events favor floodplain mollusc diversity. Hydrobiologia 621, 63–73

IUSS Working Group WRB (2015) World Reference Base for Soil Resources 2014, update 2015: International soil classification system for naming soils and creating legends for soil maps. FAO, Rome.

Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED (1996) A global analysis of root distributions for terrestrial biomes. Oecologia 108:389–411. doi:10.1007/BF00333714

Jonard M, Misson L, Ponette Q (2006) Long-term thinning effects on the forest floor and the foliar nutrient status on Norway spruce stands in the Belgian Ardennes. Canadian Journal of Forest Research, 36: 2684–2695

Jonard M, Augusto L, Morel C, Achat DL, Saur E (2009) Forest floor contribution to phosphorus nutrition. Experimental data. Annals of Forest Science 66:510. doi:10.1051/forest/2009039

Jonard M, Furst A, Verstraeten A, Thimonier A, Timmermann V, Potocic N, Waldner P, Benham S, Hansen K, Merila P, Ponette Q, de la Cruz, Ana C, Roskams P, Nicolas M, Croise L, Ingerslev M, Matteucci G, Decinti B, Bascietto M, Rautio P (2015) Tree mineral nutrition is deteriorating in Europe. Global change biology 21:418–430. doi:10.1111/gcb.12657

Kang J, Hesterberg D, Osmond DL (2009) Soil Organic Matter Effects on Phosphorus Sorption. A Path Analysis. Soil Science Society of America Journal 73:360. doi:10.2136/sssaj2008.0113

Kimmins JP (1997) Forest ecology a foundation for sustainable development. Prentice-Hall, Inc., Upper Saddle River, New Jersey. 596 p

Kuo S (1996) “Phosphorus”. In Methods of Soil Analysis: Chemical Methods, Part 3 Edited by: Sparks, D.L. 869–919. Madison, WI: Soil Science Society of America. SSSA No. 5

Lang F, Bauhus J, Frossard E, George E, Kaiser K, Kaupenjohann M, Krüger J, Matzner E, Polle A, Prietzel J, Rennenberg H, Wellbrock N (2016) Phosphorus in forest ecosystems. New insights from an ecosystem nutrition perspective. Journal of Plant Nutrition and Soil Science 179:129–135. doi:10.1002/jpln.201500541

Lang F, Krüger J, Amelung W, Willbold S, Frossard E, Bünemann EK, Bauhus J, Nitschke R, Kandeler E, Marhan S, Schulz S, Bergkemper F, Schloter M, Luster J, Guggisberg F, Kaiser K, Mikutta R, Guggenberger G, Polle A, Pena R, Prietzel J, Rodionov A, Talkner U, Meesenburg H, Wilpert K von, Hölscher A, Dietrich HP, Chmara I (2017) Soil phosphorus supply controls P nutrition strategies of beech forest ecosystems in Central Europe. Biogeochemistry 127:255. doi:10.1007/s10533-017-0375-0

Leuschner C (1998) Water extraction by tree fine roots in the forest floor of a temperate Fagus-Quercus forest. Annals of Forest Science:141–157

Marin CT, Bouten IW, Dekker S (2000) Forest floor water dynamics and root water uptake in four forest ecosystems in northwest Amazonia. Journal of Hydrology 237:169–183. doi:10.1016/S0022-1694(00)00302-4

McLaren TI, McLaughlin MJ, McBeath TM, Simpson RJ, Smernik RJ, Guppy CN, Richardson AE, Plant Soil (2016) 401: 23-38. https://doi.org/10.1007/s11104-015-2610-6

Minotta G, Pinzauti S (1996) Effects of light and soil fertility on growth, leaf chlorophyll content and nutrient use efficiency of beech (Fagus sylvatica L.) seedlings. Forest Ecology and Management 86:61–71. doi:10.1016/S0378-1127(96)03796-6

Murphy J, Riley JP (1962) A modified single solution method for determination of phosphate in natural waters. Anal. Chim. Acta. 26: 31-36

Pare D, Bernier B (1989) Origin of the phosphorus deficiency observed in declining sugar maple stands in the Quebec Appalachians. Can. J. For. Res. 19: 24-34

Polglase PJ, Attiwill PM, Adams MA (1992) Nitrogen and phosphorus cycling in relation to stand age of Eucalyptus regnans F. Muell. Plant and Soil 142:177–185. doi:10.1007/BF00010964.

Porder S, Ramachandran S (2012) The phosphorus concentration of common rocks—a potential driver of ecosystem P status. Plant and Soil 367:41–55. doi:10.1007/s11104-012-1490-2

Saggar S, Parfitt RL, Salt G, Skinner MF (1998) Carbon and phosphorus transformations during decompositions of pine forest floor with different phosphorus status. Biology and Fertility of Soil 27: 197–204.

Salifu KF, Timmer VR (2001) Nitrogen retranslocation response of Picea mariana seedling to nitrogen supply. Soil Science Society of America Journal 65: 905–913

Spohn M, Ermak A, Kuzyakov Y (2013) Microbial gross organic phosphorus mineralization can be stimulated by root exudates – A 33P isotopic dilution study. Soil Biology and Biochemistry 65:254–263. doi:10.1016/j.soilbio.2013.05.028

Talkner U, Meiwes KJ, Potočić N, Seletković I, Cools N, Vos B de, Rautio P (2015) Phosphorus nutrition of beech (Fagus sylvatica L.) is decreasing in Europe. Annals of Forest Science 72:919–928. doi:10.1007/s13595-015-0459-8

Vitousek P (1982) Nutrient cycling and nutrient use efficiency. Am.Nat. 119: 553-572

Vogt K A, Grier CC, Vogt DJ (1986) Production, Turnover, and Nutrient Dynamics of Above- and Belowground Detritus of World Forests. Advances in Ecological Research 15 :303-377 https://doi.org/10.1016/S0065-2504(08)60122-1Walker T, Syers J (1976) The fate of phosphorus during pedogenesis. Geoderma 15, 1–19

Walker TW, Syers JK (1976) Fate of phosphorus during pedogenesis. Geoderma 15: 1-19.

Wardle DA, Bardgett RD, Klironomos JN, Setälä H, Van Der Putten WH, Wall DH (2004) Ecological linkages between aboveground and belowground biota. Science 304(5677) :1629-1633

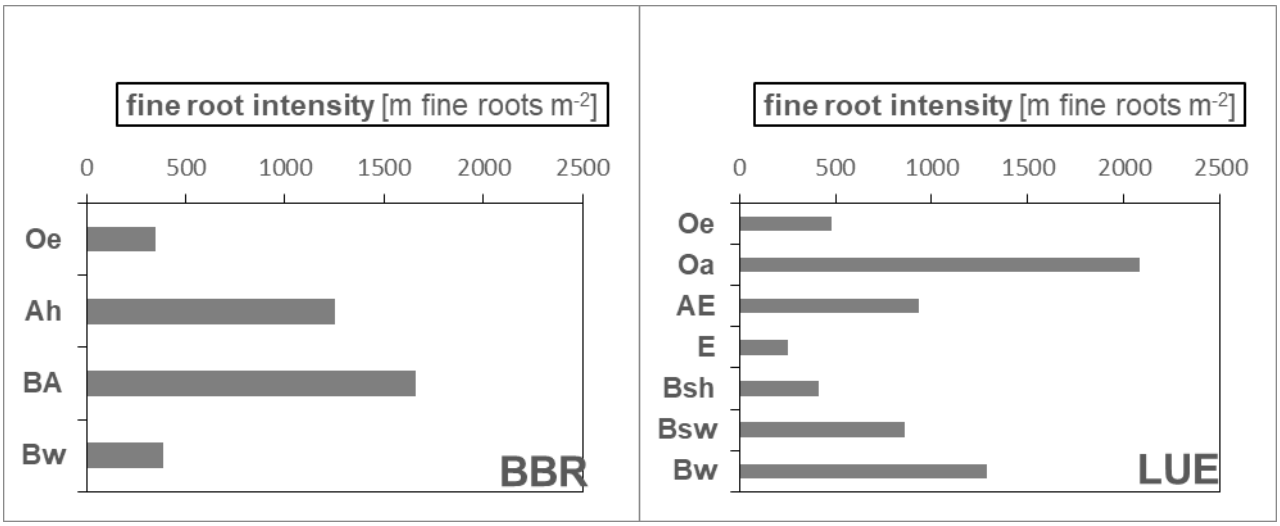
Wood T, Bormann FH, Voigt GK (1984) Phosphorus cycling in a northern hardwood forest: Biological and chemical control. Science 223: 391-393

7 Supplementary

Table A1-S1 Site characteristics of Lüss (LUE) and Bad Brückenau (BBR). MAT = mean annual temperature, MAP = mean annual precipitation, Pi = inorganic P, TP= total P, bioavailable Pi = PO4-P citr.; C=C-total (C-tot≙Corg) soil horizons had been adjusted to our pot distinction: BBR: O (+15-0cm: Oi-3cm;Oe-12cm), A (0 – -14cm: Ah), B (-14 - -33: BA) LUE: O (+15-0cm: Oi-4cm, Oe-5cm, Oa-4cm), A (0 – -12cm: AE-7cm, E-5cm), B (-12 - -35: Bsh-8cm, Bsw-15cm) (Data calculated from: Lang et al. 2017)

site	**BBR**			**LUE**		
horizon	**O**	**A**	**B**	**O**	**A**	**B**
pH (H_2O)	5.4	4.0	4.7	3.6	3.7	4.1
C [mg g^{-1}]	487	136	63	449	46	11
N [mg g^{-1}]	16.9	7.5	4.7	16.8	1.8	0.5
TP [mg kg^{-1}]	1449	3199	3033	709	146	126
bioavailable Pi [mg kg^{-1}]	324	43	35	583	53	29
C:N	29	18	13	27	25	22
C:P	336	42	21	633	315	87
N:P	12	2	1.5	24	12	4

Figure A1-S1 Horizon-specific fine root length densitiy [m fine root m^{-2}] at the field sites. Results from quantitative pit sampling by Lang et al. 2016 (BBR = Bad Brückenau, LUE = Lüss)



Determination of root length density (RLD) at field sites

Volume-based soil sampling (up to 1 m depth) at the sites LUE and BBR was carried out according to the "quantitative pit" method developed by Hamburg (1984) and adjusted by Vadeboncoeur et al. (2012). Sampled layers of the forest floor (Oe, Oa) and of the mineral soil represent single diagnostic horizons. The volume quantification of the different soil layers was carried out based on photogrammetry (Grims et al. 2014) in combination with a geographic information system software (Haas et al. 2016). Further details on the soil sampling procedure are given in Lange et al. (2017).

For the determination of fine root length, we sued static image analysis. Therefore, fine roots (< 2 mm) were manually separated from othe soil constituents, carefully washed with deionized water, dried (105°C) and weighted. Afterwards, the fine roots of each soil horizon or sampling layer were carefully cut in pieces of 0.5-5 cm length. After mixing of the sample three aliquots of around 100 fine roots parts were collected, weighted and all roots of one aliquot were spread on a light table without any overlap of single roots. The roots were

photographed (Nikon 1AW1) and the pictures were converted and analysed using the software ImageJ (Version 1.49t) (Schindelin et al. 2012, Schneider et al. 2012). The total root length of the aliquot (l) was calculated according to Eq. S1

$$l = \frac{\sum_n^1 p}{2} \qquad \text{(Eq. S1)}$$

where p is the perimeter of a single root and n is the number of roots in the aliquot. Using the weights of the aliquots we calculated a mean specific roots length (SRL) (m g-1). The SRL was used to calculate the total length of all fine roots contained in the soil sample.

Grims M, Atzberger C, Bauer T, Strauss P (2014) Low-cost Terrestrial Photogrammetry as a Tool for a Sample-Based Assessment of Soil Roughness Preiswerte terrestrische. Photogrammetrie als Werkzeug zur Bestimmung der Rauigkeit von Böden. Photogrammetrie-Fernerkundung-Geoinformation 2014(5):313–323

Haas J, Hagge Ellhöft K, Schack-Kirchner H, Lang F (2016) Using photogrammetry to 1045 assess rutting caused by a forwarder—A comparison of different tires and bogie tracks. Soil and Tillage Research 163:14–20

Lang F, Krüger J, Amelung W, Willbold S, Frossard E, Bünemann EK, Bauhus J, Nitschke R, Kandeler E, Marhan S, Schulz S, Bergkemper F, Schloter M, Luster J, Guggisberg F, Kaiser K, Mikutta R, Guggenberger G, Polle A, Pena R, Prietzel J, Rodionov A, Talkner U, Meesenburg H, von Wilpert K, Hölscher A, Dietrich HP, Chmara I (2017) Soil phosphorus

supply controls P nutrition strategies of beech forest ecosystems in Central Europe. Biogeochemistry 136(1):5-29

Schindelin, J, Arganda-Carreras, I. & Frise, E. et al. (2012), "Fiji: an open-source platform for biological-image analysis", Nature methods 9(7): 676-682

Schneider, C. A.; Rasband, W. S. & Eliceiri, K. W. (2012), "NIH Image to ImageJ: 25 years of image analysis", Nature methods 9(7): 671-675

Vadeboncoeur MA, Hamburg SP, Blum JD, Pennino MJ, Yanai RD, Johnson CE (2012) The quantitative soil pit method for measuring belowground carbon and nitrogen stocks. Soil Science Society of America Journal 76(6):2241–2255

A2 A novel oxygen stable isotope approach to study phosphate in xylem sap

Abstract

Despite the pivotal role of the xylem sap in terms of nutrient transport to locations of metabolic demand, knowledge on phosphorus (P) and its origin in xylem sap is lacking. Here we present a novel approach on the stable isotope composition of oxygen (O) of inorganic phosphate ($\delta^{18}O_{Pi}$) in xylem sap of beech trees (*Fagus sylvativa* L.). Our aims were to (i) assess Pi and Po concentrations in xylem sap, and to (ii) test the effect of a lipid removal agent (LRA) and DAX8 pretreatments on Pi and Po concentrations as well as on $\delta^{18}O_{Pi}$ values. On average, we found inorganic P (Pi) and organic P (Po) concentrations of 0.99±0.21 and 0.27±0.29 mg P l^{-1} in xylem sap, respectively. If we concentrated Pi based on anion exchange membranes, we found an incorporation of ^{18}O from isotopically spiked chemicals. Therefore, interactions with resins and/or Po biased $\delta^{18}O_{Pi}$ measurements. LRA did not remove Po quantitatively and even worse, concomitantly removed Pi.

Because the addition of DAX8 minimized interferences, we recommend the use of DAX8 before O isotope analysis of phosphate in xylem sap. $\delta^{18}O_{Pi}$ values of xylem sap measured according to this protocol ranged between -2 and +15‰. Partly negative $\delta^{18}O_{Pi}$ values of xylem sap deviating from all known $\delta^{18}O_{Pi}$ values in soil as a source of plant P uptake likely indicate enzymatically mediated, plant-internal transformation reactions of Po to Pi. Therefore, the O isotope approach in phosphate proved to be a promising tool to reveal plant-internal P cycling.

Keywords: ^{18}O spike; beech; *Fag sylvatica* L.; phosphorus cycling; phosphorus uptake; xylem sap

1 Introduction

Phosphorus (P) is an essential agent in a variety of vital processes like the build-up of DNA, RNA, and cell membranes, the energy transfer via free nucleotides and the carbon metabolism. Therefore, P is of paramount importance for plant growth and ecosystem performance (Jonard *et al.*, 2015, Scheerer *et al.*, 2018). Based on the decrease of foliar P concentrations during the last decades, P is suspected to limit the growth of trees in forests and thus, forest productivity (Jonard *et al.*, 2015, Prietzel & Stetter, 2010). An understanding of P uptake and P cycling in trees is necessary to develop management recommendations for improved forest P nutrition.

However, in contrast to N, there are knowledge gaps concerning the chemical composition of P in xylem sap - the solution transported from the roots to the leaves - and the source of P uptake from soil (Rennenberg & Herschbach, 2013). To the best of our knowledge, very few studies were published on the chemical composition of P in xylem sap. In fact, it is biased whether Po is even a component of xylem sap. Schachtmann et al. (1998) describe that Pi in xylem sap is transported solely as Pi. Whereas Maizel et al. (1956), Gout et al. (1990) and Netzer et al. (2017) also describe Po as a component in xylem sap. Netzer *et al.* (2017) described twofold organic P (Po) as compared to inorganic P (Pi) concentrations of xylem sap. They also describe a proportion of Pi in the xylem sap from remobilization of organic P from wood and bark storage organs to cover the high amount of P needed for leaf growth and development. P internal cycling in plants from P sources to areas of metabolic demand (sinks) is well described in literature. (Tsvetkova et al., 2007, Zambrosi et al., 2012, Pfahler et al., 2017, Bauke et al.,2021) Since P is stored as organic P it needs to be mobilized via enzymatic hydrolysis.

The stable isotope composition of oxygen associated to phosphorus ($\delta^{18}O_P$) is altered by enzymatic processes an could therefore be used to trace these processes. Two studies report that the majority of Po is present as organic esters in the form of phosphocholine (PC) in xylem sap of barley and tomato (Maizel *et al.*, 1956, Martin & Tolbert, 1983). The actual mechanisms of P transport in xylem sap via remobilization from stored Po into Pi, direct Po transport or even enzymatic mediated turnover along these pathways is still unknown.

As an external P source, a great proportion of Pi in xylem sap originates from the uptake of $H_2PO_4^-/HPO_4^{2-}$ in soil solution. Pi is released from the solid soil matrix into soil solution as a result of dissolution of P-containing minerals and of mineralization of soil organic matter (SOM). The differentiation of these processes could help inform foresters to improve P nutrition of forests by modifying either mineral-bond P or the composition/activity of the microbial community involved in SOM mineralization in soil. Yet, methods to identify different processes preceding P uptake into xylem sap are missing. The ratio of stable isotopes of O in phosphate ($\delta^{18}O_{Pi}$) might represent a promising approach. To the best of our knowledge, no data of isotopic exchange kinetics in plant tissue are available in the published literature. $\delta^{18}O_{Pi}$ values have been used to reveal the release of phosphate by extracellular and intracellular enzymes (Liang & Blake, 2006, von Sperber *et al.*, 2014 & 2017), the importance of microbial P cycling soil (Angert *et al.*, 2011, Tamburini *et al.*, 2012, Hacker *et al.*, 2019), and organic P pools in soil and plants (Pfahler *et al.*, 2013 & 2017,Tamburini *et al.*, 2018, Helfenstein *et al.*, 2018). However, the presence of Po might hamper the application of this approach for xylem sap. Apart from P, xylem sap is a complex mixture of a variety of different constituents: N, K, Mg, Ca, S, B, Zn, Cu, sugars, phytohormones or polyvalent heavy metal cations complexed with organic acids, amino acids and peptides (White, 2012). This matrix including Po compounds might interfere with the analysis of $\delta^{18}O_P$ in xylem sap.

The preparation of samples for $\delta^{18}O_P$ analysis includes several dissolution- and reprecipitation steps finally yielding silver phosphate. The purity of the precipitated silver phosphate is of decisive importance since small amounts of Po can bias $\delta^{18}O_P$ values (Tamburini *et al.*, 2010, Weiner *et al.*, 2011). However, methods commonly used to remove Po (strong oxidizing agents, high temperatures, strong basic conditions) may bias $\delta^{18}O_{Pi}$ values (Lécuyer, 2004). Tamburini *et al.* (2010) recommended the use of a DAX8 resin prior to the precipitation of silver phosphate for soil extracts that are colored mostly caused by dissolved organic matter (DOM). In soil, DOM is a complex mixture of mostly high-molecular-weight aromatic and aliphatic compounds (i.e. humic and fulvic acids), polysaccharides, and proteins (Schulze, 2005). Thus, the chemical composition of Po in xylem sap likely differs substantially from that in soil potentially requiring a modified pretreatment. For example,the removal of low-molecular-weight Po molecules including phosphocholine before $\delta^{18}O_P$ analyses seems advisable.

