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# Phosphorus forms and dynamics as influenced by land use changes in the sub-humid Ethiopian highlands

Dawit Solomon [a,*], Johannes Lehmann [a,1], Tekalign Mamo [b],
Florian Fritzsche [a], Wolfgang Zech [a]

[a] *Institute of Soil Science and Soil Geography, University of Bayreuth, Universitätsstr. 30, D-95440 Bayreuth, Germany*
[b] *Ethiopian Agricultural Research Organization, Debre Zeit Agricultural Research Center, PO Box 32, Debre Zeit, Ethiopia*

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## Abstract

In undisturbed tropical forest ecosystems, the phosphorus (P) cycle is essentially "closed" with minimal short-term losses or gains of P. The forms and dynamics of soil P, however, can be greatly affected by land use changes, which often involve changes in vegetation cover, biomass production and nutrient cycling in the ecosystem. Sequential extraction and $^{31}$P nuclear magnetic resonance (NMR) spectroscopy were used to investigate the influence of land use changes on the amount and structural composition of P in the sub-humid highlands of southern Ethiopia. Samples were collected from surface soils (0–10 cm) of natural forest, tea plantations and cultivated fields (25 years) at Wushwush and from *Podocarpus* dominated natural forest, *Cupressus* plantations and cultivated fields (30 years) at Munesa sites. Significantly lower ($P < 0.05$) amounts of total P were found following clear-cutting and long-term cultivation (31% and 39%), and establishment of plantations (21% and 22%) at Wushwush and Munesa, respectively. The largest depletion of sodium bicarbonate-extractable organic P ($NaHCO_3$–$P_o$) (74% and 77%) and sodium hydroxide-extractable $P_o$ (NaOH–$P_o$) (67% and 67%) due to cultivation occurred in the sand, followed by the silt (52% and 56%, $NaHCO_3$–$P_o$ and 32% and 53%, NaOH–$P_o$) and the clay (33% and 42%,

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[*] Corresponding author. Current address: Department of Crop and Soil Sciences, Bradfield and Emerson Hall, Cornell University, Ithaca, NY 14853, USA. Tel.: +1-607-254-1236; fax: +1-607-255-8615.

*E-mail address:* DS278@cornell.edu (D. Solomon).

[1] Current address: Department of Crop and Soil Sciences, Bradfield and Emerson Hall, Cornell University, Ithaca, NY 14853, USA.

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$NaHCO_3$–$P_o$ and 28% and 35%, NaOH–$P_o$) size separates from the two sites, respectively. $^{31}P$ NMR spectroscopy revealed that orthophosphate monoesters were the major organic P compounds (27–66%) followed by orthophosphate diesters (9–27%) and teichoic acids (7–11%). Unknown organic P species accounted for 3–8% (unknown A) and 3–5% (unknown B), whereas phosphonates made up 2–3%. The proportion of diester-P decreased in the order: natural forests (24% and 27%) > plantations (15% and 13%) > cultivated fields (10% and 9%) at Wushwush and Munesa, respectively. The percentages of teichoic acid, unknown A, unknown B and phosphonates also decreased, whereas the proportions of orthophosphate monoesters increased following land use changes. Greater decline in proportions of diester-P and teichoic acids were found in the silt than in the clay size separates, which may be attributed to stabilization of microbially derived organic P structures by closer association with clay minerals and/or sesquioxides. The results of sequential extraction and $^{31}P$ NMR spectroscopy indicate that continuous cultivation with little or no P input not only decreased the amount, but also influenced the structural composition and bioavailability of P in these tropical soils. Thus measures have to be designed for replenishment and subsequent maintenance of soil P stocks, to ensure sustainable crop production in sub-humid highland agroecosystems of southern Ethiopia.

*Keywords:* Cultivation; Deforestation; Plantation; $^{31}P$ NMR; Soil P; Tropical highland ecosystem

## 1. Introduction

Phosphorus (P) is among the most limiting nutrients for food production in the sub-humid and humid tropical highlands of East Africa (Sanchez et al., 1997). This deficiency is mainly caused either by the inherent characteristics of the parent material or by the strong sorption of $PO_4^{3-}$ to Al and Fe-(hydr)oxides, which turns large proportions of total soil P into unavailable forms. The problem is further exacerbated by nutrient mining due to the low-input agriculture practiced in the region.

In most natural ecosystems, the P cycle is essentially “closed” with minimal short-term losses or gains of P (Magid et al., 1996). In these ecosystems, the amounts and chemical nature of soil P are primarily determined by a combination of the major soil forming factors: parent material, climate, topography, soil biota and time. The distribution of P into various organic and inorganic forms thus reflects the history, present structure and functioning of the natural ecosystems. The forms and dynamics of soil P, however, can be greatly affected by agricultural management practices (Tiessen et al., 1983; Guggenberger et al., 1996; Turrión et al., 2000), which often involve dramatic changes in vegetation cover, biomass production, soil organic matter (SOM) level and nutrient cycling in the ecosystem (Magid et al., 1996).

Several investigations have examined the long-term dynamics of soil organic matter and related nutrients such as P in natural or managed ecosystems. However, most of the detailed studies have been conducted in temperate environments (Anderson et al., 1981; Bowman et al., 1990; Guggenberger et al., 1994). Thus there is a need to improve knowledge about the nature and turnover of organic matter and associated nutrients in the tropics (Magid et al., 1996).

In the sub-humid highlands of southern Ethiopia, deforestation of the remnant natural forests and their conversion into low-input agriculture or establishment of plantations has caused extensive soil degradation (Stoorvogel et al., 1993). Although much of the Ethiopian highlands have a high potential for crop production, almost no detailed work has been done on the status, form and dynamics of P in the soils, with most studies focusing on the plant-available inorganic P (Tekalign and Haque, 1987; Duffera and Robarge, 1999). Since available P in soil is not a single entity, a “complete account or budget” of the P forms present in the soil have to be obtained in order to determine the fate of applied P fertilizers. This can be achieved by characterizing both labile and refractory inorganic P pools in the soil environment. In addition, characterizing soil organic P pools is central for understanding the P cycle in low-input tropical agroecosystems where organic P contributes significantly to P nutrition of crops.

Hedley et al. (1982) developed a sequential extraction technique of soil P into various inorganic and organic fractions, based on their bioavailabiliy. Application of sequential extraction, coupled with particle size fractionation, allows the interpretation of specific extractable fractions in terms of functional P pools and to investigate P dynamics in natural and managed ecosystems (Hedley et al., 1982; Tiessen et al., 1983; Turrión et al., 2000; Solomon and Lehmann, 2000).

Sequential extraction permits the assessment of the biochemical and geochemical cycling of P, but it does not provide information on the structure of P compounds in the soil. $^{31}$P nuclear magnetic resonance (NMR) spectroscopy, however, is a useful tool for characterizing the structural composition of P in alkali extracts of soils (Newman and Tate, 1980), and can support the conclusions based on sequential extraction (Taranto et al., 2000). The diagnostic property in $^{31}$P NMR spectroscopy is based on the chemical shift of the P nuclei (Robinson et al., 1998). The nuclei of different classes of P species such as orthophosphate monoester-P, orthophosphate diester-P, phosphonates, pyrophosphates and inorganic orthophosphate P exhibit distinct chemical shift values, which can be used to determine the proportion of the respective P species relative to the total P in the extract. Liquid-state $^{31}$P NMR spectroscopy has been applied to characterize P forms as a function of climate, cultivation, fertilizer history or environmental conditions (Tate and Newman, 1982; Hawkes et al., 1984; Zech et al., 1985; Condron et al., 1990; Guggenberger et al., 1996; Sumann et al., 1998; Solomon and Lehmann, 2000).

Therefore, the objectives of the present study were: (i) to evaluate the forms and distribution of operationally defined labile and non-labile soil P pools using sequential extraction and liquid-state $^{31}$P NMR spectroscopy, and (ii) to assess the influence of land use changes on the amount and structural composition of P in bulk soils and size separates of soils from the sub-humid tropical environment of the southwestern Ethiopian highlands.

