THE INFLUENCE OF PHOSPHORUS FERTILISER FORMS AND RHIZOSPHERE PROCESSES ON THE PHOSPHORUS NUTRITION OF TEA (*Camellia sinensis* L.)

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To the Memory of

My late Parents

For their

Enormous love, care and guidance

ABSTRACT

The understanding of the phosphorus (P) nutrition of tea, has mainly derived from trials evaluating yield response to applied P fertilisers. The literature indicates that the fertiliser P requirement of tea is generally low (below 15 kg ha^{-1} yr^{-1}), inspite of the generally high P fixing capacity of the Ultisols used for growing tea. Very little published information is available on the reactions of P fertilisers in tea soils and on the chemistry of P in the tea rhizosphere to explain this low P requirement of tea.

Because the tea soils are highly acidic (4.5 - 5.5) a locally mined, low cost, sparingly soluble phosphate rock (Eppawala phosphate rock, EPR) has been recommended as a P fertiliser for tea in Sri Lanka. But there is no experimental information available on its suitability for tea when compared to soluble P fertilisers.

The main objective of this thesis is to study the mechanisms involved in P utilisation from the tea rhizosphere, when both soluble (triple superphosphate) and sparingly soluble EPR fertilisers are used. An existing technique used to study rhizosphere processes of annual crops was modified to study the chemical processes involved in P utilisation from the rhizosphere of camellia plants, which are of the same family as tea. The depletion of soil and fertiliser P in slices of soil away from the rhizoplane were measured using a sequential chemical P fractionation procedure. The technique allowed isolation of soil slices at increasing distances from the rhizoplane and characterisation of the depletion pattern of soil P forms in the camellia rhizosphere. Subsequently this technique was used to study the rhizosphere processes in tea and other crops normally grown in association with tea.

A glasshouse study conducted to compare the mechanisms of P utilisation of tea (clone TRI 2025) with calliandra, Guinea grass and beans showed that all species depleted resin-P and NaOH-P_i in their rhizospheres. In contrast to other species, tea accumulated NaOH-P_o (organic-P) in the rhizosphere. All plant species acidified their rhizospheres and the magnitude of acidification is in the order of Guinea grass > bean and tea > calliandra. The higher acidification in the rhizosphere compared to the bulk soil caused more EPR dissolution near the roots.

Another glasshouse trial which examined the P utilisation efficiencies of tea clones showed that TRI 2023 and TRI 2025 had a higher external P efficiency than S 106 due to greater root surface area and P uptake per unit root surface area. But the internal efficiencies were not significantly different between the clones. All tea clones acidified the rhizosphere and the magnitude of acidification is of the order : TRI 2023 > TRI 2025 > S 106. The dissolution of EPR in the rhizosphere also followed the same order. All three clones accumulated NaOH-P_o in the rhizosphere.

Rhizosphere pH of tea (clone TRI 2025) decreased compared to the bulk soil, when N was supplied as the NH_4^+ [$(NH_4)_2SO_4$] or the NH_4^+ + NO_3^- [NH_4NO_3] form and it increased when N was supplied as the NO_3^- [$Ca(NO_3)_2$] form. The $(NH_4)_2SO_4$ treatment caused the highest dissolution of EPR in the rhizosphere, whereas the $Ca(NO_3)_2$ treatment showed the lowest in accordance with the magnitude of pH decline. Cation-anion balance in the plants showed that whatever form of N was applied, plants utilised more NO_3^- than NH_4^+. High nitrification rates in the rhizosphere were probably responsible for this inspite of the addition of a nitrification inhibitor.

A glasshouse trial with young tea plants (TRI 3072) showed that the agronomic effectiveness of the sparingly soluble EPR was equal to or better than the readily soluble TSP (triple superphosphate) fertiliser. This was due to the high rate of EPR dissolution in the acid soil. About 75% of the applied EPR was dissolved in the soil during the 10 month period of the study. The results also showed that the borax soil P test used to predict the P requirement of tea, as currently used in Sri Lanka, was the best of the six soil P tests investigated. This test has the advantage of requiring only one calibration curve relating yield and soil P values in estates fertilised with both soluble and sparingly soluble PR fertilisers.

This thesis contributed new knowledge regarding P uptake processes in the rhizosphere of tea plants.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Phosphorus (P) is one of the most important macro-nutrients for tea (*Camellia sinensis* L.), influencing growth, yield and quality (Gogoi et al., 1993; Ranganathan et al., 1982; Yongming et al., 1989). Phosphorus is essential for a wide range of plant metabolic functions (Mengel and Kirkby, 1987), most importantly carbon fixation during photosynthesis. As P is indispensable for the biochemical and physiological reactions in plant tissues, fertiliser or manure P application is essential to obtain sustainable yields. The Ultic soils on which tea is commonly grown contain very low concentrations of 1 - 4 μg P g^{-1} soil (borax-extractable P, Wickremasinghe, 1986) in their native status. Young tea shoots (two leaves and a bud) that are removed regularly by plucking for processing into drinking tea have a higher P concentration (0.36 - 0.29% P) than the older leaves (0.21 - 0.20% P) (Hasselo, 1965), suggesting a higher physiological demand for P in growing tissue. This P removal (6.8 kg P ha^{-1} yr^{-1}) in young tea shoots must be replaced by frequent application of P fertilisers to the soil.

Organised research on the response of tea to applied P fertilisers in Sri Lanka was started in the 1930s' (Eden, 1934). Though symptoms that are typical of P deficiency in tea are unlikely to be observed under field conditions (Biswas et al., 1984; Sengupta et al., 1986), sub-optimum levels of soil P have been shown to reduce tea yields (Eden, 1976). Experimental evidence on Sri Lankan Ultisols showed that there is no yield response to repeated annual application of P fertiliser beyond 15 kg P ha^{-1} yr^{-1}. Results of experiments in other tea growing countries also showed that maximum response to P was around 15-20 kg P ha^{-1} yr^{-1} (Willson et al., 1975). Whilst P remains essential for growth, the reason for this low P requirement in tea is not fully understood. The high P fixation capacities of Sri Lankan tea soils, due to low pH and

the presence of Fe and Al oxides, cause a higher fraction of applied P fertiliser to remain unavailable to plants, resulting in high amounts of residual-P (Golden et al., 1981) in frequently fertilised soils. As P-diffusivity in soils is very low (Barber, 1995) the availability of P for plant uptake is dependent on the inorganic P concentration gradient and the diffusion conditions in soil around the fine roots of tea plants. Despite the high P fixation in many tea soils, tea is found to grow well in many parts of the world with low rates of P fertiliser application. One reason for this may be its ability to utilise pools of soil P that are not traditionally considered as plant available, especially in the rhizosphere. A variety of mechanisms have been proposed to account for the increased mobilisation of soil P in the rhizosphere of various crop species. These mechanisms include the exudation of reducing substances (Gardner et al., 1982), hydrolysis of organic P by microbial and plant phosphatases (Eivazi and Tabatabai, 1977; Helal and Sauerbeck, 1984; Tarafdar and Jungk, 1987), action of VA-mycorrhiza (Young et al., 1986) and excretion of organic acids (Moghimi et al., 1978). Several studies have witnessed soil pH changes in the rhizosphere as a major factor in the dissolution/desorption of soil P (Gardner et al., 1982; Hedley et al., 1982ac; Riley and Barber, 1971). Previous studies showed that tea plants secrete various organic acids (Jayman and Sivasubramaniam, 1975; Xiaoping, 1994), which could dissolve native soil P.

Most developing countries are interested in utilising low-cost locally available phosphate rock (PR) resources for sustainable crop production. In Sri Lanka, an estimated 40 million metric tons of an apatite bearing rock deposit was discovered in Eppawala (Jayawardena, 1976). This material is now ground and used as a direct P fertiliser for many perennial crops in Sri Lanka (Dahanayake et al., 1995). Eppawala phosphate rock (EPR) is recommended by the Tea Research Institute of Sri Lanka for tea plantations, but its agronomic value has been tested little. It was felt that the low pH and high rainfall in the tea-growing areas assist EPR to dissolve, thus releasing sufficient P to the plants. The regular application of EPR in previously well fertilised soils will not improve dry matter yield. Therefore a soil testing procedure is needed to identify these conditions to help minimise the wasteful use of P fertiliser, in particular EPR.

Various soil tests are being used to determine the soil P status in different tea-growing countries of the world, but these tests have not been developed based on sound research correlating soil test values to dry matter yield, or the P uptake of tea plants. Therefore there is a need to find an easy and effective soil P test to determine the P needs for tea.

In the recent past new tea clones were developed and selected by many countries (Alam, 1994; Anandappa, 1986; Astika, 1994; Othieno, 1994), for higher yields, increased resistance to pests and diseases and drought tolerance etc., but hardly any information is available on the efficiency of P utilisation by these clones from different P sources. Perennials like calliandra (*Calliandra colothrysus* L.), which is grown as a shade tree and Guinea grass (*Panicum maximum* L.), which is largely found in abondanded tea fields are known to grow well in Sri Lankan tea soils. Beans (*Phaseolus vulgaris* L.) which are a fast growing leguminous vegetable crop are also grown in these soils. Very little is known about the P acquisition characteristics of these plants as compared with new tea clones grown in the same soil and with different P sources. Such information on P uptake by tea and companion plants may be useful in the efficient management of soil and fertiliser P in strongly weathered acidic Ultisols.

1.2 OBJECTIVES OF THE THESIS

The research reported in this thesis attempts to fill some of the gaps in the knowledge on P availability to tea from native and fertiliser P sources. The specific objectives of the research are:

1. To develop a suitable technique to study root induced changes on soil pH and P fractions in the rhizosphere and P uptake processes in tea plants.
2. To compare the rate of EPR dissolution and P mobilisation in the rhizosphere of different tea clones and other plants cultivated in tea soils.
3. To determine the effect of different forms of N supply on EPR dissolution and P mobilisation in the tea plant rhizosphere,.

4. To compare different soil tests in predicting P uptake and yield of tea plants fertilised with EPR and TSP (triple superphosphate).

CHAPTER 2

LITERATURE REVIEW

2.1 TEA CULTIVATION IN SRI LANKA

Tea (*Camellia sinensis* (L.) O. Kuntze) is a perennial crop cultivated commercially as a monoculture on large scale plantations in many humid tropical countries of the world (Willson and Clifford, 1992). Tea belongs to the genus Camellia which comprises as many as 82 species distributed mainly in South-East Asia. It is consumed as a beverage after brewing. The popularity of tea is increasing throughout the world because of its positive effects on human health (Ganguly, 1993).

Tea cultivation originated in Southern China (The People's Republic of China) around 300 BC. The habit of tea drinking as a ritual and as a common habit was started in China and spread to Japan around 1000 AD (Blofeld, 1985). In 1834, the British started cultivating tea in India by importing tea plants and experts from China. The first commercial tea plantation in Sri Lanka was started by James Taylor, a Scot in 1867, on a 19 acres of land in Loolecondera estate, Hewaheta in Central province. Later, tea became a timely venture as the coffee leaf rust disease destroyed the entire coffee cultivation in Sri Lanka within 25 years of its appearance. Tea proved to be a more durable and profitable resource and rapidly became the major plantation crop in Sri Lanka (Humble, 1991). In 1871, 100 ha of tea were planted and it increased to 6000 ha in 1881. The area of cultivation and the corresponding tea production increased remarkably with time. The major tea-growing areas of Sri Lanka are presented in Figure 2.1. The first consignment of Ceylon (now Sri Lanka) tea was exported in 1872. Today Sri Lanka is one of the biggest tea producing countries and the largest tea exporter in the world. Tea is cultivated on approximately 0.2 million ha planted with varying proportions of seedlings and improved, vegetatively propagated (VP) tea clones.

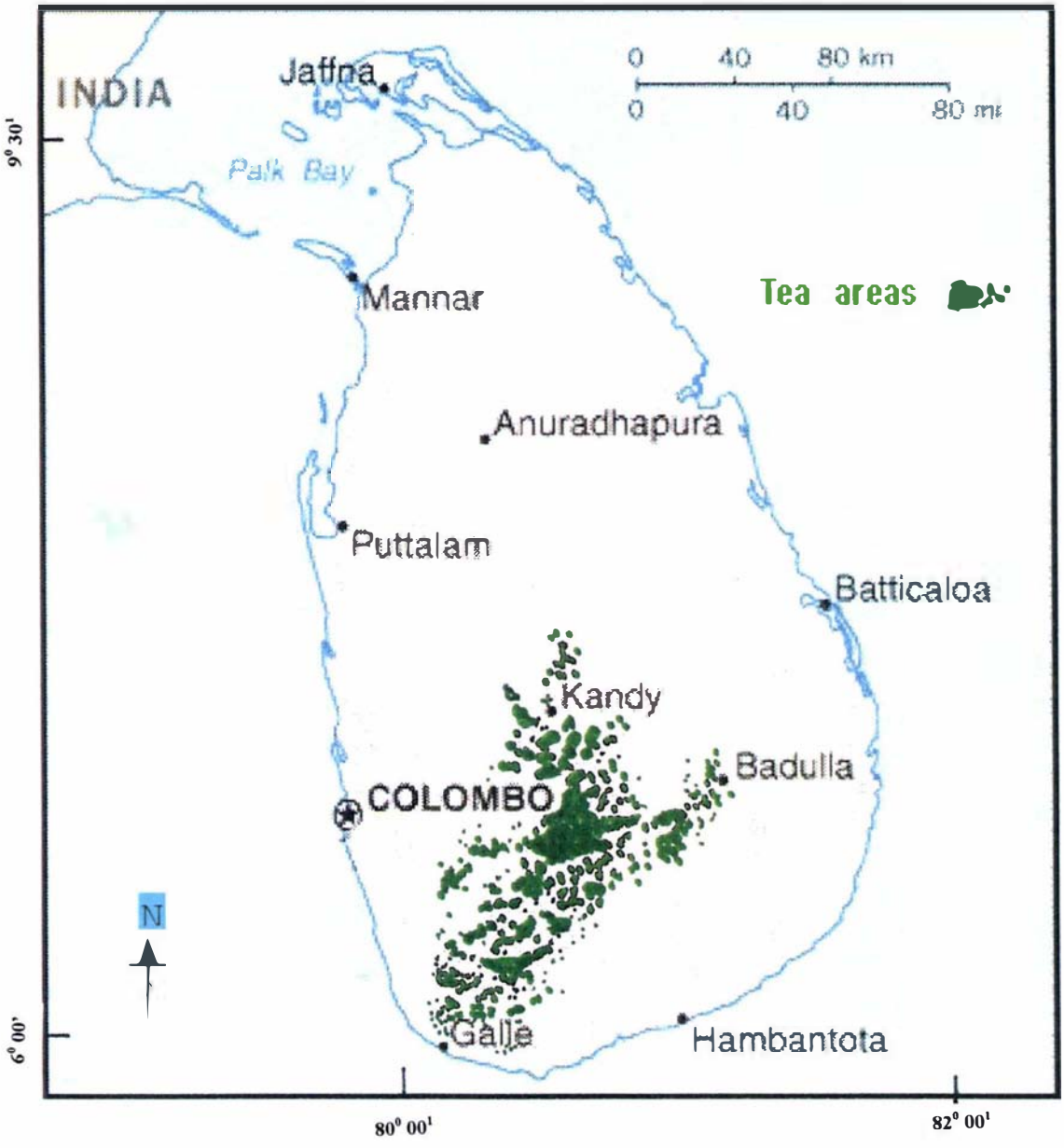

Figure 2.1 Tea growing areas of Sri Lanka

Tea production increased steadily until the mid 1960s', and thereafter consistently declined, until it reached its lowest figure of 179 million kg processed tea (779 kg ha^{-1} yr^{-1}) in 1983. Tea yields again increased and recorded the highest production of 240.7 million kg processed tea (1085 kg ha^{-1} yr^{-1}) in 1991 (Figure 2.2). The increase in total production at the early stages of the tea industry (1940 - 1960) was mainly due to the use of high yielding new tea clones coupled with a rapid expansion of the land area under cultivation. The subsequent decline in production during the 1980s was due to mismanagement, as a result of the then government policy of fragmentation of tea estates and changes in land ownership. Neglect of cultural practices, poor land care and senility of the tea bushes also played a part. The average productivity (processed tea yield ha^{-1}) of tea land has increased over time, although it is still lower compared to many other tea producing countries (Table 2.1). If only privately owned lands are considered the productivity per ha^{-1} in Sri Lanka is the highest in the world. The privately owned tea lands are about 42% of the total cultivated area of tea (Jayakodi, 1996). The key reasons for the relatively low productivity (yield ha^{-1}) in the state-owned tea plantations in Sri Lanka are predominance of seedling tea, high vacancies of plants, senility, low labour productivity, low and untimely inputs of fertiliser and poor agronomic care (Humble, 1991).

2.1.1 Economics and social impact of tea industry

Tea has been of predominant importance to the Sri Lankan economy for more than 130 years and still maintains a very influential position. The area under tea cultivation covers about 10% of the total cultivated land area in Sri Lanka, and the share of tea exports in total export earnings of Sri Lanka was about one-third in the 1980s (Bodhidasa, 1989; Bogahawatta and Herath, 1984). A substantial amount of revenue comes to the government from taxes levied on the tea industry (Ron, 1986). Tea is probably the most labour-intensive industry in Sri Lanka, employing about 3.21 persons ha^{-1}. This results in direct employment of approximately 0.5 million workers, who are complimented by at least an additional 0.2 million people dependent on the tea industry by way of product development, packing, transport, marketing, export, research, extension etc.

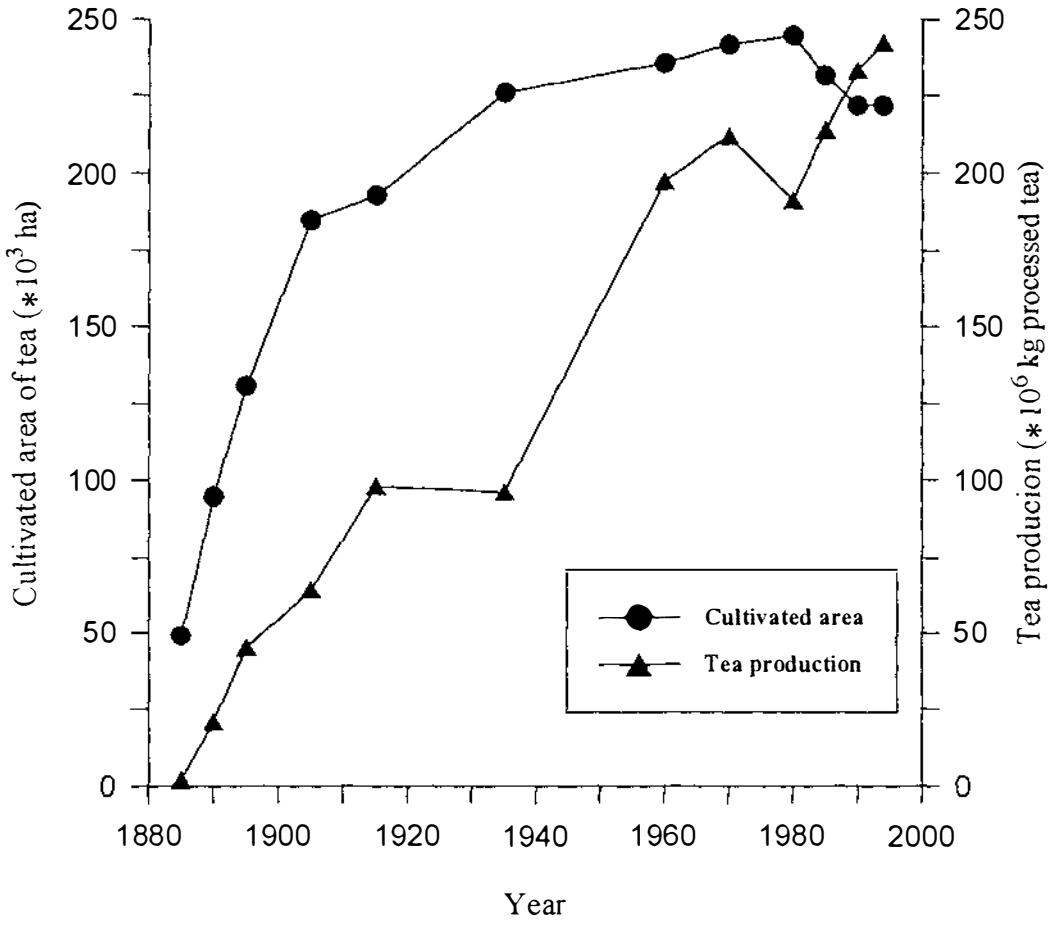

Figure 2.2 The area of cultivated tea land and tea production in Sri Lanka

Table 2.1 Average productivity of tea in the major tea growing countries (Kelegama et al., 1995)

Country	**Yield (kg processed tea ha^{-1} yr^{-1})**
Sri Lanka	
state owned estates	1268
privately owned estates	2442
India	
North	2127
South	2300
Kenya	2237
Indonesia	1645
Malawi	1929

A comparison of the profitability of tea cultivation in some tea growing countries of the world is given in Table 2.2. Compared to the other two major tea producing countries, India and Kenya, Sri Lankan labour output from tea plantations is deplorably low, especially in the state owned estates leading to high costs of production. The gross profit ha^{-1} is also very low from Sri Lankan tea lands, when the mean values of state and private production are considered.

2.2 CLIMATIC AND SOIL REQUIREMENTS OF TEA

Tea has wide soil and climatic adaptability. It grows in a range of climates and soils in many parts of the world. At present tea is grown on a commercial scale in countries all around the world from far north as Georgia (USSR) 43^0 N Lat., to far south as Corrientes (Argentina) 27^0 S Lat.. Tea is also grown at various altitudes ranging from sea level to about 2300 m above sea level in Kenya, Malawi, Japan and Indonesia. Tea cultivation is now beginning to occur in Australia and New Zealand as well.

2.2.1 Climate

Tea is grown in conditions ranging from Mediterranean type climates to the hot humid tropics (Carr and Stephens, 1992). Tea plants require a warm humid climate. An annual rainfall of 2500 - 3000 mm with even distribution throughout the year, without marked seasonality is the ideal condition. The minimum annual rainfall requirement is 1200 mm (Watson, 1986). The ideal ambient temperature (mean monthly) is considered to be 18 - 25^0 C. Mean monthly temperatures lower than 13^0 C (average for the coldest month) or higher than 30^0 C (average for the hottest month) will affect tea production. It certainly cannot withstand frost. It varies in its ability to withstand wind (Watson, 1986). Tea growing areas in Sri Lanka are classified into three main groups based on altitude (Watson, 1986). The areas under 600 m altitude are referred to as low-country, and those above 1200 m are categorised as up-country. The areas between 600 m and 1200 m are called mid-country. These areas are further subdivided into wet, intermediate and dry zones based on the amount of annual rainfall.

Table 2.2 The profitability of tea cultivation in some tea growing countries (Kelegama et. al., 1995)

Index	**Unit**	**Sri Lanka**		**India**		**Kenya**
		State estates	**Private estates**	**North**	**South**	
Plucker intake	kg day^{-1}	13.52	24.59	26.22	25.24	48.00
Labour ha^{-1}	No.	3.21	2.70	2.67	2.50	2.20
Cost of production	US \$ kg^{-1}	1.87	1.54	1.52	1.39	0.94
Revenue	US \$ ha^{-1}	2574	4957	4318	4669	4338
Gross profit	US \$ ha^{-1}	203	1196	1085	1472	2438

2.2.2 Soils

2.2.2.1 Soil mineralogy and classification

Tea grows in a wide range of soil types in tropical, sub-tropical and temperate climates, and the soils are derived from diverse parent materials (Eden, 1976; Mann, 1935). The mineralogy and the type of soils differ greatly not only between countries, but also within a country (Table 2.3). Most tea soils are highly weathered and strongly acidic and the major clay minerals present in these soils are kaolinite, gibbsite and geothite. These soils are broadly categorised as Ultisols or Oxisols according to the US soil taxonomy because they are in the advanced stage of weathering.

2.2.2.2 Physico-chemical properties

Soils of diverse origin and different morphological characteristics support viable tea cultivation in different countries (Child, 1953; Eden, 1976; Harler, 1971; Mann, 1935). Generally tea prefers a deep, permeable and well drained soil. In a given locality, soil characteristics such as soil depth <50 cm, gravelliness >50% and rockiness >20% impose severe limitations for successful growth of tea (Watson, 1986).

Some important chemical properties of the tea growing soils in different countries are given in Table 2.4. The most important soil chemical property for the good growth of tea is optimum pH. Generally the soil pH (H_2O), where tea is cultivated, varies from 3.3 - 6.0. The optimum range of soil pH (H_2O) for tea plants is 5.0 - 5.6 (Othieno, 1992). The maintenance of soil pH between 4.5 - 5.5 is preferred in Sri Lanka (Anon, 1989). Tea is considered as a calcifuge and does not grow well in soils of high base saturation. However it still needs a certain amount of Ca for satisfactory growth and for maintenance of high yield levels (Eden, 1976; Ranganathan and Natesen, 1985). Tea is also known to take up large quantities of Al (Chenery, 1955; Foy et al., 1978), which is relatively easily available in most acid soils where tea is cultivated.

Table 2.3 Mineralogy and classification of tea soils of the world

Country	Main soil types or soil classification	Clay minerals	Reference
India Darjeeling district Cachar district South India	Sedimentary Peaty Latosols	Kaolinite, some illite and montmorinolite.	Ramanathan and Krishnamoorthi (1979), Subramanian and Mani (1981)
Sri Lanka	Red-yellow podzolic, Reddish-brown latosolic, Immature brown loam.	kaolinite, gibbsite, geothite, small amount of illite.	De Alwis and Panabokke (1972)
Bangladesh	Alluvial	kaolinite, small amounts of mica and gibbsite.	Karim et al. (1981)
East Africa	Latosolic		Scott (1962)
Malawi	Alluvial		Ranganathan and Natesen (1985)
Kenya	Volcanic ash		Ranganathan and Natesen (1985)
Tanzania	Volcanic ash		Ranganathan and Natesen (1985)
Russia	Red soils, Podzols.		Jourbitzky and Strausberg (1966), Dey (1972)
Taiwan	Reddish-brown, lateritic, Red-yellow podzolic, Yellow brown earth.		Chu (1975)
Japan	Volcanic ash, Red yellow soils.		Ranganathan and Natesan (1985)
China	Red soils		Ranganathan and Natesan (1985)

Table 2.4 Chemical properties of some major tea-growing soils of the world

Country (area)	Soil depth (cm)	pH (water) (1:2.5)	Organic C%	Total N%	C/N	Total P ($\mu g\ g^{-1}$ soil)	CEC ($cmol_c\ kg^{-1}$)	Ex. bases ($cmol_c\ kg^{-1}$) K	Mg	Ca	Available-P[1] ($\mu g\ g^{-1}$ soil)	Reference
India												
Assam	0-30	4.7	1.7	0.10	10		5.8	1.0	0.4	0.8	15	Dey (1969)
Anamallais	0-15	4.9	7.5	0.28			11.2	0.5	1.7	8.8	22	Ranganathan (1976)
Kenya												
Kericho	0-5	5.1	8.5	0.60	8			2.1	2.5	0.9	14	Othieno (1973)
	5-10	4.5	8.0	0.58	8			1.9	1.4	0.6	8	Othieno (1973)
	10-15	4.6	7.1	0.50	8			1.7	0.7	0.2	7	Othieno (1973)
	0-23	4.7	3.7	0.19			20.8	3.1	2.0	0.5	4	Dey (1969)
Malawi	0-23	5.3	3.0	0.17	11		2.7	1.2	1.3	2.5	14	Dey (1969)
Sri Lanka												
St. Coombs	0-15	4.5	2.6	0.22	12	500	10.1- 13.3	0.2	0.1	0.6	73	Jayman and
Hantana	0-15	4.4	1.4	0.13	11	500	5.0 - 7.2	0.1	0.2	1.0	170	Sivasubramaniam
Passara	0-15	3.9	3.1	0.20	15	400	1.1 - 1.9	0.1	0.1	0.3	19	(1981),
Kottawa	0-15	5.2	1.6	0.13	13	80	3.4 - 3.9	0.2	0.1	0.2	17	Wickremasinghe
Ratnapura	0-15	4.7	1.7	0.13	14	700	1.3 - 2.6	0.1	0.1	0.1	25	(1986).
Taiwan	1st horizon	4.4	1.5	0.09	10			0.2	0.1	1.7	2	Chu (1975)

[1]The available P in Sri Lankan soils refers to Borax-extractable P (Beater, 1949); methods used were not reported for other countries.

Most tea soils are highly weathered and contain an abundance of variable charge colloidal minerals, such as oxides and hydrous oxides of Fe and Al and 1 : 1 type clay minerals. The soils are generally low in CEC (usually <10 $cmol_c\ kg^{-1}$). The soils have low base saturation and high Al saturation (often >40%). The excess weathering in these soils reduce plant-available P levels in the soil solution, because of high P sorption capacities associated with high contents of Fe and Al oxides (Sanchez, 1976). The overall effect is that it leads to an infertile medium for plant growth. Therefore the soils need to be managed properly to maintain a high fertility status, which will lead to high tea yields.

2.3 SOIL PHOSPHORUS

2.3.1 Plant available P

Tea meets its' P requirements from native soil P sources and from the added P of fertilisers. Among the macro-nutrients, P is the most susceptible to fixation by highly weathered acidic soils. Crops usually remove proportionately less P from added P fertiliser or native soil P in the short-term compared to other nutrients. In acid soils phosphorus fixation occurs by the removal of P from the soil solution either by sorption on Fe and Al oxides, or precipitation reactions with soluble Fe and Al, which are abundant in acid soils (Golden et al., 1981). Generally P availability declines rapidly as soil pH falls below 5.0 (Apthorp et al., 1987; Fox, 1979; Parfitt, 1977; Sanchez, 1976).

Plant-available P concentrations in tea soils vary widely with location, reflecting changes in soil and management practices (Table 2.4). It is difficult to compare the available soil P values reported in the literature as the methods of extraction are different from one country to the other. The plant-available soil P concentrations in Sri Lankan tea soils (0 - 15 cm) as determined by borax extraction (Beater, 1949) varies from 17 - 170 $\mu g\ g^{-1}$ soil (Wickremasinghe, 1986). These soil P levels are found to be significantly higher than the P in adjoining forest soils (0.9 - 8.7 $\mu g\ g^{-1}$ soil) and

result from continuous additions of P fertilisers to tea plantations over a long period of time (Wickremasinghe, 1986).

The P in soils is generally considered to be made up of the following three fractions, which are in dynamic equilibrium as shown in Equation 2.1 (Mengel and Kirkby, 1987):

Soil solution P $\Leftrightarrow$ Labile P $\Leftrightarrow$ Non-labile P ..... [Equation 2.1]

The soil solution P fraction is the phosphate dissolved in soil solution and it is the immediate source of P for plant uptake by roots. The labile P fraction is the solid phosphate, which is held on soil surfaces, in rapid equilibrium with soil solution phosphate. It consists of freshly precipitated Fe and Al phosphates and P adsorbed to the surface of soil minerals. It can be determined by means of isotopic exchange (Larsen, 1967). The non-labile-P is mostly composed of organic P and various mineral P compounds that are rather resistant and may release P only very slowly into the labile P pool. These definitions are very broad and it is extremely difficult to distinguish clearly between these pools from the P fractionation procedure.

Phosphorus may enter into the soil solution by desorption of P_i (inorganic-P) associated with the solid phase, by mineralisation of P_o (organic-P) or dissolution of lattice P (apatite). The phosphate concentration in the soil solution itself is very low and in fertile arable soils is about 10^{-5} - 10^{-4} *M* which is equivalent to about 0.3 - 3 μg ml^{-1} (Hossner et al., 1973). Fox et al. (1974) showed that most annual crops require 0.2 μg P ml^{-1} in soil solution for optimum growth. To achieve this concentration in Sri Lankan Ultisols the P adsorbed to the soil colloids was determined to be 300 to 600 μg P g^{-1} soil (Loganathan and Fernando, 1980). Thus the amount of P in soil solution in these soils is about 3000 to 6000 times less than that in the labile P fraction at field capacity moisture content. In acid soils, soil solution P exists as HPO_4^{2-} and $H_2PO_4^-$. The ratio of these two ionic species in the soil solution is strongly pH dependent. The increase of H^+ concentration shifts the equilibrium to the more protonated form according to Equation 2.2.

$HPO_4^{2-} + H^+ \Leftrightarrow H_2PO_4^-$ ....[Equation 2.2]

2.3.2 Characterisation of soil-P forms

The concentration of the various soil P fractions can be determined by using sequential extraction methods, which removes labile P first followed by the more stable P forms. A widely used sequential extraction method was developed by Chang and Jackson (1957). This sequential procedure is based on consecutive extractions with different chemical reagents, each designed to remove a particular form of P. It employs NH_4Cl to extract labile-P_i followed by NH_4F to dissolve specifically P associated with Al. This is followed by a NaOH extraction to dissolve Fe-bound P_i, and finally dithionite-citrate extraction to dissolve occluded P_i forms. HCl is used to dissolve Ca-bound P_i and the final residue is analysed by Na_2CO_3 fusion for the determination of total P. This procedure presented many interpretational problems such as the precipitation of P_i during fluoride extraction, the separation of Al and Fe associated P_i, and the ill-defined nature of the reductant-soluble or occluded P (Williams and Walker, 1969). A modified P fractionation was developed by Hedley et al. (1982b) and a general description of the various soil P fractions separated by this method is shown in Table 2.5. This sequential extraction aimed at quantifying labile-P (plant available-P), Fe + Al-associated P_i, Ca-associated P_i, as well as labile and more stable forms of P_o.

No information is available in the literature on the concentration of inorganic P fractions in tea soils determined according to the procedure of Hedley et al. (1994) but information on P fractionation according to the Chang and Jackson (1957) method is available and this is presented in Table 2.6. The results reveal that the majority of P in tea soils is found as organic-P and Fe and Al bound P (Bhattacharyya and Dey, 1978; Golden et al., 1981; Yongming et al., 1989). The concentrations of the Fe-P fraction in all soils were higher than the Al-P concentrations. These fractions are traditionally considered as plant unavailable and very little is known with respect to the relative importance of these fractions in supplying P to tea. The magnitude of H_2SO_4-P_i (apatite-P) in tea soils depends on the history of PR fertiliser application

Table 2.5 The chemical nature of the various soil P fractions (Hedley et al., 1982b)

P fraction	**Chemical nature of P**
Resin-P	Inorganic P in solution and weakly sorbed P
$NaOH\text{-}P_i$	Inorganic P associated with Fe and Al hydrous oxides
$NaOH\text{-}P_o$	Organic P associated with Fe and Al hydrous oxides
$H_2SO_4\text{-}P_i$	Predominantly calcium phosphates or "apatite like P"
Residual-P	Recalcitrant inorganic and organic P

Table 2.6 Phosphorus fractions (μg P g^{-1} soil) in tea soils of different countries determined according to the method of Chang and Jackson (1957)

Country	Al-P	Fe-P	Ca-P	Organic P	Reductant soluble P	Solution P	Total P	Reference
Sri Lanka								Golden et al. (1981)
St. Coombs (up country)	59	164	48	344			615	
Kottawa (low country)	30	36	18	148			232	
India[1]								Dey and Bhattacharyya (1980)
Upper Assam	45	98	54	186	225		608	
Mid and Lower Assam	39	77	52	129	169		466	
Cachar	40	68	47	211	174		540	
Dooars	128	182	202	355	292		1159	
Darjeeling	63	132	89	392	314		990	
China[2]								Yongming et al. (1989)
Yinde	68	199	31	183		3.0	484	
Nanchang	11	109	19	258		0.8	398	
Guiyang	5	83	8	208		0.4	304	
Langxi	5	40	6	100		0.8	152	
Changsha	24	305	19	283		3.8	635	

[1] The P fractions for Indian soils were reported in units of kg ha^{-1}. These units were converted to μg P g^{-1} soil assuming that the bulk density of soil = 1.1 Mg m^{-3}.

[2] P fractions reported are for medium yielding tea plantations.

(rates and duration of application and on the length of time taken following the application of phosphate rock prior to the collection of soil samples for analysis) because it mainly extracts the undissolved phosphate rock in these highly acid soils. The residual P fraction is less important from the plant nutrition point of view, because it is very resistant to further decomposition and P release to plants.

2.3.3 The concept of plant-available P

Since plant-available P is more a fundamental concept than a measurable quantity, its determination in soil is not a straight forward task. Only a measure of P that relates to the pool of P that is plant available can be measured. Such relationships have been developed by regressing measured soil P concentrations against plant yields or plant P concentration values for various crops (Chien et al., 1990a; Loganathan and Nalliah, 1977; Naidu et al., 1991) including tea (Jayman and Sivasubramaniam, 1980). The plant available P is a very small percentage of the total soil P pool and it is related to the quantity of P that can be potentially utilised by crops growing in the soil. Generally "extractable" may be a term preferred to "plant available" because the latter term implies that a certain quantity of P is present in a soil and can be absorbed by plants at a particular time. Practically this is not the case, because the quantity of P utilised by plants growing in a soil may be greater or less than that of the concentration of extractable P on a sample of soil collected at a particular time. However soil testing procedures can provide an accurate "relative index" of the quantity of P that plants may utilise from a soil, but really, can not provide an absolute measure of it. Therefore the term "extractable" is rather more appropriate than "available" soil P when plant P uptake is considered.

Tea plants may differ from other crops in their ability to extract P from soils due to differences in their root system caused by clonal variations, VAM (vesicular arbuscular mycorrhizae) associations, growth rates and root secretion of organic acids etc:. As tea is adapted to acidic conditions in highly weathered soils containing P-fixing soil minerals it is believed to have specific mechanisms by which it is able to utilise P that is normally considered non-available to other crops.

2.3.4 Soil tests for plant-available P

An agronomic test for plant-available P should be designed to be simple enough for routine application. It should extract sufficient P from the plant-available pool and at the same time it should not extract significant amounts of P that are not plant-available. The principle used to achieve this is by using moderately acidic or alkaline extractants, which are able to release P that is not strongly sorbed to the soil mineral phase. Specific anions are introduced to compete with P for sorption sites, or alternatively to decrease the solubility of cations (e.g. Al, Fe) that bind P to the soil.

The most common methods used to determine plant-available P are the alkaline bicarbonate extraction method of Olsen et al. (1954) developed for alkaline soils and the acid ammonium fluoride extraction method of Bray and Kurtz (1945) developed for acid soils. The bicarbonate extraction method, though developed specifically for alkaline soils, has been used successfully on a wide range of both acidic and alkaline soils for several crops having widely different growth characteristics (Loganathan et al., 1982; Smyth and Sanchez, 1982a). A rationale for the use of the bicarbonate anion as an extractant is that plant roots produce CO_2 which forms bicarbonate in the soil solution of calcareous soils. Therefore bicarbonate simulates the action of plant roots, thus giving a more appropriate measure of plant-available P.

The acid ammonium fluoride method of Bray and Kurtz (1945) has been widely used on acid and neutral soils (Fixen and Grove, 1990; Tiessen and Moir, 1993). The relatively low acid strength and the extraction mechanism make this method unsuitable for calcareous or strongly alkaline soils, which would partially neutralise the acidity and eliminate the standard test conditions. There are two Bray and Kurtz extraction methods ; Bray-1 uses 0.03 *M* NH_4F + 0.025 *M* HCl (1 : 7 soil : solution) with a 5 minute shaking period and Bray-2 uses 0.03 *M* NH_4F + 0.1 *M* HCl (1 : 7 soil : solution) with 30 minutes shaking (Bray and Kurtz, 1945). Bray-1 test extracts mostly Al bound P whereas the Olsen test removes in addition to Al bound P, Fe bound P (Le Mare, 1991) and some organic-P. Therefore, the Olsen method may provide higher P values in acid soils compared to the Bray-1 test, especially in tea soils which have higher organic-P and Fe-P than Al-P concentrations (Table 2.6).

In another approach to determining plant-available P, a sink for solution P_i in the form of an anion-exchange resin is used to extract P from soil : water suspensions. The P sorbed by the resin is subsequently desorbed using NaCl and measured. The amount of P measured is assumed to simulate root extraction of P from soil. Several different methods of resin extraction have been developed and tested, using different anionic forms, soil : water : resin ratios and times and methods of shaking, enclosure in bags or mixing with the soil suspension (Barrow and Shaw, 1977). A simplest method uses Teflon based anion and cation exchange resin membranes, which can be cut into strips and used repeatedly (Saggar et al., 1990). This method has shown to be superior to the Olsen method in predicting P availability from pasture fertilised with phosphate rocks (Saggar et al., 1992a)

Soil P tests used in tea soils are mostly the tests that are suitable for acidic soil conditions. Bray-1 extraction method was reported to be used in India (Dey and Bhattacharryya, 1980) and China (Yongming et al., 1989) for routine analysis of plant-available P and borax extraction at pH 1.5 is currently being used in Sri Lanka (Jayman and Sivasubramaniam, 1980). Jayman and Sivasubramaniam (1980) reported that Borax-P was highly correlated with leaf P concentration in tea but P extracted by other extractants failed to correlate with leaf-P concentration. They however did not report what the other extractants were (see later discussion on the borax test in Chapter 7).

2.4 PHOSPHORUS NUTRITION OF TEA WITH SPECIAL REFERENCE TO SRI LANKA

When compared with nitrogen (N), P nutrition of tea plants received scant attention in the past. Although the quantity of N required by tea is more than any other soil nutrients, sub-optimal levels of P in soil can cause reductions in tea yields (Eden, 1976). As P is very mobile within the plant and translocated to the sites where photosynthetic activity is high, it is found in high concentrations in young shoots in tea plants. These young shoots are regularly harvested at 4 - 10 day intervals in commercial plantations and consequently tea removes large amounts of nutrients (40

kg N, 13 kg K and 4 kg P per 1000 kg^{-1} processed tea) from the soil. The quantity of nutrients assimilated by different parts of the tea plant in Sri Lanka is shown in Table 2.7. A comparison of P removal by tea with other perennials and a range of annual crops is presented in Table 2.8. Tea removes less P per ha compared to most short-term agricultural crops (annuals) because the short-term crops need large amounts of P in a short period for their rapid growth. The other perennial crops reported in Table 2.8 however removed similar or less amounts of P compared to tea with the exception of sugarcane and coconut. Sugarcane removes a large amount of P from soil due to its higher rate of bio-mass production compared to the other perennials.

2.4.1 Chronology of P fertiliser use on tea in Sri Lanka

The importance of P fertilisers in tea cultivation was realised quite early in Sri Lanka (Eden, 1934). As early as 1896 some tea growers realised that fertiliser application increases yield. At early stages the main fertiliser sources were of organic origin i.e. oil seed cakes and bones (Eden, 1934). Later, fertiliser use became very popular, but it did not appear to be properly organised, and fertilisers were applied at most biennially (Eden, 1934).

The first statistically designed field experiment on tea in Sri Lanka was laid down in 1931 by Eden (Eden, 1934). The objective of the experiment was to determine the yield responses of mature tea plants to varying levels of N, P and K using two 3^3 factorial experiments. The findings of these experiments led him to recommend NPK fertiliser mixtures for Sri Lankan tea plantations. Subsequently these fertiliser mixtures were changed from time to time by other workers based on other field and glasshouse trial results. The chronological order of the fertiliser recommendations for tea in Sri Lanka with special reference to P is presented in Table 2.9.

The fertiliser mixtures used for tea in Ceylon (now Sri Lanka) before 1930s' were a pruning mixture high in N and P and a general mixture high in N. The latter was applied for subsequent manuring operations in the pruning cycle. At that time the use of large quantities of P and K in the pruning mixture was considered important for

Table 2.7 Mean dry matter distribution and nutrient removal by 100 kg processed tea and [1]per ha yr^{-1} of land in Sri Lanka (after Eden, 1949)

Organ	Mean dry matter distribution (%) in the plant	N		P		K	
		kg per 100 kg processed tea	kg ha^{-1} yr^{-1}	kg per 100 kg processed tea	kg ha^{-1} yr^{-1}	kg per 100 kg processed tea	kg ha^{-1} yr^{-1}
Tips[2]	3						
Flush[3]	21	4.0	71	0.4	6	1.3	23
Foliage	23	2.7	48	0.2	3	1.1	19
Wood	53	2.4	42	0.3	5	1.5	27
Total	100	9.1	161	0.9	14	3.9	69
Permanent removal		6.4	113	0.7	11	2.8	50

[1] Processed tea yield of 1761 kg ha^{-1} yr^{-1} (national average) was used in the calculation

[2] young shoots that are removed at the beginning of the pruning cycle to maintain an easy and even plucking table for harvesting

[3] bud and two leaves removed at harvest

Table 2.8 Comparison of P removal by different agricultural and tree crops

Species	Age (yr)	grain/dry matter yield (kg ha^{-1}) or dry matter accumulation (kg ha^{-1} yr^{-1})	P removal by plants from soil per year or cropping season (kg ha^{-1})	Reference
Annual crops				
Corn	<1	5,000	15	Hanway and Olson (1980)
Sorghum	<1	4,000	10	Hanway and Olson (1980)
Soybean	<1	1,800	13	Hanway and Olson (1980)
Wheat	<1	2,400	9	Hanway and Olson (1980)
Oats	<1	1,600	7	Hanway and Olson (1980)
Barley	<1	1,900	7	Hanway and Olson (1980)
Peanut	<1	2,763	9	Nelson (1980)
Rice	<1	4,980	13	Nelson (1980)
Tobacco	<1	1,120	15	Panabokke (1967)
Forest Trees				
Radiata pine	0-10	17,000	3.5	Will (1968)
Radiata pine	10-35	15,600	0.5	Will (1968)
Loblolly pine	10-15	7,000	1.3	Switzer and Nelson (1972)
Jack pine	20-30	2,900	0.2	Foster and Morrison (1976)
Scots pine	20-30	2,400	1.3	Malkonen (1974)
Plantation crops				
Mature tea		1,000	6.8	Eden (1949)
Rubber		1,120	3.4	Panabokke (1967)
Coconut[1]		3,000	10.3	Panabokke (1967)
Sugarcane		54,300	27	Nelson (1980)

[1] yield refers to nuts with husk

Table 2.9 Chronology of P fertiliser recommendations for tea in Sri Lanka

Year	Composition of fertiliser mixture	composition (parts by weight)	N P K equivalent kg ha^{-1} yr^{-1} N	P	K	Reference
Pre-1930	**Pruning mixture**					
	Fish guano	220				
	Blood meal	70				
	Nitrate of potash	80	35	29	24	Eden (1934)
	Phosphate rock	80				
	Concentrated superphosphate	50				
Pre-1930	**General mixture**					
	Ground nut cake	200				
	Fish guano	200				
	Blood meal	100	68	17	23	Eden (1934)
	Ammonium sulphate	100				
	Muriate of potash	50				
	Superphosphate	100				
1946	**T_{500} basic mixture**					
	Ground nut cake	430	34	9	6	Norris (1946)
	Saphos phosphate[1]	60				
	Muriate of potash	10				
1952	**T_{500} fertiliser mixture**					
	Ammonium sulphate	320	74	15	35	Lamb (1952)
	Saphos phosphate	105				
	Muriate of potash	75				

[1] a blend of phosphate rocks from Egypt (12.1% total P)

Table 2.9 continued

Year	Composition of fertiliser mixture	composition (parts by weight)	N P K equivalent kg ha^{-1} yr^{-1} N	P	K	Mg	Reference
1961	**T_{700} fertiliser mixture**						
	Ammonium sulphate	500	103	12	50		Tolhurst (1961a)
	Saphos phosphate	100					
	Muriate of potash	100					
1961	**T_{725} fertiliser mixture**						
	Ammonium sulphate	500	103	12	62		Tolhurst (1961a)
	Saphos phosphate	100					
	Muriate of potash	125					
1961	**T_{750} fertiliser mixture**						
	Ammonium sulphate	500	103	12	75		Tolhurst (1961a)
	Saphos phosphate	100					
	Muriate of potash	150					
1961	**T_{200} fertiliser mixture**		1st yr				
	Ammonium sulphate	100	124	36	74	22	Tolhurst (1961b)
	Saphos phosphate	50	2nd yr				
	Muriate of potash	25	155	45	93	27	
	Kieserite	25					
1983	**T_{750} fertiliser mixture**		3-4th yr				
	Ammonium sulphate	500					Wickremasinghe and Krishnapillai (1986)
	Saphos phosphate	100	240	27	115	17	
	Muriate of potash	100					
	Kieserite	50					

Table 2.9 continued

Year	Composition of fertiliser mixture	composition (parts by weight)	Processed tea Yield slab kg ha^{-1} yr^{-1}	N P K equivalent kg ha^{-1} yr^{-1} N	P	K	Reference
1983	U_{346} Urea Eppawala phosphate rock (14.5% total P) Muriate of potash	 174 72 100	< 800	80	9	50	Wickremasinghe and Krishnapillai (1986)
1983	U_{709} Urea Muriate of potash Eppawala phosphate rock	 438 103 168	 800-1000 1800-2000 2500-3000	 120 220 300	 8 16 22	 50 90 124	Wickremasinghe and Krishnapillai (1986)

recovery of the tea bush after pruning. Application of superphosphate at the rate of 49 kg P ha^{-1} yr^{-1} was on record at that time (Eden, 1934). During the period of the 2nd world war, inorganic fertiliser imports were affected and the use of NPK fertiliser mixtures got drastically reduced. A basal fertiliser mixture T_{500} containing low levels of K and P compared to N was introduced during that time (Norris, 1946). This mixture was used till 1950s and it resulted tea crops in some estates becoming K deficient (Portsmouth, 1953). After the war, regular supply of NPK fertilisers for tea was restored. A balanced NPK fertiliser mixture to replace the amounts of nutrients removed by the harvested crop was given priority at that time. Eden (1949), by considering the relative distribution of plant nutrient concentrations in tissues and the dry matter weights of the tissues estimated the amounts of NPK removed in 100 kg of tea crop (Table 2.7). He assumed that the flush and wood were permanently removed at the end of the cycle. On that basis a well managed privately owned tea land in Sri Lanka with an average yield of 2442 kg ha^{-1} yr^{-1} of processed tea (Table 2.1) was estimated to remove 156 kg N, 17 kg P and 70 kg K ha^{-1} yr^{-1} at harvest. Thus the amount of P that is removed from the soil is rather low compared to the other major nutrients.

In 1952, Lamb (1952) proposed a new fertiliser mixture T_{500} on the basis of nutrient removal by the tea crop. At that time it was felt that fixation of phosphate into insoluble forms could be a serious problem. As a remedy, saphosphosphate (a blend of Egyptian phosphate rocks) in the mixture was provided in double the quantity of P that was likely to be removed in the crop and prunings. Afterwards, Tolhurst (1961a) found that phosphate fixation cannot be a serious problem in tea soils and it did not limit P supply to tea plants. He introduced a fertiliser mixture T_{700}, which was still above the replacement level for P in crop and prunings, but lower than that provided by T_{500}. He recommended T_{700} for up-country tea soils which supplied 12 kg P and 50 kg K for every 100 kg of N applied. He suggested modified fertiliser mixtures with higher proportions of K for mid and low country tea soils to arrest K deficiency caused by leaching loses which are common in these soils. These fertiliser mixtures were T_{725} and T_{750} having 25 and 50% more K than T_{700}.

The need for a young tea fertiliser mixture came to light with the popularity of VP clonal tea plants, which are markedly fertiliser responsive. To obtain high growth rates and best yields Tolhurst (1961b) recommended T_{200} mixture containing higher levels of P and K in relation to N for young tea because VP plants were reported to have a higher yield potential associated with higher fertiliser responsiveness compared to seedling tea (Anandappa, 1986). Subsequently, based on the results of factorial NPK fertiliser trials and economics, Tolhurst (1965) suggested that P could be further reduced to 10 kg P ha^{-1} yr^{-1} or even omitted for one or two years. This may be due to annual application of P fertiliser over long period, which led to a build-up of P reserves in these soils. Fernando et al. (1969) also showed that for high yielding tea clones, P and K need not be increased in the same proportion as N. They recommended application rates of 9 and 13 kg P ha^{-1} yr^{-1} for seedling and clonal tea respectively.

In 1960's sterameal (an organic fertiliser) was used to supply P for tea nurseries. Visser and Khel (1961) compared the efficiency of inorganic P mixtures (superphosphate) with that of organic mixtures containing sterameal having the same composition of N, P and K on nursery tea plants. They found that there was no significant difference between the organic and inorganic fertiliser treatments in tea growth and reported that the inorganic mixture was preferable due to its cost-effectiveness and easiness of application in nurseries. Subsequently, Tolhurst and Visser (1961) found that having superphosphate in the mixture was a disadvantage due to its reactivity with soluble ammonium sulphate and potassium sulphate to form an insoluble residue of calcium sulphate. This caused several practical difficulties, including fertiliser storage and deposition of fertiliser residues on the foliage. They remedied this by substituting monoammonium phosphate for superphosphate and recommended a completely soluble nursery mixture T_{65} (Table 2.10). This mixture was found to be very effective for tea nursery plants and is still widely accepted among tea growers in Sri Lanka. A few years later due to a shortage of monoammonium phosphate in the country, Tolhurst and Richards (1965) revised T_{65} nursery mixture and recommended T_{55} instead (Table 2.10). They suggested mixing of superphosphate with soil at the rate of 222 - 446 g m^{-3} of nursery soil to supply the

Table 2.10 The fertiliser mixtures used for tea nurseries in Sri Lanka

Year	Composition of fertiliser mixture	Composition (parts by weight)	Nutrient composition (%) of the mixture (N:P:K:Mg)	Reference
1961	**T_{65}**			
	Ammonium sulphate	15	10.9 : 4.71 : 9.17 : 2.21	Tolhurst and Visser (1961)
	Monoammonium phosphate	20		
	Potassium sulphate	15		
	Epsom salt	15		
1961	**T_{55}**			
	Ammonium sulphate	35	13.11 : 0 : 7.21 : 1.74	Tolhurst and Richard (1965)
	Potassium sulphate	10		
	Epsom salt	10		
1977	**T_{57}**			
	Ammonium sulphate	30	10.84 : 0 : 9.04 : 2.35	Ayadurai and Sivasubramaniam (1977)
	Potassium sulphate	13		
	Epsom salt	14		

basal requirement of P for tea at the nursery, followed by spraying a solution of T_{55} containing N, K and Mg.

In 1977, Ayadurai and Sivasubramaniam (1977) formulated a nursery mixture T_{57} with the same components as in T_{55} but with higher proportions of K and P than in T_{55}. Though Tolhurst and Richards (1965) recommended mixing of triple superphosphate along with T_{55} as an alternative to T_{65}, Ayadurai and Sivasubramaniam (1977) showed that mixing Eppawala phosphate rock (EPR, a locally mined apatite deposit; see section 2.5) with T_{57} is a better substitute than the former because EPR was cheaper. Furthermore they did not notice any significant difference between the soluble P fertiliser and EPR on dry matter and P uptake in tea plants.

Until the end of 1970s, the main source of N fertiliser used for tea in Sri Lanka had been $(NH_4)_2SO_4$. Field experiments comparing the effect of different forms of N fertilisers on tea yield had shown that there was no significant yield difference between urea and $(NH_4)_2SO_4$ treatments (Bhavanandan and Manipura, 1969; Sandanam et al., 1980; Watson and Wettasinghe, 1972). Based on these results, urea based fertiliser mixtures (U_{346} and U_{709}) were recommended for mature tea (Wickremasinghe and Krishnapillai, 1986). During the period of the 1970s a urea plant was built in Sri Lanka, so urea was available locally. The use of locally produced urea was found to be much cheaper than the imported $(NH_4)_2SO_4$. Studies were also undertaken to test the local EPR as a source of P for tea. Sivasubramaniam et al. (1981) examined the chemical characteristics of this phosphate rock and found that it contained a higher total P content (14.5%) than the imported phosphate rock (saphosphosphate) (12.1%) (See section 2. 5). Subsequently EPR was recommended as the source of P in urea based fertiliser mixtures for tea (Sivasubramaniam et al., 1981).

2.5 EPPAWALA PHOSPHATE ROCK (EPR)

EPR is now an important source of P for tea plantations. For this reason the nature of EPR and factors affecting the plant availability of P from PRs are reviewed. In 1971 the Geological Survey Department of Sri Lanka discovered a phosphate rock deposit, estimated to be about 40 million tonnes at Eppawala in the North Central Province of Sri Lanka (Jayawardena, 1976). The weathering of the apatite rich parent carbonate rocks and associated gneisses and migmatites (rocks consisting of thin alternating layers or lenses of granite type and schist) at this site has given rise to a thick non-carbonate and phosphate rich weathering profile (Dahanayake et al., 1995). This profile is being mined to exploit its phosphate ore. It has two dominant minable components that can be recognised with the naked eye.

(i) greenish primary apatite crystals and
(ii) brownish aluminous-ferruginous-siliceous secondary phosphate matrix.

The X-ray diffraction analysis of the primary apatite crystals reveals the presence of hydroxyl chlorapatite with unit cell *a*-axis dimensions ranging from 9.46 Å to 9.80 Å. Fluorapatite or Francolite (carbonate apatite with unit cell *a*-axis values from 9.35 Å to 9.37 Å), a more soluble variety resulting from weathering, is also found within and around the primary apatite crystals. The primary crystals have P contents varying from 15.3-18.3%. Their solubilities in 2% citric acid varies from 2.3 to 2.5% P. Neutral ammonium citrate (NAC) solubility varies from 1.0 to 1.2 % P (Dahanayake et al., 1995).

The aluminous-ferruginous-siliceous secondary phosphate matrix (dominantly formed of fluorapatite with accessory hydroxyl apatite, crandallite, geothite, hematite, ilmenite, laterite and quartz) constitutes about 30 to 60% of the total deposit

depending on the location of the deposit. The P content in the secondary phosphate matrix can vary from 0 to14.5%(Dahanayake et al., 1995). The solubility of this matrix in 2% citric acid varies from 1.5 to 2.1.

The product available in the market is a mixture of the primary apatite crystals and the secondary phosphate matrix. The chemical composition of these two fractions is given in Table 2.11.

2.6 FACTORS AFFECTING P AVAILABILITY FROM PR

2.6.1 Characteristics of PRs

McClellan and Gremillion (1980) classified PRs into three broad classes according to their mineralogical composition; Fe-Al phosphates, Ca-Fe-Al phosphates and apatite group of minerals. In terms of the weathering sequence, Fe and Al phosphates are often the most weathered stable end products in a sequence, wherein apatites are the least weathered (Khasawneh and Doll, 1978). The apatites in igneous and metamorphic PR deposits are relatively inert, being coarse-grained with few internal surfaces. They contain fewer impurities as accessory minerals. Thus, the P content of these deposits is relatively high. This factor is important in the production of soluble and partially soluble P fertilisers from PR, but is of no concern when determining the reactivity of the PR when applied directly to soil as fertiliser. Sedimentary PRs, on the other hand, contain apatite minerals that are microcrystalline. They consist of fairly open, and loosely consolidated aggregates of microcrystals with relatively large specific surface areas. Usually they are carbonate apatites and also contain varying amounts of accessory minerals (Khasawneh and Doll, 1978).

The PRs of sedimentary origin are chemically more active than those of igneous and metamorphic origin (Sanyal and De Datta, 1991) and so can be used directly as a fertiliser on acid soils receiving high rainfall (White et al., 1989). The effectiveness of PRs differs widely in chemical reactivity or solubility in acid soils due to differences in mineralogy and chemistry. Isomorphous substitution of a divalent planar CO_3 group for a trivalent tetrahedral PO_4 group creates a charge imbalance and a packing void, which weakens the crystal structure of apatite. It decreases the *a* value from 9.37 Å, which is a characteristic of pure fluorapatite. This results in a reduction of crystal size and an increase in the specific surface area of the apatite. As the number of moles of

Table 2.11 Chemical analysis of the primary apatite and aluminous-ferruginous-siliceous phosphate matrix of Eppawala phosphate rock deposit (Dahanayake et al., 1995)

Composition (%)	Primary apatite	Matrix
P	17.69	14.51
Ca	38.68	31.16
F	2.03	2.07
Si	0.08	0.07
Al	0.02	0.77
Fe	0.22	8.36
Na	0.09	0.15
K	<0.01	<0.01
Mg	0.06	0.10
Cl	1.86	1.12
Total S	0.27	0.20
Mn	0.01	0.18
Ti	<0.01	0.62
(Al + Fe) = R	0.24	9.13

OH or CO_3 per mole of PO_4 increases, the standard free energy of reaction of the apatite with acidic solutions becomes more negative, and its dissolution increases (Bolan et al., 1993; Chien, 1977; Khasawneh and Doll, 1978; McClellan and Gremillion, 1980). Therefore the degree of CO_3 substitution is considered to be one of the important factors which determines the solubility of apatite and the dissolution rate of PR in acid soils (Caro and Hill, 1956). The most reactive PRs are those having a molar PO_4/CO_3 ratio less than 5 (Hedley et al., 1990).