Our aims were to (i) assess Pi and Po concentrations in xylem sap, and to (ii) test the effect of a lipid removal agent (LRA) and DAX8 pretreatments on Pi and Po concentrations as well as on $\delta^{18}O_{Pi}$ values. For the latter, we used no pretreatment as the control and included the purification of Pi in xylem sap and standard P solutions (solely Pi and 1:1 or 1:2 mixture of Pi and Po) for O isotope analysis. We checked the potential influence of treatments on $\delta^{18}O_P$ values via ^{18}O-spiked and non-spiked chemicals used for sample preparation.

2 Methods

2.1 Xylem sap extraction

We used sampling campaigns at four sites to assess Pi and Po concentrations in xylem sap. Xylem sap originated from three temperate beech forest sites: the site “Lüss” (LUE) is located in Lower Saxony, northwestern Germany in the Lüneburg Heath (52°83’ N, 10°36’ E), the site “Bad Brückenau” (BBR) is located in Bavaria, southeastern Germany in a midrange mountain area of the Rhön (50°35’ N, 9°92’ E). Twigs were harvested from the mid tree top by tree climbers. From these twigs, xylem sap was obtained in quantities of 20 – 50 ml from twigs < 12 mm diameter by means of Scholander pressure bomb extraction (Soilmoisture 3000 Series Plant Water Status Console). Samples were collected in July 2017.

At an additional site, one beech tree was felled and xylem sap was extracted at the 10th of April, located near the level 2 ICP (International Co-operative Program on Assessment and Monitoring of Air Pollution Effects on Forests) forest-monitoring site Baltmannsweiler (BMW), southeast Germany. The extraction of xylem sap was done according to the displacement method of Glavac et al. (1989). A minimum amount of 0.8 mg Pi was required for subsequent isotope analysis. Accordingly, we harvested an adult beech tree (*Fagus sylvatica* L.) and cut the trunk into segments of 1.2 m length. Ten 1.2 m long trunk segments were needed to gain 13 l xylem sap which we considered sufficient for isotope analysis. For the extraction of xylem sap, 0.05 m of bark and cambium at the uppermost und lowermost part of each segment were removed to prevent contamination with phloem constituents. The trunk segments were rotated by 180° and thus arranged in a vertical position against the direction of growth. The lowermost end of the tree segment was thoroughly washed by distilled water. Subsequently, a rubber collar was fixed at the uppermost end of each trunk segment where bark and cambium had been removed and filled by approximately 1.5 l distilled water. A water-soluble

fluorescent dye (Fluorescein sodium salt, Sigma Aldrich) was added to the water in excess of its coloring capacity (> 1 g l^{-1}). The water-dye mixture forced a downward flux of xylem sap and we collected xylem sap at the lowermost part of the trunk segments and replaced the collection container every ten minutes. During xylem sap extraction, the collecting container was externally cooled by ice and the xylem sap obtained was transferred to a collecting vessel stored in a mobile freezer (-20°C). The first 100 ml of sample were discarded to exclude contaminating cell residues from cutting. Upon the first occurrence of the dye in the collection container, the sample was discarded as well and this marked the point in time where xylem sap was recovered completely. The aliquots of xylem sap were combined to one composite sample which was transferred to the laboratory and stored at -80°C. Because relatively large volumes were required for the O isotope approach, we used xylem sap of this second sampling campaign for the experiment in the laboratory as described below.

2.2Treatments

In addition to xylem sap, we prepared three standard solutions: a Pi standard ("Pi Std" - 3 mg P l^{-1} KH_2PO_4; Sigma Aldrich), a 1:1 mixture of Pi and Po ("1:1 Pi:Po" = 3 mg KH_2PO_4-P l^{-1} + 3 mg $CaO_3POCH_2CH_2N(Cl)(CH_3)_3 \cdot 4H_2O$ l^{-1}; Sigma Aldrich), and a 1:2 mixture of Pi and Po ("1:2 Pi:Po" = 3 mg KH_2PO_4-P l^{-1} + 6 mg $CaO_3POCH_2CH_2N(Cl)(CH_3)_3 \cdot 4H_2O$ l^{-1}; Sigma Aldrich).

Xylem sap and the three artificial standard solutions (initial solutions) then followed the scheme displayed in Figure 1. We used two agents to remove Po prior to Pi purification for O isotope analysis: a lipid removal agent (LRA) and a removal agent for DOM commonly present in soil (DAX8). Purification of Pi without pretreatment served as a control. Due to the relatively small amounts of Pi in xylem sap (0.6 mg l^{-1}) and the targeted 0.2 to 0.3 mg Pi for sufficient silver phosphate mass, the DAX8 treatment and the control without pretreatment were

prepared in amounts of one liter. Throughout all steps we monitored the sample volume to enable mass balance calculations.

LRA treatment: A specific lipid-adsorbing synthetic calcium silicate hydrate (Sigma Aldrich – SUPELCO Lipid Removal Adsorbent – 13358-U) was added to an aliquot of all initial solutions. After shaking for 2h, the samples were centrifuged for 60 seconds at 3500rpm (ROTANTA 460 rs, Hettich Lab Technologies, Tuttlingen, Germany) and subsequently vacuum filtered through 0.22 µm Polyethersulfone (PES) membrane filters (Millipore Express PLUS). Another 10 ml distilled water were used for washing. The filtration solution ("LRA supernatant") was used to purify Pi as described below. Please note that we used a volume of 100 ml and concentrations of 1.5 mg l^{-1} Pi and 1.5 or 3.0 mg l^{-1} Po in the 1:1 and 1:2 Pi:Po mixtures, respectively, for the LRA treatment.

DAX8 treatment: We prepared a DAX8 resin (Sigma Aldrich – Superlite DAX8 resin) slurry by cleaning the resin with HPLC grade methanol for 15 minutes followed by rinsing five times with distilled water. An aliquot of all initial solutions was treated with 100 ml DAX8 resin slurry. After two hours of shaking under cooled (4°C) conditions, the solution-slurry mixture was vacuum filtered through 0.22 µm PES membrane filters (Millipore Express PLUS, Merck, Tullagreen, Ireland). The filtration solution ("DAX8 supernatant") was used to purify Pi as described below.

All solutions with or without pretreatment were split into two aliquots and processed either with or without ^{18}O-spiked chemicals ($\delta^{18}O_{H_2O}$ = +100‰ achieved by the dilution of > 98% ^{18}O; Rotem – HYOX ^{18}O) to yield pure silver phosphate. In total, 72 samples (4 initial solution types x 3 treatments/control x 2 water types x 3 replicates) were processed for isotope analysis.

For purification of Pi, we used 20 anion exchange resin (AER) membranes (6.25 cm x 6.25 cm x 20 ≙ 781.25 cm^2; 551642S, VWR International GmbH, Bruchsal, Germany) that were added

to each of the 72 samples following the method of Weiner *et al.* (2011). We used AER in order to quantitatively recover Pi from the xylem sap and standard solutions, as already demonstrated for soil extracts by Cooperband *et al.* (1999) and Cheesmann *et al.* (2010). After shaking for 16 hours, the AER membranes were separated from the supernatant solution and the latter was discarded. Inorganic P was desorbed twice from AER by addition of 50 ml 0.5M HNO_3 for 30 min ("AER eluate"). The AER eluate was subsequently used for Ag_3PO_4-precipitation described by Tamburini *et al*. (2010). In brief, the mineral precipitation and dissolution of ammonium phospho-molybdate was followed by mineral precipitation and dissolution of magnesium ammonium phosphate. After removal of cations and proof of the absence of chloride, silver phosphate was precipitated.

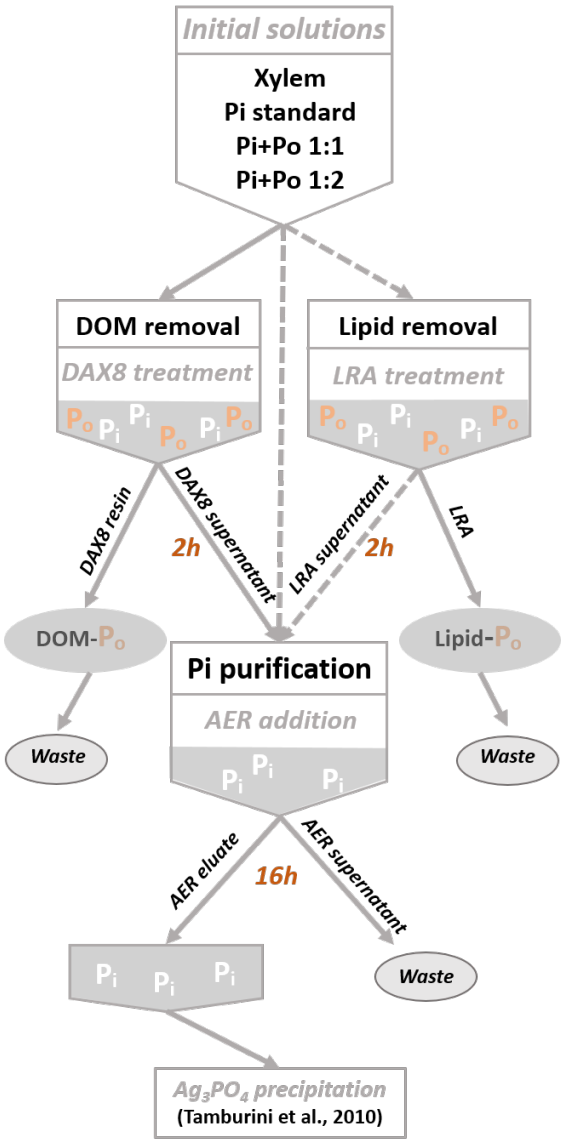


Figure A2-1: Processing of xylem sap prior to phosphate purification by silver phosphate precipitation.

2.3Chemical analyses

Concentrations of Pi were determined spectrophotometrically with an optical spectrophotometer (UV VIS, Specord 200, Analytic Jena AG, Jena, Germany) at λ = 660 nm, using the method of Murphy and Riley (1962). Total dissolved P concentrations were measured by means of Inductively Coupled Plasma/Optical Emission Spectrometry (ICP-OES, PerkinElmer Optima 5300 DV, Germany) at λ P 213.617. Limits of detection for UV-VIS 0.02

mg P l^{-1} and 0.05 mg P l^{-1} for ICP-OES. Organic P concentrations were calculated as the difference between total P and Pi concentrations (Toor et al., 2006). Negative values as a result of larger concentrations of Pi as compared to total P were set to zero (13 % of samples). This concerns mainly results from the AER eluate after DAX8 treatment due to high relative standard deviation of 13%.

The analysis of O isotope ratios was carried out by means of an TC/EA (PYRO cube) coupled in continuous flow to an IRMS (IsoPrime100, both Elementar Analysensysteme; Hanau, Germany). Three triplicate subsamples of each sample were weighed in silver capsules together with a small amount of glassy carbon powder (Alfa Aesar, type 1). Calibration and drift-corrections were accomplished by repeated measurements of two international benzoic acid standards, IAEA 601 and IAEA 602 ($\delta^{18}O$ =+23.3‰ and +71.4‰, respectively; distributed by the International Atomic Energy Agency, Vienna, Austria), and one internal Ag_3PO_4 standard ($\delta^{18}O$ =+10.2‰). The standard deviation of triplicate measurements was ± 0.6‰.

2.4Statistical analyses

Differences between treatments were tested by t-tests for dependent samples. Subsequently each treatment was tested this way against the prior treatment following the analytical concept of Figure 1, except for the AER supernatant, which was tested against the initial solution, the DAX8 supernatant or the LRA supernatant depending on which pretreatment before Po removal was used. If the prerequisite for statistical analyses was violated (non-normal distribution), Wilcoxon tests were performed instead. Statistical analysis was carried out by IBM SPSS Statistics 22.

3 Results

3.1 Concentrations of Pi and Po in xylem sap

Across sites, Pi and Po concentrations ranged from 0.13 to 4.8 and 0.19 to 6.7 mg P l^{-1}, respectively (Fig. A2-2). On average, we found 0.98 ± standard error of 0.21 mg Pi l^{-1} and 2.56 ± standard error (SE) of 0.38 mg Po l^{-1}. Accordingly, the mean Pi:Po ratio was 0.38 ± 0.3. Pi and Po concentrations of xylem sap (Pi: 0.97 mg Pi l^{-1} ± 0.13 SE; ; Po: 1.30 mg Po l^{-1} ± 0.32 SE) used for testing the O isotope approach (site BMW) were in the lowermost part of the concentration range as compared to the whole data set.

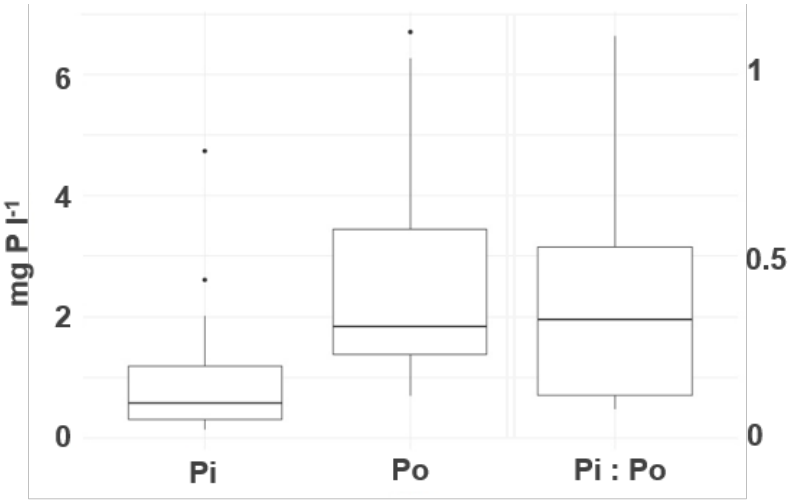


Figure A2-2: Inorganic (Pi) and organic phosphorus (Po) concentrations, as well as Pi:Po ratio in xylem sap. The right y axis belongs to Pi:Po ratio. n=20

3.2No pretreatment

If we did not include any pretreatment, Pi originally in initial solutions was recovered completely after desorption from AER (Fig. A2-3a). The recovery was 96, 103, and 103% for xylem sap, the Pi standard, and the 1:1 Pi:Po mixture, respectively. Less than 8% of Pi in the initial solution remained in the supernatant solution after AER addition in all treatments, corroborating the complete sorption of Pi to the AER (Fig. A2-3a). In case of the 1:2 Pi:Po

mixture, significantly more Pi was found after desorption from the AER as compared to the initial solution (Fig. A2-3a) resulting in a recovery of 119%.

After desorption from AER, we did not recover any Po in the Pi standard solution (0.00 mg Po). We completely recovered Po present in the initial solution in the AER supernatant for xylem sap (465%; high figures caused by concentrations close to the limit of detection) and in the 1:1 Pi:Po mixture (100%). We found a small though significant decrease of Po after desorption from AER as compared to Po in initial solution for the 1:2 Pi:Po mixture (Fig. A2-4a; -15%). Despite the nearly complete recovery of Po in the AER supernatant, we found Po in the eluate after AER addition for all solutions initially containing Po (Fig. A2-4a). This additional Po represented 300, 15, and 9% of the Po initially present in the xylem sap and the 1:1 and 1:2 Pi:Po mixtures, respectively.

The difference between ^{18}O-spiked and non-spiked aliquots of xylem sap were not significant (9.4 ± 3.9‰; p = 0.067). $\delta^{18}O_{Pi}$ values of the ^{18}O-spiked aliquot were significantly higher than those of the non-spiked aliquots for the Pi standard solution as well as for the 1:1 Pi:Po mixture (p=0.002; Fig. A2-5a). In detail, ^{18}O-spiked and non-spiked aliquots differed by 4.8 ± 0.4‰ (Pi standard solution) and 8.3 ± 1.6‰ (1:1 Pi:Po mixture).

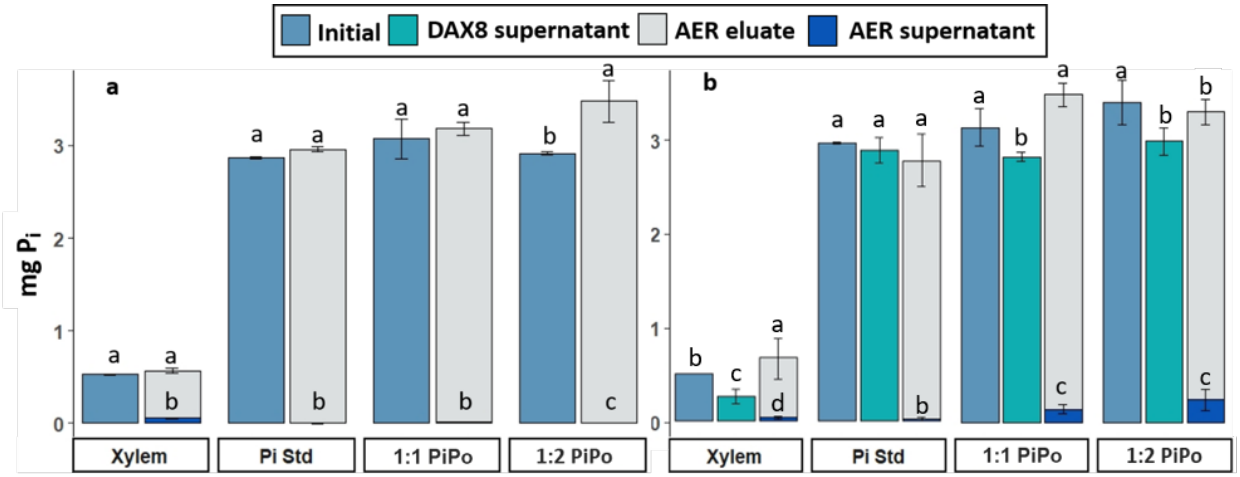


Figure A2-3: a) Inorganic phosphorus (Pi) in initial solution, in supernatant solution after anion exchange membrane (AER) addition, and in AER eluate after desorption. b) Pi in initial solution, in supernatant after DAX8 addition, in AER supernatant solution, and in AER eluate after desorption. Xylem = xylem sap; Pi Std = Pi standard; 1:1 Pi:Po = 1:1 mixture of Pi and Po standards; 1:2 Pi:Po = 1:2 mixture of Pi and Po standards. Whiskers refer to the standard deviation. Different letters above bars indicate significant differences (AER eluate and AER supernatant were tested separately against initial solution in 3a and against DAX8 supernatant in 3b).

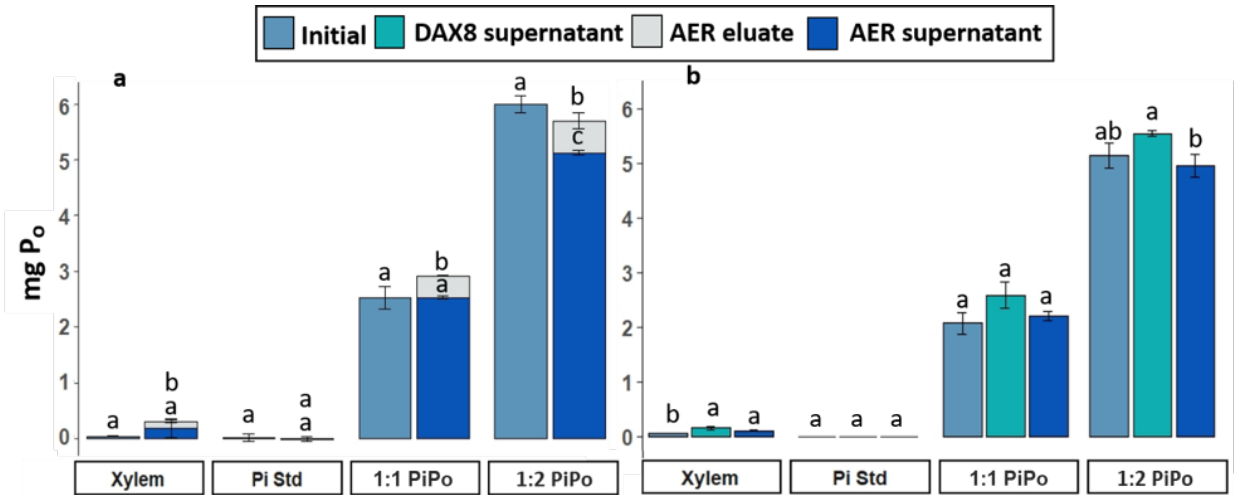


Figure A2-4: a) Organic phosphorus (Po) in initial solution, in supernatant solution after anion exchange membrane (AER) addition, and in AER eluate after desorption. b) Po in initial solution, in supernatant solution after DAX8 treatment, in AER supernatant solution, and in AER eluate after desorption. Xylem = xylem sap; Pi Std = Pi standard; 1:1 Pi:Po = 1:1 mixture of Pi and Po standards; 1:2 Pi:Po = 1:2 mixture of Pi and Po standards. Whiskers refer to the standard deviation. Different letters above bars indicate significant differences (AER eluate and AER supernatant were tested separately against initial solution in 4a).