## 2. Materials and methods

### 2.1. Site description

The study was conducted in the southwestern highlands (Wushwush) and southern rift valley escarpment (Munesa) of Ethiopia. Wushwush is located at 7°19′N to 36°07′E. The altitude of the area is 1900 m above sea level. Mean annual temperature is 18 °C with an average annual precipitation of 1800 mm. Geologically, the area is associated with Jimma Volcanics with abundant rhyolites and trachybasalts. Soils of the area are classified as Plinthic Alisols (FAO-Unesco, 1997) with clayey texture and dark reddish brown color. The Wushwush natural forest is mainly composed of *Olea africana* Mill., *Syzygium guineense* Guill. and Perr. Gmel., *Cordia africana* Lam., *Croton macrostachys* Hochst. ex Rich. and *Ficus vasta* Hayne. The Munesa site is located 7°35′N–38°45′E. Mean annual temperature is 19 °C, with an average annual precipitation of 1250 mm. Parent materials of the Munesa area are of volcanic origin, principally trachytes and basalts with ignimbrites and pumices at the rift valley floor. The escarpment extends from about 2100 to 3200 m, and the plain descends gradually to the Rift Valley lakes at about 1600 m above sea level. The soils of the area are classified as Humic Nitisols (FAO-Unesco, 1997) with clayey texture and very dark reddish brown color. Basic physical and chemical characteristics of the soils at both sites are shown in Table 1. The natural vegetation of the Munesa forest ranges from the *Arundinaria alpina* Schun., *Hagenia abyssinica* J.F. Gmel., *C. macrostachys* Hochst. ex Rich., *Podocarpus falcatus* Thunb. Mirb., *O. hochstetteri* Baker. dominated forest on the escarpment to *Acacia* woodlands (*Acacia tortilis* Hayne, *Acacia abyssinica* Hochst. ex Benth. and *Acacia seyal* Delile) in the semi-arid lowlands.

The land use systems studied at the Wushwush site were natural forest, tea plantations (35 years old) and fields cultivated for 25 years. While at the Munesa site, *Podocarpus* dominated natural forest, *Cupressus* plantations (25 years old) and 30 years cultivated fields were investigated. There is no well-established fertilization program in the tea plantation. Depending on fertilizer availability, up to 100 kg ha$^{-1}$ urea or NPK (20:10:10) was applied every 3–5 years as a side dressing. In the cultivated fields of both sites, maize (*Zea mays* L.) was grown without fertilizer inputs. However, during the intermittent dry periods, sorghum (*Sorghum bicolor* L. Moench) was grown at Munesa. The depth of cultivation at the Wushwush and Munesa sites varies between 10 and 12 cm. Crop residues were normally collected and used as animal feed.

### 2.2. Sampling

After considering the depth of cultivation in the region, we used a core sampler and collected composite samples in three replicates from the upper 10

Table 1
Physical and chemical characteristics of surface soils of the southern Ethiopian highlands

| Land use | Sand | Silt | Clay | Bulk density (mg $m^{-3}$) | pH |  | OC[a] | N | C/N | CEC ($cmol_c$ $kg^{-1}$ soil) | BS (%) | $Fe_d^b$ | $Fe_o^c$ | $Al_d$ | $Al_o$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | (g $kg^{-1}$ soil) |  |  |  | ($H_2O$) | (KCl) | (g $kg^{-1}$ soil) |  |  |  |  | (g $kg^{-1}$ soil) |  |  |  |
| *Wushwush* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Natural forest | 103 | 327 | 570 | 0.69 | 6.4 | 5.5 | 85 | 7.8 | 10.8 | 37.0 | 69 | 6.7 | 0.60 | 0.96 | 0.25 |
|  | (0.10)[d] | (0.10) | (0.10) | (0.05) | (0.08) | (0.05) | (0.20) | (0.02) | (0.11) | (0.11) | (0.01) | (0.05) | (0.02) | (0.04) | (0.05) |
| Tea plantation | 102 | 330 | 568 | 0.87 | 5.7 | 4.5 | 42 | 4.0 | 10.4 | 24.7 | 35 | 6.5 | 0.70 | 0.97 | 0.32 |
|  | (0.10) | (0.33) | (0.30) | (0.03) | (0.11) | (0.04) | (0.10) | (0.03) | (0.06) | (0.30) | (0.01) | (0.02) | (0.03) | (0.02) | (0.08) |
| Cultivation | 70 | 337 | 593 | 0.89 | 5.7 | 4.7 | 38 | 3.7 | 10.2 | 26.4 | 46 | 5.8 | 0.83 | 0.77 | 0.30 |
|  | (0.21) | (0.30) | (0.10) | (0.08) | (0.05) | (0.04) | (0.10) | (0.03) | (0.08) | (0.15) | (0.05) | (0.01) | (0.02) | (0.03) | (0.07) |
| *Munesa* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Natural forest | 130 | 347 | 523 | 0.62 | 7.6 | 6.8 | 103 | 8.2 | 12.6 | 52.2 | 98 | 2.5 | 0.30 | 0.38 | 0.15 |
|  | (0.11) | (0.24) | (0.10) | (0.10) | (0.05) | (0.07) | (0.30) | (0.10) | (0.28) | (0.12) | (0.12) | (0.03) | (0.02) | (0.04) | (0.01) |
| *Cupressus* plantation | 231 | 326 | 443 | 0.77 | 7.4 | 6.6 | 71 | 7.1 | 10.0 | 40.4 | 96 | 2.5 | 0.36 | 0.32 | 0.18 |
|  | (0.19) | (0.55) | (0.10) | (0.05) | (0.15) | (0.08) | (0.20) | (0.08) | (0.10) | (0.15) | (0.04) | (0.09) | (0.02) | (0.07) | (0.08) |
| Cultivation | 229 | 285 | 486 | 1.04 | 5.6 | 4.5 | 38 | 3.3 | 11.8 | 26.5 | 51 | 3.4 | 0.71 | 0.40 | 0.21 |
|  | (0.17) | (0.49) | (0.10) | (0.06) | (0.09) | (0.05) | (0.10) | (0.09) | (0.12) | (0.05) | (0.01) | (0.03) | (0.08) | (0.03) | (0.05) |

[a] OC, organic carbon.

[b] $Fe_d$ and $Al_d$, dithionite-citrate-bicarbonate extractable Fe and Al.

[c] $Fe_o$ and $Al_o$, oxalate extractable Fe and Al.

[d] Values in parentheses indicate standard errors of the mean ($n = 3$).

cm of the different land use systems in April 1998. This helps to minimize differences, which may arise due the dilution of SOM due to mixing of the surface soil with that of the subsoil through cultivation. We selected three representative sites from each land use and collected three subsamples (200 $cm^3$ core volume at each subsite) in a radial sampling scheme (Wilding, 1985). The three subsamples were then bulked into one sample. The spacing between the subsites on the radii ranged from 5 to 10 m depending on the size of the patches. The samples were air-dried and sieved ($<2$ mm) prior to fractionation and chemical analysis.

### 2.3. Particle size fractionation

Particle size fractionation was done on $<2$ mm material (bulk soil) according to Amelung et al. (1998). After removing visible root remnants, 30 g of soil was ultrasonically treated with an energy input of 60 J $ml^{-1}$ using a probe type sonicator (Branson Sonifier W-450) in a soil/water ratio of 1:5 (w/v). The coarse sand fraction (250–2000 μm) was isolated by wet sieving. In order to completely disperse the remaining material in the $<250$ μm suspension, ultrasound was again applied with an energy input of 440 J $ml^{-1}$ in a soil/water ratio of 1:10 (w/v). The clay fraction ($<2$ μm) was separated from the silt (2–20 μm) and fine sand (20–250 μm) fractions by repeated centrifugation. The silt fraction was separated from the fine sand fractions by wet sieving. Coarse and fine sand separates were combined and all fractions were dried at 40 °C before grinding them for chemical analysis. The recovery of size separates after ultrasonic dispersion, wet sieving and centrifugation ranged from 968 to 979 g $kg^{-1}$ of the initial soil mass.

### 2.4. Chemical analysis

Carbon and nitrogen contents of bulk soils and particle size separates were analyzed by dry combustion with a C/H/N/S-analyzer (Elementar Vario EL). The pH-($H_2O$) and pH-(KCl) were determined in 1:2.5 soil/water (w/v) suspension using a glass electrode. Cation-exchange capacity (CEC) was determined with 1 M $CH_3CO_2NH_4$ ($pH=7.0$) according to Avery and Bascomb (1974) (Table 1). Dithionite-citrate-bicarbonate extractable aluminum and iron ($Al_d$, $Fe_d$) were determined after double extractions at 70 °C for 15 min, as described by Mehra and Jackson (1960). Oxalate-extractable aluminum and iron ($Al_o$, $Fe_o$) were determined using atomic absorption spectroscopy (Varian AAS-400) after extraction for 2 h with 0.2 M ammonium oxalate at $pH=3$ in the dark (Blume and Schwertmann, 1969).

