Phosphorus in Agroforestry Systems: a Contribution to Sustainable Agriculture in the Zona da Mata of Minas Gerais, Brazil

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Phosphorus in Agroforestry Systems: a Contribution to Sustainable Agriculture in the Zona da Mata of Minas Gerais, Brazil

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To the small-scale farmers in Brazil and the world, especially those from the Zona da Mata of Minas Gerais and to Rita Martins de Paiva (in memoriam) and José Rafael Cardoso (in memoriam)

CONTENTS

ABSTRACT

The Zona da Mata is a region situated in the domain of the Atlantic Coastal Rainforest in the south-east of the state of Minas Gerais, Brazil. This domain stretches along the Brazilian coast from north to south and ranks among the top five of the 25 biodiversity hotspots, the richest and most threatened reservoirs of plant and animal life on Earth. Originally, forest covered the region but now only about 7.5 % of the original vegetation remains. Most of the trees were cut for wood and the area is nowadays used for agriculture. In general, the agroecosystems in the Zona da Mata show a decreasing productivity due to the increasing intensity of soil use, with practices inadequately adapted to the environment. In 1993, farmers and researchers searching for a more sustainable agriculture started implementing or improving agroforestry coffee (cash-crop) systems in the region. The natural environmental conditions of the region are favourable for growing trees, as illustrated by the fact that the entire area was originally covered with forest. The main goals with agroforestry were 1) land regeneration and conservation; 2) decrease of external input to agriculture; 3) increase or maintenance of production level; and 4) improvement of productivity. To reach these goals, a better understanding of nutrient recycling in the systems is required. The work presented here aims to contribute to such better understanding and focuses on the effect of agroforestry on phosphorus (P) cycling. Phosphorus may be the major nutrient in relatively short supply in most natural ecosystems, and the primary limiting nutrient for crop production in highly weathered tropical soils.

This P deficiency is mainly caused by strong adsorption of $H_2PO_4^-$ to aluminium (Al) and iron (Fe) (hydr)oxides, which turns large proportions of total P into a form that is unavailable to plants. The main strategy to cope with P deficiency in the tropics has been the addition of fertilisers. At the same time, the global reserves of apatite, which is needed for producing P fertilisers, are limited and known reserves may be exhausted in about 100 years with the current growth of P usage. More sustainable strategies need to be developed to utilise applied and native soil P more effectively in order to reduce P fertiliser demands. Agroforestry is considered one of these strategies.

The hypothesis of this study is that agroforestry modifies P dynamics through various pathways, transforming part of the unavailable inorganic P to a form that is available to agricultural crops, for instance coffee. One pathway is through the association of trees with arbuscular mycorrhizal fungi (AMF). These fungi generally improve plant growth by enhancing the uptake of nutrients, especially P. Differences in mycorrhizal association are expected when crops are in association with trees, compared to monoculture, and this would lead to differences in P cycling. For instance, more roots in deeper layers would increase the mycorrhizal activity in depth in the agroforestry systems relative to the conventional systems. The core questions of this thesis were: 1) Do agroforestry systems modify P dynamics in the soil? 2) Do these modification vary with depth? 3) Do agroforestry systems increase P cycling and thereby release soil P that is otherwise unavailable to the crop? 4) How does this process work? The specific objectives of the thesis were a) to characterise and compare P pools at different depths in soils from agroforestry coffee systems and conventional coffee systems from the Zona da Mata, and b) to study mechanisms involved in the P cycling, in particular the increase of availability of soil P by mycorrhizal plants.

Chapter 1 introduces the thesis. Chapter 2 describes the participatory processes and the range of methods by which agroforestry systems took hold in Zona da Mata. Chapter 3 characterises the soil inorganic and organic P (Pi and Po) pools in two agroforestry and two conventional coffee systems and compares the soil P pools at different depths in these two coffee cultivation systems. The agroforestry fields had consistently higher ratios of Po to total labile P than the conventional fields. Chapter 4 evaluates the various Pi and Po compounds at different depths in agroforestry and conventional coffee cultivation systems, using ^{31}PNMR (phosphorus 31 nuclear magnetic resonance) and a methodology adapted to tropical soils to extract P for ^{31}PNMR analyses. The fraction of total P consisting of Po and the fraction of diester in the whole spectra were higher in the agroforestry coffee fields than in the conventional coffee fields. The amount of Po and of diester decreased less with depth in agroforestry fields than in the conventional fields. Chapter 5 reports on the vertical distribution of AMF spores related to the distribution of roots in soils from agroforestry and conventional coffee fields. The data suggest that abundance and activity of mycorrhizal fungi in agroforestry systems were higher and located in a larger soil volume than in conventional systems. Chapter 6 analyses

whether AMF could take up P from pools unavailable to plants (an Al-resistant maize variety) from acid soils with highly fixed P and either with high or low organic matter content. The mycorrhizal plants performed better in the hostile environment (low pH, high Al^{3+}), taking up P whereas the non-mycorrhizal plants failed completely. Chapter 6 also describes a new methodology, the double pot-double compartment approach, which can be used to study nutrient uptake by mycorrhiza and subsequent transfer to the plants. Chapter 7 is a summary of Chapter 6, emphasising that Al resistance and P uptake from soil with high Al toxicity are not related, contrary to what is commonly assumed. Chapter 8 presents a modeling exercise to verify the suggestions derived from Chapters 3 to 6. The model confirms that the ratio Po to labile P should be higher in agroforestry than in conventional coffee systems, as shown in Chapter 3.

The final chapter evaluates the main outcomes and pitfalls of each chapter and of the thesis as a whole. It also presents a framework to investigate further aspects of phosphorus cycling in the agroforestry systems in the Zona da Mata and how this research can be expanded to other areas of the Atlantic Coastal Rainforest. The different research endeavours throughout the research period offered partial answers to the research questions raised. The results are consistent with the hypothesis that agroforestry systems influence the dynamics of P through the conversion of part of the Pi into Po, which is probably a consequence of higher biological activity in the agroforestry systems. However, the rate and the impacts of this change on P cycling and the efficiency of P use of the crops in the long term needs to be further examined and understood before reaching a comprehensive evaluation of the importance of agroforestry in soil P utilisation.

CHAPTER 1

INTRODUCTION

The research area and description of the problems

The Atlantic Coastal Rainforest sensu stricto is defined by an area of dense and open evergreen forest that stretches along the Brazilian coast and extends 300 kilometres inland (Ab'Saber, 1969; Veloso et al., 1991). The northern and southern limits are located respectively in the states of Rio Grande do Norte (about 5° S) and Rio Grande do Sul (about 30° S). In past eras, the Brazilian Atlantic forests covered around one million square kilometres, corresponding to almost 12 % of the country's area (Dean, 1998). Due to its relative accessibility, deforestation started just after colonisation by the Europeans and by the nineteenth century most of the forest had been cut. Today, the Brazilian Atlantic forest occupies about 7.5 % of the original cover and it has become one of the most notorious examples of radical destruction of tropical forests (Myers et al., 2000). The remaining forest is critical for biodiversity, since it contains numerous endemic species (found nowhere else), including 73 species of mammals, of which 21 species and subspecies are primates, 160 species of birds, and 165 species of amphibians (Moffat, 2002). It is the habitat for endangered animal species such as the birds *Ara chloroptera* (Psittacidae, arara-vermelha-grande) and *Cyanopsitta spixii* (Psittacidae, ararinha-azul), mammals such as *Chrysocyan brachyurus* (Canidae, lobo-guará), *Panthera onca* (Felidae, onça pintada) and the monkeys *Brachyteles arachnoides* (Cebidae, mono-carvoeiro), *Cebus apella* (Cebidae, macaco prego) and *Leontopithecus rosalia* (Callithicidae, mico-leão-dourado) and plant species such as *Caesalpinia echinata* (Leguminosae, pau-brasil), *Plathymenia foliolosa* (Leguminoseae, vinhático), *Carniana strellesnis* (Lecythidaceae, jequitibá) and *Melanoxylum brauna* (Leguminoseae, braúna, Dean, 1998). The Atlantic Forest ranks among the top five of the 25 richest and most threatened reservoirs of plant and animal life on Earth (so-called biodiversity hotspots, Myers et al., 2000). Thus, conserving the remaining forest cover is essential, but reversing environmental degradation of the region by sustainable management is also paramount.

The region of the Zona da Mata (about 36,000 km^2) is situated in the Atlantic Coastal Rainforest in the south-east of the state of Minas Gerais (Figure 1). Some 30% of the human population of Zona da Mata live in rural areas (BDMG, 1989). The region has a tropical highland climate (average temperature 18 °C, average precipitation 1500 mm, with 2 to 4 dry months) and is undulating, with average altitudes of 200-1800 m (Valverde, 1958). In the past, forest covered almost the entire region, but nowadays, only little more than 7% of the original forest remains. The dominant soil types in the Zona da Mata are Oxisols, which are deep and well drained, but acidic and low in nutrient availability.

Non-native exploitation of the region dates back to the mid-19th century with the expansion of coffee (*Coffea arabica* L.) production from the east, and the settling of migrants from the declining neighbouring gold-mining area (Valverde, 1958). In Zona da Mata, coffee took but a few decades to cause great ecosystem damage. Coffee cropping replaced the forest and broke the nutrient recycling in the ecosystem. Moreover, coffee was (and is) cultivated on hills, where soil erosion was accelerated, leading to land degradation. This resulted in coffee farms occupying new and more fertile areas, thus in further deforestation, while some of the old coffee fields were subsequently used as pasture or for production of staple food crops such as corn, beans and sugar cane (Valverde, 1958).

Nowadays, farmers mainly cultivate pasture and coffee, often intercropped with corn and/or beans. Coffee is the main cash crop. Other significant crops are sugar cane, cassava and rice. Since the sixties, governmental policies have been promoting Green Revolution-type technologies, which were only partially adopted due to the environmental and socio-economical constraints of smallholder production in the region. The introduction of Green Revolution elements into the peasant economy has contributed to significant environmental deterioration (biodiversity loss, agrochemical pollution, erosion due to deforestation, degradation of water resources, etc.), as well as to the weakening of family farming as an economic enterprise (indebtedness, dependency on single crops, competition with large commercial enterprises, etc.). In general, in the Zona da Mata the agroecosystems show a decreasing productivity due to the increasing intensity of soil use, with practices inadequately adapted to the environment, for instance coffee crops on steeps slope without soil conservation measures (Ferrari, 1996).

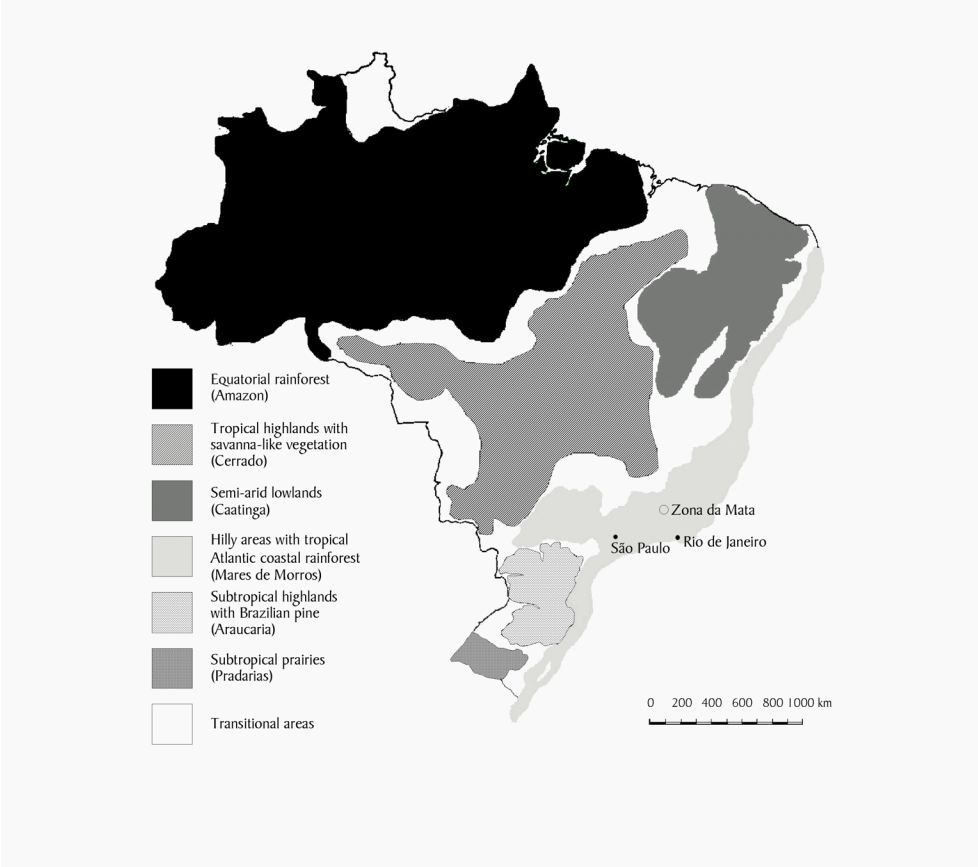

Figure 1. Morphoclimatics domains of Brazil (adapted from Ab'Saber, 1969).

In spite of this, smallholder production has maintained its vital importance within the region, mainly through the production of food crops for domestic consumption (Ferrari, 1996). During the 1980s, a strong movement of small producers and farm labourers developed, which did not only lead to the creation of new unions representing their interests (Rural Workers Unions), but also to the organisation of smallholders and agricultural wage earners at various levels and in different entities. The Centre for Alternative Technologies of the Zona da Mata (CTA-ZM) emerged in this context. CTA-ZM is a NGO whose social basis consists of local smallholder and farm labourer unions from within the region. It is active in 21 municipalities, corresponding to the area of influence of 14 local Unions. Since 1995, a group of researchers and teachers from the Federal University of Viçosa (UFV) have been working jointly with this NGO, local unions and associations in activities such as soil management, agroforestry, organic fertilising, animal breeding, and environmental education.

The agricultural model proposed by the farmers, CTA-ZM and UFV group strives towards farming households being freely organised to produce, process and market their products through an economically, socially, technologically and environmentally sustainable agriculture. This approach of sustainability, central to this thesis, aims to maintain biotic and abiotic environmental factors, to conserve the biological diversity and to seek, through use of participatory methodologies, to reverse environmental degradation and to improve social conditions.

The ecological and socio-economic problems in the Zona da Mata are not simply caused by a lack of knowledge on the part of the land users. As in other parts of the world, these problems are interrelated and derived from the historical conditions of the agriculture (Rosset and Altieri, 1997). Both ecological and socio-economic problems require urgent and integrated solutions.

Agroforestry as a possible solution

Agroforestry can be defined as a form of multiple cropping which satisfies the following basic conditions a) at least two plant species that interact biologically, b) at least one of the plant species is a woody perennial, and c) at least one of the plant species is managed for forage, annual or perennial crop production (Sommariba, 1992). Other definitions of agroforestry incorporate environmental, social and economical aspects, such as the one by Leakey (1998): integrated land use that, through the capture of intra-specific diversity and the diversification of species on farm, combines increases in productivity and income generation with environmental rehabilitation and the creation of biodiverse agroecosystems. Agroforestry systems have a range of functions in the agroecosystems. The principal ecosystem functions of agroforestry trees at the scale of a farm are a) food production, b) nutrient cycling, c) erosion control, d) water cycling, e) increase of biodiversity and f) micro-climate regulation. At the scale of a watershed/landscape/region: a) decreased poverty, b) erosion and sedimentation control, c) decreased deforestation and desertification, d) water cycling e) increase of biodiversity. At the global scale: a) greenhouse gases and climate regulation (carbon sequestration), b) increase of biodiversity, and c) rural poverty alleviation (Izac and Sanchez, 2001).

Young (1997) stated that the need for agroforestry systems is particularly great in densely populated, sloping regions in the humid and sub-humid tropics. The soils in such areas have often been degraded by erosion. Typically, the forest cover has been cleared extensively for timber, charcoal and agriculture, and over-cutting degrades what remains.

The Zona da Mata fully meets these criteria. The farmers have been confronted with decreases of crop productivity and been tempted to out-migration. Coffee cultivated on hills without appropriate soil management led to a drastic reduction of soil fertility due to erosion. The natural environmental conditions of the region, such as soil depth, light and water are favourable for growing trees, as proven by the fact that the area was originally covered with forest. Thus, it is expected that agroforestry systems can be used to restore soil conditions. Moreover, coffee has favourable characteristics for agroforestry. In its original habitat, coffee naturally occurs in native forests. The period of flowering, when coffee requires more light, coincides with the dry season, in which the agroforestry trees lose their leaves. A side effect of this is that coffee trees do not compete for water with other species. Coffee production increases when grown in habitats suitable for sustaining pollinators, for instance, honey bees in shade-grown coffee (Roubik, 2002).

In spite of these naturally promising features, intercropping with trees was not a significant aspect of the land use systems until very recently. In 1993, UFV researchers, CTA-ZM staff and small-scale farmers started to develop agroforestry systems using participatory processes, involving various steps that were fine-tuned en route. In the 1994/1995 season, 39 small agroforestry experiments were started involving 33 farmers in 11 municipalities. These experiments mainly concern a "perennial crop combination" (classification according to Young, 1997), with coffee being the crop.

The main goals of these agroforestry systems are: (1) land regeneration and conservation; (2) diversification of production; (3) decrease of the need of input; (4) increase or maintenance of production; and (5) improvement of productivity. Young (1997) formulated 12 hypotheses on functioning of agroforestry in soil management (Box 1). The goals mentioned above are in line with the 1st, 11th and 12Ath hypotheses. From 1996 to 1999, a participatory process to monitor and evaluate local development activities including agroforestry, that were developed by CTA-ZM, UFV and the Rural Workers Union, was carried out as a pilot study in one municipality of Zona da Mata (Araponga). The monitoring process (Chapter 2) showed that the agroforestry systems are effective to conserve land in the Zona da Mata (see Hypothesis 1, Box 1), however, a better understanding of the underlying processes was needed to improve nutrient recycling in the systems (Hypothesis 7), thus decreasing the need for fertiliser and increasing or maintaining production and productivity (Hypothesis 12A). Therefore, farmers requested UFV researchers for 'more academic research on soil quality and nutrient recycling'. For better understanding of nutrient cycling (Hypothesis 7), some aspects of other Hypotheses need also be investigated such as the level of organic matter and biological activity (Hypothesis 3), nitrogen fixation (Hypothesis 5), nutrient inputs (Hypothesis 6), nutrient cycling (Hypothesis 7), the roles of litter (Hypothesis 9) and roots (Hypothesis 10). Some aspects of these hypotheses have been examined in

Box 1. Twelve hypotheses about soils and agroforestry (Young, 1997, p. 20).

1. Agroforestry systems can control runoff and soil erosion, thereby reducing loss of water, soil material, organic matter and nutrients.
2. Agroforestry can augment soil water availability in land-use systems.
3. Agroforestry systems can maintain soil organic matter and biological activity at levels satisfactory for soil fertility.
4. Agroforestry systems can maintain more favourable soil physical properties than conventional agriculture, through maintenance of organic-matter and the effect of tree roots.
5. Nitrogen-fixing trees can substantially increase nitrogen inputs to agroforestry crops.
6. Trees can increase nutrient inputs to agroforestry crops by retrieval from lower soil horizons and weathering rock.
7. Agroforestry systems can lead to more closed nutrient cycling than agriculture, and hence to more efficient use of nutrients.
8. Agroforestry systems can control the development of soil toxicities, or reduce existing toxicities.
9. The development of tree litter and pruning can substantially contribute to maintenance of soil fertility.

9A. Release of nutrient from the decomposition of tree residues can be synchronised with the requirements for nutrient uptake of associated crops (the synchrony hypothesis, as applied to agroforestry).

10. In the maintenance of soil fertility under agroforestry, the role of roots is at least as important as that of above-ground biomass.

10A. Tree-root systems can take up nutrients, which would otherwise be lost by leaching (the root-leaching or 'safety-net' hypothesis).

11. Agroforestry systems can be employed to reclaim eroded and degraded land.
12. The trees in agroforestry systems can acquire environmental resources which the crop alone would not acquire, thereby increasing the total biological productivity of the systems (the resource uptake hypothesis).

12A. The favourable effects of agroforestry systems upon soils can be achieved without reducing production, thereby leading to sustainable land use (the production hypothesis).

Zona da Mata by Franco (2000), Campanha (2001), Carvalho and Ferreira Neto (2001), Mendonça et al. (2001) and Neves (2001).

The work presented here focuses on phosphorus. The impetus for the work was the farmers' request for more insight into nutrient cycling (Chapter 2). However the decision to work specifically on phosphorus was mine, for reasons explained in the following section. Central to my work are Hypotheses 3, 7 and 10 (Box 1).

Phosphorus

Phosphorus (P) may be the major nutrient in relatively short supply in most natural ecosystems, and the primary limiting nutrient for crop production in highly weathered tropic soils (Nye and Bertheux, 1957; Van Breemen, 1993; Linquist et al., 1997). The Oxisols of the agroforestry systems in Zona da Mata are such highly weathered tropical soils. Therefore, I decided to focus the research on P.

The P deficiency in tropical soils is especially caused by strong adsorption of $H_2PO_4^-$ to Al- and mainly Fe-(hydr)oxides, which turns large proportions of total P into a form that is unavailable to plants (Fontes and Weed, 1996). According to Tiessen (1995) an inefficient way to satisfy the crops' P demand is by the exclusive use of inorganic fertilisers, which are usually expensive imports and often have relatively low use efficiencies in the field due to P transformations in soil. Moreover, should usage of P fertilisers continue to grow at recent rates, known reserves will be used up in about 100 years (Stevenson and Cole, 1999). Therefore, P is a key factor in sustainable agriculture. One basic sustainable strategy is to recycle the large amounts of P that are present in waste materials, such as sewage sludge. Since P in soils is present in pools varying in availability, and pools with the lowest availability are the biggest in Oxisols (Novais and Smyth, 1999), another strategy is exploit this soil reserve of poorly available P through the use

of P-efficient plants (Stevenson and Cole, 1999). The poorly available P could be made available to the main crop after decomposition of the residues of P-efficient plants (Richardson, 2001). Organic inputs, such as plant residue, enhance nutrient cycling, mineralisation rates, and the transformation of inorganic forms of phosphorus into more available organic ones (Sanchez, 2002).

It has often been thought that the P cycle is improved by agroforestry systems through recycling litter and prunings, increased uptake from soil through the association of trees with mycorrhiza, ability of trees to draw upon otherwise unavailable soil P, including the P from deeper soil layers (Grubb, 1989; Van Breemen, 1993; Young, 1997; Radersma, 2002). Lal (1991), however, stated that there are popular myths about nutrient recycling in agroforestry systems and, in reality, there are major obstacles to make use of the potential benefits of such a system. According to Sanchez (1995), agroforestry cannot supply the P inputs required by crops. Nevertheless, if sustainability of P use has to be achieved, the potentials of plants to improve recycling of native and applied P has to be explored (Stevenson and Cole, 1999). This can be combined with better nutrient cycling within urban-rural agroecosystems (Van Noordwijk, 1999). Palm (1995) pointed to the obvious need to channel research efforts to accelerating P cycling, improving P availability in intercropping systems and using the potential of plant materials to release P.

In tropical environments, organic P (Po) may provide a major source of available P, playing a substantial role in nutrient recycling (Linquist et al., 1997). The amounts of Po in agricultural soils are usually from 30 to 80% of total P (Dalal, 1977). However, the study of Po in tropical soils needs further attention (Cross and Schlesinger, 1995). Phosphorus research in Brazil has strongly emphasised inorganic P (Pi) while Po has been virtually ignored (Novais and Smyth, 1999), partly because Po is difficult to analyse.

Estimation of the various P pools in the soil, including Po, is usually undertaken by P fractionation (Tiessen and Moir, 1993). The procedure comprises the sequential extraction of Pi and Po with increasingly aggressive reagents. This allows characterisation of the different fractions of Pi and Po that are supposed to be differentially available to plants (Tiessen and Moir 1993; Novais and Smyth, 1999). Following the procedure by Tiessen and Moir (1993), the fractions, named according to the extractants, are a) Resin-Pi and $NaHCO_3$-Pi and -Po as very labile P, b) NaOH-Pi and -Po as moderately labile P, reflecting P transformations during one and or more seasons respectively (Tiessen and Moir 1993), c) 1M HCl fraction representing primary Ca-associated P, d) the concentrated HCl fraction representing the stable pool that does not readily participate in P transformations (Agbenin and Tiessen, 1995) and e) the residue pool digested with H_2SO_4 and H_2O_2. The residue is unlikely to contain anything but highly recalcitrant or inert Pi (Tiessen and Moir 1993).

Whereas P fractionation deals with the extractability of P in the soils, it does not characterise the chemical compounds present. Traditionally, chromatography techniques have been used to identify specific organic P compounds in the soil (Cade-Menun and Preston, 1996). Nowadays, ^{31}PNMR spectroscopy, a less complex and more direct technique, has been used to estimate various inorganic and organic P compounds in soil both qualitatively and quantitatively (Newman and Tate, 1980; Cade-Menun and Preston, 1996). Although promising, the ^{31}PNMR technique still faces several methodological problems, such as the influence of paramagnetic ions (mainly iron and manganese), which are especially important in tropical soils (Cade-Menun and Preston, 1996). The most common inorganic compounds (Pi) found in soils are the orthophosphate and pyrophosphate, and the most common organic compounds (Po) polyphosphate, phosphanate, orthophosphate monoester and orthophosphate diester (Newman and Tate, 1980).

In soil, the P transformation and redistribution into different forms is the net result of the transformations in a highly dynamic P cycle, where micro-organisms play a major role (Stewart and Tiessen, 1987). The general cycle of P in soils is depicted in Figure 2. Micro-organisms can solubilise, take-up and immobilise P (Stewart & Tiessen, 1987). Among the micro-organisms, the importance of mycorrhiza in increasing P uptake by plants is well documented (Bolan, 1991).

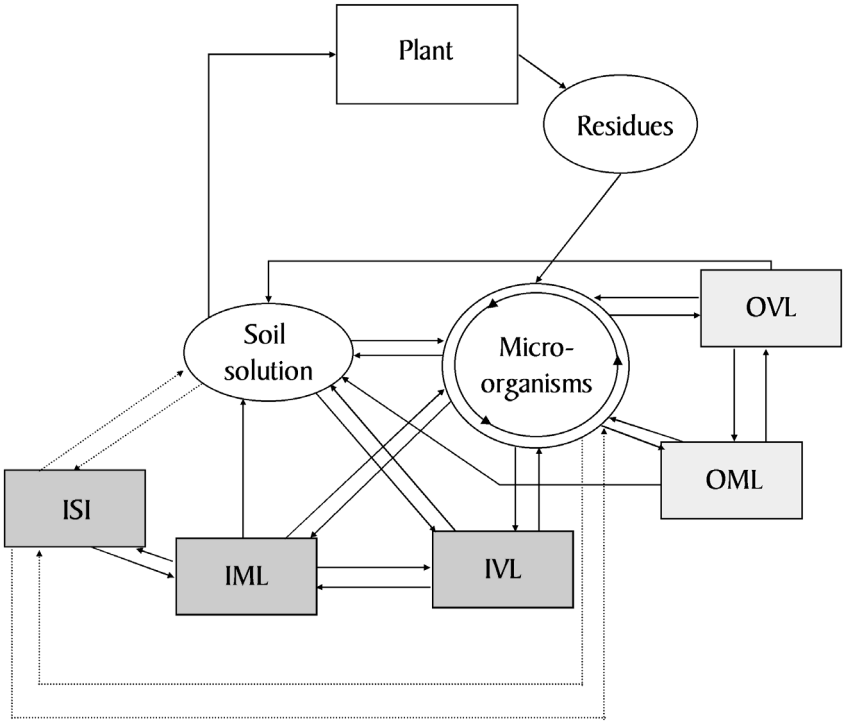

Figure 2. General cycle of P in soils (adapted from Stevenson and Cole, 1999). The P is portioned into pools that vary in availability to plants. IVL (inorganic very labile), IML (inorganic moderately labile), ISI (inorganic stable or inert), OVL (organic very labile) and OML (organic moderately labile). Very labile can be considered available to plants in short terms, for instance for annual crops. Moderately labile is available to plants in medium terms, for instance, perennial crops. Stable or inert is only available in long term or not available at all. Bold arrows represent very labile pools and dashed arrows represent the stable or inert pools.

Mycorrhiza

Mycorrhizal fungi are universally predominant micro-organisms in plant-soil environments (Brundrett, 1991). The majority of tropical plants depend for their nutrition on mycorrhizas, which form mutual symbioses with plant roots (Brundrett, 1991). Tropical trees typically form symbioses with arbuscular-mycorrhizal fungi (AMF, Siqueira and Saggin-Junior, 2001; Onguene and Kuyper, 2001).

AMF generally improve plant growth by enhancing the uptake of nutrients, especially P (Bolan et al., 1987, Marschner, 1995). In soils with strong P-fixing capacity, or where P is not adequately supplied, plant demand for this nutrient exceeds the rate at which it diffuses into the root zone, resulting in zones of P depletion surrounding roots. The AMF help overcome this problem by extending their external hyphae from root surfaces to areas of soil beyond the P depletion zone, thereby exploring a greater volume of the soil than is accessible to the unaided root (Hayman, 1983). AM fungal hyphae are smaller in diameter than plant roots and therefore have a greater surface area per unit volume. This large surface area makes the fungi much more efficient than roots in the uptake of P. Moreover, the smaller diameter of AMF hyphae allows them to explore micropores in the soil that are not accessible to roots (Bolan, 1991).

Although controversial, it also has been suggested that mycorrhiza may benefit plant growth by increasing the availability of P from non-labile sources (Bolan et al., 1987). The controversy is mainly due to several studies showing that mycorrhizal and non-mycorrhizal plants appeared to use the same labile P sources (Sanders and Tinker, 1971; Mosse et al., 1973). However, other studies demonstrated that mycorrhizal plants can obtain P from normally unavailable inorganic and organic P sources (Bolan et al., 1987; Jayachandran et al., 1989; Koide and Kabir, 2000).

Work Hypothesis and Objectives

Phosphorus is present in soils in different pools of Pi and Po that are supposed to be differentially available to plants (Tiessen and Moir 1993; Novais and Smyth, 1999). The hypothesis of this study is that in agroforestry systems a part of the unavailable inorganic P is made available to agricultural crops, for instance coffee, by modifying P dynamics along various pathways. Some trees take up part of the inorganic P that is not available to agricultural crops and transform it into available inorganic P or organic P, which can be used by crops after mineralisation. One reason is that tree roots use more soil volume than crop roots. A second reason is the mycorrhizal association of trees. Differences in mycorrhizal association are expected when crops are alone or in association with trees, because of the greater plant species diversity and larger amounts of roots are expected in the soil. A third mechanism is the exudation of organic acid/anions by tree roots releasing adsorbed P or precipitated P. The P released may then either be taken up directly by agricultural crops or may first be taken up by the tree and then made available after residue decomposition. If not taken up by crop roots, micro-organisms or tree roots, the mineralised P can again be adsorbed to soil particles or precipitate. However, newly adsorbed P desorbs more easily than "aged" P in the soil, as the formation of more stable forms of P in the soil depends on long-term transformations (Tiessen and Moir, 1993).

This brings me to the core questions of this thesis:

1. Do agroforestry systems modify P dynamics in the soil?
2. Do these modification vary with depth?
3. Do agroforestry systems increase P cycling and thereby release soil P that is otherwise unavailable to the crop?
4. How does this process occur?

The specific objectives of the thesis are a) to characterise P pools at different depths in soils from agroforestry coffee systems and conventional coffee systems (to allow comparisons) from Zona da Mata of Minas Gerais, and b) to study mechanisms involved in the P cycling, in particular the increase of availability of soil P by mycorrhizal plants.

Outline of the Thesis

Chapter 2 describes the participatory processes and range of methods by which agroforestry systems took hold in the Zona da Mata. It discusses some of the key benefits and problems of agroforestry systems encountered during the first five years. Chapter 2 also shows how the starting point of the research questions presented in this thesis emerged from the participatory monitoring and evaluation of the agroforestry systems in Araponga, Zona da Mata.

A first step in the study of P dynamics is the estimation of the various P pools in the soil, including organic P, which is usually done by P fractionation. In Chapter 3, I characterise the soil inorganic and organic P pools in two agroforestry and two conventional coffee systems and compare the soil P pools at different depths in these two coffee cultivation systems.

In Chapter 4, I evaluate the various inorganic and organic P compounds at different depths in agroforestry and conventional coffee cultivation systems, using ^{31}PNMR (phosphorus 31 nuclear magnetic resonance) and a methodology adapted to tropical soils to extract P for ^{31}PNMR analyses.

In the next two chapters, I deal with AMF symbiosis. In order to study the status of the agroforestry and conventional coffee systems with respect to mycorrhiza, I consider the number of AMF spores as an indicator of mycorrhiza incidence in soil. In Chapter 5, I report the vertical distribution of AMF spores related to the distribution of roots in soils from agroforestry and conventional coffee systems. The objective of Chapter 6 was to analyse whether AMF could take up P from unavailable pools to plants from acid soils with highly fixed P and either with high or low organic matter content. Furthermore, I describe a new methodology, the double pot-double compartment approach, which can be used to study nutrient uptake by mycorrhiza and subsequent transfer to the plants. Chapter 7 is a summary of Chapter 6, emphasising the separate role of Al resistance in plant and mycorrhizas in uptake of P from an Oxisol by an Al resistance maize variety.

Chapter 8 presents a modeling exercise to verify the suggestions from Chapters 3 to 6. The model is a simplified version of the models NUTCYC (Nutrient Cycling) and DYNAMITE (Dynamics of Nutrients And Moisture In Tropical Ecosystems). Because the main objective with the model was to examine how the system of coffee cultivation affects

the distribution of soil P over organic and inorganic pools, the model is restricted to simple calculations of the major P flows between soil and plant.

In the final chapter (General Discussion), I evaluate the main outcomes and the pitfalls of each chapter and of the thesis as a whole. I also summarise some of the research that has been undertaken with agroforestry systems in Zona da Mata by researchers from UFV, to which I relate my own findings. I propose a framework to research further aspects of phosphorus in the agroforestry systems in the Zona da Mata during my future work at the Federal University of Viçosa and how this research can be expanded to other regions of Atlantic Coastal Rainforest. This framework has two major aspects, one related to the direct implications of the P cycling in agroforestry for the farmers. Another aspect is related to interactions between soil chemistry and soil biology regarding the behaviour of P in the rhizosphere.

CHAPTER 2

CONTINUAL LEARNING FOR AGROFORESTRY SYSTEM DESIGN: UNIVERSITY, NGO AND FARMER PARTNERSHIP IN MINAS GERAIS, BRAZIL

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Abstract

This article discusses a long-term participatory process used to develop agroforestry systems in the Zona da Mata, Minas Gerais, Brazil. This area has various characteristics considered appropriate for establishing such systems, which, if well managed, contribute to sustainable land use. In 1993, university researchers, NGO staff and small-scale farmers started to develop agroforestry systems using a participatory process, involving various steps and fine-tuning. In 1994/1995, 39 small experiments were started involving 33 farmers in eleven municipalities. The participatory approach was effective, as almost all the farmers involved continue with their experiments, while others have joined. However, although results in terms of soil conservation are promising, expectations regarding increase in production and reduction of input have not yet been met, causing tensions and forcing adjustments. The benefits and problems encountered during the first five years are discussed, in order to highlight the complexity of participatory development of agroforestry systems and the need for their continual development.

List of abreviations	
NGO	non-governmental organisation
CTA-ZM	Centre for Alternative Technologies of the Zona da Mata
UFV	Federal University of Viçosa
PRA	Participatory Rural Appraisal
D&D	Diagnosis and Design

Introduction

In the last century, scientists widely considered farmers ignorant of proper land use despite their having lived with agriculture for millennia (Bekele-Tesemma, 1997). The dominance of Western scientific practice, norms and reasoning, or *episteme* knowledge (Marglin, 1991), marginalised farmers' insights from contributing to scientific practice. Farmers' experience-based, *techne* knowledge was not only regarded as inferior but sometimes not as knowledge at all (Marglin, 1991). Nowadays this view is being modified (cf Brokensha et al., 1980; Röling, 1992; Chambers, 1997). Recognition has grown that two different knowledge systems[1] can make unique and complementary contributions (Martin and Sherington, 1997; Loader and Amartya, 1999), and with it the appreciation for building bridges between these fundamentally different ways of 'understanding perceiving, apprehending and experiencing reality' (Marglin, 1991:112). Hence the keen interest in participatory approaches that facilitate close interaction between scientists and farmers, for modifying existing land care systems, including the adoption of agroforestry systems (Cornwall et al., 1994; Lal, 1991; Buck et al., 1999; Sanchez, 1995; Young, 1997).

Participation by the resource managers is particularly essential in the case of agroforestry systems, as the complexity and variability of the systems (Scherr, 1991) make the relevance and acceptability of proposed land use changes often highly site-specific (Cooper et al., 1996). Farmers have substantial and invaluable experiential knowledge about their systems (Rocheleau, 1999; Scherr, 1991; Nair, 1998), whereas researchers have poor understanding of farmers' agroforestry strategies, lack basic technical information about agroforestry systems, and lack

[1] Despite the usefulness in our case for distinguishing these knowledge systems, others, such as Leeuwis (1996), criticise the simple dichotomy, arguing that both systems have characteristics of the other.

locally-validated agroforestry technologies (Scherr, 1991). Thus, if farmers are involved, they can help focus the research, identify pertinent research questions, and speed up the iterative research process (Rusten and Gold, 1991; Sinclair and Walker, 1999). Experience with agroforestry systems suggest that participatory research mobilises, empowers and induces technical innovation (Rocheleau, 1999). However, as we shall explain below, 'participation' is often viewed simplistically by technical researchers. It is not a simple matter of some group discussions out of which emerges a collectively agreed plan that is then implemented. Instead, we have found that agroforestry system design is an evolving learning process that requires continual adaptation and even reversals of core principles. This is only possible through a methodologically diverse and participatory trajectory, an example of which forms the backbone of this article.

Though the design process is the subject of debate, the basic merits of agroforestry are widely accepted. Farmers and scientists involved in agroforestry value these systems for two core properties: land regeneration and degradation prevention. Regeneration results from tree cover which has been shown to improve soil fertility (Lal, 1991; Sanchez, 1995; Palm, 1995; Copper et al., 1996; Young, 1997), although the rate with which this occurs varies greatly depending on species and biophysical conditions for growth. Thus trees are incorporated in many initiatives that aim to reclaim degraded land (Cooper et al., 1996; Chambers and Leach, 1989; Young, 1997; Guijt and Race, 1998). More fundamental is the potential of agroforestry systems to avoid land degradation and contribute to sustained land use (Sanchez, 1995; Cooper et al., 1996; Young, 1997; Huxley, 1999). Altieri (1995) states that the basic tenets of a sustainable agroecosystem are conservation of renewable resources, adaptation of the crop to its environment, and maintenance of a high but sustainable level of productivity. Designing such agroecosystems means ensuring two fundamental ecosystem functions in agricultural fields: (1) biodiversity of microorganisms, plants and animals, and (2) biologically mediated recycling of nutrients. Agroforestry systems have been shown to enhance both these functions (Lal, 1991; Altieri, 1995; Sanchez, 1995).

Young (1997) states that the need for agroforestry systems is particularly great in densely populated, sloping regions in the humid and sub-humid tropics. The soils in these areas have often been degraded by erosion. Typically, the forest cover has been cleared extensively for timber, charcoal and agriculture, and over-cutting degrades what remains, due to the extra pressure on small remaining patches (construction material, fuelwood etc). Where forest has been replaced by pasture, these fields degrade quickly and fodder shortage becomes endemic. Deforestation and erosion also reduce the base flow of rivers. These symptoms frequently contribute to outward migration of land users, as productivity declines to levels that are not economically viable. In such environments, agroforestry can help control runoff and erosion (Lal, 1991; Sanchez, 1995), improve pasture and support small watershed management, besides contributing to diversifying and sustaining of productivity by timber and non-timber products.

The Zona da Mata (Brazil, see Figure 1, Chapter 1, Introduction), the region we are discussing in this paper, fully meets these criteria. It is a sub-humid environment with steep hills (see Section 2 below), largely degraded due to removal of much of its forest cover, with farmers confronting productivity problems and tempted by out-migration. Fortunately, as the original land cover testifies, the Zona da Mata was once dense forest (Dean, 1996). So existing biophysical conditions, such as soil depth, light and water, are favourable and will not hinder tree growth. However, an aptitude for tree cover will not necessarily ensure successful agroforestry.

Despite this potential, inter-cropping with trees was not important in local land use systems until recent years. The reasons for the lack of trees on farms in Zona da Mata are not well researched. However, our experiences lead us to suggest these include a range of factors, such as: colonisation and its search for cash crops that first led to deforestation of lands previously managed by indigenous peoples; varietal development of full sun resistant coffee; farmers' poor understanding of nutrient recycling and researchers' inadequate focus on appropriate technologies; socio-economic constraints such as labour shortage; and more recently Green Revolution-oriented government policies that have virtually forced monoculture.

In 1993, several Rural Workers Unions[2] in the Zona da Mata, assisted by an agriculture-focused NGO (non-governmental organisation), CTA-ZM (Centre for Alternative Technologies of the Zona da Mata) and several researchers from the UFV (Federal University of Viçosa), started to implement agroforestry systems[3], working as closely as possible with existing examples of trees on farms. Working in partnership has been critical. As noted by others, parties acting on their own will probably fail to find relevant and viable solutions to local agricultural problems (Reijntjes, 1992; Hinchcliffe et al., 1999; Thrupp, 1996), while partnerships have all the advantages of groups: enhanced creativity, more complete picture of a situation, recognition of incorrect solutions, etc (Johnson and Johnson, 1994). The UFV contributes its theoretical insights and rigorous methodologies for understanding micro-processes of soil fertility. NGO staff offers its general insights into agricultural processes, its facilitation skills to engage the three parties, and its strategic socio-political analysis to ensure the research is relevant. Finally, the farmers add their in-depth insights into local ecological conditions and dynamics, their clearly expressed economic needs and ecological concerns, and last but not least, their fields. All parties contribute their time to collective meetings and individual experimentation, thus leading to a highly complementary and integrated partnership. However, group-based partnerships also had their limitations in the agroforestry design process as we shall discuss below.

Two assumptions underpin the long-term success of our partnership: (1) that agroforestry systems are a sustainable practice for the region and (2) that a participatory methodology is fundamental for successful establishment of such systems. This paper takes a critical look at our simple assumptions by describing the participatory processes and range of methods by which agroforestry systems took hold in Zona da Mata, recounting some of the key benefits and problems encountered during the first five years. In doing so we aim to encourage more reflection on the complexity of participatory agroforestry system design. Success requires long term commitment to a participatory trajectory, and the willingness and capacity to use diverse methods from both the hard and soft traditions (Guijt et al, 2000). After describing the region, we discuss the methodology as it evolved, highlighting the contribution of each step to developing a more viable agroforestry system. The steps were: a) survey for assessing basic feasibility, b) PRA for assessing core problems and possible solutions, c) D&D for deepening agroforestry option and identifying specific local tree knowledge; d) consolidation meetings for finalising initial experimentation design, and e) collective monitoring for evaluating interim technical and process results. We present initial findings about the uptake of technology. Finally, we discuss the challenges posed by a participatory endeavour – not to detract from its merits – but to highlight the importance of site-specific analysis and ongoing dialogue to allow for the inevitable evolution of agroforestry systems.

The evolving regional landscape and its problems

The Zona da Mata is situated in the south east of the Brazilian state of Minas Gerais, in the Atlantic Coastal Rainforest domain (Ab'Saber, 1969, Figure 1, Chapter 1, Introduction). It has a tropical highland climate: average temperature 18 °C, average precipitation 1500 mm, with 2-4 dry months per year. The landscape is steep, with slopes ranging from 20 to 45 % and with average altitudes ranging from 200 to 1800 m (Golfari, 1975). In the past, forest covered almost the entire region but little more than 7 % of the original vegetation remains today (Dean, 1996). The dominant soil types are Oxisols, which are deep and well drained, but acidic and poor in nutrient availability. Some 30% of the population lives in rural areas (BDMG, 1989).

Today's agricultural production in the Zona da Mata has three main characteristics: long-term land use, small-scale production systems, and traditional agricultural practices. The most important crops are pasture and coffee, often inter-cropped with maize and/or beans. Other crops are sugar cane, cassava and rice.

[2] Rural Workers Unions (*Sindicatos de Trabalhadores Rurais*) are elected bodies at the municipal level, with legislative status through Brazil. They represent the interests of their members who are mainly small scale farmers – interests include land tenure, health rights, cooperative initiatives, etc.

[3] This was part of a long-term commitment to the region to develop sustainable local municipal development plans, and thus involves other initiatives than agroforestry development. However, for the purposes of this paper we will restrict ourselves to discussing the efforts related to agroforestry development.

Land cover in the region has typically passed through a cycle that started with Atlantic Coastal Rainforest being replaced by coffee plantations. This broke nutrient recycling in the forest ecosystem and drastically reduced soil fertility within a few decades, mainly due to erosion and loss via harvesting. This process aggravated deforestation as coffee farmers, in search of fertile land, occupied new, forested areas. Meanwhile, pastures and staple food crops such as maize, beans and sugar cane replaced old coffee fields.

More recently, government policies promoted the adoption of Green Revolution-type technologies and practices, which contributed to significant environmental deterioration (biodiversity loss, agrochemical pollution, erosion due to deforestation, degradation of water resources, etc) and a weakening of the 'family farm' as an economic enterprise (indebtedness, dependency on single crops, competition with large commercial enterprises, etc) (cf Staver, 1999). In spite of this, smallholder production has maintained its vital importance within the region, mainly in the production of food crops for domestic consumption (Gomes, 1986; Ferrari, 1996). In summary, most agroecosystems in the region today have low productivity due to a history of (increasingly) intense soil use, with practices not well adapted to the environment, for instance coffee crops without soil conservation (Ferrari, 1996).

Initial insights and first design steps

Given the existing environmental potential and environmental and socio-economic problems, around 1990, UFV and CTA-ZM researchers started speculating about the suitability of agroforestry systems for the region. However, with its feasibility an unknown, the first step was to develop an initial overview of existing systems, particularly how trees were used on farms. Furthermore, even if agroforestry systems were shown to be feasible, it was unclear if farmers would prioritise land use problems sufficiently to be willing to invest significantly in land use improvement.

Thus a survey of existing systems was followed by extensive Participatory Rural Appraisal (PRA) and Diagnosis and Design (D&D) processes and focused discussions on the roles of trees in production systems, via exchange visits to other areas, eventually leading to the initial design of agroforestry experiments. These steps are detailed below.

Understanding the baseline with an agroforestry survey

Observations and informal contacts between farmers and the researchers had already indicated some inter-cropping practices with trees. In 1992, the survey was undertaken in one small watershed in the region (São Bartolomeu) to identify existing agroforestry systems. Several sites were visited by the UFV and NGO researchers, who interviewed farmers. The interviews mainly covered the local economic and landscape history, state of natural resources, objectives of the agroforestry systems, management practices, crop and inter-cropping species and the inter-cropping design. The farmers also shared their impressions of the performance of their agroforestry systems.

The survey revealed the existence of seven agroforestry systems of three broad types: four perennial-crop combinations (classification according to Young, 1997) including coffee (*Coffea arabica*), two home gardens and one pasture with trees. One farmer established two of the perennial-crop combinations in 1980 and 1984, and consisted of coffee with mainly native trees. In the older of these two systems, 14 trees were found belonging to 12 species of perennials, besides coffee, on 390 m^2. In the youngest system, eight trees were found belonging to six species of perennials. Six other species besides coffee were found on 214 m^2. The species found are listed in Table 1. During the interview, the farmer stated the main characteristics of these two systems, which compared favourably to the characteristics of conventional coffee in the region (Table 2).

These experiences with agroforestry systems, however few within an area as large as the Zona da Mata, gave us initial confidence in the feasibility of agroforestry systems for the region. While clearly giving us, as researchers, initial insights, the dialogue with the farmers had only just started. It was their learning process and our collective design that now needed to be given shape.

Table 1. Trees and shrubs used in agroforestry systems in Zona da Mata, Minas Gerais, Brazil (listed per family names and species with local names in parentheses).

Family	Species (local name)
Anacardiaceae	*Mangifera indica* (manga)[a]
	Miracroduon urundeuva (Aroeira*)*
	Schinus terebentifolium (aroeirinha)
	Spondias sp. (umbú) [a]
Annonaceae	*Annona muricata* (graviola) [a]
	Rollinia silvatica (araticum)
Araucariaceae	*Araucaria angustifolia* [1] (pinheiro brasileiro) [a]
Apocynaceae	*Aspidosperma polyneurum* (guatambu)
	Peschiera affinis (esperta)
Bignoniaceae	*Jacaranda micrantha* (caroba)
	Sparatosperma sp.[2] (cinco folhas)
	Tabebuia impetiginosa (ipê roxo)
	Tabebuia schysotricha (ipê mulato)
	Tabebuia serratifolia[l] (ipê amarelo)
Bixaceae	*Bixa orellana* (urucum) [a]
Bombacaceae	*Bombax* sp. (castanha Mineira)
	Chorisia speciosa (paineira)
Caprifoliacea	*Sambucus nigra* (sabugueiro)
Caesalpinaceae	*Caesalpinia peltophoroides* (sibipiruna)
	Hymeneae courbaril (jatobá) [a]
	Schizolobium parahyba[l] (breu)
	Senna macranthera (fedegoso)
	Peltophorum dubium (canafístula)
Caricaceae	*Carica papaya*[l] (mamão) [a]
Casuarinaceae	*Casuarina equisetifolia*[l] (casuarina)
Cecropiacea	*Cecropia hololeuca*[2] (embaúba)
Compositae	*Vanillosmopsis erythropapa* (candeia)
	Vernonia polyanthes (cambará)
Ebenaceae	*Diospyrus kaki* (caqui) [a]
Elaeocarpaceae	*Muntingia calabura* (calabura)
Esterculiacea	*Dombeya wallichii* (astrapeia)
Euphorbiacea	*Alchornea triplinervia* (folha-de-bôlo*)*
	Joanesia princeps (cotieira)
	Hyeronima alchorneoides (liquerana)
	Manhihot esculenta (mandioca) [a]
	Ricinus communis (mamona)
Fabaceae	*Cajanus cajan* (guandu) [a]
	Flemingia sp. (flemingia)
	Dalbergia nigra (jacarandá caviúna)
	Machaeriun stiptatun (canela-de-velho)
	Machaeriun nictitans (jacarandá-bico-de-pato)
Labiatae	*Coleus barbatus* (boldo)
Lauraceae	*Persea gratissima* (abacate) [a]
	Persea pyrifolia (maçaranduba)
Malvaceae	*Gossypium hirsutum* (algodão) [a]
	Hibiscus rosa-sinensis (graxa)
Melastomaceae	*Tibouchina granulosa*[l] (quaresmeira)

(Table continued on next page)

Table 1 (continued)

Family	Species (local name)[a]
Meliaceae	*Cedrela fissilis* (cedro)
	Melia azedarach (cinamomo)
	Toona ciliata (cedro australiano)
Mimosaceae	*Annadenanthera peregrina* (angico vermelho)
	Enterolobium contortisiliquum (orelha-de-macaco)
	Calliandra calothyrsus (caleandra)
	Inga vera (ingá)
	Piptadenia gonocantha (jacaré)
Moraceae	*Artocarpus integrifolia* (jaca) [a]
	Morus alba (amora) [a]
Musaceae	*Musa* sp.[1] (banana) [a]
Myrsinaceae	*Rapanea ferruginea* (pororoca)
Myrtaceae	*Campomanesia xanthocarpa* (gabiroba)
	Eugenia malaccensis (jamelão) [a]
	Eugenia pitanga[1] (pitanga) [a]
	Myrcia jaboticaba (jabuticaba) [a]
	Psidium araça (araça)
	Psidium guajava (goiaba) [a]
	Sysigium jambos (jambo) [a]
Palmae	*Bactris gaesipaes* (pupunha) [a]
	Cocus nucifera (côco-da-baia) [a]
	Euterpe edulis (palmito jussara) [a]
	Syagrus romanzofianum (côco-babão)
Pinaceae	*Pinus* sp.[1] (pinus)
Rhamnaceae	*Ovenia dulcis* (ovênia)
Rosaceae	*Moquilea tomentosa* (oiti)
	Eriobotrya japonica[1] (ameixa) [a]
	Pirus communis (pêra) [a]
	Prunus persica (pêssego) [a]
Rutaceae	*Citrus* sp. (limão-cravo) [a]
	Citrus sp. (Mexerica) [a]
	Citrus sinensis (laranja) [a]
	Citrus sp. (turanga) [a]
	Dictyoloma vandellianum (brauninha)
Solanaceae	*Solanum argenteum* (capoeira branca)
	Solanum lycocarpum (lobeira)
Sapindaceae	*Litchi sinensis* (lixia) [a]
Tiliacea	*Luehea speciosa* (açoita cavalo)
Ulmaceae	*Trema micrantha* (crindiúva)
Verbenaceae	*Aegiphila sellowiana* (papagaio)
	Cytharexyllum mirianthum (pau-de-viola)

[1] and [2] species present in agroforestry systems established in 1980 and 1984 found during the survey; [1] species also found in the agroforestry systems monitored in the Zona da Mata, Minas Gerais, Brazil; [a] fruits and/or crop

Understanding the context with PRA and D&D

After the survey, an extensive participatory analysis of one municipality (Araponga) in the region took place to understand the wider context of agroecosystems, followed by a more specific analysis of agroforestry systems. These analyses became a pilot study for farmers in other municipalities. Araponga is one of the municipalities where CTA and UFV are active. It was chosen as the location for the appraisal because the Rural Workers Union there had requested more support from CTA, already specifying their own need for more information about local land use problems.

Instead of the extractive data-gathering ritual of traditional research, the UFV researchers and CTA staff opted to use Participatory Rural Appraisal (PRA) to analyse the agroecosystems, in line with similar developments elsewhere

Table 2. Comparison between conventional coffee and agroforestry, as expressed by a farmer with the two oldest agroforestry systems in Zona da Mata, Minas Gerais, Brazil.

Conventional	Agroforestry (established in 1980 and 1984)
Coffee trees need to be pruned when 10-12 years old, or younger[1]	Coffee trees were not pruned until the age of 15 - 19 years
Production fluctuates per year[2]	Production is more constant
Non-homogeneous maturation, resulting in lower quality and more work in harvesting	Homogenous maturation resulting in better quality and less work in harvesting
More soil erosion	Less soil erosion
Less biodiversity[3]	More biodiversity
More use of fertiliser	Less use of fertiliser

[1] Trees bred to sustain full sun and that receive regular fertiliser must be pruned sooner, as they grow faster but are less vigorous; [2] See Table 7 for production levels; [3] Counted as number of species per surface area.

(Dorward et al., 1997; Martin and Sherington, 1997; Loader and Amartya, 1999). This approach encourages and allows local people to determine much of the agenda, to gather and analyse information, and to plan actions (Chambers, 1994; 1997). PRA centres around semi-structured interviews or focus group discussions, and uses visualised analysis techniques such as preference ranking, mapping and modelling, seasonal and historical calendars, institutional diagrams, matrix scoring, and impact flow diagrams. When done well[4], these facilitate information sharing, analysis, and action among stakeholders (Chambers, 1994; Dorward et al., 1997; Thompson and Guijt, 1999). The tasks of UFV and CTA were to convene farmers, trigger discussions, guide the use of methods, and to document.

Thus, in 1993, a PRA process (see Table 3) was undertaken in Araponga to involve farmers in the process of discussing, evaluating and planning agroecosystems. One of the biggest problems pointed out by the participating farmers was the low productivity of their land, which they attributed to soil conservation problems such as erosion. Although this was not a novel insight in itself, being a well-known problem in the region, the PRA process allowed farmers to discuss and describe it to the researchers and NGO staff, and not the other way around, as it is more common. The farmers prioritised land use problems and selected a committee composed of farmers, NGO staff and researchers to present some land conservation proposals to overcome with the problem. The committee suggested agroforestry for reclaiming the degraded land in Araponga. Thus, PRA was not undertaken to 'implant' the importance of agroforestry in farmers' minds. Rather, agroforestry emerged as a proposal suitable for the land degradation problems raised by the farmers during the appraisal.

