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# Organic phosphorus in soil water under a European beech (*Fagus sylvatica* L.) stand in northeastern Bavaria, Germany: seasonal variability and changes with soil depth

KLAUS KAISER*, GEORG GUGGENBERGER and LUDWIG HAUMAIER
*Institute of Soil Science and Soil Geography, University of Bayreuth, 95440 Bayreuth, Germany; *Author for correspondence (e-mail: klaus.kaiser@uni-bayreuth.de; phone: +49 921 55 2178; fax: +49 921 55 2246)*

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**Abstract.** Organically bound phosphorus (P) is a mobile form of phosphorus in many soils and thus its dynamics relevant for the leaching and cycling of this element. Despite its importance, little is known about the chemical composition of dissolved organic P. We studied the concentrations, fluxes, and chemical composition of organic P in forest floor leachates and soil solutions in a Rendzic Leptosol under a 90-year-old European beech (*Fagus sylvatica* L.) forest over a 27-month period (1997–1999). The chemical composition of organic P was analysed using XAD-8 fractionation and $^{31}$P-nuclear magnetic resonance (NMR) spectroscopy. Organic P was the dominant P form in forest floor leachates as well as in porewaters of the mineral soil. The largest concentrations of organic P were observed during summer and peaked (330–400 $\mu$g dissolved organic P l$^{-1}$) after rain storms following short dry periods, concurrently with the concentrations of organic carbon (OC). Because of high rainfall, fluxes of organic P (and C) were greatest in autumn although concentrations of organic C and P were lower than in summer. In forest floor leachates, the hydrophilic fraction of dissolved organic matter contained 83 ± 13% of the bulk organic P. In soil solutions from 90 cm depth, organic P was almost exclusively in the hydrophilic fraction. Because of the low retention of the hydrophilic fraction of dissolved organic matter in the mineral soils, concentrations of organic P in soil water remained almost constant with depth. Consequently, organic P contributed > 95% of the total P leached into deeper subsoils. The overall retention of organic P in the weakly developed mineral soils was little and so the average annual fluxes of organic P in subsoils at 90 cm depth (38 mg m$^{-2}$) comprised 67% of those from the forest floors (57 mg m$^{-2}$) during the study period. Hence, organic P proved to be mobile in the studied soil. $^{31}$P-NMR spectroscopy confirmed the dominance of organic P species in soil water. Signals due to inorganic P occurred only in spectra of samples collected in winter and spring months. Spectra of samples from summer and autumn revealed traces of condensed phosphates. Due to low P contents, identification of organic P species in samples from winter and spring was not always possible. In summer and autumn, monoester and diester phosphates were the dominant organic species and varied little in their relative distributions. The distribution of organic species changed little from forest floor leachates to the subsoil solutions indicating that the composition of P-containing compounds was not influenced by sorptive interactions or biological transformation.

## Introduction

In forest ecosystems, organic forms comprise major or even dominant proportions of nitrogen (N), phosphorus (P), and sulphur (S) dissolved in throughfall, forest floor leachates, and soil percolation waters (Homann et al. 1990; Qualls et al. 1991; Qualls and Haines 1991; Hedin et al. 1995; Michalzik et al. 2001). Organic forms are often more important in the transport of N and P than are inorganic species, especially in the deeper mineral soil, thus controlling the nutrient leaching from rooting zones (Qualls and Haines 1991; Hedin et al. 1995). Translocation and leaching of organic nutrient species exceed not only those of inorganic species, organic nutrient species are also more mobile in soil than organic carbon (C) (e.g., Qualls and Haines (1991) and Andersson et al. (1999)). Some of the reasons for the preferential leaching of organic nutrient species are due to little uptake by plant roots (Helal and Sauerbeck 1984) and occurrence in the mobile hydrophilic fraction of dissolved organic matter (Andersson et al. 1999; Kaiser 2001).

The production of soluble organic C, N, P, and S is sensitive to changes of the soil environment such as temperature and pH (Smith et al. 1998; Andersson et al. (1999, 2000)) and so varies seasonally (Kalbitz et al. 2000; Michalzik et al. 2001). Because seasonal variations in the amount of dissolved organic C and N coincide with changes in the chemical composition of organic species (Michalzik and Matzner 1999; Kaiser et al. 2001a), it is reasonable to assume that there are also changes in the chemistry of other organic nutrients.

While organic N has received increasing attention over the past decade (see review by Michalzik et al. (2001)), studies on organic P in soil waters of forest ecosystems are still few (Fahey and Yavitt 1988; Qualls et al. (1991, 2000); Qualls and Haines 1991; Kaiser et al. 2000). One of the reasons for this could be that, in contrast to N, distinguishing between soluble inorganic and organic P is complicated. Total P in soil solutions can be analysed relatively easily using inductively coupled plasma–optical emission spectroscopy (ICP–OES) or persulfate–UV digestion followed by photometric determination of orthophosphate. Total P comprises orthophosphate, pyrophosphate, condensed phosphates (di-, tri-, and other polyphosphates), and organic P species. Molybdenum blue reagent is largely sensitive to orthophosphate (Gerke 1992), but under strong acidic conditions required for the formation of the colour complex, release of P due to hydrolysis of labile organic sugar phosphates may occur after prolonged sample storage (Denison et al. 1998). Phosphorus determined by this method can not be attributed exclusively to inorganic P species and is consequently commonly referred as molybdenum blue-reactive P. The difference between molybdenum blue-reactive P and total P, sometimes called unreactive P, represents both organic P species and condensed inorganic phosphates (Ron Vaz et al. 1993). Exact determination of dissolved organic P by the difference between molybdenum blue-reactive P and total P is valid only if hydrolysis of sugar phosphates can be excluded and condensed phosphates are negligible. Nevertheless, this difference gives at least an estimate of dissolved organic P (Qualls et al. 1991; Smith et al. 1998).

Few attempts have been made to characterise the chemical composition of organic P and to use this information to identify the processes governing its production and transport in soils (Williams and Edwards 1993; Chardon et al. 1997). $^{31}$P-nuclear magnetic resonance (NMR) spectroscopy offers a detailed examination of organic and inorganic P species in samples that contain sufficient P. While widely used to determine P forms in soils and sediments (Newman and Tate 1980; Dai et al. 1996; Gressel et al. 1996; Carman et al. 2000), this technique has been used only to analyse the composition of dissolved P in aquatic systems (Nanny and Minear 1997; Clark et al. 1999; Kolowith et al. 2001). These studies showed that monoesters and diesters are the predominant organic P forms in freshwater and seawater.

In a 27-month field experiment in an old-growth European beech (*Fagus sylvatica* L.) forest (Kaiser et al. (2000, 2001a, 2001b)) we combined the determination of concentrations and fluxes of dissolved organic P with XAD-8 fractionation and $^{31}$P-NMR spectroscopy. Changes in composition, concentrations, and fluxes of organic P over time and with soil depth were used to deduce the mechanisms of its production in the forest floor and its transport in the mineral soil.

## Materials and methods

### *Field experiments*

*Sites*. The experiments were carried out at a 90-year-old European beech (*Fagus sylvatica* L.) forest located on a hilltop 520 m above sea level near Betzenstein, NE-Bavaria, Germany (49°44′ N, 11°23′ E). Ground vegetation was little throughout the year and was dominated by *Anemone nemorosa* L. in early spring and later on by *Mercuralis perennis* L. The soils, strongly aggregated and rich in organic C, derived from Upper Kimmeridgian dolomite. They were classified as Rendzic Leptosols (FAO–Unesco 1990) or Lithic Rendolls (Soil Survey Staff 1994). The forest floor was mull-type and covered the mineral soil at variable thickness throughout the entire experimental period. Trees and ground vegetation rooted exclusively in the mineral soil. The mean annual temperature during the study period was 8.2 °C and the mean annual precipitation 956 mm. Snowfall contributed $< 5\%$ of the precipitation during the experimental period. More detailed information on the experimental site, the forest floor, and the climatic conditions during the experimental period is given in Kaiser et al. (2001a). Chemical and physical properties of the mineral soils are summarised in Table 1.

