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ORIGINAL PAPER

Foliar P nutrition of European beech (*Fagus sylvatica* L.) depends on the season but remains unaffected by co-cultivation with silver fir (*Abies alba* Mill.)

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Abstract

Beech (*Fagus sylvatica*) and silver fir (*Abies alba*) are often cultivated in mixed stands and, hence, compete for water and nutrients. Besides nitrogen (N), also phosphorus (P) is an important nutrient for growth and development. Beech trees in Central Europe grow on both P-poor and P-rich soils, thereby showing similar growth and low variation in foliar P. The central aim of the present study was to test the hypothesis that variations in foliar P contents of beech are driven by seasonal changes rather than by the competition with silver fir. It was further hypothesized that P contents in silver fir needles depend on needle age and forest site. To test these hypotheses, P contents and P fractions, i.e. organic-bound P (P_{org}) and inorganic phosphate P (P_i), were measured in the foliage of beech trees from pure beech and mixed beech/silver fir plots as well as in needles of silver fir of the mixed plots. The forest sites investigated are located in Central Europe in the Black Forest, Germany, and in Croatia near the south-eastern distribution limit of beech and are all poor in plant-available soil P. The analyses showed that the main driver of P contents and P fractions in beech leaves at all forest sites is the season and that competition with silver fir had no effect. Hence, the present results demonstrate the high plasticity of beech trees to adapt to both poor plant-available soil P and competition with silver fir. Total P contents of silver fir needles were higher at the Croatian site compared to the Black Forest sites and originated from higher foliar P_i contents. One third of the P present in current-year needles in late summer was remobilized and exported until the needles reached the age of 1 year. The difference in P contents between current-year and 1-year-old needles can be seen as the amount of P resorbed from 1-year-old needles in summer during the generation of new needles to support the P demand of current-year needles for growth and development.

Keywords *Fagus sylvatica* · *Abies alba* · Competition · Phosphorus nutrition · Seasonal changes

Introduction

Beech (*Fagus sylvatica* L.) is the dominating broad leaf climax tree species of Central European forests (Distribution map of Beech (*Fagus sylvatica*) EUFORGEN 2009,

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www.euforgen.org. http://www.euforgen.org/species/fagus-sylvatica/) and constitutes its potential natural vegetation (Ellenberg 1988). Its distribution is restricted by both, too much water availability in regions prone to flooding, and too low water availability in dry habitats. The latter determines the southern distribution limit of beech (Jump et al. 2006). Accompanying tree species of old-growth beech forests include the conifer tree species silver fir (*Abies alba*) and *Picea abies* as well the deciduous tree species *Acer platanoides*, *Acer pseudoplatanus*, *Carpinus betulus*, *Fraxinus excelsior*, *Quercus petraea*, *Quercus robur* and *Tilia cordata* (Brunet et al. 2010). At its southern distribution limit, beech is partially or completely replaced by more drought-tolerant oak species in Western Europe (Hanewinkel et al. 2013). In the south-eastern parts of Europe, beech is replaced by oriental beech (*Fagus orientalis*) (Houston et al. 2016) and by silver fir. This natural structure of beech forests in south-eastern Europe is mostly preserved until today (Ivanković et al. 2011). However, while beech forests in Croatia have been naturally regenerated, nowadays the absence of regular mast crop is one of the biggest problems for assisted natural regeneration (Gavranović et al. 2018). A recent study showed that both, beech and silver fir, benefit from admixture in radial growth (Schwarz and Bauhus 2019). This positive effect was, however, more pronounced in silver fir. Besides water, tree growth needs sufficient availability of nutrients such as nitrogen (N) that can restrict tree growth in the temperate zone, whereas phosphorus (P) is the limiting nutrient, for example, of tropical rainforests (Townsend et al. 2007; Dalling et al. 2016). However, beech forests of the temperate growing zone in Central Europe are well adapted to N limitation (Rennenberg et al. 2009; Rennenberg and Schmidt 2010), but due to high anthropogenic N deposition, N limitation can turn into saturation, where, as a consequence, P can become limiting for beech growth (Jonard et al. 2015; Talkner et al. 2015). In addition, nutrient competition between tree species may determine species composition in forests depending on climate (Rennenberg 2014; González de Andrés 2019). Beech trees in a Central European mixed beech/silver fir forests did not seem to compete for nitrogen with *A. alba* trees (Magh et al. 2018a, b). Near the southern distribution limit of beech, total N contents of beech leaves were even slightly enhanced, when growing together with silver fir at sufficient water supply (Magh et al. 2018b). Similar effects were observed for *Picea sitchensis* when grown together with *Pinus contorta*, *Pinus sylvestris* or *Larix kaempferi* (Rothe and Binkley 2001). Also, other studies revealed that—depending on the combination of tree species—foliar nutrient content of a distinct nutrient remained unaffected or increased in mixed compared to pure forest stands (reviewed in Rothe and Binkley 2001).

As constituents of amino acids, proteins, DNA and RNA, membrane lipids, and further metabolic, regulatory, and signalling compounds, nitrogen (N), sulphur (S), and phosphorus (P) are essential macronutrients for growth and development. Coniferous and deciduous trees of temperate Central Europe, where soils are mostly limited in N availability (Rennenberg et al. 2009; Rennenberg and Schmidt 2010), have developed different strategies to meet their demand for growth and development. In this context, tree and ecosystem internal nutrient cycling during the annual growth constitutes an important strategy to maintain nutrient contents in growing and developing tissues. *Fagus sylvatica* is a deciduous tree species with determinate leaf development, which stores and mobilizes N (Gessler et al. 1998), S (Herschbach and Rennenberg 1995, 1996) and P (Netzer et al. 2017, 2018a) in and from branch bark and wood. The deciduous poplar with continuous leaf development stores and mobilizes S (Dürr et al. 2010; Malcheska et al. 2013) and N (Schneider et al. 1994b; Wildhagen et al. 2010; Rennenberg et al. 2010), but not P (Netzer et al. 2018b; Watanabe et al. 2018) in and from these tissues. Instead, the P demand of poplar is fulfilled mostly by P_i uptake from the soil (Netzer et al. 2018b; Plassard 2018). In contrast to deciduous trees, coniferous tree species are reported to store N and S in and subsequently mobilize them from older needle generations to supply current-year needles (Schupp and Rennenberg 1988, 1992; Schupp et al. 1992; Schneider et al. 1994a; Blaschke et al. 1996; Näsholm 1994; Miesel 2012; Wyka et al. 2016). For P similar results have been reported for *Pinus taeda* and *Pinus sylvestris* (Zhang and Allen 1996; Helmisaari 1992), while Manghabati et al. (2019) questioned P storage in older needle generations.

Plant-available P in the soil has been found to affect leaf/needle P content (Fäth et al. 2019). P contents in spruce needles and beech leaves also varied depending on soil pH with higher P contents in the foliage of trees grown in acidic soil compared to soil with neutral pH (Braun et al. 2010; Manghabati et al. 2018a). About one third of the potential beech forest area in Central Europe is located on calcareous soil (Dannenmann et al. 2016), exhibiting pH values higher than 6 (Prietzel et al. 2016). This might result in low foliage P contents due to diminished soil P_i availability and, hence, low P_i uptake (Manghabati et al. 2018a; Braun et al. 2010; Shen et al. 2011). As a consequence, the P content in foliage depends on the bedrock and the soil type developed from the bedrock that together with leaching during soil ageing determines P stocks of the soil (Hinsinger 2001; Vitousek et al. 2010) and, hence, foliar P contents (Lang et al. 2017; Zederer and Talkner 2018). Still, forest ecosystems developed strategies to produce equal amounts of biomass largely independent of plant-available P in the soil (Lang et al. 2016, 2017). In beech, this seems to be achieved by tree internal processes of P storage and (re)mobilization (Netzer et al. 2017, 2018a).

