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DISSERTATION

PHOSPHORUS CYCLING DURING DECOMPOSITION OF PLANT RESIDUES IN WEATHERED SOILS FROM THE TROPICS: INFLUENCE OF PLANT FACTORS

Submitted by

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In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

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Fort Collins, Colorado

Spring 2001

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY ANA M. SALAS ENTITLED PHOSPHORUS CYCLING DURING DECOMPOSITION OF PLANT RESIDUES IN WEATHERED SOILS FROM THE TROPICS: INFLUENCE OF PLANT FACTORS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Co-adviser

Adviser

Department Head

ABSTRACT OF DISSERTATION

PHOSPHORUS CYCLING DURING DECOMPOSITION OF PLANT RESIDUES IN WEATHERED SOILS FROM THE TROPICS: INFLUENCE OF PLANT FACTORS

Increasing interest in incorporation of plant residues into soils in tropical cropping systems has focused attention on the identification of plant quality factors controlling P cycling. In this study the primary objective was to evaluate the influence of selected plant characteristics on P availability during decomposition. Residues of sorghum (*Sorghum bicolor*) and crotalaria *(Crotalaria juncea*) having the same C:P ratio but differing in the content of water extractable Pi, enzymatic activities and quality of C compounds were compared for their ability to enhance the resin P and Bray extractable P levels in two acid soils (an Alfisol and an Ultisol) from the tropics. In order to elucidate the mechanisms involved, soil phosphatase activities, pattern of P release from the residues, as well as microbial P dynamics were investigated during laboratory incubations. The role of changes in microbial community composition and immobilization of P in decomposing residues were also evaluated for their contribution to explain differences in turnover of microbial P and P availability.

Covariance analysis revealed that crotalaria residue had on average, 75 and 40 % higher Bray P for low (330) and high C:P (630) ratio, respectively, as compared with sorghum residue. To investigate this substrate dependency several mechanisms were tested. Results indicated a relationship between plant phosphatase activity and soil phosphatase activity only

for the Ultisol. Pattern of release of P from the residues showed that both residues released more than 90% of the total P in the first 5 days of decomposition; no differences existed between residues types. Plant enzymatic activity and release of P from residues could not satisfactorily account for differences in P availability from the different residues.

Dynamics of microbial biomass P significantly differed between residues. Sorghum residue showed significantly higher values of microbial P and the rate of microbial P loss was lower as compared with crotalaria. This suggests that P immobilization and turnover of microbial P was affected differently by the residue type. Different patterns of P immobilization in decomposing residues supported this conclusion. P immobilization in the sorghum residue was 2-4 times higher than in crotalaria, and represented up to 30% of the total plant P added. This immobilization of P in the decomposing residues could account for observed differences in P availability between the residues.

Residue types also affected soil fungal biomass. A correspondence between colonization of the Particulate Organic Matter (POM) by soil fungi and P accumulation in the decomposing residues confirmed that changes in soil microbial community structure can affect the pattern of P immobilization. Phosphorus cycling was thus influenced by changes in turnover of decomposer communities driven by residue types. Although the mechanisms shaping microbial communities were not completely understood, results point out the significance of plant factors, other than C:P ratio, in determining short-term P availability from plant residues.

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DEDICATION

I dedicate this dissertation to my family, my parents: Carlos Julio and Ana Mireya, and my son Carlos Julio. Without their support, it would have not been possible to assume the challenges of motherhood while being graduate student.

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CHAPTER I

PHOSPHORUS CYCLING

Chemical transformations in weathered soils from the tropics

A significant proportion of the agricultural areas in the tropics are located on fragile and infertile acid soils. These soils, comprising about 1650 million hectares, commonly represent more than 50% of the total area in many developing countries (IBSRAM, 1985). Phosphorus deficiency has been largely considered one of the most important limiting factors of the productivity in these areas. Intensive weathering leading to the disappearance of primary phosphate minerals has contributed to the formation of secondary minerals with high selectivity for phosphate anions. These losses of phosphate from the system, accompanied by increasing sorption reactions with secondary minerals, have been used to explain phosphorus deficiency in tropical areas (Sanchez and Uehara, 1980).

A broad range of P sorption capacity has been reported for soils. Sanchez and Uehara (1980) found values ranging from 15 to 1500 mgP kg^{-1}, however lower values have been reported for medium- to fine-textured agricultural soils from the northeast of Brazil (117 to 350 mgP kg^{-1}) (Agbenin and Tiessen, 1994) and in the Cerrado (700 mgP kg^{-1}, Goedert 1983). Phosphorus sorption reactions in weathered soils are strongly influenced by the amount and reactivity of the different components in the clay fraction. Free Al and Fe oxides, as well as

hydroxylated Al and Fe minerals, have been shown to be responsible for the increased P sorption capacity of weathered soils from the tropics (Sanchez and Uehara, 1980).

Although oxides and hydroxides of Fe and Al have been commonly considered to be responsible for P sorption in weathered soils, significant differences exist in their ability to retain P (Parfitt 1978, Barrow 1985). Based on studies using pure minerals, most of the differences in reactivity of these surfaces have been attributed to the presence of coordinated OH surface groups that constitute reactive sites for P sorption, or the existence of amorphous phases with larger reactive surface. A greater P sorption capacity of goethite (2.50 μ mol m^{-2}), as compared with hematite (0.97 μ mol m^{-2}), has been attributed to its larger proportion of the total area containing reactive sites having OH surface groups (Barrón et al. 1988). Similarly, decreasing crystallization of Al oxides increased the number of available sites for P sorption (Sims and Ellis, 1983). A summary of the influence of these factors is represented by Sollins (1991) who ordered pure minerals according to their increasing P sorption capacity: montmorillonite < hematite = kaolinite < gibbsite < goethite < ferrihydrite < allophane.

Differences in reactivity shown by pure minerals can only partially explain differences in P sorption capacity in soils because of the influence of texture and the coexistence of different mineral types, stages of crystallization, interaction with other soil minerals and intensity of weathering. In many cases, amorphous Al compounds have been found to satisfactorily explain differences in P fixation in some tropical areas (Le Mare, 1982). In some other cases, free Fe oxides have better explained the phosphate sorption capacity with correlation

coefficients higher than with amorphous Fe compounds (Singh et al. 1983). The presence of certain type of oxides can be very significant for P fixation even if its quantity is relatively low. Al oxides played a more significant role than Fe oxides in P sorption of semiarid soils of Brazil, even though their contents were three to ten times lower than Fe oxides (Agbenin and Tiessen, 1994).

The two main mechanisms of P fixation into slightly soluble forms are precipitation and adsorption reactions with Fe and Al compounds and amorphous colloids of low silica-sesquioxide ratios present in acid soils. In precipitation reactions, the solubility product of the less soluble P compound in the solid phase controls the formation and dissolution of the precipitate, and therefore the concentration of P in solution (Sposito, 1984). Most of the solubility equilibrium experiments have tried to identify P solid phases controlling the P concentration using solubility isotherms of well characterized compounds. Early studies revealed the formation of X-ray analogs of variscite [$Al(OH)_2H_2PO_4$)] and Na-montebrasite [$AlOHNaPO_4$] when $Al_2O_3 \cdot n\ H_2O$, synthetic allophanes, and allophanic soils were reacted with Na o-phosphates, $Na_{3-A}H_APO_4$ of varying acidity A (Veith and Sposito, 1977a). The presence of Na montebrasite was indirectly inferred from analysis of the formation curves and from measurements of the amount of Na bound with Al and P. This approach based on the solubility products have been considered a valuable tool in delineating the kind of P compounds controlling the phosphate activity in soil solution, but special attention should be given to the assumptions for its application in soil systems. As mentioned by Olsen and Khasawneh (1980), this physicochemical approach assumes that ionic products represent solubility products, when they can actually represent super- or under-saturated conditions.

Similarly, solid P phases in soils are not in standard state because isomorphous substitution occur widely in soil minerals and perfect crystalline forms of soil P are unlikely to occur in the soil environment (Olsen and Khasawneh, 1980).

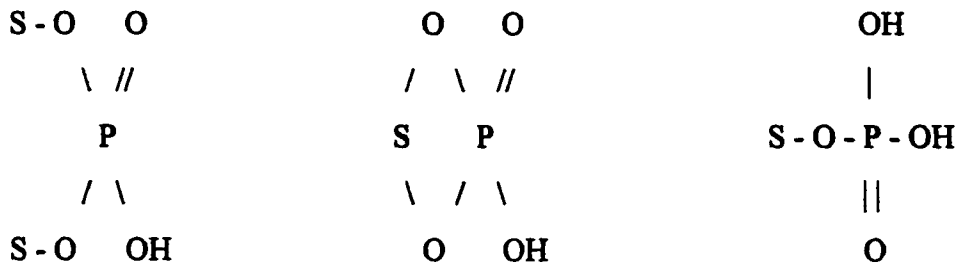
More direct evidence of the precipitation process was obtained in studies conducted in the goethite/phosphate system by combination of scanning auger, XPS, scanning SIMS and TEM techniques (Martin et al., 1988). They investigated the formation of the mineral griphite after reaction of naturally-occurring goethite with 10^{-3} M KH_2PO_4. These authors pointed out that adsorption of phosphate can be the precursor to precipitation reactions and this new phase is unlikely to be detected by the x-ray diffraction or analytical scanning electron microscopy because of the small size of the crystallite. Although these direct methods have revealed the presence of phosphate minerals, the low representability at the micro metric scale level and the need for clean minerals have limited their utilization to predict the contribution of precipitation reactions to the total P fixation in weathered soils (Frossard et al.1995).

Along with precipitation, adsorption reactions have been the subject of many studies and important contributions about the mechanisms involved have been derived from the utilization of pure oxides and hydroxylated Al and Fe minerals. Adsorption of P by kaolinite, gibbsite and pseudobohemite was studied by Muljadi et al. (1966) who suggested, based on adsorption isotherms, the existence of three regions according to the affinity of P to three energetically different reactive sites. They suggested that the mechanism of adsorption in regions I and II was the exchange of phosphate for OH groups associated with a positively charged Al atom. Adsorption in region III was thought to take place through the penetration of P into some less

crystalline phase of the clay surface. Later, Hingston et al (1972) suggested that phosphate is capable of exchange with either OH_2 or OH^- and become coordinated to the metal ion at the surface and be specifically adsorbed. According to these authors, ligand exchange occurs when phosphate having a specific affinity for metal atoms in hydroxylated minerals, enters the coordination layer of the surface and becomes adsorbed in proportion to their activity in the aqueous solution. They showed that phosphate anions can be adsorbed beyond the neutralization of positive surface sites and even on surfaces having net negative surface charge. They proposed that phosphate anions can be adsorbed onto a negatively charge after dissociation of their own protons, which then react with surface hydroxyl to form water molecules that in turn, can be readily replaced by the anions.

The previously described ligand exchange mechanism was corroborated by Rajan et al. (1974). The authors suggested three types of reactive sites based on changes in the molar ratio of hydroxyl released to phosphate adsorbed. At low P concentration, adsorption occurs by displacing aquo groups from Lewis acid sites, Al-OH_2. At higher P concentrations, hydroxyl groups (Al-OH) are displaced and at very high concentrations binuclear hydroxyl bridges (Al-OH-Al) are broken and new adsorption sites are created. Binuclear surface complexes with Fe, Fe-O-($P=O_2$)-O-Fe has been used to explain the irreversibility of the adsorbed phosphate (Parfitt et al. 1975), and its formation has been directly confirmed by X-ray photoelectron spectroscopy (Martin and Smart, 1987). Results from experiments of adsorption kinetics, hydroxyl ion release, infrared spectroscopy as well as stereo chemical calculations, confirmed the ligand exchange mechanism for phosphate adsorption by hydroxilated minerals (Goldberg and Sposito, 1985). The main phosphate surface complexes

as suggested by Rajan et al (1974) can be represented as followed:



Surface complexes resulting from ligand exchange of phosphate are characterized for not having water molecules between the surface Lewis acid site and the adsorbed anion. These complexes referred to as inner-sphere are considered quite stable, showing mainly covalent or ionic bonding character (Sposito, 1989). A quantitative description of these phosphate-surface hydroxyl reactions in soils have been made by the utilization of chemical models initially developed for reference hydrous oxide minerals (Goldberg and Sposito 1984 a, b). The most common is the constant capacitance model which describes the ligand-exchange of o-phosphate by mean of six parameters: two surface protonation-dissociation constants, three o-phosphate surface complexation constants, and a capacitance density parameter. This chemical adsorption model has been recently coupled to precipitation-dissolution and convective-dispersive models of P transport to develop more complicated models to describe inorganic P transformations in ecosystems studies (Grant and Heaney, 1997).

Although precipitation and adsorption reactions have been considered in many cases as two separate mechanism of P fixation in the soil, their differentiation is difficult. Direct observations indicating that adsorption can be the precursor of precipitation reactions suggest that they may occur as a continuum and a clear separation does not exist between the two

processes (Martin et al., 1988). Sometimes it is preferred to consider the combination of precipitation and adsorption in *sorption reactions*. A common way to express the sorption of P in soils is through linear square regression of the points in a plot derived from the Langmuir equation. Although this isotherm seems to apply on the basis of a statistical analysis, it cannot be used to determine whether adsorption or precipitation is occurring during anion sorption reactions (Veith and Sposito, 1977b). Some authors suggest that isotherms should be restricted to soil surfaces where adsorption is the main mechanism, so the parameters could be interpreted in terms of surface reactions (Bar-Yosef et al. 1988). In spite of the limitations in the appropriate interpretation of the parameters, isotherms are normally used to describe the combined sorption reactions and to estimate changes in P sorption characteristics of the soils.

Along with sorption reactions, the desorption of P from the soil can be equally important since most of the P taken up by the plants is derived from the solid phase of the soil. Desorption of P can normally be assessed by using chemical extractants, dilute electrolyte solutions or by lowering the solution P concentration through the utilization of P sinks, such as, anionic resin or iron oxide coated filter papers (Menon et al. 1996/1997, Parfitt et al. 1994). Another approach to estimate the P desorption is through isotopic exchange of the adsorbed ^{31}P with ^{32}P. When $^{32}PO_4$ is introduced into a soil-solution suspension, the specific activity of phosphate in solution ($^{32}P/^{31}P$) is equal, at any given time, to the specific activity of the exchangeable phosphate located in the solid phase (Larsen, 1967). Fardeau et al. (1985) considered the available P as a pool that can be more or less rapidly exchanged with the P in the solution according to kinetic parameters derived from isotopic exchange. This

approach was modified so it could be applied to weathered soils by using a double isotopic dilution (Tran et al. 1988, Salcedo et al. 1991). It also has been used to estimate the P buffering capacity of the soil by measuring the proportion of the added radioactivity that remains in soil solution after 1 minute of isotopic exchange (r_1/R_0). Most weathered soils from the tropics have values of r_1/R_0 ratios lower than 0.1, indicative of very high P buffer capacity (Frossard et al. 1993).

The above discussions highlight some of the chemical reactions explaining the high retention of P in weathered acid soils from the tropics. Along with P sorption reactions, some additional factors might contribute to the phosphorus deficiency in these soils. Diffusion-related mechanisms may cause the decline of available P in highly aggregated soils. P in solution can be initially sorbed outside of aggregates and be diffused into aggregates very slowly due to very small intra-aggregate diffusion coefficients ($1.5 \times 10^{-12} cm^2 s^{-1}$) (Linquist et al. 1997). This slow intra-aggregate diffusion can limit the availability of interior aggregate P. Losses of P by leaching and erosion can also decrease the P availability in these soils (Dowdle and Uexküll, 1988). In spite of their high P fixing capacity, leaching of P has been attributed to rapid infiltration and percolation through macro- and meso-pores after frequent events of rain (Sollins, 1991). Loss of the top soil layer can remove significant amounts of inorganic and organic P that can be readily available for the plants. Losses of microbial P have accounted for much of the total P reduction from the top soil layer in slash burn systems (Hands et al. 1995).

Many research efforts have been devoted to develop strategies to overcome the low P availability in agricultural systems from the tropics. Results have indicated that consecutive applications of P fertilizers (up to 140 kg P ha^{-1}) and liming might be required to maintain adequate levels of available P for annual crops (Beck and Sanchez, 1994). Effectiveness of phosphate fertilizers can however decrease with time (Barrow, 1980), and a decline in P availability and yield after one year of large P application suggests a low residual effect of soluble P fertilizers in those areas (Linquist et al. 1996).

More recently, emphasis has been given to develop alternative strategies that can be applied to low inputs management systems. Among them, utilization of alley cropping systems and crop rotations with green manures have been considered largely beneficial because of the improvement in the amount of organic matter returned to the soil as mulch and its implications on nutrient cycling. The positive effect of the incorporation of plant residues on nitrogen cycling has been widely recognized in tropical areas (Vanlauwe et al. 1998). It also has a significant effect on the biological transformations affecting the short- and long term phosphorus cycling (Hands et al. 1995 and Maroko et al. 1999). Plant residues are not only a potential source of P for subsequent crops, they can also prevent P fixation by keeping the element in organic forms (McLaughlin et al 1988) that are less susceptible to be irreversibly sorbed in the soil surface. Incorporation of high quality plant residues has been comparable or more effective source of available P than highly soluble P fertilizers in increasing P availability in weathered soils from the tropics (Nziguheba et al. 1998). Studies conducted in the last decades in tropical areas have pointed out the significance of the transformations of the organic P pools and their importance to supply significant amounts of available P to

crops in agricultural systems (Stewart and Tiessen 1987, Tiessen et al. 1992, Beck and Sanchez 1994). In the last section of this review emphasis will be given to the biological components of the P cycle, how these components might be affected by incorporation of plant residues and their implications on phosphorus availability in tropical areas.

Biological transformations during decomposition of plant residues

To understand the main changes in the P cycle following the incorporation of plant residues it is necessary to integrate the chemical reactions with the biological transformations taking place in the soil environment. Since immobilization, mineralization and redistribution of P in the solid phase of the soil occur simultaneously, both types of P transformations should be considered as interrelated. A conceptual model containing chemical and biological components will thus be useful to describe the main processes and their relationships. The model of the P cycle proposed by Stewart and Tiessen (1987) will be employed as it describes the main chemical and biological components and will help to illustrate the changes resulting from the incorporation of plant residues (Figure 1).

Release and microbial utilization of phosphorus from decomposing plant residues

At early stages of decomposition plant residues release significant amounts of organic and inorganic P compounds. Due to autolysis of the plant cells and hydrolysis of phosphate esters by plant phosphatases, significant amounts of P can be released from dried plant materials even without microbial activity (Martin and Cunnigham, 1972). Fifty to 85 % of

the plant P may be in soluble forms, especially as inorganic P (Bromfield and Jones 1972, Chisholm, et al 1981). The transfer of P from residues to the soil has been shown to be very rapid. Appearance of over 50% of residue-derived P in the soil inorganic pools after 11 days has been attributed to the presence of soluble inorganic P in the residues (Friesen and Blair, 1988). Studies of phosphorus cycling using ^{32}P-labelled medic residues in wheat-pasture rotations have also indicated that up to 90% of the plant P can be released during the first 7 days of decomposition (McLaughlin et al. 1988b).

This flush of soluble P can enter the available pool and/or be adsorbed by soil surfaces if microbial populations and active roots are not well established to act as active sinks of available P. Once the microbial populations start growing, probably favored by the presence of C substrates in the residues, the demands for P will be increased resulting in immobilization of Pi. The amount of Pi being incorporated by microbes has been shown to follow Michaelis-Menten kinetics and it is a function of the microbial population size and the internal requirements of P of the microbes (Beever and Burns, 1980). Immobilization of Pi by microbes has been reported when soils enriched in P are supplemented with cellulose under incubation conditions (Chauhan et al., 1981). Similarly, Lee et al. (1990) observed that glucose additions increased the quantity of microbial P in a highly weathered soil. Phosphorus immobilization can also occur during decomposition of plant residues. Early studies suggested that after rapid mineralization of residues, P can be used for synthesis of microbial organic phosphates (Cosgrove, 1977). Decomposition studies carried out using labelled medic residues have shown that plant P, mostly in inorganic forms, can be rapidly assimilated by microbes. Twenty five percent of plant P was incorporated in microbial

biomass after only seven days, while over 50% was present in organic forms after 90 days of decomposition (McLaughlin et al. 1988a, b). The authors thus concluded that P released from decomposing plant materials may not be available for plant uptake, as microbes can assimilate the P released from residues. At this stage of decomposition, it is probable that competition between microbes and soil sorption reactions will determine the final P availability from crop residues.

Competition between microbes and solid phase for the P released from residues can be influenced by several factors. Due to unequal distribution of the residues in the soil, it is probable that P released from residues will be localized in *discrete microsites* surrounding the decomposing residues, reducing thus the contact between the available P and the soil surfaces. Higher microbial activity in these *microsites* can probably favor the microbial utilization of the released P. Recent studies suggesting that microbes can effectively utilize P sorbed on Fe and Al minerals can give additional advantages to the microbes in the competition for the released P. Bioavailability of sorbed Pi and glucose 6-phosphate on short-range ordered Al precipitates was investigated as sources of P to support the growth of microbial communities from Brazilian Oxisols (Shang et al. 1996). The results indicate that these bound phosphates provide a sufficient source of P to support the growth of *Enterobacter* and *Pseudomonas* cultures. Incubation studies using culture media and suspended pure minerals have shown that microbes can utilize from 38-46% of the sorbed P in variable charge minerals (He and Zhu, 1998). The results varied according to the type of mineral, having the highest microbial utilization of sorbed P in kaolinite and goethite (41-46%), and the lowest values for the amorphous Al oxides (38%). This could also represent a competitive advantage for

microbes over roots, considering that the previous values of microbial utilization of sorbed P are significantly higher than those normally reported (5-15%) for utilization of soil P by crops under field conditions (Blair and Bolan, 1978).

Differences in growth rate of microbial populations and plant roots can also be critical in determining the accessibility to the *discrete microsites*, and thus the competition for available P. Under optimal conditions of soil moisture and aeration, it is probable that microbial growth will not be limited because of the high nutrient and C availability and the relative even distribution of the soil microbes that facilitates the microbial colonization of the residues. Microbial inoculum already present in the residues can also participate in early decomposition of the residues (Tester, 1988). In roots by contrast, their growth might be affected by physiological and soil factors. Even after a complete root system is developed, it is probable that unequal distribution of the residues may limit the accessibility of the roots to the microsites containing the P released from residues. The more even distribution of the microbes throughout the soil would lend them a significant competitive advantage over plant roots for P uptake from residues (McLaughlin et al. 1988b). Although there is not much information about the factors regulating the competition, it is believed that microbes should play an important role in the P cycling during decomposition of plant residues. Dynamics of this active pool might ultimately define the short and long-term P availability from plant residues.

Biological transformations taking place in residues, without the participation of soil chemical reactions, can give additional insights about the P transformations occurring during

decomposition of residues in the soil environment. Early studies comparing undecomposed and partially decomposed phalaris (without soil) showed that there was an accumulation of acid-insoluble organic P in the litter during decomposition (Jones and Bromfield, 1982). This accumulation of organic P was attributed to the microbial utilization of the P initially contained in soluble forms in the litter. Similarly, incubation studies comparing litters of a secondary forest at different stages of decomposition showed that significant changes occurred in the distribution of the organic and inorganic P forms, although the total amount of P in the litter remained relatively constant (Mueller-Harvey and Wild, 1986). While at the beginning of the experiment, about 50% of the total P in the litter was in inorganic forms, it represented less than 20% after 8 months of incubation. The transfer of P was mainly from inorganic P (386 μg P g^{-1} soil) to organic P forms contained in the LiOH extractable (91 μg P g^{-1} soil), residual (163 μg P g^{-1} soil) and acid soluble esters fraction (122 μg P g^{-1} soil) of the litter. Characterization by thin layer chromatography of lipid extracts obtained from litter at the beginning and end of incubation, revealed the synthesis of phosphatidyl ethanolamine and phosphatidyl choline of microbial origin during litter decomposition (Mueller-Harvey and Wild, 1986).

Results from litter incubations without soils suggest an accumulation of organic P in the residues associated with microbial activity in the decomposing residues. Conversion of plant P into organic P compounds is also supported by changes in the composition of phosphate esters during decomposition of the litter (Mueller-Harvey and Wild, 1986). Most of the organic P in leaves, as well as in microorganisms, occurs mostly in RNA, DNA and phospholipids (Table 1). In decomposed litter by contrast, no nucleotide phosphate was

detected and very small amounts of phospholipids and soluble P-esters were extractable. Most of the Po was difficult to extract and remained unidentified. These results suggested that organic P accumulated in the litter during decomposition might be composed of sub products of microbial activity that become selectively stabilized, and therefore, do not conserve the composition of the microbes from where they were derived. Results from litter incubations corroborated previous findings indicating that the dynamics of the microbial biomass P is an important component of the soil P cycle during decomposition of plant residues.

Transformations of organic P accumulated during decomposition of plant residues

Understanding the mechanisms by which the plant P is utilized by the microbes and posteriorly returned to the available P pool is critical to assess the final availability of P from plant residues. Mechanism of P uptake by active microbial populations growing in nutrient media (Beever and Burns, 1980) could potentially be extrapolated to soil microbes decomposing plant residues. Release of P from microbial cells is however, less understood and only limited information is available in the literature. Ryszkowski et al. (1989) estimated that P associated with different components of the below-ground food webs contributes significantly to the total P flowing in the system during a year. According to the authors, depending on cropping systems, P retained in the fungal biomass can fluctuate between 40 - 108 kg P ha^{-1} , while the bacterial biomass contained 18 - 49 kg P ha^{-1}.

The question arising at this point concerns the turnover of the microbial P synthesized during decomposition of residues and how it can eventually become available for the plants Predatory and non-predatory interactions are believed to be responsible for the death and lysis of the microbial cells. One approach to assess turnover of this microbial P is through the knowledge of mass flow through the soil food web. In this case, P flow at each trophic level is estimated from seasonal changes in populations and data on P composition of the different components of the soil food web. This approach was used by Cole et al. (1977) who estimated that the turnover of microbial P is not rapid, averaging one or twice per year. Magid et al. (1996) simulated the soil food web based on data of changes in populations obtained at the Loevinkhoefe experimental farm. The P cycling at the various trophic levels was estimated, and the total annual P flow through all the functional groups was 12 and 15 kg P ha^{-1} y^{-1}, for conventional and integrated managed systems, respectively.

Several factors have been identified which influence the turnover of microbial P, and consequently could influence the P availability from plant residues. Activity of the grazers can regulate the sequence and flow of biomass being released from different decomposers (Coleman et al. 1978). Although fungi and bacteria seemed to be the major components in terms of biomass, and P flow, higher trophic levels in the food web (e. g. amoebae and nematodes) can determine the predatory death rate of fungi and bacteria, and thus play an important role in the P cycling (Cole et al.1978, Elliott et al. 1980). These predatory interactions are affected by the habitable pore space (Elliott, 1980). Soil sites differ in the type and intensity of biological activity and therefore, they should also differ in phosphorus flows. Comparing macro- and microaggregates, significantly higher amounts of microbial C

has been found in macro aggregates, whereas microbial biomass P was greater in the micro aggregates (Elliott, 1986, Singh and Singh, 1995). This was interpreted as differences in levels of grazing and/or differences in biological (fungi-dominated and bacteria-dominated) components of the soil food web between these two size classes of aggregates.

Other trophic interactions might be equally important during decomposition of plant residues. The role of earthworms and termites has been of particular interest, especially in tropical areas. Short-term plant availability of P derived from litter was increased two to three-fold upon digestion by surface casting earthworm (Mansell et al. 1991), and alkali-extractable P was significantly higher in the cast than in the surrounding soil (Guggenberg et al. 1996). Decomposition and patterns of phosphorus release from different forestry and agricultural litters have been studied under humid tropical conditions using litter bags with different mesh size (0.5, 2 and 7 mm) to allow differential access of the soil fauna (Tian et al., 1992). Increasing mesh size of the litter bags significantly increased the release of phosphorus and other nutrients, suggesting that soil faunal activities enhance mobilization of nutrients contained in the residues.

Once the microbes die, whether because of predatory interactions as indicated above, or because non predatory interactions, significant amounts of organic P are released following the lysis of the microbial cells. The organic P compounds released from the microbes can be then hydrolyzed by the soil phosphatases or be preferentially stabilized in the soil matrix (Stewart and Tiessen, 1987). Phosphodiesters are likely to participate in biochemical and biological reactions since they are less subject to sorption reactions onto the soil surfaces

(Tate, 1985). Phosphomonoesters, such as inositol hexa- and penta-phosphate, can be strongly adsorbed to iron oxide rich soils (Anderson et al. 1974 , McKercher and Anderson, 1989). This selective stabilization of microbial sub products has been shown to occur also during decomposition of litters. Results from incubation with different litters have indicated an accumulation in the soil of significant amounts of microbially derived inositol penta- and hexa-phosphate, which were almost absent in the litter at the beginning of the experiment (Mueller-Harvey and Wild, 1986). Some of the inositol phosphates being stabilized in the soil are frequently in isomeric forms that appeared not to be plant derived, but may be epimerized from plant forms by microbes (Cosgrove, 1977).

The organic phosphorus accumulated in the soil during the decomposition of plant residues can finally be mineralized when Pi supply is limiting and phosphatase enzymes are induced (McGill and Cole, 1981). Phosphatases responsible for the hydrolysis of a wide range of organic P compounds can be produced by microorganisms and plants (Dalal 1982, Tarafdar and Claassen 1988). Their activity is a function of soil properties such as, pH, water content and oxides or humic substances which can form stable complexes with the phosphatases (Nannipieri 1984, Pant and Warman 2000). Phosphatase activity can also vary according to the location of the enzymes at different soil sites. Stabilized extracellular phosphatases made resistant to thermal degradation and proteolysis by association with soil colloids may differ in activity from phosphatases associated with intracellular components located in dead cells or cell debris (Nannipieri et al. 1996).

