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Phosphorus in Preferential Flow Pathways of Forest Soils in Germany

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Abstract: The transport of nutrients in forest soils predominantly occurs along preferential flow pathways (PFP). This study investigated the composition of phosphorus (P) forms in PFPs and soil matrix in several temperate beech forests with contrasting soil P contents in Germany. The PFPs were visualized using dye tracer experiments. Stained and unstained soil was sampled from three profile cuts per plot and analyzed for P fractions. The results show that labile P concentrations were highest in the O-layer and had the same range of values at all sites (240–320 mg·kg^{-1}), although total P (TP) differed considerably (530–2330 mg·kg^{-1}). The ratio of labile P to TP was significantly lower in the P-rich soil compared to the medium and P-poor soils. By contrast, the ratio of moderately labile P to TP was highest at the P-rich site. The shifts in P fractions with soil depth were generally gradual in the P-rich soil, but more abrupt at the others. The contents of labile and moderately labile P clearly differed in PFPs compared to soil matrix, but not statistically significant. The studied soils are characterized by high stone contents with low potential for P sorption. However, indications were found that labile organically bound P accumulates in PFPs such as biopores.

Keywords: phosphorus fractions; forest soil; preferential flow; PFP

1. Introduction

Phosphorus (P) in forest soils is distributed highly variably, both between and within soil types, with respect to contents, speciation, availability, and sources of P [1]. Soils contain various P forms, generally differentiated in organically bound (P$_o$) and inorganic (Pi) P. Organic P occurs as orthophosphate monoesters (e.g., inositols, mononucleotides, sugar phosphates), orthophosphate diesters (phospholipids, teichoic acids, nucleic acids, and their degradation products), and phosphonates. Inorganic P is bound in minerals (e.g., apatite) and is present in dissolved form as ortho-, pyro-, and/or polyphosphates [2,3]. The total content and stock of soil P varies from very low (<100 mg·kg^{-1} and <10 g·m^{-2}) to P-rich (>3000 mg·kg^{-1} and >1000 g·m^{-2}), and the mineral P supply and P speciation is strongly dependent on the parent material and pedogenesis [4,5]. Whereas P supply from the mineral phase is the main source of available P in P-rich soils, P availability for plants in P-poor soils is considerably influenced by P speciation [4]. These differences in P supply drive P fluxes in the systems and determine the dominating strategy of ecosystem nutrition [6]. Besides those internal factors, external environmental and anthropogenic impacts (as forest management, N deposition, etc.) substantially influence the distribution of P and nutrient cycling in temperate forest soils [7–10].

The predominant transport pathway for nutrients in forest soils is along preferential flow pathways (PFP), bypassing large parts of the soil matrix [11–13]. An established method to investigate PFPs and their role in water and nutrient transport are tracer experiments [14–17]. Previous tracer studies indicated that PFPs in forest soils can be stable for decades, resulting in differences in biological and chemical parameters between PFPs and the soil matrix, particularly when preferential flow

along root channels dominates the flow system [12,18]. Accumulation in stable flow paths has been found in previous studies for radionuclides, SOC, N [15,18], and also for C, Ca, Mg, and Fe [12]. Backnäs et al. [19] investigated P fractions in PFPs as compared to the soil matrix in a Podzol under forest in Southern Lapland, Finland. The results indicated that the leaching of P through PFPs occurred primarily along stone surfaces, where PFPs are larger than PFPs related to coarse grains or roots. Thus, P sorption may be limited due to potentially faster water flow, weaker lateral mass exchange and less sorption interaction than in smaller PFPs [20]. By contrast, P accumulation might occur in small preferential pores with decelerated water flow and longer contact time between the solution and inner pore surfaces [19]. As a result, such PFPs with high potential to accumulate P increase the likelihood for elevated P concentrations in export fluxes [21]. However, there are very few data available regarding P accumulation and transport in forest soils. Therefore, this study investigated the composition of P forms in PFPs and the soil matrix in four temperate forest ecosystems in Germany. Furthermore, the influence of different initial conditions regarding the content of total and available P at the sites on the distribution of P forms in the soil profiles and in PFPs was examined. The correlation between P fractions and chemical parameters was tested to identify the most important factors influencing the occurrence of P fractions in the investigated soils.

2. Materials and Methods

2.1. Site Description

Field experiments and sampling were conducted at four beech dominated forests (*F. sylvatica* L.) across Germany with similar stand age (110–120 years). The four study sites Lüss (LUE), Vessertal (VES), Mitterfels (MIT), and Bad Brückenau (BBR) are core sites of the German DFG Priority Program SPP 1685 which aims at testing the overall hypothesis that the P-depletion of soils drives forest ecosystems from P acquiring systems (efficient mobilization of P from the mineral phase) to P recycling systems (highly efficient cycling of P) [6]. Further, all sites are long-term monitoring sites in Germany and part of the EU-ICP Forests Level II Program (International Co-operative Programme on the Assessment and Monitoring of Air Pollution Effects on Forests). The sites were selected with regard to their considerable different availability of mineral P following the sequence BBR > MIT > VES > LUE. The BBR site was defined as P-rich site, LUE as P-poor site, and MIT and VES as medium P-supplied sites. Three sites (VES, MIT, and BBR) are located in the low mountain ranges, whereas LUE is in the lowlands. The sites differ in parent material, soil type, and climate (Table 1). Soil properties are summarized in Table 2. For better comparisons, the soil horizons were aggregated for each site considering their physical and chemical properties to: O—organic layer, A—topsoil horizon, B—subsoil horizon, C—lower subsoil horizon containing weathered parent material.