### 2.5. Sequential fractionation

A modified version of the sequential extraction technique of Hedley et al. (1982) and Tiessen and Moir (1993) was used to fractionate P from the bulk soils and sand, silt and clay size separates with minor modification for the final step: the remaining soil residue, after a sequential extraction with 0.5 M sodium bicarbonate ($NaHCO_3$), 0.1 M sodium hydroxide (NaOH), 1 M hydrochloric acid (HCl), and concentrated HCl, was heated at 560 °C in a furnace and subsequently the P was extracted after shaking the residue with 30 ml of 0.5 M $H_2SO_4$ for 16 h. Inorganic P ($P_i$) in 0.5 M $NaHCO_3$, 0.1 M NaOH, 1 M HCl, concentrated HCl, and 0.5 M $H_2SO_4$ was determined after pH adjustment by the molybdate–ascorbic acid method (Murphy and Riley, 1962). Since some of the extracts were very dark in color, de-coloring was essential for accurate inorganic P determination (Adams and Byrne, 1989). This was done by adding 200 mg of activated charcoal (Darco-60) to the extracts, shaken end-over-end for 5 min and P was measured from the supernatant. Total P in $NaHCO_3$ and NaOH was determined using an inductively coupled plasma-optical emission spectrometer (ICP-OES, Integra XMP), and organic P ($P_o$) was calculated as the difference between total and inorganic P.

The $NaHCO_3$-extractable inorganic and organic P fractions were considered as labile P loosely sorbed on the surfaces of Fe and Al oxides and some microbial P (Bowman and Cole, 1978). The 0.1 M NaOH-extractable P contained secondary inorganic P bound to Fe and Al compounds and clay edges and organic P associated with humic and fulvic acids (Syers et al., 1969; Tiessen and Moir, 1993). Therefore, it was considered as moderately labile. Dilute HCl-extractable P was taken as inorganic P associated with Ca (Tiessen and Moir, 1993). The concentrated HCl was intended to remove very stable residual inorganic P. Afterwards, the highly recalcitrant P was extracted by 0.5 M $H_2SO_4$ after combustion at 560 °C.

### 2.6. Nuclear magnetic resonance spectroscopy

For liquid-state $^{31}P$ NMR measurements, bulk soil and silt and clay separates were extracted three times with a solution containing 0.1 M NaOH and 0.4 M NaF at a ratio of soil to extraction solution of 1:5 (w/v). Sample size was adjusted to yield 200 mg of organic C in the extracts. The extraction procedure followed the outline of Schnitzer (1982), modified by Sumann et al. (1998). The 0.1 M NaOH was used in order to reduce possible hydrolysis of organic ester P (Condron et al., 1985; Leinweber et al., 1997). Samples were shaken for 24 h on a rotary shaker and centrifuged at $17{,}700 \times g$ for 10 min. Each time, the supernatant was decanted and stored in a refrigerator at 4 °C. Finally, the clear supernatants were dialyzed (molecular weight cutoff, MWCO, 12,000–14,000 u) and freeze-dried.

One hundred and fifty milligrams of freeze-dried materials were dissolved in 3 ml of 0.5 M NaOD in $D_2O$ in 10 mm NMR tubes and analyzed within 12 h by $^{31}P$ NMR spectroscopy. Liquid-state $^{31}P$ NMR spectra were acquired using a Bruker Avance DRX 500 NMR spectrometer (Bruker Instruments) (11.7 T;

202.5 MHz) without $^1H$ decoupling at a temperature of 298 K. An accusation time of 0.1 s, a 90°-pulse and a relaxation delay of 0.2 s were used. Spectra were recorded with a line broadening of 20 MHz after collecting 8000 scans. Preliminary measurements with different relaxation times showed that such a delay would give better resolution of spectra. Chemical shifts were measured relative to 85% orthophosphoric acid in a 5-mm tube that was inserted into the 10-mm sample tube before the measurement of each sample. Intensities of signals were determined by electronic integration of peaks and signal assignments were done according to Newman and Tate (1980), Condron et al. (1990) and Makarov (2000, personal communication). The total P in the dialyzed extract was determined after ignition (560 °C) and dissolution of the residues in 0.5 M $H_2SO_4$. Inorganic P was measured directly from the extract, and organic P was calculated as the difference of the two. Phosphorus from the dialyzed extracts was extracted by 0.5 M $H_2SO_4$ and determined by the molybdate–ascorbic acid method (Murphy and Riley, 1962).

### 2.7. Statistics

Statistical analysis of the data was carried out on the replicates by one-way analysis of variance (ANOVA). If the main effects were significant at $P < 0.05$, a post hoc separation of means was done by univariate least significant difference (LSD) test. Statistical analysis was conducted with STATISTICA for Windows 5.1 (Statistica, 1995).

## 3. Results and discussion

### 3.1. Phosphorus fractions and contents in bulk soils

The total P content in the bulk soils of the Wushwush and Munesa sites ranged from 927 to 1343 mg $kg^{-1}$ soil and 874 to 1426 mg $kg^{-1}$ soil, respectively (Table 2). These values are comparable to those obtained for forest-derived soils of Nigeria (Uzu et al., 1975) and Brazil (Agbenin and Tiessen, 1995). However, our results are higher than those reported by Udo and Ogunwale (1977) for Alfisols in the savanna zone of Nigeria and by Solomon and Lehmann (2000) for Luvisols under the savanna woodlands of northern Tanzania.

In many tropical soils recently cleared from forest vegetation, a substantial amount of soil P is present in organic form. In the studied sub-humid tropical ecosystems, organic P ($NaHCO_3$–$P_o$ and NaOH–$P_o$) comprised on the average 35% of the total soil P, NaOH–$P_o$ being the dominant fraction representing 87% of the total organic P pool. (Figs. 1 and 2). These values are in a good agreement with the results for forest-derived tropical soils under humid environ-

Table 2

Total P contents (mg $kg^{-1}$ soil) in bulk soils and size separates of soils of the southern Ethiopian highlands

| Total P | Wushwush | | | Munesa | | |
|---|---|---|---|---|---|---|
| | Natural forest | Tea plantation | Cultivation | Natural forest | *Cupressus* plantation | Cultivation |
| Bulk soil | 1343a[a] | 1057b | 927c | 1426a | 1119b | 874c |
| Sand | 249a | 176b | 148c | 155a | 102b | 80c |
| Silt | 970a | 866b | 786c | 802a | 674b | 527c |
| Clay | 1591a | 1429b | 1315b | 1498a | 1289b | 1120b |

[a] Letters along the row indicate significant differences between the means of the different land use systems of each site at $P < 0.05$ ($n = 3$).

ments (Udo and Ogunwale, 1977; Uzu et al., 1975; Sattell and Morris, 1992). However, they are higher than the values reported for soils under arid and semi-arid climates (Udo and Ogunwale, 1977; Agbenin and Tiessen, 1995; Solomon and Lehmann, 2000). The relatively higher proportion of organic P at our sites might be attributed to a much larger biomass production under the sub-humid tropical environment compared to the arid and semi-arid conditions,

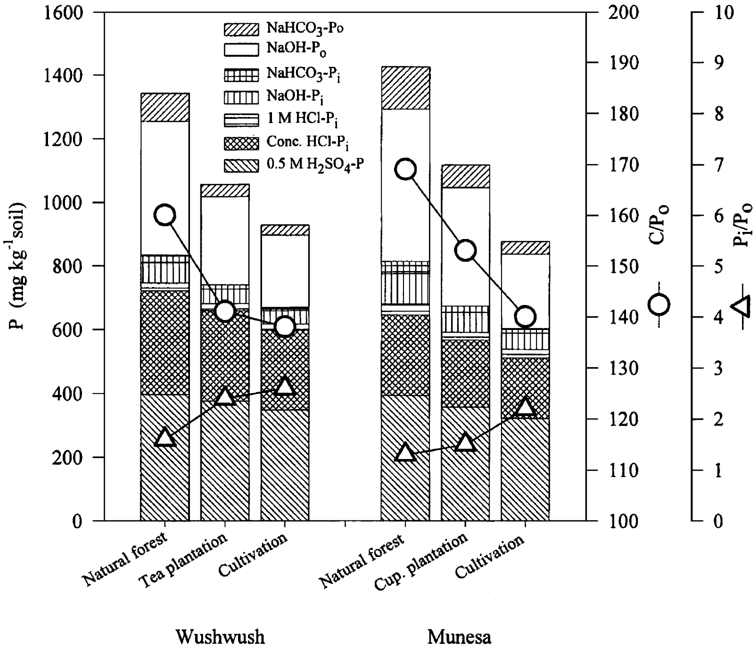

Fig. 1. Amounts of the different P fractions and $C/P_o$ and $P_i/P_o$ ratios in bulk soils of the different land use systems. Cup. plantation, *Cupressus* plantation.