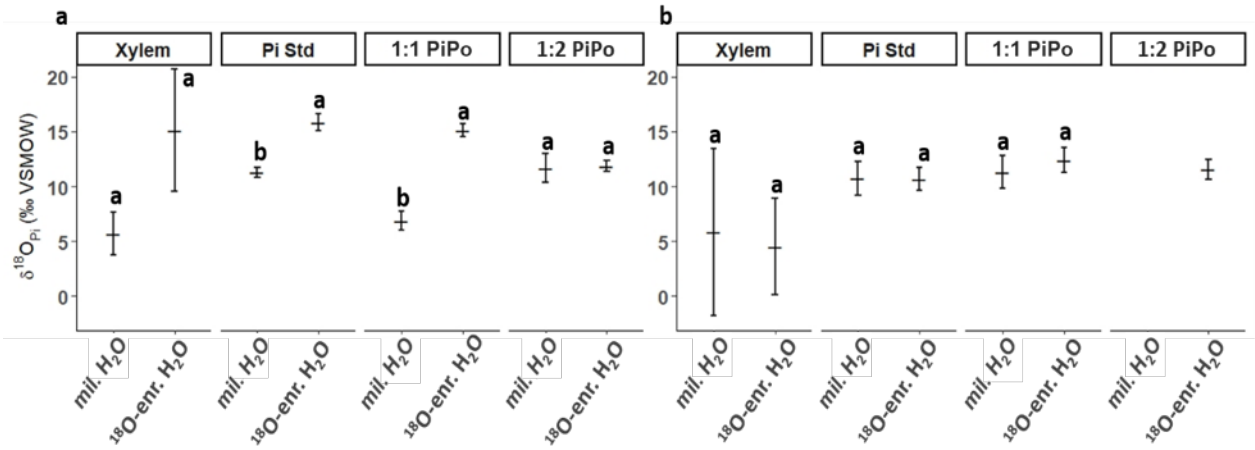


Figure A2-5: $\delta^{18}O$ values of inorganic P ($\delta^{18}O_{Pi}$) relative to standard mean ocean water (VSMOW) after processing of AER eluates for silver phosphate precipitation without (a) and with DAX8 treatment (b). Xylem = xylem sap; Pi Std = Pi standard; 1:1 Pi:Po = 1:1 mixture of Pi and Po standards; 1:2 Pi:Po = 1:2 mixture of Pi and Po standards; mil. H2O and 18O-enr. H2O = solutions for silver phosphate precipitation were prepared with millipore water (-7 ‰ VSMOW) and 18O-enriched water (+100 ‰ VSMOW), respectively. Whiskers refer to the standard deviation. Different letters above bars indicate significant differences. Missing values (b)

3.3 LRA treatment

More Pi was found after xylem sap was treated with LRA as compared to Pi initially present, but this effect was likely due to concentrations close to the limit of detection (Fig. A2-S1a: 0.02 to 0.07 mg P). By contrast, Pi was hardly recovered in the LRA supernatant indicating adsorption to LRA in all standard solutions (Fig. A2-S1a). In the case of xylem sap, Po initially present was reduced by 89% by the addition of LRA (Fig. A2-S1b). This was not the case for the 1:1 and 1:2 Pi:Po mixtures: Po was hardly sorbed to the LRA because 86% and 89% of Po were retained in the LRA supernatant as compared to the initial solution (Fig. A2-S1b). Combined with the adsorption of > 80% Pi by the addition of LRA to the initial solution (Fig. A2-S1) Pi:Po ratios changed from 1:1 to 1:11 and 1:2 to 1:21.

The ^{18}O-spiked aliquots showed significantly higher $\delta^{18}O_{Pi}$ values as compared to non-spiked aliquots in xylem sap (difference of 20.5 ‰; Fig. A2-S2). However, one must keep in mind that the high adsorption of Pi to the LRA resulted in very small amounts of Pi (< 0.2 mg P for

standard solutions and 0.04 mg P for xylem sap) rendering the interpretation of $\delta^{18}O_{Pi}$ values impossible.

3.4DAX8 treatment

In xylem sap, Pi decreased if DAX8 was added as compared to Pi initially present (-48%) and increased again after separation by means of the AER (+28%; Fig. A2-3b). By contrast, Pi stayed constant in the DAX8 supernatant as compared to the initial solution in case of the Pi standard and the 1:2 Pi:Po mixture (Fig. A2-3b). The significant difference in Pi between the DAX8 supernatant and the initial solution for the 1:1 Pi:Po mixture was in the range of the measurement error (Fig. A2-3b; -10%). Similarly, the small though significant decrease of Pi after addition of AER to the DAX8 supernatant and subsequent desorption as compared to Pi in initial solution for the Pi standard solution was within the measurement error (Figure A2-3b; -7%).

We found significantly more Po after DAX8 addition compared to initial solution in xylem sap (+260%, Fig. A2-4b) but we must keep in mind that large figures relate to low concentrations. For both Pi:Po mixtures, Po was not sorbed to DAX8 since we found no significant differences between the initial solution and the supernatant after DAX8 addition (Fig. A2-4b). Thereafter, Po was again recovered completely (= no significant difference) in the supernatant after AER addition in case of xylem sap and the 1:1 Pi:Po mixture. The small though significant decrease of Po in the AER supernatant compared to the DAX supernatant solution in case of the 1:2 Pi:Po mixture was within the measurement error (Fig. A2-4b; -11%). If DAX8 was used prior to AER purification of Pi, we observed no significant difference between aliquots processed either with or without ^{18}O-spiked solutions (Fig. A2-5).

4 Discussion

4.1 Concentrations of Pi and Po in xylem sap

The ranges of 0.13 to 4.8 mg Pi l^{-1} and 0.19 to 6.7 mg Po l^{-1} in xylem sap found in our study matched with 0.5 to 70 mg Pi l^{-1} and 0.5 to 80 mg Po l^{-1} reported in the literature (Bollard, 1960, Saur *et al.*, 1995, Prima-Putra & Botton, 1998, Netzer *et al.*, 2017). The high variability of Pi and Po concentrations in xylem is mainly caused by season via its effect on P availability in soil, as well as P and water uptake and subsequent transport driven by photosynthetic activity (Dambrine *et al.*, 1995, Peuke & Rennenberg, 2004, Netzer *et al.*, 2017). Xylem sap further processed for O isotope analysis in phosphate contained small concentrations of Pi and Po (Fig. A2-2). Such small concentrations very likely are caused by the sampling time. We sampled xylem sap in early spring before budbreak, when xylem pressure was at its seasonal peak (Cochard *et al.*, 2001). High xylem pressure is a result of potential-driven water uptake and thus, likely diluted concentrations of xylem constituents in our study.

4.2Method development for O isotope analysis of phosphate in xylem sap

If we did not remove Po before purification for O isotope analysis, we completely recovered Pi of the initial Pi standard solution after desorption from the AER (Fig. A2-3a). However, despite complete Pi recovery and in the absence of Po we found an incorporation of ^{18}O from ambient water into recovered Pi (Fig. A2-5). We can only speculate that a very small amount of Pi with a highly ^{18}O-enriched isotope signature was released from functional groups of the AER.

Although we nearly completely recovered Po in the AER supernatant for those solutions that contained Po (in xylem, 1:1 and 1:2 Pi:Po mixtures), we found additional Po after desorption

from the AER (Fig. A2-4a). This additional Po could either represent Po released from the resin material or Po present in the initial solution. The first explanation is corroborated by the 1:1 Pi:Po mixture because we found more Po than was initially present (Fig. A2-4a). This process was associated with the incorporation of ^{18}O from ambient water (Fig. A2-5a). Therefore, the release of Po from the resin seems to be associated with the incorporation of O atoms from ambient water e.g., during UV-induced hydrolysis of C-P bonds (Sandy et al. 2013, Jaisi et al. 2016) likely present in the AER. Subsequently, ^{18}O-enriched Po was coprecipitated in silver phosphate after the purification procedure. The second explanation is corroborated by results of the 1:2 Pi:Po mixture, since Po not recovered in the AER supernatant was found in the AER eluate (Fig. A2-4a). Similarly, Weiner *et al.* (2011) and Cheesman *et al.* (2010) performed soil extractions and found that Po was sorbed to the AER concomitantly with Pi. We did not detect a difference in $\delta^{18}O_{Pi}$ values of the 1:2 Pi:Po mixture which could be related to similar $\delta^{18}O_P$ values of the inorganic and organic standard material (Liang & Blake 2006, 2009; von Sperber et al. 2014). In addition, given the fact that we found more Pi than initially present in the AER eluate (Fig. A2-3a), we cannot rule out that a proportion of Po was hydrolyzed. Our results highlight that interactions with resins and/or Po biased $\delta^{18}O_{Pi}$ measurements if no pretreatment was performed.

The intended removal of Po by LRA resulted in sorption of more than 80% of Pi initially present in the standard solutions (Fig. A2-S1). Furthermore, contrary to our expectations LRA did remove only small amounts (< 10%) of phosphocholine from the 1:1 and 1:2 Pi:Po mixtures. Therefore, we discourage the use of LRA to remove Po in xylem sap before O isotope analysis in phosphate.

If DAX8 was used to remove Po, we recovered Pi completely in all solutions corroborating the negligible influence of DAX8 on Pi (Joshi *et al.*, 2016). The only exception was the decrease of

Pi after DAX addition for xylem sap (Fig. A2-3b). As Escoda *et al.* (1999) describe Fe-phosphate-citrate complex formation in the soil solution, a formation of such Pi containing ligand bound complexes seems also plausible in xylem sap (Hell & Stephan, 2003). Thus, explaining the drop of Pi in DAX8 supernatant as compared to initial solution. Pi in complexes such as the Fe(III)-citrate or phytoferritin (Frossard *et al*., 2000) could have been sorbed to DAX8 and therefore a proportion of Pi was removed concomitantly with the removal of the DAX8 resin.

However, we found Po in the supernatant of all three solutions that contained Po (xylem, 1:1 and 1:2 Pi:Po mixtures, Fig. A2-4b). Therefore, DAX8 did not remove Po. By contrast, Jaisi (2016) reported a reduction of Po ranging from 28 to 6% by the addition of DAX8. However, as opposed to the complex mixture of Po compounds extracted from soil by Jaisi (2016), we used a well-defined compound namely phosphocholine as a surrogate for Po species in xylem sap (Maizel *et al.*, 1956, Martin & Tolbert, 1983). Phosphocholine represents a small molecule (molecular weight of 184 Da) and thus, was obviously not captured by DAX8 which is known to adsorb highly condensed molecules up to 150.000 Da.

Nevertheless, the presence of phosphocholine did not compromise O isotope measurements in phosphate. $\delta^{18}O_{Pi}$ values of standard solutions (Pi standard, 1:1 and 1:2 Pi:Po mixtures) were, on average, 11.4 ± 0.6‰ irrespective of whether or not ^{18}O-spiked chemicals were used. Similarly, $\delta^{18}O_{Pi}$ values of xylem sap did not differ between ^{18}O-spiked and non-spiked chemicals (Fig. A2-5). Therefore, O was not incorporated from ambient water into phosphate if DAX8 was used prior to preparation for O isotope analysis. Therefore, we recommend the use of DAX8 before O isotope analysis of phosphate in xylem sap to produce reliable $\delta^{18}O_{Pi}$ values.

$\delta^{18}O_{Pi}$ values in xylem sap ranged from -2‰ to +15‰ (Fig. A2-5). 2 out of 6 (33%) xylem samples were characterized by negative $\delta^{18}O_{Pi}$ values. Soil (+6 to +25‰) and plant (+3.5 to

+57‰) P pools are commonly characterized by more positive $\delta^{18}O_{Pi}$ values than those we observed. (Tamburini *et al.*, 2014, von Sperber et al., 2023) To the best of our knowledge negative $\delta^{18}O_{Pi}$ values in soil xylem sap have never been reported before. One explanation relates to isotope fractionation of O during root uptake. To the best of our knowledge negative $\delta^{18}O_{Pi}$ fractionation factors for plant root uptake have never been reported before. Vargas *et al*. (2017) describe a fractionation of oxygen isotopes ($\delta^{18}O_W$) during root water uptake in a field study, while other authors assume this effect to be negligible (Meunier *et al*., 2017; Barbeta *et al*., 2020). Noteworthy Barbeta *et al*. (2020) describe the physiological processes related to fractionation within plants and during root water uptake to be still poorly understood. In the same manner Bauke et al. (2021) describe root and shoot $\delta^{18}O_W$ values as consistently lower than those of the ambient nutrient solution outside the plant tissue. In addition, Bauke et al. (2017) reported of TCA extractable $\delta^{18}O_P$ values on root and shoot tissue of *Triticum aestivum,* while the root $\delta^{18}O_P$ values being always significantly lower than shoot $\delta^{18}O_P$ values and manly reflecting the $\delta^{18}O_P$ signal of the ambient nutrient solution. Even our results are at the lowermost range ever reported for plant $\delta^{18}O_P$ values, we have no option to compare them due to the lack of $\delta^{18}O_P$ data on plant xylem sap. Furthermore, enzymes could serve as an explanation. Extracellular enzymes are associated with a negative isotope fractionation of up to -39 ‰ (Liang & Blake, 2006; von Sperber *et al.*, 2014). In line, Bollard (1960) and Purcino & Lynd (1986) describe enzymatic activity in xylem for nitrogen transport systems. Gout et al. (1990) consider acid phosphatase activity responsible for phosphocholine hydrolysis in xylem sap in areas of metabolic demand. Therefore, we suggest that the negative $\delta^{18}O_{Pi}$ values in xylem sap originate from extracellular enzyme-mediated transformation reactions of Po to Pi.

Furthermore Phloem/xylem exchange is also described in some papers (Van Bel, 1990, White & Ding, 2023). Also Pfahler et al. (2017) and Qin et al. (2018) describe the influence of arbuscular mycorrhizal fungi as an $\delta^{18}O_{Pi}$ altering agent during plant P uptake.

5 Conclusions

We used phosphocholine as a surrogate for Po in xylem sap. However, this simplification might not well reflect the speciation of Po in xylem sap. Therefore, further studies should aim at revealing the Po compounds in xylem sap by means of e.g., ^{31}P-NMR. The pre-treatment of samples with a DAX resin did not bias isotope measurements and thus, proved useful for reliable $\delta^{18}O_{Pi}$ values in xylem sap. In contrast to the positive $\delta^{18}O_{Pi}$ values in soil reported in the literature, we found low and partly negative $\delta^{18}O_{Pi}$ values in xylem sap that were likely linked to the activity of extracellular enzymes. The assessment of phosphatase enzymes in xylem sap and of the associated isotope fractionation factors could build the basis for a conceptual model of plant-internal P cycling in the future. Nevertheless, plant-internal P cycling strongly depends on season and thus, requires highly temporally resolved experiments.

6 Acknowledgements

The authors gratefully acknowledge funding by the German Research Foundation (DFG) (Oe516/6-2 in the priority program SPP 1685 ‘‘Ecosystem Nutrition: Forest Strategies for Limited Phosphorus Resources’’). Furthermore, we are grateful for support by the forest ranger Helmut Glowania in Baltmannsweiler – Stuttgart/Esslingen, Germany, to retrieve the trunk of an adult beech tree.

7 Authors

YO conceived the idea of the study, SH conducted the study. The manuscript was jointly written by SH and YO.

8 References

Angert, A, Weiner, T, Mazeh, S, Tamburini, F, Frossard, E, Bernasconi, SM, Sternberg, M. 2011. Seasonal variability of soil phosphate stable oxygen isotopes in rainfall manipulation experiments. *Geochimica et Cosmochimica Acta* **75**: 4216–4227.

Barbeta, A, Gimeno, TE, Clav ́eL, Fr ́ejaville, B, Jones, SP, Delvigne, C, Wingate, L,Og ́ee, J, 2020. An explanantion for the isotopic offset between soil and stemwater in a temperate tree species. *New Phytologist* **227**: 766–779.

Bauke, S, Schnepf, A, Sperber, C, Orlowski, N, Lewandowski, H, Selzner, T, Tamburini, F, Amelung, W. 2021. Tracing uptake and translocation of phosphorus in wheat using oxygen isotopes and mathematical modeling. *New Phytologist.* **230**. 10.1111/nph.17307.

Blake, RE, O'Neil, JR, Surkov, AV. 2005. Biogeochemical cycling of phosphorus Insights from oxygen isotope effects of phosphoenzymes. *American Journal of Science* **305**: 596–620.

Bollard, EG. 1960. Transport in the Xylem. *Plant Physiology* **11**: 141–166

Chang, SJ, Blake, RE. 2015. Precise calibration of equilibrium oxygen isotope fractionations between dissolved phosphate and water from 3 to 37°C. *Geochimica et Cosmochimica Acta* **150**: 314–329

Cheesman, AW, Turner, BL, Reddy, KR. 2010. Interaction of Phosphorus Compounds with Anion-Exchange Membranes. Implications for Soil Analysis. *Soil Science Society of America Journal* **74**: 1607-1612

Cochard, H, Lemoine, D, Améglio, T, Granier, A. 2001. Mechanisms of xylem recovery from winter embolism in Fagus sylvatica. *Tree Physiology* **21**: 27–33.

Cooperband, LR, Gale, PM, Comerford, NB. 1999. Refinement of the Anion Exchange Membrane Method for Soluble Phosphorus Measurement. *Soil Science Society of America Journal* **63**: 58–64.

Dambrine, E, Martin, F, Carisey, N, Granier, A, Hällgren, J-E, Bishop, K. 1995. Xylem sap composition. A tool for investigating mineral uptake and cycling in adult spruce. *Plant and Soil* **168**: 233–241.

Escoda, ML , Florencio de la Torre, Salvado, V. 1999. The formation of mixed ligand complexes of Fe(III) with phosphoric and citric acids in 0.5 M NaNO aqueous solutions. *Polyhedron* **18**: 3269–3274.

Saur, E, Brechet, C, Lambrot, C, Masson, P. 1995. Micronutrient composition of xylem sap and needles as a result of P-fertilization in maritime pine. *Trees* **10**: 52–54.

Frossard, E, Bucher, M, Mächler, F, Mozafar, A, Hurrell, R. 2000. Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *Journal of the Science of Food and Agriculture* **80**: 861-879.

Gout, E, Bligny, R, Roby, C, & Douce, R. 1990. Transport of phosphocholine in higher plant cells: 31P nuclear magnetic resonance studies. *Proceedings of the National Academy of Sciences of the United States of America* **87**: 4280–4283.

Glavac, V, Koenies, H, Jochheim, H, & Ebben, U. 1989. Mineralstoffe im Xylemsaft der Buche und ihre jahreszeitlichen Konzentrationsveränderungen entlang der Stammhöhe. *Angewandte Botanik* **63**: 471-486.

Hacker, N, Wilcke, W, Oelmann, Y. 2019. The oxygen isotope composition of bioavailable phosphate in soil reflects the oxygen isotope composition in soil water driven by plant diversity effects on evaporation. *Geochimica et Cosmochimica Acta* **248**: 387-399

Helfenstein, J, Tamburini, F, Sperber, C von, Massey, MS, Pistocchi, C, Chadwick, OA, Vitousek, PM, Kretzschmar, R, Frossard, E. 2018. Combining spectroscopic and isotopic techniques gives a dynamic view of phosphorus cycling in soil. *Nature communications* **9**: 3226.