After the proposal was formulated, a complementary and more focused analysis was needed to analyse the proposal in more detail with interested farmers. Known by some as a 'topical PRA', (Dunn, 1994) this analysis also aimed to recover their specific knowledge about trees and their use in the diverse agroecosystems in Araponga.

The theory of Diagnosis and Design (D&D) guided the next step. Following a lead set by the International Centre for Research in Agroforestry (ICRAF) in the early 1980s, it has become common practice to precede research in a new region with a diagnosis and design survey (Raintree, 1987; Sanchez, 1995). D&D, which uses similar techniques as PRA, is focused exclusively around the design of appropriate agroforestry systems (ICRAF, 1983).

The D&D was undertaken in 1994 in two small watersheds in Araponga (São Joaquim and Boné). The sequence followed and methods used (see Table 4) was adapted from several authors, among them ICRAF (1983) and Montagnini (1992). The construction of watershed maps provided specific information about forest cover and land use, while seasonal calendars identified land management practices. With the matrix, it was possible to identify local tree species with good potential for agroforestry. The causal diagramming allowed better analysis of the causes and consequences of limited tree availability in the two micro-watersheds.

4 This is an important point as many examples exist of poorly carried out PRA.

Table 3. Steps in the Participatory Rural Appraisal (PRA) undertaken in Araponga, Minas Gerais, Brazil.

Stage	Method	Outputs
Pre-analyses	• Literature study and organisation of the available data on environment and development of the municipality	• First insights about the region • Information about local agroecosystems
Use of some PRA tools with farmer union leaders	• Venn diagram • Map • Seasonal diagram	• Information about agroecosystems from farmers union leaders, discussion about this information
Plan the next stages	• Meetings with farmer union leaders	• Draft plan of community work • Key topics to guide interviews: production, migration, health and education
Semi-structured interviews with farm families	• During one day, some houses in one community (normally a small watershed) were visited and the family interviewed (11 communities in total) • Interview guide was based on main topics as suggested by farmer union leaders and by Altieri's checklist of issues for studying agroecosystems (1995) • In the evenings, after the interviews, the contents were discussed with the farmers	• Information about local agroecosystems from farmers, and discussion with them
Large meeting	• Meeting with 350 farmers involved in the interviews and others interested in joining	• Main problems were identified and discussed collectively • Choice of the representatives of the communities to participate in the next stage
Small meeting	• Meeting with representatives of the communities chosen in the large meeting to prioritise the problems to be resolved	• Prioritisation of land use problems • Creation of the 'healthy land' committee composed of farmers, researchers and NGO staff
'Healthy land' committee	• Regular meetings	• Decision to adopt agroforestry as a practice for sustainable land use

Consolidating insights into agroforestry and selecting species

Several steps were taken to consolidate insights of the farmers, NGO staff and UFV researchers into agroforestry, such as excursions to visit agroforestry systems, group meetings and visits by Ernst Götsch (Table 5). Götsch is well known in Brazil for developing a complex agroforestry system based on natural succession (Nowotny and Nowotny, 1993) in Pirai do Norte, Bahia (Brazil). As this area also falls within the Atlantic Coastal Rainforest system, Götsch's input was considered logical and suggested by the NGO to the farmers (see 3.4).

During this phase, farmers selected the species she or he wanted for their on-farm experiments. The species chosen generally had the following characteristics: native trees with which farmers were familiar; proven compatibility with coffee; easy availability of seeds or seedlings; fast growing and resistant to pruning. The two last characteristics were considered particularly important for ensuring soil cover and for high biomass production. Compatibility with coffee is important because it is the cash crop in the region and it is cultivated in the hills where problems with erosion are more common.

The initial design

In 1994, following the dialogue, debate and collective designing from the PRA and D&D processes, the first agroforestry plots were established. From 1994 to 1997, 39 on-farm experiments were established, involving 33 small-scale farmers in 25 communities in 11 municipalities of the Zona da Mata of Minas Gerais. Thirty-seven of the systems focused on coffee and two were based on pastures. Coffee has favourable characteristics for agroforestry (cf

Table 4. Approach for the adapted Diagnosis and Design (D&D) in two mini watersheds in Araponga, Minas Gerais, Brazil.

Stages	Methods	Outputs
Analysis of conclusions from the PRA process	• Meetings	• The decision to undertake D&D in two watersheds to discuss agroforestry as an alternative land use in depth with farmers
Use some PRA methods for specific questions	• Maps • Seasonal calendar • Matrix • Causal diagramming	• Specific information and discussion about trees and their potential
Discussion about concepts, advantages and disadvantages of agroforestry systems	• Two meetings, one in each watershed	• Establishment of two agroforestry plots, one in each watershed

Staver 1999). It occurs naturally in semi-deciduous native forest in Ethiopia, the microclimatic conditions of which are reproduced in agroforestry systems. The period of flowering when more light is required coincides with the dry season. During this time the trees lose their leaves or are pruned. A side effect of this is that coffee trees do not compete for water with other species.

The experiments involved about 9300 coffee trees. The spacing between the coffee plants was generally 3x1.5 m, with trees planted mainly between coffee tree rows. The total area of coffee was around 41,800 m^2, thus each small-scale experiment occupied on average 1000 m^2.

Besides the existing coffee trees, the number of additional trees planted was around 9,400, notwithstanding any natural regeneration of trees that occurred. Currently evergreen trees are pruned at least once per year, at the start of the dry season in May or June.

The design of the systems was strongly influenced by Ernest Götsch (see Appendix A). As his approach is to cover the soil as soon as possible, plant species were planted very closely in the beginning, and mainly pioneer species were used.

Monitoring and evaluating the systems

With a design, the process of agroforestry establishment has, of course, only just started. Before making any claims about success, changes in the design and impacts must be tracked. In line with the participatory diagnosis and collective design process followed thus far, we opted for a collective monitoring effort.

Participatory monitoring and evaluation

Monitoring has two general purposes: to support decision-making and planning, and to improve accountability – while in the case of participatory monitoring, it can enhance local capacity to record and analyse change and thus to support community-based initiatives. However, when farmers and academically-trained scientists interact to monitor collectively, scientific rigour is put to the test as farmers, and their *techne* ways of understanding, may reject the norms that derive from the *episteme* tradition (see Introduction, this article). Thus academic rigour can be compromised in exchange for a more equal partnership and for results that are more meaningful locally (see Appendix B). The converse is also true, with in some cases, laboratory analyses or experiments under controlled conditions can complement information (Abbot and Guijt, 1998). Compromises in both directions were certainly made in our work in Araponga.

In Araponga, where the PRA and D&D took place, a participatory process to monitor and evaluate the activities that were developed by CTA, UFV and the Rural Workers Union was carried out from 1996 to 1999, as a pilot study[5]. Among the twenty-eight development activities that had been initiated in the municipality after the PRA

[5] The process was developed in partnership with the International Institute for Environment and Development (IIED), which guided the methodological development (c.f. Guijt, 1998; Abbot and Guijt, 1998; Sidersky and Guijt, 2000).

Table 5. Steps to consolidate insights into agroforestry in Zona da Mata, Minas Gerais, Brazil.

Inputs	Where	Objectives	Outputs
Excursion organised by CTA with 20 farmers from Zona da Mata to several agroforestry systems	• Neighbouring state of Espírito Santo.	• To see agroforestery experiences, and learn about advantages and disadvantages.	• Increased awareness amongst the farmers and NGO/UFV staff of a) diversity of agroforestry systems and b) their viability.
Four meetings.	• One meeting in each of the three micro-regions where agroforestry work was to take place (Muriaé, Araponga and Carangola). • One collective meeting.	• To share insights from the survey, PRA and D&D and the excursion. • Exchange own experiences with inter-cropping of trees.	• Consolidated farmers' motivation to pursue agroforestry. • Enabled farmers to design their specific experiments (species and practices).
Visits by Ernest Götsch.	• Zona da Mata.	• To learn from his experience.	• Technical capacity building of farmers and NGO/UFV staff about options for agroforestry systems. • Collective decision to implement highly diversified systems.

process (see Footnote 3), the farmers chose six activities to monitor and evaluate. Agroforestry was one of the activities chosen, as the farmers perceived it as a critical innovation in their agroecosystems that merited more analysis. Therefore the monitoring process was not oriented exclusively around the agroforestry systems but around the wider sustainable municipal development plans. Of the 39 agroforestry experiments in Zona da Mata, 20 were initiated in Araponga and five were selected for monitoring, three 4-5 year old perennial-crop combinations including coffee and two 1-2 year old home gardens. Together these comprise around 7.4 hectares.

As a first step in indicator selection, the farmers, UFV and CTA re-examined their expectations of the agroforestry experiments. No less than 29 objectives emerged (see Table 6)! To keep the monitoring feasible, key objectives were selected by consensus for which indicators were identified. The selected objectives and their respective indicators were: 1. soil coverage: percentage of soil covered; 2. production costs: costs of the variable expenses (labour, fertiliser, limestone etc); 3. diversity of the production: number of species per system; 4. production: amount of each product (coffee, fuelwood, etc) harvested.

One year after monitoring started, two additional objectives and their respective indicators were included: (1) improved soil health:[6] soil analyses; and (2) nutrient recycling: soil analyses, as farmers perceived the need for more detailed soil analysis. However, farmers did not specify what type of soil analysis they desired and it is UFV's task to develop ideas. UFV researchers think that to fulfil farmers' requests for 'more academic research on soil quality and nutrient recycling', more than soil analysis alone will be needed. Currently, one of the authors (Cardoso) is focusing on the dynamics of organic phosphorus and on arbuscular mycorrhiza, while another (Fiorini) is examining erosion processes.

In the five agroforestry systems being monitored, the percentage of soil covered was measured using a wooden frame (with four quadrants about 1 m^2 in total) placed on the ground (see Appendix B). The percentages of soil covered were estimated within the wooden frame. Farmers registered, in notebooks and on questionnaires that had been designed together, all the costs of the variable expenses during 15 months. In the same period, the numbers of plant species per system were counted and listed at least two times. The farmers, with UFV and CTA support, also measured the production of the systems.

Table 6. Collective objectives of agroforestry in Araponga (Minas Gerais, Brazil), arranged from most immediately achievable (1) to most long term (9).

Time frame for Achievement	Objectives
1	• protect springs and water tables • cover soil • avoid/reduce erosion • fertilise soil • ensure shading • recycle nutrients • reduce/avoid chemical inputs
2	• improve water infiltration • reduce evaporation • increase biological nitrogen fixation
3	• increase biodiversity • reduce weeds
4	• regulate temperature • maintain ecosystems • reduce presence of pests/diseases • reduce costs (including labour costs)
5	• improve quality of product • maintain or increase production • diversify production • keep production fairly constant
6	• value women's work and role • value local knowledge • increase understanding of ecosystems • reduce pressure on remaining forest
7	• return to original forest cover and pure air
8	• conserve nature • obtain 'strong soil' (sustained production) • strengthen farmers' organisations
9	• improve quality of life

Results of the collective monitoring

Monitoring data showed that the percentage of soil covered in the agroforestry systems was 67%, as compared to 16% in the conventional coffee systems and 37% in the conventional pasture. In the first 15 months of monitoring, the five farmers worked a total of 452 days in the agroforestry systems (ranging from 33 to 142 days per farmer). In the same period they used a total of 1495 kgs of fertiliser (ranging from zero to 925 kilos), 2590 kgs of limestone (ranging from 250 to 850 kilos), 100 litres of bio fertiliser (in only one system), 200 kgs of manure (ranging from zero to 120 kilos) and 450 additional coffee seedlings (in one system). The farmers counted the presence of 86 species of trees and shrubs, belonging to 38 families (see Table 1). They also counted 41 species of weeds, 12 species of annual or bi-annual food crops, five species of green manure, and three species of grass. During the period of monitoring, 25 species that had originally been planted were removed from various experiments and another 41 were included. The total production obtained in the first 15 months was 414 kgs of coffee, 180 kgs of maize, 3.5 m^3 of fuelwood, 37 pineapples, 15 kgs of green manure seed, two kgs of *Raphanus sativus* (radish). While inadequate at this stage for deriving statistically valid analyses, farmers valued these numbers to gain an integrated picture of 'cost-

[6] Farmers used the term '*terra forte*', or strong earth

benefit', and to compare their own performance with others. The less technical value of this information obtained from joint monitoring is discussed in the conclusions.

Undoubtedly, diversification within the systems is very high (see Table 1), thus care had to be taken in deriving general conclusions from the data. During the evaluation of the agroforestry systems that was based on the monitoring data, the farmers, CTA staff and UFV researchers reached four essential conclusions. First, the agroforestry systems were effective in covering the soil and thus reducing erosion. Second, most farmers cannot afford the amount of labour required as an initial investment as production has remained low in these first years. Therefore, additional external investments are necessary to initiate agroforestry systems[7] and ways must be found to manage the systems with less labour. Third, it is necessary to improve nutrient recycling, make better use of fertilisers, and to use plants that are more adapted to the soil conditions and with a quicker capacity to produce. Fourth, the diversity of production in the agroforestry systems, moving farmers away from an economically fragile monoculture livelihood, seems to be stable or increasing but insufficient data are available for conclusions. Finally, all agreed that the monitoring process had been critical for all to become aware of the problems with agroforestry. Their curiousity provoked, the farmers decided to include two more objectives to be monitored and evaluated after the first year, both related to soil nutrients.

Of great interest to us, as researchers, was also the unambiguously problematic and contradictory data that had emerged in the regular exchange visits and meetings that accompanied the monitoring (and which continue to date). While ecologically beneficial, the agroforestry proposal was not increasing production as planned and thus seemed to be economically unfeasible. Armed with data they had collected themselves, the farmers were confident about debating these contradictions and challenging the researchers and the NGO about the agroforestry systems they had introduced in Araponga.

Facing up to design problems

As part of the continual learning, frequent field visits to the agroforestry plots and yearly evaluations with the experimenting farmers have been essential for exchanging experiences, assessing performance and re-designing the systems.

In 1999, 13 representatives of farmers from six municipalities, UFV researchers and CTA staff attended the yearly assessment meeting. Those at the meeting agreed with the initial conclusions that had emerged from the monitoring data in Araponga after five years of agroforestry: agroforestry systems were very effective in protecting the soil but required more labour, production had not yet met expectations, and fertiliser use had decreased only slightly. For an expected annual production of 0.7 kg of unshelled coffee per tree, the amount of fertiliser normally recommended, according to regional soil analysis, is over 300gms of 20-5-20 (N-P-K) (IBC, 1986; CFSEMG, 1989), besides other fertilisers. The oldest agroforestry systems found in the survey (established in 1980 and 1984) reached that production with less fertiliser (see Table 7), thus, the perspective is that, with time and good management, the need for fertiliser will decrease.

During the meeting, the farmers reminded UFV and CTA of the five main goals of their agroforestry systems that had been monitored: (1) land regeneration; (2) diversification of production; (3) decrease of input, including labour; (4) increase or maintenance of production; and (5) improve productivity. All agreed that modifications were essential if all these goals were to be achieved. For instance, more fast-producing species were suggested, as was the use of more deciduous trees or sub-perennial species that do not require pruning and thus minimised labour. It was still unclear how to improve the nutrient recycling to reduce fertiliser use. Some farmers had modified their systems, of which removal of some species was a clear indicator. For example, in one of the monitored cases, the farmer removed several non-leguminous trees and introduced more *Inga vera*, a leguminous tree, so has a fertilising function. Furthermore, although evergreen, it continually loses and renews many leaves. This favours light in the system, decreasing the need to prune and therefore requiring less labour.

[7] Using the labour data to argue their case, the farmers and CTA-ZM succeeded in accessing local development funds to compensate the farmers for the labour invested in establishing agroforestry plots.

Table 7. Levels of chemical fertiliser and coffee productivity according to farmers in the Zona da Mata (Minas Gerais, Brazil).

Age of system	Chemical fertiliser per coffee tree		Productivity (unshelled)
	Fertiliser [1]	Amount [1] (g/tree)	Kg/tree
19 years[2]	N-P-K 20-5-20	130	Each year:0.7
15 years[3]	N-P-K 20-5-20	130	Each year:0.7
4 years	N-P-K 20-5-20	50	1999: 0.3
	Ammonium sulphate + KCl (5%)	100g	
4 years	Superphosphate + KCl	30	1998: 0.2

[1] The amount of fertiliser N-P-K advised for conventional production systems for an expected annual production of 0.7 kg, is more than 300 g/trees; [2] See Table 2 for overall comparison with conventional farming system; [3] ibid

Thus far, despite the uncertainties and slow improvement related to some of the original goals (soil coverage, lower production costs, greater production diversity, higher production), the farmers remain motivated to invest in developing more viable agroforestry. They are maintaining and adapting their systems, and above all, are learning from their daily experiences and from each other. Only two of the initial 33 farmers who started have abandoned agroforestry and an additional 21 have joined. Uncertainties such as those confronted by farmers in Araponga, especially low production, have ruined far less complex proposals than agroforestry. Yet in Araponga, the farmers are continuing with agroforestry. Our analysis is that this results from the extensive participatory processes that has slowly been improving understanding of the problems, possible solutions and strategies.

Conclusions

The experiences in Zona da Mata offer insights at two levels: the effectiveness of agroforestry and the process of developing appropriate agroforestry systems.

The agroforestry systems have proven effective at conserving land in the Zona da Mata. However, to improve nutrient recycling in the systems, better understanding of the underlying processes is needed. Studies are also needed to evaluate better the other effects of agroforestry systems, like changes in soil biology, weed composition, competition among plants, shifts in biodiversity, etc. This will help to understand the mechanisms involved in nutrient recycling and the outputs and inputs of the systems. More effort is also needed to find ways of increasing production more quickly, or of offsetting the initially high investments required of the farmers.

The rising numbers of interested farmers and the perseverance of the early starters, despite the problems encountered with low productivity and high labour input, testify that participation in the design, monitoring, evaluation and adjustment of the agroforestry systems is an interesting option. The first stages of the process enabled identification of farmers' knowledge about their agricultural systems, analysis of the information (particularly the context in which agroforestry was proposed as an alternative form of land use), discussion of causes of local problems and possible solutions. However, a good design process alone does not guarantee successful agroforestry. The participatory approach we are pursuing has become a dynamic learning process, which requires modifications as work progresses, farmers learn, and family and economic circumstances change - a process without a time horizon. Thus, the latest cycle of modifications in the systems design that have emerged are a welcome and essential part of the process.

The participation of farmers, UFV researchers and CTA staff ensured the implementation of an effective analysis, design and monitoring approach. The academic researchers from UFV adapted their inherited methodologies to ensure more local relevance. On the other hand, farmers have demanded more academic research from UFV, highlighting the need for more methodological complementarity from researchers than is usual (cf Walker et al., 1999). The data that emerges from this will, no doubt, lead to further adjustments in their systems.

Finally, we feel the process highlights an interesting dilemma for agricultural science. Many researchers nowadays recognise that a farmer participatory process has considerable value when designing agroforestry systems (ICRAF,

1983; Sanchez, 1995; Young, 1997; Huxley, 1999). Yet according to these authors, few researchers are working with farmers to monitor and evaluate the systems, which is essential for a continual learning for agroforestry system design. Joint monitoring will require some convergence on norms, data collection methods, and data interpretation – if western scientific methods were to dominate monitoring efforts, the quality of farmer participation could justifiably be questioned.

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Appendixes

Appendix A. Core ideas of Ernest Götsch (Nowotny and Nowotny, 1993).

1. To take nature in Atlantic Coastal Forest as a model, which means:

- Biodiversity – ensuring high diversity in ecosystems;
- Succession – the dynamic process in the forest is composed of four stages: pioneer species, species at the onset of the secondary stage, species at the end of the secondary stage, climax.

2. To reproduce the mechanisms of nature more quickly:

- Start with pioneers, use as many species as possible and plant the species as close together as possible to cover the soil quickly and to create high levels of biomass, to increase nutrient recycling.
- Pruning: (1) severe prune to renew pioneer species before their maturation phase to ensure their regeneration; (2) clean prune to eliminate pioneer species in maturation phase.
- Introduction of other species according to the stages of the system.

Appendix B. Querying Quadrats (IIED, CTA-ZM and STR Araponga, 1997; Abbot and Guijt, 1998).

During a workshop in Araponga, the farmers, NGO staff, union representatives, and university academics were deciding which method could assess 'the percentage of vegetation cover' (one of the indicators chosen for monitoring agroforestry plots). Besides using a wooden frame (with 4 quadrats about 1m^2 in total, to be placed on the ground in several sites within the agroforestry plot and estimate visually the surface area covered by vegetation), the academics suggested a form to fill in the percentages. While the wooden frame was acceptable, the farmers thought the form would be too complicated. The academics then suggested a form with pre-drawn quadrats, which the farmer could shade to depict the area under vegetation. Again, it was rejected as too alien to the farmers' way of registering, as they have great reluctance to use pen and paper. They agreed to use wooden sticks or rulers, on which the farmer scratches a mark to indicate the estimated percentage of vegetation cover in terms of a certain segment of the ruler. Each farmer was to use a new stick for each measuring event. When the farmers meet for the agroforestry project, they bring their marked sticks, register the measurements on paper, and discuss the findings and their significance for their plots. However, in the end, all farmers resorted to observations based on the wooden framework. Scientists might well debate the accuracy of a scratch mark on a wooden stick compared with written percentages on a piece of paper. However, if the paper-based method had been imposed, the reliability of the information would probably have been low because of the reluctance of the farmers to use this approach. In this case, participation probably ensured a more realistic version of 'rigorous' data collection.

CHAPTER 3

INORGANIC AND ORGANIC PHOSPHORUS IN OXISOLS UNDER AGROFORESTRY AND CONVENTIONAL COFFEE (*Coffea arabica* L.) SYSTEMS IN MINAS GERAIS, BRAZIL

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Abstract

Phosphorus (P) is a primary limiting nutrient for crop production in weathered tropical soils. The deficiency is mainly caused by sorption of phosphate to Al- and Fe- (hydr)oxides. We hypothesise that the distribution of soil P among various pools is influenced by land use. Our objective was to characterise the soil inorganic and organic P (Pi and Po) pools and to compare the different pools at different depths in agroforestry and conventional coffee cultivation systems. The study was carried out in the Atlantic Coastal Rainforest domain, Brazil, with Oxisols as the dominant soil type. Soils were collected from four coffee (*Coffea arabica* L.) fields, two with agroforestry and two conventional (full-sun, monoculture) systems. Three profiles were sampled per field, at depths of 2-3, 10-15 and 40-60 cm. A simplified sequential P fractionation was carried out, using resin, $NaHCO_3$, NaOH, diluted HCl and concentrated HCl as extractants. Sum-P ranged from 370 to 830 mg kg^{-1}. Concentrated HCl extracted the largest portion (74 %), followed by NaOH (22.5 %). Labile P ranged from 13 to 40 % of Sum-P. The major part (62 %) of the labile fraction was Po. In the agroforestry fields, the amount of Po decreased less with depth and the percentage of Po in labile pools was higher than in conventional fields. This suggests that agroforestry maintains larger fractions of P available to agricultural crops by influencing the dynamics of P through the conversion of part of the Pi into Po, thereby reducing P losses to the unavailable pools.

Introduction

Phosphorus is an important nutrient in relatively short supply in most natural ecosystems, and the primary limiting nutrient for crop production in highly weathered tropical soils (Linquist et al., 1997). The deficiency is mainly caused by strong adsorption of $H_2PO_4^-$ to Al- and Fe- (hydr)oxides, which turns large proportions of total P into a form that is unavailable to plants (Fontes and Weed, 1996). Various standardised tests are being used to estimate the fraction of P available to plants (Neufeldt et al., 2000). However it has been suggested that in highly weathered tropical soils, plants have access to P fractions, in particular to moderately labile organic P, not accounted for in these standard P tests (Beck and Sanchez, 1994).

In tropical environments, organic P (Po) may be a major source of available P (Linquist et al., 1997; Beck and Sanchez, 1994). In Brazil, there has been a strong emphasis on the study of inorganic P while organic P has been virtually ignored (Novais and Smyth, 1999). With the increasing cost and scarcity of P fertiliser, especially for resource-poor farmers (Ae et al., 1990), it is necessary to increase the availability of soil P by accelerating the rate of P cycling. These developments ask for a better understanding of the P dynamics associated with decomposition of organic residues, and for soil management strategies that utilise applied and native soil P more effectively, thus reducing P fertiliser demands (Rheinheimer et al., 1999). Evidently, the study of organic P in tropical soils needs further attention (e.g. Cross and Schlesinger, 1995)

Estimation of the various P pools in the soil, including organic P, is usually done by P fractionation. Nowadays, the main fractionation procedure used is that of Hedley (Tiessen and Moir, 1993). The procedure comprises the sequential extraction of inorganic P and organic P with increasingly aggressive reagents. This allows characterisation of the different pools of Pi and Po that are supposed to be differentially available to plants (Beck and Sanchez, 1994; Tiessen and Moir, 1993; Novais and Smyth, 1999). However, any chemical fractionation can at best approximate biological functions (Novais and Smyth, 1999) and, therefore, different definitions of the 'labile' pools are found in the literature. Some authors only consider the Pi and Po extracted by resin and $NaHCO_3$ as labile, and the Pi and Po

extracted by NaOH and HCl as recalcitrant or unavailable P (Cross and Schlesinger, 1995). Other authors argue that forms of P extractable with sodium hydroxide are involved in long-term transformations of P in the soil, and contribute to labile P (Neufeldt et al., 2000; Magid et al., 1996). Fractions that are labile in the tropical soils are not necessarily labile in temperate soils, and what is labile in the long term may not be labile in the short term (Magid et al., 1996). From a perspective of long-term P transformation, which is relevant for perennial crops such as the coffee (*Coffea arabica* L.) studied here, NaOH fractions can be considered as labile. In this study, we therefore consider Resin-Pi and $NaHCO_3$-Pi and -Po as labile P, and NaOH-Pi and -Po as moderately labile P, reflecting P transformations during one and more seasons respectively (Tiessen and Moir 1993; Agbenin and Tiessen, 1995; Lilienfein et al., 2000; Neufeldt et al., 2000). The 1M HCl pool represents primary Ca-associated P, and the concentrated HCl pool represents the recalcitrant pool that does not readily participate in P transformations (Agbenin and Tiessen, 1995).

Transformations and availability of soil P not only depend on soil characteristics, but also on (interactions with) plants and on associations of plants with micro-organisms (Magid et al., 1996; Novais and Smyth, 1999). Microbial activity plays a major role in nutrient turnover in general and in P transformation and redistribution into different forms in particular (Stewart and Tiessen, 1987). Micro-organisms can solubilise, take-up and immobilise P. The importance of mycorrhiza in increasing nutrient uptake by plants, especially phosphorus, is well documented (Bolan, 1991). Changes in the quantity and nature of Po are the net result of the transformations in a highly dynamic P cycle (Stewart and Tiessen, 1987). The addition of organic and inorganic P fertilisers can lead to build-up of organic P in soils, as a consequence of increasing activities of soil biomass. Immobilisation of P depends on the availability of Pi and of organic substrates to soil biota (Stewart and Tiessen, 1987; Magid et al., 1996).

It has been suggested that intercropping with plant species that are efficient in P acquisition, due to special plant characteristics and/or special interactions with micro-organisms, may increase the availability and supply of P to the main crop. One form of intercropping is agroforestry, which includes trees as a component of the agroecosystem. These trees may increase P supply by retrieving nutrients from lower soil horizons (Young, 1997), and increase P availability through accelerating P cycling by (1) improving the chemical and physical quality of soils, and (2) by enhancing microbial activity (Cooper et al., 1996).

The hypothesis of the present study is that agroforestry modifies the distribution of soil P among the various pools. Some trees, for instance, take up part of the inorganic P that is not available to agricultural crops, and transform it to organic P, which, after being released and mineralised in the soil environment can be used by the crops. This process can take place not only close to the surface but also in deeper layers of the soil. Thus, the objective of this paper is (i) to characterise the inorganic and organic P pools of an Oxisol in two medium-aged and two old coffee fields with either agroforestry or conventional cultivation systems; and (ii) to compare the soil P pools at different depths in these two coffee cultivation systems.

Materials and methods

Study sites

We studied the effect of different cropping practices on soil P distribution in the Zona da Mata, Brazil, as part of ongoing research on agroforestry systems. The Zona da Mata is situated in the Southeast of the state of Minas Gerais, in the Atlantic Coastal Rainforest domain. The area has steep slopes (20 to 45%) with altitudes ranging from 200 to 1800 m, and a tropical highland climate (average temperature 18°C, average precipitation 1500 mm, and one dry period of 2-4 months). The parental rock is gneiss and the dominant soil types are deep and well drained Oxisols, acid and poor in available nutrients. In the past, forest covered almost the entire region, but nowadays only little more than 7% of the original vegetation remains. The most important crops are pasture and coffee, often inter-cropped with maize and/or beans. Other crops are sugar cane, cassava and rice. Cardoso et al. (2001a) give details on pedology, agriculture and sociology.

Soil samples were collected from two on-farm agroforestry coffee fields of 15 and 19 years, denoted by AM (medium-aged) and AO (old) respectively, and two on-farm conventional coffee (full-sun, monoculture) fields of 15-20 and 20-24 years old, denoted by CM and CO, respectively. Because this was an on-farm study, an exact match of the ages of the fields was impossible.

Samples were taken in a landscape that is representative of the region, with slopes at the collection sites ranging from 21 to 36% and altitudes from 685 to 720 m. All fields were located in the same watershed and the agroforestry fields were adjacent to each other. The conventional fields were one and five km apart from the agroforestry fields. The sites of AM, AO and CM were fallow before they were implanted with agroforestry or conventional coffee, the site of CO was pasture.

Since the farmers only use manual tools, no more than the first five centimetres of the soil were supposed to be mixed by annual management (minimum tillage). Fertilisers were applied in the form of compounds (N-P-K), at rates of 130 (AM and AO), 300 (CM) and 100 (CO) grams of 20-5-20 per coffee tree per year. Fertiliser was always applied under the coffee canopy, i.e., not between rows. Limestone was broadcast at a rate 0.6 ton per ha per year in AM and AO. In CO the rate was 100 g per tree, under the canopy, once every four years. In CM, limestone was applied for the last time 4-5 years ago but the rate and method are not known. Chicken manure was applied in AM in the year of the soil sampling at a rate of around 3000 kg per ha. In CO, about 3600 kg of chicken manure per hectare per year were applied during 10-14 years, the last time it was applied 10 years before we sampled the soil. The manure was applied between coffee rows, where the sampling took place. Herbicides were applied in the conventional fields.

Around 2000 coffee trees per ha were present in AM, AO and CM, and around 1300 in CO. AM and AO had around 370 agroforestry trees per ha. The genera of plant species observed around the sampled spots in AM and the AO were: *Araucaria, Sparatosperma, Tabebuia, Schizolobium, Carica, Casuarina, Cecropia, Commelina, Tibouchina, Musa, Eugenia, Pinus, Eriobotrya* (Cardoso et al., 2001a).

Sampling and analysis

Samples were collected in June 1999 (dry season), in three profiles, approximately 10 m apart, in between the coffee rows, at depths of 2-3 cm, 10-15 cm and 40-60 cm. The trees in the agroforestry systems were also located between coffee rows. Approximately one litre of soil was collected from each layer, air-dried and passed through a two-millimetre sieve.

Using routine procedures, the soils were analysed for texture (pipette method); pH (in water), organic matter (Walkley-Black method); calcium and magnesium (extractor KCl, 1 mol); and phosphorus and potassium (extractant Mehlich-1).

The sequential P fractionation followed the flow diagram of Figure 1, based on Tiessen and Moir (1993), but without filtering and centrifugation at 5000 rpm at room temperature, as proposed by Beck and Sanchez (1994). Each sample was done in duplicate and the results were averaged. Inorganic P in the extracts was determined colorimetrically with the molybdate-ascorbic acid (Murphy and Riley, 1962). Total P was also measured in this way, after digestion of the $NaHCO_3$ and NaOH extracts. The amount of organic P in these extracts was calculated as the difference between total and inorganic P. The soil residue after extraction with concentrated HCl was not further digested in $H_2SO_4+H_2O_2$, because this extract is unlikely to contain anything but highly recalcitrant Pi (Tiessen and Moir, 1993). We refer to the sum of all fractions as Sum-P instead of total P because we did not include the last step of Tiessen and Moir (1993), i.e., digestion with $H_2SO_4+H_20_2$. Phosphorus pools were both expressed as fractions (part of Sum-P) and as concentrations (mg kg^{-1} soil).

Statistical analyses

Statistical analyses were performed using Statistica (StatSoft Inc.). Analysis of variance, followed by a LSD (least significant difference, $p < 0.05$) post-hoc test, was used to test for differences among treatments, after sequential Bonferroni corrections (Rice, 1988). The relative data (mg P/mg P) were arcsin (sqrt) transformed prior to statistical analyses to satisfy normality and homogeneity of variance assumptions. In these investigations we compared "on-farm" fields that differed in management practices (agroforestry and conventional coffee systems), age (medium-aged and old) at three depths. As explained in Cardoso et al. (2002a), within field replicates were considered as sufficiently independent replicates for a proper analysis of variance.

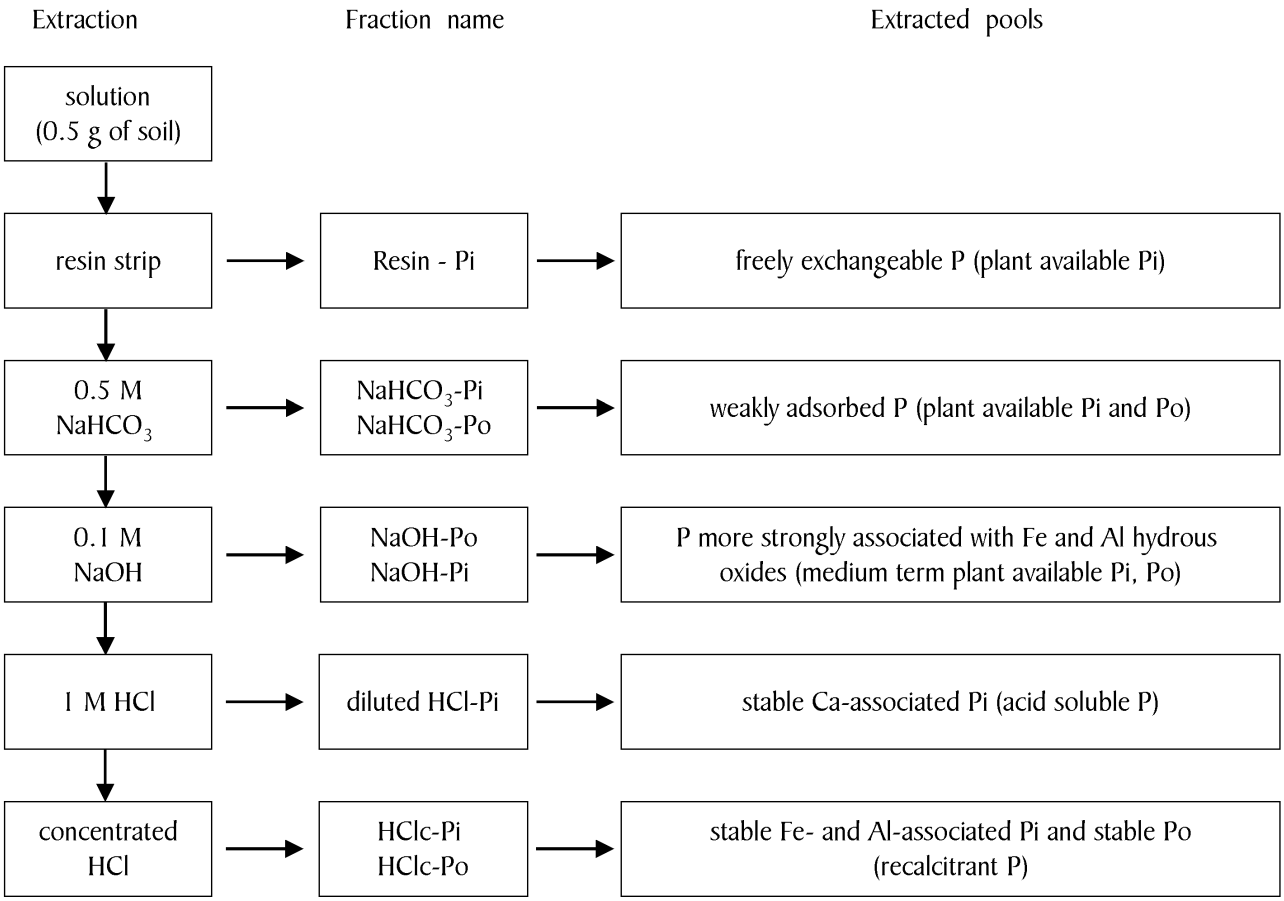

Figure 1. Flow diagram of the P fractionation method as developed by Tiessen and Moir (1993) and modified by Neufeldt et al. (2000) and Lilienfein et al. (2000).

Results

The characteristics of the soils and post hoc comparisons (LSD) are shown in Table 1, and can be interpreted as follows, according to the general criteria of Soil Fertility Committee of Minas Gerais States, Brazil (CFSEMG, 1989). Soils were clayey and acidic; the levels of organic matter were high in 2-3 cm and medium in deeper layers, except in the CO system, which showed low levels in the deepest layer. Exchangeable cations (calcium, magnesium and potassium) in the agroforestry fields were high at the surface and medium to low in the deeper layers. P-Mehlich is low at all depths in all fields. Table 2 shows the results of the statistical analysis of the effects of system, age and depth on soil characteristics as shown in Table 1.

Table 3 shows the average values and the post-hoc comparisons of Sum-P, the labile P, the sum of organic P (Sum-Po) and the mass fractions of P in each extract analysed per depth (2-3 cm, 10-15 cm and 40-60 cm) in the systems (AM, AO, CM, CO). For the same depths and systems Table 4 shows the total carbon:sum-Po (C:Po). Organic P in concentrated HCl was not detected, probably because it was very low, as demonstrated by Lilienfein et al. (1996) for Brazilian Oxisols. Sum-P ranged from 370 to 830 mg kg^{-1}. Concentrated HCl extracted the largest portion of Sum-P (74 % on average), followed by NaOH (22.5 %). Resin, diluted HCl and $NaHCO_3$ extracts contained on average 0.5, 0.7 and 2.3 % of Sum-P, respectively. The organic P fractions, $NaHCO_3$-Po and NaOH-Po, were on average 1.3 % and 14 % of Sum-P.

Table 1. Average (n = 3) of soil characteristics of medium-aged and old agroforestry coffee fields (AM and AO) and of medium-aged and old conventional coffee fields (CM and CO), at three depths (2-3 cm, 10-15 cm and 40-60 cm). Significant differences are indicated by different letters (P<0.05, LSD, with sequential Bonferroni corrections) either among fields (rows) or among depths within fields (columns).

Soil characteristics	Depths	AM	AO	CM	CO
clay[1]	2-3	44 b	43 b	39 b	56 a
	20-30	65 a	49 b	45 b	65 a
	40-60	66 a	45 b	47 b	64 a
sand[1]	2-3	46 a	37 ab	41 a	34 bc
	20-30	31 bc	34 ac	36 ab	25 c
	40-60	30 b	34 ab	35 ab	25 bc
pH (H_20)	2-3	5.0 a	5.0 a	4.8 ab	4.6 b
	10-15	5.0 a	5.0 a	4.8 a	4.7 ab
	40-60	4.9 ab	4.8 ab	5.1 a	4.6 b
organic matter[2]	2-3	40.6 a	40.0 a	39.1 a	38.9 a
	10-15	26.5 ab	22.6 ab	25.4 ab	21.7 ab
	40-60	17.2 b	15.9 b	17.2 b	11.2 b
exchangeable cations[3] : Ca^{2+} + Mg^{2+} + K^{+}	2-3	55.8 a	68.5 a	20.9 b	23.0 b
	10-15	20.7 b	25.7 b	12.2 bc	7.8 c
	40-60	11.4 bc	7.5 c	7.9 c	5.5 c
P Mehlich-1[4]	2-3	3.1 b	4.5 ab	5.3 a	3.8 ab
	10-15	1.2 cd	1.1 cd	2.2 c	1.5 c
	40-60	0.7 d	0.6 d	0.3 d	3.0 bc

[1]%, [2]g kg^{-1}, [3]$mmol_c$ kg^{-1}, [4]mg kg^{-1}.

Table 5 shows significant effects (ANOVA) of system, age and depth on Sum-P, Labile-P, Sum-Po, Sum-Po : Labile-P, fractions of P and C:Po. There were effects of system and age, and a significant system x age interaction on Sum-P, which can be ascribed to the high values in CO. The labile pool (Resin-Pi + $NaHCO_3$-Pi, -Po + NaOH-Pi, -Po) showed significant effects of system, age, depth and a system x depth interaction. Labile P was higher in AM and CO than in the other fields (Table 3). In general, labile P decreased with increasing depth for the conventional but not for the agroforestry systems (Table 3). Labile P was on average 26% of Sum-P (range 13 - 40%). Organic P represented on average 62% of the labile P (range 38 – 76%, Figure 2).

There were significant effects of depth and significant interactions of system and depth on Sum-Po, $NaHCO_3$-Po and NaOH-Po (Table 5). The concentration of Po did not change in depth in the agroforestry fields, but was higher in the first layers than in the deep layers of the conventional fields (Table 3). System, age and depth had significant effects on the ratio organic P : labile pools (Table 5, Figure 2).

There were significant effects of system, age, and depth on Resin P with the amount of P in the layer close to the surface being higher than the amount in the other layers (Table 3). Within each depth (Table 3) the amount of P in the

Table 2. Significance levels (ANOVA) of the effects of system (agroforestry or conventional), age (medium or old), depth (2-3 cm, 10-15 cm and 40-60 cm) and interactions on soil characteristics.

Factors	Clay	Sand	pH	Exchangea-ble cations	P mehlich	Organic matter
system	ns	ns	**	***	**	ns
age	ns	**	*	ns	ns	ns
depth	***	***	ns	***	***	***
system x age	***	*	ns	ns	ns	ns
system x depth	ns	ns	ns	***	ns	ns
age x depth	*	ns	ns	ns	ns	ns
system x age x depth	ns	ns	ns	ns	**	ns

Significance levels: *: (P<0.05), **: (P<0.01), ***: (P<0.001), ns: not significant, with sequential Bonferroni corrections.

Table 3. Concentration of phosphorus (P) in the various extracts. Sum-Po is $NaHCO_3$-Po + NaOH-Po, Labile P is Resin-Pi + $NHCO_3$-Pi and -Po + NaOH-Pi and -Po, and Sum-P is Labile-P + 1M HCl-Pi + concentrated HCl-Pi. Average values (n = 3) at three depths (2-3, 10-15 and 40-60 cm) in medium-aged and old agroforestry coffee fields (AM and AO) and conventional coffee fields (CM and CO). Significant differences are indicated by different letters ($P < 0.05$, LSD with sequential Bonferroni corrections) either among fields (rows) or among depths within fields (columns).

P fractions[1]	Depths	AM		AO		CM		CO	
Resin-Pi[2]	2-3	2.6	b	6.0	a	5.7	a	6.4	a
	10-15	0.7	b	1.7	b	2.0	b	2.7	b
	40-60	0.7	b	1.5	b	0.0	b	2.5	b
$NaHCO_3$-Pi	2-3	5.2	d	4.7	d	12.7	a	8.8	b
	10-15	0.8	e	2.9	de	8.2	c	3.1	de
	40-60	0.7	e	2.9	de	6.8	c	6.5	bc
$NaHCO_3$-Po[3]	2-3	8.5	a	8.7	a	11.9	a	9.8	a
	10-15	7.0	a	7.4	a	7.3	a	6.9	ab
	40-60	4.8	ab	6.8	ab	2.2	b	2.0	b
NaOH-Pi	2-3	47.9	c	29.5	de	55.6	c	90.2	b
	10-15	28.2	d	20.8	de	24.2	de	54.3	c
	40-60	41.9	cd	24.3	e	13.7	e	116.8	a
NaOH-Po	2-3	95.0	ab	67.7	bc	68.8	b	129.5	a
	10-15	88.5	ab	48.7	c	51.4	bcd	119.5	a
	40-60	117.2	a	46.3	cd	24.9	d	75.3	c
HCl-Pi 1M	2-3	2.4	bc	6.0	b	11.8	a	1.6	c
	10-15	1.8	cd	2.0	d	6.1	b	0.3	cd
	40-60	1.7	bc	4.3	bcd	5.4	b	1.0	c
HClc-Pi[4]	2-3	282.8	c	439.8	bc	300.0	c	534.8	ab
	10-15	254.1	c	414.1	bc	334.1	c	572.8	ab
	40-60	246.4	c	445.6	b	318.0	bc	624.5	a
Sum-P	2-3	444.3	b	562.3	b	466.5	b	781.0	a
	10-15	381.1	b	497.5	b	433.3	b	759.6	a
	40-60	413.1	b	531.6	b	371.0	b	828.7	a
Sum-Po	2-3	103.5	ab	76.3	bc	80.6	b	139.3	a
	10-15	95.5	ab	56.1	c	58.7	bc	126.4	a
	40-60	122.0	a	53.1	cd	27.1	d	77.4	bc
Labile-P	2-3	159.1	cd	116.5	e	154.7	de	244.6	a
	10-15	125.2	df	81.4	ef	93.1	f	186.5	b
	40-60	165.1	c	81.7	eg	47.6	g	203.1	b

[1]mg P kg soil^{-1}; Pi, [2]inorganic P; [3]Po, organic P; [4]HClc, concentrated HCl

resin fractions did not differ among the fields except for 2-3 cm where AM was lower than the others.

There were effects of system (agroforestry or conventional) and depth on $NaHCO_3$-Pi and NaOH-Pi (Table 4). The conventional fields contained higher concentrations of $NaHCO_3$-Pi than the agroforestry fields and the first layers in general contained higher concentrations of $NaHCO_3$-Pi than the other layers (Table 3). The effect of system on NaOH-Pi was mainly due to the CO field, which had a higher amount than the other fields (Table 3).

Diluted HCl extractable P showed effects of systems, age and depth with the first layers showing higher amounts of Pi than other layers (Table 3). The CM had in general higher amounts of diluted HCl-Pi in every layer than the other fields (Table 3). The values of concentrated HCl are higher in CO than in the other fields (Table 3), which caused an effect of system and age. No effects of depth were found.

C:Po was on average 202, ranging from 82 to 367 (Table 4). The post hoc comparisons (Table 4) did not show any difference among systems, age or depth except for the lowest layer of CM, which causes some significant effects shown in Table 5. Po correlates well with carbon contents for all fields (AO, $r = 0.77$, $p < 0.01$; CM, $r = 0.83$, $p < 0.005$; CO, $r = 0.74$, $p < 0.025$) except for AM ($r = 0.50$). When looking at the separate pools as Po-$NaHCO_3$

Table 4. Carbon : Sum organic Po (C:Po) at three depths (2-3, 10-15 and 40-60 cm) in medium-aged and old agroforestry coffee fields (AM and AO) and conventional coffee fields (CM and CO). Significant differences are indicated by different letters ($p < 0.05$, LSD, with sequential Bonferroni corrections) either among fields (rows) or among depths within fields (columns).

C : Po	Depths	AM	AO	CM	CO
	2-3	228b	304b	282ab	162b
	10-15	161b	234b	251ab	100b
	40-60	82b	173b	367a	84b

and Po-NaOH, the AM showed a strong correlation between carbon with Po-$NaHCO_3$ ($r = 0.89$, $p < 0.005$) but no correlation with Po-NaOH ($r = 0.56$).

Discussion

In general, the differences in soil characteristics (Table 1) are considered slight according to the levels used to interpret soil fertility in Minas Gerais (CFSEMG, 1989). Differences between systems were found for exchangeable cations (Table 1). The agroforestry systems had higher values than the conventional systems, especially in the top layers. This has to be attributed to the fact that limestone was broadcast in agroforestry systems and was applied around coffee trees in the conventional fields. The application rates of limestone were higher in the agroforestry than in the conventional systems, and erosion was probably less, both contributing to a higher impact of the limestone.

All systems contained similar amounts of soil organic matter if the same depths are compared. The amount of organic P in the soil is roughly correlated to the amount of carbon, but inositol is known to obscure this correlation (Dalal, 1977). Since NaOH probably extracts compounds related to ortho-P monoesters, mainly inositol (Robinson et al., 1998), we do not expect a clear correlation between C and Po extracted with NaOH. Nevertheless, we found a significant correlation in all fields except for the AM.

Sum-P values were similar in all layers within field (Table 3). Because all systems were subject to minimum tillage, this even distribution is likely to be the result of soil mixing by soil fauna. Sum-P was highest in CO (760-830 mg kg^{-1}), probably as a result of large applications of chicken manure in the past. Although Pi is immobile in Oxisols, the addition of manure to soil triggers the movement of P, including inorganic forms (Novais and Smyth, 1999) and mineralisation of transported organic P occurs (James et al., 1996). High amounts (mg) of P in CO are mainly found in the recalcitrant fraction (concentrated HCl) followed by NaOH-Pi fractions, which can be due to inorganic P in the chicken manure or due to mineralisation of the Po also present. The values of the Sum-P in the younger fields (AM and CM, 370-470) are rather similar to the 255 to 471 mg kg^{-1} (excluding the residue-P) found by Lilienfein et al. (2000), and the 225 to 386 mg kg^{-1} of Neufeldt et al. (2000), but higher than the amounts found by Tiessen and Moir (1993) in differently textured sand Brazilian Oxisols (110 to 130 mg kg^{-1}). The difference is due to difference in soil texture. Based on soil survey reports concerning the first 20 cm of soil and assuming soil density as 1.3 kg dm^{-3}, Resende (1997) calculated the average of total P (sulphuric acid method) in Brazilian soils to be 153 mg kg^{-1}, which equals 1800 kg of P_2O_5 (20 cm depth) ha^{-1}. This amount of P is not considered low, but most of it is unavailable for crops, although part of it is used by other plants than agricultural crops, for instance Inga sp, Erythrina, Cajanus etc. (Ae et al., 1990; Rheinheimer et al., 1999). Brazilian soils with these amounts of P can therefore be considered as a P reserve (Resende, 1997), certainly if one takes into account that these soils (mainly Oxisols), are often more than 20 meters deep and can potentially be explored by deep tree roots, as is the case in agroforestry systems.

No effect of system was found on either of the organic fractions ($NaHCO_3$-Po or NaOH-Po) or their sum (Sum-Po). Although higher inputs of organic material (leaves, roots etc) are to be expected in the agroforestry fields, and this could increase the amount of organic P, the cycling rate is also expected to be high which could increase the turnover. Therefore, a large difference in P pools among fields was not expected. However, the significant effect of depth and the significant interaction between system and depth do suggest that trees had an impact on organic P distributions. The amount of organic P decreases less with depth in the agroforestry systems than in conventional systems (Table 2). This can be an indication of the impact of agroforestry systems in P transformation on deeper soil layers compared to the conventional systems.

Table 5. Significance levels (ANOVA) of the effects of system (agroforestry or conventional), age (medium or old), depth (2-3 cm, 10-15 cm and 40-60 cm) and interactions on soil phosphorus (P) mass fractions. Sum-Po is $NaHCO_3$-Po + NaOH-Po, Labile P is Resin-Pi + $NHCO_3$-Pi and -Po + NaOH-Pi and -Po, and Sum-P is labile P + 1M HCl-Pi + concentrated HCl-Pi.

Effects	Sum-P	Labile-P	Sum-Po	Sum Po: Labile-P[1]	Resin- Pi[2]	$NaHCO_3$		NaOH		HCl (1M)-Pi	HClc[3]-Pi	C:Po[4]
						Pi	Po	Pi	Po			
system	***	***	ns	***	*	***	ns	***	ns	*	***	ns
age	***	***	ns	**	***	ns	ns	***	ns	***	***	*
depth	ns	***	***	***	***	***	***	***	**	***	ns	*
system x age	***	***	***	ns	ns	***	ns	***	***	***	ns	***
system x depth	ns	***	***	**	ns	ns	**	*	**	ns	ns	*
age x depth	ns	ns	ns	**	ns	ns	ns	***	ns	ns	ns	ns
system x age x depth	ns	***	ns	*	ns	ns	ns	***	ns	*	ns	ns

[1]Sum Po:Labile, ratio of the sum of organic P to labile P; [2]Pi, inorganic P; [3]HClc, concentrated HCl; [4]C:Po, Total C:Sum Po. Significance levels: *: ($P < 0.05$); **: ($P < 0.01$); ***: ($P < 0.001$); ns: not significant, with sequential Bonferroni corrections.

The ratio of labile P to Sum-P is low. Within the labile pool, a larger fraction is organic than inorganic. The agroforestry systems had consistently higher fraction of Po than the conventional systems. The larger percentage of organic P within the labile pool and the significant effect of agroforestry systems on the absolute amount of organic P in 40-60 cm are likely due to tree biomass additions. Using ^{31}PNMR (Phosphorus Nuclear Magnetic Resonance), we showed that in our study area the agroforestry coffee systems the ratios of organic P to total P and of diester to monoester phosphates are higher than in conventional coffee growing systems (Cardoso et al., 2002b). We also showed that agroforestry fields had a higher amount of roots and more mycorrhizal fungal spores in the deeper soil layers than conventional fields (Cardoso et al., 2002a). Moreover, we observed more earthworms in the agroforestry fields than in the conventional fields during the soil sampling.

The largest P fraction was found with concentrated HCl. This is in agreement with other data for tropical Oxisols (Novais and Smyth, 1999; Cross and Schlesinger, 1995; Tiessen and Moir, 1993). The second largest fraction of P was extracted with NaOH. This is in agreement with the data of Tiessen and Moir (1993) for Brazilian Oxisols, but in disagreement with the results of Lilienfein et al. (2000) for a cerrado Oxisol in Minas Gerais. They found the largest fraction in NaOH, followed by concentrated HCl. For this soil type, Lilienfein et al. (1996) found low resin-P, which is in agreement with the present study, and no diluted HCl-P. The large amount of P in the recalcitrant fractions (HCl) emphasises the need to develop sound management strategies to utilise native soil P more effectively, which can perhaps be achieved by manipulation of plants, such as in agroforestry systems, associated with micro-organisms (Richardson, 2001).