Reports specifically about the mineralization of plant organic P added to soils are quite few. Differentiation between mineralization of soil organic P and plant organic P usually requires the utilization of labelled plant materials which allow only short-term studies (less than 12 weeks). Dalal (1979) reported net P mineralization rates of 0.008 and 0.009 day^{-1} for roots and tops of ^{32}P-labelled white clover (*Trifolium repens* L.), respectively, as measured by the ^{32}P utilized by oat (*Avena sativa* L.). White and Ayoub (1983) studied mineralization of labelled *Vicia faba* residues with varying C:P ratios (123, 251, 506) applied at a rate of 8 ton C ha^{-1}. A mineralization rate of 0.008 day^{-1} was observed only for the residue with the lowest C:P ratio. This mineralization rate represented an increase of 2 μg P g^{-1} soil in the soil extractable Pi over 42 days. This value was small as compared with the total extractable P in the soil-residue mixture at the beginning of the experiment (38.4 μg P g^{-1} soil). This study thus revealed that, in spite of the low C:P ratio of the residue, significant amounts of P can be either sorbed or immobilized by microbes and therefore, can not be immediately available for subsequent crops.

Assessing phosphorus cycling during decomposition of plant residues

The transfers of P resulting from additions of plant residues have usually been estimated by measuring the changes in P associated with different organic and inorganic soil pools. One approach has been the utilization of chemical procedures that differentiate organic and inorganic P fractions based on solubility of the soil P compounds. Sequential fractionation procedures have been normally used for this purposes. Friesen and Blair (1988) used the Chang and Jackson fractionation procedure to evaluate the transfers of inorganic P after

incorporation of labelled plant residues. Fifty percent of the added plant P was found in inorganic P pools, mainly in the Al-P fraction, after only 11 days of decomposition. The remaining P was presumably localized in organically bound P forms, including microbial P.

More recent P fractionation procedures have allowed the separation of several organic P fractions with differences in availability. The P fractionation procedure developed by Hedley et al. (1982) describes changes in readily available (Resin, $NaHCO_3$), moderately resistant (NaOH) and highly resistant organic and inorganic P fractions (HCl, hot concentrated HCl, residual P). This method has been successfully used to study transformations of organically and geochemically bound P during pedogenesis (Tiessen et al. 1984). Cross and Schlesinger (1995) recently used the procedure to analyze data from 17 studies including nine soil orders. The authors concluded that the contribution of biological versus geochemical processes to the phosphorus cycling varied with pedogenesis. The biologically active or organically bound P, measured by the sum of $NaHCO_3$-Po , sonic-Po and NaOH-Po fractions, represented an increasing proportion of the total P with soil development. While five percent of the total P was present in these labile organic fractions in the Entisol, the percentage increased to 35% in Oxisols. Short- and long-term dynamics of these organic P fractions could significantly contribute to the total available P in weathered soils.

Application of the Hedley fractionation procedure has led to inferences about the changes in P forms following additions of organic sources of P. Under incubation, addition of organic amendments (manure, alfalfa and wheat straw) to high P fixing soils, significantly affected the distribution of P among the different P pools in soils (Iyamuremye

et al. 1996a). Both , NaOH-Pi and NaOH-Po, increased with manure or alfalfa residues. Since the NaOH-Pi fraction is considered to be sites for P sorption; it was concluded that rich-P organic materials can decrease future sorption of P in soils because of the reduction in sorption sites. Incorporation of tithonia residues increased the resin P, bicarbonate P, microbial P and sodium hydroxide inorganic P in an Alfisol from western Kenya (Nziguheba et al. 1998). Combination of tithonia residues with triple super phosphate (1:1 on total P basis) was comparable to the soluble fertilizer in improving the P availability after 16 weeks of incorporation under field conditions. The efficient response of the organic source was attributed to the rapid release of P from the residues and reduction in the P sorption capacity resulting from competition with organic ligands produced during decomposition.

Although P fractionation procedures have been widely used to describe P transformations, they have some limitations that should be carefully considered. Among the deficiencies of the fractionation procedures are: i) associated errors in the estimation of some P fractions (e. g. Microbial P, total and organic P) (Potter et al.1991, Rubaek and Sibbesen, 1993), ii) hydrolysis of Po and transfers of P among P fractions during the extraction procedure, iii) they do not give information about the nature of the P compounds separated in each fraction, and iv) they may not discriminate between P fractions differing in biological activity (Magid et al. 1996).

Some of these limitations can have implications, particularly when describing P transformations in ecosystems characterized by receiving significant amounts of organic inputs. Recent studies have indicated that after addition of plant residues, significant

amounts of organic P with rapid turnover can be located in the light particulate organic matter (Maroko et al.1999). This organic P, although biologically unstable, would be eventually considered as resistant since its release would take place after breaking up the cellulose with the hot acid digestion in the last step of the fractionation procedure. Errors associated with determination of the P fractions can be significant considering the amount of P normally added as plant residues. A common amount of P added annually as plant residues, 10 μg P g^{-1} soil (assuming a bulk density of 1.4 g cm^{-3}, a soil depth of 10 cm and a residue rate of 10 Mg dry matter ha^{-1} with 0.15% P), can be close to the standard error of the difference in means (~ 7.5 μg P g^{-1} soil) normally found for the sum of the P fractions (Nziguheba et al. 1998). Inferences about P transformations will be thus, difficult to achieve on a short-term basis, specially for those organic fractions having the highest experimental errors (eg. NaOH-Po). Finally, since extraction-based procedures separate fractions based on solubility of organic and inorganic P compounds, no discrimination can be made between plant- and soil- derived P, making it difficult to assess the extent of P transformations of plant residues occurring during decomposition. Transformation of readily available Po compounds contained in residues (eg. RNA and phospholipids) to soil phosphomonoesters requires the utilization of complementary techniques.

Characterization of soil P by ^{31}P-NMR is an useful tool that generally complements the information given by the sequential extraction procedures. Several inorganic and organic P species can be identified by ^{31}P-NMR (i.e., inorganic orthophosphate, phosphomonoesters, phosphodiesters, polyphosphate, pyrophosphate, phosphonate and teichoic acids) (Newman and Tate 1980, Condron et al. 1990). This technique has been used to describe the sequence

of mineralization of organic P compounds in temperate soils. Condron et al. (1990), examining soils from native and cultivated areas concluded that phosphodiesters are mineralized more readily than phosphomonoesters, which tend to accumulate in the soil. Recent mineralization studies of soils amended with RNA and Fe-phytate have confirmed this sequence in woodland and pasture (Taranto et al. 2000). In soils amended with RNA, more than 70% of the P added was recovered as orthophosphate in anion resins after 8 months of incubation. In the Fe-phytate treatment on the other hand, 80% of the added P was recovered in organic forms extracted with NaOH. This sequence of mineralization of organic P compounds has also been found in decomposing litters. Incubation studies during 18 months using pine litter bags have showed that nucleic acids, initially present in 9% of the total signal, were absent by the end of the experiment, while the amount of phosphomonoesters remained closely unchanged (Gressel, et al. 1997).

Although paramagnetic species, such as Fe, can affect the detection of some inorganic phosphate compounds by ^{31}P-NMR (Bedrock et al. 1995), this technique can still supply valuable information about the changes in organic P compounds resulting from increasing incorporation of organic matter in weathered soils. Guggenberger et al. (1996) studied the changes of organic P fractions following the establishment of grass/legume pastures in a native savanna located in Carimagua, Colombia. They found that organic P species associated with humic and fulvic acids increased significantly with the rate of organic inputs in the pasture. A decrease in the monoester:diester P ratio in the humic fraction under pasture (1-3:1), as compared with values reported for this fraction in temperate soils (4-20:1), indicated an accumulation of labile organic species. This enrichment in labile organic P species in the

humic fraction was attributed mainly to the presence of microbially derived products that were accumulated in the pasture.

Another approach to assess the P cycling during decomposition of plant residues is based on estimates of P transfers among different organic and inorganic soil pools defined by functional criteria rather than by chemical extraction. Among the soil processes, phosphorus uptake by decomposers and subsequent turnover of the organic P synthesized during decomposition have been considered one of the largest annual P flows in the soil (Cole et al. 1977, Harrison1985). Comparing the different P flows in the soil-plant system, the P flow from labile inorganic pools to decomposers (2.4-3.1 g P m^{-2}), from decomposers to labile organic P (2.1-3.2 g P m^{-2}) and its subsequent mineralization (3.0-4.3 g P m^{-2}), have been found significantly higher than the annual P uptake by live roots (0.5-1.2 g P m^{-2}) in natural grasslands (Cole et al., 1977). This reflects that growth and death cycles of organisms as well as mineralization of newly synthesized Po can play an important role in the P cycle and those transfers can not be properly inferred by utilization of conventional chemical procedures.

In this functional perspective, emphasis is given to the processes as well as to the characterization of the pools in the P cycle. During decomposition, initial release of P from plant residues depends on the P solubility of the residues and subsequently, on the rate of decomposer growth (Blair and Boland, 1978). Large amounts of P are required by decomposers during plant residue decomposition. The ratio of cellulose decomposition to organic P formed may vary from 64 to 115 g C/g organic P (Chang 1940, cited by Cole et al. 1977). This phosphorus taken up by microbes during decomposition can be derived from

either plant materials or from different sources of soil available Pi. The contribution of each source will depend on the P content of the residues and the soil P availability. Results using medic residues have indicated that up to 30% of the microbial biomass P can be derived from decomposing plant residues and the remaining P is derived from soil available forms (McLaughlin et al. 1988a). Although relatively low, this percentage could be higher in soils with low P availability in which decomposing plant residues can be the main source of P for microbial growth. Once the P is incorporated into the microbial biomass, the rate of microbial death and subsequent mineralization of organic P compounds will finally determine the P cycling and availability of P from plant residues (Blair and Boland 1987, Tate and Salcedo, 1988).

A quantitative representation of the P transfers following the incorporation of plant residues has been possible by the utilization of labelled residues. Early studies measuring the utilization of ^{32}P- ^{14}C- labelled white clover by growing oat plants indicated that 38 % of the applied plant P can be mineralized and utilized by oats after 10 weeks (Dalal, 1979). Half lives of 12.4 and 8.1 weeks for mineralization of P and C, respectively, indicated a delay between the C and P mineralization. Between 32 to 38 % of plant C was mineralized before net P mineralization was observed. This delay was attributed to the accumulation of ^{32}P in less available organic and inorganic forms as revealed by higher amounts of ^{32}P remaining in the soil at the end of the experiment. Inability to differentiate the ^{32}P remaining in the residue from that released during decomposition made it difficult to establish whether the P mineralization was limited by the release of P from the residue or by the turnover of microbial P and microbial by products.

A more detailed P budget for soils under wheat-pasture rotations was made using ^{33}P-labelled medic residues (McLaughlin et al. 1988b). ^{33}P data from ignited and non ignited soil samples suggested a rapid incorporation of residue P in the soil organic pool. Forty percent of the plant residue P was incorporated into soil organic forms after only seven days of decomposition and approximately 50% was found after 90 days of incubation. Since most of the P in the plant (> 70 %) was originally present in inorganic forms, it was concluded that a major portion of the Po accumulated in the soil during decomposition was microbially derived rather than of plant origin. Subsequent turnover of this microbial P and its subsequent mineralization would thus determine the net mineralization of the P contained in plant residues. Estimates of net P mineralization made by Frossard et al. (1995) using the previous data supports that conclusion. Since the insoluble part of the residue might have a C:P ratio greater than 300, the authors predicted that mineralization of P is likely to occur mainly from microbial biomass P with substrates having C:P ratios close to 30-50. Net mineralization of P from residues was estimated to occur only after the 4th generation of decomposers on the medic residues.

Long-term effects of the incorporation of plant materials has been less documented in weathered areas from the tropics. Tiessen et al (1992) reported 20-30% higher levels of NaOH Po following an increase in organic matter accumulation after 8 years of bush fallow. Consecutive additions of plant residues and other organic materials could eventually promote accumulation of P in organic forms which are less susceptible to irreversible sorption by chemical reactions in these soils. Recent studies have suggested that these labile organic P forms are responsible for maintaining the levels of available P. Beck and Sanchez (1994)

utilizing pathway analysis showed that 44% of the variability in resin Pi resulting from long-term incorporation of corn residues and mucuna as cover crop could be explained by changes in labile Po extracted with bicarbonate. Enhancement in the available P associated with increasing amounts of organic inputs has also been observed in grass/legume pastures following native savanna in Oxisols from Colombia (Guggenberger et al. 1996). After 15 years of continuous grass/legume pasture, P associated with humic and fulvic acid increased by more than 40%, most of which was in the form of labile diester P of microbial origin. This enhancement in the labile organic P was very significant especially for plant nutrition, since this organic reserve (18.1 kg P ha^{-1}) exceeded the ready available inorganic P measured by the Bray solution (3.1 kg P ha^{-1}).

Continuous mulching of nutrient-rich foliage derived from leguminous trees in alley cropping systems has also been considered an alternative to improve P cycling in tropical areas. A long-term experiment established in an alley cropping system using two leguminous trees, *Gliricidia sepium* and *Erytrina fusca* grown in association with maize and beans, illustrates the main changes in P budget resulting from consecutive additions of organic inputs (Hands et al. 1995). Preliminary results after three years of continuous mulching with legume pruning revealed that total P cycling through all vegetation and P returns in plant residues were greater in the alley cropping system as compared with the conventional monoculture of maize and beans. Both, greater plant P uptake and greater residue return under the alley cropping system favored a greater return of P (19.5 kg P ha^{-1}) to the soil in comparison with the monoculture system (11.4 kg P ha^{-1}). This increased return of P to the soil in the alley system was partially responsible for an increase of 30 % in the total available soil P.

The previous studies showed that incorporation of plant residues can significantly modify the short- and long-term phosphorus cycle by stimulating the biological transformations that compete with chemical reactions in weathered soils. Although evidence has indicated that this effect can have a significant impact on the productivity and sustainability of those phosphorus-deficient areas, little information is known about the main mechanisms involved. Some studies have attributed the improvement in P availability following the addition of residues to reduction in the P sorption retention characteristic resulting from ligand exchange reactions with organic acids produced during decomposition (Hue 1991, Iyamuremye et al.1996b, Ohno and Erich, 1997). Other studies have suggested that improved microbial activity following addition of residues can promote the allocation of plant derived P in labile organic forms preventing its fixation in soil minerals (Friesen and Blair 1988, McLaughlin et al. 1988b). Although these two effects may be equally important, the microbial transformations following the decomposition of residues have been less studied. Factors regulating the transfer of plant P to the microbes and the subsequent turnover of this microbial P seem to be critical to understand the P cycling during decomposition of plant residues. Among these factors, characteristics of the plant residues might be of particular importance since they usually determine the type and activity of microbial populations decomposing the plant residues. In the present study the main objective was to evaluate the effect of plant characteristics on short-term P dynamics, giving special emphasis to study the biological and biochemical transformations and their contribution to explain differences in P availability from plant residues.

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Table 1. Distribution of organic P compounds in litter compared with reported values in leaves, bacteria and fungi.

	Litter[1]	Leaves[1]	Bacteria[2]	fungi[2]
Lipid P	3	8	15	20
Inorganic P-water soluble	58	70		
LiOH extractable	6	0		
Water soluble-P esters	5	5		
RNA	Not	16	65	58
DNA	Detected			
Other LiOH soluble organic P	9	0		
Residual P	16	0		
Monoesters P			20	22

[1] Litter after 8 months of decomposition, Mueller-Harvey and Wild (1986)
[2] Magid et al. 1996.

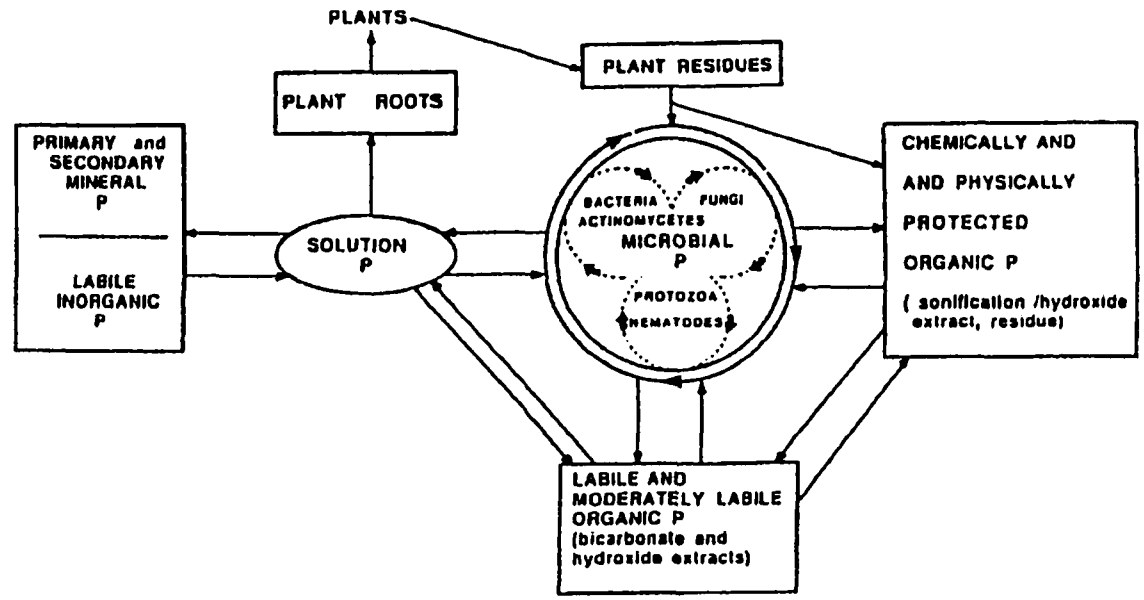

Figure 1 Schematic illustration of the measurable components of the phosphorus cycle (Taken from Stewart and Tiessen, 1987)

CHAPTER II

CHANGES IN PHOSPHORUS AVAILABILITY DURING DECOMPOSITION OF PLANT RESIDUES AS AFFECTED BY PLANT QUALITY FACTORS

1 Introduction

Many studies have been devoted to study phosphorus soil deficiency in highly weathered soils of the tropics. Factors linked to the existence of high P retention capacity and low amount of available P contribute to the explanation of this deficiency. Alternatives to enhance phosphorus cycling in agroecosystems have received much attention as a strategy to reduce fertilizer dependency in the tropics. Specifically the role of biological processes has become of particular interest because some organic fractions can account for 44-88% of the available soil P and their turnover can contribute to explain many of the differences in P taken up by the plant in tropical areas (Tiessen et al. 1984, Beck and Sanchez 1994).

One method used to stimulate phosphorus cycling in the tropics is the incorporation of plant residues as part of integrated management practices in crop rotation systems (Hands et al. 1995). Residues can supply significant amounts of P that can become available through complex reactions in which residues are used by microorganisms as a source of energy and phosphate can be released directly, or by enzymatic transformations mediated by microbial

activity (Blair and Bolan, 1978). Changes in labile and moderately labile soil P fractions have been associated with the mineralization of plant residues (Iyamuremye et al. 1996, Nziguheba et al.1998). The contribution of this process to plant nutrition has been measured by the recovery of P applied as plant residues in subsequent crops. Phosphorus recovery values ranging from 5.4 to 38 percent (Dalal 1979, McLaughlin et al. 1988a, Friesen and Blair 1988) show that even after short periods of time (less than 10 weeks), plant residues can supply significant amounts of P for plant nutrition.

Increased use of plant residues in cropping systems has focused attention on the identification of plant quality factors controlling nutrient cycling. The influence of many plant biochemical factors in determining the rate of decomposition and mineralization of N has been extensively recognized (Heal et al.1997), but relatively few evaluations have addressed the influence of plant factors on P cycling. Phosphorus content and C:P ratio have been used as indicators of residue quality and to predict P availability from residues. Singh and Jones (1976) reported that organic materials containing more than 0.3% P decrease P sorption capacity and increase P mineralization. Also it has been established that plant residues with C:P ratio lower than 200 increase P availability, while residues with C:P ratio greater than 200 are able to induce P immobilization (Dalal, 1979; White and Ayoub, 1983; Umrit and Friesen 1994). However, studies indicating different responses for plant materials having the same P content and C:P ratio (Friesen and Blair, 1988), as well as significant amounts of P mineralized from residues with relatively high C:P ratio (700) (Umrit and Friesen, 1994), indicate that some other plant characteristics might be influencing the release and P mineralization from plant residues.

Plant characteristics can potentially affect P cycling in different ways. Residues can differ in phosphorus composition which can directly determine the total amount and rate of release of P to the soil (Blair and Bolan, 1978; White and Ayoub, 1983). Residues can also indirectly affect P cycling through their influence on microbes mediating different processes taking place during decomposition, such as P immobilization and P mineralization. Finally, decomposition of plant residues can release low molecular weight organic acids that can modify the solubility of the soil P by affecting P sorption characteristics of the soil and the formation of Al- and Fe-P precipitates (Ohno and Crannell, 1996; Iyamuremye et al. 1996). Studies have usually emphasized the effect of residues on soil chemical reactions and less attention has been given to the biological and biochemical transformations occurring during litter decomposition and their influence on P availability. Among the few studies considering the biological transformations, most of them have highlighted the influence of the C:P ratio of plant materials in determining P availability (White and Ayoub, 1983; Umrit and Friesen, 1994; Nziguheba et al. 1998). Some other plant characteristics have been less studied. Plant residues are known to differ in the content of soluble P (25-70 % of the total P) (Chisholm, et al. 1981) and phosphatase activities (Dalal 1982), which can directly affect the release of P from residues. Carbon compounds present in the residues could influence the biochemical P mineralization by affecting the microbial demands for P (McGill and Cole, 1981) and probably by regulating the accessibility of enzymes to the substrate (Gressel et al. 1996). Also, carbon compounds could affect P cycling by shaping different microbial communities according to their degradative abilities. These microbial communities probably differ in their chemical composition and biomass turnover.

The contribution of these plant factors (soluble P, enzymatic activity and C compounds) in determining the P cycling as well as the mechanisms involved have not often been considered in studies of litter decomposition. Most attention has been given to the influence of the C:P ratio. In our study the first objective was to examine selected plant factors influencing the short-term P availability during decomposition of plant residues. Special emphasis will be given to the evaluation of plant factors that might be even more important than the C:P ratio in explaining differences in P availability. In order to study the possible mechanisms involved, our second objective was to study the effect of the residues on soil phosphatase activities, P immobilization and turnover of microbial P. To address these objectives plant materials differing in phosphatase activity , C:P ratio and quality of the C compounds were used to conduct decomposition studies in two acid soils representative of weathered soils from the tropics.

2. Materials and methods

2.1 *Soil and plant characteristics*

Incubation experiments were conducted using the top layer (0-15 cm) of two weathered soils representative of semiarid agricultural areas located in the central and eastern part of Venezuela. The main soil characteristics of the Ultisol and Alfisol are given in table 1. Plant materials of sorghum (*Sorghum vulgare)* and crotalaria (*Crotalaria juncea)* differing in P contents and enzymatic activities were obtained by using two different P rates (equivalent to 27 and 54 kg P ha^{-1}) and three harvesting times (45, 60 and 90 days after planting) under

greenhouse conditions. After harvesting, the plant material was air dried and ground to < 1mm using a Wiley grinder. Different analyses were conducted to characterize the plant materials. Total C and N were determined on finely ground samples using a LECO CHN-1000 analyzer. Total P was obtained by nitric-perchloric digestion. The extractable inorganic P was obtained after cold water extraction for 1hr (150 mg air-dried plant sample and 25 ml distilled water) and filtration with filter paper Whatman #42. In this water extract, the total amount of dissolved organic C was determined using an infrared analyzer (Shimadzu TOC-500) after acidification of the extracts. Blanks without plants were included in the analysis. The cellulose and lignin contents in the residues were determined on ground samples using the acid detergent fibre method (Van Soest 1967). To estimate extracellular phosphatase activities of the residues, samples from the different treatments were mixed with sterile acid-washed sand at a rate of 1% (w/w) and distilled water was added to have a final moisture content of 12%. Immediately after mixing of residues with sand, phospho monoesterase and phospho diesterase activities were assessed. Briefly, the p-nitrophenol released after hydrolysis of added p-nitrophenyl phosphate and bis-p-nitrophenyl phosphate was measured to determine, respectively; phospho monoesterase and phospho diesterase activity, following the procedure described by Tabatabai (1982). Blanks for each plant residue were prepared by adding the substrate immediately before the colorimetric analysis to take into account the absorbance attributable to dissolved organic matter or non hydrolyzed p-nitrophenyl. Also a control without residues was included. All the analysis were performed in triplicate and the treatment means were used for further comparisons.

2.2 *Incubation experiments*

Two main experiments were conducted to evaluate the influence of selected plant quality factors on P cycling and to establish how these factors could explain observed differences in short-term P availability during residue decomposition. In the first experiment, emphasis was placed on identifying plant factors and their contribution to explain variations in P availability as well as potential mechanisms involved. Based on these results, a second experiment was designed to test the hypothesis that changes in microbial community structure during decomposition of plant residues can potentially affect the short-term P availability by modifying the turnover of the microbial P.

2.2.1 **Influence of plant factors on P cycling**

Incubation protocol

Sixty days decomposition studies were conducted using the two soils and different treatments of plant residues resulting from the combination of two P rates and different harvesting times as described previously. Samples of 40 g air-dried soil sieved at 4mm were mixed in triplicate with plant materials incorporated at a rate of 2% (w/w). Two additional treatments were included: 1) Only inorganic P uniformly applied in solution (KH_2PO_4) at a rate of 30 μgP g^{-1} soil (referred to as Pi); and 2) A mixture of cellulose in powder form (9900 μgC g^{-1} soil) and inorganic P, to give a C:P ratio 330 (referred to as cell+Pi). For each soil, controls without plant residues were also included. A total of six sampling times were used: 1, 5, 15,

30, 45 and 60 days. Hoagland's solution containing macro and micro nutrients (except P) was uniformly applied using a syringe in order to bring the soil moisture content to 65% of the water holding capacity which is equivalent to 15 and 20 % w/w, for the Ultisol and Alfisol, respectively. Since the treatments with residues had higher water holding capacity, adjustments with distilled water were made to bring the final moisture to 17.5 and 25% w/w for the Ultisol and Alfisol, respectively. Nutrient solution containing inorganic P was utilized for the treatments with and without cellulose. Incubations were conducted at 25 °C in sealed 1-l Mason jars which were opened to the air twice a week. Bulk density measured in the controls (1.2 and 1.0 Mg m^{-3} for the Ultisol and Alfisol, respectively) was used to calculate the water filled pore space (WFPS), which was found to be 64 % of the total soil porosity for both soils. To facilitate the initial equilibration of the soil with the solution, the experimental units were kept at 5 °C for 16 hours previous to the initiation of incubation. According to the rate of plant material and the P content of the residues, the total amount of P added fluctuated between 15 and 30 μgP g^{-1}. A completely randomized experimental design was used and destructive sampling occurred at each sampling.

Soil P availability indexes

At each of the sampling times, soil samples were analyzed for readily available P and strongly sorbed inorganic P using anion exchange resins and chemical extraction, respectively. Two grams of moist soil was shaken for 16 hrs (150 reciprocations min^{-1}) with 30 ml of distilled water and four 204-U-386 anion resin strips converted to the bicarbonate form (Parfitt et al. 1994). Desorption of the P from the resins was done by shaking the resins for 2hrs at 150

reciprocation min^{-1} with 0.5M NaCl. The more strongly sorbed P was extracted using air-dried soil samples and the Bray (0.03N NH_4F + 0.025N HCl) solution as described in Olsen and Sommers (1982). The P concentration in solution was analyzed colorimetrically by the molybdate blue method (Murphy and Riley, 1962). These measures of P availability obtained at different sampling times were used to perform correlation analysis with the plant characteristics.

Microbial biomass P and phosphatases

Since the incorporation of plant residues is believed to affect the dynamics of soil enzymes and patterns of P immobilization during decomposition, some treatments were selected to monitor changes in microbial P and acid phosphatases. Treatments with low (330) and high (630) C:P ratio of both plant residues, as well as the combination of inorganic P with and without cellulose, were utilized to determine microbial biomass P by chloroform fumigation and resin extraction according to the method of Kuono et al. (1995). Chloroform released P was calculated as the difference between the chloroform and the non- chloroform treated samples with corrections for the recovery of the added inorganic P. Microbial biomass P was estimated by dividing the chloroform released P by a factor K_{pi} 0.41 (Brookes et al. 1982), which represents the extractable part of microbial biomass P. Given similar recoveries of ^{32}P found for labelled bacteria and fungi populations when using either sodium bicarbonate or resin exchange methods (McLaughlin et al.1986), it is assumed that the factor K_{pi}, initially suggested for extraction with sodium bicarbonate, can be satisfactory used in resin extraction of the released microbial P. In addition to microbial P, microbial activity and

decomposition of residues was estimated by measuring the cumulative evolution of CO_2 during the incubation. This was performed by sampling 1ml of gas from the head-space of the Mason jars and injection into an infrared CO_2 analyzer (LI-COR, Model LI-6252). After sampling the head space, Mason jars were aired before further incubation.

Moist soil samples were analyzed for phospho monoesterase and phospho diesterase activities at pH 5.0 (Tabatabai, 1982). As described in the characterization of plant residues, blanks were prepared by adding the substrate immediately before the colorimetric measurements. Also, standards of p-nitrophenol were prepared in the presence of the soil to take into account the incomplete extraction of p-nitrophenol from the soil as described by Sarathchandra and Perrot (1981).

P isotherms

Phosphorus isotherms were plotted to evaluate the influence of these plant residues on the P retention characteristics of the evaluated soils and how that could contribute to explaining the P availability. These isotherms were developed for both soils at the end of the experiment using the basic procedure of Fox and Kamprath (1970) and the treatments with low C:P ratio and the control. A preliminary study using the controls showed that the equilibrium was reached for both soils after 48 hs of continuous agitation, therefore this equilibrium time was used. Data from the isotherms were fitted to the Langmuir equation to estimate the parameters of adsorption affinity (k) and maximum adsorption (b), as well as the amount of P sorbed at a concentration of P in solution of 0.2 μgP ml^{-1}.