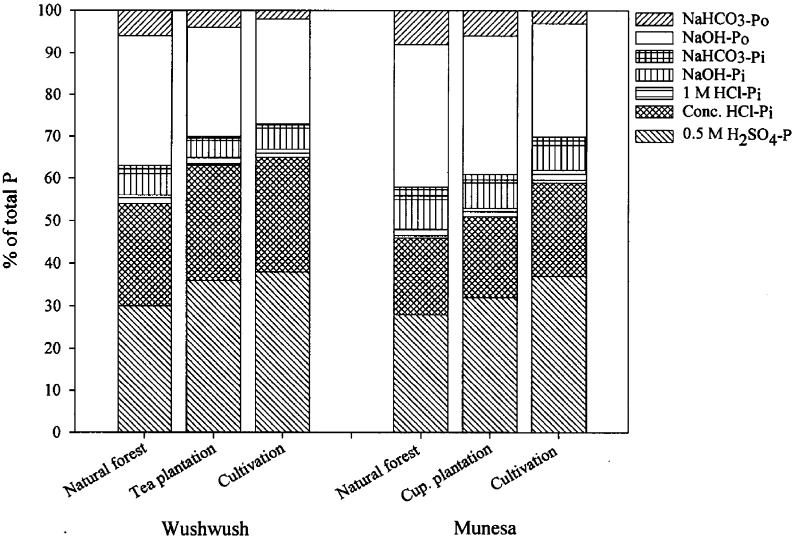

Fig. 2. Percentage of organic and inorganic P fractions in bulk soils of the different land use systems. Cup. plantation, *Cupressus* plantation.

where lower precipitation and higher temperature limits plant growth, and thereby, leads to lower levels of organic matter in the soils.

The ratio of organic C to organic P ($C/P_o$) was used to estimate the mineralization potential of organic P in the soils under investigation. The $C/P_o$ ratios of these soils ranged from 138 to 160 at the Wushwush and from 140 to 169 at the Munesa sites (Fig. 1). Since the $C/P_o$ ratios of these surface soils were below 200, mineralization of organic P could readily occur (Dalal, 1977), leading to an increase in the level of available inorganic P, provided the P thus released is not fixed in unavailable forms by Fe and Al oxides and hydroxides or clays.

The amount and distribution of the various inorganic P fractions at the Wushwush and Munesa sites are shown in Figs. 1 and 2. The labile ($NaHCO_3–P_i$) and moderately labile ($NaOH–P_i$) inorganic P fractions constitute, on the average 2% (10–39 mg $kg^{-1}$ soil) and 6% (42–95 mg $kg^{-1}$ soil) of the total P at both sites, respectively. The inorganic P associated with Ca (1 M $HCl–P_i$), made up 2% (19–34 mg $kg^{-1}$ soil) of the total soil P. The recalcitrant P fractions (both concentrated $HCl–P_i$ and 0.5 M $H_2SO_4–P$) comprised on the average 55% of the total P, 60% of it being from the $H_2SO_4–P$ fraction. These values show that in these clayey tropical soils, the refractory inorganic P fractions (1 M $HCl-P_i$, concentrated $HCl-P_i$ and 0.5 M $H_2SO_4-P$) were the major inorganic P pool, comprising 89% of the total inorganic P pool, while the plant-available or labile and moderately labile inorganic P fractions were generally small and made up only 11% of the total inorganic P pool. Our results concur positively with the results of Udo and Ogunwale (1977) for Alfisols and Oxisols of Nigeria, Neufeldt et al. (2000) for Oxisols of Brazil and Solomon and Lehmann (2000) for Luvisols of Tanzania.

### *3.2. Phosphorus forms and contents in particle size separates*

Sequential fractionation of P from particle size separates of the Wushwush and Munesa soils showed that the bulk of total P and organic P fractions ($NaHCO_3$–$P_o$ and NaOH–$P_o$) in these soils were concentrated in the clay, followed by the silt and sand size separates (Figs. 3 and 4). The C/$P_o$ ratios decreased in the order: sand (126–185) > silt (71–154) > clay (52–66). Furthermore, the $P_i/P_o$ ratio decreased with decreasing particle size, reflecting stabilization of organic P in finer size separates. The largest amount of organic P in these soils was thus associated with the clay size separates, with the largest amount of organic C, N and S and with the lowest C/N (Solomon et al., 2000, unpublished data) and C/$P_o$ ratios. These results suggest that at least for the soils under study, the dynamics of organic P is closely related to the dynamic of soil organic matter.

The amounts of both alkali- ($NaHCO_3$–$P_i$, NaOH–$P_i$) and acid-extractable (1 M HCl–$P_i$, concentrated HCl–$P_i$ and 0.5 M $H_2SO_4$–P) inorganic P fractions significantly increased ($P < 0.05$) in the order: sand < silt < clay, the highest being in the clay size separates (Figs. 3 and 4). The enrichment of P in the clay

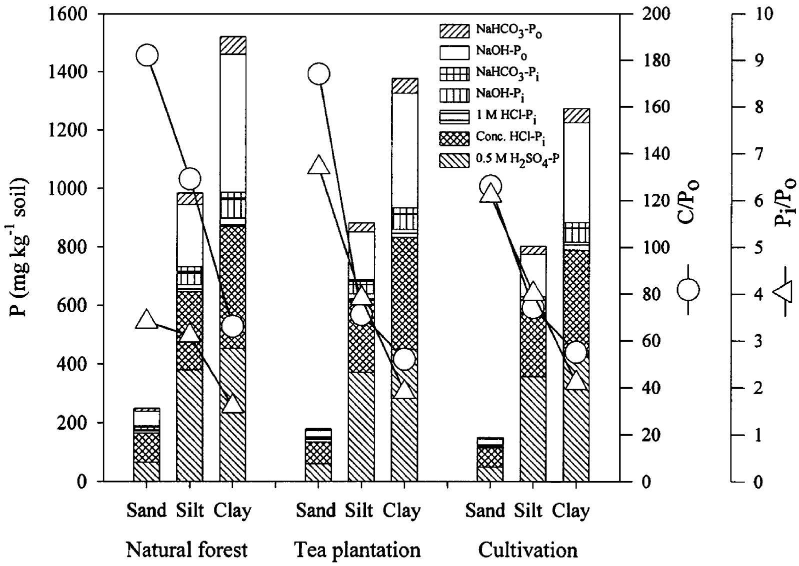

Fig. 3. Amounts of the different P fractions and C/$P_o$ and $P_i$ /$P_o$ ratios in particle size separates of the different land use systems at the Wushwush site.

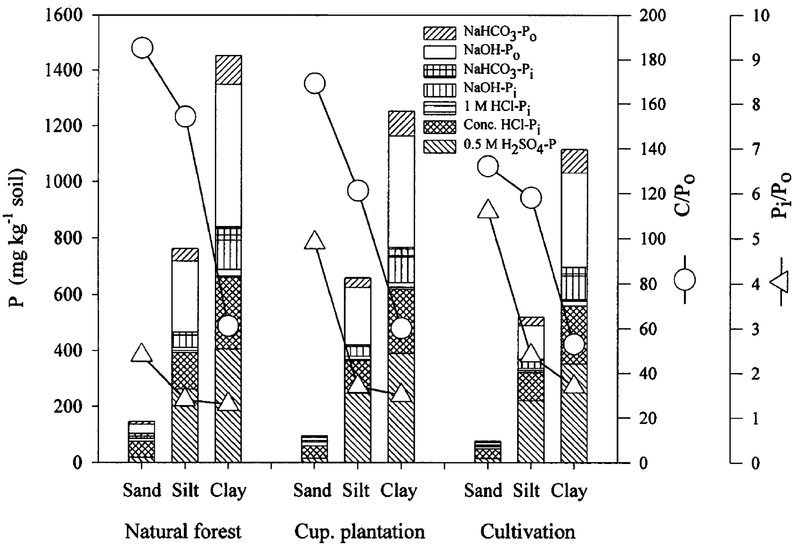

Fig. 4. Amounts of the different P fractions and $C/P_o$ and $P_i/P_o$ ratios in particle size separates of the different land use systems at the Munesa site. Cup. plantation, *Cupressus* plantation.

could be ascribed to the large amount of sesquioxides and to the high specific surface area of these size separates compared to the silt and sand size separates. Therefore, these results suggest that in the soils under investigation, the clay size separates can be considered as the major reservoir of both organic and inorganic P fractions.

