Improving phosphorus availability in two upland soils of Vietnam using *Tithonia diversifolia* H.

Phan Thi Cong[1,3] & Roel Merckx[2]

[1]*Institute for Agricultural Sciences of South Vietnam, 121 Nguyen Binh Khiem Street, District 1, Hochiminh City, Vietnam.* [2]*Laboratory of Soil and Water Management, Department of Land Management, Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, 3001 Heverlee, Belgium.* [3]*Corresponding author**

Abstract

Phosphorus was added to two acidic upland soils (a Cambisol and a Ferralsol) at two rates (9 mg P kg^{-1} and 145 mg P kg^{-1}) either in an inorganic P form (KH$_2$PO$_4$) or as a green manure (*Tithonia diversifolia* H. at 2.5 g kg^{-1} and 40 g kg^{-1}). The effect of P source on the chemical availability of P was assessed in an incubation experiment by measuring resin extractable P, soluble molybdate reactive (DMR-P) and unreactive P (DMU-P). Soil pH and extractable Al were monitored during the incubation period of 49 days. Green manure addition caused an immediate and sustained increase in soil pH and an immediate and sustained decrease in extractable Al. Labile P (resin P + DMR-P + DMU-P) was increased more by P added as a green manure than when added in inorganic form in one soil (Ferralsol), while it decreased or did not differ in the other one (Cambisol). In both soils, the concentrations of soluble DMU-P were frequently higher where *Tithonia* had been added. The effects of green manure amendment on physical factors governing the phosphorus supply through diffusive transport were also investigated. Aggregate size distribution was substantially changed by green manure amendment due to a shift in the percentage of microaggregates (<250 μm in diameter) to larger sizes. Changes in soil aggregation as a consequence of green manure amendment led to a reduction in specific surface area (SSA) of the whole soil. Coupled with the large increase in effective cation exchange capacity caused by green manure amendment in both soils, and the decrease in SSA, there was an increase in the net negative surface charge density in both soils. In summary, at a large addition rate – and in addition to the well-known effect derived from the extra supply in P, green manure amendment may improve the chemical availability and diffusive supply of P through the following mechanisms: (i) an increase in soil pH increasing the solubility of phosphate sources; (ii) a decrease in extractable Al reducing the fixation of added P; (iii) increased macro-aggregation and reduced specific surface area and porosity leading to fewer sorption sites for P and hence enhanced diffusion rates; and (iv) increased negative charges and reduced positive charges at the soil surface resulting in a net increase in repulsive force for P. The induced changes in most measured soil properties were smaller in the Ferralsol than in the Cambisol.

Introduction

Historically, green manures have been used for their fertiliser value (Joachim and Kandiah, 1929; Joffe, 1955; Nagarajah and Amarasiri, 1997) and the addition of green manure to soil is recognised as playing an important role in maintaining soil organic matter content while securing adequate soil nutrient concentrations (Cakmak, 2002; Singh et al., 1992). Apart from these roles, green manures may help in soil structure improvement, soil moisture retention through mulching, and nutrient recycling (Oades,

---

*FAX No: +84 88 29 76 50.
E-mail: congphanthi@hcm.vnn.vn

1984; Sanchez, 1976; Tisdall and Oades, 1982; White, 1997).

Among different plant species used as green manures, *Tithonia diversifolia* has been suggested as particularly promising. The plant is widespread in many regions in the uplands of South Vietnam. Besides having typically high concentrations of N, P, K and S, *Tithonia* biomass also contains high concentrations of Ca and Mg (Buresh et al., 1997; Palm et al., 1999).

The processes leading to an increased biological availability of P can be grouped into two categories. Firstly, there are the direct mechanisms that increase the 'chemical' availability of P [by increasing the amounts of P (organic or inorganic) in the soil solution or weakly sorbed to soil surfaces] and secondly, those mechanisms that increase the supply of P to the root by facilitating diffusive transport. The latter mechanisms relate primarily to soil physical properties such as aggregation or porosity and physico-chemical ones like sorption.

There are several reports of green manure amendments, especially with *Tithonia diversifolia*, improving the 'chemical' availability of soil P (Blair and Boland, 1978; Easterwood and Sartain, 1990; Fox et al., 1990; Hue, 1991; Nziguheba et al., 1998). Several mechanisms have been postulated for this improvement: release of P from the decomposition of the amended green manures (Singh et al., 1992; Palm et al., 1999); reduced P sorption due to 'blocking' of P sorption sites by organic compounds (Hue et al., 1986, 1991); complexation of Al by organic compounds leading to P release from Al-P complexes (Appelt et al., 1975); and increased soil pH leading to increased P concentrations in soil solution. However, Guppy (2003) was unable to measure any consistent effects of added plant materials on soil P sorption properties. He concluded that any increase in chemical P availability due to amendment with plant materials was due to mineralisation of P from the added plant materials.

Green manure addition may also affect P availability by improving diffusive supply of P. Diffusion in soil is governed by the 'effective diffusion coefficient', conventionally defined (Nye, 1968) as:

$$D_e = D_l \theta f (dC_l/dC_s), \qquad (1)$$

where D_e is the effective diffusion coefficient [m²/s], D_l is the diffusion coefficient in water [m²/s], θ is the volumetric water content, f is the tortuosity factor, and dC_l/dC_s is the inverse of the differential buffer capacity of the soil with C_l the concentration of P in soil solution and C_s the concentration of P on the solid phase.

The tortuosity factor in Equation 1 is a measure of the ease of movement of P through the soil solution as affected by the continuity of liquid films. It is therefore a function of soil porosity and the distribution of pore diameters, and a change in these soil properties will result in a change in the diffusive supply of P. The C_l/C_s term, or the inverse P buffer capacity of the soil in Equation 1, is determined by the number of P sorption sites in the soil and the strength of bonding between the sorbed P and soil surfaces. The former is a function of the specific surface area (SSA) of a soil, and SSA has been related to the extent of phosphorus sorption in different soils (Parfitt, 1977; Linquist et al., 1997). For example, Linquist et al. (1997) reported that phosphorus sorption increased 4 to 5 times (from 55 to 245 mg P kg⁻¹) when mean aggregate diameter decreased from 3400 to 375 μm. They found that phosphorus release was linearly correlated to the reactive mass of the aggregates.

An important physico-chemical property determining the strength of bonding of sorbed P (the other determinant of P buffer capacity) is the surface charge density (charge per unit specific surface area). Increasing net negative charge per unit area will result in increasing repulsion of the negatively charged orthophosphate ion, resulting in a larger proportion of P remaining in solution. Amending soils with green manures has been shown to increase the mean diameter of soil aggregates (Hamblin and Greenland, 1977; Tisdall and Oades, 1982) and to increase cation exchange capacity (Helling et al., 1964). From Equation 1, both effects would be expected to enhance diffusion of P.