The objective of the present study was to address the following research questions: (A) Does the geographic forest location impact the foliar P nutrition of beech (*Fagus sylvatica*) and admixed silver fir (*Abies alba*)? (B) Is the foliar P nutrition of beech negatively affected by co-cultivation with silver fir? (C) Are 1-year-old silver fir needles storage organs for P? It was hypothesized that (1) plant-available soil P_i affects the P content of beech leaves in both pure beech and mixed beech/silver fir forest stands as well as of silver fir needles in the mixed forest stands; (2) beech and silver fir compete for soil P in the mixed forest stands thereby affecting foliar P contents of beech leaves compared to the pure stands; (3) P is re-mobilized from older needle generations to fulfil the P demand of current-year needles of silver fir indicating P storage in fully developed needles.

Materials and methods

Field site description

Foliar P contents of European beech (*Fagus sylvatica L.*) and silver fir (*Abies alba Mill.*) were investigated at three European forest sites, previously described in detail (Magh et al. 2018a, b; Prietzel et al. 2016; see also Table 1). The Emmendingen (EMM) and the Conventwald (CON) sites are located at the foothills of the Black Forest (distance ~ 15 km from each other) in Germany near Freiburg, while the third forest site is located near the city of Gospic in Croatia (CRO) near the south-eastern distribution limit of European beech (Ellenberg and Leuschner 2010; Fang and Lechowicz 2006). At all sites, pure beech and mixed beech/silver fir stands were separated. The EMM mixed forest stand is composed of 70% beech, 15% silver fir and 15% larch. The CON forest was divided into two stands: with the “pure beech” stand consisting of beech (55%), silver fir (10%), and spruce (35%), while the “mixed beech” stand consists of beech (35%), silver fir (35%), spruce (5%), Douglas-fir (10%),

Table 1 Characteristics of the “Emmendingen”, “Conventwald”, and “Croatia” forest sites

Location	Emmendingen (EMM) (Germany)	Conventwald (CON) (Germany)	Gospic (CRO) (Croatia)
Longitude/latitude	48° 08′52.4″ North 7° 54′ 20.2″ East	48° 01′ 15.11″ North 7°58′ 18.42″ East	Mixed: 44° 33′ 58.6″ North 15° 13′ 03.1″ East Pure: 44° 32′ 26.2″ North 15° 13′ 21.0″ East
Elevation asl. (m)	400[e]	700–840[a,e]	800–900[e]
Annual precipitation (mm) (period)	1100 (1987–2014)[e]	1777 (1971–2001)[e]	2230 (1987–2010)[e]
Mean annual air temperature (°C) (period)	9.6 (1987–2014)[e]	7.3 (1971–2001)[e]	7.5 (1987–2010)[e]
Stands age (years)	40–60[e]	Pure/mixed 67–102/120[e]	Pure/mixed 30–40/70–90[e]
Tree height (average) (m)	24[e]	29[e]	Pure/mixed 15/25[e]
Soil texture	Sandy loam[e]	Loam[e]	Silty clay[e]
Soil pH (H_2O)			Pure/mixed
Ah	3.9 (0–15 cm)[c]	3.6 (0–10 cm)[b]	5.4/6.4 (0–5 cm)[c]
Bv1	3.8 (15–40 cm)[c]	3.8 (10–45 cm)[b]	4.46/7.4 (5–25 cm)[c]
Bv2	3.8 (40–100 cm)[c]	4.0 (45–90 cm)[b]	5.0/7.4 (25–45 cm)[c]
Citrate extractable	Pure/mixed	Pure/mixed	Pure/mixed
	44/72 (0–5 cm)[d]	131–135/94–95 (0–5 cm)[d]	110 ± 39/53 ± 29 (0–5 cm)[d]
P_{tot} (mg kg^{-1} DW)	16/48 (5–10 cm)[d]	74–75/113–114 (5–10 cm)[d]	23 ± 22/20 ± 12 (5–10 cm)[d]
	28/13 (10–25 cm)[d]		
Citrate extractable	Pure/mixed	Pure/mixed	Pure/mixed
	14/35 (0–5 cm)[d]	48–51/28 (0–5 cm)[d]	98 ± 45/34 ± 26 (0–5 cm)[d]
P_i (mg P kg^{-1} DW)	3/7 (5–10 cm)[d]	13/26–30 (5–10 cm)[d]	14 ± 18/5 ± 5 (5–10 cm)[d]
	5/3 (10–25 cm)[d]		

[a] Lang et al. (2017), [b] Prietzel et al. (2016), [c] Dannenmann, M., Institute for Meteorology and Climate Research—Atmospheric Environmental Research, KIT, Germany, [d] Lang, F., Soil Ecology, Albert-Ludwigs-University Freiburg, Germany. All other data were taken from [e] Magh et al. 2018b. Soil P data are taken from composite samples in EMM and CON and from separate samples ($n = 5$) at CRO (see Methods)

larch (5%), and other species (10%). The "mixed beech" stand of the CRO site is composed of beech (38%), silver fir (50%), and other tree species; the "pure beech" stand consists of beech (57%), silver fir (40%), and other species (3%) (Magh et al. 2018b). At all three forests sites trees were selected within one plot of roughly 100–200 m^2 in the pure beech and the mixed beech/silver fir stands. In these plots, the composition of species was as follows: for the pure beech plots, there were solely beech trees present, while in the mixed beech plot beeches and silver firs were present in equal share (50:50).

Important soil characteristics and climate conditions of these forest sites are presented in Table 1. Basic information of the forest sites specified by longitude/latitude is taken from Magh et al. (2018a, b); these include elevation asl., annual precipitation, mean annual air temperature, stand age, tree height, and soil texture. Additional information on elevation for the CON forest was taken from Prietzel et al. (2016).

Harvest of beech leaves and silver fir needles

Leaves from beech trees similar in size as well as needles from silver firs were harvested in late spring 2016, when beech leaves were fully developed, 31st of May to 4th of June at EMM, 6th to 10th of June at CON and 5 days starting from 22nd of June at the CRO field site. Due to the higher elevation, bud break and full leaf development was later at the CRO compared to the EMM site. The second harvest was performed in late summer 2016, 24th of September to 7th of October at EMM, 26th of September to 30th of September at CON and 3rd to 12th of September at the CRO field site. Within one forest site, trees of similar age and height were selected, while between the field sites age ranged from 50–70 (EMM), over 100–120 (CRO) to 140–180 (CON) years (Schwarz and Bauhus 2019). At each sampling campaign, professional tree climbers collected branches in the upper sun crown of approx. 30–45 cm length from beech and silver fir trees. Branches were harvested between 10 am and 2 pm to limit diurnal metabolic variation and variation in light availability. From beech branches, three morphologically intact leaves were collected. Current- and previous-year needles were excised from twigs of silver fir. Leaves and needles were immediately shock-frozen in liquid nitrogen in the field and stored at − 20 °C until further analyses.

Soil sampling

Soil samples from the EMM, CON and the CRO forest sites were collected during the dormancy period between December 2017 and April 2018. Soil samples from 0 to 5 cm, 5 to 10 cm and 10 to 25 cm depth were taken randomly at three sampling spots of both the pure beech and the mixed beech/silver fir plots using a 30 cm corer (5 cm in diameter). In Croatia, the soil type is a brown soil on limestone and dolomite, horizon profile Amo-B(rz)-R. For each core, the soil below the organic layer was split into sections of 0–5 cm, 5–10 cm, and 10–25 cm depth. At each plot, three samples per soil depth were taken at the EMM and the CON forest, whereas five core samples were taken and analysed for the CRO plots. Larger stones (> 2 cm), branches, and roots were removed. Air-dried soil samples were sieved (passed through 2 mm mesh width) and stored at room temperature until further analyses, i.e. pH and including analysis of citrate-extractable phosphate (P_i) and total P (P_{tot}) (see next chapter).