Table 1. Overview over the study sites.

	LUE	VES	MIT	BBR
Location	52°50′32″ N 10°16′06″ E	50°36′26″ N 10°46′20″ E	48°58′35″ N 12°52′49″ E	50°21′38″ N 9°55′71″ E
Elevation (m a.s.l.)	115	810	1023	809
MAT (°C)	8.0	5.5	4.5	5.8
MAP (mm)	779	1200	1229	1031
Parent material	Pleistocene sands	Trachyandesite	Gneiss	Basalt
Soil type (WRB *)	Dystric skeletic Cambisol	Hyperdystric skeletic folic Cambisol	Hyperdystric skeletic chromic Cambisol	Hyperdystric folic Cambisol

LUE: Lüss (Lower Saxony); VES, Vessertal (Thuringian Forest); MIT, Mitterfels (Bavarian Forest); BBR, Bad Brückenau (Bavarian Rhön Mountains); MAT, Mean annual temperature; MAP, Mean annual precipitation; * World Reference Base for Soil Resources 2014, World Soil Resources Reports 106, FAO, Rome, Italy.

Table 2. Soil properties at four study sites in beech forests in Germany.

Study Site/ Horizon	Depth (cm)	Texture	pH (CaCl$_2$)	C (%)	N (%)	Al$_{ox}$	Al$_{di}$	Fe$_{ox}$ (g·kg^{-1})	Fe$_{di}$	P$_{ox}$	TP
Lüss (LUE)											
O	9 to 0		2.8	36.63	1.59	0.73	0.79	0.87	1.30	0.15	0.49
A	0 to −12	Loamy sand	3.0	1.69	0.07	0.18	0.37	0.29	2.17	<0.04	0.06
B	−12 to −52	Loamy sand	3.7	0.90	0.04	1.04	1.00	1.40	3.21	<0.04	0.08
C	−52 to −100	Sand	4.3	0.12	0.01	0.50	0.41	0.15	1.01	<0.04	0.04
Vessertal (VES)											
O	4 to 0		3.4	32.17	1.72	4.11	1.20	1.28	1.75	0.30	0.86
A	0 to −3	Sandy loam	3.0	7.49	0.45	2.75	1.68	3.14	7.46	0.53	0.70
B	−3 to −65	Sandy loam	4.1	3.03	0.20	9.44	7.40	5.13	9.23	0.83	0.98
C	−65 to −100	Loamy sand	4.1	0.37	0.03	4.64	2.83	0.59	5.55	0.16	0.32
Mitterfels (MIT)											
O	4 to 0		3.5	44.12	2.19	0.95	0.87	1.28	1.45	0.34	0.88
A	0 to −4	Silt loam	3.0	35.50	2.05	3.32	3.83	3.58	4.97	0.47	1.14
B	−4 to −80	Silt loam	3.9	5.45	0.34	6.26	5.62	8.05	12.17	0.53	0.91
C	−80 to −100	Loamy sand	4.3	1.09	0.09	5.73	4.63	3.61	10.89	0.35	0.66
Bad Brückenau (BBR)											
O	3 to 0		4.6	27.33	1.68	5.53	5.51	15.27	21.85	1.29	2.24
A	0 to −7	Silty clay	4.2	17.57	1.28	7.77	8.30	21.15	31.52	1.51	2.45
B	−7 to −30	Silt clay loam	4.3	7.45	0.54	10.58	10.83	24.46	35.41	1.84	2.62
C	−30 to −65	Loam	4.5	4.60	0.34	10.70	9.57	18.87	25.92	1.63	2.39

Horizon: O—organic layer, A—topsoil horizon, B—subsoil horizon, C—lower subsoil horizon containing weathered parent material. Values for C and N are total contents (for C this equals organic C); Al$_{ox}$, Fe$_{ox}$, and P$_{ox}$—ammonium oxalate-extractable contents; Al$_{di}$ and Fe$_{di}$—dithionite-extractable contents; TP—total P contents.