In this paper we report on the effects of amendment with *Tithonia diversifolia* on soil properties that affect either the chemical availability of P (e.g. pH and extractable Al) or its diffusive supply (e.g. aggregate size distribution, porosity and surface charge density).

Materials and methods

Plant characteristics

Tithonia diversifolia was collected from hedges along paths and around fields in Da Loan village, Duc Trong district, Lam Dong province, Vietnam. Green leaves and twigs were cut, sun-dried and then oven-dried at 60 °C. The material was ground into fine powder (< 0.5 mm). Ground dry material was analysed for

Table 1. Nutrient concentrations in *Tithonia diversifolia* plant material used in the aerobic incubation. Dissolved molyddate-reactive P (DMR-P) is the P fraction extracted by 1 mM $CaCl_2$ which reacts with molybdate following the Murphy and Riley (1962) method. Dissolved molybdate-unreactive P (DMU-P) is the P fraction calculated from the difference between total P determined by potassium persulphate digestion (Rowland and Haygarth, 1997) and the DMR-P

Element	Concentration (mg kg^{-1})
N	37.5
P total	3.62
P extractable by 1 mM $CaCl_2$	2.04
DMR-P	1.92
DMU-P	0.12
K	24.7
S	3.3
Ca	15.7
Mg	3.6

Table 2. Selected characteristics of the Da Loan (Ferralic Cambisol) and Duc Lieu (Rhodic Ferralsol) soils

Parameter	Da Loan	Duc Lieu
pH_{CaCl2} (1:2.5)	4.41	4.39
pH_{KCl} (1:2.5)	3.89	4.04
Org C (mg kg^{-1})	16.4	26.5
ECEC (cmol$_c$ kg^{-1})	2.96	1.67
Olsen P (mg kg^{-1})	15.2	2.6
Bray-II-2 (mg kg^{-1})	21.2	3.0
pH buffer capacity (cmol$_c$ kg^{-1} pH^{-1})	0.288	0.251
Fe$_{ox}$[a] (mg kg^{-1})	5,867	4,402
Al$_{ox}$[b] (mg kg^{-1})	3,120	2,916
Silt (%)	24	15
Clay (%)	40	48

[a]Fe$_{ox}$: oxalate extractable Fe.
[b]Al$_{ox}$: oxalate extractable Al.

Table 2. Samples were air-dried and passed through a 2-mm sieve.

Soil incubation

Two hundred gram samples of dry soil were weighed into plastic beakers (ϕ = 12 cm) provided with caps. Finely ground *Tithonia* was added at 0, 2.5 and 40 g kg^{-1} soil on a dry weight basis and thoroughly mixed with the soil. Similarly, two rates of inorganic P (9 and 145 mg P kg^{-1}) as KH_2PO_4 (equivalent to the total amounts of P added to the soil at the two rates of *Tithonia*) were added to another set of tubes. There were 3 replicates per treatment. The soils were kept at 45% of their water holding capacity and incubated at 25 °C. Samples were taken at 0, 1, 2, 3, 7, 14, 28 and 49 days after commencement of the incubation, dried, and analysed for pH_{CaCl2}, pH_{KCl}, and extractable Al, using methods described later. Cation exchange capacity (CEC), anion exchange capacity (AEC), and specific surface area were determined on samples taken at 7 and 49 days. Aggregate size distribution was determined on undried soil samples taken at the same two times. The final samples taken at 49 days were also analysed for resin extractable P (Resin P), dissolved molybdate reactive P (DMR-P) and dissolved molybdate unreactive P (DMU-P).

Soil analyses

Soil pH_{CaCl2} and pH_{KCl} were determined on 1:2.5 soil-water suspensions by shaking 10 g (<2 mm) soil samples in 25 mL of 1 mM $CaCl_2$ or 1 M KCl for 30 min. Organic C was determined using the Walkley

total P by the Murphy and Riley (1962) procedure following wet digestion (AOAC, 1985). To obtain a measure of soluble plant P concentration, 0.2 g dried, ground plant material was shaken in 20 mL 1 mM $CaCl_2$ on a reciprocal shaker for 16 h. The obtained suspension was centrifuged at 23,600 g for 15 min and the supernatant filtered through a 0.45 μm membrane filter (Millipore). Phosphorus in the extract was determined following the Murphy and Riley procedure (1962). The so-identified P fraction is called 'dissolved molybdate reactive phosphate' (DMR-P). The total amount of P present in the 1 mM $CaCl_2$ extract was determined by digestion with potassium persulphate in an autoclave at 1 atm pressure for one hour (Rowland and Haygarth, 1997). The difference between total P and MRP is designated 'molybdate unreactive phosphate' (DMU-P). This fraction of P is assumed to consist of large molecular weight and/or organic forms of P. The characteristics of the plant material used in the experiments are listed in Table 1.

Soil sampling and pre-treatment

Surface (0–15 cm) samples were collected from two soils: a Ferralic Cambisol located at Da Loan village, Duc Trong district, Lam Dong province, and a Rhodic Ferralsol located at Duc Lieu village, Bu Dang district, Binh Phuoc province. Soil characteristics are given in

and Black (1934) method. Exchangeable cations were determined in the supernatant by atomic absorption spectrophotometry after percolating the soil with 1 M ammonium acetate buffered to pH 7. Exchangeable acidity was determined by shaking a 5 g soil sample in 50 mL distilled water for 30 min then titrating with 0.01 M NaOH. Effective CEC was calculated by summation of exchangeable Ca, Mg, Na, K and exchangeable acidity. Olsen P was extracted by shaking 2.5 g of soil in 50 mL of 0.5 M $NaHCO_3$ at pH 8.5 for 30 min and then filtering. Bray-II-P was extracted by shaking 5 g of soil in 35 mL of 0.1 M HCl and 0.03 M NH_4F for 1 min followed by filtering. The supernatants of these two extracts were then analysed for P by the Murphy and Riley (1962) colorimetric method. Particle size distribution was determined by the pipette method, following pre-treatment with H_2O_2 oxidation to remove organic matter and dispersion with $(NaPO_3)_6$. Oxalate-extractable iron and aluminium (Fe_{ox} and Al_{ox}) were extracted with an acid 0.2 M ammonium oxalate solution at a soil: solution ratio 1:20 for 2 h on an end-over-end shaker. Iron and Al were determined in the extracts by atomic absorption spectrophotometry. Aluminium was extracted with 1 M KCl at a soil-to-solution ratio of 1:10 for 1 h on a reciprocal shaker at 160 rpm. The samples were then centrifuged at 23,600 g for 15 min. The supernatant was filtered through 0.45 μm Millipore membranes and Al concentration determined by conventional atomic absorption spectrophotometry.