Determination of soil citrate-extractable P

Citrate-extractable P was determined according to König et al. (2005) and Manghabati et al. (2018b). For this purpose, an aliquot of 5 g dried mineral soil was extracted with 100 ml 1% citric acid. The suspension was shaken for 16 h at room temperature and was filtered using P-free paper filter (Munktell 131, Munktell Filter AB, Sweden). P_i in the extracts was determined as described by Murphy and Riley (1962) using a continuous flow analyser (Skalar SAN++, Skalar Analytical B. V., The Netherlands). Total P in citrate extracts was measured using ICP-OES (Spectro Ciros, SPECTRO Analytical Instruments GmbH, Kleve, Germany).

Analyses of foliar P fractions

Beech leaves and silver fir needles were ground to a homogenous fine powder with a mortar and pestle in liquid nitrogen. Phosphate contents were determined in aqueous extracts as described by Netzer et al. (2016). Shortly, approx. 25 mg of frozen homogenized foliar powder was suspended in 1 ml ddH$_2$O containing 100 mg purified PVPP (polyvinylpolypyrrolidone) (Loomis and Battaile 1966). Samples were shaken in a cold room at 8 °C for 1 h. Afterwards, extracts were boiled in a water bath (96 °C) for 10 min. After cooling down on ice (10 min), the extracts were centrifuged twice (21500 g, 4 °C, and 10 min) to obtain a clear supernatant. Inorganic phosphate contents were determined by the molybdenum blue test of Murphy and Riley (1962) as previously described (Netzer et al. 2016). For dry weight mass determination, aliquots of powdered leaf and needle samples were oven-dried at 60 °C until weight constancy.

Total P (P_{tot}) in the foliage was extracted from dried powdered leaf/needle material using a modified method of Lambers et al. (2012) as reported by Netzer et al. (2017). For this purpose, approx. 20 mg of the homogeneous dry foliage powder was weighed into glass vials and digested in 1 ml of an acid mixture (HNO_3: $HClO_4 = 3:1$ v/v) at room temperature for 12 to 21 days. The remaining digests were diluted

1:10 (ddH_2O). 500 µl of the diluted digests was adjusted to pH 1.5 by adding 560 µl NaOH (1 M). Phosphate contents were determined as described for P_i using the molybdenum blue test (Murphy and Riley 1962) adapted by Netzer et al. (2016) for the application to the multimode reader. The contents of organic-bound P (P_{org}) were calculated by subtracting inorganic phosphate P (P_i) from total P (P_{tot}).

Determination of total foliar N and C

Total N and total C contents were measured as previously described (Simon et al. 2010). Aliquots of 1.0–1.5 mg oven dried leaf and needle material were weighed into tin capsules (IVA Analysentechnik, Meerbusch, Germany). Total N and C content was measured with an element analyser (NA2500, CE Instruments, Milan, Italy).

Statistics

Statistically significant differences were investigated between the three forest sites (EMM, CON, and CRO) for the leaves of beech trees of the pure and the mixed plots as well as for current (0yn) and 1-year-old (1yn) needles of silver fir at different seasons. In order to determine significant differences of each parameter between different sites (i.e. EMM, CON, CRO) within the same season, plot, and needle age, one-way ANOVA followed by the post hoc Tukey test was performed with SigmaPlot 12.0 software (Systat Software GmbH, Erkrath, Germany). The linear mixed model was applied on the data in order to analyse differences between stands (i.e. pure stand and mixed stand), between needle ages (i.e. current-year needles and 1-year-old needles), and between seasons (i.e. early summer and late summer) at each site (i.e. EMM, CON, CRO) as described in Magh et al. (2018b) using R (R core team 2017). A detailed list of all R packages used is given in Table S1. Needle age and season were treated as fixed factors, whereas the tree number served as random factor due to the repeated measurements on the same trees. Total C, total N, total P, P_i, P_{org} and the ratios of P_i/total P, N/P, C/P, N/P_i, C/P_i, C/N, and DW/FW (dry to fresh weight ratio) were the dependent variables of each model. Normal distribution and homoscedasticity were checked by the Shapiro–Wilk test and Levene's test, respectively. Raw data were transformed by either cube root or $\log_{10}$ transformation if these assumptions were not meet. Subsequently, transformed data were fitted again and the assumptions were checked as above. Significant differences were assessed by pairwise Tukey-adjusted comparisons of the means with "ls-means" in R. Supplemental Tables S2 and S3 provide an overview on the number of replicates analysed for beech leaves and silver fir needles, respectively.

Results

Total citrate-extractable soil P as well as citrate-extractable P_i was in a similar range at all forest locations (Table 1). At the CRO site, total P and P_i tended to be higher in the pure compared to the mixed beech forest plot in consistency with higher soil pH in the pure compared to the mixed forest plot (Table 1).

P contents in beech leaves reveal a seasonal pattern

In leaves of beech trees from both plots, i.e. of the pure and mixed stand, total P (P_{tot}) contents were lower in late summer than in early summer (Fig. 1a) at all three forest sites (CON, EMM, and CRO). The decline in P_{tot} was caused by reductions in organic-bound P (P_{org}), but not by changes in phosphate (P_i) (Fig. 1b, c). Consequently, the P_i as a percentage of P_{tot} (P_i%) increased in late summer. This increase was significant at the EMM forest for beeches of the mixed plot only (Fig. 1d).

Seasonal differences in beech leaf P affect nutrient ratios

For both plots, i.e. the plot of the pure and mixed forest stand, total C contents were highest in beech leaves of the CON forest (approx. 480 mg g^{-1} DW) and lower in beech leaves of the EMM and CRO forests (approx. 460 mg g^{-1} DW). Because total C contents in beech leaves did not change with the season in both, pure and mixed plots (Figure S1A), the C/P ratios of the leaves were affected by reductions in P_{tot} and, thus, were higher in late than in early summer (Fig. 2a). As P_i contents of beech leaves were not affected by the season, the C/P_i ratios remained mostly similar during the seasons (Figure S1B).

Total N contents in beech leaves were lowest at the CON mixed plot (approx. 21 mg g^{-1} DW), and higher but similar at the EMM and CRO mixed plots (approx. 27 (EMM) and 25 (CRO) mg g^{-1} DW) (Figure S2A). As observed for the C/P ratios, the decline in P_{tot} in late summer resulted in higher N/P ratios in late compared to early summer (Fig. 2b). These findings were independent of the beech forest sites (CON, EMM, CRO) and stand composition (pure vs. mixed beech plot). Similar to the C/P_i ratios, the N/P_i ratios remained mostly unaffected by the season. At the EMM forest, the N/P_i ratios were higher compared to the CON and CRO forests, especially in the mixed plots in early summer (Figure S2B). Mean C/N ratios (over all stand plots and seasons) were highest for the leaves from CON beech trees (mean over all samples and season: 21.6)