Fractionation of P, as done here, is a static tool to study P and is not sufficient to draw firm conclusions on organic P transformation. So far, we can conclude that in the agroforestry fields the amount of organic P decreases less with

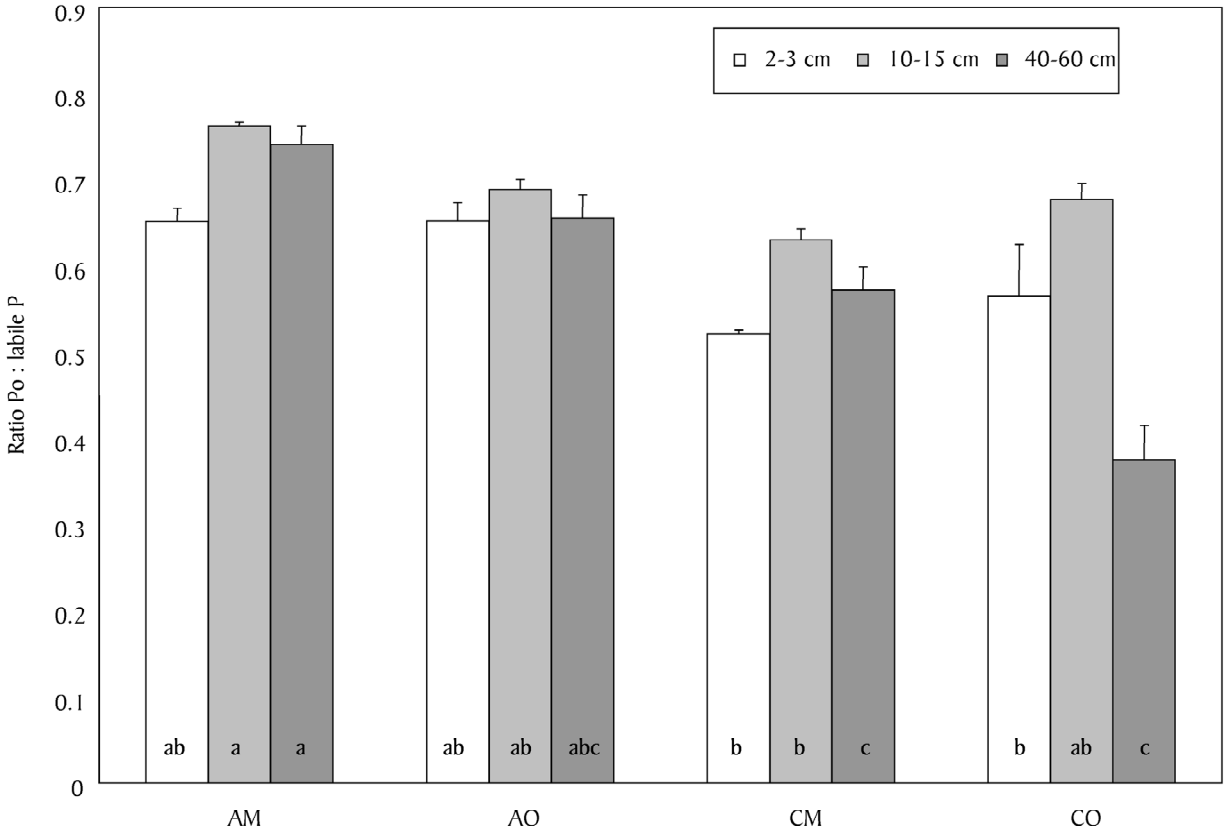

Figure 2. Average (and s.e., n = 3) organic P ($NaHCO_3$ + NaOH) as fraction of the total labile P (Resin + $NaHCO_3$ + NaOH) in medium-aged and old agroforestry coffee fields (AM and AO) and conventional coffee fields (CM and CO) at three depths (2-3, 10-15 and 40-60 cm). Bars with the same letters among depths within fields or per depth among fields are not significantly different at the 0.05 level, with sequential Bonferroni corrections.

depth and the percentage of organic P in labile pools was higher in agroforestry fields than in conventional fields. This suggests that agroforestry influences the dynamics of P through the conversion of part of the inorganic P into organic P. Because the rate of cycling is higher for organic P than for inorganic P, agroforestry would maintain larger fractions of P available to agricultural crops, thereby reducing P losses to the unavailable pools. However, the rate and the impacts of these changes on P cycling and efficiency of P use of the crops in the long-term need to be further examined and understood for full evaluation of the importance of agroforestry in soil P utilisation. Moreover, detailed studies are required for a better understanding of the P transformation in the soil through microbial activity.

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CHAPTER 4

ANALYSIS OF PHOSPHORUS BY ^{31}PNMR IN OXISOLS UNDER AGROFORESTRY AND CONVENTIONAL COFFEE SYSTEMS IN BRAZIL

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Abstract

Phosphorus (P) is the primary limiting nutrient for crop production in highly weathered tropical soils. The deficiency is mainly caused by strong adsorption of $H_2PO_4^-$ to Al- and Fe- (hydr)oxides, which turns large proportions of total P into a form that is unavailable to plants. Soil management modifies P dynamics. Some plants, including trees used in agroforestry systems, are known to accelerate P cycling. We used ^{31}PNMR to evaluate the inorganic (Pi) and organic P (Po) compounds in Oxisol from two agroforestry (15 and 19 years old) and two conventional (full sun, monoculture, ca. 15-20 and 20-24 year) coffee systems, at three different depths (2-3 cm, 10-15 cm and 40-60 cm). We hypothesised that the amounts of 1) organic P and 2) diester are higher in agroforestry fields than in conventional coffee fields and 3) the organic P and the diester decrease less with depth in the agroforestry systems than in the conventional systems. The soils were sampled from on-farm experiments in the Atlantic Coastal Rainforest, Brazil. The soil P was extracted with NaOH 0.5 M + EDTA 0.1 M. Resin chelex-X100 was used to remove the paramagnetic ions. The total P in the NaOH-EDTA extract was measured through ICP and the Pi by the ammonium molybdate-ascorbic acid method. Po was calculated as the difference between total P and Pi. The amount of Po was higher, the decrease of Po with depth was more sharp and the Po : total P lower in the conventional systems than in the agroforestry systems. Based on literature and standards, ^{31}PNMR signals were interpreted as inorganic orthophosphate, orthophosphate monoester (inositol phosphates and mononucleotides), orthophosphate diester (phospholipids, nucleic acids and teichoic acid) and pyrophosphates. The proportion of organic P (Po) was on average 47 %, consisting of monoester (95 %) and diester (5 %). The amounts of diester phosphates did not differ between systems, but the proportion of diester to total spectra areas was higher and the decrease of diester with depth was less in the agroforestry than in the conventional systems. The proportions of inorganic P to total P consisted on average of 45 % orthophosphate and 8 % pyrophosphate. Our results suggest that agroforestry systems influence the dynamics of P through the conversion of part of the inorganic P into organic P. The effect was higher in deeper layers. Because the rate of cycling is higher for organic P than for inorganic P and for diester than for monoester, and because the P in deep layers is normally less available to crop plants, agroforestry would maintain larger fractions of P available to agricultural crops, thereby reducing P losses to the unavailable pools. The rate and the impacts of these changes on P cycling and efficiency of P use of the crops in the long-term need to be further examined and understood, for full evaluation of the importance of agroforestry in soil P utilisation.

Introduction

Phosphorus (P) is a major nutrient in relatively short supply in most natural ecosystems, and the primary limiting nutrient for crop production in highly weathered tropical soils (Linquist et al., 1997). The deficiency is mainly caused by strong adsorption of $H_2PO_4^-$ to Al- and Fe- (hydr)oxides, which turns large proportions of total P into a form that is unavailable to plants (Fontes and Weed, 1996). With the increasing cost and scarcity of soluble P, especially for resource-poor farmers (Ae et al., 1990), it is necessary to increase the availability of soil P and to accelerate cycling of available P. This can be done by intercropping, as in agroforestry systems, using the potential of some non-crop plants to release P from recalcitrant pools, thus making it available to crops (Ae et al., 1990; Palm, 1995). For instance, the Brazilian native palm tree Bactris gaesipaes (palm beach) was found to accelerate P cycling in agroforestry systems (McGrath et al., 2000).

Young (1997) stated that the need for agroforestry systems is particularly great in densely populated, sloping regions in the humid and sub-humid tropics. The soils in these areas have often been degraded by erosion. Typically, the forest cover has been cleared extensively for timber, charcoal, agriculture, and over-cutting degrades what remains. These symptoms frequently contribute to outward migration of land users, as productivity declines to levels that are not economically viable.

In agroforestry systems, trees may increase phosphorus supply by retrieving nutrients from lower soil horizons (Young, 1997). Trees may also increase phosphorus availability through accelerating P cycling by (1) enhancing the chemical and physical quality of soils, and (2) enhancing the biological quality of the soil through increasing microbial activity, for instance, associations with mycorrhiza (Cooper et al., 1996). Microbial activity plays a major role in nutrient turnover in general and in P transformation and redistribution into different inorganic and organic forms in particular (Stewart and Tiessen, 1987).

The most common forms of P found in soils are the inorganic pools (Pi) orthophosphate and pyrophosphate, and the organic compounds (Po) polyphosphate, phosphanate, orthophosphate monoester and orthophosphate diester (Newman and Tate, 1980). Among the organic compounds, monoester is certainly predominant (Dalal, 1977). The monoester phosphates form relatively insoluble complexes with metal ions such as Fe or Al, eventually protecting them from degradation (Anderson, 1980), they are therefore considered moderately labile (Tate and Newman, 1982). The diester phosphates, although found in small amounts in soil, are reported to be more chemically labile than monoester phosphorus (Tate and Newman, 1982), with higher accessibility to microbial or enzyme attack in the soil environment than monoester forms (Dai et al., 1996). Because the diester is considered more labile and more accessible to plants than monoester, the diester to monoester ratio is a measure of organic P lability (Chapuis-Lardy et al., 2001; Turrión et al., 2001). An increase in the ratio of diester to monoester indicates increases in the microbial activity (Turrión et al., 2001), because phosphate diester is considered to be derived primarily from microbial origin (Guggenberger et al., 1996). Microbial activity as well as phosphate diester are dependent on vegetation type and agricultural practice, increasing with increased inputs of plant residues (Hawkes et al., 1984; Bedrock et al., 1994), which is the case in agroforestry systems.

In tropical environments, organic P may be a major source of available P, and may play an essential role in nutrient recycling (Udo and Ogunwale, 1977; Linquist et al., 1997). In soil Po transformations, micro-organisms play a key role (Stewart and Tiessen, 1987). For instance, micro-organisms can solubilize sparingly soluble Pi (Richardson, 2001) and protect the immobilized P against physico-chemical adsorption (a problem in tropical soil) through the gradual release of it via microbial turnover (Magid et al., 1996). The addition of organic material leads to increased microbial activity and the formation of organic phosphorus (Dalal, 1977). Therefore, it is expected that soil management that increases the addition of organic material, like in agroforestry systems, increases the formation of Po. Thus, it is important to study the Po in tropical soils under different management to develop strategies for more sustainable use of the native and applied P.

With the objective to use the potential of the trees to increase the availability of nutrients, especially of phosphorus (Cardoso et al., 2001a), in 1993, several Rural Workers Unions started to implement agroforestry coffee systems in the Zona da Mata of Minas Gerais (Brazil), where the present fieldwork was done. The farmers were assisted by an agriculture-focused non-governmental organisation (NGO), Centre for Alternative technologies of the Zona da Mata (CTA-ZM) and researchers from the Federal University of Viçosa (UFV). The Zona da Mata fully meets the criteria raised by Young (1997) as a region where agroforestry is greatly needed. It is a sub-humid environment with steep hills, largely degraded due to removal of much of its forest cover, with farmers confronted by productivity problems and being tempted to migrate outwards. As the original land cover testifies, the Zona da Mata was once dense forest (Dean, 1996), so existing biophysical conditions, such as soil depth, light and water, are favourable and will not hinder tree growth. Moreover, coffee, the cash-crop of the region, has favourable characteristics for agroforestry (Staver, 1999). It occurs naturally in semi-deciduous native forest in Ethiopia, the microclimatic conditions of which are reproduced in agroforestry systems. Despite this potential, inter-cropping with trees was not important in local land use systems until recent years and only little experience with agroforestry coffee systems existed in the region. The varietal development of full-sun resistant coffee is one of the reasons for agroforestry not being a common practice in the region (Cardoso et al., 2001a).

Since the area has been under forest climax vegetation in the past, it is expected that the ratio of organic P (Po) to total P in soils of cropped fields is lower than e.g. in soils under forest. In the agricultural systems, a major part of P absorbed by the crop is removed from the fields, and thus does not return to the soil. Because we expect an increased

addition of organic material to the soil leading to higher microbial activity under agroforestry, we hypothesised that the amounts of 1) organic P and 2) diester within the organic P are higher in agroforestry coffee systems than in conventional (full-sun, monoculture) coffee systems in the Zona da Mata.

The amount of roots is generally higher in the agroforestry fields than in the conventional systems, also in the deepest layers of agroforestry fields (Cardoso unpubl. data). The larger amount of roots can trigger higher microbial activity, also in the deepest layers. Furthermore, we found (unpubl. data) that the numbers of spores of arbuscular mycorrhizal fungi were higher in deeper layers and lower in layers close to the surface in the agroforestry fields than in the conventional fields. Therefore we also hypothesised 3) that the organic P and diester within the organic P decrease less with depth in the agroforestry fields than in the conventional fields.

To test our hypotheses, we used ^{31}PNMR (phosphorus 31 nuclear magnetic resonance). Although important, the study of organic P in tropical soils has received little attention (Cross and Schlesinger, 1995), one of the reasons being that organic P is not easily analysed. Even though the study of organic P has received more attention with the advent of the ^{31}PNMR during the last two decades, only few ^{31}PNMR analyses of tropical soils are found in the literature.

Traditionally, chromatography techniques have been used to identify specific organic P compounds in the soil (Cade-Menun and Preston, 1996). Nowadays, ^{31}PNMR spectroscopy, a less complex and more direct technique, has been used to estimate various inorganic P and organic P compounds in soil both qualitatively and quantitatively (Newman and Tate, 1980; Cade-Menun and Preston, 1996; Turrión et al., 2000). Although promising, the ^{31}PNMR technique still has several methodological problems to overcome, such as the influence of paramagnetic ions (mainly iron and manganese), which are especially important in tropical soils, because they contain large quantities of those paramagnetic ions.

The objective of this research was to evaluate the various inorganic and organic P compounds at different depths in agroforestry and conventional coffee cultivation systems, using ^{31}PNMR and a methodology suitable to extract P for ^{31}PNMR analyses that is adapted to tropical soils. Working with the same soil, Cardoso et al. (2001b) found that the fraction of Po of the total available pools was higher in the agroforestry fields than in the conventional fields. This result suggests that some trees take up part of the inorganic P that is not available to agricultural crops, and transform it to organic P, which, after being released and mineralised in the soil, becomes available for use by the crops. This process can take place not only close to the surface, but also in deeper layers of the soil and is mediated by soil microbes.

Material and methods

Site description

Our study was carried out in the Zona da Mata, Brazil, as part of ongoing research on agroforestry systems. The Zona da Mata is situated in the Southeast of the state of Minas Gerais, Brazil, in the Atlantic Coastal Rainforest domain (Ab' Saber, 1969). The area has a tropical highland climate (average temperature 18°C, average precipitation 1500 mm, and one dry period of 2-4 months). The parental rock is gneiss. The topography is steep with slopes ranging from 20 to 45% and average altitude ranging from 200 to 1800 m (Golfari, 1975). The dominant soil types in the Zona da Mata are Oxisols, which are deep and well drained, but acidic and poor in available nutrients. The most important crops are pasture and coffee, the latter often being inter-cropped with maize and/or beans. Other crops are sugarcane, cassava and rice (see Cardoso et al., 2001a, for more pedological, agricultural and sociological details).

Soils were collected from two on-farm agroforestry and two on-farm conventional (full sun, monoculture) systems planted with coffee (*Coffea arabica*). The trees of the agroforestry systems were planted between coffee rows. The agroforestry fields were 15 and 19 years of age, further referred to as AM (medium-aged) and AO (old) respectively. The conventional fields were 15-20 and 20-24 years of age, from now on called CM (medium-aged) and CO (old) respectively. Because this was an on-farm study, an exact match of the age of the systems was impossible. The distance among coffee trees was 3.5 m (between rows) x 1.5 m (in rows) or 4 m x 2.0 m. The AM, AO and CM had

around 2000 coffee trees per hectare whereas the CO had around 1300 coffee trees per hectare. Besides coffee trees, each agroforestry field had around 370 trees per hectare. Plant species identified around the sampled spots are listed in Table 1. The farmer mainly harvested coffee berries whereas agroforestry trees were planted for soil conservation and nutrient cycling. To allow more light and air circulation within the agroforestry fields, the low parts of the tree crowns (except coffee) were pruned in winter, leaving the leaves and branches in the field. Moreover, some species such as *Tabebuia serratifolia* and *Schizolobium parahyba* lose their leaves in the winter, which also favours light and air circulation in the system. Spontaneous vegetation was cut down once a year in the agroforestry fields and three times a year in the conventional fields. Other research in the Zona da Mata showed that 67 % of the soil was covered by vegetation in agroforestry fields and 16 % in conventional fields (Cardoso et al., 2001a).

Since farmers only used manual tools, no more than the first five centimetres of the soil were mixed by annual preparation (minimum tillage). Thus, effects of P fertilisation were not expected in the deeper layers collected and analysed in this work. The amounts of N-P-K (20-5-20), limestone and chicken manure that were applied to the fields are summarised in Table 2.

Samples were taken in a landscape that was representative of the region, with slopes at the collection sites ranging from 21 to 36% and the altitudes from 685 to 720 m. All systems were located in the same watershed and the agroforestry systems were adjacent to each other. The conventional systems are one and five km apart from the agroforestry systems respectively. The sites of AM, AO and CM were fallow before they were implanted with agroforestry or conventional coffee, whereas the site of CO was used as pasture.

Sampling and analysis

Samples were collected in June 1999 (dry season), in three profiles (= replicates), approximately 10 m apart, in between the coffee rows, at depths of 2-3 cm, 10-15 cm and 40-60 cm. The agroforestry trees in AM and AO were also located between coffee rows. The position of the profiles was chosen together with the farmers and was expected to be representative of the fields. Approximately one litre of soil was collected from each layer, air-dried and passed through a two-mm sieve for routine analyses. Afterwards the samples were grained and sieved at 0.5 mm.

The soils were analysed according to De Fillipo and Ribeiro (1977) and EMBRAPA (1997) procedures for texture (pipette method), pH (in water), organic matter (Walkley-Black method), calcium and magnesium (extraction with 1 M

Table 1. Plant species identified in the medium-aged (AM) and old agroforestry coffee (AO) fields and in the medium-aged (CM) and old conventional coffee fields (CO) in Zona da Mata, Minas Gerais, Brazil.

Family	Species	AM	AO	CM	CO
Araucariaceae	*Araucaria angustifolia*[1]		X		
Asteraceae	*Bidens pilosa*[2]				X
	Vernonia polyanthes[2]				X
Bignoniaceae	*Sparattosperma* sp.		X		
	Tabebuia serratifolia	X	X		
Caricaceae	*Carica papaya*[1]		X		
Casuarinaceae	*Casuarina equisetifolia*		X		
Cecropiaceae	*Cecropia hololeuca*		X		
Commelinaceae	*Commelina virginica*[2]	X	X	X	
Leguminosae	*Schizolobium parahyba*	X	X		
Malvaceae	*Sida rhombifolia*[2]				X
Melastomaceae	*Tibouchina granulosa*		X		
Musaceae	*Musa* sp.[1]		X		
Myrtaceae	*Stenocalix pitanga*[1]	X			
Pinaceae	*Pinus* sp.		X		
Rosaceae	*Eriobotrya japonica*[2]	X	X		

[1] Fruits and/or crop; [2] spontaneous vegetation.

Table 2. Levels of chemical fertiliser (N-P-K), limestone and chicken manure applied on the agroforestry medium-aged (AM) and old (AO) and in the conventional medium-aged (CM) and old (CO) coffee systems in Zona da Mata (Minas Gerais, Brazil).

	N-P-K[1] (20-5-20)	Limestone	Chicken Manure[4]
AM	130	0.6 ton[2]	3000
AO	130	0.6 ton[2]	0
CM	300	unknown[3]	0
CO	100	100 g [3]	3600

[1] g coffee tree^{-1} year^{-1}; [2] ton ha^{-1}, every year, broadcast; [3] g coffee trees^{-1}, every 4-5 years; last application in CM 4-5 years prior sampling, in CO in the year of sampling; [4] kg ha^{-1} year^{-1} between coffee rows, applied in AM once, in the year of sampling; in CO during 10-14 years, the last application was 10 years prior to sampling.

KCl), and phosphorus and potassium (extractant Mehlich-1, dilute double 0.05 mol L^{-1} HCl in 0.0125 mol L^{-1} H_2S0_4). Total P in soil was the sum of the P pools analysed (Cardoso et al., 2001b) according to the procedure of Tiessen and Moir (1993). The pools are Resin-Pi, 0.5 M $NaHCO_3$-Pi and Po, 0.1 M NaOH-Pi and Po, 1 M HCl-Pi, concentrated HCl-Pi. The soil residue after extraction with concentrated HCl was not further digested in H_2SO_4+H_2O_2, because this extract is unlikely to contain anything but highly recalcitrant Pi (Tiessen and Moir, 1993). No organic P was detected in concentrated HCl (Cardoso et al., 2001b).

^{31}PMMR measurements

In most ^{31}PNMR studies, phosphorus has been extracted by alkali extracts such 0.5 M NaOH (Dai et al., 1996). However, NaOH-EDTA seems to extract more organic phosphorus and cause less hydrolysis of P compounds than NaOH alone (Cade-Menun and Preston, 1996). The use of NaOH-EDTA also increases the extraction of paramagnetic ions such as Fe and Mn that cause broadening and distortion of ^{31}PNMR spectra (Cade-Menum and Preston, 1996). The use of chelating resin (chelex) has been proposed as a method to remove the paramagnetic ions and improve the spectra (Adams and Byrne, 1989; Robinson et al., 1998, Rheinheimer et al., 2002). Chelex is a chelating cation exchange resin that shows a high preference for Fe and other polyvalent metal ions over cations as Na or K (Adams and Byrne, 1989). For these reasons, we decided to use the method summarised in Figure 1. It was based on contributions of several authors (Cade-Menun and Preston, 1996, Robinson et al., 1998) and was adapted to tropical soils by Rheinheimer et al. (2002).

Two strips (9 x 62 mm) of BDH nº 55164 anion exchange membrane were shaken for 16 hours in centrifuge bottles containing three grams of soil and 30 ml of deionized water. The resin extraction serves to remove readily extractable inorganic P and to improve the ^{31}PNMR spectra (Robinson et al., 1998). The resin procedure was repeated with one strip, after which the samples were centrifuged and the supernatant discarded. To the remaining pellets, 30 ml of NaOH 0.5 M + EDTA 0.1 M (from now on called NaOH-EDTA) was added and shaken for 16 hours. Subsequently, the samples were centrifuged. An aliquot of 25 ml was transferred from the supernatant to a plastic snap-cap bottle with 3 cm^3 of chelex-X100, shaken for 6 hours and filtered (Whatman nº 42). The filtrates from the three replicates were mixed, and an aliquot of 45 ml was freeze-dried. The remaining solution was used to analyse total and inorganic P. Inorganic P in the extract was quantified, after removal of organic matter through acidification and centrifugation (Tiessen and Moir 1993), by the ammonium molybdate-ascorbic acid method (Murphy and Riley, 1962). Total P (in the NAOH-EDTA extract) was measured using inductively coupled plasma (ICP). Organic P was taken as the difference between total and inorganic P. The chemical analyses using ICP and Murphy and Riley method were done to allow for comparison with the proportion of P compounds measured with ^{31}PNMR analyses. After filtration, the chelex-X100 was checked for P removed from the extracts.

Each freeze-dried product was dissolved in two ml of D_2O ("heavy water"), shaken for 4 min, kept at room temperature for two hours and then centrifuged. The supernatant was collected and the ^{31}PNMR spectra were recorded with a Bruker DPX-300 NMR spectrometer operating at 121.49 MHz using a 90 degree pulse length, a relaxation delay of 2 seconds, an acquisition time of 0.96 sec and a total accumulation time of 50 min and 42 sec.

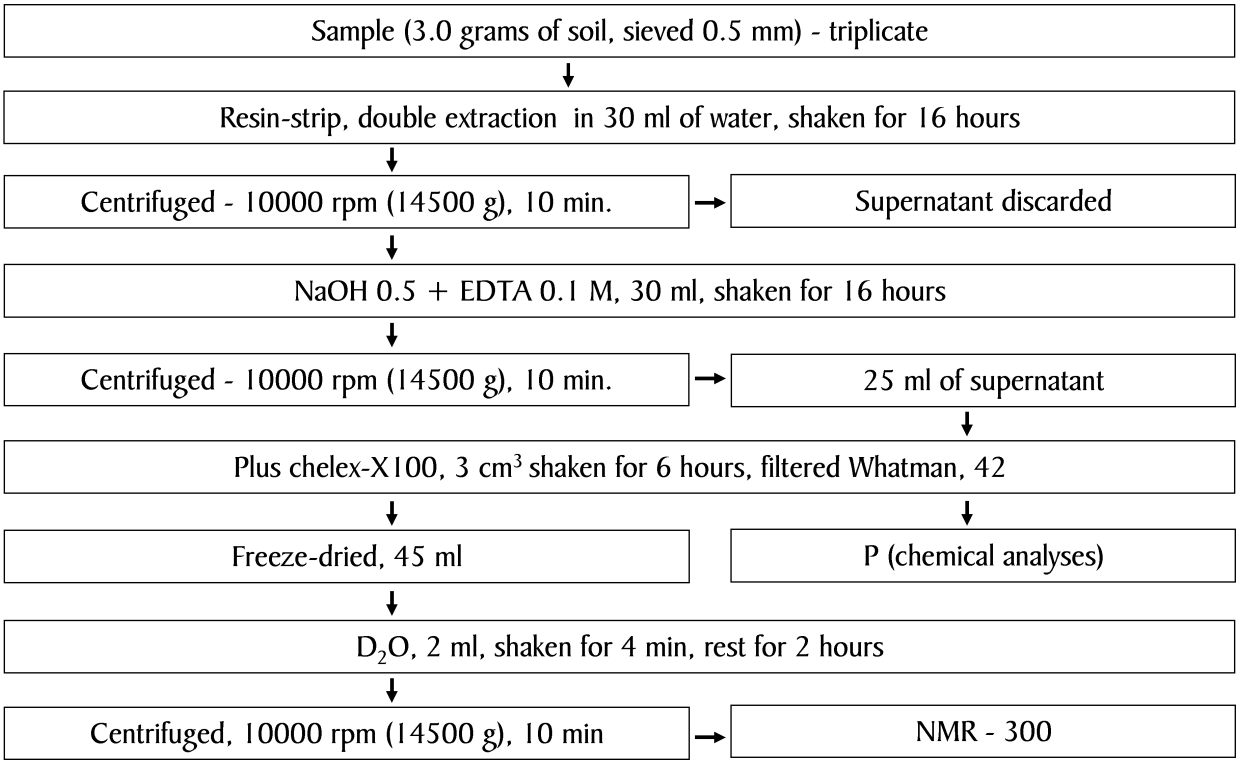

Figure 1. Flow diagram of the extraction method (adapted from Rheinheimer et al., 2002).

The chemical shifts were measured relatively to 85 % orthophosphoric acid using a 58.6 mM/l internal methylenediphosphonic acid trisodium salt tetrahydrate (98 %) standard. This gave a reference line at $\delta = 18.0$ ppm. Quantification was achieved by instrumental integration of the peaks. Interpretations of the spectra were based on literature assignments (Newman and Tate, 1980; Cade-Menun and Preston, 1996; Dai et al., 1996; Rheinheimer et al., 2002) and standards (orthophosphate, glucose-6-phosphate and pyrophosphate). These include: inorganic orthophosphate at $\delta = 5.3$-8 ppm; orthophosphate monoesters (inositol phosphate, sugar phosphates and mononucleotides) at $\delta = 3$-6.2 ppm; orthophosphate diesters (teichoic acid) at $\delta = 0.36$-3 ppm; orthophosphate diesters (phospholipids, DNA and RNA) at $\delta = 1.5$-(-2.0) ppm; and pyrophosphate at $\delta = -2.8$-(-6.0) ppm.

Statistical analyses

Statistical analyses were done using analysis of variance with sequential Bonferroni corrections (Rice, 1988), followed by planned comparisons, tested for significance at the 5 % level (Statistica, StatSoft Inc., 1997). Percentages of phosphorus were arcsine sqrt transformed prior to statistical analyses to satisfy normal distribution and homogeneity of variance assumptions (Sokal and Rohlf, 1995). In these investigations we compared "on-farm" fields that differed in management practices (agroforestry and conventional coffee systems), age (medium-aged and old) at three depths. Using criteria suggested by Hairston (1989) we concluded that individual samples could be treated as independent replicates for a proper ANOVA (testing against error, not testing against interaction). Hairston suggested that the F-ratio of the mean square between fields divided by the mean square within a field is an indication of whether the use of individual samples as replicates is (if $F \leq 1$) or is not (if $F > 1$) justified. We therefore analysed soil data most likely to be unaffected by the various treatment factors. We used soil texture (sand), pH and exchangeable cations in the layer 40-60 cm.

Results

Selected soil characteristics and planned comparisons are presented in Table 3 and can be evaluated as follows, according to the criteria of Soil Fertility Committee of Minas Gerais States, Brazil (CFSEMG, 1989). The soils were clayey and acidic in each system and at all depths; the levels of organic matter were high in the first layers and medium in deeper layers, except in the CO system, which showed a low level in the deepest layer; the concentrations of exchangeable cations (calcium, magnesium and potassium) in the agroforestry systems were high at the surface and medium to low in the deeper layers; Available phosphorus (P-Mehlich) was low at all depths in all systems. Total P in the soil ranged from 370 to 830 mg kg^{-1}. Table 4 shows the significance levels (ANOVA) of the effects of system (agroforestry or conventional), age (Medium or Old), depth (2-3 cm, 10-15 cm and 40-60 cm) and interactions on soil characteristics shown in Table 3. There were effects of system and age, and a significant system x age interaction on Total-P, which can be ascribed to the high values in CO.

Table 5 shows the concentration of total P, Pi and Po (mg kg^{-1} soil) and relative proportions (%) of Po in NaOH-EDTA extracts characterised by chemical analysis. The average Po fraction was 52 %. Table 6 shows the significance levels (ANOVA) of the effects of system (agroforestry or conventional), age (medium or old), depth (2-3 cm, 10-15 cm and 40-60 cm) and interactions on soil P, Pi and Po and ratio Po : P (data shown in Table 5). Conventional fields showed higher amounts of P, total Pi and Po than agroforestry fields (Table 6, $p < 0.01$). The interaction systems x depths was significant for Po (Table 6, $p < 0.001$). The Po decreased more sharply in the conventional fields than in the agroforestry fields. In the agroforestry fields there were no differences in Po between the 10-15 and 40-60 cm, whereas in the conventional fields the Po in 40-60 cm layers was lower than in the 10-15 cm layers (planned comparisons, Table 5). The fraction of Po to total P was higher for the agroforestry fields than for the conventional fields (Table 6, $p < 0.001$). There was an effect of depth on the Po : P ratio (Table 6, $p < 0.001$); This ratio decreased from the 10-15 cm layers to the 40-60 cm layers in the conventional fields, whereas it remained the same in the agroforestry fields (planned comparisons, Table 5). No P was found to be held by the chelex-X100.

Table 3. Mean (n = 3) soil characteristics of agroforestry medium-aged (AM) and old (AO), conventional medium-aged (CM) and old (CO) coffee systems, at three depths (2-3 cm, 10-15 cm and 40-60 cm).

	Depth	AM	AO	CM	CO
clay[1]	2-3	44.0 c[5]	43.0 c	39.0 c	56.0 b
	20-30	65.0 a	49.0 c	45.0 bc	65.0 a
	40-60	66.0 a	45.0 bc	47.0 b	64.0 a
sand[1]	2-3	46.0 a	37.0 bc	41.0 ac	34.0 b
	20-30	31.0 c	34.0 b	36.0 bc	25.0 d
	40-60	30.0 cd	34.0 b	35.0 bc	25.0 d
pH (H_20)	2-3	5.0 bc	5.0 b	4.8 c	4.6 c
	10-15	5.0 b	5.0 b	4.8 bc	4.7 c
	40-60	4.9 b	4.8 b	5.1 a	4.6 bc
organic matter[2]	2-3	40.6 a	40.0 a	39.1 a	38.9 a
	10-15	26.5 ab	22.6 ab	25.4 ab	21.7 ab
	40-60	17.2 b	15.9 b	17.2 b	11.2 b
exchangeable cations[3]	2-3	55.8 a	68.5 a	20.9 b	23.0 b
	10-15	20.7 b	25.7 b	12.2 c	7.8 c
	40-60	11.4 c	7.5 c	7.9 c	5.5 c
P Mehlich-1[4]	2-3	3.1 b	4.5 ab	5.3 a	3.8 b
	10-15	1.2 cd	1.1 d	2.2 c	1.5 cd
	40-60	0.7 d	0.6 d	0.3 d	3.0 b
total P_{soil}[4]	2-3	444.3 c	562.3 b	466.5 c	781.0 a
	10-15	381.1 c	497.5 b	433.3 c	759.6 a
	40-60	413.1 c	531.6 b	371.0 c	828.7 a

[1] %, [2] g kg^{-1}, [3] $Ca^{2+} + Mg^{2+} + K^{+}$, $mmol_c$ kg^{-1}, [4] mg kg^{-1}; [5] Numbers with the same letters either within fields and among depths (columns) or within depths among fields (rows) are not significantly different ($p < 0.05$, planned comparisons).

^{31}PNMR provides information on the forms of phosphorus in the EDTA-NaOH extract. Figures 2 and 3 show representative ^{31}PNMR spectra of the alkali extracts of agroforestry (AM and AO) and conventional (CM and CO) coffee systems at three different depths (2-3 cm, 10-15 cm and 40-60 cm). Signals were interpreted as inorganic P (Pi) orthophosphate at δ = 6.8 ppm and pyrophosphates at δ = -2.9 ppm.; as organic P (Po) orthophosphate

Table 4. Significance levels (ANOVA) of the effects of system (agroforestry or conventional), age (Medium or Old), depth (2-3 cm, 10-15 cm and 40-60 cm) and interactions on soil characteristics (data in Table 3).

	Clay	Sand	pH	Organic Matter	Exchangea-ble Cations	P-Mehlich	Total P_{soil}
system	ns	ns	**	ns	***	**	***
age	ns	**	*	ns	ns	ns	***
depth	***	***	ns	***	***	***	ns
system x age	***	*	ns	ns	ns	ns	***
system x depth	ns	ns	ns	ns	***	ns	ns
age x depth	*	ns	ns	ns	ns	ns	ns
system x age x depth	ns	ns	ns	ns	ns	**	ns

Significance levels: *: ($p < 0.05$), **: ($p < 0.01$), ***: ($p < 0.001$), ns: not significant, with sequential Bonferroni corrections.

Table 5. Mean (n = 3) total P, inorganic P (Pi) and organic P (Po, Total P – Pi) concentrations, and relative proportions (%) of Po to total P in NaOH-EDTA extracts, in agroforestry medium-aged (AM) and old (AO), conventional medium-aged (CM) and old (CO) coffee systems, at three depths (2-3 cm, 10-15 cm and 40-60 cm).

	Depth	AM	AO	CM	CO
total P[1,2]	2-3	139.0 b[5]	97.7 d	140.7 b	194.5 a
	10-15	85.8 c	62.3 e	81.9 e	121.7 b
	40-60	94.3 c	68.7 e	37.3 f	175.7 a
Pi[1,3]	2-3	60.7 c	40.7 de	64.3 c	104.1 b
	10-15	37.4 df	25.5 f	30.1 f	57.0 c
	40-60	47.8 cd	31.3 ef	18.6 f	128.4 a
Po[1]	2-3	78.3 ab	57.0 c	76.4 b	90.4 a
	10-15	48.4 ce	36.8 e	51.8 cd	64.7 d
	40-60	46.5 e	37.5 e	18.7 f	47.3 e
Po : Total P[4]	2-3	56.3 ab	58.3 ab	54.3 b	46.5 c
	10-15	56.4 a	59.1 a	63.3 a	53.2 ac
	40-60	49.3 ab	54.6 ab	50.1 b	26.9 d

[1]mg kg^{-1}; [2]Measured with inductively coupled plasma spectroscopy; [3]Measured with the Murphy and Riley (1962) method; [4]%; [5]Numbers with the same letters either within fields and among depths (columns) or within depths and among fields (rows) are not significantly different ($p < 0.05$, planned comparisons).

Table 6. Significance levels (ANOVA) of the effects of system (agroforestry or conventional), age (Medium or Old), depth (2-3 cm, 10-15 cm and 40-60 cm) and interactions on total P, organic P (Po), inorganic P (Pi) and the Po : P ratio measured in the NaOH-EDTA extracts (data in Table5).

	Total P	Pi	Po	Po : P
system	***	***	**	***
age	***	***	ns	**
depth	***	***	***	***
system x age	***	***	***	***
system x depth	*	**	***	**
age x depth	***	***	ns	ns
system x age x depth	***	***	ns	ns

Significance levels, * ($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$) or not significant (ns), with sequential Bonferroni corrections.

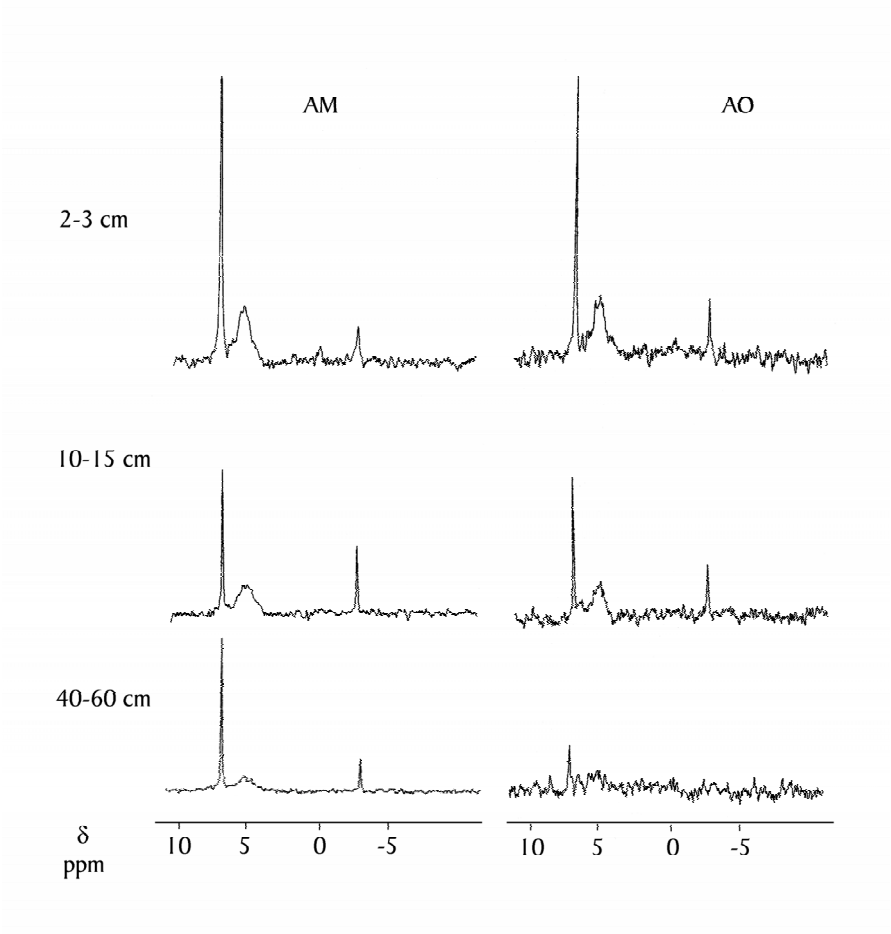

Figure 2. Representative ^{31}PNMR spectra of the alkali extracts of agroforestry (AM and AO) coffee systems, at three different depths (2-3 cm, 10-15 cm and 40-60 cm).

monoester (a group comprising inositol phosphates and mononucleotides) at δ = 5.1 ppm and orthophosphate diester at δ = 1.5 ppm (teichoic acid) and at δ = -0.4 ppm (phospholipids and nucleic acids).

Table 7 shows the amounts of these compounds and the proportions of each compound in relation to the total spectral area measured by ^{31}PNMR (between parenthesis). The amounts of these compounds were calculated by multiplying the proportions measured by ^{31}PNMR with amounts of total P in the NaOH-EDTA extracts measured by chemical analysis (Table 5). The proportion of organic P (Table 7) was on average 47 %, consisting of orthophosphate monoester (on average 95 %) and orthophosphate diester (on average 5 % consisting of 4.5 % phospholipids and nucleic acids and 0.5 % teichoic acid). The ratio of diester to monoester was on average 0.06 (Table 7). The proportions of inorganic P to total P (Table 7) were on average 45 % for Orthophosphate and 8 % for pyrophosphate. Table 8 shows the significance levels (ANOVA) of the effects of system (agroforestry or conventional), age (medium or old), depth (2-3 cm, 10-15 cm and 40-60 cm) and interactions on the concentration orthophosphate monoester, orthophosphate diester, orthophosphate and pyrophosphate and fractions (%) of diester in the whole spectral area and ratio diester : monoester (D : M), (Table 7).

There were significant effects of systems on the inorganic orthophosphate compound ($p < 0.01$) and on the fraction of diester compounds in the whole spectral area, (marginally significant, $p = 0.055$, Table 8). Significant effects of age were found in the inorganic orthophosphate ($p < 0.001$) and monoester ($p = 0.03$, Table 8). Significant effects of depth were found in all compounds in the soils, except pyrophosphate, as well as in the fraction of diester and the diester : monoester ratio (Table 8). The conventional systems contained higher levels of inorganic P than the agroforestry fields. This was mainly due to the CO field (age effect) and first layer of the CM field. The agroforestry fields showed higher proportions of diester than the conventional fields. The concentrations and also the fractions of diester and diester : monoester ratio decreased with depth. The decrease of diester was less sharp with

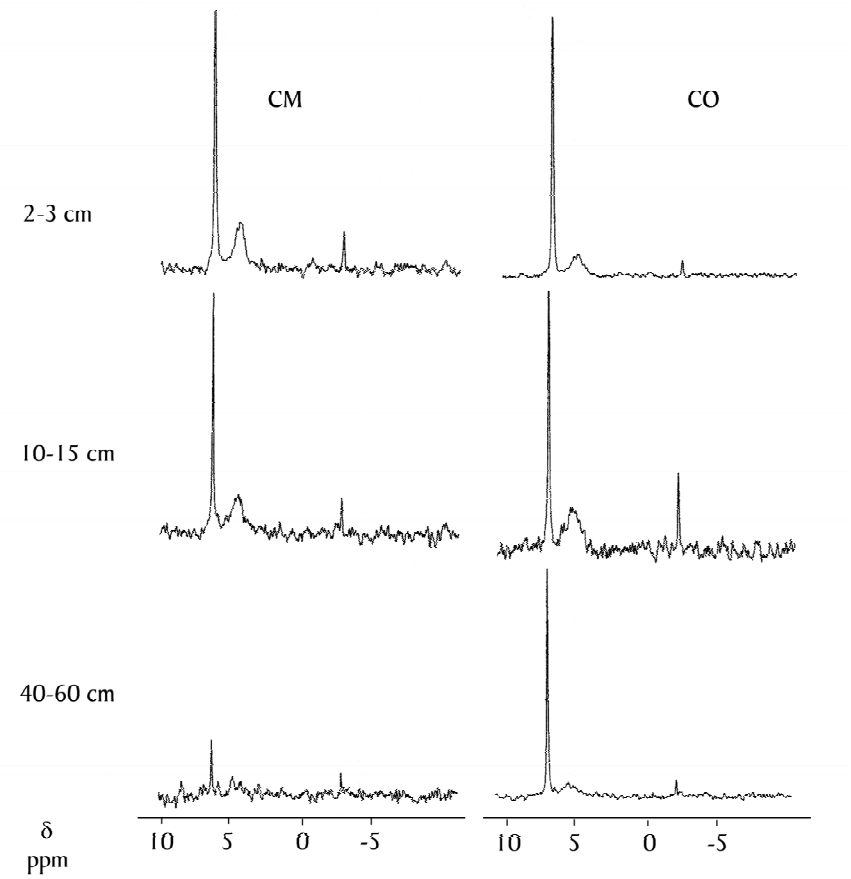

Figure 3. Representative ^{31}PNMR spectra of the alkali extracts of conventional (CM and CO) coffee systems, at three different depths (2-3 cm, 10-15 cm and 40-60 cm).

depth in the agroforestry fields than in the conventional fields. The fractions of diester and the diester : monoester ratio decreased within layers in the conventional fields and remained the same in the agroforestry fields (Table 7).

Figure 4 shows a significant correlation ($R^2 = 0.47$, $p < 0.001$) between the proportions of organic P to total P in the NaOH-EDTA extracts characterised by chemical analysis and ^{31}PNMR spectroscopy. Figure 5 shows a significant correlation ($R^2 = 0.49$, $p < 0.001$) between Total P extracted by NaOH-EDTA and total P soil. On average, NaOH-EDTA extracted 20 % (ranging from 11 – 35 %) of total P soil. Figure 6 shows a significant correlation ($R^2 = 0.82$, $p < 0.001$) between Total P extracted by NaOH-EDTA and P extracted by $NaHCO_3$ plus P extracted by NaOH. On average, NaOH-EDTA extracted 81 % (ranging from 60-100 %) of the P extracted by $NaHCO_3$ plus NaOH.

Discussion

We compared "on-farm" fields that differed in management practices (agroforestry and conventional coffee systems) and age. According to the criteria used for soil fertility evaluation in Minas Gerais (CFSEMG, 1989) as well as the statistical analyses the differences in soil characteristics among the farms were considered slight (Tables 3 and 4). Certainly, the differences were smaller at greater depths where there was little influence of the application of fertiliser and limestone than close to the surface (Table 3).

The amount of P extracted with NaOH-EDTA correlated well with the amounts found with the P fractionation following the procedure of Tiessen and Moir (1993). However, the amounts found with both procedures differ. The low (20 %) P recovery by NaOH-EDTA in relation to total P soil can be explained by a large amount of recalcitrant P

Table 7. Mean (n = 3) concentration of various P forms extracted by NaOH-EDTA estimated from ^{31}PNMR spectra. Values in parentheses are percentage of the whole spectral area allocated to a particular P form.

	Depth	AM		AO		CM		CO	
Organic P									
orthophosphate	2-3	54.0 ab	(38.8)	46.3 b	(47.3)	51.3 ba	(36.5)	61.4 a	(31.8)
monoester[1]	10-30	42.4 dc	(49.2)	32.0 c	(51.3)	41.8 bcd	(50.8)	53.0 ad	(43.6)
	40-60	43.5 ac	(46.6)	39.4 bc	(60.4)	18.0 e	(47.9)	50.2 ac	(28.6)
orthophosphate	2-3	5.3[3,4]a	(3.8) a	3.6 a	(3.8) a	5.9 a	(4.2) a	6.3[3] a	(3.2) a
diester[1]	10-15	2.8 ab	(3.2) a	1.5 ab	(2.6) a	1.9[3] b	(2.5) a	1.6 b	(1.3) a
	40-60	1.7 b	(1.8) ab	2.6 ab	(3.4) a	0.0 b	(0.0) bc	0.7[3] b	(0.4) c
D : M[2]	2-3	0.10 a		0.08 a		0.12 a		0.10 a	
	10-15	0.07 a		0.05 a		0.05 a		0.03 a	
	40-60	0.04 ab		0.06 ab		0.00 b		0.01 b	
Inorganic P									
orthophosphate[1]	2-3	69.6 cb	(50.0)	38.9 c	(39.8)	76.8 b	(54.5)	119.2 a	(60.9)
	10-15	32.6 de	(37.9)	20.0 e	(31.9)	32.5 de	(39.8)	58.5 c	(48.0)
	40-60	43.8 d	(45.9)	23.0 e	(31.4)	13.9 e	(37.3)	115.3 a	(65.6)
pyrophosphate[1]	2-3	10.2 a	(7.4)	8.9 a	(9.1)	6.7 a	(4.8)	7.6 a	(4.1)
	10-15	8.0 ab	(9.7)	8.8 a	(14.2)	5.6 a	(6.9)	8.6 a	(7.1)
	40-60	5.3 bc	(5.7)	3.7 c	(4.8)	5.5 ac	(14.8)	9.5 ab	(5.4)

[1] mg kg^{-1} (between parentheses %); [2] D : M = diester : monoester ratio. [3] In those layers the teichoic acid is included (AM = 0.2; CM = 0.7; AO = 0.5 and 0.2); [4] Numbers with the same letters either within fields and among depths (columns) or within depths and among fields (rows) are not significantly different ($p < 0.05$, planned comparisons; except for the diester, where $p < 0.1$ among fields, depth 40-60cm).

Table 8. Significance levels (ANOVA) of the effects of system (agroforestry or conventional), age (medium or old), depth (2-3 cm, 10-15 cm and 40-60 cm) and interactions on orthophosphate monoester (Po), orthophosphate diester (Po), fractions (%) of diester in the whole spectral area, the diester : monoester ratio, orthophosphate (Pi) and pyrophosphate (Pi) measured by ^{31}PNMR spectroscopy (data shown in Table 7).

	Ortho-phosphate Monoester	Ortho-phosphate Diester	Ortho-phosphate Diester (fractions)	D : M	Ortho-phosphate	Pyrophos-phate
system	ns	ns	‡	ns	***	ns
age	*	ns	ns	ns	***	ns
depth	***	***	**	***	***	ns
system x age	***	ns	ns	ns	***	*
system x depth	*	ns	ns	ns	*	*
age x depth	*	ns	ns	ns	***	ns
system x age x depth	ns	ns	ns	ns	***	ns

Significance levels, * ($p < 0.05$), * ($p < 0.01$) and ***($p < 0.001$), not significant (ns) or ‡ ($p = 0.055$).

extracted by concentrated HCl and not extracted by NaOH-EDTA. Moreover, the P recovery was very high (on average 81 %) and correlated very well with the P extracted by $NaHCO_3$ plus NaOH extractants, which were supposed to extract similar pools as NaOH-EDTA. The pools extracted by $NaHCO_3$ and NaOH are more active in the soil than the pool extracted by concentrated HCl and contain the majority of organic P (Tiessen and Moir, 1993). The low amounts of organic P in the concentrated HCl extractant were confirmed by Cardoso et al. (2001b).

The chemical analysis and ^{31}PNMR spectroscopy provided similar results with respect to the separation of organic

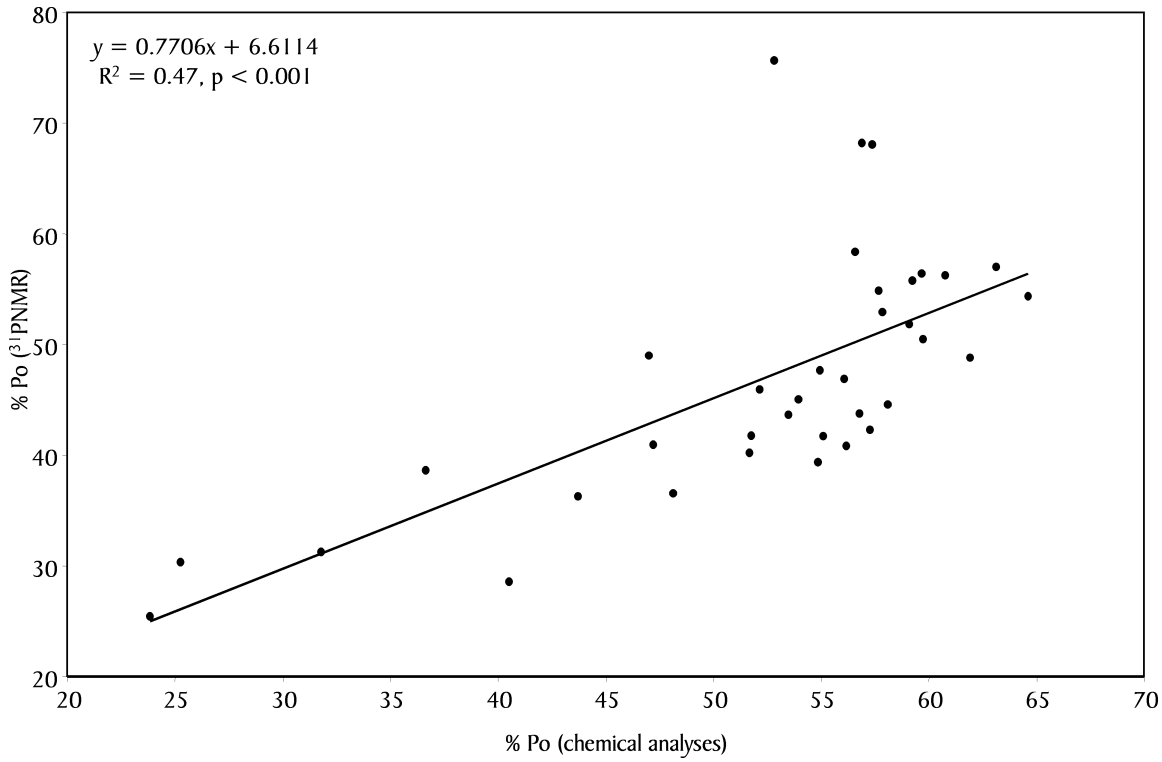

Figure 4. Correlation between the proportion of organic P to total P extracted by NaOH-EDTA as characterised by chemical analysis and ^{31}PNMR spectroscopy

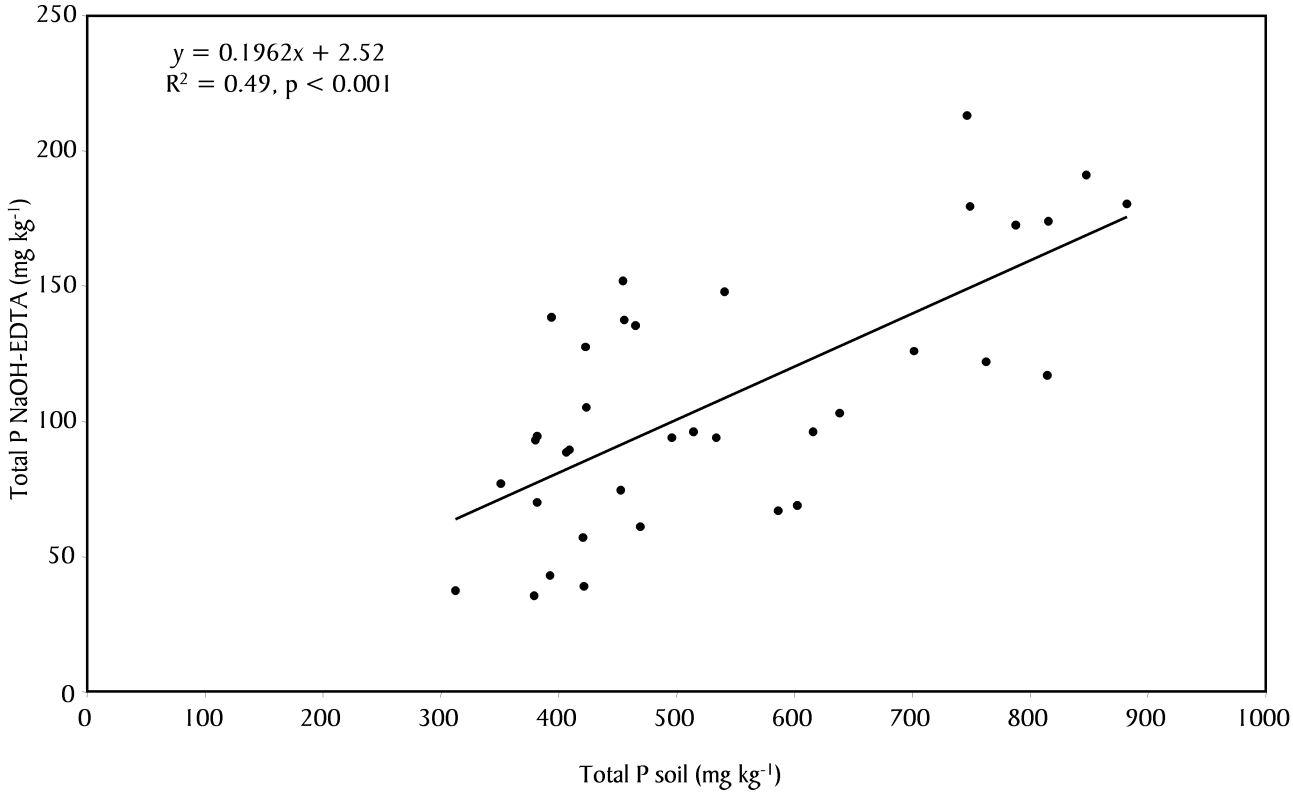

Figure 5. Correlation between the proportion total P extracted by NaOH-EDTA and total P in the soil (sum of the P fractions, Cardoso et al, 2001b, following the procedure of Tiessen and Moir, 1993).

from inorganic P. Differences between the two methods have been reported in the literature and are generally attributed to uncertainties resulting from the indirect chemical method used for evaluating organic P and possible noise in the NMR spectra (Adams and Byrne, 1988; Dai et al., 1996; Guggenberger et al., 1996). The lower levels of organic P detected by PNMR than by chemical analyses may be indicative for some of the larger phosphorus containing molecules not being detected by ^{31}PNMR (Bedrock et al., 1994). Similar results of chemical analysis and NMR analysis (Figure 4) may be an indicator that no significant chemical changes of Po compounds took place during NMR analysis (Zhang et al., 1999). Moreover, our spectra had little noise. These facts seemed to indicate that the methodology used was suitable for ^{31}PNMR analysis in tropical soils.