*Instrumentation and sampling*. Three 50 × 50 m plots were selected with a minimum distance between each other of 200 m. Each plot was equipped with four stainless steel zero-tension lysimeters (∅ = 127 mm, mesh size 0.01 mm) installed directly beneath the forest floor. The zero-tension lysimeters were pre-washed with deionised water. In each plot, we installed four disk tension lysimeters (inner diameter 90 mm) at 15 and 30 cm depth made from glass sinter plates with a pore

*Table 1.* Properties of soil horizons of Rendzic Leptosols under a 90-yr-old European beech (*Fagus sylvatica* L.) forest (taken from Kaiser et al. (2001a, 2001b)). The chemical properties refer to the fine earth fraction (< 2 mm). CEC = cation exchange capacity; $CO_3$-C = carbonate carbon; OC = organic carbon; $Al_o$, $Fe_o$ = oxalate-extractable Al and Fe; $Fe_d$ = dithionite–citrate–bicarbonate-extractable Fe; TP = total phosphorus. Samples were taken in October 1997.

| Horizon | Depth cm | $pH_{CaCl_2}$ | CEC mmol$_c$ kg$^{-1}$ | $CO_3$-C g kg$^{-1}$ | OC | Clay[a] | $Al_o$ | $Fe_o$ | $Fe_d$ | TP[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| Oi | 5–0 | 5.8 | n.m. | – | 481 | – | – | – | – | 1.1 |
| A1 | 0–10 | 7.1 | 422 | 4 | 112 | 230 | 5.3 | 3.6 | 8.4 | 1.0 |
| A2 | 11–25 | 7.3 | 284 | 24 | 69 | 210 | 4.7 | 3.3 | 7.7 | 0.8 |
| C | 26–95 | 7.6 | 63 | 70 | 11 | 70 | 1.4 | 1.6 | 5.0 | 0.4 |

n.m. = not measured

[a] the dominant clay mineral was illite.

[b] determined after digestion at 650 °C for 5 hours and extraction with 0.5 M $H_2SO_4$.

size < 16 $\mu$m. The porous plates were mounted on grooved polyacrylic plates. The zero-tension lysimeters and the tension lysimeters were installed laterally from pits by inserting them into a 25 to 30 cm deep pre-cut slit and connected to 2-l glass bottles below them. At 90 cm depth, four ceramic suction cups with a pore size < 40 $\mu$m (Simplex K-100, UMS GmbH, München, Germany) were installed in each plot using an auger and fitted to 2-l glass bottles. Disk tension lysimeters and suction cups were conditioned with a diluted water extract of the forest floor. The sampling equipment was equilibrated in the soil for 6 months and solution sampled during that time discarded. Suction cups removed from the soil after the end of the experimental period indicated neither sorption nor release of organic or inorganic compounds in laboratory tests. On each plot, four tensiometers (Simplex T-100, UMS GmbH, München, Germany) were installed at 10, 20, 60, and 100 cm depths. Zero-tension and tension lysimeters, suction cups, and tensiometers were placed at a mean maximum distance between trees to avoid influence from stemflow. Tensions were measured weekly with a pressure conductor (Infield 5, UMS GmbH, München, Germany). The vacuum at the tension lysimeters and suction cups was regulated according to the measured tension of the soil water. We collected soil water from August 1997 to November 1999 at 7-day intervals during the growing season (April–November) and at a minimum of 14-day intervals during the dormant season (December–March). Samples were measured for volume, combined per plot and depth, filtered through 0.45-$\mu$m polysulfone membrane filters (Supor-450, Pall Gelman Science, Ann Arbor, MI), and stored frozen at −18 to −30 °C. In preliminary experiments, this procedure had no effect on the concentrations and composition of C and P. The amount of water leached from the forest floor was calculated by normalising the volume of the sample to the area of the lysimeter.

The amount of rainfall was determined using 12 rainfall collectors made of plastic funnels (⌀ = 200 mm) fitted to 2-l bottles placed on clearings close to the experimental plots (< 400 m). The amount of throughfall was determined using 12 rainfall collectors placed in each plot. The volume of rainwater and throughfall was measured at the same intervals as the lysimeters and suction cups were sampled. The amount of rain and throughfall was calculated by normalising the volume of rainwater to the area of the rainfall collectors.

To determine the amount of litterfall, we placed four polyethylene nets (size 1 × 1 m, mesh-size 0.1 mm) close to each experimental plot. The nets were sampled parallel to the lysimeters and suction cups. The litter was dried at 40 °C, weighed, and ground in a coffee mill. An aliquot was further ground to < 0.01 mm. Samples were analysed for C using a CN-analyser (Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany, or NC 2500, ThermoQuest-Fisons, Milan, Italy). Phosphorus was extracted with 0.5 M $H_2SO_4$ after digestion at 650 °C for 5 hours. Determination of P in the extracts was done by inductively coupled plasma–optical emission spectrometery (ICP–OES, Integra XMP, GBC Scientific Equipment Pty Ltd., Dandenong, Victoria, Australia).

### *Dissolved organic matter sample treatment*

Aliquots of the filtered solution samples were analysed for organic C using Pt-catalysed, high-temperature combustion (680 °C) followed by infrared detection of $CO_2$ (TOC-5050, Shimadzu Corp., Tokyo, Japan). Before determination of organic C, the inorganic C was removed by adjusting the pH of the solution to 2 using concentrated $H_3PO_4$ and sparging with $CO_2$-free synthetic air at a flow rate of 50 ml $min^{-1}$ for 2 min. No flocculation of organic matter was observed on acidification of the samples. Samples were analysed for total P (photometric determination of $H_2PO_4^-$ after persulphate–UV digestion, SAN Plus, Skalar Analytical B.V., Breda, The Netherlands, or ICP–OES, Integra XMP) and molybdenum blue-reactive P (SAN Plus). Tests proved that the two methods used to determine total P gave similar results. The photometric method was used at concentrations below the detection limit of ICP–OES (0.05 mg $l^{-1}$). Since the samples were stored frozen and the analyses were done promptly after thawing, release of P from labile sugar phosphates should have been negligible (Denison et al. 1998). Based on the assumption that molybdenum blue-reactive P represents exclusively orthophosphate and that condensed phosphates are only trace compounds (see results of the $^{31}$P-NMR analyses), dissolved organic P was calculated as the difference between total P and molybdenum blue-reactive P.

A part of the sample solutions (40 ml) was acidified to pH 2 with 1 M HCl, then pumped through small glass columns (4 ml) filled with Amberlite XAD-8 resin (Rohm & Haas Co., Philadelphia, PA) to determine the distribution of organic C and P among two operationally defined fractions, the so-called hydrophilic fraction and the so-called hydrophobic fraction (Aiken and Leenheer 1993; Raastad and Mulder 1999). Columns effluents, representing the hydrophilic fraction, were promptly analysed for organic C and P (see above), and the amount of organic C and P in the hydrophobic fractions was calculated by difference between organic C and P in the bulk sample and in the hydrophilic fraction. Routine tests showed that acidification to pH 2 had no effect on the distribution of P forms in the bulk sample.

Volume-weighted portions of the sample solutions were combined into three-monthly samples representing the following periods: September to November 1997 (Autumn 97), December 1997 to February 1998 (Winter 97/98), March to May 1998 (Spring 98), June to August 1998 (Summer 98), September to November 1998 (Autumn 98), December 1998 to February 1999 (Winter 98/99), March to May 1999 (Spring 99), June to August 1999 (Summer 99), and September to November 1999 (Autumn 99). The sample designations follow the meteorological definition of seasons. The combined samples were treated with a strongly acidic cation exchanger (AG-MP 50, BioRad Laboratories, Richmond, CA) in order to remove all other cations than $H^+$, and a portion was freeze-dried and homogenised by grinding. The ash contents of the freeze-dried samples were $< 5\%$.

### *Calculation of fluxes*

We calculated fluxes of the dissolved organic C and P from the forest floor into the mineral soil from the amounts of seepage water collected by the zero-tension lysimeters beneath the forest floor and the concentrations therein.