### 3.3. Land use effects on the status and forms of P in bulk soils

In the sub-humid highlands of southern Ethiopia, clear-cutting of the indigenous forests and their conversion into agricultural fields or plantations significantly reduced ($P < 0.05$) the amount of total P. Total soil P declined by 31% at the Wushwush and 39% at the Munesa sites due to clearing and long-term cultivation. Compared to the continuously cropped fields, smaller losses of total soil P were found in the tea (21%) and *Cupressus* (22%) plantations (Fig. 1).

In a study conducted in Brazil, Tiessen et al. (1983) found out that the $NaHCO_3$–$P_o$ fraction remained unchanged, while the NaOH–$P_o$ fraction was depleted during cultivation and cautioned that the term labile, as associated with certain extractants, should not be universally applied to temperate and tropical soils. However, in the present study, a greater proportion of organic P was lost from the $NaHCO_3$–$P_o$ (65% at Wushwush and 70% at Munesa) than from the NaOH–$P_o$ fraction (46% at Wushwush and 51% at Munesa) following long-term cultivation confirming the potential of sequential extraction technique in predicting P stability in these sub-humid tropical soils. Compared to the cultivated fields, relatively lower losses of organic P were observed in the tea (56%, $NaHCO_3$–$P_o$ and 34%, NaOH–$P_o$) and *Cupressus* (46%, $NaHCO_3$–$P_o$ and 22%, NaOH–$P_o$) plantations. In general, our results indicate that in addition to the $NaHCO_3$–$P_o$ fraction, the NaOH–$P_o$ fraction appears to represent an important source of mineralizable organic P pool in these tropical highland ecosystems.

The greater loss of organic P observed in the continuously cropped fields compared to the plantations may be attributed to the increase in mineralization of organic P following forest clearing and continuous cropping and to the export of P along with crop or animal products. On the contrary, lower depletion of organic P in surface soils of the plantations may be ascribed to the active biocycling of P due to the presence of a perennial plant cover with deeper-reaching plant roots and to the better crop residue management through the use of pruned tea plant parts as a mulch and to surface litter accumulation after senescence observed in the *Cupressus* plantations. The $C/P_o$ and $P_i/P_o$ ratios in the bulk soils were also influenced by changes in management practices (Fig. 1). The average $C/P_o$ ratio of these soils decreased in the order: natural forests (160 and 169) > plantations (141 and 153) > cultivated fields (138 and 140), while the average $P_i/P_o$ ratio increased in the order: natural forest (1.4 and 1.3) < plantations (2.4 and 1.5) < cultivated fields (2.6 and 2.2) at the Wushwush and Munesa sites, respectively.

Stewart and Tiessen (1987) state that under temperate regimes, the decline in total soil organic matter level will be accompanied by a smaller decline in organic P compared to C. However, under tropical conditions where organic matter and associated materials are frequently stabilized in biomass and recent dead materials, the breakdown of this organic matter will cause C, N and P losses in equal proportions (Dalal, 1977; Stewart and Tiessen, 1987). The depletion of organic P (49% and 55%) as a result of cultivation was, however, smaller than the losses of organic C (55% and 63%) and N (52% and 60%) at the Wushwush and Munesa sites. The apparent stability of organic P relative to organic C and N observed in these soils could possibly be explained by the framework of the dichotomous system of nutrients suggested by McGill and Cole (1981). According to this hypothesis, nutrients that are stabilized by the direct association with C (e.g. organic N and C-bonded S) are mineralized as a result of C oxidation to provide energy. However, those elements existing primarily as ester (e.g. organic P and ester $SO_4$–S) may also be stabilized through the reactions of esters with soil components and their mobilization by extracellular enzymes is strongly related to the need and the supply of the end product (such as P) rather than the requirement for energy sources. This suggestion is also supported by our results of organic S fractions, where we have observed greater depletion of C-bonded S than ester S following land use changes (Solomon et al., 2001). The lower depletion of organic P compared to organic C and N may also reflect a more conservative cycling of organic P than organic C and N, which can be more easily lost by leaching and volatilization.

Tiessen et al. (1983) and Condron et al. (1990) reported that cultivation of native soils resulted in a large reduction of alkali-extractable P, while the acid-extractable P increased or remained unchanged in temperate agroecosystems. In the studied sub-humid tropical agroecosystems, however, low-input agricultural management led to significant reduction ($P < 0.05$) of both alkali- ($NaHCO_3$–$P_i$ and NaOH–$P_i$) and acid-extractable (1 M HCl–$P_i$, concentrated HCl–$P_i$ and 0.5 M $H_2SO_4$–P) inorganic P fractions (Fig. 1). After 25 years of cultivation at the Wushwush site, on the average, 61% of the $NaHCO_3$–$P_i$ and 35% of the NaOH–$P_i$ fractions were lost. While at the Munesa site, 30 years of continuous cropping led to the depletion of 65% and 47% of the $NaHCO_3$–$P_i$ and NaOH–$P_i$ fractions, respectively (Fig. 1). Lower losses of both $NaHCO_3$ (48% in tea and 52% in *Cupressus*) and NaOH (29% in tea and 34% in *Cupressus*) extractable inorganic P fractions were observed from the plantations than from the continuously cultivated fields. The average proportions of acid-extractable inorganic P fractions, lost as a result of land use changes from the two sites were, however, smaller than the losses from the alkali-extractable P. These results corroborate the suggestion by Neufeldt et al. (2000) that in non-fertilized tropical agroecosystems, stable inorganic P pools may also contribute to the plant-available P pool following the progressive depletion of labile P fractions.

### *3.4. Land use effects on the status and forms of P in size separates*

Agricultural management practices significantly ($P < 0.05$) reduced the amount of organic P in particle size separates of soils at the Wushwush and Munesa sites (Figs. 3 and 4). The largest depletion of $NaHCO_3$–$P_o$ (74% and 77%) and NaOH–$P_o$ (67% and 67%) fractions due to cultivation occurred from the soil organic matter associated with the sand size separates of the two sites, respectively. The organic matter associated with the silt size separates of the Wushwush and Munesa sites lost on the average 52% and 56% of the $NaHCO_3$–$P_o$, and 32% and 53% of the NaOH–$P_o$ fractions, while the reduction from the clay-bound soil organic matter amounted to 33% and 42% of the $NaHCO_3$–$P_o$ and 28% and 35% of the NaOH–$P_o$ fractions, respectively. Compared to the cultivated fields, however, relatively lower average losses of $NaHCO_3$–$P_o$ (67% and 69%) and NaOH–$P_o$ (57% and 64%) fractions were obtained from the soil organic matter associated with the sand, the silt (42% and 45%, $NaHCO_3$–$P_o$ and 21% and 19% NaOH–$P_o$) and the clay (21% and 16%, $NaHCO_3$–$P_o$ and 17% and 22% NaOH–$P_o$) size separates of the tea and *Cupressus* plantations, respectively. These results support the suggestion by Tiessen et al. (1983) and Neufeldt et al. (2000) that in addition to the $NaHCO_3$-extractable organic P, a substantial amount of NaOH-extractable organic P from the soil organic matter associated with the silt and clay size separates is quite labile and may undergo significant changes during one or more growing seasons.

The C/$P_o$ ratios of size separates of the natural forests were higher than the C/$P_o$ ratios of corresponding cultivated fields and plantations (Figs. 3 and 4). This is in accordance with the general pattern of C/N and C/S ratios of size separates of these soils (Solomon et al., 2000 unpublished data). The highest $P_i/P_o$ ratios were found in the size separates of the cultivated soils, whereas the lowest $P_i/P_o$ ratios were obtained in size separates of the corresponding forest's soils of both sites.

Low-input agricultural management greatly influenced the amount of both alkali- and acid-extractable inorganic P fractions in size separates of the soils under investigation. The highest proportion of inorganic P was lost from the sand, followed by the silt and clay size separates (Figs. 3 and 4). Compared to the acid-extractable inorganic P, however, a notable proportion of inorganic P was lost from the alkali-extractable inorganic P fractions of the different size separates due to land use changes. At the Wushwush and Munesa sites, on the average, 81% and 85% of the $NaHCO_3$–$P_i$ and 49% and 56% of the NaOH–$P_i$ fractions were lost from the sand size separates following long-term continuous cultivation, respectively (Figs. 3 and 4). The silt size separates lost on the average 42% and 50% of the $NaHCO_3$–$P_i$ and 33% and 35% of the NaOH–$P_i$ fractions, while the losses from the clay separates amounted to 26% and 31% ($NaHCO_3$–$P_i$) and 22% and 19% (NaOH–$P_i$) at the two sites, respectively. The loss of alkali-extractable inorganic P from the sand (55% and 69%, $NaHCO_3$–$P_i$, 38% and 43% NaOH–$P_i$), silt (20% and 27%, $NaHCO_3$–$P_i$, 21% and 24% NaOH–$P_i$) and clay (16% and 28%, $NaHCO_3$–$P_i$, 15% and 13%, NaOH–$P_i$) size separates of the tea and *Cupressus* plantations, respectively, were lower compared to the losses from similar size separates of the corresponding cultivated fields.