As the extent of CO_3 substitution in an apatite molecule is difficult to measure, the chemical reactivity of PRs are empirically assessed according to their solubility in selected chemical extractants. The solubility of PR in 2% citric acid, 2% formic acid (Chien and Hammond, 1978ab) and neutral ammonium citrate (McClellan and Gremillion, 1980) has been used in New Zealand, Europe and USA and Australia, respectively as a measure of the reactivity of PRs. In New Zealand PRs dissolving ≥30% of the total P in 2% citric-acid are classified as reactive PR and the others as less reactive PR (White et al., 1989). The EPR in Sri Lanka has 2% citric acid solubility of 1.5% P (Dahanayake et al., 1995) and NAC solubility of <2% P (Riggs and Syers, 1991). The % of total P in EPR soluble in 2% citric-acid is about 14%. Therefore EPR is classified as a less reactive PR according to the New Zealand classification. Many of the PR sources in tropical countries have been shown to be relatively unreactive according to numerous agronomic trials (Hammond et al., 1986b).

The dissolution of PR occurs at the surface of the PR particle (Barrow, 1990). Decreasing the particle size by fine grinding will increase the surface area of PR particles exposed to the soil and therefore it increases the rate of P release from the PR, but fine grinding is not a substitute for reactivity. Reducing PR particles to a size less than 100 mesh (150 μm) by grinding is generally not warranted as finer particles do not increase agronomic effectiveness greatly (Khasawneh and Doll, 1978). The use of finely ground PRs may result in handling problems for mechanised agriculture, but would pose fewer problems for the more labour intensive agricultural systems like tea cultivation in developing countries.

2.6.2 Soil properties

The major component of most PRs is apatite, the dissolution of which in an acid soil can be described by the following equation (Khasawneh and Doll, 1978).

$$Ca_{10}(PO_4)_6F_2 + 12\ H^+ \Leftrightarrow 10\ Ca^{2+} + 6H_2PO_4^- + 2F^- \quad \text{[Equation 2.3]}$$

According to this equation, the driving force for the dissolution of PR in soils is the supply of H^+ and the removal of reaction products Ca^{2+}, $H_2PO_4^-$ and F^- from the site of dissolution. The ability of the soil to provide H^+ to drive the dissolution process is therefore essential for the agronomic effectiveness of PR. Many field and laboratory studies show that low soil pH (Bolan and Hedley, 1989; Kanabo and Gilkes, 1987; Rajan et al., 1996; Tambunan, 1992; Utomo, 1995; Zaharah and Sharifuddin, 1995) with high P buffer capacity (Rajan et al., 1996; Smyth and Sanchez, 1982b) contribute to enhanced PR dissolution. In high P-fixing allophanic soils in New Zealand, Bolan and Hedley (1990) reported an increase in P dissolution, from 29.3% to 83.5%, 18.2% to 78.9% and 12.5% to 60.3% in North Carolina phosphate rock, Jordan phosphate rock and Nauru phosphate rock respectively, by decreasing pH from 6.5 to 3.9. Smyth and Sanchez (1982b) observed that dissolution rates of North Carolina PR and Patas de Minas PR increased in a group of acidic Oxisols in Cerrado, Brazil with increase in P-sorption capacities. They showed that the maintenance of low concentrations of P in soil solution is considered as an important driving force for PR dissolution. Tea soils certainly have high acidity (pH 4.5 - 5.5 in water, Table 2.4) as well as high P fixing capacities (Gohain, 1988; Golden et al., 1981) to promote PR dissolution. Although PR dissolution increases with increases in P fixation the agronomic effectiveness of the PR, measured relative to triple superphosphate (TSP) or single superphosphate (SSP), may not be higher, but may be less, in a high P fixing soil than in a low P fixing soil because the fixed P is not readily available to plants (Hammond et al., 1986a).

The mass action of ions in equation 2.3 also indicates that PR dissolution would be favoured by soil conditions that maintain low concentrations of Ca already dissolved in the soil solution. Mackay and Syers (1986) showed that low levels of exchangeable

Ca associated with reduced Ca saturation of the soil's CEC, which in turn would lower Ca concentration in the soil solution, promote an increase in PR dissolution. Acid tea soils have low levels of exchangeable Ca and low Ca saturation which is expected to increase PR dissolution.

2.6.3 Crop species

Phosphate rocks (PR) are considered to be more effective in supplying P to perennial plantation crops like tea, coffee, rubber, sugarcane and pastures compared to short-term annual crops such as wheat, maize or millet (Chien et al., 1990a; Sale and Mokwunye, 1993). Examples of previous studies carried out on P utilisation from different PRs by various crop species and the major conclusions made in these studies are presented in Table 2.12.

Numerous studies indicate that annual crops require a higher rate of P supply during their rapid vegetative growth phase compared to perennials, and this need is readily met by the P supplying characteristics of water soluble P fertilisers rather than the sparingly soluble PRs (Bolan and Hedley, 1995; Palmer and Jessop, 1982). Unlike annual crops, perennial crops like tea would obtain their P requirement over a considerably longer period at a slower rate and this requirement can be met by application of selected PR fertilisers. Furthermore, the slow rate of P release from PR may be an advantage in high P fixing soils, because a significant % of P released from the PR fertiliser is taken-up by the crop before it gets fixed to any significant degree compared to soluble P fertilisers where a higher proportion of P may get fixed (Chien and Menon, 1995).

Actively growing plant roots are reported to have a stimulating effect on PR dissolution. The root induced acidification, where H^+ build up around the root surface, will result in an increase in the rate of dissolution of PR particles (Aguilar and Van Diest, 1981; Bolan et al., 1997; Hinsinger and Gilkes, 1995; Paauw, 1965; Trolove et al., 1996b). The plant induced processes of acidification will be discussed in a separate section in this review. Plants with higher root densities in the surface layers of the soil where the PR is concentrated would be expected to acquire

Table 2.12 Examples of previous research conducted on P utilisation from phosphate rocks by different plant species

Plant species	Phosphate rock	Observations	Reference
Oats	Florida	Soil pH of 6.0 or below is necessary for satisfactory utilisation of PRs	Ellis et al. (1955)
Wheat and red clover	Florida	P uptake from PR by clover is greater than wheat	Murdoch and Seay (1955)
Buckwheat and alfalfa	Tunis, Morocco, Curacao, South Carolina, Florida, Idaho, Tennessee, Montana, Virginia	Availability of P from PRs was related to the carbonate content of the PRs	Armiger and Fried (1957)
Potato	Gafsa and Florida	P availability from PR increased with decrease of soil pH	Paauw (1965)
Wheat, rye, lettuce, barley, cauliflower, cabbage, maize, pearl millet, clover, lupin and tomato	Reno and Langfos	P availability from PR is greater for dicotyledons than monocotyledons. The uptake of Ca by the former is an important factor determining their ability to utilise PR	Diest et al. (1971)
Squash, soybean, barley and wheat	Virginia	Dissolution of PR is related to the rate of Ca removal by plant roots	Johnston and Olsen (1972)
Wheat, paspalum, maize, soybean and buckwheat	Maranhao, Alvorada, Mali and Patos de minas	Buckwheat is more efficient than others in the utilisation of P. Plant P uptake is related to the cation : anion uptake ratio	Van Ray and Van Diest (1979)
Maize	Togo	Ground PR increased dry matter yield over unground PR. Acidulation of PR with elemental S increased PR solubility and its effectiveness	Mokwunye (1979)

Table 2.12 continued

Plant species	Phosphate rock	Observations	Reference
Ryegrass	Chatham Rise	Dry matter yield depends on the physical form (powder or pellet), rate and time of application of the PR. Powdered form was more effective in increasing dry matter yield	Mackay et al. (1980)
Soybean and alfalfa	Morocco and Mali	P uptake from PRs is related to the acidification caused by the uptake of excess cation over anions	Aguilar and Van Diest (1981)
Buckwheat, ryegrass, rhodegrass, bean and maize	Mali, Mexico and Florida	Plants enhance solubilisation of PR through higher cation over anion uptake pattern	Bekele et al. (1983)
Wheat	Christmas Island, North Carolina, Khouribga, Gafsa, Sechura, Central Florida, Tennessee, Pesca, Kadjari, Huila, Idaho, Jhamar Kotra, Tapira, Cargill and Missouri	P availability from PRs related to their solubility in chemical extracts such as acidic (pH 3) or neutral ammonium citrate or 2% citric acid. Tracer technique provided more reliable index of P availability than these two extractants	Kucey and Bole (1984)
Brown top, white clover, subterranean clover, perennial rye grass, lotus	Chatham Rise	Residual effects of the PR in the field depends on the soil type, P sorption capacity. The presence of $CaCO_3$ in close proximity decrease the rate of PR dissolution due to increase pH and Ca concentration in the solution film immediately surrounding the PR particles.	Mackay et al. (1984)

Table 2.12 continued

Plant species	Phosphate rock	Observations	Reference
Ryegrass	Huila, Pesca, Sechura, Gafsa, North Carolina, Central Florida and Tennessee	Dissolution and plant P availability from PR was related to the carbonate content of the PRs	Anderson et al. (1985)
Pueraria	Morocco and Mali	P uptake from PR is related to the acidification caused by excess cation over anion uptake	De Swart and Van Diest (1987)
Rape	Mali	Solubilisation of PR in the presence of plant roots is achieved by release of organic acids by roots	Hoffland et al. (1989)
Maize	Huila, Capinota	Agronomic effectiveness of partially acidulated Huila PR and Huila PR compacted with TSP were similar to TSP. Partial acidulation of Capinota PR was only half as effective as TSP. Capinota PR compacted with TSP was as effective as TSP. Partial acidulation of slightly to moderately reactive PR with high Fe_2O_3 content is less effective. Only way to make profitable use of the PR is to mix with water-soluble P sources. Compaction is one such way	Menon and Chien (1990)
Ryegrass, wheat, maize	Sechura	Ryegrass acquired more P than wheat or maize from PR fertilised soil due to its higher root density	Chien et al. (1990b)

Table 2.12 continued

Plant species	Phosphate rock	Observations	Reference
Ryegrass	Sechura, North Carolina, Gafsa, Youssoufia, Arad, Khouribga, Jordan, Zin, Mexico, Nauru, Florida	P extracted form PRs by 2% citric acid, 2% formic acid and neutral ammonium citrate correlated well between themselves (R^2 0.82 - 0.99). Formic-P was the best predictor of the agronomic effectiveness of PRs. Citric-P was a poorer indicator of the reactivity of PRs.	Rajan et al. (1992)
White lupin, narrow leaf lupin	North Carolina	Rhizosphere acidification and removal of P and Ca by plant uptake enhanced PR dissolution. White lupin dissolved twice as much PR as narrow leaf lupin	Hinsinger and Gilkes (1995)
Lotus, white clover	North Carolina	Lotus had higher internal and external P efficiency than white clover in PR fertilised soil due to greater root length	Trolove et al. (1996a)
Corn	Sukulu Hills	Dry matter yield decreased with increase in P-fixation capacity of the soil.	Butegwa et al. (1996)
Lotus	North Carolina, Jordan	Release of H^+ and the removal of dissolved products of PRs by plant uptake enhanced PR dissolution	Bolan et al. (1997)

dissolved P from the PR at a higher rate, because there would be a greater likelihood that the root encountering localised 'high concentration pockets' of soluble P adjacent to the PR. This proposal is supported by evidence from glasshouse pot trials using reactive Sechura PR, where ryegrass acquired more P than wheat or maize, because of its higher root density (Chien et al., 1990b). Plants promote P dissolution by increasing the root density in the vicinity of PR particles (Kirk and Nye, 1986). This is attributed to the reductions of the concentration of Ca and P by plant uptake. In pot trials using a loess soil (1.4 mg P 100 g^{-1} soil), Flach et al. (1987) showed that the Ca uptake pattern (finger millet > pearl millet > maize) followed the order of crop response to applied PRs (Zimpan PR from Mexico 15% total P, 15% of total P soluble in 2% citric acid; Khouribga PR from Morocco 14% total P, 25% of total P soluble in 2% citric acid).

2.6.4 Moisture

The soil water regime is an important factor controlling PR dissolution, because PRs will not dissolve in dry soils (Sale, 1990). PR particles need to be surrounded by moisture films to enable the dissolved products such as Ca^{2+} to diffuse away from the dissolving surface and to permit the inward diffusion of H^{+} towards the PR surface. Incubation studies confirmed that PR dissolution declines as soil moisture content is decreased (Gregg et al., 1987). Reactive PRs (RPR) are recommended for pasture in Australia and New Zealand in areas having rainfall > 800 mm with even distribution throughout the year and soil pH <6 because under these conditions RPR is expected to dissolve at a satisfactory rate to supply adequate P to plants (White et al., 1989). Tambunan (1992) observed that North Carolina phosphate rock (NCPR) was more effective than TSP in increasing maize yields in an acidic Ultisol of Indonesia having higher moisture content compared to soils in a dry area because NCPR dissolution was greater in soils with higher moisture content. Most tea-growing areas in the major tea producing countries receive an annual rainfall of approximately 2000 mm or more and pH <6 and therefore, RPRs or even less reactive PRs should dissolve to supply adequate amount of P to maintain soil P levels satisfactory for uptake by tea plants.

2.6.5 Management practices

Phosphate rock dissolution in soils is influenced by management practices in a number of ways. Studies have shown that broadcasting and incorporating the PR in the surface soil will result in greater PR effectiveness than placing the PR in a concentrated band in the soil (Hammond et al., 1986b; Khasawneh and Doll, 1978). Reduction of PR dissolution when PR is placed as bands is partly attributed to the overlapping diffusion zones around the closely spaced PR particles, which hinders the movement of H^+ to the surface of PR particle and movement of Ca and phosphate ions away from the particle (Barrow, 1990). The build-up of dissolved products at the surfaces of these particles would limit PR dissolution. This was clearly demonstrated by Hughes and Gilkes (1986) in their incubation studies.

Liming is normally practised to reduce Al toxicity and increase Ca^{2+} in acid soils. Liming will both reduce the supply of H^+ and increase the supply of Ca^{2+} in the soil solution (Hammond et al., 1986b). The outcome would be a lower rate of dissolution of PRs applied to these lime amended soils. To remedy the negative effects of lime on PR dissolution, PR has to be applied long before liming. This would allow PR to dissolve before the pH and Ca status of the soil are raised by lime application.

2.7 RESPONSE OF TEA PLANTS TO P FERTILISERS

The response in tea growth to P fertilisers is more common in nurseries or on young tea plants in the field than on mature tea. The higher growth rate and the smaller volume of soil explored by the roots in the early stages of growth compared to a mature stage may be the reason for the higher P demand by nursery and young plants. Green (1965) showed that mixing of superphosphate with soil in the planting hole had increased the pruning weights (dry weight of first 3 young leaves and soft wood) of young plants by an average of 37% within a year compared to untreated plants. The mixing of P fertiliser benefited the plant by greater root growth, which eventually led to an increased uptake of water and nutrients by the tea bush to synthesise more dry matter.

The agronomic effectiveness of EPR was compared with saphosphosphate (a blend of Egyptian phosphate rocks) in a glasshouse trial in Sri Lanka at an application rate of 15 kg P ha^{-1} yr^{-1} by measuring the yield and P uptake of 12 month old tea plants (clone TRI 2025) (Sivasubramaniam et al., 1981). The dry matter yield and P uptake at the end of one year of the trial did not differ significantly between any of the two P treatments or the control (no P addition) treatment. The lack of response to P fertiliser may have been due to high residual P levels in the soil as a result of lavish quantities of P applied to the estate in the past from which this soil was collected. Sivasubramaniam et al. (1981) did not report the P status of the soil they used in the trial, but Wickremasinghe et al. (1986) reported that these soils had 25.6 μg P g^{-1} soil of borax extractable-P which is considered to be high according to the classification of Beater (1949). Sivasubramaniam et al. (1981) found that malic acid is a more appropriate solvent to be used to asses the solubility of PR in soil because tea roots excrete significant quantities of malic acid, which can dissolve native P sources in the soil (Jayman and Sivasubramaniam, 1975; Xiaoping, 1994). They also found that malic acid extracted more P from soils treated with EPR compared to soils treated with imported PR and this led them to conclude that EPR is equally or more effective than the imported PR.

Dey and Bhattacharyya (1980) showed that continuous application of P fertiliser (40 kg P ha^{-1}) to a sandy loam soil in Borbhetta, India over a long period (22 yrs) had resulted in an increase in leaf P concentration from 0.28% (no P fertiliser treatment) to 0.39%. This response could be due to the low levels of plant-available P in these soils (Bray and Kurtz extractable P of 4 - 8 μg g^{-1} soil). Similar results were observed by Tolhurst (1963) on mature tea in a long term (17 yrs) field trial in an acid Ultisol in Sri Lanka. Tolhurst's trial showed that both Saphosphosphate and TSP increased leaf P concentration from 0.25 to 0.30% when P rates increased from 15 to 29 kg ha^{-1} yr^{-1} but there was no difference between the two P sources on leaf P concentration and shoot yield.

Willson (1969) estimated the amount of P required by mature tea plants yielding 5050 kg processed tea ha^{-1} in Kenya on a 3 year pruning cycle (Table 2.13). He quantified the removal of P by crop and the amount of P circulated as leaf fall and prunings. He

Table 2.13 Circulation of phosphorus in tea plants yielding 5050 kg processed tea ha^{-1} in a three year pruning cycle (calculated from the data of Willson, 1969)

Plant process	**Organ or tissue involved in the process**	**Amount of P absorbed or removed by plants (kg ha^{-1} pruning $cycle^{-1}$)**
P absorption by plants from soil	Roots	80
P removal from plants and return to soil	Prunings Leaf fall	59 4
P removal from estate	Young shoot harvest	18

reported that tea plants absorbed nearly 80 kg P ha^{-1} during a 3 year cycle and 18 kg P ha^{-1} is removed as flush (bud and two leaves) at harvest. From the total removal, about 75% of P was removed with prunings at the end of the cycle. In Sri Lanka, the prunings are normally buried into tea fields as a standard practise to recycle the nutrients and therefore the P in prunings are returned to the soil.

Traditionally soluble P fertilisers are used to supply P to most agricultural crops. Odhiambo (1987) studied the effect of single superphosphate (SSP) and diammonium phosphate (DAP) mixed at different rates (0, 9, 18, 35 and 70 kg P ha^{-1}) with the soil in planting holes on the growth of clonal tea in Kenya. He found that replanted clonal tea treated with 15 - 30 g SSP or 10 - 20 g DAP per planting hole giving an equivalent of 18 - 35 kg P ha^{-1} showed the highest plant vigour and survival rate. The application of P above this range showed no benefit to the plants.

Ranganathan (1971 - 1980) reported the results of a field trial on mature tea in an acid soil in South India comparing soluble P fertilisers (ammonium phosphate, diammonium phosphate, superphosphate), Suphala (a NPK fertiliser mixture, 15 : 6.5 : 12.5) and an unnamed sparingly soluble phosphate (Table 2.14). During the trial period some of the P treatments were changed [i.e. inclusion of dicalcium phosphate and/or Myssoorie PR (total P% 11.3 w/w and citric-acid soluble P 26% of total P) instead of Suphala]. The results of this trial showed that the form of P had no significant effect on processed tea yield during three pruning cycles.

The soluble P fertilisers are much expensive because their manufacture requires high capital investment and more energy. Therefore direct application of finely ground PRs to tea soils could be considered as a cheap option than the use of soluble P fertilisers. Many tea growing countries in the humid tropics are therefore attracted by the possibility of using PRs particularly those that are indigenous. In general, PR materials are found to be most effective in P-deficient acid soils (Chien and Hammond, 1989; Rajan et al., 1996). Nevertheless, PR sources vary widely in their agronomic effectiveness depending on their chemical characteristics, soil and climatic conditions and crop growth (Bolan et al., 1990; Chien and Menon, 1995; Khasawneh

Table 2.14 Effect of P sources on processed tea yield (kg ha^{-1} yr^{-1}) in South India (Ranganathan, 1971-1980)

P Source	Pruning cycle-1			P Source	Pruning cycle-2			P Source	Pruning cycle-3		
	1971-72	**1972-73**	**1973-74**		**1974-75**	**1975-76)**	**1976-77**		**1977-78**	**1978-79**	**1979-80**
Control	2070	2083	1485	Control	3416	2803	3662	Control	2346	4479	4596
Suphala	2214	2368	1752	Dicalcium phosphate	4015	3062	3882	Myssoorie PR	2313	4736	5104
Diammonium phosphate	2260	2314	1716	Diammonium phosphate	3893	2807	3740	Diammonium phosphate	2187	4608	4813
Ammonium phosphate	2358	2418	1734	Ammonium phosphate	3955	3033	3902	Ammonium phosphate	2384	4797	5057
Rock phosphate	2513	2457	1688	Rock phosphate	3943	2933	3770	Rock phosphate	2522	4970	5314
Superphosphate	2427	2316	1743	Superphosphate	3876	2899	3799	Superphosphate	2493	4820	4997
CD (p=0.05)	173	244	214		388	120	147		148	246	302
Plucking rounds yr^{-1}	52	44	28		45	49	51		32	51	51

and Doll, 1978). The factors that affect P availability from PR was adequately discussed in Section 2.6.

Several key issues still remain unresolved with respect to the P nutrition of tea. Further intensive research is imperative to fill these knowledge gaps and to obtain a more comprehensive understanding of the different processes involved in P uptake by tea from soils treated with PR especially the locally available EPR. Most research by previous workers has been on the growth response of tea to applied P fertilisers, but these studies did not examine adequately the reactions of fertilisers in tea soils, mechanisms of P uptake by tea roots and the relationship between soil P forms and P uptake by tea. In order to study the mechanisms of P uptake one needs to understand the P chemistry in the zone where the roots are active i.e. the rhizosphere. No such rhizosphere studies are known to have been conducted with tea, though studies on other crops have been documented and they are reviewed in the next section.

2.8 ROOT-SOIL INTERFACE (RHIZOSPHERE)

The zone immediately surrounding the root has been termed the rhizosphere and this zone has properties significantly different from that of the bulk soil (Armstrong and Helyar, 1992; Darrah, 1993; Gahoonia et al., 1992; Hedley et al., 1994; Hinsinger and Gilkes, 1996; Jungk, 1996; McKenzie et al., 1995; Tarafdar and Jungk, 1987). The term "rhizosphere" has evolved from the very narrow definition first used by Hiltner in 1904 to describe the narrow zone of intense bacterial activity around legume roots (Darrah, 1993). Currently it is extended to a broader definition of "the zone of soil surrounding the root which is affected by it". Depending on plant species, the width of the rhizosphere zone has shown to extend a few mm to several cm from the root surface (Bolan et al., 1997; Darrah, 1993). The rhizoplane is an elaborate word for the root surface.

Plant root processes influence chemical, biochemical and physical conditions within the rhizosphere. Several complex mechanisms are involved in the uptake of nutrients by the root, particularly P, from the rhizosphere. Plants preferentially absorb P,

depleting rhizosphere labile-P levels (Dormaar, 1988; Hedley et al., 1994; Trolldenier, 1992; Trolove et al., 1996b). The mobility of soil P to the roots is primarily by diffusion (Barber, 1995). The rate of plant P uptake depends on the steepness of the concentration gradient of P between the rhizoplane and the bulk soil (Barber, 1995; Jungk, 1996). Rhizosphere pH which is altered by the uneven uptake of cations and anions (Gahoonia et al., 1992; Gijsman, 1990abc; Haynes, 1990; Nye, 1981) can influence phosphate solubility and uptake (Bolan et al., 1997; De Swart and Van Diest, 1987; Trolove et al., 1996b). Plant roots release enzymes, such as phosphatase, which can mineralise organic-P compounds through hydrolysis (Eivazi and Tabatabai, 1977; Gahoonia and Nielsen, 1992; Tarafdar and Jungk, 1987). Bacteria and fungi which are actively engaged in P transformation in soils are about 20 - 50 times more abundant in the rhizosphere compared to the bulk soil because of abundant quantities of carbon compounds produced by roots in this zone (Newman, 1978; Rovira, 1979). Due to the high microbial activity in this zone labile inorganic P can get immobilised onto the organic P fraction (Armstrong and Helyar, 1992; Darrah, 1993; Trolove et al., 1996b). The abundant quantity of organic compounds in the rhizosphere can also influence other forms of P (e. g. P fixed to inorganic minerals) in the soil.

It is now well known that soil fertility information obtained from routine soil chemical analysis do not always correlate well with data on plant growth and nutrient uptake (Binkley, 1986; Mahendrappa et al., 1986). This may be partly due to the fact that soil samples collected for routine soil analysis are missing a small, but distinctly different and important soil fraction, the soil in the rhizosphere, which greatly influences the plant nutrient availability to plants (Curl and Truelove, 1986). Therefore a better understanding of the P status of the rhizosphere and its relationship to the P status of bulk soils is required to interpret routine soil analytical values.

Although many workers have attempted to describe some of the P transformation processes in the rhizosphere, the understanding of the intricacies of this unique zone is still at its infancy. A significant drawback limiting research on the rhizosphere is the technical difficulty of drawing soil samples from this zone. The problems are associated with sampling small amounts of rhizosphere soils for analysis and determining the line of demarcation between the rhizosphere and bulk soil. Attempts

were made by several workers to develop suitable techniques to study the rhizosphere processes in annual crops. Some examples include, separation of soil-root zone by a nylon mesh (Dormaar 1988; Gahoonia et al., 1992; Helal and Sauerbeck 1984; Kuchenbuch and Jungk, 1982) and porous plastic envelopes where roots have no physical contact with the soil (Brown and Ul-Haq, 1984). Other methods are more practically suited to field studies. These include gentle shaking (Häussling and Marschner, 1989; Hendriks and Jungk, 1981; Kirlew and Bouldin, 1987; Majidi and Persson, 1993) or brushing (Clemensson-Lindell and Persson, 1992; Häussling and Marschner, 1989) of roots collected from the field to free adhering rhizosphere soil. However literature reports on rhizosphere research on perennial tree crops are very scanty, more so under field conditions.

2.8.1 Rhizosphere acidification

Plant roots acquire most of their essential mineral nutrients in cationic (NH_4^+, Ca^{2+}, Mg^{2+}, K^+, Na^+ and most micronutrients) or anionic (NO_3^- Cl^-, SO_4^{2-}, $H_2PO_4^-$) forms. Ions with no apparent physiological role, such as various forms of Al, may also be taken-up in large quantities by the roots in acid soils. It has been reported that tea plants take-up large quantities of Al (Sivasubramaniam and Talibudeen, 1971). However neither the function nor the charge of Al species taken-up in tea plants is clearly understood. The simultaneous uptake of several charged species in different proportions cause imbalance with respect to charge within the plant and therefore, in order to maintain electroneutrality within the plant cells, roots excrete charged ions back into the soil. If excess anions over cations are taken-up by plants, roots generally excrete OH^- or HCO_3^- causing a rise in rhizosphere pH. If excess cations over anions are taken-up then roots generally excrete H^+ causing a decrease in rhizosphere pH (Gijsman, 1990b,c; Haynes, 1990). This H^+/OH^- excretion is stoichimetrically equivalent to the charge imbalance (Breteler, 1973; Gijsman, 1990b; Hedley et al., 1982a; Troelstra, 1983; Troelstra et al., 1985).

Considerable differences (0.5 - 2.0 pH units) between rhizosphere and bulk soil pHs have been reported for rape (Hedley et al., 1982a), red spruce (Smith and Pooley,

1989), rice (Hedley et al., 1994) and rye grass and white clover (Trolove et al., 1996b). Many researchers have linked pH changes primarily to N nutrition, arguing that N is the nutrient taken up in greatest quantities. Plants take up N in three main forms - as a cation (NH_4^+), as an anion (NO_3^-) or as a neutral N_2 molecule (N_2 fixation). Depending upon the form of N taken up and the mechanisms of assimilation in the plant, excess of cation or anion uptake may occur in plants (Haynes, 1983; Haynes and Goh, 1978; Kirkby and Knight, 1977). Therefore pH changes in the rhizosphere are largely as a consequence of uptake of predominantly NH_4^+ (rhizosphere acidification) or NO_3^- (rhizosphere alkalisation) (Bekele, 1983; Darrah, 1993). Gijsman (1990bc) grew Douglas fir (*Pseudotsuga menziesii*) in strongly acid soil fertilised with NH_4^+, NO_3^- or combination of both and found that the pattern of pH changes in the rhoizosphere correlated well with the form of N taken up. When NH_4^+ was used, rhizosphere pH decreased and when NO_3^- form was used the rhizosphere pH increased and for the combination of NH_4^+ plus NO_3^- forms the rhizosphere pH increased moderately compared to the bulk soil. A schematic representation of H^+ and OH^- generation during the uptake and assimilation of different forms of N into amino acids and subsequent dissociation of these amino acids are shown in Figure 2.3.

It has been reported that tea preferentially absorbs NH_4^+ compared to NO_3^- owing to the lower activity of nitrate reductase in roots (Ishigaki, 1978; Xan and Jianyun, 1994). According to Ishigaki (1978) and Xan and Jianyun (1994), in soils containing both NH_4^+ and NO_3^-, tea plants should preferentially absorb NH_4^+ leaving much NO_3^- in the soils unassimilated, which is then prone to leaching. However they did not report any experimental evidence to show that tea took-up more NH_4^+ in preference to NO_3^-.

In the case of N_2 fixation by legumes, the neutral N_2 is assimilated into protein and no charge imbalance is generated across the soil/root interface. Many legumes, however, commonly export H^+ into their rhizospheres when actively fixing N_2 (Nyatsanga and Pierre, 1973). This acidity is generated by the assimilation of uncharged CO_2 into amino acids, which contain carboxylic acid groups with low pKa values. The H^+ generated within legume roots comes from the dissociation of H^+ from these carboxyl

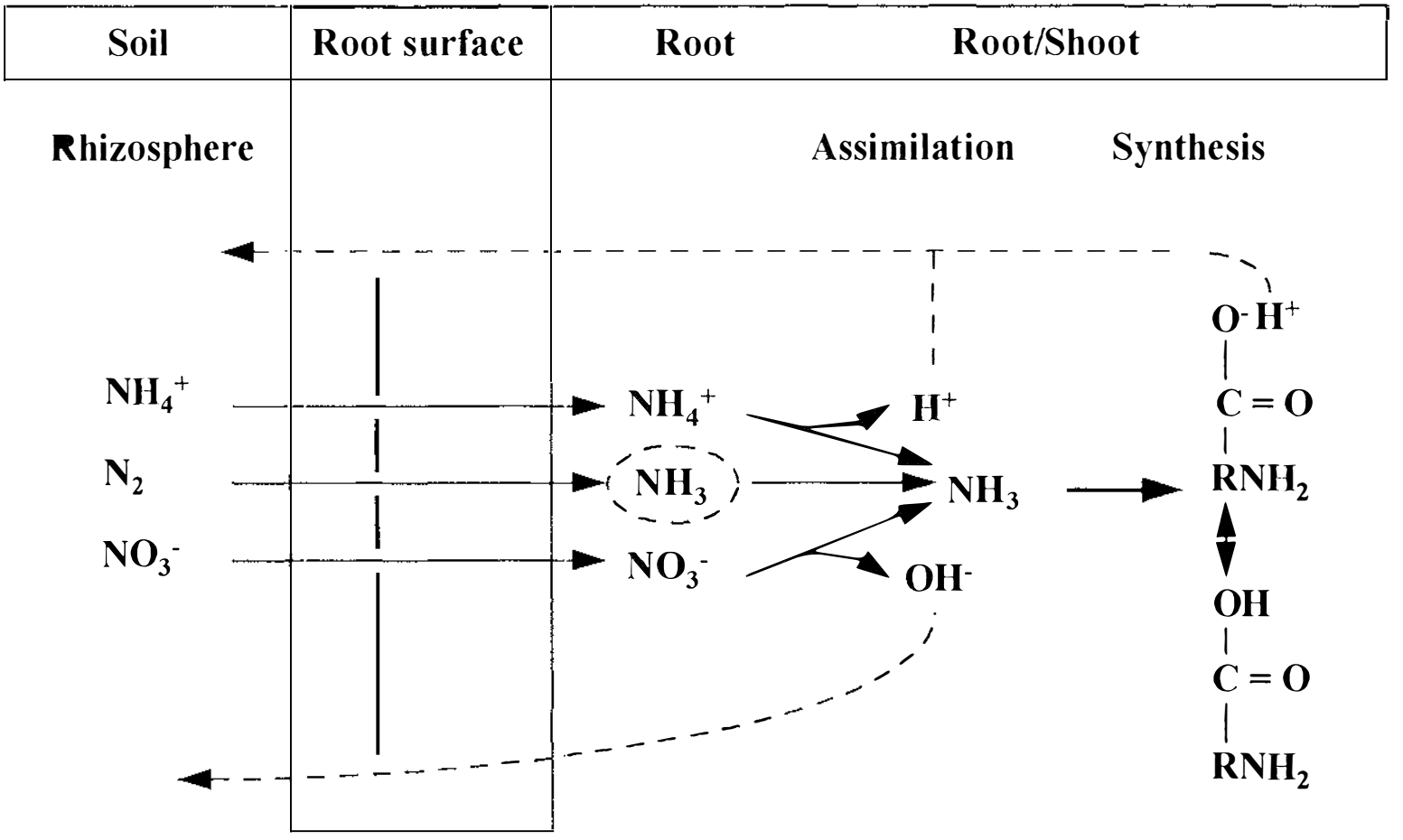

Figure 2.3 Proton (H^+) and hydroxyl (OH^-) generation during uptake and assimilation of different forms of N into amino acids and subsequent dissociation of amino acids (Bolan et al., 1991).

groups. The acidity generated by N_2 fixing legumes has been found to be equivalent to the excess uptake of cations over anions by the plant and it varies from 0.2 - 0.7 mol H^+ per mol of fixed N (Jarvis and Robson, 1983; Nyatsanga and Pierre, 1973). Some tropical legumes, however do not acidify their rhizosphere as much as temperate legumes do when actively fixing N_2 (Israel and Jackson, 1978). This is partly due to the fact that their NH_3 assimilation products appear to be ureides (allantoin and allantoic acid), which have high pK values (e.g. allantoin pKa 8.96) and therefore are unlikely to dissociate releasing protons at the cytoplasmic and xylem pHs. Permanent soil acidity can also be generated under temperate legumes because the N cycle is uncoupled (Bolan et al., 1990). In the litter or dung mineralisation subcycle H^+ produced during nitrification is not neutralised fully by OH^- release through NO_3^- uptake by plants because some NO_3^- may be leached.

2.8.2 Relationship between cation-anion uptake and rhizosphere pH

The difference (C-A) between accumulated amounts of inorganic cations (NH_4^+, Na^+, K^+, Ca^{2+}, Mg^{2+} : total = C) and inorganic anions ($H_2PO_4^-$, NO_3^-, SO_4^{2-}, Cl^- : total = A) in plant tissues is a measure of the organic-anion or carboxylate content in the plant (Wit et al., 1963). The form of N (NH_4^+ or NO_3^-) taken up however must be estimated. Since micronutrients are only present in very small quantities, these ions are generally not considered in the calculation of (C-A). A higher amount of cation uptake over anion uptake (higher C-A) is accompanied by the excretion of H^+ and a higher uptake of anions over cations is accompanied by excretion of HCO_3^- or OH^- (Haynes, 1990). These excretions are reflected in the rhizosphere soil pH. In the case of 100% NO_3^- nutrition the OH^- efflux can be expressed as:

$$OH^- \text{ efflux} = N_{org} + S_{org} - (C\text{-}A) \quad \text{[Equation 2.4]}$$

where N_{org} is organic N uptake in the plant, S_{org} is organic S uptake in the plant and all parameters are in units of meq per plant (Troelstra, 1983). Under certain circumstances this OH^- efflux can be negative (i.e., H^+ efflux). Since S_{org} can be

estimated on an equivalent basis as 5.4% of the N_{org} (Dijkshoorn and Van Wijk, 1967) the equation 2.4 can be approximated as :

OH^- efflux = $1.054 * N_{org}$ - (C-A) ..... [Equation 2.5]

In the case of 100% NH_4^+ nutrition, H^+ efflux can be expressed as;

H^+ efflux = N_{org} - S_{org} + (C-A) ..... [Equation 2.6]

or H^+ efflux = $0.946 * N_{org}$ + (C-A) ....[Equation 2.7]

When both NH_4^+ and NO_3^- are present in soil, organic N will originate from NO_3^- as well as NH_4^+. If the total organic-N is *a* meq plant^{-1}, the following fractions may be defined for the utilised NO_3^- and NH_4^+, respectively: *X* and *a-X* meq plant^{-1}.

Based on the fact that electroneutrality is maintained both inside and outside the plant, it can now be stated as:

N_{org} (originating from NO_3^-) + S_{org} + NO_3^- + Cl^- + $H_2PO_4^-$ + SO_4^{2-} - OH^- efflux = N_{org} (originating from NH_4^+) + K^+ + Ca^{2+} + Mg^{2+} + Na^+ - H^+ efflux

or

OH^- efflux - H^+ efflux = X + 0.054 a + A - (a-X) - C

or

net OH^- efflux = 2 X - 0.946 a - (C-A)

or net H^+ efflux = (C-A) + 0.946 N_{org} - 2 X.....[Equation 2.8]

2.8.3 Factors affecting P availability in the rhizosphere

Ample evidence has accumulated over the last several years to show that plant roots can significantly alter the chemical conditions in the rhizosphere and thereby influence the acquisition of mineral nutrients (Barber, 1995; Darrah, 1993; Hinsinger, 1994). This review considers the factors that are controlling the plant acquisition of P from the rhizosphere.

Plants draw P from the soil solution around the roots and this is buffered by re-equilibration of solution P with P held in other forms. The soil solution P concentration is governed by P in Fe and Al precipitates, P absorbed to soil minerals and organic P. The equilibrium between the dissolution of precipitated P and solution P is governed by the solubility product of the precipitated phase (Barber, 1995). In soils fertilised with PR, the depletion of solution P by plant uptake will cause more PR dissolution in the rhizosphere to replenish P that is removed from the rhizosphere (Hinsinger and Gilkes, 1995). Similarly depletion of solution P will cause desorption of adsorbed P (Barber, 1995; Mengel and Kirkby, 1987) and mineralisation of organic-P to maintain the equilibrium (Bolan et al., 1997; Jungk, 1996; Tarafdar and Jungk, 1987).

2.8.3.1 pH

Plant induced rhizosphere acidification results in an increase in the rate of dissolution of the PR particles in the rhizosphere (Hinsinger and Gilkes, 1995; Kirk and Nye, 1986; Trolove et al., 1996b) and thereby increases the availability of phosphate to the plants in soils treated with PR. Trolove et al. (1996b) observed more PR dissolution in the rhizosphere of lotus plants treated with NCPR fertiliser and explained this as due to lower pH in that zone compared to that in the bulk soil. Gahoonia et al. (1992) showed that the dissolution of calcium bound-P in a luvisol increased due to acidification resulting from NH_4^+ nutrition whereas ryegrass utilisation of P sorbed to Fe and Al increased by NO_3^- induced alkalisation in an oxisol.

Plant roots through their effect on pH can influence the adsorption and desorption of P by soils (Hinsinger and Gilkes, 1996), dissolution of Fe and Al phosphate (Armstrong and Helyar, 1992) and thereby control P availability to plants.

2.8.3.2 Release of organic acids and their chelating action

The roots of many plant species release various organic compounds into the soil. They can be subdivided into three groups (Jungk, 1996).

1. Mucilage, consisting of high molecular polysaccharides and polygalacturonic acid
2. Sloughed off cell from root cap
3. Low molecular organic acids, and complexing agents.

The mucilage and sloughed off cells from the root cap do not seem to help nutrient uptake in any specific manner. Among these three groups, the third one which comprises organic acids is more important in influencing the solubility of soil phosphate particularly Fe and Al bound P (Gerke and Jungk, 1991). The mechanisms reported include the effect of protons, but apparently more important is the complexation of metals and ligand exchange of adsorbed phosphate by carboxyl groups (Jones and Darrah, 1994). The release of dicarboxylic and tricarboxylic acids (citric, malic and other acids) from the apical parts of the roots into the rhizosphere helps P mobilisation. The release of these acids was found in several dicotyledon plant species, particularly in leguminosae members (Grierson, 1993; Hoffland et al., 1989). Grasses do not seem to secrete appreciable amounts of these acids. It was reported that tea roots secrete significant amounts of citric and malic acids which can dissolve native phosphate compounds in the rhizosphere soil (Jayman and Sivasubramaniam, 1975; Xiaoping, 1994). The secretion of these acids are greatly enhanced in some plants when they are subjected to phosphate starvation. Hedley et al. (1982a) observed rape plants secrete organic acids from roots when they are starved of P. Hoffland et al. (1992) showed that rape plants are efficient users of phosphate rock, due to their release of citric and malic acids as a response to P stress. Fox et al.

(1990) and Jones and Darrah (1994) showed that the citrate and malate secretion by roots could significantly increase the concentration of phosphate in soil solution and thus provide a higher flux from soil to the roots.

2.8.3.3 Phosphatase enzyme activity and mineralisation of organic-P

About 50% of the total soil phosphate occurs in organic forms, most of which derived from plant residues and, in part, synthesised by soil micro-organisms from inorganic sources (Sanyal and De Datta, 1991). The contribution of organic P (P_o) is considered as an important source for plant P nutrition in tropical countries (Sanchez, 1976), but it is commonly believed that P_o has no direct effect on the P nutrition of plants. Organic P has to be mineralised before being absorbed by plants and this is done through hydrolysis by a group of phosphatase enzymes produced by micro-organisms. It has been found that the phosphatase enzyme activity immediately outside the plant roots is significantly higher than that in the bulk soil (Dinkelaker and Marschner, 1992; Tarafdar and Jungk, 1987). The higher phosphatase enzyme activity causes hydrolysis of P_o to produce plant-available inorganic P. The activity of phosphatase enzymes in tea roots was studied by Xiaoping et al. (1989) in pot trials on Red Earth soils in China and the results showed that the activity of these enzymes was greater in soils near the roots compared to those that are distant from the root surface. They also showed that the enzyme activity was positively correlated with organic C and P_o status of the soil.

The P_o component of the 0.1 *M* NaOH extract is believed to contain some labile organic compounds such as RNA, nucleotides and glycerophosphates. These organic compounds have been found to be readily mineralisable and subsequently available for plant uptake (Bowman and Cole, 1978; Tarafdar and Claassen, 1988). Adams and Pate (1992) observed that inositol phosphate was as efficient as the inorganic-P source KH_2PO_4 in supplying P to lupins when grown in a sand culture. In acid forest soils, Häussling and Marschner (1989) found that readily hydrolysable P_o was depleted in the rhizosphere of 60 to 100 year old Norway spruce (*Picea abies* L. Karst) grown in a Cambisol while P_i concentrations were unaffected or even

increased. This could be due to root-mediated production of acid phosphatases in these soils, which may have hydrolysed P_o into P_i (Dinkelaker and Marschner, 1992). Though readily soluble P_o comprised a large proportion of the 0.1 *M* NaOH extract of soils, Armstrong and Helyar (1992) did not observe any utilisation of this fraction by semi-arid pasture grasses from South-western Queensland. This was explained as due to accumulation of P_o at a similar rate to that of plant uptake of mineralised P_i.

2.8.3.4 Mycorrhizal association

The association of mycorrhizal fungi (VAM, vesicular arbuscular mycorrhizae) is known to improve the phosphate supply to plants if available phosphate in the soil is low (Tinker, 1984). It was found that more phosphates per volume of soil was extracted by the fungus at the root surface. The efficiency of VAM in transferring P from soil into plants was mainly attributed to the structure of the mycelium (Barea, 1991; Bolan, 1991). It is likely that plants that are heavily infected with VAM are better able to acquire dissolved P from PR because of greater volume of soil into which the mycorrhizal root system can extend, compared to non-mycorrhizal roots. Mycorrhizal hyphae could extend to a distance greater than the usual P depletion zone in the rhizosphere soil so that VAM hyphae could extract P from a soil zone beyond the reach of the roots. The increase in plant growth by mycorrhizal association is largely due to increased absorption of nutrients from the soil solution. It has been reported that the rate of nutrient uptake in mycorrhizal associated plants is faster than non-mycorrhizal associated plants (Smith et al., 1985; Son and Smith, 1988). For example Sanders and Tinker (1973) observed that the rate of inflow of P into mycorrhizal roots was much higher ($17 * 10^{-14}$ moles cm^{-1} s^{-1}) than that of non-mycorrhizal associated plants ($3.6 * 10^{-14}$ moles cm^{-1} s^{-1}).

Sainz and Arines (1988) measured different fractions of P in an acid soil after growing red clover with and without mycorrhiza. They found that both mycorrhizal and non-mycorrhizal associated plants decreased the concentration of inorganic-P in the soil but did not affect the concentration of organic-P and suggested that both mycorrhizal and non-mycorrhizal associated plants obtained their P requirements from the

inorganic source of P in the soil. However change of P in one fraction due to plant uptake can alter another fraction, which makes it very difficult to identify the sources of P utilised by the plant.

Benefits have been reported from the association of VAM (endo-mycorrhiza) with tea plants in India (Barthakur et al., 1987), China (Zhi, 1993) and in Japan (Morita and Konishi, 1989), but no information is available on the extent of this association in different tea clones and its influence on the efficiency of plant P uptake from soils.

2.9 SUMMARY AND RESEARCH NEEDS

Previous studies on the P nutrition of tea were largely carried out on tea yield responses to P fertilisers in glasshouse and field trials. Most trials showed that tea yield responses to applied P fertilisers were inconsistent or irregular and in many cases there was no significant response at all. In these experiments, the soil P status or the fate of applied P was not reported adequately and therefore it was not possible to determine whether the soils had adequate plant-available P before application of P fertilisers and to what degree the plant available P pool increased with the application of P fertiliser.

Tea soils are highly acidic (pH 4.0 - 5.5) and are rich in oxides and hydroxyoxides of Fe and Al which are known to fix P. This led to the belief that high rates of P fertilisers needed to be applied to obtain yield responses in tea. But in practice rates as low as 5 - 15 kg P ha^{-1} yr^{-1} seem to be sufficient to reach maximum yield though the yield increase was small. Very little work has been carried out to understand the reasons behind the complexity of the P chemistry of tea soils and the mechanisms of P uptake by tea plants. It has been suggested that tea roots secrete organic acids and these acids may be dissolving some of the P fixed to soil so as to make them available to the roots. In order to test this hypothesis, studies need to be conducted on the chemistry of P in soils close to the roots (i.e. in the rhizosphere).

Currently a locally available PR mined at Eppawala (EPR) in the North Central province of Sri Lanka is being used as the sole source of P fertiliser for mature tea in Sri Lanka, but without adequately testing its agronomic suitability for tea. EPR is considered to be a PR of low reactivity according to its citric acid solubility. Whether it will dissolve adequately in the highly acidic tea soils especially in the rhizosphere is a question that needs to be answered with proper research studies. More research is required in determining its suitability as P fertiliser for tea.

The literature shows that different soil P tests have been used in various tea growing countries to predict availability of soil P to tea, without adequately testing their suitability in comparison to other soil tests. Research is needed to compare the various soil P tests and to choose the most suitable tests by correlating soil test values with shoot dry matter yield and plant P uptake.

CHAPTER 3

A TECHNIQUE FOR STUDYING RHIZOSPHERE PROCESSES IN TREE CROPS : SOIL PHOSPHORUS DEPLETION AROUND CAMELLIA (*Camellia japonica* L.) ROOTS[1]

3.1 INTRODUCTION

The review of literature presented in Chapter 2 shows that root-soil interactions in the rhizosphere markedly affect P availability to plants (Marschner et al.,1987).

The conditions at the root-soil interface are considerably different from, and influence plant growth more, than those at a distance from the root. For this reason, many researchers have been interested in studying the characteristics of this zone, the rhizosphere, relative to those of the bulk soil (Armstrong and Helyar, 1992; Gahoonia et al., 1992; Hedley et al., 1994; Hinsinger and Gilkes, 1995; Marschner et al., 1987; Wang et al., 1995). The rhizosphere is a narrow soil cylinder (about 0 - 2 mm radius) surrounding the root and therefore, it is technically difficult to study the root induced chemical changes in this zone. One problem is the small amount of rhizosphere soil available for chemical analysis and another is the determination of the line of demarcation between the rhizosphere and the bulk soil.

Different techniques for studying chemical changes in the rhizosphere had been developed in the past for annual crops, grasses and legumes (Gahoonia et al., 1992; Hedley et al., 1994; Jungk and Claassen, 1989; McLaughlin and James, 1991; Youssef and Chino, 1988). Some of these studies assumed that soil particles adhering to the roots are representative of rhizosphere soil and the soil distant from the roots was bulk soil and not influenced by roots (Ohno, 1989; Riley and Barber, 1971).

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[1]**Zoysa, A K N, Loganathan P and Hedley M J 1997 A technique for studying rhizosphere processes in tree crops : soil phosphorus depletion around camellia (*Camellia japonica* L.) roots. *Plant and Soil*, 190, 253-265.**

The practical difficulty of getting samples at known distances from the rhizoplane (root surface) is a significant obstacle in this approach. In other studies this problem was overcome by growing plants in soil in a cropping device based on the early work of Kuchenbuch and Jungk (1982), where a planar mat of roots was physically separated from the soil by a polyester mesh. Thin sections of soils at various distances from the mesh (rhizoplane) were sliced and chemically analysed to determine root induced chemical changes (Gahoonia and Nielsen, 1991; Hedley et al., 1994; Wang et al., 1995; Youssef and Chino, 1989). The above studies on annual crops, grasses and legumes showed that there were marked differences in soil pH and the concentration of the different P forms between soil layers within a few mm from the root surface and the bulk soil. Nevertheless much less is known about the rhizosphere processes in tree crops especially in the field due to the absence of a dependable method for sampling the rhizosphere soil.

3.2 OBJECTIVES

The objectives of the investigation reported in this chapter are:

1. To modify the rhizosphere study container (RSC) technique of Kuchenbuch and Jungk (1982) to investigate rhizosphere processes in tree crops under glasshouse and field conditions.
2. To study rhizosphere acidification and soil and fertiliser P depletion patterns around the fine roots of camellia (*Camellia japonica* L.) under glasshouse and field conditions.
3. To investigate the fate of applied P fertilisers in the rhizosphere and bulk soil.

3.3 MATERIALS AND METHODS

3.3.1 Soils

An alluvial soil (0 - 10 cm depth) lying beyond the reach of flood water in the North Island of New Zealand was used in this study. The soil carries the soil type name Karapoti silt loam. It belongs to the Recent Order in the New Zealand classification system and is classified as a Dystric Eutrochrept in the US soil Taxonomy. Some important physico-chemical characteristics of the soil are presented in Table 3.1.

3.3.2 Glasshouse trial

The soil was air-dried, passed through a 2 mm sieve and amended with four P fertilisers: North Carolina phosphate rock (NCPR, particle size 34.1% > 250μm; 52.5% 150-250 μm; 13.4% < 150 μm, total P 13%, all water insoluble), single superphosphate (SSP, total P 9%, 80% total P water soluble), monocalcium phosphate (MCP, 24% total P, all water soluble) and diammonium phosphate (DAP, 20% total P, all water soluble) at the rate of 200 μg P g^{-1} soil. To ensure that N and K deficiencies did not restrict plant growth, urea and KCl were applied at the rate of 100 μg N and K g^{-1} soil respectively.

Rhizosphere study containers described by Kuchenbuch and Jungk (1982) and Hedley et al. (1994) were used to study the rhizosphere processes. An RSC is a two compartment device, made-up of two (PVC) cylinders, the upper compartment having an internal diameter of 82 mm and 25 mm effective depth and the lower compartment having an internal diameter of 74 mm and 50 mm depth. The two compartments were separated by a 24 μm pore-diameter polyester mesh. The upper compartment was packed with 130 g soil (bulk density; 1.0 Mg m^{-3}) and the lower compartment with 242 g of soil (bulk density; 1.1 Mg m^{-3}). Eight month old seedlings of Tom Thumb, a variety of camellia (*Camellia japonica* L.) propagated from cuttings, were transplanted into the upper compartment of the RSCs (Figure 3.1a). Plant roots in the upper compartment striking the polyester mesh were unable to penetrate the mesh and

Table 3.1 The physico-chemical characteristics of the experimental soil

Character	Unit	Value
Soil pH	1 : 2.5 w/w (0.01 $CaCl_2$)	5.0
pH buffer capacity (at pH 4-5)	mmol H^+ kg^{-1} pH^{-1}	21
Organic C	%	2
Olsen P	µg g^{-1} soil	30
CEC[1]	$cmol_c$ kg^{-1}	15
Ex. Ca	$cmol_c$ kg^{-1}	7
Ex. Mg	$cmol_c$ kg^{-1}	2
Ex. K	$cmol_c$ kg^{-1}	2

[1] 1 *M* NH_4OAc pH 7 extraction (Blackmore et al., 1987)

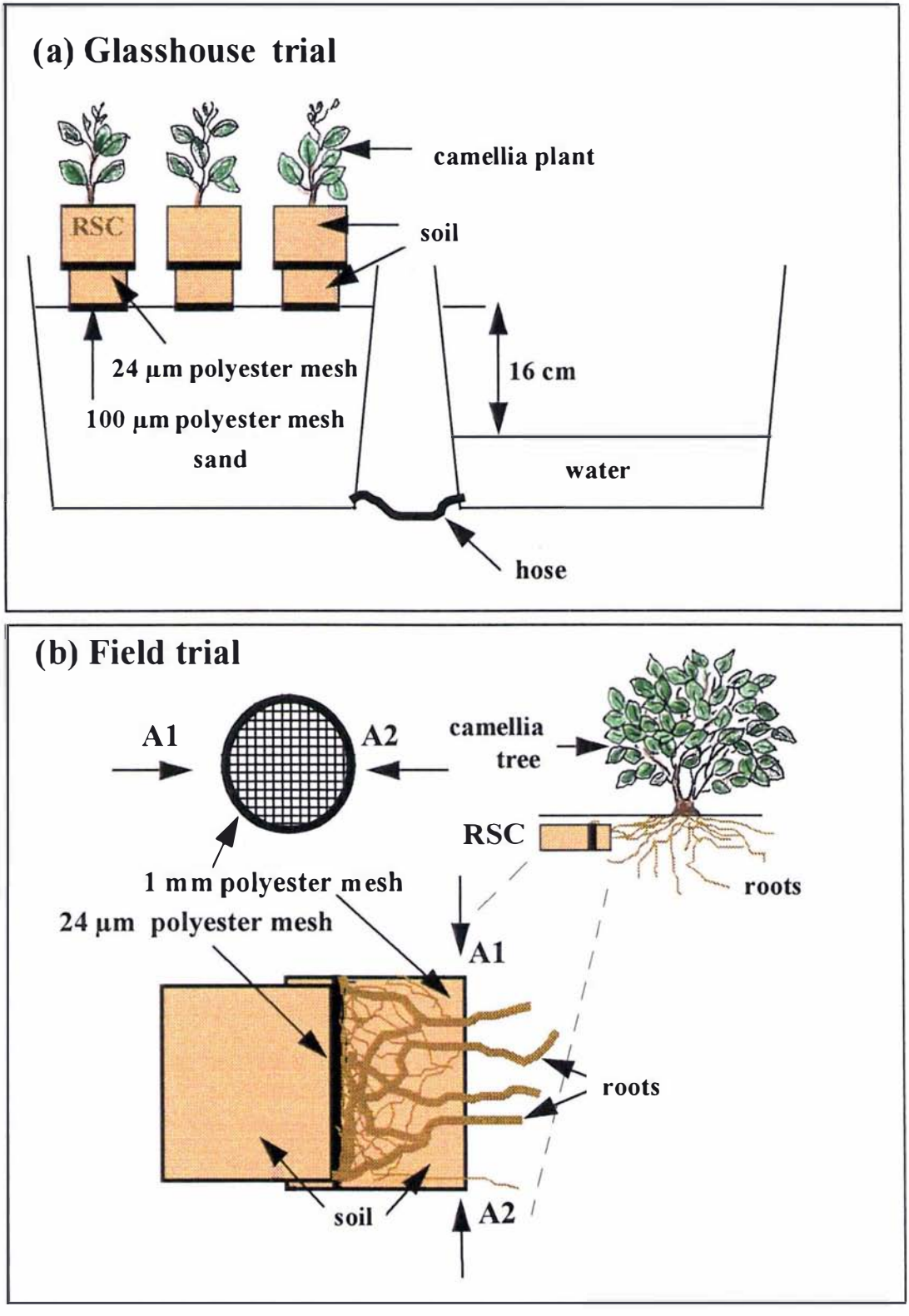

Figure 3.1 Schematic representation of Root Study Container (RSC) technique used in the (a) glasshouse and in the (b) field trials.

therefore grew horizontally along the mesh forming a root mat (Figure 3.2). The soil below the polyester mesh therefore represents the rhizosphere and the zone of transition demarcating the bulk soil. The RSCs were placed on a sand bed, which was kept moist by a water reservoir (Figure 3.1a). The watertable was fixed at 160 mm below the base of the RSCs. This enabled the RSCs to be kept at a constant water potential of approximately -1.6 kPa. Ten ml of 1% urea solution was added to all RSCs at the end of the seventh week. The trial examined the effects of four forms of P fertilisers on soil pH and soil P fractions in the rhizosphere of camellia plants. These treatments plus a control (with no P fertiliser added) were replicated five times and arranged in a randomized complete block design in a glasshouse maintained at 28^0 C maximum and 13^0 C minimum temperatures.

At the end of 56 days, plant shoots were cut 5 mm above the soil surface. The soil in the lower compartment was sliced into thin sections with a piston microtome (Figure 3.3) starting at the inter-cell boundary. The first four sections were sliced at a 0.5 mm thickness and a second set of 6 slices were taken each at 1 mm thickness in order to study the root induced changes in soil slices with increasing distance from the rhizoplane. The effect of the P fertilisers on soil pH and P fractions in the absence of plants was also measured in replicated RSCs treated with the four P fertilisers and a control (with no P fertiliser).

3.3.3 Field trial

In the field trial the RSCs were modified by mounting a 1 mm pore-diameter polyester mesh at the opening on one side of one of the compartments (Figure 3.1b, 3.4 and 3.5) to allow plant roots to enter the soil inside this compartment. Phosphate fertilisers tested were NCPR and SSP. These, urea and KCl fertilisers were mixed with the soil at the same rates as in the glasshouse trial and the RSC containers were filled with this soil. In the control treatment the soil was mixed only with N and K fertilisers (No P fertiliser). A deep vertical hole was made into the soil 30 cm away from the base trunks of >10 yr old camellia trees by cutting the roots with a sharp blade and carefully removing the soil. The RSCs were buried horizontally in the hole

Figure 3.2 Bottom view of the root mats formed above the polyester mesh in root study containers (RSCs)

Figure 3.3 Front view of the piston microtome

Figure 3.4 Root Study Container (RSC) modified for field situation

Figure 3.5 A root study container buried near a mature camellia tree in the field

to allow new roots to grow and penetrate through the 1.0 mm polyester mesh into the RSC compartment facing the tree roots (Figures 3.1b and 3.5). The treatments were replicated five times. At the end of six months, the soil around the RSCs was dug out and RSCs were removed by cutting the roots entering the RSCs a few mm outside the 1 mm mesh. The soil in the compartment on the tree side was sliced 1 mm above the inter-cell boundary (24 μm polyester mesh) to obtain measurements of root length and weight at the boundary. Root radius was determined using the formula $\sqrt{(M/\pi\rho L)}$ (M : root weight, π : 22/7, ρ : density of roots, L : length of root) assuming it is a cylindrical tube of constant radius. These roots were responsible for the changes in soil characteristics on the other side of the mesh. The roots were removed from the soil, gently washed and root lengths measured using a Comair root length scanner. Rhizosphere soil in the RSC compartment away from the roots was sampled using a piston microtome as described in the glasshouse trial.

3.3.4 Plant and soil analysis

Plant samples were dried at 60^0 C and ground to <1 mm. Both shoot and root samples were analysed for total P (Jackson, 1958). The soils were air-dried and analysed for available soil-P (Olsen et al., 1954), cation exchange capacity (CEC) and exchangeable cations (1 *M* NH_4OAc buffered at pH 7.0, Blackmore et al., 1987), soil organic C (Walkley and Black, 1934) and exchangeable NH_4^+ and NO_3^- (1 *M* KCl extraction, Markus et al., 1985). Soil pH was measured in 0.01 *M* $CaCl_2$ (0.5 g soil : 1.25 ml). pH buffer capacity was determined by $Ca(OH)_2$ titration method (Bolan et al., 1986). The amount of NCPR dissolution in the soils was determined by the method of Tambunan et al. (1993). The % of P dissolved from EPR was calculated as follows.

$$\% \text{ dissolution of P} = 100 \frac{[1 - 0.5\,M\,H_2SO_4 \text{ extractable P } [(\text{soil} + \text{PR fertiliser}) - (\text{soil alone})]]}{\text{fertiliser P added}}$$

3.3.5 Soil P fractionation

Beginning with 0.5 g of <1 mm air dry soil the following soil P fractions were determined sequentially by the procedure of Hedley et al. (1994). (1) Resin-P, by shaking end-over-end for 16 h at 25^0 C in 30 ml of deionised water containing a strip each of anion (AER) and cation (CER) exchange resin membrane (approximately 0.5 meq of exchange capacity per strip), then removing the strips and recovering P from them by eluting with 0.5 *M* NaCl. (2) NaOH-P_i, by adding 3.3 ml of 1 *M* NaOH to the suspension from step (1) (i.e. final concentration 0.1 *M* NaOH) and reshaking as above. (3) NaOH-P_o, by digesting 5 ml of the NaOH extract in 4 ml of conc. H_2SO_4 and 0.5 ml of H_2O_2 and subtracting NaOH-P_i from the digested P. (4) H_2SO_4-P_i, by adding 30 ml of 0.5 *M* H_2SO_4 to the soil residue from step (2) and re-shaking as above. (5) Residual-P, by refluxing the soil residue from step (4) in 8 ml conc. H_2SO_4 at 350^0 C for 3 h, cooling, adding 0.5 ml H_2SO_4 and reheating, and repeating this step until the residue remained white on further reheating. The digests were finally diluted to 50 ml with deionised water. Phosphate concentrations in all the extracts and digests were determined by colorimetry (Murphy and Riley, 1962).