2.2. Field Experiments

At each study site a test plot of 1 m^2 was established at a minimum distance of 2 m from any trees. On the first day, the plots were irrigated with 30 L of tracer-water solution containing 3 g·L^{-1} of Brilliant Blue FCF (within 1 hour) to activate rapid flow paths (PFPs) in the soils. Brilliant Blue dye was used due to its good visibility, retardation behavior in soil, and its non-toxicity to the environment [16,17]. After the dye tracer irrigation, the plots were sprinkled with 2 L of water to wash off the dye residues from the organic layer [19], and then covered overnight with a tarpaulin to exclude disturbances (e.g., rainfall). The irrigated plots were excavated on the following day by successively preparing five horizontal profile cuts per plot (at 20, 35, 50, 65, and 80 cm from the plot border) down to 1 m depth and 1 m width (see Figure 1). Stained (flow region) and unstained (non-flow region) soil was sampled respectively from the three inner cuts at 35, 50, and 65 cm from all morphological horizons. The amounts of soil material per sample varied because the thickness of soil horizons and stained/unstained parts per horizon differed considerably. The samples were sieved (<2 mm), dried at 40 °C, and then stored in plastic bottles until analysis. For the analysis of C, N, and total P the samples were finely grounded.
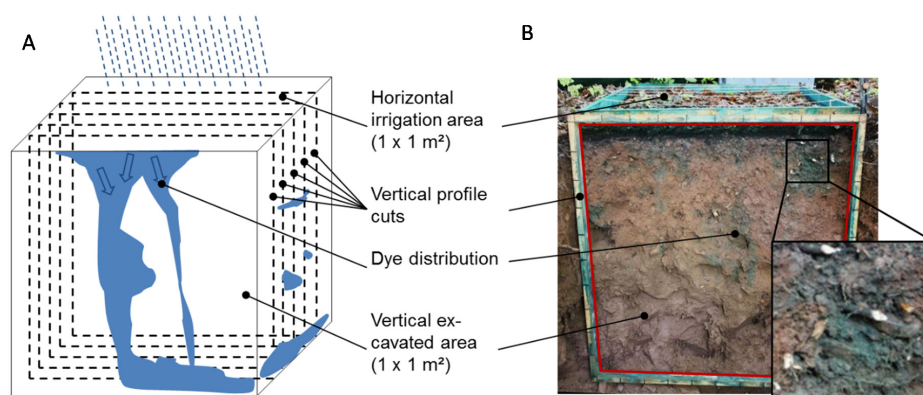

Figure 1. Scheme of tracer experiments (**A**): Dye application at the soil surface (1 m^2), covering overnight, and excavation of five vertical (1 m^2) profile cuts (example on the right (**B**)). The black framed enlargement shows staining of flow pathways along roots and stone surfaces.

2.3. Chemical Analyses

The P fractions in stained and unstained soil samples were analyzed using the Hedley fractionation method [22,23]. This method uses increasingly strong extractants to determine P forms with differing availability for plants. All samples were extracted with a replicate. Additionally, certified reference soil material from the Wageningen Evaluating Programs for Analytical Laboratories, International Soil-analytical Exchange (WEPAL-ISE 865 and 884) and blanks were treated equally to the samples for quality control of the extraction procedure.

In the fractionation procedure, a subsample of 0.5 g soil was first shaken in distilled water containing an anion exchange resin (Dowex 22, 16–50 mesh, Sigma-Aldrich, Taufkirchen, Germany) in netting bags (PP-405/230-41, Bückmann, Mönchengladbach, Germany) [24]. The following extractants applied were: 0.5 M sodium bicarbonate ($NaHCO_3$-P), 0.1 M sodium hydroxide ($NaOH_{-i}$), 1 M hydrochloric acid (HCl_{dil}-P), hot concentrated 3.5 M hydrochloric acid (HCl_{conc}-P), and finally nitric acid digestion of the residual P fraction (residual P). After each extraction step, the amount of inorganic P in the extract solution was measured using the method of Murphy and Riley [25] and O'Halloran and Cade-Menun [26]. For the determination of total P (TP) in $NaHCO_3$, $NaOH$, and HCl_{conc}, the extracts were oxidized in an autoclave using ammonium persulfate. Organically bound P (P_o) was calculated as the difference between TP and P_i [23]. The P concentrations in all extracts were measured colorimetrically with a spectrophotometer (UV-mini 1240, Shimadzu Deutschland GmbH, Duisburg, Germany). For a detailed description of the procedure applied here see Tiessen and Moir [23].

According to the concept of Hedley fractionation, the different P fractions represent (1) surface-adsorbed readily available P, (2) strongly-bonded or adsorbed moderate available P, and (3) very strongly-bonded or inaccessible mineral P with very low availability [22,23,27]. Therefore, the analyzed P fractions were classified for further data analyses as labile P (resin-P_i + $NaHCO_3$-$P_{i,o}$), moderately labile P ($NaOH$-$P_{i,o}$ + HCl_{dil}-P_i), and stable P (HCl_{conc}-$P_{i,o}$ + residual-P) (cf. [28]). The initial TP content of all samples was determined using the same method as the residual P fraction, by digestion with concentrated nitric acid and measurement with ICP-OES (CIROS, SPECTRO Analytical Instruments GmbH, Kleve, Germany). Total C and N contents were measured after dry combustion of grounded samples with a CN analyzer (Vario EL III Elementar GmbH, Hanau, Germany). The soils contained no inorganic C, thus soil organic C (SOC) equals total C. The concentrations of C in PFPs were not corrected for any C added as part of the Brilliant Blue dye. The C content of the dye is 56%, and the amount of total added C in the experiments was 50 g C m^{-2}. Bundt et al. [15] conducted comparable experiments with a dye-derived addition of 75 g C m^{-2} and found a resulting increase of C with a maximum of 2.5 % SOC in in PFPs in 0–9 cm depth, and a maximum of 3.5 % SOC in PFPs in 50–100 cm depth. In the current study, less dye was applied to the plots, and thus the change in C content in PFPs is expected to be very low. Amorphous metal sesquioxides (Al_{ox}, Fe_{ox}, Mn_{ox}) and P_{ox} were determined by extraction with acid 0.2 M ammonium oxalate (100 mL added to a subsample of 2 g). Dithionite-extractable (Fe_{di}, Al_{di}, Mn_{di}) was analyzed with 50 mL Na-citrate-dithionite in a subsample of 0.5 g (modified after Holmgren, 1967). The measurement of element contents in the extracts was conducted with ICP-OES.