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

### 3.5.1. Distribution of P structures in alkali extracts of bulk soils

The various forms of P compounds in the NaOH–NaF soil extracts were characterized by liquid-state $^{31}$P NMR spectroscopy according to differences in the chemical environments around the P nuclei, causing differences in the resonance frequencies. The resonance around $\delta = 19.0$ ppm was assigned to phosphonates. Intense signals between $\delta = 4.9$–$5.3$ ppm represent orthophosphate monoesters, a diverse P form comprising inositol phosphate, sugar phosphate and mononucleotides (Newman and Tate, 1980). The signals around $\delta = 1.5$ ppm may originate from teichoic acids, an orthophosphate diester which consists of glycerol or sugar molecules linked by phosphate groups (Condron et al., 1990). Resonance between $\delta = 0.3$–$1.0$ ppm and $\delta = -0.5$–$2.2$ ppm have not yet been identified (Sumann et al., 1998; Makarov, 2000, personal communication), thus they were assigned as unknown A and B. Orthophosphate diesters (phospholipids and DNA), resonate at $\delta = 0.0$–$0.2$ ppm. Signals accounting for inorganic orthophosphate P appeared around $\delta = 6.1$ ppm, while the peaks around $\delta = -4.7$ ppm were assigned as pyrophosphates. The $^{31}$P NMR spectra from these tropical soils did not show the presence of polyphosphates which usually appear around $\delta = -20.0$ ppm. The NaOH–NaF extracted 22–36% of the total P in bulk soils and particle size separates of these soils. This is in good agreement with the proportion of alkali-extractable P by sequential extraction from these tropical soils and with the results of Zech et al. (1985) for Vertisols of Mexico, Gil-Sotres et al. (1990) for Cambisols of Spain and Solomon and Lehmann (2000) for Luvisols of Tanzania.

The stacked $^{31}$P NMR spectra (Figs. 5 and 6) and the relative proportions of the different P forms in bulk soils (Table 3) indicate that orthophosphate monoesters were the major group of organic P compounds in these tropical soils. They accounted for 27–66% of total alkali-extractable P. Orthophosphate diesters represented 9–27%, while teichoic acids comprised 7–11% of the alkali-soluble P.

The ratios of orthophosphate diester-P to orthophosphate monoester-P (excluding teichoic acids) were calculated for bulk soils and size separates (Table 3). In the bulk soils, the ratios of diester-P to monoester-P ranged from 0.16–0.58 at the Wushwush and 0.13–1.00 at the Munesa sites. These ratios correspond to those reported by Guggenberger et al. (1996) for Oxisols of Colombia. However, the diester-P to monoester-P ratios at our sites were higher than the values reported for native grassland and cultivated soils of New Zealand by Hawkes et al. (1984) and for native soils of the Great Plains of North

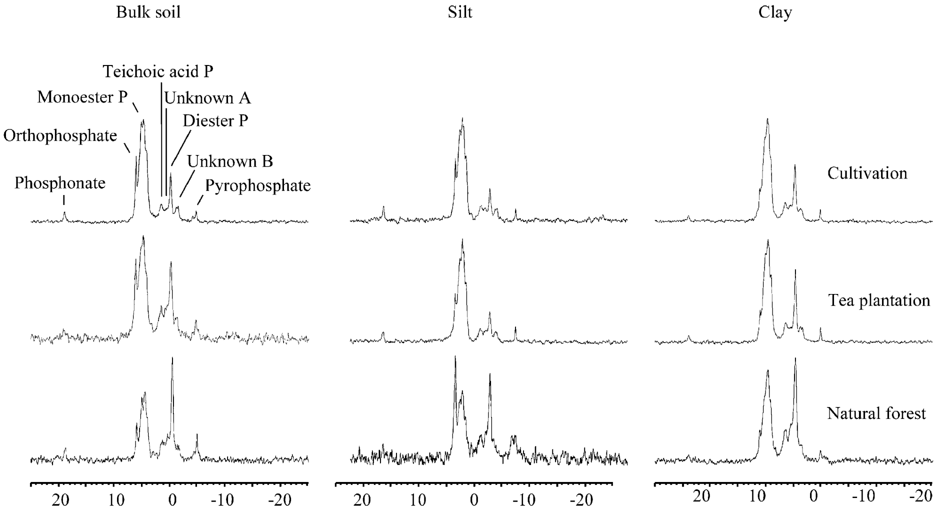

Fig. 5. $^{31}$P NMR spectra of alkali extracts of bulk soils and size separates of the different land use systems at the Wushwush site.

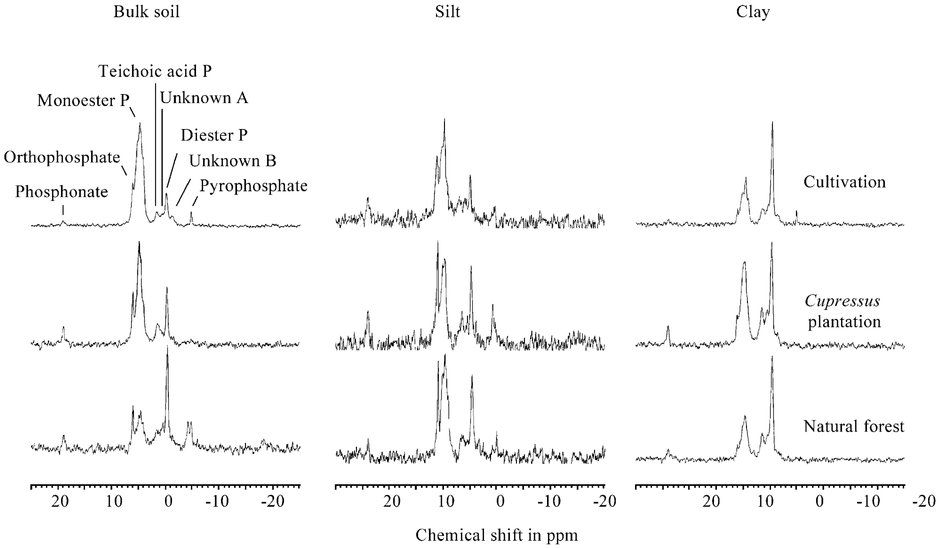

Fig. 6. $^{31}$P NMR spectra of alkali extracts of bulk soils and size separates of the different land use systems at the Munesa site.

America by Sumann et al. (1998), indicating the prominence of orthophosphate diesters in these forest-derived tropical soils.

The resonance of unknown P compounds were previously reported by Hawkes et al. (1984), Adams and Byrne (1989), Guggenberger et al. (1996), Miltner et al. (1998), Sumann et al. (1998) and Möller et al. (2000). Guggenberger et al. (1996) and Miltner et al. (1998) suggested that the peaks between $\delta = -0.5$–$2.0$ and $\delta = 0.1$–$1.0$ ppm belong to diester-P structures which have not yet been identified. Attempts to identify these compounds by the artificial addition of ribonucleic acid (RNA) into the NaOD solution resulted in a chemical shift of $\delta = 0.3$–$0.5$ (Sumann et al., 1998; Makarov, 2000, personal communication). However, it is uncertain whether RNA persists long enough in NaOH–NaF solution, since Tate and Newman (1982) suggested that it might be hydrolyzed under alkaline conditions. The unknown organic P compounds, i.e. unknown A and B, accounted for 3–8% and 3–5% of the alkali-extractable P, respectively.

