

M.-B. Turrión · B. Glaser · D. Solomon  
A. Ni · W. Zech

## Effects of deforestation on phosphorus pools in mountain soils of the Alay Range, Khyrgyzia

Received: 10 March 1999

**Abstract** The amount, quality and turnover of soil P is heavily influenced by changes in soil management. The objective of this study was to investigate the effects of deforestation and pasture establishment on the concentrations, forms and turnover rate of soil P in mountain soils of the Alay Range, Khyrgyzia. A sequential extraction was applied to distinguish soil P pools. We used particle-size fractionation to follow the dynamics of different P pools in soils under forest and pasture and  $^{31}\text{P}$ -NMR spectroscopy to investigate the structure of alkali-soluble P forms. In the A horizons of the forest soils, total soil P concentration was  $1093 \text{ mg kg}^{-1}$ , organic P ( $\text{P}_o$ ) representing 46% of the total P. Deforestation followed by pasture establishment not only increased significantly ( $P < 0.01$ ) the total P concentration ( $1560 \text{ mg kg}^{-1}$ ) but also the contribution of  $\text{P}_o$  to total P was increased by 17%. Pasture soils had significantly higher P pools than forest soils except highly labile inorganic P ( $\text{P}_i \text{ NaHCO}_3$ ) and primary  $\text{P}_i$  ( $\text{P}_i \text{ HCl}_{\text{dil}}$ ). Both in forest and pasture soils stable P increased with decreasing particle size (coarse sand 50%, clay 80% of total P) and primary P decreased with decreasing particle size. Phosphate monoesters and diesters represented 80% of P identified by  $^{31}\text{P}$  NMR. Low monoester to diester ratios in the alkali extracts of forest and pasture soils indicate low microbial activity. This is consistent with high C/ $\text{P}_o$  ratios and high stable  $\text{P}_o$  concentrations in the fine earth of forest and pasture.

**Key words** Effects of deforestation · Mountain soil · Particle-size fractionation · Phosphorus fractionation · Phosphorus pools

### Introduction

In natural ecosystems, the concentrations and chemical nature of soil organic P ( $\text{P}_o$ ) are mainly determined by a combination of the major soil-forming factors: time, parent material, climate, topography and organisms (Smeck 1985). The distribution of P in different organic and inorganic forms reflects the history, present structure, and function of an ecosystem (Magid et al. 1996). The nature and dynamics of soil organic matter (SOM) and associated nutrients, including P, in the soil is different under different kinds of land use. The study of the dynamics of soil P deals with overall changes in the concentrations and chemical nature of P, which results from the cumulative effects of continued annual or seasonal nutrient cycling processes. The latter includes a combination of chemical and biological processes which control the balance between P immobilisation and  $\text{P}_o$  mineralisation. Even though  $\text{P}_o$  undergoes extensive stabilisation in soils, there is also an active turnover of  $\text{P}_o$  fractions (Magid et al. 1996). The long-term dynamics of  $\text{P}_o$  in the soil environment are closely related to the dynamics of organic C, N and S (Stewart and Tiessen 1987). Despite recent advances, a great deal remains to be understood about the nature and dynamics of soil  $\text{P}_o$  and principally the relationships between the cycling of  $\text{P}_o$  and other soil organic constituents and nutrients (Magid et al. 1996; Turrión et al. in press).

A sequential fractionation has been used to separate plant-available or labile forms of P from various refractory P pools in soils (Tiessen and Moir 1993). In this manner, the fractions chemically less resistant to hydrolysis, which are thought to have greater bioavailability and turnover in the soil system, are separated from stable inorganic ( $\text{P}_i$ ) and  $\text{P}_o$  fractions. The advantage of this fractionation is that information can be obtained about short- and long-term P availability by quantifying  $\text{P}_o$  and  $\text{P}_i$  fractions of varying degrees of availability to plants (Schmidt et al. 1997), although ecological interpretations of these chemically defined fractions are

B. Glaser · D. Solomon · A. Ni · W. Zech  
Institute of Soil Science and Soil Geography,  
University of Bayreuth, D-95440 Bayreuth, Germany

M.-B. Turrión (✉)  
C.S.I.C. Apt. 257, E-37071 Salamanca, Spain  
e-mail: beltur@gugu.usal.es, bturrión@agro.uva.es  
Fax: +34-923219609

**Table 1** Interpretation of different forms of phosphorus following the sequential extraction procedure proposed by Tiessen and Moir (1993)

| P form                           | Extracts                                     | Interpretation  |
|----------------------------------|--|---|
| Highly labile P <sub>i</sub>     | P <sub>i</sub> (NaHCO <sub>3</sub> )         | Readily plant-available P (Wager et al. 1986)   |
| Highly labile P <sub>o</sub>     | P <sub>o</sub> (NaHCO <sub>3</sub> )         | Labile P <sub>o</sub> , easily mineralisable (Bowman and Cole 1978)   |
| Moderately labile P <sub>i</sub> | P <sub>i</sub> (NaOH)                        | Minus plant-available P, associated with amorphous and some crystalline Al, Fe phosphates (Tiessen et al. 1983)                                     |
| Primary P <sub>i</sub>           | P (HCl <sub>dil</sub> )                      | Ca-associated P   |
| Stable P <sub>i</sub>            | P (HCl <sub>conc</sub> ) + P residual        | P derived from not alkali-extractable organic debris (Tiessen and Moir 1993; Magid et al. 1996); highly recalcitrant P (Cross and Schlesinger 1995) |
| Stable P <sub>o</sub>            | P <sub>o</sub> (NaOH) + P <sub>o</sub> (HCl) | More stable form of P <sub>o</sub> , involved in long-term transformations (Bowman and Cole 1978; Tiessen and Moir 1993)                            |

controversial (Cross and Schlesinger 1995). Our terminology (Table 1) was based on the work of Tiessen and Moir (1993) and the literature review of Cross and Schlesinger (1995). On the other hand, the procedure suggested by Tiessen and Moir (1993) does not provide information on the structural composition of the various forms of P in soil (Guggenberger et al. 1996a). Since the work of Newman and Tate (1980), <sup>31</sup>P NMR analysis has been used in studies to examine the structural composition of alkali-soluble P (Hawkes et al. 1984; Condron et al. 1985).