2.2.2 Microbial community structure and turnover of microbial P

Results from the first experiment suggested that plant residues significantly influenced the P availability by affecting the P immobilization and turnover of microbial P. In this second experiment, the hypothesis tested was that decomposition of these two kinds of plant residues differentially affects the turnover of microbial P by modifying the microbial community structure of the decomposers. More specifically, changes in bacteria versus fungal biomass would lead to differences in turnover of microbial P. For this reason, a decomposition study was conducted using the two soils and residues of sorghum and crotalaria having low C:P ratio (treatments with 27 kg P ha^{-1} and harvesting time 45 day, according to Table 2). Triplicates of the treatments prepared in the same way as described in the first experiment were incubated for 15 and 30 days after which, moist soil samples were taken for microbial counting to estimate the bacterial and fungal biomass. Controls without residues and the treatment containing cellulose and inorganic P were also included for both soils.

Bacterial and fungal biomass

The procedure for preparation the soil smears and capturing the images for microscopic counting of bacteria and fungi was adopted from that previously described by Frey et al. 1999. Briefly, two sets of soil smears, one each for bacterial and fungal staining were prepared for microbial counting. Bacteria were manually counted from soil smears stained with DTAF (5-(4, 6-dichlorotriazin-2-yl)aminofluorescein) using epifluorescence microscopy (1000 X magnification) according to Bloem et al. 1995. Images of the microscope field were

captured using the Optronics cooled CCD camera (model DEI-470) and Adobe Photoshop image capturing software. Twenty images randomly collected from each smear were used for counting of bacterial cells. Two smears were counted per soil sample and the average bacterial abundance was used for biomass calculations. Measurements of widths and lengths of individual cells were made randomly on a subset of collected images of each treatment by using the IPLab Spectrum image analysis software (Signal Analytics Corporation, Vienna, VA). Bacterial biovolume per cell (V) was calculated from average widths and lengths (L) using the equation $V = \Pi/4 \times W2 \times (L - W/3)$ (Bloem et al., 1995). Bacterial biomass C was estimated by assuming a specific C content of 2.2×10^{-3} g C μm^{-3} (Bratbak and Dundas, 1984). Fungal hyphae were stained with calcifluor M2R fluorescence brightener (Bloem et al., 1995) and observed at 400X magnification using the filter set for UV illumination following. Thirty images were randomly collected from each smear. Fungal hyphal lengths and widths were measured as described by bacteria. Since hyphal widths varied significantly across treatments, hyphal width was taken into account for the calculations of biovolume. Total fungal biomass C was estimated by multiplying fungal biovolume by the specific C content 1.3×10^{-3} g C μm^{-3} (van Veen and Paul, 1979). The proportion of fungal biomass was calculated as a fraction of the total microbial biomass (fungal + bacteria biomass).

In order to determine the differences in fungi inoculum present on the two residue types, samples of both plant materials (<1 mm) were utilized to grow fungi under selective agar media. Fifteen pieces of plant material were transferred to sterile petri plates containing rose Bengal-streptomycin agar media (Johnson and Curl, 1972). A total of 10 petri plates were

used for each plant residue. The number of colony forming units in each petri plate was recorded after 7 and 10 days of incubation.

2.3 *Statistical analysis*

Correlation analysis for determining the relationship between plant biochemical factors and P availability was performed at all sampling times on both soils using the SAS general linear models procedure version 6.12 (PROC GLM, SAS Institute,1990). Plant characteristics affecting the available P were selected based on the significance of the correlation coefficients. In order to assess the influence of plant factors other than C:P ratio on P cycling, covariance analysis for Bray-P and Resin-P was performed using the C:P ratio as the covariate. Mean comparisons between residue types was performed for selected low (330) and high (630) C:P ratio for both soils. Analysis of variance of a factorial design was used by combining all dates in a multivariate repeated measures. At each sampling time mean comparisons among treatments were performed using the Tukey's HSD at $P < 0.05$. In the second experiment, data for individual sampling and soil were subjected to one-way analysis of variance and mean separation tests were performed by Tukey's $HSD_{0.05}$. Microbial biomass data were log transformed and proportions were arcsine-squared-root transformed before performing the analysis to meet requirements of normality and homogeneity of variances.

3. Results and discussion

3.1 Influence of plant quality factors on P cycling

3.1.1 *Plant factors and P availability indexes*

The main characteristics of plant materials indicate differences in total and extractable inorganic P, C:P ratio, phospho monoesterase and phospho diesterase activities, and in carbon compounds among the different treatments used in this experiment (Table 2). Correlation analysis between plant characteristics and Bray extractable P for the two soils showed significant correlations for some variables, most of which fluctuated with incubation time (Table 3). As expected, the total P content and the C:P ratio were highly correlated with Bray extractable P in both soils at most of the sampling times, while the extractable inorganic P was highly significant only at early stages of decomposition. Increasing amounts of inorganic P in the residues (50-73% of total P) resulted in higher levels of Bray extractable Pi during the first five days of incubation. After that initial period, it is probable that utilization of readily available inorganic P by microbial populations could have a higher influence, in comparison to the initial solubility of the plant P, in determining the soil P availability. Phosphorus immobilization by microbes has already been shown to take place after addition of carbon substrates (Chauhan et al. 1981, Lee et al.1990) and plant residues (Blair and Bolan 1978). In a tracer study, McLauhling et al. (1988b) recovered 25% of the plant residue ^{33}P in the microbial biomass after only 7 days of residue incorporation. This rapid P immobilization was attributed to the ability of the growing microorganisms to utilize

readily soluble inorganic P compounds as well as adsorbed soil P. More recent studies have shown that microbes can effectively utilize organic and inorganic P compounds adsorbed on Al precipitates present in weathered soils with variable charge (Shang et al. 1996, He and Zhu, 1998).

In addition to the amount and form of phosphorus already present in the plant, correlations analysis showed that other plant characteristics can eventually affect the soil P availability during decomposition. Highly significant positive correlations were found for acid phospho moesterase and phospho diesterase activities in both soils (Table 3). Both enzymes have been shown to participate in the hydrolysis of soil organic P compounds of plant and microbial origin (Eivazi and Tabatabai, 1977). The high correlations found suggest that differences in the ability to hydrolyze monoesters and diester organic P compounds shown by the residues could potentially affect P cycling during decomposition. However, the extent to which the enzymes contained in the residues seemed to affect P cycling depended on the soil type. In the Ultisol, the phospho monoesterase activity showed significant correlations during most of the incubation period. In the Alfisol, on the other hand, significant correlations were obtained for both enzymes only at the end of the incubation period. This variability in the influence of the enzymes contained in the residues might indicate differences in interactions of the enzymes with the soil environment, and/or some differences in mechanisms regulating P cycling in both soils. It has been shown that enzymes released by aboveground plant residues and plant roots may undergo several types of transformations in the soil. They may become stabilized by forming humus-enzyme or clay-enzyme complexes (Nannipieri et al. 1988) or they may be degradated by soil microorganisms or by native soil proteases (Dick et

al. 1983). In this study it is probable that interactions of the enzymes with clay fraction may have modified the initial enzymatic activity in the Alfisol. The fact that highly significant correlations were found only at relatively advanced stages of decomposition, especially in the Alfisol, suggests that the activity of the enzymes was more critical in regulating the hydrolysis of organic P compounds of microbial origin synthesized late in the decomposition than those compounds existing initially in the residues.

Correlations analysis also indicates that availability of carbon compounds existing in the plant residues could potentially affect P availability (Table 3). Highly significant positive and negative correlations coefficients for cellulose and dissolved organic carbon, respectively, in both soils suggest that readily available carbon in the residues might decrease the P availability. Although quite a few studies have been devoted to evaluate the relationship between decomposition and P mineralization, two different approaches have been considered. First, release and mineralization of P from OM has been considered to depend on microbial demand for P and subsequent hydrolysis by phosphatase production (McGill and Cole, 1981). In this approach, increased microbial demand for P resulting from increased consumption of C during decomposition favors the hydrolysis and release of P from the residues. Taking this idea further, recent conceptual models of plant litter degradation (Sinsabaugh and Moorhead 1994) suggest that decomposition occurs by microbial optimization of C, N and P supplies through synthesis of extracellular enzymes. In the second approach, on the other hand, hydrolysis of organic P in litter is considered to occur progressively as decomposition takes place and it is limited by the accessibility of the enzymes to the substrate rather than by the need for P in the system (Gressel et al. 1996). Both perspectives (i.e. release of P

independently of the C moitie versus release of P restricted to the sites of decomposition) predict a direct positive relationship between release of P in the litter and decomposition process. Thus, it would be expected that for plant residues differing in quality of C compounds, the one having the better quality should have a higher release of plant P, and potentially, higher P availability.

Results from our study do not support this prediction since, as mentioned earlier, a negative relationship was found between C availability and Bray extractable P (Table 3). This apparent inconsistence suggest that C compounds might have another effect, in addition to regulating the initial release of P from the residues, and this effect could significantly affect the P availability, at least at the beginning of the decomposition process. Analysis of these decomposing litters showed that most of the P contained in these residues (90%) was released during the first ten days independently of the differences in quality of the C compounds existing in the plant materials (third chapter). This seems to indicate that the effect of the quality of C compounds on P availability was not due to differences in the release of P from the residues. Instead, it is believed that quality of C compounds could affect the P immobilization and microbial turnover following the release of P from the residues. Two possible hypotheses are proposed to explain our findings. First, an increase in C availability can induce larger P immobilization by increasing the size of the microbial populations. Second, an increase in C availability could eventually induce changes in microbial community structure resulting in changes in microbial turnover. Both of these effects could potentially affect the P availability from plant residues.

The influence of plant quality factors on Bray extractable P can be more closely examined by comparing the different treatments at the end of the incubation period (Figure 1). Comparisons made between residue types at the same C:P ratio suggest that plant characteristics not directly associated with the total amount of P can affect P availability. Covariance analysis performed to compare residues types at low (330) and high (630) C:P ratio showed a significantly higher Bray P for the crotalaria residues in both soils (Table 4). Comparing crotalaria with the sorghum residue, there was an increase in Bray extractable P of 107 and 57 % at low C:P ratio, and an increase of 97 and 19 % at high C:P ratio for the Ultisol and Alfisol, respectively. The fact that this increase in available P is of the same magnitude as that attributable to changes in C:P ratio (330-630) might suggest that the influence of other plant factors affecting the P availability can become equally important as the amount of P.

3.1.2 *Phosphorus immobilization and phosphatase activity*

As previously described, correlations analysis allowed us to identify two plant characteristics that potentially affect the P availability, which are: 1) the level of enzymes, and 2) the availability of carbon compounds contained in the residues. To examine more closely how these plant characteristics could have influenced the available P and the mechanisms involved, the two residue types at low (330) and high (630) C:P ratio were used to investigate the dynamics of the soil enzymes and the pattern of P immobilization. Phosphorus immobilization estimated by microbial P is consistent with the previous hypothesis about the influence of the quality of C compounds (Figure 2). The crotalaria residue, having more resistant C

compounds (Table 2), showed significantly lower values of microbial P ($P < 0.01$) as compared with the sorghum treatment for most of the sampling times in both soils. This is consistent with higher values of resin P (Figure 2) observed for crotalaria in most of the sampling times. The lower P immobilization in the crotalaria residue also corresponded with its lower decomposition as measured by evolution of CO_2 (Figure 3). Comparing both residue types, the treatment with crotalaria showed 30 and 21 % less cumulative evolution of CO_2 at the end of the incubation in the Ultisol and Alfisol, respectively.

The fact that lower decomposition corresponded with lower P immobilization and higher P availability indicates that C compounds contained in these residues may have influenced the P cycling by affecting the microbial utilization of P. If the influence of the C compounds were exclusively due to a physical protection in the release of P from the residues, as suggested by Gressel et al. (1996), a higher P availability would be expected in the residue having a higher decomposition. In contrast, we found that an increasing demand of P by the microbes as the decomposition increased, significantly decreased available P. It is important to note, that some exceptions occurred to this observation, especially for resin extractable P before 10 days of incubation. In this case, the resin P in the treatment with sorghum was significantly higher than that of crotalaria ($P<0.01$) in both soils, probably reflecting the differences existing in extractable inorganic P between the two types of residue. When comparing any of the plant residues with the treatment cellulose+Pi, it can be seen that a significant amount of P remained resin extractable in both soils (Figure 2), indicating again that the microbial demands for P were reduced by the lower quality of the C substrate. The microbial P results for the treatment cellulose+Pi (Figure 2), supports these conclusions,

especially in the Alfisol where the microbial P was significatively less. However, the lack of contact between the C source (powder) and the Pi (solution), and the differences in soil P diffusion, may have also affected the microbial utilization of the inorganic P in this treatment. Results for the high C:P ratio showed the same trend described for the low C:P ratio treatments (data not shown), with the only difference being that resin P for both residues had consistently lower values than the control.

Microbial biomass results obtained by fumigation procedures using chloroform can be affected by several factors. Fumigation with chloroform can solubilize non-microbial P compounds, especially phospholipids contained in the plant residues, and might result in overestimation of the microbial P (McLaughlin and Alston,1985). In our experiment, microbial P analysis performed immediately after the addition of plant residues to the dry soil confirmed a solubilization of 7% of plant P (2.1 μg P g^{-1}) during fumigation with chloroform and no differences were observed between plant residues (data not shown). We estimate that this effect might be more significant at early stages of decomposition where some P might be remaining in the plant residues, but it could become negligible as the residues are decomposed. Analysis of these decomposing litters showed that most of the plant P was released in the first 10 days (third chapter). Also, since this overestimation seems to equally affect both residues, the comparisons between residue treatments would not be affected.

Other aspects to be considered in the microbial biomass estimates are the assumptions and the conversion factors utilized to calculate the microbial P from the amount of P extracted after fumigation, ie., the so-called *k* values. The k_{Pi} value representing the extractable part of

microbial biomass P is normally assumed to be a constant value, 0.40, but it can vary from 0.33-0.57 according to differences in enzymatic autolysis of the microbial cells (Hedley and Stewart 1982, Brookes et al. 1982, McLaughlin et al. 1986). Overestimations of microbial P have been shown to occur when using this average value in acid soils dominated by fungi (Joergensen et al. 1995) because of the higher extraction of P shown by fungi cells (Hedley and Stewart 1982, Eberhardt et al.1996). Assuming that fungal biomass became increased by the addition of sorghum residues in our experiment, the utilization of a higher k_{Pi} (0.57 instead of 0.40) would still give significant differences in microbial P between the two residue types. Finally, the incomplete recovery of microbial P attributed to sorption reactions of inorganic P by the soil particles (Morel et al. 1996) could also have affected the absolute results of microbial biomass in each of the soils. We speculate that the correction factors for each soil may have contributed to alleviate the problem, and the effect if any, would be more likely between soils. The relative differences between treatments within each soil would not be affected.

From previous discussions it seems that differences in P immobilization between plant residues can satisfactorily explain observed differences in P availability in both soils. In contrast, fluctuations in soil enzymatic activity during the incubation are not always consistent with the predictions derived from correlation analysis. Significant positive correlations between phospho monoesterases and phospho diesterases contained in the residues and the available soil P for both soils (Table 3), suggested an enhancement of the plant enzymes on the hydrolysis of organic P. Results of soil enzymatic activities measured during the experiment indicated that the initial effect associated with the residues can be substantially

modified depending upon the soil type. In the Ultisol, phospho monoesterase activity of the crotalaria treatment was significantly higher than sorghum in three of the six sampling times (Figure 4), following the same trend as given initially by the residues alone (Table 2). In this case, the behavior of the soil phospho monoesterase was determined by the activity of the enzyme present in the residues. In the Alfisol on the other hand, the differences in enzymes levels between the two residues existed only at the beginning and were not maintained after 15 days when there was a shift in soil phospho monoesterase activity (Figure 4). After 15 days, the treatment with sorghum had significantly higher soil monoesterase activity ($P < 0.001$) than that with crotalaria, contradicting the trend given by the residues alone (Table 2). The fact that the enzymes contained in the residues can not be directly related to the changes in available P suggests that more complex factors should be considered, such as, stabilization and disintegration of the enzymes during decomposition (Dick et al. 1983). More recently studies have shown that phosphatases, particularly phospho monoesterases, can be stabilized by adsorption on positively charged surfaces commonly existing in weathered soils (Pant and Warman, 2000). Given the higher clay content in the Alfisol, it is probable that phosphatases may become stabilized more easily in this soil.

3.1.3 *Turnover of microbial P*

In addition to the differences in P immobilization between the two residue types, differences in the dynamics of microbial P between the two types of plant residues suggest that these treatments also differ in the turnover of microbial P (Figure 2). Losses of microbial P, estimated by subtracting the value of microbial P obtained after 60 days from the respective

value obtained at the maximum of microbial P (Figure 2), suggests a slower loss of microbial P in sorghum as compared with crotalaria residue. In the Ultisol, losses of microbial P between 15 and 60 days were 3.9 ug P g^{-1} soil (13.8-9.9) with sorghum, and 6.7 ug P g^{-1} soil (9.3-2.6) for crotalaria. In the Alfisol, losses of microbial P between 30 and 60 days were 5.4 ug P g^{-1} soil (19.1-14.1) and 10.1 ug P g^{-1} soil (14.2-4.1), for the sorghum and crotalaria residues, respectively. Changes in turnover of microbial biomass after the incorporation of plant litter have been attributed to shifts in microbial community structure during decomposition (Holland and Coleman, 1987; Lundquist et al. 1999) and/or creation of soil sites with different microbial and grazing activity (Chotte et al. 1998). Differences in availability of the carbon substrates contained in the litter, their interaction with soil particles, as well as the presence of inhibitory compounds are some of the mechanisms proposed to explain differences in microbial community structure resulting during decomposition (Ladd et al.1995).

3.2 **Microbial community structure and turnover of microbial P**

To determine if the observed differences in turnover of the microbial P could be attributed to changes in community structure induced by these plant residues, fungal and bacterial biomass analysis were performed by microbial counting in the second incubation study. The bacterial abundance for the Alfisol ranged from $3.9 \times 10^8 \pm 0.4 \times 10^8$ g^{-1} soil in the control to $8.3 \times 10^8 \pm 0.4 \times 10^8$ g^{-1} soil for the treatment with crotalaria; while in the Ultisol, the abundance for these treatments ranged from $4.4 \times 10^8 \pm 0.3 \times 10^8$ g^{-1} soil to $9.0 \times 10^8 \pm 0.7 \times 10^8$ g^{-1} soil, respectively. No significant differences were found in biovolume of bacterial cells between

residue types, although biovolume did vary across soil types. The average bacterial biovolume for the treatments with plant residues was 0.58 ± 0.06 and 0.99 ± 0.09 μm^3, for the Alfisol and Ultisol, respectively; while the average values without residues were 0.29 ± 0.02 and 0.31± 0.02 μm^3, respectively.

Results of microbial biomass obtained by direct counting showed significant changes in total biomass and composition of microbial biomass in response to the different treatments evaluated (Figure 5). Incorporation of plant residues significantly increased the total microbial biomass (bacterial + fungal C) after 15 and 30 days of incubation in both soils. Comparing the two plant residues, consistently higher values of total microbial biomass were found in the sorghum residue treatment, especially after 30 days of incubation (Figures 5B and 5D), which support previous conclusions about the higher degree of decomposition in this litter treatment. Composition of microbial biomass was also very sensitive to the addition of plant residues and the effect was strongly influenced by the soil type. In the Ultisol, the utilization of crotalaria seemed to promote bacterial growth as shown by the relatively high contribution of bacteria biomass to the total biomass after 15 and 30 days (Figure 5A and 5B). In contrast in the sorghum residue, there was a significant increase in fungal biomass C, which changed the proportion of fungi from 0.15 in crotalaria, to 0.39 in the sorghum residue (Table 5). This switch in microbial composition resulting from the two residue types is also clear in the Alfisol, except that the contribution of the fungal biomass is even higher in this soil (Figure 5C and 5D). The proportion of fungal biomass after 30 days of incubation was 0.79 for the treatment with sorghum residues, while it was only 0.46 for the treatment receiving crotalaria (Table 5).

The main response shown by the residue treatments was strongly affected by the microbial composition existing in each soil. The influence of soil type is evident by the increasing contribution of fungal biomass as the soil pH decreased ($P < 0.001$). In the Ultisol, for the control with pH of 6.5, only 9% of the total biomass C at 30 days was composed of fungi, while in the Alfisol, with pH of 4.8, the fungi represented 35% of the total microbial biomass C (Table 5). The increasing proportion of fungi in acid soils has been attributed to their ability to survive low pH better than bacteria (Swift et al., 1979) and utilize available C substrates more efficiently due to their higher C assimilation (Holland and Coleman, 1987; Sakamoto and Oba, 1994). The enhanced efficiency in substrate utilization might be an advantage for fungal growth, specially in acid soils containing aluminum, iron, and manganese oxides where it has been found that stabilization reactions can significantly reduce the availability of the organic matter for the microbes (Miltner and Zech, 1998). Although characterization of oxides was not part of our study, we believe that stabilization reactions of the organic matter might have been more intense in the Alfisol given its higher clay content and exchangeable Al in this soil as compared to the Ultisol.

The correspondence between the previously described changes in microbial community structure and the changes in turnover of microbial P confirms that plant residues can affect differently the P cycling. Apparently, an increase in the fungal biomass during decomposition of sorghum residues could have decreased the turnover of the microbial P, reducing thus the soil available P. In previous studies, increasing dominance of fungi has been suggested to slow decomposition of incorporated crop residues, mainly attributed to a greater C assimilation efficiency and slower biomass turnover for fungi relative to bacteria (Holland and Coleman,

1987; Sakamoto and Oba, 1994). The influence of changes in microbial community structure have been documented for decomposition and N cycling (Franzluebbers et al. 1995; Lundquist et al. 1999; Wardle et al. 1999, Blagodatskaya and Anderson, 1999). It would also be expected to affect the P cycling since differences in turnover of microbial biomass P and hydrolysis of organic P in microbial by products might differ among decomposer groups. In addition, turnover of microbial P can also be affected by fungal immobilization of P in decomposing plant residues (third chapter). Hyphae growing inside partially decomposed plant residues might have different chemical composition and predation relationships than those growing far from the residues in the bulk soil. Although these effects might be transitory and no information is available about its influence at later stages of decomposition (longer than 60 days), it could be significant in determining the short-term P cycling of plant residues, particularly in green manures, where rapid changes in nutrient availability are expected.

Different factors have been suggested to drive changes in microbial community structure during litter decomposition. It is commonly accepted that differences in quality of the substrates existing in decomposing litter can promote succession of different saprophytic communities and changes in abundance of specific microbial groups according to their degradative abilities (Beare et al. 1992; Wardle and Lavelle 1997). Bacteria, having high growth rates and low yield of biomass per unit of substrate, can better proliferate when there is high nutrient and substrate availability, while fungi can be more competitive utilizing relatively more resistant plant material compounds (Beare et al. 1992, Lundquist et al.1999). Plant residues also might contain phenolic compounds which can inhibit the decomposer

community (Swift et al. 1979). Finally, plant residues might differ in their initial inoculum. Microorganisms already contained in the residues can rapidly colonize the residues at early stages of decomposition given the contact with the energy and nutrient sources (Tester 1988).

The factors mentioned above can partially account for the differences in microbial structure observed in our study. At early stages of decomposition of plant residues (15 days), especially in the Ultisol, a large proportion of the total microbial biomass was composed of bacteria in both residues types, and this bacterial predominance decreased at later stages of decomposition (30 days). This supports previous findings showing a succession in decomposer communities according to the availability of C substrates, with fungi becoming more abundant a later stages of decomposition. While this effect could explain differences in the sequence of utilization of different substrates, it does not account for the observed differences between the two plant residues at any sampling time. Because the higher fungal biomass in the sorghum residue occurred consistently in both soils and from early stages of decomposition (after 15 days), it is concluded that sorghum residue offered better conditions for fungal growth. At early stages of decomposition, relatively higher amounts of dissolved organic C (DOC) could have favored the proliferation of 'sugar fungi'. Increasing water-soluble C has been shown to favor some fungi populations because of their ability to withstand high osmotic pressure (Griffiths et al. 1999). Estimates of DOC, based on the rate of residues and their DOC contents (Table 2), revealed higher loading rates (2430 ugC g^{-1} soil) for sorghum as compared to crotalaria (1350 ugC g^{-1}). At later stages of decomposition (after 30 days), when the easily available substrates probably have been depleted, a

significantly higher fungal colonization was observed in the partially decomposed sorghum residues (third chapter), which may again indicate a better quality of C compounds remaining in this residue. This high quality of the sorghum is supported by its lower cellulose (33%) and lignin (4.6%) content as compared to crotalaria (47% and 7.9%, respectively). At this point it is important to note that these changes in microbial structure could have also been due to the presence of inhibitors compounds or differences in predatory relationships, both of which effects were not measured in this study. The potential influence of initial inoculum in determining differences in community structure was discarded since no differences in fungal growth were observed between the two residues when using a fungi-selective media (data not shown).

4 Summary

Different characteristics of plant residues influenced the phosphorus cycling and the short-term P availability. As expected, the characteristics associated with the total amount and solubility of phosphorus compounds contained in the residues were very influential in determining short-term P availability measured by anion exchange resin or Bray extraction. However, differences of up to 107% in available P for residues having the same C:P ratio suggested that other factors might also influence the P cycling during decomposition of plant residues. Results from correlation analysis suggested that the level of acid phosphatases and the quality of carbon substrates contained in the residues could similarly affect the response. It is believed that phosphatases can play a major role in the hydrolysis of organic P compounds derived from microbial by products rather than in the initial release of plant P.

Availability of the C compounds in the residues on the other hand, can affect the P cycling by modifying the decomposer community structure and the turnover of microbial P. A significantly higher proportion of the total biomass C composed of fungi in the sorghum residue, could satisfactorily explain the consistently slower turnover of microbial P and lower P availability shown by the sorghum residue. Stronger correspondence between the turnover of microbial P and the changes in available P observed in the plant residues, suggests that the influence of microbial activity might be relatively more significant than phosphatases in determining P cycling under these conditions. Although this effect might be transitory, our results suggest that differences in P cycling associated with residue types could significantly modify the short-term P availability and the effectiveness of the residues to enhance phosphorus supply in crop rotations. The fact that factors other than the C:P ratio can influence the P availability from residues could explain some of the inconsistencies previously reported in the literature. Further research, is needed to elucidate the role that changes in microbial structure and turnover of microbial P can play in determining P cycling and its impact on productivity of agroecosystems in the short and long-term basis.

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Table 1. Selected characteristics of the soils utilized in the incubation studies.

Soil characteristics	ULTISOL	ALFISOL
Taxonomic group	Typic Haplustult	Typic Paleudalf
Texture		
clay, %	5	12
sand, %	90	70
silt, %	5	18
pH (1:1, soil: water)	5.8	4.8
Total organic carbon, g kg^{-1}	6.5	8.7
Resin extractable Pi, μgP g^{-1}	2.2	0.5
Bray I extractable P, μgP g^{-1}	7.5	7.8
Total P, μgP g^{-1}	155	285
Sorbed P from isotherms [1], μgP g^{-1}	1.7	40
Total cation exchangeable, NH_4AcO pH 5.0, $cmol_c kg^{-1}$	3.1	5.8
Exchangeable Al in KCl, $cmol_c kg^{-1}$	0.05	0.7

[1] Sorbed P required to maintain a soil solution P concentration of 0.2 μgP ml^{-1}, obtained from isotherms according to the Fox and Kamprath (1979) method.

Table 2. Characterization of sorghum and crotalaria residues that were grown using two P rates and harvested at three different stages of growth.

Sorghum	Harvesting time	Total C	Total N	Total P	Water Extrac. Pi	C:P ratio	C:N ratio	cellulose	lignin	D O C[1]	phospho monoesterase	phospho diesterase
P rate kg P ha^{-1}	(days)	---------- mg g^{-1} plant----------			mg P g^{-1} plant			%	%	mg C g^{-1} plant	μg p-nitrophenol mg^{-1} plant h^{-1}	
27	45	430	27.8	1.30± 0.07	0.95± 0.01	332	16	33	4.6	117	15.1	5.3
27	60	450	13.1	0.71± 0.07	0.40± 0.01	637	34	31	4.5	115	11.8	3.4
27	90	450	7.5	0.45± 0.03	0.16± 0.01	1005	60	33	4.9	111	7.3	1.6
54	45	440	14.2	1.22± 0.02	0.81± 0.03	360	31	34	4.9	93	12.9	4.2
54	60	450	10.8	0.81± 0.02	0.48± 0.01	558	42	29	4.6	139	10.4	2.8
54	90	450	5.4	0.50± 0.04	0.16± 0.01	910	83	35	5.2	94	6.7	1.2
Crotalaria												
27	45	410	23.2	1.22± 0.07	0.78± 0.05	336	18	47	7.9	57	60.2	21.3
27	60	460	13.8	0.77± 0.07	0.43± 0.03	597	33	53	9.2	66	39.9	17.7
27	90	460	9.3	0.72± 0.05	0.41± 0.01	638	49	50	9.4	68	25.7	9.3
54	45	450	23.9	1.41± 0.01	0.88± 0.05	320	19	48	7.4	63	63.7	14.7
54	60	460	14.6	1.03± 0.03	0.64± 0.01	446	32	54	8.6	55	32.2	8.9
54	90	450	10.5	1.03± 0.01	0.72 ± 0.02	435	43	54	7.4	58	30.1	5.1

[1]DOC: Dissolved organic Carbon obtained by cold water extraction.

Table 3. Correlation coefficients between plant biochemical parameters and P availability measured by Bray P extraction at different sampling times in the two soils.