Hell, R, Stephan, UW. 2003. Iron uptake, trafficking and homeostasis in plants. *Planta* **216**: 541–551.

Jaisi, DP, Li, H, Wallace, AF, Paudel, P, Sun, MJ, Balakrishna, A, and Lerch, RN. 2016. Mechanisms of Bond Cleavage during Manganese Oxide and UV Degradation of Glyphosate: Results from Phosphate Oxygen Isotopes and Molecular Simulations. *Journal of Agriculture and Food Chemisrty* **64:** 8474-8482.

Jonard, M, Furst, A, Verstraeten, A, Thimonier, A, Timmermann, V, Potocic, N, Waldner, P, Benham, S, Hansen, K, Merila, P, Ponette, Q, de la Cruz, Ana C, Roskams, P, Nicolas, M, Croise, L, Ingerslev, M, Matteucci, G, Decinti, B, Bascietto, M, Rautio, P. 2015. Tree mineral nutrition is deteriorating in Europe. *Global change biology* **21**: 418–430.

Joshi, SR, Li, X, Jaisi, DP. 2016. Transformation of Phosphorus Pools in an Agricultural Soil. An Application of Oxygen-18 Labeling in Phosphate. *Soil Science Society of America Journal* **80**: 69-78.

Lécuyer, C. 2004. Oxygen Isotope Analysis of Phosphate (Chapter 22). *Handbook of stable isotope analytical techniques* **1**: 482-496.

Liang, Y, Blake, RE. 2006. Oxygen isotope composition of phosphate in organic compounds: Isotope effects of extraction methods. *Organic Geochemistry* **37**: 1263–1277.

Liang, Y, Blake, RE. 2006. Oxygen isotope signature of Pi regeneration from organic compounds by phosphomonoesterases and photooxidation. *Geochimica et Cosmochimica Acta* **70**: 3957–3969.

Liang, Y, Blake, RE. 2009. Compound- and enzyme-specific phosphodiester hydrolysis mechanisms revealed by δ18O of dissolved inorganic phosphate: Implications for marine P cycling. *Geochimica et Cosmochimica Acta* **73**: 3782–3794.

Maizel, JV, Benson, AA, Tolbert, NE. 1956. Identification of Phosphorylcholine as an important constituent of plant saps. *Plant Physiology* **31**: 408–409.

Martin, BA, Tolbert, NE. 1983. Factors Which Affect the Amount of Inorganic Phosphate, Phosphorylcholine, and Phosphorylethanolamine in Xylem Exudate of Tomato Plants. *Plant Physiology* **73**: 464–470.

Meunier, F, Rothfuss, Y, Bariac, T, Biron, P, Richard, P, Durand, JL, Couvreur, V, Vanderborght, J, Javaux, M, 2017. Measuring and modeling hydraulic lift of Lolium multiflorumusing stable water isotopes. *Vadose Zone Journal* **17**:1–15.

Murphy, J, Riley, JP. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* **27**: 31–36.

Netzer, F, Schmid, C, Herschbach, C, Rennenberg, H. 2017. Phosphorus-nutrition of European beech (Fagus sylvatica L.) during annual growth depends on tree age and P-availability in the soil. *Environmental and Experimental Botany* **137**: 194–207.

Peuke, AD, Rennenberg, H. 2004. Carbon, nitrogen, phosphorus, and sulphur concentration and partitioning in beech ecotypes (Fagus sylvatica L.). Phosphorus most affected by drought. *Trees* **18**: 639–648.

Pfahler, V, Dürr-Auster, T, Tamburini, F, M. Bernasconi, S, Frossard, E. 2013. ^{18}O enrichment in phosphorus pools extracted from soybean leaves. *New Phytologist* **197**: 186–193.

Pfahler, V, Tamburini, F, Bernasconi, SM, Frossard, E. 2017. A dual isotopic approach using radioactive phosphorus and the isotopic composition of oxygen associated to phosphorus to understand plant reaction to a change in P nutrition. *Plant Methods* **13**: 75.

Prietzel, J, Stetter, U. 2010. Long-term trends of phosphorus nutrition and topsoil phosphorus stocks in unfertilized and fertilized Scots pine (*Pinus sylvestris*) stands at two sites in Southern Germany. *Forest Ecology and Management* **259**: 1141-1150.

Prima-Putra, D, Botton, B. 1998. Organic and inorganic compounds of xylem exudates from five woody plants at the stage of bud breaking. *Journal of Plant Physiology* **153**: 670–676.

Purcino, AAC & Lynd, JO. 1986. Soil fertility effects on growth, nitrogen fixation, nodule enzyme activity and xylem exudates of lablab purpureus (L.) sweet, grown on a typic eutrustox. *Communications in Soil Science and Plant Analysis* **17**: 1331-1354

Qin, Y, Duan, G, Zhao, Z, Tian, H, Solaiman, ZM. 2018. ^{18}O-labeled phosphate applied to soil appears in the shoots of maize after uptake by roots but not after uptake by an arbuscular mycorrhizal fungus. *Mycorrhiza* **28(8)**: 787-793. doi: 10.1007/s00572-018-0849-5. Epub 2018 Jun 27. PMID: 29951862.

Rennenberg, H, Herschbach, C. 2013. Phosphorus nutrition of woody plants: many questions - few answers. *Plant Biology* **15**: 785-788.

Sandy, EH, Blake, RE, Chang, SJ, Jun, Y, and Yu, C. 2013. Oxygen isotope signature of UV degradation of glyphosate and phosphonoacetate: Tracing sources and cycling of phosphonates. *Journal of Hazardous Materials* **260**: 947-954.

Saur, E, Lambrot, C, Loustau, D, Rotival, N, & Trichet, P. 1995. Growth and uptake of mineral elements in response to sodium chloride of three provenances of maritime pine. *Journal of Plant Nutrition* **18**: 243-256.

Schachtmann, DP, Reid, RJ, Ayling, SM. 1998. Phosphorus Uptake by Plants: From Soil to Cell. *Plant Physiol.* **116:** 447–453.

Scheerer, U, Netzer, F, Bauer, AF, Herschbach, C. 2018. Measurements of 18 O-Pi uptake indicate fast metabolism of phosphate in tree roots. *Plant biology* **21**: 565-570.

Schulze, WC. 2005. Protein analysis in dissolved organic matter: What proteins from organic debris, soil leachate and surface water can tell us – a perspective. *Biogeosciences, European Geosciences Union* **2**: 75–86.

Sperber, C von, **Kries, H, Tamburini, F, Bernasconi, SM, Frossard, E. 2014.** The effect of phosphomonoesterases on the oxygen isotope composition of phosphate. *Geochimica et Cosmochimica Acta* **125**: 519–527.

Sperber, C von, **Lewandowski, H, Tamburini, F, Bernasconi, SM, Amelung, W, Frossard, E. 2017.** Kinetics of enzyme-catalysed oxygen isotope exchange between phosphate and water revealed by Raman spectroscopy. *Journal of Raman Spectroscopy* **48**: 368–373.

Tamburini, F, Bernasconi, SM, Angert, A, Weiner, T, Frossard, E. 2010. A method for the analysis of the δ18O of inorganic phosphate extracted from soils with HCl. *European Journal of Soil Science* **61**: 1025–1032.

Tamburini, F, Pfahler, V, Bünemann, EK, Guelland, K, Bernasconi, SM, Frossard, E. 2012. Oxygen Isotopes Unravel the Role of Microorganisms in Phosphate Cycling in Soils. *Environmental Science & Technology* **46**: 5956–5962.

Tamburini, F, Pfahler, V, Sperber, C von, **Frossard, E, Bernasconi, SM. 2014.** Oxygen Isotopes for Unraveling Phosphorus Transformations in the Soil–Plant System: A Review. *Soil Science Society of America Journal* **78**: 38-46.

Tamburini, F, Pistocchi, C, Helfenstein, J, Frossard, E. 2018. A method to analyse the isotopic composition of oxygen associated with organic phosphorus in soil and plant material. *European Journal of Soil Science* **69**: 816–826.

Toor, SG, Hunger, SJ, Peak, DJ, Sims, T, Sparks, DL. 2006. Advances in the Characterization of Phosphorus in Organic Wastes. Environmental and Agronomic Applications. *Acta Scientiarum. Agronomy* **89**: 1–72.

Tsvetkova, G, Georgiev, G. 2007. Changes in phosphate fractions extracted from different organs of phosphorus starved nitrogen fixing pea plants. *J Plant Nutr. :* **30**(10–12):2129–40.

Sperber, C, Pistocchi, C, Weiler, M, Tamburini, F, 2023. Oxygen isotope ratios of phosphates in the soil-plant system: Limitations and future developments. *European Journal of Soil Science,* **74**(6), e13434. https://doi.org/10.1111/ejss.13434

Van Bel, AJE, 1990. Xylem-Phloem Exchange Via the Rays. The Undervalued Route of Transport. *Journal of Experimental Botany* **41**; 631–644: https://doi.org/10.1093/jxb/41.6.631

Vargas, AI, Schaffer, B, Yuhong,0 L, da Sternberg, SL, 2017. Testing plant use ofmobile vs immobile soil water sources using stable isotope experiments. *NewPhytologist* **215**: 582–594.

Weiner, T, Mazeh, S, Tamburini, F, Frossard, E, Bernasconi, SM, Chiti, T, Angert, A. 2011. A method for analyzing the $\delta^{18}O$ of resin-extractable soil inorganic phosphate. *Rapid Communications in Mass Spectrometry* **25**: 624–628.

White, PJ. 2012. Long-distance Transport in the Xylem and Phloem (Chapter 3). In: *Marschner's Mineral Nutrition of Higher Plants* **3**: 49–70.

White, PJ. Ding, G. 2023. Long-distance transport in the xylem and phloem (Chapter 3) This chapter is a revision of the third edition chapter by P.J. White, pp. 49–70. *Marschner's Mineral*

Nutrition of Plants (Fourth Edition) **73-104**: DOI: https://doi.org/10.1016/B978-0-12-384905-2.00003-0.

Zambrosi, FCB, Mattos, D, Boaretto, RM, Quaggio, JA, Muraoka, T, Syvertsen, JP, 2012. Contribution of phosphorus (^{32}P) absorption and remobilization for citrus growth. *Plant Soil*.; **355**(1–2):353–62.

9 Supplementary

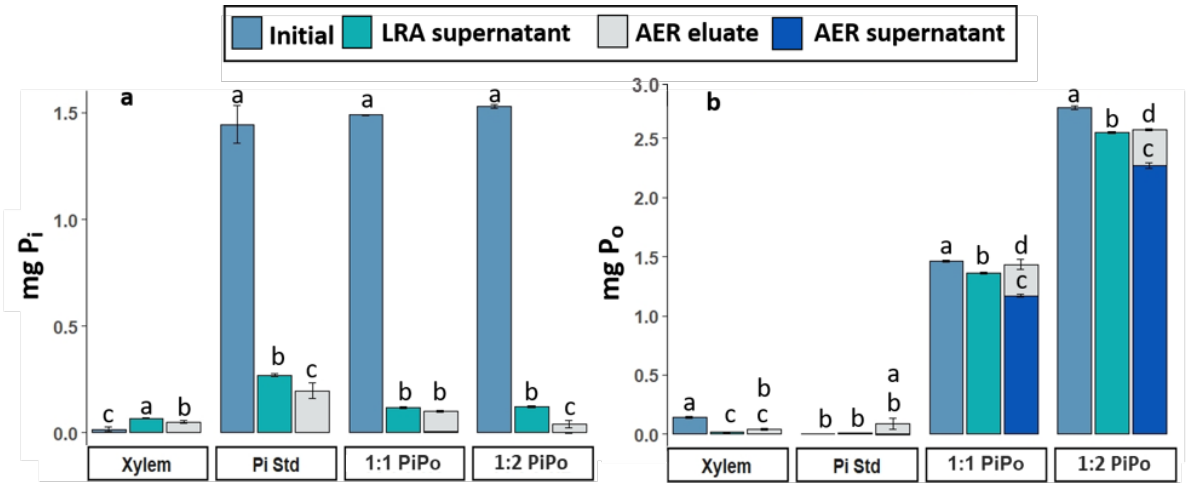


Figure A2-S1: a) Inorganic phosphorus (Pi) and b) organic phosphorus (Po) in initial solution, in supernatant solution after LRA treatment, in AER supernatant solution and in AER eluate after desorption. Xylem = xylem sap; Pi Std = Pi standard; 1:1 Pi:Po = 1:1 mixture of Pi and Po standards; 1:2 Pi:Po = 1:2 mixture of Pi and Po standards. Whiskers refer to the standard deviation. Different letters above bars indicate significant differences (AER eluate and AER supernatant were tested separately against LRA supernatant in S1b). Note different scales of y axes.

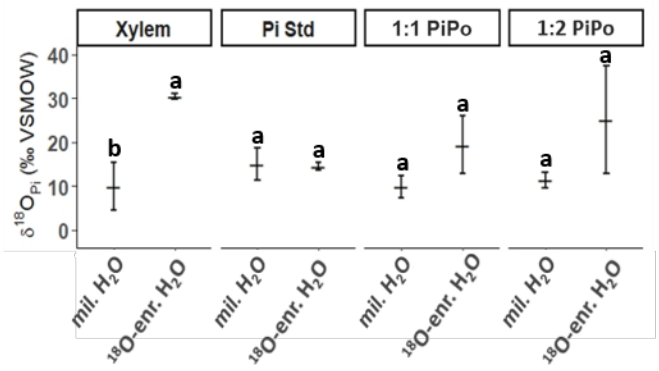


Figure A2-S2: $\delta^{18}O$ values of inorganic P ($\delta^{18}O_{Pi}$) relative to standard mean ocean water (VSMOW) after processing of AER eluates for silver phosphate precipitation with LRA treatment. Xylem = xylem sap; Pi Std = Pi standard; 1:1 Pi:Po = 1:1 mixture of Pi and Po standards; 1:2 Pi:Po = 1:2 mixture of Pi and Po standards; mil. H_2O and ^{18}O-enr. H_2O = solutions for silver phosphate precipitation were prepared with millipore water (-7 ‰ VSMOW) and ^{18}O-enriched water (+100 ‰ VSMOW), respectively. Whiskers refer to the standard deviation. Different letters above bars indicate significant differences.

A3 Impacts of fertilization on biologically cycled P in xylem sap of *Fagus sylvatica* L. revealed by means of the oxygen isotope ratio in phosphate

Abstract

Studies on the effect of high atmospheric N deposition report inconsistent results on forest productivity and N cycling which might be related to P availability in soil and subsequently affect tree P nutrition. We wanted to test the effects of (i) site i.e., a P-poor versus a P-rich site and of (ii) fertilization (N, P, N+P) on inorganic P (Pi) and organic P (Po) concentrations as well as on biologically cycled phosphate (inferred from the O isotope signature after adding an ^{18}O-enriched label) in xylem sap. We measured Pi and Po concentrations and the O isotope signature in phosphate ($\delta^{18}O_{Pi}$) in xylem sap of beech (*Fagus sylvatica* L.) trees two and 14 days after addition of ^{18}O-enriched water to the organic layer in a full factorial fertilization experiment (control, +N, +P, +NP) at two sites differing in P availability.

Higher P concentrations in xylem sap at the P-rich than at the P-poor site originated from accelerated biological P cycling indicated by incorporation of ^{18}O from the isotope label into phosphate in xylem sap shortly after labeling. At this site, $\delta^{18}O_W$ values of xylem sap after label application remained close to background $\delta^{18}O_W$ values of soil solution. We speculate that in contrast to P uptake, trees took up water from deeper (non ^{18}O-labeled) soil layers. At the P-poor site, the ^{18}O label was recovered both in xylem sap water and phosphate in xylem sap, the latter only after 14 days.

These results imply that trees relied on the organic layer for P acquisition and water uptake. However, biological processes associated with an incorporation of ^{18}O from the label were

slower at the P-poor than at the P-rich site. P addition (P, NP) increased Pi concentrations in xylem sap at the P-rich site. Based on $\delta^{18}O_{Pi}$ values in xylem sap, the additional P originated both from the fertilizer and from accelerated biological P cycling in soil.

We conclude that P-poor sites likely suffer more from climate change in case of an increased frequency of droughts because as opposed to P-rich sites both water and nutrient uptake will be affected.

1 Introduction

Phosphorus (P) is an essential agent in a variety of vital processes like the build-up of DNA, RNA, and cell membranes, the energy transfer via free nucleotides and the carbon metabolism. Therefore, P is of paramount importance for plant growth and ecosystem performance (Marschner, 1995; Scheerer et al., 2018). Based on the decrease of foliar P concentrations during the last decades, P is suspected to limit the growth of trees (*Fagus sylvatica* L; *Pinus sylvestris* L.) in forests and thus, forest productivity (Prietzel and Stetter, 2010; Jonard et al., 2015).

The drivers for P limitation in European forest ecosystems are mainly associated with anthropogenic activities. In particular, continuously high deposition of atmospheric N and increased CO_2 concentrations due to climate change accelerate forest growth, consequently resulting in enhanced P demands by trees (Jonard et al., 2015; Talkner et al., 2015). However, the effect of increased N supply on forest growth reported so far are not consistent. Magill et al. (2004) found that forest growth was increased by N fertilization whereas N fertilization had not effect in several other studies (Nadelhoffer et al., 2004; Finzi, 2009; Lovett et al., 2013). Given an unbalanced N and P deposition together with the crucial role of phosphorus (P) in plant development, seminatural ecosystems are gradually moving from N to P limitation (Peñuelas *et al*., 2012, 2013). Gaudio et al. (2015) describe N limitation in forests to become crucial under climate scenarios where the turnover rate of organic matter is slow and N deposition continues to decrease. Consequently, other nutrients such as P could also become limiting in the future or are already limiting in nutrient-poor ecosystems (Jonard *et al*., 2012). For soils, Mo et al. (2015) found that N addition resulted in an overall decline in nutrient availability (except N), which seemed to result from a decline in litter decomposition and increased soil acidification. Waldrop and Firestone (2004) reported that excess N reduced the

production of enzymes involved in decomposition, particularly in lignin degradation. Thus, soil respiration and decomposition were reduced, while soil-organic matter accumulation in fertilized soils was increased (Pregitzer et al., 2007; Nave et al., 2009; Janssens et al., 2010). Therefore, positive growth responses of trees to N fertilization might be constrained by negative fertilization effects on decomposition rates. In addition, missing effects of N fertilization might be due to the fact that elements other than N limit forest growth.

Jonard et al. (2015) as well as Talkner et al. (2015) consider P as the upcoming limiting factor in nutrient poor forest ecosystems which yet has to be approved by P fertilization trials (Polglase et al., 1992; Clarholm, 1993). The effect of P fertilization likely depends on the type of nutrient acquisition in ecosystems. In “acquiring ecosystems”, e.g. on young, nutrient-rich or just P-rich soils, P is mobilized from P-containing minerals in the soil and subsequently taken up by trees (Odum, 2014; Lang et al., 2016). In forests on old, highly weathered and/or P-poor soils, trees mainly rely on the mobilization from organically bound P in soil organic matter (“recycling ecosystems”; Hauenstein et al., 2018). Augusto et al. (2017) consider the parent material as the principal component controlling P availability in soils, beside the progression of pedogenesis, or climatic controls. This view is supported by Porder and Ramachandran (2012), who showed that parent material serves as principal control of bioavailable P in soil. Similar findings of forest ecosystem adaptions towards a “recycling” system along decreasing nutrient supply from different parent materials are described by several authors (Lang et al. 2016, 2017; Hansson et al. 2020; Legout et al. 2020). In case of such P-poor soils, P fertilization increased forest growth/productivity (Blevins et al., 2006; Trichet et al., 2009; Turner and Lambert, 2015). This effect was likely driven by soil processes exemplary described by Mo et al. (2015) who found that the addition of P to soils containing very little available P resulted in a significant ‘priming’ effect, which stimulated microbial activity and nutrient turnover of

litter, whereas no effect of P addition was observed in soils containing sufficiently available P. This is in line with Bergkemper et al. (2016) who described a shift in bacterial communities which led to increased organic P acquisition on P-poor soils, whereas in P-rich soils inorganic P acquisition dominated. By contrast, several authors reported the downregulation of enzyme activity slowing down P mobilization from organic P after the addition of water soluble mineral P to soil irrespective of initial P availability in soil (DeForest et al., 2012; Marklein and Houlton, 2012; Shaw and DeForest, 2013). Whether these P fertilization effects on P availability in soil translate into tree P uptake via xylem sap has hardly been studied (Prietzel and Stetter, 2010). We expect that N and P fertilization effects on forest growth and nutrient availability in soil are reflected in P uptake by trees i.e., P concentrations in xylem sap. In xylem sap, the addition of water-soluble mineral P to soil likely increases Pi concentrations by direct uptake of fertilizer P and by increased uptake from biologically cycled P originating from a stimulation of biological activity in soil.