Fluxes with the porewater in the mineral soil were calculated from porewater concentrations and simulated water fluxes. The simulation of water flux was carried out using soil water transport models (WHNSIM, Huwe (1992), HYDRUS-2D, Simunek et al. (1996)). Input variables were meteorological data of the sites and physical properties of the soils. Root distribution and density was estimated from soil pits and the evapotranspiration was estimated from literature (Peck and Mayer 1996; Köstner 2001) in conjunction with the meteorological data. Measured water fluxes from the forest floor were used instead of precipitation as input data. The validation of the models was done according to measured soil water tensions. Simulated water fluxes were cross-checked by calculating water fluxes from the measured tension gradients. All three methods provided comparable water fluxes for the mineral soils. To be consistent with previously published data the calculation of the fluxes of dissolved organic C and dissolved P was based on water fluxes simulated using WHNSIM.

### *Liquid-state $^{31}P$-NMR spectroscopy*

Freeze-dried, bulk three-month samples were analysed for P species using liquid-state $^{31}$P-NMR spectroscopy. The $^{31}$P-NMR spectra were obtained on an Avance DRX 500 spectrometer (Bruker Analytik GmbH, Karlsruhe, Germany) at 202 MHz. About 100 to 150 mg samples were dissolved in 3 ml of 0.5 M NaOD in a 10-mm NMR tube. To avoid alkaline hydrolysis of labile compounds such as RNA (Makarov et al. 2002 (in press)), samples were measured promptly after dissolution. At a pulse angle of 90°, 0.2-s pulse delay, 0.1-s acquisition time, and no $^1$H-decoupling, about 8,000 scans were accumulated for each sample. The signal-to-noise ratio was improved by using a line-broadening of 20 Hz. Chemical shifts were given relative to the resonances of 85% $H_3PO_4$ and signal assignments were made based on literature (e.g., Newman and Tate (1980) and Condron et al. (1990)). The signal areas were calculated by electronic integration.

## Results and discussion

### *Water percolation through the forest floor and mineral soil*

The amounts of seepage water from the forest floor reflected rainfall patterns (Kaiser et al. 2001a). On average, about 75% of the rainfall and 93% of the throughfall percolated through the forest floor. These figures suggest that the sampling of forest floor seepage by zero-tension lysimeters provided a good estimate of the input into the mineral soil. Seepage water from the forest floor represented on average 80% in 1997/98 and 71% in 1998/99 of the amount of summer rainfall. The almost complete ground coverage by the forest canopy during summer and early autumn resulted in the interception of a large part of the rainfall even during heavy rainstorms. The largest amounts of seepage water occurred during autumn 1998 (Table 2) due to huge amounts of rainfall and limited interception after litterfall (Kaiser et al. 2001a).

In summer and early autumn, the calculated amount of percolation water in the subsoil was far less than that of the seepage from the forest floor and comprised $<$ 30% of the rainfall most of the time because of the consumption of water by the forest stand (Table 2). After litterfall in October–November, amounts of percolation water in subsoil approached the amount of seepage from the forest floor. During the remaining dormant period (November–April), percolation water in the subsoil and seepage water from the forest floor were similar. The proportion of the yearly gross precipitation that reached the subsoil was 47% in 1997/98 and 44% in 1998/99.

### *Dissolved organic phosphorus concentrations and fluxes*

Organic P was by far the largest contributor to total P dissolved in forest floor leachates and in mineral soil solutions. The proportions of organic P were lowest during winter and spring (61–77% of total P) in forest floor leachates while in summer and autumn, the contribution of organic P raised to $>$ 81% of total P. These values are at the upper end of the range of published proportions of organic P in forest floor leachates (Qualls et al. (1991, 2000)) or in water extracts of forest litter layers (Huang and Schoenau 1998; Smith et al. 1998).

With increasing soil depth, inorganic P decreased strongly and was not detectable in most of the solutions from the mineral subsoil. Decreases in inorganic P can result from various processes such as uptake by roots (Helal and Sauerbeck 1984), adsorption to Al and Fe oxides and carbonates, and precipitation with Ca ions (Anderson et al. 1974; Cross and Schlesinger 1995). Thus, P in subsoil solutions

*Table 2.* Seasonal fluxes of water, dissolved organic carbon (DOC), and dissolved inorganic and organic phosphorus ($P_{inorg}$ and DOP) from the forest floor and in the mineral subsoil at 90 cm depth under a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols. In addition, the annual fluxes for the periods December 1997 to November 1998 (1997/1998) and December 1998 to November 1999 (1998/1999) are given.

| Sampling period | Forest floor | | | | Subsoil at 90 cm depth | | | |
|---|---|---|---|---|---|---|---|---|
| | water[a] | DOC | $P_{inorg}$ | DOP | water[b] | DOC | $P_{inorg}$ | DOP |
| | mm | g $m^{-2}$ | mg $m^{-2}$ | | mm | g $m^{-2}$ | mg $m^{-2}$ | |
| Seasonal fluxes | | | | | | | | |
| Dec 1997–Feb 1998 | 110 | 1.22 | 1.1 | 3.4 | 95 | 0.56 | 0.7 | 3.0 |
| Mar–May 1998 | 102 | 2.13 | 2.6 | 5.8 | 91 | 0.87 | n.d. | 5.2 |
| Jun–Aug 1998 | 118 | 7.92 | 2.3 | 21.3 | 42 | 1.51 | 0.1 | 9.1 |
| Sep–Nov 1998 | 433 | 8.07 | 3.4 | 21.5 | 280 | 3.20 | n.d. | 20.6 |
| Dec 1998–Feb 1999 | 153 | 1.42 | 2.3 | 4.3 | 131 | 0.62 | 0.5 | 4.0 |
| Mar–May 1999 | 165 | 2.43 | 3.2 | 8.1 | 116 | 1.09 | n.d. | 7.5 |
| Jun–Aug 1999 | 115 | 4.44 | 2.5 | 20.1 | 23 | 0.81 | n.d. | 5.2 |
| Sep–Nov 1999 | 154 | 6.05 | 3.5 | 29.6 | 103 | 2.46 | n.d. | 21.9 |
| Annual fluxes | | | | | | | | |
| 1997/1998 | 873 | 19.34 | 9.4 | 52.0 | 565 | 6.03 | 0.9 | 37.9 |
| 1998/1999 | 587 | 14.34 | 11.5 | 62.1 | 373 | 4.98 | 0.5 | 38.6 |

n.d. not detectable

[a] measured.

[b] calculated (WHNSIM).

was predominantly organically bound. This is in agreement to previous studies in forest ecosystems (e.g., Qualls and Haines (1991) and Qualls et al. (2000)).

The concentrations of dissolved organic P in forest floor leachates showed a strong temporal variation (Figure 1). The temporal course was similar to that of dissolved organic C (Kaiser et al. 2001a). Higher concentrations of organic P occurred in summer and autumn (Figures 1 and 2). The average concentrations of organic P in summer–autumn exceeded those in winter–spring by a factor of 7 (Kaiser et al. 2000). The concentrations of organic P peaked (330–400 $\mu$g $l^{-1}$) after rainstorms that followed short dry periods in summer. The close correlations between organic C and P (Figure 3) suggests that the production of soluble organic P at the study site was directly linked to that of soluble organic C which seems to be controlled by microorganisms (Kaiser et al. 2001a).