According to Table 3, phosphonates made up 2–5% of the alkali-extractable P in the soils under investigation. The occurrence of phosphonates in the soil was first reported by Newman and Tate (1980). These P forms were reported to be the product of protozoan metabolism (Bedrock et al., 1994). The presence of phosphonates in the soil was often related to cool, moist and acidic conditions, where bacteria containing phosphonatase enzyme capable of breaking the C—P bonds in phosphonates are less numerous (Tate and Newman, 1982; Hawkes et al., 1984; Zech et al., 1985). However, the presence of phosphonates was also reported in well-drained savanna soils under isohyperthermic temperature

Table 3
Distribution of P compounds (as the proportion of total peak area of $^{31}$P NMR spectra) and diester to monoester ratios in alkali extracts of bulk soils from the southern Ethiopian highlands

| Land use | Phosphonates (%) | Orthophosphate (%) | Monoester (%) | Teichoic acid (%) | Unknown A (%) | Diester (%) | Unknown B (%) | Pyrophosphate (%) | Diester/Monoester |
|---|---|---|---|---|---|---|---|---|---|
| *Wushwush* | | | | | | | | | |
| Natural forest | 2.3 | 6.8 | 40.9 | 9.4 | 6.2 | 23.6 | 4.7 | 6.1 | 0.58 |
| Tea plantation | 1.8 | 11.1 | 46.9 | 9.3 | 7.0 | 15.0 | 5.3 | 3.6 | 0.32 |
| Cultivation | 2.0 | 8.8 | 61.2 | 7.1 | 3.6 | 10.0 | 5.0 | 2.3 | 0.16 |
| Mean[a] | 2.0 | 8.9 | 49.7 | 8.6 | 5.6 | 16.2 | 5.0 | 4.0 | 0.33 |
| *Munesa* | | | | | | | | | |
| Natural forest | 3.0 | 6.8 | 26.5 | 11.4 | 7.6 | 26.5 | 5.1 | 13.1 | 1.00 |
| *Cupressus* plantation | 5.0 | 9.9 | 54.1 | 10.2 | 3.2 | 13.1 | 2.7 | 1.8 | 0.24 |
| Cultivation | 1.5 | 7.9 | 66.1 | 6.8 | 3.2 | 8.7 | 3.6 | 2.2 | 0.13 |
| Mean | 3.2 | 8.2 | 48.9 | 9.4 | 4.7 | 16.1 | 3.8 | 5.7 | 0.33 |

[a] Mean values of P species in bulk soils.

regimes (Guggenberger et al., 1996) and in microbially active soils with slightly acid to slightly alkaline pH under mesic and thermic temperature regimes (Sumann et al., 1998). Because phosphonates originate only from microorganisms, their presence under the above mentioned conditions could be attributed to lysis of cells as a result of drying (Sumann et al., 1998).

The resonance of inorganic P forms was not prominent in the $^{31}$P NMR spectra of the alkaline extracts (Figs. 5 and 6). The proportion of inorganic orthophosphate represented only 7–11% of the total alkali-extractable P, whereas pyrophosphate (with the exception of the soil of natural forest at Munesa) accounted for 2–6% of the total alkali-extractable P at the two sites. The presence of up to 23% residual inorganic P after a thorough dialysis were also reported by Guggenberger et al. (1996), Miltner et al. (1998) and Möller et al. (2000). The presence of these residual inorganic P compounds in the NaOH–NaF extracts of these soils may be attributed to orthophosphate ($PO_4^{3-}$) and pyrophosphate ($P_2O_7^{4-}$) bound to organic matter via Fe and Al bridges, which could be large enough not to be removed by dialysis (Guggenberger et al., 1996). Bedrock et al. (1995) reported that inorganic orthophosphate, which is found in complex association with paramagnetic species, such as Fe may not be fully detected by $^{31}$P NMR. Because of the possibilities of the loss of signal, due to the presence of the paramagnetic centers and variable amounts of alkali-extractable free inorganic P which could be lost through dialysis from the bulk soil and particle size separate samples, the remaining strongly adsorbed inorganic P was not considered to evaluate the influence of land use changes in the present study.

### 3.5.2. Distribution of P structures in alkali extracts of size separates

$^{31}$P NMR spectra of alkali extracts of the size separates showed that orthophosphate monoesters were the dominant P forms both in the silt (35–67%) and clay (34–63%) size separates (Figs. 5 and 6 and Tables 4 and 5). Orthophosphate diesters represented 7–19% in the silt and 14–34% in the clay separates, while the proportion of teichoic acids in the extracts varied between 6–11% in the silt and 8–14% in the clay size separates of soils at the Wushwush and Munesa sites. These results show that in both bulk soils and size separates, NaOH–NaF extractable organic P species were mainly ester phosphates (C—O—P) bound to soil organic matter, and thus were the integral parts of organic molecules (Stewart and Tiessen, 1987).

Comparisons of the $^{31}$P NMR spectra in the size separates indicate that orthophosphate diesters were enriched in the clay compared to the silt size separates, while the largest proportion of orthophosphate monoesters were found in the silt size separates. This could also be shown by the higher average diester-P to monoester-P ratios (without teichoic acids) in the clay (0.32 and 0.79) than in the silt size separates (0.20 and 0.25) of soils at Wushwush and Munesa, respectively. Gressel et al. (1996) indicated that much of the alkali-extractable orthophosphate monoester-P originates from hydrolyzed plant-derived

Table 4
Distribution of P species (as the proportion of total peak area of $^{31}P$ NMR spectra) and diester to monoester ratios in alkali extracts of size separates of the Wushwush site

| Separate | land use | Phosphonates (%) | Orthophosphate (%) | Monoester (%) | Teichoic acid (%) | Unknown A (%) | Diester (%) | Unknown B (%) | Pyrophosphate (%) | Diester/ Monoester |
|---|---|---|---|---|---|---|---|---|---|---|
| Silt | natural forest | 2.7 | 17.2 | 34.6 | 9.2 | 5.1 | 19.1 | 2.8 | 9.3 | 0.55 |
| | tea plantation | 2.1 | 7.9 | 67.0 | 6.6 | 2.9 | 7.7 | 3.5 | 2.3 | 0.12 |
| | cultivation | 2.9 | 9.9 | 65.6 | 6.0 | 3.3 | 6.9 | 4.1 | 1.3 | 0.11 |
| | mean[a] | 2.6 | 11.6 | 55.7 | 7.3 | 3.8 | 11.2 | 3.5 | 4.3 | 0.20 |
| Clay | natural forest | 1.3 | 3.7 | 45.7 | 10.8 | 7.5 | 25.1 | 3.9 | 2.0 | 0.53 |
| | tea plantation | 1.5 | 4.0 | 60.7 | 7.7 | 3.7 | 15.1 | 5.3 | 2.0 | 0.25 |
| | cultivation | 1.1 | 4.0 | 63.1 | 8.4 | 3.9 | 13.6 | 4.3 | 1.6 | 0.22 |
| | mean[a] | 1.3 | 3.9 | 56.5 | 9.0 | 5.0 | 17.9 | 4.5 | 1.9 | 0.32 |

[a] Mean values of P species in silt and clay size separate.

Table 5

Distribution of P species (as the proportion of total peak area of $^{31}P$ NMR spectra) and diester to monoester ratios in alkali extracts of size separates of the Munesa site

| Separate land use | | Phosphonates (%) | Orthophosphate (%) | Monoester (%) | Teichoic acid (%) | Unknown A (%) | Diester (%) | Unknown B (%) | Pyrophosphate (%) | Diester/Monoester |
|---|---|---|---|---|---|---|---|---|---|---|
| Silt | natural forest | 6.3 | 14.9 | 37.2 | 11.2 | 3.8 | 14.1 | 2.9 | 9.6 | 0.38 |
| | *Cupressus* plantation | 6.7 | 15.3 | 49.1 | 11.3 | 2.5 | 12.1 | 1.0 | 2.0 | 0.25 |
| | cultivation | 2.7 | 14.1 | 53.1 | 7.9 | 4.3 | 8.3 | 4.8 | 4.8 | 0.16 |
| | mean[a] | 5.2 | 14.8 | 46.5 | 10.1 | 3.5 | 11.5 | 2.9 | 5.5 | 0.25 |
| Clay | natural forest | 3.6 | 2.3 | 34.4 | 13.9 | 6.7 | 34.4 | 4.6 | n.d.[b] | 1.00 |
| | *Cupressus* plantation | 3.3 | 3.2 | 37.4 | 10.7 | 7.3 | 33.0 | 5.1 | n.d. | 0.88 |
| | cultivation | 2.2 | 3.6 | 46.8 | 11.3 | 5.0 | 24.8 | 3.2 | 3.0 | 0.53 |
| | mean | 3.0 | 3.0 | 39.5 | 12.0 | 6.3 | 30.8 | 4.3 | 1.0 | 0.80 |

[a] Mean values of P species in silt and clay size separate.

[b] n.d., not detected.

phospholipids (including glycerophosphate, inositol phosphate, choline phosphate and others) that are associated with cuticle and middle lamella tissue and cellular membranes. In contrast, the major source of orthophosphate diesters in soils are microbially derived nucleic acids and phospholipids (Tate and Newman, 1982; Robinson et al., 1998). Moreover, Bedrock et al. (1994) and Sumann et al. (1998) reported that the modest amount of orthophosphate diester-P produced by plants is very labile, whereas the diester-P produced by microbes is more likely to be stabilized on mineral surfaces (Miltner et al., 1998), possibly due to the closer proximity of microbes to those surfaces. Therefore, the larger proportion of orthophosphate diesters in clay than in the silt size separates may suggest the enrichment of microbially derived organic P structures in clay, while the higher proportion of orthophosphate monoesters observed in the silt than in the clay size separates of these soils may indicate the enrichment of plant-derived organic P compounds in the coarser size separates.