To distinguish biological P mobilization processes from other processes, recent studies used the ratio of stable oxygen (O) isotopes in phosphate ($\delta^{18}O_{Pi}$) (Pistocchi et al., 2018; Hacker et al., 2019). This is possible because only biological processes involve an exchange between O atoms in phosphate and O atoms in ambient water (Blake et al., 2005), while no such exchange takes place during the dissolution of P-containing minerals and the desorption of $H_2PO_4^-$ /HPO_4^{2-} from charged surfaces (Liang and Blake, 2007). Particularly if the O isotope signature of ambient water is modified via isotope labeling by ^{18}O-enriched- or ^{18}O-depleted water, mobilization processes or distinct P sources can be identified (Hacker et al., 2019). Accordingly, an incorporation of O atoms from ^{18}O-enriched ambient water into phosphate by biological activity in soil should be visible in $\delta^{18}O_{Pi}$ values of xylem sap once phosphate has been taken up by trees. Given the assumption that an acquiring ecosystem relies much less

on nutrient release from organic matter via biological activity than a recycling ecosystem does, different contributions of biologically cycled phosphate i.e., different extents of ^{18}O-enrichment, in xylem sap can be expected. Similarly, a stimulation of biological activity in the soil by P fertilization should be reflected in a greater contribution of biologically cycled phosphate particularly so in the recycling ecosystem.

Our objectives were to test the effects of (i) site i.e., an acquiring versus a recycling ecosystem, and of (ii) fertilization (N, P, N+P) on Pi and Po concentrations as well as on biologically cycled phosphate (inferred from the O isotope signature after adding an ^{18}O label) in xylem sap.

2 Methods

2.1 Study sites

In the frame of the priority program SPP 1685 'Ecosystem Nutrition, Forest Strategies for limited Phosphorus Resources' (Lang et al. 2016), two temperate beech forest sites were selected for the purpose of this study. The site "Bad Brückenau" (BBR) is located in Bavaria, southeastern Germany in a midrange mountain area of the Rhön (50°35' N, 9°92' E). The potential natural vegetation at the site BBR is Hordelymo-Fagetum (Lang et al., 2017. The forest stand comprises 99% *Fagus sylvatica* L. and 1% *Acer pseudoplatanus* L. Mean stand age for beech is 137 years with an average height of 26.8 m, a mean (breast height) diameter of 36.8 cm and a number of 335 tress per hectare. At this site, mull-like Moder forest floors on a dystric skeletic Cambisols developed on alkaline igneous rocks/metamorphites (Haußmann and Lux, 1997).

The order of organic soil horizons (IUSS 2015) was as follows, for LUE: Oi (3 cm) - Oe (4 cm) – Oa (1 cm) and for BBR: Oi (3 cm) – Oe (3 cm). Mean annual air temperature and the mean sum of annual precipitation are 5.8 °C and 1031 mm, respectively (Level II ICP-forest monitoring;

Lorenz, 1995). At the site BBR yearly atmospheric N deposition was 13.8 kg N ha^{-1} (measured between 2009-20012; personal communication Dietrich, H.-P., LWF-Bayern). For more detailed site characteristics see Lang et al. (2017). During the experimental course, soil temperature at a depth of 0.05 m were, on average, of 13.0 ± standard deviation (SD) 0.4°C. During this period of time, 26.6 mm throughfall were reported (personal communication Dr. Mesenburg, NW-FVA). The site "Lüss" (LUE) is located in Lower Saxony, northwestern Germany in the Lüneburg Heath (52°83' N, 10°36' E). The potential natural vegetation at the site LUE is Luzulo-Fagetum (Lang et al., 2017). The forest stand comprises 91% *Fagus sylvatica* and 9% *Quercus petrea (Mattuschka) Liebl.*. Mean stand age for beech is 132 years with an average height of 27.3 m, a mean (breast height) diameter of 27.5 cm and a number of 480 tress per hectare. Organic layers and soil types are classified as a mor-like Moder forest floor on hyperdystric folic Cambisols developed from Pleistocene sands. The mean annual air temperature is 8° C and the annual sum of precipitation amounts to 779 mm (Level II ICP-forest monitoring; Lorenz, 1995). During the experimental course, the mean soil temperature mean at a depth of 0.06 m was 9.3 ± SD 0.1 °C and 23.8 mm throughfall were observed (personal communication Mr.Dietrich, LWF-Bayern). At the site LUE yearly atmospheric N deposition was 10 kg N ha^{-1} (measured between 2009-20012; personal communication Dietrich, H.-P., LWF-Bayern). Sites were selected according to their bioavailability of P, thus showing the ecosystem adaptions to different parent materials.

We assume the influence of the climate conditions to be important but not fundamental for the development and adaptions of the forest ecosystems on these sites.

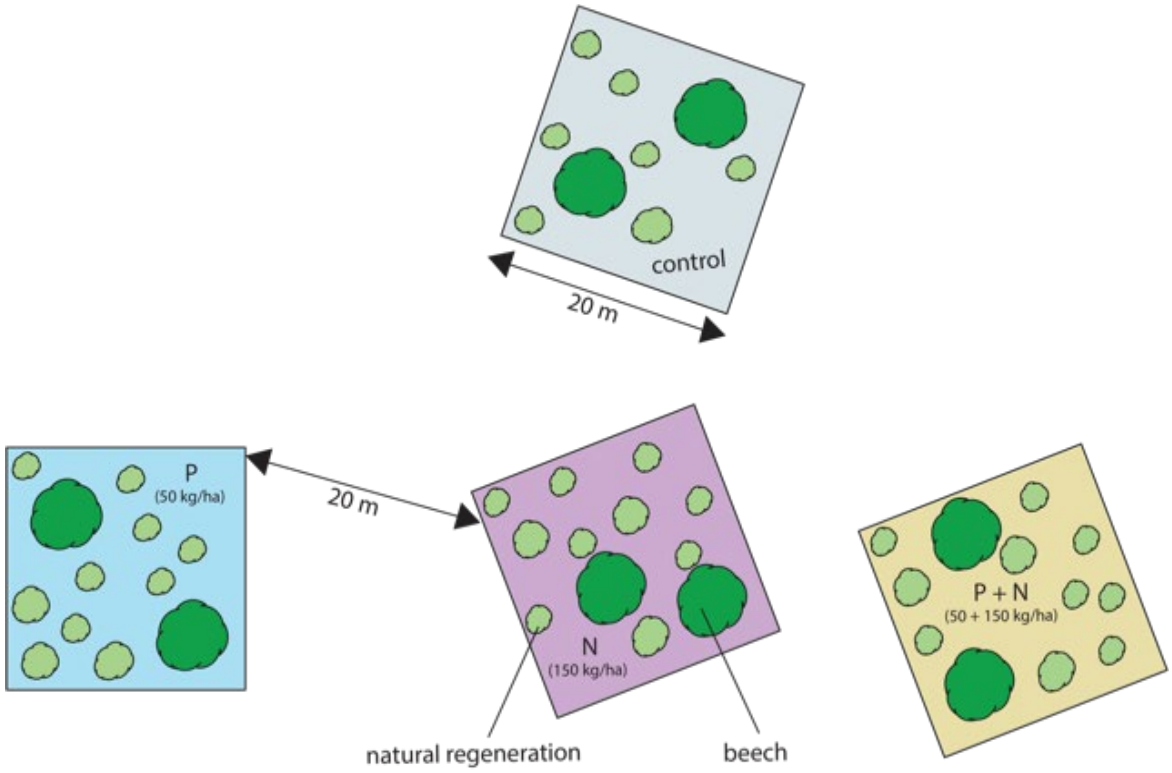


Figure A3-1: Sketch of the experimental design of the fertilizer treatments (done in triplicate). The amount of fertilizer applied is given in brackets in kg ha^{-1} $year^{-1}$. Nitrogen (N) and phosphorus (P) were applied as NH_4NO_3 and KH_2PO_4, respectively. Note that the control and N treatment received KCl to ease comparison with the P treatment which included the addition of K as well. Courtesy of Jaane Krüger.

2.2 Fertilization experiment

At both sites, a full-factorial fertilization experiment was established in April-May 2016. The experiment comprised a control and three fertilization treatments: N addition (150 kg N ha^{-1} $year^{-1}$ as NH_4NO_3), P addition (50 kg N ha^{-1} $year^{-1}$ as KH_2PO_4), N+P addition (combination of N and P addition). Control and treatment plots (20 m x 20 m) were established in triplicates which were randomly distributed in the stands while keeping a minimum distance of 20 m between plots (Fig. A3-1). Each plot contained at least two individuals of adult beech trees

(*Fagus sylvatica* L.) with a diameter at breast height of 46.8 ± SD 3.65 cm at the site BBR and 48.8 ± SD 5.81cm at the site LUE and an approximate age of 120 years.

The control and the N addition treatments received KCl to account for addition of K in the P-containing treatments. The addition of N was split into three parts (50 kg N ha^{-1} each) in spring, summer, and autumn to account for the seasonal demand of trees and to reduce leaching losses. The addition of P took place once a year along with the N addition in spring. The fertilizer addition was carried out in dissolved form by means of garden sprayers. The experiment was conducted in Juli/August 2017. We acknowledge that by restricting the fertilization to N and P, we did exclude the potential limitation of forest growth by elements other than N and P which was speculated by (Jonard et al., 2012). We tried to reach a conservative estimate of maximum N input accounting for the next decade of atmospheric N deposition. Nevertheless, similar to many other forest fertilization experiments, our fertilization approach did not take into account direct interactions between the fertilizer and the canopy which would have occurred had we manipulated atmospheric deposition directly.

2.3 Labeling by ^{18}O-enriched water

The isotope labeling took place 19.06.-21.06.2017 at the site BBR and 12.06-14.06.2017 at the site LUE. We used an area of 10.8 m^2 around each tree individual for the application of ^{18}O-enriched water. This area was formed by two circles with an identical center in the middle of each tree with radii of 0.4 m and 1.9 m. We prepared ^{18}O-enriched water by diluting tap water with ^{18}O-enriched water (>98 atom% ^{18}O, Hyox ^{18}O, rotem, Arava , Israel) aiming at $\delta^{18}O$ values of soil solution of around +40 ‰. Assuming thermodynamic equilibrium fractionation (Chang & Blake, 2015), $\delta^{18}O_{Pi}$ values of ++61.7 ‰ would result (see calculations for realized $\delta^{18}O_W$ values of soil solution in Fig. A3-S1). We applied the ^{18}O-enriched water using a syringe (100ml,

Vektenxi, amazon.de) that was composed of a 12 cm long needle with a closed tip and five outlet holes (1 mm diameter). We injected the ^{18}O-enriched water into the Oe horizon and the injection depth was adapted to the site-specific forest floor structure in order to be centered in the Oe horizon. To assure homogeneous application of the label, the area was divided into eight subareas. 60 injections of 2.5 ml water were allocated randomly to each subarea. In total, 1.2 l of ^{18}O-enriched water was applied in the prescribed area around each tree individual and this equals 2.1% (BBR) and 1.7% (LUE) of the average soil water content in the organic layers. To check for resulting labeling of soil water, organic layer samples were taken and stored in gas-tight vials until isotope analysis.

2.4 Xylem sap extraction and sample purification

In total, we sampled 48 trees (2 sites x 4 treatments x 2 time steps x 6 replicates). Two time steps were necessary to cover the potential duration until ^{18}O-enriched phosphate originating from incorporation of ^{18}O from the water label in soil were taken up by the trees. To follow the uptake of ^{18}O-enriched phosphate, we extracted xylem sap. To this end, branches (diameter of 0.8 - 2 cm) were harvested by tree climbers from the middle canopy. Directly after harvesting, we removed 2 cm of bark and cambium to prevent contamination with phloem constituents and tightly pulled a PE hose over the twigs. The twigs were placed in the Scholander pressure bomb (Soilmoisture 3000 Series Plant Water Status Console). The pressure was applied as N_2 gas (Grade 3). This procedure was repeated until a minimum amount of 30ml xylem sap was extracted. Directly after sampling the xylem sap was filtered through 1.2 µm PET syringe filters (CHROMAFIL Xtra PET-120/25, MACHEREY-NAGEL GmbH &

Co. KG, Düren, Germany). The samples were stored in a portable fridge at 4°C. Aliquots were split for $\delta^{18}O_W$ (1 ml) and $\delta^{18}O_{Pi}$ analyses.

Because some organic P compounds might be rapidly transformed into Pi in xylem sap, we processed xylem sap samples each day directly after returning from the field. We purified Pi following the protocol of Weiner et al. (2011) and added anion exchange resin membranes (VWR International GmbH, Bruchsal, Germany) to the xylem sap samples. After 16 h of shaking, the membranes were removed, rinsed with H_2O and eluated by HNO_3. Eluates were again stored in a portable fridge at 4°C until preparation for isotope analysis in the laboratory. In the laboratory, the eluate was used for precipitation of silver phosphate as described by Weiner et al. (2011). In brief, the mineral precipitation and dissolution of ammonium phospho-molybdate was followed by mineral precipitation and dissolution of magnesium ammonium phosphate. After removal of cations and proof of the absence of chloride, silver phosphate was precipitated.

2.5 Chemical analyses

Concentration of inorganic P (Pi) in xylem was determined spectrophotometrically with a continuous flow analyzer (CFA, AA3, XY2, Seal-Analytic, Norderstedt, Germany) at λ = 660 nm, using the method of Murphy and Riley (1962). Concentration of total P in xylem as well as total dissolved P concentrations in soil extraction solutions were measured by means of Inductively Coupled Plasma/Optical Emission Spectrometry (ICP-OES, PerkinElmer Optima 5300 DVGermany) at λ P 213.617. Organic P concentrations (Po) were calculated as the difference between total P and Pi concentrations (Toor et al., 2006). Limits of detection were 0.02 mg P l^{-1} for CFA and 0.05 mg P l^{-1} for ICP-OES.

Soil water for O isotope analysis was gathered by cryoextraction (Orlowski et al., 2013). $\delta^{18}O$ values of soil water extracts as well as of xylem sap were measured by cavitiy ring down

spectroscopy (PICARRO Inc., 480, Oakmead Parkway, Sunnvale, CA, 94085, US) The analysis of O isotope ratios of silver phosphate was carried out by means of a TC/EA (PYRO cube) coupled in continuous flow to an IRMS (IsoPrime100, both Elementar Analysensysteme; Hanau, Germany). Three triplicate subsamples (if enough silver phosphate was available) of each sample were weighed in silver capsules together with a small amount of glassy carbon powder to promote CO formation during combustion (Thermofisher scientific, Type 1, Kandel, Germany). The purity of the precipitated silver phosphate was ensured by the close match of standards and samples regarding the regression of O yield on analyte weight (Figure S2). Calibration and drift-corrections were accomplished by repeated measurements of two international benzoic acid standards, IAEA 601 and IAEA 602 ($\delta^{18}O$ =+23.3‰ and +71.4‰, respectively; distributed by the International Atomic Energy Agency, Vienna, Austria), and one internal Ag_3PO_4 standard ($\delta^{18}O$ = +10.2‰). The standard deviation of triplicate measurements was ± 0.6‰.

2.5 Calculations and statistical analyses

General site effects were derived from a comparison of control plots between sites. In these cases, differences between sites were tested using a student's t test. For single time steps and the complete design (i.e. including all treatments), we used a repeated measures ANOVA with site as between-subject factor and treatment (fertilization) as within-subject factor. Furthermore, we tested whether calculated differences between treatments (Δ) significantly deviated from zero based on a t test against zero. For time series, a repeated measures ANOVA was conducted with site as between-subject factor and time step as within-subject factor. If the prerequisite for statistical analyses was violated (non-normal distribution), Wilcoxon tests were performed instead. Statistical analysis was carried out by IBM SPSS Statistics 22.

3 Results

3.1 Site effects on P concentrations in xylem sap

Across control plots of both sites, Pi and Po concentrations ranged from 0.1 to 4.8 and 1.2 to 6.7 mg P l^{-1}, respectively. On average, we found Pi concentrations of 1.1 ± standard error (SE) 0.4 mg P l^{-1} and Po concentrations of 3.4 ± SE 0.6 mg P l^{-1}. In general, the variability of P concentrations in xylem was high at both sites (coefficient of variation [CV] 41% in BBR and 63% in LUE). The mean Pi:Po ratio was 0.33 ± SE 0.08. We found significantly higher Ptot, Pi, Po concentrations and Pi:Po ratios in BBR than in LUE (Fig. A3-2). In LUE, Po concentrations were 4.3 times higher than Pi concentrations (Fig. A3-2).

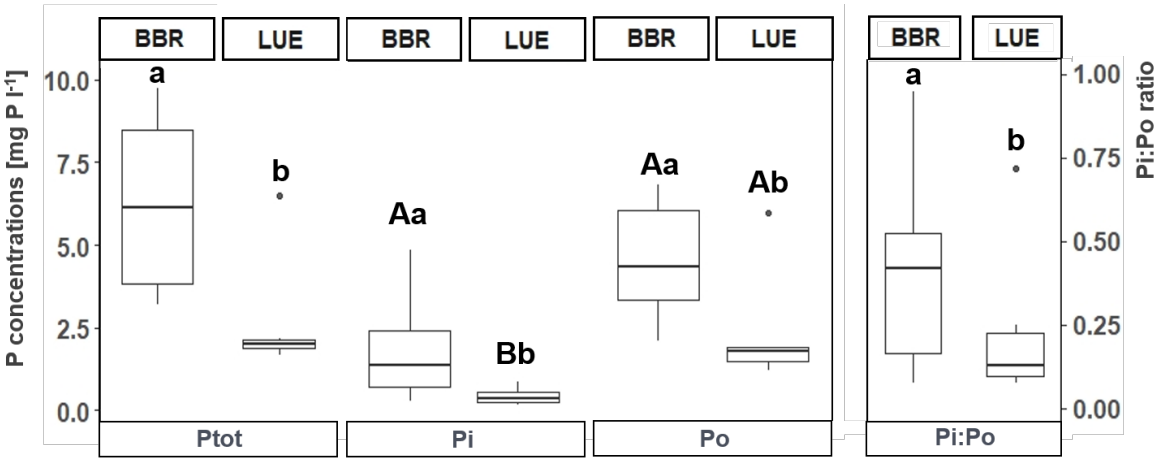


Figure A3-2: Total (Ptot), inorganic (Pi) and organic (Po) phosphorus concentrations (mg P l^{-1}), and Pi:Po ratios in xylem sap of trees in the control plots. Note that Pi:Po ratios refer to the second y axis. For ease of reading, samples were pooled from both sampling periods but treated separately for statistical analyses. Lowercase letters indicate differences between sites whereas uppercase letters indicate significant differences between Pi and Po concentrations of a given site. BBR = Bad Brückenau; LUE = Lüss.