Physical fractionation of soil according to particle size combined with degradative chemical techniques has shown to be a potent tool for evaluating land-use-induced changes in SOM (Guggenberger et al. 1994). This fractionation is based on the concept that SOM fractions associated with particles of various size classes and mineralogical composition differ in structure and function and, therefore, play different roles in SOM turnover (Tiessen and Stewart 1983; Anderson and Paul 1984; Christensen 1992). Particulate organic matter (POM) is assumed to be associated with the sand fraction, SOM with medium-term turnover with the clay fraction, and SOM with long-term turnover with the silt fraction (Guggenberger et al. 1995; Zech and Guggenberger 1996; Glaser et al., in press).

The objective of this study was to investigate effects of deforestation followed by pasture establishment on concentration, forms, and turnover of soil P in mountain soils of the Alay Range, Khyrgyzia.

## Materials and methods

The study site is located in the Alay Range, Khyrgyzia (40°02'N; 72°39'E) at about 3000 m above sea level; the climate is semiarid with mean annual precipitation of 900 mm and mean annual temperature of -0.1 °C. The parent material consists of granitic and granodioritic rocks. Soils can be classified as Borolls. They are, generally, stratified with silty surface horizons and sandy loamy subsurface layers, with high stone content, especially on steep slopes. The natural forest is dominated by *Juniperus turkestanica*. Most of the stands were cleared but recently the remaining relicts have been heavily disturbed by grazing and uncontrolled logging, despite being protected.

To investigate the effects of deforestation on different P pools, we selected according to a radial sampling scheme (Wilding 1985) undisturbed samples of humus layers of the forest stands and five mineral undisturbed soil samples from the A horizon (0–10 cm). Samples were collected after removal of the organic forest floor below the canopy of dense Juniper microstands. For comparison, five mixed pasture soil samples (0–10 cm) were selected from about 150–200 m spacing from forest stands having the same inclination, aspect, and parent material. Soil samples were taken from the A horizon because P changes due to deforestation were expected in this superficial horizon.

### Particle-size separation

From each sample, the fine earth (<2 mm) and the following particle-size fractions were analysed separately: coarse sand (250–2000 µm), fine sand (20–250 µm), silt (2–20 µm) and clay (<2 µm). The procedure was carried out as described by Glaser et al. (in press).

### Sequential extraction and P analyses

A sequential extraction similar to the procedure of Tiessen and Moir (1993) was done to distinguish between different soil P pools extracted from A horizons of forest and pasture soils and their particle-size fractions. Briefly, 0.25 g of soil was extracted sequentially by adding 15 ml of 0.5 M NaHCO<sub>3</sub>, 0.1 M NaOH, 1 M HCl and shaking for 16 h. The supernatant of each extract was filtered through an ashless filter after centrifugation at 8000 rpm for 10 min. Then 15 ml of 10 M HCl was added and the samples were heated for 10 min at 90 °C and incubated at room temperature for 1 h. Then suspensions were filtered through an ashless filter and the bottles rinsed with 20 ml of deionised water. The soil residue was combusted for 16 h at 560 °C and subsequently shaken for 16 h with 15 ml of 0.5 M H<sub>2</sub>SO<sub>4</sub>. From each extract, a 1–2 ml aliquot was used for P<sub>i</sub> determination according to a modified molybdenum-blue method of Murphy and Riley (1962). In each extract total P was determined using ICP-OES and P<sub>o</sub> was calculated by difference.

Total P, total P<sub>o</sub> and total P<sub>i</sub> in the soil samples and humus layers were determined according to Saunders and Williams (1955).

### <sup>31</sup>P-NMR spectroscopy

<sup>31</sup>P NMR analysis was used to obtain information on the structural composition of alkali-soluble P. Extracts for <sup>31</sup>P-NMR analysis were obtained using the method of Newman and Tate (1980). For this purpose, 6.7 g of finely ground samples (<2 mm) were dispersed ultrasonically with 90 ml of 0.5 M NaOH and the suspension was centrifuged at 10,000 rpm for 120 min at 0 °C. The result-

ing supernatant was concentrated to about 2 ml at 40 °C in a rotary evaporator, then 1 ml of D<sub>2</sub>O was added. <sup>31</sup>P-NMR spectra were recorded on a Bruker AM 500 NMR spectrometer (11.7 T; 202.5 MHz) without proton decoupling at a temperature of 280 K. An acquisition time of 0.1 s, a 90° pulse and a relaxation delay of 0.2 s were used. The spectra were recorded with a line-broadening factor of 20 Hz. Chemical shifts were measured relative to 85% H<sub>3</sub>PO<sub>4</sub> in a 5 mm tube inserted into a 10 mm sample tube before the measurement of each sample. Peak assignments were according to Newman and Tate (1980) and Condrón et al. (1990). Intensities of signals were determined by integration. The mean signal to noise ratio was 66 for the most intensive peak and 10–14 for the less intensive peaks.

Total P in the 0.5 M NaOH extract was determined after ignition (550 °C, 16 h) and dissolution of the residues in 0.1 M H<sub>2</sub>SO<sub>4</sub>. P<sub>i</sub> was measured directly in the extracts, and P<sub>o</sub> in these fractions was calculated as the difference (Saunders and Williams 1955). In all cases, P was analysed by a modified molybdenum-blue method of Murphy and Riley (1962).

#### Statistical analyses

All data were statistically analysed using Excel 8.0 or Statistica 5.1 for WinNT 4.0. Data were tested for normality using the David test. The homogeneity of the variances of the normally distributed data was tested with an *F*-test. Significant differences between the mean measurements among forest and pasture were tested subsequently with an adequate *t*-test (Hartung et al. 1993; Sokal and Rohlf 1995). The non-parametric *U*-test (Mann-Whitney) was applied for these samples unless the prerequisites for the *t*-test were accomplished (Sokal and Rohlf 1995). Differences between size fractions within one land use system were tested with MANOVA followed by a Scheffé test. Logarithmic transformation was applied if data were not normally distributed or the variances were heterogeneous.