The central factors controlling soil Po transformations are the concentration of P in the soil solution and the activities of the soil biomass (Stewart and Tiessen, 1987). Our agroforestry systems are expected to have more microbial activity than the conventional systems. Each system had received mineral fertilisation (including P) which was expected to influence the concentration of P in the soil solution, but mainly in the upper centimetres of the soil, since minimum-tillage was used. The CM system had received more fertilisation than the others, whereas the CO system had higher total P content than the other systems (Table 3). Moreover, Campanha (2001) found that, although litterfall was higher in the agroforestry systems, the amount of P in the litterfall was the same as in the conventional systems. This was attributed to the higher content of P in the coffee leaves of the conventional system and because full-sun coffee trees lost more leaves than coffee in agroforestry systems. Taken together, these factors may explain why the conventional fields had more total Pi (Tables 5 and 6) in the NaOH-EDTA than the agroforestry fields. Inorganic P may also increase the content of organic P in the soils (Stewart and Tiessen, 1987). This can be the reason why we found more organic P in the conventional systems than in the agroforestry systems. Therefore, our data do not support our first hypothesis, that the amount of organic P is higher in agroforestry coffee fields than in the conventional coffee fields. We decided to look at the ratio of organic to total P due to the possible build up of organic P due to higher amount of inorganic P in conventional systems. The higher proportion of organic P relative to total P

in the agroforestry systems compared to the conventional systems was most likely due to the effect of agroforestry systems on the dynamics of phosphorus in the soils, through the conversion of inorganic P into organic P (Cardoso et al., 2001b). The significant interaction (Table 6) between system and depth on Po, with a smaller decrease of organic P in depth in the agroforestry fields than in the conventional fields supports our third hypothesis. This suggested that in depth, the agroforestry fields had a higher impact on P dynamics than the conventional fields.

The dominance of monoesters within the organic P forms is consistent with most studies involving alkali extraction (Tate and Newman, 1982; Dai et al., 1996; Rheinheimer et al., 2002). The fraction of diester phosphate (phospholipids, nucleic acids and teichoic acids) was lower (on average 2.5%) than other reports, but is consistent with those found by Rheinheimer et al. (2002) for Brazilian Oxisols. These authors also found less than 4% phospholipids and nucleic acids and no teichoic acids peaks.

The ratio of diester to monoester found was on average 0.06 (Table 7). Which is consistent with the literature (Dai et al., 1996: 0.0 – 2.0; Rheinheimer et al., 2002: 0.05 – 0.12). The lack of effect of system on the amount of diester (Tables 8) did not allow us to confirm our second hypothesis. However, the diester ratios (%) were higher in the whole spectral area (Tables 7 and 8). The slower decrease of diester with depth in the agroforestry fields than in conventional fields is in line with our third hypothesis. These effects may be due to a higher production of plant material and higher microbial activity, especially in deeper layers of agroforestry fields than of conventional fields. Indeed, more roots and more arbuscular mycorrhizal spores have been found in deeper layers of agroforestry fields than in conventional fields and more earthworms were observed in the agroforestry fields than in conventional fields during the soil sampling (Cardoso unpubl. data). Moreover, the D : M ratio decreased less sharply with depth in the agroforestry fields. This ratio may also be an indicator of higher microbial activity over the soil profile in the agroforestry systems than in the conventional fields.

The fraction of pyrophosphate in total P was on average 8 % (Table 7), higher than the average found by Rheinheimer et al. (2002, 4%) and in agreement with the average found by Chapuis-Lardy et al. (2001) for Brazilian

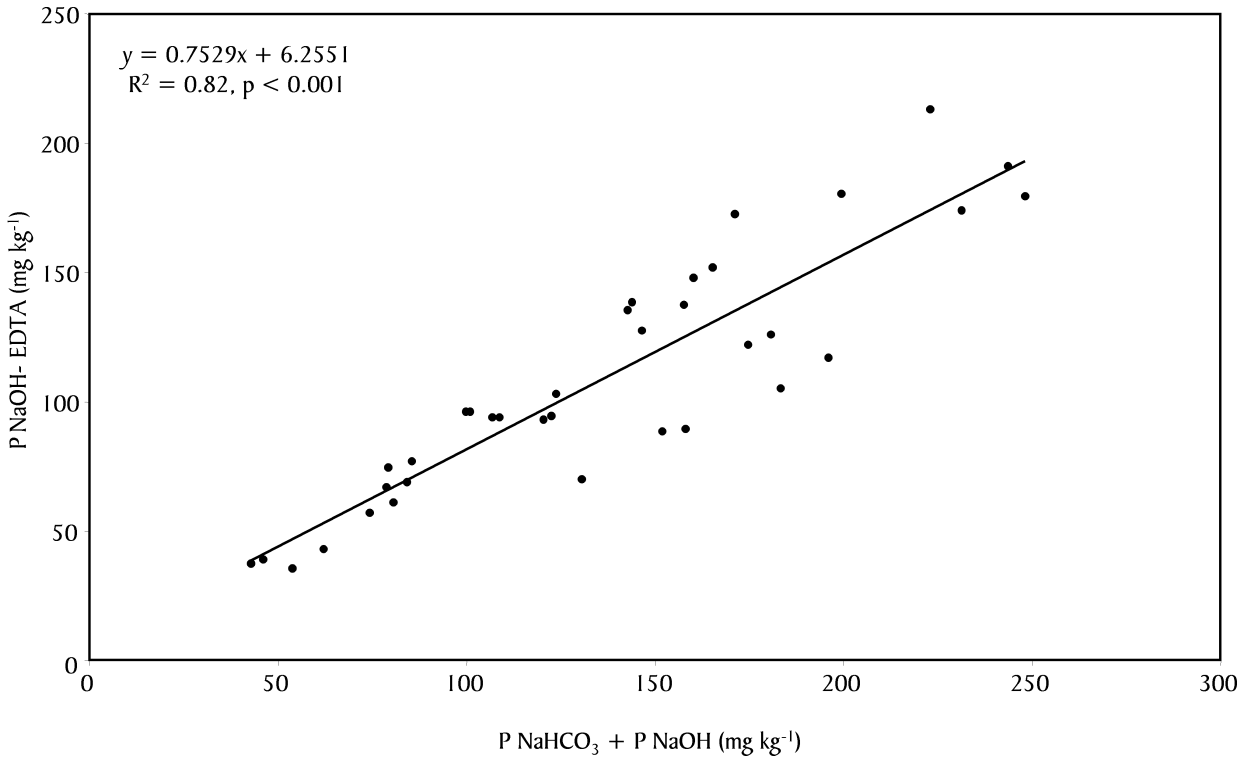

Figure 6. Correlation between the proportion total P extracted by NaOH-EDTA and P extracted by 0.5 M $NaHCO_3$ plus P extracted by 0.1 M NaOH (Cardoso et al., 2001b, following the procedure of Tiessen and Moir, 1993).

Oxisols. Pyrophosphate may originate from hydrolysis of organic esters during the alkali extraction (Condron et al., 1985). However, the extraction method we used is assumed not to cause such hydrolysis (Cade-Menun and Preston, 1996). This would mean that the soils studied here contain high amounts of pyrophosphates. Some authors claim that this is the result of microbial activity in the soil (Condron et al., 1985; Bedrock et al., 1994), but Dai et al. (1996) stated that microbial activity could lead to rapid enzymatic hydrolysis of pyrophosphates, resulting in low concentrations in the soil. Hence, it is difficult to draw firm conclusions from the high levels of pyrophosphate in our samples.

On the basis of our results, it may be concluded that the results of ^{31}PNMR confirmed and extended the results obtained by conventional P extraction procedures (Cardoso et al., 2001b). The procedure used yields clear and reproducible ^{31}PNMR spectra with clear peaks of P compounds. There is evidence that the ratios of organic P to total P and of the diester to the whole spectra were higher in the agroforestry coffee fields than in the conventional coffee fields. Moreover the amount of organic P and of diester decreased less with depth in agroforestry fields than in the conventional fields. Our results suggest that agroforestry influenced the dynamics of P through the conversion of part of the inorganic P into organic P. This influence increased with depth. Because the rate of cycling is higher for organic P than for inorganic P and for diester than for monoester, and because the P in deep layers is normally less available to crop plants, the agroforestry systems would maintain larger fractions of P available to agricultural crops, thereby reducing P losses to the unavailable pools. The rates and the impacts of these changes on P cycling and efficiency of P use of the crops in the long-term need to be further examined and understood, for full evaluation of the importance of agroforestry in soil P utilisation.

Acknowledgements

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CHAPTER 5

VERTICAL DISTRIBUTION OF SPORES OF ARBUSCULAR MYCORRHIZAL FUNGI IN OXISOLS FROM AGROFORESTRY AND CONVENTIONAL COFFEE SYSTEMS IN BRAZIL

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(submitted to *Agroforestry Systems*)

Abstract

We investigated the vertical distribution of arbuscular mycorrhizal fungi (AMF) spores in Oxisols under agroforestry and conventional coffee systems in on-farm experiments in Brazil. Our working hypothesis is that the incidence of spores in agroforestry differs from that in conventional (full-sun, monoculture) coffee systems due to the occurrence of more roots in the deeper soil layers in the former system. The number of AMF spores was considered as an indicator of mycorrhiza incidence in soil. Spores were extracted from 0-1, 2-3, 5-7.5, 10-15, 20-30, 40-60 cm soil-depths in agroforestry and conventional coffee systems, of three different age groups (young, medium-aged and old), using centrifugation methods and counted. Fine roots were collected and dry-weighed from 0-30 cm in young and old systems and from several depths in medium-aged systems. Soils were characterised with respect to texture, pH, organic matter, calcium, magnesium, phosphorus and potassium. Soils were clayey, acidic, with medium to high levels of organic matter, low to high exchangeable cations and low levels of phosphorus. Compared to conventional fields the agroforestry fields showed higher numbers of spores and higher amounts of roots in the deeper soil layers and lower numbers of spores closer to the soil surface. Higher numbers of spores in the deeper soil layers may be explained by higher amount of roots and may be an indicator of higher incidence of mycorrhiza in agroforestry than in conventional coffee systems. Higher mycorrhizal incidence at deeper soil layers in the agroforestry system may change the dynamics of phosphorus cycling in soil, making this nutrient more available to plants.

Introduction

The merits of agroforestry systems in reducing land degradation are widely accepted (Sanchez, 1995; Young, 1997). Agroforestry systems can increase soil nutrient availability and accelerate phosphorus (P) cycling because the deeper tree roots can (1) retrieve nutrients from lower soil horizons (Young, 1997), (2) enhance the chemical and physical quality of soils and (3) increase soil microbial activity (Cooper et al., 1996).

The need for agroforestry systems is particularly great in densely populated, sloping regions in the humid and sub-humid tropics (Young, 1997), for example in the Zona da Mata, Minas Gerais, Brazil. Coffee is the main cash crop in the region and it is cultivated in the hills where problems with soil erosion are accelerated leading to land degradation problems. In 1993, several Rural Workers Unions, assisted by the Centre for Alternative Technologies of the Zona da Mata (CTA/ZM), a Non-Governmental organisation (NGO) and researchers from the Federal University of Viçosa (UFV) started to establish and improve agroforestry coffee systems in the Zona da Mata (Cardoso et al., 2001a). In Zona da Mata, the main objectives of using agroforestry systems are the conservation of non-renewable resources (e.g. soil) and the maintenance of sustainable levels of productivity (Cardoso et al., 2001a). The environmental conditions in the region (i.e. soil depth, light and water) are favourable for growing trees, proven by the fact that the area was originally covered in forest.

Recently (1998), the agroforestry coffee systems in the Zona da Mata were monitored and evaluated (Cardoso et al., 2001a), they were considered to be effective in improving soil conservation when compared to conventional coffee systems which adopt a monocropping approach to production. To improve the efficiency of nutrient recycling in these agroforestry systems, however, a better understanding of the underlying processes is still needed. For this, studies are necessary to evaluate the nutrient dynamics and indicative changes in soil biology in these systems (Cardoso et al., 2001a). Studies on the nutrient dynamics in the same region have already shown differences in the composition of the P pools between agroforestry and conventional coffee systems suggesting that the agroforestry

system have influenced the dynamics of P through the conversion of part of the inorganic P into organic P (Cardoso et al., 2001b; Cardoso et al., 2002b). The activity of soil micro-organisms is known to influence the dynamics of soil P through the conversion of inorganic to organic P. Mycorrhizal fungi are universally predominant micro-organisms in the plant-soil environment and their importance in plant-soil nutrient dynamic processes, especially P, are well known (Brundrett, 1991). The majority of tropical plants are dependant on mycorrhizas, which form mutualistic symbioses with plant roots, for their nutrition (Brundrett, 1991). Tropical trees typically form symbioses with arbuscular-mycorrhizal fungi (AMF) (Siqueira and Saggin-Junior, 2001; Onguene and Kuyper, 2001). Propagules of AMF in the soil, from which the mycorrhizal symbioses can be formed, comprise spores, colonised fragments of root and external mycelia (Lambais, 1996). The majority of fungi that form arbuscular mycorrhizas produce these soil-borne spores (Lambais, 1996). In several cases, a positive correlation between spore numbers and root colonisation has been found (Fischer et al., 1994; Onguene and Kuyper, 2001). Other authors report that sporulation is positively correlated with the growth of mycorrhizal plants (Hetrick and Bloom, 1986; Giovannetti et al., 1988). The spores of AMF are, therefore, considered as an indicator of mycorrhizal incidence.

The majority of studies consider spores and mycorrhizal roots to be concentrated near the soil surface (Brundrett, 1991) at a depth of 0-20 cm. Sampling thus usually occurs only in this top layer of soil where plant roots are most common (Zajicek et al., 1986). Studies evaluating the numbers of spores of AMF in the deeper layers of soil are largely focused on arid ecosystems or dune environments (Zajicek et al., 1986). The vertical distribution of mycorrhizal spores is, however, equally important in agroforestry systems which support trees that have roots in the deeper soil layers. The specific objective of this work is, therefore to investigate the vertical distribution of spores of AMF related to the distribution of roots in soils from agroforestry and conventional coffee systems in order to contribute to a better understanding of the nutrient dynamic processes in the agroforestry coffee systems of Zona da Mata, Minas Gerais, Brazil. Our working hypothesis is that the incidence of spores in agroforestry coffee system differs from that in conventional coffee system due to the occurrence of more roots in the deeper soil layers in the former system. Spores were extracted, counted and fine roots were collected and dry-weighed from different soil-depths in both systems.

Materials and Methods

Site characteristics

The study was carried out in the Zona da Mata, which is situated in the Southeast of the state of Minas Gerais, Brazil, in the Atlantic Coastal Rainforest domain. This region has a tropical highland climate with an average temperature of 18 °C, an average annual precipitation of 1500 mm, and one dry period of 2-4 months. The topography is steep, with slopes ranging from 20 to 45 % and with average altitudes ranging from 200 to 1800 m. In the past, forest covered almost the entire region, but nowadays only about 7 % of the original vegetation remains. The dominant soil types in the Zona da Mata are Oxisols (USDA classification), which are deep and well drained, acidic and have a low nutrient availability. Pedological, agricultural and sociological details of the region are given by Cardoso et al. (2001a).

The local farmers use only manual tools for soil management. The soil is therefore subjected to only minimal tillage (approximately 5 cm depth). Fertilisers are applied in the agroforestry and conventional coffee systems at an annual rate of 150 g of N:P:K (20:5:20) per coffee tree. Limestone is broadcast every year, 0.6 per hectare in AF_{Old} and AF_{Medium}. In the other fields it is applied around coffee trees, every 3-5 years at rates of three tons per hectare. The sites were used either as pasture or natural fallow before they were planted to agroforestry or conventional coffee.

Soil sampling and analyses

Soils were collected from both agroforestry and conventional (full-sun, monoculture) coffee systems of three different ages. Three agroforestry coffee (*Coffea arabica* L.) fields were sampled: 1) 4-5 years old (AF_{Young}), 2) 15 years old (AF_{Medium}) and 3) 19 years old (AF_{Old}) and three conventional coffee (*C. arabica* L.) fields: 1) 5 years old (C_{Young}), 2) 15-20 years old (C_{Medium}) and 3) 20-24 years old (C_{Old}). This was an on-farm study so an exact match of the ages of the

fields was impossible. The fields are, however, located in the same climate and class of soil, so the number of spores can be expected to be a consequence of soil management/use. The samples were collected in June (dry season), in three soil profiles, approximately 10 m apart, in between the coffee rows. This corresponds to the position of the trees in the agroforestry fields. Samples were taken at six depths (0-1, 2-3, 5-7.5, 10-15, 20-30 and 40-60 cm). Approximately one litre of soil was collected at each depth, air-dried and passed through a 2 mm sieve.

An inventory was made of plants around the soil profiles and, except for the coffee plants, these are listed in Table 1. All plant species, except *Pinus* sp., including coffee, belong to families or genera that are demonstrated to form arbuscular mycorrhizas (Siqueira and Saggin-Júnior, 2001; Janos, 1996).

The soils were characterised by texture, pH, exchangeable cations, labile phosphorus and organic matter (Table 2).

Spores of AMF

The spores of AMF were extracted from each soil sample (0-1, 2-3, 5-7.5, 10-15, 20-30 and 40-60 cm) using a wet-sieving/gradient centrifugation technique (Brundrett et al., 1996). This technique was repeated twice with each soil sample because in heavy clay soils some spores may be buried in the soil microaggregates or encircled by clay particles. The number of spores collected, for each soil sample, was counted in a gridline petri dish under a stereomicroscope at x 400 (WILD Heerbrugg M7A, Switzerland). Only apparently healthy spores (those which contained cytoplasm when viewed under transmitted light) were counted.

Table 1. Plant species identified in the young, medium-aged and old agroforestry (AF) and in the young, medium-aged and old conventional coffee systems (C) in the Zona da Mata, Minas Gerais, Brazil. Plants are listed by family and species with local names in parentheses.

	Species (local name)	AF_{Young}	AF_{Mediu}	AF_{Old}	C_{Young}	C_{Medium}	C_{Old}
Araucariaceae	*Araucaria angustifolia* (Bertol.) Kuntze (pinheiro-brasileiro)[a]			X			
Asteraceae	*Bidens pilosa* Linn. (picão)				X		X
	Vernonia polyanthes Less. (cambará)						X
Bignoniaceae	*Sparattosperma* sp. (cinco folhas)			X			
	Tabebuia serratifolia Rolfe. (ipê amarelo)		X	X			
Bombacaceae	*Bombax* sp (castanha mineira)	X					
Bromeliaceae	*Ananas sativus* Schult.f. (abacaxi)[a]	X					
Caricaceae	*Carica papaya Linn.* (mamão)[a]	X		X			
Casuarinaceae	*Casuarina equisetifolia* Linn. (casuarina)			X			
Cecropiaceae	*Cecropia hololeuca* Miq. (embaúba)			X			
Commelinaceae	*Commelina virginica* Forst. f. (trapoeraba)		X	X		X	
Euphorbiaceae	*Joannesia princeps Vell.* (cotieira)	X					
	Manhihot esculenta Crantz (mandioca)[a]	X					
	Ricinus communis Linn. (mamona)	X					X
Lauraceae	*Persea gratissima* Gaertn. f. (abacate)[a]	X					

(Table continued on next page)

Table 1 (continued)

Family	Species (local name)	AF_{Young}	AF_{Mediu}	AF_{Old}	C_{Young}	C_{Medium}	C_{Old}
Leguminosae	*Cajanus cajan* (L.) Millsp.) (guandu)[a]	X					
	Calliandra calothyrsus Meissn. (caleandra)	X					
	Inga vera Willd. (ingá)	X					
	Schizolobium parahyba (Vell. Conc.) S. F. Blake (breu)		X	X			
	Senna macranthera (Colladon) H. Irwin & Barneby (fedegoso)	X					
Malvaceae	*Sida rhombifolia* Linn. (vassoura)						X
	Hibiscus esculentus Linn. (quiabo)[a]	X					
Melastomaceae	*Tibouchina granulosa* Cogn. (quaresmeira)			X			
Musaceae	*Musa sp* (banana)[a]			X			
Myrtaceae	*Stenocalix pitanga* (Berg) Kiaersk (pitanga)[a]		X				
Pinaceae	*Pinus sp* (pinus)			X			
Poaceae	*Pennisetum purpureum* Shum. (capim elefante)	X					
	Digitaria horizontalis Willd. (mulambo)				X		
	Trichachne insularis Ness (gengibre)				X		
Rosaceae	*Eriobotrya japonica* Lindl. (ameixa)[a]		X	X			
	Prunus persica Stokes (pêssego)[a]	X					
Sapindaceae	*Litchi sinensis* J. F. Gmel. (lixia)[a]	X					
Verbenaceae	*Aegiphila sellowiana* Cham. (papagaio)	X					

[a]Fruits and/or crop.

Root sampling

Fine roots, diameter $\leq$ 2 mm were sampled from 0-30 cm depth in each system, but in the agroforestry and conventional fields of medium-age, the roots were sampled in the same six layers as the soil. For each root sample, approximately one litre of soil was collected and washed over a 0.5 mm sieve. To assess whether the roots were mycorrhizal (on a presence/absence basis), they were cleared (10 % KOH) and stained (Trypan blue) using the technique of Brundrett et al. (1996). The level of mycorrhizal colonisation in the roots was not measured because of difficulty in obtaining individual root samples from specified plant species – hence spores were used as a measure of the incidence of AMF.

The fresh weights of the roots were measured. The roots were then dried at 70 °C for 48 hours before their dry weights were measured.

Statistical analysis

Two related issues are pertinent here, viz, what the relevant form of replication is (Mead, 1988) and whether replicates are truly independent (Hairston, 1989). Hairston suggested that the F-ratio of the mean square between a field divided by the mean square within a field is an indication of whether the use of individual samples as replicates is (if $F \leq 1$) or is not (if $F > 1$) justified. We therefore analysed soil data most likely to be unaffected by the various treatment factors. We used soil texture (sand), pH and exchangeable cations in the layer 40-60 cm.

Statistical analyses were performed using the Statistica package. Analysis of variance was used to test the difference in root weights from 0-30 cm among systems and at different depths between AF_{Medium} and C_{Medium} systems. For root weights from 0-30 cm the analysis of variance was followed by an LSD post-hoc test. The numbers of spores were natural log transformed to satisfy normal distribution parameters and homogeneity of variance assumptions. The t-test was used to test differences between two means.

Results

The characteristics of the soils are shown in Table 2, and can be evaluated according to the criteria of the Soil Fertility Committee of Minas Gerais State, Brazil: the soils were clayey and acidic in all fields and at all depths. The levels of exchangeable cations (calcium, magnesium and potassium) are high or medium in the top soil layers and low in the deeper soil layers. The contents of both available P and organic matter were highest in the top soil layers, decreasing to the deeper soil layers in each of the six coffee fields sampled.

Root biomass

Figure 1 shows the dry weight of roots in both the agroforestry and conventional coffee systems of medium age. The dry weights of roots from AF_{Medium} and C_{Medium} were different among depths ($p < 0.0001$) and between coffee system ($p = 0.0005$). They were higher in the agroforestry coffee system than in the conventional coffee system. The interaction between depth and system was also significant ($p = 0.0036$). In the conventional coffee system the dry weight of roots decreased more sharply with depth than in the agroforestry coffee system.

The dry weights of the roots sampled from 0-30 cm are shown in Figure 2. There was a significant effect of coffee system ($p = 0.003$) and age ($p = 0.003$), as well as a significant interaction ($p = 0.006$), on the dry weight of roots sampled at 0-30 cm. The agroforestry coffee system had signifcantly more roots in the top 30 cm of soil compared to the conventional coffee systems at all ages except the AF_{Young} and C_{Young} fields which did not significantly differ (Figure 2). The AF_{Medium} field had significantly more roots than either the AF_{Young} or the AF_{Old} fields (Figure 2). The C_{Young} field had signifcantly more roots than either the C_{Medium} or C_{Old} fields (Figure 2).

Mycorrhizal colonisation

The roots sampled from all soil depths in all fields were colonised by AMF.

Spores of AMF

The numbers of spores of AMF (g^{-1} soil) for the conventional and agroforestry coffee systems are shown in Figure 3. Significantly higher numbers of spores of AMF were found in the surface layers of soil (0-1, 2-3 and 5-7.5 cm depth) in the conventional coffee fields compared to those in the surface layers of soil in the agroforestry coffee fields. In the deeper soil layers (40-60 cm depth), agroforestry coffee fields had significantly higher numbers of spores of AMF than in the conventional coffee fields (Figure 3). The Linear regressions of the numbers of the spores of AMF (log transformed) in the soil for agroforestry and conventional coffee systems are shown in the top right-hand corner of Figure 3 (Figure 3a). The conventional and agroforestry coffee systems showed significant differences in both intercept ($p = 0.0129$) and slope ($p < 0.0001$). All agroforestry fields as well as all conventional fields were taken together (Figure 3a) because the intercepts and slopes of conventional and agroforestry coffee systems were the same when different fields of the same age were compared, i.e, C_{Old} had a higher intercept and a less steep slope than AF_{Old}, C_{Medium} than AF_{Medium}, C_{Young} than AF_{Young} (data not shown). The difference was not significant for the intercept of C_{Old} vs A_{Old}. In all fields, the number of spores decreased markedly below a soil depth of 15 cm (Figure 3a). In the agroforestry fields, the number of spores of AMF ranged from 79 to 89 % (0-15 cm) and 12-21 % (20-60 cm). In the conventional fields these ranges were 88 to 97 % (0-15 cm) and 3-12 % (20-60 cm).

Table 2. Soil characteristics of the young, medium-aged and old agroforestry (AF) and the young, medium-aged and old conventional coffee systems (C), at three soil depths. Numbers in parentheses are s.e. (n = 3).

Soil characteristics	Depths (cm)	AF_{Young}		AF_{Medium}		AF_{Old}		C_{Young}		C_{Medium}		C_{Old}	
clay (%)	2-3	38	(2.19)	44	(2.96)	43	(2.19)	36	(0.88)	39	(2.60)	56	(2.40)
	20-30	54	(1.67)	65	(2.33)	49	(2.08)	52	(1.45)	45	(3.79)	65	(0.33)
	40-60	54	(2.60)	66	(0.88)	45	(1.33)	56	(2.33)	47	(1.76)	64	(2.33)
sand (%)	2-3	47.3	(0.67)	45.7	(0.67)	37	(1.73)	51	(1.15)	41	(3.06)	34	(2.89)
	20-30	34.7	(0.88)	31	(1.53)	34	(1.15)	37.3	(0.88)	36	(3.61)	25	(0.58)
	40-60	35.3	(0.88)	29.7	(0.67)	34	(0.58)	33.3	(0.88)	34.7	(4.63)	25.3	(1.20)
pH (H_20)	2-3	5.0	(0.03)	5.0	(0.15)	5.0	(0.06)	5.0	(0.06)	4.8	(0.06)	4.6	(0.00)
	10-15	5.2	(0.10)	5.0	(0.12)	5.0	(0.07)	5.0	(0.03)	4.8	(0.09)	4.7	(0.00)
	40-60	5.2	(0.15)	4.9	(0.09)	4.8	(0.07)	5.2	(0.03)	5.1	(0.00)	4.6	(0.09)
exchangeable cations : Ca^{+2} + Mg^{+2} + K^{+} ($mmol_c\ kg^{-1}$)	2-3	33.8	(0.82)	55.8	(0.11)	68.5	(0.47)	42.3	(0.47)	20.9	(0.38)	23.0	(0.36)
	10-15	26.2	(0.90)	20.7	(0.12)	25.7	(0.42)	13.7	(0.15)	12.2	(0.33)	7.8	(0.08)
	40-60	15.8	(0.95)	11.4	(0.38)	7.5	(0.04)	5.1	(0.08)	7.9	(0.16)	5.5	(0.06)
P Mehlich-1 ($mg\ kg^{-1}$)	2-3	1.86	(0.24)	3.08	(0.68)	4.52	(0.52)	2.17	(0.30)	5.26	(0.38)	3.85	(0.73)
	10-15	0.94	(0.24)	1.22	(0.29)	1.13	(0.13)	1.11	(0.06)	2.23	(0.41)	1.46	(0.12)
	40-60	0.66	(0.14)	0.70	(0.10)	0.61	(0.20)	0.33	(0.07)	0.33	(0.17)	2.99	(0.22)
organic matter ($g\ kg^{-1}$)	2-3	62.1	(6.90)	40.6	(5.88)	40.0	(1.79)	48.1	(1.43)	39.1	(3.93)	38.9	(4.78)
	10-15	46.9	(4.91)	26.5	(3.25)	22.6	(3.78)	32.6	(3.27)	25.4	(2.99)	21.7	(1.86)
	40-60	38.2	(2.64)	17.2	(5.39)	15.9	(2.09)	21.8	(1.89)	17.2	(4.47)	11.2	(1.87)

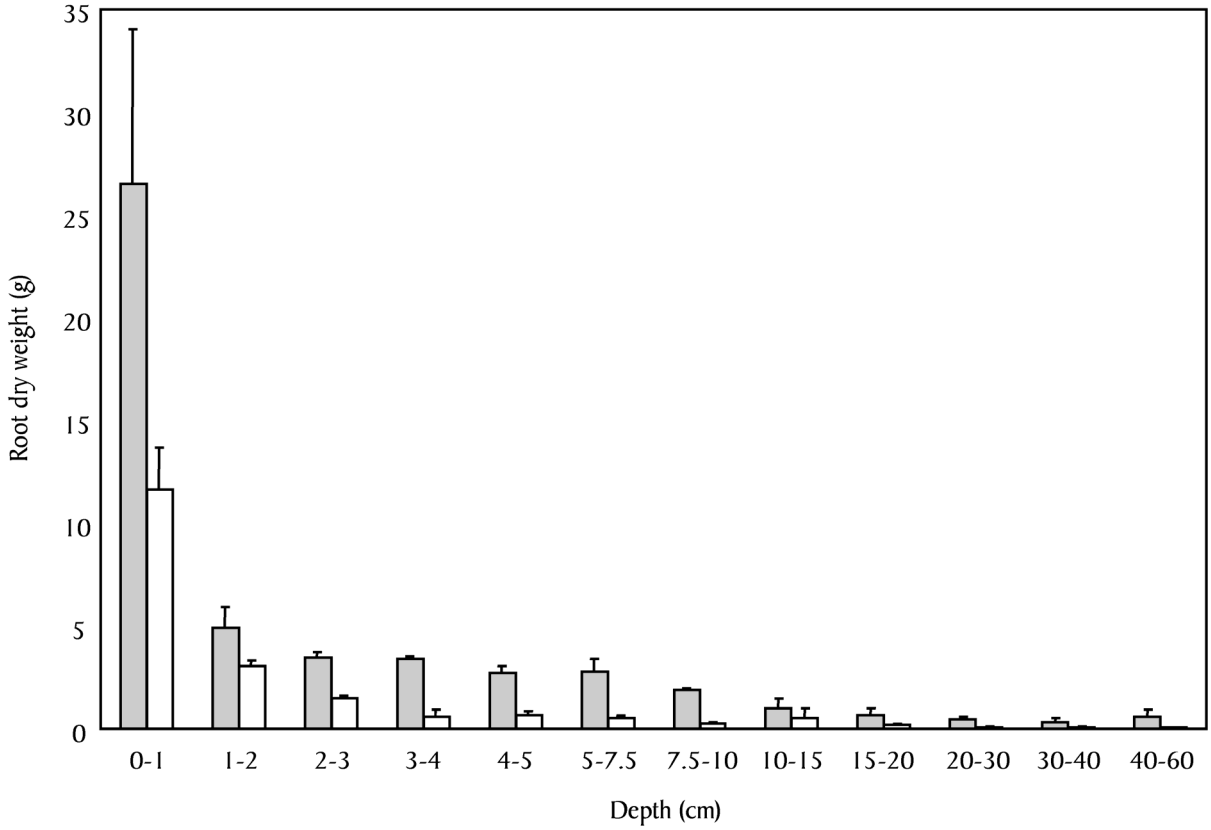

Figure 1. The dry weight of roots (g) in the agroforestry (gray bars, regression equation: $23.95*(depth)^{-1.5}$, $R^2 = 0.90$) and conventional coffee systems (white bars, $13.52*(depth)^{-2.0}$, $R^2 = 0.92$) of medium-age at different soil depths.

The relationship between root weight and numbers of spores of AMF at different soil depths for the fields AF_{Medium} and C_{Medium} are shown in Figure 4 (AF_{Medium} , $R^2 = 0.64$, C_{Medium}, $R^2 = 0.91$). There were more spores of AMF per gram of roots in the conventional than in the agroforestry coffee systems (Figure 4).

Discussion and Conclusions

In these investigations we compared "on-farm" coffee systems that differed in management practice (agroforestry and conventional) and age. Such "on-farm" investigations cannot always be easily fitted into the framework of controlled experiments due to inherent high levels of variation in data from field work (Huxley and Mead, 1988). Despite this the F-ratio for the soil texture and exchangeable cations was smaller than 1. Though the F-ratio for the pH was larger than 1, the difference in pH was minor and according to the criteria used for soil fertility evaluation in Minas Gerais the differences in soil characteristics among the farms are considered slight (Table 2). Certainly those differences become lower at deeper soil depths where there is less or no influence of fertiliser and limestone additions than closer to the soil surface. We therefore concluded that individual samples could be treated as independent replicates. The samples were taken 10 m apart and spore abundance is unlikely to show positive covariation at this distance. Therefore, from a biological perspective, these samples are also independent. Thus, qualitative and quantitative differences concerning spores of AMF have not likely been caused by differences in soil characteristics between systems, but probably by differences in soil use and tree management.

The numbers of spores of AMF (ranging on average from 2 to 130 g^{-1} soil) found in the coffee systems are not as low as expected in undisturbed humid tropical soils with evergreen rainforest, which are considered to contain few AMF spores (Janos, 1996). MacGee et al. (1997) considered a density of spores from 3.6 to 212 spores g^{-1}, to be high, and assumed that 5 spores g^{-1} soil would be required to initiate maximum levels of colonisation, considering that

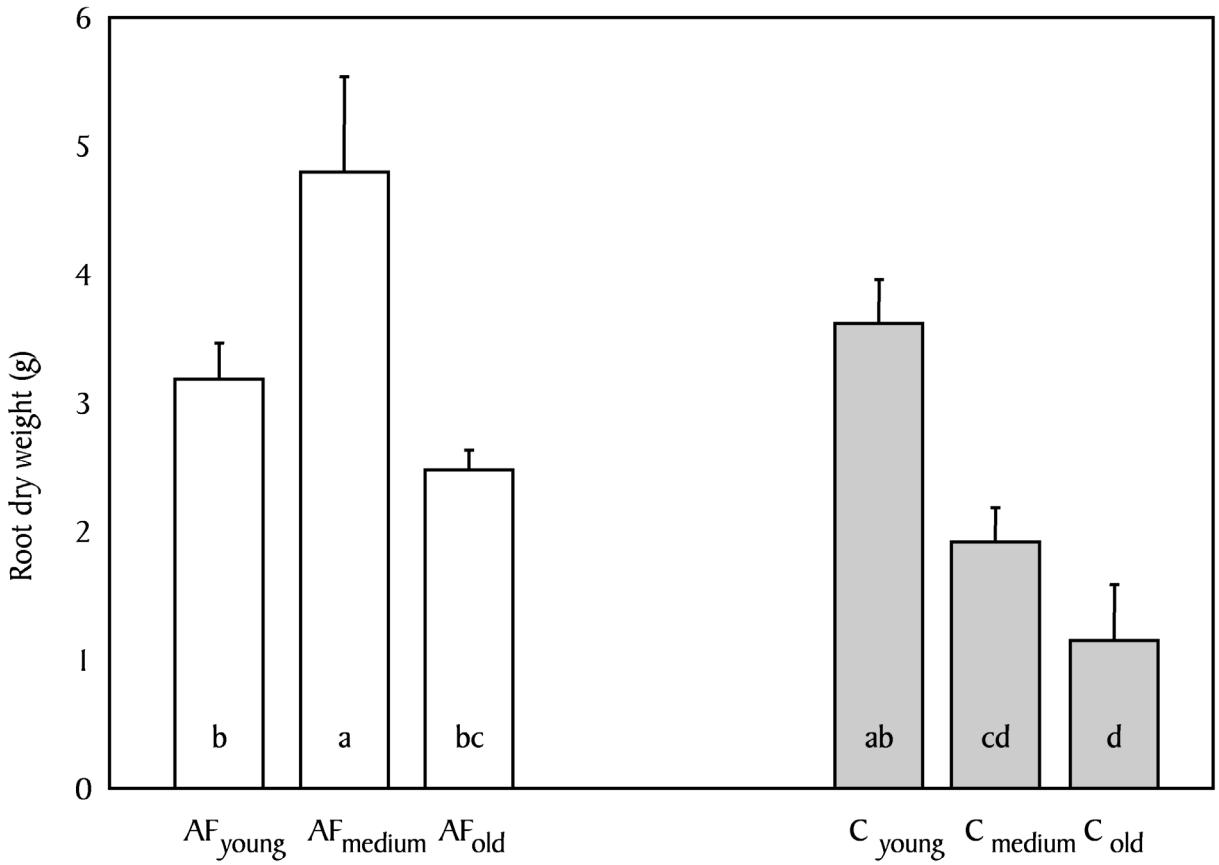

Figure 2. The dry weight of fine roots (g kg^{-1} soil) from 0-30 cm depth for the young, medium-aged and old agroforestry (AF) and the young, medium-aged and old conventional coffee systems (C). Bars with the same letters are not significantly different at P = 0.05 (n = 3).

normally only 5 % of the spores germinate. Saggin Júnior and Siqueira (1996) state that 0.5 spores g^{-1} soil is enough for good root colonisation in coffee seedlings. However, no information is available for mature plants in the system for the region studied here. The high spore numbers in these coffee systems can be explained by the low levels of disturbance in the fields studied here, where minimal tillage management is practised. The specific environmental conditions for this part of Atlantic Coastal Rainforest, where a dry period occurs, are another possible cause for the high numbers of spores of AMF. The sampling was done in the dry season (June), when a higher abundance of spores can be expected (Guadarrama and Álvarez-Sánches, 1999).

Clearly, the numbers of spores of AMF and the amount of roots decline with increasing soil depth in both coffee systems (Figure 3 and Figure 1). Similar results were found by Kabir et al. (1998). There were significantly higher numbers of spores of AMF close to the soil surface in the conventional than the agroforestry coffee systems. A wide range of environmental factors can influence AMF spore production. Spore production can be influenced by the root length of the plant. The amount of roots in the surface soil layers is higher in the agroforestry coffee fields than in the conventional coffee fields and may reduce the need for uptake of P by mycorrhizas, hence their colonisation and subsequent spore production. The main difference found in the soil analyses concerns the exchangeable cations. The AF_{Medium} and AF_{Old} fields showed higher amounts of exchangeable cations at the surface compared to the other fields. This can be attributed to the more frequent use of limestone in the AF_{Old} and AF_{Medium} than in the other fields, combined with environmental conditions in agroforestry fields, such as less erosion, also contributing to a higher impact of the limestone. Limestone has been shown to increase the growth of crop plants in Oxisols (Novais and Smyth, 1999) and may contribute to the increased root growth by plants in the surface soil layers of the agroforestry coffee fields. The coffee systems also showed some differences in plant-available P, although there were no

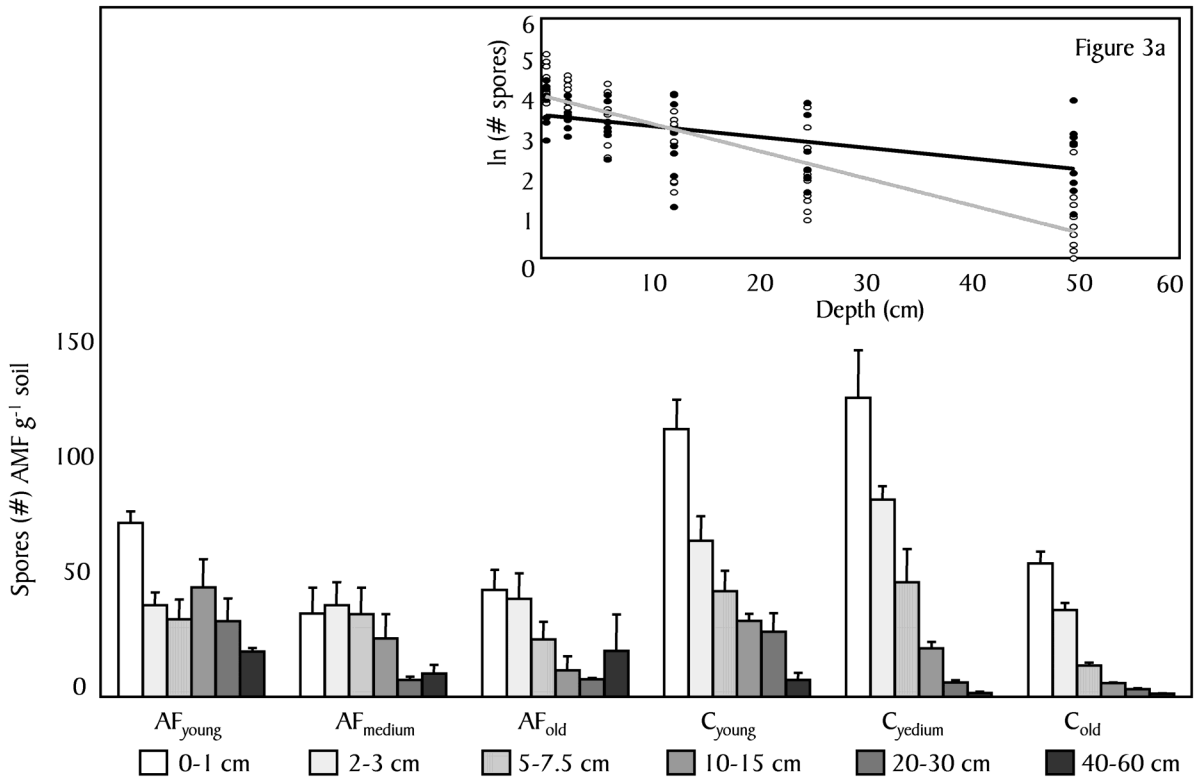

Figure 3. The numbers of spores of AMF (g^{-1} soil) for the young, medium-aged and old agroforestry (AF) and the young, medium-aged and old conventional coffee systems (C) at different soil depths. The figure in the top right-hand corner (a) is the regression analysis of the natural logarithm (ln) of the number (#) of spores of AMF as a function of soil depth in the agroforestry (● black line: 0.0269*depth + 3.5873, $R^2 = 0.30$, $p < 0.001$) and conventional coffee systems (❍ gray line: -0.068*depth + 4.0792, $R^2 = 0.71$ ($p < 0.001$). Both intercepts ($p = 0.0129$) and slopes ($p < 0.0001$) differ significantly at $p = 0.05$.

consistencies with system or age. Plant-available P was always low, according to the standard provided by Soil Fertility Committee of Minas Gerais. For each depth at all sites organic matter is fairly similar except in the AF_{Young} field.

Environmental differences are, however, also important factors determining spore production by AMF. It is known that high temperature and high light intensities can increase AMF sporulation (Hetrick, 1984; Brundrett, 1991; Guadarrama and Álvarez-Sánches, 1999). These characteristics could be responsible for the higher amounts of spores of AMF close to the soil surface in the conventional than in the agroforestry coffee systems. Due to lower shade levels increasing both temperature and light conditions at the soil surface, more spores are expected in the top soil layers of conventional than in agroforestry coffee systems.

The numbers of spores of AMF declined less sharply with increasing soil depth in the agroforestry than in the conventional coffee systems. Our data seem to corroborate the idea that the main reason for having more spores in the deeper soil layers in the agroforestry as compared to the conventional coffee fields is the presence of more roots in the deeper soil layers in the agroforestry fields. More roots occur in deeper layers of the medium-aged agroforestry field than in those of the medium-aged conventional field, for example (Figure 2). The spores found in the deeper soil layers must have been the result of colonisation of roots by AMF, followed by spore production, because light and temperature differences are negated at the deeper soil layers and roots are a necessary condition for growth and sporulation of AMF (Janos, 1996; Brundrett, 1991).

Root colonization, another indicator of mycorrhizal incidence, is very difficult to verify in systems with a high diversity of tree species as it is impossible to obtain individual root samples from identified plant species. However, sporulation and root colonisation are two phenomena that are often closely related, so factors that stimulate or inhibit sporulation stimulate or inhibit colonisation as well (Daft and Nicolson 1972). Our results indeed show a strong relationship between weight of the roots and number of spores for deeper soil layers of the medium-aged agroforestry

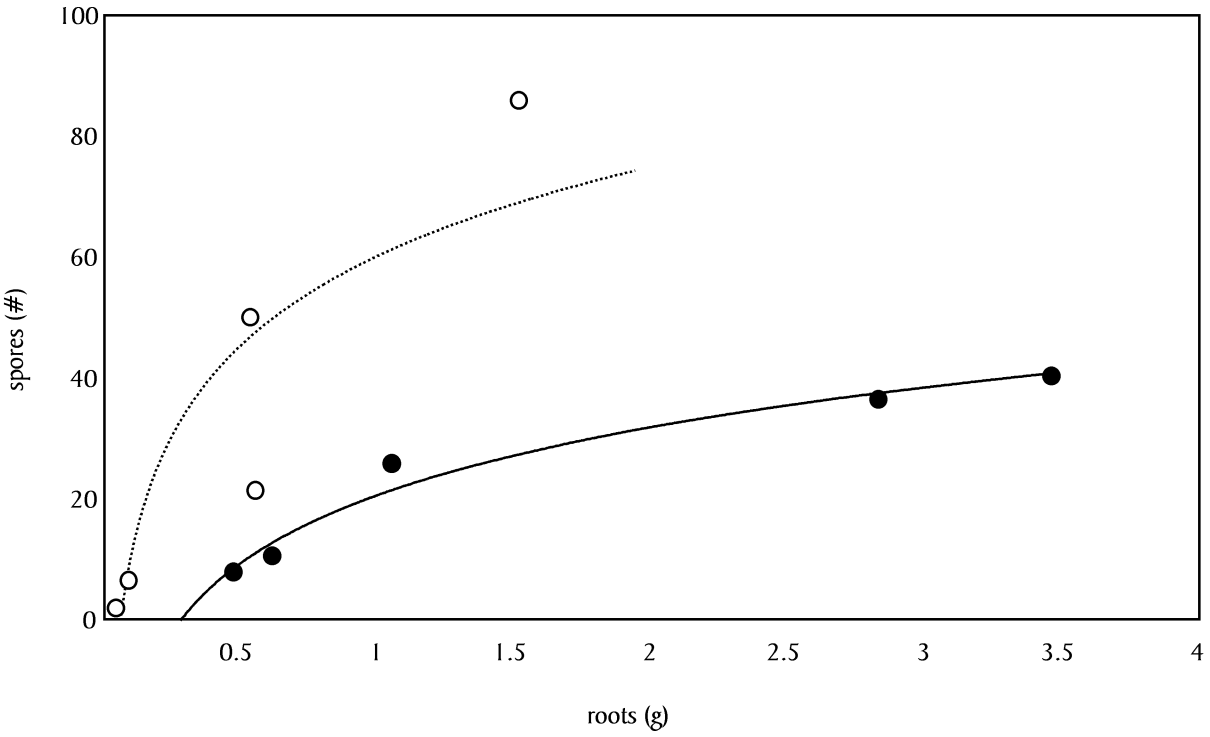

Figure 4. The relationhip between the root fresh weight and the number of spores of AMF (g^{-1} soil) at different soil depths for agroforestry (● full line, regression equation: 16.21*ln (roots) + 20.61, R^2 = 0.97) and conventional coffee systems (❍ dashed line, 21.29*ln (roots) + 60.20, R^2 = 0.79) of medium-age.

and conventional coffee systems (Figure 4). For the time being, we cannot explain why the number of spores per gram of roots is higher in C_{Medium} than in AF_{Medium}. A possible explanation could be a trade-off between below-ground carbon allocation to fine roots and reproductive mechanisms of mycorrhizal fungi; a difference in plant species composition between different systems (Table 1); differences in fungal turnover rates in both systems, etc. Our data do not yet allow an evaluation of these various alternative explanations.

In conclusion, we found spores of AMF in all soil layers in both coffee systems but spore numbers were relatively low in the deeper soil layers. The vertical distribution of spores under agroforestry and conventional coffee systems was different. The agroforestry fields showed higher number of spores in the deeper soil layers and the conventional fields showed higher numbers of spores closer to the soil surface. Higher numbers of spores in the deeper soil layers in the agroforestry than in conventional coffee systems may be an indicator of higher incidence of mycorrhiza in deeper layers of agroforestry fields than in conventional fields. This may increase P recycling from the deeper layers in the agroforestry fields and may change the dynamics of P in the soil, for instance changes in the pools of P (Cardoso et al., 2001b). In some Brazilian soils the amount of P is not so low but mainly present in unavailable forms (Cardoso et al., 2001b). In such soils, a higher level of mycorrhizal activity in the deeper soil layers may be important to make more P available to the plant, thus increasing the efficiency of nutrient recycling processes in these coffee systems.

Acknowledgements

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CHAPTER 6

PHOSPHORUS ACQUISITION BY MYCORRHIZAL AL-RESISTANT MAIZE IN STRONGLY P-FIXING SOILS. THE DOUBLE POT-DOUBLE COMPARTMENT APPROACH

Irene M. Cardoso, Claire L. Boddington, Bert H. Janssen, Oene Oenema, Thomas W. Kuyper

Abstract

We analysed if AMF could take up P from pools that are normally considered unavailable to plants, a controversy present in the literature. We cultivated (90 days) in acid P fixing soils (A and B horizons), in the greenhouse, an aluminium resistant maize variety inoculated with *Glomus* or uninoculated, supplied with nutrient solution without phosphorus. We used the double compartment - double pot approach (here described) with an inner compartment made of fine or coarse mesh. The fine mesh limited the access of plant roots to more volume of soil. During the growth of the plants we measured the shoot sizes five times. When harvested, we quantified the fresh and dry weights and the P content of the shoots and roots, the root colonisation and the root and mycelium lengths from the outer compartment. Soil P fractionation was done prior and after treatments. All measured parameters were higher for the mycorrhizal than the non-mycorrhizal treatments and higher for plants grown in the A than in the B soil horizon. Whether roots could access more soil volume did not cause any significant effect. P in the A and B soil horizons (~270 mg soil kg^{-1}) were differently distributed among the pools. Mycorrhizal plants depleted the Resin-Pi and $NaHCO_3$-Pi, used some of the NaOH-Pi and used neither the recalcitrant nor the organic P fractions. A P balance showed that non-mycorrhizal plants could not acquire P from the soil and P found in the plants was from the seeds. The maize Al-resistant variety relied completely on mycorrhizas to acquire P.

Introduction

Low phosphorus (P) availability limits plant growth in many acid soils of the humid tropical lowlands (Sanchez and Lorgan, 1992). The P deficiency is mainly caused by strong adsorption of $H_2PO_4^-$ to aluminium (Al) and iron (Fe) (hydr)oxides, which turn large proportions of total P into a form that is unavailable to plants (Fontes and Weed, 1996). The main strategy to cope with P deficiency in the tropics has been the addition of fertilisers. At the same time, the global reserves of apatite, which is needed for producing P fertilisers, are limited and known reserves may be exhausted in about 100 years with the current growth of P usage (Stevenson and Cole, 1999). Sound management strategies need to be developed to utilise applied and native soil P more effectively to reduce P fertiliser demands (Lehmann et al., 2001). To develop such strategies it is necessary to obtain a better understanding of the mechanisms involved in the P uptake by plants, especially in acid soils.

By definition, acid soils have a relatively high activity of hydrogen ions (H^+). Low pH per se is often not the cause of reduced plant growth, but it is the association of low pH with the toxicity of Al and manganese (Mn) and deficiencies of P, calcium (Ca), magnesium (Mg), and potassium (K) (Marschner, 1995). The ability of plants to grow in acid soils may be determined by their symbiosis with arbuscular mycorrhizal fungi (AMF) and the adaptability of AMF to low pH conditions (Koslowsky and Boerner, 1989; Siqueira et al., 1984)

AMF generally improve plant growth by enhancing the uptake of nutrients, especially P (Bolan et al., 1987). The various mechanisms proposed to account for this increased nutrient uptake include a wider physical exploration of the soil, increased translocation of P into mycorrhizal hyphae and modification of the root environment (Hayman, 1983). It has also been suggested that mycorrhizas may benefit plant growth by increasing the availability of P from non-labile sources. In many studies, mycorrhizal and non-mycorrhizal plants appear to use the same labile P sources (Sanders and Tinker, 1971; Mosse et al., 1973), however, other studies demonstrate that mycorrhizal plants can obtain P from normally

unavailable inorganic and organic P sources (Bolan et al., 1987; Jayachandran et al., 1989; Li et al., 1991, Koide and Kabir, 2000). Whether mycorrhizal plants take up P from sources unavailable to non-mycorrhizal plants, therefore, remains controversial.

On the one hand, the experiments that show that mycorrhizas do not facilitate P uptake from sources unavailable to plants normally involve labelling soils with radioactive P (^{32}P) or testing, under a particular amount of P application, between different sources of P (Bolan et al., 1987). However, there are problems with the interpretations of the results of these experiments because a) forms of P in soils that differ in their availability to mycorrhizal and non-mycorrhizal plants are uniformly labelled by the addition of ^{32}P and b) the extent to which mycorrhizas increase growth and P uptake has been shown to vary with the amount of P application (Bolan et al., 1987).

On the other hand, studies that show that mycorrhizal plants can obtain P from normally unavailable P sources to non-mycorrhizal plants add either organic or inorganic P sources to artificial medium or soil (Bolan et al., 1987; Jayachandran et al., 1989; Jayachandran et al., 1992; Koide and Kabir, 2000). In these studies, doubts arise if mycorrhizas can utilise P that is naturally fixed or organically bound in the soils (Koide and Kabir, 2000). For instance, added substrates like phytates are readily mineralised, whereas endogenous soil organic P (Po) and Po in plant residues are not (Joner et al., 2000). The use of natural substrates with analyses of different P pools (P fractionation) prior to and after a treatment can be an alternative methodology. P extractions and analyses are laborious and many reports related to P nutrition and mycorrhizas do not present such data (Joner et al., 2000).

The objective of the present work is to analyse if AMF can take up P from soil nutrient pools that are normally considered to be unavailable to plants when grown in acid soils with highly fixed P and with different amounts of organic matter content. Furthermore, we describe a new methodology, the double compartment - double pot approach, to be used for the study of nutrient uptake by mycorrhizas and subsequent transfer to the plants. The double (or more) compartment is a technique common in studies on nutrient uptake by mycorrhizas where mesh of different pore sizes is used to limit the access of plant roots or hyphae of mycorrhizal fungi to certain compartments (Kotari et al., 1991; Li et al., 1991; Rasmussen et al., 2000). In several studies using the double compartment approach the soil nutrients supplied are mixed with the soil. Mixing nutrients with the soil has disadvantages: a) the investigator is never sure if the nutrients supplied are available to the plants because reactions in the soil may make nutrients unavailable, and b) the nutrients added to the soil may disturb the original chemical soil parameters for example pH and base saturation (Brunt, 1982).

The double pot technique is a method used to assess the nutritional stress of plants grown in different soils. It was developed by Janssen (1974) as a sequel to the technique used by Bouma (Janssen, 1990). The main principle of this technique is that of the missing element. The major and trace elements are added to a nutrient solution except the element that is the object of study (Brunt, 1982). The roots of the plants growing in the soil compartment (upper pot) pass through a mesh that forms the bottom of the pot and reach the nutrient solution in a container (lower pot) placed under the upper pot. With the double pot approach the nutrients other than the nutrient to be tested are available to plants (nutrient solution in the lower pot) without disturbing the natural conditions of the soil. When a nutrient is omitted from the solution, plants can take it up only from the soil (Janssen, 1990). In this way, the nutrients are supplied to the plants without mixing them with the soil, and the effect of the missing nutrient can be tested. Thus the double compartment - double pot approach assures that either mycorrhizal fungi or the roots (depending on the compartment) take up the limiting nutrient in its natural state.

When assessing the nutrient stress in plants using the double pot technique, the sum of the leaf areas is used as a growth parameter. This parameter is well suited for species in the Gramineae as there exists a close relationship between dry matter production and the sum of the leaf areas (Brunt, 1982). The advantage of this is that plants do not need to be destructively harvested during the experiment, avoiding the need for many replicates (Janssen, 1990). For studies on nutrient uptake by mycorrhizal hyphae, the sum of the leaf areas can also be suitable, if P concentrations remain

unchanged, as a growth parameter to study the contribution of mycorrhizas to plant growth during the experiment, for instance, to find the beginning of the plant responsiveness to mycorrhizas.