3.4 RESULTS AND DISCUSSION

3.4.1 Effect of P fertilisers on soil pH

The soils fertilised with readily soluble P fertilisers (DAP, MCP and SSP) had lower pHs than the control treatment in both rhizosphere and bulk soil in the glasshouse as well as in the field trials, while NCPR had a higher soil pH than the control treatment (Figure 3.6a, b, c).

The decrease in pH observed for the MCP and the SSP treatments is probably due to the acidity produced when the dihydrogen phosphate in MCP and SSP dissociates to produce monohydrogen phosphate in the soil (Giroux et al., 1984). Hedley et al. (1994) also observed pH reduction in soils treated with MCP compared to soils with

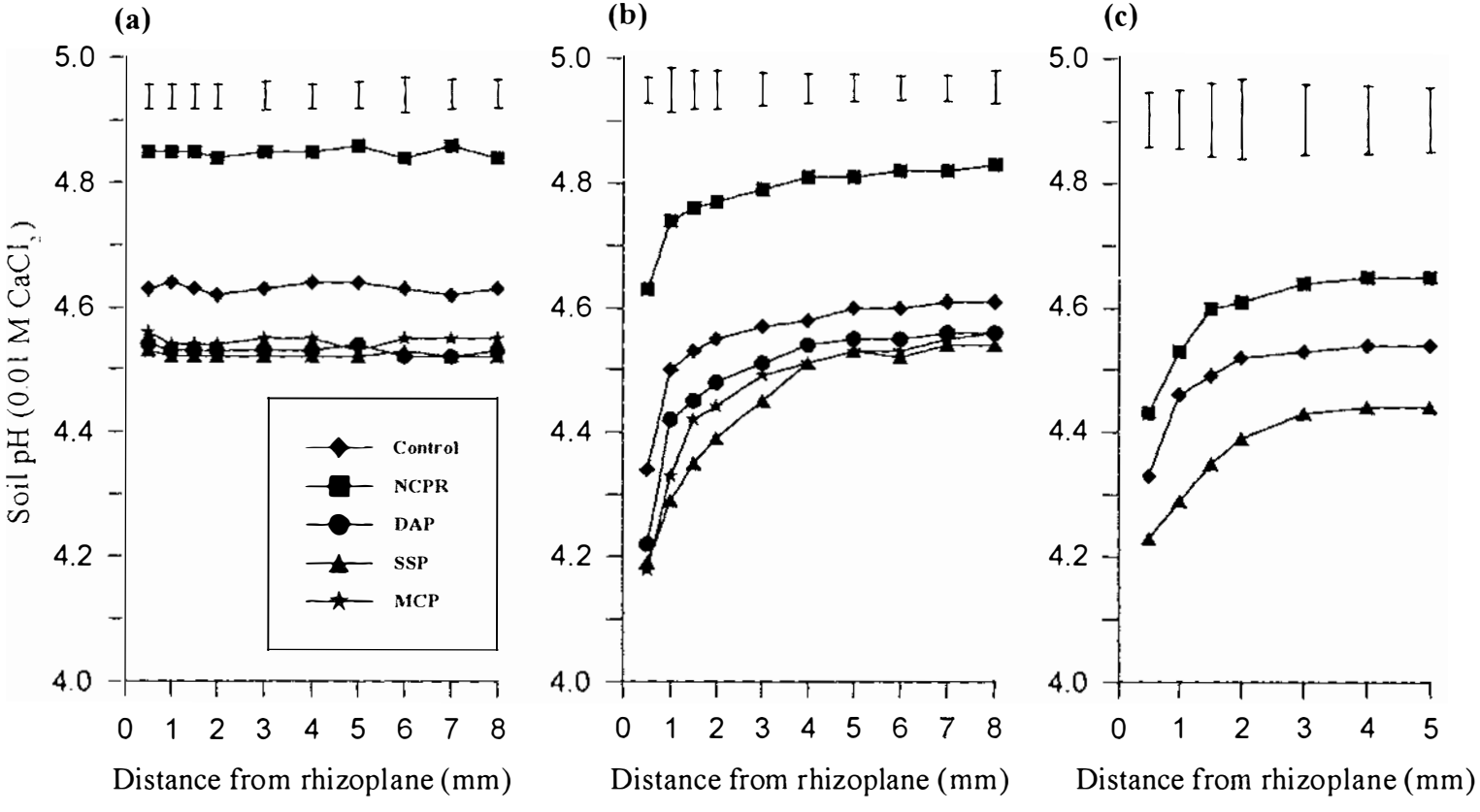

Figure 3.6 Effect of P fertiliser forms on soil pH (0.01 M $CaCl_2$) in camellia rhizosphere in the glasshouse and the field trials (a) without plants - glasshouse trial (b) with plants - glasshouse trial and (c) with plants - field trial. Vertical bars correspond to Lsd at $p < 0.05$.

no fertilisers. The release of protons from nitrification of NH_4^+ in DAP may have been the reason for the decrease in pH in the DAP treatment. The increase in soil pH observed for the NCPR treatment compared to the control treatment is due to the consumption of protons during the dissolution of phosphate rock and carbonate in apatite structure and in the accessory minerals (Loganathan et al., 1995). In a yellow-grey earth(Aeric Fragiaqualf)soil under pasture Manoharan et al. (1995) also reported that application of NCPR increased soil pH and DAP decreased soil pH over the control (no P) treatment but SSP application had no effect on soil pH.

3.4.2 Effect of plant roots on soil pH

A reduction in soil pH of 0.2 - 0.4 units was observed near the roots of camellia plants compared with the bulk soil in both the glasshouse and field trials for all treatments (Figure 3.6b, c). No change in soil pH was observed with distance from the polyester mesh in the lower compartment in the soils with no plants (Figure 3.6a). This clearly showed that the soil pH changes were induced by plant roots. The extrusion of protons by roots to maintain electroneutrality in plant tissues as plant roots take up an excess of cations over anions is considered to be the dominant cause for rhizosphere acidification (Barber 1995; Haynes, 1990). The nature of ions excreted to maintain electroneutrality in non-legumes is usually governed by the plant's N nutrition. A higher uptake of NH_4^+ causes H^+ release and higher NO_3^- uptake causes OH^- release to the rhizosphere (Gahoonia et al., 1992; Gijsman, 1990bc; Nye, 1981). Proton extrusion may also be associated with the release of low molecular weight organic acid anions (Hoffland, 1992; Hoffland et al., 1989; Liu et al., 1990). Tea plants (*Camellia sinensis* L.), which belong to the same family as camellias, are reported to secrete significant quantities of malic acid from their roots (Jayman and Sivasubramaniam, 1975; Xiaoping, 1994).

The pH drop in the rhizosphere of NCPR treated soil was lower than those in the soluble P fertiliser treated soils in the glasshouse trial. This is because higher proton consumption from NCPR dissolution buffered the pH change in the rhizosphere. Differences in pH drop between treatments in the rhizosphere soils were not observed

in the field trial. NCPR dissolution decreased with distance from the rhizoplane (Figure 3.7) because the rhizoplane was the source of H^+, which diffused out into the bulk soil.

The amount of H^+ consumed in dissolving NCPR in the rhizosphere and bulk soil was estimated using the amount of NCPR dissolved and from the relationship that 2 moles of H^+ are consumed for every mole of P dissolved (Loganathan et al., 1995). Similarly H^+ consumption for this dissolution was predicted by using the soil's pH buffering capacity, the observed pH increase in the rhizosphere and in the bulk soil of the NCPR treatment over the control treatment. The predicted and estimated H^+ consumption in NCPR dissolution was found to be in better agreement when the P-fractionation scheme of Tambunan et al. (1993) was used to measure the extent of NCPR dissolution, than when the P-fractionation procedure involving resin strips was used (Table 3.2). [This is caused by an over estimation of P-dissolution when the resin extraction was used (Trolove et al., 1996b) as the initial step in the fractionation scheme. This discrepancy in P dissolution between the two procedures is discussed in the next section]. Similar estimations carried out for NCPR dissolution under field conditions did not show a good agreement between the predicted and actual H^+ consumption for either of the two fractionation schemes (Table 3.2). This may be due to removal of NCPR dissolution products such as F and Ca by percolating rainfall and/or acidity intrusion into the RSCs from soil surrounding the RSCs in the field trial. These processes are not likely to occur in the closed experimental system used in the glasshouse trial.

3.4.3 Effect of P fertilisers on soil P fractions

At the end of the respective study periods, the P fractionation of bulk soil (3 - 8 mm from the rhizoplane - an area not significantly influenced by P depletion by plant roots) in the unfertilised (control) treatment in both the glasshouse and in the field trial showed that NaOH-P_i and H_2SO_4-P_i (120-158 $\mu g\ g^{-1}$ soil) had larger P fractions than resin-P, NaOH-P_o and residual-P (16-65 $\mu g\ g^{-1}$ soil) (Table 3.3, Figures 3.8 - 3.11). Application of P fertilisers increased all soil P fractions compared to control

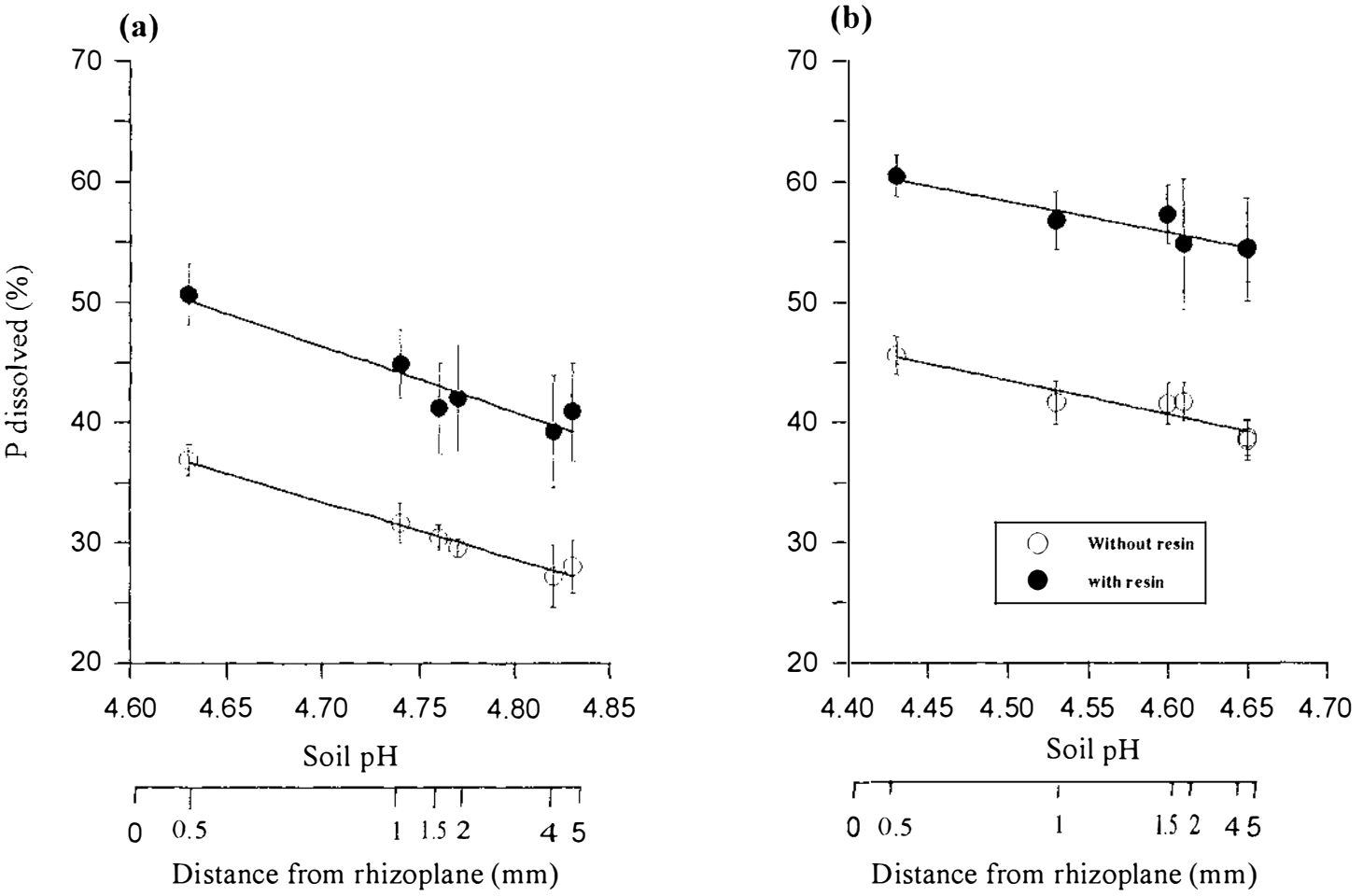

Figure 3.7 Effect of soil pH (0.01 M $CaCl_2$), distance from rhizoplane and method of P extraction (with and without resin) on NCPR-P dissolution in the (a) glasshouse and (b) field trials. Vertical lines correspond to standard errors of means.

Table 3.2 Estimated and predicted H^+ consumption for dissolution of NCPR fertiliser in the rhizosphere and bulk soil in the glasshouse and the field trials

Distance from rhizoplane (mm)	NCPR dissolution (%)	Estimated H^+ consumption from NCPR dissolution (μmol H^+ g^{-1} soil)	Observed pH increase in NCPR treatment over unfertilised control	Predicted H^+ consumption due to pH rise (μmol H^+ g^{-1} soil)
Glasshouse trial				
(a) TEA/NaCl (pH 7) extraction of soil as the first step in P fractionation				
0-0.5	36.9	6.16 ± 0.22	0.29	5.79 ± 0.21
0.5-1.0	31.6	5.28 ± 0.28	0.24	5.08 ± 0.20
1.0-1.5	30.4	5.07 ± 0.17	0.23	4.78 ± 0.14
1.5-2.0	29.5	4.92 ± 0.12	0.22	4.70 ± 0.33
6.0-7.0	27.3	4.54 ± 0.43	0.21	4.57 ± 0.24
7.0-8.0	28.0	4.67 ± 0.37	0.22	4.54 ± 0.37
(b) Resin extraction of soil as the first step in P fractionation				
0-0.5	50.7	8.46 ± 0.86	0.29	5.79 ± 0.21
0.5-1.0	44.9	7.49 ± 0.94	0.24	5.08 ± 0.20
1.0-1.5	41.2	6.87 ± 1.26	0.23	4.78 ± 0.14
1.5-2.0	42.0	7.01 ± 1.47	0.22	4.70 ± 0.33
6.0-7.0	39.3	6.56 ± 1.56	0.21	4.57 ± 0.24
7.0-8.0	40.9	6.83 ± 1.38	0.22	4.54 ± 0.37
Field trial				
(a) TEA/NaCl (pH 7) extraction of soil as the first step in P fractionation				
0-0.5	45.6	7.62 ± 0.06	0.10	2.00 ± 0.83
0.5-1.0	41.7	6.98 ± 0.06	0.07	1.40 ± 0.81
1.0-1.5	41.5	6.96 ± 0.07	0.11	2.20 ± 0.11
1.5-2.0	41.7	6.98 ± 0.06	0.09	1.85 ± 0.12
3.0-4.0	38.5	6.40 ± 0.06	0.11	2.26 ± 0.24
4.0-5.0	38.8	6.42 ± 0.03	0.11	2.36 ± 0.24
(b) Resin extraction of soil as the first step in P fractionation				
0-0.5	60.0	9.88 ± 0.07	0.10	2.00 ± 0.83
0.5-1.0	56.8	9.29 ± 0.12	0.07	1.40 ± 0.81
1.0-1.5	57.3	9.41 ± 0.17	0.11	2.20 ± 0.11
1.5-2.0	54.8	8.86 ± 0.42	0.09	1.85 ± 0.12
3.0-4.0	54.6	8.85 ± 0.11	0.11	2.26 ± 0.24
4.0-5.0	54.4	8.84 ± 0.28	0.11	2.36 ± 0.24

Table 3.3 P-fractions in unfertilised bulk soil (mean of soil slices 3-8 mm from rhizoplane) and % recovery[1] of added P fertiliser in the various P fractions in the glasshouse and the field trials

	Glasshouse trial					Field trial		
P-fraction	Unfertilised bulk soil P ($\mu g\ g^{-1}$ soil)	Fertiliser P recovery (%) NCPR	DAP	SSP	MCP	Unfertilised bulk soil P ($\mu g\ g^{-1}$ soil)	Fertiliser P recovery (%) NCPR	SSP
Resin-P	35	15.6	14.0	13.6	12.8	48	8.7	13.9
NaOH-P_i	151	30.6	33.2	37.8	32.3	158	37.9	53.5
NaOH-P_o	50	- 12.0	-7.7	-12.2	-13.9	65	-0.3	-4.5
H_2SO_4-P_i	138	62.3	47.4	46.4	45.5	120	43.1	20.4
Residual-P	16	6.7	6.5	7.9	10.8	25	10.4	15.2
Total-P	390	103.2	93.4	93.5	87.5	416	99.8	98.5

[1] $\frac{(\text{P fraction in fertilised soil - P fraction in unfertilised soil})}{\text{Fertiliser P added to soil}} * 100$

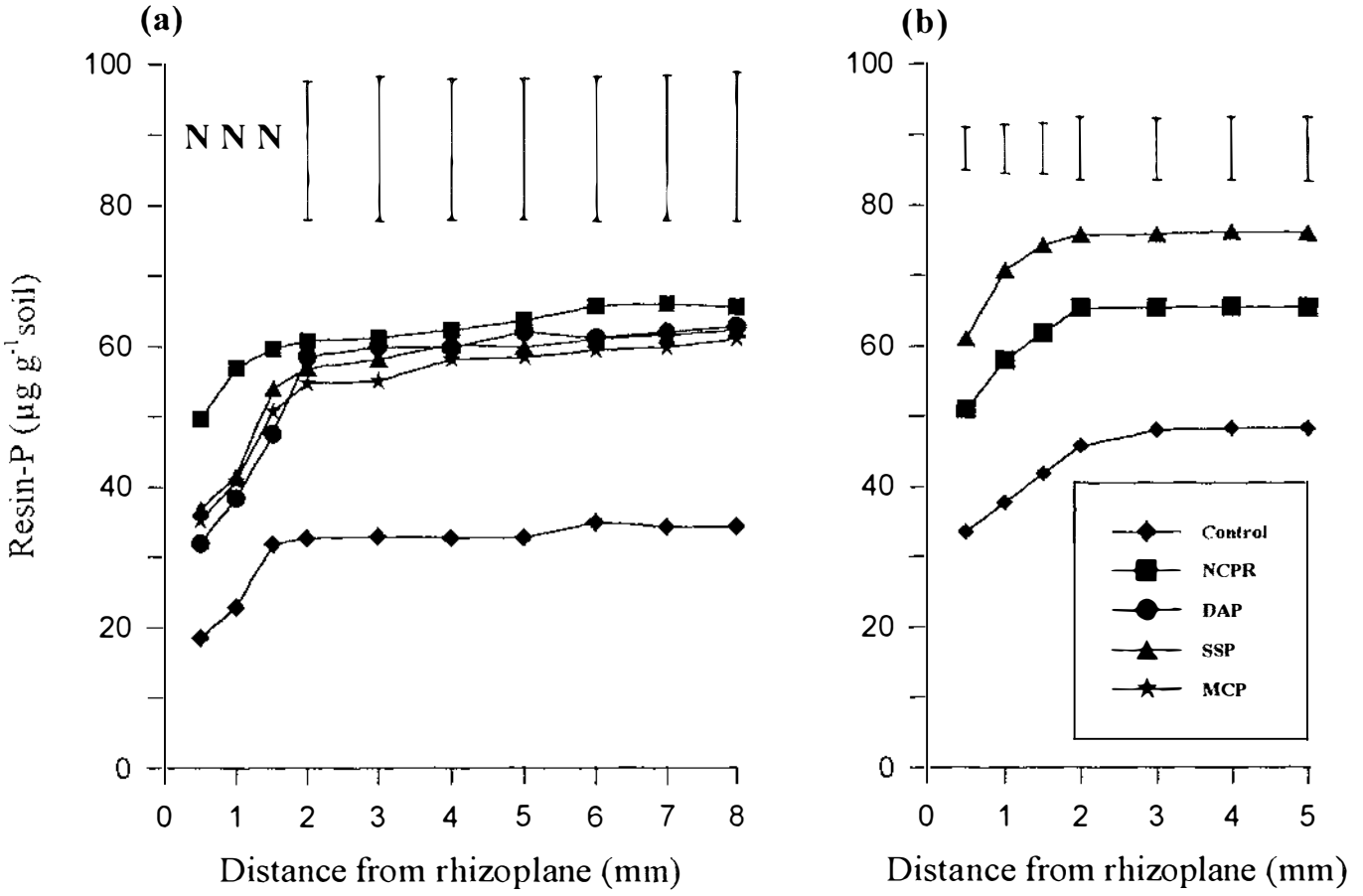

Figure 3.8 Effect of P fertiliser forms on resin-P in camellia rhizosphere in the glasshouse and in the field trials (a) with plants - glasshouse trial and (b) with plants - field trial. Vertical bars correspond to Lsd at $p < 0.05$ and N represents statistically nonsignificant at $p < 0.05$.

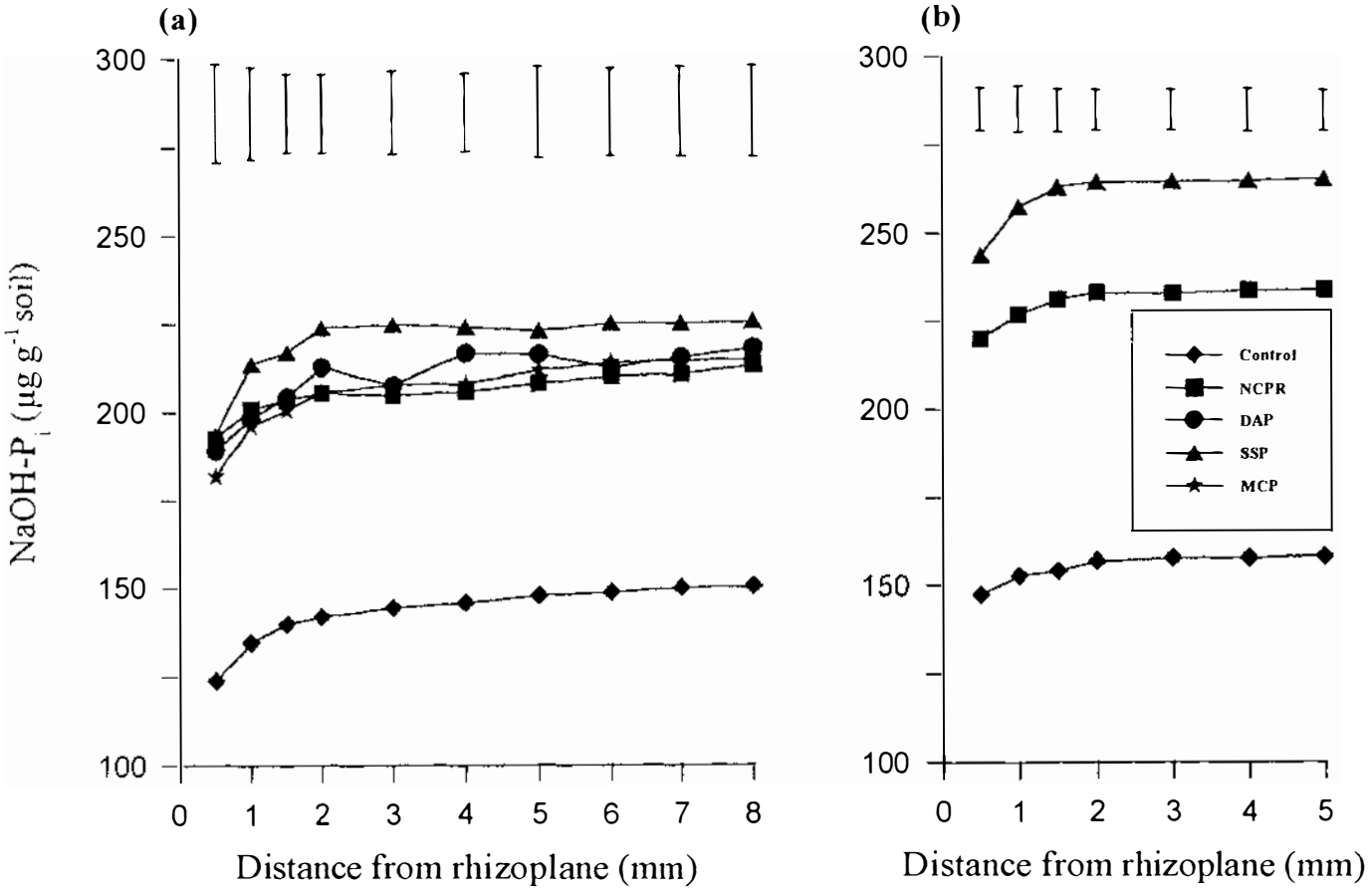

Figure 3.9 Effect of P fertiliser forms on NaOH-P_i in camellia rhizosphere in the glasshouse and in the field trials (a) with plants - glasshouse trial (b) with plants - field trial. Vertical bars correspond to Lsd at $p < 0.05$.

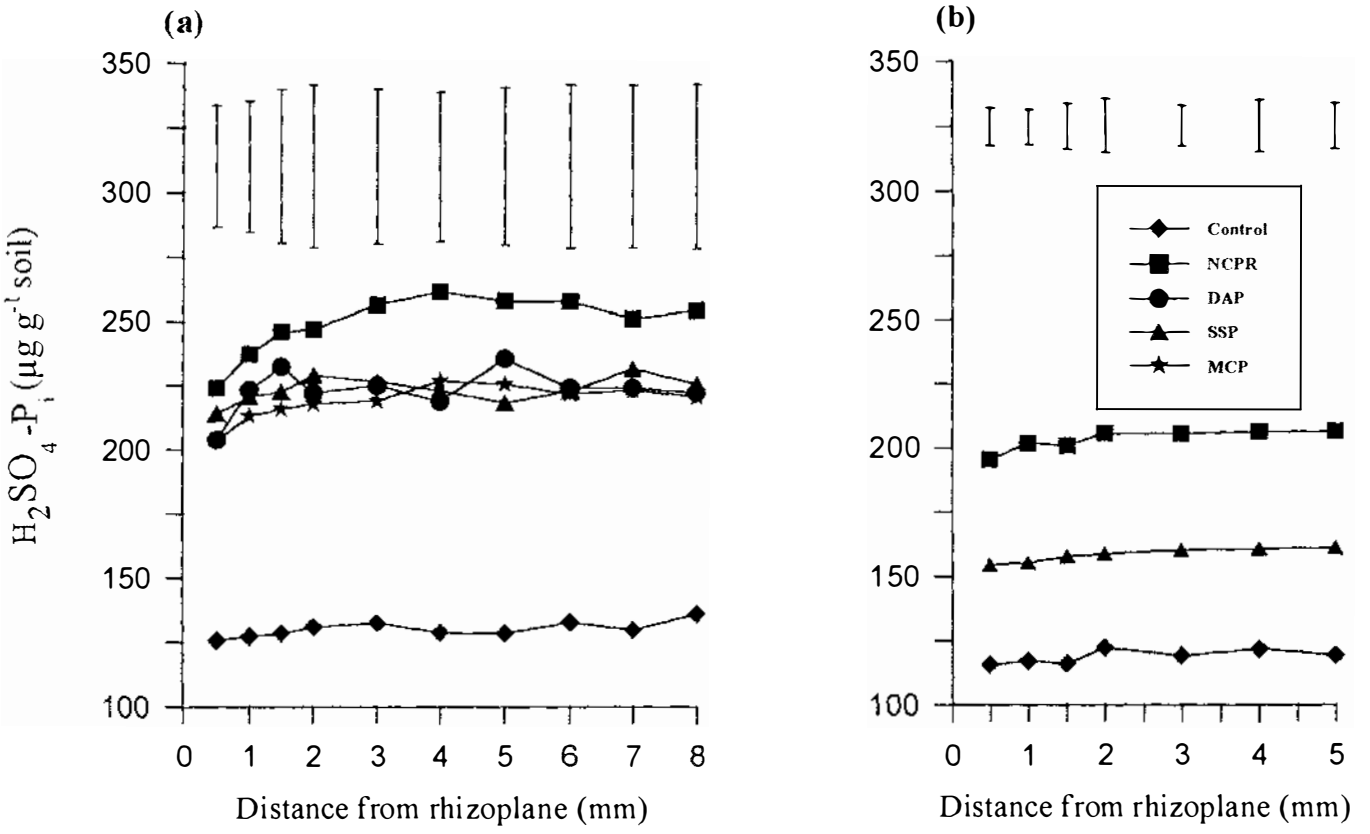

Figure 3.10 Effect of P fertiliser forms on H_2SO_4-P_i in camellia rhizosphere in the glasshouse and in the field trials (a) with plants - glasshouse trial and (b) with plants - field trial. Vertical bars correspond to Lsd at $p < 0.05$.

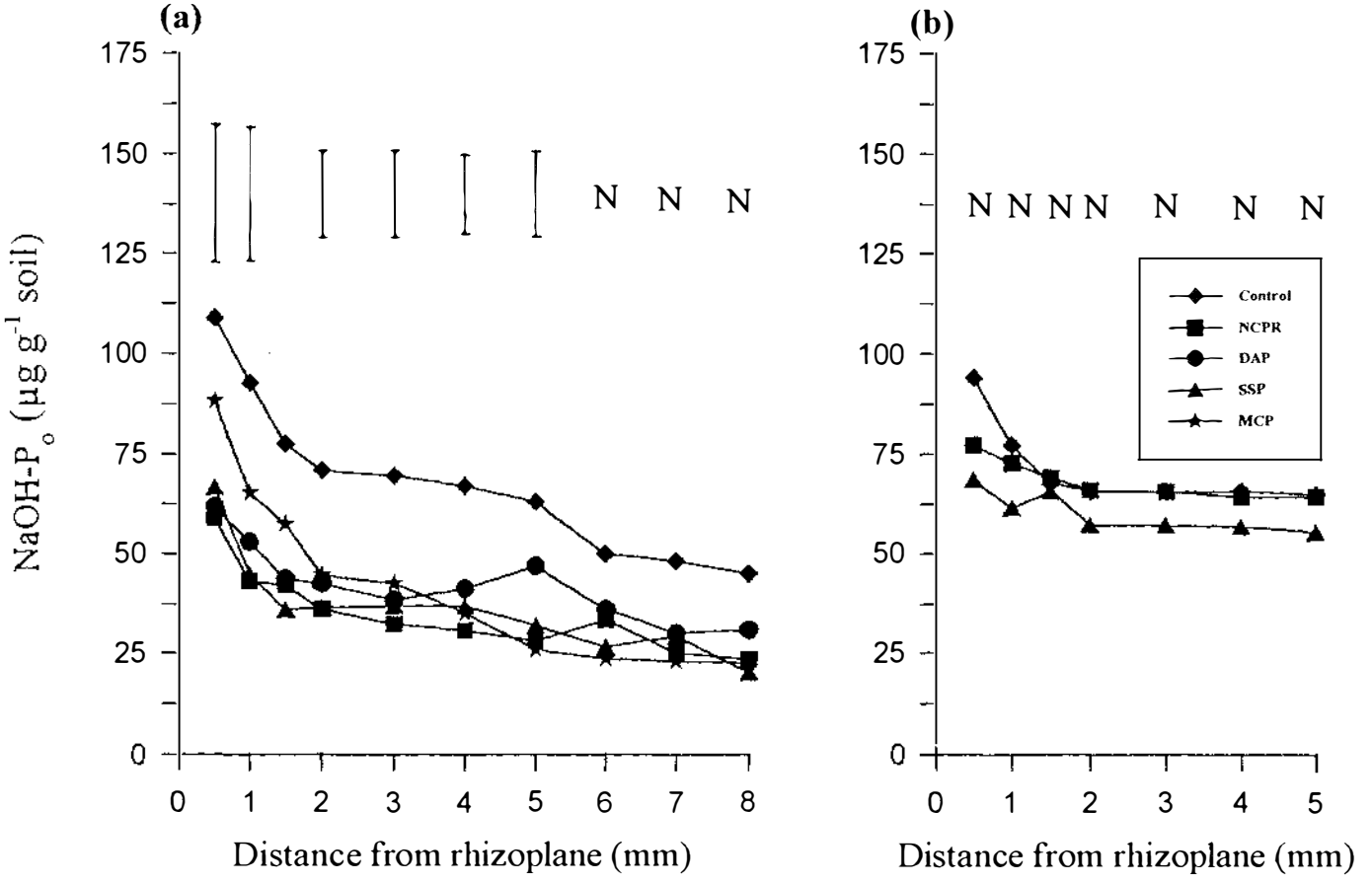

Figure 3.11 Effect of P fertiliser forms on NaOH-P$_o$ in camellia rhizosphere in the glashouse and in the field trials (a) with plants - glasshouse trial and (b) with plants - field trial. Vertical bars correspond to Lsd at $p < 0.05$ and N represents statistically nonsignificant at $p < 0.05$.

treatment (Figure 3.8, 3.9, 3.10) except NaOH-P_o in both trials (Figure 3.11b and c). In the glasshouse trial (Figures 3.8 - 3.11) P fertiliser form had no significant effect on the extent to which soil P fractions increased. In the field trial however, significant differences were observed in the fractions of NaOH-P_i and H_2SO_4-P_i between P fertiliser treatments. H_2SO_4-P_i was highest for NCPR treatment in both glasshouse and field trials compared to the control treatment (Figure 3.10). The recovery of fertiliser P in the bulk soil in both trials was close to 100% (88 - 103% in the glasshouse trial and 99 - 100% in the field trial, Table 3.3).

The majority of added fertiliser P was recovered in the NaOH-P_i or H_2SO_4-P_i fractions, which are not immediately available to plants (Table 3.3). Rapid chemical reactions change the dissolved constituents of soluble fertilisers (MCP, DAP and SSP) into compounds similar to those of "native" soil-P i.e. NaOH-P_i (Fe and Al bound P) or NaOH-P_o (Golden et al., 1991; Hedley et al., 1994; Perrott, 1995). The recovery of large amounts of H_2SO_4-P_i in DAP, SSP and MCP treated soils was not expected since accumulation of P in these fractions as reaction products of soluble P has not been evident in low pH soils with lower Ca saturation (Hedley et al., 1994; Tambunan et al., 1993). Perhaps the higher exchangeable Ca in the soils may have precipitated calcium phosphates over time. In NCPR treated soils undissolved NCPR residue dominated the H_2SO_4-P_i fraction.

The estimated NCPR dissolution in glasshouse soils showed that nearly 41% was dissolved in the bulk soil when a resin strip was used as the initial step, while only 28% was dissolved when the P-dissolution scheme of Tambunan et al. (1993) where TEA/NaCl (pH 7) was used as the initial step to remove exchangeable Ca (Figure 3.7). The discrepancy in the amounts of P dissolution was due to the differences of pH in the equilibrium medium of the initial steps of the P fractionation in the two schemes. In the scheme where the soil was agitated with resin strips for 16 hr the soil suspension pH was that of the acidic soil (pH 4.8) and this would have dissolved some residual NCPR during extraction (Trolove et al., 1996b). In the Tambunan et al. (1993) scheme where a buffered neutral pH medium was used to equilibrate the soil, the neutral pH did not dissolve any NCPR (Loganathan et al., 1995). Differences in

NCPR dissolution by the two methods was observed in the field trial too (Figure 3.7).

3.4.4 Effect of plant roots on soil P fractions

The roots exerted a profound effect on the phosphate chemistry of the surrounding soil. In both trials, reductions in resin-P, NaOH-P_i and H_2SO_4-P_i concentrations were observed near the rhizoplane for all treatments (Figure 3.8, 3.9, 3.10). The zone of these reductions did not extend to more than 2 - 3 mm from the rhizoplane. As in the case of soil pH, no change in the concentration of these fractions was observed at any distance from the polyester mesh in RSC without plants suggesting that roots were the cause for the depletion of these fractions.

The resin-P depletion in the rhizosphere of camellia roots created a P concentration gradient between the bulk soil and the root surface. This gradient is the driving force for a flux of P diffusing towards the rhizosphere from the bulk soil and the desorption/ dissolution of solid phase P forms. The rate at which P moves to the root is reduced if the soil P buffering capacity is high. An additional factor influencing the depletion of H_2SO_4-P_i fraction will be the rate at which H^+ ions can diffuse from their source at the rhizoplane into the surrounding soil (Nye, 1972). Resin-P and NaOH-P_i were the dominant forms of P depleted from the rhizosphere in soil fertilised with soluble P fertilisers in both trials. Plant and microbial P uptake could be considered as the major causes for the low resin-P in rhizosphere soil rather than P-fixation by Fe and Al because the NaOH-P_i fraction, which is a measure of Fe-P and Al-P, also decreased. A significant decrease of H_2SO_4-P_i near the rhizoplane in NCPR treated soil was due to the higher dissolution of NCPR at the low pH encountered in the rhizosphere. These results show that camellia roots possess the ability to utilise P from difficultly available P forms in the soil i.e. NaOH-P_i and H_2SO_4-P_i. Hedley et al. (1994) also reported similar P depletions in the NaOH-P_i and H_2SO_4-P_i fractions by upland rice in tropical soils. Attempts to model the uptake of P by these plants must consider the depletion of these insoluble P forms in rhizosphere soil as well as the diffusion of more labile P forms to plant roots.

Phosphorus fractionation of soils from the glass house trial with resin extraction as the initial step showed that the proportion of NCPR that dissolved near the rhizoplane and in the bulk soil (7 - 8 mm) were 51% and 41% respectively (Table 3.2). The corresponding figures for the field trial were 60% and 54%. As mentioned in the earlier section, P fractionation with an initial resin extraction resulted in higher NCPR dissolution than when NaCl/TEA (pH 7) was used for the initial extraction (Tambunan et al., 1993). The NCPR dissolution by the latter method was nearly 13 - 15% lower compared with the former method in both the rhizosphere and bulk soil of the glasshouse and field trials (Figure 3.7). It also showed that 6 - 8% more P was dissolved from NCPR in the 0 - 1.5 mm zone of the rhizosphere compared to the bulk soil in both trials. As well as the lower pH of the rhizosphere soil, the removal of dissolved P and Ca by plant uptake also contributes to the higher P dissolution of NCPR in the rhizosphere.

Unlike the observations made with clover by Tarafdar and Jungk (1987) and with rape by Gahoonia and Nielsen (1992) an accumulation of NaOH-P_o in the rhizosphere was observed in my study for all the treatments in both glasshouse and field trials (Figure 3.11a and b). Armstrong and Helyar (1992) also observed an increase in NaOH-P_o in the rhizosphere of several grass species in Mulga shrublands of South Western Queensland. Trolove et al. (1996a) have also observed this with pastoral legumes. The deposition of P_o near the rhizoplane could be due to the assimilation of P_i by the microorganisms, which use the organic anions secreted by camellia roots. Xiaoping (1994) reported that *Camellia sinensis* L. secretes large amounts of low molecular weight organic acids such as oxalic, succinic, malic and citric. These organic anions excreted by camellia roots may be acting as a source of carbon for the growth and multiplication of the micro-organisms in the rhizosphere. The P that is organically bound in microbial tissues may be released at a later time and become available for plant uptake following mineralisation (Gahoonia and Nielsen, 1992). Generally, phosphatase enzyme activity is higher at the root-soil interface than in the bulk soil (Tarafdar and Jungk, 1987). This enzyme is expected to hydrolyse NaOH-P_o and convert it to inorganic P, thus decreasing NaOH-P_o in the rhizosphere. It appears in this study that the rate of immobilisation of P_i is faster than the conversion of P_o to P_i.

This may be due to a high concentration of P_i in the rhizosphere and low rate of plant uptake of P_i. Hedley et al. (1994) also observed that an increase of phosphatase activity near the rhizoplane was not matched by an increased P_o depletion.

3.4.5 Comparison of rhizosphere P depletion with P uptake processes

Two P_i uptake processes occur, plant P uptake and conversion of P_i to P_o in the rhizosphere soil. Both are a function of root activity, because the P depletion or transformation of P_i to P_o did not occur to any significant extent at more than 3 mm from the roots. Net plant P uptake from the rhizosphere can be estimated from the total amount of P depleted in the RSC compartments ($\mu g\ RSC^{-1}$). The P depleted from lower RSC compartment was estimated (Table 3.4) by taking the difference between the total amount of P in the slices near the root (0 - 3 mm) and total amount of P at 3 - 8 mm from the root (bulk soil - uninfluenced by rhizosphere processes). Root activity in the unfertilised treatment and soluble P fertiliser treatments depleted most of the P from resin-P (30 - 38%) and NaOH-P_i (50 - 60%) pools, while the contribution from the H_2SO_4-P_i pool was only 15%. Root activity in the NCPR treatments depleted P mostly from the H_2SO_4-P_i fraction (44%) while depletion of other forms were comparatively low (28% resin-P and 28% NaOH-P_i). Total plant P uptake was estimated by subtracting the initial P content from the final P content of the plants by destructive sampling of plants of similar size to those of test plants at the start of the trial.

About 40 - 60% of plant P uptake in the soluble P fertiliser treatments was derived from the rhizosphere in the lower compartment, whereas it was 37% in the NCPR and control treatments (Table 3.4). The plant P uptake unaccounted for by the P depletion in the lower compartment came from the P depletion in the upper compartment. Almost all the fine roots in the upper compartment were found lying on the mesh, where the lower and upper halves of the surface of these roots were assumed to have caused equal amounts of P depletion in the soil. Therefore the total P depletion in the soil is expected to be about twice that in the lower compartment, which agrees reasonably well with the amount of P removed by the plant.

Table 3.4 Comparison of phosphorus depletion from soil in the lower compartment of RSC with plant P uptake in the glasshouse trial (values represent the mean of 5 replicates)

	Control	NCPR	DAP	SSP	MCP
Depletion ($\mu g\ RSC^{-1}$)					
Resin-P	265	311	477	503	479
NaOH-P_i	526	328	567	613	713
H2SO4-P_i	121	556	224	192	236
Total	912	1195	1268	1308	1428
Accumulation ($\mu g\ RSC^{-1}$)					
NaOH-P_o	530	507	431	470	496
Net depletion ($\mu g\ RSC^{-1}$)	382	688	837	838	932
Initial plant P content ($\mu g\ pot^{-1}$)	3907	6240	5878	6661	4691
Final plant P content ($\mu g\ pot^{-1}$)	4980	8060	7560	8760	6200
Net P uptake ($\mu g\ pot^{-1}$)	1073	1820	1682	2099	1509

Both NCPR and SSP significantly increased total root length and decreased root radius, which led to an increase in root surface area (Table 3.5). Total root surface area is an important property for effective contact between soil nutrients and root surface (Barber, 1995). The results show that depletion of P by camellia trees per unit surface area of roots, increased with P fertiliser application, the increase being statistically significant for SSP application (Table 3.5). Thus the greater P uptake was caused by P fertiliser addition concentrating the pool of plant available P (see resin P_i Figure 3.6a, b; Table 3.4) and greater root growth in the zone of P fertilisation. This effect of P fertiliser on root growth has been reported by Barber (1995) and Föhse and Jungk (1983).

3.4.6 Limitations of the RSC technique

When using the technique to study the long-term effects of tree roots on chemical changes in the rhizosphere care should be taken to avoid penetration of roots of weeds and other plants into the RSC. Also the interruption of root growth and proliferation of lateral rootlets at the RSC mesh boundary causes a planar root surface which puts a high P-demand on the soil. This allows small changes in the chemistry of the rhizosphere to be detected, but causes larger chemical changes than would be expected from the radial geometry around single roots in the field. The difference in the radial and planar effects is mainly in the magnitude of the measured changes in the rhizosphere and not on the rhizosphere processes. Similarly proliferation of roots may result at lower soil moisture than might occur around single roots. In addition the angle at which the RSC is buried in the soil may affect the moisture status inside the RSC if water infiltration is intercepted.

Table 3.5 Effect of P fertiliser forms on root growth within 1 mm of the mesh and P depletion in the field trial (values represent the mean of 5 replicates)

Treatment	Root length (cm)	Root radius (cm)	Root surface area (cm^2)	P depletion per root surface area[1] ($\mu g\ cm^{-2}$)
Control	180	0.039	44.1	8.5
NCPR	400	0.025	62.9	9.8
SSP	380	0.026	56.4	12.3
Lsd ($P<0.05$)	192	0.006	15.6	2.3

[1]P depletion in the RSC containing no roots was multiplied by 2 to include P depletion in the RSC containing roots

3.5 CONCLUSIONS

The root study container (RSC) technique of Kuchenbuch and Jungk (1982) adopted for camellias under glass house conditions and modified for the field situation gave similar results and provided useful information on the rhizosphere processes involved in P utilisation by camellia seedlings and mature trees.

The chemistry of phosphorus in the camellia rhizosphere differs distinctly from that of the bulk soil. Camellia roots induce acidification in their rhizosphere. Plant induced acidification in the rhizosphere created conditions conducive to the dissolution of the sparingly soluble NCPR fertiliser.

Addition of P fertilisers increased resin-P, NaOH-P_i (Fe and Al bound P) and H_2SO_4-P_i (Ca bound P) in the soils. Camellia plants utilised NaOH-P_i and H_2SO_4-P_i, which are traditionally considered as not readily available for plants. The accumulation of NaOH-P_o in the rhizosphere suggests that soluble inorganic P was transformed into organic P forms as a result of increased microbial activity in the rhizosphere promoted by the secretion of carbon exudates from the roots. This can be of considerable benefit to the plant on a long-term, because immobilization of organic P by the microbial biomass would concentrate P in the rhizosphere and subsequent mineralisation of this organic P would release this immobilised P into plant available forms.

Phosphorus fertilisation increased P uptake due to increased root growth and increased concentration of plant available P pool in the rhizosphere.

CHAPTER 4

EPPAWALA PHOSPHATE ROCK DISSOLUTION AND TRANSFORMATION IN THE RHIZOSPHERE OF TEA (*Camellia sinensis* L.) COMPARED TO OTHER SELECTED PLANT SPECIES

4.1 INTRODUCTION

Plant species differ in their ability to utilise soil and fertiliser P due to differences in the chemical characteristics of their rhizospheres (Aguilar and Van Diest, 1981; Fried, 1953). Several complex mechanisms are involved in plant P uptake from the rhizosphere soil depending on the plant species (Bekele et al., 1983; Diest et al., 1971). Plants vary in the proportions of the uptake of cations and anions resulting in changes in rhizosphere pH, which influences phosphate solubility and P uptake by the plant (Nye, 1981). The release of enzymes by plant roots, which hydrolyse labile organic P compounds in the rhizosphere and increase plant-available P may also vary with plant species (Gahoonia and Nielsen, 1992; Tarafdar and Jungk, 1987).

Tea (*Camellia sinensis* L.) is mostly grown in highly weathered acidic ultisols of the humid tropics (Ranganathan and Natesen, 1985). These soils contain significant quantities of Fe and Al oxides and hydroxyoxides (Golden et al., 1981; Karim and Rahman, 1980), which are known to fix P. The low pH and high P fixing capacity of these soils are favourable for the dissolution of phosphate rocks (PR) (Bolan and Hedley, 1989; Chien et al., 1990b; Mackay et al., 1986). Therefore tea is generally fertilised with sparingly soluble PR fertiliser sources (Ranganathan, 1977a; Sivasubramaniam et al., 1981). In Chapter 3 it was shown that camellia (*Camellia japonica* L.) plants, which are of the same family as tea, when grown in a Dystric Eutrochrept soil from New Zealand, were able to dissolve more North Carolina phosphate rock (NCPR) in the rhizosphere (37% of added P) in 56 days compared to that in the bulk soil (27% of added P). In this Chapter the rate of dissolution of a Sri

Lankan PR, Eppawala phosphate rock (EPR) in the rhizosphere of tea grown in a highly acidic Sri Lankan Ultisol is compared with that in the bulk soil.

Plants normally grown in association with tea have diverse growth habits. Calliandra (*Calliandra calothyrsus* L.), a leguminous tree crop is grown in tea fields to provide shade and organic material, which helps to conserve soil moisture (Anon, 1990). Guinea grass (*Panicum maximum* L.) is usually found on abandoned sloping tea lands, which helps to control soil erosion. Bean (*Phaseolus vulgaris* L.) is a common leguminous vegetable crop cultivated in the same soil type in adjoining farm gardens. Generally the supply of P for tea is from sparingly soluble EPR and for beans it is provided by a soluble P fertiliser. The intention of including crops of differing growth habits in this study was to provide information on the effect of slow and fast growing plants, as well as leguminous and non-leguminous species, on rhizosphere acidification and to test how best these plants could utilise P from the locally mined sparingly soluble PR, Eppawala phosphate rock (EPR). There is no information available in the literature on the relative efficiencies of these crops in utilising nutrients from acidic Ultisols particularly in the soils of tea-growing areas.

4.2 OBJECTIVES

The objectives of the study reported in this chapter are:

1. To compare root induced changes in pH and P fractions in the rhizosphere of tea and other plants.
2. To quantify the release of H^+ into the rhizosphere by these plants and assess their influence on PR dissolution in the rhizosphere.
3. To understand the diversity of mechanisms involved in P uptake and the utilisation efficiency of these plants which have diverse growth habits.

4.3 MATERIALS AND METHODS

The soil used in this study was a Rhodustult (De Alwis and Panabokke, 1972) collected from the top 75 mm depth after removing the litter layer of soil in a tea estate in the Southern part (Kottawa) of Sri Lanka. The selected physico-chemical properties of the soil are presented in Table 4.1. The air-dried soil was passed through a 2 mm sieve and mixed with Eppawala phosphate rock (EPR, particle size 5.2% > 250 μm; 39.2% 150 - 250 μm; 39.9% 150 - 75 μm; 15.7% < 75μm, total P 14.5%, citric acid (2%) soluble P 1.97%, almost insoluble in water and locally mined in Sri Lanka) at the rate of 200 μg P g^{-1} soil. Urea and KCl fertilisers were mixed with the soil at the rate of 200 μg N or K g^{-1} soil before planting. The root study container (RSC) technique used for camellias in the glasshouse described in Chapter 3 was used in this study (Figure 4.1). The upper and lower compartments were packed with 135 and 242 g soil (bulk density of 1.1 Mg m^{-3}) respectively. Vegetatively propagated 5 months old tea (TRI 2025) plants, 5 months old calliandra (*Calliandra calothyrsus* L.) plants, Guinea grass (*Panicum maximum* L.) cuttings and bean (*Phaseolus vulgaris* L.) seeds were planted in the upper compartment.

The four plant species, treated with EPR and a control without EPR fertiliser were considered as treatments. The treatments were replicated 4 times and arranged in a randomised block design in a glasshouse maintained at 12^0 C minimum and 26^0 C maximum temperatures at St. Coombs, Sri Lanka. Four RSCs (fallow) having the same N, P and K treatments as the planted pots, but this time with no plants, were used to study any changes in the soil in the absence of plants. All RSCs were placed on a fine sand bed, which was kept moist by a water reservoir. The water level in the reservoir was fixed at 160 mm below the base of the RSCs. This enabled the RSCs to be maintained at a constant water potential of approximately -1.6 kPa.

4.3.1 Soil and plant sampling

After 35 days of planting, plant shoots were cut 5 mm above the soil surface. The increase in plant dry matter during the trial period was estimated by taking the difference between final and initial dry weights of the seedlings.

Table 4.1 Selected physico-chemical properties of the soil used in the study

Property	Unit	Value
Sand	%	60
Silt	%	15
Clay	%	25
Soil pH	Soil : water (1 : 2.5 w/w)	4.5
Bulk density	Mg m^{-3}	1.1
pH buffering capacity (at pH 4-5)	mmol H^+ kg^{-1} pH^{-1}	30
Organic C	%	1.70
Effective CEC[1,2]	$cmol_c$ kg^{-1}	4.01
Ex. Ca	$cmol_c$ kg^{-1}	0.21
Ex. Mg	$cmol_c$ kg^{-1}	0.31
Ex. K	$cmol_c$ kg^{-1}	0.15
Ex. Na	$cmol_c$ kg^{-1}	0.11
Ex. Al	$cmol_c$ kg^{-1}	1.35
Olsen-P	µg g^{-1} soil	20
P-fixing capacity[2]	%	93

[1]Exchangeable (Ca + Mg + K + Na + Al + H)

[2]Blackmore et al. (1987)

Figure 4.1 The arrangement of plant species in the glasshouse experiment (A - Guinea grass, B- Calliandra, C - Bean and D - Tea)

The soil in the lower compartment was sliced in thin sections as described in Chapter 3. A 2.0 mm thick soil slice in the upper compartment immediately above the inter-cell boundary (24 μm polyester mesh) was also sampled and root length was measured according to the line intercept method described by Newman (1966). Root surface area was determined by using the formula $2\sqrt{(\pi ML/\rho)}$ (where π: 22/7, M: weight of roots, L: length of roots and ρ: density of roots) and assuming that a root is a cylindrical tube with a constant radius. The true density of roots was determined from the volume and weight of the roots. The root volume was determined by the amount of water displaced when the roots were immersed in water. The lower surface area of these roots was considered responsible for the observed changes in pH and soil P fractions in the lower compartment. The bottom view of RSCs showing roots of various plant species are presented in Figure 4.2, 4.3, 4.4 and 4.5.

4.3.2 Plant and soil analysis

Plant samples were separated into shoots and roots and dried at 60^0 C, weighed and ground to powder. The plant materials were dry-ashed at 550^0 C, and thereafter the ash was taken up in 0.05 *M* HCl solution and analysed for P by the vanadamolybdate method (Jackson, 1958), and Ca by atomic absorption spectrophotometry. The initial nutrient composition and dry weights of the plants were determined by destructive sampling of ten randomly selected plants of similar size to the test plants at the beginning of the study. The soil was air-dried and analysed for pH (1 : 2.5 soil : water ratio using a pH meter). Resin-P, NaOH-P_i, NaOH-P_o, H_2SO_4-P_i and residual P were determined by the sequential P fractionation scheme of Hedley et al. (1994) described in Chapter 3. The amount of EPR dissolution in the soil was determined by the method of Tambunan et al. (1993) as described in Chapter 3. The statistical analyses were performed according to the procedures of the SAS systems (SAS Institute, 1985).

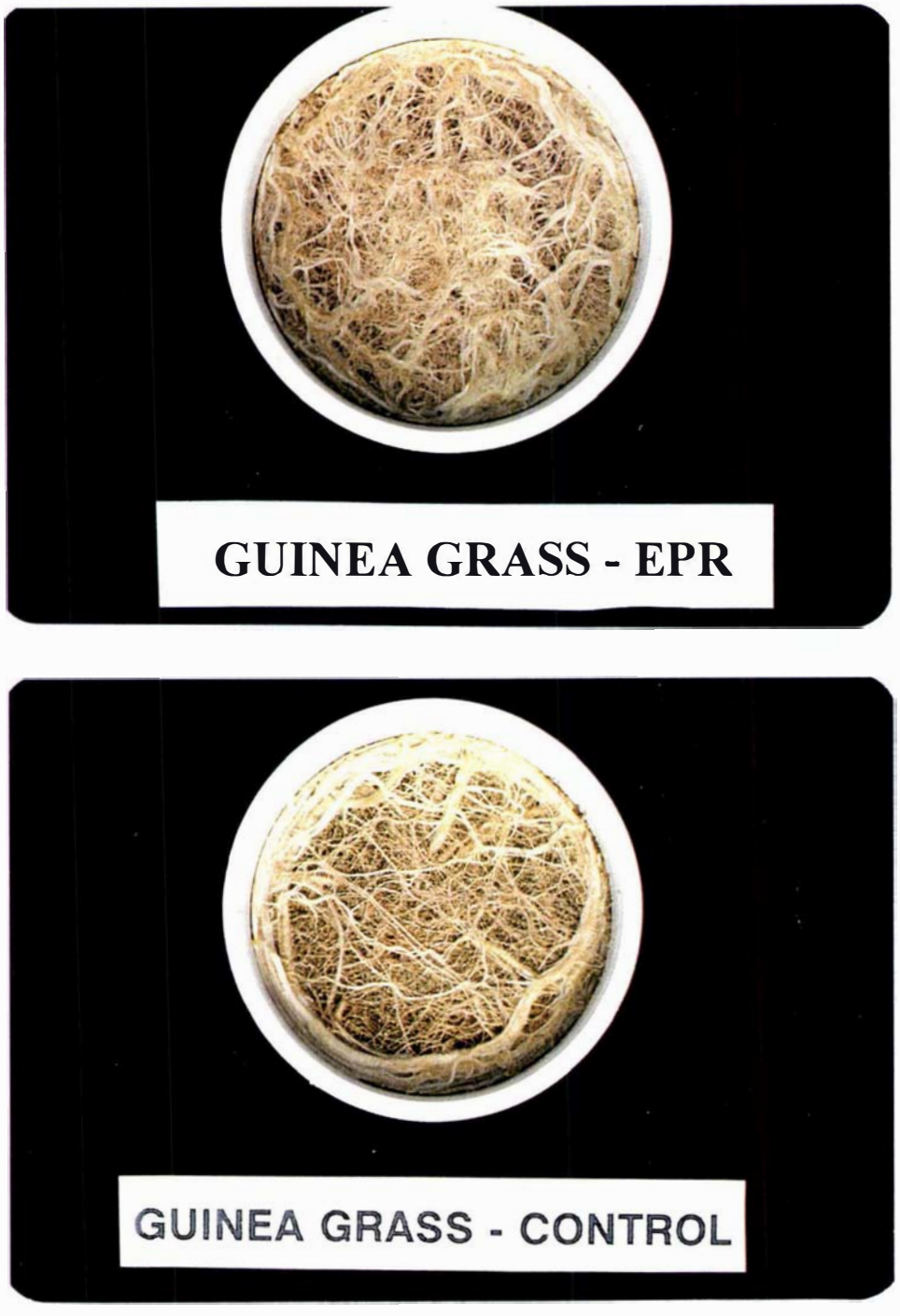

Figure 4.2 Roots mats formed on the polyester mesh for Guinea grass in EPR and control treatments

Figure 4.3 Roots mats formed on the polyester mesh for bean in EPR and control treatments

Figure 4.4 Roots mats formed on the polyester mesh for calliandra in EPR and control treatments

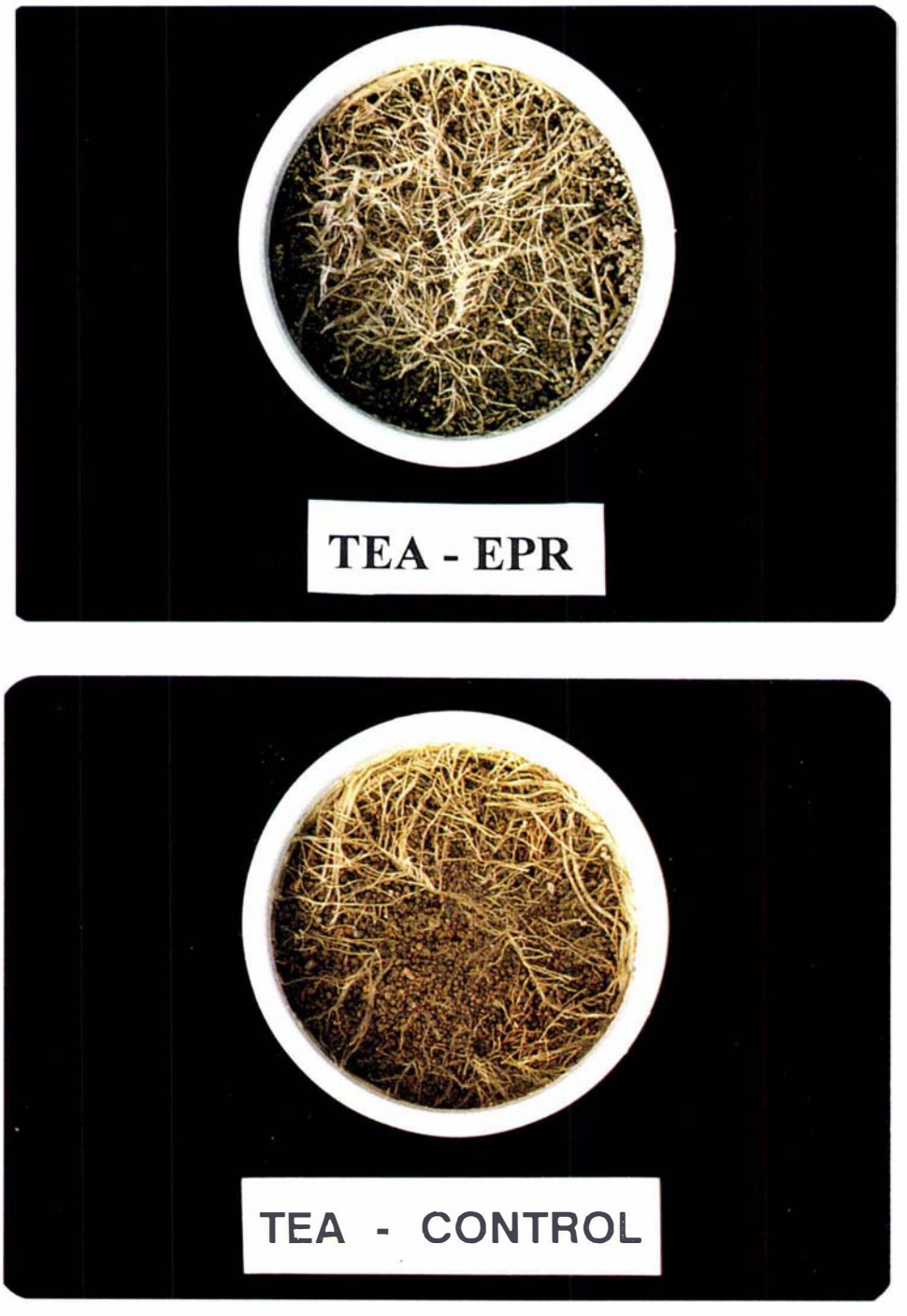

Figure 4.5 Roots mats formed on the polyester mesh for tea in EPR and control treatments

4.4 RESULTS AND DISCUSSION

4.4.1 Effect of plant species and EPR on growth characteristics

Significantly higher ($p < 0.05$) dry matter yields (final dry matter - initial dry matter) were obtained for bean and Guinea grass compared to tea and calliandra plants, whether EPR was applied to the soil or not (Table 4.2), with bean showing the highest dry matter production. Bean being a short-term vegetable crop and Guinea grass being a fast growing grass species produced larger amounts of dry matter than the other two plant species within a short period. Tea and calliandra on the other hand are perennials and their dry matter production was less due to a slower rate of growth. The shoot : root ratio was in the same order as dry matter production (bean > Guinea grass > calliandra and tea).