## Results and discussion

### Effects of deforestation on concentration and forms of P

Total P concentrations in the fine earth of A horizons of forest and pasture soils were 1093 mg kg<sup>-1</sup> and 1566 mg kg<sup>-1</sup>, respectively (Table 2). P<sub>o</sub> represented 46% of total P in forest soils and 63% in pasture soils. Deforestation followed by pasture establishment not only increased significantly (*P* < 0.01) the total P concentration but also the contribution of P<sub>o</sub> to total P was increased by 17%. Our experimental sites in the Alay Range of Khyrgyzia may have been enriched with P due to the accumulation of animal excrement. Despite the fact that forest grazing plays an important role, the animals spend most of their time on pasture land depositing there high amounts of excrement. Similar total P concentrations were reported by Guggenberger et al. (1996a) in spruce and mixed deciduous forest soils in the prealpine region of Bavaria, P<sub>o</sub> comprising 70% of total P, whereas Magid (1993) reported P<sub>o</sub> representing 15% of total P in beech forest soils in Denmark. Condrón et al. (1996) found a significant decrease in soil P<sub>o</sub> concentration in New Zealand soils under *Pinus radiata* compared with soils under grass, suggesting that the presence of *P. radiata* stimulates net mineralisation of

**Table 2** Concentrations and standard deviations (*n* = 5) of different P<sub>o</sub> and P<sub>i</sub> pools in fine earth from forest and pasture soils in [mg P kg<sup>-1</sup>]. Relative contribution in [%] of each P form to the total P. Significant differences between forest and pasture are indicated by \* *P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001, ns not significant

|                                       | Forest     | Pasture    |     |
|---------------------------------------|------------|------------|-----|
| P <sub>i</sub> (NaHCO <sub>3</sub> )  | 33 ± 11    | 40 ± 9     | ns  |
|                                       | 3%         | 3%         |     |
| P <sub>o</sub> (NaHCO <sub>3</sub> )  | 57 ± 9     | 242 ± 30   | *** |
|                                       | 5%         | 15%        |     |
| P <sub>t</sub> (NaHCO <sub>3</sub> )  | 90 ± 10    | 282 ± 20   | *** |
|                                       | 8%         | 18%        |     |
| P <sub>i</sub> (NaOH)                 | 30 ± 3     | 61 ± 5     | **  |
|                                       | 3%         | 4%         |     |
| P <sub>o</sub> (NaOH)                 | 275 ± 25   | 477 ± 38   | **  |
|                                       | 25%        | 30%        |     |
| P <sub>t</sub> (NaOH)                 | 305 ± 14   | 538 ± 21   | *** |
|                                       | 28%        | 34%        |     |
| P (HCl <sub>dil</sub> )               | 340 ± 85   | 153 ± 24   | *** |
|                                       | 31%        | 10%        |     |
| P <sub>i</sub> (HCl <sub>conc</sub> ) | 127 ± 58   | 222 ± 27   | **  |
|                                       | 12%        | 14%        |     |
| P <sub>o</sub> (HCl <sub>conc</sub> ) | 171 ± 45   | 285 ± 89   | **  |
|                                       | 16%        | 18%        |     |
| P <sub>t</sub> (HCl <sub>conc</sub> ) | 298 ± 52   | 507 ± 58   | *** |
|                                       | 28%        | 32%        |     |
| Presidual                             | 60 ± 3     | 86 ± 11    | *** |
|                                       | 5%         | 6%         |     |
| P <sub>total</sub>                    | 1093 ± 111 | 1566 ± 157 | **  |

soil P<sub>o</sub> which may be attributed to elevated levels of phosphatase enzymes from mycorrhizal fungi (particularly ectomycorrhiza) associated with tree roots.

Total P amounts were 1.3 ± 0.1 Mg P ha<sup>-1</sup> dm<sup>-1</sup> in forest soils compared to 2.1 ± 0.4 Mg P ha<sup>-1</sup> dm<sup>-1</sup> in pasture soils, indicating a P accumulation of about 44% after deforestation. It is of interest to note that this accumulation corresponds principally to P<sub>o</sub>, whose content in forest soils was 0.5 ± 0.1 Mg P ha<sup>-1</sup> dm<sup>-1</sup> and in pasture soil 1.2 ± 0.3 Mg P ha<sup>-1</sup> dm<sup>-1</sup>, but not to P<sub>i</sub> (0.8 ± 0.2 Mg P ha<sup>-1</sup> dm<sup>-1</sup> in forest soil and 0.9 ± 0.2 Mg P ha<sup>-1</sup> dm<sup>-1</sup> in pasture soil). Considering the incorporation of organic matter of the litter and humus layers into the mineral A horizon of the deforested soil when pasture was established, the accumulation of P in the pasture soil is 35%. On the other hand, pasture installation led to a loss of about 30% total organic carbon (TOC) and to an accumulation of about 20% of N compared to the native *Juniperus turkestanica* forest stands (Glaser et al. in press). The different pattern of soil P after deforestation in comparison to TOC and N is possibly due to the preferential stabilisation of reactive P groups with soil components or due to the fact that P is recycled more conservatively than C and N, which are more easily lost by leaching and volatilisation.

Interpretation and concentrations of different organic and inorganic forms of P in the fine earth of the investigated soils are shown in Tables 1 and 2, respectively. The differences in the concentrations of highly labile P (P<sub>t</sub> NaHCO<sub>3</sub>) between forest and pasture soils

were mainly due to  $P_o$  ( $P_o$  NaHCO<sub>3</sub>) representing 72% of this pool in forest soil and 90% in pasture soil, because we observed no significant differences in the concentrations of highly labile  $P_i$  ( $P_i$  NaHCO<sub>3</sub>) between forest and pasture soils (Table 2). This is probably due to the incorporation of  $P_o$  from animal excrement into the topsoil horizon under pasture, and also may explain the higher contribution of highly labile P ( $P_i$  NaHCO<sub>3</sub>) to total P of pasture (18%) in comparison to forest soils (8%).

The concentration of Ca-associated P ( $P_i$  HCl<sub>dil</sub>) was significantly ( $P < 0.001$ ) higher in the topsoil horizon of forest than in the pasture soil, representing 31% of total P in forest and only 10% in pasture soils (Table 2). This indicates a higher contribution of  $P_i$  from primary minerals in the forest soil. A lower pH of 5.6 in the pasture soil caused a progressive weathering of Ca-bound P from primary P minerals. Tiessen et al. (1984) and Sanyal and Datta (1991) found that soils with high pH values tend to have lower proportions of P in easily extractable organic and inorganic forms, and relatively higher acid P contents due to the higher P solubility at lower pH. In addition, input of Ca-phosphates by dry deposition in the forest ecosystem may play an important role in this semiarid environment with pronounced deflation and significant dust emissions.

The pasture soils had significantly ( $P < 0.01$ ) higher concentrations of stable  $P_i$  ( $P_i$  HCl<sub>conc</sub> + P residual) compared with the forest soils (Table 2). The relative contribution to total P, however, was about 20% in both soils (Fig. 1).

The concentration of stable  $P_o$  ( $P_o$  NaOH +  $P_o$  HCl) was significantly ( $P < 0.01$ ) higher in pasture than in forest (Table 2), representing almost 50% of total P in both soils (Fig. 1).