The relatively higher proportion of teichoic acids, which originate exclusively from the cell wall of Gram-positive bacteria in the clay than in the silt size separates (Sumann et al., 1998) also indicate the enrichment of bacterially derived organic P in the clay size separates, further strengthening the suggestion mentioned above. The proportion of unknown A was 3–5% in the silt and 4–8% in the clay, whereas unknown B made up 1–5% of the silt and 3–5% in the clay size separates. There were no relative enrichments of phosphonates in the extracts obtained from the clay separates, although phosphonates are thought to be of microbial origin (Bedrock et al., 1994). They accounted for 2–7% and 1–4% of the alkali-extractable P in the silt and clay separates of these soils, respectively. Inorganic orthophosphate comprised 8–17% of the silt and 2–4% of the clay size separates, while pyrophosphate represented 2–10% and 0–2% of the alkali-soluble P in the silt and clay size fractions, respectively.

### *3.5.3. Land use effects on P structures in alkali extracts of bulk soils*

The relative signal intensity of $^{31}$P NMR spectra of NaOH–NaF extracts (Figs. 5 and 6) clearly demonstrated the influence of land use on the different P species present in these tropical soils. According to Table 3, the relative proportions of diester-P decreased in the order: natural forests (24% and 27%) > plantations (15% and 13%) > cultivated fields (10% and 9%) at the Wushwush and Munesa sites, respectively. Moreover, lower percentages of teichoic acid, unknown A, unknown B and phosphonates were also found in the bulk soils of cultivated fields than in the natural forests. In contrast, the proportion of orthophosphate monoesters increased in the order: natural forest (27% and 41%) < plantations (47% and 54%) < cultivated fields (61% and 66%) at the Wushwush and Munesa sites, respectively. Orthophosphate diesters ($(RO)\ (R'O).PO_2^-$) have a lower charge density than the orthophosphate monoesters and their OH groups bound to P are considerably shielded by the two organic moieties from ionic interactions (Miltner et al., 1998). This makes them accessible to microbial or enzymatic attack in the soil environment (Tate, 1984), thus explaining the smaller proportions present in the clear-cut and long-term cultivated fields or plantations compared to the orthophosphate monoesters. In contrast, orthophosphate monoesters ($RO.PO_3^{2-}$) represent a more resistant group of organic P compounds to microbial and enzymatic attack than the orthophosphate diesters. The higher recalcitrance of orthophosphate monoesters could more likely be attributed to the strong binding through the reactive —$OPO_3^{2-}$ to positively charged sites on organic matter, clays, sesquioxides or to free cations present in the soil solution (Stewart and Tiessen, 1987).

The various P species identified by $^{31}P$ NMR spectroscopy can be used to distinguish P compounds with different stabilities against microbial decomposition. Hinedi et al. (1988) indicated that under suitable soil environments, orthophosphate diesters (including teichoic acids) could be converted to orthophosphate monoester, while monoester-P remains stable. In the soils under investigation, the ratios of diester-P to monoester-P decreased in the order: natural forest (0.58 and 1.00) > plantations (0.32 and 0.24) > cultivated fields (0.16 and 0.13) at the Wushwush and Munesa sites, respectively. These results also indicate the preferential depletion of the more labile orthophosphate diester-P compared to orthophosphate monoester-P as a result of land use changes. In general, our results support the suggestion by several authors (Tate and Newman, 1982; Guggenberger et al., 1996; Solomon and Lehmann, 2000) that organic P compounds such as orthophosphate diesters (including teichoic acids) and phosphonates may apparently represent more labile organic P pools which could readily replenish available P through mineralization.

Pyrophosphate occurs in the soil in inorganic P form or alternatively as an ester which is hydrolyzed during extraction (Condron et al., 1985). In the soils under investigation, relatively higher proportions of pyrophosphate were found in the natural forests than in the plantations or in the cultivated fields of both sites. Condron et al. (1985) stated that this P species is believed to be biologically active. During enzymatic hydrolysis, pyrophosphate can be hydrolyzed to inorganic orthophosphates in soils (Dai et al., 1996), which may be responsible for the observed lower proportion of pyrophosphate in the plantations or in the cultivated fields compared to the natural forest sites.

### *3.5.4. Land use effects on P structures in alkali extracts of size separates*

$^{31}P$ NMR spectra also revealed substantial change in the proportions of the various P species extracted from the size separates following land use changes. The proportions of orthophosphate diesters in silt size separates of the continuously cultivated fields decreased by 64% and 41%, whereas the proportions of teichoic acids in these separates dropped by 35% and 30% at the Wushwush and Munesa sites, respectively (Tables 4 and 5). The diester-P to monoester-P ratios (without teichoic acids) in the silt size separates decreased in the order: natural forest (0.55 and 0.38) > plantations (0.12 and 0.25) > cultivated fields (0.11 and

0.16) at the Wushwush and Munesa sites, respectively. Compared to the silt size separates, however, lower decline in the proportion of the orthophosphate diesters (46%, at Wushwush and 28%, at Munesa) and teichoic acids (22%, at Wushwush and 19%, at Munesa) were observed in the clay size separates following forest clearing and cultivation. The ratios of diester-P to monoester-P in the clay size separates also increased in the order: cultivated fields (0.22 and 0.53) < plantations (0.25 and 0.88) < natural forests (0.53 and 1.00), the highest being in the clay size separates of the forest-derived soils of the two sites, respectively. The greater decline in the relative proportions of diester-P and teichoic acids found from the silt than the clay size separates may be attributed to stabilization of microbially derived labile organic P structures by closer association with clay minerals and/or by oxides and hydroxides (Guggenberger et al., 1996; Solomon and Lehmann, 2000). Generally, a significantly lower proportion of pyrophosphate was also found in the silt and clay size separates of soils of the plantations and cultivated fields compared to the same size separates of the soils from the corresponding natural forests.

## 4. Conclusions

Agricultural management led to marked depletions of both alkali- ($NaHCO_3$–P and NaOH–P) and acid-extractable (1 M HCl–P, concentrated HCl–P and 0.5 M $H_2SO_4$–P) P fractions, indicating that in these low-input tropical highland agroecosystems, stable P pools act not only as a sink but also contribute to the plant-available P following the progressive depletion of the labile P fractions.

The greater proportion of organic P was lost from the $NaHCO_3$–$P_o$ than in the NaOH–$P_o$ fraction in the bulk soils and size separates, due to land use changes confirming the potential of sequential extraction technique in predicting apparent P stability in the soils under investigation.

Lower losses of P were found from the plantations than in the cultivated fields, which may be ascribed to the active biocycling of P due to the presence of a perennial plant cover with deeper-reaching plant roots and to the better crop residue management through the use of pruned tea plant parts as a mulch, and to surface litter accumulation after senescence observed in the *Cupressus* plantations.

The lower depletion of organic P compared to organic C and N, observed in these soils, may reflect a more conservative cycling of organic P in these sub-humid tropical highland ecosystems than organic C and N, which can more easily be lost from the ecosystem by leaching and volatilization.

Land use changes led to the decline in the proportions of orthophosphate monoesters, teichoic acid, unknown A, unknown B and phosphonates, suggesting that organic P compounds such as orthophosphate diesters, teichoic acids and phosphonates may apparently represent more labile organic P species, which could readily replenish available P. In contrast, the proportion of the more stable organic P species (orthophosphate monoesters) increased following land use changes compared to the natural forests. Thus it is possible to suggest that in the soils under study, long-term agricultural management not only influenced the structural composition but also the relative bioavailability of organic P compounds.

A greater decline in the proportions of diester-P and teichoic acids was found from the silt than the clay size separates, which may be attributed to the stabilization of microbially derived organic P structures by closer association with the clay minerals and/or by oxides and hydroxides.

In general, the results of sequential extraction and $^{31}$P NMR spectroscopy indicate that clear-cutting of the indigenous natural forests followed by continuous cropping or establishment of plantations with little or no P input not only decreased the amount but also significantly influenced the structural composition and bioavailability of P in bulk soils and size separates of these tropical soils. Thus, measures have to be designed for replenishment and subsequent maintenance of soil P stocks, to ensure sustainable crop production in the sub-humid highland agroecosystems of southern Ethiopia.

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