EPR application did not increase dry matter yield significantly in any of the plant species (Table 4.2). The P fertiliser application however increased shoot P concentration significantly ($p < 0.05$) in bean and Guinea grass.

4.4.2 Effect of EPR fertiliser on soil pH

Application of EPR had no significant effect on soil pH (Figure 4.6). In contrast to EPR, NCPR application significantly increased soil pH in a soil with lower pH buffering (Chapter 3). The dissolution of EPR in the bulk soil is only about 10% P (Figure 4.7) compared to 27% P dissolution from NCPR in the trial reported in Chapter 3. Therefore the consumption of soil acidity for EPR dissolution is much lower than that for NCPR dissolution and hence caused an insignificant rise in the bulk soil pH for EPR treatment. The lower reactivity of EPR (13.5% of total P dissolved in 2% citric acid) compared to NCPR (32% of total P dissolved in 2% citric acid, White et al., 1989) and the absence of accessory carbonate minerals in the apatite structure of EPR (Dahanayke et al., 1995) (NCPR has 11.7% $CaCO_3$, Syers et al., 1986) may have been the reason for the lower effect of EPR on soil pH.

Table 4.2 Comparison of plant dry matter yield and shoot : root weight ratio and P concentration of tea with those of other plant species

Plant species	Treatment	Plant dry matter (g)			Shoot : Root ((by weight)	P concentration (%)	
		Shoot	Root	Total		Shoot	Root
Tea	-P	0.42	0.26	0.68	1.62	0.147	0.062
Calliandra	(Control)	0.27	0.16	0.43	1.68	0.115	0.078
Guinea grass		2.80	0.98	3.78	2.85	0.072	0.072
Bean		5.17	0.81	5.98	6.38	0.024	0.103
Lsd ($p < 0.05$)		0.22	0.21	0.33	0.88	0.005	0.004
Tea	+ P	0.49	0.39	0.88	1.26	0.156	0.075
Calliandra	(EPR)	0.34	0.20	0.54	1.70	0.125	0.093
Guinea grass		3.29	1.34	4.63	2.45	0.102	0.109
Bean		5.54	0.99	6.53	5.59	0.052	0.114
Lsd ($p < 0.05$)		0.28	0.18	0.44	0.52	0.005	0.002

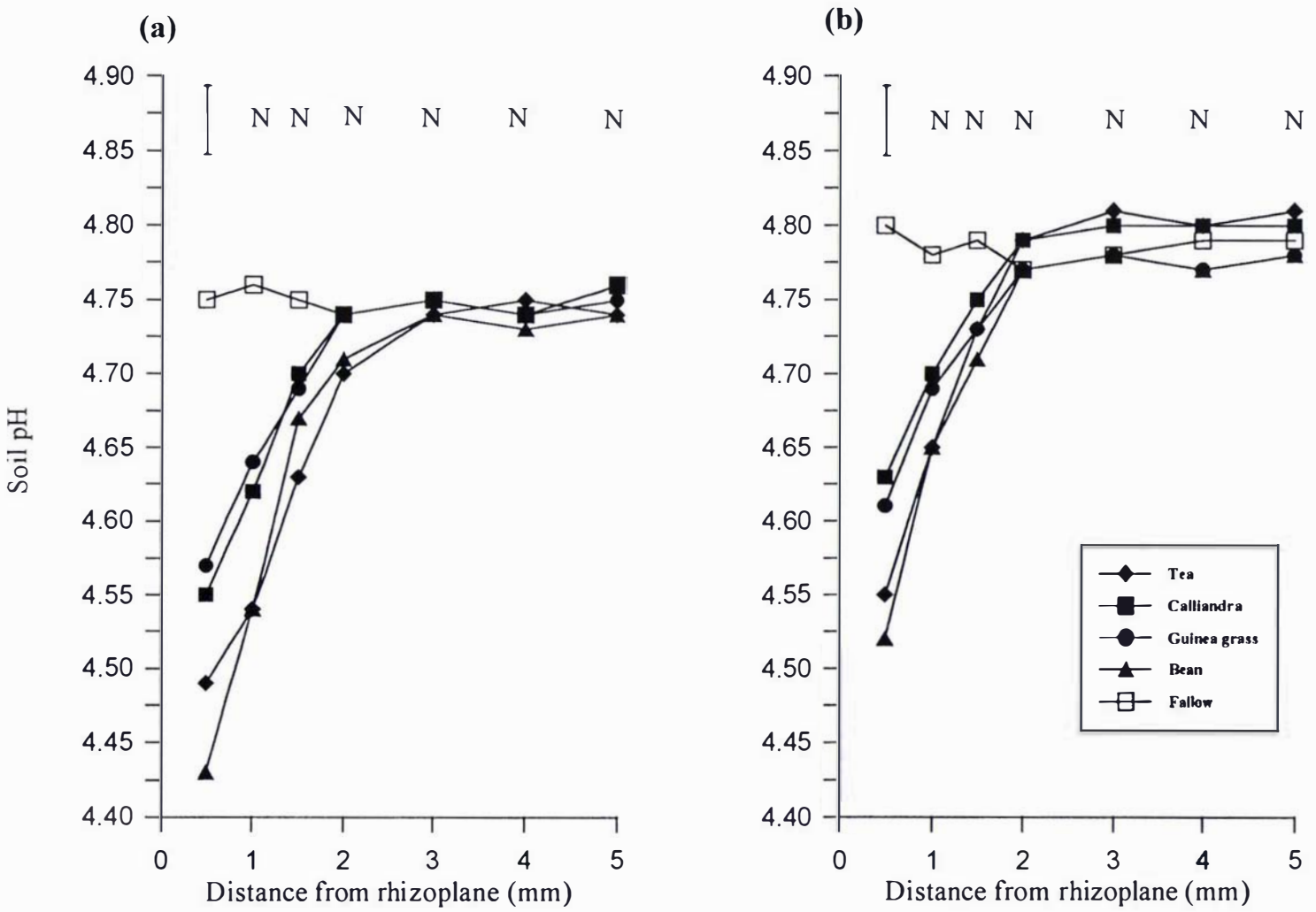

Figure 4.6 Effect of plant species on rhizosphere pH (1 : 2.5 w/w H_2O) of (a) control and (b) EPR fertilised soils. Vertical bars and N represent Lsd at $p < 0.05$ and treatments that are not statistically significant at $p < 0.05$ respectively.

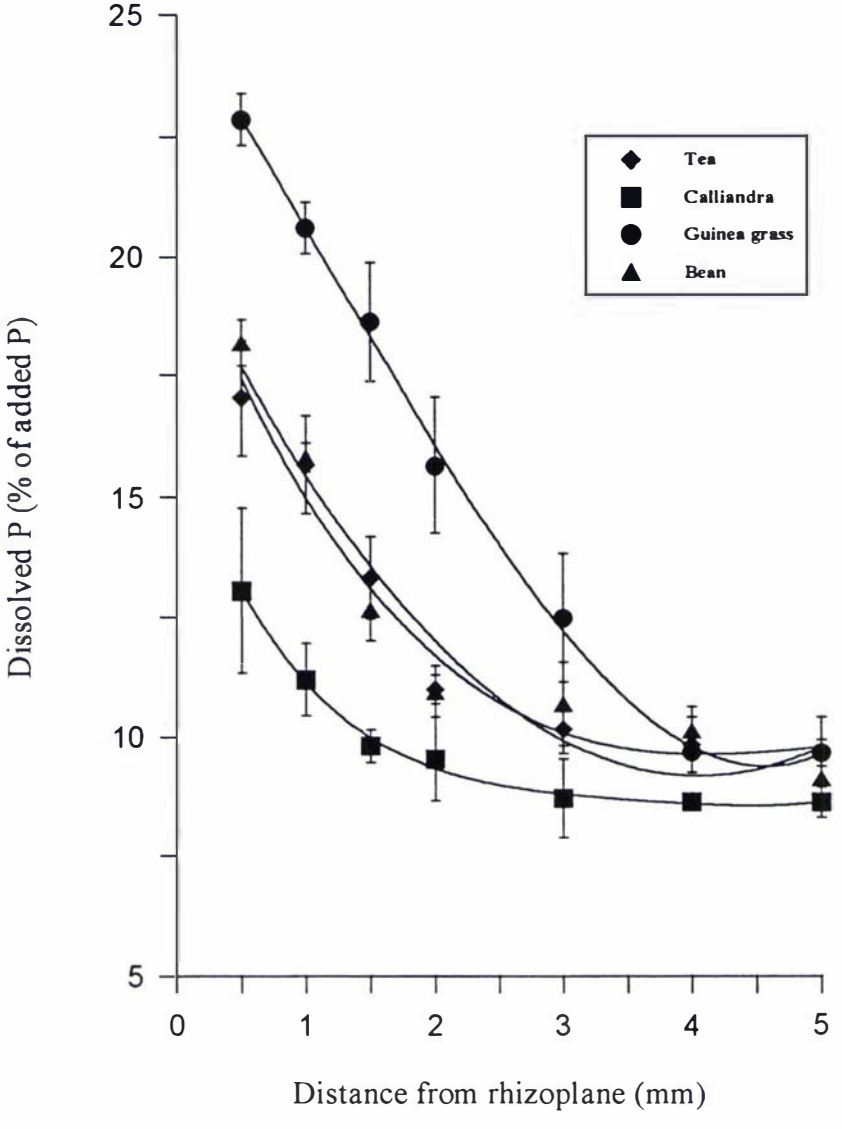

Figure 4.7 Effect of plant species on EPR dissolution in the rhizosphere. Vertical bars represent standard errors of the means.

4.4.3 Effect of plant roots on soil pH and EPR dissolution

Soil pH decreased in the rhizosphere compared to that in the bulk soil for all plant species, but the magnitude of reductions varied distinctly among the plant species (Figure 4.6). The magnitudes of the pH reductions in the rhizoplane of the plant species treated with EPR were 0.31, 0.26, 0.18 and 0.21 for bean, tea, Guinea grass and calliandra respectively. These results show that all four plant species released H^+ in the rhizosphere. Part of the H^+ released was consumed in the dissolution of PR in soils and the balance of H^+ caused pH reductions in the rhizosphere. The amounts of H^+ released into the rhizosphere (0 - 3 mm) soil in the lower compartment of RSC was calculated by adding the H^+ release calculated from the pH decrease in the rhizosphere compared to the bulk soil and the amounts of H^+ that were consumed for the dissolution of EPR in that zone (Table 4.3). The amounts of H^+ released into the rhizosphere corresponding to the pH decrease was estimated, by taking the difference in pH in the bulk soil and each of the rhizosphere soil slices. Then multiplying the respective pH differences by the soil pH buffering capacity and the weight of the corresponding soil slice, and summing the values for all the soil slices within the 0 - 3 mm distance zone from the rhizoplane. The amount of H^+ consumed in dissolving EPR in the rhizosphere and bulk soil was estimated using the amount of EPR dissolved and from the relationship that 2 moles of H^+ were consumed for every mole of P dissolved (%P in EPR/atomic weight of P * 1/100 * 2 = 0.00933 μmol H^+ μg^{-1} EPR dissolved). Mineralogical analysis of EPR using XRD showed that EPR has no detectable amounts of free carbonates ($CaCO_3$ or $MgCO_3$) (Tazaki et al., 1987), therefore all acids consumed in the dissolution of EPR were assumed to be due to the reaction of acids with the apatite in EPR. This calculation was also done for each soil slice within the 0 - 3 mm distance zone from the rhizoplane and the values summed-up to obtain the H^+ consumption for EPR dissolution in the rhizosphere.

Guinea grass released the highest amount of acidity into the lower compartment of RSC compared to all the other plant species, but because of its larger root surface area (Table 4.3) - the amount of acidity produced per unit surface area of roots is lowest for Guinea grass. Guinea grass produced the lowest reduction in pH measured

Table 4.3. The total acid production by roots of different plant species treated with EPR into the rhizosphere of the lower compartment

Plant species	pH drop in the rhizosphere (0-3 mm) compared to bulk soil[1]	Observed H^+ production within 0-3 mm of the rhizosphere in excess of bulk soil[2] (μmol H^+)	Average of EPR dissolution within 0-3 mm of the rhizosphere in excess of bulk soil[3] (P%)	H^+ consumption for EPR dissolution within 0-3 mm of the rhizosphere in excess of bulk soil[4] (μmol H^+)	Total H^+ production in (0-3 mm) of the rhizosphere in excess of bulk soil[5] (μmol H^+)	Surface area of roots within 0-2 mm above the mesh (cm^2)	Amount of H^+ released per unit surface area of roots[6] (μmol H^+ cm^{-2})
Bean	0.26 ± 0.01	15.8 ± 0.99	13.28 ± 1.60	6.69 ± 0.53	22.49 ± 2.5	526	0.0427
Tea	0.24 ± 0.01	15.3 ± 0.52	13.45 ± 1.31	6.67 ± 0.59	22.00 ± 2.4	270	0.0814
Guinea grass	0.17 ± 0.01	10.1 ± 0.79	18.05 ± 1.82	17.83 ± 1.30	27.99 ± 3.5	1196	0.0234
Calliandra	0.17 ± 0.02	9.6 ± 1.27	10.67 ± 0.66	4.66 ± 0.86	14.31 ± 2.3	256	0.0558

[1] Weighted mean pH of all slices within 3-5 mm from the rhizoplane minus weighted mean pH of all slices within 0-3 mm from the rhizoplane.

[2] Sum total of (change of pH in a rhizosphere soil slice compared to bulk soil {3-5 mm} * soil pH buffering capacity {30 μmol H^+ g^{-1} pH^{-1} soil} * weight of that soil slice) for all slices in the rhizosphere (0-3 mm).

[3] %P dissolved in the rhizosphere in excess of bulk soil.

[4] Amount of EPR dissolved (μg EPR g^{-1} soil) * amount of H^+ required to dissolve 1 μg EPR (0.00933 μmol H^+ μg^{-1} EPR - see text 4.4.3) * soil weight.

[5] Observed H^+ release in the 0-3 mm of the rhizosphere calculated from pH drop plus the amount of H^+ consumption during EPR dissolution in the rhizosphere soils.

[6] Total H^+ released into the rhizosphere divided by the surface area of roots lying above the mesh within 0-2 mm * 0.5. [The multiplication factor 0.5 is used because only the lower half of the surface area of the roots on the mesh is responsible for the H^+ release in the lower compartment].

in water in the rhizosphere. This is because more PR dissolved in the Guinea grass rhizosphere compared to that in other crops (Figure 4.7) and this caused more consumption of H^+ for PR dissolution giving rise to a higher pH in the Guinea grass rhizosphere.

Bean, being a legume obtains its N requirement through atmospheric N_2 fixation and very little through NO_3^- uptake. The low NO_3^- uptake by this plant may have caused a high cation-anion balance in the plants producing high acidity in the rhizosphere (Haynes, 1992). The total amount of acidity produced by bean is only second to Guinea grass. Calliandra is also a legume like bean, but it did not produce as much acidity as bean. This may be due to the low growth rate and smaller root surface area of calliandra (Table 4.3). The acidity produced per unit root surface area of calliandra is however not much different from that of bean. Tea, inspite of being a non-legume reduced rhizosphere soil pH more than calliandra. The acidity produced per unit root surface area of tea is the highest among the four crops. This may be because tea plants either took up NH_4^+ more than NO_3^- from the soil as suggested by Ishigaki (1978) or tea roots excreted significant amount of organic acids (Xiaoping, 1994) or both these reasons. Ishigaki (1978) reported that tea roots have a low concentration of nitrate reductase and therefore prefer to take up NH_4^+ to NO_3^-. This suggestion has been tested and the results are presented in Chapter 6.

The H^+ released by plant roots into the rhizosphere contributed to more PR dissolution in the rhizosphere compared to that in the bulk soil (Figure 4.7). The dissolution of PR was highest in the rhizosphere of Guinea grass (23% P dissolved near the rhizoplane) and lowest for calliandra (13% P dissolved near the rhizoplane). The higher dissolution of EPR in Guinea grass was attributed to the higher amount of acid release into the rhizosphere of these plants as a result of higher amounts of roots on the mesh. The higher amounts of Guinea grass roots may also have removed higher amounts of P and Ca, the dissolved products of EPR, causing more EPR dissolution compared to that in the rhizosphere of other plant species. A limitation in the calculation of acid release in the rhizosphere is that pH was determined in water. Changes in soil solution chemistry may have an effect on pH, without differences in

exchangeable acidity occurring. In future studies it may be more appropriate to measure the rhizosphere pH and determine pH buffering capacity in 0.01 *M* $CaCl_2$.

4.4.4 Effect of EPR addition and plant roots on soil P fractions

Increases in the P fractions in bulk soil treated with EPR (the zone unaffected by plant roots) compared to unfertilised bulk soil, accounted for nearly 100% of the added EPR (Table 4.4). The dominant P fraction in the EPR treated bulk soil was H_2SO_4-P_i (76 - 80%). According to the findings of Tambunan et al. (1993), this indicated that the majority of the added P remained as undissolved EPR in the soils at the end of the trial.

The amounts and forms of the P fractions extracted from soil slices close to the mesh boundary of planted and unplanted RSCs were compared. Planted RSCs showed distinct changes of P form and amount in soil 0 - 3 mm from the mesh (Figure 4.8, 4.9, 4.10 and 4.11). Similar plant induced effects on the rhizosphere chemistry in relation to P uptake were reported for camellia (*Camellia japonica* L.) in Chapter 3. In the rhizosphere, the amount and nature of the P fractions varied with the plant species. The absence of plants (fallow RSCs) showed no difference in any of the P forms with distance from the polyester mesh. Therefore any difference observed in the P fractions between the bulk soil and the rhizosphere was due to the influence of plant roots.

Phosphorus uptake by plant and microorganisms depleted resin-P (P in soil solution and P loosely sorbed to soil minerals) near the roots (Figure 4.8). The steep resin-P depletion profiles in the rhizosphere of bean and Guinea grass compared to calliandra were considered to be caused by the former growing at a faster rate and having a greater amount of roots at the inter-compartment boundary. Steep resin-P depletion profiles in the tea rhizosphere were due to plant P uptake as well as conversion of P_i to P_o (NaOH-P_o) by the active microbial population in the tea rhizosphere (see next paragraph). The depleted P is replenished by desorption of P bound to Fe and Al oxides (NaOH-P_i) (Figure 4.9) and dissolution of EPR (as indicated by a reduction in

Table 4.4 The P fractions in the control treatment and % recovery[1] of added P from EPR in the bulk soil

P-fraction	Control ($\mu g\ g^{-1}$ soil)	P recovery from EPR (%)
Resin-P	10.5 ± 1.1	3.3 ± 0.5
$NaOH\text{-}P_i$	68.2 ± 2.5	8.5 ± 1.2
$NaOH\text{-}P_o$	68.0 ± 2.1	4.7 ± 1.2
$H_2SO_4\text{-}P_i$	34.2 ± 1.1	78.5 ± 2.8
Residual-P	74.2 ± 3.1	2.2 ± 0.8
Total-P	255.1 ± 5.2	97.2 ± 2.1

[1] P recovery % = $\frac{\text{(amount of P in fertilised soil - amount of P in control soil)}}{\text{amount of fertiliser P added to the soil}} * 100$

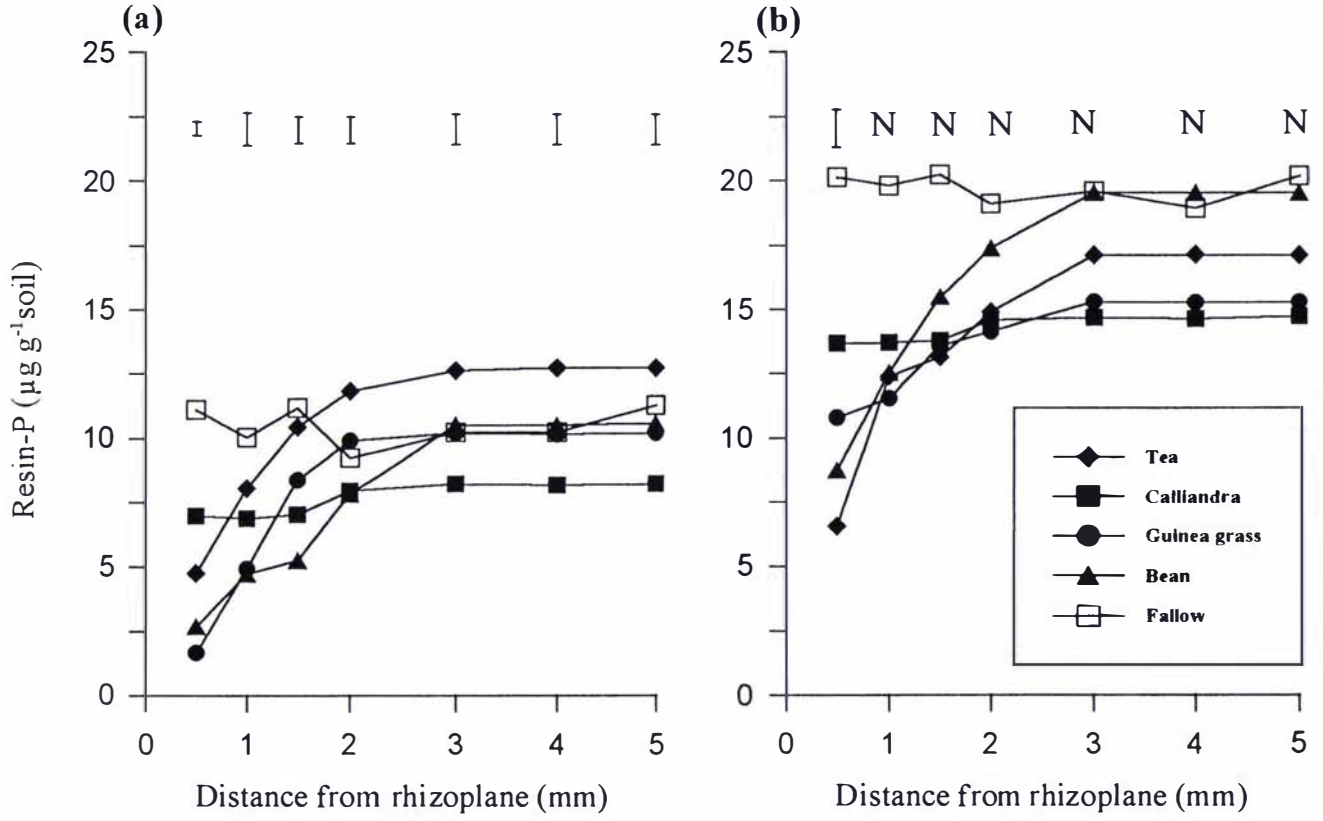

Figure 4.8 Effect of plant species on resin-P in the rhizosphere of (a) control (no EPR added) and (b) EPR fertilised soils. Vertical bars and N represent Lsd at $p < 0.05$ and treatments that are not statistically significant at $p < 0.05$ respectively.

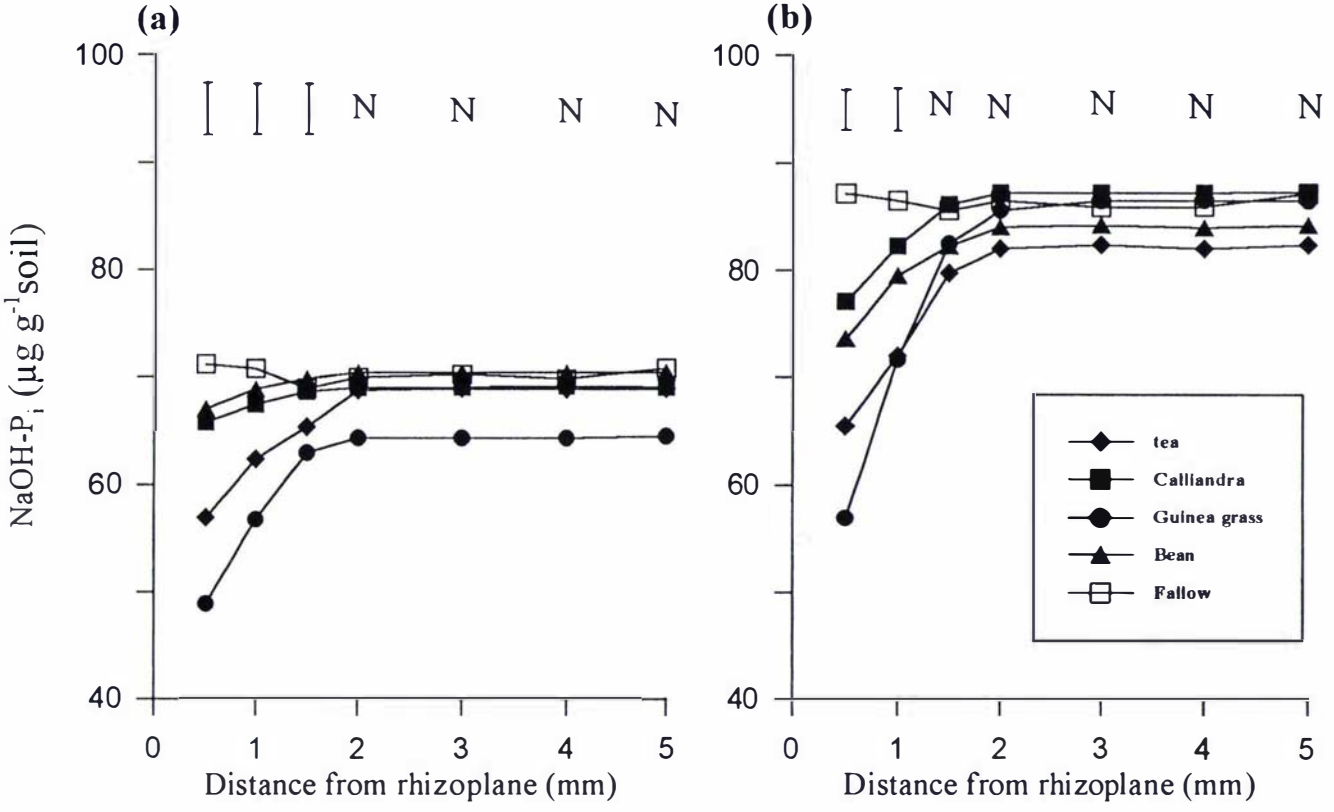

Figure 4.9 Effect of plant species on NaOH-P_i in the rhizosphere of (a) control (no EPR added) and (b) EPR fertilised soils. Vertical bars and N represent Lsd at $p < 0.05$ and treatments that are not statistically significant at $p < 0.05$ respectively.

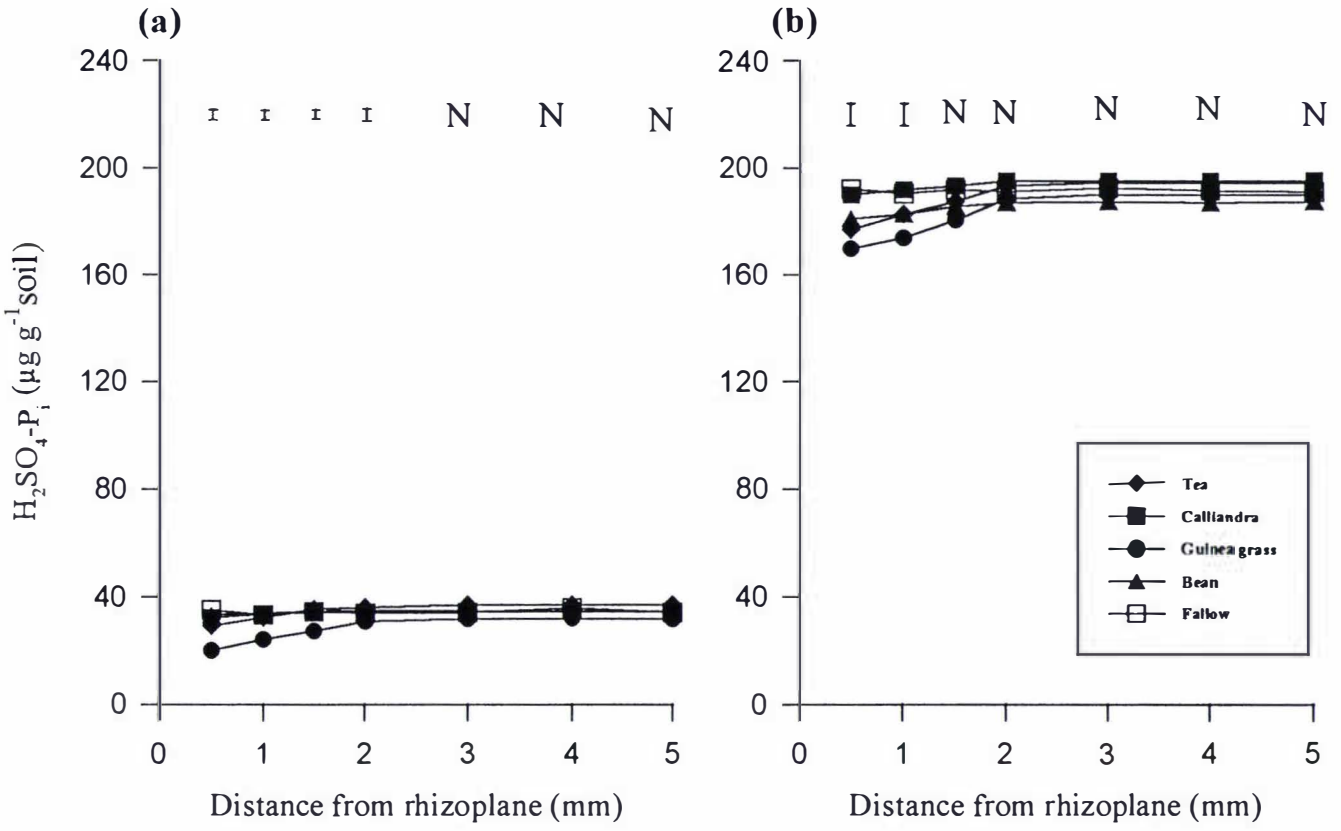

Figure 4.10 Effect of plant species on H_2SO_4-P_i in the rhizosphere of (a) control (no EPR added) and and (b) EPR fertilised soils. Vertical bars and N represent Lsd at $p < 0.05$ and treatments that are not statistically significant at $p < 0.05$ respectively.

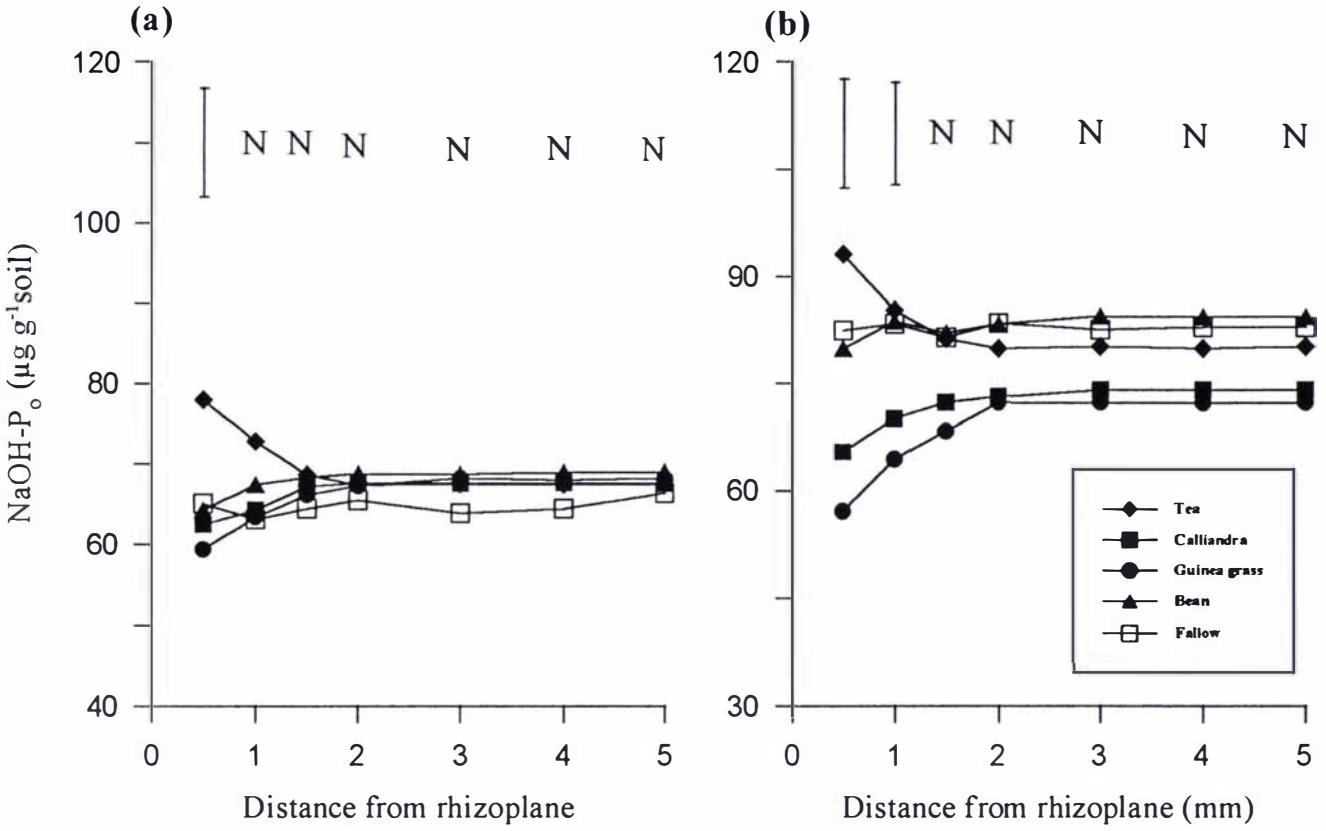

Figure 4.11 Effect of plant species on NaOH-P_o in the rhizosphere of (a) control (no EPR added) and (b) EPR fertilised soils. Vertical bars and N represent Lsd at $p < 0.05$ and treatments that are not statistically significant at $p < 0.05$ respectively.

H_2SO_4-P_i) (Figure 4.10). Low pH is however expected to increase P fixation by Fe and Al oxides and thereby increase NaOH-P_i. This may be the reason for less NaOH-P_i depletion in the bean rhizosphere, which had a lower pH than that in the Guinea grass rhizosphere. Among the plant species Guinea grass had the highest pH in the rhizosphere (Figure 4.6) and therefore the lowest P fixation in the rhizosphere soil resulting in the greatest NaOH-P_i depletion. The NaOH-P_i was also significantly depleted in the tea rhizosphere (Figure 4.9) even though the rhizosphere pH was significantly lower than that in the Guinea grass rhizosphere (Figure 4.6). Tea roots are known to secrete significant quantities of organic anions (Xiaoping, 1994), which may help in the release of fixed P by ligand exchange. The chelating action of some of these anions on Fe and Al may also enhance the release of adsorbed P (Earl et al., 1979; Nagarajah et al., 1968).

Except tea, in all the other three plant species P mobilised from NaOH-P_i and H_2SO_4-P_i fractions must have diffused across the rhizosphere towards the root surface, where it was taken up by the plant. Unlike other plant species, however NaOH-P_o accumulated in the rhizosphere of tea (Figure 4.11), suggesting that part of the depleted P_i forms were converted into organic P due to the assimilation of P_i by microorganisms, which use the organic anions secreted by the tea roots (Xiaoping, 1994) as a carbon (energy) source. No root hairs were visible in tea roots, when viewed through an optical microscope (1000 magnification). Therefore the accumulation of NaOH-P_o in the rhizosphere is unlikely to be due to organic P derived from root hairs. Armstrong and Helyar (1992) with several grass species in Australia and Trolove et al. (1996b) with pastoral legumes in New Zealand and the work reported on camellia in Chapter 3 also showed an increase of NaOH-P_o in the rhizosphere. In the present study, all other plant species except tea depleted P_o in the rhizosphere. This may be due to the rapid mineralisation of NaOH-P_o by phosphatase enzymes released into the rhizosphere by these plants. Tarafdar and Jungk (1987) and Gahoonia and Nielsen (1992) observed that phosphatase enzyme activity was higher at the root-soil interface than in the bulk soil for clover and rape plants and this was associated with depletion of NaOH-P_o in the rhizosphere of these plants. The accumulation of P_o in the vicinity of roots may be of considerable benefit for tea plants because it may be a useful mechanism in regulating a constant supply of P through

remobilisation of the organic P forms into inorganic P forms in periods of P deficiency in soils in the long run.

4.4.5 Comparison of rhizosphere P depletion with plant P uptake

The P depletion in the lower compartment of RSC was estimated by taking the difference between the total amount of P (μg P g^{-1} soil) in a slice near the root plane (0 - 3 mm) and the total amount of P at 3 - 5 mm from the rhizoplane (bulk soil). Total P depletion was calculated by summing the P depletion for all slices in the 0 - 3 mm zone. In all plant species, net P depletion in the soil of control treatment was lower than in EPR fertilised soil (Table 4.5). Among the plant species, the net P depletion was greater in Guinea grass compared to all other species for both EPR fertilised and control (no EPR) treatments. This was associated with a greater root surface area at the inter-compartment boundary, which absorbed more P from the rhizosphere in the lower compartment of RSC. Plant P uptake was estimated, by subtracting the initial P content of seedlings from the final P content of the plants by destructive sampling of seedlings of similar size to those of test plants at the beginning of the trial. The amount of P taken up by plants that were derived from the lower compartment varied with the plant species. Tea and calliandra plants derived 30 - 40% whereas Guinea grass and calliandra removed 12 - 20% of P from the lower compartment. The plant P uptake unaccounted for by P depletion in the lower compartment must have come from the P depletion in the upper compartment. The proportion of plant P derived from the soil in lower RSC was predicted by determining the fraction of the total roots influencing depletion of P in lower compartment. It was assumed that roots within 0 - 2 mm distance above the polyester mesh in the upper RSC could cause P depletion from the lower RSC. It was further assumed that only half of the surface area of these roots caused P depletion in the lower RSC and the other half caused depletion in upper RSC. The comparison of predicted and observed P depletions of the plant species showed that the predicted P depletion varied between -9 and 26% from the observed P depletion. These variations can be attributed to the overlapping of the depletion profiles of rhizospheres and interactions between root processes in P mobilisation. The possible errors that may

Table 4.5 Comparison of P depletion by different plant species from soil in the lower compartment of RSC with plant P uptake

Plant species	Tea		Calliandra		Guinea grass		Bean	
Treatment	Control	EPR	Control	EPR	Control	EPR	Control	EPR
Observed P depletion (μg RSC^{-1})								
Resin-P	111	134	23	17	104	75	168	184
NaOH-P_i	153	187	33	97	160	315	38	124
H_2SO_4-P_i	108	237	18	64	168	318	18	97
NaOH-P_o	0	0	53	90	106	178	44	63
Total	372	558	127	268	538	886	268	468
Observed P accumulation (μg RSC^{-1})								
NaOH-P_o	116	118	0	0	0	0	0	0
Observed net P depletion (μg RSC^{-1})	256	440	127	268	538	886	268	468
Total surface area[1] of boundary (0-2 mm above mesh) roots (cm^2)	245	270	225	256	1037	1196	415	526
Plant P uptake (μg $plant^{-1}$)	782	1070	443	622	2735	4825	2085	4023
Total root surface area[1] (cm^2)	297	359	307	317	2097	2745	1854	2011
Plant P uptake per root surface area (μg cm^{-2})	2.6	3.0	1.4	1.9	1.3	1.7	1.1	2.0
Predicted total P depletion[2] (μg RSC^{-1})	323	402	146	237	676	1051	233	526
Deviation of Predicted P depletion from observed (%)	26	-9	15	-11	26	19	-13	12

[1] Root surface area = $2\sqrt{(\pi ML/\rho)}$
where π - 3.1428, M - Fresh weight of roots, L - length of roots, ρ - density of roots

[2] $\frac{\text{Plant P uptake}}{\text{Total root surface area}} * \text{boundary root surface area} * 0.5$
[The factor 0.5 is used because only half the root surface area was assumed to cause depletion in the lower RSC]

have taken place in the measurements of root length will also have contributed to this effect.

4.4.6 External and internal efficiency of P utilisation

Plant species differ in their P needs and it is reported that they adopt various mechanisms to acquire P to meet their requirements. These mechanisms vary with plant species and the differences in P requirements are met by their external and internal P efficiencies. The external P utilisation efficiency is the ability of the plant to extract P from the soil and it is represented by the total plant P uptake. The increase in root surface area, rhizosphere acidification, removal of dissolved P and other products of PR from the reaction site, and the secretion of organic acids from roots are important factors that determine plant's external P utilisation efficiency. On the other hand the internal P efficiency is defined as the amount of dry matter synthesised per unit P taken up by the plant. A species that could produce high amounts of dry matter with small amounts of absorbed P would be internally more efficient. However it is possible that the P efficiency measurements are influenced by the differences in the type (seeds, seedlings or cuttings) and age of planting material, growth rates and duration of the trial. Therefore care must be taken in interpreting these results because their validity is confined to the conditions of these experiments.

4.4.6.1 External efficiency of P utilisation

The external efficiency of P utilisation of the plant species studied were in the order of Guinea grass > bean > tea > calliandra irrespective of P fertiliser addition. The higher external P efficiency of Guinea grass and bean (Table 4.6) is associated with a greater root surface area and not due to greater P uptake per unit root surface area (Table 4.5). Trolove et al. (1996a) also observed that higher external efficiency of white clover varieties was due to greater root surface area and not due to greater P uptake per unit surface area. Unlike other plant species, tea showed a significantly higher P uptake per unit surface area, and it may be because tea plants have mechanisms to extract more P from soil per unit surface area of roots compared to the others.

Table 4.6 Comparison of external and internal efficiencies of P in plants with and without added P fertiliser

Plant species	Treatment	External efficiency[1] (mg P)			Internal efficiency[2] (mg dry matter mg^{-1} P)		
		Shoot	Root	Total	Shoot	Root	Total
Tea	-P	0.621	0.161	0.782	537	331	868
Calliandra	(Control)	0.319	0.124	0.443	623	354	977
Guinea grass		2.024	0.711	2.735	1025	359	1384
Bean		1.246	0.839	2.085	2478	390	2868
Lsd ($p < 0.05$)		0.314	0.176	0.393	88	49	50
Tea	+P	0.773	0.296	1.070	462	364	826
Calliandra	(EPR)	0.431	0.192	0.622	551	330	861
Guinea grass		3.364	1.461	4.825	683	277	960
Bean		2.891	1.132	4.023	1377	246	1623
Lsd ($p < 0.05$)		0.304	0.184	0.456	76	52	95

[1] Plant P uptake by the respective tissue

[2] $\frac{\text{Dry matter production of respective tissue}}{\text{Total P uptake}}$

Extrusion of organic acids by tea roots (Jayman and Sivasubramaniam, 1975; Xiaoping, 1994) thereby causing dissolution of EPR as well as desorption of P from soil (depletion of NaOH-P_i - see section 4.4.4) may well be some of those mechanisms.

4.4.6.2 Internal efficiency of P utilisation

Significant differences between the plant species were observed in their internal P efficiencies for both control and EPR treatments. Bean plants had the highest internal efficiency and tea showed the lowest of the four plant species (Table 4.6). The plants with higher growth rates (bean, Guinea grass) could convert absorbed P into dry matter quickly compared to plant species having a slower growth rate (tea, calliandra). For all plant species the internal P efficiency was higher in the absence of EPR fertiliser (control treatment) than with it. In a classical model of plant dry matter response to the increased supply of P, the highest amount of dry matter production per unit of P absorbed by the plant was at the lowest level of P supply. This implies that as the soil P concentration increases, the P uptake and dry matter production also increases, but the rate of increase in dry matter production per unit of P supplied decreases for each additional unit of P absorbed.

The above results show that genetic variability within and among plant species could be utilised to develop, and screen, new varieties that are suitable for a location and also could utilise locally available resources, such as phosphate rock, for sustainable production of the crops. However limitations of this method such as the initial weight of planting materials, differences in growth rates and the length of trial period may need to be taken into account when comparing plant species. The internal P efficiency index needs careful interpretation. Some apparently P efficient species may have severe P stress and not yield useful seed or marketable products. The practical potential of the above findings was studied in detail using 3 different tea clones and the results are presented in Chapter 5.

4.5 CONCLUSIONS

All plant species studied acidified their rhizospheres. The magnitude of acidification varied with plant species and the extent of root growth. The plant induced rhizosphere acidification increased dissolution of EPR in that zone compared to the bulk soil. Guinea grass with the largest root mass caused the highest acidification in the rhizosphere and resulted in the highest EPR dissolution. But the rate of acidification per unit surface area of Guinea grass was the lowest among the four plant species, with tea producing the highest rate of acidification per unit surface area.

It was common for all plant species to deplete resin-P and NaOH-P_i in the rhizosphere. Except for tea, all other species depleted NaOH-P_o in the rhizosphere, with tea this fraction increased. These differences can be associated with the activities of phosphatase enzyme released to the rhizosphere from the roots of these plants or due to the relative rate of root C release. The organic P accumulation in the tea rhizosphere is probably due to P immobilisation by the increased microbial activity caused by the abundance of carbon exuded by the tea roots.

Guinea grass and bean plants are externally more P efficient than tea and calliandra. This was largely caused by the differences in root surface areas. The plants which have greater root surface area could extract more P from the soil compared to those that have lower root surface area. The internal P efficiencies were in the order of bean > Guinea grass > calliandra > tea and it reflects the ability of each species to convert absorbed P into dry matter based on their genetic diversity. However the validity and use of this information on P efficiency of different plant species lies within the limits of the conditions under which the experiment was conducted. Long-term glasshouse and field trials are required to test these findings.

CHAPTER 5

PHOSPHORUS UTILISATION EFFICIENCY AND DEPLETION OF PHOSPHATE FRACTIONS IN THE RHIZOSPHERE OF THREE TEA (*Camellia sinensis* L.) CLONES[1]

5.1 INTRODUCTION

Tea is cultivated in many parts of the humid and sub-humid tropical regions of the world, mainly in acid soils having a pH (H_2O) of 4.5 - 5.5 (Othieno, 1992; Ranganathan and Natesan, 1985). At these low pHs, aluminium (Al) is highly soluble and reacts with P to form insoluble Al-P complexes. Furthermore most tea soils are highly weathered and they contain large amounts of Fe and Al oxides and hydroxyoxides (Golden et al., 1981; Karim and Rahman, 1980), which are known to fix P. The precipitation of Al-P and the fixation of P in soils can cause a reduction in plant availability of P, from both native and fertiliser P sources. Phosphorus fractionation of Sri Lankan tea soils revealed that most of the applied P fertilisers are recovered as insoluble Fe and Al phosphates (Golden et al., 1981). Despite the high P fixation in these soils, tea plants do not generally suffer from P deficiencies. This suggests that tea plants have some mechanisms by which they are able to utilise the fixed soil P. The studies reported in Chapter 3 using camellia (*Camellia japonica* L.) plants, which is of the same family as tea, and also comparison of tea clone TRI 2025 with other plant species presented in Chapter 4 showed that tea plants can modify the root environment by acidifying the rhizosphere.

The possibility of exploiting genotype differences for improving nutrient efficiency has received much attention in recent times (Föhse et al., 1988; Gahoonia and Nielsen, 1996; Godwin and Blair, 1991). Phosphorus efficient genotypes can be useful for

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[1] **Zoysa A K N, Loganathan P and Hedley M J (1998) Phosphorus utilisation efficiency and depletion of phosphate fractions in the rhizosphere of three tea (*Camellia sinensis* L.) clones. *Nutrient Cycling in Agroecosystems* (in print).**

maintaining high productivity in low input agriculture. From a mineral nutrition point of view, a genotype is more efficient than others if it mobilises and absorbs more P from soils (external P efficiency) and/or makes better use of the absorbed P to produce greater biomass (internal P efficiency). Improvement of P efficiency by genetic selection of plants seems possible. Breeding of new crop genotypes with improved P efficiency may be a supplementary alternative to reducing the input of traditional amendments to soils such as the application of fertiliser (Batten, 1992).

New tea clones have been developed in many countries to increase yield, tolerance to drought and resistance to pests and diseases (Alam, 1994; Anandappa, 1986; Astika, 1994; Barbora; 1994). But there is no published information available on their relative efficiencies in utilising soil P. The external efficiency of P in plants is largely influenced by the size and distribution of the root system (O'Toole and Bland, 1987), the formation of root hairs (Föhse et al., 1991), root induced changes in the rhizosphere (Gahoonia and Nielsen, 1992; Marschner et al., 1987), the kinetics of P uptake parameters (Nielsen and Barber, 1978), soil moisture (Gahoonia et al., 1994) and the plant's ability to associate with mycorrhiza (Morita and Konishi, 1989; Zhi, 1993).

The slow diffusion of P results in depletion of P from the soil near the absorbing roots of the tea plants (Chapter 4). Therefore variation of P depletion profiles in the rhizosphere may provide information on the external efficiencies of P uptake in plants. The rhizosphere study container (RSC) technique described in Chapter 3 is used in this chapter to study the rhizosphere processes involved in P uptake by three tea clones having different genotypes.

5.2 OBJECTIVES

The objectives of the study reported in this chapter are:

1. To study the differences in the root induced changes in soil P fractions and soil pH in the rhizosphere of the three tea clones.

2. To determine the P utilisation efficiencies and screen the tea clones according to their ability to exploit soil and fertiliser phosphorus.

5.3 MATERIALS AND METHODS

The soil used in this study was a Rhodustult (Red yellow podsolic soil; De Alwis and Panabokke, 1972) collected from Kottawa, Sri Lanka. The physico-chemical characteristics of this soil was presented in Table 4.1, Chapter 4. The air-dried soil was passed through a 2 mm sieve and mixed with either triple superphosphate (TSP, total P 20% and 85 - 95% of total P soluble in water) and Eppawala phosphate rock (EPR, particle size 5.2% > 250 μm; 39.2% 150 - 250 μm; 39.9% 150 - 75 μm; 15.7% < 75 μm, total P 14.5%, citric acid (2%) soluble P 1.97%, locally mined in Sri Lanka) at rates of 200 μg P g^{-1} soil. Nitrogen (N) and potassium (K) fertilisers were mixed with the soil at the rate of 200 μg N and K g^{-1} soil in the form of urea and KCl respectively before planting.

The rhizosphere study container (RSC) technique described in Chapter 3 is used in this study. The upper and lower RSC compartments were packed with 135 g soil (bulk density 1.1 Mg m^{-3}) and 242 g soil (bulk density 1.1 Mg m^{-3}) respectively. Vegetatively propagated 5 month old tea plants of clones S 106, TRI 2025 and TRI 2023 were planted in the upper compartment of the RSC. These tea clones were selected for the study based on their differences in yield potential, resistance to pest and diseases or tolerance to drought (Anon, 1994abcd).

The treatments used in this study were the three tea clones, two P fertilisers and a control (no P treatment). These treatments were replicated 4 times and arranged in a randomised complete block design in a glasshouse maintained at 12^0 C minimum and 26^0 C maximum temperatures at St. Coombs, Sri Lanka. Four replicated RSCs without plants (fallow) were also included in the experiment to study the changes in soil P fractions in the absence of tea plants. All RSCs were kept on a fine sand bed which was kept moist by a water reservoir. The water level in the reservoir was fixed

at 160 mm below the base of the RSCs and soil moisture in pots were maintained approximately at a constant potential of -1.6 kPa.

5.3.1 Soil, plant and root sampling

After fifty six days of plant growth, the plant shoots were cut 5 mm above the soil surface. The soil and plant roots were sampled according to the methods described in Chapter 4 section 4.3.1.

5.3.2 Plant and soil analysis

Plant samples were separated into shoots and roots, dried at 60^0 C weighed and ground to powder. Both shoot and root samples were analysed for total N by Kjedhal digestion (Jackson, 1958). Soil pH, soil P fractionation and plant P concentrations were determined as described in Chapter 4 section 4.3.2.

5.4 RESULTS AND DISCUSSION

5.4.1 Dry matter yield, P and N concentration of tea clones

At the end of 56 days of plant growth, TRI 2023 and TRI 2025 had significantly ($p < 0.05$) greater shoot and root dry matter yields than S 106 for the two P fertiliser treatments as well as control treatment (Table 5.1). The differences in dry matter production in the tea clones could be attributed to their genetic variability. Application of P and types of P fertiliser showed no significant effect on dry matter yield.

The shoot P concentrations were higher in TRI 2023 and TRI 2025 compared to S 106 in all the treatments (Table 5.1). This may be attributed to increased root growth, which helped root exploration of a larger soil volume than the roots of S 106. The lower P concentration in the roots of TRI 2023 and TRI 2025 in soils treated with P fertiliser may be due to a dilution effect caused by increased root weight. The plants did not show any N deficiency symptoms during the course of the study. The N

Table 5.1 Dry matter yield, N and P concentration in shoots and roots, and P utilisation efficiencies of three tea clones

Clone	Treatment	Shoot			Root			Total root surface area (cm^2)	Plant P uptake per root surface area ($\mu g\ cm^{-2}$)	External P efficiency (mg P $plant^{-1}$)	Internal P efficiency (Shoot dry wt g per mg total P uptake)
		Dry wt (g)	N%	P%	Dry wt (g)	N%	P%				
S106	Control	0.482 a	1.51 a	0.117 a	0.372 a	1.26 a	0.049 a	242	3.22	0.779 a	620 a
TRI 2025		1.240 b	1.73 b	0.126 b	0.957 b	1.51 b	0.052 a	257	3.95	2.031 b	611 a
TRI 2023		1.525 b	1.77 b	0.130 b	1.177 b	1.43 b	0.067 b	305	3.41	2.718 c	562 b
S106	EPR	0.525 a	1.56 a	0.127 a	0.405 a	1.29 a	0.079 b	402	5.05	1.015 a	519 a
TRI 2025		1.394 b	1.89 b	0.136 a	1.076 b	1.45 b	0.056 a	484	5.07	2.455 b	569 a
TRI 2023		1.670 b	1.75 a	0.149 b	1.289 b	1.54 b	0.065 a	579	4.63	3.296 c	518 a
S106	TSP	0.544 a	1.65 a	0.130 a	0.420 a	1.36 a	0.074 a	568	4.78	1.041 a	528 a
TRI 2025		1.493 b	1.78 a	0.146 b	1.153 b	1.45 a	0.055 a	702	4.69	2.679 b	559 a
TRI 2023		1.704 b	1.76 a	0.152 b	1.316 b	1.57 b	0.066 a	781	4.28	3.340 c	512 a

Numbers within each cell followed by common letters indicate treatment means are not significantly different at $p < 0.05$ according to DMRT

concentrations of mature leaves were 3.6 - 4.1% which is considered adequate for satisfactory growth of tea (3.5 - 4.5% leaf N, Bonheure and Willson, 1992).

5.4.2 External and internal P utilisation efficiency of tea clones

The external efficiency of P utilisation of the tea clones is in the order TRI 2023 > TRI 2025 > S 106 regardless of whether P fertilisers were applied or not (Table 5.1). In all three clones the fertiliser treatments had no effect on the external efficiency of P. Higher external efficiency of P in TRI 2023 and TRI 2025 over S 106 is due to greater root surface area and greater P uptake per unit surface area in the two TRI clones (Table 5.1; Figure 5.1). The higher external efficiency of P in TRI 2023 compared to TRI 2025 is however due to a greater root surface area in TRI 2023 and not due to a higher P uptake per unit surface area as observed in white clover varieties (Trolove et al., 1996a). These results suggests that TRI 2023 and TRI 2025 have a higher genetic potential to increase root growth, which can explore more soil volume to utilise P. These two clones have higher ability to absorb P per unit root surface area than S 106 probably because of greater acidification of the rhizosphere (Figure 5.2), root exudation and/or mycorrhizal fungus association. It has been observed that tea roots have vesicular-arbuscular-mycorrhiza (VAM) relationships (Barthakur et al., 1987; Zhi, 1993), but there is no information available on the extent of this association in different tea clones. Further research in this area would help to understand the differences in P utilisation efficiencies of tea clones.

In general, internal P utilisation efficiency was similar among the tea clones (Table 5.1). In all three clones however the internal P efficiency was significantly higher in the absence of any P fertiliser treatments (control treatment) than when P fertilisers were applied as observed for tea clone TRI 2025, bean, Guinea grass and calliandra in Chapter 4.