Seasonal solution fluxes from the forest floor into the mineral soil were considerably different for inorganic and organic P (Table 2). Fluxes of organic P in summer and autumn were 3.5 to 8.5 times higher than those in winter and spring. This increase was presumably induced by the increased microbial activity and production in summer and autumn (Kaiser et al. 2001a). Interestingly, fluxes of inorganic P showed little changes with seasons. Increased microbial activity should result not only in an increase of soluble organic matter but also in mineralised P. Increased

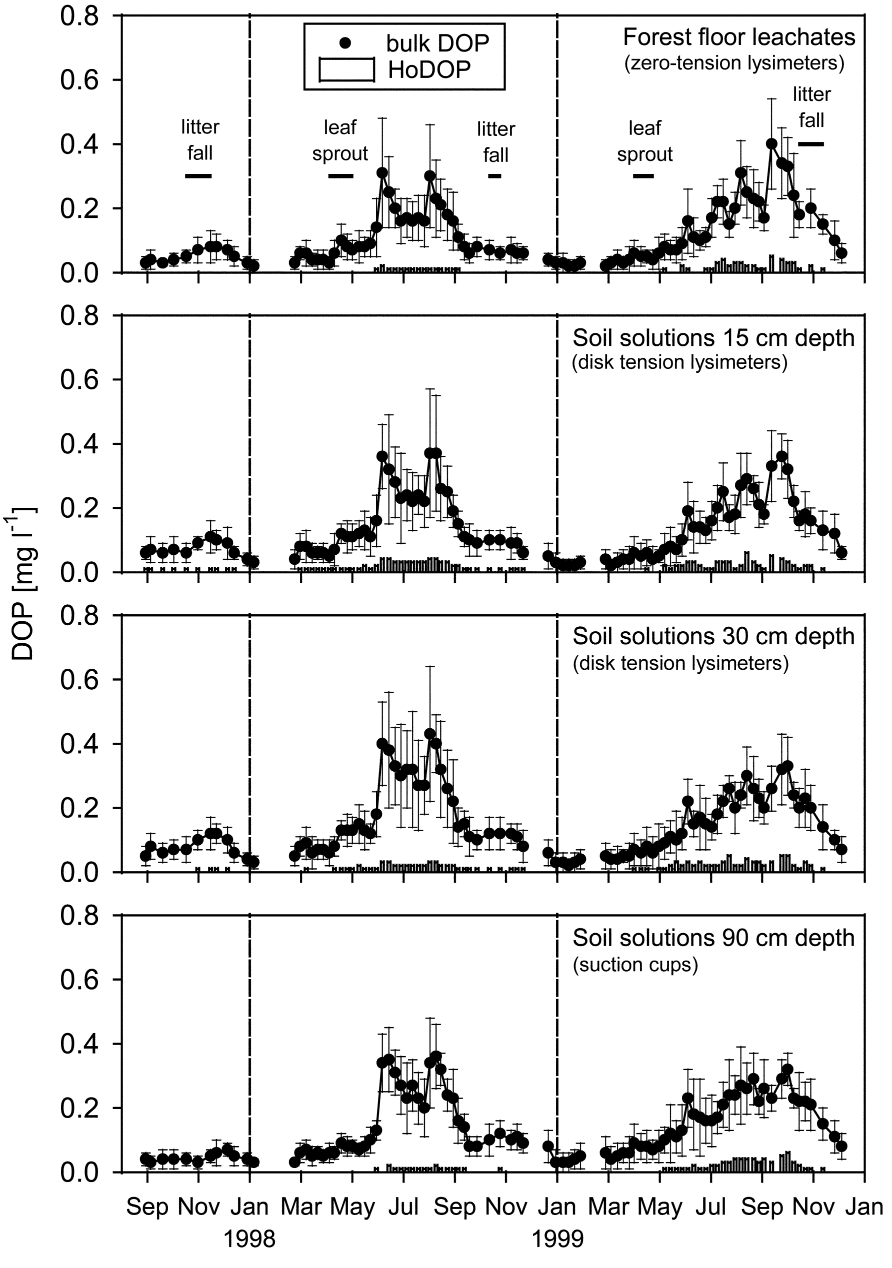

*Figure 1.* Temporal course of the concentrations of bulk dissolved organic phosphorus (bulk DOP) and of organic phosphorus in the hydrophobic fraction of dissolved organic matter (HoDOP) in forest floor leachates and mineral soil solutions (15 cm, 30 cm, and 90 cm depth) under a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols during the sampling period August 1997 to November 1999. Error bars for bulk DOP represent the standard deviation as a measure of variation between three plots.

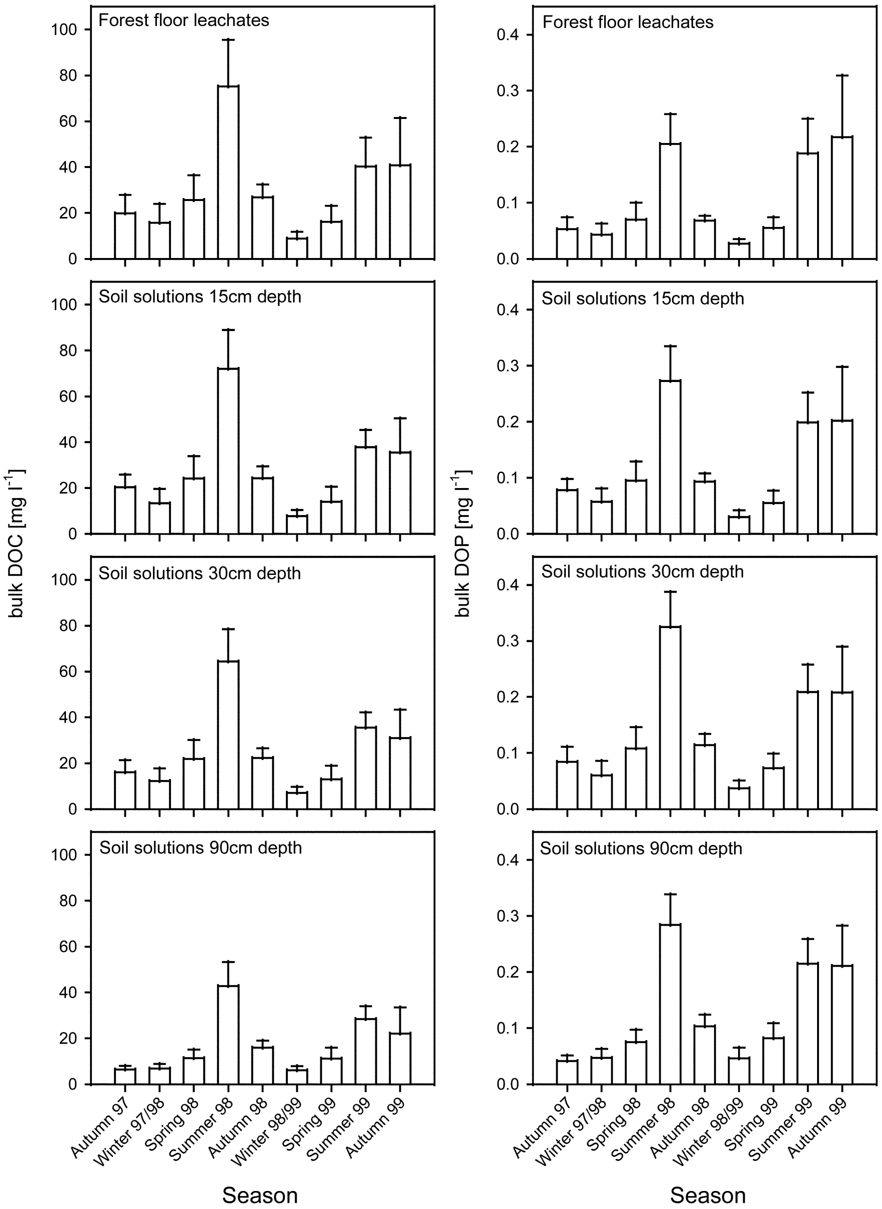

*Figure 2.* Seasonal mean bulk concentrations of dissolved organic carbon (DOC) and dissolved organic phosphorus (DOP) in forest floor leachates and mineral soil solutions (15 cm, 30 cm, and 90 cm depth) under a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols. The error bars represent the standard deviation as a measure of temporal variation during each three-month period (n ⩾ 6).