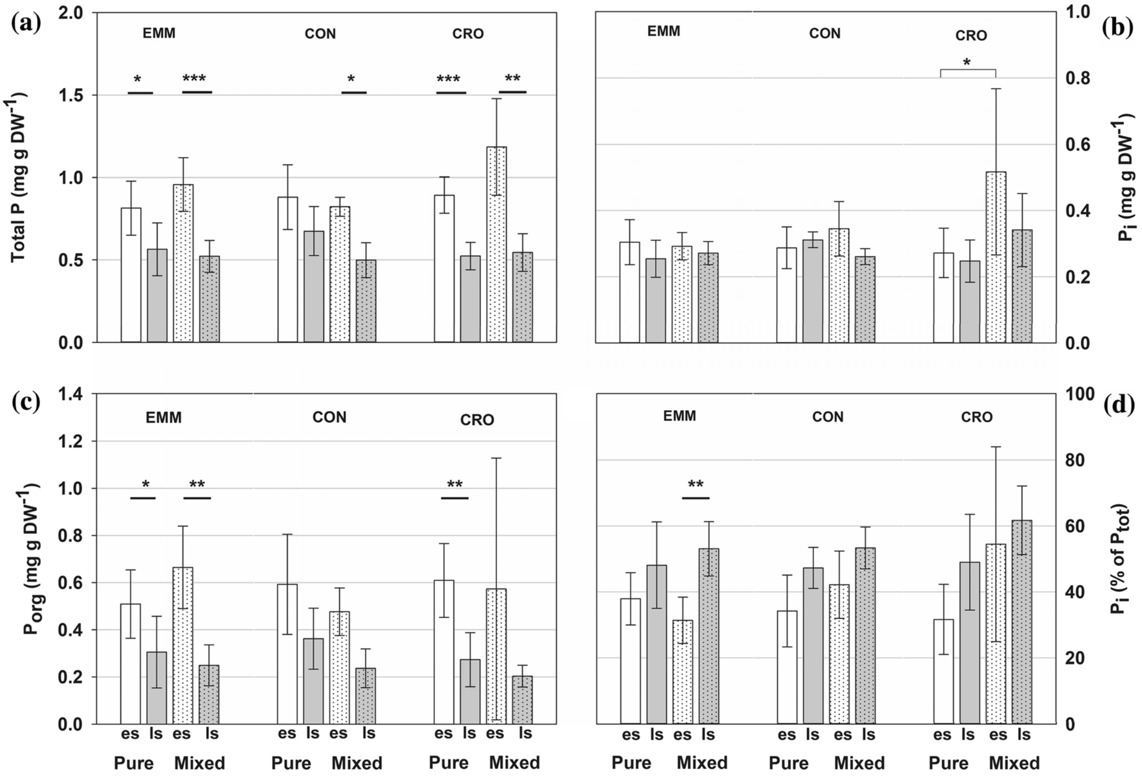


Fig. 1 P contents and fractions of beech leaves of the three forest locations in dependency on season and forest site. Total P (**a**), phosphate (P_i) (**b**), organic-bound P (P_{org}) (**c**), and the P_i as a percentage of P_{tot} (P_i%) (**d**) of beech leaves are presented for the EMM, CON, and CRO forest site in dependency on the seasons, i.e. early summer (es) and late summer (ls) and the plots, i.e. mixed beech plots (Mixed) versus pure (Pure) beech plots. White bars: early summer pure beech plot; grey bars: late summer pure beech plot; white dotted bars: early summer mixed beech forest plot; grey dotted bars: late summer mixed beech forest plot. Data are mean ± S.D. The number of replicates is given in Supplemental Table S2. Different letters indicate significant differences between forest sites. Small letter for early summer in pure beech plots; capital symbols for late summer in pure beech plots; capital letters for early summer in mixed beech forest plots; small symbols for late summer in mixed beech forest plots. Significant differences between seasons are indicated by a line and stars (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) for leaves of the pure and mixed plots of each forest site. Significant differences between pure and mixed plots at the respective season are indicated by box brackets subjected by stars (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). The absence of the respective letter of a line or a box bracket indicates that statistically significant differences were not found

because of the highest C and lowest N contents compared to both other forest sites (Fig. 2c).

P contents of silver fir needles are affected by geographic forest location, season and needle age

Effects of forest location

Higher total P contents in silver fir needles were observed at the CRO compared to the EMM and CON mixed plots with significantly higher P_{tot} contents in current-year needles in early summer and 1-year-old needles in late summer (Fig. 3a). The P_i content was higher in silver fir needles at the CRO (nearly doubled) compared to the EMM and CON mixed plots in current-year needles during late summer, and in the 1-year-old needles independent of season (Fig. 3b). The differences in P_{tot} did not result in consistent differences regarding the P_i as a percentage of P_{tot} (P_i%) (Fig. 3d). Organic-bound P in silver fir needles showed high variability (Fig. 3c).

Effects of season depend on the forest location

At the CON forest, the P_{tot} content of silver fir needles showed clear seasonal changes. Independent of needle age, P_{tot} was significantly lower in late compared to early summer (Fig. 3a). In contrast, at the CRO forest P_{tot} of 1-year-old needles increased, although not significantly, from early to late summer. This increase in P_{tot} was caused by P_{org}, whereas P_i remained unchanged. The P_i as a percentage of P_{tot} (P_i%) significantly increased from early to late summer in 1-year-old needles of the CON mixed plot (Fig. 3d).

Differences in foliar P contents are related to needle age

Tendentially, the P_{tot} content of current-year needles was higher than in 1-year-old needles independent of the season (Fig. 3a). This behaviour was also identifiable for P_i in early summer although significant only for the EMM mixed plot. At the EMM mixed plot, as described, P_{tot} was lower in 1-year-old compared to current-year needles, which originated from changes in P_{org} and is reflected by increasing P_i%.

The P contents of silver fir needles in relation to total C

Total C was similar in silver fir needles over all forest sites, seasons and needle ages (Fig. 4a). Consequently, the differences observed for the C/P and the C/P_i ratios were caused by changes in total P and P_i as already found for beech.

Effects of forest location

C/P ratios of silver fir needles at the CRO mixed plot (~ 820) were similar, independent of needle age, but on average lower compared to the EMM (~ 1094) and CON (~ 1195) mixed plots (Fig. 4b). In general, similar to the C/P ratios, the C/P_i ratios were lowest in needles of the CRO mixed plot. This effect was significant for 1-year-old needles independent of season.

Effects of season

Independent of age, silver fir needles showed seasonal differences with higher C/P ratios mediated by lower total P contents in late compared to early summer at the CON mixed plot (Fig. 4b). In current-year needles, the C/P_i ratio tended to be lower during early compared to late summer at each forest location caused by enhanced P_i contents; yet this effect was not significant (Fig. 4c).

Effects of needle age

Needle age-dependent differences were most obvious for both early and late summer. In late summer, the C/P was significantly higher for 1-year-old needles compared to current-year needles, significant for the EMM and the CON mixed plots. The C/P_i ratio was higher in 1-year-old than in current-year needles in early summer, significant, however, only for the EMM mixed plot.