In this study none of the clones had the combination of both high internal and external P use efficiencies. A combination of both these traits is expected to further benefit the plant. In essence, an ideal clone should be externally efficient to extract more P from

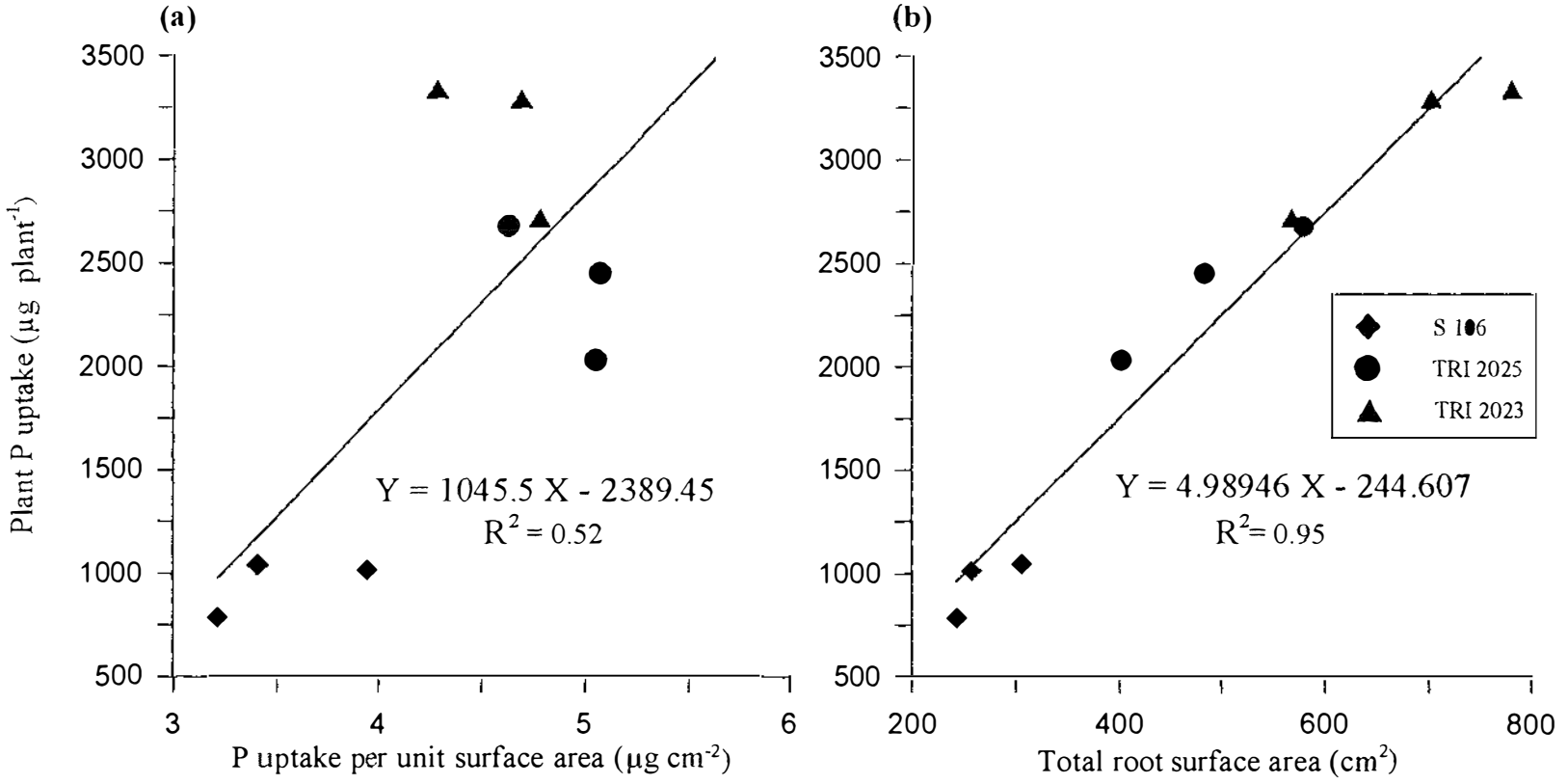

Figure 5.1 Effect of (a) P uptake per unit surface area and (b) total root surface area on P uptake by tea clones

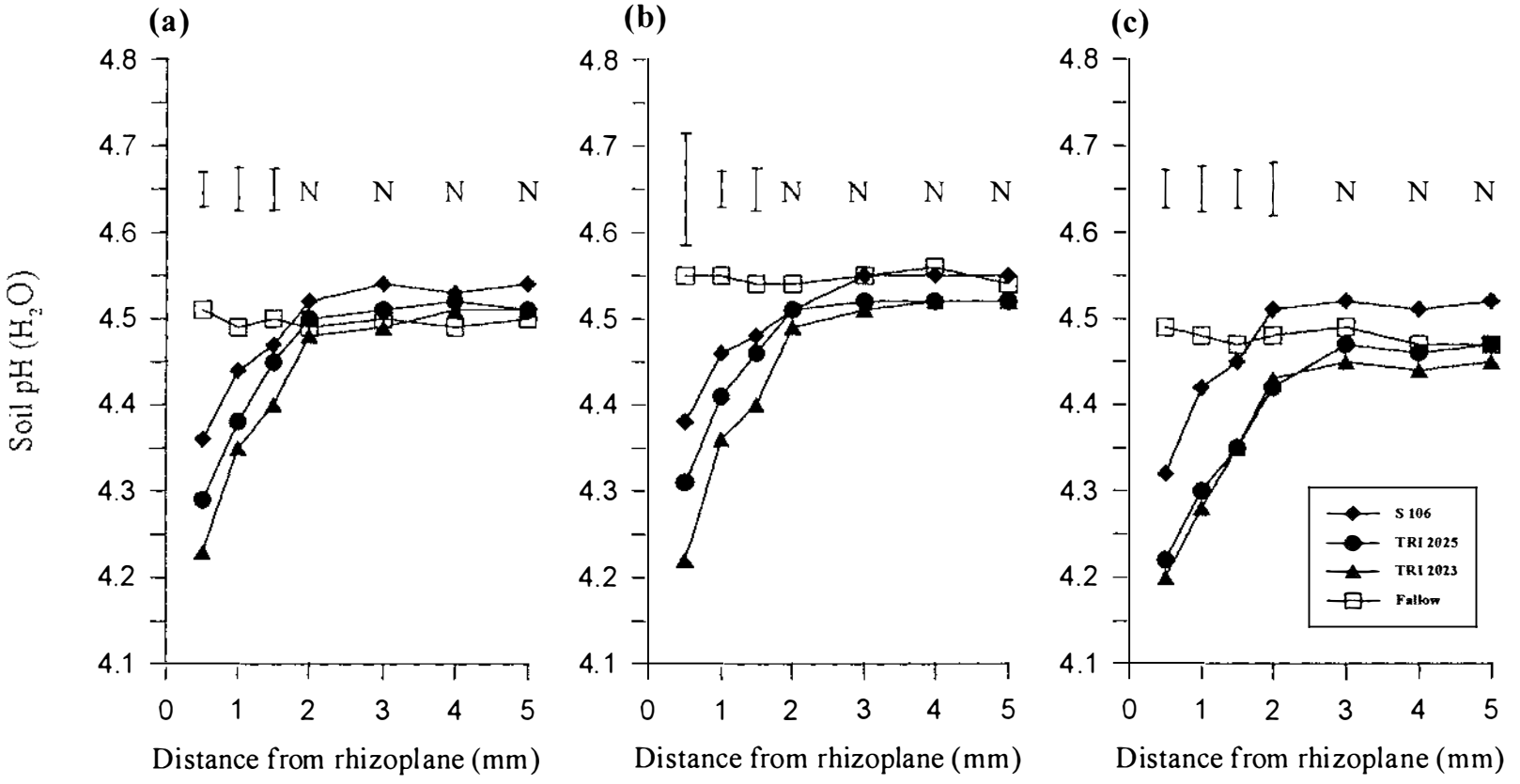

Figure 5.2 Effect of tea clones on rhizosphere soil pH in (a) Control (b) EPR and (c) TSP treatments. Vertical bars correspond to Lsd at $p < 0.05$ and N represents treatments are not statistically significant at $p < 0.05$.

the soil (P acquisition) and internally efficient to produce more dry matter from the absorbed P (P use efficiency). Therefore in the event of tea clonal screening, plant breeders should try to combine these two characters to obtain maximum yield with minimum P input.

5.4.3 Effect of P fertilisers and tea clones on soil pH

Soil pH decreased in the rhizosphere of all tea clones (Figure 5.2), but there was no change in soil pH more than 3 mm away from the rhizoplane, nor in the soil without tea plants (fallow). The pH changes in the rhizosphere were due to the influence of plant root activity. The decrease of soil pH in the rhizosphere varied with the tea clone. TRI 2023 had the highest pH decrease (0.25 - 0.30 units) whereas S 106 showed the lowest pH decrease (0.12 - 0.20 units). TRI 2025 decreased rhizosphere pH by 0.19 - 0.29 units. Greater shoot and root mass was associated with lower rhizosphere pH. The acidity released into the rhizosphere spread to a distance of about 3 mm from the rhizoplane for all the clones. The differences between clones in the release of acidity into the rhizosphere may be linked to the maintenance of electroneutrality in plant tissues after the excessive uptake of cations over anions (C-A) (Barber, 1995; Haynes, 1990). Acidification of the rhizosphere has sometimes been attributed to exudation of low molecular weight organic acids (Hoffland, 1992; Hoffland et al., 1989; Liu et al., 1990). Tea plants are reported to secrete significant quantities of malic and citric acids from their roots (Jayman and Sivasubramaniam, 1975; Xiaoping, 1994). The pK_1 values of citric and malic acids are 3.14 and 3.4 respectively (Weast, 1971), which are much lower than the cytoplasmic pH (6 - 7). Therefore these acids must leave the root cell cytoplasm in dissociated salt forms along with H^+ to maintain electroneutrality within the cells.

Dissolution of phosphate rocks is known to increase soil pH (Loganathan et al., 1995), but in our experiment the EPR treatment had no significant influence on soil pH (Figure 5.2). The dissolution of EPR in the rhizosphere (0-3 mm) ranged from 17 - 26% P and in the bulk soil (3 - 5 mm) it ranged from 12 - 14% P (Figure 5.3). The extent of EPR dissolution in the bulk soil was however much lower than North

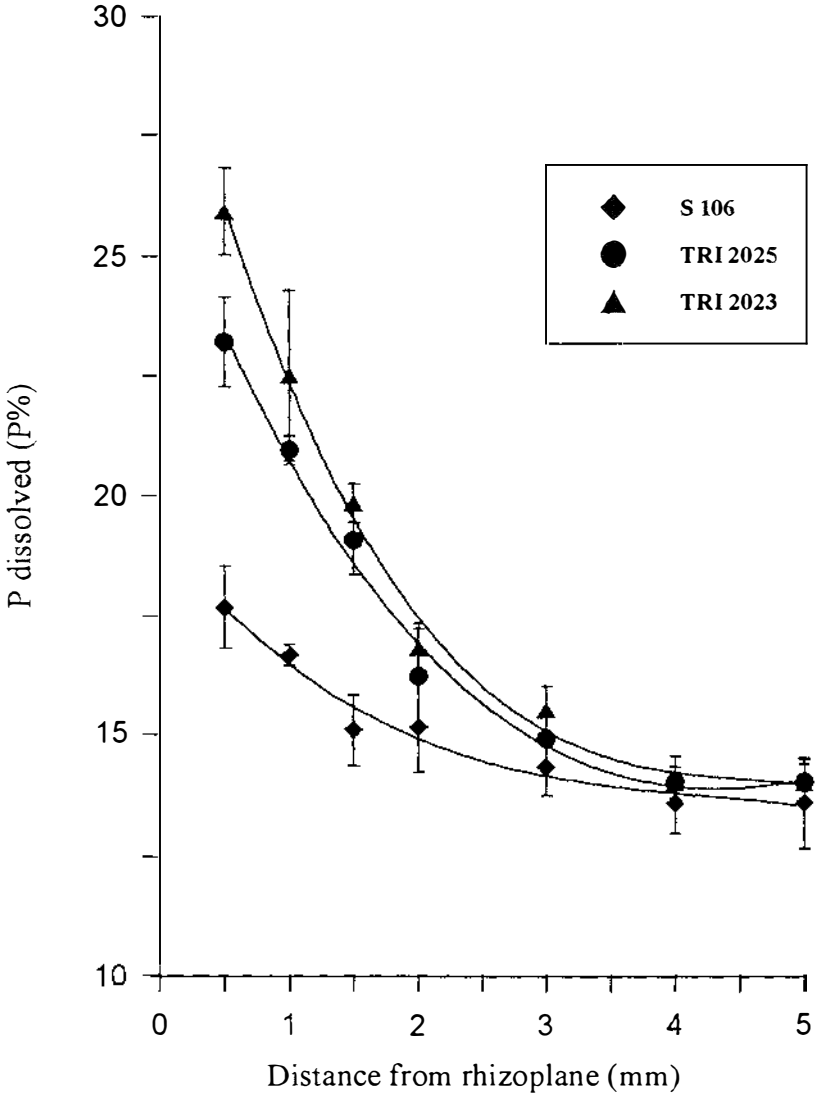

Figure 5.3 Effect of clonal differences on EPR dissolution in the rhizosphere. Vertical bars represent standard errors of the means.

Carolina phosphate rock (NCPR) dissolution (30 - 32%) observed in an earlier trial, where a significant pH increase was observed in bulk soil for the NCPR treatment compared to control (Chapter 3). Another reason for the absence of any noticeable pH rise in EPR treatments is that this PR has no accessory carbonate minerals associated with it (Dahanayake et al., 1995) unlike NCPR and many other reactive phosphate rocks (Syers et al., 1986).

The higher dissolution of EPR in the rhizosphere for all clones is because of lower pH in the rhizosphere compared to the bulk soil. TRI 2023 and TRI 2025 produced significantly more EPR dissolution than S 106 (Figure 5.3). The amount of EPR dissolution near the rhizoplane was in the range of 23 - 26% in TRI 2023 and TRI 2025 whereas in S 106 it was 18%. This is due to the secretion of more protons into the rhizosphere by TRI 2023 and TRI 2025 than S 106, as observed from the pH differences in the rhizosphere of these clones.

The amount of H^+ released into the inner rhizosphere - an area very close to the rhizoplane (0 - 0.5 mm zone) was calculated by adding both H^+ released due to pH decrease in the rhizosphere compared to the bulk soil and the amounts of H^+ that were consumed for the dissolution of EPR in that zone (see Table 5.2 foot notes for details of this calculation). The amount of H^+ consumed in dissolving EPR in the rhizosphere and the bulk soil was determined as described in Chapter 4., section 4.4.3. The calculations showed that less than 12% of the acid released was used up in dissolving EPR (Table 5.2). This indicates that the presence of H^+ in the vicinity of EPR particles is not the sole factor causing dissolution of EPR. The removal of dissolved products of EPR (P, Ca and F) from the reaction site by plant uptake, soil adsorption and leaching are also key factors controlling EPR dissolution in the rhizosphere.

5.4.4 Effect of P fertilisers on soil P fractions

The P fractionation of bulk soil with no P fertiliser additions showed that the soil used in the study had much higher NaOH-P_i, NaOH-P_o and residual-P than resin-P and H_2SO_4-P_i (Table 5.3) indicating that the labile and Ca bound P are low in these soils

Table 5.2 The comparison of observed acidity release in the rhizosphere of tea clones in EPR treated soil and the predicted acid release based on EPR dissolution

Clone	pH decrease near rhizoplane (0-0.5 mm) compared to bulk soil[1]	H^+ release within 0-0.5 mm of the rhizoplane[2] (μmol H^+ g^{-1} soil)	EPR dissolution within 0-0.5 mm of the rhizoplane soil (P%)	H^+ consumption for EPR dissolution within 0-0.5 mm of the rhizoplane[3] (μmol H^+ g^{-1} soil)	EPR dissolution in the bulk (3-5 mm) soil (P%)	H^+ consumption for EPR dissolution in bulk soil[3] (3-5 mm) (μmol H^+ g^{-1} soil)	Difference in the amount of H^+ used for dissolution of EPR in bulk and the rhizoplane (0-0.5 mm) soils[4] (μmol H^+ g^{-1} soil)	Total H^+ production in the (0-0.5 mm) rhizoplane[5] (μmol H^+ g^{-1} soil)
S 106	0.17 ± 0.05	4.95 ± 1.62	17.67 ± 1.86	2.28 ± 0.11	13.60 ± 1.91	1.75 ± 0.12	0.53 ± 0.04	5.48 ± 1.62
TRI 2025	0.19 ± 0.03	7.98 ± 1.16	23.21 ± 1.94	2.99 ± 0.12	14.06 ± 1.46	1.81 ± 0.06	1.18 ± 0.17	9.16 ± 0.99
TRI 2023	0.30 ± 0.02	12.70 ± 0.76	25.93 ± 1.90	3.34 ± 0.12	14.27 ± 1.37	1.84 ± 0.05	1.50 ± 0.08	14.20 ± 0.77

[1]Difference in pH (H_2O) of 0-0.5 mm soil slice and the weighted mean pH of all slices within 3-5 mm from the rhizoplane.

[2] Change of pH in the rhizosphere compared to bulk soil * soil pH buffering capacity (30 μmol H^+ g^{-1} pH^{-1} soil).

[3]Amount of EPR dissolved (μg EPR g^{-1} soil) * amount of H^+ required to dissolve 1 μg EPR (0.00933 μmol H^+ μg^{-1} EPR - see text).

[4] H^+ consumption for EPR dissolution in 0-0.5 mm zone of the rhizosphere minus H^+ consumption for EPR dissolution in the bulk soil (3-5 mm).

[5] H^+ released in the 0-0.5 mm from the rhizoplane plus the difference in the amount of H^+ used for dissolving EPR in the bulk and the rhizosphere soils.

Table 5.3 P fractions in the control soil (no P fertiliser added) and % recovery[1] of added P from EPR and TSP treated bulk soil (3 - 5 mm) after 56 days of plant growth

P-fraction	Control bulk soil ($\mu g\ g^{-1}$ soil)	S 106		TRI 2025		TRI 2023	
		EPR	TSP	EPR	TSP	EPR	TSP
		(P%)		(P%)		(P%)	
Resin-P_i	13	7	10	6	9	7	9
NaOH-P_i	113	8	66	8	69	14	73
NaOH-P_o	61	6	10	6	10	6	7
H_2SO_4-P_i	28	78	9	77	9	71	8
Residual-P	72	2	4	1	2	2	3
Total-P	287	101	99	98	99	100	100

[1]P% recovery = $\frac{\text{(P fraction in fertilised soil - P fraction in control soil)}}{\text{amount fertiliser P added to the soil}} * 100$

(Golden et al., 1981). The small amounts of labile P in these soils is due to high P fixation (Table 4.1, Chapter 4) and the low concentrations of Ca in the soil. The recovery of fertiliser P in the bulk soil was 98 - 101% with 66 - 73 % of the P from TSP fertiliser converted into NaOH-P_i and 71 - 78% of the P from EPR fertiliser remaining in the H_2SO_4-P_i fraction.

Most of the applied P fertilisers were recovered in NaOH-P_i and H_2SO_4-P_i fractions, which were traditionally considered as pools of P that are not easily available for plant uptake. Triple superphosphate had increased the readily available and weakly sorbed fractions of P (resin-P). These fractions were short-lived in soil, because of rapid chemical reactions with Fe and Al oxides and hydroxyoxides transformed this dissolved P into NaOH-P_i (Fe-P and Al-P) (Golden et al., 1991; Hedley et al. 1994; Perrott, 1995). The recovery of large amounts of H_2SO_4-P_i (Ca-P) in soils treated with sparingly soluble EPR fertiliser was as expected because the P in the apatite mineral in EPR is mainly bound to Ca.

5.4.5 Effect of tea clones on soil P fractions

There was a significant reduction of resin-P, NaOH-P_i, and H_2SO_4-P_i up to 3 mm from the rhizoplane in all the tea clones compared to the bulk soil (Figures 5.4, 5.5 and 5.6) as observed for tea clone TRI 2025 and other crops in Chapter 3 and 4. The reduction of resin-P in the rhizosphere was significantly greater ($p < 0.05$) in TRI 2023 and TRI 2025 compared with S 106 and the reduction of NaOH-P_i in the rhizosphere was significantly greater ($p < 0.05$) in TRI 2023 compared to the other two clones. The depletion of these P fractions is due to plant uptake and microbial immobilisation of P_i. Plant P uptake and immobilisation of P_i into NaOH-P_o are the major causes of the depletion of resin-P in the rhizosphere soil of all tea clones, rather than P fixation by Fe and Al, because NaOH-P_i fraction which is a measure of Fe-P and Al-P also decreased. The significant differences in the H_2SO_4-P_i among the tea clones near the rhizoplane in EPR treated soil was due to the differences in the amount of EPR dissolution caused by the differences in rhizosphere pH (Figure 5.2).

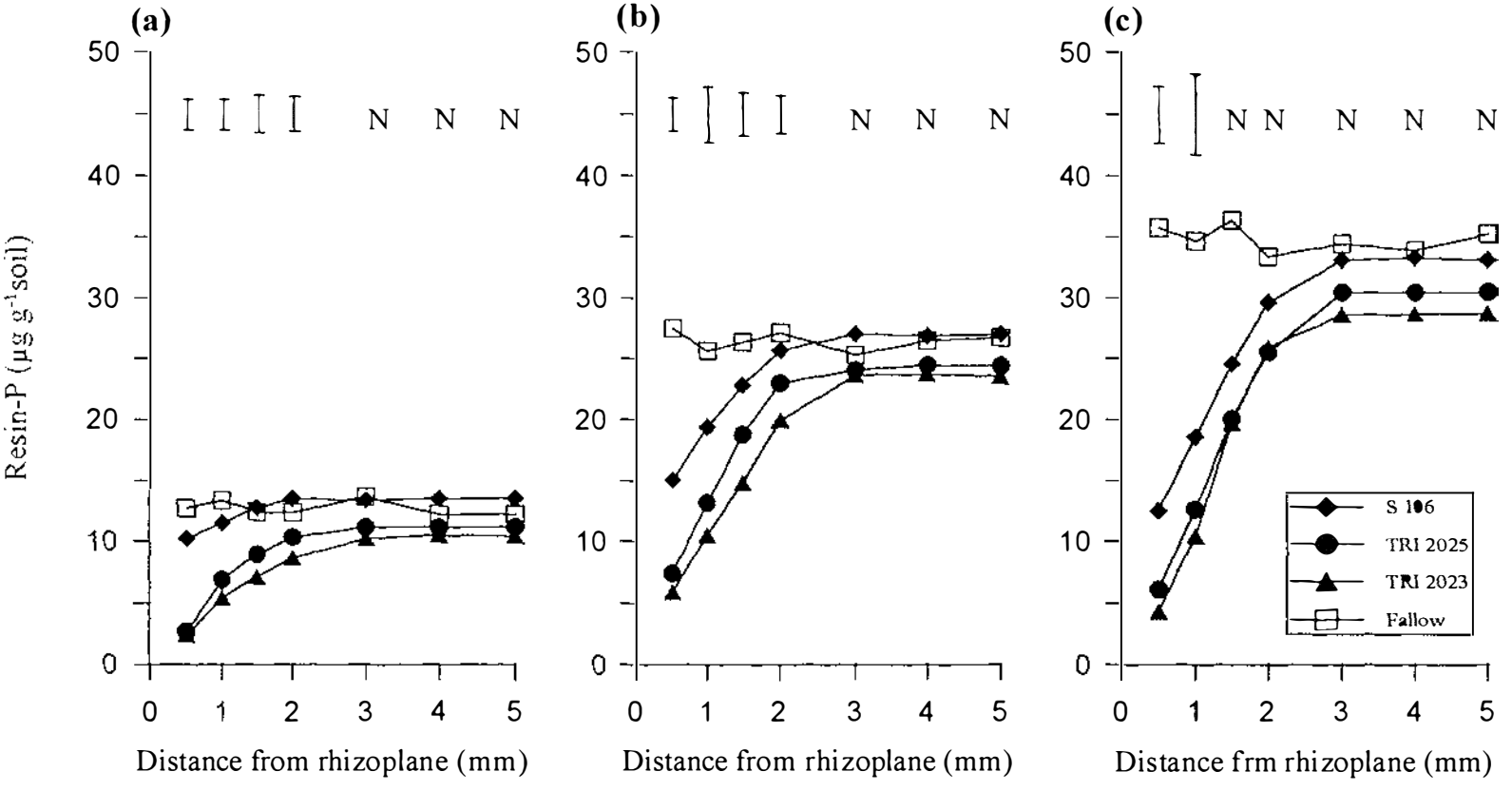

Figure 5.4 Effect of tea clones on resin-P in soil with (a) Control (b) EPR and (c) TSP treatments. Vertical bars correspond to Lsd at $p < 0.05$ and N represents treaments not statistically significant at $p < 0.05$.

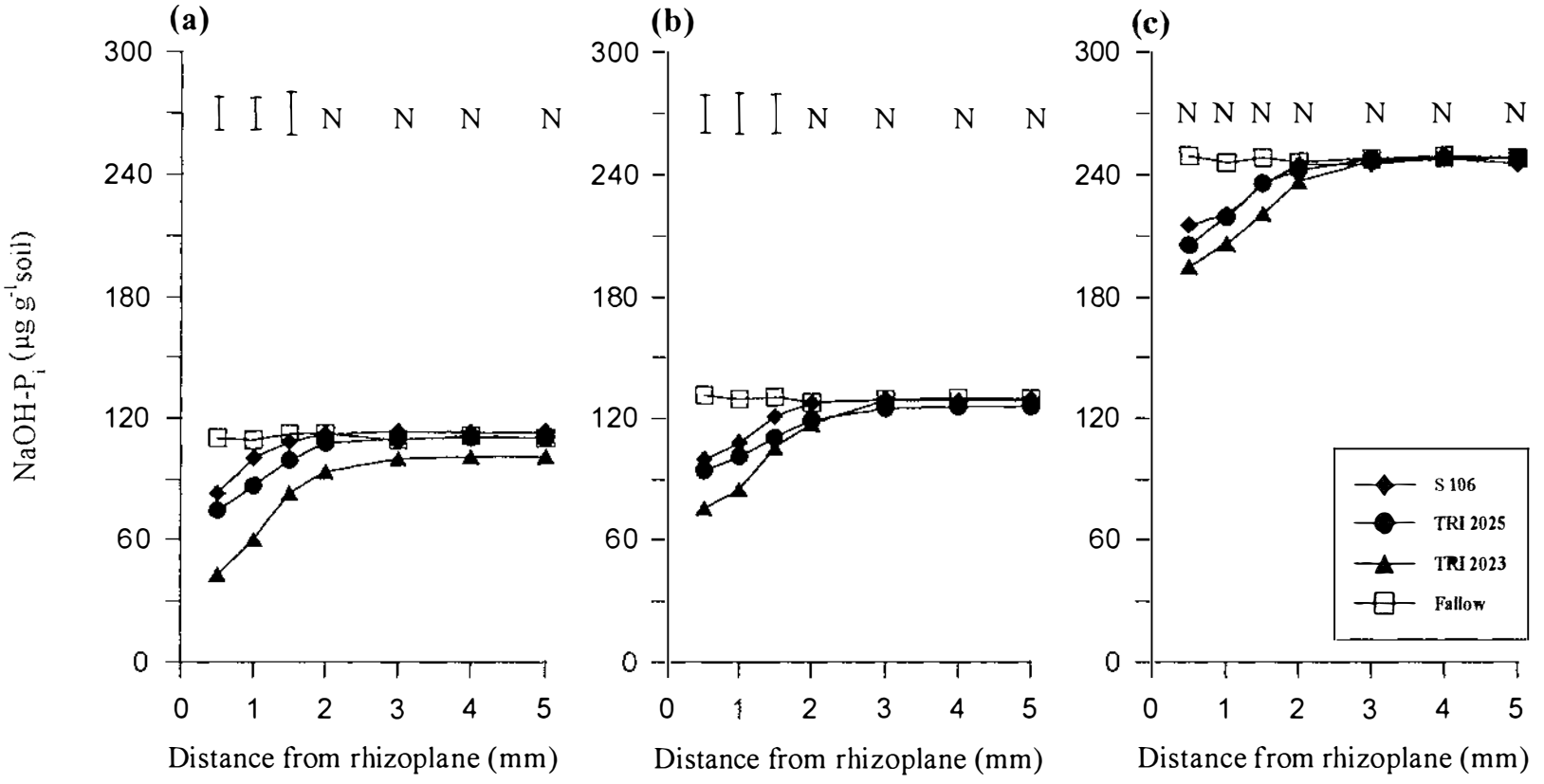

Figure 5.5 Effect of tea clones on NaOH-P_i in soil with (a) control (b) EPR and (c) TSP treatments. Vertical bars correspond to Lsd at $p < 0.05$ and N represents treatments not statistically significant at $p < 0.05$.

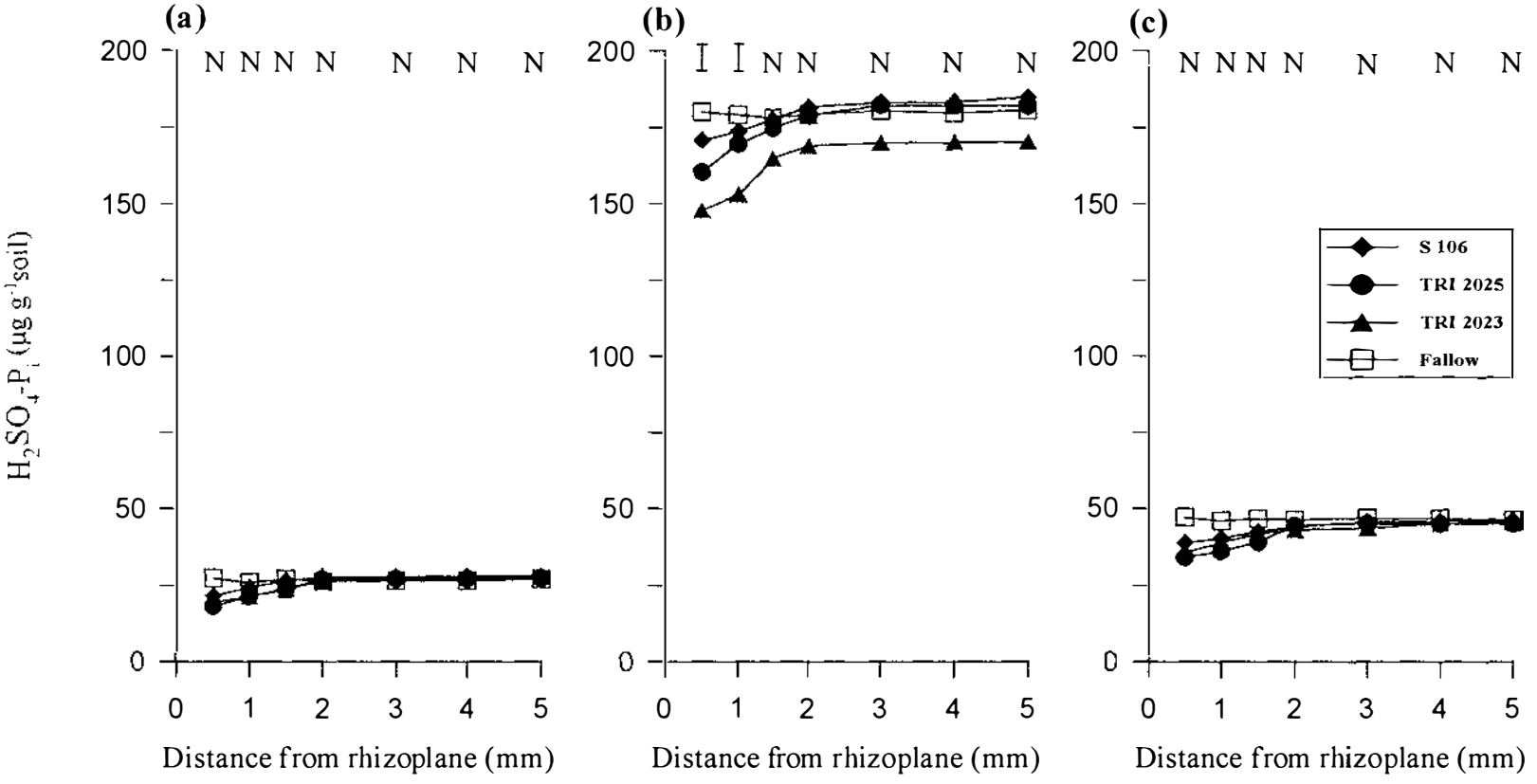

Figure 5.6 Effect of tea clones on H_2SO_4-P_i in soil with (a) control (b) EPR (c) TSP treatments. Vertical bars correspond to Lsd at $p < 0.05$ and N represents treatments not statistically significant at $p < 0.05$.

Steeper P depletion profiles in the rhizosphere of TRI 2023 and TRI 2025 compared to that in S 106 are a result of their faster growth rates causing a higher demand for P by these clones. The dry matter yield as well as the plant P concentrations were higher for TRI 2023 and TRI 2025 clones. The depletion of resin-P in the rhizosphere is partly replenished by the desorption of P forms that are bound to Fe and Al oxides ($NaOH\text{-}P_i$) depending on the P buffering capacity of the soil. The desorption of Fe and Al bound P could be either by ligand exchange with organic anions secreted by the tea roots or by the chelation of these anions with Fe and Al abundantly found in acid soils. Xiaoping (1994) observed that tea plant roots secrete significant quantities of citrates and malates. It would be of practical significance to investigate the variability among tea clones in their ability to secrete organic anions because they exert a strong influence on the potential of tea clones to extract P from difficultly available P fractions in the soil.

$NaOH\text{-}P_o$ accumulated in the rhizosphere of all the tea clones (Figure 5.7) as already observed for camellia in Chapter 3 and tea clone TRI 2025 in Chapter 4. The application of EPR and TSP fertilisers increased the $NaOH\text{-}P_o$ fraction in the rhizoplane compared to the unplanted control treatment. The possible reasons for the increase of $NaOH\text{-}P_o$ in the rhizosphere were reported in Chapter 4.

5.4.6 Comparison of rhizosphere P depletion with plant P uptake

In the bulk soil of the lower RSC compartment the concentration of any of the P fractions was not significantly different between treatments with or without plants. Therefore the entire P uptake by the plants must have come from the rhizosphere soil around all roots. An attempt was made to predict rhizosphere P depletion in the lower compartment of the RSC by calculating the proportion of the total plant P uptake that is derived from P depletion in this compartment as described in Chapter 4 section 4.4.5. The predicted rhizosphere P depletion of these clones agrees reasonably well with the measured P depletion in the soil (Table 5.4). The possible reasons for the variation between the estimated (predicted) and measured (observed) P depletion values are explained in Chapter 4.

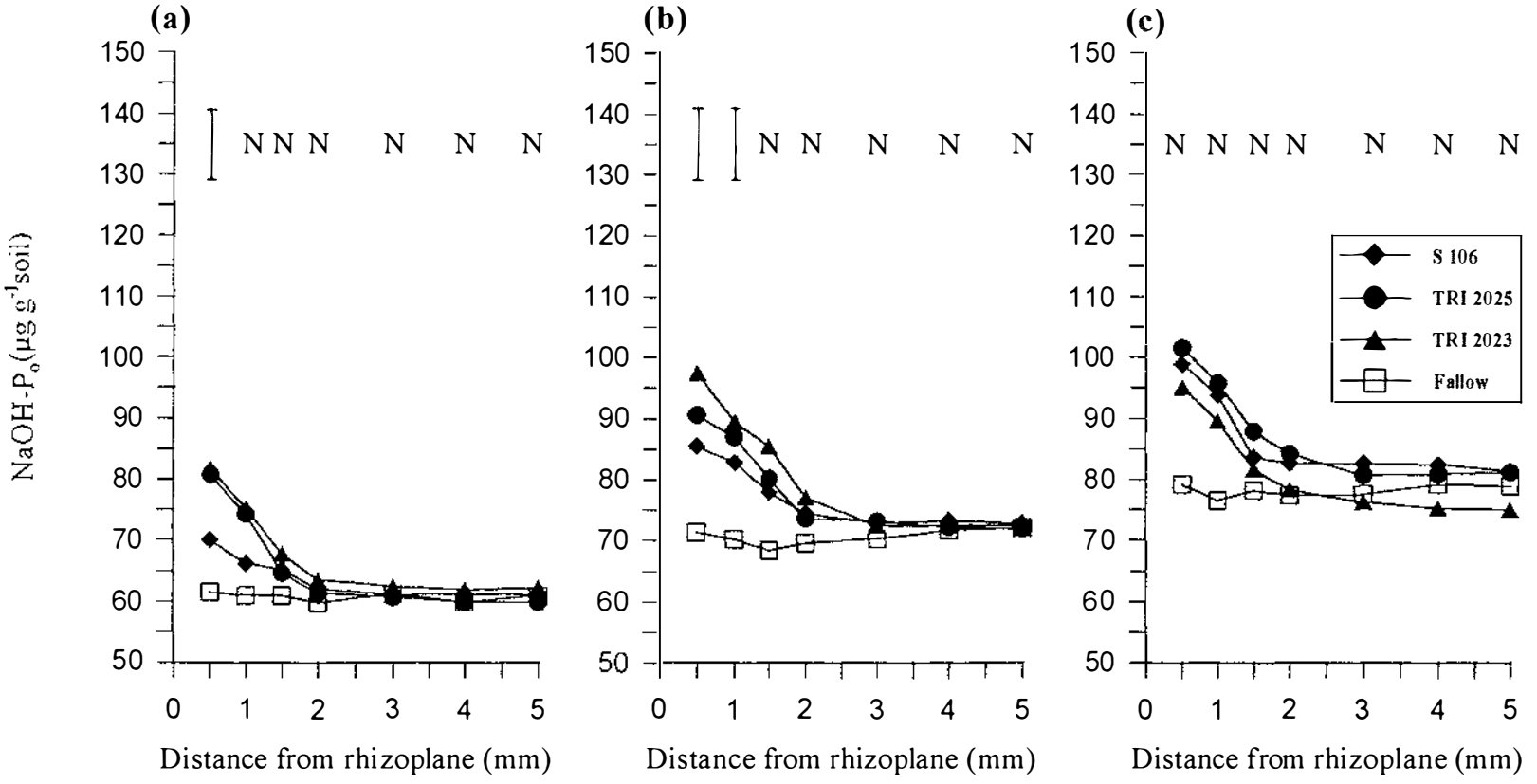

Figure 5.7 Effect of tea clones on NaOH-P_o in soil with (a) control (b) EPR and (c) TSP treatments. Vertical bars correspond to Lsd at $p < 0.05$ and N represents treatments not statistically significant at $p < 0.05$.

Table 5.4 Comparison of observed P depletion in the rhizosphere with predicted P depletion estimated from plant P uptake

Clone	S 106			TRI 2025			TRI 2023		
Treatment	Control	EPR	TSP	Control	EPR	TSP	Control	EPR	TSP
Observed depletion in lower RSC ($\mu g\ RSC^{-1}$)									
Resin-P	38	177	288	135	229	370	107	256	326
$NaOH\text{-}P_i$	270	310	345	569	496	576	737	776	794
$H_2SO_4\text{-}P_i$	75	223	127	141	264	170	126	332	116
Total	382	710	760	845	989	1116	970	1364	1236
Observed accumulation in lower RSC ($\mu g\ RSC^{-1}$)									
$NaOH\text{-}P_o$	135	326	301	254	272	292	238	351	255
Observed net depletion in lower RSC ($\mu g\ RSC^{-1}$)	247	384	459	591	717	824	732	1013	981
Total surface area of boundary roots (●-2 mm above mesh) (cm^2)	170	206	229	288	330	367	352	393	441
Plant P uptake ($\mu g\ plant^{-1}$)	778	1015	1041	2031	2454	2679	2718	3296	3341
Predicted total P depletion in lower RSC[1] ($\mu g\ RSC^{-1}$)	273	406	392	727	837	850	842	923	943
Deviation of Predicted P depletion from observed (%)	10	8	-15	23	17	3	15	-9	-4

[1] $\frac{\text{(Plant P uptake)}}{\text{(Surface area of all roots)}} * \text{(boundary root surface area)} * 0.5$

(see Table 5.1 for plant P uptake and total surface area of roots)
[The factor 0.5 is used because only half the root surface area was assumed to cause depletion in the lower RSC]

The magnitude of P depletions are in the order of TRI 2023 > TRI 2025 > S 106. The differences in P depletions are in line with the differences in surface area of the roots in the boundary zone (0 - 2 mm above the mesh) (Table 5.4) and growth rates (Table 5.1).

5.5 CONCLUSIONS

TRI 2023 and TRI 2025 had significantly higher dry matter production than S 106. The external P efficiency of TRI 2023 and TRI 2025 was higher than S 106, mainly due to greater root surface area and greater P uptake per unit surface area. TRI 2023 had higher external efficiency than TRI 2025 as a result of higher root surface area, but not due to higher P uptake per unit surface area. The higher P uptake per unit surface area in TRI 2023 and TRI 2025 clones may be due to their higher root acidification, root exudation of organic compounds and/or mycorrhizal association and these aspects needs further investigation. In all clones, P fertilisers had no influence on external P efficiency, but internal P efficiency was highest in soils receiving no P fertiliser.

All three tea clones induced acidification of the rhizosphere. This caused increased dissolution of EPR in the rhizosphere compared to that in the bulk soil. Approximately 18 to 26% of EPR dissolved in 56 days of tea growth. This suggests that though EPR is considered a less reactive PR according to its citric acid solubility, in the vicinity of tea roots appreciable amounts of this PR will dissolve to supply P to the plants. The rhizosphere acidification and EPR dissolution were in the order TRI 2023 > TRI 2025 > S 106. All clones depleted resin-P and NaOH-P_i, but increased NaOH-P_o in the rhizosphere. The rate of depletion of resin-P and NaOH-P_i was in the order of TRI 2023 > TRI 2025 > S 106. The accumulation of NaOH-P_o in the rhizosphere is probably due to transformation of P_i into P_o by the enhanced microbial activity in this zone.

The above findings could be utilised in future tea breeding programmes to develop new tea clones having both higher external and internal P efficiencies combined with

other desirable characteristics. With the rising cost of P fertilisers, the potential of using P efficient tea clones is an attractive alternative for sustainable tea production.

The results demonstrated that short-term rhizosphere studies can be used to obtain quick information on the screening of plant varieties/clones for their P utilisation efficiencies.

CHAPTER 6

EFFECT OF FORMS OF NITROGEN SUPPLY ON MOBILISATION OF PHOSPHORUS FROM EPPAWALA PHOSPHATE ROCK AND ACIDIFICATION IN THE RHIZOSPHERE OF TEA (*Camellia sinensis* L.)[1]

6.1 INTRODUCTION

In Chapters 3, 4 and 5 it has been shown that soil pH in the rhizosphere differs markedly from that of the bulk soil for tea and many other crops and this causes differences in PR dissolution in the two zones. Generally, rhizosphere pH decreases when NH_4^+ forms of N fertiliser are used and increases when NO_3^- forms are used, because of the release of H^+ and OH^- or HCO_3^- respectively to the soil to maintain electroneutrality within the plant cell (Gahoonia et al., 1992; Kirkby and Mengel, 1967; Youssef and Chino, 1988). In ryegrass seedlings, rhizosphere pH decreased by 1.6 units as a result of NH_4^+ supply and it increased by 0.6 units with NO_3^- supply (Gahoonia et al., 1992). The forms of nitrogen (N) supply also exert a strong influence on the plant availability of P in the rhizosphere soil through their influence on pH, which determines the rate of dissolution of PR and P fixation by soil colloids (Barrow, 1984).

In Chapter 5, it was shown that rhizosphere pH decreased by 0.2 - 0.3 units compared to that of the bulk soil for three clones of tea, which were fertilised with urea. This was believed to be due to plant uptake of NH_4^+ formed by the ammonification of urea and the root exudation of organic anions. The uptake of NH_4^+ and excretion of organic anions cause the release of H^+ to maintain electroneutrality within root cells. It is not clear however, if tea roots will continue to depress rhizosphere pH if NO_3^- or $NO_3^- + NH_4^+$ forms of N are applied instead of NH_4^+ forms. Tea plants may prefer to

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[1] **Zoysa A K N, Loganathan P and Hedley M J 1998 Effect of forms of nitrogen supply on mobilisation of phosphorus from a phosphate rock and acidification in the rhizosphere of tea (*Camellia sinensis* L.) *Australian Journal of Soil Research* (in print).**

use predominantly NH_4^+-N (Xan and Jianyun, 1994) even in the presence of NO_3^--N.

Trials have been conducted in many countries to investigate the use of calcium ammonium nitrate (CAN), a N fertiliser which creates less soil acidity, because the continuous use of $(NH_4)_2SO_4$ for tea leads to increased soil acidity (Bonheure and Willson, 1992; Sandanam et al., 1980; Watson and Wettasinghe, 1972). All these studies however revealed that CAN produced lower tea yields than $(NH_4)_2SO_4$. The reduction in yield could have been caused by the lower availability of P from PR, and the lower availability of Mn at the higher pH of CAN fertilised soil (Harler, 1968). Tea is a calcifuge, preferring low pH and low Ca saturated soils and this may explain the lower tea yield with CAN (Watson and Wettasinghe, 1972). These studies however did not give any information on the effect of N forms on soil P fractions and pH either in the bulk soil or in the rhizosphere of tea.

6.2 OBJECTIVES

The study reported in this chapter was designed to investigate the following objectives:

1. To investigate the effect of NH_4^+-N and NO_3^--N separately [$(NH_4)_2SO_4$, $Ca(NO_3)_2$] and in combination [NH_4NO_3] on the pH in the rhizosphere of tea seedlings.
2. To study the effect of the form of N supply on the mobilisation of P from EPR in tea rhizosphere.
3. To investigate which form of N is preferred by tea plants.

6.3 MATERIALS AND METHODS

The physico-chemical characteristics of the soil (Rhodustult) used in this study were presented in Chapter 4 - Table 4.1. The soil was air-dried and passed through a 2 mm sieve and mixed with Eppawala phosphate rock (EPR - for specification details see section 4.3) and KCl at the rate of 200 μg P or K g^{-1} soil. Three sources of N

treatment and a control (no N fertiliser) treatment were used. The N sources were $(NH_4)_2SO_4$ (100% NH_4^+-N), $Ca(NO_3)_2$ (100% NO_3^--N) and NH_4NO_3 (50% NH_4^+-N and 50% NO_3^--N). The N fertilisers were mixed separately with the soil at the rate of 200 μg N g^{-1} soil. All soils were mixed with a nitrification inhibitor dicyandiamide (DCD) at the rate of 45 μg g^{-1} soil at planting to reduce as much as the possible transformation of NH_4^+ to NO_3^-.

The modified root study container (RSC) technique described in Chapter 3 was used for this trial. The upper and lower compartments were packed with 135 g soil (bulk density 1.1 Mg m^{-3}) and 242 g soil (bulk density 1.1 Mg m^{-3}), respectively. A single vegetatively propagated four-month-old tea plant (clone TRI 2025) was planted in each of the upper compartments. The treatments (three N forms and control) were replicated four times and arranged in a randomised complete block design in a glasshouse maintained at 12^0 C minimum and 26^0 C maximum temperatures at St. Coombs, Sri Lanka. Four replicated pots without plants but having the same treatments (three N forms and control) were also included to study the changes in the soils due to fertilisers in the absence of tea plants. The RSCs were placed on top of a sand bed connected to a water reservoir and the water table was fixed at 160 mm below the base of the RSCs as in the trials in earlier chapters. This enabled the RSCs to be maintained at a constant water potential of approximately -1.6 kPa. The trial set-up in the glasshouse is shown in Figure 6.1

6.3.1 Soil, plant and root sampling

Sixty days after transplanting plants to RSCs, the shoots were cut 5 mm above the soil surface and dried at 60^0 C, weighed and ground to powder. The soil in the lower compartment was sliced into thin sections with a piston microtome, dried and ground as described in Chapters 4.

Root sampling and measurements of root mass, length, volume and surface area were also as described in Chapter 4.

Figure 6.1 Plant growth system in the glasshouse

6.3.2 Soil and plant analysis

Shoot and root samples were analysed for total N by Kjeldhal digestion (Jackson, 1958). The plant materials were dry-ashed at 550^0 C and the ash was taken up in 0.05 *M* HCl solution and analysed for K and Na by flame emission spectrophotometry, Ca and Mg by atomic absorption spectrophotometry, P by the vanadomolybdate method (Jackson, 1958) and Al by the aluminon method (Jayman and Sivasubramaniam, 1974). Plant materials were extracted with hot water and the concentration of Cl measured with a Cl electrode (Adriano and Doner, 1982) and SO_4 by turbidometry (Buttlers and Chenery, 1959). The initial nutrient composition and dry weights of the plants were determined on a sample of 10 randomly selected plants of similar size and foliar characteristics to the test plants at the beginning of the experiment. Soil pH, amount of EPR dissolution and soil P fractionation were carried out as described in Chapter 3.

6.4 RESULTS AND DISCUSSION

6.4.1 Effect of N forms on N and P uptake by tea

All forms of N significantly ($p < 0.05$) increased shoot dry matter yield and shoot : root dry matter ratio over the control treatment, but only $(NH_4)_2SO_4$ and NH_4NO_3 treatments significantly ($p < 0.05$) increased the root dry matter yield (Table 6.1). However, root, shoot and total dry matter yield and shoot : root ratios were not significantly affected by the form of N fertiliser. The increase in dry matter yield obtained with all N forms compared to the control treatment is similar to the results obtained in field trials, where tea yields have always increased with N application because the N uptake rate of tea is higher than most other crops (Eden, 1976; Tolhurst, 1968). The increase in total dry matter yield is due both to increases in root and shoot growth, although the shoot growth response to fertilisers is higher than the root growth response. Adding $(NH_4)_2SO_4$ significantly ($p < 0.05$) increased N and P concentrations and uptake in both roots and shoots compared to the control treatment. But the NH_4NO_3 treatment only significantly ($p < 0.05$) increased shoot and root N and

Table 6.1 Effect of N forms on plant shoot and root dry matter[1], shoot : root ratio, tissue N, P concentrations and uptake by tea plants

Treatment	Shoot					Root					Total dry matter yield	Shoot : Root dry matter ratio
	Dry matter ($g\ pot^{-1}$)	N (%)	N uptake ($mg\ plant^{-1}$)	P (%)	P uptake ($mg\ plant^{-1}$)	Dry matter ($g\ pot^{-1}$)	N (%)	N uptake ($mg\ plant^{-1}$)	P (%)	P uptake ($mg\ plant^{-1}$)	($g\ pot^{-1}$)	ratio
Control	0.93	1.36	12.81	0.116	1.08	1.06	1.03	10.96	0.046	0.47	1.99	0.88
$(NH_4)_2SO_4$	1.77	1.51	26.68	0.132	2.33	1.56	1.45	22.67	0.054	0.84	3.33	1.13
NH_4NO_3	1.92	1.43	27.39	0.126	2.42	1.55	1.55	24.23	0.053	0.82	3.47	1.24
$Ca(NO_3)_2$	1.69	1.49	25.26	0.114	1.92	1.32	1.31	17.33	0.040	0.52	3.01	1.28
Lsd ($p < 0.05$)	0.36	0.14	5.85	0.010	0.47	0.39	0.18	7.09	0.008	0.23	0.59	0.20

[1] The difference in the dry matter yield at harvest minus initial dry matter content

P uptake and root N concentration compared to the control treatment. The $Ca(NO_3)_2$ treatment only significantly ($p < 0.05$) increased shoot N and P uptake and root N concentration.

A significantly lower P concentration and P uptake in the $Ca(NO_3)_2$ treatment compared with the NH_4NO_3 and $(NH_4)_2SO_4$ treatments may be due to the lower solubility of EPR (Figure 6.2) at the higher pH encountered in the rhizosphere of the $Ca(NO_3)_2$ treatment (Figure 6.3a). It could also be due to the competition of anions, NO_3^- from the $Ca(NO_3)_2$ supply and increased OH^- concentration resulting from the increased rhizosphere pH in this treatment. The increased pH would have increased the valancy of the phosphate ions ($H_2PO_4^-$ to HPO_4^{2-}) which are taken by plants at a lower rate than the lower valancy ions (Tisdale, 1985). Additionally the Ca in $Ca(NO_3)_2$ may have also reduced N assimilation as tea is a calcifuge, which does not perform well in soils high in Ca. Higher Ca concentrations in the soil solution may also have reduced the phosphate rock dissolution (see next section).

6.4.2 Effect of N forms on pH and P fractions in bulk soil

A significant reduction in pH of the $(NH_4)_2SO_4$ treated bulk soil (3-5 mm from mesh - outside the influence of the roots) in both planted and unplanted pots compared to the control treatment, indicated that some nitrification occurred despite using an inhibitor (Figure 6.3). Soil pH (Figure 6.3) was lower in $(NH_4)_2SO_4$, NH_4NO_3 and control and the dissolution of EPR fertiliser was higher (Figure 6.2) in the bulk soil (3 - 5 mm away from root surface - zone not influenced by roots) compared to the $Ca(NO_3)_2$ treatment. This increase in EPR dissolution with the $(NH_4)_2SO_4$ treatment resulted in increased resin-P and NaOH-P_i in the bulk soil (Table 6.2). The higher amounts of H_2SO_4-P_i in the bulk soil of the $Ca(NO_3)_2$ treatment compared to other treatments resulted from a lower EPR dissolution (8% P dissolution for $Ca(NO_3)_2$ treatment compared to 16% P dissolution for $(NH_4)_2SO_4$ treatment) at the higher soil pH of this treatment (Figure 6.3). In addition to the higher pH, the $Ca(NO_3)_2$ treatment supplied soluble Ca to the soil which may have also reduced EPR dissolution (Mackay et al., 1986).

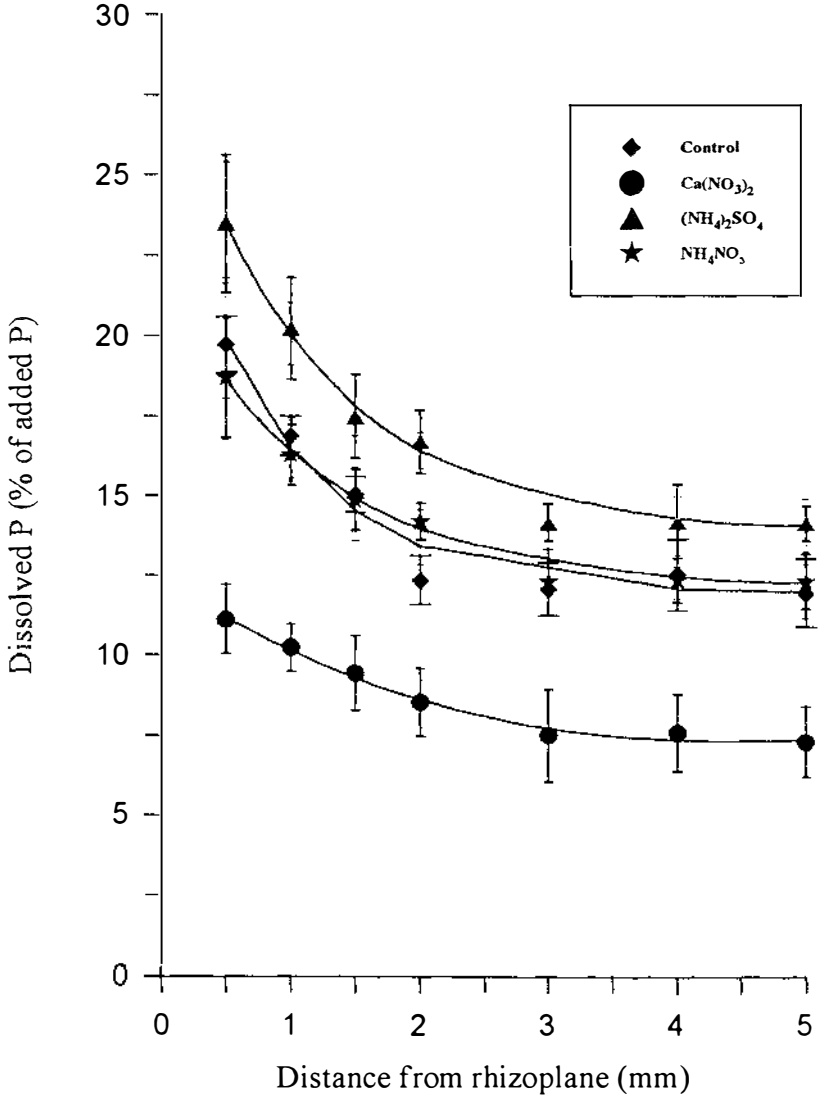

Figure 6.2 Effect of nitrogen forms on P dissolution from EPR. (Vertical bars represent standard errors of the means).

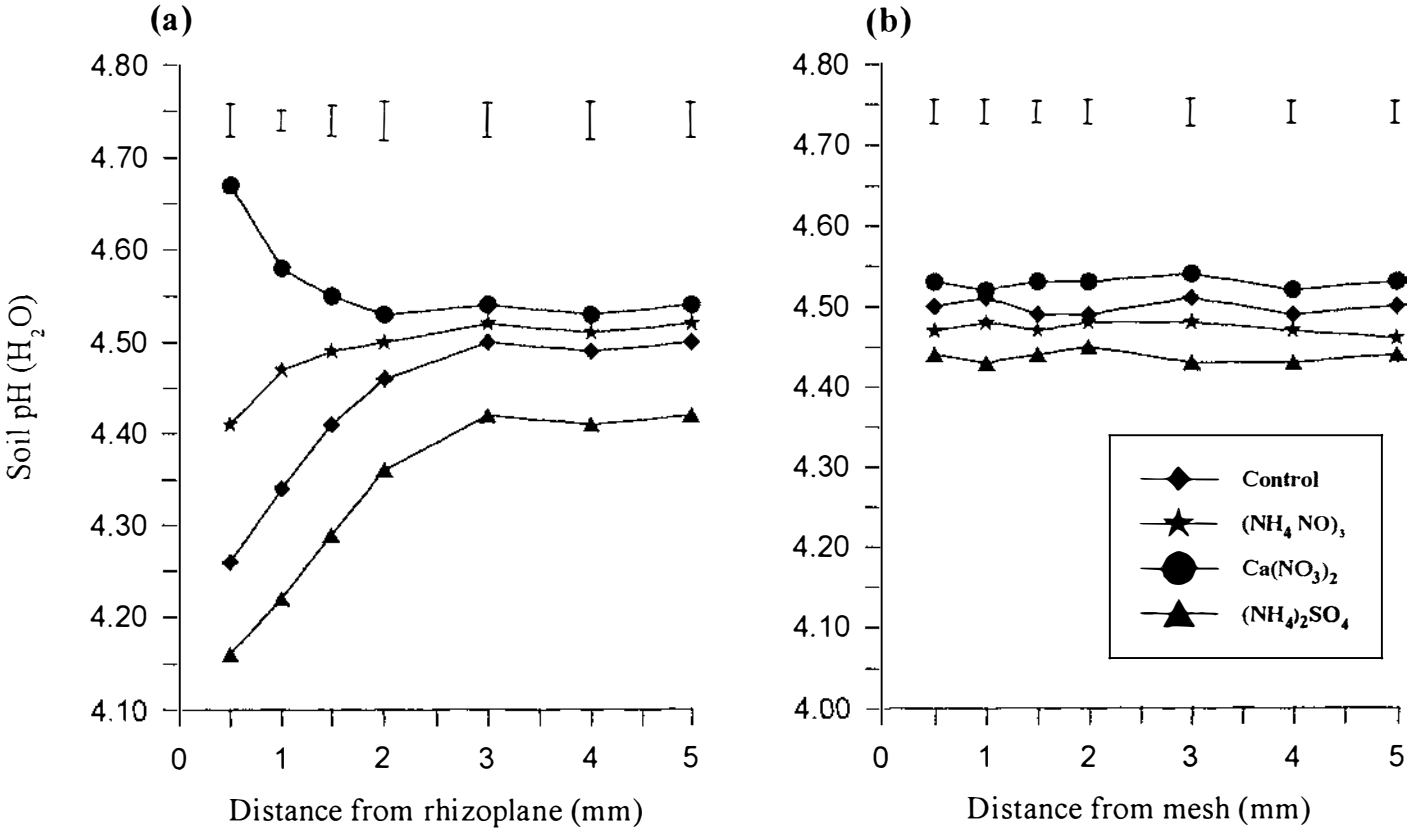

Figure 6.3 Effect of nitrogen fertiliser forms on soil pH (a) with plants and (b) without plants (Vertical bars represent Lsd for treatment means at $p < 0.05$).

Table 6.2 Phosphorus fractions in control soil (without EPR) and the % recovery of added EPR-P in bulk soils (3-5 mm) for various N treatments

P fraction	Unfertilised bulk soil ($\mu g\ g^{-1}$ soil)	EPR Fertiliser recovery (P%)[1]			
		Control (No N fertiliser)	$Ca(NO_3)_2$	$(NH4)_2SO_4$	NH_4NO_3
Resin-P	12	2	2	5	4
NaOH-P_i	141	9	2	15	10
NaOH-P_o	57	2	2	2	3
H_2SO_4-P_i	28	79	95	78	83
Residual-P	60	5	-	1	1
Total-P	298	97	101	101	101

[1] $\frac{(\text{P fraction in fertilised soil - P fraction in control soil})}{\text{Fertilised P added to soil}} * 100$

In the non-rhizosphere (bulk) soil (3 - 5 mm), between 97 - 101% of P was recovered by the P fractionation from the applied EPR fertiliser for all treatments (Table 6.2) with NaOH-P_i and H_2SO_4-P_i fractions (146 - 224 $\mu g\ g^{-1}$ soil) being greater than resin-P, NaOH-P_o and residual-P fractions (17 - 64 $\mu g\ g^{-1}$ soil). This agrees with the observations made in the trials reported in Chapters 4 and 5.

6.4.3 Effect of N forms on rhizosphere pH

Adding NH_4^+ fertiliser lowered rhizosphere soil pH, whereas NO_3^- fertiliser increased rhizosphere soil pH (up to 3 mm from the rhizoplane), compared to the control treatment (Figure 6.3a). In the absence of plant roots pH changed little with distance from the mesh (Figure 6.3b). Compared to the bulk soil, the pH of rhizosphere soil was 0.29 units lower in the $(NH_4)_2SO_4$ treatment, 0.11 units in the NH_4NO_3 and 0.24 units in the control treatments. In contrast, adding $Ca(NO_3)_2$ increased rhizosphere pH by 0.13 units compared to that in the bulk soil. These results show that the form of N supply to tea roots exerts a strong influence on their rhizosphere pH.

The effect of the form of N supply on rhizosphere pH is due to the difference in the uptake of NH_4^+ and NO_3^- by plants; uptake of NH_4^+ results in production of H^+ and NO_3^- results in excretion of OH^- and HCO_3^- (Troelstra, 1983; Troelstra et al., 1985). The $(NH_4)_2SO_4$ fertiliser being solely an NH_4^+ type produced the highest reduction in rhizosphere pH whereas NH_4NO_3, which contains both NH_4^+ and NO_3^- produced the smallest pH drop. The increase in rhizosphere pH compared with bulk soil pH in the $Ca(NO_3)_2$ treatment is consistent with excretion of OH^- and HCO_3^- to counter balance the NO_3^- uptake. The pH increase (0.13 pH units) however is about 50% of the pH decrease observed for the $(NH_4)_2SO_4$ treatment (0.29 pH units), even though the same rate of N was supplied to the plants. A possible reason for this is the rhizosphere acidification caused by the exudation of organic anions and associated protons by the roots. Tea plants secrete significant amounts of malic and citric acids from their roots (Jayman and Sivasubramaniam, 1975; Xiaoping, 1994). The organic acids (or organic anions plus protons) released may have acidified the rhizosphere regardless of the

form of N supply. Furthermore greater amounts of organic acids may have been excreted in the presence of NO_3^- as observed for rape roots by Hoffland et al. (1989).