	Correlation coefficient Sampling time (days after incubation)					
Biochemical parameter	1	5	15	30	45	60
			ULTISOL			
C:P ratio	-0.95***	-0.92***	-0.85**	-0.67*[1]	-0.72*	-0.70*
Water extr. Pi	0.85**	0.71*	NS	NS	NS	NS
Monoesterase	NS	NS	0.79**	0.87**	0.90***	0.91***
Diesterase	NS	NS	NS	NS	0.68*	0.71*
Cellulose	NS	NS	NS	0.71*	0.75*	0.85**
DOC [2]	NS	NS	NS	-0.71*	-0.76**	-0.77**
Lignin	NS	NS	NS	NS	NS	NS
Lignin:P ratio	NS	NS	NS	NS	NS	NS
			ALFISOL			
C:P ratio	-0.70*	-0.66*	-0.78**	-0.79**	-0.75*	-0.77**
Water extr. Pi	0.91***	0.67*	NS	NS	NS	NS
Monoesterase	NS	NS	NS	NS	0.89***	0.91***
Diesterase	NS	NS	NS	0.66*	0.70*	0.71*
Cellulose	NS	NS	NS	NS	0.65*	0.75*
DOC [2]	-0.78**	-0.77**	-0.74*	-0.66*	-0.66*	-0.70*
Lignin	NS	NS	NS	NS	NS	NS
Lignin:P ratio	NS	NS	NS	NS	NS	NS

[1] Probability of significance for the correlation coefficient: *** $P \leq 0.001$, ** $0.01 < P < 0.001$, * $0.01 < P < 0.05$ and NS (no significative) $P \geq 0.05$.
[2] Dissolved organic C obtained by cold water extraction.

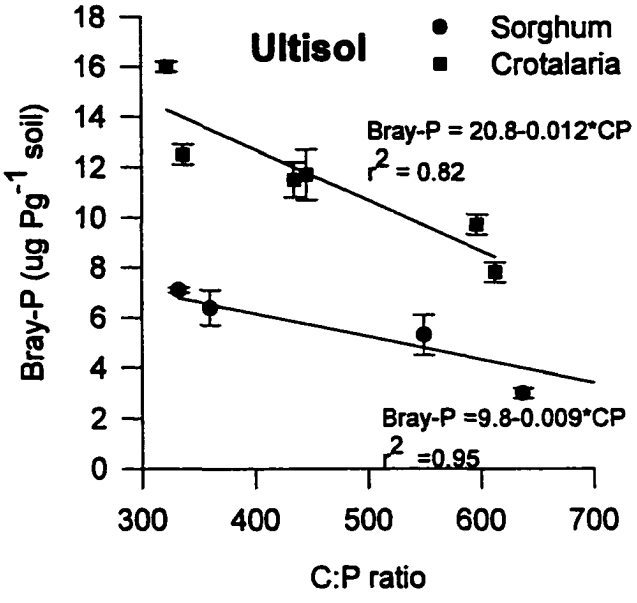
Table 4. Estimates of Bray extractable P and resin P at low (330) and high (630) C:P ratio obtained by covariance analysis after 45 days of incubation in both soils.

Covariate	Bray P		Resin P	
	C:P		C:P	
Residue type	330	660	330	660
	ULTISOL			
	(μg P g^{-1} soil)			
Crotalaria	14.1	8.1	4.7	1.2
Sorghum	6.8	4.1	2.7	1.6
Significance of difference between means at each C:P	0.0001	0.001	0.025	0.64
	ALFISOL			
Crotalaria	14.8	7.5	4.0	1.6
Sorghum	9.4	6.3	2.1	1.7
Significance of difference between means at each C:P	0.0001	0.10	0.03	0.9

Table 5. Proportion of total microbial biomass C composed by fungi for the different treatments in the two soils evaluated.

		Proportion of total microbial biomass C composed by fungi	
		days after incubation	
Soil	Treatments	15	30
Ultisol	Control	0.03 b*	0.09 c
	Cell+Pi	0.36 a	0.51 a
	Crotalaria	0.04 c	0.15 c
	Sorghum	0.28 b	0.39 b
Alfisol	Control	0.06 c	0.35 c
	Cell+Pi	0.35 b	0.72 a
	Crotalaria	0.45 b	0.46 b
	Sorghum	0.78 a	0.79 a

* Means followed by the same letter within soil and sampling time are statistically different according to the Tukey mean test (P 0.05)



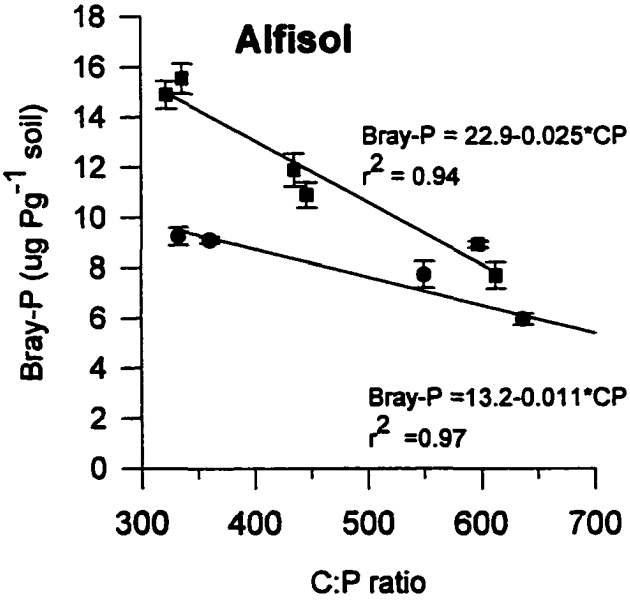


Figure 1. Effect of plant residue type and C:P ratio on the Bray-P after 45 days of incubation in the Ultisol and Alfisol.

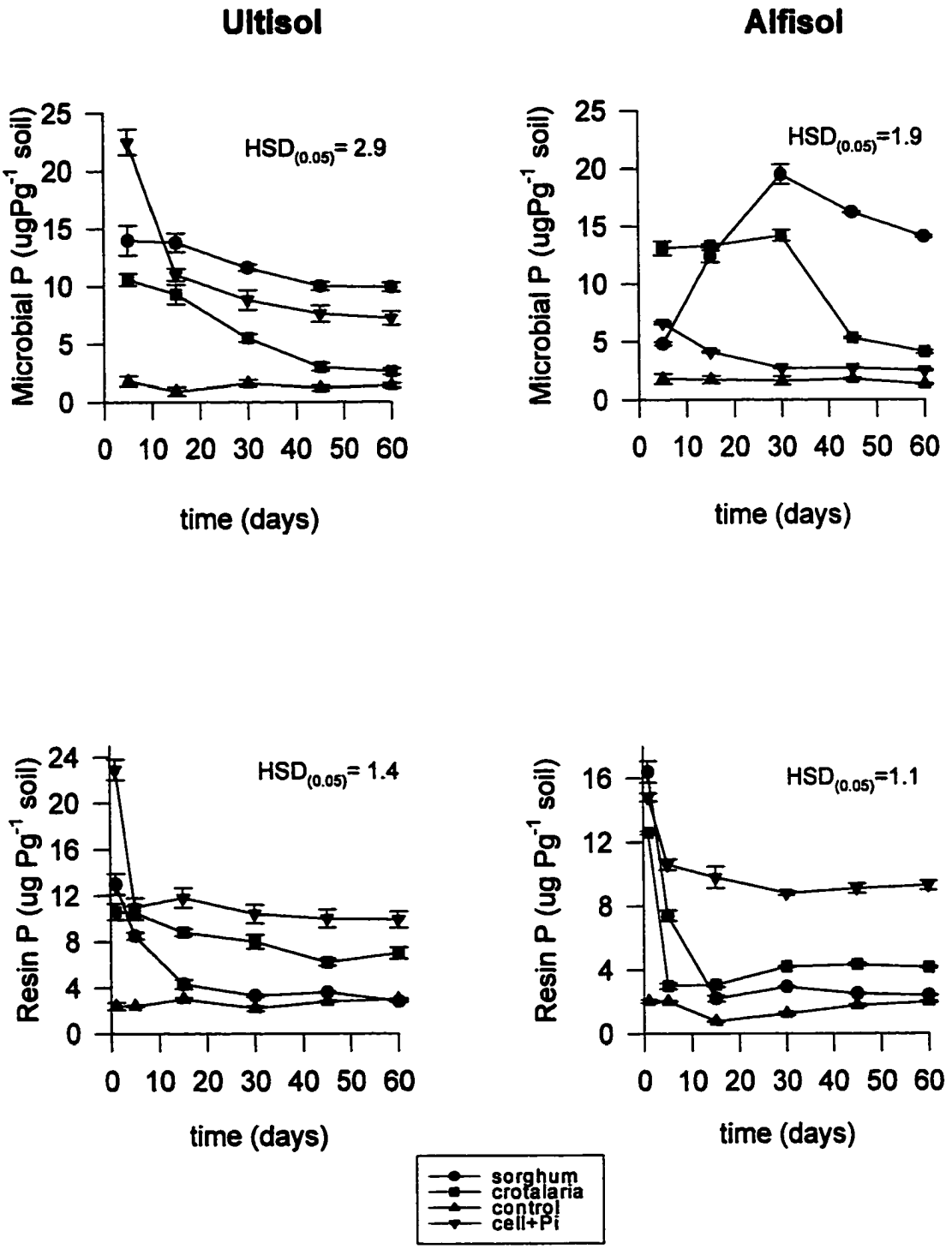


Figure 2. Changes in microbial biomass P and resin P after incorporation of plant residues and mixture of cellulose + Pi in the Ultisol and Alfisol.

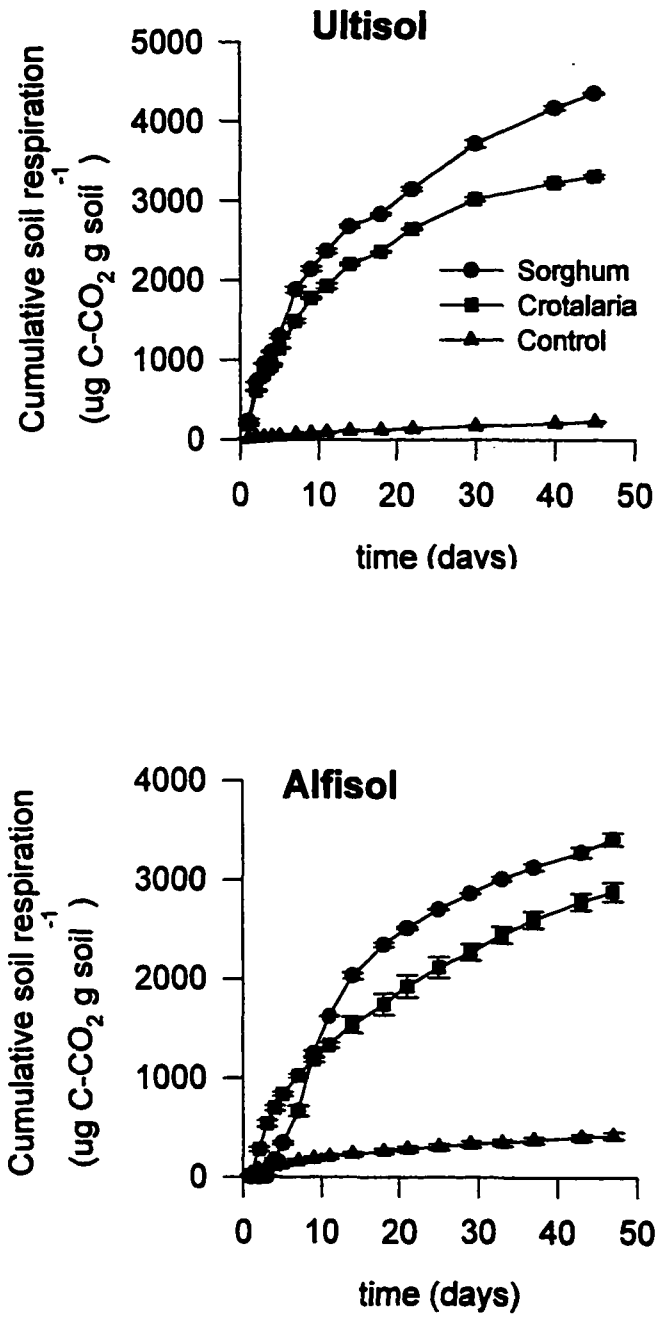


Figure 3. Cumulative respiration during decomposition of plant residues with low C:P ratio in the Ultisol and Alfisol.

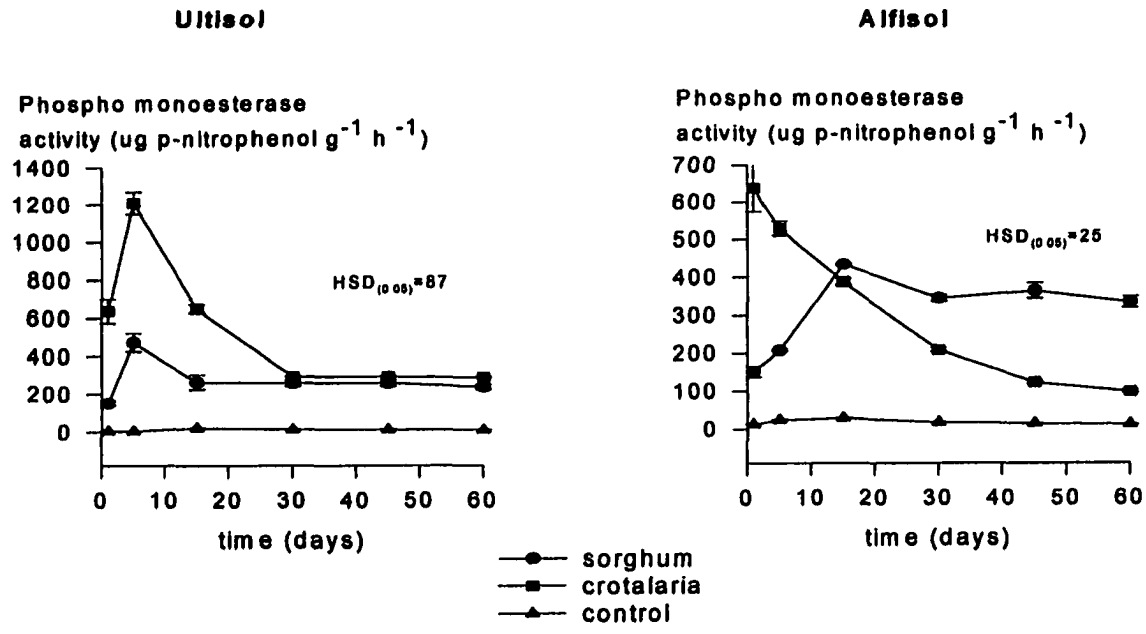


Figure 4. Soil phospho monoesterase activity during decomposition of plant residues with low C:P ratio in the Ultisol and Alfisol

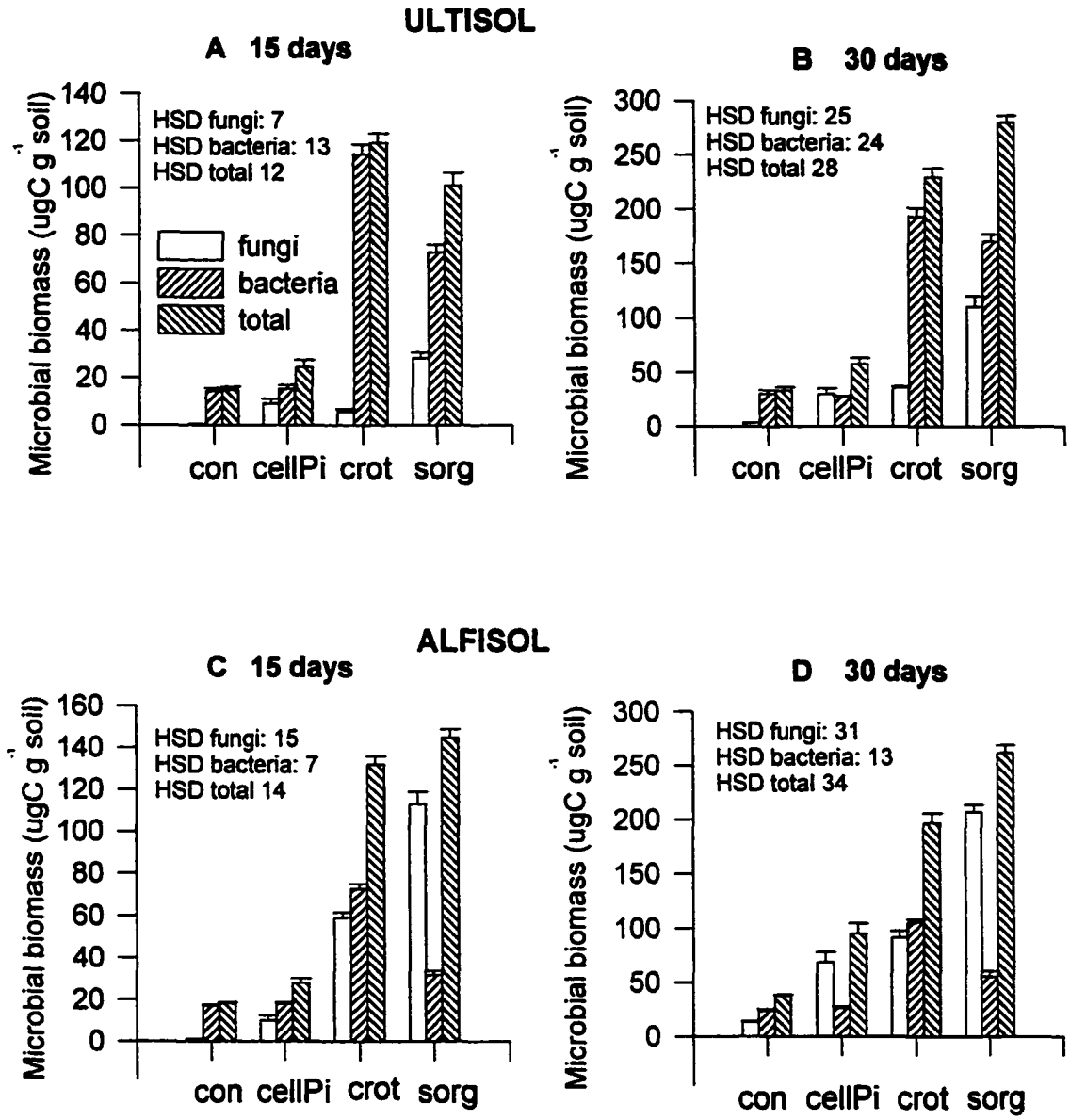


Figure 5. Microbial biomass composition as influenced by substrate additions at 15 and 30 days of incubation in the soils evaluated. Con=control, cell+Pi= cellulose + inorganic P, crot= crotalaria, sorg=sorghum.

CHAPTER III

PARTICULATE ORGANIC MATTER: ITS ROLE IN PHOSPHORUS CYCLING DURING DECOMPOSITION OF PLANT RESIDUES

1. Introduction

Incorporation of plant residues in crop rotations with either legumes or native vegetation is an option to build up nutrient availability in many agroecosystems from the tropics. The ability of plant residues to supply nutrients to subsequent crops is of special interest in the management of soil fertility in those areas given the possibility of reducing fertilizer dependency. A lot of attention has been given to the nitrogen supplying of residues to subsequent crops (Vanlauwe et al. 1998a) and efforts have been devoted to determine plant, biological and environmental factors that influence the release and mineralization of nitrogen from plant residues (Vanlauwe et al.1996, Vanlauwe et al. 1998b, Bending et al. 1998). Relatively fewer studies have been conducted to evaluate the effect of the incorporation of plant residues on the P cycle and its contribution to plant nutrition (Dalal 1979, McLaughlin et al 1988a, Umrit and Friesen 1994). Most studies have focused on the effect of plant residues on chemical reactions, particularly, their effect on P sorption characteristics and inorganic P fractions of the soil (Iyamuremye et al. 1996, Nziguheba et al. 1998). Quite a few have considered the biological transformations associated with the release of P from the

residues and the accumulation and turnover of organic P during decomposition of plant residues (McLaughlin et al 1988c). Intensification of cropping systems increases the amount of residue and litter in the top soil, resulting in accumulation of organic P which makes the availability of P less predictable by using chemical extraction methods of inorganic P. Utilization of chemical P fractionation procedures based on solubility of different P compounds might not discriminate well among organic P pools having differences in turnover. The potential contribution of the residue P and phosphorus transformations following the decomposition of residues may not be properly assessed by traditional available inorganic P soil testing (Bowman and Halvorson, 1997).

Availability of phosphorus from plant residues is affected by the initial release of P from the residues and microbial transformations occurring during decomposition of plant residues (Blair and Boland, 1978). Utilization of radioactive tracers to follow the transformation of plant residues in soils has revealed that release of P from the residues is relatively rapid (Friesen and Blair, 1988). Phosphorus immobilization by microorganisms, turnover of the microbial P and mineralization of microbial by products seem to be the major processes regulating P cycling, and P availability from plant residues (Dalal 1979, McLaughlin et al. 1988b). Recent decomposition studies using litter bags have indicated that a significant part of microbial immobilization of P takes place inside decomposing plant residues (Schomberg and Steiner 1999, Mafongoya et al. 2000), where microbial turnover and P mineralization might differ from the processes occurring outside the residues, in the bulk soil. This immobilization of P in decomposing residues increases the amount of P in the litter up to 70% when using agricultural residues (Tian et al. 1992, Somda and Powell 1998, Schomberg

and Steiner 1999), and from 20 to 200 % in tree leaves (McTiernan et al. 1997, Conn and Dighton 2000). Although the effect of microbial immobilization on nutrient availability has been better documented for nitrogen (Hart and Firestone 1993, Knowles et al.1993), some reports have suggested that also immobilization of P in residues could be responsible for a decrease in P availability from added plant residues (Schomberg and Steiner 1999, Mafongoya et al. 2000).

The role of soil fungi in the immobilization of nutrients in decaying litters has been mentioned in previous studies. Nitrogen immobilization in many different types of litter has been attributed to the translocation of the element from the soil inorganic pool by soil fungi (Hart and Firestone 1993, Bedrock et al. 1998, Frey et al. 2000). A similar mechanism has been suggested to explain the immobilization of phosphorus in decaying tree leaves (Conn and Dighton, 2000). These authors found that immobilization of P in tree litters closely followed the hyphal colonization of the litter. Although immobilization of P in residues has been suggested to occur in agroecosystems (Friesen and Blair 1988, Tian et al. 1992, Schomberg and Steiner 1999), no evidence have been presented regarding the participation of the soil fungi, and the factors influencing this process in agricultural systems. This lack of information can be partially due to difficulties in sampling the decomposed residues in agricultural systems as well as the assessment of the fungal immobilization of P in this material. The fact that agricultural plant residues are in contact with the soil limits the utilization of the resin bag approach to estimate the P accumulation in residues partially or completely incorporated into the soil. Even for residues applied at the surface, the presence of soil contaminants has been

shown to produce a high variability in the amount of P accumulated in litter bags (Schomberg and Steiner, 1999).

Particulate organic matter (POM) has been considered an active OM pool that participates in the decomposition and release of nutrients in cultivated areas (Cambardella and Elliott 1992, Magid et al.1996). It also has been used as an indicator of the portion of added plant material that remains undecomposed in the soil (Muller et al. 1998). It has advantages over the litter bag approach because the POM allows evaluation of residues that are in contact with the soil particles. Recent studies indicate that the dynamics of this macroorganic matter can significantly influence the short-term changes in P cycling after crop rotations with either legumes or natural fallow (Maroko et al. 1999 and Vanlauwe et al. 2000), suggesting that P contained in this OM pool can have a significant influence on P availability. Changes in phosphorus associated with particulate organic matter could eventually indicate the pattern of release of P from plant residues and any immobilization of P in the decomposing litters. The main objective of our study was to evaluate the changes in phosphorus associated with particulate organic matter during decomposition of plant residues and to determine their contribution to explain short-term phosphorus availability from plant residues. From the previous chapter it was concluded that residues can significantly differ in soil P availability presumably due to differences in turnover of microbial P. In this study, it is hypothesized that immobilization of P in plant residues could eventually reduce the available P from plant residues by affecting the turnover of the microbial P. To evaluate the influence of some plant factors that, in addition to the C:P ratio, could potentially influence the P immobilization in the residues, plant materials having the same C:P ratio but from different species were utilized

to follow the changes in accumulation of P in the POM. Finally, in order to establish the possible role of soil fungi in the immobilization of P, measurements of fungal colonization in decomposing residues were conducted at several stages of decomposition.

2. Materials and methods

2.1 *Soils and plant materials*

Incubation experiments were conducted using the top layer (0-15 cm) of two weathered soils representative of semiarid agricultural areas located in the central and eastern part of Venezuela. Summarizing the main soil characteristics, the Ultisol (Typic Haplustult) has 5% clay, 90% sand and 5% silt, pH (1:1 soil water suspension)= 5.8, resin extractable Pi= 2.2 μg P g^{-1} and Bray I extractable P= 7.5 μg P g^{-1} and total P= 255 μg P g^{-1}. The Alfisol (Typic Paleudalf) has the following characteristics:12% clay , 70% sand and 18% silt, pH (1:1 soil water suspension)= 4.8, total organic C= 8.7 g kg^{-1}, resin extractable Pi= 0.5 μg P g^{-1}, Bray I extractable P= 7.8 μg P g^{-1} and total P 185= μg P g^{-1}. The Ultisol, considered low P fixing soil by means of P isotherms, requires an amount of sorbed P of only 1.7 μg P g^{-1} to maintain a P concentration in solution of 0.2 μg P ml^{-1}, while the Alfisol, with medium P fixing capacity requires an amount of sorbed P of 40 μg P g^{-1} to reach the same level of P in solution.

Plant residues of sorghum (*Sorghum bicolor* (L) Moench) and crotalaria (*Crotalaria juncea),* having relatively high quality, were used to conduct decomposition studies under controlled

conditions. The main characteristics of the sorghum residue are: C:N ratio 16, total P =1.31 mg P g^{-1} plant, extractable Pi= 0.95 mg P g^{-1} plant, C:P ratio 332, cellulose 33%, lignin 4.6% and dissolved organic C= 117 mg C g^{-1} plant. The crotalaria residue has a C:N ratio 18, total P= 1.23 mg P g^{-1} plant, plant extractable Pi= 0.78 mg P g^{-1} plant, C:P ratio 336, cellulose 47%, lignin 7.9% and dissolved organic C= 57 mg C g^{-1} plant. Plant materials were air dried and cut in 1-cm pieces previous to being gently ground (< 1000 μm) using a Wiley grinder. The percentage of material retained between the 500 and 1000 μm sieve was 85 and 90% for the sorghum and crotalaria residues, respectively. Detailed characteristics of the soils and plant materials as well as the methods used for their characterization can be found in the second chapter of this dissertation.

2.2 *Preliminary experiments*

Separation of Particulate Organic Matter for P analysis

A series of preliminary experiments were conducted to evaluate different methods to separate the particle organic matter (POM) for phosphorus analysis. Conventional methods for obtaining the POM are based on the utilization of sodium hexametaphosphate (Na-HMP) for dispersion of the aggregates (Cambardella and Elliot, 1992), which make them not suitable for use in P analysis. Alternative methods include the utilization of distilled water (Maroko et al. 1999), Na exchange resins (Dalal and Mayer 1986) and Na_2CO_3 solution (Vanlauwe et al. 1998b). Two aspects limit the utilization of these methods for studies of POM-P during decomposition of plant residues. The first one is the potential solubilization of organic P

compounds contained in the POM when using alkaline solutions (Na_2CO_3); and secondly; the incomplete recovery of the POM due to its allocation in non dispersed micro and macro soil aggregates which cannot be separated from the sand by flotation procedures.

Preliminary tests indicated that NaCl solutions could effectively disperse the soil aggregates with no solubilization of the organic matter. Given this behavior and the possibility to be easily implemented in the laboratory, this solution was chosen for further comparisons with the HMP. In order to select the best conditions for the soil dispersion, several concentrations of NaCl were compared in terms of their ability to disperse the aggregates in the two soils evaluated. Briefly, for each soil (sieved < 2mm), four 25 g- sets of dry soil were weighed in triplicate into 50 ml-plastic containers. Thirty ml of NaCl solution with increasing concentrations (0.001, 0.01, 0.05 and 0.1 M) were added for soil dispersion. The soil suspension was shaken for 2 hs in a circular shaker at 150 rpm, after which it was passed through a 53 μm sieve and rinsed with distilled water until a clear solution was obtained. The material remaining in the sieve was dried at 100 °C and weighed, and the percentage of material in the sand fraction (>53 μm) was calculated. The degree of dispersion of the NaCl solutions was assessed by comparisons with the dispersion obtained using 0.5 % Na hexa metaphosphate (Na-HMP) for 18 hs at the same soil:solution ratio. Any increase in the percentage of soil in the sand fraction, as compared with the values obtained using the Na-HMP, was used as a indicator of an incomplete dispersion of the soil aggregates.

After selecting the most effective concentration of NaCl based on its ability to completely disperse the soils, the effect of this solution on the recovery of C from added plant residues

was evaluated for both soils. Sorghum and crotalaria air-dried residues were mixed in triplicate with 20 g of dry soil at a rate equivalent to 2 % wt. basis. This mixture of soil and plant was immediately used for soil dispersion with 0.05M NaCl following the same procedure as described above. Controls for each soil and plant residue were also prepared for dispersion with Na-HMP. The sand fraction and the POM obtained after sieving was transferred into a pre-weighed aluminum pan to be dried at 60 °C for 48 hs and the dried weight recorded. This fraction was further finely grounded by hand using a porcelain mortar and pestle, and samples were taken for total C analysis using a LECO CHN-1000 analyzer. The recovery of plant C in the POM was calculated for both soils based on the C concentration and the weight of the sand fraction.