Our hypothesis is that the double compartment - double pot approach combined with P fractionation (Tiessen and Moir, 1993) allows detection of differences in soil P pools after growing plants either or not inoculated with AMF in the soil in its natural condition, with a guaranteed supply of other elements. To test our hypothesis, we used a mixture of three species of AMF (*Glomus etunicatum, Glomus claroideum, and Glomus clarum*) inoculated onto an Al-resistant maize variety (*Zea mays* L.) growing in either the A or the B horizon of an Oxisol. We used the double compartment (inner and outer) approach to restrict the amount of soil explored by the roots, and the double pot (upper and lower) approach to supply plants with nutrient solution without P. A factorial design of two soil horizons (A and B), with and without mycorrhizas (+M and -M) and with the inner compartment made of fine or coarse mesh (F or C) was used.

Materials and Methods

Double compartment – double pot approach

The double compartment – double pot is described below. Figure 1A gives an overview and Figures 1B, 1C and 1D give some details of the pots and compartments

The upper pot with double compartment

Pots of 8 x 9 x 7.5 cm were used. A central hole (4 cm) was made in the bottom of each pot (Figure 1B). Cylinders (8.5 x 3.9 cm) were then made either from fine or from coarse nylon mesh (30 μm pore size or one mm pore size respectively, Figure 1C). The fine mesh allows mycorrhizal hyphae to pass through but prevents root penetration. The coarse mesh allows both mycorrhizal hyphae and roots to pass through. To connect the cylinder to the pot, small PVC rings were prepared. The PVC ring was glued inside the mesh cylinder. The mesh cylinder with the ring glued to it had a bottom of coarse gauze to avoid loss of soil (Figure 1C). The mesh cylinder with the PVC ring inside was placed in the central hole of each pot (Figure 1C). The bottom and the wall of the pot were covered with the coarse mesh. The mesh in the bottom of the pot served to avoid soil loss through the small holes. The mesh on the wall of the pot creates a rough surface avoiding an empty space between the soil and the wall of the pot where roots could grow (Figure 1C). In this way we had two compartments: one outside of the mesh cylinder (outer compartment) and one inside the mesh cylinder (inner compartment, Figure 1A).

The outer compartment was filled with 200 g of soil from either the A or the B horizon of a Brazilian Oxisol. Some soil characteristics are presented in Table 1. The field capacity of the soils were measured (moisture content at pF 2). The soils were air-dried, sieved through a 2 mm sieve and then sterilised using irradiation at 25 Kgy. For the mycorrhizal treatments, the inner compartments were filled with 30 g of soil from either the A or the B horizon, mixed with 10 g of mycorrhizal inoculum (soil with pieces of roots, hyphae and spores) from INVAM (Morton et al., 1993). The mycorrhizal species used were a mixture of *Glomus etunicatum* (BR 149), *Glomus claroideum* (BR 147A) and *Glomus clarum* (BR 147B). These species were originally isolated from Brazilian soils, therefore we believed they were suitable to the acid soils used in our experiment. For the non-mycorrhizal treatment the inner compartments were filled with the same mix of soil (30 g) and soil-inoculum (10 g) but after the soil-inoculum had been autoclaved. In this way, the soil material in the inner pots was the same for all treatments. A filtered extract (Blue ribbon filter paper No. 5893, Schleicher and Schüll, 3354 Dassel, FRG) of the soil-inoculum was added to the soil in the inner compartments of the non-mycorrhizal treatment to introduce comparable microflora other than mycorrhizal fungi (Kothari et al., 1991).

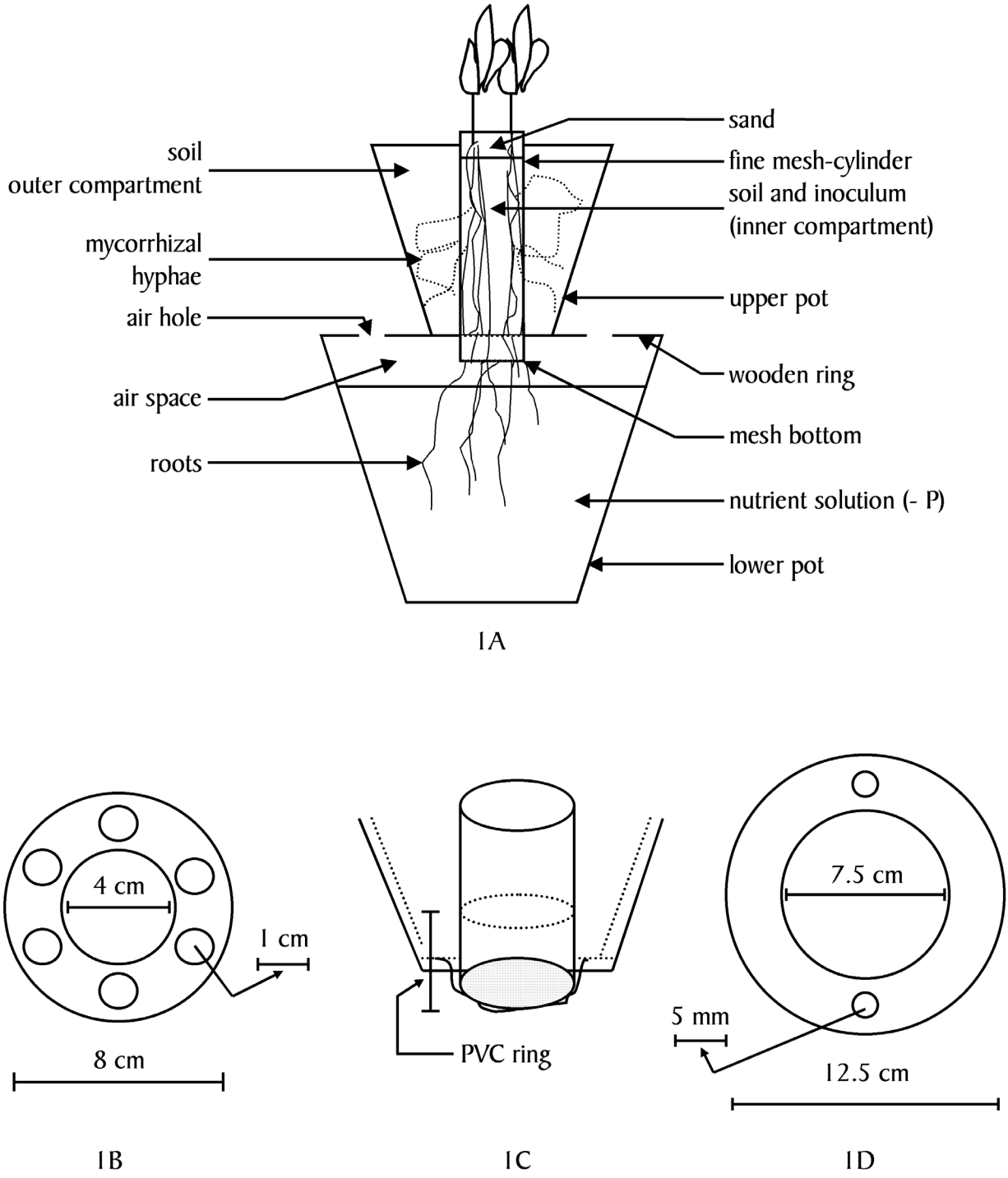

Figure 1. The double pot - double compartment set up; A) overview of the pots; B) the central and small holes in the bottom of the upper pot; C) the PVC ring glued in the mesh-cylinder (inner compartment) inside the upper pot with gauze on the bottom and wall; D) the wooden-ring with the air holes.

Table 1. Characteristics of the A and B soil horizons (Nunes, 1998).

Soil Horizons	Textural fractions[1]		pH (1:2.5) H_2O	Exchangeable[2] cations	Acidity[2]		Iron Oxide[3] (Fe_2O_3)	Organic Carbon[3]
	Sand	Clay		(Ca^{++}, Mg^{++}, K^{+})	Al^{3+}	H^{+}		
A	32	62	4.2	0.29	3.3	16.0	118.0	51.7
B	24	71	4.6	0.10	0.1	2.2	136.0	15.6

[1]%, [2]$cmol_c\ kg^{-1}$; [3]$g\ kg^{-1}$

Each inner compartment was filled to a level two cm below the level of the soil in the outer compartment. A one cm layer of sand (autoclaved) was added on top of the soil in the inner compartment, three seeds of maize were put on top of this layer and the seeds were covered with another one cm layer of sand. The maize used was an Al resistant variety (CMS 36) adapted to Oxisols (Lopes et al., 1987). The seeds were soaked overnight in de-ionised water before sowing. After sowing, the pots were weighed and covered until germination. After germination and plant thinning (one plant was taken out), a one cm thick layer of gravel (size varying from 3 to 5 mm) was placed on top of the soils to avoid excessive evaporation (the weight of the gravel was added to the weight of the pot). The pots were watered daily to maintain their moisture content at field capacity, corrections were made for increases in plant weight (Brunt, 1982).

The lower pot

Pots of 1000 ml (lower compartment, Figure 1A) were filled with 900 ml of a nutrient solution. The nutrient solution contained all the essential elements except P (Table 2). The nutrient solution was replaced weekly. A wooden ring with two holes, to allow air into the nutrient solution, was placed on top of each lower compartment (Figure 1D).

Each upper pot was placed on top of a lower pot (Figure 1A) and the pots were kept in the greenhouse (average temperature 30 °C, humidity 70-80 %, natural light) from June to September 2001, in The Netherlands.

Table 2. Nutrient solution concentration in the lower pot (Brunt, 1982)

Stock solutions	Molarity	g $litre^{-1}$
$Ca(NO_3)2.4H_2O$	1.5	354
KNO_3	1.0	101
$NH_4H_2PO_4$	2.0	230
$MgSO_4.7H_2O$	0.75	185
KCl	2.0	149
KH_2PO_4	1.0	136
K_2SO_4	0.5	87
$CaCl_2.6H_2O$	0.5	110
NH_4NO_3	1.0	80
$Mg(NO_3)2.6H_2O$	0.5	128
$MgCl_2.6H_2O$	0.5	102
NH_4Cl	1.0	53
Fe-EDTA		35
H_3BO_3		2.86
$MnCl_2.4H_2O$		1.81
$CuSO_4.5H_2O$		0.16
$ZnSO_4.7H_2O$		0.22
$(NH_4)6Mo_7O_{24}.4H_2O$		0.04

Experimental design

A factorial design of two soil horizons (A and B), with and without mycorrhizas (+M and -M) and with the inner compartment made of fine or coarse mesh (F or C) was used. The treatments from now on are reported as either A or B soil horizons, mycorrhizal plants, fine mesh (A_{+MF} or B_{+MF}); mycorrhizal plants, coarse mesh (A_{+MC} or B_{+MC}); non-mycorrhizal plants, fine mesh (A_{-MF} or B_{-MF}); non-mycorrhizal plants, coarse mesh (A_{-MC} or B_{-MC}). Each treatment was replicated three times and the experiment was arranged in a completely randomised design.

Shoot sizes

The sum of shoot sizes, from the leave base (soil) to the apex, was measured after 15, 25, 40, 70 and 90 days of growth. The plants were harvested after 90 days.

Dry weights of roots and shoots

When harvested, the fresh weights of the shoots and roots of plants were measured. The roots were separated into those from the nutrient solution compartment, the outer compartment and the inner compartment. The shoots and roots from the nutrient solution were then dried at 70 °C for 48 hours before their dry weights were measured. The dry weights and fresh weights of the roots from the nutrient solution were correlated to calculate the dry weight of the roots from the outer and inner compartment.

Root lengths

The lengths of the roots from the outer compartment were measured photomechanically by a Comair root length scanner (Hawker De Havilland, Australia).

Mycorrhizal mycelium and root colonisation

Four soil samples were collected equidistantly from the bulk soil in each pot, mixed, air-dried and sieved (2 mm). Extra-radical mycelium was extracted from 5 g of each sample and the hyphae which were aseptate in appearance and between 1.0-13.4 μm in diameter were quantified as AMF (Boddington et al., 1999).

The roots from the outer compartment were cleared in 10 % KOH in a water bath at 90 °C, for one hour, kept 15 minutes in 1 % HCl and then stained with acid fuchsin in a water bath at 60 °C, for one hour, before their fractional colonisation was measured (Brundrett et al., 1996).

P in Plant

The shoots and roots from the nutrient solution were ground and P was extracted using 0.01 M calcium chloride and quantified using molybdenum blue by Segmented-Flow Analysis (Houba et al., 2000).

The Mycorrhiza phosphorus responsiveness (MPR) was calculated as:

$MPR = [(\text{Shoot } P_M - \text{Shoot } P_{NM})/\text{shoot } P_M]*100;$

With M = mycorrhizal plants; NM = non-mycorrhizal plants (Zhu et al., 2001).

P fractionation

After the plants were harvested, the soils were sampled from the rhizosphere (soil adhering to the roots after gently removing the roots from the soil) when roots were present in the outer compartment, or close to the inner compartment when roots were absent in the outer compartment. The soil samples were air dried and sieved (0.5 mm) and submitted to a P fractionation procedure according to the flow diagram in Figure 2 (Tiessen and Moir, 1992). The P fractionation was also performed on the sterilised soils used in the experiment (A and B soil horizons) prior to the treatments. The P

fractions are Resin-Pi, $NaHCO_3$-Pi, $NaHCO_3$-Po, NaOH-Pi, NaOH-Po, concentrated HCl-Pi (HClc-Pi), HClc-Po and Residue-Pi (Figure 2).

Statistical analyses

Statistical analyses were performed using Statistica (StatSoft Inc). Analysis of variance, followed by planned comparisons, was used to test for differences among treatments per soil horizon. Fractional colonisation was arcsin sqrt transformed to satisfy normal distribution and homogeneity of variance assumptions. When necessary a t-test was used to compare means.

Results

Shoot sizes

Figures 3 (A soil horizon) and 4 (B soil horizon) show the sum of shoot sizes measured at 15, 25, 40, 70 and 90 days for the different treatments. There were significant effects of time ($p < 0.001$) for plants grown in the A and B soil horizons, and of mycorrhizas ($p < 0.001$) for plants grown in the A soil horizon. No effect of mesh size was observed. The interaction between mycorrhizas and time was significant for plants grown in the A soil horizon ($p < 0.001$). The mycorrhizal treatments started differentiating from the non-mycorrhizal treatments after 40 days growth for plants grown

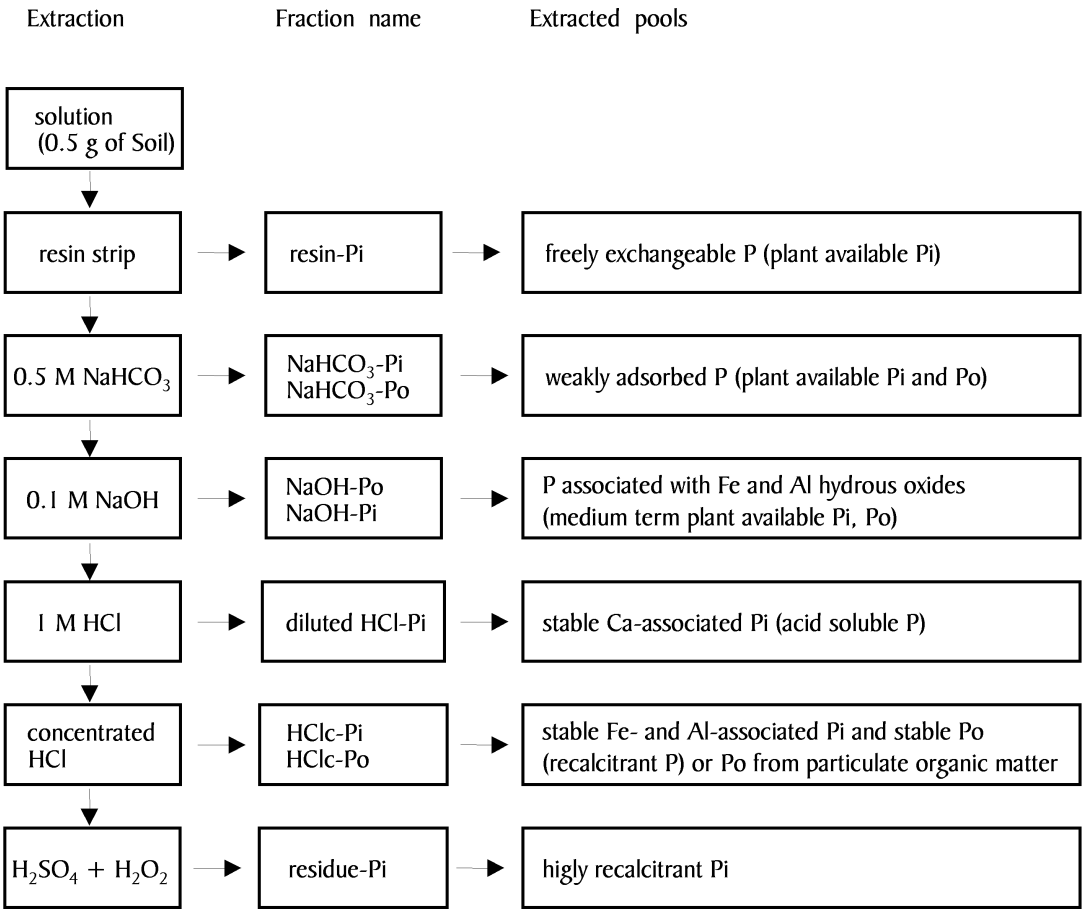

Figure 2. Flow of P fractionation (Tiessen and Moir, 1993).

in the A soil horizon (Figure 3) and after 90 days growth for plants grown in the B soil horizon (Figure 4). The shoots in A_{+MF} and A_{+MC} treatments were significantly larger than in the A_{-MF} and A_{-MC} treatments after 70 days growth and plants in the B_{+MF} treatment were significantly larger than those in either the B_{-MF} or B_{-MC} treatments after 90 days growth (Figures 3 and 4). The shoots of plants grown in the A soil horizon were significantly larger than those grown in the B soil horizon for the mycorrhizal treatments after 70 days growth ($p < 0.05$). There was a linear relationship between the dry weight of the plant and the shoot size after 90 days growth for plants grown in the A soil horizon ($R^2 = 0.98$, $p < 0.001$, $n = 12$) and for the B soil horizon ($R^2 = 0.74$, $p < 0.001$, $n = 12$).

Plants and mycorrhizal measurements after harvesting

Table 3 shows the average of the dry weight (g) of shoots and roots, the ratio of root dry weight shoot dry weight, the P content (mg plants pot^{-1}) and P concentration (g plant kg^{-1}) in the shoots and roots of plants growing in either the A or B horizon, with (+M) or without (-M) mycorrhiza, with fine (F) or coarse (C) mesh in the inner compartment. Table 4 shows the root lengths (m), mycelium length (m g $soil^{-1}$), and the fractional mycorrhizal colonisation in the roots, in the outer compartment. Table 5 shows the analyses of variance of the data showed in Tables 3 and 4.

Dry weight of shoots and roots

There were significant effects of both soil horizon and mycorrhizal treatment, no effect of mesh size and a significant interaction between soil horizon and mycorrhizal treatment for the dry weight of shoots and roots (Table 5). The mycorrhizal plants had a significantly higher shoot and root dry weight than the non-mycorrhizal plants for both the A and

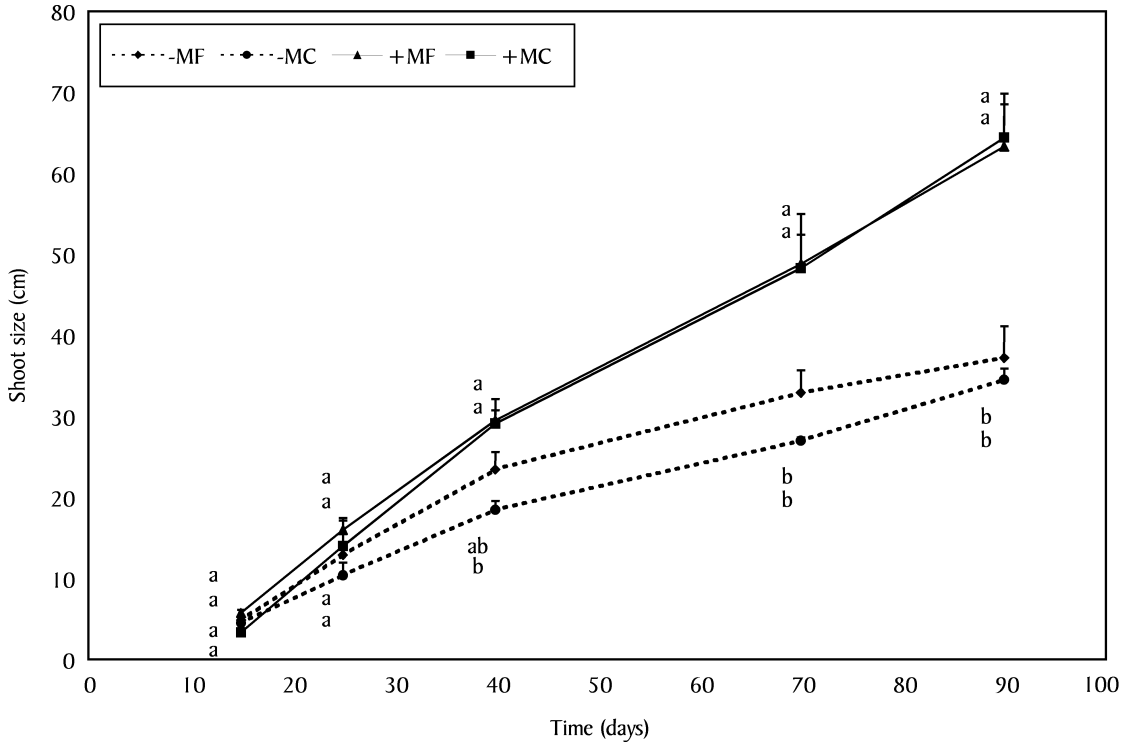

Figure 3. Average (n = 3) of the shoot sizes at 15, 25, 40, 70 and 90 days for plants grown in the A soil horizon. Treatments are -MF (non-mycorrhizal and fine gauze), -MC (non-mycorrhizal and coarse gauze), +MF (mycorrhizal and fine gauze), +MC (mycorrhizal and coarse gauze). Averages with the same letters (vertically) are not significantly different at the 0.05 level.

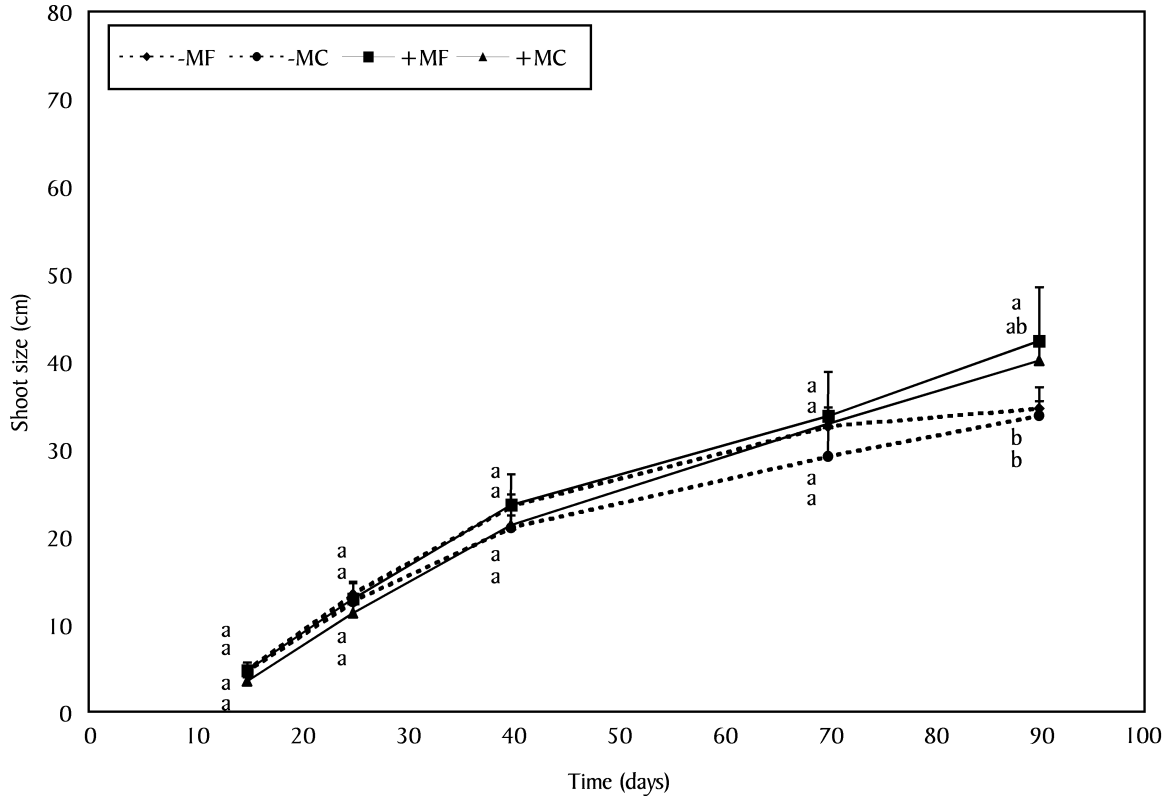

Figure 4. Average (n = 3) of the shoot sizes at 15, 25, 40, 70 and 90 days for the B soil horizon. Treatments are -MF (non-mycorrhizal and fine gauze), -MC (non-mycorrhizal and coarse gauze), +MF (mycorrhizal and fine gauze), +MC (mycorrhizal and coarse gauze). Averages with the same letters (vertically) are not significantly different at the 0.05 level.

B soil horizons, with or without roots growing in the outer compartment. There were no significant differences (Table 3) in either the shoot or root dry weights between the mycorrhizal (+MF and +MC) or between the non-mycorrhizal (-MF and –MC) treatments independently of roots in the outer compartment for plants grown either in the A or B soil horizons. There were significant differences among mycorrhizal treatments (+MC and +MF) and non-mycorrhizal treatments (-MC and -MF) in the shoot and root dry weights of plants grown in the A or B soil horizon, except that the difference between the B_{-MF} and B_{+MC} treatments for root dry weight was not significant at $p < 0.05$ (Table 3). There was an effect of soil horizon and no effect of mycorrhizal treatment on the root to shoot ratio (Table 5). The B soil horizon showed a higher root : shoot ratio than the A soil horizon (Table 3).

Root lengths

There were effects of soil horizon and mycorrhizal treatment on the root length in the outer compartment (Table 5). The planned comparisons (Table 4) showed that the lengths of the roots were significantly higher in the mycorrhizal treatments than in the non-mycorrhizal treatments. The root length was significantly higher for plants grown in the A soil horizon than for plants grown in the B soil horizon (Tables 4 and 5). The amount of roots in the +MC treatment was significantly higher than in the -MC treatment for both A and B soil horizons, but for the B soil horizon the significance was only marginal (Table 4, $0.05 < p < 0.1$).

Mycorrhizal mycelium and root colonisation

The length of the mycorrhizal mycelium differed between soil horizons in the outer compartment, no effect of mesh size and no significant mesh x soil horizon interaction (Table 5). The lengths of the mycelium in the outer compartment were significantly higher in the A soil horizon than in the B soil horizon. Within the soil horizons, planned comparisons (Table 4) showed that there were neither differences in the lengths of mycelium between the treatments with plants grown in the A soil horizon (A_{+MF} and A_{+MC}) nor with plants grown in the B soil horizon (B_{+MF} and B_{+MC}). There was a significant difference in the mycorrhizal root colonisation (Table 4 and 5) in the outer compartment between plants grown in the A soil horizon (higher) and the B soil horizon (lower).

P content and P concentration in shoots and roots and mycorrhizal P responsiveness

There were effects of soil horizon and mycorrhizal treatment, of the interaction between soil horizon and mycorrhizal treatment and no effect of mesh on the P content (mg plant pot^{-1}) in the shoots and roots (Table 3). The mycorrhizal treatments in both the A and B soil horizons had significantly more P in the shoots and roots than the non-mycorrhizal plants, whether or not roots were present in the outer compartment (Table 3), but P in the roots in the B_{-MF} treatment was not significantly lower than the B mycorrhizal treatments. The mycorrhizal plants grown in the A soil horizon had significantly more P in the shoots and roots than plants grown in the B soil horizon (Tables 3 and 5). The P concentration (P g kg^{-1} of plant) was the same for shoots and roots in the A and B soil horizons except that the concentration of P in the shoots in the B_{-MC} treatment was lower than P in the B mycorrhizal treatments (Table 3), which made the mycorrhizal effect on P shoot concentration (Table 5).

The mycorrhizal phosphorus responsiveness (MPR) was 64 % for plants grown in the A soil horizon with fine mesh in the inner compartment [A(-MF,+MF)], 70 % for plants grown in the A soil horizon with coarse mesh in the inner compartment [A(-MC, +MC)], 45 % for plants grown in the B soil horizon with coarse mesh in the inner compartment [B(-MF)/(+MF)], and 48 % for plants grown in the B soil horizon with coarse mesh in the inner compartment [B(-MC)/(+MC)]. No statistical test was executed for MPR, since it was calculated based on average P in shoots of non-mycorrhizal and mycorrhizal treatments (McGonigle and Fitter, 1990). However, if we correct for P coming from the plant seeds, the responsiveness is 100% for both horizons.

P fractionation

Figure 5 shows the Total P and P fractions (%) in the A and B soil horizons, prior to the treatments. The readily labile Pi

Table 3. Average (n = 3) shoot and root dry weight, root : shoot dry weight, phosphorus content in the shoots and roots, and P concentration of shoot and roots of plants grown in either the A or B soil horizon, with (+M) or without (-M) mycorrhizas, with fine (F) or coarse (C) mesh in the inner compartment. Numbers in rows either within the A or B soil horizons with the same letters are not significantly different at the 0.05 level (planned comparisons).

		A+MF	A+MC	A-MF	A-MC	B+MF	B+MC	B-MF	B-MC
dry weight[1]	Shoots	4.34a	4.47a	1.70b	1.45b	2.12a	2.24a	1.37b	1.39b
	Roots	1.13a	1.07a	0.54b	0.43b	0.79a	0.70ab	0.55bc	0.50c
root : shoot dry weight		0.27a	0.25a	0.32a	0.29a	0.37a	0.33a	0.41a	0.37a
P content[2]	Shoots	2.42a	2.39a	0.87b	0.71b	1.36a	1.37a	0.75b	0.68b
	Roots	0.60a	0.66a	0.29b	0.26b	0.42a	0.45a	0.35ab	0.27b
P concentration[3]	Shoots	0.56a	0.54a	0.51a	0.49a	0.64a	0.61a	0.55ab	0.49b
	Roots	0.53a	0.62a	0.52a	0.60a	0.55a	0.64a	0.64a	0.54a

[1]g; [2]mg plant pot^{-1}; [3]g plant kg^{-1}

(Resin and $NaHCO_3$, Tiessen and Moir, 1992) was 3 % in the A soil horizon and 1 % in the B soil horizon. The moderately labile Pi (NaOH-Pi) was 15 % in the A soil horizon and 13 % in the B soil horizon. The recalcitrant Pi pool (concentrated HCl and Residue) was 58 % in the A soil horizon and 81 % in the B soil horizon. The organic P was 24 % in the A soil horizon and 5 % in the B soil horizon. Table 6 shows the average (mg kg^{-1} of soil) of P fractions and the Sum-Pi, Sum-Po and Total-P (Sum Pi + Sum-Po) in the soil from A soil horizon after treatments. The lower part of the table shows the analysis of variance of these data. There was a significant effect of mycorrhizal treatment on the Resin-Pi, $NaHCO_3$-Pi, NaOH-Pi, Sum-Pi and Sum-Po fractions in the A soil horizon. There was no significant effect of mesh size. The interaction between mycorrhiza and roots was significant in the NaOH-Pi fraction. The planned comparisons (Table 6) showed significantly less phosphorus in the Resin-Pi, $NaHCO_3$-Pi, NaOH-Pi and Sum-Pi fractions and more phosphorus in the Sum-Po fraction in the soil grown with mycorrhizal plants than in the soil grown with non-mycorrhizal plants. The differences among treatments were not significant in the P fractions of the B soil horizon after treatments (data not shown).

P balance

To trace back the source of the P found in the plants, we made some calculations (Table 7) using the results from A horizon presented in Table 6 and Figure 5. The possible contribution from the soil in the pots for plant P acquisition (200 g of A soil horizon in the outer compartment, 30 g of A soil horizon and 10 g of soil with the inoculum in the inner

Table 4. Average (n = 3) root length, mycelium length, and root colonisation of plants grown in soil in the outer compartment. Plants were grown in the A or B soil horizon, with (+M) or without (-M) mycorrhizas, with fine (F) or coarse (C) mesh in the inner compartment (A_{-MF} and B_{-MF} are not shown because there was neither roots nor mycelium in the outer compartments). Numbers in rows either within the A or within the B soil horizon with the same letters are not significantly different at the 0.05 level (planned comparisons).

	A+MF	A+MC	A-MC	B+MF	B+MC	B-MC
root[1]	0.0	41.4a	16.20b	0.0	22.4b[4]	6.5b[4]
mycelium[2]	7.4a	8.6a	x	0.6a	0.8a	x
root colonization[3]	x	44.2	0.0	x	19.5	0.0

[1]m; [2]m soil g^{-1}; [3]%; [4]significant difference between B_{-MC} and B_{+MC} p = 0.1; x = not applicable

Table 5. Analyses of variance of shoot, root dry and root : shoot dry weight, P content and P concentration of shoot and root, length of root and mycelium and root colonization of data presented in Table 3 and 4. The factors are either the A or B soil horizon, with (+M) or without (-M) mycorrhiza, with fine (F) or coarse (C) mesh in the inner compartment, with soil horizon x mycorrhiza interaction.

	Dry weight			P content		P concentration		Length		Root colonization
	Shoot	Root	Root : shoot	Shoot	Root	Shoot	Root	Root	Mycelium	
factors										
soil horizon	***	**	**	***	*	ns	ns	*	***	**
mycorrhiza	***	***	ns	***	***	*	ns	**	x	x
mesh	ns	ns	ns	ns	ns	ns	ns	x	ns	x
soil horizon x mycorrhiza	***	***	ns	**	**	ns	ns	ns	x	x

Significance levels, *(P<0.05), **(P<0.01), ***(P<0.001) or not significant (ns). Interactions not shown are not significant; x = not aplicable

compartment) and from the seeds was calculated using the formula:

$P_{acquisition} = (\Delta Pi + 0.17) - \Delta Po + 1.14$

ΔPi = soil inorganic P prior minus after treatment

ΔPo = soil organic P after minus prior treatment

To make this calculation we made some basic assumptions and considerations: (i) Since the difference was not significant between the A_{-MF} and $A_{-MC,}$ they were averaged (A_{-MFC}) and used together in the calculations. The same was done for the A_{+MF} and A_{+MC} treatments (A_{+MFC}); (ii) the inorganic P fractions that showed significant differences among treatments (Table 6) were Resin-Pi, $NaHCO_3$-Pi and NaOH-Pi, therefore these fractions were considered to contribute to plant P acquisition during the experiment. (iii) there was a significant mycorrhizal effect on the Sum-Po, the mycorrhizal treatments had significantly more organic P than the non-mycorrhizal treatments (Table 6), thus we assumed that some organic P was immobilised during the experiment (the fractions that were probably built up during the experiment were $NaHCO_3$-Po and HCl-Po). (iv) the possible contribution of each fraction in the soil to plant P acquisition (ΔPi) was calculated as P in the fraction prior to treatment minus the averaged P in the fraction after treatment ($A-A_{-MFC}$ and $A-A_{+MFC}$). When ΔPi value was negative it was considered zero, because negative means that there was no P uptake from the fraction. Because some Po was immobilised during the experiment, ΔPo was calculated as Po in the fraction after treatment minus the averaged Po in the fraction prior to treatment ($A_{-MFC}-A$ and $A_{+MFC}-A$). (v) The fractionations of P in the soil with inoculum (10 g) prior to treatment was also analysed (data not shown). No Po was found in the soil with inoculum. The only P fraction present in the soil with inoculum (Psi) found that probably contributed to plant P uptake was the NaOH-Pi (0.17 mg), (vi) it was assumed that this fraction was depleted during the experiment and (vii) the P in the seeds (two seeds, 1.14 mg) was also assumed to be used by the plants during the experiment.

We considered that the $NaHCO_3$-Po and HCl-Po were built up during the experiment and not the NaOH-Po. The

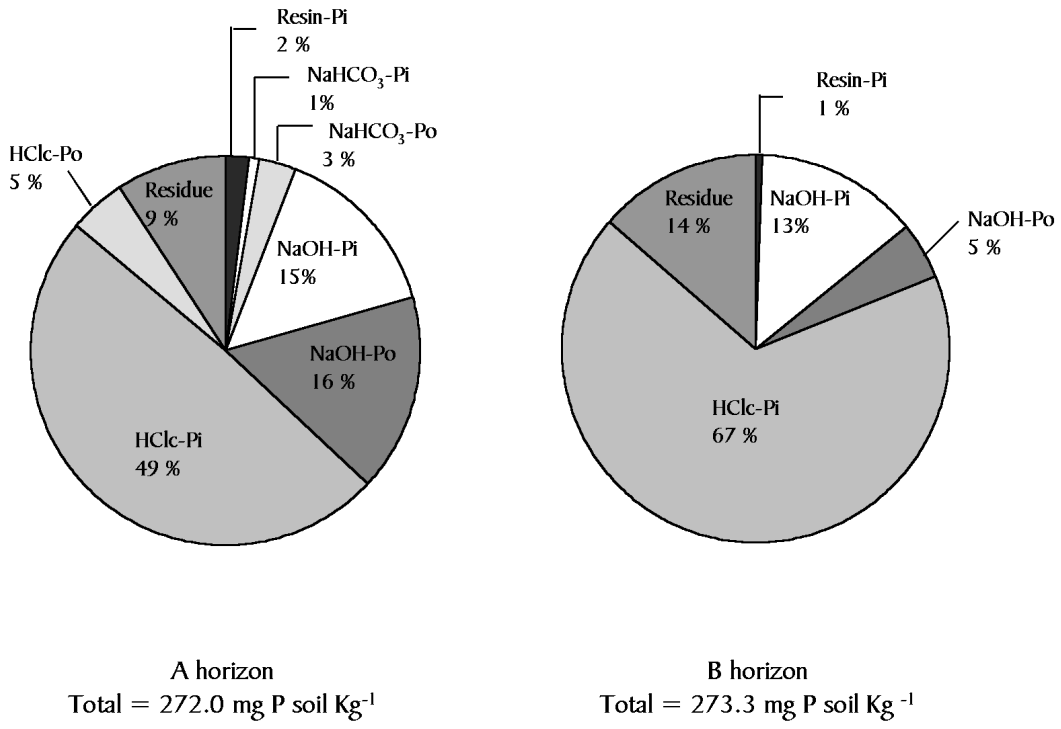

Figure 5. P fractions (%) and total P (mg soil kg^{-1}) in the A and B soil horizons prior to treatments.

$NaHCO_3$-Po may be considered as P-diester and major sources of P-diester in the soil include microbially-derived nucleic acids and phospholipids (Robinson et al., 1998), therefore the $NaHCO_3$-Po built in the mycorrhizal treatments could be from hyphae present in the soil. The HCl-Po may come from particulate organic matter that is not alkali extractable, for instance small pieces of roots (Turión et al., 2000). Therefore, the presence of roots in the treatments with roots in the outer compartment (A_{+MC} and A_{-MC}) could contribute to the build up of HCl-Po in the soil. The NaOH-Po may be considered as a P monoester, the major source of which in the soil is inositol phosphate (Robinson et al., 1998). Inositol phosphates are released in soil at a much slower rate than many other esters and its accumulation in the soil is mainly because it is stabilised in soil particles (Anderson, 1980). It can be from plant origin (mainly in the seeds) or synthesised in situ by micro-organisms (Anderson, 1980). The plants used the P present in the seeds and if some inositol phosphate were synthesised by the micro-organisms, we neglected it.

In the non-mycorrhizal treatments (A_{-MFC}) the P from the soil (ΔPi_{soil}) plus P from the inoculum minus ΔPo was negative [(ΔPi_{soil} + 0.17) – ΔPo]. Negative means that the plant did not acquire any P from the soil, thus we considered negative as having zero contribution to plant P uptake, and that the P in the plant was due to the seeds (1.14 mg). In the non-mycorrhizal treatments the P content in the plants pot^{-1} ($A_{-MFC,}$ Table 3) was on average 1.07 mg and the difference was not significant (t-test, p = 0.337) between the potentially available P and P in the plants pot^{-1}.

In the mycorrhizal treatments (A_{+MFC}) the available P uptake from the soil (ΔPi- ΔPo) plus inoculum plus seeds (ΔPi – ΔPo + 0.17+ 1.14) was on average 3.40 mg. The P in the plants pot^{-1} was on average 3.03 mg. No difference (t-test, p = 0.508) was found between the potentially available P to plant uptake. The difference between available P (Pss) in the mycorrhizal and non-mycorrhizal treatments [($^{+}$MPss)-($^{-}$MPss), 1.973 mg pot^{-1}] was similar to the difference between the P in the Plant (Ppl) in the mycorrhizal and non-mycorrhizal treatments [($^{+}$MPpl)-($^{-}$MPpl), 1.966 mg pot^{-1}]. Therefore, with our calculation we showed that for the A soil horizon the P in non-mycorrhizal plants came mainly from the seeds, whereas the difference in P uptake between mycorrhizal and non mycorrhizal plants is due to uptake from the soil.

The P in the non-mycorrhizal plants grown in the B soil horizon was on average 1.03 mg, very similar to P in the non-mycorrhizal plants grown in the A soil horizon (1.07, Table 7). Therefore we can conclude that also non-mycorrhizal plants grown in the B soil horizon did not acquire P from the soil.

Table 6. Average (n = 3) of P fractions (Resin-Pi, $NHCO_3$-Pi, -Po, NaOH-Pi, -Po, HClc-Pi, -Po, Residue-Pi) and the Sum-Pi, Sum-Po and Total-P in the A horizon after treatments with (+M) or without (-M) mycorrhiza, with fine (F) or coarse (C) mesh in the inner compartment. The effect of treatments and interactions are given in the lower part of the table. Columns with the same letters do not differ at 0.05 level (planned comparisons).

	Resin	$NaHCO_3$		NaOH		HCl_c [3]		Residue	Sum		Total P[6]
	Pi[1]	Pi	Po[2]	Pi	Po	Pi	Po	Pi	Pi[4]	Po[5]	
	mg kg $soil^{-1}$										
A_{+MF}	0.0b	0.0b	9.2	31.0b	51.2	134.1	17.8	26.7	191.7 b	78.3a	270.0
A_{+MC}	0.7b	0.0b	10.4	34.2bc[7]	53.7	133.4	14.6	26.7	195.0 b	78.7a	273.8
A_{-MF}	4.3a	3.3a	7.1	44.1a	46.4	136.0	12.7	26.7	214.4 a	66.3b	280.7
A_{-MC}	4.2a	2.2a	9.4	39.2ac	51.0	132.8	15.9	25.4	203.7ab	76.3a	280.1
Factors											
Mycorrhiza	***	***	ns	***	ns	ns	ns	ns	*	**	ns
Mesh	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mycorrhiza x mesh	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns

HCl 1M fraction: not shown because no P was found in this extract; [1]Pi, inorganic P; [2]Po, organic P; [3]HClc, concentrated HCl; [4]Sum-Pi = Resin-Pi + $NHCO_3$-Pi + NaOH-Pi + HClc-Pi + Residue-Pi; [5]Sum-Po = $NaHCO_3$-Po + NaOH-Po + HClc-Po; [6]Total P = Sum-Pi + Sum-Po. [7]significant different between A_{-MC} and A_{+MC} p = 0.0509; Significance levels, *(P<0.05), **(P<0.01), ***(P<0.001) or not significant (ns).

Discussion

Double pot – double compartment approach

Our results show that the double pot - double compartment system was suitable for our experiment because (i) by measuring shoot sizes it was possible to monitor the effect of mycorrhiza colonisation on plant growth without destructively harvesting the plants; (ii) the roots in the lower pot could take up the necessary nutrients for plant growth (except for P which was absent in the nutrient solution). The P concentration (Table 5) was similar for plants growing in the A and B horizon, therefore no other nutrients besides P limited the plant growth, and (iii) we could block root growth but allow hyphal exploration of the soil with the fine mesh, and with the coarse mesh the roots could grow into the outer compartment.

Contrary to our hypothesis, we did not find differences in plant growth when root growth was not limited vs root growth limited to the small compartment only. In order to suggest an explanation for this a P-balance could provide a meaningful insight. With the P balance we showed that the non-mycorrhizal plants in our experiment did not take up P, although maize is not obligatorily mycorrhizal (Kothari et al., 1991), and the lack of effect of mesh size is not surprising.

Non P acquisiton by non-mycorrhizal plants

Surprisingly, the roots did not take up P from the fractions that are considered available in the short term to plants, i.e., Resin-Pi and $NaHCO_3$-Pi, -Po (Tiessen and Moir, 1993). The A and B soil horizons contained Resin-Pi and the A soil

Table 7. P balance. Inorganic and organic P (Pi and Po) fractions (Resin-Pi, $NaHCO_3$-Pi, -Po, NaOH-Pi and HCl-Po) in the pot filled with A soil horizon (230 g) prior and after treatments. A_{-MFC} and A_{+MFC} are the averages of the non-mycorrhizal (-M) and Mycorrhizal (+M) treatments, with fine (F) or coarse (C). ΔPi is the Pi fractions prior minus Pi after treatments. ΔPo is the Po fractions after minus Po prior to treatments. Sum-Pi is ΔPi + 0.17 mg (P in 10 g of soil with inoculum). ΔP is Sum-Pi minus Sum-Po. Pss is ΔP plus P in seeds (1.14mg). The Ppl is the average P in plants (A_{-MFC} and A_{+MFC}, Table 3). The difference found between the available P in the pot (Pss) and the P in the plants (Ppl-Pss), the difference in the P in the pot in the mycorrhizal and non-mycorrhizal plants [($^{+}$MPss)-($^{-}$MPss)], and the difference in the P in the plants in the mycorrhizal and non-mycorrhizal [($^{+}$MPl)-($^{-}$MPpl)] were calculated. See text for further details.

	Resin	$NaHCO_3$		NaOH	HCl
	Pi	Pi	Po	Pi	Po
A horizon			mg pot^{-1}		
prior to treatments	1.265	0.529	1.863	9.154	2.852
after treatments					
A_{-MFC}	0.98	0.63	1.90	9.58	3.30
A_{+MFC}	0.08	0.00	2.26	7.50	3.73
	ΔPi		ΔPo	ΔPi	ΔPo
$\Delta(A_{-MFC})$	0.285	0.000[1]	0.037	0.000[1]	0.448
$\Delta(A_{+MFC})$	1.185	0.529	0.397	1.654	0.878
	Calculations (mg P pot^{-1})				
	Sum-Pi	Sum-Po	ΔP	Pss	Ppl
A_{-MFC}	0.455	0.485	0.000[1]	1.14a[2]	1.07a
A_{+MFC}	3.538	1.275	2.263	3.40a	3.03a
	Difference between soil P depletion and P in the plants (mg pot^{-1})				
	Ppl-Pss	($^{+}$MPss)-($^{-}$MPss)		($^{+}$MPpl)-($^{-}$MPpl)	
A_{-MFC}	0.07				
A_{+MFC}	-0.37	1.973		1.966	

[1]Negative Pi and ΔP were considered zero (no contribution from the soil to the plant); [2]The same letters in a row are not significantly different, t-test, $p > 0.5$.

horizon also contained $NaHCO_3$-Pi,-Po (Figure 7). The mycorrhizal-plants depleted these sources of available P, as shown in the P fractionation for the A soil horizon (Table 5). From this, it is clear that mycorrhizas used an available source that plants did not use. Probably mycorrhizas can perform better in the acid soil environment than the plant roots. The maize variety used in our experiment is aluminium resistant, inbred in soil with a high acidity (Lopes et al., 1987). This variety produced about 250 pmol, whereas an Al-sensitivity variety produced about 75 pmol citric acid root tip^{-1} $hour^{-1}$ when in presence of 40 μM Al (Mariano and Keltjens, 2001). These results suggested that citric acid accounted for the resistance exhibited. Moreover, the amount of exchangeable Al^{3+} in the B soil horizon is low (Table 2). An aluminium sensitive maize variety showed problems in root development when grown in the A soil horizon and no problem in the B soil horizon (unpublished observations). This is an evidence that the Al was a problem for the roots of aluminium sensitive plants grown in the A soil horizon but not in the B soil horizon. The plant roots from the aluminium resistant variety used in our experiment did not show any problem in roots growth in the A soil horizon. Therefore, the Al^{3+} concentration alone does not explain why the plant roots could not take up P.

The organic acids/anions such as citric acid are considered to be involved in a response of plant roots to P starvation (Neumann and Römheld, 1999). But for the tropical soils, this is rather controversial since the organic acid/anions may increase the acidity of the rhizosphere, the plants exude rather small fluxes of organic acids and they have low mobility in the soil and are confined to the root surface (Hinsinger, 2001; Jones, 1998). Therefore, if citric acid played a role in Al resistance, it did not play any role in P acquisition. In the variety used here, the mechanisms to cope with Al toxicity and P deficiency seem not to be interrelated as suggested in the literature (Marschner, 1995).

Because of the amount of P in the available fractions (Resin and $NaHCO_3$), especially in the A soil horizon (16 mg P $soil\ kg^{-1}$) we expected P in the soil solution to be higher than the minimum concentration normally taken up by roots. For instance, for *Centrosema pubescens* and *Paspalum notatum* the amount below which roots can not take up P but mycorrhiza can is 3 mg kg^{-1} $NaHCO_3$-P (Hayman, 1983). In sterile conditions, the maize variety we used can take up P from soil solution with high P concentration (Eduardo Mariano, personal communication). Thus, the variety used in our experiment, although more Al-resistant is sensitive to lower P and relies on mycorrhiza to take P up in acid conditions.

P acquisition by mycorrhizal plants

Colonisation by arbuscular mycorrhizal fungi resulted in consistently larger shoot size, larger dry weight of plants and higher amounts of P in plants. These results were also higher in the plants grown in the A soil horizon than in the B soil horizon. In the A soil horizon the mycelium length and root colonisation were higher than in the B soil horizon, so was the mycorrhiza P responsiveness (MPR). Apparently the mycorrhiza developed better in the A soil horizon than in the B soil horizon. The A soil horizon had similar clay content, more exchangeable Al, a higher H^+ concentration and, therefore a lower pH than the B soil horizon, however, the A soil horizon had around three times higher organic matter content than the B soil horizon. Probably the higher organic matter content in the A soil horizon was the responsible for the better performance of mycorrhiza. The better physical properties due to organic matter such as increasing soil porosity reduced the mechanical resistance to hyphae growth through the soil (Joner and Jakobsen, 1995). Moreover, the A soil horizon had higher amount of labile P (Resin and $NaHCO_3$ fractions), which could have sped up the growth of the plants in the beginning by allowing more photosynthesis and thus a higher carbon excess to the mycorrhiza.

The mycorrhizal-plants also used some of the NaOH-Pi presented in the soil, as shown for the A soil horizon (Table 6). This fraction is presumably bound to Fe and Al (Hydr)oxides (Tiessen and Moir, 1993). This could be due to a shift in the equilibrium of adsorption-desorption being the driving force for the diffusion of phosphate towards the soil solution enabling the roots (or mycorrhiza in our case) to access less available P (Hinsinger, 2001). However, the transfer ratio from NaOH-Pi to more available fractions is about 0.033 $year^{-1}$ (Noij et al., 1993). Thus, the amount of NaOH-Pi being transferred via desorption to more available pools in our experiment account for only 5 % of the P changes in NaOH-Pi fractions prior and after the experiment. Therefore, we agree with Hinsinger (2001) that because of the poor reversibility

of P adsorption it is likely that the mycorrhiza could directly use the least mobile fractions of soil inorganic phosphorus. A possible explanation for the mechanism used by mycorrhizal is a change in rhizosphere pH. The alkalinization of the root rhizosphere resulted in depletion of NaOH-P (Gahoonia and Nielsen, 1992). Mycorrhizas are reported to increase pH in the rhizosphere for electrochemical reasons (Bledsoe and Zasoski, 1983). Maybe in our experiment the mycorrhiza, contrary to the roots, promoted certain alkalinization of the mycorhizosphere. Increasing pH decreases the solubilities of Fe- and Al-hydr(oxide), thereby increasing the solubility of Fe- and Al-phosphate (Lindsay and Moreno, 1960). NaOH-Pi fraction is considered available only in the medium term (more than one growth season) to plant roots. However, the mycorrhiza could use part of it in the short term (3 months).

In our short-term experiment, the mycorrhiza-plant could neither use the more recalcitrant source of Pi, i.e., the HCl_c-Pi or Residue-Pi, nor the organic P (Table 6). Some organic P was built up in the soil (Table 6), one of the steps (immobilisation in microbial biomass) of organic P cycling (immobilisation/mineralisation). Since AMF does not have saprotrophic activity (mineralisation of organic matter), their strategy can be to capture nutrients recently mineralised by other microorganisms, thus contributing to a closer cycling of P by intercepting P fixation by soil particle (Joner et al., 2000), rather than use organic P already stabilised in the soil. For instance, the inositol (Po) produced by micro-organisms can be used by mycorrhiza, through phosphatase activity, before being fixed by the soil particle (NaOH-Po fraction). This seems to be possible since in artificial medium it was shown (Jayachandran et al., 1992; Koide and Kabir, 2000) that mycorrhizal can hydrolyse phytate (inositol).

In the long term (relevant for perennial crops) the use of NaOH-Pi together with the ready available fractions (Resin-Pi and $NaHCO_3$-P) could shift the equilibrium of adsorption-desorption being the driving force for the diffusion of phosphate towards the soil solution, thus making the more recalcitrant forms available to plants. It is necessary to better understand the mechanism that allows promoting sound management strategies to use the native P, including the recalcitrant forms, since these can be the largest pools in some acid soils. The recalcitrant pools were up to 218 mg kg $soil^{-1}$ (80 %), in the soils used in our experiment (Figure 5). This means, approximately 2300 kg P_2O_5 ha^{-1}, at the first 20 cm of the soil. Considering the deepness of some acid soils, this is a considerable source.

Conclusions

We concluded that in our experiment the organic pools were irrelevant to mycorrhizal functioning. The mycorrhizal plants could cope better with the hostile environment (low pH, high Al^{3+}) taking P up whereas the non-mycorrhizal plants failed completely. In the short term, the mycorrhizal-plant used the fraction of P (NaOH-Pi) considered available to plants only in the medium term. Therefore, mycorrhiza in our experiment did more than simply shortening the distance that P ions must diffuse to plant roots. One mechanism proposed in the literature (Bolan et al., 1987) is that mycorrhizal fungi may break the bond between Fe and P, releasing P. Such mechanism deserves further studies, specially in tropical soils with high amounts of iron-phosphate.

We also conclude that the double pot and double compartment experiment is suitable for the study of nutrient uptake by mycorrhiza and subsequent transfer to the plants.

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CHAPTER 7

AL-RESISTANT MAIZE ONLY ACQUIRES P FROM ACIDIC TROPICAL SOILS THROUGH MYCORRHIZAL ASSOCIATION

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Abstract

The main problems for development of sustainable agriculture production in acidic tropical soils are aluminium (Al) toxicity and phosphorus (P) deficiency. P is present in tropical soils but strongly adsorbed and unavailable to plants. Current knowledge suggests that Al resistance and P uptake are correlated. We show that this is not the case: Al-resistant maize plants were unable to take up P from acidic tropical soil, even with low Al concentrations. Plants were able to take up P when associated with arbuscular mycorrhiza. Therefore, selection of crops for Al-resistance should be accompanied by studies of mechanisms of P-uptake, such as the association with mycorrhizas.