uptake of inorganic P by plants and microorganisms apparently balanced increased

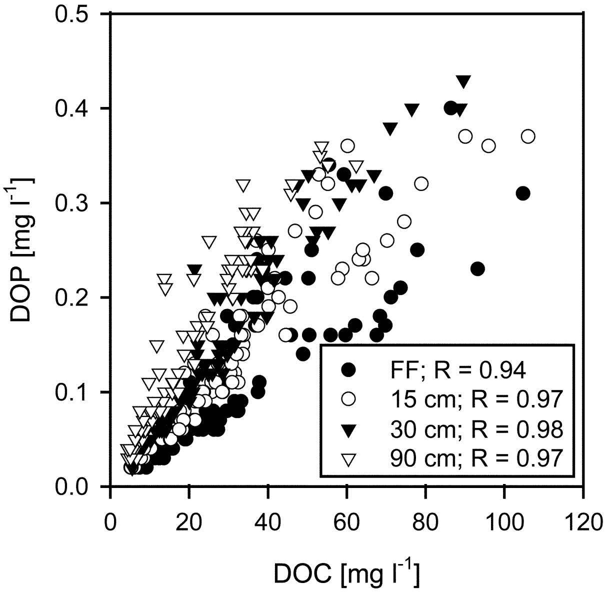

*Figure 3.* Relationship between bulk dissolved organic carbon (DOC) and bulk dissolved organic phosphorus (DOP) in forest floor leachates (FF) and mineral soil solutions (15 cm, 30 cm, and 90 cm depth) under a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols (n $\geqslant$ 91). Note that since the data were not normally distributed, Spearman correlation coefficients are given for each soil compartment.

mineralisation in summer and autumn. We found beech tree roots exclusively in the mineral soil, and during late summer and autumn ground vegetation was sparse. Thus, uptake of inorganic P must be due to microbial biomass production, which suggests rapid recycling of inorganic P within the forest floor.

Annual fluxes of inorganic P in forest floor leachates (Table 2) were at the lower end of the range of inorganic P fluxes reported for undisturbed temperate coniferous and deciduous forests. In contrast, annual fluxes of organic P ranged at the upper end of reported values (e.g., Qualls et al. (1991, 2000)).

The concentrations of dissolved organic P in the mineral soil remained fairly constant with depth (Figures 1, 2 and 4). Concentration patterns in the mineral soil reflected those of forest floor leachates, although the amplitudes of the temporal changes in the concentrations decreased with depth. As for the forest floor leachates, dissolved organic C and P in the mineral soil solutions were strongly correlated at all depths (Figure 3). Since dissolved organic C decreased with depth (Figure 4), the relationship between organic C and P became steeper in the subsoil (Figure 3). This means that P in dissolved organic matter increased during the passage through the mineral soil. Release of P-rich organic compounds, e.g., from roots (Helal and Sauerbeck 1984), is one possible reason. Another explanation is that organic P is concentrated in the mobile hydrophilic fraction of dissolved organic matter while the most sorptive and therefore largely immobile hydrophobic fractions of dissolved

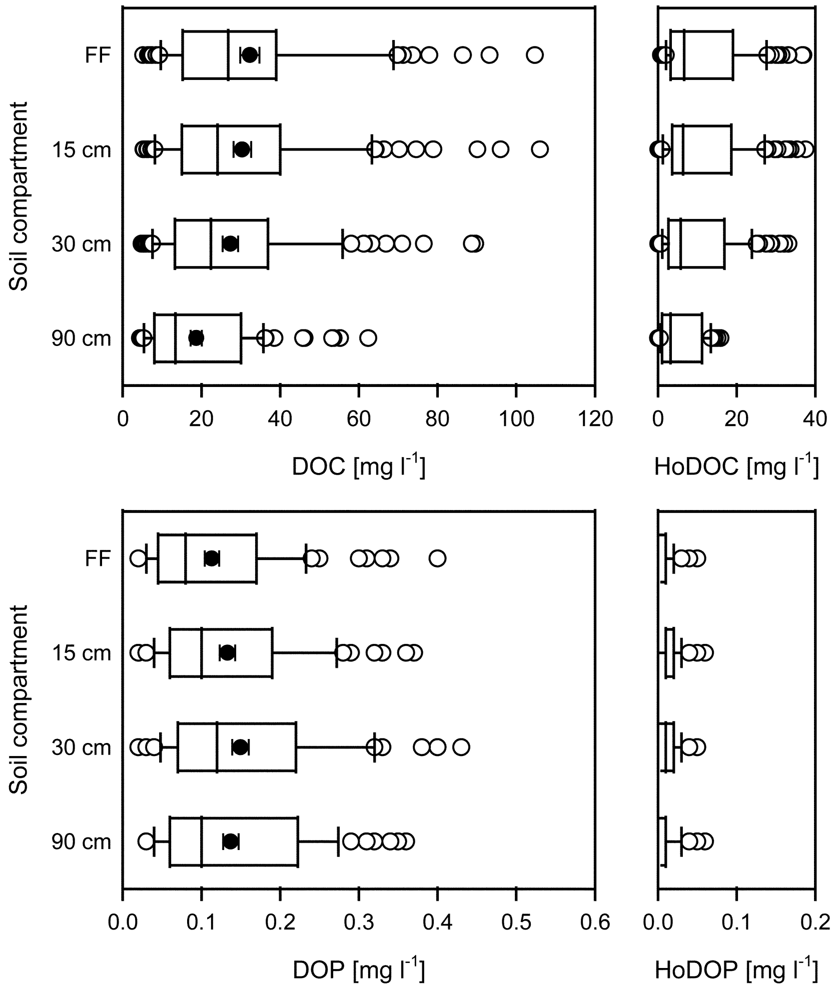

*Figure 4.* Depth distribution of the concentrations of bulk dissolved organic carbon (DOC) and bulk dissolved organic phosphorus (DOP) and of organic carbon and phosphorus in the hydrophobic fraction of dissolved organic matter (HoDOC and HoDOP) in forest floor leachates (FF) and mineral soil solutions (15 cm, 30 cm, and 90 cm depth) under a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols during the sampling period August 1997 to November 1999. The extents of the boxes indicate the 25th and 75th percentiles, the lines inside the boxes the 50th percentile. Whiskers represent 10th and 90th percentiles and outliers are given as open symbols. The error bars given for the mean concentrations of bulk DOC and bulk DOP (●) indicate the standard error ($n \geqslant 91$).

organic matter contain little P (Qualls and Haines 1991; Kaiser 2001). This is similar to other organically bound nutrients such as N and S (Qualls and Haines 1991; Andersson et al. 1999; Nilsson et al. 2001; Solinger et al. 2001).

In general, the observed depth distributions of both organic C and P were different from the results of most other studies in temperate forest ecosystems where dissolved organic P (and C) strongly decreased with depth (e.g., Qualls et al. (2000)). In contrast to those studies, the soils in our study were weakly developed. The contents of strongly sorbing Al and Fe oxides were small and the accumulation of organic C, especially in the topsoil, was high (Table 1) compared to the developed acidic soils in previous studies. Consequently, the soils had little capacity to sorb organic matter (Kaiser et al. 2001b). Moreover, pH values in mineral soil solutions were neutral to slightly alkaline (7.1–7.9), which reduces the sorption of dissolved organic matter by mineral surfaces (e.g., Tipping (1981) and Jardine et al. (1989)). Sorption of organic matter might also be affected by competitive effects of $HCO_3^-$ which was present in the mineral soil solutions at high concentrations (8–45 mg $l^{-1}$).

Results of this study indicate that organic P dominated the fluxes of dissolved P in subsoil solutions at all times of the year (Table 2). Organic P was therefore almost exclusively responsible for P leaching from the rooting zone. This is in agreement with previous findings on the impact of organic species on the leaching of nutrients in forest ecosystems (Qualls and Haines 1991; Hedin et al. 1995; Qualls et al. 2000). Because of the strong fragmentation of the underground dolomite rocks resulting in pronounced flow funnels it seems reasonable to assume that dissolved organic P leaving the rooting zone may be transported further down to shallow groundwater systems.

Due to little retention by the weakly developed mineral soil, fluxes of dissolved organic P in the subsoil were primarily controlled by the production of soluble compounds in the forest floor. The fluxes were larger than those of dissolved organic and total P reported for disturbed and undisturbed temperate forest ecosystems (Fahey and Yavitt 1988; Yanai 1991; Qualls et al. 2000). The average annual flux in the subsoil at 90 cm depth comprised 73% of that from the forest floor in 1997/98 and 62% in 1998/99 (Table 2).