2.4. Statistical Analysis

The statistical significance of differences between P contents of different chemical fractions in flow pathways and matrix were tested using the R software package (version R i386 3.1.0, R Foundation for Statistical Computing 2014, Vienna, Austria). Given that most of the data sets exhibited non-normal distributions (tested by Shapiro-Wilk test and q-q-plot), the non-parametric Wilcoxon rank-sum test was used to statistically compare mean values. The statistical tests were conducted pair-wise including all data of the four sites, and at horizon level due to the hierarchical structure of the data (profiles, cuts, horizons). For correlations between chemical parameters and P fractions, the Spearman's rho correlation coefficients were calculated and tested for significance.

3. Results and Discussion

The soil P fractions classified as labile, moderately labile, and stable P for PFP and soil matrix samples are shown in Figure 2. The values represent mean values for the three vertical cuts per profile. For the forest floor (O-layer), it was not possible to distinguish between stained and unstained material, due to the homogeneous distribution of the dye tracer from the irrigation. At the sites VES and MIT, the tracer did not reach the C horizon. Thus, the presented results contain no soil matrix data for the O-layer and no PFP data for the lower subsoil.

The TP values differed at the four study sites in their contents (45–2700 mg·kg^{-1}) and distribution within the soil profiles (Figure 2, TP = total bar length). Whereas TP at the P-poor site (LUE) was clearly highest in the O-layer and strongly reduced in the mineral soil, the P-rich soil (BBR) showed the lowest values in the O-layer and had a slight increase in the subsoil. However, the amount of TP in all horizons at the P-rich site was much higher compared to the other sites, and was more or less equally distributed through the profile. The summarized P fractions were compared to the initial TP contents in order to determine the recovery rate of the analytical method. Considering all samples, the TP recovery was very high, with a correlation coefficient of 0.98.
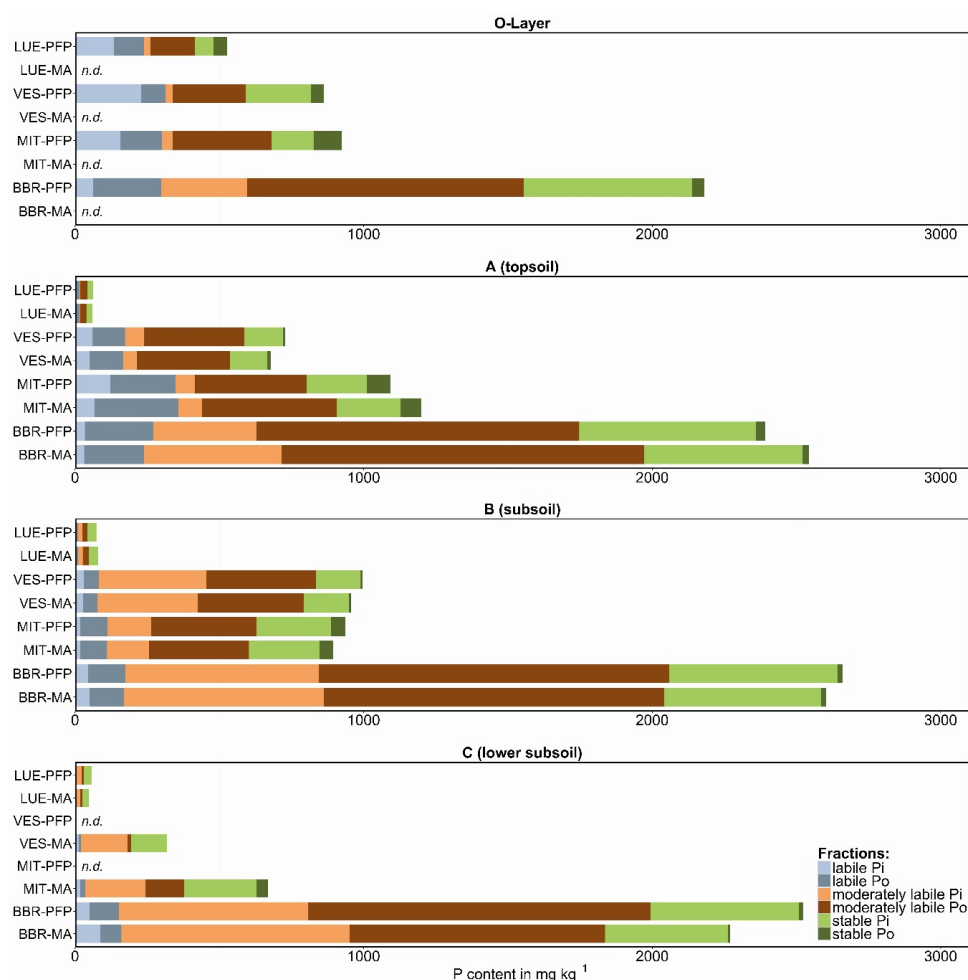