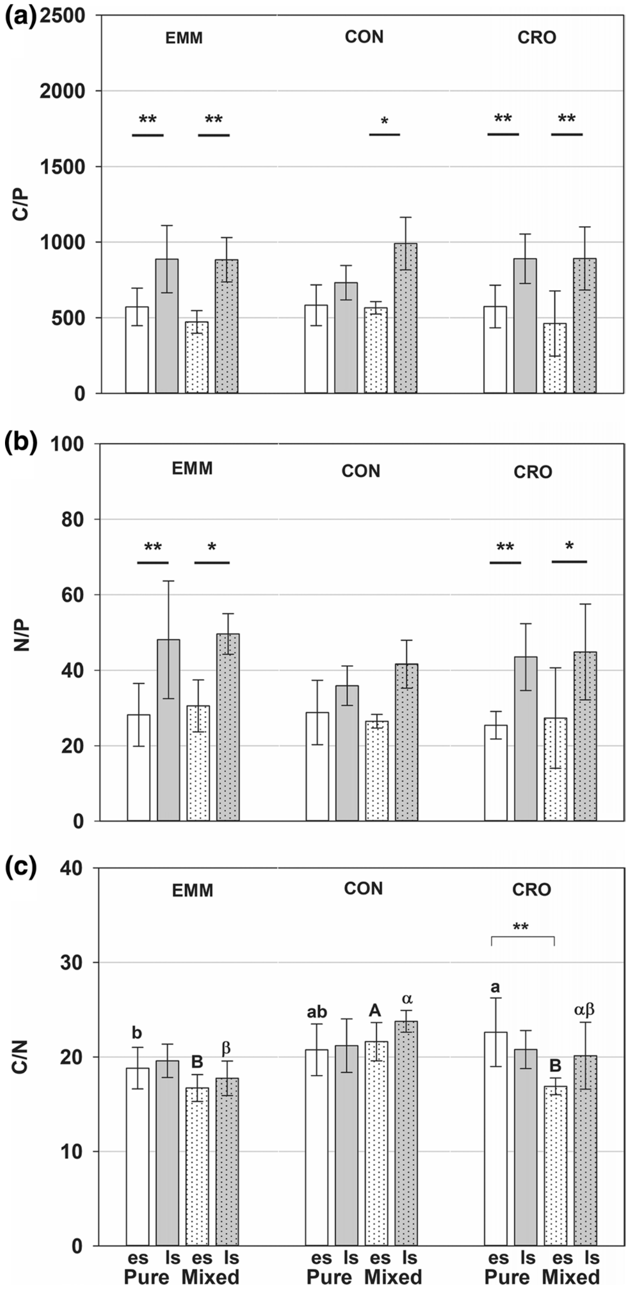


Fig. 2 Nutrient ratios in beech leaves of the three forest locations in dependency on season and forest site. C/P (**a**), N/P (**b**), and C/N (**c**) ratios of beech leaves are presented for the EMM, CON, and CRO forest site in dependency on the seasons, i.e. early summer (es) and late summer (ls) and the plots, i.e. mixed beech plots (Mixed) versus pure (Pure) beech plots. White bars: early summer pure beech plots; grey bars: late summer pure beech plots; white dotted bars: early summer mixed beech forest plots; grey dotted bars: late summer mixed beech forest plots. Data are mean ± S.D. The number of replicates is given in Supplemental Table S2. Different letters indicate significant differences between forest sites. Small letter for early summer in pure beech plots; capital symbols for late summer in pure beech plots; capital letters for early summer in mixed beech forest plots; small symbols for late summer in mixed beech forest plots. Significant differences between seasons are indicated by a line and stars (*$p<0.05$, **$p<0.01$; ***$p<0.001$) for leaves of the pure and mixed plots of each forest location. Significant differences between pure and mixed plots at the respective season are indicated by box brackets subjected by stars (*$p<0.05$; **$p<0.01$; ***$p<0.001$). The absence of the respective letter of a line or a box bracket indicates that statistically significant differences were not found

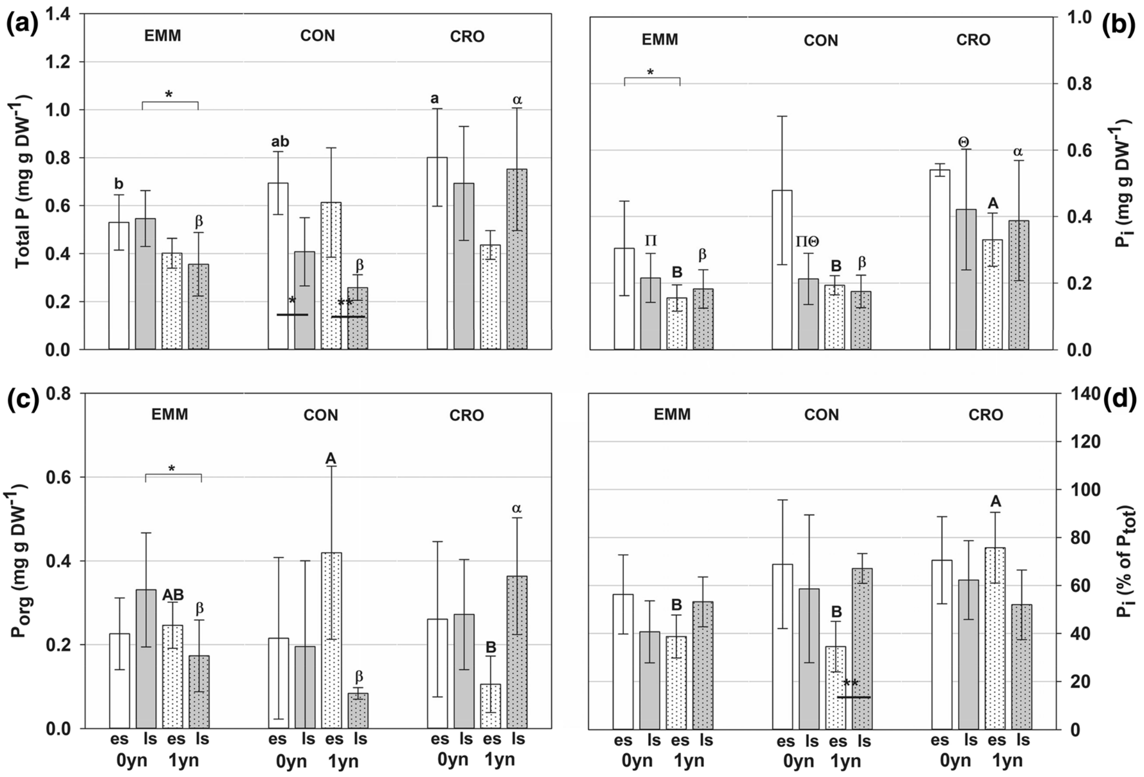


Fig. 3 P contents and fractions in silver fir needles of the three forest locations in dependency on needle age and forest site. Total P (**a**), phosphate (P_i) (**b**), organic-bound P (P_{org}) (**c**), and the P_i as a percentage of P_{tot} (P_i%) (**d**) of silver fir needles are presented for the EMM, CON, and CRO forest sites in dependency on the needle ages, i.e. early summer (es) and late summer (ls) and the needle age, i.e. current-year needles (0yn) versus 1-year-old needles (1yn). White bars: early summer current-year needles; grey bars: late summer current-year needles; white dotted bars: early summer 1-year-old needles; grey dotted bars: late summer 1-year-old needles. Data are mean ± S.D. The number of replicates is given in Supplemental Table S3. Different letters indicate significant differences between forest sites. Small letter for early summer of current-year needles; capital symbols for late summer of current-year needles; capital letters for early summer of 1-year-old needles; small symbols for late summer of 1-year-old needles. Significant differences between seasons are indicated by a line and stars (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) for current and 1-year-old needles at each forest site. Significant differences between current and 1-year-old needles at the respective season are indicated by box brackets subjected by stars (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). The absence of the respective letter of a line or a box bracket indicates that statistically significant differences were not found

The P contents of silver fir needles in relation to total N

Total N contents were recalculated from Magh et al. (2018b) (Fig. 5a). Total N in needles of silver fir ranged between 11 and 18 mg g^{-1} DW. In early summer, foliar N was greater in current-year needles than in 1-year-old needles for the EMM and CRO mixed plots. As total C was mostly unaffected, the C/N ratio behaviour was reverse to the pattern of total N (Figure S3). The N/P and N/P_i ratios were not consistently affected due to changes in both, P and N contents.

Effects of forest location

During early summer higher N contents were found at the EMM compared to the CON and CRO mixed plots in current and 1-year-old needles. Due to the higher P_{tot} contents in the silver fir needles from the CRO mixed plot, the N/P ratio in these needles ranged around 20. At the EMM and CON mixed plots, the needle N/P ratios showed high variability and strong P deficiency, especially in 1-year-old silver fir needles with N/P ratios higher than 40 (Fig. 5b). The N/P_i ratios showed a similar pattern. The significant lowest ratios were found at the CRO mixed plot. Highest N/P_i ratios in silver fir needles were found at the EMM mixed plot (Fig. 5c).

Effects of season

At all three forest sites, total N was higher in early than in late summer for current-year needles (Fig. 5a); this difference was significant for the EMM and the CRO mixed plots. One-year-old needles showed higher contents in late than in early summer; however, this effect was not significant.