Aluminium (Al) toxicity and phosphorus (P) deficiency are the primary problems for crop production in acidic soils (Hocking, 2001). Sustainable strategies, dealing with both problems in tropical soils are necessary to secure food production by small-scale poor farmers in the tropics. Phosphorus deficiency is mainly caused by strong adsorption of $H_2PO_4^-$ to Al- and Fe- (hydr)oxides, which makes it unavailable to plants (Fontes and Weed, 1996). It is generally agreed that high Al resistance is a key factor in the adaptation of crop plants to acid soils (Marschner, 1995), although low P availability limits plant growth in these soils (*Sanchez and Logan, 1992*) and plants should therefore also be highly efficient in utilising applied P and native soil P (Marschner, 1995; Lehmann et al., 2001). Current knowledge suggests that the mechanism responsible for Al resistance in plants facilitates P uptake from acidic soils (Hocking, 2001; Marschner, 1995), although it is not clear how (Hocking, 2001; Marschner, 1995; Koslowsky and Boener, 1989; Zoysa et al., 1999). Al counteracts P acquisition, higher Al resistance is therefore assumed to improve plant P acquisition as well. Organic anions exuded by plant roots are often considered part of an important mechanism to cope with Al toxicity and low P availability (Hocking, 2001; Ae et al., 1990; Haynes and Mokolobate, 2001; Ström et al., 2002), but the extent to which such organic anions improve the bio-availability of soil inorganic P is rather controversial (Hissinger, 2001) and the ecological significance of the organic anions is questionable (Ström et al., 2002; Jones, 1998).

Arbuscular mycorrhizal fungi (AMF) can also improve plant growth, both by enhancing nutrient acquisition, especially P (Sanders and Tinker, 1971), and by overcoming Al toxicity (Clark, 1997).

We evaluated P acquisition from the A and B horizons of an acidic Brazilian Oxisol by an Al-resistant maize variety, which was obtained in Brazil by screening for Al resistance in a highly acid soil (Lopes et al., 1987; Appendix A). In the presence of 40 μM Al, the maize variety exuded approximately 250 pmol citric acid and 130 pmol malate root apex^{-1} hour^{-1}, whereas an Al-sensitive variety exuded approximately 75 pmol root apex^{-1} hour^{-1} (Delhaize et al., 1993; Ryan et al., 1993). The high production of citric acid by the Al-resistant variety and the fact that organic anions play a role in Al resistance (Ma et al., 1997) suggest a correlation between the organic acid release and Al resistance.

We grew maize plants from seeds in pots with sterilised soil from either the A or B horizon (Appendix B). The A horizon has high (33 $mmol_c$ kg^{-1}) and the B horizon has low exchangeable Al (1 $mmol_c$ kg^{-1}), hence if Al would hamper P acquisition, this is expected to occur in the A horizon only. Both horizons have low P availability. Plants were supplied with a nutrient solution, excluding P, in a separate pot under the upper pot (Janssen, 1990), and the roots grew into this solution (Figure 1). Roots were confined to a small portion of the upper pot using a fine mesh cylinder (1/6 of the total pot volume), or were allowed to grow throughout the pot using a coarse mesh cylinder (Kothari et al., 1991). The fine mesh serves to limit the amount of soil available to the plant roots. Plants were either

inoculated with mycorrhizas (Morton et al., 1993) or left untreated. AMF hyphae could access all soil through both mesh sizes. After a period of 12 weeks, there was a significant effect of horizon ($p < 0.05$) and mycorrhiza ($p < 0.001$), but not of mesh size, on the dry weight and P contents of shoots and roots (Appendix C, Figure 2). Planned comparisons (Appendix D) showed that dry weight and P content of shoots and roots of mycorrhizal plants were significantly higher than of non-mycorrhizal plants within each horizon ($p < 0.05$). Between horizons, the differences were significant for mycorrhizal plants ($p < 0.001$), but not for non-mycorrhizal plants (Figure 2). This shows that the mycorrhizas were paramount in taking up P.

Root growth was not inhibited by Al toxicity when roots were not confined by fine mesh, as plants grown in the A horizon (higher acidity) had the longest roots (Table 1, $p < 0.01$). The mycorrhizal mycelium lengths (Appendix E, Table 1) did not differ among treatments with more or less soil being exploited by roots (coarse or fine mesh), but there was more mycelium in the A than in the B horizon ($p < 0.001$, Table 1). Root colonisation by mycorrhizas (Brundrett et al., 1996) was more than twice as high in the A horizon (44.2 %) as in the B horizon (19.5 %). The mycorrhizal P responsiveness (Zhu et al., 2001) (MPR = $[(\text{Shoot } P_M - \text{Shoot } P_{NM})/\text{shoot } P_M] \times 100$) was on average 67 % for the A horizon and 46 % for the B horizon. However, if we correct for P coming from the plant seeds, the responsiveness is 100% for both horizons.

Prior to the experiment, P fractionation (Appendix F) showed that the soils contained similar amounts of total P but that the distribution over pools differed between horizons (Figure 3a,b). The size of the pools after the experiment did not change in the non-mycorrhizal treatments, consistent with a lack of acquisition of P by plant roots (Figure 3c),

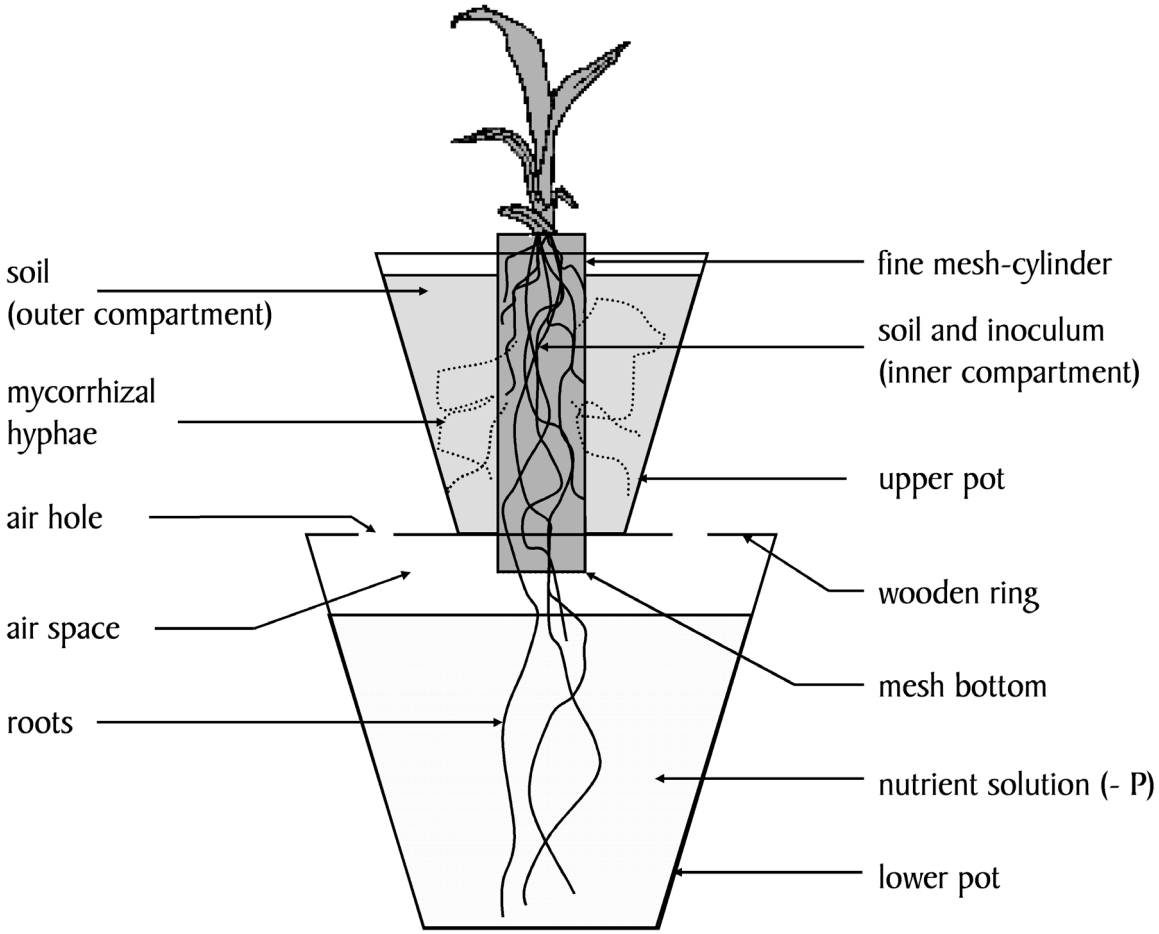

Figure 1. The double pot - double compartment set-up used in the experiment. The size of the upper pot was 8 x 9 x 7.5 cm, the inner compartment was 8.5 x 3.9 cm and the lower pot was 1000 ml. See materials and methods for further explanation.

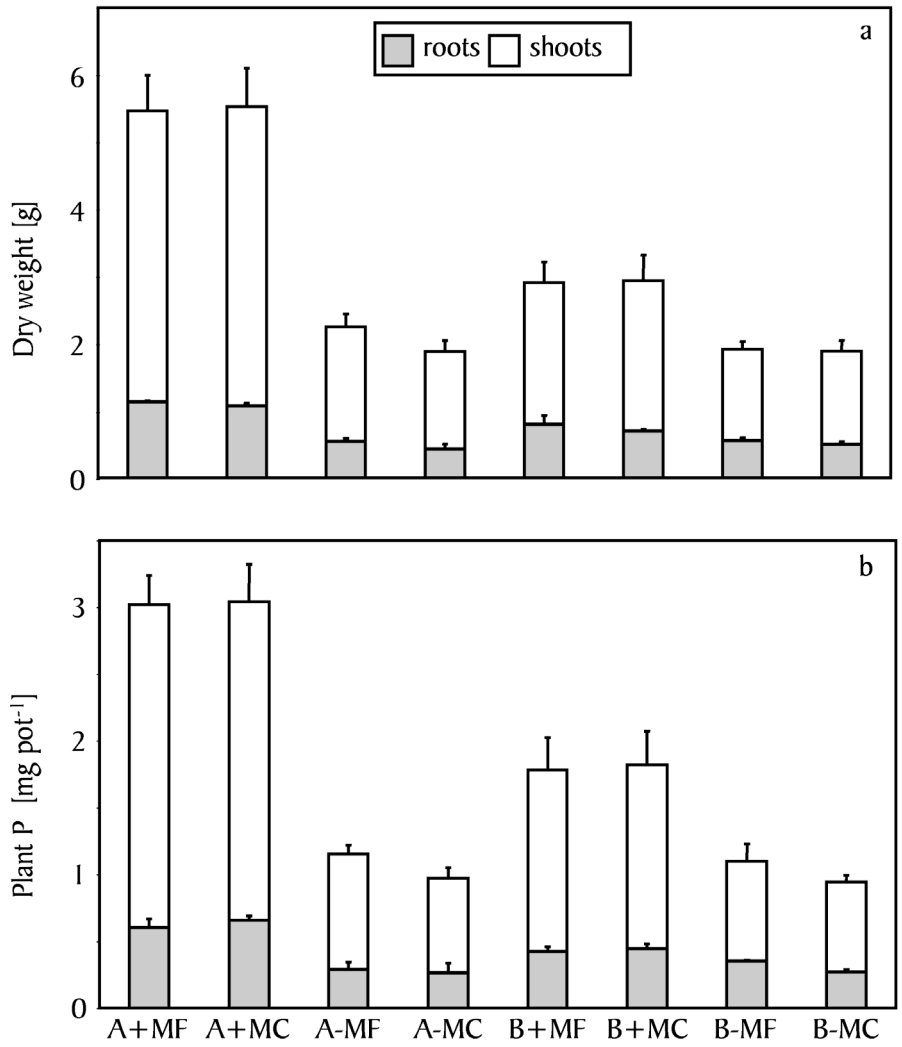

Figure 2. Average (+ s.e.) dry weight (a) and P content (b) of roots and shoots of plants grown in the A or B horizon of an Oxisol. Treatments on the x-axis are indicated as follows: A, B refer to the soil horizon; +/-M is with or without mycorrhiza; F/C refers to Fine or Coarse mesh of the inner root compartment. Fine mesh restricts root growth to the inner compartment, whereas coarse mesh does not.

whereas mycorrhizal plants depleted the resin-inorganic P (Pi) and $NaHCO_3$-Pi in the A horizon (Figure 3d). These fractions are considered readily available both for plants and mycorrhizas. Moreover, about 20 % of the NaOH-Pi, considered moderately available to plants, was taken up by mycorrhizal plants. No P was acquired from the recalcitrant (concentrated HCl-Pi and Residue-Pi) and organic fractions. Furthermore, total organic P was higher in the mycorrhizal treatments than in non-mycorrhizal treatments. Thus, some of the soil organic pools increased during the experiment, probably due to mycelium and roots turnover. The pools used by the mycorrhizal plants were relatively small in the B horizon, and we could therefore not detect differences between pools before and after the experiment. Together with the low content of organic matter, the relatively small plant-available P pools (Figure 3b) may have caused the low amount of mycelium in the B horizon, which probably resulted in the smaller size of plants grown in this horizon (Figure 2a).

Based on the P fractionation before and after the experiment, a P balance was calculated for the mycorrhizal and non-mycorrhizal treatments in the A horizon. The amount of P that disappeared from the soil in treatments with mycorrhizas was approximately 2.26 mg, whereas the seeds contained 1.14 mg P, a total of 3.40 mg per pot. The P found in the mycorrhizal plants was 3.03 mg per pot, which is not significantly different from this total (t-test, p =

Table 1. Average (n = 3) root length and mycelium length of plants growing in the soil in the outer compartment. Plants grew in the A or B horizon, with (+M) or without (-M) mycorrhizas, with fine (F) or coarse (C) mesh in the inner compartment.

	A+MF	A+MC	A-MF	A-MC	B+MF	B+MC	B-MF	B-MC
Root[1]	0.0	41.4	0.0	16.2	0.0	22.4	0.0	6.5
Mycelium[2]	7.4	8.5	0.0	0.0	0.6	0.8	0.0	0.0

[1]m; [2] m soil g^{-1}

0.508). No change in soil P was measured in the soil with non-mycorrhizal plants, and the amount of P found in the plants (1.07 mg) was similar to that from the seeds.

We conclude that the roots from the Al-resistant variety failed to acquire P from the soils in the absence of mycorrhizas, even from readily available fractions in the A horizon (Resin-Pi and $NaHCO_3$-Pi). The P content of the non-mycorrhizal plants in the B horizon and non-mycorrhizal plants in the A horizon was very similar (Figure 2b), indicating that non-mycorrhizal plants growing in the B horizon also did not acquire P. Hence, the plants relied completely on mycorrhizal activity for P acquisition, even when exchangeable Al was low. To our knowledge, this is the first evidence that a maize variety is completely dependent on mycorrhizas (MPR = 100%). Furthermore, the mechanisms to cope with Al toxicity and P deficiency are not necessarily interrelated, contrary to suggestions from the literature (Marschner, 1995). If citric acid plays a role in the Al resistance of this variety, it is not important for direct P acquisition. Considering that P limits growth, we cannot support the view that high Al resistance alone is the key to adaptation of crop plants to acidic soils. Plant breeders should therefore not only select for Al-resistant varieties, but

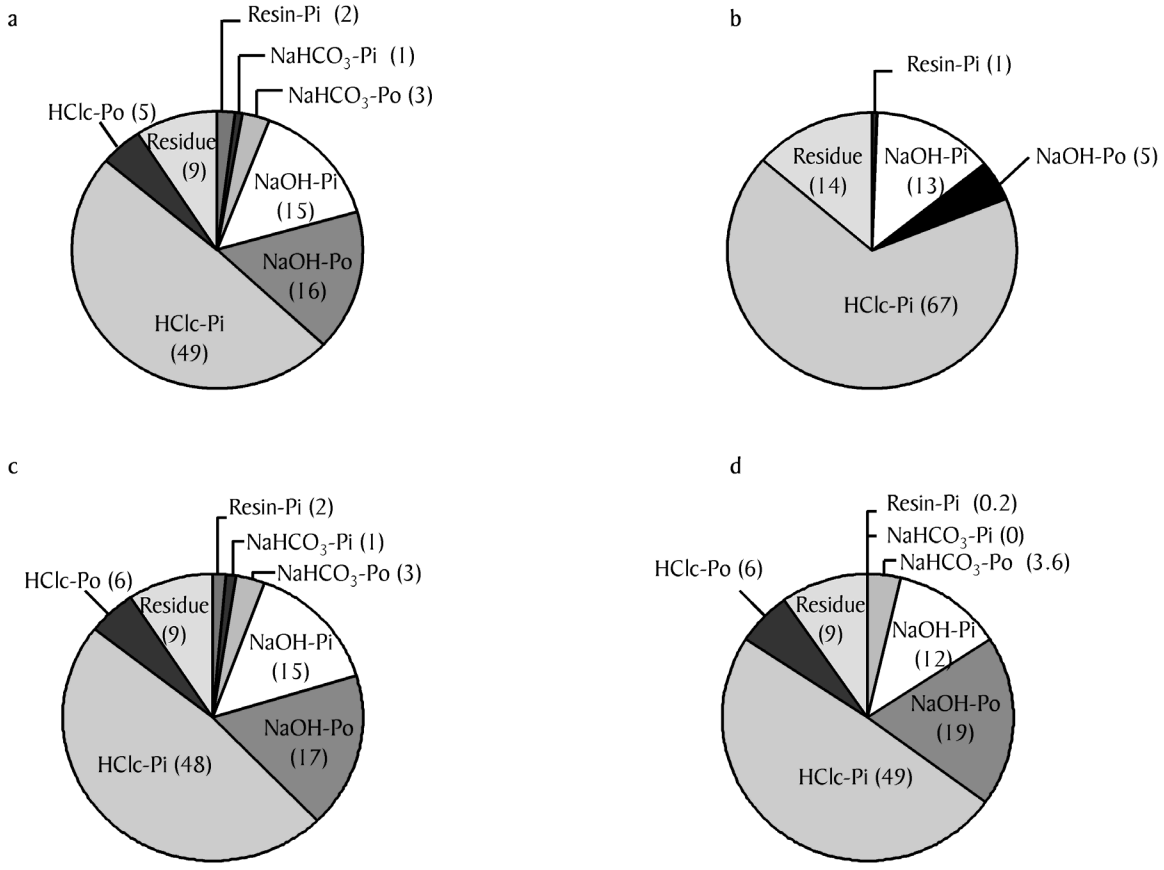

Figure 3. P fractions (%) prior to the experiment in the A horizon (a) and the B horizon (b), as well as after the experiment for the A horizon without (c) and with (d) mycorrhiza. The total P in the A and B horizons was 272.0 and 273.3 mg $(kg\ soil)^{-1}$ respectively. Differences in the Resin, $NaHCO_3$-Pi and NaOH-Pi fractions, as well as in total Po were significant between soils from mycorrhizal and non-mycorrhizal plants ($p < 0.05$).

should also consider mechanisms of plants to take up P from acid soils, such as the association with mycorrhizas (Sanders and Tinker, 1971).

Al toxicity and low P availability limit plant growth in about one-third of the area of tropical soils (1.5 billion ha) (Sanchez and Logan, 1992). The most important strategies to cope with these problems are the use of lime and P fertilisers. The financial problems associated with these strategies are especially pressing for the poor farmers who normally use such soils. Furthermore, it is difficult to incorporate lime deeper than 30 cm, whereas sub-soil acidity is potentially growth-limiting (Marschner, 1995). Moreover, global reserves of apatite, needed for producing P fertilisers, may be exhausted in about 100 years with the current growth of P usage (Stevenson and Cole, 1999). It is therefore essential to understand the mechanisms that allow plants to deal with Al toxicity and low P availability for the development of sustainable soil management strategies that are necessary to overcome problems of food security in the tropics (Sanchez, 2002).

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We thank Willem Menkveld, Arie Brader and Peter Pellen for help in the greenhouse, Simon Maasland for help with preparation of the pots and Jaap Nelemans and Willeke van Tintelen for support in the lab. Sidney Netto Parentoni from Embrapa (CNPMS) is acknowledged for the maize seeds and Joe Morton (INVAM) for the inoculum of AMF. Thanks also to CAPES, the Brazilian sponsor of I.M.C. and E.D.M.

Appendix A

Seedlings of two maize genotypes (CMS 36 and BR 106), contrasting in Al^{3+} sensitivity, were grown axenically in Petri dishes. The divided-root-chamber technique (Delhaize et al., 1993; Ryan et al., 1993) was used to study the exudation of organic anions by root apices. $AlCl_3$ was added to reach a concentration of 40 μM L^{-1}. The solutions containing the root exudates were analysed for citric and malic anions using enzymatic methods (Delhaize et al., 1993) with small modifications.

Appendix B

The Al-resistant (CMS 36) maize variety was grown in the A (pH = 4.2, organic carbon = 51.7 g kg^{-1}) and B horizon (pH = 4.6, organic carbon = 15.6 g kg^{-1}) of an acidic Brazilian Oxisol for 90 days (June to August) in a greenhouse (80 % humidity, 30 °C, natural light) in The Netherlands. We merged a double pot (Janssen, 1990) with a double compartment (Kothari et al., 1991) set-up (Figure 1). Treatments were soil from the A or B horizon (A or B), with or without mycorrhizas (-M and +M) and with the inner compartment made of fine (F, 30 μm pore size) or coarse (C, 1 mm pore size) mesh. The fine mesh allowed mycorrhizal hyphae but not roots to grow into the outer compartment, whereas both roots and mycorrhizas could pass through the coarse gauze. Each treatment was in triplicate and the experiment was arranged in a completely randomised design. The moisture content in the soil was kept at field capacity. Prior to the experiment, all soils were air-dried, sieved (2 mm) and sterilised (gamma irradiation, 25 kgy). The outer compartment of the upper double-compartment pot was filled with 200 g of the A or B horizon. For the mycorrhizal treatments, the inner compartment was filled with 30 g of the same soil, mixed with 10 g of mycorrhizal inoculum (soil with pieces of roots, hyphae and spores) from INVAM (Morton et al., 1993), consisting of a mixture of *Glomus etunicatum* (BR 149), *Glomus claroideum* (BR 147A) and *Glomus clarum* (BR 147B) isolated from Brazilian soils. In non-mycorrhizal treatments, the inner compartment was filled with the same mix of soil and soil-inoculum but the soil-inoculum was autoclaved (121 °C, 20 minutes). In this way, the soil material in the inner pot was the same for all treatments. Moreover, a filtered extract (Blue ribbon filter paper No. 5893, Schleicher and Schüll, 3354 Dassel, FRG) of the soil-inoculum was added to the inner compartment of the non-mycorrhizal treatment to introduce comparable microflora other than mycorrhizal fungi (Kothari et al., 1991). Three maize seeds were sown and one plant was taken out after germination. The pots were placed on top of a lower pot with a 900 ml nutrient solution (minus P). Roots could grow into the solution through a mesh in the bottom of the upper pot. The solution was renewed weekly.

Appendix C

Upon harvest, shoots and roots were weighed separately. The roots were separated into those from the nutrient solution compartment, from the outer compartment and roots from the inner compartment. Shoots and the roots from the nutrient solution were subsequently dried (48 h at 70 °C) and weighed again. The regression equation of dry vs fresh weights of the roots from the nutrient solution was used to estimate the dry weight of the roots from the outer and inner compartment. The length of

roots from the outer compartment was measured photomechanically by a Comair root length scanner (Hawker De Havilland, Australia). Shoots and roots from the nutrient solution were ground and P was extracted using 0.01 M calcium chloride and quantified using molybdenum blue by Segmented-Flow Analysis (Houba et al., 2000).

Appendix D

We used ANOVA, followed by planned comparisons, to test for differences among treatments within and between horizons and between mycorrhizal and non-mycorrhizal plants (Statistica, StatSoft Inc., 1997). When necessary, t-tests were used to compare means.

Appendix E

Roots from the outer compartment were cleared in 10 % KOH (1 h at 90 °C), incubated in 1 % HCl (15 min), stained with acid fuchsin (1 h at 60 °C), and the fractional colonisation was measured (Brundrett et al., 1996). Four soil samples were collected equidistantly from the bulk soil in each pot, mixed, air-dried and sieved (2 mm). Extra-radical mycelium was extracted from five grams of each sample and the AMF hyphae, which were aseptate in appearance and between 1.0 - 13.4 μm in diameter, were quantified (Boddington et al., 1999).

Appendix F

After plant harvest, soil was sampled from the rhizosphere (soil adhering to the roots after gently removing the roots from the soil) when roots were present in the outer compartment, or close to the inner compartment when roots were absent from the outer compartment. Samples were air-dried and sieved (0.5 mm) and submitted to a P fractionation procedure (Tiessen and Moir, 1993). The fractions are named after the extractants used, i.e. Resin-Pi, $NHCO_3$-Pi, $NHCO_3$-Po, NaOH-Pi, NaOH-Po, HClc-Pi, HClc-Po and Residue-Pi. Total P is the sum of all fractions.

CHAPTER 8

CYCLING OF P IN AGROFORESTRY AND CONVENTIONAL COFFEE SYSTEMS ACCORDING TO A SIMPLIFIED VERSION OF THE MODEL DYNAMITE

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Abstract

Models can be a considerable aid to understand the relations between soils, plants and other components of agroforestry systems. The role of phosphorus (P) dynamics in conventional and agroforestry coffee cultivation systems was modelled and compared to P-dynamics in the steady state in undisturbed forest. The model used here is a simplified version of the models NUTCYC and DYNAMITE. A new aspect is that the influence of mycorrhiza on P uptake is explicitly considered. The major objective of our modelling exercise was to examine how agroforestry and conventional (full-sun, monoculture) coffee systems affect the distribution of soil P over organic and inorganic pools. The model is confined to simple calculations of the major P flows between soil and plant. The compartments in each of the ecosystems are a) soil containing organic and inorganic P pools, and soil solution; b) vegetation consisting of one or more of the vegetation types: coffee trees, (agro-)forestry trees, weeds. In the soils, the organic pools are very and moderately labile. The inorganic pools are very and moderately labile, and stable and inert. The coffee trees consist of wood, leaves, and fruits, the (agro-)forestry trees of wood and leaves, whereas the weeds have only leaf material. Roots have not been included. The model cannot be considered as a mechanistic model. Calculations were based on empirical relationships; the time step was one year. The situation under original forest was taken as a starting point. Inputs of P are from atmospheric deposition, and organic and inorganic fertilisers. Outputs occur via erosion and harvest of coffee berries. The internal P flows were calculated as a fraction of the pool from where the flow departed. Most of the values of the coefficients were derived from the results described in foregoing chapters of this thesis, or from the literature. Some values had to be calculated as the 'unknowns' in algebraic equations formulated on the basis of known state variables and flows in original forest. In the end of the calculations, forest kept the same P values as in the start point (steady state); total P was 2000 kg ha^{-1} for forest, 2019.2 kg ha^{-1} for the agroforestry systems, 2205.0 kg $ha^{-1}$for the conventional systems. The difference on total P among the systems is due to harvest and differences in fertilisation. The total organic P was 500 kg ha^{-1}, 235.5 kg for the agroforestry systems and 178.8 kg for the conventional systems. The total labile P was 630 kg ha^{-1} for the forest, 420.4 kg for the agroforestry systems and 423.2 kg for the conventional systems. Hence, the ratio Po : labile P was 0.79 for the forest, 0.56 for agroforestry coffee systems and 0.42 for conventional coffee systems. These ratios are qualitatively similar to the results presented in Chapter 3. The model calculations suggested that indeed agroforestry converts part of the inorganic P into organic P. In conclusion, our modelling exercise was useful to integrate available information and further substantiate the claim that agroforestry influences P dynamics through conversion of Pi to Po. Missing information also comes to light, mainly the explicit role of the micro-organisms in the P flows among pools. With future research, the model can be improved. Suggestions are made what processes and rates need to be better assessed for model improvement.

Introduction

Agroforestry systems have several service functions, such as the production of fuelwood, fodder and fruit. However, the major service function of agroforestry systems is its role in soil management, including control of erosion and maintenance and improvement of soil fertility (Young, 1997). Particularly, trees in agroforestry systems may increase P supply by retrieving nutrients from lower soil horizons (Young, 1997), and increase P availability through accelerating P cycling by (1) improving the chemical and physical quality of soils, and (2) by enhancing microbial activity (Cooper et al., 1996).

Phosphorus is an important nutrient in relatively short supply in most natural ecosystems, and the primary limiting nutrient for crop production in highly weathered tropical soils (Linquist et al., 1997). In this thesis (Chapter 3) we hypothesised that the distribution of soil P among various pools is influenced by agroforestry. To test our hypothesis, we characterised the soil inorganic and organic P (Pi and Po) pools and compared the different pools at different

depths in agroforestry and conventional (full-sun, monoculture) coffee (*Coffea arabica* L.) systems. A simplified sequential P fractionation was carried out. Our results showed that the agroforestry systems had consistently higher ratios of Po to total labile P than the conventional fields. The ratio of Po : labile P was on average 0.69 in the agroforestry fields and 0.56 in the conventional fields (Chapter 3). This was considered to be an indication of the impact of agroforestry systems in P transformation compared to the conventional systems.

However, fractionation of P, as done in Chapter 3, is a static tool to study P and is not sufficient to draw firm conclusions on organic P transformation. A full description of P cycling in soils and plants requires an understanding of the chemical, physical and biological processes influencing the behaviour of the various forms of P in the soil profile (Jones et al., 1984).

Models can be a considerable aid to understanding the relations between soils, plants and other components of agroforestry systems, in particular in studying the relations between these components over time (Young, 1997). Thus, models can be useful to study P transformations in agroforestry systems. A model, in general, is an important tool for a quantitative description of the numerous interactions and feedback processes (Janssen et al., 1990). To design models has the advantages to a) take the available information together and to integrate it in a way that the missing information comes to light, b) allow indication (through sensitivity and uncertainty analysis) which parameters and processes are of vital importance and c) in one step further to use the models for (management) scenario analyses (Noij et al., 1993).

The sequential extraction results (Chapter 3) were therefore integrated in a modelling exercise, to better understand the impact of agroforestry systems in P transformations.

The model used here is a simplified version of the models NUTCYC (Nutrient Cycling) and DYNAMITE (Dynamics of Nutrients And Moisture In Tropical Ecosystems) (Noij, 1988; Janssen et al., 1990; Noij et al., 1993). NUTCYC is a model where primary production is limited by P-availability. For both conventional and agroforestry systems on the highly P-fixing Oxisols in our study area, P-limitation is very likely to occur. DYNAMITE is a more complex model where primary production can be limited by either nutrients (P, N, K) or water (soil moisture). In the simplified model of DYNAMITE presented here, we assume that P limits primary production. Models where primary production is P-limited are inherently more complex than models where nitrogen is the factor that limits primary production, as was also experienced in the later version of the CENTURY model (Parton et al., 1988). This is due to the fact that two interrelated P-cycles must be taken into consideration, viz. a cycle of decomposition of organic matter and mineralisation of phosphorus, and a cycle of P-transformations of inorganic phosphorus, driven by adsorption, fixation and desorption characteristics. Both cycles interact, and understanding these interrelationships and assessment of exchange rates between pools of both subcycles is essential for modelling P-behaviour and resultant plant growth.

Because the major objective of our modelling exercise was to examine how the system of coffee cultivation affects the distribution of soil P over organic and inorganic pools, the model is confined to simple calculations of the major P flows between soil and plant. A new aspect compared to NUTCYC and DYNAMITE is that in the simplified version the influence of mycorrhiza on P uptake is explicitly considered.

Chapter 6 of the thesis provides evidence for the importance of arbuscular mycorrhizal fungi (AMF) for P-uptake (in fact, in that experiment all P was taken up through AMF), while Chapter 5 suggests that abundance and activity of AMF in agroforestry systems are higher and located in a larger soil volume than in conventional systems. For that reason we tried to include explicitly mycorrhizal functioning in our simplified model.

General design of the simplified model

Both NUTCYC and DYNAMITE use undisturbed ecosystems, i.e. under steady state conditions where inputs (through atmospheric deposition and mineral weathering) equal outputs (through erosion and leaching) as the reference system; nutrient dynamics and plant productivity in agricultural systems are considered as derived from the forest systems (Janssen et al., 1990).

Compared to undisturbed ecosystems, agro-ecosystems have a major output in the form of harvested products (in our case coffee berries is the main product). Agro-ecosystems can therefore only be in a steady state (or can be

sustainable) with extra inputs through the use of artificial fertilisers and organic amendments (for instance manure and mulch). Another way to increase inputs is to enlarge the soil volume exploited by plants root with mycorrhiza, if this is accompanied by an upward movement of nutrients. For sustainability in the long term, output through harvests must necessarily balance inputs. However, for shorter term calculations, primary production can remain constant (and the system hence sustainable) if 1) pool size is very large; 2) annual output is small compared to pool size; 3) internal cycling / transformation rates can be increased, so that effects on pool size do not translate into changes in P-availability.

In our model the following ecosystems are considered:

1. Original forest (FOREST, Figure 1);
2. Coffee cultivation systems, with a subdivision into

- Agroforestry coffee cultivation with agroforestry trees in between the coffee tree rows (AFC, Figure 2)
- Conventional coffee cultivation with weeds in between the coffee tree rows (CCW, Figure 3).

The original forest is assumed to be in steady state and this system is considered to be the reference system to which the two agricultural systems are compared. As the climax forest had not been studied in our area, the model could only be run approximately. This was based on using rounded estimates of the P pools determined in the present research and on rate constants given in Noij et al. (1993). On this basis the model is initialised, and various coffee systems are derived from that steady state.

The model DYNAMITE contains a module where water availability (soil moisture) limits primary productivity. In our simplified model we omitted this part of the cycle. This is equivalent to the condition where there is no effect of agricultural system (compared to the forest condition) on water availability. It is likely, however, that canopy opening will lead to soil warming, reduced water availability (or increased water logging at times of heavy rainfall as there is less

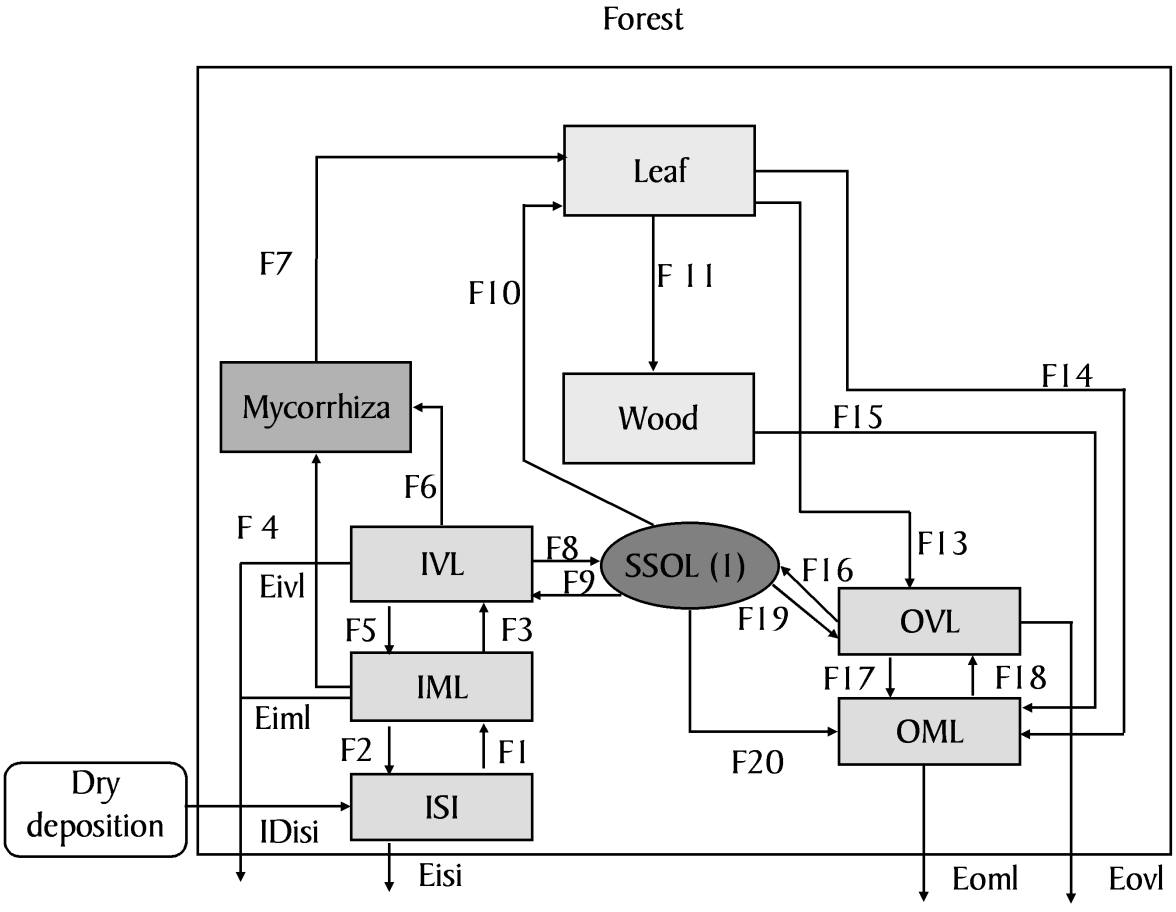

Figure 1. Forest in steady state. Schematic representation of the P processes incorporated in the simplified version of the models NUTCYC and DYNAMITE (Noij, 1988; Janssen, 1990; Noij et al., 1993). As explained in the text the letter F followed by numbers are the fluxes among the P pools, soil solution (SSOL), mycorrhizas and plant.

canopy interception), especially in seasonal, whereas agricultural systems will also affect the depth of the soil layers and the water content stored there. Differences in soil temperature, which affects the decomposition of organic matter and the mineralisation of phosphorus, between different coffee systems will also likely affect the model. The whole climatic issue is not treated here, but subsequent model improvements should take the effect of agroforestry on the hydrological cycle, temperature and water availability into account (Radersma, 2002).

Each of the ecosystems contains two compartments, viz.

1. Vegetation. Vegetation can contain one or more components (agroforestry trees, coffee and weeds). In the region, conventional coffee is often intercropped with annuals. As this was not the case in the fields sampled in our work (Chapter, 3, 4 and 5) we did not consider a system with annual crops. However, in further improvement of the model it is important to include annual crops, since it means more P leaving the systems through harvesting. The (agro-) forestry trees consist of wood and leaves, the coffee trees of wood, leaves, and fruits, whereas the weeds have only leaf material. In view of the purpose of this modelling exercise, there was no need to explicitly consider the roots of trees and herbs. However, mycorrhizal functioning is explicitly treated.

2. Soil. Soil contains organic and inorganic P pools and P in the soil solution (SSOL).

The organic pools in the soil are 'organic very labile' (OVL) and organic moderately labile' (OML). The inorganic pools in the soil are 'inorganic very labile' (IVL), inorganic moderately labile' (IML) and inorganic stable and inert (ISI). The meaning of the SSOL in the model is not the same as in real world. In the real world, SSOL contains only very little P and is replenished a few times per day. In the model we consider SSOL as a transit pool for all inorganic P that is dissolved and organic P that is mineralised in the course of one year. A same amount of P leaves SSOL through microbial immobilisation, through uptake by trees and weeds, and through adsorption to IVL. The soil solution

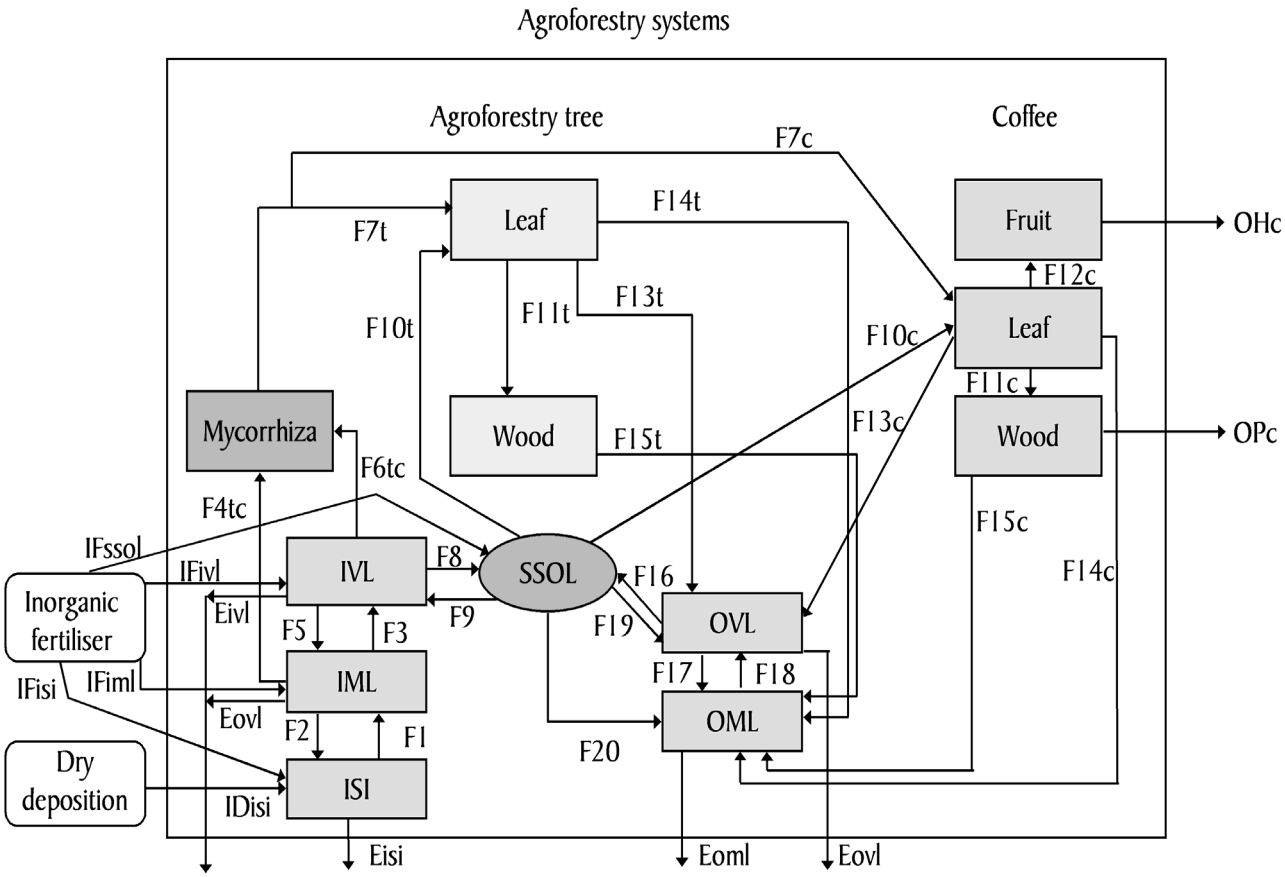

Figure 2. Agroforestry coffee systems. Schematic representation of the P processes incorporated in the simplified version of the models NUTCYC and DYNAMITE (Noij, 1988; Janssen, 1990; Noij et al., 1993). As explained in the text the letter F followed by numbers are the fluxes among the P pools, soil solution (SSOL), mycorrhizas and plant.

functions as the link between inorganic and organic P pools. Another way to (further) link these pools would be through the explicit inclusion of the microbial biomass (this is now only implicit in the model – see below). Beck (1991, see Figure 4) linked both kinds of P pools through a relationship between the inorganic P pool (as assessed by diluted HCl) and organic P pools through microbiological activity. However, this link was introduced for modelling purposes and does not (yet) have a biologically meaningful mechanistic underpinning.

The definition of the organic P pools is derived from the decomposability (as dependent on apparent initial age) of the organic materials. These pools have both a conceptual and operational aspects, measurable through the study of carbon dynamics. Operationally, these pools are not exactly equivalent to the OVL and OML pools as defined in Chapters 3 and 4. Further studies are needed to better link both ways of defining organic P pools. The conceptual difference can be seen from a comparison of Figure 2 (Chapter 1) and Figures 1, 2 and 3 in this chapter. In the latter model the saprotrophic part of the microbial biomass is treated implicitly as part of OVL and OML, whereas the mycorrhizal biomass is not linked to organic P pools. In the conceptual model of Chapter 1 the microbial biomass is explicit. Here the micro-organisms are considered as a wheel that rotates in the soil, simultaneously consuming and releasing P in the soil solution and transferring P among the pools (Stevenson and Cole, 1999).

Our model cannot be considered as a mechanistic model. Calculations are based on empirical relationships. The time step is one year. In each time step erosion is calculated first. Thereafter most other processes take place simultaneously, but immobilisation and adsorption of SSOL P have priority to uptake by the plants.

State variables

The situation under original forest is taken as a starting point. The relative pool sizes (as percentage of total P) in FOREST are derived from the chemical fractionation of the soils in agroforestry fields, as described in Chapter 3. Table 1 shows the pools and the corresponding chemical fractions as well as the relative distribution of P and the pool sizes assuming a total pool size of 2000 kg P per ha. The total size is based on an rounded average value of 500 mg P per kg soil, and a total soil mass of $4*10^6$ kg ha^{-1}, roughly representing the soil layer from 15 to 45 cm with a bulk

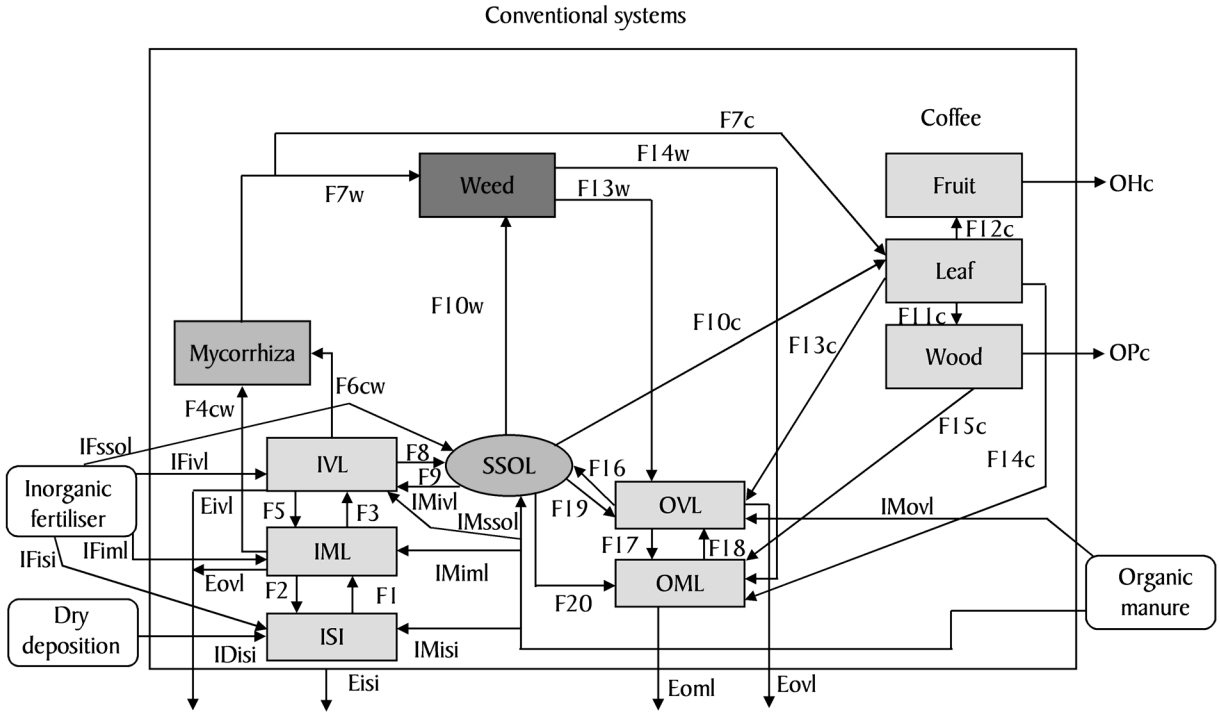

Figure 3. Conventional (full-sun, monoculture) coffee systems. Schematic representation of the P processes incorporated in the simplified version of the models NUTCYC and DYNAMITE (Noij, 1988; Janssen, 1990; Noij et al., 1993). As explained in the text the letter F followed by numbers are the fluxes among the P pools, soil solution (SSOL), mycorrhizas and plant.

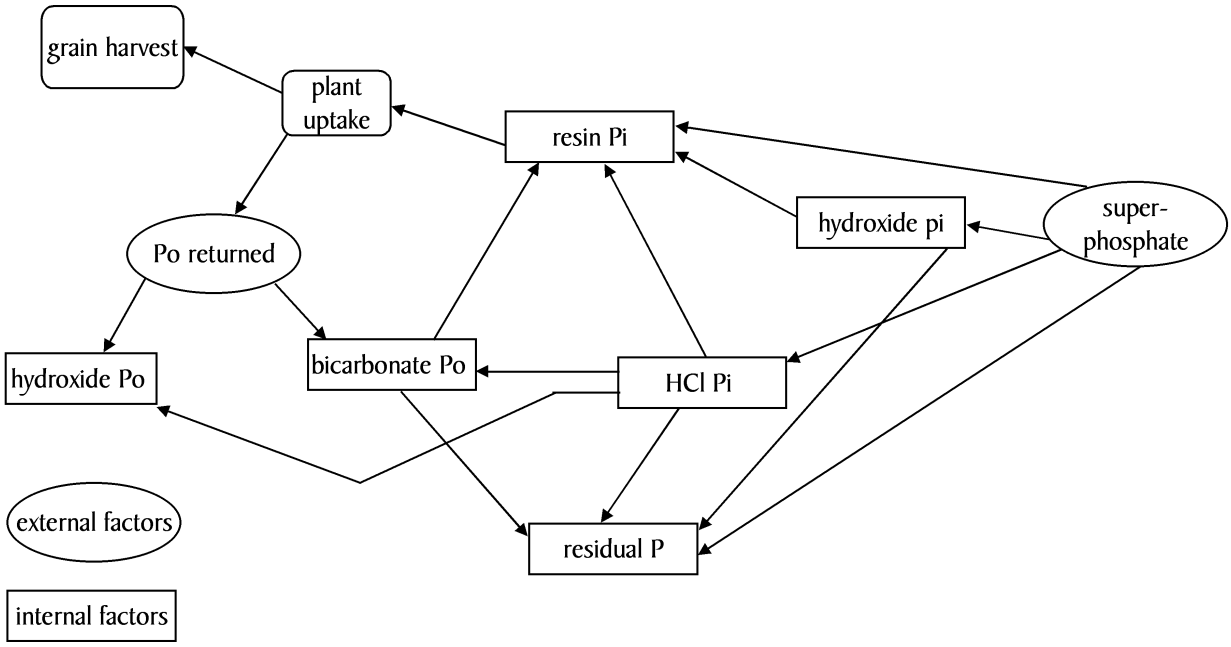

Figure 4. Relationships between the soil inorganic and organic P pools (Beck, 1991).

density of 1.33 g cm^{-3}. The fractions of inorganic and organic pools are 75% and 25% for total P, about 2.5 % of very labile P and 68.5 % of stable and inert P. According to Table 1, OML consists of NaOH Po + HCl Po. It should be noticed that no HCl Po was detected in our soils (Chapter 3).

Inputs and outputs of P to the ecosystems

Inputs of P to the ecosystems may be in the shape of atmospheric deposition, weathering of primary materials, and organic and inorganic fertilisers. Outputs from the ecosystem may occur via leaching, erosion and harvest of coffee berries. In Oxisols, weathering of primary minerals is negligible. Because of the very low P concentrations in the soil solution, no leaching of P is considered.

As atmospheric deposition consists of P present in dust or volcanic ash, it is described as a P flow to the ISI pool (IDisi). Its value often is below 1 kg per ha per year (Poels, 1989; Stoorvogel et al., 1997). In our calculations we take a default value of 1 kg per ha per year. Because the ratio of inorganic to organic P in the soil equals 1500:500, the fate of dust P is modelled in such a way that 0.25 kg per ha per year enters the 'closed organic cycle' of the ecosystem (see below).

In FOREST (steady state), the output through erosion must equal the input through deposition. Given a total soil P

Table 1. Soil P pools, corresponding extraction methods, relative distribution of P and P pool sizes as used in the model calculations.

Pools[1]	Extraction	P proportion[2]	P pool size[3]
IVL	Resin + $NaHCO_3$-Pi	0.5	10
IML	NaOH Pi	6.0	120
ISI	HCl Pi + residue[4]	68.5	1370
OVL	$NaHCO_3$-Po	2.0	40
OML	NaOH-Po + HCl-Po	23.0	460

[1]I = inorganic; V = very; L = labile; M = moderately; SI = stable or inert; O = organic; [2] %; [3] Kg ha^{-1}; [4]In Chapter 3 residue was not measured.

supply of 2000 kg/ha and an annual input of 1 kg per ha, erosion is set at an annual fraction of 0.0005 of soil P in forest. For agroforestry systems the same value is used, and for conventional systems it is set at 0.001. Two times more erosion in the conventional systems is a conservative value, since it was shown that for the region studied here, the value is at least 4 times more (Franco, 2000).

In the appendix, we discuss the approach to make a justified allocation of inorganic fertiliser P (IF standing for Input Fertilisers) among IVL (IFivl), IML (IFiml), ISI (Ifisi) and SSOL (IFssol).

On the basis of the studies by Gerritse and Zugec (1977), organic P fertilisers (IM stands for Input Manure) are estimated to consist of organic P for 20 %, and of inorganic P for 80%. The inorganic part is subdivided (as for inorganic fertiliser, see appendix) to IVL (IMivl), IML (IMiml), ISI (IMisi) and SSOL (IMssol). The organic part is considered to be equally resistant to decomposition as leaf material (see appendix), and hence is allocated to OVL (IMovl).

Internal P flows in FOREST (steady state)

Calibration procedure

The internal P flows are calculated as a fraction per year (coefficients) of the state variable from where they depart, so we do not make the calculations with rate constants of first order reactions. Most of the values of the coefficients were derived from the results described in foregoing chapters of this thesis, or from other literature. Some values had to be calculated as the 'unknowns' in algebraic equations formulated on the basis of known state variables and flows in FOREST.

The discussion below follows the numbering of the flows shown in Figure 1. First the coefficients of the flows in the inorganic part of FOREST are solved and next the organic part of the system is described. In the case of soil pools, the calculations start with the erosion of the particular pool during that time step. The coefficients of the other flows starting refer always to the pool sizes corrected for erosion. They are denoted by ISIae, IMLae, IVLae, OVLae and OMLae, where 'ae' means 'after erosion'.

In a closed ecosystem, the internal nutrient cycle can be described as a sequence of fluxes between organic pools only. In such a system there is no role for inorganic nutrient pools. The subsequent fluxes are from soil solution to vegetation (uptake), from vegetation to soil organic pools (litter fall), and finally from soil organic pools to again the soil solution (mineralisation).

The system FOREST is not perfectly closed, as there is an input of dust from outside and an output via erosion. To compensate for the erosion of the organic pools, 0.25 kg P per ha per year must enter the 'closed organic cycle' of the ecosystem. This happens via nutrient uptake from the inorganic pools.

Inorganic part of the model

In the forest, because the forest system is in steady state, the state variables do not change in size. For each of them the sum of inputs equals the sum of outputs. Table 2 gives an overview of the inputs and outputs for the three inorganic pools. It contains known as well as unknown values of fluxes and coefficients.

The sizes of ISIae, IMLae and IVLae are easily found as the differences between ISI and Eisi, IML and Eiml, and IVL and Eivl, respectively. They are: 1369.315 kg, 119.94 kg and 9.995 kg P per ha.To solve the other unknowns, we start with fluxes F1 and F2, describing the flows between ISI and IML. From Table 2 it follows:

$$1 + IMLae * C2 = 0.685 + ISIae * C1 \qquad \text{Eq. 1}$$

There are two unknowns and just one equation. If we, for the time being, consider F1 as the net flux between the two pools, which implies that F2 = 0, and hence C2 = 0, F1 and the corresponding C1 can be calculated (Equation 2a,b):

$$F1 = 1 - 0.685 = 0.315 \qquad \text{Eq. 2a}$$

Table 2. Inputs and outputs of P for each inorganic soil pool. Internal fluxes are denoted by F and the numbering is as in Figure 1. Known flux sizes are in kg P ha^{-1} $year^{-1}$.