The fluxes of organic C from the forest floor represented 7% of the C in litterfall in 1997/98 and 6% in 1998/99. For organic P, the respective values were 8% and 11%. The fluxes of organic P in the subsoil at 90 cm represented 6% of the P in litter in 1997/98 and 7% in 1998/99. The mean annual atmospheric input of P was low (20 mg $m^{-2}$). Steady-state conditions assumed, this loss must be compensated by weathering of the P-rich dolomite.

### *Fractions of dissolved organic phosphorus*

Dissolved organic P in forest floor leachates and mineral soil solutions was concentrated in the hydrophilic fraction of dissolved organic matter (Figures 1 and 4). On average, 83 ± 13% of the organic P in forest floor leachates was in the hydrophilic fraction. The respective proportion for subsoil solutions at 90 cm depth was 91 ± 7%. In forest floor leachates, the C:P ratio of the hydrophilic fraction averaged 228 ± 78 and that of the hydrophobic fraction 1718 ± 1002. The respective values for subsoil solutions at 90 cm depth were 108 ± 26 and 735 ± 419.

The proportions of organic P in the hydrophilic fraction in winter and spring months were larger (86–100%) than those in summer and autumn (73–98%; Figure 1). This coincided with increases of organic C in the hydrophobic fraction in summer and spring (Kaiser et al. 2001a). The concentrations of organic P in the hydrophilic fractions of dissolved organic matter were in agreement with the results of Qualls et al. (1991) and Qualls and Haines (1991), who found about two thirds of the organic P in hydrophilic fractions. The smaller contribution of the hydrophobic fraction to organic P in our study was comparable with the small proportion of organic C in the hydrophobic fraction. The proportion of organic C in the hydrophobic fraction in forest floor leachates averaged 34% of the bulk organic C and 23% in the mineral soil solutions at 90 cm depth. In the study of Qualls and Haines (1991), proportions of organic C in hydrophobic fractions ranged from 35 to 57%.

Organic P in hydrophobic fractions in solutions of the upper mineral soil (15 cm depth) was higher compared to forest floor leachates (Figure 3). This was possibly a function of release of P-rich hydrophobic organic compounds, i.e., during the degradation of particulate organic matter, and by mineralisation of hydrophilic compounds. Earthworms frequently transport leaf fragments into the mineral soil at the studied site and thus provide a constant supply of easily degradable subsurface organic matter, in addition to root litter. Hydrophilic fractions of organic matter in soil waters are labile while the hydrophobic fractions seem to be largely refractory (Qualls and Haines 1992; Boissier and Fontvieille 1993).

At depths beneath 15 cm, soil solution organic P in the hydrophobic fraction decreased (Figure 4), which coincided with a strong decrease of organic C in the hydrophobic fraction (Kaiser et al. 2000). The decrease of C and P in the hydrophobic fraction can be assigned to sorption to the surfaces of Fe and Al oxides (Kaiser 2001). However, the decrease was small as compared with the strong retention of the hydrophobic fraction in deeply weathered soil rich in Al and Fe oxides (e.g., Qualls and Haines (1992)).

We consider the large accumulation of organic P within the hydrophilic fraction to be one reason for the almost constant concentrations of dissolved organic P with depth (see above). This fraction sorbs only little to mineral surfaces and is therefore mobile in soil. Previous studies have already pointed out the importance of the hydrophilic fraction for the preferential transport of organically bound nutrients in soil as compared to organic C (Qualls and Haines 1991; Andersson et al. 1999; Kaiser 2001; Nilsson et al. 2001). Despite the decrease of organic P in the hydrophobic fraction in the deeper subsoil, concentrations of bulk dissolved organic P were constant or slightly increased with depth (Figure 4). This may have been due to decreasing amounts of percolation water caused by plant uptake (Table 2).

### *Carbon-to-phosphorus ratios of dissolved organic matter*

The close correlations between organic C and P in the forest floor leachates and in mineral soil solutions (Figure 3) suggested that production and transport of both are intimately related to each other. The almost constant C:P ratios of bulk dissolved organic matter in forest floor leachates during the first year of the experimental period (1997/98) underline this (Figure 5). We assume that there was no decoupling

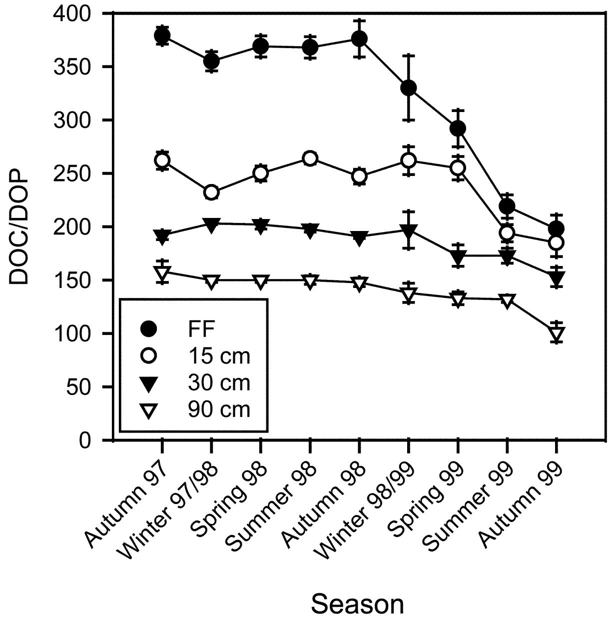

*Figure 5.* Seasonal mean ratios of dissolved organic carbon to dissolved organic phosphorus (DOC/DOP) in forest floor leachates (FF) and mineral soil solutions (15 cm, 30 cm, and 90 cm depth) under a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols. The error bars represent the standard deviation as a measure of temporal variation during each three-month period (n $\geqslant$ 6).

in the production of dissolved organic C and P. Soluble organic compounds both rich and poor in P seemed to be produced and released into soil water at similar rates. This is different from the observations made by Michalzik and Matzner (1999) who reported large seasonal differences in the production of organic C and N.

From winter 1998/99 to autumn 1999, C:P ratios of bulk dissolved organic matter in the forest floor leachates decreased continuously, which suggested an enhanced production of soluble organic matter rich in P. One probable reason may be increased mineralisation of C. The reduced fluxes of organic C in 1998/99 (Kaiser et al. 2001a) agree with this assumption. Chapman et al. (1997) observed decreasing ratios of organic C to organic P in water extracts of mineral soils with decreasing soil-to-solution ratios. Thus, the different solubility of organic matter rich or poor in P also might contribute to the decrease in C:P ratios. Except for autumn, water percolation through the forest floor in 1998/99 was similar or larger than in 1997/98 (Table 2).

C:P ratios of bulk dissolved organic matter in mineral soil solutions decreased with depth but followed the same course as those in forest floor leachates. This was the result of the preferential retention of the hydrophobic fraction of dissolved organic matter low in P. In general, the ratios of organic C to organic P were smaller compared to the ratios found for soil waters by Qualls and Haines (1991) and Qualls et al. (2000). This could be due to preferential mineralisation of C as discussed above.

### *Composition of dissolved organic matter according to liquid-state $^{31}$P-NMR spectroscopy*

The dominance of organic P in forest floor leachates and subsoil solutions was confirmed by the $^{31}$P-NMR spectra (Figures 6 and 7). In the forest floor leachates, orthophosphate represented > 10% of total P in samples collected during winter and spring and < 5% in summer and autumn samples (Table 3). This agrees well with the distribution of organic and inorganic P measured in the water samples (see above). Other inorganic forms such as pyrophosphate and probably inorganic condensed phosphates (polyphosphates) were present at considerable amounts only in the sample of winter 1998/99 (Figure 7, Table 3). The low contribution of condensed phosphates confirms the assumption that the difference between bulk P and phosphate P represents organic P. Phosphonates were not detected. The dominance of phosphate monoesters and diesters compares well to findings on organic P forms in seawaters and freshwaters (Nanny and Minear 1997; Clark et al. 1999; Kolowith et al. 2001). Because of small contents of P, some of the spring samples yielded only poor spectra (Figures 6 and 7). Thus, the interpretation of results of the dormant season is limited mainly to the winter samples.