Figure 2. Phosphorus fractions summarized as labile (resin-P$_i$ + NaHCO$_3$-P$_{i,o}$), moderately labile (NaOH-P$_{i,o}$ + HCl$_{dil}$-P$_i$), and stable P (HCl$_{conc}$-P$_{i,o}$ + residual-P) for PFP (preferential flow pathways) and MA (soil matrix) samples; n.d., no data available; study sites in the sequence P-poor to P-rich are: LUE, Lüss, Lower Saxony; VES, Vessertal, Thuringian Forest; MIT, Mitterfels, Bavarian Forest; BBR, Bad Brückenau, Bavarian Rhön Mountains.

3.1. Labile P Fractions

Despite the large difference in TP between the sites, labile P (here defined as resin-P_i + NaHCO$_3$-$P_{i,o}$) was in the same range of values, at least for the medium P-supplied (VES, MIT), and the P-rich (BBR) sites. In the O-layer, the P-poor site (LUE) also had comparable values for labile P as the other sites. The share of labile P to TP in the O-layer was highest at the P-poor site (45 %), slightly lower at the medium sites (36 % and 33 %), and lowest in the P-rich site (14 %) (Figure 3a.1). There was a steep decrease in the ratio labile P to TP with soil depth at the P-poor to medium sites; and a more gradual decrease with depth at the P-rich site.
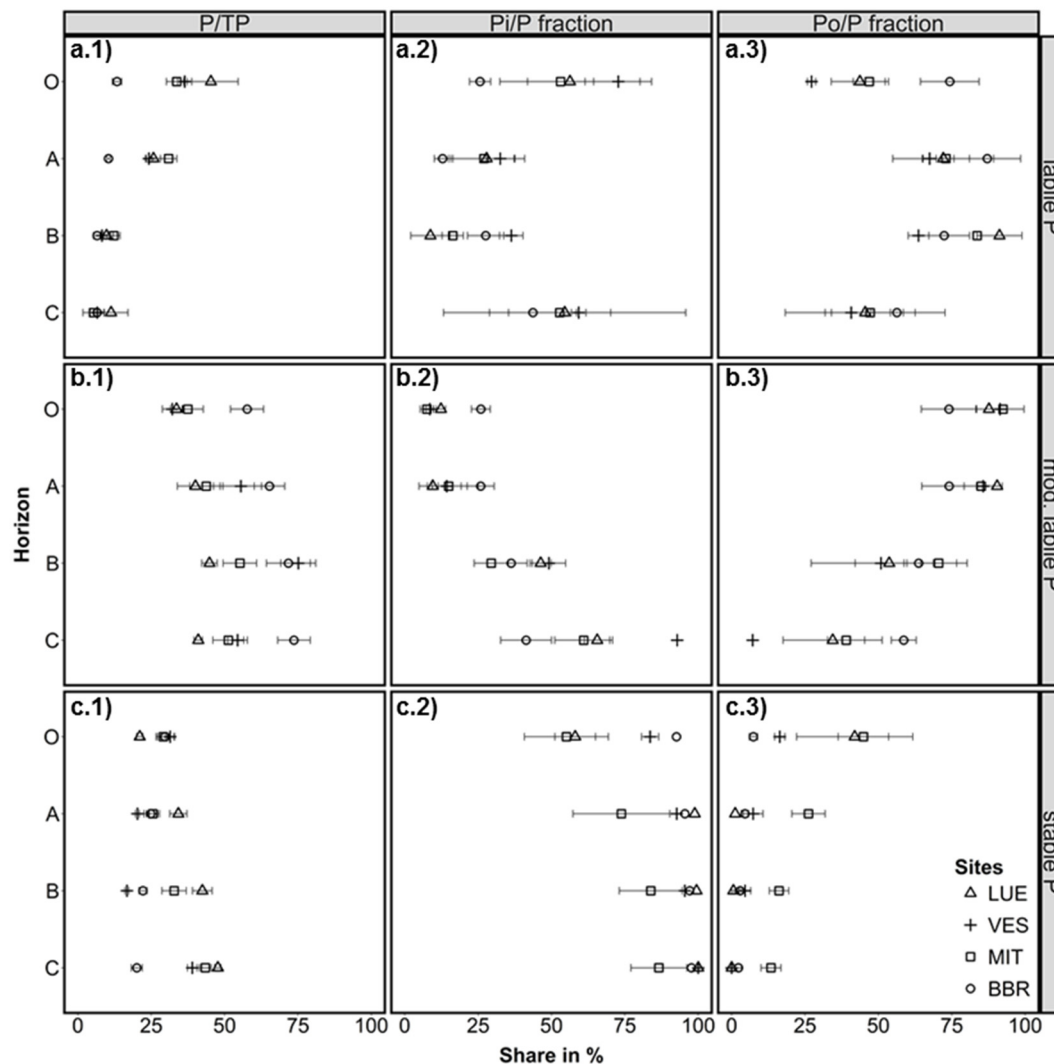