For the determination of resin extractable P, anion exchange membrane sheets (125 × 125 mm; BH 15 1TD from BDH Laboratory Supplies) were cut into strips of 42 × 15 mm. At each sampling date, two grams of air-dried soil were weighed into a 100-mL nalgene bottle with 50 mL of 1 mM $CaCl_2$ and one strip of anion exchange membrane. The bottles were placed on an end-over-end shaker for 16 h at 25 °C. After 16 h, the membrane was removed and repeatedly rinsed with Milli-Q water to remove soil particles. The membrane was put into a 100-mL nalgene bottle with 25 mL of 0.5 M HCl and shaken for 16 h to extract adsorbed phosphorus. The membrane was then transferred into a bottle containing 25 mL of 0.5 M HCl and shaken for 1 h. This last procedure was repeated twice to ensure that all the P sorbed on the membrane was extracted. The so obtained three HCl extracts were analysed separately for P. The sum of the three is taken as the resin extractable P. After removing the resin membrane, the soil suspension was centrifuged at 23,600 g for 15 min and the su-

pernatant filtered through a 0.45 μm membrane filter (Millipore). Phosphorus concentrations in the extracts were determined following the Murphy and Riley (1962) or Van Veldhoven and Mannaerts (1987) colorimetric procedures, depending on the concentration range. The so-identified P fraction is called 'dissolved molybdate reactive phosphate' (DMR-P) since it is detectable after forming a complex with ammonium molybdic acid. The total amount of P present in the 1 mM $CaCl_2$ extract was determined by digestion with potassium persulphate in an autoclave at 1 atm pressure for one hour. The difference between total P and MRP is likewise called 'dissolved molybdate unreactive phosphate' (DMU-P). As in the case of DMU-P in the plant material, this fraction of P is assumed to contain P of large molecular weight and/or organic forms of P and in this case is not recovered on the resin membrane. The sum of resin P, DMR-P and DMU-P is then referred to as the total 'labile P' fraction extracted from the soil, allowing comparisons of the effects of individual treatments on available soil P.

Aggregate size distribution was determined following manual wet-sieving by the method described by Elliott (1986). In short, soils were first put on the largest sieve submerged in distilled water in a plastic container. The sieve was then gently moved 3 cm vertically, 50 times in a period of at least 2 min. Material remaining on the sieve was back-washed into an aluminium container and dried at 60 °C in a ventilated drying oven. Soil passing a particular sieve was transferred to the next finer sieve and the process repeated. Since the amount of soil that passed through the 53 μm sieve was very small compared with the total soil mass (between 2% to 7% of total soil mass in the Duc Lieu soil), no further fractionation was done on sizes <53 μm. The material was allowed to settle, centrifuged and oven dried. Floatable material was removed and discarded during the sieving process. In total, seven size fractions were obtained: >2000 μm, 1000–2000 μm, 500–1000 μm, 250–500 μm, 100–250 μm, 53–100 μm and < 53 μm.

Surface charge was characterised by the cation and anion adsorption method described by Zelazny et al. (1996), and involving equilibration of the soil with 1 mM KCl, with displacement of the adsorbed K by 0.5 M $NaNO_3$ following shaking and centrifuging. The concentrations of K in the supernatants before and after displacement were used to calculate the negative surface charges of the soil. Because the anion exchange capacity of these soils is very low, the difference in chloride concentration between the replacing

solution and the equilibrating solution was not large enough to be accurately detected by routine methods for Cl analysis. Therefore 0.2 mL of a labelled ^{36}Cl solution (370 Bq ml^{-1} 1 mM KCl) was added to the suspension of each sample. The tubes were shaken for 4 h. The samples were centrifuged, decanted and weighed to determine the volume of the entrained solution. The supernatants were analysed for ^{36}Cl by liquid scintillation counting, and the isotopic dilution principle used to calculate sorbed Cl$^-$ as a measure of AEC.

Specific surface area was determined on a subset of samples of total soil and of two aggregate fractions (1000–2000 μm and <53 μm) taken at 49 days, using a Coulter Omnisorp 100 instrument following the Brunauer, Emmett and Teller (BET) method (Greenland and Mott, 1978). Nitrogen was used as the adsorbate and nitrogen gas adsorption isotherms at liquid nitrogen temperature (77K) were obtained with a laboratory-built volumetric apparatus equipped with a Datametrics 0–1000 Tor pressure gauge. Dry soil samples were kept intact without any grinding. Samples were degassed overnight at 60 °C at a 0.03 Tor vacuum. The sample was typically maintained at a constant temperature in liquid nitrogen. Adsorbed gas volume and relative pressure p/p_o (ranged between 0.05 to 0.25) were recorded every 30 sec. Specific surface areas were calculated with the linearised BET equation.

Statistical analysis

Analyses of variance and correlation analyses were performed using the SAS software (SAS, 1988).

Results

Soil pH

The pH$_{CaCl2}$ of Da Loan soil slightly decreased in the control and in the 2.5 g kg^{-1} *Tithonia* amended soils in the first three days and reverted to the initial pH later (Figure 1a and b). From 7 days onwards, the soil pH continued to increase up to 14 days and then remained almost constant. Soil pH$_{CaCl2}$ increased dramatically in the soil that received large amounts of *Tithonia* (40 g kg^{-1}) during the first week after incubation and then increased more slowly after 14 days. At day 49, pH$_{CaCl2}$ of the Da Loan soil was increased by 0.3 units (from 4.4 to 4.7) at small *Tithonia* amendment dose and increased by 1.7 units (from 4.4 to 6.1) at

the large amendment dose. The pH$_{CaCl2}$ of Duc Lieu soil increased immediately after *Tithonia* addition in all treatments resulting in an increase of 0.2 units at the small amendment and 1.2 units at the larger dose (Figure 1a).