The observed acid release by tea roots based on pH changes and the predicted acid production based on EPR dissolution for the different N treatments were calculated as described in Chapter 4 and presented in Table 6.3. The greatest acidity release in the rhizosphere was for the $(NH_4)_2SO_4$ treatment (123 μmol H^+) and the smallest was for the NH_4NO_3 treatment (30 μmol H^+). In contrast, the plants treated with $Ca(NO_3)_2$ released 7 μmol OH^- or HCO_3^- into the rhizosphere. Despite the pH rise in the rhizosphere compared to the bulk soil in the $Ca(NO_3)_2$ treatment, EPR dissolution in the rhizosphere was still higher than that in the bulk soil. This may be due to the removal of dissolved products of EPR (P and Ca) by plant or microbial uptake, which would have increased the dissolution of EPR in the tea rhizosphere.

6.4.4 Effect of plant roots on P fractions in the soil

In the absence of plant roots there was no change in any form of P with distance from the mesh (Figure 6.4b, 6.5b, 6.6b and 6.7b). Therefore any difference observed in the P-fractions between bulk soil and rhizosphere was assumed to be due to either P uptake by plant roots or increased microbial activity in the rhizosphere resulting from organic carbon exudation by the roots (Tinker, 1980). Higher microbial activity in the rhizosphere can immobilise some soluble P (Helal and Sauerbeck, 1991), whereas organic carbon exudates can reduce the P fixation by Fe and Al oxides (Nagarajah et al., 1968).

The profile of soil P depletion, which could be used as a measure of P mobilisation was markedly affected by the forms of N added. Resin-P depletion was greatest for the $NH_4(SO_4)_2$ treatment and the lowest in the $Ca(NO_3)_2$ and control treatments (Figure 6.4a). The greater resin-P depletion in the $(NH_4)_2SO_4$ treatment results from higher P fixation by Fe and Al oxides at the lower soil pH of the $(NH_4)_2SO_4$ treatment (Barrow, 1984). In contrast less NaOH-P_i was depleted in the rhizosphere of the $(NH_4)_2SO_4$ treatment compared to the $Ca(NO_3)_2$, NH_4NO_3 and control treatments

Table 6.3 The observed acid release by tea roots based on pH changes and the predicted acid production based on EPR dissolution in the lower compartment of RSC

Treatment	pH drop (-) or rise (+) in the rhizosphere (0-3 mm) compared to bulk soil[1]	Observed H^+ production (+) or consumption (-) within 0-3 mm of the rhizosphere[2] (μmol H^+)	Average of EPR dissolution within 0-3 mm of the rhizosphere (P%)	H^+ consumption for EPR dissolution within 0-3 mm of the rhizosphere[3] (μmol H^+)	EPR dissolution in the bulk (3-5 mm) soil (P%)	H+ consumption for EPR dissolution in bulk soil[3] (3-5 mm) (μmol H^+)	Difference in the amount of H^+ used for dissolution of EPR in the bulk soil and the rhizosphere (0-3 mm) soils[4] (μmol H^+)	Total H^+ production (+) or consumption (-) in (0-3 mm) of the rhizosphere[5] (μmol H^+)
Control	- 0.24 ± 0.01	+42.53 ± 8.45	15.2 ± 1.21	33.64 ± 1.13	12.23 ± 0.01	22.34 ± 0.15	11.30 ± 0.22	+ 53.83 ± 0.52
$(NH_4)_2SO_4$	- 0.29 ± 0.01	+107.89 ± 1.01	18.40 ± 1.19	45.36 ± 1.59	14.16 ± 0.01	30.58 ± 0.03	14.78 ± 0.22	+ 122.67 ± 0.42
NH_4NO_3	- 0.11 ± 0.02	+17.90 ± 1.87	15.26 ± 0.78	37.90 ± 1.30	12.32 ± 0.01	25.47 ± 0.20	12.43 ± 0.35	+ 30.33 ± 0.37
$Ca(NO_3)_2$	+ 0.13 ± 0.03	-15.03 ± 0.62	9.35 ± 0.43	22.94 ± 0.84	7.42 ± 0.05	15.33 ± 0.09	7.61 ± 0.11	- 7.42 ± 0.21

[1] Difference in pH (H_2O) between 0-3 mm soil slice and the weighted mean pH of all slices within 3-5 mm of the rhizoplane.

[2] Sum total of (change of pH in the rhizosphere soil slice compared to the bulk soil {3-5 mm} * soil pH buffering capacity {30 μmol H^+ g^{-1} pH^{-1} soil} * weight of that soil slice) for all slices in the rhizosphere (0-3 mm).

[3] Amount of EPR dissolved (μg EPR g^{-1} soil) * amount of H^+ required to dissolve 1 μg EPR (0.00933 μmol H^+ μg^{-1} EPR - see text) * soil weight.

[4] H^+ consumption for EPR dissolution in 0-3 mm zone of the rhizosphere minus H^+ consumption for EPR dissolution in the bulk soil (3-5 mm).

[5] H^+ released in the 0-3 mm of the rhizosphere plus the difference in the amount of H^+ used for dissolving EPR in the bulk and the rhizosphere soils.

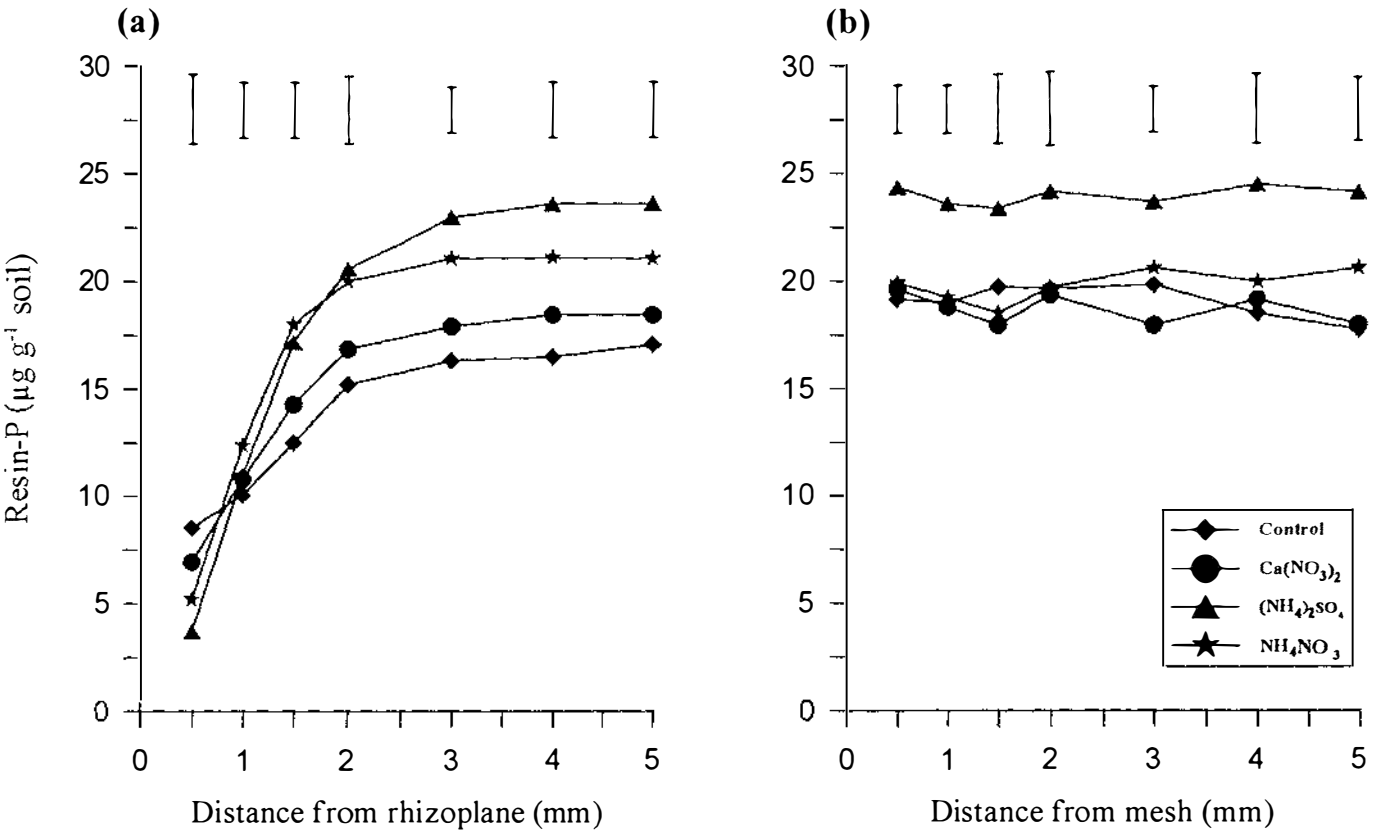

Figure 6.4 Effect of nitrogen forms on resin-P (a) with and (b) without plants. Vertical bars represent Lsd for treatment means at $p < 0.05$.

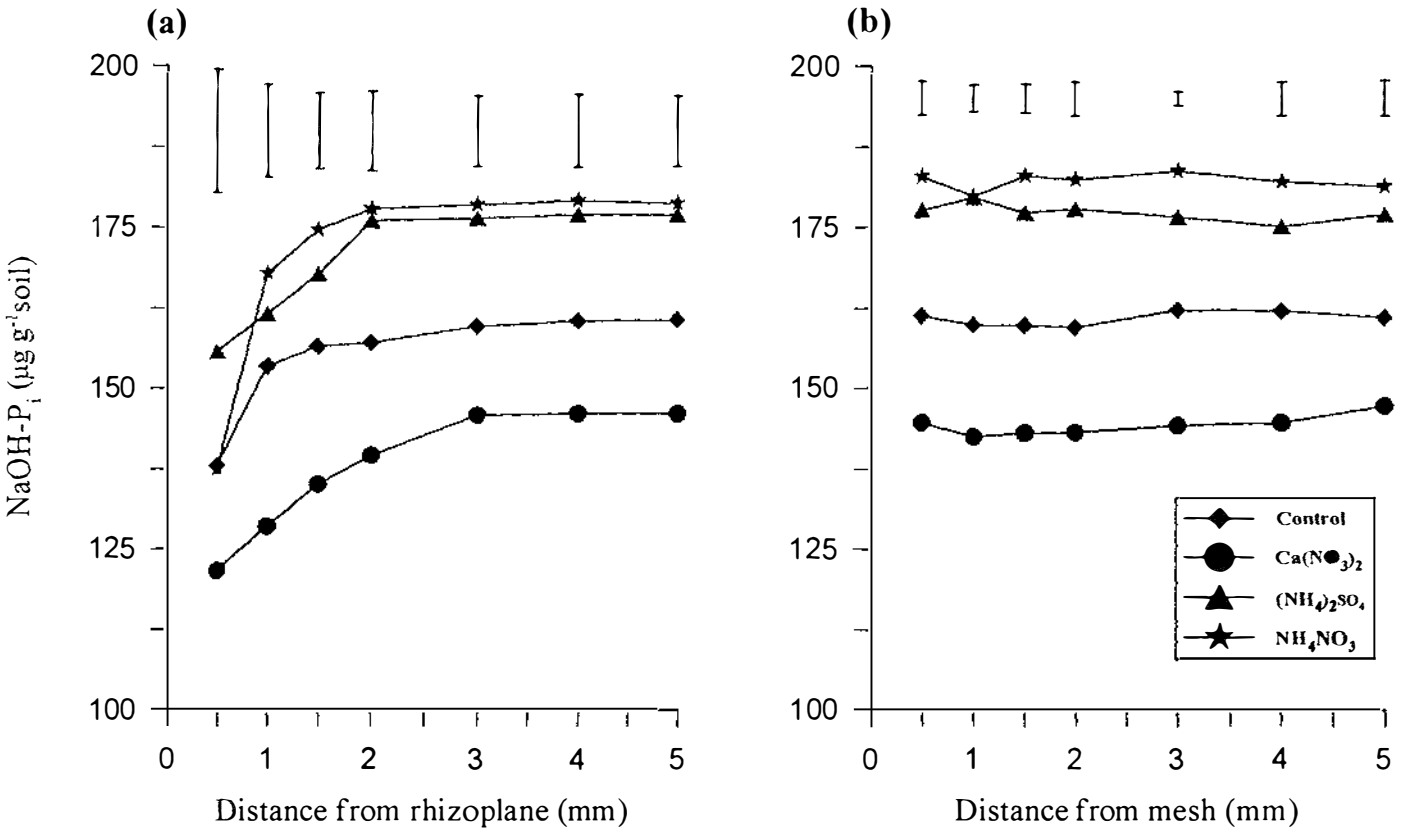

Figure 6.5 Effect of nitrgen forms on NaOH-P_i in soil (a) with and (b) without plants. Vertical bars represent Lsd for treatment means at $p < 0.05$.

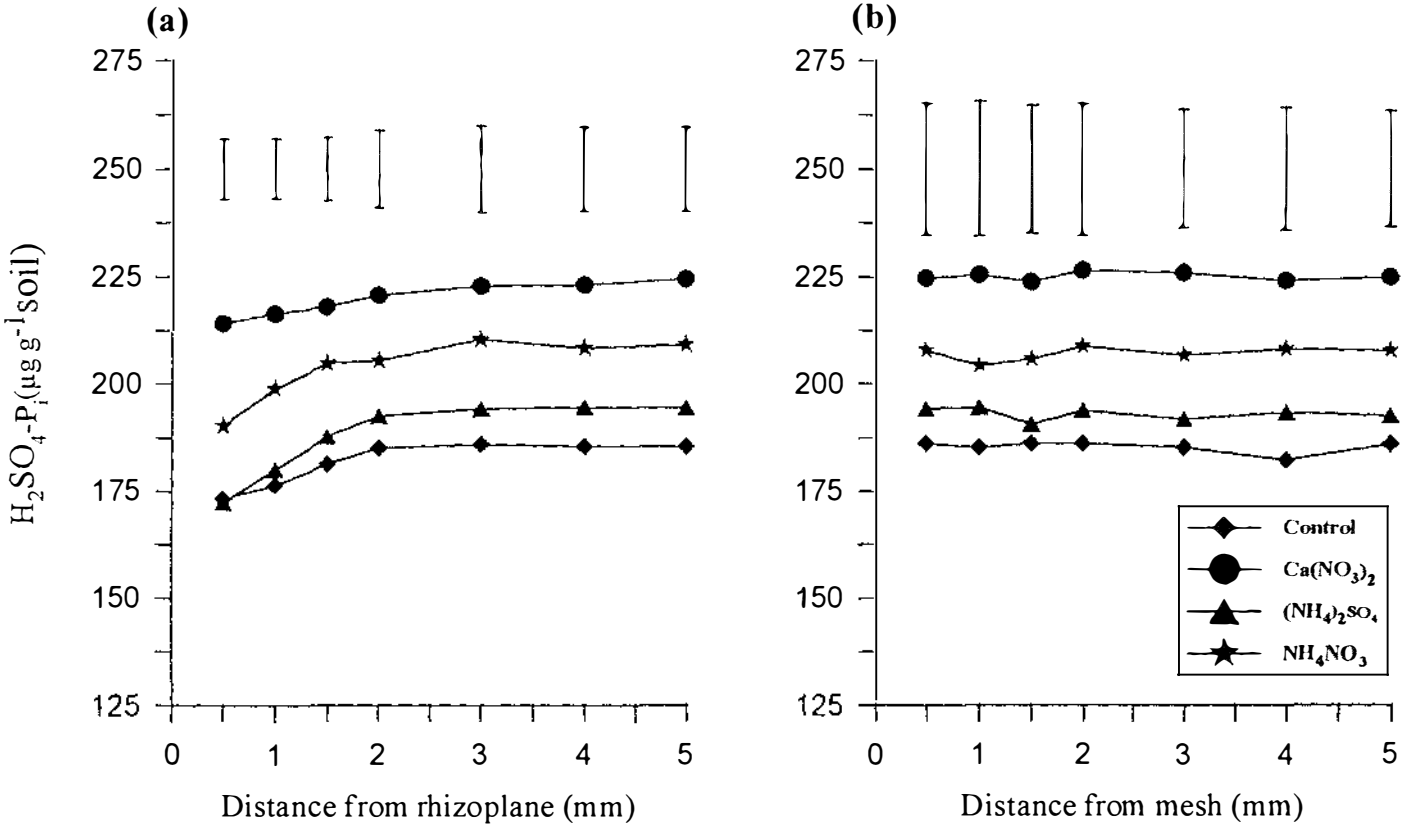

Figure 6.6 Effect of nitrogen forms on H_2SO_4-P_i (a) with and (b) without plants. Vertical bars represent Lsd for treatment means at $p < 0.05$.

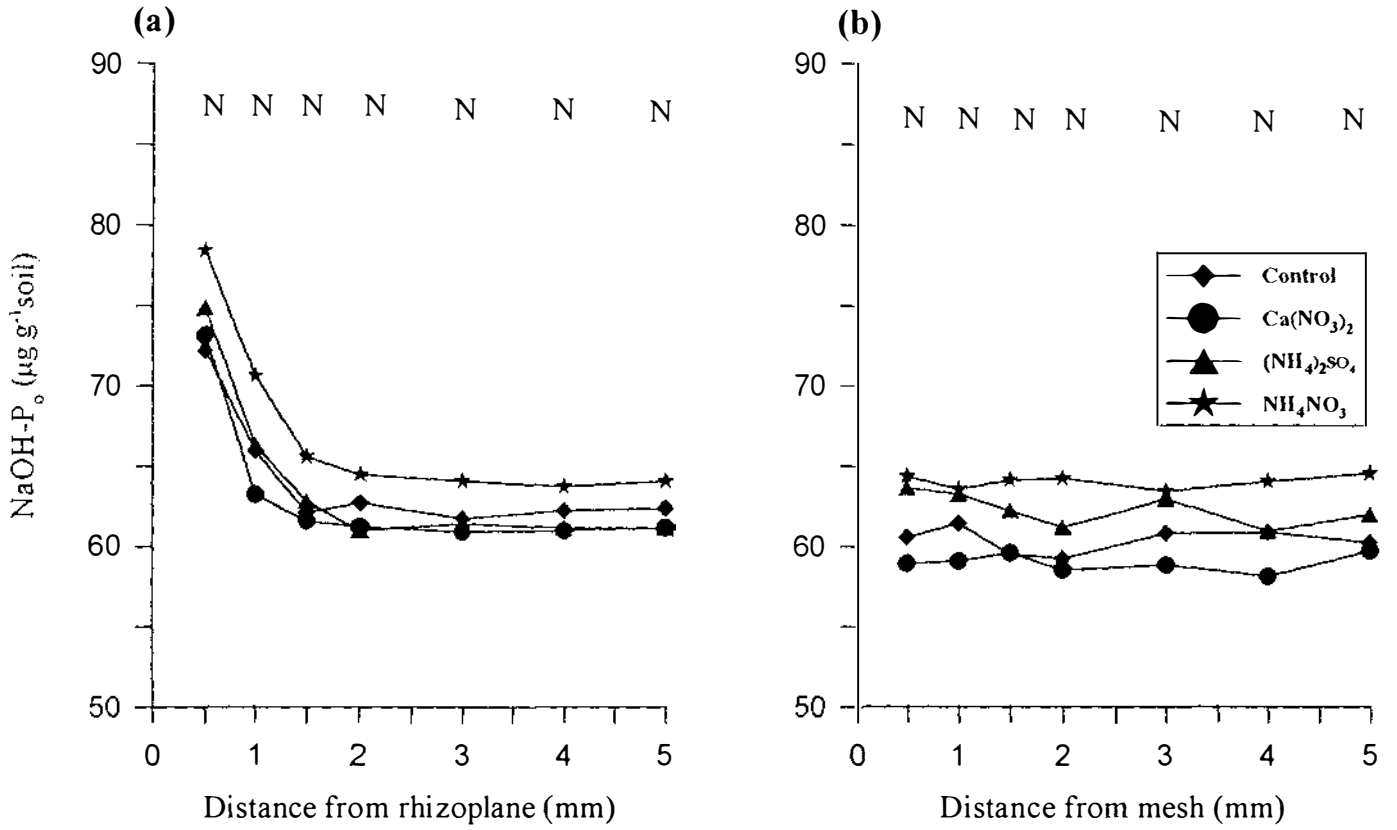

Figure 6.7 Effect of nitrogen forms on NaOH-P_o in soil (a) with and (b) without plants. The N shows that treatments are not statistically significantly different at $p < 0.05$.

(Figure 6.5a and Table 6.4). This cannot be explained by differences in plant P uptake as plant P uptake in the $(NH_4)_2SO_4$ and NH_4NO_3 treatments were similar (Table 6.1).

Excretion of OH^- and/or HCO_3^- from roots due to NO_3^- uptake in the $Ca(NO_3)_2$ treatment may have released phosphate ions adsorbed to Fe and Al oxides in the soil by ligand exchange with the OH^- and HCO_3^- (Gahoonia et al., 1992). Also the higher amounts of organic acids excreted in the presence of NO_3^- compared to the NH_4^+ source of N (Hoffland et al., 1989) may have dissolved more fixed P in the soil because of the organic anions complexing with Fe and Al (Earl et al., 1979; Nagarajah et al., 1968) thereby increasing resin-P and reducing NaOH-P_i.

The H_2SO_4-P_i depletion was lowest in the $Ca(NO_3)_2$ treatment (23% of total-P depletion) compared with all other treatments with $(NH_4)_2SO_4$ having the highest depletion (34% of total depletion) (Figure 6.6; Table 6.4). The higher rhizosphere pH and Ca input in the $Ca(NO_3)_2$ treatment may have reduced the dissolution of EPR in the soil thus lowering H_2SO_4-P_i depletion.

An accumulation of NaOH-P_o was observed within 2 mm from the rhizoplane for all treatments (Figure 6.7a). Higher concentrations of NaOH-P_o in the rhizosphere compared to the bulk soil may be due to the transformation of labile P_i into P_o by microbial utilisation of P_i as discussed in Chapter 4. However there was no difference in NaOH-P_o concentration between N sources suggesting that the source of N or the pH changes due to these sources had no significant effect on the utilisation of P_i by microorganisms in the rhizosphere.

An attempt was made to predict rhizosphere P depletion using plant P uptake. Plant P uptake, observed total P depletion and predicted total P depletion in the rhizosphere were estimated for each treatment as explained in Chapters 4 and 5. The predicted P depletions in the rhizosphere were lower than the observed P depletion by 10 - 26% (Table 6.4). In a similar experiment, but with different tea clones fertilised with urea, the predicted P depletion varied between -15 and 23% of the observed P depletion (Chapter 5). The reasons for the differences between observed and predicted P depletions were given in Chapter 4.

Table 6.4 Comparison of observed P depletion in the soil in the lower compartment of RSC with predicted P depletion calculated from plant P uptake

	Treatments			
	Control	$(NH_4)_2SO_4$	NH_4NO_3	$Ca(NO_3)_2$
Observed depletion in the lower RSC ($\mu g\ RSC^{-1}$)				
Resin-P	283	525	376	327
NaOH-P_i	503	587	755	790
H_2SO_4-P_i	377	580	442	333
Total	1163	1692	1573	1450
Observed accumulation in the lower RSC ($\mu g\ RSC^{-1}$)				
NaOH-P_o	200	262	303	222
Observed net depletion in the lower RSC($\mu g\ RSC^{-1}$)	963	1430	1270	1228
Surface area of boundary (0-2 mm above mesh) roots (cm^2)	236	290	306	266
Plant P uptake ($\mu g\ plant^{-1}$)	1524	3173	3247	2450
Total root surface area (cm^2)	251	386	435	358
Plant P uptake/root surface area ($\mu g\ cm^{-2}$)	6.1	8.2	7.5	6.8
Predicted Total P depletion in the lower RSC[1] ($\mu g\ RSC^{-1}$)	716	1192	1142	912
Deviation of predicted P depletion from observed (%)	-26	-17	-10	-26

[1] $\frac{\text{Plant P uptake}}{\text{Total root surface area}} * \text{boundary root surface area} * 0.5$

[The factor 0.5 is used because only half the root surface area was assumed to cause depletion in the lower RSC]

6.4.5 Nutrient uptake and electroneutrality in plant tissues

Plants generally absorb different amounts of cations and anions. The excess positive charge created by greater uptake of cations (NH_4^+, Na^+, K^+, Ca^{2+}, Mg^{2+} : total C) than inorganic anions ($H_2PO_4^-$, NO_3^-, Cl^-, SO_4^{2-} : total A) in the plant tissue (C-A) is balanced by formation of organic anions (e.g. carboxylate) in the plant (Troelstra, 1983). Tea plants absorb large quantities of Al from acid soils (Sivasubramaniam and Talibudeen, 1971), but because the form and the charge of the absorbed Al ions are not known it was not included in the (C-A) calculation as many others did in their work (Gijsman, 1990b; Troelstra, 1983).

The fractions of N taken up as NO_3^- and NH_4^+ by the plants were approximately estimated by matching the proton release into the rhizosphere soil with (C-A) calculated for different proportions of NH_4^+ and NO_3^- taken up by the plants (Table 6.5). Such matching showed that in the $(NH_4)_2SO_4$ treatment, the proportions of N taken up as NH_4^+ and NO_3^- were probably around 29% and 71% respectively whereas in the $Ca(NO_3)_2$ treatment it was 23% and 77%.

The proportion of NO_3^- taken up by the plants was also estimated by using the following formula (Troelstra et al., 1985):

$$X = \frac{(C\text{-}A) + 0.946\, N_{Org} - H^+ \text{efflux}}{2}$$

where all parameters are in units of μeq per plant. X is the concentration of organic N (N_{org}) contributed by NO_3^- taken-up by the plant (the balance contribution to N_{org} is from NH_4^+ taken-up by the plant), C-A is the cation - anion concentration difference in plants, H^+ efflux is the acidity released by the roots into the rhizosphere soil. The plant uptake ratios of NH_4^+ : NO_3^- was calculated from the X values estimated using the above formula (Table 6.5) These ratios agreed very well with the corresponding ratios calculated in the preceding paragraph by matching NH_4^+ and NO_3^- uptake with charge balance in the plants. It should be noted however that these calculations rely on the accuracy with which the rhizosphere pH change and the soil pH buffering

Table 6.5 Effect of different N forms and assumed ratios of NH_4^+ : NO_3^- uptake on the net release of H^+ to the rhizosphere (0-3 mm) by tea roots

Assumed ratios of NH_4^+ : NO_3^- taken-up by plant	(Cations - Anions) uptake in the plant[1] (μeq plant^{-1})			
	Control	$(NH_4)_2SO_4$	NH_4NO_3	$Ca(NO_3)_2$
0 : 100	-606	-2398	-2592	-1867
20 : 80	43	-569	-620	-353
22 : 78	108	-386	-423	-201
25 : 75	205	-112	-127	26
28 : 72	303	162	168	253
30 : 70	368	345	365	404
32 : 68	433	528	563	556
45 : 55	855	1677	1844	1541
47 : 53	919	1860	2041	1691
50 : 50	1017	2174	2337	1919
52 : 48	1082	2317	2543	2071
55 : 45	1433	2591	2829	2297
100 : 0	2641	6745	7265	5704
Total H^+ release (+) or OH^- release (-) in the rhizosphere of all roots[2] (μeq H^+ plant^{-1})	114	326	86	-20
Predicted ratio of (NH_4 : NO_3) uptake by the plant	23 : 77	29 : 71	26 : 74	23 : 77
Calculated (NH_4 : NO_3) uptake using the imperical formula[3]	24 : 76	33 : 67	29 : 71	27 : 73

[1] Σcharge of total cation uptake (μeq) by plant - Σcharge of total anions uptake (μeq) by plant (NH_4^+ and NO_3^- calculated from N_{org} in plant according to the ratios in column 1).

[2] $$\frac{\text{9th column in Table 6.3} * \text{Total root surface area}}{\text{Surface area of roots above mesh (0-2 mm)} * 0.5}$$

(The factor 0.5 is used because the acidity produced in the lower RSC is assumed to be due to half of the surface area of the roots on of the roots on the mesh).

[3] $$X = \frac{(C\text{-}A) + 0.946\ N_{org} - H^+ \text{efflux}}{2}$$

where X represents the contribution of NO_3^- to Organic-N (Troelstra et al., 1985). See text for units and explanation of the equation.

capacity (pH_{bc}) are determined. Changes in pH with ionic strength and pH_{bc} with time may affect this calculation.

The high proportion of NO_3^- compared to NH_4^+ taken up in the $(NH_4)_2SO_4$ treatment suggests that a large proportion of NH_4^+ in the $(NH_4)_2SO_4$ treatment may have been nitrified inspite of the nitrification inhibitor used in this study. Tolhurst (1955) reported significant nitrification in Sri Lankan tea soils even at a pH as low as 3.7. Sandanam et al. (1978) reported a recovery of 39% of fertiliser N as NO_3^- from acid tea soils treated with $(NH_4)_2SO_4$ fertiliser within 27 days of incubation at 20 - 22^0 C at 35% (w/w) moisture content. Therefore it is quite possible that a significant proportion of the NH_4^+ added in the present trial would also have been converted to NO_3^- within the trial period of 60 days despite addition of dicyandiamide to stop nitrification.

Nitrification rates may even be higher in the rhizosphere than in the bulk soil due to the higher activity of nitrifying bacteria in the rhizosphere, as a result of abundant energy provided by carbon exudates from the roots. Rovira and McDougall (1967) reported that the activity of nitrifying bacteria (nitrosomonas and nitrobacter) in the rhizospheres of wheat, maize and lucerne was much higher compared to that in the bulk soil. It is possible that the higher activity of these bacteria may have nitrified much of the NH_4^+ in the fertilisers. This would have reduced pH in the rhizosphere of $(NH_4)_2SO_4$ and NH_4NO_3 treated soils - even though the plants took-up predominantly NO_3^- from these fertilisers causing the roots to release OH^- or HCO_3^- into the rhizosphere. Nitrification produces two moles of H^+ per mole of N whereas NO_3^- uptake produces one mole of OH^- per mole of N. The net result is therefore rhizosphere acidification.

In contrast to the results reported in this Chapter, Xan and Jianyun (1994) reported that tea plants preferentially absorb NH_4^+ compared to NO_3^-, because tea roots have less nitrate reductase activity to reduce any NO_3^- taken-up. However, Selvendran (1970) reported that there were appreciable amounts of nitrate reductase enzyme in the active white roots of tea. In the present study the soils may have had mainly the

NO_3^- form of N and low amounts of NH_4^+-N even when N was supplied in the NH_4^+ form and therefore the plants had access mainly to NO_3^--N. As the soils in this trial were not analysed at the end of the trial to determine the relative proportions of soil NH_4^+ and NO_3^-, it is not possible to verify the above suggestion.

6.5 CONCLUSIONS AND IMPLICATIONS

The form of N supplied to tea had a marked influence on the rhizosphere pH and on the uptake and utilisation of soil P and P from EPR fertiliser. The supply of NH_4^+-N decreased rhizosphere pH resulting in higher P dissolution and adding NO_3^- increased rhizosphere pH, which reduced P dissolution in the rhizosphere. Irrespective of the form of N, phosphate rock dissolution in the rhizosphere was always greater than in the bulk soil.

Exchangeable (resin-P) and weak alkali (0.1 *M* NaOH-P_i) extractable soil P forms were depleted in the rhizosphere due to plant and microbial uptake of P. Weak alkali extractable organic P (0.1 *M* NaOH-P_o), accumulated and this may be due to the transformation of P_i into P_o, by the high microbial activity in the rhizosphere compared to that in the bulk soil. The depletion of NaOH-P_i in the rhizosphere was lowest when NH_4^+-N was supplied to the plant due to the higher P fixation caused by the lower rhizosphere pH compared to when NO_3^--N was supplied to the plant.

The cation-anion balance studies in the plant indicated that tea removed more NO_3^--N than NH_4^+-N from the soil irrespective of the form of N added to the soil. These results together with the results of the forms of N supply on rhizosphere pH suggest that nitrification of NH_4^+ was reasonably rapid relative to N uptake. The calculation of the proportion of N taken-up as NH_4^+ and NO_3^- in this study was based on several assumptions. These results require confirmation through further investigation, perhaps using ^{15}N labeled NH_4^+ and NO_3^- fertilisers, higher doses of split application of nitrification inhibitors and measuring NH_4^+ and NO_3^- concentration in the soils at different periods during plant growth. Alternatively, the plants could be fed continuously with dilute solutions of N and nitrification inhibitors in the experiments.

CHAPTER 7

THE FATE AND EFFECTS OF PHOSPHATE FERTILISERS ON PHOSPHORUS AVAILABILITY TO TEA (*Camellia sinensis* L.) IN A HIGHLY ACIDIC ULTISOL IN SRI LANKA

7.1 INTRODUCTION

Tea soils frequently present problems that have constrained the development of a successful sustainable tea production, due to conditions associated with high soil acidity and deficiency in plant-available P caused by fixation of P by Fe and Al oxides and hydroxyoxides (Bhattacharyya and Dey, 1983; Golden et al., 1981). As these soils have very low pHs (pH in water <5.5) and receive high rainfall (>2000 mm), phosphate rock (PR), when applied to these soils, is expected to dissolve and supply adequate amounts of P to plants (White et al., 1989). Therefore direct application of finely ground locally available PR may be an economically attractive alternative to the use of more expensive imported soluble P fertilisers. The rate of dissolution of PRs in soil, and hence the potential availability of P to plants, depends on the properties of the PR and on soil factors such as pH, P sorption capacity (Bolan and Hedley, 1989; Chien et al., 1980; Mackay et al., 1986), exchangeable Ca content and CEC (Mackay et al., 1986; Robinson and Syers, 1991). Although the supply of acidity is a prerequisite for PR dissolution it may not necessarily result in an increase in plant-available P (Syers and Mackay, 1986) because low soil pH also causes high P fixation resulting in a decrease in plant-available P (Apthorp et al., 1987; Sanchez, 1976).

In recent times many countries have been attracted by the possibility of using PRs, to increase agricultural production, particularly those having indigenous PRs (Sale and Mokwunye, 1993). In mid 1970s a large PR deposit estimated to be about 40 million metric tones (Jayawardene, 1976) was discovered at Eppawala in the North-central province of Sri Lanka (Chapter 2, section 2.5). This PR (Eppawala phosphate rock, EPR) is now recommended as a P fertiliser for direct application to many crops

including mature tea in Sri Lanka (Dahanayake et al., 1995) in spite of inconclusive experimental evidence available on the agronomic effectiveness of EPR on tea.

Mature tea plants seldom showed yield responses to the application of soluble and sparingly soluble P fertilisers (Willson and Clifford, 1992). Eden (1949) reported that the application of a PR fertiliser (saphosphosphate - a blend of Egyptian PRs) at a rate of 15 kg P ha^{-1} yr^{-1} gave the maximum yield response by tea on an Ultisol in Sri Lanka with no further response accruing from higher rates of application. In India, a comparison of the yield responses to a range of easily soluble and sparingly soluble P fertilisers showed that all P forms applied at a rate of 26 kg P ha^{-1} yr^{-1} increased tea yield over the control (no P fertiliser) treatment but no significant difference in yield between the P forms (Ranganathan, 1971-1980). In Sri Lanka, EPR was compared with imported saphosphosphate as P fertiliser for young tea at an application rate of 15 kg P ha^{-1} under glasshouse conditions and found that there were no yield responses to either fertilisers (Sivasubramaniam et al., 1981). Therefore it was not possible to compare the relative agronomic effectiveness of the two P fertilisers. There is also no published information on the reactions and transformation of P applied in fertilisers to tea soils in any of the tea growing countries. The present experiment was therefore designed to test the effect of Eppawala phosphate rock (EPR) and Triple superphosphate (TSP) applied at different rates on the dry matter yield of tea and the transformation of fertiliser P in a Sri Lankan acid soil.

Soil P tests are important tools for assessing the availability of soil P to plants and determining P fertiliser requirements of plants. This aids efficient use of P fertilisers, limits wastage of fertiliser materials and minimises pollution hazards in soils and water bodies. Many soil tests have been developed in the past to estimate the pool of plant-available P in soils. The performance of different tests is influenced by soil properties, the climate and the crop grown. The Olsen-P test ($NaHCO_3$, pH 8.5) has been used for wheat in India (Gattani and Seth, 1973) and Bolivia (Waugh and Manzano, 1971) and for coconut in Sri Lanka (Loganathan et al., 1982). This method is also successful in predicting soil P availability to pasture in soils fertilised with soluble P fertilisers in New Zealand (Saunders et al., 1987) and Australia (Colwell, 1963). In acid soils, Bray-1 and Bray-2 tests have been found to be suitable in estimating plant-available P

for a number of crops (Fixen and Grove, 1990). An acid borax extractant (pH 1.5, Beater, 1949) has been used in Sri Lanka to predict P availability to tea (Jayman and Sivasubramaniam, 1980), but no experimental evidence is available on which to base a comparison of the suitability of the borax test with other soil test methods.

7.2 OBJECTIVES

The objectives of the study reported in this chapter are:

(1) To determine the chemical changes in the P fractions in a highly acidic Sri Lankan Ultisol fertilised with two forms of P fertilisers (EPR and TSP) over 10 months of tea seedling growth.
(2) To determine the extent of plant-induced dissolution of EPR in the acid soil.
(3) To compare the agronomic effectiveness of EPR with TSP in the above trial.
(4) To determine the most suitable soil test that can predict dry matter yield of tea in the above trial.
(5) To determine whether leaf P concentration is a good index of P supply to tea plants.

7.3 MATERIALS AND METHODS

The soil used in the study was collected in St. Coombs estate(1382 m amsl), Talawakelle, Sri Lanka, where tea has been cultivated for over 50 years. The soil belongs to the Red Yellow Podsolic Great Soil Group (Rhodustult according to US Soil Taxonomy, De Alwis and Panabokke, 1972). Selected properties of the soil are presented in Table 7.1. After removing the surface litter, the soil was collected from a 0 - 15 cm depth, air-dried at room temperature, lumps broken and passed through a 2.0 mm sieve. Approximately 4.5 kg of air-dried soil was then weighed into each of 107 plastic pots.

Eight month old tea plants (TRI 3072) of similar size were removed from nursery bags and soil adhering to roots were carefully removed by immersing in water. The

Table 7.1 Selected properties of the soil (Rhodustult) used

Soil property	Unit	Value
Sand	%	49
Silt	%	23
Clay	%	28
pH (Soil : H_2O 1:2.5 w/w)		4.55
Organic C	%	2.12
Effective CEC[1]	$cmol_c\ kg^{-1}$	4.07
Total N	%	0.16
Ex. Na	$cmol_c\ kg^{-1}$	0.12
Ex. K	$cmol_c\ kg^{-1}$	0.17
Ex. Ca	$cmol_c\ kg^{-1}$	0.16
Ex. Mg	$cmol_c\ kg^{-1}$	0.35
Ex. Al	$cmol_c\ kg^{-1}$	1.29
pH buffer capacity (at pH 4-5)	$mmol\ H^+\ kg^{-1}\ pH^{-1}$	17
Resin-P	$\mu g\ g^{-1}$ soil	2
Olsen-P	$\mu g\ g^{-1}$ soil	40
Bray-1 P	$\mu g\ g^{-1}$ soil	4
Borax-P	$\mu g\ g^{-1}$ soil	6
P-fixing capacity[2]	%	95

[1]Exchangeable Ca + Mg + Na + K + Al + H

[2]Blackmore et al. (1987)

plants were weighed and then planted in pots. Each pot received either Triple superphosphate (TSP, total P 20% and 85-95% of total P dissolved in water) or Eppawala phosphate rock (particle size 5.2% > 250 μm; 39.2% 150 - 250 μm; 39.9% 150 - 75 μm; 15.7% < 75 μm, total P 14.5%, citric acid (2%) soluble P 1.97%, almost insoluble in water) as the P source. Triple superphosphate and EPR were applied evenly on the surface of the soils at the rates of 0, 10, 20, 30, 40, 50 and 60 kg P ha^{-1} (10 kg P ha^{-1} is equivalent to 6 μg P g^{-1} soil assuming a bulk density of 1.1 Mg M^{-3} and 0-15 cm soil depth) at the beginning of the trial. The treatments were replicated six times. Nitrogen (N) and potassium (K) were applied on to the soil surface at rates of 120 kg N and K ha^{-1} as $(NH_4)_2SO_4$ and KCl respectively in four split applications, with the first application at the time of planting and the final application nine months later.

Duplicate pots were filled with the same quantity of soils receiving the same P treatments, but without tea plants (fallow pots) to study the effect of the P treatments on soil P status in the absence of plants. Nitrogen and potassium were also applied to these pots as was done in the case of planted pots.

All the pots were arranged in a completely randomised design and kept in a glasshouse maintained at 13^0 C minimum and 25^0 C maximum at the St. Coombs estate in Sri Lanka. The soil in the pots was kept at field capacity moisture content throughout the trial period. At the end of 5 months, three replicates of each treatment with plants were dismantled for soil and plant analyses. Soil samples were also taken from the unplanted pots (fallow pots) for analysis at the same time. After 10 months the remaining plants were harvested, plant and soil samples were taken for analysis as in the first harvest. The plant growth system in the glasshouse is shown in Figure 7.1.

7.3.1 Plant and soil analyses

Plant samples were oven-dried at 60^0 C and ground to < 1.0 mm. Both shoot and root samples were analysed for total P by the vanadomolybdate method (Jackson, 1958). Soil samples were air-dried and analysed for plant-available soil P according to the

Figure 7.1 Tea plants growing in pots in the glasshouse study

methods of mixed cation and anion resin strip test (Saggar et al., 1990), Olsen test (Olsen et al., 1954), Bray-1 test (Bray and Kurtz, 1945), borax extraction (Beater, 1949), 2% citric acid extraction and 2% malic acid extraction (Sivasubramaniam et al., 1981) (see Table 7.2). Exchangeable cations were extracted by 1 *M* NH_4OAc buffered at pH 7.0 and determined by the method of Blackmore et al. (1987) and soil organic C by the method of Walkley and Black (1934). Soil pH, pH buffering capacity, P fractionation and the amount of EPR dissolution in the soil was determined as described in Chapter 3. The statistical analyses were carried out using the Statistical Analysis System (SAS) software package (SAS, 1985).

7.3.2 Relative agronomic effectiveness (RAE)

The agronomic effectiveness (RAE) of EPR, relative to the standard TSP was calculated from the yield and P uptake response relationship according to the "vertical" comparison method (Saggar et al., 1993) using equations 7.1, 7.4 and 7.5 described below.

(a) The RAE of EPR was calculated from the cumulative dry matter yield at each harvest using the definition :

$$\text{RAE (\%)} = \frac{\text{Yield with EPR (averaged over all rates of P) - Control}}{\text{Yield with TSP (averaged over all rates of P) - Control}} * 100 \quad ..(7.1)$$

(b) The RAE was calculated from cumulative dry matter yield and P uptake for both P sources at five and ten months after P fertiliser application using a Mitscherlich-type equation described as:

$$Y = a + b\,[1 - \exp(-cX)] \quad .....(7.2)$$

where Y is the yield (g pot^{-1}) at a P application rate of X (kg P ha^{-1}), a and b are parameters that describe the yield on an unammended soil and the maximum yield increment when the nutrient (P) is not limiting. The parameter b is considered to be

Table 7.2 Summary of soil P tests used

P-test	Extractant	pH	Soil : solution ratio	Time of extraction
Olsen	0.05 *M* $NaHCO_3$	8.5	1:20	30 min
Bray-1	0.03 *M* NH_4F + 0.025 *M* HCl	3.0	1:7	5 min
Borax extract	0.00015 *M* $Na_2B_4O_7$ buffered with c. H_2SO_4 (98%)	1.5	1:10	30 min
Anion and cation exchange resin strips	Resin strips saturated with HCO_3^- and Na^+	~ 6.8	1:30	16 hrs
Citric acid	2% Citric acid	2.5	1:10	1 hr
Malic acid	2% Malic acid	1.5	1:10	1 hr

the responsiveness of the plant to the nutrient P added to the soil and parameter *c* describes the steepness of the response curve (Bennett and Ozanne, 1972).

The Mitscherlich equation is often used to describe plant growth responses to nutrients (Campbell and Keay, 1970; Bennett and Ozanne, 1972; Spencer et al., 1980; Saggar et al., 1993). This form of response curve implies that each successive increment in nutrient supply produces a diminishing increment in the yield.

The Equation 7.2 can be rewritten as

$$Y - a = b\,[1 - \exp(-cX)] \quad \text{..... (7.3)}$$

The RAE of EPR relative to TSP at any one of the rates of P (10, 20, 30 ,40 ,50 and 60 kg P ha^{-1}) was estimated using Equation (7.4),

$$\text{RAE (\%)} = \frac{b_1\,[1 - \exp(-c_1X)]}{b_2\,[1 - \exp(-c_2X)]} * 100 \quad \text{..... (7.4)}$$

Where the subscripts 1 and 2 refers to EPR and TSP respectively.

The effectiveness of EPR relative to TSP was also calculated from the ratio of the initial slopes represented as a product of maximum response (b) and the rate constant (c) of the fitted models (Barrow, 1985; Chien et al., 1990a) i. e.

$$\text{RAE (\%)} = (b_1c_1 / b_2c_2) * 100 \quad \text{..... (7.5)}$$

7.4 RESULTS AND DISCUSSION

7.4.1 EPR dissolution in soil

The dissolution of EPR was higher in the soil with tea plants compared to that in the unplanted soil. Plant induced dissolution of PR is now well documented (Bekele et al.,

1983; Bolan et al., 1997; Hoffland et al., 1989; Chapters 3, 4 , 5 and 6). One of the reasons for higher dissolution of EPR in the presence of plants is rhizosphere acidification (Chapters 4, 5 and 6). Trolove et al. (1996b) also observed a higher dissolution of North Carolina Phosphate rock (NCPR) in the rhizospheres of white clover and lotus due a to higher acid production by roots in that zone. Other reasons are the removal of PR dissolution products, Ca and P by plant uptake and root secretion of organic acids (Jayman and Sivasubramaniam, 1975; Xiaoping, 1994) as discussed in Chapters 3, 4, 5 and 6.

The proportion of EPR dissolved was greater at lower rates of EPR application compared to that at higher rates (Figure 7.2) though the amount of EPR dissolution was higher at higher rates (Table 7.3). This was because at lower rates of EPR additions, there were proportionately higher amounts of acidity available for dissolution and also more sinks for the removal of the dissolved products P, Ca and F per unit of applied EPR. The amount of EPR dissolution in the soil was significantly higher at the 10 month sampling compared to that at the 5 month sampling, however significant differences ($p < 0.05$) could be observed only among rates that were below 20 kg P ha^{-1} (Figure 7.2). At the 10 months sampling, in the presence of plants, more than 50% of added EPR has dissolved at P application rates < 30 kg P ha^{-1}. This suggests that at least 50% of EPR has the potential to dissolve in tea soils under field conditions, when applied at the rate of 20 kg P ha^{-1} yr^{-1} currently recommended by the TRI (Tea Research Institute) of Sri Lanka. In line with the results of this experiment, Tambunan (1992) observed in field trials on Ultisols of Indonesia under Calopogonium the dissolution of NCPR and MPR (Moroccan phosphate rock) added at the rate of 80 kg P ha^{-1} had increased with increased contact time of PR and soil. For example after 545, 360 and 180 days the dissolution of NCPR was 98, 82 and 40% respectively.

As the experimental soil is highly acidic (pH 4.55) and exchangeable Ca levels (0.16 $cmol_c$ kg^{-1}) and % Ca saturation of the exchange complex (2.5%) are low, it provided favourable conditions for PR dissolution (Bolan and Hedley, 1989; Mackay et al., 1986). Though the initial rate of PR dissolution is high, with time it decreased because

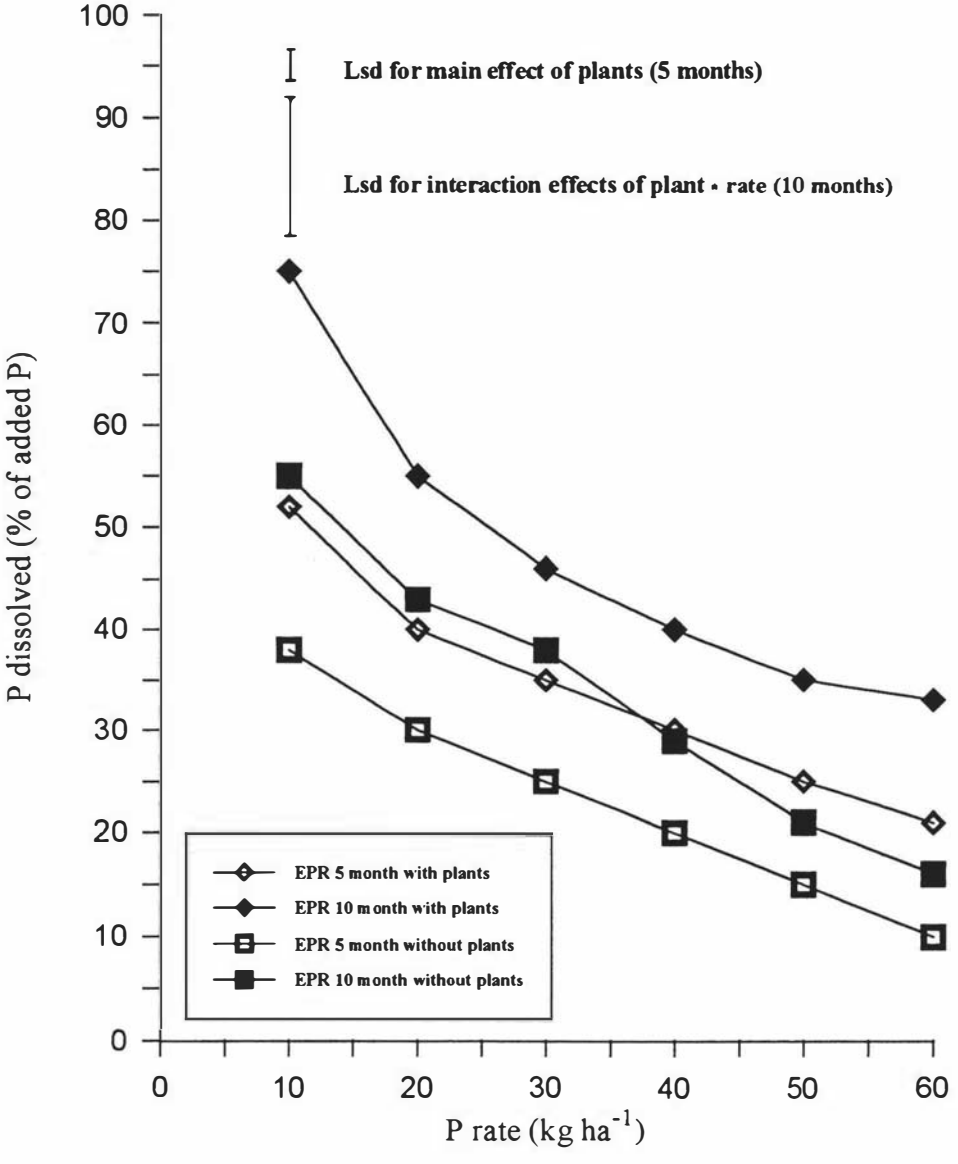

Figure 7.2 Effect of time and EPR fertiliser rate of addition on P dissolution in soil with and without plants. Vertical bars correspond to Lsd at $p < 0.05$.

Table 7.3 The H^+ consumption for EPR dissolution in soil with and without tea plants

Rate of EPR application (kg P ha^{-1})	Experimental condition	Amount of EPR dissolved after 5 months (μg P g^{-1} soil)	H^+ consumption[1] for EPR dissolution in soil after 5 months (μmol H^+ g^{-1} soil)	Amount of EPR dissolved after 10 months (μg P g^{-1} soil)	H^+ consumption[1] for EPR dissolution in soil after 10 months (μmol H^+ g^{-1} soil)	Contribution of soil pH increase due to EPR dissolution[2]
10	With tea plants	3.12 ± 0.17	0.20 ± 0.011	4.50 ± 0.22	0.26 ± 0.035	0.015
20		4.81 ± 0.24	0.31 ± 0.015	6.60 ± 0.48	0.43 ± 0.031	0.025
30		6.28 ± 0.26	0.41 ± 0.017	8.35 ± 0.21	0.54 ± 0.014	0.032
40		7.16 ± 0.21	0.46 ± 0.014	9.72 ± 0.36	0.63 ± 0.024	0.037
50		7.49 ± 0.43	0.48 ± 0.028	10.52 ± 0.23	0.68 ± 0.015	0.040
60		7.62 ± 0.28	0.49 ± 0.018	11.91 ± 0.44	0.77 ± 0.029	0.045
10	Without tea plants	2.28 ± 0.08	0.15 ± 0.005	3.32 ± 0.12	0.21 ± 0.008	0.012
20		3.59 ± 0.21	0.23 ± 0.014	5.16 ± 0.16	0.33 ± 0.010	0.019
30		4.54 ± 0.25	0.29 ± 0.016	6.80 ± 0.24	0.44 ± 0.016	0.025
40		4.88 ± 0.31	0.31 ± 0.020	6.97 ± 0.26	0.45 ± 0.017	0.026
50		4.61 ± 0.39	0.30 ± 0.026	6.21 ± 0.32	0.40 ± 0.021	0.023
60		3.67 ± 0.27	0.24 ± 0.017	5.69 ± 0.31	0.37 ± 0.020	0.021

[1]Amount of EPR dissolved (μg EPR g^{-1} soil) * amount of H^+ required to dissolve EPR (0.00933 μmol H^+ μg^{-1} EPR - see text)

[2] Amount of H^+ consumed for dissolution of EPR divided by the pH buffering capacity

the dissolution products, Ca and P accumulate and soil pH increases near the PR particles in the soil (Hammond et al., 1986b).

7.4.2 Effect of P fertilisers on soil pH

At the beginning of the trial, the soil pH was 4.55 (H_2O) and it significantly decreased during the trial period for both P treatments (EPR and TSP) in pots without plants (Figure 7.3). This may be due to nitrification of NH_4^+, which was derived from $(NH_4)_2SO_4$ added as N fertiliser at the beginning of the trial and subsequently at each 3 month interval. Another reason for the decline in pH could be due to the increase in ionic strength caused by the addition of KCl and $(NH_4)_2SO_4$ and the mineralisation of organic matter; these processes would have reduced the pH measured in H_2O. The EPR dissolution would have consumed some of the acidity, but this seems to be of a lower order of magnitude (Table 7.3) compared to the acid generating process discussed above. The reduction in soil pH was however not statistically significant in the pots with tea plants. This may be due to NO_3^- uptake by the plants resulting in the release of OH^- or HCO_3^- to the soil, which neutralised part of the acidity produced in the soil through nitrification. Another reason could be that the ionic strength in the presence of plants may have been lower because of plant uptake of ions and therefore the pH measured in H_2O would have been influenced to a lesser extent in soils with plants compared to those without plants.

The amount of acidity consumed for EPR dissolution in the soil with and without plants at the 5 and 10 month samplings was determined as described in Chapter 4, section 4.4.3 (Table 7.3). At both sampling times the acid consumption for EPR dissolution in the presence of plants was greater than in their absence.

7.4.3 Effect of forms and rates of P fertilisers on soil P fractions

At the end of the trial (at 10 months) approximately 100% of the added fertiliser P was recovered in the various soil P fractions from the unplanted pots for both P fertiliser treatments (Table 7.4). The lower recovery of P from soils in planted pots is

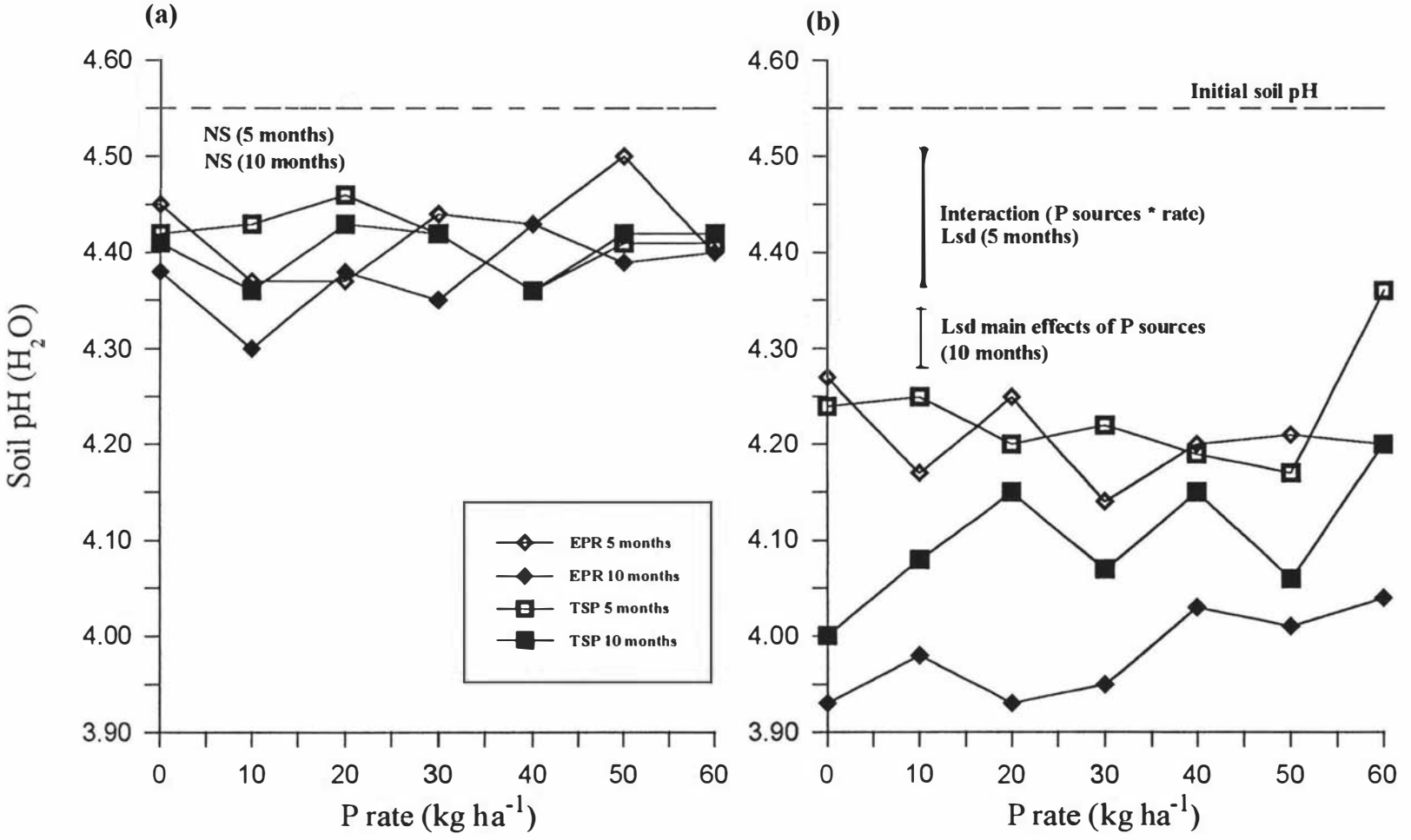

Figure 7.3 Effect of EPR and TSP on soil pH (H_2O) after 5 and 10 months of application (a) with and (b) without tea plants. Vertical bars correspond to Lsd at $p < 0.05$ and NS represents treatments not statistically significant at $p < 0.05$.