To further investigate the effect of the POM separation procedure on the amount of phosphorus recovered from plant residues, a short-term incubation was conducted using both plant residues and the Alfisol. For each plant residue, two sets of six experimental units were prepared by mixing samples of 40 g of dry soil with the required amount of plant material to incorporate an equivalent rate of 2% wt. basis. Distilled water was uniformly added into the soil by mean of plastic syringe to bring the soil up to 65% of the water holding capacity. One set of six experimental units was immediately sampled to evaluate the initial recovery of plant P in the POM, and the remaining six units were placed in Mason jars to be incubated at 25 °C for 30 days. At the sampling time, 20g of moist soil were dispersed with NaCl as previously described, and the sand fraction containing the POM was separated by using a 53 μm sieve and rinsing the material with distilled water until the solution gets clear. Moist soil samples were taken from the incubations for gravimetric estimates of soil water moisture.

The remaining moist soil after 30 days of incubations was used for characterization of fungal colonization in the POM.

The separation of POM from sand was further investigated in three of the experimental units by flotation using Na polytungstate (Na-PT, specific gravity = 1.85 g cm^{-3}) following the basic procedure as described by Cambardella and Elliot (1992). Since no information was available about the effect of the Na-PT on the recovery of P in the POM, flotation with water was also included as a reference. Thus, half of the samples (three) was used for water flotation and the other half was used for flotation with Na-PT. Flotation of the POM was done by carefully transferring the material remaining in the sieve to 50ml graduated conical centrifuge tubes containing 35 ml of either Na-PT or distilled water. The suspended material was mixed by slow reciprocal shaking by hand (10 strokes) and slowly placing the centrifuge tube in vertical position. Following centrifugation at 2500 rpm for 45 minutes, the material was aspirated onto a 20-μm mesh nylon filter, rinsed throughly with distilled water to remove the excess of Na-PT and then transferred to a pre weighed Al pan to be dried at 60 °C for 24 hs. The dried material was weighed and ground by hand with a porcelain mortar and pestle. A subsample of 250 mg of the POM was used for nitric perchloric digestion and P analysis using the colorimetric method (Murphy and Riley, 1962). The amount of P in the POM (POM-P) was calculated for the different plant treatments, methods of POM flotation and sampling times. The P recovery of plant P was thus estimated at the beginning of the experiment by relating the POM-P at time zero to the total P added as plant residues.

Assessing fungal colonization of the Particulate Organic Matter

In order to estimate the fungal colonization of the POM, preliminary experiments were conducted to evaluate the optimal conditions for staining fungi in the POM as well as the sampling procedure. Previously incubated soil samples from the C recovery experiment were utilized to obtain POM according to the procedure described above using Na-PT. After flotation and separation of the POM, the fresh material retained on the nylon filter was transferred to an Al pan and a subsample was used to do preliminary tests for staining the fungi using trypan blue. The staining procedure was basically the same as that described by Phillips and Hayman (1970), developed to assess the parasitic and vesicular-arbuscular mycorrhizal infection in roots. Given that the method was originally developed for roots, and that the optimal concentration of the base as well as the duration of the clearing pretreatment can vary greatly according to the plant issue (Brundrett et al. 1996); a lower concentration of KOH (2.5% instead of 10%) was used. Several time periods for the clearing pretreatment (5, 10, 15, 30 and 60 minutes) at 90 °C were tested. Sets of twenty POM pieces of sorghum cleared at different times were carefully washed with distilled water and immersed in 0.1 M HCl for 1 h. After this period, the POM pieces were stained with trypan blue (0.05%) in lactophenol at 60 °C for 15 min. The stained POM samples were then placed in destaining solution to remove the excess of trypan blue and were transferred to microscope slides for observation under the microscope at a 50X magnification. Images of the microscopy field were captured using the Optronics cooled CCD camera (Model DEI-470) and Adobe Photoshop image capturing software. Visible hyphal lengths and POM area were measured in each POM piece by using the IPLab Spectrum image analysis software. Fungal

colonization in the POM was calculated by dividing the total fungal length by the POM area. Average area of the POM without any treatment was also recorded by observations of thirty POM pieces under the microscope. The optimum time of clearing was selected by comparing the fungal colonization and POM area among the different treatments. The treatment showing the least effect on the POM area and fungal colonization, as well as the best resolution, was selected.

The optimal sample size for fungal colonization was estimated by statistical procedures considering the variability in the POM measurements obtained for both plant residues after 30 days of incubation. To estimate the variability in fungal colonization, POM samples were obtained from the C recovery experiment using the Alfisol. Thirty POM pieces were stained per plant residue using the trypan blue procedure and the clearing pretreatment with KOH for 10 minutes. Total visible hyphal length, average hyphal width and area were measured for each POM piece as described above. The variability in the POM weight was also assessed by weighing five sets of 30 POM pieces (dried at 60 °C) using a 5-digits balance. Fungal colonization of the POM in both residues was calculated by dividing the hyphal length by the POM area. Results were used for calculation of the standard deviation for both plant residues.

2.3 *Main experiments*

2.3.1 *Phosphorus dynamics in Particulate Organic Matter*

To evaluate the dynamics of phosphorus in the particulate organic matter, decomposition studies were conducted using crotalaria and sorghum residues in two soils. For each soil,

experimental units were prepared by mixing 40 g of air-dried soil (sieved at 4mm) with each of the two plant materials incorporated at a rate of 2% (w/w). Controls without plant residues were prepared for the Ultisol and the Alfisol. Triplicates of the different treatments were prepared and a total of six sampling times were used: 0, 5, 15, 30, 45 and 60 days of incubation. Hoagland's nutrient solution containing macro- and micronutrients (except P) was uniformly applied by means of a syringe in order to bring the soil moisture to 65% of the water holding capacity; or 15 and 20 % w/w, for the Ultisol and Alfisol, respectively. According to the rate of plant material and the P content of the residues, the total amount of P added was 24.6 and 26.0 μg P g^{-1} soil, for the crotalaria and sorghum residues. Treatments and their replicates were randomly assigned within each sampling time and experimental units were destructively sampled at each sampling.

At each sampling time, soil samples were taken for separation and analysis of POM. As previously described in the preliminary experiments, moist soil samples were dispersed and POM was separated by flotation using Na-PT. Moist soil samples from the incubations were also taken for determining the soil moisture content. After drying the POM at 55 °C, its weight was recorded and the material was finely grounded by hand in a mortar and pestle for element analysis. Subsamples were taken for determination of C and N concentrations by dry combustion using a CHN-1000 analyzer (Leco Corp.). P concentration was determined using the colorimetric method (Murphy and Riley, 1962) after nitric-perchloric digestion of POM subsamples. The amount of C, N and P contained in the POM (POM-C, POM-N and POM-P) per unit of soil (or POM) weight was determined for both plant residue treatments and all sampling times.

2.3.2 *Fungal colonization of the Particulate Organic Matter*

To evaluate the role of fungi in the immobilization of phosphorus in decomposing plant residues, fungal colonization in the POM was measured at different stages of decomposition of crotalaria and sorghum residues. Irradiated crotalaria and sorghum residues were used in addition to the non irradiated plant residues to establish the contribution of the plant inoculum to the fungal colonization of the plant residues. The irradiation treatment was done by exposing the plant materials to *gamma* radiation (2.5 M rad) during 16 hrs. The experimental procedure was similar to that previously described for the POM-P experiment, except that only the Alfisol was used for the experiment. Five treatments resulted from two residue types, two irradiation treatments and the control (without residue). Sampling to obtain the POM was done after 0, 5, 15 30 and 45 days of incubation. The separation of the POM from the soil as well as its preparation for fungal analysis was performed as indicated in the preliminary experiments. Based on results of the sampling tests, twenty POM pieces were utilized to measure the POM area as well as the hyphal length and average hyphal width after staining with trypan blue. Fungal colonization was calculated by dividing the hyphal length by the POM area. The POM remaining was dried at 60 °C and its weight recorded. Fungal colonization on POM weight basis was estimated by taking into account the average weight of twenty (dried) POM pieces and their total area. Similarly, estimates of fungal colonization on soil weight basis were obtained, considering the weights of the total POM and the dried soil. Finally fungal biomass C in the POM was estimated by multiplying fungal biovolume by the specific C content of the fungi (0.132 g C cm^{-3}), as suggested by Bloem et

al. (1995). Previous results indicated that variability in the weight of 20 POM pieces was relatively small, with coefficient of variations that were less than 20 % for all sampling times.

Statistical analysis

For the preliminary experiments the data were analyzed as a completely randomized design using a one-way ANOVA. Pair-wise mean comparisons were performed by using the Tukey's Honestly Significant Difference test and paired-t test ($P < 0.05$). Estimates of sample size required to have a 95% confidence interval for the difference between means of fungal colonization was made by using pooled values of standard deviations and power calculations computed for two sample t-tests. Regression analysis were also performed to evaluate any possible interdependency of fungal colonization on POM area. For the main experiments data were analyzed as a completely randomized two factorial design with interaction. Within soil, treatments and sampling times were considered the factors. Data of fungal colonization as well as fungal biomass in POM were log transformed prior to analysis to meet normality and homogeneity of variance requirements for ANOVA. Mean comparisons were performed at each sampling time using the Tukey's Honestly Significant Difference ($P < 0.05$). All the analysis were done using the GLM and REG procedure of the SAS program (SAS, 1990).

3. Results and discussion

3.1 *Separation of Particulate Organic Matter for P analysis*

Solutions of different concentrations of NaCl were evaluated for their ability to disperse the soil. Results indicate that the percentage of soil present in the sand fraction of the Alfisol significantly increased as the concentration of the NaCl solution changed from 0.1 to 0.001 M (Table 1). Comparing this percentage for the NaCl solutions with the value given for Na-HMP indicates that the utilization of concentrations of 0.01 M and 0.001M significantly reduced the dispersion of the soil which potentially could affect the POM recovery. In the Ultisol on the other hand, with a low amount of clay (5%), the soil dispersion was not affected by the concentration of NaCl, and the results are comparable to that obtained using the Na-HMP. A NaCl concentration of 0.05 M seemed to be adequate for soil dispersion in both soils. Results showing the same recovery of plant C in the POM using either NaCl 0.05 M or Na-HMP (Table 2), also confirm the potential use of the former solution to recover the POM from these soils. The ability of the NaCl (0.05 M) solution to completely disperse the soil was not affected by the presence of decomposing plant residues since the percentage of sand fraction after 30 days of incubation using plant residues, was close to the value obtained without residues, for both soils (data not shown).

Recovery of plant P in the POM was evaluated after separation of the POM from the sand fraction using flotation procedures. Because the flotation procedure might potentially affect the recovery of P in POM, the Na polytungstate (Na PT) was compared with water flotation

in the Alfisol. Results indicated a recovery of 35 and 27 % of the initial P contained in the crotalaria and sorghum residues, respectively, after flotation with water (Table 3). This low recovery is closely related to the high proportion of inorganic P present in these residues, 73 and 63 % of total P, for sorghum and crotalaria residues, respectively. The recovery of the added plant P in the POM was also affected by the flotation procedure (Table 3). Utilization of Na PT significantly reduced the amount of plant P recovered in the POM at the beginning (0 days) by 14 and 16 %. It is probable that the higher osmotic potential in the Na PT could have modified the extractability of organic P compounds existing in the plant residues. In spite of the initial lower recovery of POM-P obtained with the Na PT, this solution was selected for further experiments because of its higher recovery of POM-P at later stages of decomposition of plant residues (Table 3). Water flotation showed significantly lower amounts of POM-P after 30 days of decomposition in both plant residues. Observations indicated a significant amount of non-floated POM remaining in the sand fraction of this treatment after 30 days of incubation which suggests that changes in density of the partially decomposed residues may affect its flotation in water.

3.2 *Assessing fungal colonization of the Particulate Organic Matter*

Particulate organic matter separated from the sand fraction was utilized to examine the fungal colonization of partially decomposed plant residues. A modification of the trypan blue staining procedure developed for studies of mycorrhizal infection of roots was evaluated. Basically, given the small size of the POM (average 1.50 mm^2) and its higher susceptibility to weight losses during the digestion as compared to roots, lower concentration of alkali

(2.5% instead of 10 %) was used for the clearing pretreatment and several times of digestion were tested. Results indicate a significant reduction in the POM area as the time of digestion was increased (Table 4). The POM area, expressed as percentage of the POM area without pretreatment was 97, 91, 69, 41 and 27% for 5, 10, 15, 30 and 60 minutes of digestion, respectively. Although 5 minutes of digestion did not change the area significantly, it did affect the clearing procedure of the POM, showing that this was not enough time to remove the plant pigments. The time of pretreatment also affected the measurement of fungal colonization of the POM. Digestion treatment of 30 minutes reduced the fungal colonization in 30 %. Based on the previous results, digestion with KOH (2.5% w/w) during 10 min was selected as clearing pretreatment for staining the fungi in POM. At this point it is important to note that sieving and flotation can also remove part of the hyphae growing outside of the POM. For instance, pieces of POM taken directly from the soil, without either sieving or flotation, had 35 to 50 % more hyphae growing in the POM surface (data not shown).

Microscopic observations of the fungi colonizing the POM showed some differences compared with the hyphae growing in the soil for the same treatments. The average hyphal width was higher in the POM than in the soil (Figure 1, Table 5). In the POM, the average hyphal width was 8.23 and 2.64 μm; while in the soil, the values were 2.07 and 1.99 μm for the sorghum and crotalaria treatments, respectively. This represents an increase in fungal width of 4 and 1.2 times for the hyphae growing inside of the decomposing sorghum and crotalaria residues, respectively. The results of the hyphal length on the other hand, revealed that the contribution of the fungal length in the POM is small compared with the values given in the bulk soil for both plant residues. However, considering the influence of the width in

the fungal biovolume, we estimate that the contribution of the fungal biovolume, and thus the fungal biomass, located in the POM might be significant, particularly for the treatment with sorghum residues. Based on the information given in the Table 5 for this residue, it is estimated that 1 cm of hyphae in the POM has a biovolume of $5.3 * 10^{-7}$ cm^3 while the same hyphal length in the bulk soil has a biovolume 15 times smaller ($3.4 * 10^{-8}$ cm^3).

In addition to the morphological characteristics of the hyphae growing inside the POM, the preliminary experiment was also used to evaluate the conditions for making estimates of fungal colonization in the POM. These estimates of fungal colonization will be used for biovolume and fungal biomass calculations in the POM. The sample size for fungal colonization was determined by power analysis by finding the appropriate *n* for a 95% confidence interval for the difference between the means of crotalaria and sorghum residues. Results indicate that in order to have a confidence interval narrower than 2 mm mm^{-2}, it is necessary to quantify the fungal colonization in a least 20 pieces of POM per residue treatment. Variability in the POM area did not affect the fungal colonization since no relationship was found between area and fungal colonization measurements ($r^2 = 0.018$, n= 30) after 30 days of incubation. It is probable that the small POM area (1.39 ± 0.35 mm^2 $piece^{-1}$) and its relatively low coefficient of variation (25 %) contributed to explain this lack of relationship. In larger POM particle sizes, as expected under field conditions, appropriate considerations should be taken of the influence of the POM area on the fungal colonization measurements.

3.3 *Phosphorus dynamics in Particulate Organic Matter*

Phosphorus contained in POM (POM-P) changed significantly during the decomposition process. After five days of incubation a significant decline in POM-P suggests a rapid release of P from the plant residues in both soils (Table 6). In the Ultisol, 92 and 95 % of the P initially contained in the POM was released from the sorghum and crotalaria residues, respectively. In the Alfisol, 75 and 77 % of the P contained in the POM was released during the same period. These results are partially explained by a large proportion of total P in inorganic forms (73 and 63 % of the total P for sorghum and crotalaria residues, respectively) in those plant residues. To some extent the procedure for obtaining the POM could also have contributed to facilitating the release of P from residues, although this effect was previously found to be only 15 %.

Previous studies have indicated a rapid release of P from the residues at early stages of decomposition. The presence of more than 50% of the residue-derived P in the inorganic pools after 11 days of the residue incorporation supports these findings (Friesen and Blair, 1988). McLaughlin et al. (1988c) found that 50 % of the ^{33}P applied as medic residues was extractable from the soil with $NaHCO_3$ (pH 8.5) at the beginning of the experiment. They also estimated the amount of P remaining in the residues from extraction of ^{33}P with 0.5 M H_2SO_4 in ignited and non-ignited soil samples. Their data indicated that only 4% of the ^{33}P initially added remained in the residues after 15 days of incorporation. They attributed the rapid release of P from the residues to the high proportion of inorganic P initially present in the residues and the rapid microbial utilization of Pi at early stages of decomposition. Our

results differ however, from those obtained by Dalal (1979) who found that mineralization of P from plant residues was relatively slow and closely followed the C mineralization, both of which processes were described by first-order kinetics reactions. While in that study only 37 % of the initially added plant P was recovered in a subsequent crop after 10 weeks of incorporation, in our study, most of the plant P was released in the first week. It is probable that mineralization of P in plant residues measured by recovery of ^{32}P in subsequent crops, as used by Dalal (1979), is controlled by the turnover of microbial P and subsequent mineralization of microbial by products rather than by the initial release of plant P, which seems to be relatively rapid. Our results support this prediction since estimates of loss rates of POM-P at the beginning of the decomposition (1.1 μg P g^{-1} soil d^{-1}, based on Table 6), were significantly higher than estimates of loss rates of microbial P for the same treatments (0.06 - 0.16 μg P g^{-1} soil d^{-1}, from second chapter), or P mineralization rates (0.02 μg P g^{-1} soil d^{-1}) found for plant residues (White and Ayoub 1983).

After the initial rapid release of P from the residues, an increasing accumulation of P in the POM was observed from 5 to 60 days of incubation in both soils and plant residues (Figure 2). This accumulation of P in the POM strongly depended on the plant residue ($P< 0.001$) in both soils. In the Ultisol, the POM-P (in soil basis) increased from 0.46 to 5.8 μg P g $soil^{-1}$ with the sorghum residue; while for crotalaria, the value changed from 0.36 to 2.8 μg P g $soil^{-1}$. For the same period of time in the Alfisol, the POM-P changed from 1.54 to 7.90 μg P g $soil^{-1}$ and from 1.83 to 1.90 μg P g $soil^{-1}$ in sorghum and crotalaria residues, respectively (Table 6). Thus, the amount of POM-P increased about 13 and 5 times in the sorghum residue, for the Ultisol and Alfisol; respectively, while it increased 8 times using the crotalaria

in the Ultisol. This increase in the amount of POM-P closely followed changes in the P concentration of the POM (Table 6). Phosphorus concentrations in sorghum residue POM was 15 (Ultisol) and 7 times higher (Alfisol) at the end of the experiment compared with the respective values given at 5 days of incubation. It should be noted that particularly for this residue, the amount of P as well as the P concentration of the POM at the end of the experiment were equal or higher than the values found in the POM at the beginning of the experiment (time 0).

This increasing accumulation of P in POM coincided with an increase in the amount of N in the POM (POM-N) after five days of incubation for most of the treatments (Table 7). At the end of the incubation, a significantly higher POM-N was found in sorghum as compared with the crotalaria, although both residues had closely the same values at the beginning of the experiment (time 0). In our experiment it was not possible to differentiate the N that was originally in the POM from that which was immobilized in the POM during decomposition. However, data showed a significant amount of N (109 and 81 %, for the Ultisol and Alfisol, respectively) remaining in the sorghum POM at the end of the experiment, in spite of the C losses (54 and 49%) , suggesting that immobilization of N probably occurred in this residue (Table 7). In crotalaria POM by contrast, there was a significantly lower POM-N (60 and 37 % of the initial POM-N, for the Ultisol and Alfisol, respectively). This seems to support the observation of an increased accumulation of P in the sorghum POM. Increasing accumulation of P in the POM led us to hypothesize that an immobilization of P in the litter might occur as microorganisms start utilizing recalcitrant C sources remaining in the decomposing litters, and the microbial demands for P are increased. If this hypothesis is

shown to be valid, it would be expected that differences in the amount of P accumulated in the POM could be explained by differences in the demands for P of the microbial communities colonizing the decomposing litters. To test this hypothesis in the last experiment, fungal colonization of the POM was measured during decomposition of both residues in the Alfisol.

3.4 *Fungal colonization of Particulate Organic Matter*

Our results in the Alfisol showed that fungal colonization of the decomposing litter was strongly influenced by the residue type ($P < 0.001$). From early stages of decomposition, sorghum residues had consistently higher levels of fungal colonization as measured by fungal length per unit of POM weight (Table 8). Because of the greater fungal length and the larger width of the hyphae colonizing the sorghum residue (Table 5), the fungal biomass in this litter was significantly higher than that obtained in the crotalaria residues (Table 8). The peak of fungal biomass occurred at 30 days of incubation in which 35.3 and 0.19 μg C g soil^{-1} were found for sorghum and crotalaria POM, respectively. The sequence of fungal colonization presented in Table 8 agrees with previous results indicating that fungi can colonize plant residues at later stages of decomposition given its competitive ability to utilize more resistant C compounds (Beare et al. 1992, Wardle and Lavelle 1997). These results of fungal colonization in the POM are in the range previously described for decomposing litters (30-200 m g^{-1}) using the litter bag approach (Conn and Dighton, 2000). A comparison of fungal colonization in POM at 15 and 30 days of incubation for the sorghum residue is shown in Figure 3. It is important to note, that this fungal colonization could have been underestimated to some extent, since fine hyphae (less than 2.0 μm width) can not be seen under a

magnification of 50X. Close examination of the POM at higher magnification (200 X) revealed the existence of fine hyphae (width approx. 1.5 μm) growing especially at the edges of the POM. Although the contribution of these hyphae was not assessed in this study, we speculate, based on the influence of width in the biovolume calculations, that their biomass was probably relatively small.

The origin of the fungi colonizing the litters was further investigated by the utilization of irradiated plant residues. In the sorghum residue, results indicate that the irradiated residue had significantly lower fungal colonization than non-irradiated residues at the beginning of the experiment (5 and 15 days of incubation), but after that initial period, no differences were found between the irradiation treatments (Table 8). This indicates that a fraction of the initial fungal colonization in the sorghum residue can be attributed to the fungi inoculum present in the residue which may have conferred some advantages to early colonization of this residue. After that initial period however, the soil fungi became the dominant group in the litter. In the crotalaria residue on the other hand, the irradiation treatment did not affect the fungal colonization in the POM which suggests a low initial inoculum in this residue.

A strong correspondence between the accumulation of P in POM (Table 6) and the previously described pattern of fungal colonization (Table 8) suggests that immobilization of P in these partially decomposed plant residues might be mediated by soil fungi. Immobilization of phosphorus and many other nutrients in decomposing plant residues has been largely suggested as a mechanism to facilitate the establishment of microbial communities and the decomposition of litters in many forests and agroecosystems (Melillo and Aber 1984).

Evidences have shown that this microbial translocation of nutrients from the mineral pool to the litter can be responsible for immobilization of significant amounts of nitrogen (Hart and Firestone 1993, Knowles et al.1993, Frey et al. 2000), phosphorus (Tian et al. 1992, Schomberg and Steiner, 1999), and calcium and magnesium (McTiernan et al. 1997) in the litter. The possible role of the soil fungi in this immobilization has been attributed to its ability to colonize more recalcitrant and nutrient-poor substrates remaining during decomposition given its efficient utilization of C substrates and nutrients (Paustian and Schnürer 1987, Sakamoto and Oba 1994) and ability to translocate nutrients from spatially-separated sources. Identification of N compounds of fungal origin in decomposing wheat straw by ^{15}N CPMAS (Bedrock et al. 1998) as well as experiments of fungi-exclusion using ^{15}N (Frey et al. 2000) have confirmed the participation of fungi in the mobilization of nutrients, particularly nitrogen, into the decomposing plant residues.

Immobilization of phosphorus as predicted by increasing accumulation of P in decomposing litters has been reported for several forestry and agricultural plant species (Tian et al.1992, Schomberg and Steiner 1999, Conn and Dighton 2000). Although transitional and dependent on seasonal and faunal variations, this apparent microbial immobilization has shown to increase the amount of P in the litter up to 70% in agricultural residues (Tian et al.1992, Somda and Powell 1998, Schomberg and Steiner 1999) and from 20 to 200 % in forestry litters (McTiernan et al. 1997, Conn and Dighton 2000). This P immobilization could be even higher considering that most of these studies were conducted using litter bags placed above-ground where the colonization of the residues by the soil microbes could have been reduced.

Our study thus confirms previous reports suggesting microbial immobilization of phosphorus in plant residues and highlight the possible role of fungi in this process. As plant residues become rapidly depleted in P, it is probable that fungi colonizing more recalcitrant substrates take up P from the soil surrounding the residues. Because no other source of P was used with the plant residues, it is probable that the soil environment surrounding the decomposing residues had a higher level of available P that could be easily utilized by fungi growing in the litter. Considering that the fungal colonization was strongly affected by the residue type, it would be expected that the microbial demands for P, and thus the P immobilization in the litter, could also be modified by the residue type. Our results confirm this prediction, since a consistently higher accumulation of P was found in the sorghum residue in which there was a significantly higher fungal colonization. The participation of abiotic reactions cannot be discounted in this study, but we speculate that their contribution if any, would be probably relatively small, given the low accumulation of P in the residue having low level of colonization. We also estimate that any inorganic P moving by diffusion inside the residue would probably be removed during the POM separation procedure. A difference of nitrogen, abiotic immobilization of P, that is, incorporation of inorganic P in organic compounds by chemical reactions, has not been documented in the literature.

The pattern of changes in POM-P characterized by a rapid depletion of P in the plant residues followed by a consistent increase in the accumulation of P suggests that most of the P in the decomposing residues was immobilized during decomposition and relatively less P remained from the original plant P. Considering that microbial immobilization is probably the main mechanism of P transport into the residue, it would be predicted that most of the P

accumulated in the decomposing residues was microbially derived. Estimates of P immobilization in the sorghum residue based on fungal biomass in the residue (Table 8) and the P content of fungal cells, indicates however, that allocation of P in the fungal biomass can only account for a small fraction of the total P accumulated in the POM at the end of the experiment. A fungal biomass of 26 μg C g soil^{-1} (58 μg g^{-1} soil) for the sorghum treatment at 45 days of incubation, and a P content of the fungal cells varying from 0.2 to 1.4 % dry wt (Hedley and Stewart 1982, Brookes et al. 1982) could theoretically account for only 0.1 to 0.8 μg P g^{-1} soil immobilized in the fungal biomass. This value is significantly lower than 5.27 μg P g^{-1} soil, the observed amount of P accumulated in the POM of that residue at the same period of time in the Alfisol (Table 6). This suggests that probably a large proportion of the P in the decomposing residues is composed by microbial residual products that have been accumulated in the residue during decomposition. A lower rate of mineralization of these microbial products relative to the turnover rate of microbial P could probably explain this accumulation of microbially derived P in the residues. Studies of P cycling in microcosms using ^{32}P-labelled bacterial cells (Barsdate et al. 1974, Van Veen et al. 1987) indicates that turnover of microbial P is greater than the P mineralization from microbial detrital materials which supports the above conclusion.

This apparent immobilization of phosphorus in POM might also suggest that a proportion of P added as plant residues could eventually be retranslocated into the litter at later stages of decomposition. Based on the amount of P located in the POM of the Alfisol at the end of the experiment (Table 6) and the total P added as residues, we estimate that 30 and 8 % of the P added in sorghum and crotalaria residues, can be incorporated in their respective litters

via microbial immobilization. Although these percentages seem relatively small, the absolute values of P immobilized in the sorghum (7.9 μg P g soil^{-1}) and crotalaria residue (1.9 μg P g soil^{-1}) at the end of the experiment for this soil could potentially explain the observed differences in available P between these residues (second chapter).

Sorghum residue, with high immobilization of P in the litter, showed consistently low values of Bray extractable P (9 μg P g soil^{-1}), while the opposite was found for crotalaria which had on average more available P (16 μg P g soil^{-1}). This negative relationship between P immobilization in the litter and available soil P, even for residues having closely the same low (~ 330) C:P ratio , suggests that immobilization of P in the residues might potentially affect the turnover of microbial P and the subsequent mineralization of microbial residual products (empty hyphae, dead microbial cells, etc.). Previous results indicating a significantly lower turnover of microbial P for sorghum and a comparatively lower mineralization of P in this residue (second chapter), seems to support this conclusion. It is probable that residues can offer a certain protection to the microbes against predation which could slow the turnover of microbial P in the residues. Similarly, it is probable that litter could eventually limit the accessibility of the soil enzymes to the microbial residual products localized in the residues at late stages of decomposition which could potentially decrease P availability.

From the previous results it seems that immobilization of P in plant litters can potentially affect the short-term P availability during decomposition of plant residues and this effect could be strongly dependent on the type of residue. The fact that these residues had closely the same (low) C:P ratio indicates that some other characteristics of plant residue quality

influenced the microbial colonization and therefore, the immobilization of P in the residues. A larger fungal biomass found in the litter as well as in the soil for the sorghum, in comparison with the crotalaria residue, reflects significant differences in the microbial community structure during decomposition of these residues.

Several plant biochemical factors have been suggested to affect the soil microbial communities. Soluble C compounds, C:N ratio, the presence of inhibitors and the contents of cellulose and lignin have been some of the mentioned factors that could affect the microbial activity during decomposition (Swift et al. 1979, Bending et al. 1998, Muller et al.1998). A higher amount of easily decomposable substances and lower content of lignin and cellulose in the sorghum residue might have contributed to the colonization of this residue. At early stages of decomposition, the presence of soluble C compounds could have favored the development of some species of "sugar fungi". At later stages of decomposition, as soluble C compounds have been consumed, it is probable that fungi had better abilities to colonize more recalcitrant substrates given their wide range of enzymatic capabilities and mechanisms to efficiently utilize the available C and nutrients (Paustian and Schnürer, 1987, Sakamoto and Oba 1994). The enhanced utilization of these recalcitrant sources in the sorghum residue could also be due to the "priming" effect attributed to the decomposition of more readily decomposable C (Vanlauwe et al. 1994). We speculate that in the crotalaria residue with significantly higher contents of lignin (7.9%) and cellulose (47%) relative to the sorghum residue (lignin 4.6%, cellulose 33%), the utilization of these substrates could have been limited, affecting the fungal colonization of this residue. It is also possible that microbial inhibitors were a limiting factor during decomposition, particularly in crotalaria, and could

have limited the fungal population's ability to utilize more resistant C substrates present in this residue. Water soluble phenolic acid and polyphenols have been found to affect the microbial communities during decomposition of plant residues (Palm and Sanchez, 1991, Bending et al. 1998). This possibility cannot be discarded since no information was available in this study about the presence of any of these compounds in the crotalaria residue.