Pools	Inputs (codes)	Size	Outputs (codes)	Size
ISI	IDisi	I	Eisi	0.685[1]
	F2	IMLae*C2	F1	ISIae*C1
IML	F1	ISIae*C1	Eiml	0.06[1]
	F5	IVLae*C5	F2	IMLae*C2
			F3	IMLae*C3
			F4	IMLae*C4
IVL	F3	IMLae*C3	Eivl	0.005[1]
	F9	See text	F5	IVLae*C5
			F6	IVLae*C6
			F8	IVLae*C8

[1] pool * erosion rate (1370*0.0005; 120*0.0005; 10*0.0005)

$$C1 = 0.315/1369.315 = 0.00023 \quad \text{Eq. 2b.}$$

Such a solution is satisfying under steady state conditions, but not in other situations. In a fertilised system, F2 can not be considered zero because there is fixation of P, i.e., the moderately labile P transforms in stable P. To allow for movement of P from IML to ISI, C2 must be positive, and hence C1 must be greater than calculated in Eq. 2b. The reasoning was as follows.

If there would be a perfect chemical equilibrium between ISI and IML, F1 would equal F2, and the ratio C2/C1 would be equal to the ratio ISI/IML or ISIae/IMLae (Janssen, 1999). This would result in: $C2/C1 = 1369.315/119.94 = 11.42$.

There is however not a perfect equilibrium between ISI and IML, but a dynamic equilibrium as both pools have also other flows than just F1 and F2 between them. From Eq. 1, it follows:

$$C2/C1 = ISIae/IMLae - 0.315/(C1 * IMLae) < 11.42$$

To have a starting point, we use a value of 6 for C2/C1, which is the ratio of the coefficients found for the transformations of labile P to stable P and vice versa in a former study (Janssen et al., 1987, Wolf et al., 1987). Substitution of $C2/C1 = 6$ gives C1 and C2, to be used in the model:

$C1 = 0.000485$, $C2 = 0.0029$, $F1 = 0.6639$ and $F2 = 0.3490$.

To find values for the other coefficients, we have to continue with the uptake flows F8, F6 and F4. Three uptake routes are considered: from IVL via the soil solution (F8), from IVL with the help of mycorrhiza (F6), and from IML with the help of mycorrhiza (F4). The total uptake from inorganic pools is 0.25 kg P. Hence it follows:

$$IMLae * C4 + IVLae * C6 + IVLae * C6 = 0.25 \quad \text{Eq. 3}$$

In the model it is attributed to the three flows on the basis of the results of the greenhouse experiment described in Chapter 6. Table 3 presents the calculation of the fractions of the pools that were used for the uptake routes. Next the proportion between IML and IVL of the mycorrhiza uptake fractions was calculated, and the proportion between direct and mycorrhiza of the uptake fractions from IVL. Assuming that in the field the proportions between IVL and IML, and between direct uptake and uptake via mycorrhiza are the same as in the pot (see below), the coefficients C4,

Table 3. Uptake of P from IVL and IML, directly or via mycorrhiza, as found in the greenhouse experiment, and calculation of the proportions of the coefficients C6 and C8 to be used in the model for the pools after erosion.

	Route	Pool[1]	Uptake [1]	Uptake : Pool	Coefficients	Ratio of coeficientes
IML	Mycorrhiza	9.154	1.654	0.1812	C4	
IVL	Mycorrhiza	1.792	1.429	0.7974	C6	4.400[2]
IVL	Direct	1.792	0.285	0.1590	C8	5.014[3]

[1]mg pot^{-1} in three months (greenhouse experiment, Chapter 6); [2] 0.7974/0.1812; [3]0.7974/0.1590

C6 and C8 can be calculated. From Table 3 it follows (rounded data): C6 = 5.0 * C8, and C6 = 4.4 * C4. So C4 = 5.0/4.4 = 1.14 * C8. Substitution in Eq. 3 results in:

119.94 * 1.14 * C8 + 9.995 * 5.0 * C8 + 9.995 * C8 = 0.25, and

C8 = 0.00127.

Hence C6 = 0.00637 and C4 = 0.001448. The corresponding fluxes F4, F6 and F8 can now be calculated as 0.173635, 0.063667 and 0.012698, together 0.25.

While the experiment reported in Chapter 6 showed that more than 90% of the nutrients were taken up through mycorrhizas, it should not be forgotten that in that experiment the organic P cycle would be small compared to the field situation, where litter fall and mineralisation of fresh litter provide an additional source of P to the soil solution. This partly explains why in this model the soil solution (F10, see below) is more important for nutrient uptake than the mycorrhizal part (F4 and F6). In the model we force the uptake from inorganic pools to be exactly 0.25 kg P per ha per year, and that likely is the major reason for the low influence of mycorrhizas in the model.

The two fluxes between IVL and IML, F3 and F5, and F9 the flux between soil solution (SSOL) and IVL are still unknown. They are related to each other according to Equation 4, based on the equality of inputs and outputs of IVL:

$$119.94 * C3 + F9 = 0.0005 + 9.995 * C5 + 0.063667 + 0.012698 \qquad \text{Eq. 4}$$

Flux F9, from SSOL to IVL, represents rapid adsorption of P. Its value is for the time being set at 0 in the steady state situation. This does not mean that we neglect adsorption, but that we consider F8 as the net result of desorption and adsorption in steady state. The allocation of the major part of soluble fertiliser P to IVL, IML and ISI is also meant to take the instantaneous adsorption into account. If there is no steady state there will be net adsorption or net desorption, depending on the relative values of SSOL and IVL. This is further discussed in subsection *Soil solution and P adsorption in the model*.

To find C3 and C5, we assumed that C3 is 0.033 (Noij et al., 1993, p. 30). With C3 we can solve C5 from Equation 4, which results in C5 = 0.388. It should be stressed that this is considered a provisional solution. Further elaboration is needed.

Organic part of the model

To compensate for the erosion of the organic pools, 0.25 kg P per ha per year must enter the 'closed organic cycle' of the ecosystem. This happens via nutrient release from the inorganic pools (F4, F6 and F8). The inputs and outputs within the organic part of FOREST are summarised in Table 4, in a similar way as those of the inorganic part in Table 2. The nutrients taken up are supposed to go directly from the roots to the leaves. The portion that is tapped in the stem is probably smaller than 5% (Noij et al., 1993) and neglected for the sake of simplicity. Fluxes F4 and F6 are combined in F7, the uptake via mycorrhiza; so F7 = 0.173635 + 0.063667 = 0.237302.

F10, the uptake from SSOL is different than F8, desorption of P from IVL, because there are more flows related to SSOL (F9, F16, F 19 and F20). Hence F10 cannot yet be calculated.

Nutrients leaving OVL by erosion (Eovl) amount to 0.0005 * 40 = 0.02, and hence OVLae = 39.98. Erosion of OML, Eoml, is equal to 0.0005* 460 = 0.23. The sum of Eovl + Eoml is 0.25, equal to the input in the organic part of the ecosystem via flows F4, F6 and F8. Organic P present in woody litter moves to OML (F15). It is assumed that forest trees reach an average each of 80 years. Hence C15 is 1/80 or 0.0125. Input and output of WOOD are equal and hence:

0.6 * LEAF = 0.125 * WOOD or WOOD = 48 * LEAF.

F15 can now be expressed as a function of LEAF: 0.0125 * 48 * LEAF = 0.6 * LEAF. Before leaves fall from the tree, Leaf P is redistributed in the tree to WOOD (F11) and in the case of coffee trees, also to FRUIT (F12). The corresponding coefficient C11 is set at 0.6, based on Noij et al. (1993). Leaf P is further allocated to the organic very labile P pool (F13); as leaves are easily decomposable no Leaf P is allocated to OML, so C13 is 0.4, and C14 and F14 are 0.

From Table 4 it follows that the following unknowns still have to be solved: C16, C17, C18, F9, F19, F20, LEAF and WOOD. From Table 4 in Chapter 3 it follows that the ratio organic C : organic P (henceforth denoted by C/P) is 200; this value is used for both soil organic pools. For C/P in WOOD and LEAF rounded values of 1600 and 400 were derived from Table III.3, page 163 in Noij et al. (1993). Because a fraction of 0.6 of LEAF P is redistributed, and hence only a fraction of 0.4 of the original P is remaining in leaf litter, C/P in leaf litter is (1/0.4) * 400 = 1000. If the addition of leaf carbon to the soil would be 1000 kg C, the addition of P (F13) would be 1 kg P, while the original LEAF P is 2.5 kg P.

In the appendix it is explained how the fraction organic material remaining at 1 year after addition to the soil (y_1/y_0) can be calculated with the equations by Janssen (1984, 1986) on the basis of a value of 2.18 years (Noij et al., 1993) for the standard apparent initial age of leaf material, and given an average annual temperature of 18 °C:

$$y_1/y_0 = \exp\{4.7[(2.18 + 2*1)^{-0.6} - 2.18^{-0.6}]\} = 0.386 \qquad \text{Eq. 5}$$

If the remaining organic material after one year has a C/P of 200, its P content would be 386/200 = 1.93 kg, hence

Table 4. Inputs and outputs of P for state variables in the organic part of FOREST (steady state). Internal fluxes are denoted by F and the numbering is as in Figure 1. Values refer to coefficients and fluxes that were known before the calibration of the organic part of the model. Known flux sizes are in kg ha^{-1} $year^{-1}$.

Pool	Inputs code	Size	Outputs code	Size
MYCORRHIZA	F4	0.173635	F7	0.237302
	F6	0.063667		
LEAF	F7	0.237302	F11	0.6 * LEAF
	F10	F8+F16-F9-F19-F20	F13	0.4 * LEAF
WOOD	F11	0.6 * LEAF	F15	0.0125 * WOOD
OVL[1]	F13	0.4 * LEAF	Eovl	0.02
	F18	C18* 459.77	F16	C16 * 39.98
	F19	0.93 * F13	F17	C17 * 39.98
OML[2]	F14	0.0 * LEAF	Eoml	0.23
	F15	0.0125 * WOOD	F18	C18 * 459.77
	F17	C17 * 39.98		
	F20	4.143109 * F15		
SSOL[3]	F8	0.012698	F9	See text
	F16	C16 * 39.98	F10	F8+F16-F9-F19-F20
			F19	0.93 * F13
			F20	4.143109 * F15

[1] Organic P very labile; [2] Organic P moderately labile; [3] soil solution.

0.93 kg P has to be immobilised per kg P moving from LEAF to OVL, so F 19 = 0.93 * F13.

WOOD P is 48 * LEAF P = 48 * 2.5 = 120 kg P, in the case LEAFP is 2.5 kg P. In WOOD, C/P = 1600, making WOODC is 1600 * 120 =192 000 kg per ha. Average age of forest trees is estimated at 80 years (various sources, a.o. Noij et al., 1993), so per year 192 000/80 = 2400 kg WOODC and 120/80 = 1.5 kg WOODP move from WOOD to OML. The apparent initial age of wood is 4.0 (Noij et al., 1993). Given an average annual temperature of 18 °C, the fraction of wood remaining at 1 year after addition to the soil (y_1/y_0) is calculated to be:

$$y_1/y_0 = \exp\{4.7[(4.0 + 2*1)^{-0.6} - 4.0^{-0.6}]\} = 0.643 \qquad \text{Eq. 6}$$

So 1543 (rounded) of the 2400 kg C are remaining after one year. Again assuming that the remaining organic material has a C/P of 200, its P content would be 1543/200 = 7.715 kg, which implies that 7.715 - 1.5 kg = 6.215 kg P (F20) has to be immobilised. So F20 = 6.215/1.5 = 4.143 times F15.

In the model, immobilisation has priority above uptake because saprotrophic micro-organisms are considered to be competitively superior to plants under conditions of P-limitation. The P remaining in SSOL after immobilisation (SSOLnet) is partly adsorbed to IVL (see below, F9). The difference between SSOLnet and F9 is taken up by the trees. The solution of F9 is discussed in the subsection of SSOL.

The four remaining unknowns - C16, C17, C18 and LEAF - must follow from the P and C balances of OVL, OML and SSOL. The P balances of OVL, OML and SSOL can be described with Eq. 7, 8 and 9, respectively (compare Table 4):

$$0.4 * LEAF + C18 * 459.77 + 0.93 * 0.4 * LEAF = 0.02 + C16 * 39.98 + C17 * 39.98 \qquad \text{Eq. 7}$$

$$0.6 * LEAF + C17 * 39.98 + 4.143109 * 0.6 * LEAF = 0.23 + C18 * 459.77 \qquad \text{Eq. 8}$$

$$0.012698 + C16 * 39.98 = F10 + 0.93 * 0.4 * LEAF + 4.143109 * 0.6 * LEAF \qquad \text{Eq. 9a}$$

From Eq.8a it follows:

$$LEAF = 148.995 * C18 - 12.956 * C17 + 0.0745 \qquad \text{Eq. 8b}$$

As F10, the uptake from the solution, is equal to the total uptake minus the uptake via mycorrhiza (F7), and the total uptake is equal to LEAF, it holds:

F10 = LEAF - 0.237302. After substitution of F10 in Eq. 9a, and simplifying, we get:

$$C16 * 39.98 = 3.858\,78654 * LEAF - 0.25 \qquad \text{Eq. 9b}$$

The amounts of organic C in OVL and OML (OVL C and OML C) are 200 * organic P; LEAF C = 400 * LEAF P, and WOOD C = 1600 * WOOD P. For the mineralisation (F16) of 1 kg P of organic matter with a C/P of 200, a dissimilation of 200 kg C is required (see Appendix). Immobilisation as such does not apply to C, so F19 and F20 do not exist for C, but during the process a part of the added C dissimilates. From Eq. 5 and 6, it follows that the fraction of C that dissimilates is 0.614 and 0.357 for F13 and F15, respectively. They are outgoing flows for the carbon balances of OVL and OML. The C balances can now be described with Eq. 10 and 11, respectively (compare Table 4):

$$0.4 * LEAF * 1000 + C18 * 459.77 * 200 = 0.02 * 200 + C16 * 39.98 * 200 + C17 * 39.98 * 200 + 0.614 * 0.4 * LEAF * 1000 \qquad \text{Eq. 10}$$

$$0.6 * LEAF * 1600 + C17 * 39.98 * 200 =$$

$$0.23 * 200 + C18 * 459.77 * 200 + 0.357 * 0.6 * LEAF * 1600 \quad \text{Eq. 11}$$

(Eq. 10 and 11 are only presented for the sake of completeness; they do not offer extra means to solve the unknowns.)

The mineralisation of organic P is related to the decomposition of soil organic matter. Hence the mean rate of decomposition of soil organic matter must be known to solve the remaining unknowns C16, C17 and C18. Because no data were at hand, we calculated the decomposition rate, assuming an apparent initial age of 20 years for SOM and an average temperature of 18 °C:

$$y_1/y_0 = \exp\{4.7[20.0 + 2*1)^{-0.6} - 20.0^{-0.6} = 0.9576 \quad \text{Eq. 12}$$

and the fraction of soil organic matter and soil organic carbon mineralised per year is 0.0424 or about 4.24%. In reality the decomposition may be less because part of the organic matter is fixed to particles of clay and oxides and hence less available for breakdown. Other reasons may be the lower temperature than average in the forest, and the occurrence of dry spells. Consequently, the amount of P released may also be less.

In the model mineralisation of SOM is represented by F16, for C it is equal to C16 * 39.98 * 200. The sum of C in OVL and OML is 200 * (39.98 + 459.77) = 99950 kg per ha. It follows:

$$C16 * 39.98 * 200 = 0.04236872 * 99950$$
$$C16 = 0.5296$$
$$F16 = C16 * OVLae = 0.5296* 39.98 = 21.17376782$$

Substitution of C16 in Eq. 9b yields:

$$LEAF = (0.5296 * 39.98 + 0.25)/ 3.858= 5.5533$$
So, $WOOD = 48 * LEAF = 266.557$.

The value of 267 kg P per ha is quite high for tropical rain forests (Noij et al., 1993), this must be attributed to the assumed high decomposition rate of 4.42% of total soil organic matter and the fact that we neglected F9, adsorption of P from SSOL to IVL.

Next we can calculate:

$$F13 = 0.4 * 5.553 = 2.213$$
$$F15 = 0.0125 * WOOD = 3.332.$$
$$F19 = 0.93 * F13 = 2.066$$
$$F20 = 4.143 * F15 = 13.805$$

Substitution of LEAF and C16 in Eq.7 gives

$$0.4 * 5.553 + C18 * 459.77 + 0.93 * 0.4 * 5.553 = 0.02 + 0.5296 * 39.98 + C17 * 39.98$$
$$C18 * 459.77 = C17 * 39.98 + 16.907$$

$$C18 = 0.0870* C17 + 0.0368 \quad \text{Eq. 13}$$

As F17 and F18 describe the fluxes between OVL and OML, their values depend on each other. Equations 7, 8, 10 and 11 result all in the same relationship (of Eq. 13) between C18 and C17. For each value of C17, the

corresponding value of C18 can be calculated; C18 is 0.0368 for C17 is 0. Therefore an independent relationship for either C17 or C18 is needed. It was found via the following reasoning.

The very labile organic pool is fed by inflows of materials that are easily decomposable, such as leaves, organic manure and microbial biomass. In the terminology of Janssen (1984, 1986) they have an apparent initial age between 2.0 and 2.5 years. Under the prevailing temperature of 18 °C, the fraction mineralised per year is 55-65%.

The moderately labile organic pool is fed by inflows of materials that are not very decomposable, such as wood, leaves and organic manure that has already stayed for one year in the soil. They have an apparent initial age between 4.0 and 4.5 years (under the prevailing temperature of 18 °C, each calendar year equals two decomposition years in the terminology of Janssen), corresponding with a fraction mineralised per year between 30-35%.

From the foregoing it follows that F17 consists of leaves and organic manure which are already one year in the soil. The amount of P in F17 is equal to the initial amount plus the immobilised P (no immobilisation takes place for Imovl, because manure has a low C/P):

F17 = F13 + F19 + Movl = 2.213 + 2.066 + 0 = 4.279
C17 = (F13 + F19 + Movl)/OVLae = 4.279/39.98 = 0.107.
C18 = 0.0870* C17 + 0.0368= 0.0461

F18 consists of microbial biomass and microbial products formed in OML, being as decomposable as leaves and organic manure. F18 can be calculated as:

F18 = F14 + F15 + F17 + F20 – Eoml = 0 + 3.332 + 4.279 +13.805– 0.23 = 21.186

From which follows C18 = 21.186/459.77 = 0.0461

Soil solution and P adsorption in the model

Finally SSOL and F9 must be considered. Inputs to SSOL are F8 and F16. So, SSOL = 0.0127 + 21.1738 = 21.1865. Outputs are immobilisation (F19 = 2.066 and F20 = 13.805), uptake (F10) and adsorption to IVL. In FOREST, we assume for the time being that F9 = 0. In other systems but also in the forest F9 may have a positive or negative values, depending on the ratio of IVL to SSOL, or better of IVL to SSOLnet, where SSOLnet is SSOL corrected for immobilisation. In the FOREST described here it holds:

SSOLnet = SSOL – F19 – F20
= 21.1865- 2.066 - 13.805 = 5.3160
IVLae = 9.995
IVL/SSOLnet= 1.880
So, in FOREST:
(IVLae + F9)/(SSOLnet - F9) = 1.880
F9 = 0.6528 SSOLnet - 0.3472 * IVLae Eq. 14

The result of Eq. 14 is of course 0 in FOREST. Finally F10 = SSOLnet – F9 = 5.3160– 0 = 5.3160.

In more general terms, it holds:

(IVLae + F9)/(SSOLnet - F9) = Cads,

where Cads stands for adsorption constant. Its value can be found via interpretation of fertiliser experiments. See appendix.

F10 + F7 must equal LEAF: 5.3160 + 0.2373 = 5.5533, a value equal to the earlier calculated one.

Table 5 gives an overview of all model parameters used so far and of the ones to be discussed below.

Table 5. Overview of internal flows, inputs and outputs, and (rounded) default values of the corresponding coefficients or equations to calculate the flow in the ecosystems: forest, AFC (agroforestry coffee systems) and CCW (conventional coffee systems, with weeds between coffee rows).

Ecosystem	Flow	From	To	Coefficient or Equation	Share
Internal flows					
All	F1	ISI[1]	IML	$0.485 * 10^{-3}$	
All	F2	IML[2]	ISI	$2.909* 10^{-3}$	
All	F3	IML	IVL	0.033	
Forest	F4	IML	MYCOR[15]	$1.448 * 10^{-3}$	
AFC	F4t	IML	MYCOR	$1.448 * 10^{-3}$	0.4
AFC	F4c	IML	MYCOR	$1.448 * 10^{-3}$	0.6
CCW	F4w	IML	MYCOR	$1.086* 10^{-3}$	0.4
CCW	F4c	IML	MYCOR	$1.086* 10^{-3}$	0.6
All	F5	IVL[3]	IML	0.388	
Forest	F6	IVL	MYCOR	$6.37 * 10^{-3}$	
AFC	F6t	IVL	MYCOR	$6.37 * 10^{-3}$	0.4
AFC	F6c	IVL	MYCOR	$6.37 * 10^{-3}$	06
CCW	F6w	IVL	MYCOR	$4.78 * 10^{-3}$	0.4
CCW	F6c	IVL	MYCOR	$4.78 * 10^{-3}$	0.6
All	F7	MYCOR[4]	LEAF	F4 + F6	
All	F8	IVL	SSOL	$1.27 * 10^{-3}$	
All	F9	SSOL[5]	IVL	See text	
Forest	F10	SSOL	LEAF	F8 + F16 – F19	
AFC	F10t	SSOL	LEAFt	F8 + F16 – F19	0.4
AFC	F10c	SSOL	LEAFc	F8 + F16 – F19	0.6
CCW	F10w	SSOL	LEAFw	F8 + F16 – F19	0.4
CCW	F10c	SSOL	LEAFc	F8 + F16 – F19	0.6
Forest	F11	LEAF	WOOD	0.6	
AFC	F11t	LEAFt[6]	WOOD	0.6	
AFC	F11c	LEAFc[7]	WOOD	0.05	
AFC	F12c	LEAFc	FRUIT	0.75	
Forest	F13	LEAF	OVL	0.4	
AFC	F13t	LEAFt	OVL	0.4	
AFC, CCW	F13c	LEAFc	OVL	0.2	
CCW	F13w	LEAFw[8]	OVL	1.0	
Forest	F14	LEAF	OML	0.0	
AFC	F14t	LEAFt	OML	0.0	
AFC, CCW	F14c	LEAFc	OML	0.0	
CCW	F14w	LEAFw	OML	0.0	
Forest	F15	WOOD	OML	$12.5 * 10^{-3}$	
AFC	F15t	WOODt	OML	$12.5 * 10^{-3}$	
AFC, CCW	F15c	WOODc	OML	$0.05 * 10^{-3}$	
All	F16	OVL	SSOL	0.530	
All	F17	OVL	OML	0.107	
All	F18	OML	OVL	0.046	
All	F19	SSOL	OVL	0.93 * F13	

(Table continued on next page)

Table 5 (continued)

Ecosystem	Flow	From	To	Coefficient or Equation	Share
All	F20	SSOL	OML	4.143 * F15	
Inputs					
All	Idisi[9]	ATMOS	ISI	1 kg year^{-1}	
AFC, CCW	IF[10] ivl	CF[15]	IVL	0.06	
AFC, CCW	IF iml	CF	IML	0.10	
AFC, CCW	IF isi	CF	ISI	0.79	
AFC, CCW	IF ssol	CF	SSOL	0.05	
CCW	Imovl	AM[16]	OVL	0.2	
CCW	Imoml	AM	OML	0.0	
CCW	Imivl	AM	IVL	0.048	
CCW	IM[11]iml	AM	IML	0.08	
CCW	IM isi	AM	ISI	0.632	
CCW	Imssol	AM	SSOL	0.04	
Outputs					
AFC, CCW	OH c[12]	FRUITc		1*FRUITc	
AFC	OPc [13]	WOODc		0.75	1* per 20 years
CCW	OPc	WOODc		0.75	1* per 15 years
Forest, AFC	E[14]	All pools		0.0005	
CCW	E	All pools		0.0010	

[1]Inorganic stable or inert; [2]Inorganic moderately labile; [3]Inorganic very labile, [4]Mycorrhiza; [5]Soil solution; [6]Tree; [7]Coffee; [8]Weed; [9]Inorganic P due to atmospheric deposition; [10]Input as inorganic fertiliser; [11]Input as manure; [12]Output harvest coffee; [13]Output pruning coffee; [14]Erosion; [15]Chemical fertiliser; [16]Animal manure.

Internal P flows in coffee cultivation systems

Characteristics of P cycling in coffee cultivation systems

For coffee growing the forest trees have to be removed, and coffee trees must be planted. Usually the trees are planted in rows, about 4 m or 3.5 m, and the distance within the row is 2 m or 1.5 m; the total number of coffee trees varies from 1300 to 2000 per ha. In between the coffee tree rows, the soil is occupied by weeds in the conventional systems, and planted to trees in agroforestry system. Hence, the soil pools to be exploited must be shared by coffee trees and other crops. The default values used in the model are 0.6 for coffee and 0.4 for the other crops (see most right column in Table 6).This sharing plays a role for all flows between soil pools and trees, i.e. from the flows starting from IML, IVL, and SSOL. Coffee trees produce berries, and the required P is derived from LEAFc. The coefficient is set at 0.75, a value derived from data given by Malavolta (1993). The remaining P of LEAFc is distributed among WOODc (0.05) and OVL (0.2).

Coffee trees are assumed to be pruned every 20 years in the agroforestry system and every 15 years in the conventional system. At that time 75% of the wood is cut and removed from the field (OPc). Further it is assumed that 5% of the P in WOODc, moves to OML; this is mainly from dying roots.

Only one of the conventional fields studied in this thesis (Chapter 3, 4 and 5) received organic manure. The organic manure was applied during 10 years, and the last application was about 15 years ago. Thus we considered organic fertilisation only for the conventional systems during the first 10 years, at a rate of 10 kg P ha^{-1} year^{-1}. The quantities of inorganic fertiliser P is set at 10 kg P per ha per year in both systems.

We assume that there is one large mycelium network belowground from which coffee plants as well as intercrops derive benefit. Using spore abundance as an approximation of mycorrhizal activity (Chapter 5), we estimate about 25% more spores in agroforestry than in conventional systems. In the model this would affect only F7 (F4 +F6), not F10, as all the net P in the soil solution (SSOLnet) is considered to be available for uptake (and hence actually taken up) by roots and or mycorrhizal fungi. Differences in mycorrhizal functioning between conventional and agroforestry

would have only a minor impact on the model outcomes, as the model was constrained to have only 0.25 kg P going from inorganic to organic pools through mycorrhizal activity. However, if, as conventional wisdom about mycorrhizal functioning suggests, mycorrhizal fungi and plant roots have access to the same P-sources and mycorrhizas extend the depletion zone that develops around as a consequence of the limited diffusion of P (Smith and Read, 1997), differences in mycorrhizal activity should also have an effect on SSOL. However, we are not yet able to incorporate this in the model in a way that is both biologically realistic and computationally efficient. This is due to the fact that the conceptual definition of SSOL (used in this chapter) cannot be operationalised into a measurable pool such as P-resin. This conclusion reinforces the idea that the links between the organic and inorganic P pools are critical to this model and that SSOL as the sole bridge between both pools may be too restricted. If the lower mycorrhizal activity in conventional compared to agroforestry would result in a lower uptake potential from SSOL, the model would feed back into lower primary production and lower P-return to the soil until uptake and P-mineralisation are similar again. That model would indeed support the claim that P-cycling rates in agroforestry are higher than in conventional. However, that result would be a consequence of the assumption built in the model.

In both systems coffee is supposed to take up 60 % via F4, F6 and F10 (share rate). In the agroforestry systems trees and in conventional systems weeds take up the other 40 %. These values are mainly based on the fact that there are more coffee trees in the systems than agroforestry trees and weeds. The real values are unknown for the time being.

Agroforestry coffee cultivation (AFC)

The most prominent characteristic of AFC is the presence of agroforestry trees in between the coffee tree rows. As said above the soil is shared by coffee trees and agroforestry trees in the proportion of 0.6 : 0.4. Agroforestry trees are assumed to have similar default values for the various coefficients as forest trees, including the same mycorrhizal activity (C4 and C6 in Table 5). For the model runs, the start point was set at 5 year old coffee and 15 year old agroforestry trees. The model was running for 30 years.

Conventional coffee cultivation – weed (CCW)

The most prominent characteristic of CCW is the presence of weeds in between the coffee tree rows. Also here the soil is shared in the proportion of 0.6 : 0.4 for coffee and weeds (default values). The P retained in weeds is returning to OVL (F13w) every year (Table 5). The mycorrhizal activity (C4 and C6) was set as 75% of that in AFS. Again the model was running for 30 years, and the start point for the model runs was set at 5 year old coffee, while weeds started every year.

Output of the model

Table 6 shows the amounts and proportions (in parenthesis) of the P pools at the start point of the model and after 30 time steps for the forest in the steady state, agroforestry and conventional coffee systems. In the steady state there were no changes in the P pools, indicating that the model parameters had appropriate values. Neither there were

Table 6. P pools at the start point of the model and after 30 time steps for the forest in the steady state, agroforestry and conventional coffee systems.

	IVL[1]		IML		ISI		OVL		OML	
Start point	10.0[2]	(0.5)[3]	120.0	(6.0)	1370.0	(68.5)	40.0	(2.0)	460.0	(23.0)
Forestry	10.0	(0.5)	120.0	(6.0)	1370.0	(68.5)	40.0	(2.0)	460.0	(23.0)
Agroforestry	11.7	(0.6)	173.2	(8.6)	1598.8	(79.2)	17.7	(0.9)	217.8	(10.8)
Conventional	17.6	(0.8)	226.9	(10.3)	1781.7	(80.0)	17.1	(0.8)	161.7	(7.3)

[1]IVL = inorganic very labile; IML inorganic moderately labile; ISI = Inorganic stable or inert; OVL = organic very labile; OML = organic moderately labile; [2]kg ha^{-1} (30 cm of soil); [3] % of the total (in parentheses).

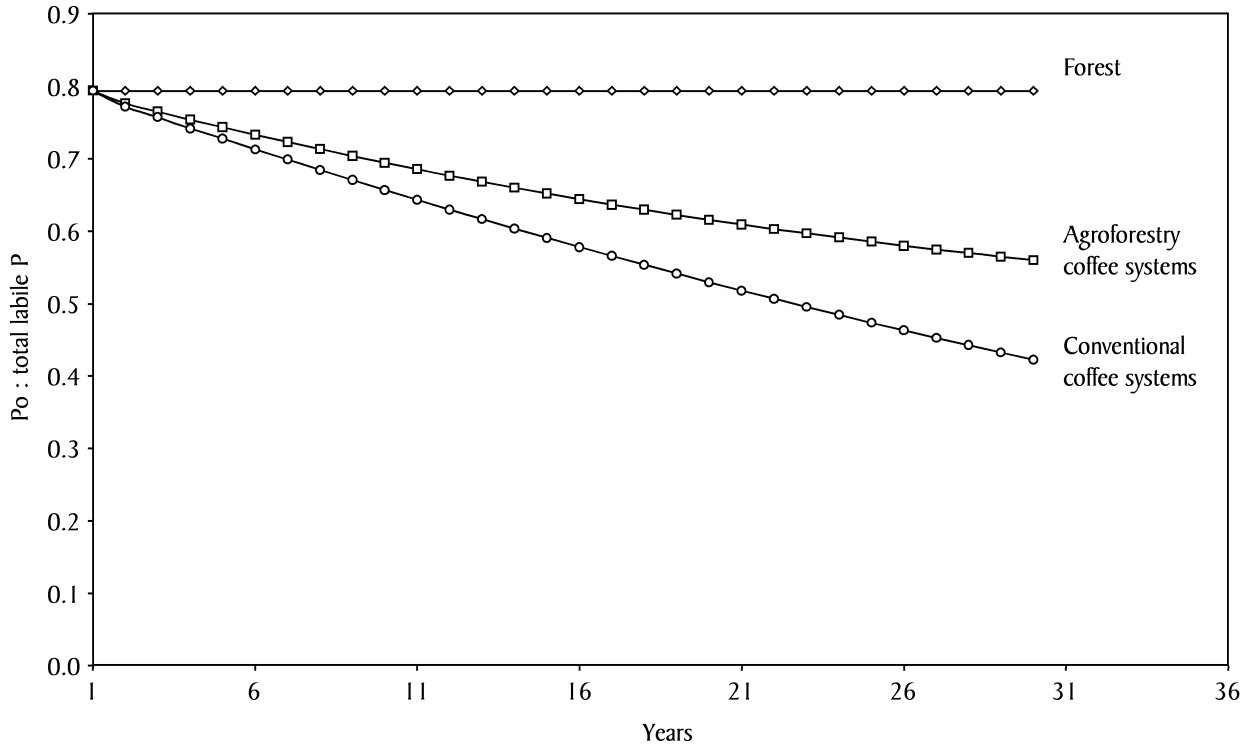

Figure 5. Ratio organic P (Po) : labile P as calculated using the simplified version of the models NUTCYC and DYNAMITE (Noij, 1988; Janssen, 1990; Noij et al., 1993).

changes in leaf and wood P.

Figure 5 shows the ratio Po : labile P as calculated using the simplified version of the models NUTCYC and DYNAMITE. The values after 30 time steps for the total P was 2019.2 kg ha^{-1} for the agroforestry coffee systems and 2205.0 kg ha^{-1} for the conventional coffee systems. Organic P (OVL and OML, Table 6) was 235.5 kg ha^{-1} for the agroforestry systems and 178.8 kg ha^{-1} for the conventional systems. Also in the model the labile P was considered as in Chapter 3 to be IVL, IML, OVL and OML. The total labile P after 30 time steps (Table 6) was 420.4 kg ha^{-1} for the agroforestry systems and 423.2 kg ha^{-1} for the conventional systems. Hence, the ratio Po : labile P was 0.79 for the forest, 0.56 for agroforestry coffee systems and 0.42 for conventional coffee systems. These values are similar to the values found in the P fractionation (Chapter 3): 0.69 (agroforestry fields) and 0.56 (conventional fields).

Discussion

The model calculation, as the chemical analyses, suggested that agroforestry indeed influences the dynamics of P through the conversion of part of the inorganic P into organic P. The difference on total P among the systems is due to harvest and differences in fertilisation. The organic pools decreased more than 50 %, this can be, as discussed before, due to a higher fraction of organic matter protected from decomposition and mineralisation and or due to a poor link between the inorganic and organic P pools considered in this modelling exercise.

As proposed in the general discussion (Chapter 9), the results of the research suggested in Figure 1 of Chapter 9 can be integrated in the model and more realistic outcomes can be reached. In the diagram depicted in the introduction (Chapter 1, Figure 2) the micro-organisms were considered as a "wheel" that rotates in the soil, simultaneously consuming and releasing P in the soil solution and transferring P among the pools (Stevenson and Cole, 1999). In the modelling exercise in this chapter the micro-organisms are implicitly considered, except mycorrhizas. Moreover, mycorrhizal decay can also contribute to increase in organic pools (Chapter 6) and that was not considered in our model.

In our model we considered the whole soil, but the processes that occur at the rhizosphere level can be different from the bulk soil (Chapter 9, General discussion). The distribution of P among pools at the start of the model was based on the P fractionation done in the soil from agroforestry systems. This is not the start point for the coffee cultivation but the situation in 1999, at the time of sampling. In the future, P fractionation in soil from neighbour forest of the agroforestry and coffee systems can be done and more real values can be used in the model.

In conclusion, our modelling exercise was useful to integrate available information and further substantiate the claim that agroforestry influences P dynamics through conversion of Pi to Po. Missing information also came to light, mainly the explicit role of the micro-organisms in the P flows among pools. With future research, the model can be improved. Points requiring special attention are:

1. The quantitative importance of the micro-organisms in the P flows among pools.
2. The origin of P (inorganic, organic) that is taken up by the vegetation. In the present model the inorganic contribution was restricted to the compensation of erosion losses of the organic P pools, which may be a substantial underestimation of the significance of mycorrhiza.
3. The allocation of P within the agroforestry trees (C11 and C12) may be used as important criteria for species screening. If recycling though pruning is desirable, plant species that allocate less P in the wood will be preferred.
4. Experimental assessment in the field of conversion rates of soil organic pools.
5. The time step is one year. It should be much shorter for mechanistic modelling.
6. The allocation of P from organic and inorganic fertilisers needs further elaboration.

With future improvement, the model can provide 1) a better comparison agricultural systems including an assessment of the relative importance of the factors that make the systems different; 2) a better evaluation of the processes and transformations in the model as possible means to improve the coffee cultivation systems. Both purposes are interrelated and may be studied simultaneously for mutual insight.

Appendix

Calculation of mineralisation of organic phosphorus

Mineralisation of organic phosphorus requires microbial conversion of the organic matter. Part of the converted matter is used for assimilation in microbial tissue and part for oxidation to gain energy (dissimilation). The often used term "organic-matter decomposition", refers, strictly speaking, to dissimilation. The quantity of decomposed or dissimilated organic matter is thus the difference between the total amount of organic matter that is converted and the amount that is assimilated by the micro-organisms.

Similarly, part of the phosphorus that is present in the converted organic material is used in microbial tissue and part is set free as inorganic phosphorus. In the case of phosphorus, the difference between conversion and assimilation is usually called mineralisation. If the converted organic matter is low in phosphorus, the amount of phosphorus that is converted may be too low to satisfy the assimilation needs of the microbes. In such cases, microbes take up inorganic phosphorus from their environment, usually being the soil solution or the moisture in the organic material. The mineralisation is then negative. This process, known as immobilisation, results in an increase of organic P in the remaining organic material.

In many mineralisation studies first-order kinetics are assumed. The change in the quantity of organic C is then described as follows:

$$dY / dt = -k * Y$$

in which t is time; Y is the quantity of C present at t; k (≥0) is the relative mineralisation rate, or simply, the rate constant. Integration yields the following equation:

$$Y_t = Y_0 * \exp(-k * t)$$

where Y_0 is the quantity of C at $t = 0$.

Because in reality k is not constant, Janssen (1984, 1986) has introduced a model with a time-dependent relative mineralisation rate. He found that k changes over time:

$$k = 2.82 * (a + f*t)^{-1.6}$$

in which k is the momentary relative mineralisation rate at t; **a** is the so-called 'apparent initial age', an index for the resistance of a substrate to mineralisation; **f** is a temperature correction factor.

Substitution of k in the first equation and subsequent integration yield:

$$Y_t = Y_0 * \exp\{4.7 * [(a + f*t)^{-0.6} - a^{-0.6}]\}$$

The essence of this model is the use of one general equation for all organic materials. The difference in resistance is accounted for by assigning different 'apparent initial ages' (**a**) to different substrates. Values of **a** used in Eq. 5, 6 and 12 were

2.18, 4.00 and 20 years for leaf and animal manure, wood, and soil organic matter, respectively. The temperature correction factor, f, is calculated with:

$f = 2^{(T09)/9}$

where T is temperature in °C. In our area with an average temperature of 18 °C, f is 2, as is used in Eq. 5, 6 and 12.

The C/P ratios assumed were 1000 for leaf litter, 1600 for wood, 200 for animal manure and soil organic matter. We assumed that after one year the organic material remaining as soil organic matter would also have a C/P of 200. The calculation of P immobilisation is illustrated in the following example of 1000 g of leaf substrate with an apparent initial age of 2.18 years and a C/P ratio 1000:

	Carbon	Phosphorus
Initial amount	1000	1.00
Remaining after one year	386	386/200 = 1.93
Immobilisation		0.93

Calculation of the allocation of fertiliser P

Inorganic P fertilisers (IF standing for Input Fertilisers) are supposed to dissolve for 80 %. The remaining 20 % is present as tri-calcium phosphate and other P-compounds that remain in the fertiliser granules (Leenaars-Leijh, 1985; Van der Eijk, 1997). Hence, a portion of 0.2 is immediately assigned to ISI (IFisi). The dissolved P remains partly in SSOL and is partly adsorbed to IVL, IML and ISI. The distribution among SSOL, IVL and ISI determines the recovery of fertiliser P. Hence we must know the recovery fraction found in field experiments to make a justified allocation of inorganic fertiliser P among IVL (IFivl), IML (IFiml) and SSOL (IFssol). Our preliminary approach was as follows.

A fraction of 0.8 enters the soil solution. We assume that the uptake of fertiliser P is essentially from the soil solution. So, if the recovery is 5%, we allocate a fraction of 0.05 to SSOL (remember that the meaning of the soil solution (SSOL) in the model is not the same as in real world: SSOL is a transit pool for all inorganic P that is dissolved and organic P that is mineralised in the course of one year). What moves to the solid phase is called ACF (adsorbed chemical fertiliser); it must be distributed among IVL, IML, and ISI. Within short time ACF is divided over IVL and IML. The part of ACF that moves from IVL to IML is called a*ACF, and the part of a*ACF that moves from IML to ISI is called a*b*ACF.

After equilibrium we get the following equations:

(a * ACF)/(ACF - a * ACF) = K1

(a*b*ACF)/(a * ACF - a*b*ACF) = K2

For K1 we take the ratio of C5/C3 being 2.719/0.227 = 11.98 and for K2 is 6, the ratio of C2/C1 (see subsection on inorganic part of the model). It follows:

a * ACF = 11.98 * ACF – 11.98 *a * ACF and a = 11.98/12.98 = 0.92

(a*b*ACF) = 6 * a * ACF – 6* a*b*ACF)and b = 6/7 = 0.86

So IVL receives (1 – 0.92) * ACF = 0.08 * ACF, IML receives (0.92 – 0.92 * 0.86) * ACF = 0.13 * ACF, and ISI receives 0.92*0.86 = 0.79 * ACF. If the fertiliser P recovery by the crop is 5%, ACF is 75% of the applied fertiliser. The total fertiliser distribution is then: to SSOL: 0.05; to IVL: 0.06; to IML: 0.10, and to ISI: 0.59 + 0.2 (the fraction remaining in the granules) = 0.79. If the fertiliser P recovery by the crop is 10%, ACF is 70% of the applied fertiliser. The total fertiliser distribution is then: to SSOL: 0.10, to IVL: 0.06, to IML: 0.09, and to ISI: 0.55 + 0.2 (the fraction remaining in the granules) = 0.75. We assumed in our model, the P recovery by the crops as 5 %. This approach is not yet perfectly in line with the calculation of C3 and C5 (Eq. 4), and F9 (Eq. 14), and needs further elaboration.

CHAPTER 9

SUMMARY AND GENERAL DISCUSSION

Summary

The Zona da Mata is a region situated in the domain of the Atlantic Coastal Rainforest in the southeast of the state of Minas Gerais, Brazil. This domain stretches along the Brazilian coast from north to south and ranks among the top five of the 25 biodiversity hotspots, the richest and the most threatened reservoirs of plant and animal life on Earth. Originally, forest covered the region but nowadays only about 7.5 % of the original vegetation remains. Most of the trees were cut for wood and the area is nowadays used for agriculture. In general, the agro-ecosystems in the Zona da Mata show a decreasing productivity due to the increasing intensity of soil use, with practices inadequately adapted to the environment. In 1993, farmers and researchers searching for a more sustainable agriculture started implementing or improving agroforestry coffee (cash-crop) systems in the region. The natural environmental conditions of the region are favourable for growing trees, as illustrated by the fact that the entire area was originally covered with forest. The main goals with agroforestry were 1) land regeneration and conservation; 2) decrease of external input to agriculture; 3) increase or maintenance of production level; and 4) improvement of productivity. To reach these goals, a better understanding of nutrient recycling in the systems is required. The work presented here aims to contribute to such better understanding and focuses on the effect of agroforestry on phosphorus (P) cycling. Phosphorus may be the major nutrient in relatively short supply in most natural ecosystems, and the primary limiting nutrient for crop production in highly weathered tropical soils. The P deficiency is mainly caused by strong adsorption of $H_2PO_4^-$ to aluminium (Al) and iron (Fe) (hydr)oxides, which turn large proportions of total P into a form that is unavailable to plants. The main strategy to cope with P deficiency in the tropics has been the addition of fertilisers. At the same time, the global reserves of apatite, which is needed for producing P fertilisers, are limited and known reserves may be exhausted in about 100 years with the current growth of P usage (Stevenson and Cole, 1999). More sustainable strategies need to be developed to utilise applied and native soil P more effectively to reduce P fertiliser demands (Lehmann et al., 2001). Agroforestry can be considered one of these strategies. The main hypothesis was that in agroforestry systems, part of the unavailable inorganic P (Pi) in soil is made available to agricultural crops by modifying P dynamics along various pathways. Some tree roots can use more soil than crop roots, have different associations with micro-organisms including mycorrhiza and change the rhizosphere through exudates, such as organic anions and phosphatase (Young, 1997). The core questions of this thesis were:

1. Do agroforestry systems modify P dynamics in the soil?
2. Do these modifications vary with depth?
3. Do agroforestry systems increase P cycling and release P that is otherwise unavailable to the crops?
4. How does this process occur?

To answer these questions, I characterised soils under agroforestry and conventional (full-sun, monoculture) coffee systems from the Zona da Mata of Minas Gerais at different depths and studied mechanisms involved in the improvement of P recycling. The different research endeavours throughout the research period offer partial answers to these questions. This chapter is a discussion of the implications of the results and the response to the hypothesis.

The outline of the research, as well as the description of the problem and area were presented in Chapter 1. Chapter 2 described the participatory processes and range of methods by which agroforestry systems took hold in the Zona da Mata. It discussed some of the key benefits and problems of agroforestry systems encountered during the first five years. Chapter 2 also showed how the starting point of the research questions presented in this thesis emerged from the participatory monitoring and evaluation of the agroforestry systems in Araponga, Zona da Mata. During the monitoring process the farmers requested for 'more academic research on soil quality and nutrient recycling'.

A first step in the study of P dynamics is the estimation of the various P pools in the soil, including organic P, which is usually done by P fractionation. With the P fractionation procedures (Chapter 3), I showed differences between agroforestry and conventional systems: the amount of organic P (Po) decreased less with depth (effect of depth in the Sum-Po) and the percentage of organic P in labile pools was higher (effect of systems in the Sum-Po : labile) in the agroforestry fields. In Chapter 4 I used ^{31}PNMR (phosphorus 31 nuclear magnetic resonance) to evaluate the inorganic (Pi) and organic P (Po) compounds in the soils. The results confirmed and extended the results of the previous chapter. I found that the ratios of organic P to total P and of diester (a labile Po compound) to total P are higher in agroforestry coffee fields than in conventional coffee fields. There were also effects of depths: agroforestry fields showed a higher ratio of organic P to total P in depth than conventional coffee fields. Moreover, the amount of diester and the ratio diester : monoester (a less labile Po compound) decreased less with depth in the agroforestry fields than in the conventional fields. These results are consistent with the hypothesis that agroforestry systems influence the dynamics of P through the conversion of part of the inorganic P into organic P, which is probably a consequence of higher biological activity in the agroforestry systems. Because organic P appears to be less readily fixed than inorganic forms and because soil biological activity is likely to lead to greater recycling into younger, more labile, P pools (Young, 1997) agroforestry would maintain higher fractions of P available to agricultural crops and would reduce P losses to the unavailable pools.

In Chapter 5, I reported on the presence of higher numbers of spores of arbuscular mycorrhizal fungi (AMF) in the deeper soil layers of the agroforestry fields than in the deeper soil layers of the conventional fields. This was probably due to the presence of more roots in deep layers in the agroforestry fields than in the conventional fields. The presence of more spores may be an indicator of higher incidence of mycorrhiza in deeper layers of agroforestry fields than in conventional fields. High activity of mycorrhiza (in deep layers) may increase P recycling from the deeper layers in the agroforestry fields and may change the dynamics of P in the soil, for instance through changing the distribution of P over the various pools (Chapters 3 and 4). The effects of agroforestry systems on P fractions, mainly in depth, found in Chapters 3 and 4 are in line with the findings in Chapter 5.

In Chapter 6, I studied one of the mechanisms that plants can use to cope with P deficiency in acid soils, i.e. the association with mycorrhiza. I used an Al-resistant maize variety. In this experiment I merged the double pot, normally used in plant nutrition experiments, with the double compartment approach, normally used in mycorrhizal experiments. The mycorrhizal plants grew better in the acid soil than the non-mycorrhizal plants. The mycorrhizal plants depleted P from the readily labile fractions (Resin and $NaHCO_3$-Pi) and acquired about 20 % of the moderately (NaOH-Pi) labile fractions whereas the non-mycorrhizal plants failed completely in acquiring P even from the labile fractions. It is currently accepted that mycorrhizal plants acquire P from the same source of available P as non-mycorrhizal plants but mycorrhizas explore more volume of soil than roots. Although controversial, it has also been suggested that mycorrhiza may benefit plant growth by increasing the availability of P from non-labile sources. In my experiment, mycorrhiza did more than just increase the soil volume explored by the roots. Furthermore, I also showed that the double pot - double compartment approach was suitable for the study of nutrient uptake by mycorrhiza and subsequent transfer to the plants.

Chapter 7 is a summary of Chapter 6, emphasising that mechanisms of Al resistance and P uptake from soil with high Al toxicity are not necessarily related, contrary to what is commonly assumed. The Al resistance was probably due to the production of high amounts of citric acid by the maize variety. Current knowledge suggests that this mechanism also facilitates P uptake from acidic soils (Hocking, 2001; Marschner, 1995). However, the roots from the Al-resistant variety failed to acquire P from the soils in the absence of mycorrhizas, even from readily available fractions in the A horizon (Resin and $NaHCO_3$-Pi). Therefore, the mechanisms to cope with Al toxicity and P deficiency were not necessarily interrelated, contrary to suggestions from the literature (Marschner, 1995). If citric acid plays a role in the Al resistance of this variety, it is not important for direct P acquisition.

In Chapter 8, the model (based on DYNAMITE model) developed as an exercise to study the distribution of soil P over organic and inorganic pools, qualitatively confirmed the results from Chapter 3 that the ratio Po to labile P is higher in

agroforestry than in conventional systems. The exercise was useful in integrating information. Missing information also come to light, mainly the role of the micro-organisms in the P flows among pools.

Results of the P fractionation of the soil (Oxisol) used in the greenhouse experiment (Chapter 6) confirmed the results of the P fractionation of the soils (also Oxisols) from the agroforestry and conventional coffee systems (Chapter 3): the amount of total P in the soils used in the greenhouse experiment (Chapter 6) was not very low, however, on average 70 % was in the concentrated HCl and residue fractions, which are considered unavailable to plant crops in the short (one season) or medium term (more than one season, Tiesen and Moir, 1993); and the second highest fraction was the NaOH-Pi pool (on average 25 %), which is considered available in the medium term (Tiessen and Moir, 1993). Diffusion is the main mechanism of P transport in soil (Novais and Smith, 1999). Thus, in the long term, the use of NaOH-Pi and the readily available fractions (Resin-Pi and $NaHCO_3$-P) by mycorrhiza (Chapter 6), will shift the partitioning of P between pools.

Thus, if trees can change the performance of mycorrhiza in the agroforestry systems as compared to the conventional systems, for instance by increasing the activity of mycorrhiza in deeper layers (presence of more spores, Chapter 5), I would expect a change in P dynamics that would make more P available to the plant. This would increase the efficiency of nutrient recycling processes in the agroforestry systems.

The rate and the impacts of this change on P cycling and the efficiency of P use of the crops in the long term needs to be further examined and understood before coming to a comprehensive evaluation of the importance of agroforestry in soil P utilisation. Some of the methods used in this thesis (P fractionation and ^{31}PNMR analysis) are too static to study P dynamics and are insufficient to draw final conclusions on P transformations. Spore number (Chapter 5) is only an indicator of mycorrhiza presence. Mechanisms of P acquisition have to be understood not only in crop plants (maize for instance, Chapters 6 and 7) but also in the native trees used in the agroforestry systems, such as the trees listed in Chapters 2 and 5. Thus, detailed studies are required for a better understanding of the P transformation in soil through biological activity. The results of these studies can be incorporated in the model (Chapter 8) to improve it.

This study forms a starting point to understand P dynamics in the agroforestry systems in the Zona da Mata of Minas Gerais. The farmers' request for more insight into nutrient dynamics (Chapter 2) cannot be answered completely yet. To do so, P studies must be integrated into existing agricultural development activities that are being carried out by the key players in the Zona da Mata: farmers, staff from CTA (Alternative Technologies of the Zona da Mata), researchers from UFV (Federal University of Viçosa), among others. I will first discuss some of the research that has been done on the agroforestry systems in the Zona da Mata in order to propose a framework for my future research on P cycling in these systems. My research suggestions will also be relevant for other regions of the Atlantic Coastal Rainforest.

Research on agroforestry systems in the Zona da Mata, Minas Gerais

Franco (2000) presented a survey and characterisation of the agroforestry coffee systems in the Zona da Mata. The objective of the survey was to look for alternative uses of riparian zones and hill slopes. The authors used semi-structured interviews with 13 farmers to understand several environmental and social-economical aspects involved in the agroforestry systems, including history of land use, soil, water availability, inputs, land tenure, available work, plant species used, etc. One of the main problems pointed out by the farmers in those systems related to poor soil fertility, caused by natural soil characteristics, low levels of external inputs, and limited understanding of the nutrient cycling process in the systems. Therefore, Franco (2000) concluded that more in depth studies on soil fertility were needed. This was confirmed by the participatory rural appraisal undertaken in Araponga (Zona da Mata, Chapter 2).

A key problem pointed out by the farmers in the participatory rural appraisal was the low productivity of their land, which they attributed to soil conservation problems such as erosion. Agroforestry was postulated as a potential solution (Chapter 2). To quantify the degree to which erosion could be controlled by agroforestry and other land use systems, a device was designed to measure rainfall erosion and a research programme set up to investigate the soil erosion problem

(Carvalho and Ferreira Neto, 2001). The device helped farmers to understand soil erosion, which together with the participatory monitoring results (Chapter 2), led them to conclude that agroforestry systems could fully succeed in conserving soil, thus motivating them to keep the trials running (Chapter 2, Carvalho and Ferreira Neto, 2001).

The analyses (Franco, 2000) of eroded soil material collected by Carvalho and Ferreira Neto (2001) showed that the P (labile) in the soil lost from agroforestry systems was on average 1.6 g/ha/year and in conventional systems was 46.5 g ha^{-1} $year^{-1}$. Therefore, the losses of P in agroforestry systems were much lower than in conventional systems (Franco, 2000).

To evaluate the effect of agroforestry systems on reclaiming degraded soils in the Zona da Mata, some soil analyses were undertaken to characterise the organic matter, the nitrogen forms and its dynamics (Mendonça et al., 2000, 2001). The authors fractionated the organic matter (humic acid, fulvic acid and humin) and measured the total organic C and N (ammonia and nitrate), the soluble C in water and in NaH_2SO_4, the C in the humic fractions, the lower molecular organic compounds, the light organic matter, the microbial biomass and the mineralised C. Some of these analyses were made with the soil samples after separation in classes of aggregation. The authors came to the general conclusions that the agroforestry systems had improved soil fertility and that light organic matter is higher in the agroforestry than in conventional systems, especially in the surface layers, but that total organic matter did not differ from the conventional systems. Neither Campanha (2001) nor I (Chapter 3) found a difference in total organic matter content when comparing agroforestry and conventional coffee systems.

Campanha (2001) aimed to compare one agroforestry and one conventional coffee system with respect to various components of the soil-plant environment. Among the components she studied were: vegetative (number of leaves, number of branches, etc) and reproductive (number of buds, number of fruits etc) growth of coffee, pests and diseases, nutritional condition of coffee plants, system temperatures, soil moisture content, amount and quality of litter fall, chemical soil characteristics (pH, exchangeable cations, organic matter etc) and non-coffee tree diameters. She concluded that a) coffee in the agroforestry field grew slower and lost fewer leaves than in conventional systems; b) the coffee berries production was higher under conventional management; c) the levels of leaf nutrients for both systems met recommended levels; d) the soil nutrients (using routine analysis) were higher in agroforestry field except P; e) the soil organic matter content was similar for both fields; and f) the agroforestry field kept more water in the soil; g) the temperature was lower within the agroforestry fields; f) the amount of litter fall was higher in the agroforestry fields. Litter in the conventional field mainly consisted of coffee leaves and in general the nutrient contents in the litter fall was higher in the conventional fields than in agroforestry field. Although less litter fall was found in the conventional field, due to its composition (coffee leaves) the amounts of P (around 5.5 kg ha^{-1} year) returned to the soil by litter fall were similar for both systems.