The samples from summer and autumn were characterised by signals of phosphate monoesters and diesters at varying intensities and signal patterns (Figures 6 and 7, Table 3). The signal patterns resemble much those of alkaline extracts of soils and of marine and lacustrine sediments (e.g., Dai et al. (1996) and Carman et al. (2000)). Signals in the monoester region are often attributed to inositol phosphates (e.g., Condron et al. (1985)). Most samples were characterised by pronounced resonances at about 4.5 ppm (Figures 6 and 7). This signal is close to one of the typical resonances of inositol hexaphosphate (4.4 ppm), therefore the presence of inositol phosphates is possible. More likely, the signals are due to mononucleotides. Prolonged intensive alkaline hydrolysis of RNA has been shown to result in a sharp signal at 4.5 ppm (Makarov et al. 2002 (in press)). Other possible contributors to signals in the monoester region are sugar phosphates and choline phosphate (Newman and Tate 1980; Condron et al. 1985). Signals in the diester region can be assigned to phospholipids (1.5–1.7 ppm and 0.6–0.7 ppm) and teichoic and nucleic acids (Newman and Tate 1980; Condron et al. 1990; Bedrock et al. 1994; Makarov et al. 2002 (in press)). The strong resonance at about 0 ppm is presumably by DNA-P (Makarov et al. 2002 (in press)). All phosphate monoesters, including the chemically refractory inositol phosphates, and diesters represent organic structures that are relatively labile for enzymatic digestion (Suzumura and Kamatani 1995; Suzumura et al. 1998; Shand and Smith 1997). Nevertheless, for coastal seawater it has been shown that labile phosphate esters attached to or within larger and refractory organic molecules are not necessarily labile (Suzumura et al. 1998).

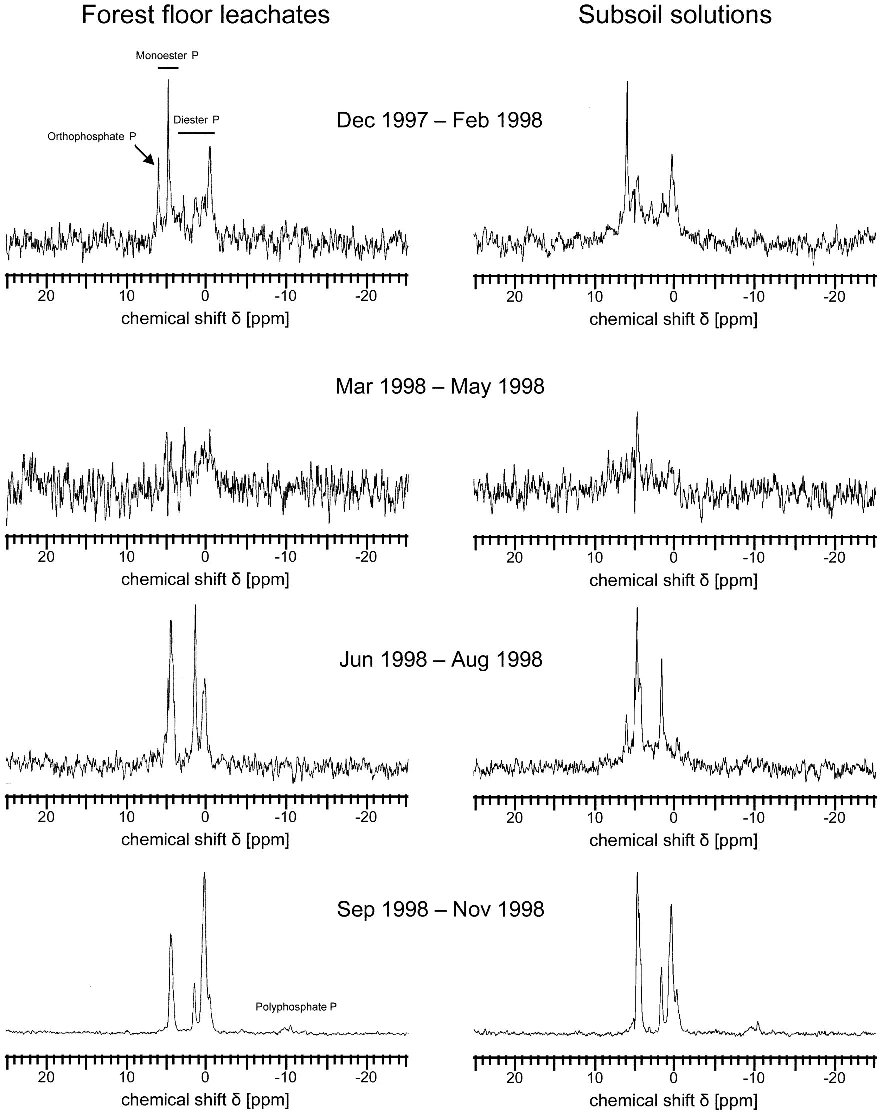

*Figure 6.* Liquid-state $^{31}$P-NMR spectra of combined three-month samples of forest floor leachates and soil solutions at 90 cm depth under a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols. Samples were collected from winter 1997/98 to autumn 1998.

The $^{31}$P-NMR spectra of samples from subsoil solutions were in most cases similar to those of samples from forest floor leachates (Figures 6 and 7, Table 3). This result points again to little changes in the composition of P forms during the percolation of water through the soil. It agrees with the largely unchanged concentrations of organic P suggesting neither pronounced sorptive interactions of P-containing organic compounds with the soil matrix nor microbial degradation. Because

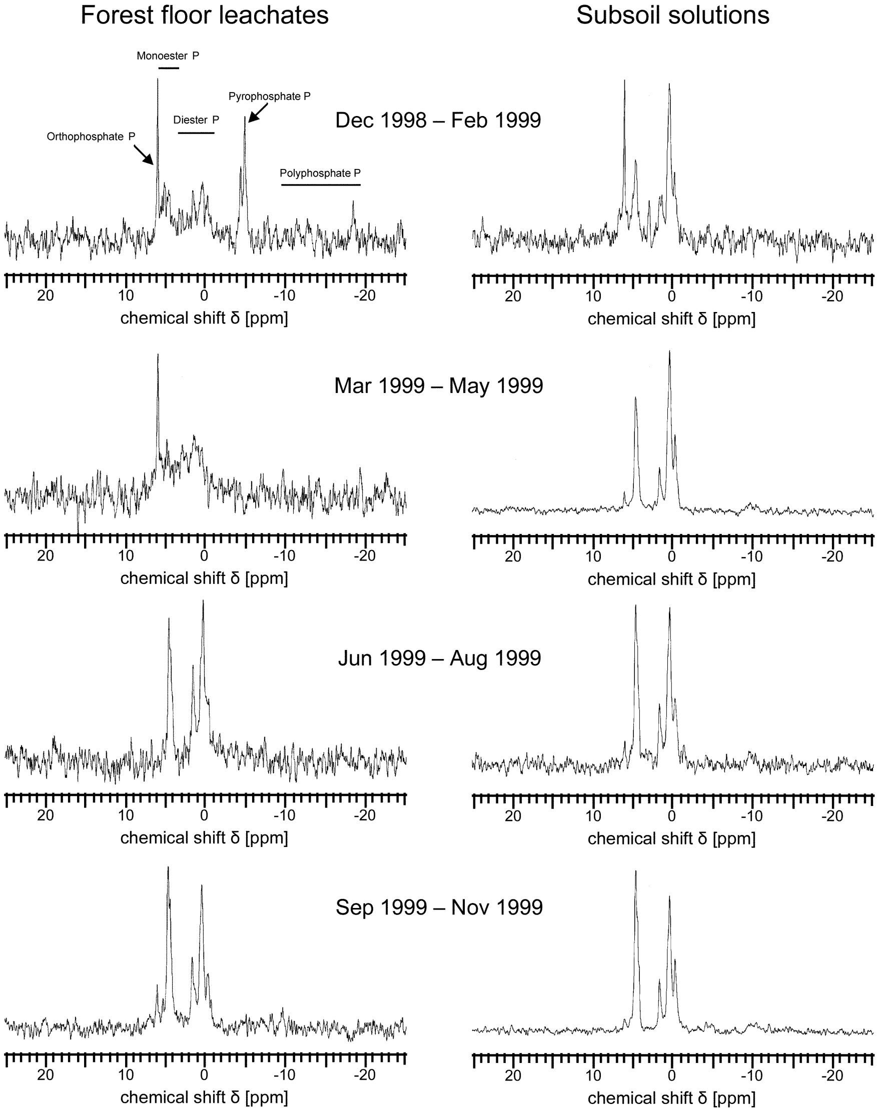

*Figure 7.* Liquid-state $^{31}$P-NMR spectra of combined three-month samples of forest floor leachates and soil solutions at 90 cm depth under a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols. Samples were collected from winter 1998/99 to autumn 1999.