4. Summary

Incorporation of plant residues can strongly influence the phosphorus contained in the particulate organic matter. Changes in this OM pool could be used to describe the initial release of phosphorus from plant residues and the microbiological transformations of the substrate remaining during decomposition. In our study, changes in the amount of P in the POM indicated a rapid initial release of P from the residues followed by an increasing accumulation of the element suggesting a microbial immobilization of P in the decomposing residues. A correspondence between the accumulation of P in the POM and its fungal colonization pointed out the possible role of the soil fungi in this immobilization. This immobilization of P in the litter could account for observed differences in short-term phosphorus availability from plant residues. However, the way plant factors are regulating this microbial immobilization of P are not completely understood and further research is needed to assess the long-term effect and the influence of changes in the soil environment derived from agricultural practices. Seasonal variations in climate, soil biological activity, cultivation, amount of residue input, and competition with plant roots are some of the factors

that could potentially modify the immobilization of P in decomposing residues under field conditions.

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Table 1. Comparison of different concentrations of NaCl utilized for soil dispersion and separation of the sand fraction (53 - 2000 μm) and POM in the two soils evaluated.

Soil Type	--------- Sand fraction, percentage of total soil weight ----------				
	Na-HMP	---------- Concentration of Na Cl (M) ----------			
		0.1	0.05	0.01	0.001
Alfisol	71± 3[\l]	70± 2	72± 3	82± 3	87± 3
Ultisol	91± 2	90± 3	90± 1	91± 2	93± 1

[\l] Average of three replicates± standard deviation.

Table 2. Carbon recovery in the Particulate Organic Matter (POM) after dispersion with NaCl (0.05M) or Na-hexametaphosphate in the soils

Soil	Percent of C added as plant residues (%)		
	Plant residue type	NaCl 0.05 M	Na-HMP
Ultisol	sorghum	73 [1]	72 NS[2]
	crotalaria	82	84 NS
Alfisol	sorghum	79	81 NS
	crotalaria	97	95 NS

[1] Average of three reps
[2] NS: No significant differences between the two methods of soil dispersion using paired-t test (P 0.05)

Table 3. Phosphorus in Particulate Organic Matter (POM-P) of two residue types after flotation of the sand fraction with distilled water (DW) or Na- polytungstate (Na-PT, specific gravity 1.85 g cm^{-3}) at different sampling times in the Alfisol.

Flotation Method	Crotalaria		Sorghum	
	Days after incubation			
	0	30	0	30
Distilled water	8.7 (35)[1]	0.6	7.0 (27)	1.1
Na-PT	7.5 (31)	1.1	5.9 (23)	2.4
$HSD_{0.05}$	0.8	0.6	0.9	0.7

[1]Values in parentheses are percentage of total P added as plant residues

Table 4. Evaluation of clearing pretreatment utilized for staining the fungi in the Particulate Organic Matter.

	---------- digestion time using KOH 2.5 % (minutes) ----------					
	5	10	15	30	60	$HSD_{0.05}$
POM area (mm^2)	1.45 (97)\[1]	1.35 (91)	1.02 (69)	0.61 (41)	0.40 (27)	0.25
Fungal colonization ($mm\ mm^{-2}$)	ND [3]	5.81	5.67	4.05	ND [2]	0.53

[1] Values in parentheses represent percent of the average POM area without pre-treatment

[2] ND: could not be determined because of the disintegration of the material

[3] ND: could not be determined because the clearing process was incomplete

Table 5. Width and length of fungal hyphae sampled from soil and POM after 30 days of incorporation of plant residues in the Alfisol

Treatments	Hyphal Width (μm)		Hyphal length (m g soil $^{-1}$)	
	Soil bulk	POM	Soil bulk	POM
Sorghum	2.07± 0.38[\2]	8.23 ± 1.03[\1]	432 ± 10[\2]	4.4 ± 0.3[\1]
Crotalaria	1.99± 0.35	2.64 ± 0.20	189 ± 14	0.3 ± 0.03
Control	1.15± 0.28	ND	85 ± 7	ND

[\1] Average of thirty POM pieces ± standard deviation.
[\2] Average obtained from previous data of soil fungi for the same treatments (Second chapter)

Table 6. Changes in phosphorus associated with the Particulate Organic Matter (POM-P) after the incorporation of plant residues in the two soils evaluated

Residue type	0	5	15	30	45	60
			ULTISOL			
			days after incubation			
			POM-P, soil basis (μg P g^{-1} soil)			
Sorghum	5.4 b[1]	0.46 a	1.52 a	3.68 a	4.64 a	5.80 a
crotalaria	6.8 a	0.36 a	0.78 b	2.32 b	2.52 b	2.80 b
control	0.67	0.43	0.5	0.59	0.57	0.51
			POM-P, POM basis (μg P g^{-1} POM)			
Sorghum	217 a	0.375	68 a	197 a	299 a	315 a
crotalaria	274 a	14 b	31 b	103 b	98 b	105 b
			ALFISOL			
			POM-P, soil basis (μg P g^{-1} soil)			
Sorghum	6.10 b	1.54 a	1.58 a	2.25 a	5.27 a	7.9 a
crotalaria	7.90 a	1.83 a	0.48 a	1.04 b	1.67 b	1.9 b
control	1.38	1.44	1.34	1.44	1.65	1.75
			POM-P, POM basis (μg P g^{-1} POM)			
Sorghum	227 b	45 b	60 a	74 a	271 a	308 a
crotalaria	268 a	66 a	15 b	23 b	77 b	81 b

[1] Means followed by the same letter within sampling time and soil are not significantly different according to the Tukey's mean test (P 0.05).

Table 7. Changes in C and N associated with the Particulate Organic Matter (POM-C, POM-N) during decomposition of plant residues in the two soils evaluated.

Residue type	days after incubation 0	5	15	30	45
ULTISOL					
			POM-C, soil basis (μg C g^{-1} soil)		
Sorghum	7248 a[1]	6670 a	5485 a	3790 b	3055 b
crotalaria	7768 a	6390 a	5710 a	4722 a	4025 a
control	1013	1021	955	899	910
			POM-N, soil basis (μg N g^{-1} soil)		
Sorghum	273 a	233 a	271 a	278 a	297 a
crotalaria	254 a	104 b	124 b	154 b	153 b
control	71	73	63	59	61
			POM-N, POM basis (mg N g^{-1} POM)		
Sorghum	7.6 a	7.9 a	11.3 a	14.8 a	16.5 a
crotalaria	6.7 a	3.9 b	5.1 b	7.6 b	9.4 b
ALFISOL					
			POM-C, soil basis (μg C g^{-1} soil)		
Sorghum	8077 b	7471 a	5401 a	5224 a	3815 b
crotalaria	9220 a	7695 a	5349 a	5375 a	4666 a
control	1263	707	712	886	737
			POM-N, soil basis (μg N g^{-1} soil)		
Sorghum	332 a	190 a	272 a	288 a	269 a
crotalaria	348 a	223 a	161 b	137 b	128 b
control	66	50	55	58	41
			POM-N, POM basis (mg N g^{-1} POM)		
Sorghum	8.9 a	5.6 a	9.4 a	9.4 a	12.3 a
crotalaria	8.9 a	7.6 a	5.3 a	5.2 b	5.0 b

[1] Treatment means followed by the same letter within soil and sampling time are not statistically different according to the Tukey's mean test (P 0.05).

Table 8. Fungal colonization and fungal biomass of Particulate Organic Matter (POM) during decomposition of irradiated and non irradiated plant residues in the Alfisol.

Treatment	days after incubation 5	15	30	45
	Fungal colonization (m g^{-1} POM)			
Non irradiated sorghum	60.8 a C\1	83.0 a B	151.0 a A	80.1 a A
Irradiated sorghum	45.6 **\2	66.6 *	152.7	77.1
Non irradiated crotalaria	1.9 b B	2.7 b B	11.7 b A	7.8 b A
Irradiated crotalaria	2.3	2.4	8.4	6.7
	Fungal biomass (μg C g^{-1} soil)			
Non irradiated sorghum	18.4 a C	19.4 a C	35.3 a A	26.3 a B
Irradiated sorghum	12.1 *	16.2	38	25.9
Non irradiated crotalaria	0.04 b A	0.05 b A	0.19 b A	0.1 b A
Irradiated crotalaria	0.05	0.05	0.14	0.09

\1 Means of non irradiated plant residues followed by the same lower case within each sampling time are not significantly different. Means of non-irradiated treatments followed by the same capital letter along sampling times are not significantly different. In both cases the criteria for mean comparisons was obtained by Tukey's test (P 0.05).

\2 Asterisks indicate a statistically significant difference (* $0.01 < P < 0.05$; ** $P < 0.01$) between irradiation treatments within each residue and sampling time according to paired t-tests.

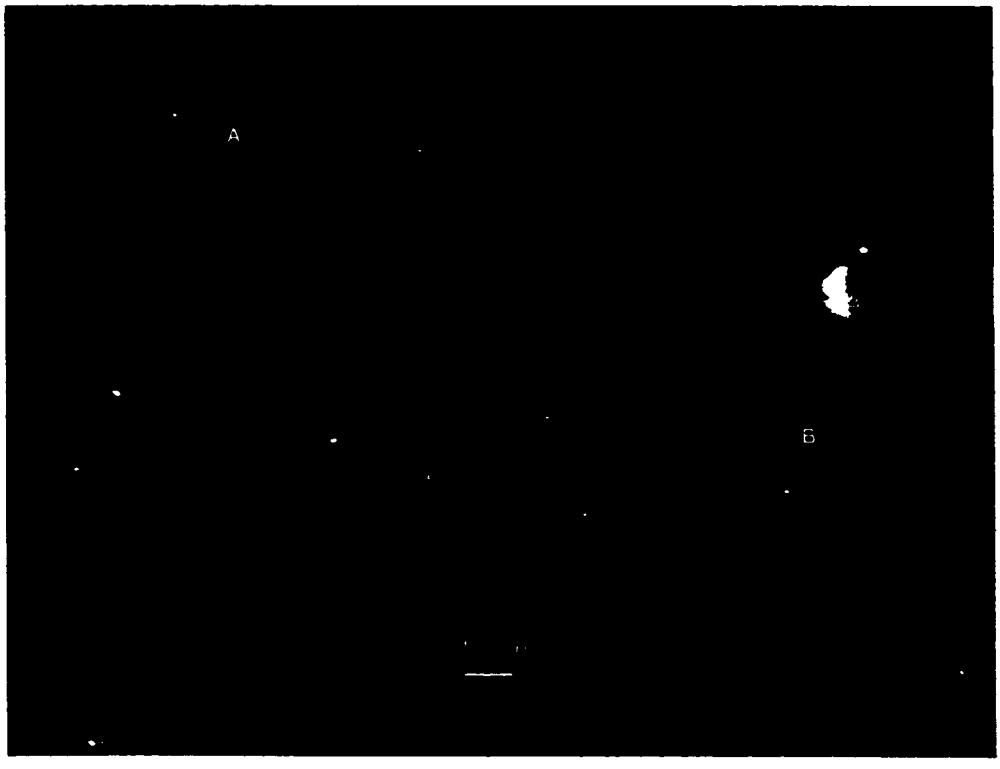

Figure 1. Fungal hyphae observed under epifluorescence microscope at 400X magnification from soil samples prepared after incorporation of sorghum residue in the Alfisol. Morphological characteristics of the hyphae found in soil bulk (A) and growing in the partially decomposed plant material (B).

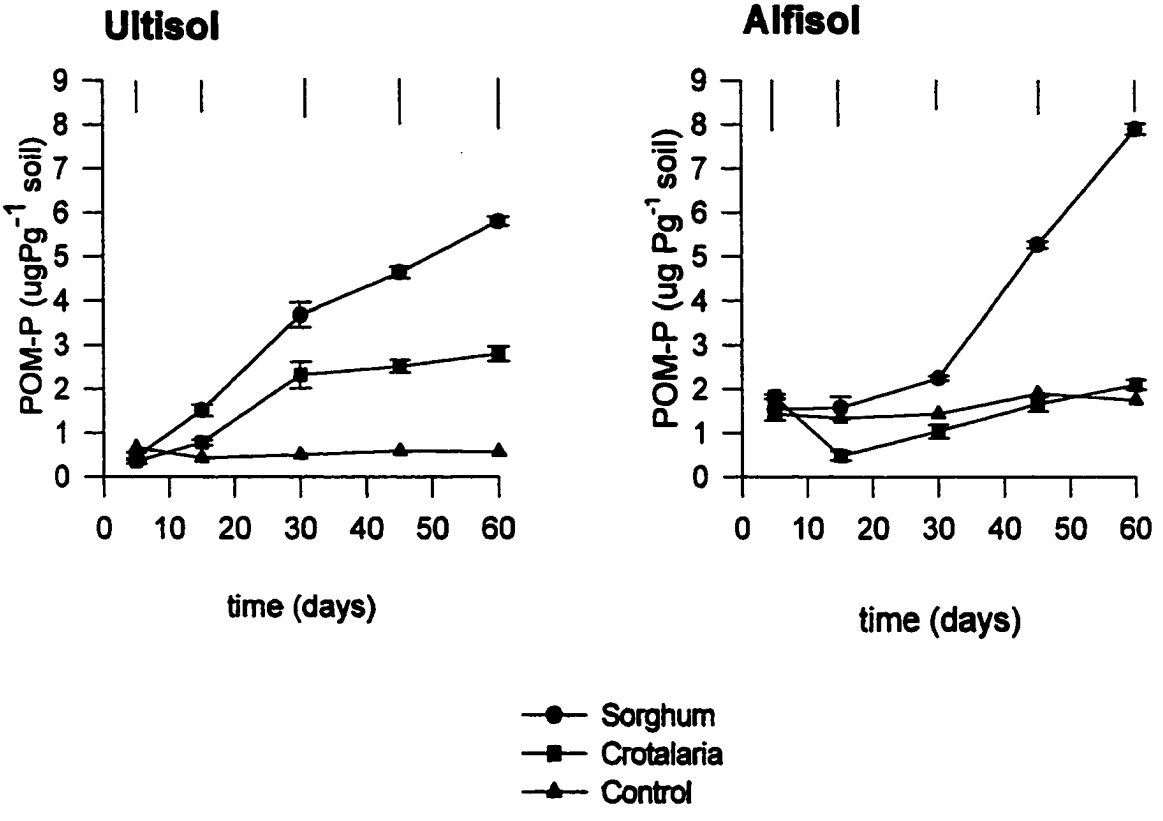

Figure 2. Changes in phosphorus associated to the Particulate Organic Matter (POM-P) after the incorporation of plant residues in the Ultisol and Alfisol. Bars represent Tukey's Honest Significant Difference (P 0.05) for mean comparisons between plant residue treatments within sampling time.

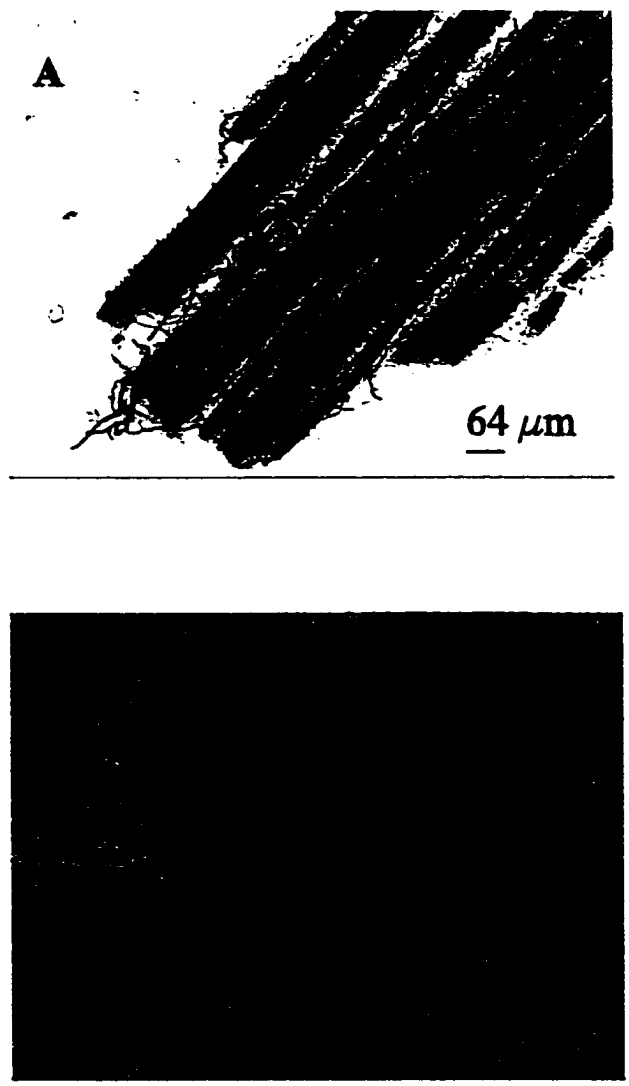

Figure 3. Fungal colonization of the particulate organic matter observed under the microscope after 15 (A) and 30 days (B) of decomposition of sorghum residue in the Alfisol.

CHAPTER IV

MODELING PHOSPHORUS CYCLING DURING DECOMPOSITION OF PLANT RESIDUES: EFFECT OF PLANT FACTORS

1. Introduction

Incorporation of plant residues is becoming an alternative management practice to enhance nutrient cycling in many agroecosystems in the tropics. Nitrogen and phosphorus availability from plant residues is receiving much attention given the possibility to reduce fertilizer dependency and nutrient losses in those areas. Attempts to estimate the release and mineralization of N from litter or plant residues have followed two main approaches. The first describes N flow tightly linked to C flows in pools having different turnover rates (Parton et al. 1987, Molina and Smith 1998). This functional approach has been successfully used to predict long-term N dynamics after the incorporation of organic amendments as well as in natural ecosystems (Paustian et al. 1992, Parton et al. 1994). In the second approach, taken by researchers interested in short-term dynamics, emphasis is given to estimate N mineralization by fitting equations that describe changes in available inorganic N (Paustian and Bonde 1987). In this approach, no attempts are made to simulate the dynamics of decomposers, and N pools are defined according to their susceptibility to be mineralized. In contrast to nitrogen, estimation of phosphorus availability from plant residues has been less

documented. Mechanistic models developed to understand chemical transformation of the element in the soil and subsequent plant uptake (Barber and Cushman 1981, Grant and Heaney 1997) do not consider the biological transformations of the soil organic pools. However, the role of biological processes in increasing the phosphorus availability is generally well accepted, and its contribution can be very significant, especially in the tropics where the turnover of some organic pools can account for 44-88% of the total available soil P (Tiessen et al. 1984).

Simulations of phosphorus cycling, taking into account simultaneously the dynamics of the inorganic and organic P pools, are quite few. Early application of models under this perspective linked P transformation in the soil to the dynamics of decomposers and mineralization of organic pools. Model predictions based on this approach have satisfactorily explained the decomposer P uptake and turnover rates of organic P pools in semiarid grasslands from Colorado and Saskatchewan (Cole et al. 1977). Phosphorus uptake by decomposers was the largest annual P flow in the simulation. Unfortunately, lack of information about seasonal fluctuation of decomposers, their turnover rates and P composition of microbial cells have limited further application of this approach.

More recently, simulation models have attempted to combine decomposition processes and P cycling. In the CENTURY model, P flows have been coupled to C and N flows, and pools are defined according to their ability to supply energy to decomposers (Parton et al. 1988). This conceptual model was extended to include several inorganic P pools and was applied to predict P transformations during decomposition of plant residues (Huffman 1991). Although

this approach has been successfully used to predict C and N cycling after incorporation of organic amendments and N fertilizers (Paustian et. al 1992), its application to predict changes in P availability during decomposition can be limited by some assumptions implicit in the model.

One critical assumption is the partitioning of P between structural and metabolic pools which can produce unrealistic P distribution in plant residues (Gijsman et al.1996). The definition of structural and metabolic P pools implies differences in composition and turnover rates of the pools which has only been appropriately tested for C and N (Marstop 1996, Brelands 1997). There is no evidence indicating that release of P from agricultural residues follows the partitioning scheme based on C turnover. Results indicating a very rapid release of plant P (> 90% of plant P) relative to the C utilization by microbes (10 % of plant C) at early stages of decomposition (second chapter of this dissertation) suggests that probably these pools might differ between the two elements.

In addition, pools and flow structure in the P model of CENTURY are commonly considered the same as those for N and C, and the three elements are linked via C:N and C:P ratios. That structure implies that organic C, N and P are stabilized and mineralized in the same way which does not consider the biochemical reactions in the P cycling (McGill and Cole 1981). Incorporation of plant residues has been shown to differentially modify the soil phosphatase activity (Dalal 1982), and therefore it could influence the potential P mineralization of the organic P pools. From the previous discussion it seems that testing alternative models considering decoupling the P model from the C and N models, as well as the incorporation

of biochemical reactions could enhance our understanding of the P cycling in agroecosystems characterized by receiving frequent additions of plant residues. In the present study, a P model decoupled from the N and C models was developed to explain the main P transformations occurring during decomposition of plant residues. The model will be used later to test hypotheses related to the influence of plant characteristics controlling P availability during decomposition of plant residues. Results of this hypothesis testing will help to identify plant factors other than the C:P ratio that can potentially affect the P cycling and to elucidate the possible mechanisms involved.

Hypotheses to be tested with the model:

1 Changes in mineralization rates of the organic P pools driven by differences in phosphatase activity occurring during decomposition can influence P availability from plant residues.

2 Content of soluble inorganic P in plant residues can affect P availability from plant residues.

3 Plant residues can affect P availability by modifying the microbial demands for P and turnover of microbial P.

2. Materials and methods

The model was built using difference equations coded in FORTRAN with a fixed time step (0.02 day) and optimization techniques for parameter estimation (Hunt et al. 1998, Hunt et

al. 1999). The data utilized to build and calibrate the model were obtained from an incubation study conducted during 60 days (See second chapter for details about experimental procedure). Plant residues of crotalaria (*Crotalaria juncea)* and sorghum (*sorghum bicolor*) differing in plant enzymatic activity, soluble inorganic P and quality of C compounds were incorporated in two acid soils (Alfisol and Ultisol) at a rate of 2%. Chemical analyses were conducted at 0, 5, 15, 30, 45 and 60 days of incubation to represent some of the state and driving variables utilized in the C, N and P sub models. In the P submodel, initial values of the state variables were inferred from P fractions obtained by sequential P fractionation procedure (Hedley et al. 1982), while phosphatase activities measured by substrate conversion (Tabatabai, 1982) were considered as driving variables. Cumulative CO_2 evolution (as described in second chapter) and inorganic KCl extractable N (Keeney and Nelson, 1982) were the soil variables used to fit the C and N submodels, respectively.

2.1 *Model description*

State variables used in the model represent the major important forms of C and P in the residue and in the soil (Figures 1 and 2). The N model contains the same pools as the C model except that it has the pool for inorganic N instead of the C sink. A brief description of the state variables utilized in the C, N and P sub models is given in Appendix A (Table A1). The C and N sub models consist of three soil organic matter pools (microbes, labile and stable) and two plant residue pools (structural and metabolic) which correspond with pools defined in other SOM models (Parton et al. 1988). In the P model, pools are represented by P forms contained in plant residues (organic and inorganic) and different soil organic and

inorganic P forms. The labile and stable organic P as well as the labile and strongly sorbed inorganic P pools correspond with the P fractions obtained by sequential extraction (Hedley et al. 1982), while the microbial P was obtained by fumigation extraction (Kuono et al. 1995).

The model establishes two pools in the plant residues. Residue C (Figure 1) is partitioned between metabolic and structural forms considering differences in substrate availability for microbial growth and plant C:N ratio. The initial partitioning of the C residue was estimated based on results of cold water extractable organic C obtained for the plant residues. Partitioning of plant N was estimated by solving numerically an equation where the whole plant C:N ratio is a weighted mean of the C:N ratio of the metabolic (6) and the structural (100) plant pool. The labile soil organic C and N pools represent the cellular contents released after the death of the microbial cells. Flow of C between the microbes and this labile pool is calculated as a function of a specific death rate and the size of the microbial pool. Excretion of organic compounds during microbial growth is not considered.

Metabolic C, structural C and labile organic C decomposition are described by Michaelis-Menten equations to represent simultaneous microbial utilization of three C sources (Pickett, 1982). Each of these decomposition flows is regulated by a reduction factor which is a function of the soil water content (swc). In this case, since the swc was kept constant at 65% of the water holding capacity, a maximum decomposition rate was assumed. In addition to the water effect, the C:N and C:P ratio of the microbes were used to regulate the decomposition rates of the structural and labile organic C pools as suggested by Huffman (1991). It was assumed that a maximum decomposition rate takes place when the C:N and

C:P ratios of the microbes are 5; in which case, the microbial growth is C-limited. When C:N and C:P ratio of the microbes reach values equal or greater than 20 and 100 , respectively; the decomposition rate is minimized (10% of the maximum) because of limitations in nutrient supply.

Once the different C substrates are consumed by the microbes during decomposition, yield coefficients are used to determine the amount of C that flows to the biomass and CO_2 forms (Parton et al. 1988). Nitrogen consumption is linked to C consumption and it is calculated based on the C:N ratio of the substrate. In order to satisfy additional N requirements for microbial growth, soil inorganic N is taken up by microbes according to a Michaelis-Menten function. This flow is controlled by a regulatory mechanism based on the difference between the actual microbial C:N ratio and a theoretical value (10) below which it is expected that the inorganic N uptake will be reduced.

Stabilization of organic C compounds is described as in the CENTURY model (Parton et al.1987). A fraction of the flow from microbes to the labile pool is stabilized according to the clay content of the soil. As the structural C is decomposed during microbial growth, a fraction of this flow becomes stabilized in the soil organic matter and in the soil particles, and which is also dependent of the clay content. Since the stabilization takes place by reactions of adsorption and polymerization in which the whole molecule is stabilized, the stabilization of N is calculated by dividing the C flow by the respective C:N ratio of the donor pools.

The phosphorus model partitions P in the plant residues between inorganic and organic pools. Inorganic P is derived from the amount of soluble inorganic P existing in the residues, while the organic P pool is calculated by difference with respect to the total amount of plant P added. In contrast to previous P models, there are not two pools of organic P in the plant residues. Instead, it is believed that since the organic P compounds in the plant residues are well localized in the plant cells (Maschner, 1995), and they are not restricted by carbon compounds, they might constitute an unique pool with relatively uniform turnover rates. The organic P in plant residues can be either mineralized directly from the substrate or it can be released to the soil solution in the organic form where it can be mineralized. Although we know of no evidence indicating predominance of any of these two pathways, it is probable that both happen at the same time since the breakdown of C substrates during decomposition might facilitate the release of organic P as well as its hydrolysis by microbial phosphatases. Once the inorganic phosphorus is in the soil solution it can be either taken up by the microbes or be sorbed into the soil particles. The net result of these two competing reactions will depend on the balance between the sorption and immobilization processes.

To estimate P immobilization, the rate of inorganic P uptake by the microbes is modeled by Michelis-Menten kinetics as suggested by Beever and Burns (1980) and a regulatory factor calculated as a function of the microbial C:P ratio. The transfer of P out of the microbial pool is calculated as a function of the specific rate of death and the size of the microbial P pool. Although the grazing of microbes was not explicitly considered in this model, this flow reflects both the action of the grazers and the non predatory death of the microbes. Once the P is released from the microbial cells it is transferred to the soil solution and the labile organic

pool according to the solubility of organic P compounds existing in the microbial cells. Since more than 70% of the organic P in microbial cells is in nucleic acids and phospholipids (Beever and Burns, 1980), which are insoluble in water, it is assumed that 70 % of the decaying microbial biomass P is transferred to the organic labile pool, and the remaining 30% goes the soil solution.

Soluble, labile and stable organic P are mineralized by soil and microbial phosphatases following the general structure previously utilized to predict the phosphorus cycling in semiarid grassland (Cole et al. 1977). A significant difference is that in this study, P mineralization rates are calculated as functions of specific rates of mineralization, size of the organic P pools and phospho monoesterase activity; while in the grassland model, the rates were calculated based on C turnover and the average C: P ratio of the pool mineralized. In the present model it is assumed that there is a correspondence between organic and inorganic P pools in the model and the P fractions obtained by sequential extraction procedures. Initial values for soil solution Pi, labile Pi and strongly sorbed Pi pools were obtained from measurements of water extractable Pi (soil:water ratio 1:1), resin P (Parfitt et al. 1994) and NaOH-Pi fraction (Hedley et al. 1982), respectively. Similar to the CNP model developed by Huffman (1991), inorganic reactions in the soil solid phase are estimated based on simple rate constants and the size of each inorganic pool.

The flows among state variables and the parameters utilized in each flow are indicated in Appendix A (Table A2). Equations utilized to calculate the flows among state variables are described in Appendix B. In order to build the model only data from the Ultisol and the

sorghum residue treatment were used. Most of the parameters used in the model were taken from the literature. Values of Vmax for NH_4 uptake and consumption of labile and metabolic carbon by microorganisms, as well as maintenance respiration rate were taken from the variable-ratio model developed to simulate growth of bacteria (Hunt et al. 1985). Yield coefficients for microbial utilization of different C substrates and equations for stabilization of labile organic C based on clay content were used as indicated in the CENTURY model (Parton et al. 1987). To take into account simultaneously the predatory and the non predatory microbial death, the specific death rate (0.05 day^{-1}) used in the CNP model (Huffman, 1991) was increased to 0.125 day^{-1}.