### P pools related to SOM turnover

In order to follow the dynamics of P within the soil, P pools were determined in particle-size fractions (Table 3).

To eliminate the effects of different total P levels in fine earth, the enrichment ratios for the different P forms,  $E_{P\text{-form}} = (\text{mg P-form kg}^{-1} \text{ separate}) / (\text{g P-form kg}^{-1} \text{ fine earth})$  were calculated for each particle size separately. In general, the repartition of P pools within

the particle-size groups showed the same tendency in soils under forest and pasture (Table 4). All P pools except the primary P were enriched in the clay fraction.

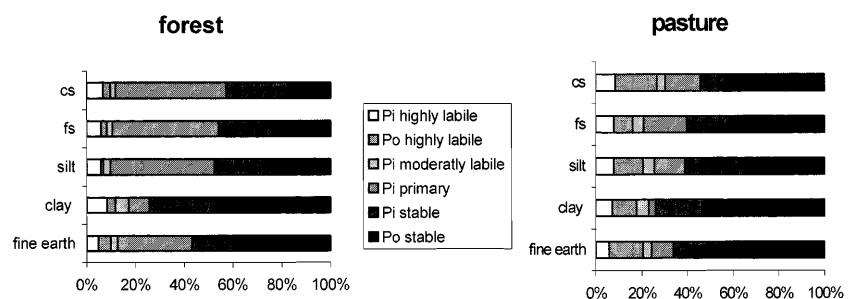
P assumed to be chemisorbed to amorphous and crystalline Al and Fe oxides ( $P_i$  NaOH) was about three times higher in the clay fraction compared to the silt fraction and to the fine earth, and seven times greater compared to the coarse sand and fine sand fraction (Table 4). These results corroborate the common interpretation of this  $P_i$  pool of lesser plant availability and stabilised by oxides (Cross and Schlesinger 1995) and consisting mainly of surface adsorbed  $P_i$ . The highest amounts of this  $P_i$  pool are therefore found in the finest particle-size fractions which have a high specific surface area (Tiessen et al. 1983). These trends are the opposite of those observed with primary P ( $P$  HCl<sub>dil</sub>), which was depleted in the clay fraction, indicating chemical weathering.

Stable  $P_i$  ( $P$  HCl<sub>conc</sub> + P residual) was enriched in the small particle-size fractions containing stable SOM (Table 4). This P pool is considered to be highly recalcitrant (Cross and Schlesinger 1995). The concentration of P extracted with hot concentrated HCl ( $P$  HCl<sub>conc</sub>) was significantly ( $P < 0.05$ ) higher in the clay fraction of forest and pasture soils than in all other fractions (Table 3). This fraction was interpreted by Tiessen and Moir (1993) as being derived from particulate organic debris, which is not alkali extractable but the hot acid would break up cellulose in the residual fraction and release P (Magid et al. 1996). Consequently an enrichment of  $P$  HCl<sub>conc</sub> was expected in the coarse sand fraction, since there is a high fine root and litter debris input as a source of POM. Therefore we can conclude that this interpretation for  $P$  HCl<sub>conc</sub> is not adequate and more studies are necessary to interpret it correctly.

As can be seen in Fig. 1, about 80% of total P in the clay fraction was represented by stable P both in forest and pasture, decreasing with increasing particle size, reaching about 50% in the coarse sand fraction. The percentage contribution of primary P to total P decreased with decreasing particle size both in forest and pasture.

The ratio of total organic C to  $P_o$  ( $C/P_o$ ) was used to estimate the mineralisation potential of  $P_o$  (Dalal 1977; Guggenberger et al. 1996b). As can be seen in Table 5, the  $C/P_o$  ratio of forest soil is almost three times greater than that of the pasture soil. The  $C/P_o$  ratio tended to

**Fig. 1** Relative contribution of  $P_o$  and  $P_i$  in fine earth and particle-size fractions of soils under forest and pasture. *cs* Coarse sand; *fs* fine sand.  $P_i$  highly labile =  $P_i$  NaHCO<sub>3</sub>;  $P_o$  highly labile =  $P_o$  NaHCO<sub>3</sub>;  $P_i$  moderately labile =  $P_i$  NaOH;  $P_i$  primary =  $P$  HCl<sub>dil</sub>;  $P_i$  stable =  $P_i$  HCl<sub>conc</sub> + P residual;  $P_o$  stable =  $P_o$  NaOH +  $P_o$  HCl



**Table 3** Concentrations and standard deviations ( $n=5$ ) of different P pools in particle-size fractions from forest and pasture [ $\text{mg P kg}^{-1}$ ]. *Cs* Coarse sand (250–2000  $\mu\text{m}$ ); *Fs* fine sand (20–250  $\mu\text{m}$ ); silt (2–20  $\mu\text{m}$ ); clay (<2  $\mu\text{m}$ ). Significant differ-

ences between forest and pasture are indicated by \*  $P<0.05$ , \*\*  $P<0.01$ , \*\*\*  $P<0.001$ , *ns* not significant. Different letters indicate significant differences ( $P<0.05$ ) between different size fractions within one land use system