3.2 Site effects on the O isotope signature of soil water, xylem sap, and phosphate of xylem sap after application of ^{18}O-enriched water

The application of ^{18}O-enriched water in the Oe horizon increased $\delta^{18}O_W$ values of soil solution to +41 ± SE 5 ‰ across sites and sampling dates and this represented a substantial increase as compared to $\delta^{18}O_W$ values before label application (BBR: -8.2 ‰ ± SD 0.9 ‰, LUE: -9.1 ‰ ± SD 0.5 ‰, Fig. A3-3a). This increase was similarly observed in BBR and LUE (compare boxplots and dashed red line in Fig. A3-3a). Despite the successful and consistent ^{18}O labeling of soil water at both sites, $\delta^{18}O_W$ values in xylem sap after labeling did not differ from those observed before label application in BBR (Fig. A3-3b). By contrast, $\delta^{18}O_W$ values of xylem sap in LUE were higher after than before labeling and significantly higher than in BBR at both sampling dates (Fig. A3-3b). In LUE, $\delta^{18}O_W$ values of xylem sap significantly decreased with time (Fig. A3-3b). At both sampling dates after the application of ^{18}O-enriched water (2 and 14 days), $\delta^{18}O_{Pi}$ values of xylem sap were significantly different to the background $\delta^{18}O_{Pi}$ values in xylem sap (mean $\delta^{18}O_{Pi}$ background = -9.15‰ in BBR and -2.26‰ in LUE). In BBR, the difference significantly decreased from 26.1 ± SE 1.7 ‰ to 7.8 ± SE 2.4 ‰ during the period from 2 to 14 days after label application (Fig. A3-4). In LUE, the difference was 9.8 ± SE 3.1 ‰ and 13.9 ± SE 2.2 ‰ 2 and 14 days after label application, respectively (Fig. A3-4). Notably, the variation in $\delta^{18}O_{Pi}$ values of xylem sap was smallest 2 days after label application in BBR (coefficient of variation [CV] 16% as opposed to 75% after 14 days), whereas in LUE $\delta^{18}O_{Pi}$ values of xylem sap varied the least 14 days after label application (CV 78 and 39% after 2 and 14 days, respectively).

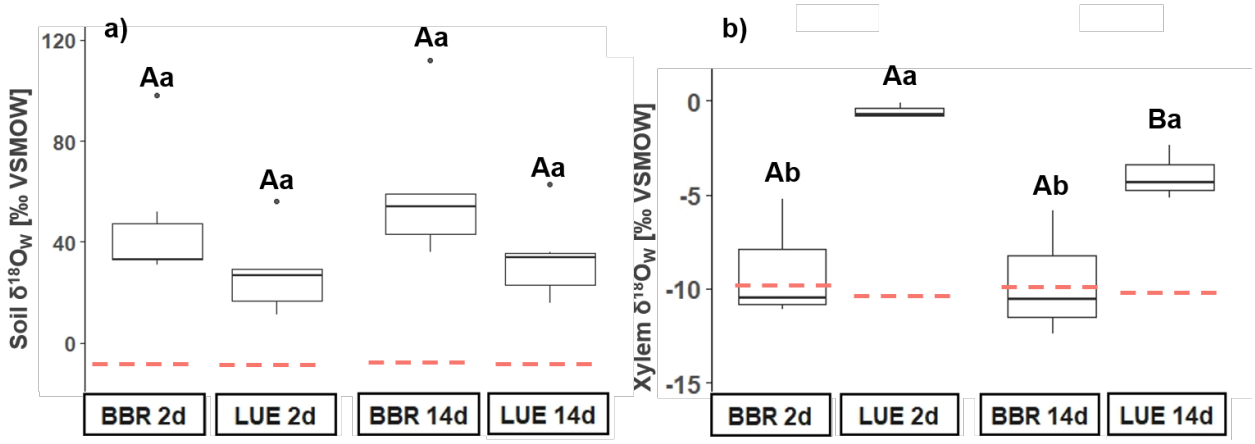


Figure A3-3: Oxygen isotope signatures ($\delta^{18}O$) of xylem sap water (xylem $\delta^{18}O_w$, a), and soil water (soil $\delta^{18}O_w$, b) of control plots on the two sampling dates (2d and 14d = 2 days and 14 days, respectively) after addition of ^{18}O-enriched water. Lowercase letters indicate significant differences between sites, whereas uppercase letters refer to significant differences between sampling dates. Red dashed lines show the background oxygen isotope signatures ($\delta^{18}O$) before ^{18}O addition (a) background: BBR: -8.2 ‰ ± SD 0.9 ‰, LUE: -9.1 ‰ ± SD 0.5 ‰, (b) background: BBR: -9.8 ‰, LUE: -10.6 ‰; BBR = Bad Brückenau; LUE = Lüss.

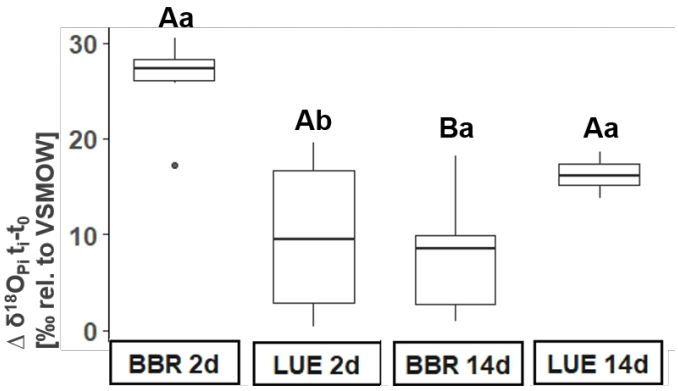


Figure A3-4: Difference between oxygen isotope signatures of inorganic phosphate ($\delta^{18}O_{Pi}$) in xylem sap before (background, t0) and after addition of ^{18}O-enriched water (ti) in the control plots. ti refers to the two time points after label application (2d and 14d = 2 days and 14 days, respectively). Lowercase letters indicate significant differences between sites, whereas uppercase letters refer to significant differences between sampling dates. BBR = Bad Brückenau; LUE = Lüss.

3.3 Fertilizer effects on P concentrations and O isotope signatures of phosphate in xylem sap

We found only few effects of fertilization on P concentrations in xylem sap. In BBR, N+P addition significantly increased Ptot and Pi concentrations while P addition resulted only in increased Pi concentrations in xylem sap of fertilized as compared to non-fertilized trees (Fig. A3-5a and A3-5b, Fig. A3-S3). As a consequence, the Pi:Po ratio of xylem sap was significantly higher in the P-fertilized than the non-fertilized trees in BBR (Fig. A3-5d). Total P concentrations in xylem sap of fertilized trees were increased as compared to non-fertilized trees by P addition in LUE (Fig. A3-5, but see Fig. A3-S3). $\delta^{18}O_{Pi}$ values of xylem sap were enriched in ^{18}O irrespective of fertilizer treatments in BBR two days and in LUE 14 days after labeling (Fig. A3-S4). Strikingly, only P fertilization (P, NP) resulted in an ^{18}O-enrichment of phosphate in xylem sap on the other sampling dates at each site (14 days in BBR and two days in LUE, Fig. A3-S4). However, $\delta^{18}O_{Pi}$ values of xylem sap were not affected by fertilization treatments (Fig. A3-S5).

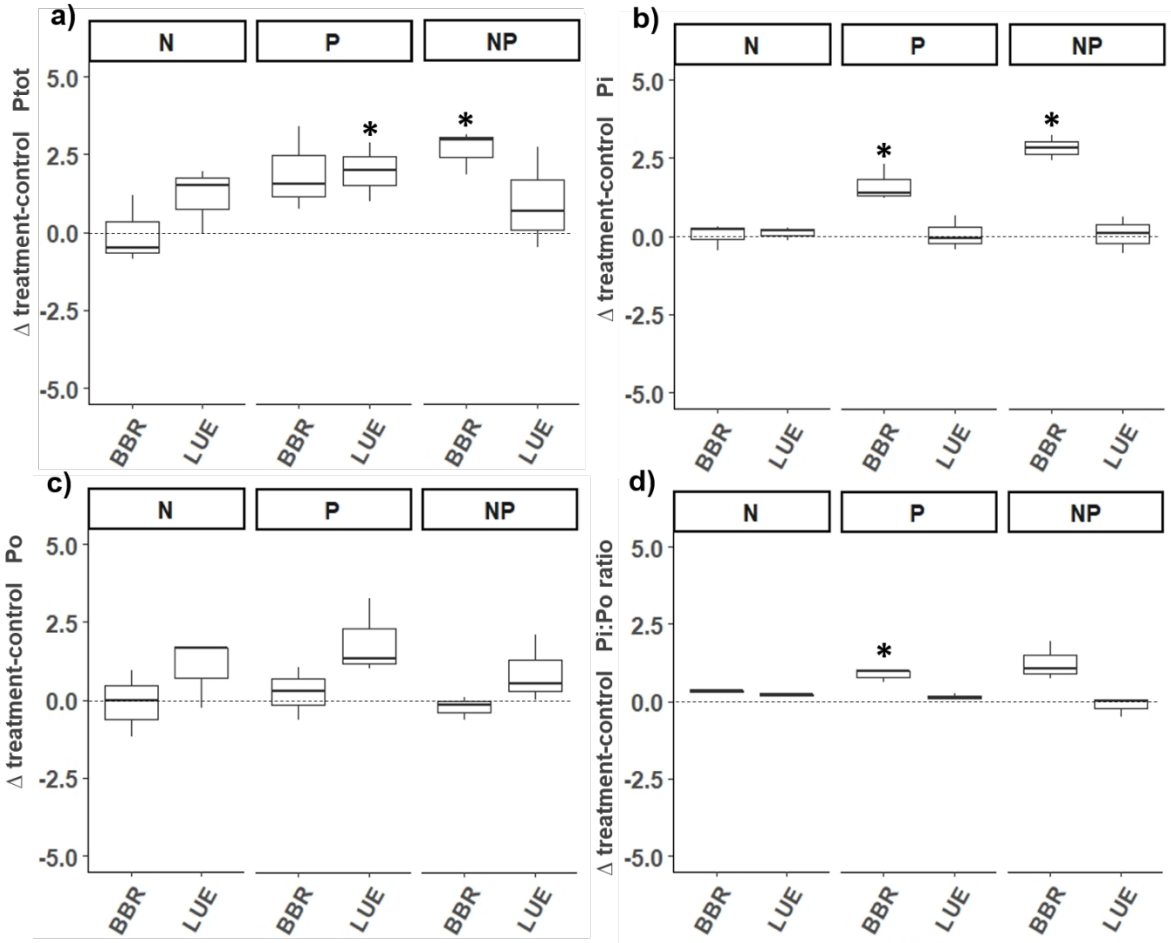


Figure A3-5: Difference in phosphorus concentrations (mg P l^{-1}) in xylem sap between fertilized trees (N, P, N+P) and non-fertilized trees (control) 14 days after application of ^{18}O-enriched water. Differences are displayed separately for total phosphorus (Ptot, a), inorganic phosphorus (Pi, b), organic phosphorus (Po, c) and the ratio of Pi to Po (d). Asterisks above boxplots indicate differences significantly deviating from zero (i.e., the control). BBR = Bad Brückenau; LUE = Lüss.

4 Discussion

4.1 Site effects on P concentrations and O isotope signatures of phosphate in xylem sap

The ranges of 0.1 to 4.8 mg P l^{-1} (Pi) and 1.2 to 6.7 mg P l^{-1} (Po) in our study were in the lower range of Pi and Po concentrations of xylem sap reported in the literature (Pi: 0.5 to 70 mg P l^{-1}; Po: 0.5 to 80 mg P l^{-1}) (Bollard, 1960; Saur et al., 1995; Prima-Putra and Botton, 1998; Netzer et al., 2017). In line with our hypotheses xylem sap total P concentrations were 2.3-fold higher in BBR than in LUE (Fig. A3-1) and this was corroborated by 10-fold higher $NaHCO_3$-P-extractable P concentrations in soil in BBR as compared to LUE (Hauenstein et al., 2018). Therefore, site-specific P availability in soil was reflected in P concentrations of xylem sap. The significantly higher Pi:Po ratios in xylem sap at the P-rich site BBR compared to the P-poor site LUE indicates an intensified uptake of inorganic P from soil solution. By contrast, the low Pi:Po ratios at the P-poor site LUE could be indicative of the uptake of Po. In line, Scheerer et al. (2019) showed that small P-containing organic molecules can be taken up directly (i.e, intact) by trees. Alternatively, low Pi:Po ratios might be indicative of tree-internal P cycling. For example, Netzer et al., (2017) described higher Po concentrations in xylem at P-poor sites postulating intensified tree internal P remobilization and enhanced internal P cycling efficiency. This is further corroborated by a larger difference in P concentrations between leaf litter and fresh leaves and thus, higher P resorption, in LUE as compared to BBR (Lang et al., 2017). In summary, not only transformations of Po to Pi in organic layers (Hauenstein et al., 2018) but also Po uptake and tree-internal cycling are decisive for P nutrition of trees at the P-poor site.

Because neither site nor date influenced $\delta^{18}O$ values of soil solution (Fig. A3-3a) and because of constant temperatures as well as negligible rainfall events during the experimental course,

we can exclude both the ^{18}O-enrichment of soil water via evaporation (Kendall and McDonnell, 1998) and the dilution of the ^{18}O tracer in soil solution via rainfall. Despite the continuous ^{18}O-enrichment of soil solution water at both sites, we observed an ^{18}O-enrichment of xylem sap in LUE only (Fig. A3-3b). Therefore, trees in LUE took up water from the organic layer to which we added the isotope label. In line, > 40% of the total fine roots (down to 1 m) are located in the organic layers in LUE (Hauenstein et al., 2018) and trees rely on the organic layer for water uptake. By contrast, 9% of the total fine roots can be found in the organic layer in BBR (Hauenstein et al., 2018) and thus, water uptake to a main proportion likely originates from deeper soil layer that we did not label. We observed differences in $\delta^{18}O$ values of xylem water between sampling dates in LUE (Fig. A3-3). These might indicate water uptake from deeper non-labeled soil layers in LUE as well. However, the absolute difference of 3.3‰ is too small to pinpoint shifting patterns during the experimental course.

We found significantly increased $\delta^{18}O_{Pi}$ values of xylem sap at both sites (Fig. A3-4). Notably, at both sites differences in $\delta^{18}O_{Pi}$ values after and before labeling were sufficiently large (+15‰) to exceed potential 'small' isotope fractionation of ca. 3 ‰ associated with active transporter-driven P uptake (Blake et al., 2005; Gross and Angert, 2015). Furthermore, the incorporation of ^{18}O into phosphate in xylem sap might originate from tree-internal biological processes. According to textbook knowledge, neither do microorganisms colonize the protoplast-free tracheids enabling xylem sap flow nor do extracellular enzymes exist in xylem sap. Therefore, xylem-internal biological processes resulting in an incorporation of ^{18}O-enriched xylem water into phosphate in xylem sap are highly unlikely. The only plausible explanation of a tree-internal ^{18}O incorporation into phosphate would be an exchange between phloem – containing P-containing metabolites produced in metabolically active plant organs such as leaves – and xylem. During metabolic reactions in leaves, O atoms from cell

water are incorporated into phosphate (Pfahler et al., 2013). Because in our study, xylem sap water was enriched in ^{18}O, this biological incorporation would be visible in xylem if there was a connection between phloem and xylem. We did not measure $\delta^{18}O$ values of cell water or $\delta^{18}O_{Pi}$ values in leaves. However, we can roughly estimate $\delta^{18}O_{Pi}$ values in metabolically active P fractions in leaves. Leaf cell water is enriched in ^{18}O caused by preferential evaporative losses of $H_2{}^{16}O$ (Dongmann et al., 1974). Gan et al. (2002) found a difference between xylem and leave mesophyll water ranging from +22.1 to +24.4‰ in cotton leaves. Based on $\delta^{18}O$ values of xylem sap water in our study (Fig.A3- 3), $\delta^{18}O$ values of leaf water of +14‰ (BBR) and +21‰ (LUE) would result. During metabolic reactions in leaves, all four O atoms in phosphate molecules are exchanged with those of cell-water-O and this reaction is accompanied by a temperature-dependent equilibrium fractionation (Pfahler et al., 2013) similar to observations of microbial metabolism (Blake et al., 2005). Applying the equation provided by Chang and Blake (2015) for a temperature of 13 and 8.5 °C (mean soil temperature in 5cm depth during the experiment in BBR and LUE, respectively), $\delta^{18}O_{Pi}$ values in leaves of 39‰ in BBR and 47‰ in LUE would result. Because estimated $\delta^{18}O_{Pi}$ values in leaves are far from those we observed and because we are not aware of studies on an exchange between xylem and phloem, we postulate that ^{18}O-enriched phosphate in xylem sap originates from the uptake of phosphate previously biologically cycled in soil rather than from tree-internal biological cycling of P. This assumption is in line with our finding that the biological incorporation of ^{18}O from ^{18}O-enriched ambient water into phosphate was visible at both sites and was in line with the ^{18}O enrichment of soil water but not of xylem sap water.

Interestingly, for the P-rich site BBR, Pi and water uptake seemed to be decoupled since phosphate contained the ^{18}O label while xylem sap did not (Fig. A3-4, Fig. A3-3). A decoupling of P and water uptake from different soil layers is plausible due to the energy-dependent

active Pi uptake (Schachtman et al., 1998). Trees can adapt the active Pi uptake to changing conditions, for example to the seasonal variation in P supply (Netzer et al., 2017). Therefore, an active uptake of Pi is not necessarily linked to water uptake. Conversely, the location of water and P uptake seem to be identical, namely the organic layer, at the P-poor site LUE since the ^{18}O enrichment of soil water, xylem sap and phosphate in xylem sap match (Fig. A3-3, Fig. A3-4). This is corroborated by the site-specific fine root distribution (Hauenstein et al., 2018) and in line with our hypotheses identifying distinct ecosysytem adaptions to P-poor sites. Nevertheless, we cannot pinpoint the exact location of water uptake due to a lack of data on $\delta^{18}O_W$ values of deeper soil layers.

Since the $\delta^{18}O_{Pi}$ values of xylem sap in BBR exhibit little variability and are highly enriched in ^{18}O already two days after label application (Fig. A3-4), ^{18}O from ambient water seem to be rapidly incorporated into phosphate which was subsequently taken up by trees in BBR. This short time scale is in line with the time scale of minutes to a few weeks until all four O atoms of a phosphate molecule are exchanged by O from isotopically labeled ambient water (Gross et al., 2015; Helfenstein et al., 2018; Sperber et al., 2017). The incorporation of ^{18}O from ambient water into phosphate is mediated by enzymes such as pyrophosphatases in microorganisms (Blake et al., 2005), or mono-/diesterases (Liang and Blake, 2006; Sperber et al., 2014). The fast occurrence of the ^{18}O label in xylem sap of BBR is corroborated by higher microbial activity (Heuck and Spohn, 2016; Pistocchi et al., 2018) as well as higher phosphatase activity (Lang et al., 2017) reported for BBR as compared to LUE. At the P-poor site LUE, a distinct occurrence of the ^{18}O label occurred after 14 days (Fig. A3-4). The slower appearance of the ^{18}O label corresponds to a slower forest floor turnover rate of 39 years in LUE compared to 5 years in BBR (Lang et al. 2017) illustrating a hampered biological activity in LUE. The slow turnover rates are linked to a greater contribution of fungi as compared to bacteria in organic

matter decomposition. This is corroborated by Zavic et al. (2018) who highlights the importance of Ectomycorrhrizal fungi (EMF) for P uptake by beech trees on P-poor sites. Similarly, Zavic et al. (2016) pinpoint the intense colonization of root tips by EMF in the organic layers. In summary, high availability of P in soil is reflected in tree P nutrition in acquiring ecosystems such as BBR. In these ecosystems, because of high biological activity, P supply of trees originates from biological processes and is not necessarily linked to water supply. The source of P supply is similar in acquiring and recycling ecosystems, however, the retarded biological processes result in less favorable P nutrition of trees in recycling as compared to acquiring ecosystems.