For the phosphorus model initial parameters for microbial P uptake were taken from previous models (Cole et al. 1977, Huffman, 1991) with adjustments made by optimization procedures in order to get a better fit to the data. Initial values of specific mineralization rates for labile and stable organic P as well as simple rate constants for the sorption-desorption reactions were taken from the CNP model (Huffman 1991). Sensitivity analysis was carried out to identify the flow parameters that contributed the most to improving the fit of the model. Based on results of sensitivity analysis, final parameters for death rate, simple rate constants and P mineralization rates were obtained by optimization procedures. Parameters were chosen to minimize f, the sum of squared relative errors, considering data of cumulative CO_2, microbial biomass P and soil labile Pi.

Data of the crotalaria residue in the Ultisol as well as data of both residue types in the Alfisol were used to further calibrate the model. Appropriate changes in state variables were made

to take into account changes in soil and plant characteristics. For each soil, initial values of inorganic and inorganic P pools were taken from P fractionation results as described for the Ultisol. Microbial biomass C and N were estimated by microbial counting, assuming a microbial C:N of 10. Microbial biomass P was obtained from chloroform fumigation as described in the second chapter. For each residue, changes in the amount of metabolic and structural C were made according to results of cold water extractable C in the residues (second chapter). In the same way, adjustments in the content of plant inorganic P were taken from results of cold water extractable inorganic P. To improve the fit of the model in each case, new parameter values of death rate, Vmax for microbial P uptake and P mineralization rates were calculated by optimization procedures. In the Alfisol, simple rate constants for inorganic P transfers between solid phase and soil solution were obtained by optimization procedures using data of the sorghum residue. Since no differences were observed in P sorption characteristics of the soil between the plant residues (Appendix C), the same rate constants for inorganic P transfer will be used for both residue types. To evaluate simulation accuracy, a combination of graphical display and statistical techniques were used as recommended by Whitmore (1991). Linear regression of observed values on simulated values with examination of r^2 and deviation of the slope from the line of perfect agreement was done using the PROG REG program, version 6.12 (SAS, 1996).

2.2 *Hypothesis testing*

Hypothesis testing was conducted to elucidate the effect of selected plant characteristics on P availability. The model initially fitted with data of the sorghum residue was used to

selectively substitute plant parameters for the corresponding values obtained for the crotalaria residue. Ability of the model to predict observed values of labile Pi in the crotalaria when changing specific set of plant parameters was evaluated using data from the Ultisol. The null hypothesis was that both residue types do not differ in any of the plant parameters tested. Three alternative hypotheses were tested for their contribution to explain differences in P availability: 1) changes in the content of inorganic P in the plant residue, 2) changes in plant enzymes affecting P mineralization rates; or 3) changes in microbial dynamics resulting from differences in quality of plant C compounds and turnover of microbial biomass (microbial death rate). Changes in these sets of variables were compared for their ability to reduce the sum of squared relative errors between the observed and predicted labile Pi for both plant residues.

3. Results

3.1 *Nominal behavior of the model*

Carbon mineralization in the Ultisol was fairly well described by the model as indicated by the high r^2 obtained when observed values were regressed on simulated values and regression lines had slopes that did not statistically differ from one (Table 1). More than 98% of the total variability in the observed cumulative C for both residues in the Ultisol, could be explained by the linear regression on the simulated values. Changes in partitioning of plant metabolic C as established by cold water extraction of plant C were able to account for differences in CO_2 evolution between the two residue types (Figure 3). The model

satisfactorily predicted 20% higher cumulative CO_2 evolution in the sorghum residue at the end of the incubation period in comparison with the crotalaria residue. Carbon mineralization was however, underestimated for the sorghum residue in the first five days, followed by overestimation in both residues for the next twenty days (Figure 3). At the end of the incubation (after 30 days) the model predicted a plateau in CO_2 evolution, while observed data for both residues suggested the existence of microbial activity during that period. Underestimation of the initial CO_2 for the sorghum residue in the Ultisol can be attributed to an underestimation of the initial soil microbial biomass in the model. In this study, initial value of soil microbial biomass C (25 ugC g^{-1} soil) was taken from results of fungal and bacterial biomass obtained by microbial counting (second chapter) which may underestimate the soil microbial biomass (Ingham and Horton, 1987). Similarly, the effect of microbial inoculum contained in the residue was not considered in the model predictions. Previous results have indicated that the presence of microbial inoculum in the sorghum residue can significantly contribute to the initial colonization of this residue (third chapter). Although microbial biomass in the plant can be relatively small, its close contact with the C substrates could have improved the C utilization and the CO_2 evolution in the sorghum residue. Overestimation of cumulative CO_2 for both residues between 5 and 23 days (Figure 3) on the other hand, was attributed to high rates of microbial maintenance respiration (0.11 day^{-1}) used in the model. When proper changes in initial microbial biomass C (60 instead of 25 ugC g^{-1} soil for the sorghum residue) and maintenance respiration (0.07 instead of 0.11 day^{-1}) were made, the model was able to better predict the cumulative CO_2, as indicated by a decrease in the sum of squared relative errors (f) from 0.64 to 0.53.

Phosphorus dynamics in the Ultisol were properly simulated by the model. High values of r^2 and regression lines with slopes that did not differ statistically from one (Table 1) indicated that the model properly described differences in microbial P and P availability for the two residue types. Regressions coefficients higher than 0.98 and 0.93 for microbial biomass P and labile Pi, respectively, showed that a high proportion of the variability in observed values for the Ultisol could be explained by the linear regression on the simulated values. In order to allow the model to fit the data of the two residue types, parameters of quality of plant C compounds, microbial death rate, Vmax for microbial Pi uptake, P mineralization rates and content of plant inorganic P were adjusted for each residue. The selection of the parameters was based on their ability to modify the P availability, and from previous information indicating that these residues differed in their content of inorganic P, phosphatase activity and turnover of microbial biomass P. Thus, appropriate changes in plant factors were made according to characterization of these plant residues (second chapter). Specific microbial death rate and Vmax for microbial P uptake were chosen because sensitivity analysis using labile Pi data showed that the model was very sensitive to these parameters, as reflected by their low f values (0.414 and 0.423, respectively, Table 2). Optimization procedures were used to find the best combination of parameter values for death rate, Vmax for microbial Pi uptake and P mineralization rates for each residue type. Mineralization rates of stable organic P pools estimated by the model (0.0027 and 0.0013 day^{-1}, for sorghum and crotalaria, respectively) are in the range (0.0014 - 0.0080 day^{-1}) reported for the literature when using plant residues (Dalal 1979, White and Ayoub 1983). Values of parameters used in the model can be found in Appendix A (Table A2).

Simulation results of microbial P dynamics and P availability for the Ultisol showed that the model was able to properly describe observed differences between residue types (Figure 4). A higher P immobilization and lower P availability was predicted for the sorghum residue (Figure 4). Simulation results of labile Pi however, showed a higher variability between observed and predicted values. The model definitively failed to predict the values of labile Pi for both residues at the first day on incubation. This could be partially explained by the fact that resin extraction of P at the beginning could have included a significant part of the inorganic P contained in the residues. Consequently, initial values of soil labile Pi should probably take into account this initial flush of P from the residues. As a result of this underestimation, data from the first day were not considered for the regression analysis.

A continuous decrease in labile Pi was predicted for both residue types along the incubation period in the Ultisol (Figure 4). Decay of microbial cells, as reflected by a rapid decrease in microbial P after 10 days (Figure 4), did not result in an increase in the labile Pi during this period. This suggests that mineralization of organic P compounds in microbial detritus can be a limiting factor regulating the short-term P availability from these plant residues. Chemical exchange reactions taking place between labile and strongly sorbed forms of P in the solid phase could also explain the decrease in short-term P availability during decomposition of plant residues as suggested by Umrit and Friesen (1994) and Iyamuremye et al. (1996). Considering these two mechanisms, it would be expected that a continuous decrease in labile Pi (from 10-14 to 3 μg P g^{-1} soil) would result in an increasing accumulation of P in either strongly sorbed inorganic P pools or in organic P pools with relatively slow turnover.

Testing which of these hypotheses might be responsible for the decrease in labile Pi can be complex, since both inorganic and organic reactions are occurring simultaneously. Utilization of the model allowed the simulation of the influence of organic and inorganic P reactions. Results indicated that both reactions can be responsible for the observed decrease in labile Pi. An increase of 30% in either the mineralization rate of the stable organic P, or in the simple rate constant between strongly sorbed and labile Pi pool, resulted in an increase of labile Pi after 30 days in both residues (data not shown). This suggests that both organic and inorganic reactions are probable controlling the dynamics of the labile Pi during decomposition of plant residues.

Accumulation of organic (Po-NaOH) and inorganic P (Pi-NaOH) at the end of the experiment (as measured by NaOH extraction following the Hedley fractionation procedure in the Ultisol) confirmed the transfer of P to more stable P pools (Table 3). Phosphorus accumulation in this fraction however, differed according to the residue type. In the crotalaria residue there was a significantly higher accumulation of P in the Pi-NaOH fraction (14.7 μgP g^{-1} soil), indicating a more intensive mineralization of P in this residue. In the sorghum residue, P accumulated in the Po-NaOH fraction (15.1 μgP g^{-1} soil). Considering that most of the P initially present in the plant has been released from the residue by the end of the experiment (third chapter), the Po-NaOH accumulated in the soil for the sorghum treatment might probably be composed of microbial byproducts that have not been mineralized. This suggests that residues might differ in the extent of P mineralization of the organic P contained in microbial by products. These results confirm previous findings indicating that mineralization of microbial P by products could control the P availability from plant residues (McLaughlin et al. 1988). The

question arising at this point is, however, whether or not the P mineralization of microbial sub products was regulated by: 1) the specific rates of P mineralization dependent on residue type; or, 2) the rate of supply of the microbial by products. These two hypotheses will be tested in the last section of this chapter.

The model was further calibrated using data of the two plant residues incorporated in the Alfisol. Soil characteristics of the Alfisol were used as initial values for soil state variables. Additional considerations were made to take into account an increased stabilization of plant residues and greater protection of plant-derived microbial biomass as the clay content increased (Ladd et al.1995, Hassink 1996). In this case, higher clay content of the Alfisol (15%) was used in a clay function regulating the flows between structural and stable C pools, and between the labile and stable C pool. Additionally, lower values of death rates (0.065 and 0.11 day^{-1}, for sorghum and crotalaria, respectively) were obtained by optimization procedures for this soil. Once the C sub model was properly fit with the CO_2 data, new parameters of P mineralization rates were estimated for each residue type in this soil. Simple rate constants for inorganic reactions between the solid phase and the soil solution pools were adjusted to fit the data of resin extractable P.

Comparisons between simulated and observed values of cumulative CO_2, microbial biomass P and labile Pi for the Alfisol are shown in Figures 5 and 6. In the Alfisol, as in the Ultisol, the model was able to represent differences in CO_2 evolution between residue types as indicated by regression coefficients and slopes of the linear regression (Table 1). The model predicted lower cumulative CO_2 in this soil, as compared with the Ultisol, specially in the

sorghum residue (Figure 5), suggesting a higher protection of the plant C substrates in this soil. Similar results were found by Sørensen et al. (1996) who found 5-10% more plant-derived CO_2 in a loamy sand soil as compared with a clay soil.

Phosphorus dynamics were not as well predicted as the C dynamics in this soil (Figure 6). Although regression analysis showed high r^2 values for most of the cases, the slopes differed from the 1:1 line (Table 1). Although the microbial P for both residues is fairly well predicted after 30 days, the model failed to predict the observed values in the first two sampling times. The model overestimated the microbial P, particularly in the sorghum residue. Similar to the CO_2 data, the observed microbial P indicates that in the sorghum residue there was a lower initial microbial activity with a maximum at 30 days of incubation. Changes in the soil microbial community composition driven by residues could explain the differences. Previous results have indicated a significantly higher proportion of the total microbial biomass composed of fungi in sorghum as compared with crotalaria residue (second chapter). Although different rates of microbial death used for each residue probably represented differences in turnover of microbial biomass between the residues, additional improvements need to be made in the model to better predict the initial microbial P uptake in the sorghum residue.

Predictions of labile Pi in the Alfisol reflected the general trend given by observed values of the two residue types (Figure 6). Compared with the Ultisol, a smaller treatment effect (less than 2 μgP g^{-1} soil) was predicted by the model in this soil. As previously indicated in the Ultisol, the model failed to represent the initial flush of Pi from the residues. However, when

comparing predictions made for more stable soil P pools with observed data, it can be seen that the model was able to predict treatment effects and differences in soil type (Table 3). Results of strongly sorbed Pi pool predicted by the model at the end of the experiment appropriately represented the changes in inorganic P extracted with NaOH (Pi-NaOH). The model simulated higher amounts of P for the crotalaria residue, following observed results given by the Pi-NaOH fraction, confirming thus a higher P mineralization in this residue.

3.2 *Hypothesis testing*

As previously indicated by results of labile Pi and strongly sorbed inorganic P, residues having the same C:P ratio significantly differed in soil P availability. In order to identify plant factors and mechanisms that could potentially explain this dependency, the model was used to test alternative hypotheses. Changes in content of plant inorganic P, phosphorus mineralization rates and microbial dynamics were tested separately for their ability to explain differences in P availability from plant residues. The procedure for hypothesis testing was based on comparisons of the sum of squared relative errors (f) between observed and predicted values including both residue types. The hypothesis with the lowest f value indicates that it contributes the most to explain the dynamics described by observed labile Pi data. The highest f value is given to the null hypothesis which states that residues do not differ, and consequently have the same parameters.

Comparing the f values of the different hypotheses (Table 4), it can be seen that differences in microbial dynamics resulting from changes in quality of C compounds and microbial death

rate (hypothesis III) contributed the most to explain the variability in the observed data for both residues. The low f value (0.85) found for the hypothesis III when using only the labile Pi data reflects that this hypothesis contributed the most to explain differences in labile Pi between residues. While hypothesis III reduced the relative error of the null hypothesis by 51%, hypotheses I and II reduced the error by only 20 and 14%, respectively (Table 4). Graphical comparison of the model predictions for each hypothesis can be seen in Figure 7. Changes in either specific P mineralization rates, or in the content of plant inorganic P did not reproduce the observed differences in P availability between plant residues.

In contrast, changes in microbial dynamics did decrease the relative error by better fitting the data of each residue. The model predicted that increasing amounts of soluble C compounds reduced the P availability by increasing the microbial demands for P. Similarly, a lower microbial death rate in the sorghum residue as compared with crotalaria, seemed to be responsible for the sustained P immobilization in this residue. This substrate dependency of the microbial turnover corresponded with observed changes in microbial community structure and fungal colonization of the decomposing residues (second and third chapter). Sorghum residue showed a consistently higher proportion of the microbial biomass composed by fungi, and a more intensive fungal colonization of the residues, which might explain slower turnover of microbial biomass in this treatment.

Hypothesis testing suggests that changes in microbial dynamics driven by different residue types can potentially influence the P availability more than changes induced in specific P mineralization rates. From these results, we speculate that the rate of supply of organic P

compounds provided by dying microbial cells could be more important than the specific (potential) P mineralization rate in controlling the actual P mineralization, at least at early stages of decomposition (less than 60 days). Apparently, slower turnover of microbial biomass P and increased microbial demands for P when using residues with high quality C could adversely affect the P availability from plant residues. Previous studies have also suggested that P mineralization of microbial by products during decomposition of residues can be a limiting factor in regulating short-term P availability (Blair and Boland 1987, McLaughlin et al. 1988). These studies however, have failed to elucidate whether the P mineralization of plant residues has been limited by the rate of supply of microbial-derived organic P or the rate of hydrolysis of organic P. These two hypotheses need to be tested experimentally, specially at different stages of decomposition where the contribution of each mechanism might be different.

4. Summary

A simulation model was developed to help understand the biological and biochemical P transformations occurring during decomposition of plant residues in soil. The model was very sensitive to plant residue parameters affecting the microbial dynamics, and the model correctly predicted changes in CO_2 evolution resulting from increasing quality of C substrates. Model predictions of microbial turnover (microbial death rate) for each residue treatment were in agreement with observed changes in soil microbial community structure following incorporation of residues. Results of hypothesis testing conducted to evaluate the influence of selected plant factors on P availability, suggested that easily decomposable C substrates can significantly reduce the P availability by increasing the microbial demands for P.

Phosphorus availability was more sensitive to changes in microbial turnover than to mineralization rates induced by the residue types. This suggests that the rate of supply of microbial products can be more determinant than the rate of hydrolysis of organic P at early stages of decomposition (less than 60 days). Soil P availability was on the other hand, surprisingly insensitive to the content of plant inorganic P. It seems that microbes can efficiently immobilize inorganic P released from the residues. These results indicate that plant factors controlling microbial dynamics could potentially affect the P availability by influencing the immobilization and release of P from microbial cells. Easily decomposable C compounds in the plant and subsequent microbial interactions among microbial populations following the incorporation of residues are believed to play a significant role in P cycling during decomposition.

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Table 1. Linear regression coefficients obtained for predicted and observed values of cumulative CO_2, microbial biomass P and labile Pi for the soils and evaluated plant residues.

		ULTISOL		ALFISOL	
State variable	Residue type	r^2 [1]	slope ± CI [2]	r^2	slope ± CI
Cumulative CO_2	sorghum	0.98	0.96 ± 0.044	0.95	1.02 ± 0.127
	crotalaria	0.99	0.98 ± 0.033	0.97	0.95 ± 0.051
Microbial biomass P	sorghum	0.99	0.95 ± 0.060	0.97	0.88 ± 0.088
	crotalaria	0.99	0.99 ± 0.069	0.94	1.21 ± 0.183
Labile Pi	sorghum	0.94	0.81 ± 0.122	0.99	1.15 ± 0.081
	crotalaria	0.95	1.02 ±0.136	0.79	0.61 ± 0.185

[1] Regression coefficients when observed values are regressed on predicted values.
[2] Slope of regression line constrained through the origin. CI denotes 95% confidence interval for the slope.

Table 2. Parameters and state variables that contributed the most to the fitness of the model obtained by sensitivity analysis using all data (n=138) or using the labile Pi data only (n= 30).

USING ALL DATA		USING Labile Pi DATA	
Parameter or state variable [1]	f [2]	Parameter or state variable [1]	f [2]
maintenance respiration	0.593	death rate	0.41
death rate	0.631	soluble/insoluble microbial P	0.42
yield of metabolic C	0.646	maintenance respiration	0.42
yield of structural C	0.651	Vmax microbial Pi uptake	0.42
plant inorganic P	0.651	plant metabolic C	0.43
Vmax microbial Pi uptake	0.656	plant structural C	0.43
organic plant P	0.659	simple rate constant labPi-solPi	0.43
mineralization of stable Po	0.659	simple rate constant LabPi-sadPi	0.43
metabolic plant C	0.661	plant inorganic P	0.44
soluble/insoluble microbial P	0.662	mineralization of labile Po	0.44
Initial f value of the model	0.664	Initial f value of the model	0.47

[1] Parameter and state variables were ordered based on their individual contributions to reduce f by changing their nominal values ± 10 %.

[2] Sum of squared relative errors, $f = \sum [(\text{predicted} - \text{data}) / \text{data}]^2$

Table 3. Organic (Po-NaOH) and inorganic phosphorus (Pi-NaOH) fractions (Hedley et al.1982) measured at the end of the experiment and compared with the predicted value of strongly sorbed Pi obtained by the model for the soils and treatments evaluated.

ULTISOL	Po-NaOH	Pi-NaOH	strongly sorbed Pi predicted by the model
	---------- μ g P g^{-1} soil ----------		
sorghum	15.1	7.3	10
crotalaria	6.9	14.7	16
control	8.9	8.4	
$HSD_{0.05}$	1.5	2.3	
ALFISOL			
sorghum	27.7	19.1	24
crotalaria	21.1	25.4	31
control	19.2	14.9	
$HSD_{0.05}$	2.1	2.8	

Table 4. Results of sensitivity analysis for hypothesis testing using all data (n=138) or labile Pi data only (n=30) in the Ultisol.

Hypothesis tested[1]	f for labile Pi data	f for all data
I.- Residues differ in P mineralization rates	1.37 (20)[2]	0.85
II.- Residue differ in the content of plant inorganic P	1.48 (14)	0.88
III.- Residues differ in microbial dynamics driven by changes in plant metabolic C and microbial death rate.	0.85 (51)	0.66
Null hypothesis: Residues do not differ in any of the previous factors	1.73	0.88

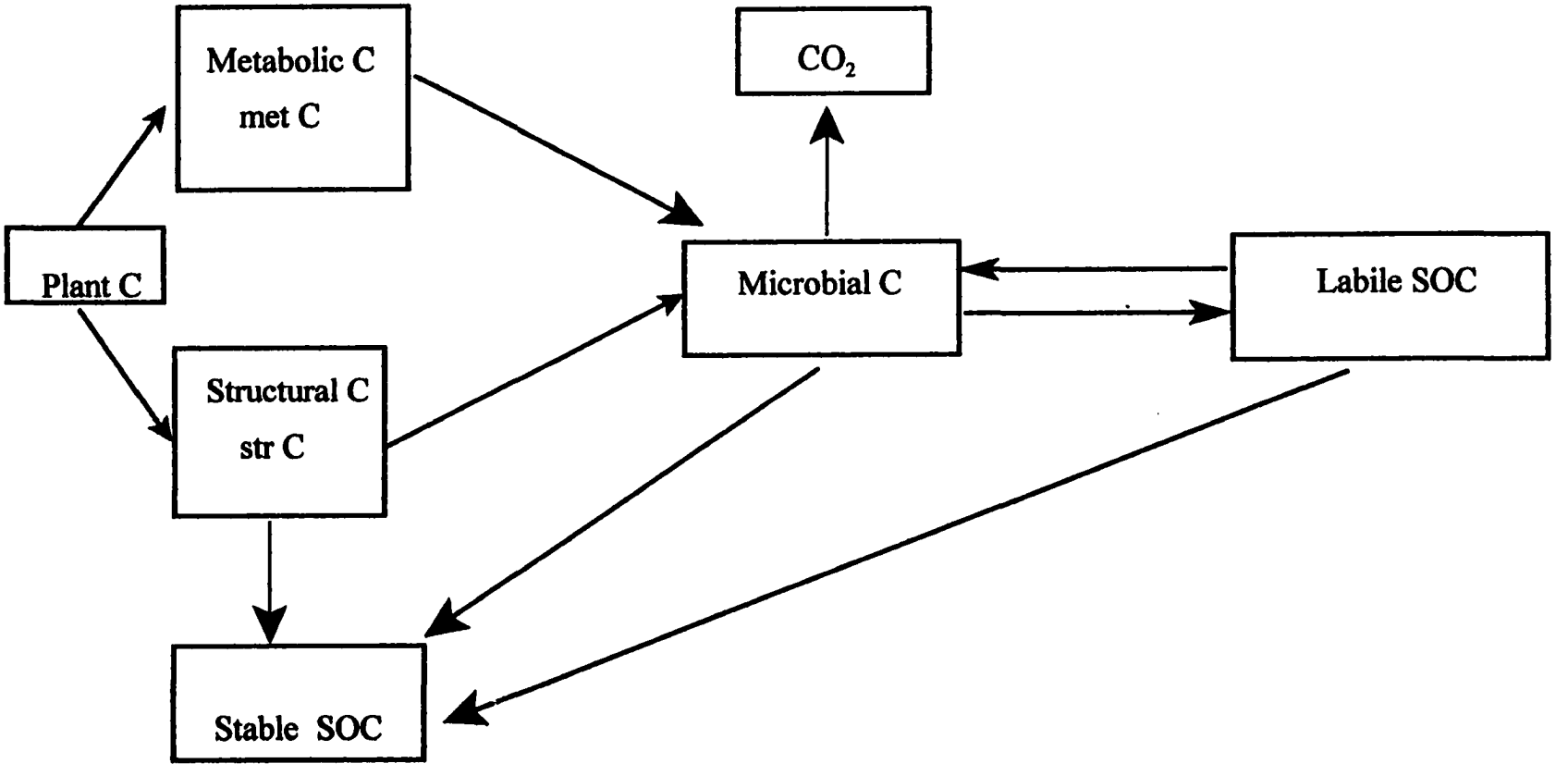
[1] Hypothesis I: Only P mineralization rates of labile (labPo) and stable organic P (stabPo) pools were changed for each residue type.
Mineralization rates of labPo: 0.031 and 0.055 day $^{-1}$ for sorghum and crotalaria, respectively.
Mineralization rates stabPo: 0.002 and 0.011 day $^{-1}$ for sorghum and crotalaria, respectively.

Hypothesis II: Only the content of plant inorganic P was changed for each residue type.
Plant inorganic P: 18 and 14 μgP g $^{-1}$ soil for sorghum and crotalaria, respectively.

Hypothesis III: Only quality of plant C and death rate were changed for each residue type.
Metabolic plant C: 2635 and 2150 μgC g^{-1} soil for sorghum and crotalaria, respectively.
Death rate: 0.125 and 0.200 day $^{-1}$ for sorghum and crotalaria, respectively.

[2] Values in parenthesis represent percentage of decrease in the sum of squared of relative error (f) as compared with the value corresponding to the null hypothesis.

Figure 1. Conceptual model for Carbon flows



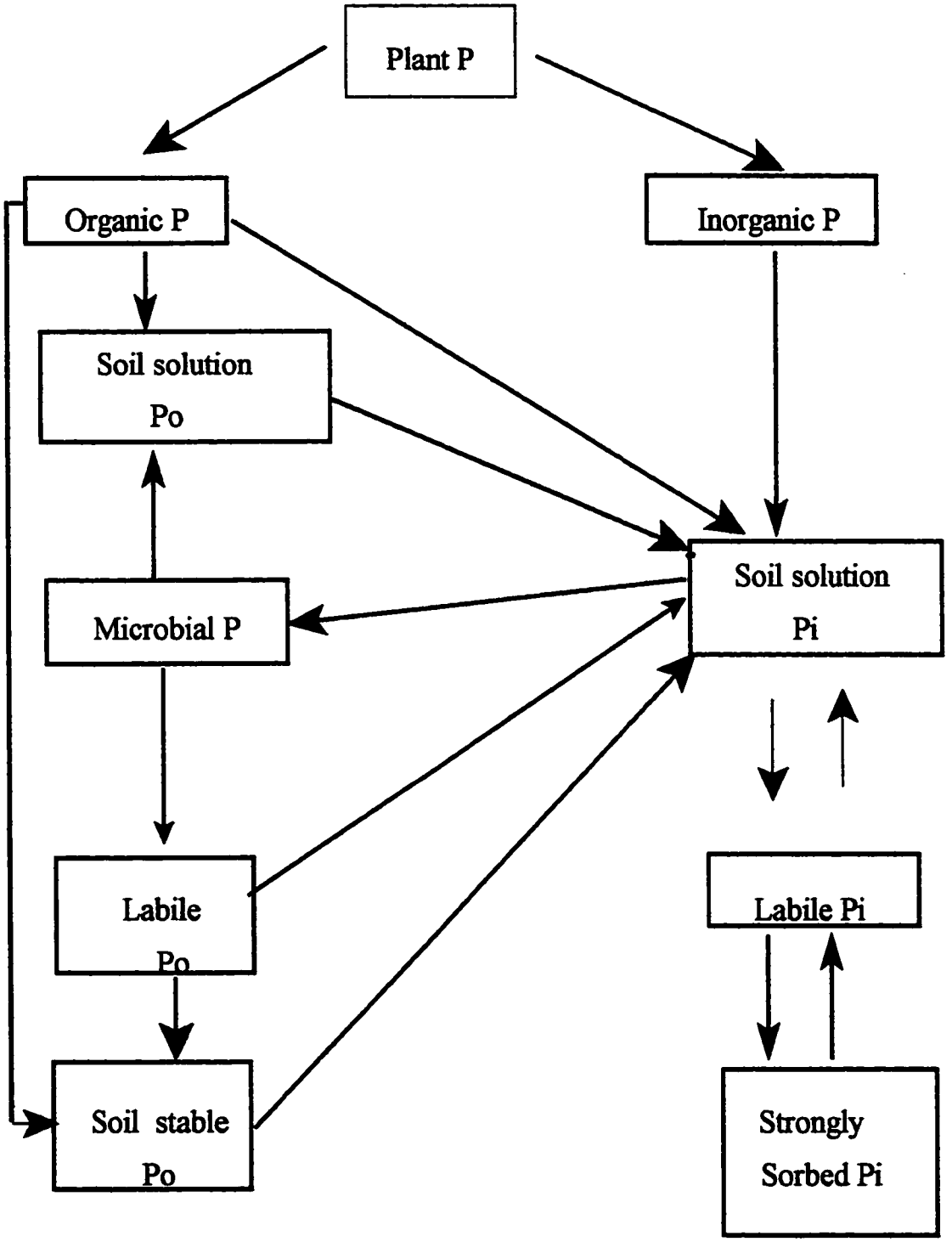


Figure 2. Conceptual model for Phosphorus flows

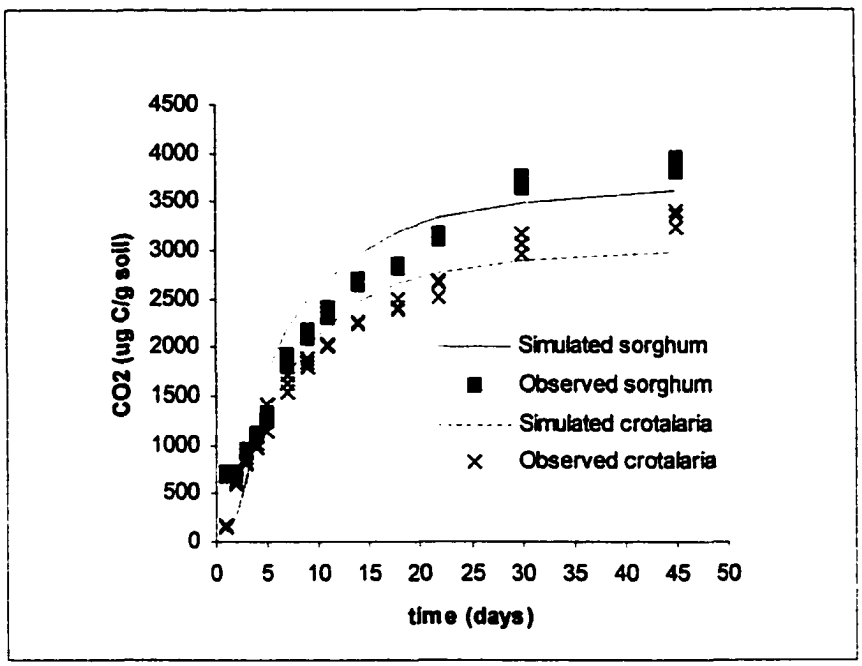


Figure 3. Cumulative CO_2 evolution during decomposition of the plant residues in the Ultisol.