|                                      | Land use | Cs           | Fs          | Silt        | Clay         |
|--------------------------------------|----------|--------------|-------------|-------------|--------------|
| $P_i$ ( $\text{NaHCO}_3$ )           | Forest   | 36 ± 8 a     | 23 ± 2 b    | 33 ± 8 ab   | 57 ± 15 a    |
|                                      | Pasture  | 62 ± 14 a    | 27 ± 3 b    | 33 ± 4 b    | 64 ± 17 a    |
| $P_o$ ( $\text{NaHCO}_3$ )           | Forest   | 25 ± 4 a     | 16 ± 2 ab   | 12 ± 3 b    | 66 ± 18 c    |
|                                      | Pasture  | 227 ± 53 a   | 71 ± 11 b   | 149 ± 13 c  | 355 ± 57 d   |
| $P_i$ (NaOH)                         | Forest   | 18 ± 4 a     | 17 ± 4 a    | 32 ± 9 b    | 104 ± 27 c   |
|                                      | Pasture  | 45 ± 14 ab   | 40 ± 14 a   | 58 ± 7 b    | 176 ± 25 c   |
| $P_o$ (NaOH)                         | Forest   | 131 ± 46 a   | 118 ± 29 a  | 151 ± 37 a  | 501 ± 171 b  |
|                                      | Pasture  | 341 ± 39 a   | 250 ± 55 a  | 262 ± 32 a  | 1225 ± 97 b  |
| $P_i$ ( $\text{HCl}_{\text{dil}}$ )  | Forest   | 376 ± 53 b   | 297 ± 97 ab | 480 ± 23 c  | 162 ± 20 a   |
|                                      | Pasture  | 194 ± 37 b   | 160 ± 22 b  | 154 ± 23 b  | 98 ± 4 a     |
| $P_i$ ( $\text{HCl}_{\text{conc}}$ ) | Forest   | 165 ± 11 a   | 98 ± 28 b   | 130 ± 34 ab | 243 ± 37 c   |
|                                      | Pasture  | 69 ± 11 a    | 86 ± 8 b    | 133 ± 39 b  | 306 ± 18 c   |
| $P_o$ ( $\text{HCl}_{\text{conc}}$ ) | Forest   | 12 ± 2 a     | 45 ± 12 b   | 110 ± 51 ab | 443 ± 32 c   |
|                                      | Pasture  | 198 ± 143 a  | 101 ± 32 a  | 86 ± 42 a   | 527 ± 35 b   |
| P residual                           | Forest   | 44 ± 16 a    | 53 ± 28 a   | 141 ± 2 a   | 244 ± 53 b   |
|                                      | Pasture  | 69 ± 11 a    | 71 ± 3 a    | 230 ± 130 b | 404 ± 115 b  |
| P total                              | Forest   | 807 ± 44 a   | 667 ± 147 a | 1089 ± 73 b | 1820 ± 396 c |
|                                      | Pasture  | 1205 ± 225 a | 806 ± 195 b | 1105 ± 69 b | 3155 ± 125 c |

**Table 4** Enrichment factors for different P forms in particle-size fractions in A horizons of forest and pasture. *Cs* Coarse sand (250–2000  $\mu\text{m}$ ); *Fs* fine sand (20–250  $\mu\text{m}$ ); silt (2–20  $\mu\text{m}$ ); clay (<2  $\mu\text{m}$ )

|                         | Land use | Cs  | Fs  | Silt | Clay |
|-------------------------|----------|-----|-----|------|------|
| $P_i$ labile            | Forest   | 1.1 | 0.7 | 1.0  | 1.7  |
|                         | Pasture  | 1.6 | 0.7 | 0.8  | 1.6  |
| $P_i$ moderately labile | Forest   | 0.6 | 0.6 | 1.1  | 3.5  |
|                         | Pasture  | 0.7 | 0.7 | 1.0  | 2.9  |
| $P_o$ labile            | Forest   | 0.4 | 0.3 | 0.2  | 1.2  |
|                         | Pasture  | 0.9 | 0.3 | 0.6  | 1.5  |
| $P_i$ stable            | Forest   | 1.1 | 0.8 | 1.4  | 2.6  |
|                         | Pasture  | 0.4 | 0.5 | 1.2  | 2.3  |
| $P_o$ stable            | Forest   | 0.3 | 0.4 | 0.6  | 2.1  |
|                         | Pasture  | 0.7 | 0.5 | 0.5  | 2.3  |
| $P_i$ primary           | Forest   | 1.1 | 0.9 | 1.4  | 0.5  |
|                         | Pasture  | 1.3 | 1.0 | 1.0  | 0.6  |

**Table 5** Carbon to phosphorus ratios ( $C/P_o$ ) in fine earth and particle-size fractions of soils under forest and pasture

|             | $C/P_o$ |         |
|-------------|---------|---------|
|             | Forest  | Pasture |
| Fine earth  | 263     | 93      |
| Coarse sand | 721     | 134     |
| Fine sand   | 669     | 211     |
| Silt        | 375     | 145     |
| Clay        | 172     | 65      |

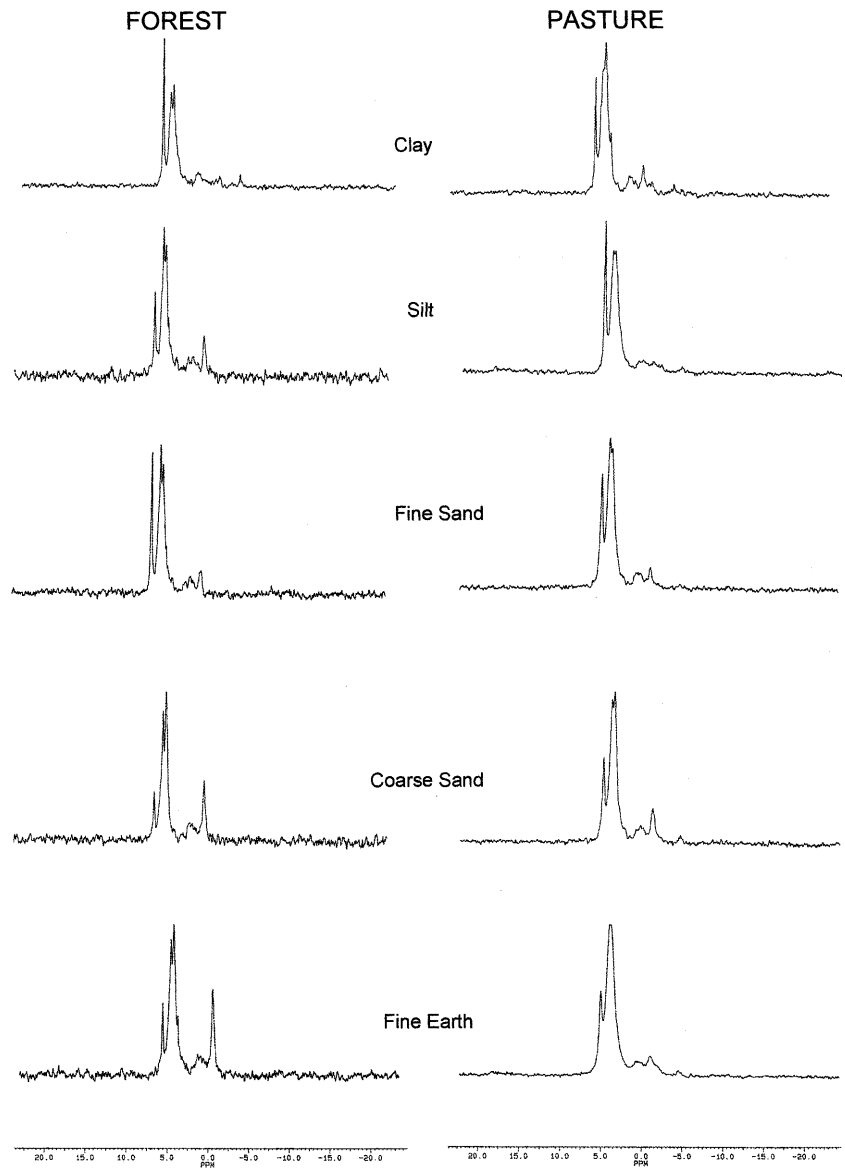
(in press) in clay than in all other fractions for the same mountain soils, indicating a higher microbial impact in clay fraction.