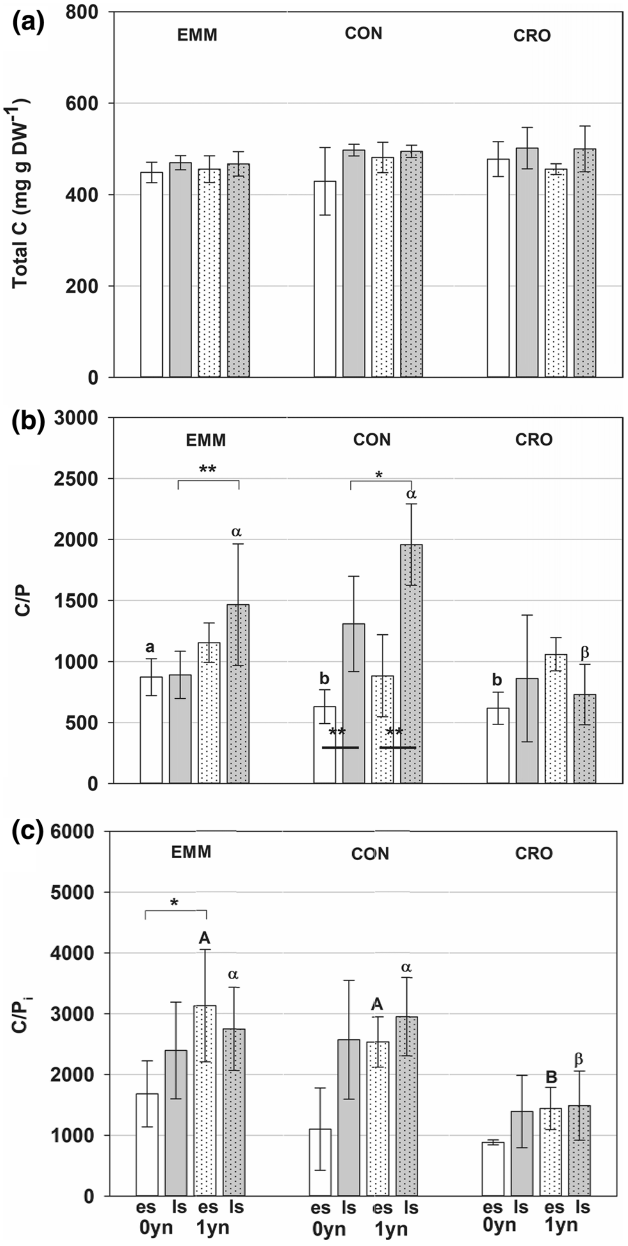


Fig. 4 Total C, the C/P, and C/P$_i$ ratios in silver fir needles of the three forest locations in dependency on needle age and forest site. Total C (**a**), C/P (**b**), and C/P$_i$ (**c**) ratios of silver fir needles are presented for the EMM, CON, and CRO forest sites in dependency on the needle ages, i.e. early summer (es) and late summer (ls) and the needle age, i.e. current-year needles (0yn) versus 1-year-old needles (1yn). White bars: early summer current-year needles; grey bars: late summer current-year needles; white dotted bars: early summer 1-year-old needles; grey dotted bars: late summer 1-year-old needles. Data are mean ± S.D. The number of replicates is given in Supplemental Table S3. Different letters indicate significant differences between forest sites. Small letter for early summer of current-year needles; capital symbols for late summer of current-year needles; capital letters for early summer of 1-year-old needles; small symbols for late summer of 1-year-old needles. Significant differences between seasons are indicated by a line and stars ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$) for current and 1-year-old needles at each forest site. Significant differences between current and 1-year-old needles at the respective season are indicated by box brackets subjected by stars ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$). The absence of the respective letter of a line or a box bracket indicates that statistically significant differences were not found

The N/P ratios did not show coherent changes because both total P and total N showed high variations. The N/P ratio of 1-year-old needles increased from early to late summer at the EMM and the CON mixed plots. This increase was due to declining total P and P_{org} contents.

Differences in foliar N and P nutrition in relation to needle age

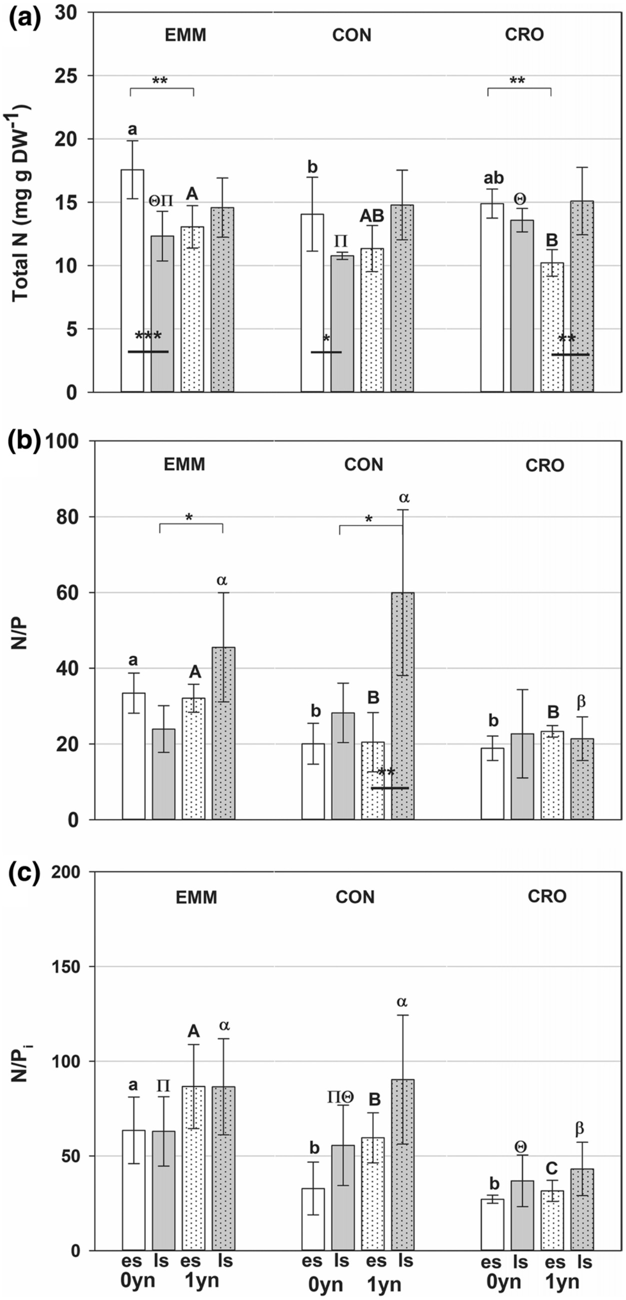
Total N contents in silver fir needles were higher in current than in 1-year-old needles during early summer (Fig. 5a). In contrast, during late summer total N in current-year needles tended to be lower than in 1-year-old needles. During early summer, the N/P ratios of current and 1-year-old needles were similar (Fig. 5b). In contrast, during late summer the N/P ratios were significantly higher in 1-year-old than in current-year silver fir needles for the EMM and the CON mixed plots. The N/P_i ratios in early and late summer were higher in the 1-year-old needles than in current-year needles at the EMM and CON mixed plots, but were similar over all ages and seasons at the CRO mixed plot (Fig. 5c).