Figure 3. Share of **a.1**) the labile (resin-P_i + NaHCO$_3$-$P_{i,o}$), **b.1**) moderately labile (NaOH-$P_{i,o}$ + HCl$_{dil}$-P_i), and **c.1**) stable (HCl$_{conc}$-$P_{i,o}$ + residual-P) fractions on total P (TP) in organic layer (O) and soil (A, B, C horizons). Share of inorganic P (**a.2**: labile P_i, **b.2**: moderately labile P_i, **c.2**: stable P_i) and organically bound P (**a.3**: labile P_o, **b.3**: moderately labile P_o, **c.3**: stable P_o) on the particular pools. Error bars: standard deviation of three samples (profile cuts). Study sites in the sequence P-poor to P-rich are: LUE, Lüss, Lower Saxony; VES, Vessertal, Thuringian Forest; MIT, Mitterfels, Bavarian Forest; and BBR, Bad Brückenau, Bavarian Rhön Mountains.

Organically bound P dominated the labile P pool in the A and B horizon for all sites, and also in the O-layer for BBR. At VES, 27% of labile P in the O-layer consisted of P_o, while MIT and LUE had approximately equal amounts of P_i and P_o (Figure 3a.2,a.3). Thus, most of the easily plant-available P_i

was located in the O-layers at the medium and P-poor sites, whereas labile P was clearly dominated by P_o at the P-richest site. This finding was unexpected, as very low P supply from the mineral phase is assumed to enhance tight P recycling in forest ecosystems [6] (and therefore the fast uptake of P_i) which would result in a higher share of P_o at the P-poor site. In a recent study conducted at the same P-rich and P-poor study sites, Bünemann et al. [29] found that biological/biochemical processes were the dominant control of P_i availability in the low-P-sorbing sandy soil of the P-poor site. By contrast organic P mineralization was negligible when compared to the high P_i availability at the P-rich study site, which has a high-P-sorbing soil and very high TP content. Further, the high contents of amorphous and crystalline sesquioxides at the P-rich site (Table 2) may act as a sink for labile P_i, when it is adsorbed onto those surfaces and precipitated (depending on pH) (cf. [30,31]).

The differences in labile P between PFP and soil matrix were marginal considering the sum of P_i and P_o of this pool (Figure 2), but the ratio of P_i to P_o differed. Thus, the medium P-supplied site showed clearly higher contents of labile P_i and less labile P_o in PFPs compared to soil matrix. By contrast, labile P_o was significantly higher in PFPs of the P-rich site. However, the variation of results for labile P_i and P_o was quite high between the three profile cuts within the soil plot (see error bars in Figure 3), which indicates that conclusions from the results have to be drawn carefully.

3.2. Moderately Labile P Fractions

The sum of moderately labile P forms ($NaOH-P_{i,o}$ + $HCl_{dil}-P_i$) was the dominant fraction in mineral soil at the P-rich sites (Figures 2 and 3). At the P-poor site, this pool was equal or slightly lower than the stable P pool. At all sites, moderately labile P was predominantly organically bound in the O-layer and topsoil. In the subsoil, the ratio of P_i to P_o shifted towards P_i (Figure 3b.2,b.3). The shifts with depth were relatively gradual in the P-rich soil, but were more abrupt at the medium and P-poor sites.

The NaOH-extracted P represents the moderately available P forms related to Al, Fe, and humic substances. In acidic forest soils on silicate parent material, NaOH-P was observed to be involved in P_i adsorption (mainly to Al-oxyhydroxides) as well as P_o adsorption (to Fe-oxyhydroxides or Al-saturated soil organic matter) [4]. The share of HCl_{dil}-extractable P (apatite-bound) was very small for most samples, which indicates highly weathered soils [32]. In the P-rich soil, HCl_{dil}-P was found to be in the range of 150–300 mg·kg^{-1}, which confirms that the supply with mineral P is still high at this site. These results are in line with findings of Prietzel et al. [4] who identified apatite-P in this P-rich soil using P *K*-edge X-ray absorption near-edge structure (XANES) spectroscopy.

The sum of moderately labile P showed a variable distribution between PFP and soil matrix (Figure 2). In the topsoils, the P-poor to medium sites (LUE and VES) had slightly higher contents of this pool in PFPs, whereas clearly higher contents in soil matrix were observed at the medium to P-rich sites (MIT and BBR). Here, both higher P_i as well as higher P_o values contributed to the increased pool contents. For the subsoils, slightly higher contents of moderately labile P in PFPs as compared to soil matrix were detected at all sites which was mainly caused by higher P_o contents, especially at the P-rich site. For the P-poor site, the differences are hardly interpretable because the chemical analyses of the very low P concentrations led to high data uncertainty.