decrease with decreasing particle size, being much higher in the forest soil. According to Dalal (1977),  $P_o$  mineralisation occurs at  $C/P_o$  ratios of <200, and P immobilisation occurs when the ratio rises up to >300. Both fine earth and all size separates of pasture showed  $C/P_o$  ratios <200, indicating P mineralisation and those of forest had  $C/P_o$  ratios >250 (except clay), indicating P immobilisation. Low  $C/P_o$  ratios were observed for the clay fractions (Table 5), indicating P mineralisation. This is in accordance with a lower C/N ratio and higher concentrations of amino sugars found by Glaser et al.

#### $^{31}\text{P}$ -NMR analysis of alkali extracts

$^{31}\text{P}$ -NMR analysis was applied to investigate the structure of alkali soluble P forms. Figure 2 shows representative NMR spectrum of the alkali extracts of forest and pasture soils and their particle-size fractions. To facilitate comparisons between soils, signal intensities of NMR spectra were converted to amounts of alkali-extractable P on the basis of total P contents of the extracts as measured by chemical analysis. Different P compounds were probably extracted by alkali to a dif-

**Fig. 2** Representative  $^{31}\text{P}$ -NMR spectrum of the alkali extracts of forest and pasture soils and their particle-size fractions



ferent extent, so the results cannot be used to calculate the amounts in the soil (Newman and Tate 1980).

Signals at  $\delta=3.0\text{--}6.2$  ppm were due to orthophosphate monoester, a group comprising inositol phosphates, sugar phosphates and mononucleotides. Diester signals at  $\delta=1.0\text{--}2.0$  ppm may arise from teichoic acid, an orthophosphate diester which consists of sugar units linked by phosphate groups (Condon et al. 1990). Orthophosphate diesters, e.g. phospholipids and DNA (Newman and Tate 1980), resonated at  $\delta=0.0\text{--}0.2$  ppm. Signals accounting for  $\text{P}_i$  peaked at  $\delta=6.5$  ppm (orthophosphate) and  $\delta=-4.4$  ppm (pyrophosphates).

The relative proportions of  $\text{P}_i$  (sum of orthophosphate and pyrophosphate) and  $\text{P}_o$  determined by  $^{31}\text{P}$ -NMR and chemical analysis were similar both for fine earth and all particle-size fractions of forest and pasture (Table 6). The relative contribution of  $\text{P}_i$  (about 16%) and  $\text{P}_o$  (about 84%) were comparable in all particle-size fractions of forest and pasture (Table 6).

**Table 6** Relative proportions [%] of  $\text{P}_i$  and  $\text{P}_o$  in 0.5 M NaOH extracts determined by chemical analysis and  $^{31}\text{P}$  NMR spectroscopy

|             | $\text{P}_i$ |     | $\text{P}_o$ |     |
|-------------|--------------|-----|--------------|-----|
|             | Chem.        | NMR | Chem.        | NMR |
| Forest      |              |     |              |     |
| Fine earth  | 11           | 8   | 89           | 92  |
| Coarse sand | 12           | 6   | 88           | 94  |
| Fine sand   | 13           | 18  | 87           | 82  |
| Silt        | 18           | 16  | 82           | 84  |
| Clay        | 20           | 23  | 80           | 77  |
| Pasture     |              |     |              |     |
| Fine earth  | 12           | 16  | 88           | 84  |
| Coarse sand | 18           | 15  | 82           | 85  |
| Fine sand   | 15           | 21  | 85           | 79  |
| Silt        | 20           | 25  | 80           | 75  |
| Clay        | 21           | 17  | 79           | 83  |

**Table 7** Concentrations and standard deviations ( $n=2$ ) of different P forms measured as  $^{31}\text{P}$  NMR in fine earth and particle-size fractions of forest and pasture [ $\text{mg P kg}^{-1}$ ]. Relative contribution [%] of each P species to the total NaOH extractable P in brackets. *tr* Trace

|                   | Land use | Fine earth   | Coarse sand  | Fine sand    | Silt         | Clay         |
|-------------------|----------|--------------|--------------|--------------|--------------|--------------|
| Ortho phosphate   | Forest   | 22 ± 2(8)    | 12 ± 1(6)    | 30 ± 3(18)   | 32 ± 9(16)   | 211 ± 17(21) |
|                   | Pasture  | 107 ± 17(15) | 67 ± 4(14)   | 88 ± 2(20)   | 111 ± 19(24) | 256 ± 44(15) |
| Monoester         | Forest   | 168 ± 1(63)  | 115 ± 7(62)  | 105 ± 7(63)  | 132 ± 4(65)  | 625 ± 57(62) |
|                   | Pasture  | 481 ± 46(66) | 300 ± 14(65) | 277 ± 12(66) | 285 ± 11(62) | 1149 ± 3(67) |
| Teichoic acid     | Forest   | 31 ± 1(12)   | 26 ± 1(4)    | 15 ± 1(9)    | 23 ± 5(11)   | 98 ± 12(10)  |
|                   | Pasture  | 64 ± 2(9)    | 47 ± 1(10)   | 39 ± 3(9)    | 36 ± 2(8)    | 149 ± 15(9)  |
| Diester §         | Forest   | 41 ± 2(15)   | 32 ± 5(17)   | 9 ± 1(5)     | 13 ± 5(6)    | 27 ± 3(3)    |
|                   | Pasture  | 47 ± 3(7)    | 38 ± 3(8)    | 23 ± 2(5)    | 17 ± 1(4)    | 90 ± 17(5)   |
| Pyrophosphate     | Forest   | tr (0)       | tr (0)       | tr (0)       | tr (0)       | 20 ± 9(2)    |
|                   | Pasture  | 6 ± 1(1)     | 7 ± 1(1)     | 5 ± 1(1)     | 6 ± 2(1)     | 30 ± 4(2)    |
| Unknown           | Forest   | 5 ± 1(2)     | 3 ± 1(1)     | 2 ± 1(1)     | 3 ± 1(2)     | 24 ± 3(2)    |
|                   | Pasture  | 14 ± 1(2)    | 6 ± 1(1)     | 4 ± 1(1)     | 6 ± 2(1)     | 45 ± 11(3)   |
| Monoester/diester | Forest   | 2.3          | 2.0          | 4.5          | 3.7          | 5.0          |
|                   | Pasture  | 4.3          | 3.6          | 4.5          | 5.4          | 4.8          |

§ Without teichoic acid

The  $^{31}\text{P}$ -NMR spectra revealed the same forms of P in the forest and pasture soils. As can be seen in Table 7, the concentrations of different P forms in the fine earth and in all particle-size fractions of pasture were always higher than in forest soils.