dissolved organic P forms are potentially labile (Macklon et al. 1997; Shand and Smith 1997; Suzumura et al. 1998) this could mean that a large proportion of organic P was bound to more refractory molecules (Suzumura et al. 1998) or that degradation of organic matter in the percolation water is minimal. Under optimised laboratory conditions, even the relatively labile hydrophilic fraction of dissolved

*Table 3.* Distribution of phosphorus forms in combined three-month samples of forest floor leachates (FF) and soil solutions at 90 cm depth (90 cm) under a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols as revealed by liquid-state $^{31}$P-NMR spectroscopy. The mean distribution for the phosphorus forms of winter–spring and summer–autumn samples are given with their standard errors (n $\geqslant$ 2) as a measure of temporal variation. Polyphosphate P includes di-phosphate P ($\sim$ − 9.5 ppm) and organic and inorganic polyphosphate P. Phosphorus moieties are listed with their respective chemical shift ranges given in parts per million (ppm).

| Sample | P moieties (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Orthophosphate P 6.2 ppm | | Monoester P 3.5 – 6.0 ppm | | Diester P − 1.0–3.5 ppm | | Pyrophosphate P − 4.8 ppm | | Polyphosphate P − 18.5–− 9.5 ppm | |
| | FF | 90 cm | FF | 90 cm | FF | 90 cm | FF | 90 cm | FF | 90 cm |
| Seasonal samples | | | | | | | | | | |
| Dec 1997–Feb 1998 | 10 | 21 | 42 | 33 | 48 | 46 | 0 | 0 | 0 | 0 |
| Mar–May 1998 | n.i. | n.i. | n.i. | n.i. | n.i. | n.i. | n.i. | n.i. | n.i. | n.i. |
| Jun–Aug 1998 | 0 | 7 | 45 | 51 | 55 | 43 | 0 | 0 | 0 | 0 |
| Sep–Nov 1998 | 0 | 0 | 27 | 41 | 63 | 54 | 1 | 0 | 4 | 5 |
| Dec 1998–Feb 1999 | 12 | 16 | 26 | 29 | 36 | 65 | 23 | 0 | 5 | 0 |
| Mar–May 1999 | n.i. | 3 | n.i. | 30 | n.i. | 64 | n.i. | 0 | n.i. | 3 |
| Jun–Aug 1999 | 0 | 3 | 31 | 36 | 69 | 57 | 0 | 0 | 0 | 5 |
| Sep–Nov 1999 | 5 | 1 | 40 | 36 | 55 | 54 | 0 | 4 | 0 | 6 |
| Mean distribution | | | | | | | | | | |
| winter–spring | 11 ± 1 | 13 ± 5 | 34 ± 8 | 31 ± 1 | 42 ± 6 | 58 ± 6 | 12 ± 12 | 0 ± 0 | 3 ± 3 | 1 ± 1 |
| summer–autumn | 1 ± 1 | 3 ± 2 | 36 ± 4 | 41 ± 4 | 61 ± 3 | 52 ± 3 | 0 ± 0 | 1 ± 1 | 1 ± 1 | 4 ± 1 |

n.i. not integrated because of poor spectrum organic matter, which contained the predominant proportion of organically bound P in our study, degrades slowly (e.g., Qualls and Haines (1992) and Boissier and Fontvieille (1993)) and so the residence time of the soil water might be too short for a marked change in composition of organic P. In addition, organic P in soil water might be replenished by release of organic matter from the soil matrix.

The large contribution of phosphate monoesters in forest floor leachates and subsoil solutions raised the question about the contribution of inositol phosphates for this would suggest that these compounds are mobile. This is in contrast with the results of Anderson et al. (1974) and McKercher and Anderson (1989) who showed that isolated inositol phosphates sorb strongly to acidic and basic soils and are therefore largely immobile. One plausible reason for the mobility of phosphate monoesters is that they are bonded to the mobile polysaccharides that dominate the hydrophilic fraction (Kaiser et al. 2001a).

In contrast to the analyses of the original water samples where inorganic P was mostly not detectable, most spectra showed small signals of orthophosphate. This might be due to hydrolysis of labile organic P structures such as diesters under the strongly alkaline conditions used to re-dissolve the freeze-dried samples (Makarov et al. 2002 (in press)). Other inorganic P forms such as pyrophosphate and condensed phosphates were not detectable in the samples of subsoil solutions suggesting that these compounds were rather immobile in the studied soil. This agrees with the strong retention of pyrophosphate and polyphosphates by soils and soil minerals (Blanchar and Hossner 1969; Hashimoto et al. 1969).

## Conclusions

1. Organically bound P represented the major P forms in forest floor leachates and mineral soil solutions. This underlines the importance of organic species in the cycling of P in forest ecosystems. Because organic P was more mobile than inorganic P, dissolved organic matter facilitates transport and leaching of P.
2. Seasonal changes in the concentrations of organic P in forest floor leachates are more pronounced than changes in the distribution between certain fractions of dissolved organic P and of different organic P species. Less seasonal changes in the composition of organic P were noted than were in the chemical composition of C (Kaiser et al. 2001a). This could mainly be due to large changes of C with the hydrophobic fraction of dissolved organic matter, which is poor in P (see Kaiser et al. (2001a)).

   The close relationship between organic C and P in forest floor leachates suggests that the production of soluble organic P relates directly to the general turnover and transformation of organic matter. Nevertheless, the decreasing DOC:DOP ratios in 1998/99 point to variations in the production of soluble organic compounds on an annual level. This can be induced by changes in the proportions of C mineralised or the microbial recycling of P. Future studies on dissolved organic matter should therefore include measurements of the soil evolution of $CO_2$ and the concurrent cycling of C and P through microbial biomass. Changes in biomarker compounds such as amino sugars suggest large variations in microbial production and shifts in the composition of the microbial community (Kaiser et al. 2001a).

3. Dissolved organic P is highly mobile in the mineral soil when concentrated in the hydrophilic fraction of dissolved organic matter. The weak retention in the mineral soil suggests that organic P is mainly attached to non-sorbing sugars and that the proportion of strongly sorbing inositol phosphates in dissolved organic matter is small. Consequently, we assume that sugar phosphates are the most important contributors to phosphate monoesters in soil water and not inositol phosphates.

   The leaching loss of organic P from soil might contribute to long-term changes in soil P fractions leading to limitations in P availability (e.g., Crews et al. (1995)). However, the number of studies is far too small to ascertain that this is a general feature of organic P in soils. To extend the data base, further ecosystem studies should include the determination of organic P in rain and soil waters.
4. Organic P forms are potentially labile. Little if any changes occurred in the concentrations of P in the hydrophilic fraction of dissolved organic matter and in the distribution of organic P structures in the mineral soil with increasing soil depth. Therefore decomposition and/or microbial transformation of organic matter dissolved in soil water must be minimal.
5. The major limitation of the approach used was that it did not allow to estimate the contribution of processes such as preferential sorption of certain fractions or release of organic matter from soil and roots to the differing transport of organic C and P. Labelling of C but especially of P in organic matter would help to distinguish between the various processes. However, the half-life of P isotopes is much too short to study incorporation and release from organic matter.

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