The pH$_{KCl}$ of soil extracts showed the same trend as pH$_{CaCl2}$ (Figure 1b). In both soils the pH$_{KCl}$ increased rapidly in all treatments during the first three days and then stabilised or kept increasing at a slower rate. In all treatments, pH$_{KCl}$ was higher in Duc Lieu soil than in Da Loan soil. The larger the amount of *Tithonia* added, the larger the pH increase. Up to 49 days after amending, soil pH$_{KCl}$ still increased in both soils and was 5.0 and 5.4 for the Da Loan and Duc Lieu soils, respectively, at the larger amendment dose. Although the increase in soil pH$_{KCl}$ at the application dose of 2.5 g kg^{-1} was small (0.03 to 0.05 units), pH$_{KCl}$ values of both soils were significantly ($P < 0.01$) higher than the control at any sampling time except for the Da Loan soil, 1 day after *Tithonia* addition.
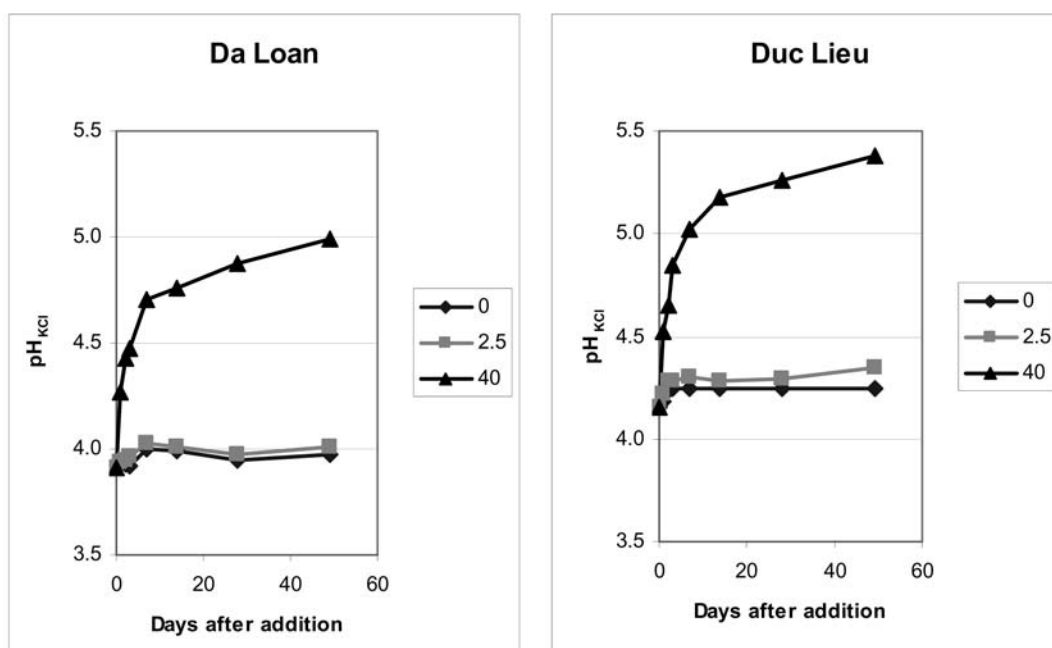
Extractable Al

Extractable aluminium concentrations (1 M KCl extract) are illustrated in Figure 2. Initially, Da Loan soil contained 2.16 cmol$_c$ Al kg^{-1}. During incubation, the concentration of extractable Al reduced slightly in the control. After three days in the treatment with 2.5 g *Tithonia* kg^{-1}, the initial extractable Al concentration decreased by 28%. This decreasing trend slowed down after 7 days and Al concentration stabilised at about 1.5 cmol$_c$ kg^{-1} thereafter. At 40 g *Tithonia* kg^{-1}, an abrupt reduction in extractable Al concentration was observed one day after addition, with a 72% decrease in extractable Al. At day three, 90% of the initial extractable Al was no longer recovered. The concentrations of extractable Al continuously decreased and were almost zero at day 49.

A similar trend was observed in Duc Lieu soil. The initial concentration of extractable Al was 0.7 cmol$_c$ kg^{-1} lower in Duc Lieu soil than in Da Loan soil. In addition, the reduction in extractable Al following *Tithonia* addition was larger in Duc Lieu soil. At the low addition rate, the extractable Al concentration was lowered to 66% of the initial concentration after a three-day incubation, demonstrating the effectiveness of *Tithonia* in lowering Al levels, even at modest additions. Large additions of *Tithonia* lowered the extractable Al concentrations of both soils to the same values, irrespective of the initial concentrations.

16
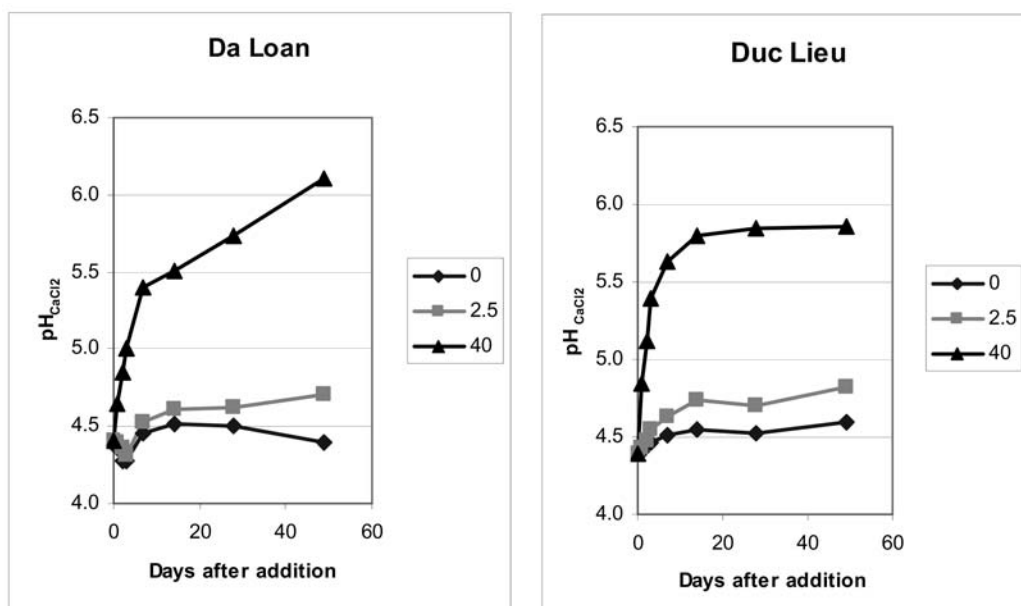
a- pH$_{CaCl2}$, 1 mM

b- pH$_{KCl}$, 1M

Figure 1. Effect of *Tithonia* amendments on (a) soil pH$_{CaCl2}$, and (b) pH$_{KCl}$ for two soils. Amendment doses were 0, 2.5 and 40 g *Tithonia* kg^{-1} soil.
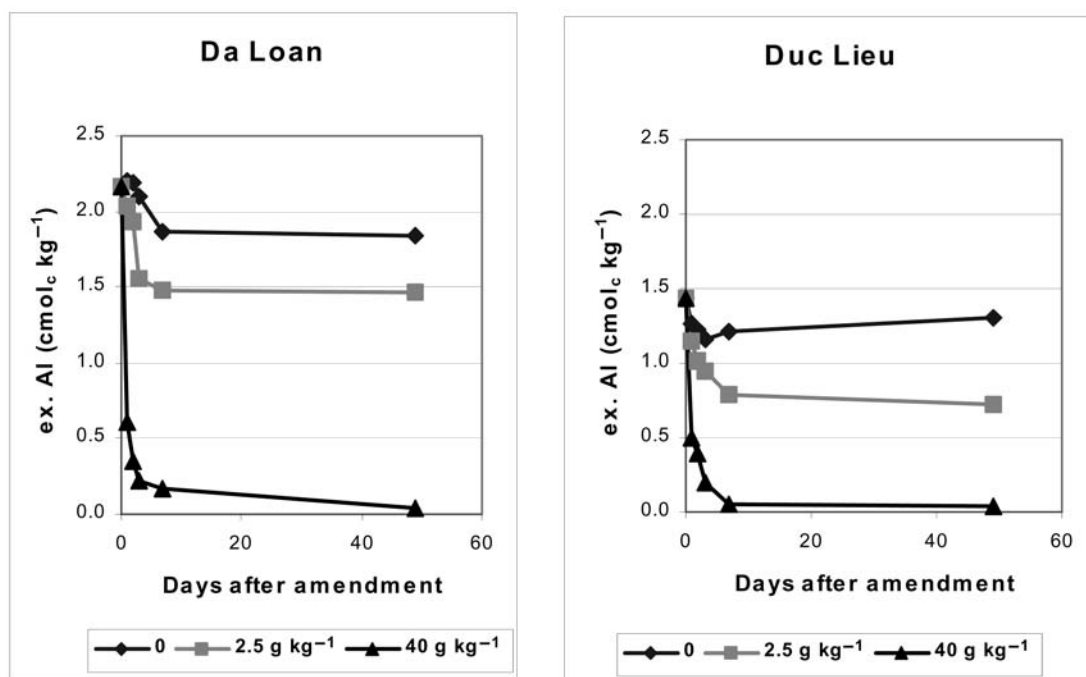
Figure 2. Effect of *Tithonia* amendment on changes in extractable aluminium in soil samples from Da Loan and Duc Lieu.