3.3. Stable P Fractions

Stable P in the soils ($HCl_{conc}-P_{i,o}$ + residual-P) followed the sequence of decreasing initial TP in the order of: BBR > MIT > VES > LUE, and was characterized by low contents of P_o compared to P_i in all soils (Figure 2). Elevated P_o portions in the O-layer were observed at the P-poor site and at the medium site MIT (Figure 3c.3), which indicates the presence of P associated with very stable organic compounds. However, the ratio of stable P to TP in the O-layer was comparable at all sites with around 30 % of TP at the P-rich to medium sites, and slightly lower at the P-poor site with 21 %. The depth distribution of this relation showed a slight decrease with depth in the P-rich soil, but an increase in the medium P-supplied soils and particularly increased in the P-poor soil. Here, almost 50 % of the

P in the subsoil is stable bound and not available for plants, which is in line with the assumption of decreasing availability of mineral-bound P from the P-rich site to the P-poor site.

The contents of stable P in PFPs and soil matrix were on the same level. Only for the P-rich soil, slightly higher values in PFPs were observed.

The distribution of P fractions revealed for the P-rich site comparable labile P concentrations as for the other sites, but a clearly lower ratio of labile P to TP, and much more P_o than P_i in this pool. Considering the assumption of negligible P_o mineralization [29], it can be assumed that the P-rich soil has enough labile P available from mineral sources and has no need for increased P_o mineralization. P_o therefore remains stable, but P_i is consumed and reduced thus relative to the amount of labile P_o. This may also explain the higher ratio of moderately labile P to TP at the P-rich site compared to the other sites. In this pool, P is moderately bound to inorganic and organic compound, and can be acquired if necessary.

3.4. Factors Affecting the Distribution of P Fractions in the Soil Profiles

The statistical test of correlations between parameters included in the first step all data from the four study sites and covered a wide range of values of chemical soil properties. The results confirmed a close relationship between labile P and the contents of soil organic C and total N, which is in accordance with previous studies [4,30,33,34] (for the correlation matrix and scatter plots see Table S1 of the supplementary material). This correlation is stronger for labile P_o (N: $R^2 = 0.98$, C: $R^2 = 0.97$) than for labile P_i (N: $R^2 = 0.94$, C: $R^2 = 0.93$). Also in agreement with previous studies [30,34], the easily available resin-P was strongly correlated with $NaHCO_3$-P_i ($R^2 = 0.79$) and $NaHCO_3$-P_o ($R^2 = 0.75$) indicating the contribution of inorganic as well as organic labile P fractions to the resin-P_i pool.

Moderately labile P was strongly related to sesquioxides (Al, Fe, Mn). In this fraction, P_i showed close correlations to Al_{ox}, Al_{di}, Mn_{ox}, Mn_{di}, and had slightly lower correlations with Fe_{ox} and Fe_{di}. Moderately labile P_i was also positively correlated with pH, but negatively correlated to the C/N ratio. By contrast, the strongest correlation of P_o in this fraction was found with Fe-oxyhydroxides, and to a lesser extent with Al- and Mn-oxyhydroxides. This strong contribution of Fe compounds to P_o adsorption, but the adsorption of mainly P_i to Al-oxyhydroxides, is in line with the study of Prietzel et al. [4] for acidic forest soils.

For stable P, a close correlation of P_i with Fe, Al, and Mn compounds was identified, whereas P_o was strongly related to C and N. This shows that P_o is occluded in stable organic compounds or particulate organic matter.

To indicate the dominant processes influencing the distribution of P_i and P_o in soils, the ratio C to P_o was calculated (Table 3). Low C/P_o values (<200) indicate the net mineralization of P, whereas high ratios (>300) are indicative for immobilization as result of microbial P_i utilization during decomposition of organic matter [35,36]. When comparing the test sites with considerable differences in soil P contents, the C to P_o ratio was highest at the P-poor site and lowest at the P-rich site. The strong reduction of C/P_o with depth at all sites can be attributed to the sharp decrease of organic C compared to P. In the O-layers, C/P_o was > 300 at the P-poor and medium sites, which indicates P immobilization (incorporation into biomass). C/P_o-values between 200 and 300, which occurred in the O-layer of the P-rich site, indicate a stable rate of cycling between P_i and P_o [35]. These findings support the hypothesis of intense P recycling at P-poor sites [6] leading to P depletion and high C to P_o ratios. The C/P_o ratio also differed considerably between PFP and soil matrix samples at all site with mostly clearly higher values in PFPs (Table 3). For the P-rich site, the difference between PFP and matrix was more pronounced in the topsoil and diminished with soil depth. In the P-poor soil, the contrasting behavior was observed: the difference increased with depth indicating stronger P_o depletion in the subsoil PFPs.