Neves (2001) also compared agroforestry and conventional coffee systems and studied vegetative growth, production, nutrition conditions of the coffee plants, soil fertility and moisture content in the soil. Her conclusions were similar to Campanha (2001) for the same parameters studied.

Mendonça and Stott (2002) measured the content of C, N, P, Ca, Mg, K, lignin, cellulose, hemicellulose and soluble polyphenols in prunings from five trees or bushes and one grass species used in the agroforestry systems in the Zona da Mata. The species they studied were *Cajanus cajan* (Leguminosae, guandu), *Solanum argenteum* (Solanaceae, capoeira branca), *Cassia ferruginea* (Leguminosae, canfístula), *Piptadenia gonoacantha* (Leguminosae, jacare), *Croton urucurana* (Euphorbiaceae, adrago) and *Milinis multiflora* (Gramineae, capim gordura). In a laboratory incubation study they also analysed the effect of the characteristics of the pruning residues on their decomposition rate. *C. cajan* contained the highest amount of P (1.23 g kg^{-1}). The N : polyphenol ratio correlated strongly ($R = 0.68$, $p < 0.001$) with the decomposition rates. The lowest N : polyphenol rates were found for *C. ferruginea* (0.15) and *C. urucana* (0.18) and the highest N : polyphenol rates were found for *C. cajan* (0.70) and *S. argenteum* (1.31). The highest C : P ratio was found for *C. ferruginea* (842) and the lowest for *C. cajan* (422). According to the authors, the high C : P ratios in the residues likely resulted in the immobilization of P by the soil microbial community during the initial stages of decomposition and so they suggested that P was the limiting nutrient in their decomposition study.

A framework to research P recycling in agroforestry systems in the Zona da Mata of Minas Gerais, Brazil

In the Zona da Mata of Minas Gerais, Brazil, on-farm experiments might provide an answer to one of the farmer's question raised during the monitoring process. The farmers asked for a better understanding of the nutrient recycling in agroforestry systems (Chapter 2). In my research, I focused on P because P is often the primary limiting nutrient for crop production in highly weathered tropical soils (Nye and Bertheux, 1957) and because sustainable ways to manage the limited amount of P available in tropical environments are needed.

The P transformations in mixed perennial agricultural systems, such as agroforestry or in native ecosystems are complex. Most P transformations are explained in terms of biological turnover of organic matter that concomitantly releases P. For understanding the dynamics and cycling of P, the entire complex of Pi and Po transformations, including mineral interactions and the organic matter dynamics, needs to be studied (Tiessen, 1993).

Agronomic practices, such as application of fertiliser, manure, compost, grazing and intercropping may affect P availability to crops either indirectly through the modification of soil properties or directly through crop impact on soil-P dynamics. The integration into the cropping system of P-mobilising plant species is a promising approach to have access to soil P fractions that are not available to the main crop (Horst et al., 2001). Agroforestry systems, which are a form of intercropping, have been suggested as an important crop system for fertility restoration and soil conservation in the Zona da Mata (Chapter 2). Screening trees that are suitable for agroforestry systems is the first step in developing viable and sustainable cropping systems.

Figure 1 presents a framework of important topics to be researched, on the "stage" of on-farm experiments, in order to better understand the P cycling and to improve the management of agroforestry systems in the Zona da Mata. This framework has two major aspects, one related to the direct implications of the P cycling in agroforestry for the farmers. Another aspect is related to interactions between soil chemistry and soil biology regarding the behaviour of P in the rhizosphere. For research on those topics to be effective and relevant, partnerships with farmers, NGO staff and groups of researchers must be extended beyond the existing partnerships (Chapter 2 and Section 2), since scientists from several other disciplines have to be involved in order to cover the range of subjects to be investigated. In Figure 1, the approaches (on farm or model) are placed in the first column, the general questions in the second column, the specific questions in the third column and the measurable factors or methods in the forth column. Obviously, to better answer some questions, more controlled experiments, for instance in greenhouses, can be done. Below I describe the topics presented in Figure 1 in more detail.

On-farm experiments and participatory approaches [1]

On-farm experimentation is considered an important tool for farmers to learn about their systems and to find their own solutions (Defoer, 2000; Sanchez, 2002). CTA, UFV and farmers use on-farm experimentation as part of a participatory approach to develop agroforestry systems in the Zona da Mata. Our type of on-farm experiments can be classified as designed and managed by farmers (Riley and Alexander, 1997) with researchers acting as facilitators. Normally the complexity of the experimental design increases with increased farmers participation (ibid). This complexity is even higher in tropical regions, where on-farm sites can vary markedly over a small area. Complexity is further exacerbated in agroforestry (Huxley and Mead, 1988). Therefore, the agroforestry on-farm experiments in the Zona da Mata are very complex.

To do research that exploits these complexities instead of reducing it by resorting to controlled conditions such as in on-station experiments (Huxley and Mead, 1988, Rocheleau, 1991), can be an interesting way to integrate the social and natural sciences. In this way, a more interdisciplinary approach can be reached. Making the most of the diversity and

[1] From now on the superscripts refer to the superscripts in Table 1.

complexity of such on-farm experiments would present an exciting avenue towards true multidisciplinarity for social scientists and natural scientists alike. To do this effectively requires methodologies that would lead to similar levels of robustness in on-farm experiments as exist in experiments under more controlled conditions. If this level of robustness cannot be achieved, it becomes difficult to involve natural scientists in on-farm experimentation teams as it would represent a doubling of their workload, i.e. to manage both the on-farm experiments and controlled experiments. Achieving acceptable levels of robustness makes it interesting for natural scientists to engage in on-farm experimentation. To do soil research in on-farm experiments with the robustness of on-station experiments might well be possible, as it is possible for geologists, field ecologists, oceanographers etc, who are accustomed to working with 'experiments' designed by nature (Rocheleau, 1991). But it requires focused methodological studies on how to simultaneously and adequately deal with farmer diversity and scientific rigour.

Research with on-farm experiments leads to information and data that demands an understanding of experimental design, statistical analysis and interpretation that reaches far beyond the standard statistical text (Riley and Alexander, 1997). This also makes it difficult to find literature that deals with natural variability in farmers' fields. Körner (2001) argued that for the two basic requirements for good science, precision and relevance, only the first has been scholarly developed (statistics) and the later has received little attention.

Huxley and Mead (1988) suggest one approach for on-farm agroforestry field investigations that embraces the variability and complexity of farmers' fields, which, in my opinion, can increase relevance of the investigation. Relevance is considered here as what might happen in the "real world" (Körner, 2001). For that, Huxley and Mead (1988) indicate five important steps: 1) identify a large population of observational units, probably spread over several farms; 2) classify each unit according to its level for each of several environmental treatment factors; 3) further classify each unit according to its level in each of several blocking factors (ecological plots, which is best done by getting farmers to select them); 4) decide whether to include interference treatments (= applied treatments) in the on-farm experiment; 5) randomly select units to represent different environmental treatments (step 2) within blocks of similar units, where blocks are defined from step 3, from a population of available quadrates (plots) of that kind and (if relevant, step 4) allocate a level of interference treatment.

CTA, UFV and Rural Farmers Unions have been working with ecological plots (step 3 above) in a process that we call participatory environmental stratification (Carvalho and Ferreira Neto, 2000), with the goal to discuss variation and potential of farmers' fields. This stratification can also be used along the lines proposed by Huxley and Mead (1988) to undertake more systematic studies about biophysical conditions in field experiments. In this way, in a participatory approach the farms can provide the experiment from where to gather the data to answer biophysical questions that are mainly investigated in more artificial and less complex environments, often in on-station experiments. The on-farm experiments are considered the baseline for the investigations proposed in Figure 1.

Dynamics and cycling [1.1]

To explain inorganic phosphorus (Pi) chemistry and transformations, P fractionation following the procedures of Hedley et al. (1982) proved to be useful (Chapter 3). However, the fractionation scheme does not provide the structural components of organic P (Po). In this respect, ^{31}PNMR has turned out to be successful (Chapter 4).

An understanding of the role of Po in the environmental P cycle must be based on an understanding of organic matter transformations and analysis of the associated P (Tiessen, 1993). Roscoe and Machado (2002) argue that physical fractionation of organic matter in tropical soils is more sensitive than chemical fractionation for evaluation of changes in the organic matter dynamics under different soil management types. Feller and Beare (1997) pointed out two reasons why chemical fractionation is not sensitive for the evaluation of these changes. First, humic and fulvic acids, in general, present low transformation rates in short term (days to decades) processes that normally occur in cultivated soil. Second, alkaline

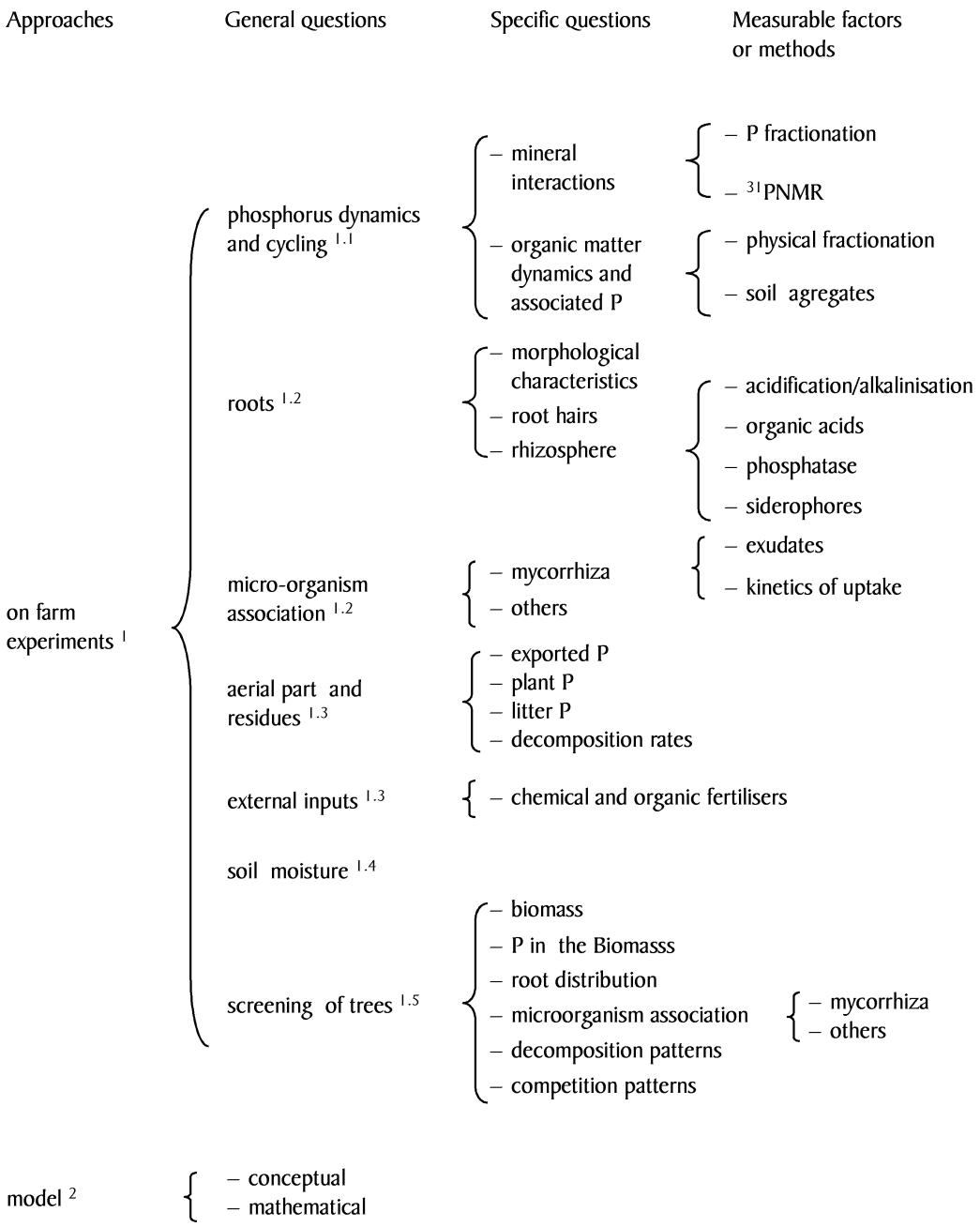

Figure 1. Overview of important topics to be investigated to better understand the P cycling and improve the management of the agroforestry systems in the Zona da Mata. The approaches (on farm or model) are placed in the first column, the general questions in the second column, the specific questions in the third column and the measurable factors or methods in the forth column. Numbers in parentheses relate to the section numbers in the general discussion. See section 9.3 for further explanation. Superscripts refer to the superscripts in the text.

or acidic extractions are highly selective and are related to the solubility of organic compounds, which triggers the extraction of similar compounds from completely different fractions of the soil organic matter. Within the physical fraction, light organic matter (density lower than 1.6-1.7 g cm-3) is more sensitive to differences in soil use and management than the chemical fraction (Roscoe and Machado, 2002, Mendonça et al., 2001). Light organic matter is an indicator of C availability in the systems, which can increase nutrient cycling (Tiessen, 1993). Since Po transformation is related to organic matter transformation, the study of P associated to physically separated fractions of soil organic matter, especially the light fraction, is therefore recommended.

Some authors have studied P pools in soil fractions of different particle size (Turrión et al., 2000), including Oxisols (Neufeldt et al., 2000). However, Oxisols are known to be highly aggregated. Thus, Roscoe and Machado (2002) argue that the fractionation according to aggregate size prior to the particle size fractionation may be more appropriate to study organic matter dynamics and the availability of its components. Since Po and organic matter transformations are inter-related, it is expected that the study of P pools in different aggregates may also be more adequate.

Mendonça et al. (2000 and 2001) have studied the organic matter dynamics in agroforestry systems in the Zona da Mata, using the approach as suggested by Roscoe and Machado (2002). To combine efforts, the studies of P (Pi and Po) dynamics must be associated with organic matter studies for a better understanding of P cycling in the agroforestry systems in the region.

Roots and micro-organisms [1,2]

P in the soil solution is in equilibrium with P sorbed to the solid phase. The equilibrium is maintained by physical-chemical reactions. The kinetics of P desorption and P diffusions are generally considered to be insufficient to supply P at a rate that is adequate for optimal plant growth in weathered tropical soils (Barber, 1980). Plants have developed a range of mechanisms that influence the availability of soil P. These include root morphological characteristics, such as the rate of root growth, total root length and degree of root-to-shoot partitioning, and the abundance and distribution of root hairs (i.e. root surface area). These characteristics effect the kinetics of P uptake at the root surface and biological and biochemical processes that occur at the root surface, and thereby influence the availability of soil P to plants (Richardson, 2001).

These biological and biochemical processes, of either plant or microbial origin, may lead to the release of exudates. The exudates may influence P availability either directly or support the growth of microbial populations within the rhizosphere (Richardson, 2001).

Among the exudates, organic acids/anions are a common constituent and are in general considered effective in releasing soil P through a number of mechanisms (Ryan et al., 2001). These mechanisms include a reduction in rhizosphere pH that occurs in response to organic acid/anion exudation, whereby H^+ ions are released as the counter ion (Ryan et al., 2001; Kirk, 1999). The organic acids/anions in the soil can also displace adsorbed P through ligand exchange reactions and chelate metal ions that would otherwise immobilise P, or by forming soluble complexes with P via metal ions (Kirk, 1999). Chelation by one or both of these means is likely to be the most important mechanism, at least in highly weathered soils containing large amounts of metal oxides (Kirk, 1999). Sequestering of Fe by siderophores may also be important for the dissociation of Fe from precipitated forms of Fe-P (Jayachandran et al., 1989).

Other processes that result in a net acidification or alkalinization of the rhizosphere similarly have potential to increase P mobilisation, which arise mostly from the release of H^+ or OH^-/HCO_3^- to counterbalance a net excess of cations or anions entering the roots (Hinsinger, 2001). Radersma (2002) showed that the tree *Cassia spectabilis* modified its rhizosphere by exuding oxalate and increasing pH. Those modifications were considered to increase P-availability and partly responsible for increases in maize growth close to the *C. spectabilis*.

Phosphatases are required for the mineralisation of Po. Various phosphatases with differing substrate specificities have been identified in plant roots and soil micro-organisms, and those being secreted as extracellular enzymes are likely to be important for the acquisition of P from Po substrates contained within soil solution (Richardson, 2001).

The symbiotic association between plant roots and mycorrhizal fungi has long been established as an important mechanism by which plants are able to acquire P from the soil (Marschner, 1995). The uptake rate of P per unit root length in mycorrhizal plants is normally considered to be two to three times higher than in non-mycorrhizal plants (Tinker et al., 1992). In non-mycorrhizal plant the depletion zone is about one cm (Marschner, 1995) whereas in mycorrhizal plants it is longer, for instance 11 cm for clover (Li et al., 1991). Mycorrhiza may produce phosphatases or other compounds, alter the kinetics of P uptake at the root surface, and may alter the pH in the rhizosphere (Marschner, 1995).

Besides mycorrhiza, various micro-organisms, including an extensive range of soil bacteria and fungi, may play a role in enhancing root growth, improving nutrient uptake, or solubilising and mineralising P (Tiessen, 1993; Marschner, 1995; Richardson, 2001). Those micro-organisms are considered important for promotion of plant growth and must be evaluated in order to understand the P cycle.

The microbial biomass in soils contain a significant amount of P, which generally accounts for one to ten percent of the total soil organic P (Hedley and Stewart, 1982). This microbial P is a dynamic component of the soil P cycle and is responsive to soil fertility status, seasonal factors and management practices (Richardson, 2001). Under favourable soil moisture and temperature conditions, micro-organisms are capable of rapidly immobilising significant amounts of P, either applied as fertiliser or supplied as a component of crop residues (McLaughlin et al., 1988). However, the availability of microbial P to plants, and its rate of turnover in soil is poorly quantified (Richardson, 2001). Mendonça et al. (2001) studied microbial biomass in the agroforestry systems in the Zona da Mata, but integration with P studies is still lacking.

Aerial parts and residues [1.3]

The potential of some plants to increase the P cycling in the agroforestry systems depends on the amount and quality of the material naturally returned to the soil or due to pruning. Thus leaf P, litter P, plant P and decomposition rates must be evaluated. Some studies on this topic in the agroforestry systems in the Zona da Mata are available (Campanha, 2001; Mendonça et al., 2001). Moreover, to make a balance of the inputs and outputs in the systems can be worthwhile to understand the P dynamics in the soil. For this, it is necessary to know the outfluxes (for instance, harvest) as well as the influxes (for instance, fertiliser) in the system.

Soil moisture [1.4]

The positive effect of intercropping a P-mobilising crop may not occur if the component crops compete for growth factors such as water, light and nutrients. Competition for water may lead to low soil moisture and reduced P transport in the soil. Moreover, Radersma (2002) showed that even if in the (sub)humid tropics, P fixing Oxisols, without water competition problems, a small decrease in soil water content cause a decrease in P-transport to roots and thereby a soil-drying induced P deficiency. Radersma (2002) found that a reduction of 2-2.5 vol. % water close to the tree line decreased aboveground biomass production of the maize crop by 30-40 %. Water per se was considered to play a minor role. The main causes of the decrease in aboveground mass production of maize were 1) that the decrease in soil water content decreased P-diffusion, and thus P-transport to the roots and P-uptake, and 2) root growth (and thus uptake of resources) is reduced because of lower aboveground biomass production caused by lower P-uptake though decreased P-diffusion. Although Neves (2001), Campanha (2001), Carvalho and Ferreira Neto (2001) and Franco (2000) studied moisture and water loss in the agroforestry systems in the Zona da Mata, P studies must be better integrated with this.

Screening of trees [1,5]

Since P is a major constraint on food and fibre production in many parts of the world, a sustained supply of P is essential to guarantee agricultural and forestry production. Some plants have developed strategies to influence the availability of soil P (Richardson, 2001). The integration of such plants into the cropping system could enhance the biological and biochemical processes that occur at the root surface, which would further increase the availability of soil P to the crop (Richardson, 2001).

There are two categories of strategies for the uptake and acquisition of P by plants, and for conservation of P within plants (Latjha and Harrison, 1995):

1. Uptake/acquisition: increase root:shoot ratio, increase root surface area, increase mycorrhizal or other micro-organisms interactions, increase root uptake rate, increase root exudation to aid solubilization of P and;
2. Conservation/use: decrease growth rates, increase growth per unit P, increase resorption/retranslocation, and increase leaf life span.

These strategies lead to the possibility that plants with the ability to use "unavailable" P can be sought and used in agricultural systems that show P limitation. Even if such plants are not crop plants, they can be used in the systems, for instance as intercrop in agroforestry systems. Thus selection for plants with P acquisition strategies from soil pools, or with high P conservation capacity, might be possible in agriculture and agroecosystems (Lajtha and Harrison, 1995)

Screening agroforestry trees for soil fertility restoration and maintenance of acid soils, is critical for developing viable and sustainable cropping systems in the humid tropics. The best alternative to use in the screening of trees is the use of local species that were able to develop mechanisms that help them to deal with soluble Al and low soil fertility levels (Kanmegne, 2000), including low available P. On high P fixing Oxisols, one of the challenges of agroforestry research is to identify species that are able to transform "unavailable-P" into forms that may be utilised by the crop, either by nutrient cycling or by direct root-effects (Radersma, 2002).

Specific criteria should be chosen for the evaluation of selected species. Criteria commonly found in the literature (Kanmegne et al., 1999; Richardson, 2001) include: a) high standing biomass; b) high nutrient concentration in the biomass; c) high decomposition and nutrient release patterns d) root morphology (for instance, large amounts of root hairs) and distribution (for instance, most roots below root crops); e) biochemical processes occurring in the rhizosphere (for instance, organic acid exudation); f) low competition with crops for growth factors such as nutrients, and water; and g) associations with mycorrhiza and other micro-organisms that improve P acquisition by the trees.

Those criteria have to be evaluated and trees with the best combination of characteristics are to be considered for integrating into the agroforestry systems. The selected trees have to be managed in way that lead an increase in a better use of the P in the systems by the crop. Moreover, the species chosen and the management of the trees have to minimise the main disadvantage of the agroforestry systems, i.e., competition for resources such as water, nutrients and light.

Modelling [2]

P cycles in soils and entire ecosystems depend on the chemistry of Pi and the transformations of organic P and hence of organic matter. Complex interrelationships exist between P and organic matter transformations. Conceptual (Tiessen et al., 1993) or mathematical (Stevenson and Cole, 1999) models may help in the analyses of soil and ecosystems data and in the planning of research approaches.

The information acquired from the research on the topics presented in Figure 1 can be integrated in the model presented in Chapter 8 in a way that unveils the missing information, allowing indication (for example, through sensitivity analysis) of the processes and parameters that are of vital importance. Finally, the model should lead to clear, testable predictions.

Agroforestry hypotheses

In the introduction (Chapter 1), I presented 12 hypotheses (groups) of the principal aspects of agroforestry in soil management (Young, 1997, Box 1 of the Introduction). In my research, I focused on hypotheses 3, 7 and 10.

Hypothesis 3 states "Agroforestry systems can maintain soil organic matter and biological activity at levels satisfactory for soil fertility". In the analyses undertaken within the context of this thesis and in other research in the agroforestry systems in the Zona da Mata (Campanha, 2000, Mendonça et al., 2001), the total organic matter did not differ in relation to the conventional systems. However, amount of light organic matter showed differences (Mendonça et al., 2001). We concluded that statements about benefits of agroforestry systems in organic matter in the tropics have to be more specific.

We were furthermore able to show that arbuscular mycorrhizal spores were present in larger amount in the deep soil layers of the agroforestry fields than in the conventional coffee fields, which we explained as a consequence of more roots in deep layers of the agroforestry systems (Chapter 5). Higher levels of spores were considered an indication of high mycorrhizal activity, which may lead to increased P cycling. These results are in line with hypothesis 3 (biological activity), hypotheses 10 (the role of roots) and 7 (nutrient cycling).

We also showed that the amount of organic P and the ratio of diester : monoester compounds decreased less with depth, and that the percentage of organic P to total P, the percentage of organic P in labile pools and the percentage of diester to total P compounds were higher in the agroforestry fields than in conventional fields (Chapters 3 and 4). These results seem to indicate that agroforestry would maintain higher fractions of P available to agricultural crops and would reduce P losses to the unavailable pools, which aligns with hypothesis 7 (nutrient cycling). Moreover, in our greenhouse experiment, we showed that in the short (three months) term, mycorrhiza used some of the moderately available inorganic P that is normally considered available to plants only after a longer period. Thus, if agroforestry systems increase the mycorrhizal activity in depth (Chapter 5) it may also increase the use of less available P pools in depth (hypothesis 3 integrated with hypothesis 7). However, in our experiment, mycorrhiza did not use the organic P pools.

Extending the horizons

The NGO (Centre of Alternative Technology in the Zona da Mata) belongs to a network called Rede-PTA (Network of Alternative Technology Projects). Nine of the NGO members of Rede-PTA are involved in developing agroforestry systems in the Atlantic Coastal Rainforest domain in Brazil. The other NGOs in Rede-PTA use similar processes as CTA to develop agroforestry systems, i.e., participatory approaches based on farmer's knowledge. Around 100 groups of farmers in 100 communities in 38 municipalities were identified by Ferreira Neto (2001) as developing experiences with agroforestry systems in the Atlantic Coastal Rain Forest. Ferreira Neto (2001) presented some of the common goals, successes, problems and challenges met by the farmers and NGO staff in these agroforestry systems. Better soil conservation and nutrient recycling are among the common goals of the agroforestry systems. The need to identify and describe trees suitable for agroforestry systems was pointed out as one challenge. Through the Rede PTA, the research on agroforestry systems in the Zona da Mata has the potential to contribute to the discussion and implementation of agroforestry systems in other regions of Atlantic Coastal Rainforest. Hopefully, the research with agroforestry in the Zona da Mata will contribute to conservation of the remains of the rainforest and to reverse the environmental degradation and social problems of the Atlantic Coastal Rainforest, which is one of the top five Hotspots (the most threatened reservoirs of plant and animal life on Earth, Myers et al., 2000).

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SAMENVATTING

De Zona da Mata is een regio in het gebied van het Atlantic Coastal Rainforest in het zuidoosten van de staat Minas Gerais in Brazilië. Dit gebied bevindt zich in een noord-zuid georiënteerde strook langs de kust, en behoort tot de top 5 van de 25 biodiversiteit-hotspots, dat zijn de rijkste (en helaas ook meest bedreigde) reservoirs van plant- en diersoorten op aarde. Het gehele gebied was aanvankelijk bedekt met regenwoud, maar daarvan is slechts 7.5 % over. De meeste bomen werden gekapt voor houtwinning het grootste deel van het gebied wordt tegenwoordig voor landbouw gebruikt. In het algemeen vertoont de productiviteit van de landbouw-ecosystemen in de Zona da Mata een dalende tendens door de toenemende intensiteit van bodemgebruik en het gebruik van methoden die niet zijn aangepast aan de omgeving.

In 1993 nam een groep boeren en onderzoekers het initiatief om een meer duurzame productiemethode te zoeken en begon met het testen van agroforestry (een landbouw-systeem waarin bomen zijn geïntegreerd) voor de teelt van koffie. Zoals de oorspronkelijke beboste staat van de regio al doet vermoeden, zijn de omgevingsfactoren in de regio geschikt voor de groei van bomen. De hoofddoelen van het initiatief waren 1) bodemverbetering en -bescherming; 2) vermindering van externe input zoals kunstmest en chemische gewasbeschermingsmiddelen (bestrijdingsmiddelen); 3) gelijkblijvende of toenemende koffieproductie; en 4) toename van de productie per oppervlakte landbouwgrond.

Om deze doelen te bereiken is het noodzakelijk om meer inzicht te verkrijgen in de processen van de voedingsstoffenkringlopen in agroforestry-systemen. Het werk gepresenteerd in dit proefschrift is bedoeld als bijdrage tot het verkrijgen van zulk inzicht en richt zich op het effect van agroforestry op de dynamiek van fosfor (P). Fosfor kan een beperkend nutriënt zijn in de natuurlijke ecosystemen en is de belangrijkste beperkende factor voor productie van landbouwgewassen in verweerde tropische bodems. Dit P-gebrek wordt voornamelijk veroorzaakt door de sterke adsorptie van $H_2PO_4^-$ aan aluminium (Al) en ijzer (Fe) (hydr)oxiden, waardoor grote hoeveelheden P voor planten niet beschikbaar zijn. Tot nu toe is de voornaamste strategie om P-tekort in de tropen te bestrijden het toedienen van (kunst)mest geweest. Echter, de wereldvoorraden van apatiet, een van de voornaamste grondstoffen voor de productie van P-meststoffen, zijn beperkt en de bekende voorraden zouden slechts toereikend zijn voor de komende 100 jaar indien het groeiende gebruik van P niet vermindert. Daarom is het noodzakelijk om duurzame strategieën te ontwikkelen voor een efficiënter gebruik van zowel toegevoegde P als van de P aanwezig in de bodem.

Agroforestry wordt beschouwd als één van deze strategieën. De belangrijkste hypothese is dat een deel van de in de bodem aanwezige anorganische P (Pi), die niet beschikbaar is voor planten, beschikbaar komt door veranderingen in de P-dynamiek via verscheidene routes. De wortels van sommige boomsoorten bereiken een groter volume van de bodem dan die van de landbouw-gewassen, hebben associaties met micro-organismen zoals mycorrhizaschimmels (schimmels die in een wederzijds voordelige symbiose met plantenwortels leven), en veranderen de rhizosfeer (de directe omgeving van de wortel) door exudatie (uitscheiding) van organische stoffen zoals anionen van onder andere citroenzuur en appelzuur en fosfatase, enzymen die fosfaat afspiltsen van organische verbindingen. Daardoor kunnen veranderingen in de P-dynamiek optreden.

De belangrijkste vragen die ik in dit proefschrift tracht te beantwoorden zijn:

1. Is de P-dynamiek in agroforestry-systemen anders dan in conventionele systemen?
2. Zijn deze veranderingen gerelateerd aan de diepte in de bodem?
3. Komt door de veranderingen in P-dynamiek in agroforestry-systemen meer P beschikbaar voor de gewassen?
4. Welke mechanismen zijn verantwoordelijk voor de veranderde P-dynamiek?

Hiertoe heb ik op verschillende diepten de bodem van zowel agroforestry-systemen als conventionele koffiesystemen in de Zona da Mata gekarakteriseerd en de mechanismen bestudeerd die de P-dynamiek beïnvloeden. Met de verschillende onderzoeken ben ik tot een gedeeltelijk antwoord gekomen op deze vragen.

De opzet van het onderzoek en de probleemstelling worden besproken in Hoofdstuk 1. In het tweede hoofdstuk beschrijf ik het participatieve proces dat is gevolgd bij het initiëren van agroforestry in de Zona da Mata. Ik bespreek ook een aantal van de voornaamste voordelen en problemen verbonden aan agroforestry die tijdens de eerste vijf jaar

werden ervaren. Het hoofdstuk laat ook zien dat de onderzoeksvragen van dit proefschrift het resultaat zijn van een interactie tussen boeren, technici en onderzoekers bij het monitoren en evalueren van agroforestry-systemen in Araponga, Zona da Mata. Tijdens het monitoren vroegen de boeren om meer fundamenteel wetenschappelijk onderzoek naar bodemkwaliteit en nutriëntenkringlopen.

Een eerste stap in het bestuderen van P-dynamiek is de schatting van de grootte van de verschillende P-fracties in de bodem, inclusief organische P (Po). Dit wordt gewoonlijk gedaan m.b.v. P-fractionering, het scheiden van fracties met behulp van extractiemiddelen van verschillende sterkte (Hoofdstuk 3). Deze fractioneringsmethoden toonden verschillen aan tussen agroforestry- en conventionele systemen: de hoeveelheid Po nam minder af met toenemende diepte en het percentage Po in labiele (potentieel voor planten beschikbare) vorm was hoger in agroforestry-systemen. De resultaten van de toepassing van de ^{31}P-NMR (phosphorus-31 nuclear magnetic resonance / fosfor-31 kernspinresonantie, Hoofdstuk 4) op bodemmonsters van beide systeemtypen bevestigden dit verschil. Bovendien werd duidelijk dat de verhoudingen van Po tot de totale hoeveelheid P en van diëster tot Po hoger waren in de bodem onder agroforestry dan onder conventioneel geteelde koffie. Bovendien nam de hoeveelheid diëster en de verhouding diëster : monoester minder snel af met toenemende diepte bij agroforestry dan bij conventionele koffiesystemen. Deze resultaten zijn overeenkomstig de hypothese dat agroforestry de dynamiek van P verandert door de omzetting van een deel van de anorganische P in Po. Dit is waarschijnlijk het gevolg van grotere biologische activiteit in de agroforestry-systemen. Doordat organische P minder snel lijkt te worden vastgelegd in voor planten niet of nauwelijks beschikbare vorm (gefixeerd) dan anorganische P en doordat biologische activiteit in de bodem waarschijnlijk leidt tot recycling van P in jongere, meer labiele P-vormen, kan dit leiden tot een hogere beschikbaarheid van P voor de gewassen en een vermindering van verlies van P door omzetting tot onbeschikbare vormen.

In Hoofdstuk 5 beschrijf ik dat er meer sporen van arbusculaire-mycorrhizaschimmels gevonden werden in de diepere bodemlagen onder agroforestry-percelen dan onder conventionele percelen. Dit werd waarschijnlijk veroorzaakt door de aanwezigheid van meer plantenwortels in diepere lagen van agroforestry-percelen dan bij conventionele velden. De aanwezigheid van meer sporen wordt beschouwd als een aanwijzing voor een talrijker voorkomen van mycorrhizaschimmels in diepere lagen onder agroforestry, en dit kan de recycling van P vanuit diepere lagen doen toenemen, waardoor de dynamiek van P in de bodem verandert, met als resultaat een andere verdeling over de verschillende vormen van P. De gevonden verschillen in P in verschillende vormen met diepte in de hoofdstukken 3 en 4 zijn hiermee in overeenstemming.

In Hoofdstuk 6 heb ik naar één van de mechanismen gekeken waarmee planten P kunnen opnemen in zure bodems met een hoge beschikbaarheid van het voor planten giftige aluminium (Al), namelijk de associatie met mycorrhizaschimmels. In een experiment heb ik twee verschillende methoden gecombineerd, te weten de "dubbele pot" methode, die normaal gebruikt wordt in onderzoek naar plantenvoedende stoffen, en de "dubbele compartiment" methode, vooral gebruikt in experimenten met mycorrhiza. Planten die waren geïnoculeerd met (inheemse) mycorrhizaschimmels groeiden veel beter in deze zure grond dan planten zonder mycorrhiza; ze namen alle P van de labiele fracties op en ongeveer 20 % van de gematigd labiele fractie, terwijl planten zonder mycorrhiza in totaal geen P opnamen, zelfs niet van de labiele fracties. Het is algemeen geaccepteerd dat planten met mycorrhiza P kunnen opnemen van dezelfde fracties als planten zonder mycorrhiza en dat het voornaamste effect van een associatie tussen planten en mycorrhizaschimmels is dat een groter volume van de bodem wordt geëxploreerd. Meer controversieel is het idee dat myccorhiza-associaties de plant tot voordeel kunnen zijn doordat ze de beschikbaarheid van P van minder labiele vormen doen toenemen. De resultaten van mijn experiment laten duidelijk zien dat de mycorrhizaschimmels meer deden dan slechts een groter volume bodem exploreren. Bovendien laten de resultaten zien dat de gecombineerde benadering van de "dubbele pot" en de "dubbele compartiment" methoden geschikt is voor het bestuderen van nutriëntopname en transport naar planten door mycorrhiza.

Hoofdstuk 7 is een samenvatting van Hoofdstuk 6, waarin benadrukt wordt dat Al-resistentie en de opname van P uit bodems met hoge Al-toxiciteit niet gerelateerd zijn, in tegenstelling tot wat algemeen wordt aangenomen. De Al-resistentie is waarschijnlijk te danken aan de productie van grote hoeveelheden citroenzuur door de mais-varieteit. Vaak wordt aangenomen dat dit mechanisme ook de opname van P in zure bodems bevordert. Ik vond echter dat de

Al-resistente maïsvariëteit wel veel citroenzuur produceert, maar niet in staat is om P uit bodems op te nemen, zelfs geen P-fracties van de A-horizon die geacht worden door planten opneembaar te zijn. Maïsplanten die geassocieerd waren met mycorrhizaschimmels konden echter wel P opnemen. De mechanismen die door planten gebruikt worden tegen Al-vergifitiging en P-gebrek hebben daarom waarschijnlijk geen rechtstreekse relatie met elkaar, in tegenstelling tot wat in de literatuur wordt vermeld.

De modeloefening in Hoofdstuk 8 bevestigt de resultaten van Hoofdstuk 3, namelijk dat de verhouding Po : labiel P hoger is in agroforestry-systemen dan in conventionele systemen. De constructie van het model was vooral nuttig omdat het verschillende informatie integreert en laat zien over welke aspecten van de P-cyclus nog onvoldoende informatie beschikbaar is. Dit laatste geldt vooral voor de rol van micro-organismen in de omzettingen van P tussen de verschillende fracties.

De resultaten van de P-fractionering van de bodem die was gebruikt in de experimenten met maïs en mycorrhiza (Hoofdstuk 6) bevestigden de resultaten van de fractionering van de grond onder agroforestry en conventionele koffieteelt (Hoofdstuk 3). De totale hoeveelheid P in de grond die gebruikt werd voor de experimenten (Hoofdstuk 6) was niet erg laag, maar gemiddeld 70 % van de P bevond zich in de fracties die geëxtraheerd werden met sterke extractiemiddelen en in de residufractie, en het wordt algemeen geaccepteerd dat deze fracties niet beschikbaar zijn voor planten op de korte (één seizoen) of middellange termijn (meer dan één seizoen). De op één na grootste fractie werd geëxtraheerd met NaOH (gemiddeld 25 %) en van deze fractie wordt aangenomen dat ze op de middellange termijn wél beschikbaar is voor planten. Aangezien diffusie het belangrijkste transportmechanisme voor P in de bodem is, zal de opname van deze anorganische fractie en de snel-beschikbare fracties door mycorrhizaschimmels leiden tot een verandering van de verdeling van P over de verschillende fracties.

Concluderend, als bomen het functioneren van mycorrhiza in agroforestry veranderen, bijvoorbeeld door de activiteit van mycorrhiza in diepere bodemlagen te doen toenemen (de grotere hoeveelheid sporen, Hoofdstuk 5, is hiervoor een aanwijzing), dan zal een verandering in de dynamiek van P optreden, die leidt tot een verhoogde beschikbaarheid van P voor planten. Dit zal de efficiëntie van de voedingsstoffenkringloop in agroforestry-systemen doen toenemen.

De snelheid en de gevolgen van deze veranderde P-dynamieka en de efficiëntie van P-gebruik door gewassen op de lange termijn moet verder worden onderzocht voordat een definitieve conclusie over het belang van agroforestry voor P-gebruik kan worden getrokken. Sommige van de methoden die gebruikt worden in dit proefschrift (P-fractionering, ^{31}P-NMR analyse) leveren een te statisch beeld op om de dynamiek van P te bestuderen en zijn ontoereikend om conclusies te trekken omtrent de omzettingsprocessen van P. Het aantal sporen (Hoofdstuk 5) is slechts een indirecte aanwijzing voor de talrijkheid van mycorrhizaschimmels. De mechanismen van P-opname moeten beter begrepen worden, niet alleen voor gewassen zoals maïs (Hoofdstuk 6 en 7), maar ook voor de inheemse bomen (Hoofdstuk 2 en 5) die gebruikt worden in agroforestry. Gedetailleerde studies zijn daarom noodzakelijk voor een beter begrip van het effect van microbiële activiteit op P-dynamiek in de bodem. De resultaten van dergelijke studies kunnen worden geïncorporeerd in het model (Hoofdstuk 8) teneinde het te verbeteren.

Mijn onderzoek is het begin van het verkrijgen van inzicht in de P-dynamiek in agroforestry-systemen in de Zona da Mata van Minas Gerais. Aan het verzoek van de boeren uit deze streek om meer inzicht te verkrijgen in de nutriëntendynamiek (Hoofdstuk 2) kan daarom nog niet volledig worden voldaan. Hiervoor is het noodzakelijk om onderzoek aan P-dynamiek te integreren met bestaande ontwikkelingen in de landbouw, die mede tot stand worden gebracht door de boeren, medewerkers van CTA-ZM (Centrum voor Alternatieve Technologie in de Zona da Mata) en onderzoekers van de Universiteit van Viçosa (UFV). In de algemene discussie (Hoofdstuk 9) presenteer ik enkele van de onderzoeken in agroforestry-systemen die gerealiseerd zijn in de Zona da Mata. Zij ondersteunen de formulering van een algemeen raamwerk voor (mijn) toekomstig onderzoek aan P-dynamiek in agroforestry-systemen in de Zona da Mata en andere regio's van de Atlantic Coastal Rainforest.

SUMARIO

A Zona da Mata está situada no domínio morfoclimático da Mata Atlântica, na região sudeste de Minas Gerais, Brasil. Este domínio acompanha a costa brasileira de norte a sul e está entre os cinco dos 25 "pontos quentes" da biodiversidade, que são as áreas de maior biodiversidade vegetal e animal e mais ameaçadas do planeta. Originalmente a Zona da Mata era coberta por florestas, restando atualmente cerca de 7.5 % da cobertura original. Os agroecosistemas que se instalaram na região apresentam produtividade decrescente devido à intensificação do uso do solo, utilizando muitas vezes manejo inadequado. Em 1993, agricultores e pesquisadores, buscando alternativas agrícolas mais sustentáveis iniciaram a implementação (ou melhoria) de sistemas agroflorestais com café (cultura de maior valor econômico) na região, acompanhando a vocação natural daquele ambiente, originalmente florestado. Os principais objetivos da implantação de sistemas agroflorestais foram: 1) regeneração e conservação dos solos; 2) diminuição do uso de insumos externos na agricultura; 3) aumento ou manutenção dos níveis de produção; e 4) melhoria da produtividade. É condição necessária para atingir esses objetivos, a melhor compreensão da ciclagem de nutrientes no solo. O presente trabalho objetivou contribuir para melhorar a compreensão desse aspecto, com ênfase no fósforo (P), por se tratar de um nutriente essencial de baixa disponibilidade na maioria dos ecossistemas e que nos solos tropicais, altamente intemperizados, se constitui no principal limitante da produção agrícola. A deficiência em P é causada principalmente pela forte adsorção do $H_2PO_4^-$ pelos (hidr)óxidos de alumínio (Al) e ferro (Fe), os quais indisponibilizam grande parte do P total para as plantas, principalmente para as plantas cultivadas. A principal estratégia para lidar com o problema da fixação de P nos trópicos tem sido a adição de fertilizantes. Entretanto, é sabido que as reservas minerais de P, necessárias para a produção de fertilizantes, são limitadas e têm previsão de se esgotar em torno de 100 anos, mantido o atual crescimento do uso de P. Nesse contexto, é necessário o desenvolvimento de estratégias sustentáveis de uso tanto do fertilizante fosfatado aplicado, como do P nativo do solo. Uma dessas estratégias consiste de sistemas agroflorestais.

A nossa principal hipótese de trabalho foi de que os sistemas agroflorestais tornam disponível parte do P inorgânico (Pi) presente nos solos, outrora não disponível para as culturas. Isto ocorre devido a diferentes mecanismos, onde se destacam: a) as raízes de algumas árvores podem explorar um volume maior de solo em relação às raízes das culturas; b) as associações entre raízes e microorganismos, incluindo micorrizas podem acontecer de forma diferente nas árvores incorporadas nos sistemas agroflorestais e; c) a rizosfera destas árvores podem apresentar características distintas, devido a liberação de diferentes exsudados, tais como ácidos orgânicos e fosfatases. As questões centrais da presente pesquisa foram:

1. Os sistemas agroflorestais modificam a dinâmica de P no solo?
2. Estas modificações acontecem em profundidade?
3. As espécies arbóreas usadas nos sistemas agroflorestais aumentam a ciclagem de P, liberando P que não estaria disponível às culturas?
4. Como tais processos ocorrem?

Para responder a essas questões, foram selecionados áreas de cafezais em sistemas agroflorestais e convencionais (pleno sol e em monocultura) da Zona da Mata de Minas Gerais, onde foram coletado solos em diferentes profundidades. Foram estudados alguns mecanismos envolvidos na melhoria da ciclagem de P nos solos. O capítulo 1 apresenta a descrição do problema, a abordagem escolhida, a caracterização da área estudada e os principais passos da pesquisa. No capítulo 2 são descritos os processos participativos e os métodos usados para implantar e monitorar os sistemas agroflorestais na Zona da Mata, assim como são discutidos algumas das vantagens e desvantagens encontrados durante os cinco primeiros anos de implantação destes sistemas. É também mostrado no capítulo 2 que durante os processos participativos de avaliação e monitoramento desses sistemas agroflorestais, os agricultores solicitaram mais pesquisas acadêmicas em qualidade do solo e reciclagem de nutrientes, suscitando as questões apresentadas nessa tese.

Os três próximos capítulos (3, 4 e 5) caracterizam o solo dos sistemas agroflorestais e convencionais, em diferentes profundidades, quanto aos compartimentos ("pools") de P orgânico e inorgânico (capítulo 3), tipo de

compostos de P (capítulo 4) e atividade de micorrizas (capítulo 5). O primeiro passo no estudo da dinâmica do P no solo é estimar os vários compartimentos de P, o que normalmente é feito por fracionamento químico. Assim, o capítulo 3 mostra as diferenças entre os sistemas agroflorestais e convencionais a partir dos procedimentos de fracionamento de P. A quantidade de P orgânico (Po) diminuiu menos em profundidade (efeito de profundidade na soma de Po) e a percentagem de Po em relação ao compartimento lábil foi maior (efeito de sistema na soma Po : lábil) nos sistemas agroflorestais em comparação com os convencionais. No capítulo 4 usou-se ^{31}PRNM (fósforo 31 Ressonância Nuclear Magnética) para avaliar os compostos de P inorgânico e orgânico presentes nos solos. Os resultados encontrados no capítulo 4 confirmam e ampliam os resultados apresentados no capítulo anterior. Encontrou-se que a taxa de Po em relação ao P total e também de diester (composto orgânico considerado lábil) em relação ao P total são maiores nos sistemas agroflorestais do que nos convencionais de café. Houve também efeito de profundidade: os sistemas agroflorestais comparados aos sistemas convencionais mostraram maior taxa de Po:P total em profundidade. Além disso, a quantidade de diester e a taxa diester:monoester (composto orgânico considerado menos lábil) decresceram menos em profundidade nos sistemas agroflorestais do que nos sistemas convencionais. Estes resultados corroboram a hipótese de que os sistemas agroflorestais influenciam a dinâmica do P devido a conversão de parte do Pi em Po, o que é provavelmente conseqüência da maior atividade biológica nos sistemas agroflorestais. Porque aparentemente o Po é fixado mais lentamente do que o Pi e porque a atividade biológica no solo recicla em formas mais jovens, mais lábil, compartimentos de P, os sistemas agroflorestais manteriam maiores quantidades de P disponíveis às culturas e reduziriam perdas de P para compartimentos menos lábeis.

No capítulo 5 reportou-se a presença de maior número de esporos de micorrizas arbuscular (MA) em maiores profundidades nos solos dos sistemas agroflorestais em relação aos sistemas convencionais, o que pode ser um indicador de maior incidência de micorrizas naqueles. Isso provavelmente se deve a presença de mais raízes em profundidade nos sistemas agroflorestais em relação aos sistemas convencionais (efeito de profundidade). A maior atividade de micorrizas em maiores profundidades pode aumentar a ciclagem de P em profundidade mudando então a dinâmica do P nos solos, por exemplo, promovendo diferenças na distribuição de P entre os vários compartimentos (capítulos 3 e 4). Os efeitos nos sistemas agroflorestais, principalmente em profundidade, encontrados no capítulo 5 estão de acordo com o resultados obtidos nos capítulos 3 e 4.

Nos dois próximos capítulos (capítulos 6 e 7) estudou-se mecanismos utilizados pelas plantas na convivência com baixa disponibilidade de P em solos ácidos. No capítulo 6 são apresentados os resultados dos estudos utilizando-se uma variedade de milho resistente a toxidez de Al (CMS 36) e sua associação com micorrizas. No experimento descrito no capitulo 6 é apresentada uma nova metodologia a de "vaso duplo-compartimento duplo", na qual associaram-se as metodologias do "vaso-duplo", normalmente usada em experimentos de nutrição de plantas, e do "compartimento-duplo", normalmente usada em experimentos com micorrizas. Nesse experimento, as plantas micorrizadas cresceram melhor em solos ácidos do que as plantas não micorrizadas. As plantas micorrizadas exauriram as formas consideradas prontamente lábeis de P (Resina e $NaHCO_3$-Pi) e usaram em torno de 20% da forma considerada moderadamente lábil (NaOH-Pi). As plantas não micorrizadas, por sua vez, fracassaram completamente em adquirir P mesmo dos compartimentos mais lábeis. É normalmente aceito na literatura que plantas micorrizadas e não micorrizadas usam P das mesmas fontes, mas que plantas micorrizadas exploram um volume maior de solo do que as plantas não micorrizadas. Embora controvertido, tem sido também sugerido que micorrizas podem utilizar P de fontes menos lábeis, não utilizadas pelas raízes de plantas sem associações com micorrizas. No experimento apresentado no capítulo 6, mostrou-se que as micorrizas foram além de só aumentar o volume de solo explorado pelas raízes. Mostrou também que a metodologia do "pote duplo-compartimento duplo" é adequada para estudos de nutrição de plantas envolvendo micorrizas.

O capítulo 7 apresentou um resumo do capítulo 6, porém enfatizou que mecanismos de resistência ao Al e aquisição de P de solos com alta toxidez de Al não são necessariamente relacionados, contrário ao que é comumente aceito na literatura. A resistência ao Al foi provavelmente devido a produção de ácido cítrico pela variedade de milho. Conhecimentos atuais sugerem que tal mecanismo também facilita aquisição de P em solos ácidos. No entanto as raízes da variedade de milho resistente ao Al fracassaram completamente em adquirir P do solo na ausência de

micorrizas, mesmo das fontes prontamente lábeis (Resina e $NaHCO_3$-Pi). Conseqüentemente, mecanismos de resistência ao Al e de convivência em ambientes de baixa disponibilidade de P parecem não estar necessariamente interligados como sugerido na literatura. Se para a variedade de milho utilizada o ácido cítrico foi importante na resistência ao Al, ele não foi importante na aquisição de P.

No capítulo 8 o modelo desenvolvido (baseado no modelo DYNAMITE) como exercício para estudar ciclagem de P, confirmou qualitativamente os resultados do capítulo 3 de que a taxa de Po em relação ao P lábil é maior nos sistemas agroflorestais do que nos sistemas convencionais. O exercício foi útil para integrar informações. A necessidade de se obter informações mais claras, principalmente aquelas que se referem ao papel dos microorganismos no fluxo de P entre os compartimentos, também vieram à tona.

Os resultados do fracionamento de P no solo (latossolo) usado no experimento da casa de vegetação (capítulo 6), confirmaram os resultados do fracionamento do P em solos (também latossolo) coletados nos sistemas agroflorestais e convencionais (capítulo 3): o P total do solo usado na casa de vegetação (horizontes A e B) não foi muito baixo, entretanto, em média 70% do P estava nas frações extraídas com HCl concentrado e no resíduo, as quais são consideradas não disponíveis para as culturas no curto prazo (uma estação de cultivo) ou médio prazo (mais de uma estação de cultivo); e a segunda maior fração foi a NaOH-Pi (em média 25%), tal fração é considerada disponível no médio prazo. A difusão é o principal mecanismo de transporte do P no solo. Assim, no longo prazo, as micorrizas (capítulo 6) podem alterar a distribuição de P entre os diversos compartimentos devido ao uso do NaOH-Pi e de frações mais lábeis (Resina-Pi e $NaHCO_3$-Pi).

Portanto, se árvores podem mudar a performance das micorrizas nos sistemas agroflorestais comparada aos sistemas convencionais, por exemplo, aumentando a atividade de micorrizas em profundidades (presença de mais esporos, capítulo 5), espera-se que ocorra uma mudança na dinâmica de P tornando-o mais disponível às plantas. Isto aumentaria a eficiência dos processos de ciclagem de nutrientes nos sistemas agroflorestais.

A taxa e o impacto destas mudanças na ciclagem de P e a eficiência de P usado pelas culturas ao longo prazo necessitam ser melhores entendidas e pesquisadas para um melhor compreensão da importância dos sistemas agroflorestais na utilização do P do solo. Alguns métodos usados nesta tese (fracionamento de P, ^{31}PRNM analysis) são muito estáticos para o estudo da dinâmica do P e são insuficientes para traçar conclusões finais a respeito das transformações ocorridas pelas formas de P do solo. Número de esporos (capítulo 5) é somente um indicador da presença de micorrizas. Mecanismos de aquisição de P devem ser entendidos não somente em plantas cultivadas (milho por exemplo, capítulos 6 e 7) mas também em árvores nativas usadas nos sistemas agroflorestais, tais como as árvores listadas nos capítulos 2 e 5. Assim, estudos mais detalhados são necessários para um melhor entendimento das transformações sofridas pelas formas de P do solo devido a ação microbiana. Os resultados destes estudos podem ser incorporados no modelo apresentado no capítulo 8 para melhorá-lo.

Os estudos aqui apresentados formam um ponto de partida para o entendimento da dinâmica de P nos sistemas agroflorestais da Zona da Mata de Minas Gerais. A demanda dos agricultores para maiores esclarecimentos sobre dinâmica de nutrientes (capítulo 2) ainda não pode ser respondida completamente. Para tal, estudos de P devem ser integrados às atividades de desenvolvimento agrícola que estão sendo realizadas por diferentes atores na Zona da Mata: agricultores, CTA (Centro de Tecnologias Alternativas da Zona da Mata) e UFV (Universidade Federal de Viçosa), dentre outros. Na discussão geral (capítulo 9) foram apresentadas algumas das pesquisas em sistemas agroflorestais que tem sido realizadas na Zona da Mata, e uma moldura geral para futuras pesquisas em ciclagem de P nesses sistemas as quais podem ser expandidas para outras regiões da Mata Atlântica.

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I was born on 12 May 1959 in Patrocínio, now with a "independent status of a distrito - village", but at that time belonging to São João do Jacutinga, a distrito (village) that belongs to Caratinga, Rio Doce Valley, Atlantic Coastal Rainforest, Southeast of Minas Gerais, Southeast of Brasil. My parents (small farmers) were José Rafael Cardoso (in memoriam) and Rita Martins de Paiva (in memoriam). I followed my first two and half years' school in Patrocinio. The rest of my primary, the secondary and the high school, I followed either in Caratinga or Belo Horizonte (the capital from Minas Gerais, 300 km away of Caratinga). As Caratinga is in the "centre of my" cardinal points, from now on all distances are relative to Caratinga. I finished my undergraduate studies in Agronomy in 1984, at the Federal University of Viçosa (200 km), Zona da Mata, Atlantic Coastal Rainforest, Southeast of Minas Gerais. From Viçosa, I went to Brasília, Cerrado domain, Central East (capital of Brasil, ~1000 km), to work as a research trainee at CPAC-EMBRAPA (Centro de Pesquisa Agropecuária do Cerrado-Empresa Brasileira de Pesquisa Agropecuária), first with a scholarship from CNPq (a Brazilian sponsor) and later from the University of Cornell (USA). In 1986, I went to Terra Roxa and Abapan, Paraná State, Araucária domain, South of Brasil (~1500 km) to work at ACARPA, the state extension service. In 1988, I worked, for a short period as an agronomist with the landless movement (MST) in Paraiba (~2000 km), domain of Caatinga in the Northeast of Brasil. In 1989, I went back to the Federal University of Viçosa to do my Msc on the subject of farmer's knowledge of soil in the Zona da Mata. In 1992, after a public examination, I started as assistant professor at the Federal University of Viçosa. My tasks included teaching, research and extension. My research and extension work was mainly done in partnership with CTA (Centro de Tecnologias Alternativa da Zona da Mata) and Small Farmers Unions. In 1998 I came to The Netherlands (10 000 km) to do my PhD, sponsored by CAPES. In November 2002 I will go back to my activities at the Federal University of Viçosa, Brasil.

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