The concentrations of extractable aluminium were highly negatively correlated ($P < 0.001$) with soil pH_{KCl} at each application dose and in both soils (Da Loan: $r = -0.94$; Duc Lieu: $r = -0.96$).

Resin P

The *Tithonia* plant material used in this study had a high total P content (0.36%) (Table 1). The portion of P that was extractable by 1 mM $CaCl_2$ amounted to 56% of the total P or 5.1 and 82 mg P kg^{-1} soil for *Tithonia* applications of 2.5 and 40 g kg^{-1}, respectively. The concentrations of resin P in the Da Loan soil at the low amendment rate were not significantly different from the untreated control, irrespective of whether *Tithonia* or inorganic P was added (Table 3). This may be due to the fact that the amount of P amended was small compared with the large amount of native P. However, resin P was significantly ($P < 0.05$) higher in the amended Duc Lieu soil even at the low application rate, where it increased from 3.2 mg P kg^{-1} in the control to 4.3 mg kg^{-1} in the *Tithonia* amended soil and 3.9 mg P kg^{-1} in the inorganic application.

At the high application rate, the resin P concentration in Da Loan soil amended with the inorganic source was 68.8 mg P kg^{-1}, significantly ($P < 0.05$) higher than in soil amended with *Tithonia* (52.8 mg P kg^{-1}). For Duc Lieu soil, the reverse was true. Soil amended with *Tithonia* had a resin P concentration of 35.3 mg P kg^{-1}, while it was 24.0 mg P kg^{-1} in the inorganically amended soil.

Dissolved molybdate-reactive P (DMR-P)

The concentration of DMR-P in centrifuged and filtered soil supernatants was very low in the untreated controls (Table 3). Amendment, either with *Tithonia* or with inorganic P, did replenish soil solution P even at the low addition rate. In Da Loan soil, the DMR-P concentration was higher for the inorganic amendment than for the *Tithonia* amendment. In Duc Lieu soil at the low application rate, DMR-P concentration was higher when P was added as *Tithonia*.

At the large amendment, DMR-P concentrations were significantly different from the untreated controls in both soils. The most important feature is that at this large application, DMR-P concentrations in both soils were higher with *Tithonia* than with the inorganic P source.

Dissolved molybdate-unreactive P (DMU-P)

At low amendment doses and in the controls, DMU-P represented a substantial proportion of the total dis-

Table 3. Extractable soil P in the two soils 49 days after addition of two rates of P as *Tithonia* or KH_2PO_4 at two different rates. Dissolved molybdate-reactive P (DMR-P) is the P fraction extracted by 1 mM $CaCl_2$ which reacts with molybdate following the Murphy and Riley (1962) method. Dissolved molybdate-unreactive P (DMU-P) is the P fraction calculated from the difference between total P determined by potassium persulphate digestion (Rowland and Haygarth, 1997) and the DMR-P

Treatment	Resin P		DMR-P		DMU-P	
	Da Loan	Duc Lieu	Da Loan	Duc Lieu	Da Loan	Duc Lieu
	$(mg\ P\ kg^{-1})$					
Control	17.0	3.2	0.02	0.02	1.40	2.95
Tithonia (9 mg P kg^{-1})	17.9	4.3	0.08	0.16	3.24	3.68
Inorganic P (9 mg P kg^{-1})	18.8	3.9	0.17	0.06	1.70	3.32
LSD (0.05)	*2.9*	*0.6*	*0.04*	*0.04*	*0.76*	*0.39*
Tithonia (145 mg P kg^{-1})	52.8	35.3	1.36	1.11	10.43	16.33
Inorganic P (145 mg P kg^{-1})	68.8	24.0	0.74	0.55	2.63	6.50
LSD (0.05)	*6.5*	*5.6*	*0.23*	*0.07*	*1.37*	*2.34*

solved P (Table 3). In Da Loan soil, the DMU-P concentration after a small inorganic P amendment was similar to the control but it increased twofold in the soil amended with *Tithonia*. In Duc Lieu soil, the DMU-P concentration of the soil amended with *Tithonia* at the low addition rate was significantly higher than in the untreated control. There was a significant ($P < 0.05$) difference in DMU-P between the organic and inorganic treatments in Da Loan soil but this difference was not significant in Duc Lieu soil.

When inorganic P was added at the high rate, DMU-P was not significantly different, either from the control or from the soil receiving a small amendment in Da Loan soil. When *Tithonia* was added at the high rate, the DMU-P concentrations were significantly higher and amounted to 10 mg P kg^{-1} in Da Loan soil and 16 mg P kg^{-1} in the Duc Lieu soil.

Comparison of P sources

In the Da Loan soil, levels of labile P were either the same (low application rate) or lower (high application rate) in the *Tithonia*-amended soil than in the soil amended with inorganic P. At both application rates in the Duc Lieu soil, labile P in the *Tithonia*-amended soil was higher than with P added in inorganic form (Table 3). Resin P showed similar trends to labile P. In the case of DMU-P, there was a consistent trend of larger values in *Tithonia*-amended soils than in inorganic P-amended soils.

Aggregate size distribution

The most notable feature of the Da Loan soil (Cambisol) was the large proportion (64.4%) of microaggregates ($<250\ \mu$m) in the control treatment (Table 4). Aggregates with a size between 1000 and 2000 μm formed only 10% of the total weight. After 7 days incubation, aggregate size distribution of the soil changed due to *Tithonia* addition (Table 4). With 2.5 g *Tithonia* added per kg soil, the proportion of aggregates with size $<53\ \mu$m significantly reduced from 30% to 22% after one week of incubation. When added in large amounts (40 g kg^{-1}) *Tithonia* produced a considerable change in the aggregate-size distribution as larger aggregates were rapidly formed after one week of incubation. Fractions smaller than 53 μm were reduced to 1/5 of the control. Aggregates larger than 2000 μm, which were by definition absent before incubation, were present in a significant amount (33%). Similar trends were observed in the samples taken 49 days after the start of the incubation (Table 4).