Table 7.4 The % recovery[1] of added P in soil P fractions in the unfertilised (control) and EPR and TSP treated soils at the end of the trial

Rate of P application (kg ha^{-1})		With or without plants	Resin-P (μg g^{-1} soil)	NaOH-P_i (μg g^{-1} soil)	NaOH-P_o (μg g^{-1} soil)	H_2SO_4-P_i (μg g^{-1} soil)	Residual-P (μg g^{-1} soil)	Total-P (μg g^{-1} soil)	Total P recovery from applied P (%)
EPR	0	**With plants**	1.9	197.5	114.8	34.2	120.5	468.9	----
	10		2.5	199.7	116.3	35.1	119.5	473.0	70
	20		3.3	202.3	115.9	34.2	121.4	477.1	69
	30		4.3	205.9	117.5	40.2	118.2	486.1	96
	40		5.3	206.9	117.6	42.5	119.4	491.7	95
	50		6.4	208.7	118.5	44.2	120.7	498.5	99
	60		8.0	210.2	118.5	44.6	119.7	500.9	89
EPR	0	**No plants**	2.8	209.7	122.8	35.1	124.2	494.6	----
	10		3.8	213.7	123.9	37.6	122.1	501.0	106
	20		4.9	215.2	125.3	40.6	121.0	506.9	102
	30		5.4	216.1	124.7	45.2	123.5	514.9	112
	40		6.8	217.3	124.8	48.9	123.0	520.8	109
	50		8.8	217.0	126.3	52.2	123.2	527.5	109
	60		10.1	218.3	128.3	52.0	124.2	533.0	107
TSP	0	**With plants**	1.9	197.5	114.8	34.2	120.5	468.9	----
	10		2.6	200.4	114.4	34.0	121.4	472.7	65
	20		3.4	206.6	115.4	35.7	117.0	478.0	76
	30		4.3	207.9	116.6	36.2	118.7	483.7	83
	40		5.5	209.7	117.5	37.3	121.8	491.7	95
	50		6.2	211.0	119.7	36.5	124.2	497.5	96
	60		8.2	215.0	121.1	37.5	120.4	502.3	93
TSP	0	**No plants**	2.8	209.7	122.8	35.1	124.2	494.6	----
	10		3.7	216.5	124.1	35.1	121.6	501.0	106
	20		4.9	220.4	126.2	36.1	120.1	507.6	108
	30		5.1	224.8	127.1	37.5	119.9	514.5	110
	40		6.0	229.6	127.1	36.9	122.9	522.4	116
	50		7.5	234.3	128.1	39.0	117.7	526.7	107
	60		9.4	237.0	131.2	37.7	118.9	534.2	110

[1] $\%P\ recovery = \frac{(\Sigma\ P\ fractions\ in\ fertilised\ soil - \Sigma\ P\ fractions\ in\ unfertilised\ soil)}{amount\ of\ fertiliser\ P\ added\ to\ the\ soil} * 100$

due to P uptake by plants. The changes caused by P fertiliser application in the individual soil P fractions, resin-P, NaOH-P_i, NaOH-P_o and H_2SO_4-P_i at the 5 and 10 month sampling times are shown in Figures 7.4, 7.5, 7.6 and 7.7 respectively. Unlike EPR, the highly soluble TSP fertiliser had quickly dissolved and mobilised into various soil P fractions. The applied TSP was mainly recovered as NaOH-P_i whereas the majority of EPR was recovered as undissolved EPR in the H_2SO_4-P_i fraction as observed in Chapter 5. In the TSP treatment water initially moves into TSP granules and dissolves monocalcium phosphate forming a metastable triple point solution containing dicalcium phosphate and free phosphoric acid. The solution coming out of the granule has a pH of 1 to 1.5 (Sanchez, 1976). In acid soils Fe and Al in the solution and the exchange phase are abundant and they react with P to form relatively insoluble Fe and Al phosphates (Coleman et al., 1960). These P forms are extracted by NaOH in the P fractionation scheme.

In both P fertiliser treatments the concentration of all P fractions increased with increasing rates of P application, but the rate of increase per unit increase of P application is highest for NaOH-P_i in the TSP treatment and for H_2SO_4-P_i in the EPR treatment (Figure 7.5, 7.7). Trolove et al. (1996b) also found an increase in the resin-P and NaOH-P_i fractions in MCP (the chemical components in TSP) treated bulk soil compared to the control treatment, whereas in NCPR treated soil only resin-P and H_2SO_4-P_i increased. My results compared well with the results obtained from a trial in Sri Lanka on a sandy soil, where long-term (10 yrs) annual application of concentrated superphosphate to coconut, had significantly increased Fe + Al bound P (NaOH-P_i) concentration, whereas application of a PR increased the concentration of P bound to Ca (H_2SO_4-P_i) compared to the no fertiliser treatment (Loganathan and Nalliah, 1977). In an acid (pH 4.6) Ultisol in Cavinti, Philippines, Hedley et al. (1994) recovered only 5% of P from applied MCP in the resin fraction because 75-80% of P was transformed into NaOH-P_i fraction within six weeks in upland rice growth.

The labile resin-P concentration in the TSP treated soils is generally higher than that in the EPR treated soils, the differences were statistically significant ($p < 0.05$) and prominent in the unplanted soil for the majority of the rates of P fertiliser application especially at 5 months (Figure 7.4). This is due to the higher solubility of TSP

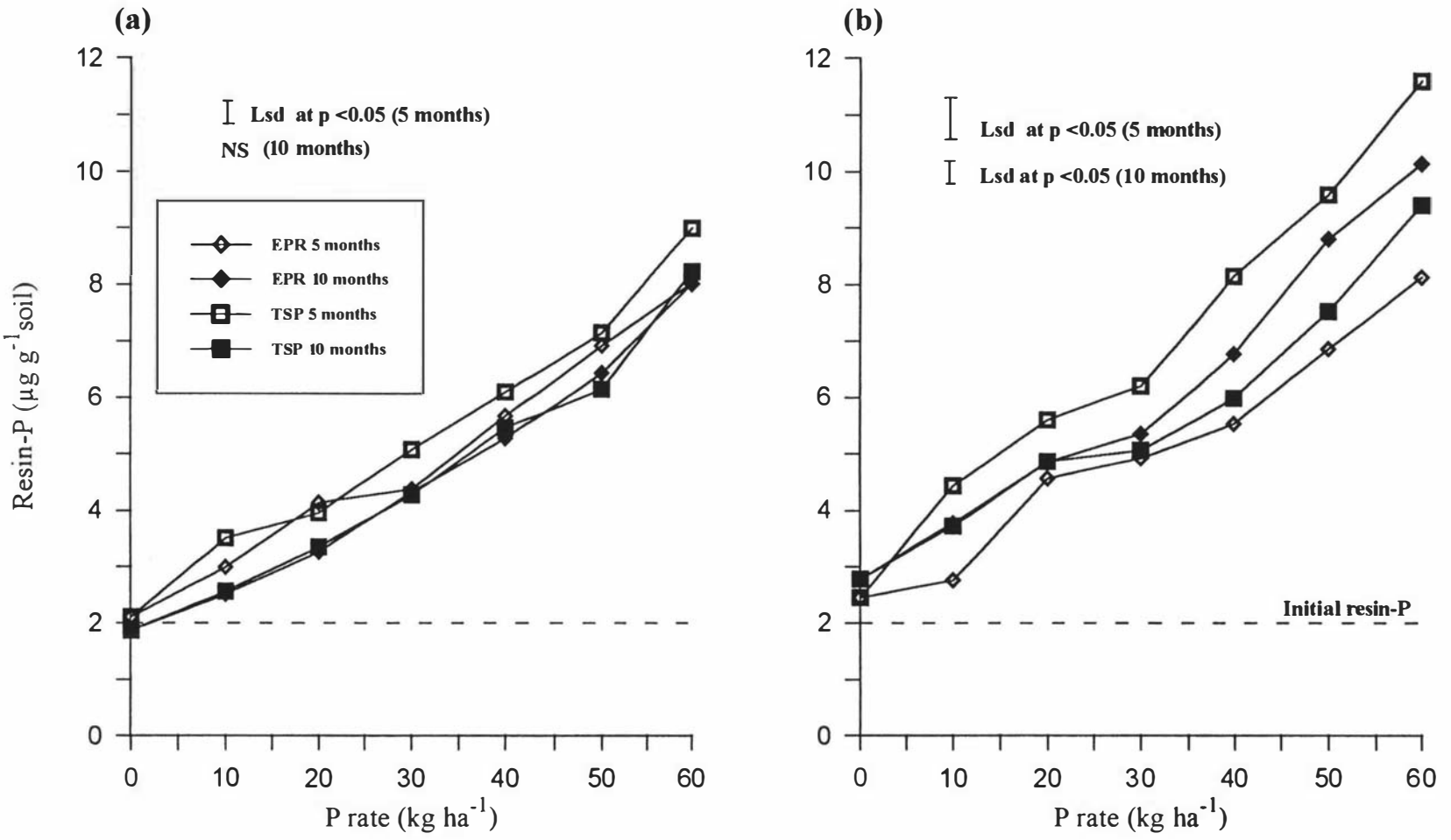

Figure 7.4 Effect of EPR and TSP fertiliser rates on resin-P in soil (a) with and (b) without tea plants. Vertical bars correspond to Lsd at p <0.05 for main effects of P sources and NS represents treatments not statistically significant at p <0.05.

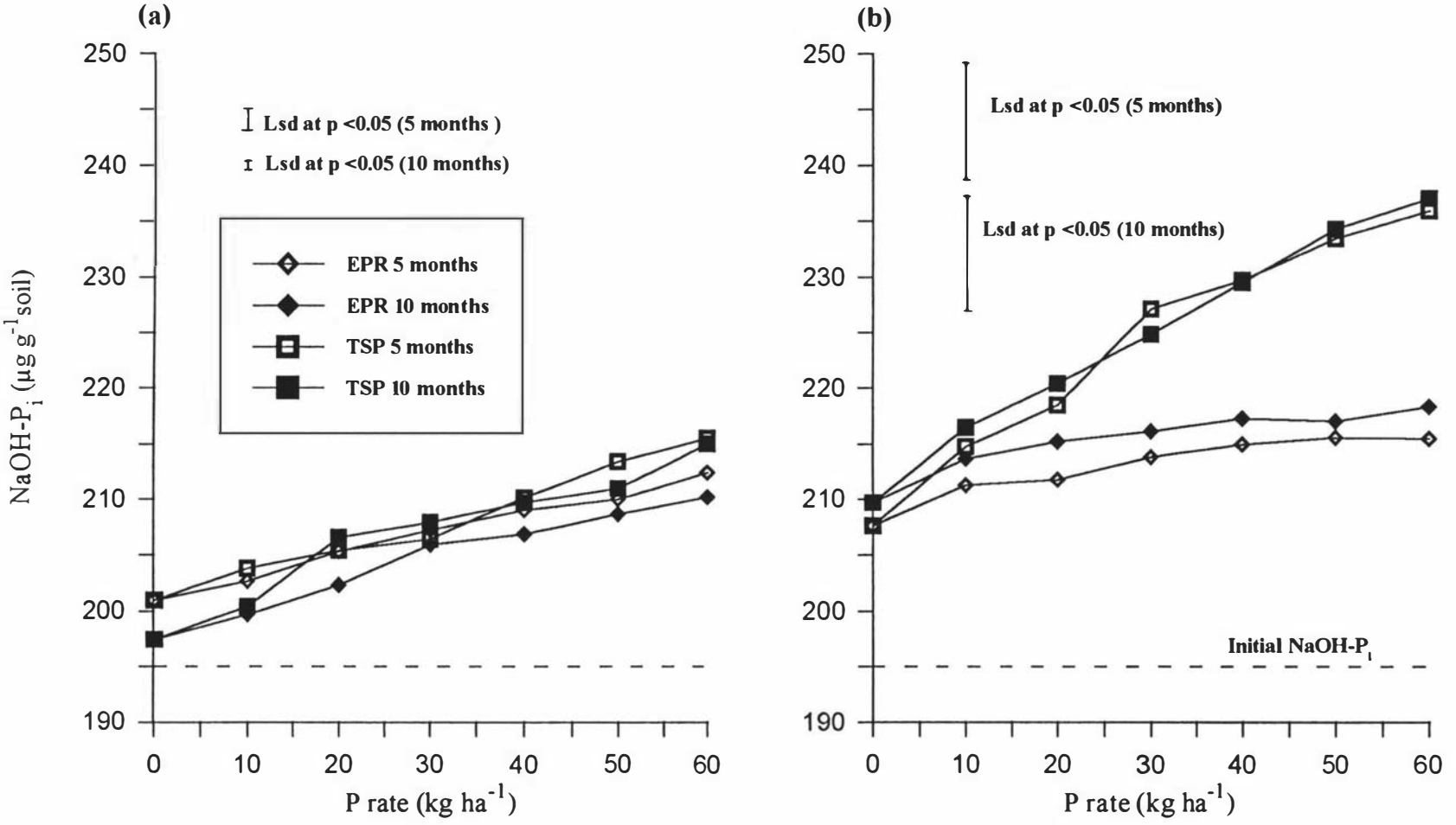

Figure 7.5 Effect of EPR and TSP fertiliser rates on NaOH-P_i fraction in soil (a) with and (b) without tea plants. Vertical bars correspond to Lsd at $p < 0.05$ for main effects of P sources in (a) and interaction effects of P sources * rates in (b) respectively.

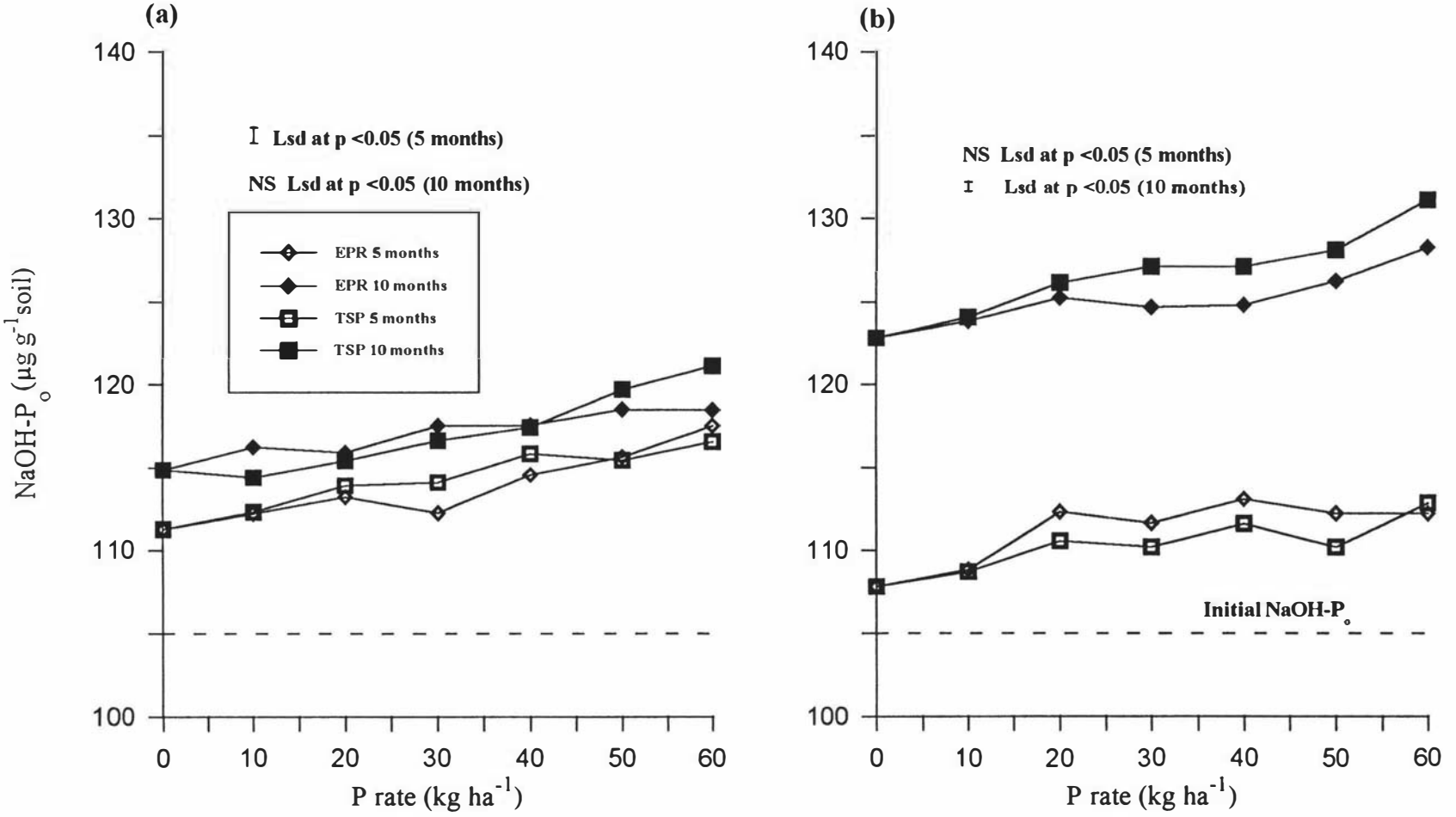

Figure 7.6 Effect of EPR and TSP fertiliser rates on NaOH-P$_o$ fraction in soil (a) with and (b) without tea plants. Vertical bars correspond to Lsd at $p < 0.05$ for main effects of P sources and NS represents treatments not statistically significant at $p < 0.05$.

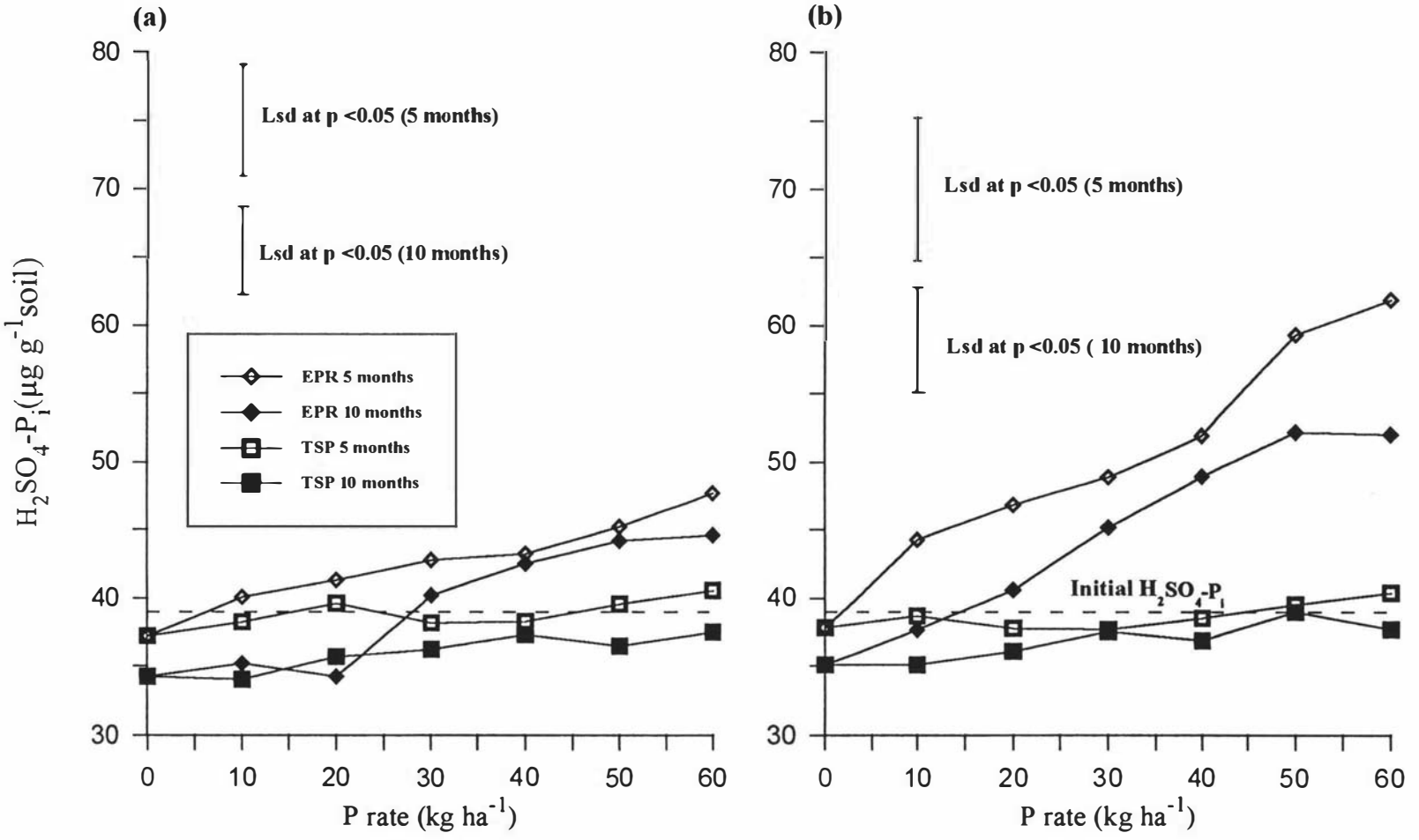

Figure 7.7 Effect of EPR and TSP fertiliser rates on H_2SO_4-P_i fraction in soil (a) with and (b) without tea plants. Vertical bars correspond to interaction (P sources * rates) Lsd at p <0.05.

fertiliser. The magnitude of the difference in resin-P concentration between the two fertiliser treatments increased with increasing rates of P application in the unplanted soil. This is because % EPR dissolution decreased with increasing rates of EPR application (Figure 7.2). The narrower difference in resin-P concentration between the two fertilisers in pots with plants compared to unplanted pots is due to a higher rate of EPR dissolution in the presence of plants (Figure 7.2) thereby increasing resin-P concentration to a level closer to that from TSP treatment.

The resin-P concentration in unplanted soil was higher at 5 months than at 10 months for the TSP treated soil whereas the values were the other way round for EPR. This is because with an increase in time TSP gets increasingly converted to NaOH-P_i and NaOH-P_o (Figure 7.6). In the EPR treatment, however, with an increase in time more EPR dissolved to increase resin-P in addition to the conversion to NaOH-P_i and/or NaOH-P_o.

The NaOH-P_i concentration, which is a measure of P fixed to Fe + Al in soils was significantly higher in the TSP treatment compared to the EPR treatment in all unplanted soils and some planted soils due to a higher supply of soluble P for P fixation from the highly soluble TSP fertiliser (Figure 7.5). The statistical significance ($p < 0.05$) of the interaction between P sources and rates, in the absence of plants, indicates that the rate of increase in NaOH-P_i concentration per unit increase in applied P varies with the source of P fertiliser. The difference in NaOH-P_i concentration between the two fertiliser treatments increased with increasing rates of P application especially in unplanted soils. This is due to a decrease in the %P dissolution of the EPR with an increase in P rates (Table 7.2).

At high rates of TSP application, NaOH-P_i concentration in the soil was lower in the presence of plants than in its absence. This may be due in part to plant utilisation of this fraction and in part to the reduction of P fixation through ligand exchange of fixed P with organic acid anions secreted by tea roots (Jayman and Sivasubramaniam, 1975) and complexation of Fe and Al by these organic anions to free fixed P_i. In the rhizosphere studies reported in Chapters 3, 4, 5 and 6 it was shown that plant roots caused a reduction in the NaOH-P_i fraction in the rhizosphere soil. Furthermore

Hedley et al. (1994) with upland rice and Trolove et al. (1996b) with clover and lotus also observed a significant depletion of the NaOH-P_i fraction in the rhizosphere due to plant uptake.

In the unplanted pots both NaOH-P_i and NaOH-P_o concentrations were higher at the 5 and 10 month sampling times than at the beginning of the trial. The decrease in soil pH with time may have increased P fixation (NaOH-P_i) (Barrow, 1984) and organic matter accumulation (Anderson, 1980).

The main factors influencing the accumulation of NaOH-P_o appear to be time, fertiliser rate and the presence or absence of plants (Figure 7.6). The NaOH-P_o concentration was markedly higher at the 10 month sampling compared to the 5 month sampling especially in unplanted soil. This may be associated with longer times for microbial activity, which may have been stimulated by the supply of K and N upto the 10 month period (two applications of N and K after the 5 month sampling). In Chapters 4, 5 and 6 it was shown that there was an accumulation of P_o in the rhizosphere of tea roots compared to that in the bulk soil. The accumulation of P was considered to be due to increased microbial activity because of an increase of carbon exudated from the roots. In contrast to this observation, Figure 7.6 shows that NaOH-P_o concentration was lower in the presence of plants than in the absence of plants for the TSP treatment at the 10 month sampling. The rhizosphere studies reported in Chapters 4, 5 and 6 were conducted for less than 2 months with the higher P addition rates of 200 $\mu g\ g^{-1}$ soil (300 kg P ha^{-1} as compared to a maximum of 60 kg P ha^{-1} in the current trial) which supplied more than sufficient P during the growth period. In the 10 month growth period of the trial reported in this Chapter, the tea plants' demand is higher and this may have resulted in some mineralisation of NaOH-P_o, thus reducing the concentration of this fraction, resin-P and NaOH-P_i fractions (Figures 7.4 and 7.5). Support for these concepts follow. At 5 months, for TSP treatment, the NaOH-P_o concentration was higher in the presence of plants. The P demand of the plants at the 5 months of growth is less, which may result in more labile P_i being consumed by the increasing microbial population of the rhizosphere. Thus increases in the NaOH-P_o fraction occurs. In wheat growing soils from Camborthid Hissar in India, ammended with organic matter, Khanna et al. (1983)

observed rapid formation (within seven weeks) of microbial organic P from the pool of inorganic-P derived from the initial decomposition of organic matter or from applied P fertiliser (dicalcium phosphate and diammonium phosphate). Subsequently, the break down of microbial tissues due to autolysis by other micro-organisms resulted in an increase in P_i (mineralisation of P_o).

The increasing rates of P application increased H_2SO_4-P_i (Ca-P) concentration in the EPR treatment. This was due to the recovery of undissolved EPR in the H_2SO_4-P_i fraction. But in the TSP treatment this is not the case (Figure 7.7), because the $Ca(HPO_4)_2$ in TSP dissolved and converted to other forms of P with very little Ca bound P remaining in the soil (Hedley et al., 1994).

7.4.4 Effect of P fertilisers on soil test extractable P

In this study various soil tests ranging from alkaline to strong acid solutions were used to extract P from the potted soils. Widely differing amounts of P were extracted by these tests from soils having the same fertiliser treatments (Figure 7.8 - 7.13). Additionally there was a marked influence of the P fertiliser source on the amount of P extracted by a P test. The differences in the amounts of P extracted was caused by differences in the extractant's mechanism of mobilising P bound to the soil.

The amounts of P extracted by all tests increased linearly with increasing rates of applied P in both fertiliser treatments (resin-P, Figure 7.8; Olsen-P, Figure 7.9; borax-P, Figure 7.10; Bray 1-P, Figure 7.11; citric acid extractable-P, Figure 7.12; and malic acid extractable-P, Figure 7.13). The extractable P values measured by all these methods were in general higher for the 10 month sampling compared to the 5 month sampling. For the EPR treatment this is due to the increased dissolution of this PR with time in both planted and unplanted soil. For the TSP treatment the increase in extractable P with time cannot be due to any increase in labile-P because the resin-P concentration was higher at 5 months than at 10 months (Figure 7.4). Except for the Olsen and borax methods of extraction the increase in extractable P with time was however small although statistically significant. Higher Olsen P values at 10 months

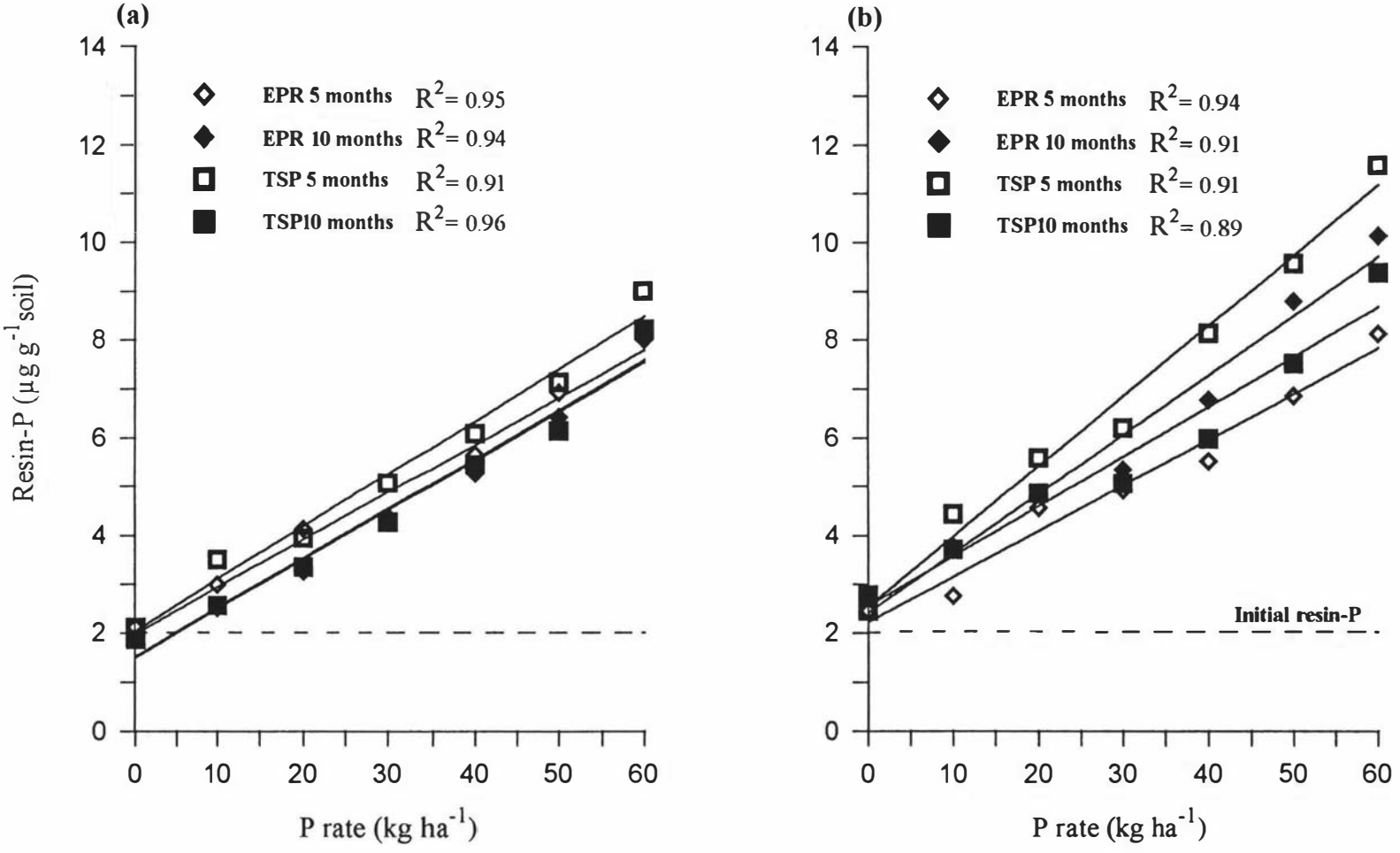

Figure 7.8 Effect of EPR and TSP fertiliser rates on Resin-P in soil (a) with and (b) without tea plants.

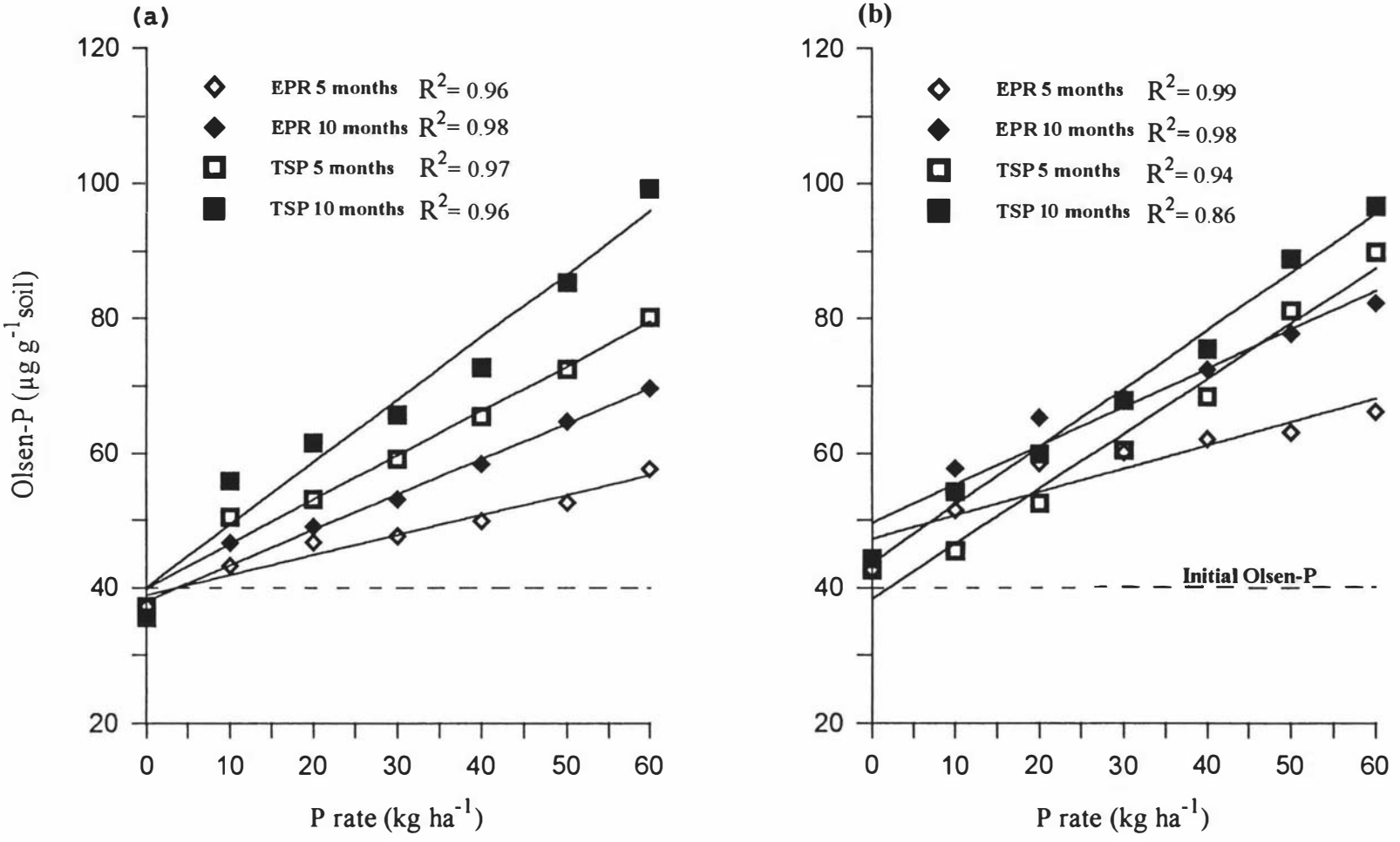

Figure 7.9 Effect of EPR and TSP fertiliser rates on Olsen-P in soil (a) with and (b) without tea plants.

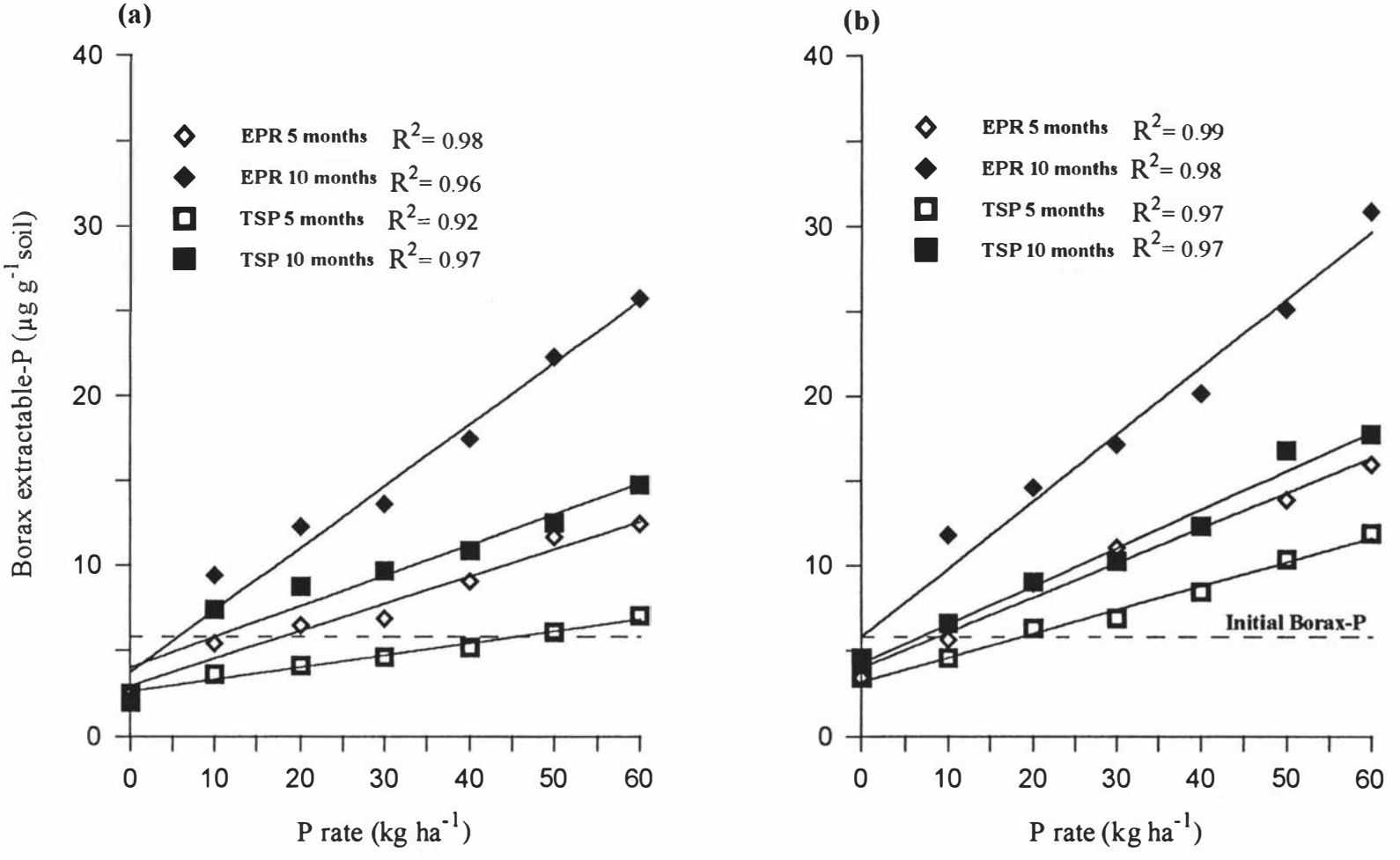

Figure 7.10 Effect of EPR and TSP fertiliser rates on Borax extractable-P in soil (a) with and (b) without tea plants.

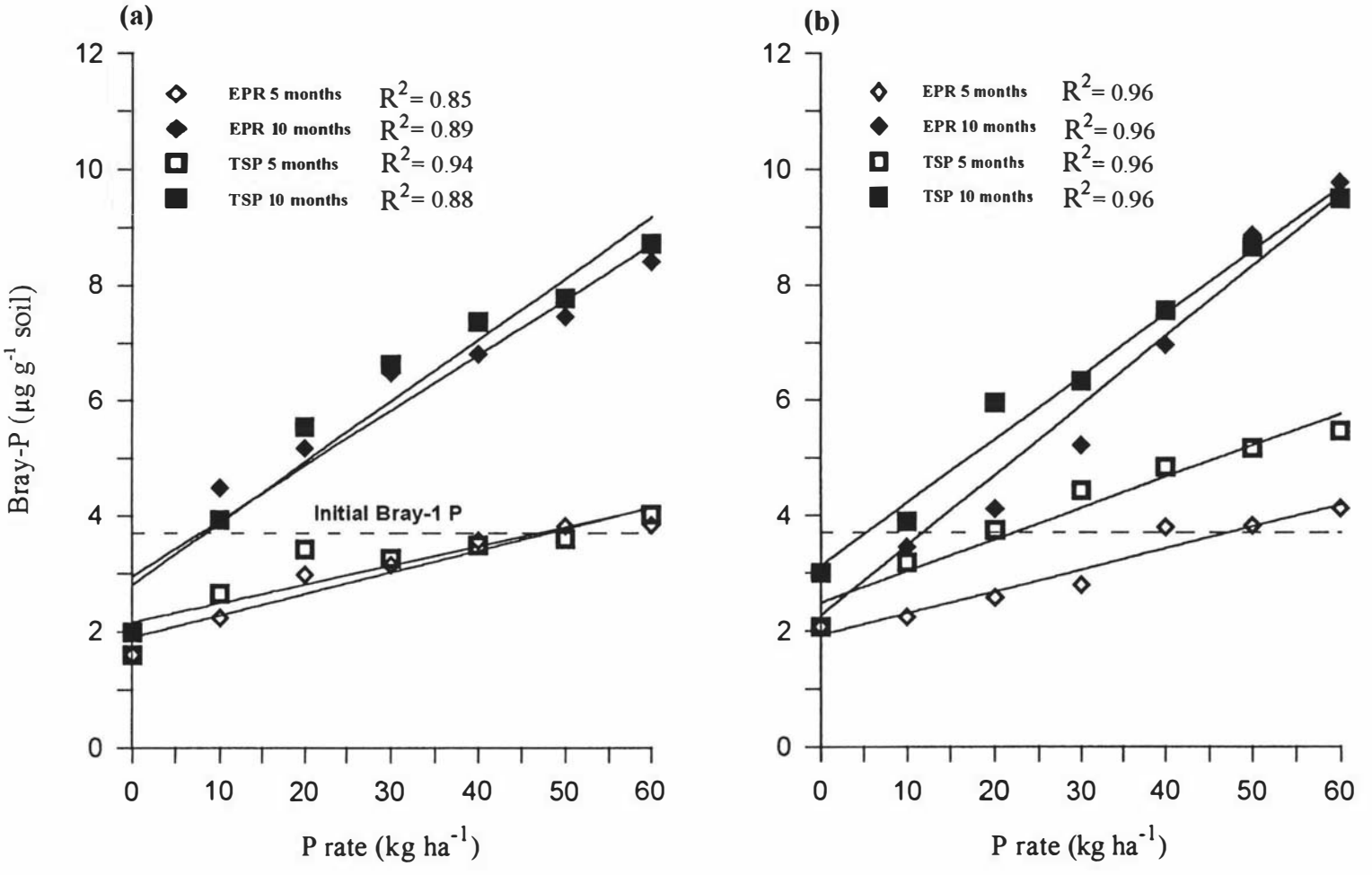

Figure 7.11 Effect of EPR and TSP fertiliser rates on Bray-1 P in soil (a) with and (b) without tea plants.

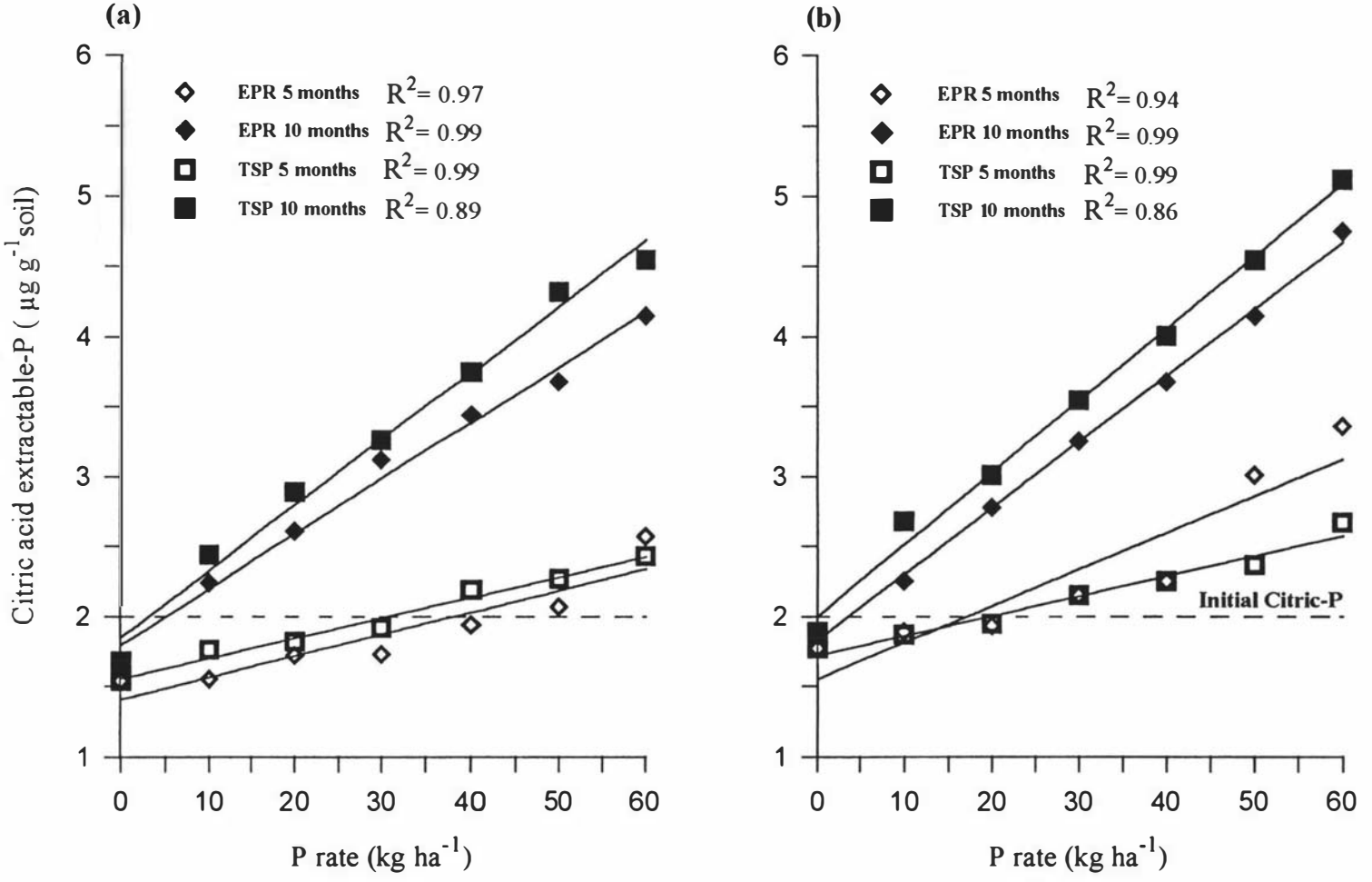

Figure 7.12 Effect of EPR and TSP fertiliser rates on Citric acid extractable-P in soil (a) with and (b) without tea plants.

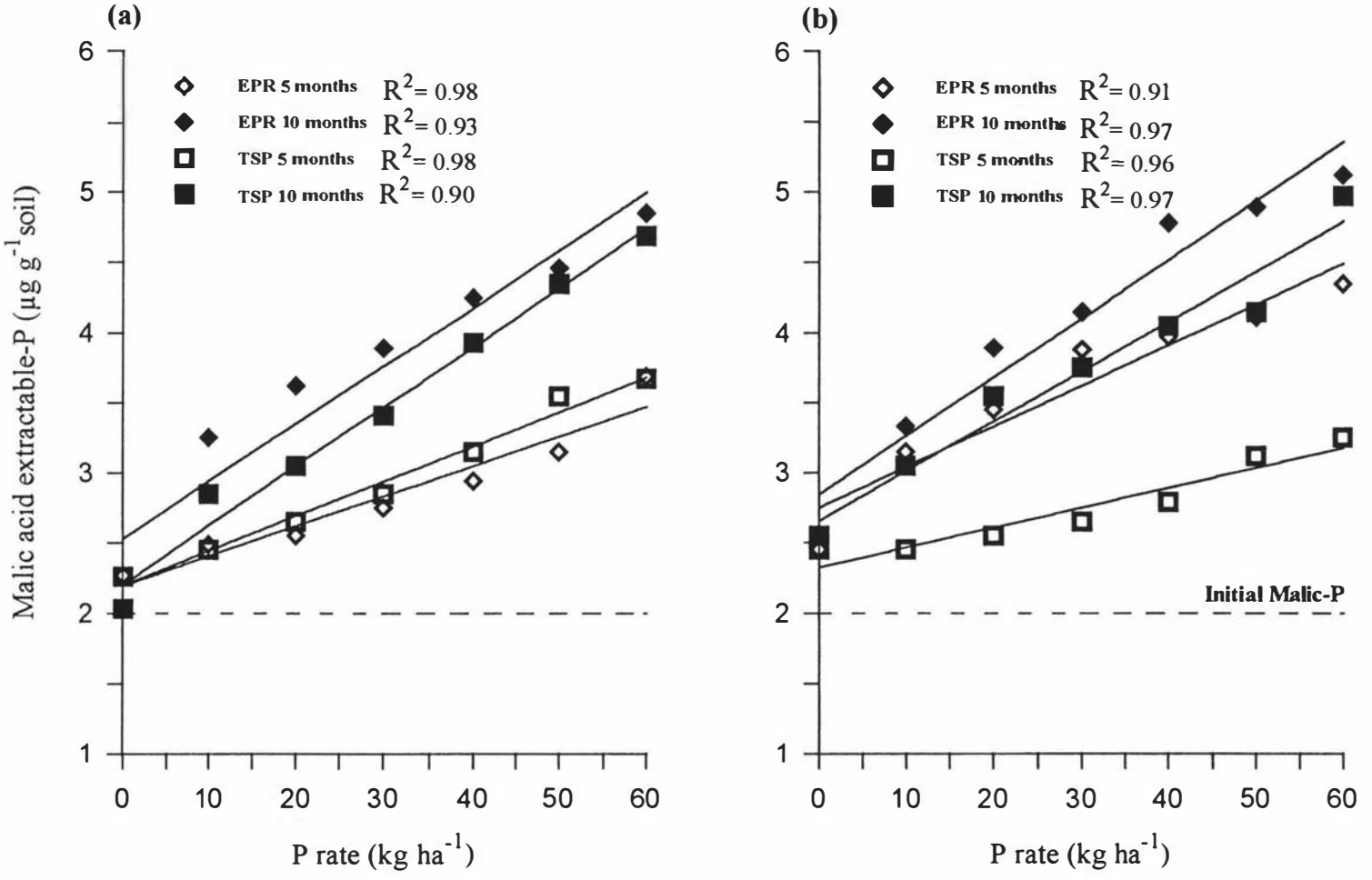

Figure 7.13 Effect of EPR and TSP fertiliser rates on Malic acid extractable-P in soil (a) with and (b) without tea plants.

compared to those at 5 months could be due to the higher amounts of NaOH-P_o at 10 months (Figure 7.6). Tambunan (1992) observed that applications of NCPR and TSP to an acidic moist Ultisol of Indonesia at rates of 140 kg P ha^{-1} upto 560 kg P ha^{-1} increased resin, Bray-1 and Olsen extractable P.

In my trial the extractable P values generally increased in the order of Olsen > borax > resin and Bray-1 > citric and malic acid. Olsen P values (35-100 μg g^{-1} soil) in the soils used in this study are very high compared to those in other tropical soils (El Swaify et al., 1985; Loganathan et al., 1982), because of the high rates of previous P applications which have raised total soil P in tea soils (mean of 616 μg g^{-1} soil, Golden et al., 1981). In contrast to tea soils, the coconut growing soils in Sri Lanka have been reported to have low amounts of total P (37 - 338 μg g^{-1} soil with a mean of 140 μg g^{-1} soil), because the latter soils have not been regularly fertilised (Loganathan et al., 1982). Olsen P concentrations in the coconut soils of Sri Lanka were reported to be an average of 2 μg g^{-1} soil (Loganathan et al., 1982), which was much lower than the values obtained in this study (Figure 7.9).

The Olsen test produced higher soil test P values than the acidic extractants, Bray-1, citric and malic acids because the Olsen method extracts both Fe and Al bound P (NaOH-P_i) and organic P, whereas the acid extractants dissolve mostly Al-P (Le Mare, 1991) or Ca-P. The other reasons for the higher P values with the Olsen extraction were due to the reduction of P fixation at the high equilibrium pH (pH 8.5) (Barrow, 1984) and desorption of fixed-P (Fe and Al bound P) by ligand exchange with HCO_3^-. The difference between the Olsen P values and those obtained by other methods was more marked, because the soil used in this study had much higher Fe-P values than Al-P values (Fe-P of 169 μg g^{-1} soil vs Al-P of 59 μg g^{-1} soil; Golden et al., 1981). The soils also had large amounts of organic P (110 - 130 μg g^{-1} soil, Figure 7.6) and it is possible that part of this organic-P (labile pool of organic-P) may have dissolved in the alkaline $NaHCO_3$ exatractant resulting in higher Olsen-test values. The lower time (5 mins) of extraction may be another reason for the low Bray-1 P values in this high P-fixing soil. Saggar et al. (1995) also showed that Bray-1 extractant gave very low recoveries of added P in a high P-fixing New Zealand soil

(Dystrandept, 4.2 μg g^{-1} soil) compared to that in a low P-fixing soil (Haplohumult, 75.5 μg g^{-1} soil) when the soils were treated with monocalcium phosphate.

The acid extractant borax dissolved greater amounts of P from all soils than the other acid extractants. The reason for higher borax extractable P values compared to the other acidic extractants (Bray-1, malic acid and citric acid) may be that the tetra borate ion ($B_4O_7^{2-}$) is more effective in extracting fixed P because of the strong energy of adsorption of $B_4O_7^{2-}$ anion to Fe and Al oxides in soils. In addition in EPR fertilised soils the pH (pH 1.5) of borax will cause increased dissolution and extraction of P from dissolved EPR residues. This may be the reason why the borax test extracted more P from EPR treated soils than TSP treated soils (Figure 7.10).

7.4.5 Effect of P fertilisers on growth and P uptake of tea plants

The application of P fertilisers increased shoot dry matter yield and total P uptake in tea plants compared to the control treatment (Table 7.5). The P rates beyond 20 kg P ha^{-1} did not significantly improve shoot dry matter yield nor shoot P uptake in any of the two P fertiliser treatments at both sampling times. There was no significant difference in shoot dry matter yield between the two forms of P fertilisers. This shows that EPR is able to dissolve at a rate sufficient to provide amounts of plant-available P equivalent to that from the completely soluble TSP fertilisers. Tambunan (1992) also found that NCPR was more effective than TSP in increasing maize yields in an acidic Ultisol from Indonesia with higher moisture content compared to soils in a dry area because soils with high moisture increased NCPR dissolution. Similar results have been reported for acid soils in many parts of the world and now there is ample evidence to support the claim that PRs are as effective as soluble P fertilisers to maintain P requirements for many crops provided the soils have conditions favourable to PR dissolution (Alston and Chin, 1974; Rajan and Gallingham, 1986; Tambunan, 1992).

Increases in shoot dry weight and P uptake in response to P fertiliser application fitted very well to Mitscherlich-type equations (Equation 7.2; Figure 7.14 and 7.15). Using

Table 7.5 Effect of EPR and TSP fertilisers and their rates on shoot dry matter yield and P uptake

	After 5 months						After 10 months					
Rate of P kg P ha^{-1}	Shoot Dry matter yield[1] ($g\ pot^{-1}$)		Leaf-P concentration[1,2] (%)		Shoot Plant P uptake[1] ($mg\ P\ pot^{-1}$)		Shoot Dry matter yield[1] ($g\ pot^{-1}$)		Leaf-P concentration[1,2] (%)		Shoot Plant P uptake[1] ($mg\ P\ pot^{-1}$)	
	EPR	TSP	EPR	TSP	EPR	TSP	EPR	TSP	EPR	TSP	EPR	TSP
0	31.85a	31.85a	0.178a	0.178a	34.28a	34.28a	40.45a	40.45a	0.177a	0.177a	43.73a	43.73a
10	35.03b	33.30ab	0.183a	0.183a	39.48b	38.27a	44.74ab	43.36a	0.182a	0.184a	50.22b	50.03b
20	35.70b	33.95ab	0.187b	0.186a	43.26b	41.22b	46.21b	44.20ab	0.186a	0.187a	52.94bc	52.32bc
30	35.88b	35.88b	0.198c	0.196b	45.21bc	44.89bc	48.31b	46.22b	0.197b	0.195b	57.94c	55.30bc
40	36.11b	35.54b	0.200c	0.200b	45.82bc	44.89bc	47.38b	45.94b	0.201b	0.199b	56.32c	57.82c
50	35.99b	35.62b	0.199c	0.200b	45.51bc	43.99bc	47.99b	45.63b	0.199b	0.201b	56.63c	56.36c
60	36.46b	35.50b	0.202c	0.199b	47.19bc	43.88bc	48.09b	45.33b	0.201b	0.198b	62.03d	55.84bc
Lsd[3] ($p < 0.05$)	NS		NS		NS		NS		NS		NS	

[1] Values representing the same letters in columns are not significantly different at $p < 0.05$ by DMRT test

[2] First mature leaf P concentration

[3] For comparison of EPR and TSP

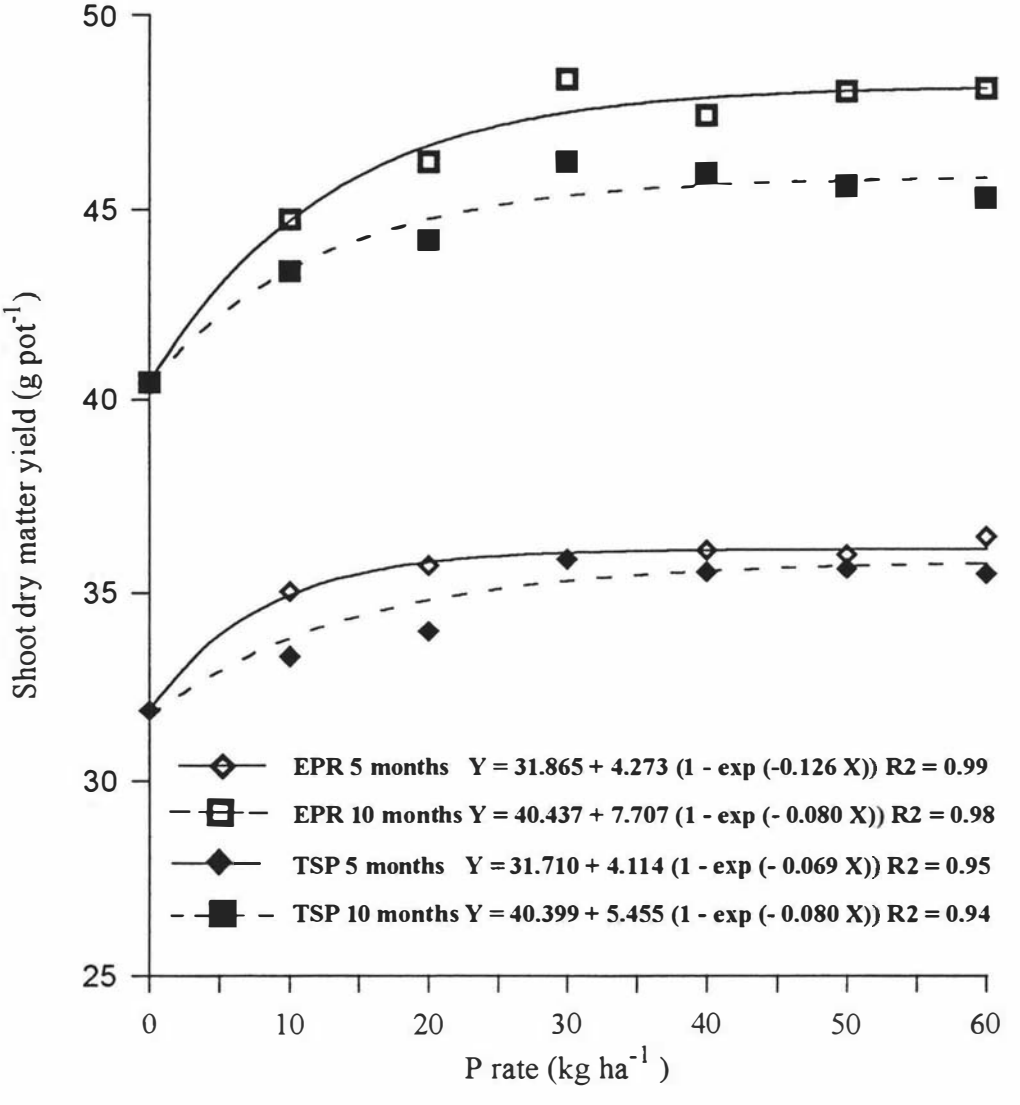

Figure 7.14 Relationship between shoot dry matter yield and the rates of EPR and TSP at 5 and 10 months after application to the soil

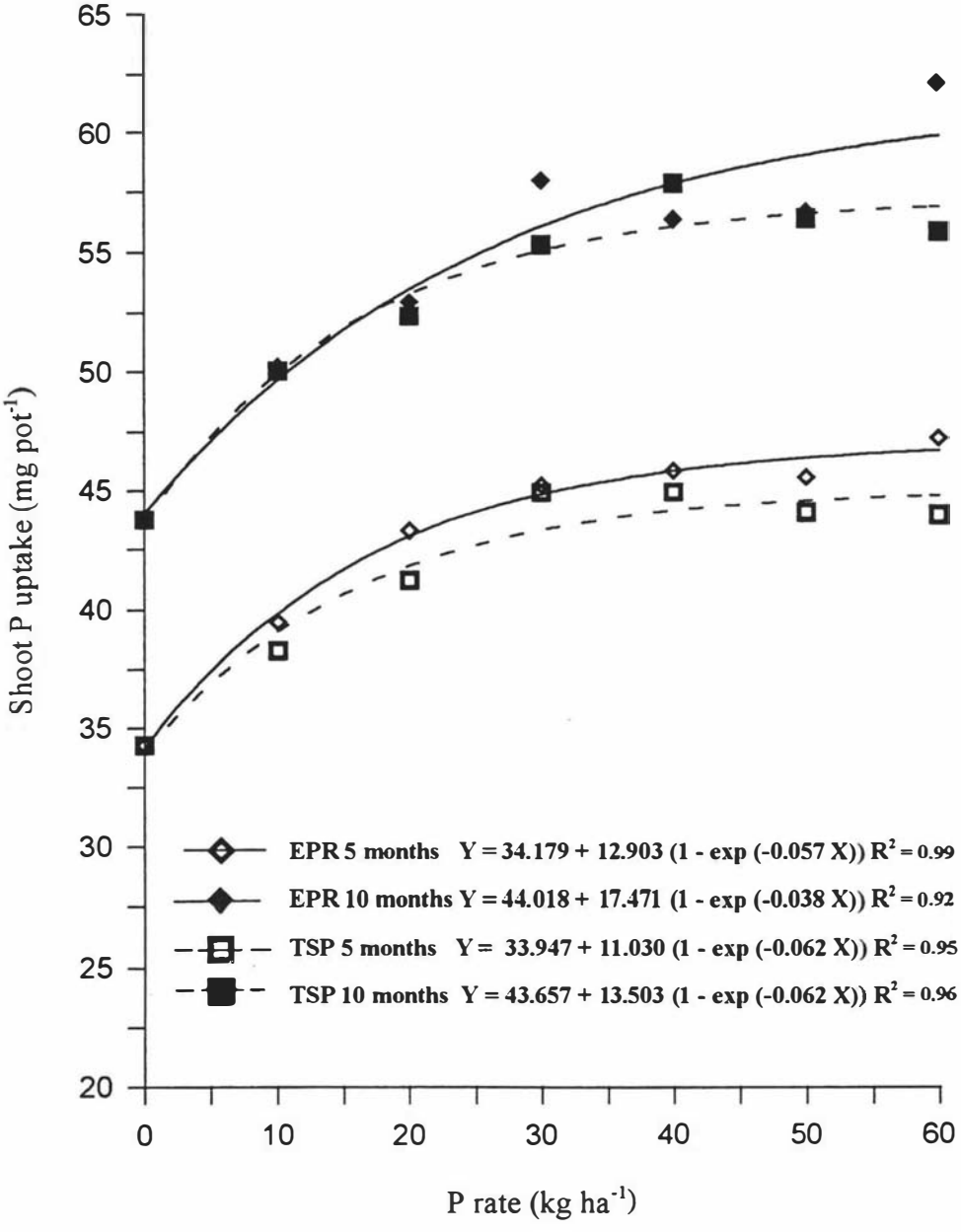

Figure 7.15 Relationship between shoot P uptake and the rates of EPR and TSP at 5 and 10 months after application to the soil

these fitted equations, the P requirement to achieve 95% of the maximum yield was calculated for both forms of P fertilisers and presented in Table 7.6. The estimated P requirement values were very low (7 - 14 kg ha^{-1}) indicating that the experimental soil is only marginally deficient in P and the response to P application was obtained only at the very lowest rate and then it diminishes to show no response at all. Sivasubramaniam et al. (1981) compared an imported phosphate rock (saphos phosphate) with EPR at rates of 0 and 15 kg P ha^{-1} in a glasshouse trial on tea and found that there was no significant yield increase due to the application of any of the P fertilisers compared to the control treatment and therefore they were not able to compare the relative agronomic effectiveness of the two P fertilisers. But they observed that malic-acid extractable P was greater in the EPR treated soil compared with the imported phosphate rock treatment and this made them to conclude that EPR is as good as the imported phosphate rock for fertilising tea. They used malic acid as the extractant because tea roots were found to excrete large quantities of malic acid (Jayman and Sivasubramaniam, 1975; Xiaoping, 1994).