Pyrophosphate was found only in small concentrations in all investigated samples comprising a maximum of 2% of NaOH-extractable P in the clay fraction. According to Condrón et al. (1985), pyrophosphate can originate from hydrolysed organic esters. Therefore, we conclude that hydrolysis due to extraction and sample preparation was negligible.

Both in the fine earth and in all particle-size fractions of forest and pasture soils, monoester P was the dominant P species, representing about 65% of NaOH-extractable P in the fine earth and in all particle-size fractions of forest and pasture. The concentrations of monoester P, however, were two to three times higher in fine earth and particle-size fractions of pasture than in forest soil. The highest concentrations of monoester P were observed in the clay fraction. The high charge density of monoesters (e.g. inositol phosphates) allows rapid adsorption on soil minerals, and extensive interaction with sesquioxides that protect free inositols from degradation (Tate 1984).

Phosphodiester comprising teichoic acid, phospholipids, and DNA represented 27% of NaOH-extractable P in the fine earth of forest and 16% in pasture. Phosphodiester have low charge densities and their phosphate groups are shielded from ionic interactions. This leaves them accessible to microbial or enzymatic attack in the soil environment (Tate 1984) and explains the small proportions present in the investigated soils. Guggenberger et al. (1996a) showed that alkali-extractable phosphodiester represent a pool of  $\text{P}_o$  that is readily available for microbial mineralisation. Phosphodiesterase catalyses the reaction of degradation of nucleic acids, and this soil enzyme has its optimum activity at buffer pH 8.0 (Browman and Tabatabai 1978).

Persistence and accumulation of phosphodiester has been shown to occur in soils where microbial activity is restrained due to acidity, waterlogging or low temperature (Makarov et al. 1995). Thus, in acid or wet soils phosphodiester represent between 10% and 36% of alkali-extractable P (Makarov et al. 1995), while in neutral, well-drained soils the percentage of phosphodiester is frequently <10% (Condrón et al. 1985). The percentages of alkali-extractable phosphodiester obtained in the present study reflect relatively low microbial activity probably due to the low mean annual temperature in these mountain soils.

The concentration of teichoic acid is about two times higher in the fine earth and in all particle-size fractions of the pasture soils compared with the forest soils. Its relative contribution to the total NaOH-extractable P, at about 10%, is comparable in both soils. Teichoic acid is found only in the cell walls of gram-positive bacteria, which make up from 20% to 40% of the dry weight of the cell walls (Zelles and Alef 1995) and can account for 85% of the total P (Anderson et al. 1977). Gehorn et al. (1984) used teichoic acid as a biomarker to estimate the biomass of gram-positive bacteria in soils and sediments. Therefore, teichoic acid belongs to the active P pool in soils.

The different P species identified by  $^{31}\text{P}$ -NMR spectroscopy can be characterised with regard to microbial degradability. Hinedi et al. (1988) showed that the diester P fraction (including teichoic acid) may be converted relatively quickly to  $\text{P}_i$  in favourable soil environments, while monoester P is much more stable. The ratio of monoester to diester P was 2.3 for forest soils and 4.3 for pasture soils (Table 7). Ratios cited in the literature vary between 1 and 20 (Hawkes et al. 1984; Condrón et al. 1990; Guggenberger et al. 1996b). The increase of the ratio of monoester to diester P in the pasture soil suggests that levels of labile  $\text{P}_o$  species decrease after deforestation and subsequent establishment of pasture. This result was consistent with the

higher C/P<sub>o</sub> ratios measured in pasture soils (Table 6).

The ratio of monoester to diester increased with decreasing particle-size fraction (Table 7). Stabilisation of microbial metabolites by association with clay minerals and oxides and hydroxides (Sanyal and de Datta 1991) may protect the diester P in the clay fraction from mineralisation and explain the higher monoester to diester ratios observed in this fraction compared with other size fractions.

## Conclusions

On the basis of our results it may be concluded that intensive pasture affects soil P pools, and thus soil fertility. P introduced into the pasture soil via animal excrement was distributed between all P pools except highly labile P<sub>i</sub> (P<sub>i</sub> NaHCO<sub>3</sub>) and primary P<sub>i</sub> (P<sub>i</sub> HCl<sub>dil</sub>). Stable P was mainly stabilised in the clay fraction. <sup>31</sup>P NMR spectroscopy reflects low microbial activity, which is probably due to the low mean annual temperature in these mountain soils. An ecological interpretation of chemically defined P pools was possible in spite of the fact that different interpretations exist in the literature. However, the interpretation for P HCl<sub>conc</sub>, as derived from particulate organic debris, which is not alkali extractable but where the hot acid would break up cellulose in the residual fraction and release P, is not adequate.

**Acknowledgements** We are indebted to Dr. Ludwig Haumaier, who recorded the <sup>31</sup>P NMR spectra. We acknowledge the kind financial support of the German Research Foundation, DFG (Ze 154/33-1). A fellowship from the Spanish Government enabled Dr. Turrión to participate in the project. Dawit Solomon was supported by the German Academic Exchange Service, DAAD. We are indebted to Dr Rico for assisting in statistical analysis. Field work was generously supported by the Academy of Sciences in Tashkent (Director Usmanov, Dr Ni) and Bishkek (Dr Lemzin).