In contrast to the Da Loan soil, 70% of Duc Lieu bulk soil (Ferralsol) was already comprised of macroaggregates ($>250\ \mu$m) at the outset (Table 5). The aggregate fraction with size $<53\ \mu$m comprised only 7%. This indicates that the soil is strongly aggregated. As expected, a small dose of *Tithonia* did not cause significant changes in any size fractions after one-week incubation, except for reducing the fraction $<53\ \mu$m (from 6.8 to 5.3%). *Tithonia*, when added at the high rate, produced a considerable change in the aggregate size distribution as larger aggregates were rapidly formed after one week of incubation. Aggre-

Table 4. Effect of Tithonia amendment on aggregate size distribution (%) in the Da Loan soil after one week and seven weeks of incubation

Amounts of *Tithonia* added (g kg^{-1})	Aggregate size (μm)						
	>2000	1000–2000	500–1000	250–500	100–250	53–100	<53
One week incubation							
0	0.0 b	10.1 b	10.8 b	14.8 a	25.5 a	8.6 a	30.3 a
2.5	2.5 b	14.5 b	14.7 ab	15.7 ab	21.8 a	8.9 a	22.0 b
40	32.6 a	29.2 a	16.3 a	7.8 b	6.1 b	1.9 b	6.2 c
Seven weeks incubation							
0	5.7 b	15.6 b	15.7 a	15.0 b	18.4 b	11.9 c	17.7 a
2.5	3.1 b	16.7 b	16.4 a	18.5 a	23.1 a	7.5 b	14.7 b
40	33.0 a	24.1 a	18.8 a	11.2 c	6 c	1.9 c	4.9 c

In a column and at each incubation period, means with the same letter are not significantly different at $P = 0.05$ according to Tukey's studentized range test.

gates larger than 2000 μm, which were not present before incubation, were formed in large amounts (28%). Significant reductions in the proportions of aggregates with size >2000 μm and 1000–2000 μm were observed when the incubation continued up to 49 days. The increase in proportion of smaller size aggregates (100–1000 μm) implied that a portion of the macroaggregates formed during the first week, broke down again when incubation continued. However, compared to the unamended control soil and for the same incubation period, treatment with 40 g *Tithonia* kg^{-1} soil still resulted in lower proportions of all fractions <250 μm (Table 5).

Specific surface area

The measured specific surface areas (SSA) of the Da Loan bulk soil and two selected aggregate sizes in treatments with and without 40 g *Tithonia* kg^{-1} are presented in Table 6. *Tithonia* amendment reduced the SSA of the total soil by 13.7%. When *Tithonia* was added, the SSA of aggregates with size 1000–2000 μm was reduced by 1.88 m^2 g^{-1} whereas this reduction was 4.87 m^2 g^{-1} for the <53 μm fraction. This indicates that *Tithonia* amendment was more effective in reducing the SSA of microaggregates than of macroaggregates. In particular, the pore volume of pores <5 nm was considerably reduced in the treatment with *Tithonia* (data not presented). This indicates that the decomposition products released from *Tithonia* plant material bound the aggregates together and so may have occluded some of the micro and mesopores so that N$_2$ gas molecules could not penetrate.

For the Duc Lieu soil, the effect of *Tithonia* amendment on the SSA of samples of total soil and aggregates 1000–2000 μm is presented in Table 6. A first observation is that the specific surface areas of Duc Lieu bulk soil and the separated fraction are approximately 50% larger than for the Da Loan soil. Duc Lieu soil contained more volume of micropores per g of soil mass than Da Loan soil, with or without *Tithonia* amendment (data not presented). The reduction in SSA of the total soil sample from Duc Lieu due to *Tithonia* addition was 4.53 m^2 g^{-1}. This value is similar to the reduction achieved in Da Loan soil indicating a dependency on the amount of residues added, irrespective soil type. However, in relative terms the reduction is smaller (9.4%). In contrast to Da Loan soil, the reduction in SSA of aggregates 1000–2000 μm (4.78 m^2 g^{-1}) in Duc Lieu soil was similar to that of the total soil (4.53 m^2 g^{-1}). This may indicate that the reduction in SSA of the total soil and the reduction in the SSA of the macroaggregates are due to similar processes.

Surface charges and surface charge density

The surface charges and charge densities of the soils after 49 days incubation are presented in Table 7. At the higher *Tithonia* addition rate, the positive surface charges (viz. AEC) were reduced by 92% in Da Loan soil but only slightly reduced in Duc Lieu soil. In contrast, the negative surface charges (viz. CEC) increased by 204% in Da Loan soil and by 345% in Duc Lieu soil at this amendment rate.

Combining charge measurements and specific surface area determination logically leads to calculation

Table 5. Effect of *Tithonia* amendment on aggregate size distribution (%) in the Duc Lieu soil after one week and seven weeks of incubation

Amounts of *Tithonia* added (g kg^{-1})	Aggregate size (μm)						
	>2000	1000–2000	500–1000	250–500	100–250	53–100	<53
One week incubation							
0	0.0 b	24.3 b	23.8 a	22.1 a	18.4 a	4.7 a	6.8 a
2.5	0.2 b	24.2 b	24.3 a	20.5 a	20.6 a	4.9 a	5.3 b
40	28.4 a	34.7 a	21.4 a	9.2 b	4.2 b	1.0 b	1.1 c
Seven weeks incubation							
0	0.8 b	29.9 b	29.3 a	17.3 b	11.8 a	5.4 a	5.4 b
2.5	0.5 b	35.5 a	24.5 b	16.7 b	12.9 a	2.7 b	7.2 a
40	4.1 a	31.8 b	28.9 ab	21.4 a	10.3 a	1.8 b	1.8 c

In a column and at each incubation period, means with the same letter are not significantly different at $P = 0.05$ according to Tukey's studentized range test.