7.4.6 Relative agronomic effectiveness (RAE) of EPR

The RAE of P fertilisers with different degrees of solubility may vary with crop species. In general, the RAE of PRs with respect to water-soluble P sources is expected to be higher for long-term or perennial crops than for short-term or annual food crops (Chien et al., 1990ab). Consistent with this, PRs have been used extensively for tree crops in Asia, particularly in Sri Lanka for tea (Wickremasinghe and Krishnapillai, 1986), rubber (Peris, 1970) and coconut (Loganathan, 1978) and for rubber and oil palm in Malaysia (Chien and Menon, 1995).

In this trial, the RAE values calculated for dry matter yield, using EPR as the test P-source relative to TSP (standard P fertiliser) are given in Table 7.7. The RAE values of EPR were calculated using three methods (Equation 7.1, 7.4 and 7.5). The results obtained using 7.1 and 7.4 show that in general the agronomic effectiveness of EPR is equal to or higher than TSP. At the 10 month sampling the RAE values were much higher compared to the 5 month sampling. The possible reason for the higher RAE of

Table 7.6 Calculated P requirement for 95% of maximum dry matter yield

P fertiliser	Growth period (months)	Growth response curves (Y - yield, X - P rate)	Calculated P requirement for 95% of the maximum yield (kg ha^{-1})
EPR	5	$Y = 31.865 + 4.273 (1 - \exp (- 0.126 X))$	7
	10	$Y = 40.437 + 7.707 (1 - \exp (- 0.080 X))$	14
TSP	5	$Y = 31.710 + 4.114 (1 - \exp (- 0.069 X))$	12
	10	$Y = 40.399 + 5.455 (1 - \exp (- 0.080 X))$	11

Table 7.7 The agronomic effectiveness of EPR relative to TSP calculated from empirical relationships

Equation used for the calculation	Rate of P kg ha^{-1}	Dry matter yield		Plant P uptake	
		5 months	10 months	5 months	10 months
7.1	all	122%	143%	118%	112%
7.4	10	149%	141%	110%	88%
	20	128%	141%	112%	97%
	30	116%	141%	113%	104%
	40	110%	141%	114%	110%
	50	106%	141%	115%	115%
	60	105%	141%	116%	119%
7.5	all	189%	141%	107%	79%

EPR relative to TSP may be due to a continuous supply of P by EPR through dissolution to meet the plant P requirement. Whereas TSP, dissolves within a short time and the dissolved P is immobilised into organic or inorganic soil P fractions with a lower P availability to tea.

Tambunan (1992) reported similar results, for field trials on a maize crop in Indonesia. The RAE of residues of NCPR applied seven months before planting maize was 26% more effective than TSP residues in a moist Ultisol at Sembawa, but 42% less effective on a dry Ultisol at Sarong. He further stated that at Sembawe the RAE of freshly applied TSP was higher than freshly applied PR, but its effectiveness decreased progressively with increasing time and became lower than that of PR after seven months. Utomo (1995) compared a locally available phosphate rock from Lamongan, East Java (total P 14.4% and 2% citric acid extractable-P 5.8%) with TSP on corn (*Zea mays* L.) growth under glasshouse conditions using an Ultisoil (pH 4.6 in water) from Sumatra and reported that the RAE of PR is almost equal to that of the TSP fertiliser. Zaharah and Sharifuddin (1995) compared the RAE of NCPR and an unreactive Chinese phosphate rock (CPR) with TSP consecutively for four cultivations of corn on an acid soil (pH 4.99 in water) of the Tebok series (Typic Kandiudult) in Malaysia. They found that the agronomic effectiveness of NCPR was equal to TSP from the second harvest and for the CPR at the third and fouth harvests.

In my experiments, the RAE values obtained using Equation 7.5 were highly variable and higher at 5 months growth than at 10 months. In Equation 7.5, RAE is calculated from the ratio of the initial slopes of the response curves (Saggar et al., 1993). Because the yield or P uptake response to P fertilisers in this study is very low, the initial slopes are low and therefore the RAE calculated from the ratios of these slopes will lead to greater errors. This may be the reason for the highly variable results obtained using this equation.

7.4.7 Relationship between dry matter yield and P uptake by plants

A Logistic model (Equation 7.6) was fitted to the data relating dry matter yield to plant P uptake at each sampling time (5 and 10 months after P fertiliser application) (Figure 7.16).

The logistic model is a sigmoid (growth) type function, which can be described by the following equation:

$$Y = a/(1 + exp(b - cX)) \quad [7.6]$$

where Y is the shoot dry matter yield and X is the plant P uptake, a is the maximum shoot dry matter yield (upper asymptote) when the plant P uptake is not limiting and c is related to the rate parameter, a higher c value indicates a rapid rise of the function between the lower and upper asymptote and b/c defines as the value of X at the point of inflection (Causton and Venus, 1981).

The models gave good fits to data at both sampling times with $R^2 = 0.97$ and 0.88 showing that the variability in the yield is satisfactorily explained by the model. This relationship provides an estimate of the internal efficiency of P use within the plant. The curvilinear relationship shows that the internal efficiency of P utilisation by tea plants is lower at high amounts of P uptake. It indicates that the amount of dry matter produced per unit of absorbed P diminishes with increasing levels of plant P uptake and this could be attributed to limitation by other factors affecting dry matter yield.

7.4.8 Relationship between dry matter yield and leaf P concentration

The shoot dry matter yield of tea plants harvested at 10 months was regressed against P concentration in the first mature leaf using a logistic type function (Figure 7.17). The first mature leaf is the one with the axil from which pluckable shoots emerge.

The rationale for taking the P concentration in the first mature leaf of tea as a diagnostic tool is that the mineral composition of this leaf varied least under the

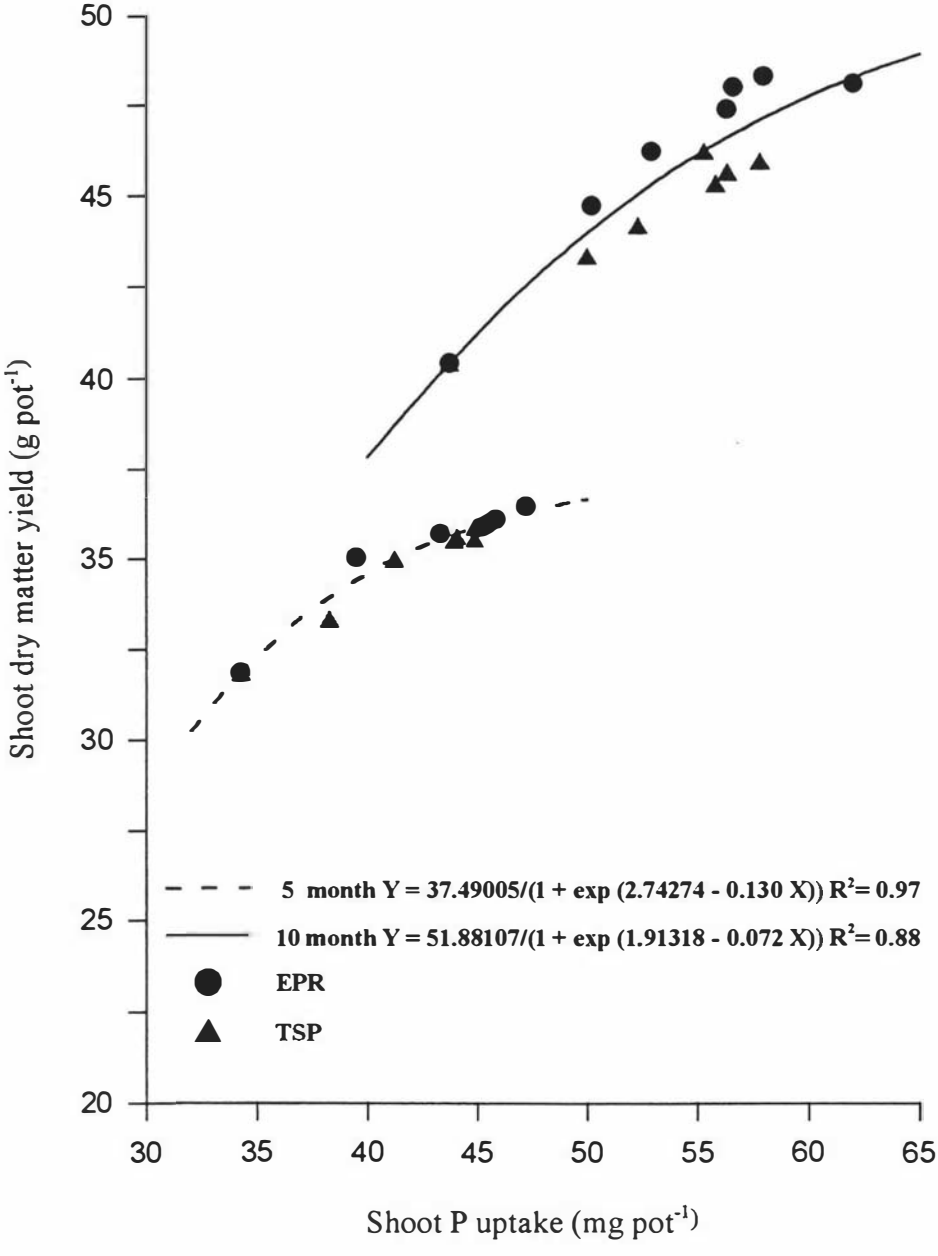

Figure 7.16 Relationship between shoot dry matter yield and P uptake by tea plants at 5 and 10 months after P fertiliser application

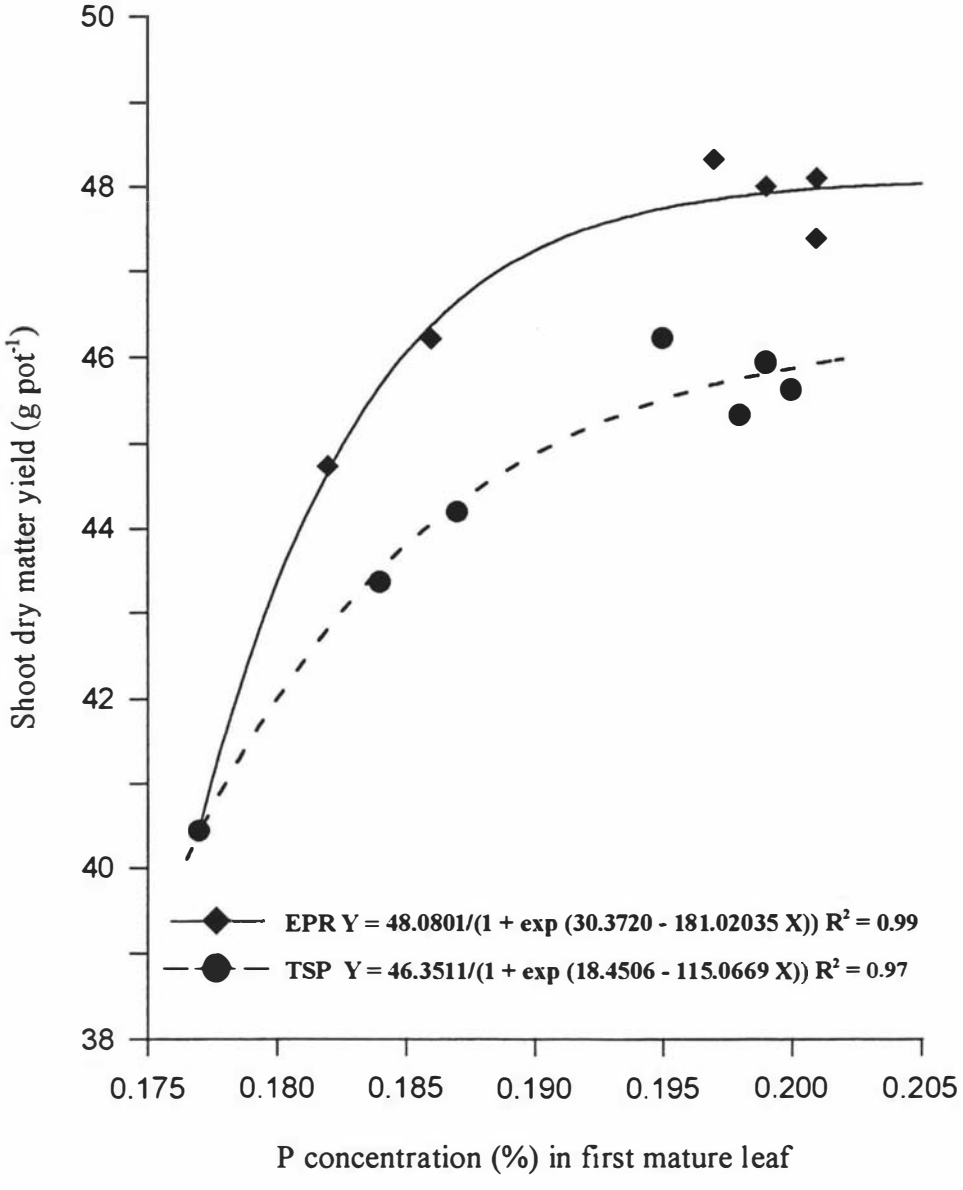

Figure 7.17 Relationship between shoot dry matter yield and P concentration in the first mature leaf for tea plants treated with EPR and TSP fertiliser

influence of changing environmental conditions (Hasselo, 1965). Wilson (1969) found that the chemical analysis of the bud or the third leaf provides a better index of the fertiliser requirements in tea plants. The validity of this claim was tested by Sivasubramaniam and Jayman (1976) and they showed that the analyses of the first mature leaf, rather than the third leaf or bud would be a more reliable index of the nutrient needs of tea plants. Hasselo (1965) also confirmed that the first mature leaf provided a better index of the nutritional status of tea plants than the older or younger leaves grown under a wide range of environmental conditions.

The shoot dry matter yield as a response to leaf-P concentration (Figure 7.17) showed very good fits to Equation 7.6 (R^2 = 0.99 and 0.97). The concentration of P (%) in the first mature leaf that was required to obtain 95% of the maximum dry matter yield was calculated from the regression equations and found to be 0.185% for EPR treatment and 0.186% for TSP treatment. These values agrees well with the 0.2% P value reported by Jayman and Sivasubramaniam (1980) for good tea growth.

7.4.9 Relationship between soil extractable-P and dry matter yield

The shoot dry matter yield harvested at 10 months was regressed against the corresponding soil P test values determined by different chemical reagents (Table 7.2) using a Mitschelich-type equation (Equation 7.2). The plots for resin-P, Olsen-P, borax-P, Bray-1, citric acid extractable P and malic acid extractable P are presented in Figures 7.18, 7.19, 7.20, 7.21, 7.22 and 7.23 respectively. The R^2 values for all plots were very high showing that any one of these soil tests can be used to predict P availability to tea. This is because all these extractants themselves are highly correlated whether all (Table 7.8) or only low rates of P application (Table 7.9, range where 95% of maximum yield was obtained) were considered.

For each soil test, critical soil P levels associated with 95% of the maximum dry matter yield (g pot^{-1}) were calculated using the regression equations (Table 7.10). These critical values of soil P were found to vary highly between the type of

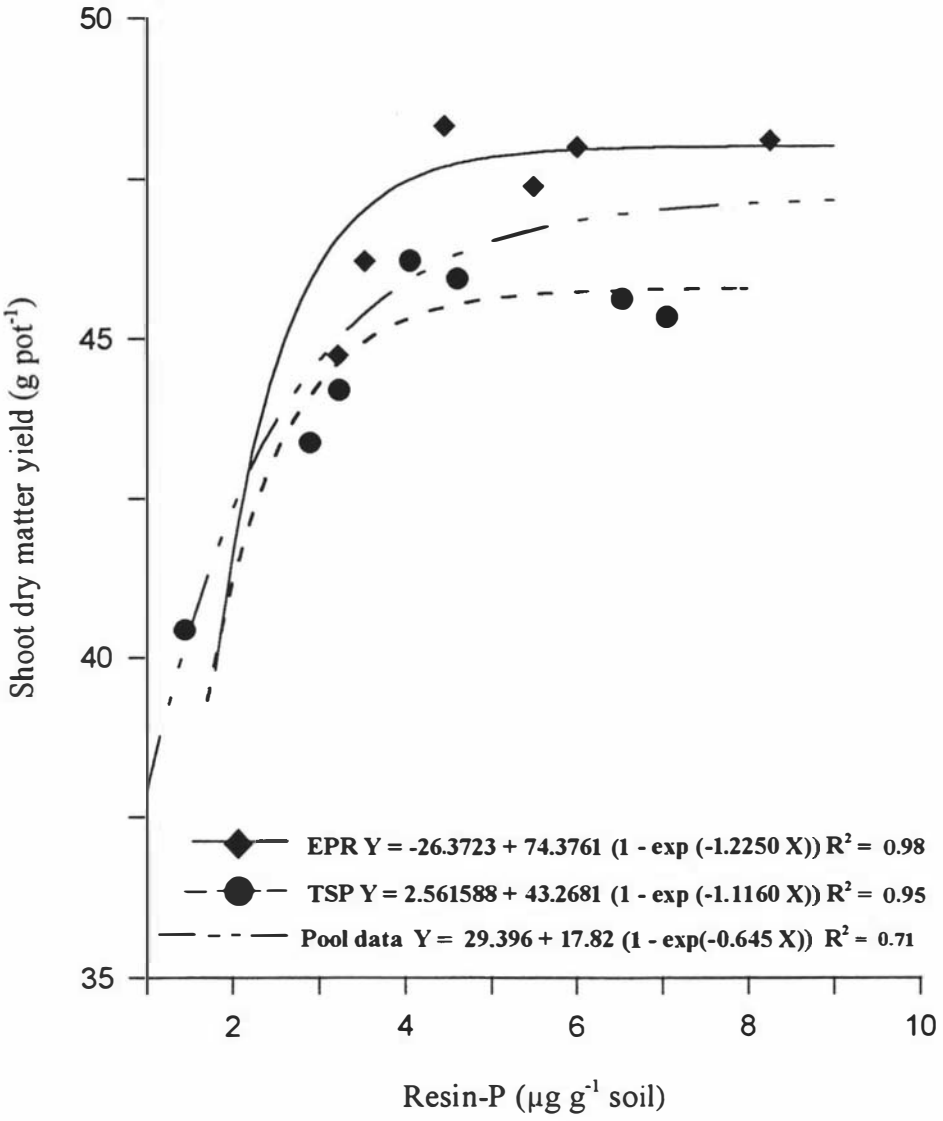

Figure 7.18 Relationship between shoot dry matter yield and resin-P for tea plants treated with EPR and TSP fertiliser.

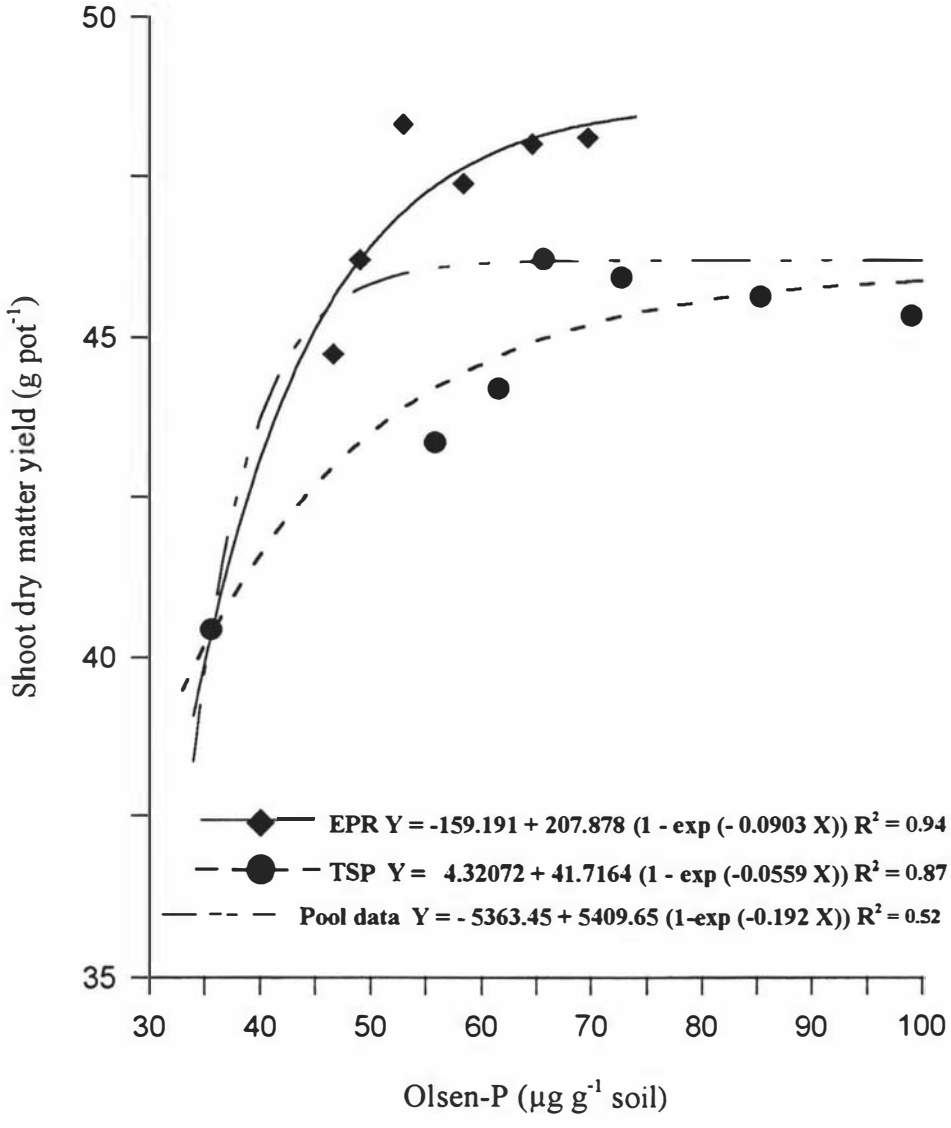

Figure 7.19 Relationship between shoot dry matter yield and Olsen-P for tea plants treated with EPR and TSP fertiliser

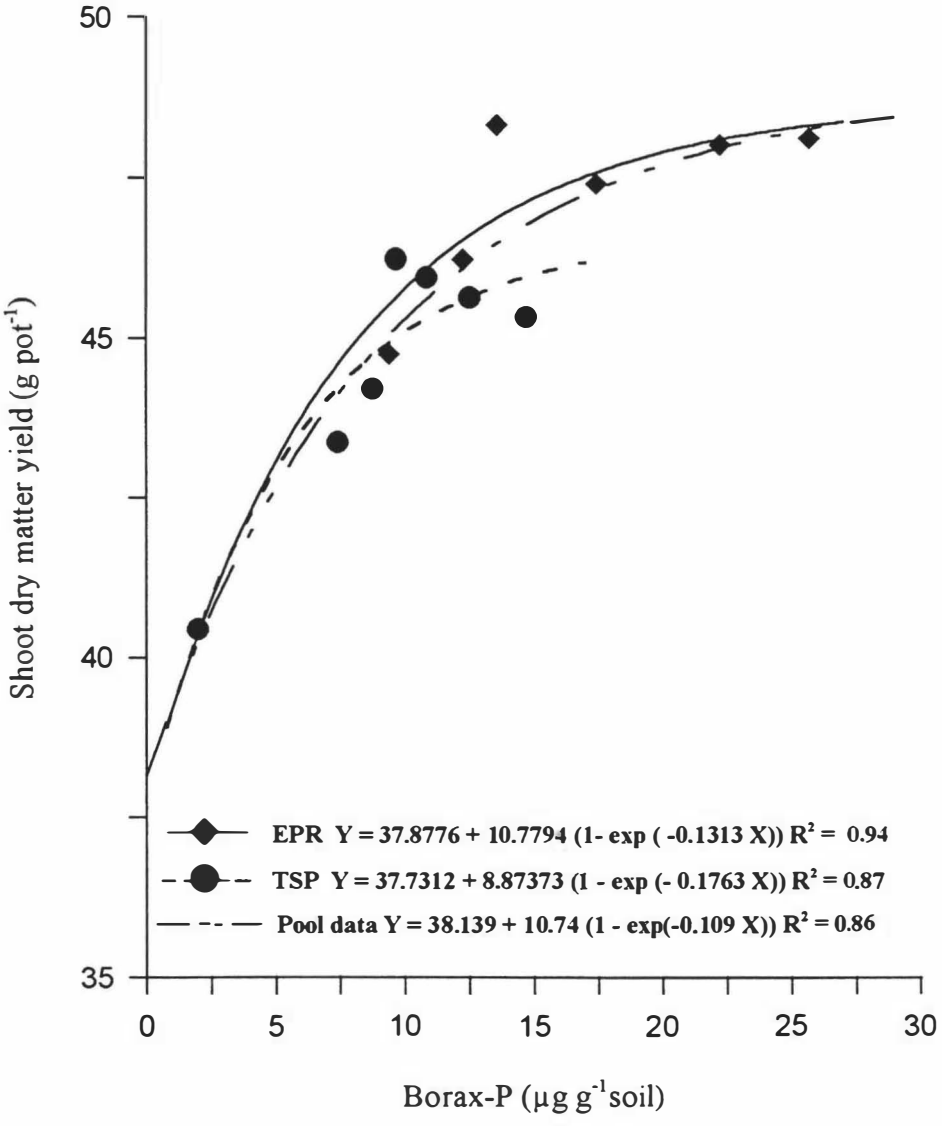

Figure 7.20 Relationship between shoot dry matter yield and borax-P for tea plants treated with EPR and TSP fertiliser

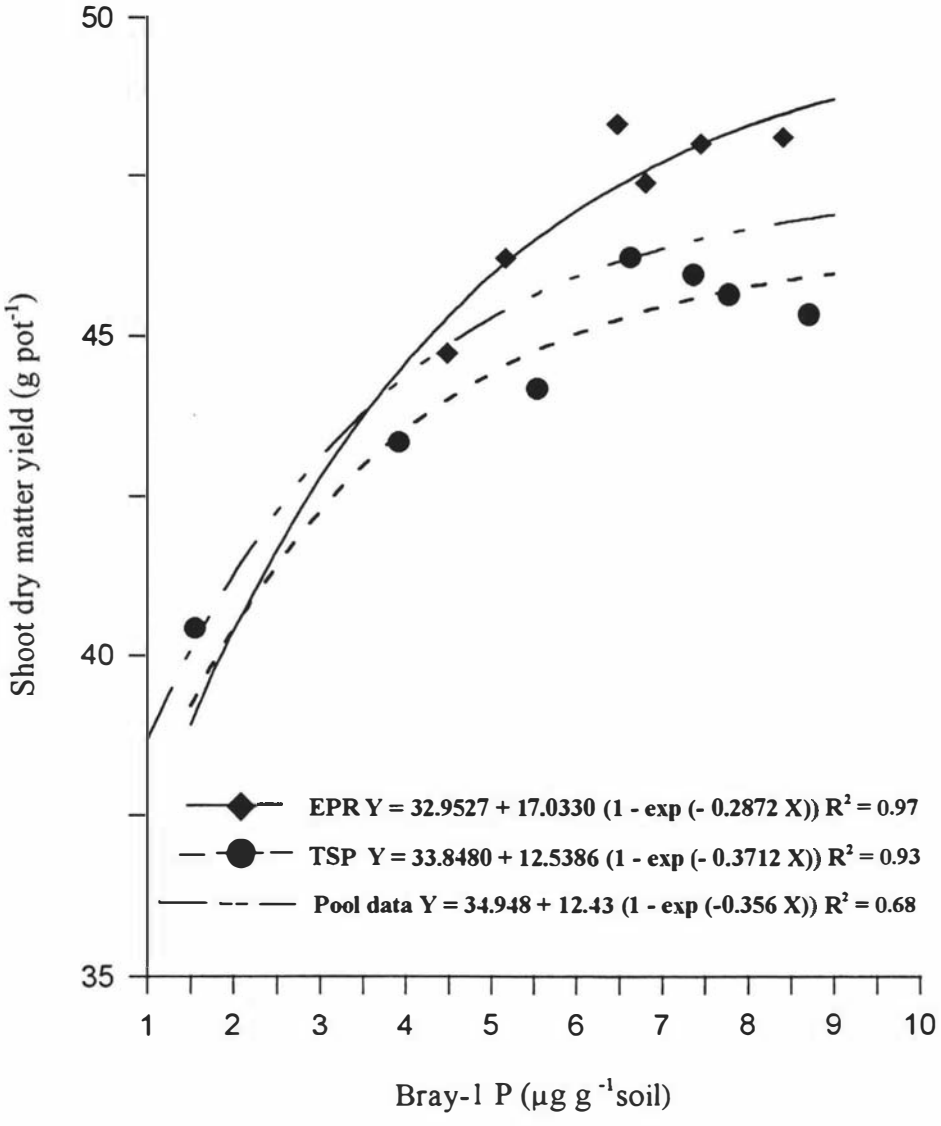

Figure 7.21 Relationship between shoot dry matter yield and Bray-1 P for tea plants treated with EPR and TSP fertiliser

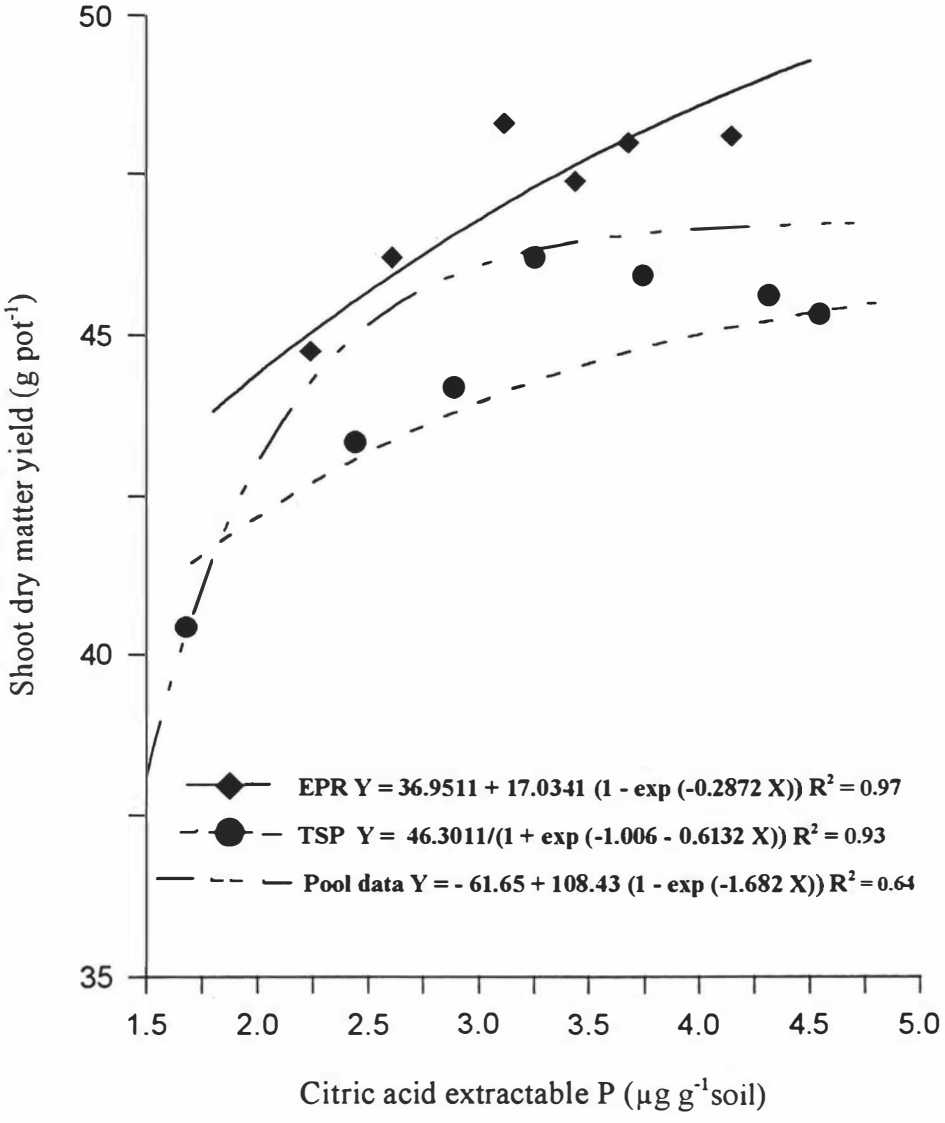

Figure 7.22 Relationship between shoot dry matter yield and citric acid extractable P for tea plants treated with EPR and TSP fertiliser

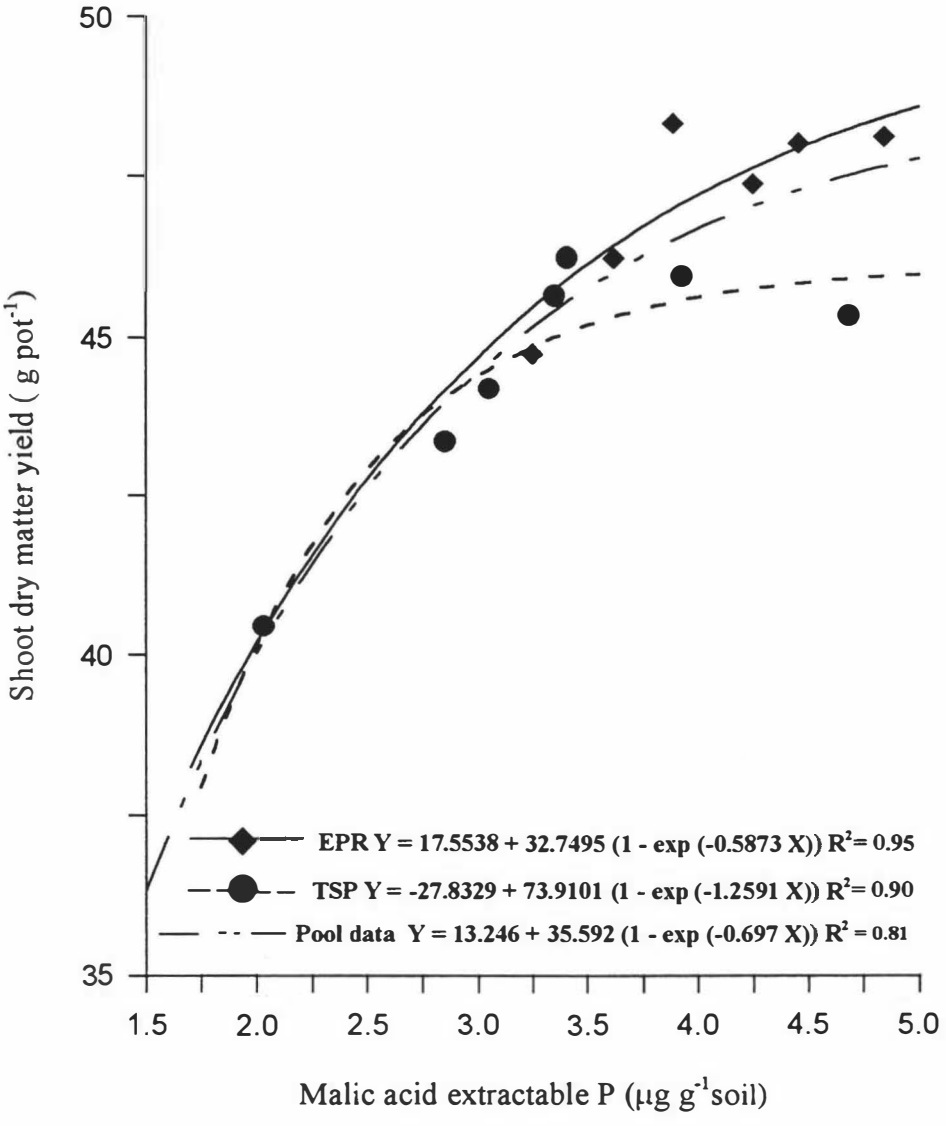

Figure 7.23 Relationship between shoot dry matter yield and Malic acid extractable P for tea plants treated with EPR and TSP fertiliser

Table 7.8 Correlation matrix for P extraction methods for all rates of (a) EPR and (b) TSP fertilisers

	(a)					
	Resin	**Bray**	**Borax**	**Citric-acid**	**Malic-acid**	**Olsen**
Resin	1					
Bray	0.957*	1				
Borax	0.984**	0.965*	1			
Citric-acid	0.981**	0.973**	0.984**	1		
Malic-acid	0.961*	0.996***	0.973**	0.972**	1	
Olsen	0.987**	0.968**	0.998***	0.999***	0.973**	1
			(b)			
Resin	1					
Bray	0.918*	1				
Borax	0.936*	0.979**	1			
Citric- acid	0.977**	0.977**	0.973**	1		
Malic-acid	0.979**	0.969**	0.976**	0.996***	1	
Olsen	0.975**	0.955*	0.985**	0.981**	0.987**	1

* Correlation coefficient significant at p = 0.05

** Correlation coefficient significant at p = 0.01

*** Correlation coefficient significant at p = 0.001

Table 7.9 Correlation matrix for P extraction methods for rates 0, 10 and 20 kg P ha^{-1} of (a) EPR and (b) TSP fertilisers

	(a)					
	Resin	**Bray**	**Borax**	**Citric -acid**	**Malic-acid**	**Olsen**
Resin	1					
Bray	0.998***	1				
Borax	0.988**	0.995***	1			
Citric-acid	0.957*	0.970**	0.989**	1		
Malic-acid	0.995**	0.998***	0.998***	0.981**	1	
Olsen	0.998***	0.999***	0.994***	0.970**	0.998***	1
	(b)					
Resin	1					
Bray	0.980**	1				
Borax	0.998***	0.968**	1			
Citric-acid	0.989**	0.998***	0.980**	1		
Malic-acid	0.998***	0.970**	0.999***	0.981**	1	
Olsen	0.999***	0.972**	0.999***	0.983**	0.999***	1

* Correlation coefficient significant at p = 0.05

** Correlation coefficient significant at p = 0.01

*** Correlation coefficient significant at p = 0.001

Table 7.10 The critical levels of plant available-P required to be in the soil to obtain 95% of the maximum dry matter yield in tea plants

Soil P-test	R^2 values of the model			Critical levels of soil P (μg g^{-1} soil)		
	EPR	**TSP**	**EPR + TSP**	**EPR**	**TSP**	**Pool data**
Resin	0.95	0.90	0.71	3	3	3
Olsen	0.94	0.86	0.52	49	52	40
Borax	0.93	0.86	0.86	11	8	13
Bray-1	0.96	0.97	0.68	7	5	5
Citric-acid	0.98	0.91	0.64	6	3	2
Malic-acid	0.94	0.88	0.81	4	3	4

extractant used, but did not differ greatly between the two fertiliser treatments for any of the extractants.

Generally P tests for diagnostic purposes are designed with several aims. An ideal test should be simple enough for routine application, extract a sufficient amount of P to be easily measurable, extract sufficient P to represent a significant portion of the potential plant uptake and it should not extract significant amounts of P that are not plant available (Fixen and Grove, 1990). Additionally a good P extractant should be able to provide values within a reasonable range, when soils are treated with different P sources. Saggar et al. (1992b) showed that the Olsen extractant was good for predicting P availability to pasture in soils treated with soluble P fertiliser, but not in soils treated with sparingly soluble P fertilisers, whereas resin-P was able to predict P availability regardless of the type of P fertilisers used.

The R^2 values for the relationship between tea shoot dry matter yield and soil test values were higher for the borax (R^2 = 0.86) and malic acid (R^2 = 0.81) extractants, when the data for EPR and TSP are pooled and analysed (Table 7.10). Therefore, for these two extractants a single calibration curve could be used for predicting P availability to tea in soils treated with EPR or TSP (Figure 7.20 and Figure 7.23). For the other extractants two calibration curves are required depending on whether EPR or TSP was used (Figure 7.18, 7.19, 7.21 and 7.22). The possibility to use a single calibration curve for both soluble and sparingly soluble P fertiliser forms in the borax test is an advantage for the analyst, because it has no influence on the tea growers preference of the form of P fertiliser used for the plantation and one calibration curve can be used when the history of P fertiliser source is unknown.

Between the borax and malic acid tests, which showed the highest R^2 values for the combined data for EPR and TSP treated soils, the borax method could be considered as the most suitable test for routine P analysis, because in addition to giving the highest R^2 values of all the extractants it provided reasonably high P values with an acceptable range to distinguish between sufficiency and deficiency of P levels in the soil. The critical borax P concentration of 13 μg P g^{-1} soil obtained in this study agrees very well with the value of 10 - 15 μg P g^{-1} soil reported by Jayman and

Sivasubramaniam (1980) as an adequate P concentration for mature tea growth in the field. Figure 7.20 also shows that for maximum tea growth, the borax P concentration is about 15 μg P g^{-1} soil.

The Olsen test though needs two calibration curves depending on the P fertilisers used, gave very high critical soil P values and therefore it can be easily measured, so soils deficient in or with sufficient P can be distinguished. As PR is the only P fertiliser used in many tea estates and likely to be used in the future too the Olsen calibration curve for PR (Figure 7.19) can be used to assess the P availability in these soils.

Irrespective of the type of P fertiliser used, the soil P concentration required to achieve 95% of the maximum shoot dry matter yield was found to be at the very beginning of the response curve in this study because initial soil P levels were high due to previous P fertiliser applications. Therefore it was not possible to classify soil test values according to a wide range of P availability classes (low, medium and high etc.). It is recommended that further glasshouse and field trials be conducted on soils with low soil P concentrations to test the findings reported in this Chapter.

7.5 CONCLUSIONS

The results of the glasshouse trial showed that application of TSP or EPR fertiliser at the lowest rate of 10 kg P ha^{-1} tested is sufficient to obtain maximum tea yield in a previously well fertilised soil. The agronomic effectiveness of EPR was equal to or slightly better than that of TSP at the 5 and 10 month periods of the trial. This shows that even though EPR is considered as a non-reactive phosphate rock according to its citric acid solubility, it dissolves at a rate fast enough to supply the P needs of tea plants in the highly acidic and high P fixing Ultisols of Sri Lanka. Therefore the low-cost locally available EPR can be recommended for use as a P fertiliser for tea in Sri Lanka.

Transformation of P from EPR and TSP fertilisers into various soil P pools varied due to differences in their solubilities. The resin-P was higher in TSP treated soil at 5

months due to its greater solubility, but at 10 months EPR produced higher resin-P due to higher dissolution with time. In the presence of tea plants, at 5 months 52% of the P from EPR applied at the rate of 10 kg ha^{-1} dissolved compared to 75% P dissolution at 10 months. The NaOH-P_i (Fe + Al bound P) concentration was significantly higher in the TSP treated soil due to the rapid conversion of easily soluble P to P forms fixed by Fe and Al oxides. In EPR treated soils, the H_2SO_4-P_i (Ca-P) concentration was greater due to the recovery of undissolved PR in the soil by this extractant. In the unplanted soil the NaOH-P_o concentration increased with time due to increased microbial activity, but during the latter part of the trial the concentration of this fraction decreased in the presence of plants due to the mineralisation of P_o into P_i and the subsequent uptake by plants.

Soil P values obtained by all the soil tests were very highly correlated with tea yield, when the data for each of the two P fertilisers was considered seperately. When the data were combined, only borax and malic acid extractants showed high R^2 values. Of these two tests, borax test gave sufficiently high P values that can be measured easily, making it simple to distinguish between sufficiency and deficiency levels. Therefore the borax test is recommended as the most suitable test to predict P availability for tea. The Olsen test, which gave the highest soil test values, also can be used in tea plantations where only one type of P fertiliser (PR in most cases) has been used previously.

For wider extrapolation of the conclusions reached in this study further research is required using glasshouse and field trials with soils of lower P status than the soil used for this study.

CHAPTER 8

SUMMARY AND CONCLUSIONS

8.1 AN OVERVIEW

The literature review (Chapter 2) indicated that the amelioration of soil phosphorus (P) deficiency is an important part of managing tea (*Camellia sinensis* L.) nutrition in the highly weathered acidic soils (Ultisols) of humid and sub-humid tropics. Evidence derived from the management of tea in Sri Lankan soils indicated that the P fertiliser requirement of tea plant is low and in most instances in soils with relatively low inputs of P at between 10 - 15 kg P ha^{-1} yr^{-1} there was no response to further applications of P.

Considering the low P requirement in previously fertilised soils the use of locally available low-cost phosphate rock (PR) is an attractive source of P for tea compared to the expensive soluble P fertilisers because PRs are soluble in tea growing soils which have pHs <5.5 and rainfall >2000 mm. In Sri Lanka, a locally mined PR (Eppawala phosphate rock, EPR) is used to meet the plant's P requirements for most perennial crops including tea. The chemical characteristics of PRs and the conditions required for their dissolution were adequately discussed. Despite the favourable conditions available for the dissolution of EPR, the high acidity in these soils can cause applied fertiliser P to get converted into forms (Fe and Al bound P) that are more stable and relatively insoluble. These P forms have been traditionally considered to be not easily available to plants.

Amidst such complexities, it is striking to note that tea grows well with low P fertiliser inputs of both soluble and insoluble forms without showing any visual symptoms of P deficiency under field conditions. Literature speculates that the ability to use sparingly soluble P forms results from the secretion of significant amounts of organic acids (citric and malic acids) by tea roots. These have been reported to be

capable of dissolving PRs in soils in the vicinity of roots. Additionally these organic anions were reported to chelate Fe and Al and release P that are bound to them.

A large volume of literature discussing the differences in chemistry and biology of the rhizosphere of many plants compared to that of the bulk soil is now available. Little is known however, on the chemical changes and the mechanisms of P uptake in the rhizosphere of tree crops including tea plants. This is mainly due to a lack of dependable techniques for sampling soils in the rhizosphere zone. The literature on the techniques currently used to study rhizosphere processes was reviewed.

Much information is now available on the factors influencing the dissolution of PR in agricultural soils. However the information available on tea plants on this subject is very scanty. The lack of information on the rate of PR dissolution in tea soils, especially in the rhizosphere zone, hampers accurate recommendations of PR fertiliser additions for tea plantations without polluting soil and water bodies by indiscriminate use.

The first objective of this thesis was to develop a suitable technique to investigate rhizosphere processes in tree crops under glasshouse and field conditions in order to study root processes involved in the P nutrition of tea plants. This technique was subsequently used to study the rhizosphere processes in tea and other crops with diverse growth habits to test the differences in the mechanisms involved in P uptake. The genetic variability in tea clones and the effect of the form of applied nitrogen (NO_3^- or NH_4^+) on P utilisation from EPR by tea plants were also investigated. Finally the agronomic effectiveness of EPR was compared with that of triple superphosphate in a glasshouse trial with tea plants. The relative suitability of soil tests in predicting P availability to tea plants in soils treated with these fertilisers were also determined.

8.2 A TECHNIQUE FOR STUDYING RHIZOSPHERE PROCESSES IN TREE CROPS

A modification of the root study container (RSC) technique of Kuchenbuch and Jungk (1982) in combination with a sequential soil P fractionation technique was used to study rhizosphere processes of P depletion around fine roots of Camellia (*Camellia japonica* L.) plants which are of the same family as tea (*Camellia sinensis* L.). The trials were carried out under glasshouse and field conditions using a top soil sample of Dystric Eutrochrept from New Zealand treated with North Carolina phosphate rock (NCPR), monocalcium phosphate (MCP), single superphosphate (SSP) and diammonium phosphate (DAP). Both glasshouse and field trials gave similar results and provided useful information on the rhizosphere processes involved in P utilisation by camellia seedlings and mature trees.

The chemical properties in camellia rhizosphere differed significantly from that of the bulk soil. Camellia roots induced acidification in their rhizosphere. Plant induced acidification in the rhizosphere created conditions conducive for the dissolution of the sparingly soluble NCPR fertiliser. The chemical fractionation of soil P showed that the plants depleted the resin-P and NaOH-P_i fractions from the rhizosphere while accumulating NaOH-P_o. The accumulation of P_o indicated a transformation of more soluble forms of P_i into P_o due to the high microbial activity in the rhizosphere.

The RSC technique proved to be a viable aid to study the rhizosphere process in tree crops in the glasshouse as well as in the field.

8.3 PHOSPHORUS CHEMISTRY IN THE RHIZOSPHERE OF TEA AND ASSOCIATED CROPS

A glasshouse study was carried out on a top soil sample from a Rhodustult in Sri Lanka using the modified RSC technique to understand how tea (clone TRI 2025) plants differ in their ability to utilise P from EPR compared to other plant species grown in the same locality. Calliandra (*Calliandra calothyrsus* L.), a leguminous tree

grown in tea fields to provide shade and organic material, Guinea grass (*Panicum maximum* L.) found abundantly in sloping tea lands which helps control soil erosion, and bean (*Phaseolus vulgaris* L.) a common leguminous vegetable grown in the same soil, were used in this study.

All plant species acidified their rhizospheres. The magnitude of acidification differed among the plant species in the order of Guinea grass > bean and tea > calliandra. The highest acidification found in Guinea grass was due to the largest root surface area of this plant among the four plant species. Guinea grass however represented the lowest rate of acidification per unit surface area, whereas tea produced the highest rate of acidification per unit surface area. The rate of EPR dissolution in the rhizosphere followed the same order as that of the acidification.

All plant species depleted resin-P, NaOH-P_i in the rhizosphere. Except for tea all other species depleted NaOH-P_o in the rhizosphere. Tea plants like camellia plants accumulated NaOH-P_o in the rhizosphere probably due to increased microbial activity caused by a large supply of carbon exudates from tea roots.

Guinea grass and bean were found to be externally more P efficient to extract P from soil compared to the other two species because they had a larger root surface area. The internal efficiency which describes the plant's ability to convert absorbed P into dry matter was in the order of bean > Guinea grass > calliandra > tea.

8.4 CLONAL VARIABILITY IN P UTILISATION

Another glasshouse study was conducted on the same soil type as the one used in the first glasshouse trial in Sri Lanka to investigate the effects of clonal differences in tea on the utilisation of P from sparingly soluble EPR and soluble triple superphosphate (TSP) P fertilisers. The tea clones used were S 106, TRI 2023 and TRI 2025.

TRI 2023 and TRI 2025 produced significantly higher dry matter yield and P uptake than S 106 for both P treatments but there was no significant difference in P uptake by any of the clones between the two P fertilisers. The external P efficiency of TRI 2023

and TRI 2025 was higher than S 106 mainly due to greater root surface area and greater P uptake per unit surface area. The reason for the higher external efficiency in TRI 2023 compared to TRI 2025 was due to higher P uptake per unit surface area and not due to higher root surface area. The higher P uptake per unit surface area may be associated with higher root acidification and root exudation of organic compounds.

All tea clones acidified their rhizospheres compared to the bulk soil in both P fertiliser treatments. The rhizosphere pH decrease among the clones was in the order of TRI 2023 > TRI 2025 > S 106. As in the previous glasshouse study more EPR dissolved in the rhizosphere compared to the bulk soil and the amount of EPR dissolution in the rhizosphere was related to pH decrease. All three tea clones depleted resin-P and NaOH-P_i but accumulated NaOH-P_o in the rhizosphere as observed in the previous glasshouse study.

The results of this study showed that the RSC technique could be successfully used to provide information on the preliminary screening of clones for their P utilisation efficiencies.

8.5 EFFECT OF FORMS OF NITROGEN SUPPLY ON MOBILISATION OF P FROM EPR

Nitrogen is the major nutrient input to tea plantations because it is constantly removed through regular harvesting of young tea shoots at 4 - 10 day intervals. The form of N supply could have a profound effect on the uptake of other nutrients notably P through its influence on rhizosphere pH. A glasshouse study similar to the one described in section 8.3 was conducted to test the effect of N forms (NH_4^+, NO_3^- or both) on the availability of P from EPR applied to tea clone TRI 2025. Ammonium sulphate, $Ca(NO_3)_2$ and NH_4NO_3 were added to the soil to supply NH_4^+, NO_3^- and $NH_4^+ + NO_3^-$ respectively.

The rhizosphere pH decreased compared to the bulk soil when N was supplied as NH_4^+ or $NH_4^+ + NO_3^-$ forms, and it increased when N was supplied as NO_3^- form. The

estimations of cation-anion charge balance in the plants showed that the plants had taken-up more NO_3^- than NH_4^+ even in the $(NH_4)_2SO_4$ treated soil. This was explained as due to high nitrification in the soils inspite of the presence of a nitrification inhibitor. The rhizosphere acidification in soils treated with $(NH_4)_2SO_4$ was considered to be mainly due to nitrification.

The dissolution of EPR in the rhizosphere increased regardless of the N forms. The $(NH_4)_2SO_4$ treatment caused highest dissolution of EPR in the rhizosphere whereas the $Ca(NO_3)_2$ treatment showed the lowest. The degree of P dissolution was in agreement with the degree of acidification in the rhizosphere as observed in previous studies. The dissolution of EPR in the rhizosphere of tea treated with $Ca(NO_3)_2$ was higher than in the bulk soil even though the rhizosphere pHs were higher. This is probably due to the plant uptake of Ca and P, the reaction products of EPR dissolution. Sequential fractionation of soil P indicated that the $(NH_4)_2SO_4$ treatment caused the highest depletion of resin-P but lowest depletion of NaOH-P_i probably due to the fixation of P by the soil at the low pHs in the rhizosphere. The concentrations of resin-P and NaOH-P_i were lower and that of NaOH-P_o was higher in the rhizosphere than that of the bulk soil as observed in the earlier studies.

8.6 AGRONOMIC EFFECTIVENESS OF EPR ON TEA

Another glasshouse trial was conducted in Sri Lanka on the same soil type as in section 8.3 to compare the agronomic effectiveness of EPR with TSP on tea. The results showed that TSP or EPR fertiliser at a rate as low as 10 kg P ha^{-1} was sufficient to obtain maximum tea yield. The agronomic effectiveness of EPR was equal to or slightly better than that of TSP at the 5 and 10 month samplings of the trial. This is due to the high rate of EPR dissolution in the highly acidic and high P fixing Ultisols which supply adequate P to meet the P needs of the tea plants. The results showed that the low-cost locally available EPR can be used profitably as a P fertiliser for tea plantations in Sri Lanka.

The composition of the soil P fractions varied according to the type of P fertiliser, reaction time in soil and the presence or absence of plants in the soil. The amount of P dissolved from EPR and the amount of TSP converted into various soil P fractions varied due to differences in their solubilities as observed in the other glasshouse trials. The resin-P was higher in the TSP treated soil at 5 months due to its greater solubility but at 10 months the EPR produced higher resin-P due to its increased dissolution over time. In the presence of tea plants, 52% of P from the EPR applied at the rate of 10 kg ha^{-1} was dissolved at 5 months compared to 75% of dissolution at the 10 month sampling. The NaOH-P_i (loosely characterising Fe + Al bound P) concentration was significantly higher in the TSP treated soil due to rapid conversion of easily soluble P to P forms fixed to Fe and Al oxides. In the EPR treated soils, H_2SO_4-P_i (Ca-P) concentration was greater due to the recovery of undissolved EPR.

Tea yields were correlated against P extracted by various soil P tests. The Olsen test extracted the highest quantity of P from tea soils (35 to 100 μg P g^{-1} soil) compared to all other extractants tested (borax, Bray-1, citric acid and malic acid; extractable P values ranged from 2 to 30 μg P g^{-1} soil). The Olsen test extracted both Fe and Al bound P, resin-P and also some labile organic P whereas the other extractants removed resin-P and mainly Al-P from these soils which are rich in Fe-P. Soil P values obtained by all soil tests were very highly correlated with tea yield when the data for each of the P fertilisers was considered separately. All tests except borax and malic acid extractions required two calibration curves depending on whether EPR or TSP fertiliser was used. Therefore they cannot be used to predict soil P availability to tea in estates where both types of fertiliser have been used. Between borax and malic acid, the former gave sufficiently high P values which could be measured easily and distinguished between the sufficiency and deficiency levels. Therefore borax test is recommended as the most suitable soil test to predict P availability to tea. Olsen test can also be used in tea plantations where only one type of P fertiliser (PR in most cases) has been used previously.

8.7 FUTURE RESEARCH AND RECOMMENDATIONS

The clonal evaluation made in the utilisation of P from EPR and native soils which indicated that TRI 2023 and TRI 2025 had high P utilisation efficiency. This needs further testing in the field in different agro-ecological regions and soil types. It is proposed that plant breeders include P utilisation efficiency trait in the selection of tea clones for different regions of the country to obtain high tea yields with low P inputs.

In all trials, P_o accumulated in tea and camellia rhizosphere but it was depleted in the rhizosphere of other crops tested. The accumulation of P_o was explained as due to the immobilisation of P_i by the highly active microbial population in the tea rhizosphere. The depletion of P_o in other crops was explained to be due to the hydrolysis of P_o by the increase concentration of phosphatase enzyme in rhizosphere compared to the bulk soil. The reasons for the differences between tea and other crops in the mobilisation and immobilisation of P_i in rhizosphere needs further investigation.

The nitrification inhibitor used in the rhizosphere study testing the effect of different forms of N on rhizosphere acidification was not completely effective. This caused difficulties in the estimation of the ratios of uptake of NH_4^+ and NO_3^- by the tea plants. To avoid such difficulties, in future studies, it is recommended that the nitrification inhibitor be split applied to the soil several times during the period of investigation. Also it is appropriate to periodically monitor the concentrations of NH_4^+ and NO_3^- in the soil to determine the degree of nitrification, if any during the trial. Perhaps using ^{15}N labelled NH_4^+ and NO_3^- fertilisers in the presence of a nitrification inhibitor would be more appropriate.

Tea plants are known to secrete significant quantities of organic acids from their roots. The role of organic acids in rhizosphere acidification and PR dissolution is now well documented for many crops. However no literature is available on the relative contribution of organic acid exudation by roots and proton release caused by the plant's uneven uptake of cation and anions towards rhizosphere acidification. Further studies on this aspect are required.

The glasshouse study reported on Chapter 7 showed that on a high P status soil EPR is as effective as TSP in supplying P to young tea plants confirming the anecdotal evidence of planters alreadey using EPR. In low P status soils the value of EPR may need further testing. The conclusion made in this study also needs further testing in the field in different agro-ecological regions of the country. These trials could also be used to further examine the suitability of borax soil test in predicting soil P availability to tea plants.

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