Discussion

Seasonality drives P contents in *Fagus sylvatica* leaves

P contents of beech leaves were driven by the seasonal demand, as shown by the observation that soil characteristics including pH, soil P, and soil N availability and competition with silver fir did not significantly affect the foliar P content of beech. At all three forest sites of the present study, EMM, CON, and CRO, plant-available P_i showed high variability, but was comparably low on average (Table 1; see also Prietzel et al. 2016; Lang et al. 2017). Citrate-extractable soil P_{tot} reached up to 800 mg kg^{-1} at the P-rich forest site Bad Brückenau, whereas it amounts to only approx. 180 mg kg^{-1} at the CON forest (Prietzel et al. 2016). The latter is still higher than the citrate-extractable P found in the present study. Hence, the forest sites of the present study can all be classified as low P forest sites. The lower citrate-extractable soil P_{tot} and P_i at 0–5 cm depth of the mixed compared to the pure beech plot at the CRO site may have originated from less decalcification. Plant-available soil P depends on the pH and is affected depending on the soil type by different pH-mediated mechanisms: (i) due to increasing binding of P_{org} and P_i to Al and Fe hydroxides with decreasing pH, (ii) due to co-precipitation of free Al and Fe ions together with P_i at decreasing pH, and (iii) due to Ca_2PO_4 precipitation at increasing pH (Hinsinger 2001; Carreira and Lajtha 1997; Penn and Camberato 2019). Such differences, however, had no influence on foliar P contents of beech in the present

Fig. 5 Total N, the N/P, and N/P_i ratios in silver fir needles of the three forest locations in dependency on needle age and forest site. Total N (**a**) (recalculated from Magh et al. 2018b), N/P (**b**), and N/P_i (**c**) ratios of silver fir needles are presented for the EMM, CON, and CRO forest sites in dependency on the needle ages, i.e. early summer (es) and late summer (ls) and the needle age, i.e. current-year needles (0yn) versus 1-year-old needles (1yn). White bars: early summer current-year needles; grey bars: late summer current-year needles; white dotted bars: early summer 1-year-old needles; grey dotted bars: late summer 1-year-old needles. Data are mean ± S.D. The number of replicates is given in Supplemental Table S3. Different letters provide significant differences between forest sites. Small letter for early summer of current-year needles; capital symbols for late summer of current-year needles; capital letters for early summer of 1-year-old needles; small symbols for late summer of 1-year-old needles. Significant differences between seasons are indicated by a line and stars ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$) for current and 1-year-old needles at each forest site. Significant differences between current and 1-year-old needles at the respective season are indicated by box brackets subjected by stars ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$). The absence of the respective letter of a line or a box bracket indicates that statistically significant differences were not found



study. P contents of beech leaves were similar at all forest sites and plots, but both, P_{tot} and P_{org}, decreased from early to late summer. It has previously been observed that P_{tot} was highest in buds and declined until early summer when leaves were fully expanded, but was not further declined by late summer (Netzer et al. 2017). The observed differences in P_{tot} at early summer between the present study and the study of Netzer et al. (2017) can be attributed to different harvesting time points in early summer. While Netzer et al. (2017) harvested beech leaves at the end of June, beech leaves were harvested at the end of May/beginning of June in the present study. During leaf maturation between spring and early summer, the foliar metabolome shows drastic changes due to differences in metabolic activities (Netzer et al. 2018a, Watanabe et al. 2013). Under these conditions, the demand of P may decline as a consequence of lowered protein synthesis and growth. This view is supported in the present study by a decline in P_{org} and an unaffected P_i pool of beech leaves. The latter may represent an equally active P pool for metabolic processes in early and late summer (Veneklaas et al. 2012). Seasonal changes within the P_{org} pool of beech leaves were mostly related to the variation in storage compounds, which were higher during dormancy in beech buds, and revealed variations in sugar-Ps and phospholipids during summer (Netzer et al. 2018a). Upon P deficiency, phospholipids may be replaced by galactolipids, sulpholipids (Lambers et al. 2012; Salter et al. 2018), and glucuronosyldiacylglycerol (GlcADG) (Okazaki et al. 2013, 2015). This replacement constitutes a strategy that keeps P_i sufficiently high for metabolic processes even at P-poor forests sites (Netzer et al. 2018a) and can explain the declining P_{org} contents of beech leaves during late summer in the present study.

Foliar P contents of beech trees remained unaffected in mixed cultivation with *A. alba*

In forest soils microbes, fungi and plants compete for water and nutrients. Nutrient competition between beech and silver fir in mixed cultivation did not affect foliar C, N and P contents of beech. Beech leaves from all forest sites showed similar P_{tot} and P_i contents in early summer as well as in late summer. The differences between early and late summer can be ascribed to seasonal changes (Netzer et al. 2017). However, effects on P_{tot} and P_i caused by an admixture of *A. alba* were not observed. This outcome corresponds to the results summarized in a review by Rothe and Binkley (2001) for *Picea* sp., where foliar nutrient contents were either higher, probably due to facilitation, or similar in mixed compared to pure forest stands. Apparently, beech trees in mixed cultivation with silver fir trees have developed strategies to avoid competition for nutrients independent of soil P and/or N availability. The difference in rooting depth between relatively shallow rooting beech and deep rooting silver fir (Köstler et al. 1968, Schmid and Kazda 2001) may at least partially explain this avoidance of competition. Avoidance strategies in N uptake have been described for beech trees between adults and their offspring in a pure beech forest (Simon et al. 2011). In the study of Simon et al. (2011), avoidance of competition for N uptake was achieved by seasonal differences in preferential N uptake by the roots. In addition, intraspecific competition depending on tree density of beech could be of importance at the pure and mixed forest plots in the present study but does not seem to affect P contents of beech leaves, as observed for the conifer *Cunninghamia lanceolata* (Dong et al. 2016; Fang et al. 2017).

The pH in the forest soil of the mixed beech forest plot at the CRO site was higher compared to the pure beech plot, accompanied by lower citrate-extractable P in the soil of the mixed stand at 0–5 cm depth (Table 1), although this did not reflect in the beech leaf P contents. In other studies, increasing soil pH mediated lower P contents in beech leaves (Braun et al. 2010, Manghabati et al. 2018a). It can be assumed that internal P recycling processes as observed for beech trees at the CON forest (Netzer et al. 2017, 2018a) kept foliar P contents similar in pure and mixed plots. Plant internal nutrient recycling strategies include nutrient storage and mobilization in branch bark and wood (Netzer et al. 2017; Herschbach et al. 2012; Rennenberg et al. 2010) and may contribute to avoid competition for nutrient uptake from the soil. By such P recycling strategies, P contents in beech leaves can be maintained at a similar level irrespective of co-cultivation with silver fir. However, the significance of each of these strategies in mixed beech/silver fir forests needs to be investigated in future studies. Still, the present results clearly demonstrate the high plasticity of beech to adapt to both nutrient scarcity and nutrient competition with silver fir.

P nutrition of *Abies alba* needles

Although the P_{org} pool revealed high variation in silver fir needles, the overall P content was similar to beech leaves over all forest sites and seasons. The higher P_{tot} content of silver fir needles at the CRO mixed plot coincides with higher P_i contents. Sufficient or even surplus of P nutrition is commonly indicated by increasing P_i contents (e.g. Veneklaas et al. 2012) and is supported by similar needle N/P ratios (approx. 20) of CRO silver fir needles. The later implies evenly balanced N and P or according to Güsewell (2004) co-limitation of N and P. In contrast, the higher N/P ratios of silver fir needles at the EMM (~ 33) and of 1-year-old needles in late summer at the CON mixed plot (60 ± 22) strongly indicate P deficiency (Güsewell 2004). Balanced N and P nutrition is indicated by N/P ratios of 6–12 for *Picea abies* and of 7–14 for *Pinus sylvestris* (Mellert and Göttlein 2012). N/P ratios above these values are thought to indicate P deficiency, but this clearly depends on the species (Güsewell 2004). Hence, the N/P ratios of *A. alba* needles at the EMM and CON mixed plots indicate P deficiency, which was also observed for the beech leaves at these forests, while the N/P ratios of silver fir needles of the CRO mixed plot indicate balanced N/P nutrition. For beech trees, foliar N/P ratios above 20 are thought to indicate P deficiency (Güsewell 2004, Mellert and Göttlein 2012), which were already observed in early summer but were strengthened in late summer and strongly supported the assumption of P deficiency for beech at all forest sites.