4.2 Fertilizer effects on P concentrations and O isotope signatures of phosphate in xylem sap

We found that P addition alone and in combination with N increased Pi concentrations in xylem sap of BBR (Fig. A3-5). Because data on tree growth or biomass are not available, it remains unclear whether the increased P uptake we observed for xylem sap was driven by fertilizer-induced increased biomass production or by growth-independent "luxury" P uptake. Other studies indicate that increased P uptake is mostly associated to growth-related increased P demand of trees (Goswami et al., 2018). At first sight, one might guess that the increased P uptake derives from the fertilizer source itself. If this was the case, the O isotope signature of the fertilizer would predominate without shifts caused by biological cycling. However, ^{18}O enrichment of phosphate in xylem sap took place (Fig. A3-4) and cannot be explained by fertilizer uptake because the fertilizer was not enriched in ^{18}O ($\delta^{18}O_{Pi}$ values = +19 ‰). Furthermore, $\delta^{18}O_{Pi}$ values were ^{18}O-enriched irrespective of treatment at the dates where the label was most intensely recovered in xylem sap at each site (BBR: two days, LUE:

14 days; Fig. A3-4, A3-S5). At the dates where label recovery was not as pronounced (BBR: 14 days; LUE two days), P fertilization (P, NP) still significantly increased $\delta^{18}O_{Pi}$ values in xylem sap which was not the case for the control and N treatment (Fig. A3-S5). Because of our interpretation of the ^{18}O-enrichment as a result of biological ^{18}O incorporation into phosphate prior to P uptake (see previous chapter on site effects), we infer that (i) fertilizer-P was cycled biologically i.e., taken up and released by soil microorganisms, and/or (ii) P fertilization stimulated biological activity in soil (Mo et al., 2015) and thereby increased biological incorporation of ^{18}O into phosphate prior to uptake by trees.

5 Conclusions

Our ^{18}O labeling approach highlights decoupled water and P uptake at the P-rich site BBR representing an acquiring ecosystem. By contrast, trees relied on the organic layer for both water and P uptake at the P-poor site LUE (recycling ecosystem). Foresters should consider that ecosystem resilience particularly at P-poor ecosystems might be strongly influenced by processes that deteriorate the organic matter and subsequently changes the forest floor structure and thereby its function as a place of water retention and nutrient retention from leaching to mineral soil layers. In this regard, ongoing N deposition and increasing soil temperatures especially in the forest floor due to climate change have the potential to considerably change forest floor turnover dynamics.

6 Acknowledgements

The authors gratefully acknowledge funding by the German Research Foundation (DFG) (Oe516/6-2 in the priority program SPP 1685 ‘‘Ecosystem Nutrition: Forest Strategies for Limited Phosphorus Resources’’). Furthermore, we are grateful for support by the forest tree climbers from Michael Furche Baumservice and Baumfällung de Vries. Also, we want to thank our Colleagues from Freiburg from the AG of Prof. Friederike Lang and all who included people who established the fertilization experiment at the forest sites.

7 Authors

YO conceived the idea of the study, SH and MN conducted the study. The manuscript was jointly written by SH and YO.

8 REFERENCES

Augusto, L., Achat, D. L., Jonard, M., Vidal, D., and Ringeval, B. (2017). Soil parent material-A major driver of plant nutrient limitations in terrestrial ecosystems. Global change biology 23, 3808–3824. doi: 10.1111/gcb.13691

Blake, R. E., O'Neil, J. R., and Surkov, A. V. (2005). Biogeochemical cycling of phosphorus Insights from oxygen isotope effects of phosphoenzymes. *American Journal of Science* 305, 596–620.

Blevins, L. L., Prescott, C. E., and van Niejenhuis, A. (2006). The roles of nitrogen and phosphorus in increasing productivity of western hemlock and western redcedar plantations on northern Vancouver Island. *Forest Ecology and Management* 234, 116–122. doi: 10.1016/j.foreco.2006.06.029

Brinkmann, N., Seeger, S., Weiler, M., Buchmann, N., Eugster, W., and Kahmen, A. (2018). Employing stable isotopes to determine the residence times of soil water and the temporal origin of water taken up by Fagus sylvatica and Picea abies in a temperate forest. New Phytol 219, 1300–1313. doi: 10.1111/nph.15255

By E. G. BOLLARD (1960). Transport in the Xylem. *Plant Physiol.*, 141–166.

Chang, S. J., and Blake, R. E. (2015). Precise calibration of equilibrium oxygen isotope fractionations between dissolved phosphate and water from 3 to 37°C. *Geochimica et Cosmochimica Acta* 150, 314–329. doi: 10.1016/j.gca.2014.10.030

Clarholm, M. (1993). Microbial biomass P, labile P, and acid phosphatase activity in the humus layer of a spruce forest, after repeated additions of fertilizers. *Biol Fert Soils*, 287–292.

Daniel P. Schachtman, Robert J. Reid, and S.M. Ayling (1998). Phosphorus Uptake by Plants: From Soil to Cell. *Plant Physiol.* 116, 447–453.

DeForest, J. L., Smemo, K. A., Burke, D. J., Elliott, H. L., and Becker, J. C. (2012). Soil microbial responses to elevated phosphorus and pH in acidic temperate deciduous forests. *Biogeochemistry* 109, 189–202. doi: 10.1007/s10533-011-9619-6

Dongmann, G., Nrnberg, H. W., Frstel, H., and Wagener, K. (1974). On the enrichment of H2 18O in the leaves of transpiring plants. *Radiat Environ Biophys* 11, 41–52. doi: 10.1007/BF01323099

Finzi, A. C. (2009). Decades of atmospheric deposition have not resulted in widespread phosphorus limitation or saturation of tree demand for nitrogen in southern New England. *Biogeochemistry* 92, 217–229. doi: 10.1007/s10533-009-9286-z

Gaj, M., and McDonnell, J. J. (2019). Possible soil tension controls on the isotopic equilibrium fractionation factor for evaporation from soil. *Hydrol. Process.* 33, 1629–1634. doi: 10.1002/hyp.13418

Gan, K. S., Wong, S. C., Yong, J. W. H., and Farquhar, G. D. (2002). 18 O Spatial Patterns of Vein Xylem Water, Leaf Water, and Dry Matter in Cotton Leaves. *Plant Physiol.* 130, 1008–1021. doi: 10.1104/pp.007419

Gaudio, N., Belyazid, S., Gendre, X., Mansat, A., Nicolas, M., Rizzetto, S., et al. (2015). Combined effect of atmospheric nitrogen deposition and climate change on temperate forest soil biogeochemistry: A modeling approach. Ecological Modelling 306, 24–34. doi: 10.1016/j.ecolmodel.2014.10.002

Goswami, S., Fisk, M. C., Vadeboncoeur, M. A., Garrison-Johnston, M., Yanai, R. D., and Fahey, T. J. (2018). Phosphorus limitation of aboveground production in northern hardwood forests. *Ecology* 99, 438–449. doi: 10.1002/ecy.2100

Gross, A., and Angert, A. (2015). What processes control the oxygen isotopes of soil bio-available phosphate? *Geochimica et Cosmochimica Acta* 159, 100–111. doi: 10.1016/j.gca.2015.03.023

Gurpal S.Toor, StefanHunger, J. DerekPeak, J. ThomasSims, Donald L.Sparks (2006). Advances in the Characterization of Phosphorus in Organic Wastes: Environmental and Agronomic Applications. *Acta Sci. Agron.* 89, 1–72. doi: 10.1016/S0065-2113(05)89001-7

Hacker, N., Wilcke, W., and Oelmann, Y. (2019). The oxygen isotope composition of bioavailable phosphate in soil reflects the oxygen isotope composition in soil water driven by plant diversity effects on evaporation. *Geochimica et Cosmochimica Acta*. doi: 10.1016/j.gca.2018.11.023

Hansson, K., Laclau, J.-P., Saint-André, L., Mareschal, L., van der Heijden, G., Nys, C., et al. (2020). Chemical fertility of forest ecosystems. Part 1: Common soil chemical analyses were poor predictors of stand productivity across a wide range of acidic forest soils. Forest Ecology and Management 461, 117843. doi: 10.1016/j.foreco.2019.117843

Hauenstein, S., Neidhardt, H., Lang, F., Krüger, J., Hofmann, D., Pütz, T., et al. (2018). Organic layers favor phosphorus storage and uptake by young beech trees (Fagus sylvatica L.) at nutrient poor ecosystems. *Plant Soil* 432, 289–301. doi: 10.1007/s11104-018-3804-5

Helfenstein, J., Tamburini, F., Sperber, C. von, Massey, M. S., Pistocchi, C., Chadwick, O. A., et al. (2018). Combining spectroscopic and isotopic techniques gives a dynamic view of phosphorus cycling in soil. *Nature communications* 9, 3226. doi: 10.1038/s41467-018-05731-2

Heuck, C., and Spohn, M. (2016). Carbon, nitrogen and phosphorus net mineralization in organic horizons of temperate forests: Stoichiometry and relations to organic matter quality. *Biogeochemistry* 131, 229–242. doi: 10.1007/s10533-016-0276-7

Janssens, I. A., Dieleman, W., Luyssaert, S., Subke, J.-A., Reichstein, M., Ceulemans, R., et al. (2010). Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geosci* 3, 315–322. doi: 10.1038/ngeo844

Jonard, M., Legout, A., Nicolas, M., Dambrine, E., Nys, C., Ulrich, E., et al. (2012). Deterioration of Norway spruce vitality despite a sharp decline in acid deposition: A long-term integrated perspective. Glob Change Biol 18, 711–725. doi: 10.1111/j.1365-2486.2011.02550.x

Jonard, M., Furst, A., Verstraeten, A., Thimonier, A., Timmermann, V., Potocic, N., et al. (2015). Tree mineral nutrition is deteriorating in Europe. *Global change biology* 21, 418–430. doi: 10.1111/gcb.12657

Kendall, C., and McDonnell, J. J. (1998). *Isotope tracers in catchment hydrology*. Amsterdam, New York: Elsevier.

Lang, F., Bauhus, J., Frossard, E., George, E., Kaiser, K., Kaupenjohann, M., et al. (2016). Phosphorus in forest ecosystems: New insights from an ecosystem nutrition perspective. *J. Plant Nutr. Soil Sci.* 179, 129–135. doi: 10.1002/jpln.201500541

Lang, F., Krüger, J., Amelung, W., Willbold, S., Frossard, E., Bünemann, E. K., et al. (2017). Soil phosphorus supply controls P nutrition strategies of beech forest ecosystems in Central Europe. *Biogeochemistry* 127, 255. doi: 10.1007/s10533-017-0375-0

Legout, A., Hansson, K., van der Heijden, G., Laclau, J.-P., Mareschal, L., Nys, C., et al. (2020). Chemical fertility of forest ecosystems. Part 2: Towards redefining the concept by untangling the role of the different components of biogeochemical cycling. Forest Ecology and Management 461, 117844. doi: 10.1016/j.foreco.2019.117844

Liang, Y., and Blake, R. E. (2006). Oxygen isotope signature of Pi regeneration from organic compounds by phosphomonoesterases and photooxidation. *Geochimica et Cosmochimica Acta* 70, 3957–3969. doi: 10.1016/j.gca.2006.04.036

Liang, Y., and Blake, R. E. (2007). Oxygen isotope fractionation between apatite and aqueous-phase phosphate: 20–45 °C. *Chemical Geology* 238, 121–133. doi: 10.1016/j.chemgeo.2006.11.004

Lorenz, M. (1995). International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests-ICP Forests-. *Water Air Soil Pollut* 85, 1221–1226. doi: 10.1007/BF00477148

Lovett, G. M., Arthur, M. A., Weathers, K. C., Fitzhugh, R. D., and Templer, P. H. (2013). Nitrogen Addition Increases Carbon Storage in Soils, But Not in Trees, in an Eastern U.S. Deciduous Forest. *Ecosystems* 16, 980–1001. doi: 10.1007/s10021-013-9662-3

Magill, A. H., Aber, J. D., Currie, W. S., Nadelhoffer, K. J., Martin, M. E., McDowell, W. H., et al. (2004). Ecosystem response to 15 years of chronic nitrogen additions at the Harvard Forest LTER, Massachusetts, USA. *Forest Ecology and Management* 196, 7–28. doi: 10.1016/j.foreco.2004.03.033

Marklein, A. R., and Houlton, B. Z. (2012). Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytol* 193, 696–704. doi: 10.1111/j.1469-8137.2011.03967.x

Marschner, H. (2008). Mineral nutrition of higher plants. Amsterdam: Acad. Press.

Mo, Q., Zou, B., Li, Y., Chen, Y., Zhang, W., Mao, R., et al. (2015). Response of plant nutrient stoichiometry to fertilization varied with plant tissues in a tropical forest. *Scientific reports* 5, 14605. doi: 10.1038/srep14605

Murphy, J., and Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* 27, 31–36. doi: 10.1016/S0003-2670(00)88444-5

Nadelhoffer, K. J., Colman, B. P., Currie, W. S., Magill, A., and Aber, J. D. (2004). Decadal-scale fates of tracers added to oak and pine stands under ambient and elevated N inputs at the Harvard Forest (USA). *Forest Ecology and Management* 196, 89–107. doi: 10.1016/j.foreco.2004.03.014

Nave, L. E., Vance, E. D., Swanston, C. W., and Curtis, P. S. (2009). Impacts of elevated N inputs on north temperate forest soil C storage, C/N, and net N-mineralization. *Geoderma* 153, 231–240. doi: 10.1016/j.geoderma.2009.08.012

Netzer, F., Schmid, C., Herschbach, C., and Rennenberg, H. (2017). Phosphorus-nutrition of European beech (Fagus sylvatica L.) during annual growth depends on tree age and P-availability in the soil. *Environmental and Experimental Botany* 137, 194–207. doi: 10.1016/j.envexpbot.2017.02.009

Odum, E. P. (2014). "The strategy of Ecosystem development," in *The Ecological Design and Planning Reader*, ed. F. O. Ndubisi (Washington, DC: Island Press/Center for Resource Economics), 203–216.

Orlowski, N., Frede, H.-G., Brüggemann, N., and Breuer, L. (2013). Validation and application of a cryogenic vacuum extraction system for soil and plant water extraction for isotope analysis. *J. Sens. Sens. Syst.* 2, 179–193. doi: 10.5194/jsss-2-179-2013

Peñuelas, J. and Sardans, J. (2012). The Role of Plants in the Effects of Global Change on Nutrient Availability and Stoichiometry in the Plant-Soil System. PLANT PHYSIOLOGY 160, 1741–1761. doi: 10.1104/pp.112.208785

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., et al. (2013). Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. Nature communications 4, 2934. doi: 10.1038/ncomms3934

Pfahler, V., Dürr-Auster, T., Tamburini, F., M. Bernasconi, S., and Frossard, E. (2013). 18 O enrichment in phosphorus pools extracted from soybean leaves. *New Phytol* 197, 186–193. doi: 10.1111/j.1469-8137.2012.04379.x

Pistocchi, C., Mészáros, É., Tamburini, F., Frossard, E., and Bünemann, E. K. (2018). Biological processes dominate phosphorus dynamics under low phosphorus availability in organic horizons of temperate forest soils. *Soil Biology and Biochemistry* 126, 64–75. doi: 10.1016/j.soilbio.2018.08.013

Polglase, P. J., Jokela, E. J., and Comerford, N. B. (1992). Phosphorus, Nitrogen, and Carbon Fractions in Litter and Soil of Southern Pine Plantations. *Soil Science Society of America Journal* 56, 566–573. doi: 10.2136/sssaj1992.03615995005600020036x

Porder, S., and Ramachandran, S. (2012). The phosphorus concentration of common rocks—a potential driver of ecosystem P status. Plant Soil 367, 41–55. doi: 10.1007/s11104-012-1490-2

Pregitzer, K. S., Burton, A. J., Zak, D. R., and Talhelm, A. F. (2007). Simulated chronic nitrogen deposition increases carbon storage in Northern Temperate forests. *Global change biology* 0, 071121035853002-??? doi: 10.1111/j.1365-2486.2007.01465.x

Prietzel, J., and Stetter, U. (2010). Long-term trends of phosphorus nutrition and topsoil phosphorus stocks in unfertilized and fertilized Scots pine (Pinus sylvestris) stands at two sites in Southern Germany. *Forest Ecology and Management* 259, 1141–1150. doi: 10.1016/j.foreco.2009.12.030

Prima-Putra, D., and Botton, B. (1998). Organic and inorganic compounds of xylem exudates from five woody plants at the stage of bud breaking. *Journal of Plant Physiology* 153, 670–676. doi: 10.1016/S0176-1617(98)80219-8

Saur, E., Brechet, C., Lambrot, C., and Masson, P. (1995). Micronutrient composition of xylem sap and needles as a result of P-fertilization in maritime pine. *Trees* 10, 52–54. doi: 10.1007/BF00197780

Scheerer, U., Netzer, F., Bauer, A. F., and Herschbach, C. (2018). Measurements of 18 O-Pi uptake indicate fast metabolism of phosphate in tree roots. *Plant biology (Stuttgart, Germany)*. doi: 10.1111/plb.12922

Scheerer, U., Trube, N., Netzer, F., Rennenberg, H., and Herschbach, C. (2019). ATP as Phosphorus and Nitrogen Source for Nutrient Uptake by Fagus sylvatica and Populus x canescens Roots. *Frontiers in plant science* 10, 378. doi: 10.3389/fpls.2019.00378

Shaw, A. N., and DeForest, J. L. (2013). The cycling of readily available phosphorus in response to elevated phosphate in acidic temperate deciduous forests. *Applied Soil Ecology* 63, 88–93. doi: 10.1016/j.apsoil.2012.09.008

Sperber, C. von, Kries, H., Tamburini, F., Bernasconi, S. M., and Frossard, E. (2014). The effect of phosphomonoesterases on the oxygen isotope composition of phosphate. *Geochimica et Cosmochimica Acta* 125, 519–527. doi: 10.1016/j.gca.2013.10.010

Spohn, M., Zavišić, A., Nassal, P., Bergkemper, F., Schulz, S., Marhan, S., et al. (2018). Temporal variations of phosphorus uptake by soil microbial biomass and young beech trees in two forest soils with contrasting phosphorus stocks. *Soil Biology and Biochemistry* 117, 191–202. doi: 10.1016/j.soilbio.2017.10.019

Talkner, U., Meiwes, K. J., Potočić, N., Seletković, I., Cools, N., Vos, B. de, et al. (2015). Phosphorus nutrition of beech (Fagus sylvatica L.) is decreasing in Europe. *Annals of Forest Science* 72, 919–928. doi: 10.1007/s13595-015-0459-8

Trichet, P., Bakker, M. R., Augusto, L., Alazard, P., and Merzeau, D. and Saur E. (2009). Fifty Years of Fertilization Experiments on Pinus pinaster in Southwest France: The Importance of Phosphorus as a Fertilizer. *Forest Science*, 390–402.