Table 6. Effect of *Tithonia* amendment (40 g kg^{-1}) on specific surface area of soil and size separate samples from Da Loan and Duc Lieu measured by the BET method

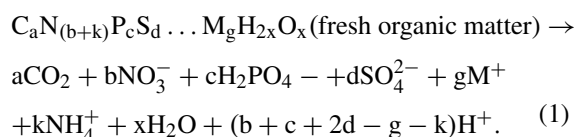|	Samples	Specific surface area (m^2 g^{-1})			
		Without *Tithonia*	With *Tithonia*	Difference	
				(m^2 g^{-1})	(%)
Da Loan	Total soil	32.25	27.83	4.42	−13.7
	1000–2000 μm	30.04	28.16	1.88	−6.0
	<53 μm	30.39	25.52	4.87	−16.0
Duc Lieu	Total soil	48.14	43.61	4.53	−9.4
	1000–2000 μm	46.76	41.98	4.78	−10.2

of surface charge density. Densities of positive and negative charges were calculated by dividing the AEC and the CEC of the soil at natural pH, respectively, by the surface area measured by the BET method. The Da Loan soil had a smaller positive charge density but a larger negative charge density than the Duc Lieu soil (Table 7). The *Tithonia* amendment reduced the positive charge density of Da Loan soil by 72% but only slightly decreased the positive charge density in Duc Lieu soil (4%). The negative charge density increased by 253% in Da Loan soil with *Tithonia* amendment at 40 g kg^{-1} soil. This increase was higher in Duc Lieu soil (390%).

Discussion

Soil pH

A general equation of the proton budget during mineralisation of plant materials was proposed by Van Breemen and Buurman (1998) where the final effect depends on (i) the composition of the plant material and (ii) the degree to which it is decomposed.

$$C_aN_{(b+k)}P_cS_d \ldots M_gH_{2x}O_x(\text{fresh organic matter}) \rightarrow$$
$$aCO_2 + bNO_3^- + cH_2PO_4 - + dSO_4^{2-} + gM^+$$
$$+ kNH_4^+ + xH_2O + (b + c + 2d - g - k)H^+. \quad (1)$$

Protons are generated during nitrification and mineralisation of P and S, while hydroxyl ions are produced during the release of metals and during ammonification. While the proton budget is determined from the values of the stoichiometric factors b, c, d, g and k, the net result on soil pH is a function of the pH buffer capacity of the soil. In this incubation study, soils were amended with *Tithonia* in a closed-system without a growing plant. Therefore, the effects of plant uptake, root exudates and leaching are not relevant and the processes responsible for the pH change are limited to the decomposition of nutrients held in the plant material and nitrogen transformations. Using a

Table 7. Effect of *Tithonia* amendment on AEC, CEC and surface charge density of soil samples from Da Loan and Duc Lieu

Soil	Amounts of *Tithonia* added (g kg^{-1})	Surface charge (cmol$_c$ kg^{-1})		Surface charge density (10^{-6} cmol$_c$ m^{-2})		Percentage change in charge density (%)	
		AEC	CEC	Positive charges	Negative charges	Positive charges	Negative charges
Da	0	0.013	2.67	0.403	82.7		
Loan	40	0.001	8.12	0.111	291.7	−72	253
Duc	0	0.024	1.00	0.503	20.8		
Lieu	40	0.023	4.45	0.484	102.0	−4	390

combination of Equation 1 and the pH-buffer curves of the two soils studied, we could reasonably predict the pH changes due to an amendment of *Tithonia* (data not presented). At the small amendment dose, the differences between the measured and the predicted pH in both soils were small and within the range of 0.02 to 0.06 pH units. When *Tithonia* was amended at a large dose, the differences amounted to 0.40 pH units. However, this discrepancy is considerably less than the observed effect of *Tithonia* addition on pH, so the results still suggest that the major underlying causes of the soil pH change were nitrogen transformations and release of metal cations as the *Tithonia* decomposed.

Labile P

The results show that organic amendment increased the organic labile fraction concomitant with the increase in addition. Inorganic amendments significantly increased this organic fraction only in Duc Lieu soil but not in Da Loan soil. The observed increase in DMR-P concentrations in both soils at the large *Tithonia* amendments is to some extent due to the large P content of *Tithonia* plant material and particularly related to the large proportion of soluble phosphorus. Since 56% of the total plant P is soluble, the amount of soluble P added to the soil at the large amendment equalled 84 mg P kg^{-1}. This amount may be rapidly sorbed to the soil very early during the incubation. The remaining 44% of the total P in *Tithonia* was in an organic form, which would contribute to the presence of DMU-P in soil solution. This labile fraction may be mineralised and may release P in an available inorganic form. It is well-known that the mineralisation process is a key factor controlling P availability in soils amended with plant materials (Blair and Boland, 1978).

In addition, the ratio between DMU-P and resin extractable P may supply useful information on the partitioning between organic and inorganic labile P pools after amendment of an organic material. In Da Loan soil, the DMU-P/resin P ratios were about 20% and 10% for *Tithonia* and inorganic P amendments, respectively. The 10% difference in the DMU-P concentrations between the two P sources is likely due to the high molecular-weight P forms derived from the organic residue. A similar trend was observed in Duc Lieu soils although the unamended control soil contained a large DMU-P concentration, which was almost as large as the concentration of resin P. For a small P-amendment, the DMU-P/ resin P ratio was as large as 85%. This ratio was larger in soil amended with *Tithonia* than with inorganic P. Therefore, the DMU-P/ resin P ratios reveal that the P source controls the ratio in both soils. Da Loan soil had smaller DMU-P/resin P ratios in all treatments compared with Duc Lieu, indicating that the dissolved molybdate-unreactive P is less important in Da Loan soil. The large DMU-P/resin P ratios in Duc Lieu soil, even at the small amendment dose, suggests an important role for the DMU-P in supplying P to plants, taking into consideration the modest resin P concentrations of this soil.

Specific surface area

The difference in specific surface area between aggregate fractions with size 1000–2000 μm and <53 μm of the original Da Loan soil was negligible (about 1%) (Table 6). This supports the common view that a macroaggregate is composed of many microaggregates. After *Tithonia* amendment, the SSA difference between the two fraction sizes was greater (about 10%). The fact that the SSA of the aggregates <53 μm was now smaller than the SSA of aggregates 1000–

2000 μm suggests that organic materials glued the microaggregates together and so blocked some sorption sites or even complete micropores as was indicated by the N_2 gas sorption data. Feller et al. (1992) reported that organic matter does indeed hide a part of the available specific surface area by binding some clay particles together. Another explanation is that the remaining microaggregate materials, not bound into macroaggregates, are likely to be the ones with smaller SSAs and consequent smaller reactivity.

In summary, a large addition of *Tithonia* reduced the specific surface area of the studied soils and separated aggregates. The SSA measured with the BET method reflected pore size distribution. The decrease in SSA could be explained by a decrease in microporosity. Therefore, addition of *Tithonia* plant material is thought to reduce the SSA of the soils through blocking of some micropores. Such action reduces the number of available sites for P sorption thus changing its distribution coefficient, and increasing the effective diffusion coefficient of the anionic P species in these soils. Coupled with the decrease in SSA caused by *Tithonia* addition, there was an increase in the net negative surface charge density. This result suggests that a decrease would occur in the sorption of any P added after the amendment due to an increased repulsion of the anions as a result of the more negatively charged surface.