Nevertheless, silver fir needles at the CRO mixed plot exhibited higher P_i contents compared to the EMM and CON mixed plots although plant-available P_i in the soil was in the same range at all three forests sites. At the CRO forest site, silver fir but not beech dominates the mixed forest stand by size and growth (Schwarz and Bauhus 2019). In order to explain this observation, various factors are conceivable, but remain speculative and need to be proved in further studies. (1) In mixed beech forests of the “Abieti-Fagetum” (Ellenberg 1988), co-existence of beech and silver fir is supposed to result from more efficient water use by both species due to different rooting depths (Köstler et al. 1968; Schmid and Kazda 2001). Nevertheless, a clear trend between sites regarding precipitation and/or temperature was not observed, except that the CRO site showed low precipitation during the vegetation period (Magh et al. 2018b). Further factors such as (2) intraspecific competition between silver fir trees (Dong et al. 2016, Fang et al. 2017), (3) nitrogen nutrition, (4) soil texture and soil pH (Sardans and Peñuelas 2004), and/or (5) mycorrhizal community composition may also significantly influence foliar P nutrition of silver firs at the CRO mixed forest plots. Yet another potential driver of the higher foliar P in silver fir needles of the CRO stand may be (6) the length of the vegetation period that is affected either by altitudes (EMM vs. CON) or by a more southerly location of the CRO forest site with shorter day length (21st of June: 17:03 h at the CON and 15:30 h at the CRO forest site (https://www.sunrise-and-sunset.com)). In addition, (7) the significant warming trend from 1990 to 2010 is accompanied by a decline in beech growth, whereas silver fir growth increased (Bosela et al. 2018). As at the CRO forest complementation of basal area index of silver fir by beech was positive (around 40%), that of beech by silver fir was negative (approx. − 30%) (Schwarz and Bauhus 2019). This observation might also be connected to the higher P_{tot}

and P_i contents of silver fir needles at the CRO compared to the CON and EMM forests observed in the present study. However, also this possible explanation needs further investigations. Moreover, multifactorial responses to climate, site characteristics, and/or competition could also result in higher P_i contents of silver fir needles at the CRO compared to EMM and CON forest stand.

Different effects of needles age on P and N contents

As found for P_{tot} in silver fir needles in the present study, declining nutrient contents with increasing needle age were found for *Abies concolor* and *Picea mariana* (Greenway et al. 1992; Hom and Oechel 1983; Miesel 2012) and indicate P remobilization from 1-year-old silver fir needles for the P supply to the growing current-year needles. Other studies with Norway spruce explained the decline of nutrient contents with needle age by nutrient dilution because of increasing needle mass (Chapin and Kedrowski 1983; Manghabati et al. 2019). The lower dry weight of current-year silver fir needles in early spring (Supplemental Figure S4) together with the declining P content is consistent with this assumption. However, when current-year needles were mature in late summer, the dry-to-fresh weight ratio reached the same amount as in 1-year-old needles (Supplemental Fig. S4). Hence, a P dilution effect between current and 1-year-old needles in late summer can be excluded, and a loss in P seems a more reasonable explanation for the reduction in total P between current and 1-year-old silver fir needles. At the EMM and the CON mixed plots, the decline in P_{tot} between current and 1-year-old needles was 35% and 37%, respectively. This proportion of P can be seen as the amount of P resorbed from the needles during ageing, i.e. until needles reach the age of 1-year. The resorbed P might be supplied to the next needle generation to fulfill the demand for growth and development as previously observed for sulphur (Schupp and Rennenberg 1988, 1992; Schupp et al. 1992; Schneider et al. 1994a; Blaschke et al. 1996). Different to the EMM and CON mixed plots, 1-year-old needles of the CRO mixed plot did not reveal any decline in total P. This lack of P mobilization coincidences with the generally higher total foliar P content of silver firs at this forest site. P resorption prior to needle abscission is a process keeping P and other nutrients within the tree (Aerts 1996; Melvin et al. 2015; Sohrt et al. 2018). The resorption efficiency seems to decline with increasing nutrition (Sohrt et al. 2018). It may thus be speculated that P resorption is not needed to fulfill the P demand of current-year needles of silver fir at the CRO mixed forest plot and that P_i uptake from the soil is sufficient to meet this demand. A similar conclusion was drawn for poplar that did not reveal P storage and mobilization in and from branch tissues (Netzer et al. 2018b; Plassard 2018). However, the P acquiring strategy of *Populus* x *canescens* seems due to an adaptation to high soil P availability in the natural habit of this tree species. Obviously under certain nutrient conditions and depending on the tree species, P resorption may be of less importance.

Although nutrient resorption has been described for other nutrients including N for conifers and deciduous trees (Helmisaari 1992; Aerts 1996), a study with *Abies pinsapo* (Blanes et al. 2013) revealed differences for N and P resorption depending on the forest site similar to the present study. Whereas in the present study the P content was lower in 1-year-old needles than in current-year needles in late summer, the opposite was seen for N. The N content seems to be higher in 1-year-old than in current-year silver fir needles at all three forest sites (EMM 117%, CON 137%, CRO 111%) and may indicate N storage. Similar results were found for *Abies pinsapo* at one of three forest sites (Blanes et al. 2013). In the study with *Abies pinsapo,* the N contents of the soil horizon analysed (though not specified) ranged from 7.6 to 32.7 µg g^{-1} and correlated with needle N (Blanes et al. 2013). In the present study, total N and inorganic N of leaf litter was similar (approx. 11 to 26 mg kg^{-1}), and the inorganic N content of the Ah horizon ranged from 4.1 to 6.8 mg kg^{-1}, but no clear correlation between foliar N and soil N was observed (Magh et al. 2018b). The foliar N/P ratio increased when the N content in needles of *Abies pinsapo* increased with needle age (Blanes et al. 2013), but did not exceed ~ 17 indicating balanced N and P nutrition. At the CRO forest site with the highest soil N in the Ah horizon, the silver fir needle N/P ratios were around 20 independent of needle age and season, but amounted to > 40 in 1-year-old needles of the EMM and the CON forest site strongly indicating P deficiency. Hence, balanced P and N nutrition at the CRO mixed plot might prevent accumulation of N in and remobilization of P from 1-year-old silver fir needles.

Conclusion

The EMM site has the highest mean annual air temperature and the lowest annual precipitation, and while the CON and CRO sites reveal similar mean air temperatures as well as amounts of rainfall, only approx. ¼ of it falls during the vegetation period at the CRO site (Magh et al. 2018b). Despite these differences in climate conditions, P contents and fractions in the foliage of beech were only affected by the season and neither by the forest site nor by co-cultivation with silver fir. It may therefore be concluded that high plasticity of beech trees leads to similar foliar P contents and fractions at all investigated forest sites. The underlying process could be internal P (re)cycling (Netzer et al. 2017, 2018a), which is supported by growth data of trees that showed increasing growth rate—measured as basal area increment—at the EMM forest, but slightly declining growth rates at the

CRO and CON forests between 2000 and 2016 (Schwarz and Bauhus 2019).

Although beech trees showed similar foliar P fractions and contents in the pure and mixed CRO plots, growth of silver fir exceeds that of beech (Schwarz and Bauhus 2019). This might relate, amongst other reasons, to drier conditions during the vegetation period, and to limited water and nutrient uptakes of beech during that time. At the CRO mixed plot, silver fir needles showed balanced N/P nutrition, as indicated by higher P_i contents and proportion of P_{tot}. Internal P_i mobilization from 1-year-old silver fir needles for the P supply of current-year needles seems not be relevant for silver fir at the CRO forest. Whether this may be an indication of a sufficient and balanced P nutrition of silver fir at the southern distribution limit of beech needs to be questioned in further studies and could relate the findings of the present study to ecological aspects. These might be related to the out-competition of beech by silver fir co-cultivation because of the acquisition strategy for P for growth of silver fir or due to higher summer drought resistance of silver fir compared to beech.

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Authors contribution CH wrote the manuscript and evaluated the data. HR and MD designed the research project and contributed to manuscript writing. MI and ML contributed to manuscript writing and soil sampling. FY and RM performed the experiments and statistics, contributed to soil sampling and manuscript writing. BD and SH performed the statistics on the linear mixed model.

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