Turner, J., and Lambert, M. J. (2015). Long-term growth responses to phosphatic fertilisers in a Pinus radiata plantation. *Australian Forestry* 78, 207–218. doi: 10.1080/00049158.2015.1071679

Waldrop, M. P., and Firestone, M. K. (2004). Altered utilization patterns of young and old soil C by microorganisms caused by temperature shifts and N additions. *Biogeochemistry* 67, 235–248. doi: 10.1023/B:BIOG.0000015321.51462.41

Weiner, T., Mazeh, S., Tamburini, F., Frossard, E., Bernasconi, S. M., Chiti, T., et al. (2011). A method for analyzing the $\delta^{18}O$ of resin-extractable soil inorganic phosphate. *Rapid Commun. Mass Spectrom.* 25, 624–628. doi: 10.1002/rcm.4899

Zavišić, A., Nassal, P., Yang, N., Heuck, C., Spohn, M., Marhan, S., et al. (2016). Phosphorus availabilities in beech (Fagus sylvatica L.) forests impose habitat filtering on ectomycorrhizal communities and impact tree nutrition. *Soil Biology and Biochemistry* 98, 127–137. doi: 10.1016/j.soilbio.2016.04.006

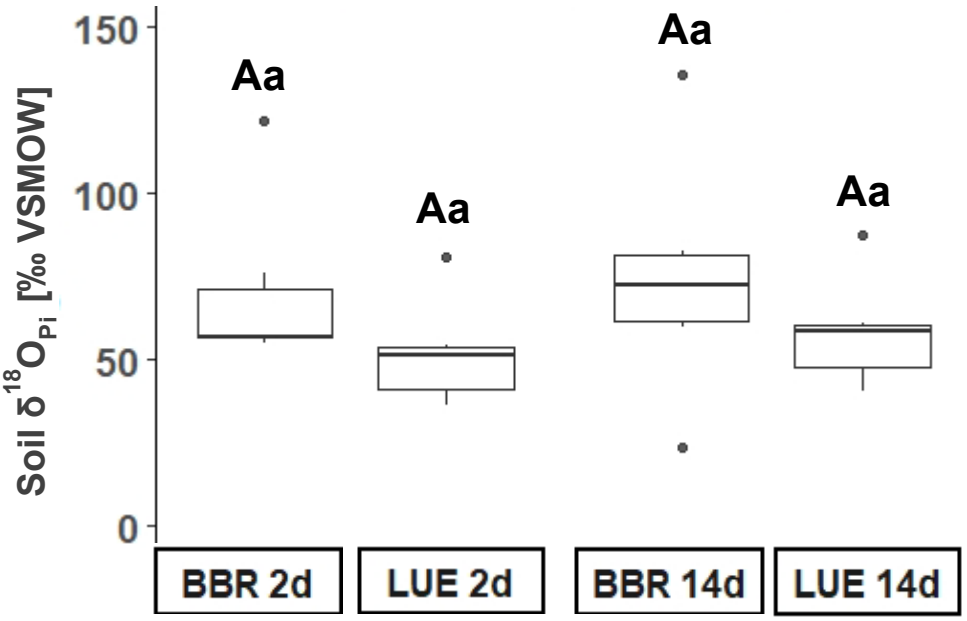
9 Supplementary

Supplementary table

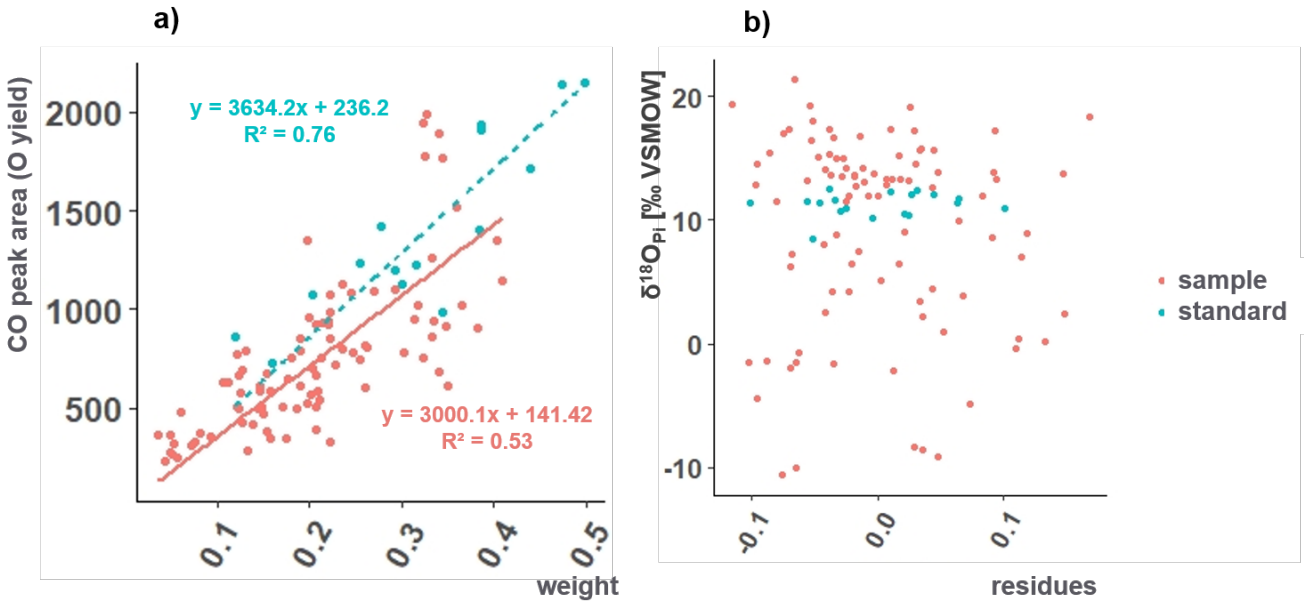
Supplementary Table A3-S1: Site characteristics of Lüss (LUE) and Bad Brückenau (BBR) MAT = mean annual temperature, MAP = mean annual precipitation, Pi = inorganic P,TP= total P, bioavailable Pi = PO_4-P citr.; C=C-total (C-tot≙Corg); (Data from: Lang et al. 2017)

site	**BBR**				**LUE**					
horizon	**O**	**Ah**	**BA**	**Bw**	**O**	**AE**	**E**	**Bsh**	**Bsw**	**Bw**
depth	**+15 - 0**	**0 - -14**	**-14 - -33**	**-33 - -75**	**+13 - 0**	**0 - -7**	**-7 - -12**	**-12 - -20**	**-20 - -35**	**-35 - -88**
pH (H_2O)	5.4	4.0	4.7	5.3	3.6	3.5	3.8	3.8	4.2	4.5
C [mg g^{-1}]	487	114	59	21	449	97	18	14	10	6
N [mg g^{-1}]	16.9	7.5	4.8	1.3	16.8	3.8	0.7	0.6	0.5	0.4
TP [mg kg^{-1}]	1449	3199	3033	2012	709	196	119	118	129	181
bioavailable Pi [mg kg^{-1}]	324	43	35	98	106	15	7	9	8	7
C:N	29	18	13	14	27	25	26	24	20	13
C:P	336	42	21	9	633	492	153	120	156	33
N:P	12	2	1.5	0.9	24	19	6	5	4	2

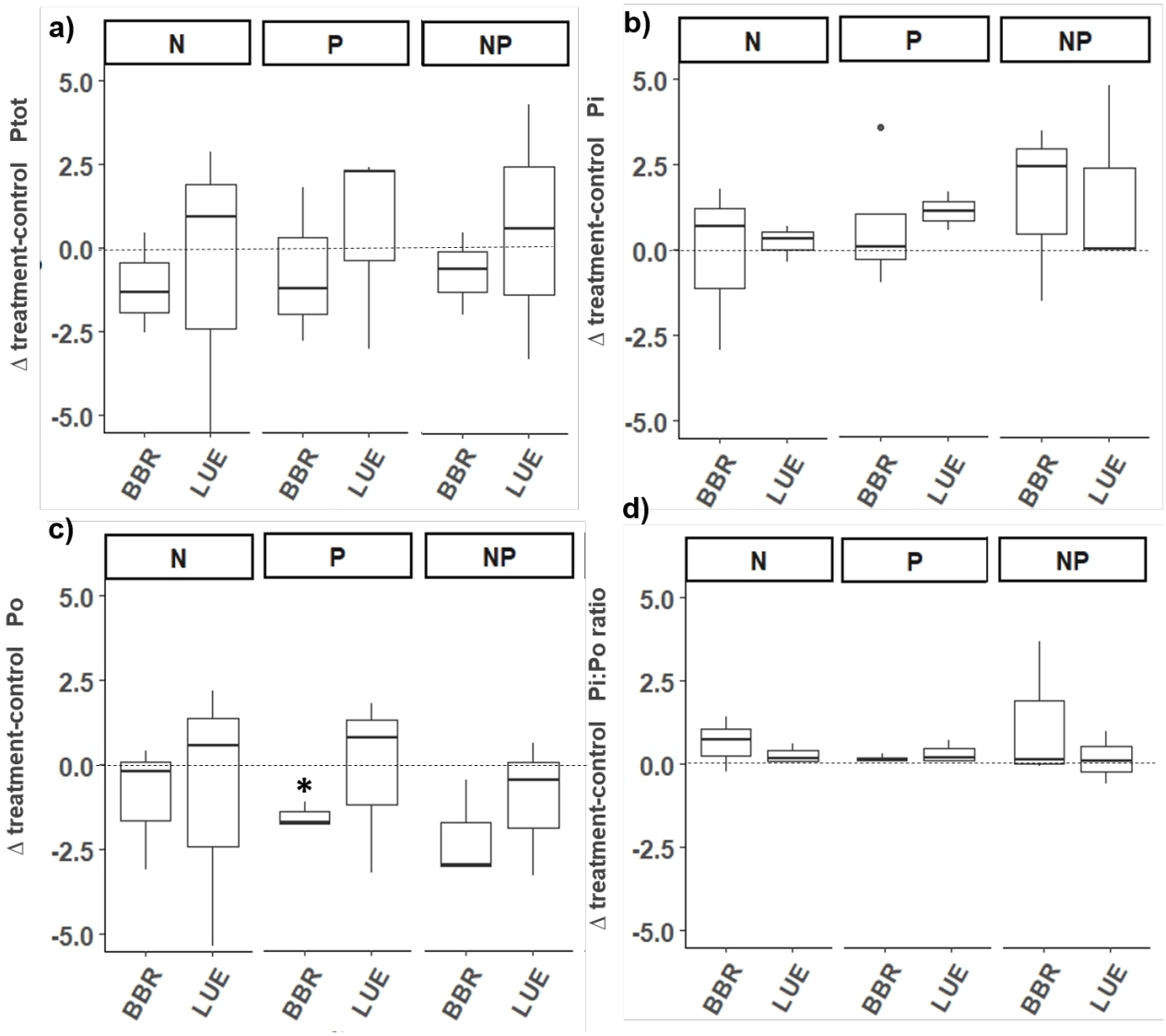
Supplementary figures



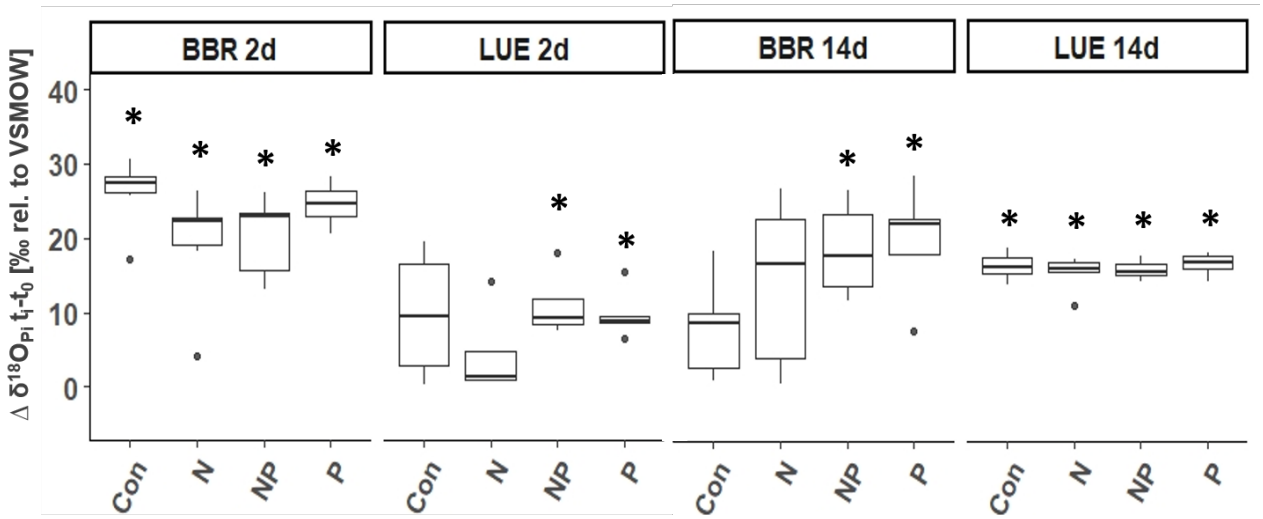
Supplementary Figure A3-S1. Expected $\delta^{18}O_{Pi}$ values of soil solution (calculated assuming thermodynamic equilibrium fractionation; the equation is given in Chang and Blake, 2015). Data is split by the two sampling dates from control plots (2d and 14d = 2 days and 14 days, respectively). Lowercase letters indicate significant differences between sites, whereas uppercase letters refer to significant differences between sampling dates. BBR = Bad Brückenau; LUE = Lüss.



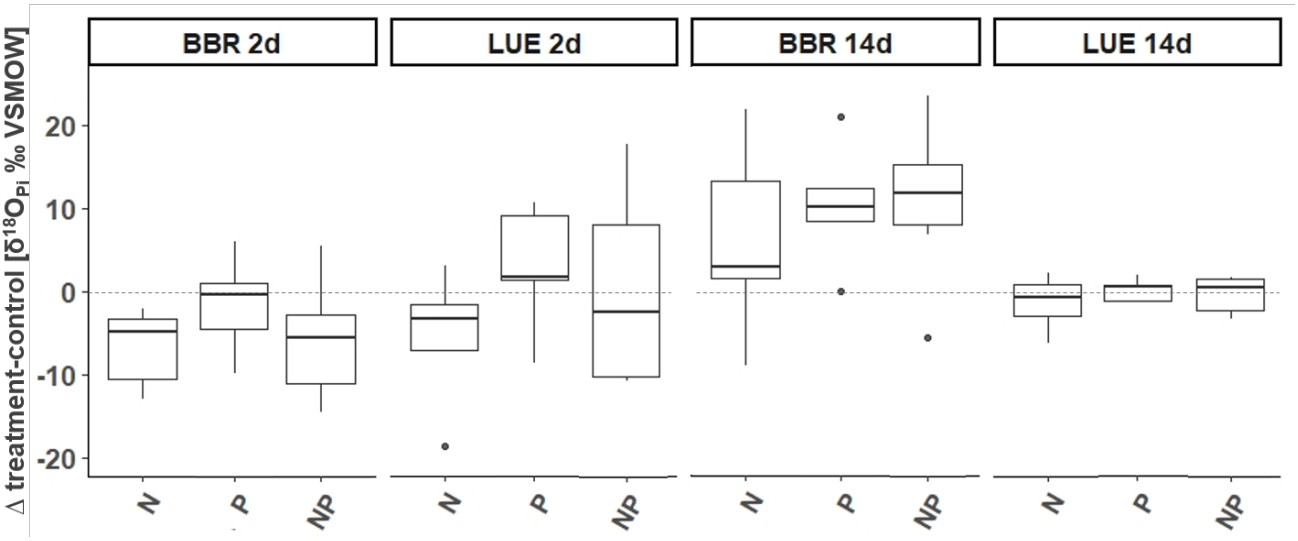
Supplementary Figure A3-S2. (a) Regression of CO peak areas on analyte weight of Ag_3PO_4 standards (red dots) and samples (green dots) as a measure of the O yield. (b) Regression of the O isotope signature ($\delta^{18}O_{Pi}$) of phosphate on residuals derived from regressions in (a).



Supplementary Figure A3-S3. Difference in phosphorus concentrations (mg P l^{-1}) in xylem sap between fertilized (N, P, N+P) and non-fertilized trees (control) 2 days after application of ^{18}O enriched water. Differences are displayed separately for total phosphorus (Ptot, a), inorganic phosphorus (Pi, b), organic phosphorus (Po, c) and the ratio of Pi to Po (d). Asterisks above boxplots indicate differences significantly deviating from zero (i.e., the control). BBR = Bad Brückenau; LUE = Lüss.



Supplementary Figure A3-S4. Difference between oxygen isotope signatures of inorganic phosphate ($\delta^{18}O_{Pi}$) in xylem sap before (background, t0) and after addition of ^{18}O-enriched water (ti). ti refers to the two time points after label application (2d and 14d = 2 days and 14 days, respectively). Asterisks above boxplots indicate differences significantly deviating from zero (i.e., from background $\delta^{18}O_{Pi}$ values). BBR = Bad Brückenau; LUE = Lüss.



Supplementary Figure A3-S5. Difference in oxygen isotope ratios of phosphate ($\delta^{18}O_{Pi}$) in xylem sap between fertilized (N, P, N+P) and non-fertilized trees (control), two and 14 days after application of ^{18}O-enriched water. No asterisks indicative of significant differences to the control are displayed because of overall non-significant results. BBR = Bad Brückenau; LUE = Lüss.

A4 Publikationen und Konferenzbeiträge

Bergkemper, F**.,** , Bünemann, E.K., **Hauenstein, S.**, Heuck, C., Kandeler, E., Krüger, J., Marhan, S.,Mészáros, E., Nassal, D., Nassal, P., Oelmann, Y., Pistocchi, C., Schloter, M., Spohn, M., Talkner, U., Zederer, D.P., Schulz, S. (2016). An inter-laboratory comparison of gaseous and liquid fumigation based methods for measuring microbial phosphorus (Pmic) in forest soils with differing P stocks. Journal of Microbiological Methods 128 , 66–68

Hauenstein, S., Neidhardt, H., Lang, F., Krüger, J., Hofmann, D., Pütz, T., Oelmann, Y. (2018). Organic layers favor phosphorus storage and uptake by young beech trees (*Fagus sylvatica* L.) at nutrient poor ecosystems. *Plant Soil* 432, 289–301. doi: 10.1007/s11104-018-3804-5

Hauenstein, S., Nebel, M., Oelamnn, Y., *(in review).* Impacts of fertilization on biologically cycled P in xylem sap of *Fagus sylvatica* L. revealed by means of the oxygen isotope ratio in phosphate. Frontiers: *Frontiers in Forests and Global Change (special issue: Forest soils)*

Hauenstein, S., Oelmann, Y., (2015). Biologisch mobilisiertes Phosphat im Wurzelbereich von jungen Buchen (*Fagus sylvatica* L.) auf zwei Standorten mit unterschiedlicher P-Verfügbarkeit. (DBG). *Poster*.

Hauenstein, S., Pütz, T., Oelmann, Y., (2016) The role of the organic layer for phosphorus nutrition of young beech trees (*Fagus sylvatica* L.) at two sites differing in soil Phosphorus availability

. (IPW8). *Poster*

Hauenstein, S., Pütz, T., Oelmann, Y., (2017) A multi-isotopic labelling approach ($^{33}P;H_2{}^{18}O$) to reveal the role of the organic layer for phosphorus nutrition of young beech trees (*Fagus sylvatica* L.) at two sites differing in soil phosphorus availability. (IUFRO). *Vortrag*

Hauenstein, S., Pütz, T., Oelmann, Y., (2017)The role of the organic layer for phosphorus nutrition of young beech trees (*Fagus sylvatica* L.) revealed by multi-isotopic labelling ($^{33}P;H_2{}^{18}O$) at two sites differing in soil phosphorus availability. (DBG). *Vortrag*

Hauenstein, S., Pütz, T., Oelmann, Y., (2017) A multi-isotopic labelling approach ($^{33}P;H_2{}^{18}O$) to reveal the role of the organic layer for phosphorus nutrition of young beech trees (*Fagus sylvatica* L.) at two sites differing in soil phosphorus availability. (Isocycles). *Vortrag*

Hauenstein, S., Pütz, T., Oelmann, Y., (2017) The role of the organic layer for phosphorus nutrition of young beech trees (*Fagus sylvatica* L.) at two sites differing in soil Phosphorus availability

. (EGU). *Poster*

Hauenstein, S., Neidhardt, H., Lang, F., Krüger, J., Hofmann, D., Pütz, T., Oelmann, Y., (2018) Organic layers favor phosphorus storage and uptake by young beech trees (*Fagus sylvatica* L.) at nutrient poor ecosystems

. (PSP6). *Poster*

Hauenstein, S., Neidhardt, H., Lang, F., Krüger, J., Hofmann, D., Pütz, T., Oelmann, Y., (2018) Organic layers favor phosphorus storage and uptake by young beech trees (*Fagus sylvatica* L.) at nutrient poor ecosystems
. (FOWITA). *Vortrag*

Hauenstein, S., Neidhardt, H., Lang, F., Krüger, J., Hofmann, D., Pütz, T., Oelmann, Y., (2019) Organic layers favor phosphorus storage and uptake by young beech trees (*Fagus sylvatica* L.) at nutrient poor ecosystems
. (IPW9). *Vortrag*

Hauenstein, S., Pütz, T., Oelmann, Y., (2019). Impacts of N/P inputs on organic P dynamics and P status in soils and plants along a nutrient gradient. (DBG). *Vortrag*