Table 3. Ratios of organic C to sum of organic P (Po) at four beech forest study sites in Germany for PFP (preferential flow pathways), Ma (soil matrix), and RC (root channel-PFP) samples.

| C/P_o | LUE | | VES | | MIT | | BBR | |
Horizon	PFP	Ma	PFP	Ma	PFP	Ma	PFP	Ma
O	1143		840		910		223	
A	512	494	155	174	593	371	139	97
B	356	274	89	66	101	93	58	51
C	204	88		170		59	42	43

Horizon: O—organic layer; A—topsoil horizon; B—subsoil horizon; C—lower subsoil horizon containing weathered parent material. Study sites in the sequence P-poor to P-rich are: LUE—Lüss, Lower Saxony; VES—Vessertal, Thuringian Forest; MIT—Mitterfels, Bavarian Forest; and BBR—Bad Brückenau, Bavarian Rhön Mountains.

For all chemical parameters, the differences between PFP and the soil matrix were tested statistically (for test results see Table S2 of the supplementary material). The most statistical differences were found for the P-rich site. Significantly higher values in PFPs occurred here for C, labile P_o, and C to TP ratio with $p < 0.01$, and for N, stable P_o and stable P_i with $p < 0.05$. For the other sites, almost no statistically significant differences of P fractions and other parameters were identified. At the P-poor site, the ratio C to TP and the C content were statistically higher in the PFPs ($p < 0.05$). At the medium sites, the content of metals differed substantially, with high contents of Fe_{ox}, Mn_{ox}, Al_{di} and Mn_{di} in PFPs, but lower values of Fe_{di} compared to matrix samples. However, these statistical tests included all data per site and have to be considered carefully due to the hierarchical structure of the data. For the same tests at the horizon level per site, the data sets were much smaller (three samples for PFP and matrix respectively) and revealed no significant differences between PFP and matrix for all parameters (Table S3). In previous studies, Hagedorn and Bundt [18] suggested that PFPs in forest soils can be stable for decades, resulting in biological and chemical gradients between PFPs and soil matrix [15]. Bogner et al. [12] confirmed this hypothesis for PFPs along root channels and found an enrichment of C, N, Ca, Mg, and Fe in PFPs compared to the soil matrix. Such an accumulation in PFPs was also documented for different P fractions in flow pathways along stones by Backnäs et al. [19]. Our study sites are characterized by very high stone contents, resulting in very heterogeneous conditions for water and solute flow. PFPs were therefore located mostly along stone surfaces with low potential for P sorption [19], and preferential flow occurred less in root channels or other biopores where P accumulation would be expected to be more prevalent. Single biopores could be identified and sampled separately, which contained decayed root material. Indications were found in these biopores of an accumulation of labile organically bound P (P_o), but could not be statistically confirmed. With respect to the distribution of subsurface flow pathways at hillslopes (midrange mountain sites), it has been shown that a predominant surface-near lateral flow does not support chemical gradients between PFPs and soil matrix. Thus, non-significant results in the statistical test are a result of specific site conditions, spatial soil heterogeneity, and analytical uncertainties with regard to very low P concentrations at the P-poor site.

Nevertheless, several factors affecting the distribution of P within the tested soils became apparent. The results showed that the labile $P_{i,o}$ pool and stable P_o were strongly related to C and N, whereas moderately labile $P_{i,o}$ as well as stable P_i correlated with metal-oxides and -oxyhydroxides (Al, Fe, Mn). At the medium to P-rich sites, where higher contents of sesquioxide in topsoil matrix compared to PFPs were observed, moderately labile P_i and P_o also was clearly higher in soil matrix. The higher P_o contents of this moderately labile pool in subsoil PFPs could be related to Fe_{ox} accumulated in the flow pathways.

4. Conclusions

As expected, considerable differences were found for the four study sites in both the P contents and the distribution of P fractions within the soil profiles. Despite these large differences, the labile P

contents in the O-layers were in the same range of values at all sites. At the site with very low supply of P from the mineral phase, the predominant portion of labile P was available in the O-layer, but was greatly reduced in the mineral soil. This indicates that for this site intense P recycling happens in the O-layer, which is also supported by a high C to P_o ratio in this layer. By contrast, labile P was available throughout the whole profile at the P-rich site. Here, the supply of plant-available P seems to be sufficient enough that there is no need for tight P recycling, and may therefore explain the very low share of labile P to TP, when compared to the P-poor site. The contents of labile and moderately labile P clearly differed in PFPs compared to soil matrix, and indicate that labile organically bound P (P_o) accumulates in PFPs with a high content of organic matter. However, these results could not be proved statistically at the tested sites. Therefore, more sites particularly for soils with a higher proportion of preferential flow through biopores should be studied to verify the indications.

Supplementary Materials: The following are available online at www.mdpi.com/1999-4907/8/1/19/s1, Table S1: Correlation matrix with Spearman's rho coefficients to test the correlation between chemical soil parameters (dataset: *n* = 123, includes data of four forest soils in Germany), Table S2: Results (*p*-values) of the non-parametric Wilcoxon rank-sum test for differences between PFPs and soil matrix samples (pair-wise test for all data per site), Table S3. Results (*p*-values) of the non-parametric Wilcoxon rank-sum test for differences between PFPs and soil matrix samples (pair-wise test at horizon level per sites).

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