## References

- Anderson AJ, Green RS, Archibald AR (1977) Specific determination of ribitol teichoic acid in whole bacteria and isolated walls of *Bacillus subtilis* W23. *Carbohydr Res* 57:7–10
- Anderson DW, Paul EA (1984) Organo-mineral complexes and their study by radiocarbon dating. *Soil Sci Soc Am J* 48:298–301
- Bowman RA, Cole CV (1978) Transformations of phosphorus substances in soil as evaluated by sodium bicarbonate extraction. *Soil Sci* 125:49–94
- Browman MG, Tabatabai MA (1978) Phosphodiesterase activity of soils. *Soil Sci Soc Am J* 42:284–290
- Christensen BT (1992) Physical fractionation of soil and organic matter in primary particle size and density separates. *Adv Soil Sci* 20:1–90
- Condon LM, Goh KM, Newman RH (1985) Nature and distribution of soil phosphorus as revealed by a sequential extraction method followed by <sup>31</sup>P nuclear magnetic resonance analysis. *J Soil Sci* 36:199–207
- Condon LM, Frossard E, Tiessen H, Stewart JWB (1990) Chemical nature of phosphorus in cultivated and uncultivated soils under different environmental conditions. *J Soil Sci* 41:41–50
- Condon LM, Davis MR, Newman RH, Cornforth IS (1996) Influence of conifers on the forms and availability of phosphorus in selected New Zealand grassland soils. *Biol Fertil Soils* 21:37–42
- Cross AF, Schlesinger WH (1995) A literature review and evaluation of the Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma* 64:197–214
- Dalal RC (1977) Soil phosphorus. *Adv Agron* 29:83–117
- Gehorn MJ, Davis JD, Glen AS, White DC (1984) Determination of the gram-positive bacterial content in soils and sediments by analysis of teichoic acid components. *J Microbiol Methods* 2:165–176
- Glaser B, Turrión MB, Solomon D, Ni A, Zech W (2000) Soil organic matter pools in mountain soils of the Alay Range, Khyrgyzia, affected by deforestation. *Biol Fertil Soils* (in press)
- Guggenberger G, Christensen BT, Zech W (1994) Land-use effects on the composition of organic matter in particle-size separates of soil: I. Lignin and carbohydrate signature. *Eur J Soil Sci* 45:449–458
- Guggenberger G, Zech W, Thomas R (1995) Lignin and carbohydrate alteration in particle-size separates of an Oxisol under tropical pastures following native savanna. *Soil Biol Biochem* 27:1629–1638
- Guggenberger G, Christensen BT, Rubæk CT, Zech W (1996a) Land-use and fertilization effects on P forms in two European soils: Resin extraction and <sup>31</sup>P-NMR analysis. *Eur J Soil Sci* 47:605–614
- Guggenberger G, Haumaier L, Thomas RL, Zech W (1996b) Assessing the phosphorus status of an Oxisol under tropical pastures following native savanna using <sup>31</sup>P NMR spectroscopy. *Biol Fertil Soils* 23:332–339
- Hartung J, Elpelt B, Klösener KH (1993) *Lehr- und Handbuch der angewandten Statistik*, 9th edn., Munich
- Hawkes GE, Powlson DS, Randall EW, Tate KR (1984) A <sup>31</sup>P nuclear magnetic resonance study of the phosphorus species in alkali extracts of soils from long-term field experiments. *J Soil Sci* 35:35–45
- Hinedi ZR, Chang AC, Lee RWK (1988) Mineralisation of phosphorus in sludge-amended soils monitored by phosphorus-31 nuclear magnetic resonance spectroscopy. *Soil Sci Soc Am J* 52:1593–1596
- Magid J (1993) Vegetation effects on phosphorus fractions in set-aside soils. *Plant Soil* 149:111–119
- Magid J, Tiessen H, Condon LM (1996) Dynamics of phosphorus in soils under natural and agricultural ecosystems. In: Piccolo A (ed) *Humic substances in terrestrial ecosystems*. Elsevier, Amsterdam, pp 429–466
- Makarov MI, Guggenberger G, Alt HG, Zech W (1995) Phosphorus status of Eutric Cambisols polluted by P-containing immissions: Results of <sup>31</sup>P NMR spectroscopy and chemical analysis. *Z Pflanzenernaehr Bodenkd* 158:293–298
- Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 27:31–36
- Newman RH, Tate KR (1980) Soil phosphorus characterization by <sup>31</sup>P nuclear magnetic resonance. *Commun Soil Sci Plant Anal* 11:835–842
- Sanyal SK, de Datta SK (1991) Chemistry of phosphorus transformations in soil. *Adv Soil Sci* 16:1–120
- Saunders WMH, Williams EG (1955) Observations on the determination of total phosphorus in soils. *J Soil Sci* 6:254–267
- Schmidt JP, Buol SW, Kamprath EJ (1997) Soil phosphorus dynamics during 17 years of continuous cultivation: A method to estimate long-term P availability. *Geoderma* 78:59–70
- Smeck ME (1985) Phosphorus dynamics in soils and landscapes. *Geoderma* 35:185–199
- Sokal RR, Rohlf FJ (1995) *Biometry. The principles and practice of statistics in biological research*, 3rd edn. Freeman, New York



- Stewart JWB, Tiessen H (1987) Dynamics of soil phosphorus. *Biogeochemistry* 4:41–60
- Tate KR (1984) The biological transformation of P in soil. *Plant Soil* 76:245–256
- Tiessen H, Moir JO (1993) Characterization of available P by sequential extraction. In: Carter MR (ed) *Soil sampling and methods of analysis*, part 2. Lewis, Boca Raton, pp 75–86
- Tiessen H, Stewart JWB (1983) Particle-size fractions and their use in studies of soil organic matter. II. Cultivation effects on organic matter composition in size fractions. *Soil Sci Soc Am J* 47:509–514
- Tiessen H, Stewart JWB, Moir JO (1983) Changes in organic and  $P_i$  composition of two grassland soils and their particle size fractions during 60–90 years of cultivation. *J Soil Sci* 34:815–823
- Tiessen H, Stewart WB, Cole CV (1984) Pathways of phosphorus transformations in soils of differing pedogenesis. *Soil Sci Soc Am J* 48:853–858
- Turrión MB, Gallardo JF, González MI (2000) Distribution of P forms in natural and fertilized forest soils of the Central Western Spain: Plant response to superphosphate fertilization. *Arid Soil Res Rehabil* (in press)
- Wilding LG (1985) Spatial variability: its documentation, accommodation and implication to soil surveys. In: Bielsen DR, Bouma J (eds) *Proceedings of a workshop of the ISSS and SSSA*. Wageningen, pp 166–187
- Zech W, Guggenberger G (1996) Organic matter dynamics in forest soils of temperate and tropical ecosystems. In: Piccolo A (ed) *Humic substances in terrestrial ecosystems*. Elsevier, Amsterdam, pp 101–170
- Zelles L, Alef K (1995) Biomarkers. In: Alef K, Nannipieri P (eds) *Methods in Applied soil microbiology and biochemistry*. Academic Press, London, pp 422–439