Implications for application rate

The high rate of *Tithonia* addition (40 g kg^{-1}) had larger effects on key soil properties affecting P availability than the low rate of addition (2.5 g kg^{-1}). While the high rate would be impractical – if at all possible - if applied to the entire cropping area, farmer's practice is to apply green manures in localised areas – either as a strip down the planting row in field crops, or in the planting hole of tree crops. The amended area therefore may comprise only about 10% of the total cropped area, and 40 g plant material kg^{-1} amended soil (only the top 10 cm) would therefore require an approximate equivalent of 4 tonnes dry material ha^{-1} only. Such an application rate can be seen as relevant and realistic.

Conclusion

Tithonia additions can be expected to increase the chemical availability and diffusive supply of P through the following mechanisms: (i) increased soil pH leading to increased solubility of phosphate, (ii) increased macro-aggregation and hence reduced specific surface area and porosity leading to fewer sorption sites for P and enhanced diffusion rates, (iii) increased negative charges at the soil surface and reduced positive charges at the soil surface resulting in a net increase in repulsive force towards P and (iv) reduced Al concentrations and hence reduced P sorption or precipitation.

In these studies, *Tithonia diversifolia* was used as an example of a green manure crop, and it is apparent that green manure residues affect many soil parameters, both physical and physico-chemical ones. The value of green manures to sustain productivity is therefore much broader than simply improving P supply through the release of their P content as they decompose and is an important management strategy for soils in the tropics.

References

AOAC 1985 Official Methods of Analysis. 40 pp.
Appelt H, Coleman N T and Pratt P F 1975 Interaction between organic compounds, minerals and ions in volcanic-ash-derived soils: II. Effects of organic compounds on the adsorption of phosphate. Soil Sci. Soc. Am. Proc. 39, 628–630.
Blair G J and Boland O W 1978 The release of phosphorus from plant material added to soil. Aust. J. Soil Res. 16, 101–111.
Buresh R J, Smithson P C and Hellums D T 1997 Building soil phosphorus capital in Africa. *In* Replenish Soil Fertility in Africa. Eds. R J Buresh, P A Sanchez and F Calhoun. pp. 111-149. SSSA Spec. Publ. 51. SSSA and ASA, Madison, WI.
Cakmak I 2002 Plant nutrient research: Priorities to meet human needs for food in sustainable ways. Plant Soil 247, 3–24.
Easterwood G W and Sartain J B 1990 Clover residue effectiveness in reducing orthophosphate sorption on Ferric Hydroxide coated soil. Soil Sci. Soc. Am. J. 54, 1345–1350.
Elliott E T 1986 Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci. Soc. Am. J. 50, 627–633.
Feller C, Schouller E, Thomas F, Bouiller J and Herbillon A J 1992 N_2-BET specific surface areas of some low activity clay soils and their relationships with secondary constituents and organic matter contents. Soil Sci. 153, 293–299.
Fox T R, Comerford N B and McFee W W 1990 Phosphorus and aluminium release from a spodic horizon mediated by organic acids. Soil Sci. Soc. Am. J. 54, 1763–1767.
Greenland D J and Mott C J B 1978 Surfaces of soil particles. *In* The Chemistry of Soil Constituents. Eds. D J Greenland and M H B Hayes. pp. 321–353 John Wiley & Sons, UK.
Guppy C 2003 Phosphorus and organic matter interactions in highly-weathered soils. PhD Thesis, School of Land and Food Sciences, The University of Queensland.
Hamblin A P and Greenland D J 1977 Effect of organic constituents and complexed metal ions on aggregate stability of some East Anglian soils. J. Soil Sci. 28, 410–416.

Helling C S, Chesters G and Corey R B 1964 Contribution of organic matter and clay to soil cation exchange capacity as affected by the pH of the saturating solution. Soil Sci. Soc. Proc., 517–520.

Hue N V 1991 Effects of organic acids/anions on P sorption and phytoavailability in soils with different mineralogies. Soil Sci. 152, 463–471.

Hue N V, Craddock G R and Adams F 1986 Effect of organic acids on aluminium toxicity in subsoils. Soil Sci. Soc. Am. J. 50, 28–34.

Joachim A W R and Kandiah S 1929 Laboratory and field studies on green-manuring under paddy-land (anaerobic) conditions. Trop. Agric. (Ceylon) 72, 253–271.

Joffe J S 1955 Green manuring viewed by a pedologist. Adv. Agro. 141–187.

Linquist B A, Singleton P W, Yost R S and Cassman K G 1997 Aggregate size effects on the sorption and release of phosphorus in an Ultisol. Soil Sci. Soc. Am. J. 61, 160–166.

Murphy J and Riley J P 1962 A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.

Nagarajah S and Amarasiri S I 1997 Use of organic materials as fertilizers for lowland rice in Sri Lanka. pp. 97–104. *In* Soil Organic Matter Studies. International Atomic Energy Agency, Vienna, Austria.

Nye P H 1968 The Use of Exchange Isotherms to Determine Diffusion Coefficients in Soil. Int. Congr. Soil Sci. Trans. 9th (Adelaide, Australia) I, 117–126.

Nziguheba G, Palm C A, Buresh R J and Smithson P C 1998 Soil phosphorus fractions and adsorption as affected by organic and inorganic sources. Plant Soil 198, 159–168.

Oades J M 1984 Soil organic matter and structural stability: mechanisms and implications for management. Plant Soil 76, 319–337.

Palm C, Nziguheba G, Gachengo C, Gacheru E and Rao M R 1999 Organic materials as sources of phosphorus. Agrofor. Forum 9, 30–33.

Parfitt R L 1977 Phosphate adsorption on an Oxisol. Soil Sci. Soc. Am J 41, 1064–1067.

Rowland A P and Haygarth P M 1997 Determination of total dissolved phosphorus in soil solutions. J. Environ. Qual. 26, 410–415.

Sanchez P A 1976 Properties and Management of Soils in the Tropics. John Wiley & Sons, New York, pp. 174–181.

SAS, 1988 SAS/STAT User's Guide, Release 6.03 edn. SAS Institute Inc Cary NC, USA.

Singh Y, Singh B and Khind C S 1992 Nutrient transformations in soils amended with green manures. Adv. Soil Sci. 20, 238–298.

Tisdall J M and Oades J M 1982 Organic matter and water-stable aggregates in soils. J. Soil Sci. 33, 141–163.

Van Breemen N and Buurman P 1998 Soil Formation. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Van Veldhoven P P and Mannaerts G P 1987 Inorganic and organic phosphate measurements in the nanomolar range. Anal. Biochem. 161, 45–48.

Walkley A and Black I A 1934 An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37, 29–38.

White R E 1997 Principles and Practice of Soil Science: The Soil as a Natural Resource, 3rd edn. 348 p. Blackwell Science Ltd., Oxford, UK.

Zelazny L W, He L and Vanwormhoudt A M 1996 Charge analysis of soils and anion exchange. *In* Methods of Soil Analysis. Part 3. Chemical Methods. Ed. DL Sparks. pp. 1231–1253. SSSA Book series no. 5.