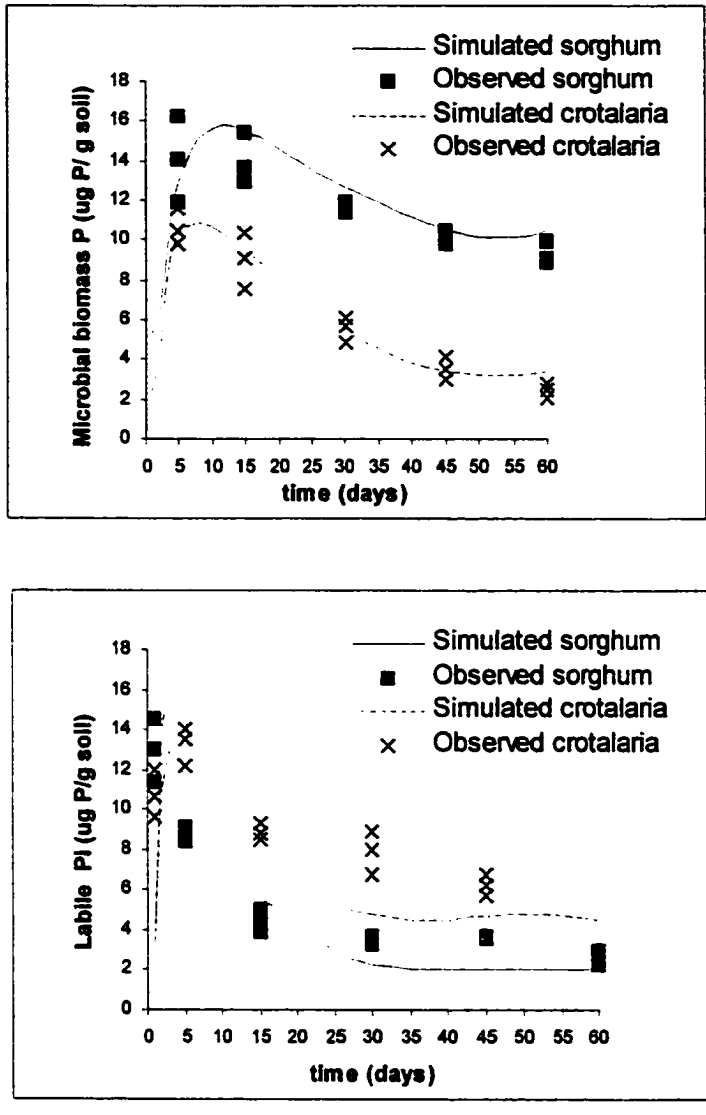


Figure 4. Observed and simulated microbial biomass P (A) and soil labile Pi (B) during decomposition of plant residues in the Ultisol.

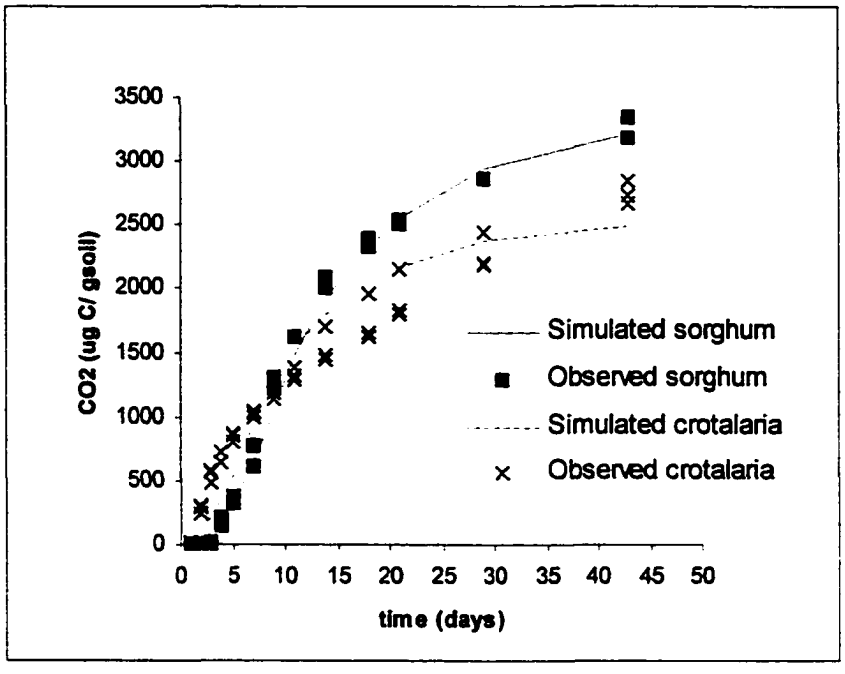


Figure 5. Observed and simulated cumulative CO_2 evolution during decomposition of plant residues in the Alfisol.

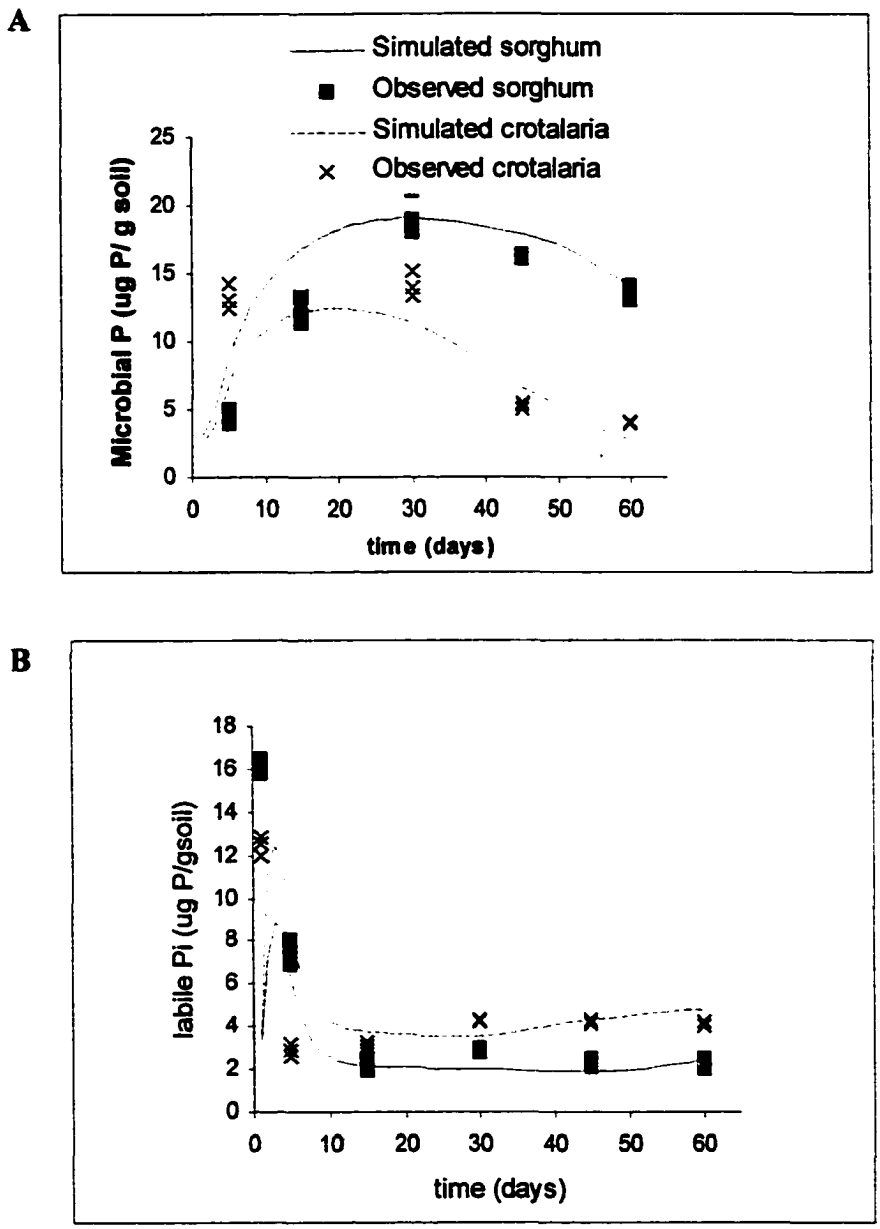
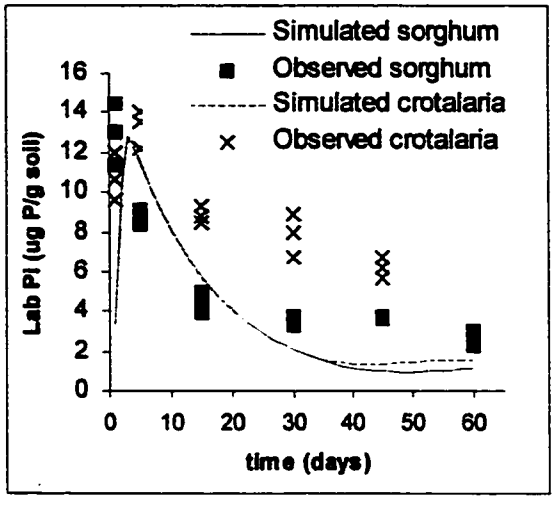
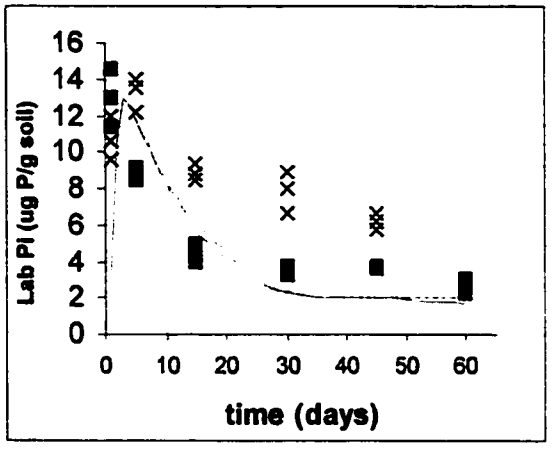


Figure 6. Observed and simulated microbial biomass P (A) and soil labile Pi (B) during decomposition of plant residues in the Alfisol.





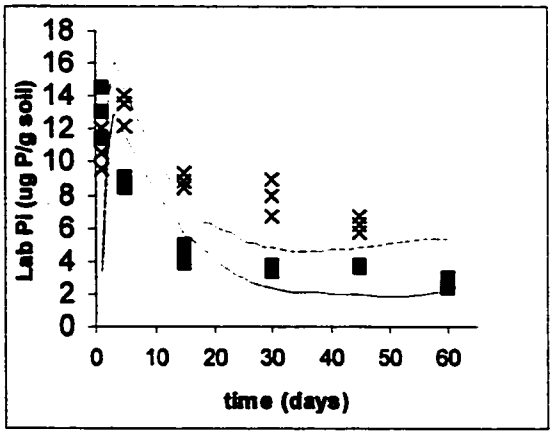


Figure 7. Predicted values of soil labile Pi when changing parameters related to: A) Specific P mineralization rates (Hypothesis I), B) content of plant inorganic P (Hypothesis II), and C) plant metabolic C & death rate (Hypothesis III) for the residue treatments in the Ultisol.

APPENDIX

A.- State variables and flow parameters used for simulating dynamics of the plant residues in the Ultisol.

B.- Fortran code utilized for simulation model.

C.- Phosphate adsorption isotherms for treatments of plant residue incorporation in the Ultisol and Alfisol.

Table A1. Initial values of soil and plant state variables utilized for the Ultisol

State Variable	Residue	Element	Value	Description of state variable
met	sorg	C	2635	Plant metabolic C (ug C/g soil)
str	sorg	C	6365	Plant structural C (ug C/g soil)
str	sorg	N	60.0	Plant structural N (ugN/g soil)
met	sorg	N	390.0	Plant metabolic N (ugN/g soil)
met	sorg	P	18.0	Plant inorganic P (ugP/g soil)
str	sorg	P	12.0	Plant organic P (ugP/g soil)
micr	sorg	C	60.0	Microbial biomass C (ugC/g soil)
micr	sorg	N	5.0	Microbial biomass N (ugN/g soil)
micr	sorg	P	2.0	Microbial biomass P (ugP/g soil)
lab	sorg	C	150.0	Labile soil organic C (ugC/g soil)
lab	sorg	N	10.0	Labile soil organic N (ugN/g soil)
lab	sorg	P	2.0	Labile soil organic P (ugP/g soil)
stab	sorg	C	4800	Stable soil organic C (ugC/g soil)
stab	sorg	N	565.0	Stable soil organic N (ugN/g soil)
stab	sorg	P	8.0	Stable soil organic P (ugP/soil)
inor	sorg	C	0.1	Initial Inorganic C
inor	sorg	N	20.0	Inorganic N in soil solution (ugN/g soil)
inor	sorg	P	0.5	Inorganic P in soil solution (ugP/g soil)
solPo	sorg	P	0.5	Organic P in solution (ugP/g soil)
labPi	sorg	P	2.4	Resin extractable Pi (ugP/g soil)
sadPi	sorg	P	8.0	Pi-NaOH (ugP/g soil)
met	crot	C	2250	Same as described for sorghum residue
str	crot	C	6750	(See above)
str	crot	N	60.0	
met	crot	N	390.0	
met	crot	P	14.0	
str	crot	P	16.0	
micr	crot	C	50.0	
micr	crot	N	5.0	
micr	crot	P	2.0	
lab	crot	C	150.0	
lab	crot	N	10.0	
lab	crot	P	2.0	
stab	crot	C	4800	
stab	crot	N	565.0	
stab	crot	P	8.0	
inor	crot	C	0.1	
inor	crot	N	20.0	
inor	crot	P	0.5	
solPo	crot	P	0.5	
labPi	crot	P	2.4	
sadPi	crot	P	8.0	

Table A2. Flow parameters used for each residue type in the Ultisol

SOURCE	DESTINATION	Flow descriptor [1]	Flow number
met sorg pl	micr sorg pl	6 9 0	1
2.5	Vmax metC (/day)		
700.0	Km metC (ugC/gsoil)		
0.7000E-01	lower soil water content (w/w)		
0.1000	low effect of water on decomposition		
0.2000	upper soil water (w/w)		
0.900	high effect of water on decomposition		
0.2000	soil water content		
######100			
str sorg pl	micr sorg pl	7 10 0	2
0.4000	Vmax strC (/day)		
0.1500E+05	Km strC (ugC/gsoil)		
5.000	lower CNmicr		
1.000	high effect of CNmicr		
20.00	upper CNmicr		
0.1000	low effect of CNmicr		
5.000	lower CPmicr		
1.000	high effect of CPmicr		
100.0	upper CPmicr		
0.1000	low effect of CPmicr		
######100			
str sorg pl	stab sorg pl	5 2 0	3
5.000	percentage of clay		
0.1000	stabilization coeficient for C and N at 5< %clay <33		
str sorg pl	stab sorg pl		
######100			
lab sorg pl	micr sorg pl	8 2 0	4
0.6000	Vmax C labile		
700.0	Km C labile		
######100			
micr sorg pl	inor sorg pl	9 4 0	5
0.6000	yield coeficient for met C use (ugCassimilated/ugC consumed)		
0.4000	yield coeficient for structural C use (ugC assimilated/ugC consumed)		
0.5000	yield coeficient for labile C use (ugC assimilated/ ugC consumed)		
0.10110	maintenance respiration rate (ugC respired/(ugCm.d))		

[1] Three digits flow descriptor: First digit indicates flow equation number as referred in FORTRAN code for calculating the rate of processes (Appendix B). Second digit indicates parameter number used in the flow equation.

```
micr   sorg     pl     lab    sorg     pl      10 3 0  6
   0.125                specific death rate at the beginning (ug C/(ugCm.d))
   0.7000               partitioning microbial unsoluble Po/total Po
       0.5000    0.80000
        ######100
   micr   sorg     pl     solPo   sorg     pl      11 0 0  7
      ######100
   lab    sorg     pl     stab    sorg     pl      12 2 0  8
    5.000               percentage of clay
    0.3700E-02          slope of function clay-stabilization fraction
      ######100
   inor   sorg     pl     micr    sorg     pl      13 9 0  9
    0.5000E-01          Vmax for microbial Ni uptake (day-1)
     5.000              Km for microbial Ni uptake (ugN/g soil)
    5.000               minimum microbial C:Nratio
    10.00               microbial C:N at which Ni uptake is reduced
    5.000               minimum microbial C:P ratio
    25.00               microbial C:P at which Pi uptake is reduced
    0.01234             Vmax for microbial Pi uptake (day-1)
    0.5000E-02          Km for microbial Pi uptake (ugP/g soil)
      0.                Pi threshold for microbial Pi uptake (ugP/g soil)
      ######100
   str    sorg     pl     solPo   sorg     pl      14 2 0  10
   0.5000E-01            rate  of release of strPo from plant to soil solution
(ugPo/(ugPo.ugCm.d)
    0.1000E-05          threshold str P for decomposition
      ######100
   str    sorg     pl     stab    sorg     pl      15 2 0  11
    5.000       percentage of clay
    0.6000E-02          slope for function clay-stab strPo at 5<%clay<33
      ######100
   str    sorg     pl     inor    sorg     pl      16 1 0  12
     11.52              mineralization rate of strPo(ugPo/(ugPo.day))
      ######100
   solPo  sorg     pl     inor    sorg     pl      17 1 0  13
    20.40               mineralization rate of solPo ( ugP/(ugPo.day))
      ######100
   lab    sorg     pl     inor    sorg     pl      18 2 0  14
    0.1000              N mineralization rate of labNo (ugNo/(ugNo.d))
    0.356E-1            P mineralization rate of labPo ( ugPo/(ugPo.day))
      ######100
   stab   sorg     pl     inor    sorg     pl      19 1 0  15
    0.265E-02           P mineralization rate of stab Po (ug Po/(ugPo.day))
      ######100
   inor   sorg     pl     labPi   sorg     pl      20 1 0  16
```

```
 1.000              simple rate constant for flow solPi to labPi
   ######100
labPi  sorg    pl     inor   sorg    pl     21 3 0  17
 0.497E-01          simple rate constant for flow lab-sol
   ######100
labPi  sorg    pl     sadPi  sorg    pl     22 1 0  18
 0.2E-01            simple rate constant for flow labPi to sadPi
   ######100
sadPi  sorg    pl     labPi  sorg    pl     23 1 0  19
 0.1050E-03         simple rate constant for flow sadsPi to labPi
   ######100
met    sorg    pl     inor   sorg    pl     24 1 0  20
0.15                specific rate of transfer of Pi from metabolic to the soil solution Pi
       0.05     1.0
   ######100
met    crot    pl     micr   crot    pl     6 9 0   1

 2.5                Vmax metC (/day)
 700.0              Km metC (ugC/gsoil)
 0.7000E-01         lower soil water content
 0.1000             low effect of water on decomposition
 0.2000             upper soil water
 0.900              high effect of water on decomposition
 0.2000             soil water content
 2.55               Initial Vmax
 0.846E-04          Final Vmax
   ######100
str    crot    pl     micr   crot    pl     7 10 0  2
 0.5000             Vmax strC (/day)
0.1500E+05          Km strC (ugC/gsoil)
 5.000              lower C:Nmicr
 1.000              high effect of C:Nmicr
 20.00              upper C:Nmicr
 0.1000             low effect of C:Nmicr
 5.000              lower C:Pmicr
 1.000              high effect of C:Pmicr
 100.0              upper C:Pmicr
 0.1000             low effect of C:Pmicr
   ######100
str    crot    pl     stab   crot    pl     5 2 0   3
 5.000              percentage of clay
 0.1000             stabilization coefficient for C and N at 5< %clay <33
   str    crot    pl     stab   crot    pl
   ######100
lab    crot    pl     micr   crot    pl     8 2 0   4
```

```
  0.6000              Vmax C labile (/day)
   700.0              Km C labile (ugC/gsoil)
     ######100
  micr   crot    pl     inor   crot    pl      9 4 0  5

  0.6000              yield coefficient for met C use (ugCassimilated/ugC consumed)
 0.3000               yield coefficient for structural C use (ugC assimilated/ugC consumed)
  0.4000              yield coefficient for labile C use (ugC assimilated/ ugC consumed)
  0.10110             maintenance respiration rate (ugC respirated/(ugCm.d))
     ######100
  micr   crot    pl     lab    crot    pl      10 3 0  6
   0.200              specific death rate at the begining (ug C/(ugCm.d))
   0.7000             partitioning microbial unsolublePo/total Po
        ######100
  micr   crot    pl     solPo  crot    pl      11 0 0  7
     ######100
  lab    crot    pl     stab   crot    pl      12 2 0  8
    5.000             percentage of clay
   0.3700E-02         slope of function clay-stabilization fraction
     ######100
  inor   crot    pl     micr   crot    pl      13 9 0  9
   0.5000E-01         Vmax for microbial Ni uptake (day-1)
    5.000             Km for microbial Ni uptake (ugN/g soil)
    5.000             minimum microbial C:Nratio
    10.00             microbial C:N at which Ni uptake is reduced
    5.000             minimum microbial CP ratio
    15.00             microbial C:P at which Pi uptake is reduced
    0.01034           Vmax for microbial Pi uptake (day-1)
   0.5000E-02         Km for microbial Pi uptake (ugP/soil)
     0.               Pi threshold for microbial Pi uptake
     ######100
  str    crot    pl     solPo  crot    pl      14 2 0  10
   0.5000E-01          rate of release of strPo from plant to soil solution
(ugPo/(ugPo.ugCm.d)
   0.1000E-05         threshold str P
     ######100
  str    crot    pl     stab   crot    pl      15 2 0  11
    5.000             percentage of clay
   0.6000E-02         slope for function clay-stab strPo at 5<%clay<33
     ######100
  str    crot    pl     inor   crot    pl      16 1 0  12
   26.57              mineralization rate of strPo (ugPo/(ugPo.day))
     ######100
  solPo  crot    pl     inor   crot    pl      17 1 0  13
   47.05              mineralization rate of solPo ( ugP/(ugPo.day))
```

```
   ######100
lab   crot    pl     inor   crot    pl      18 2 0  14
 0.1000              N mineralization rate of labNo (ugNo/(ugNo.day))
 0.555E-01           P mineralization rate of labPo ( ugPo/(ugPo.day))
   ######100
stab  crot    pl     inor   crot    pl      19 1 0  15
 0.133E-02           P mineralization rate of stab Po (ug Po/(ugPo.day))
   ######100
inor  crot    pl     labPi  crot    pl      20 1 0  16
  1.000              simple rate constant for flow solPi to labPi
   ######100
labPi crot    pl     inor   crot    pl      21 3 0  17
  0.446E-01          simple rate constant for flow lab-sol solution Pi
   ######100
labPi crot    pl     sadPi  crot    pl      22 1 0  18
 0.1948E-01          simple rate constant for flow labPi to sadPi
   ######100
sadPi crot    pl     labPi  crot    pl      23 1 0  19
 0.1342E-03          simple rate constant for flow sadsPi to labPi
   ######100
met   crot    pl     inor   crot    pl      24 1 0  20
0.1500              specific rate of transfer of Pi from metabolic to the soil solution Pi
    end end end end end end 1 0 0          end  end  end  end  end  end  0 0 0

      12 read(11,*,end=99)chfgfr,chsgfr,chplfr
       1             ,chfgto,chsgto,chplto,
       2       ftyp(fln),npm(fln),nres(fln)
```

APPENDIX B. Fortran code for rates of processes

```
  1    continue
c...intermediate variables

c      intv(ba,sg1,hipz,1) = sv(1,ba,sg1,hipz,N)/sv(1,ba,sg1,hipz,C)
       return
c  do not go to 300
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
  2    continue
c...constant flow
       flo(1,idfl,1) = parmfl(1,idfl)
c      flo(1,idfl,1) = driver(1)
       go to 300

c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
  3    continue
c...linear donor control
       flo(1,idfl,1) = parmfl(1,idfl)*sv(1,fgf,sgf,plf,1)
       go to 300

c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
  4    continue
c...Michaelis-Menten
       flo(1,idfl,1) = parmfl(1,idfl)*sv(1,fgf,sgf,plf,1)*
    1 sv(1,fgt,sgt,plt,1) /
    1  (parmfl(2,idfl) + sv(1,fgf,sgf,plf,1))
       go to 300

c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
  5    continue
c...C and N flow from structural to the stable pool
       flo(1,idfl,1)=parmfl(2,idfl)*flo(1,idfl-1,1)
       flo(1,idfl,2)=parmfl(2,idfl)*flo(1,idfl-1,2)

       go to 300

c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
  6  continue
c...C and N flow from metabolic pool to microbes
c      EW=tble(parmfl(7,idfl),2,parmfl(3,idfl))
       EW=sinsig(parmfl(7,idfl),parmfl(3,idfl),parmfl(5,idfl))
       Vmax=parmfl(8,idfl)-(parmfl(8,idfl)-parmfl(9,idfl))*time/60.
       flo(1,idfl,1)=Vmax*sv(1,met,sgf,1,1)*
    1  sv(1,micr,sgt,1,1)*EW/(parmfl(2,idfl)+sv(1,met,sgf,1,1))
```

```
      NCmet=sv(1,met,sgf,1,2)/sv(1,met,sgf,1,1)
      intv(met,sgf,1,N)=NCmet
     if(flo(1,idfl,1).lt.0.)flo(1,idfl,1)=0
      flo(1,idfl,2)=flo(1,idfl,1)*NCmet
      go to 300

c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
   7  continue
c...C and N flow from structural to microbes
      CNmicr= sv(1,micr,sgf,1,1)/sv(1,micr,sgf,1,2)
      intv(micr,sgf,1,N)=CNmicr
      CPmicr=sv(1,micr,sgf,1,1)/sv(1,micr,sgf,1,3)
      intv(micr,sgf,1,P)=CPmicr
c     ECN=tble(intv(micr,1,1,N),2,parmfl(3,idfl))
      ECN=1-sinsig(intv(micr,sgf,1,N),parmfl(3,idfl),parmfl(5,idfl))
c     ECP=tble(intv(micr,1,1,P),2,parmfl(7,idfl))
      ECP=1-sinsig(intv(micr,sgf,1,P),parmfl(7,idfl),parmfl(9,idfl))
      flo(1,idfl,1)=parmfl(1,idfl)*sv(1,str,sgf,1,1)*
    1 sv(1,micr,sgt,1,1)*
    1 (EW*ECN*ECP)**0.33/(parmfl(2,idfl)+sv(1,str,sgf,1,1))
     NCstr= sv(1,str,sgf,1,2)/sv(1,str,sgf,1,1)
      intv(str,sgf,1,N)=NCstr
      flo(1,idfl,2)=flo(1,idfl,1)*NCstr

      go to 300
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
   8  continue
c...C and N flow from labile to microbes
     flo(1,idfl,1)=parmfl(1,idfl)*sv(1,lab,sgf,1,1)*
    1 sv(1,micr,sgt,1,1)*
    1 (ECN*EW*ECP)**0.3/(parmfl(2,idfl)+sv(1,lab,sgf,1,1))
      NClab=sv(1,lab,sgf,1,2)/sv(1,lab,sgf,1,1)
      intv(lab,sgf,1,N)=NClab
      flo(1,idfl,2)=flo(1,idfl,1)*NClab

      go to 300
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
   9  continue
c...C and N flow from microbes to inor calculated as the product of the
c...previously defined flows between C sources-microbes and (1-yield)
      flo(1,idfl,1)=(1-parmfl(1,idfl))*flo(1,idfl-4,1)+
    1 (1-parmfl(2,idfl))*flo(1,idfl-3,1)+(1-parmfl(3,idfl))*
    1 flo(1,idfl-1,1)+parmfl(4,idfl)*sv(1,micr,sgf,1,1)
      flo(1,idfl,2)=flo(1,idfl,1)/intv(micr,sgf,1,N)
```

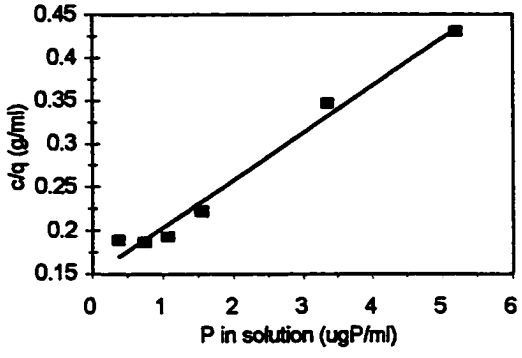
```
      go to 300
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
  10  continue
c...C,N and P flows from microbes to labile pool as a function of
c...microbial biomass and the specific rate of death

      death=parmfl(1,idfl)-(parmfl(1,idfl)-parmfl(3,idfl))*time/60.
      flo(1,idfl,1)=sv(1,micr,sgf,1,1)*death
      flo(1,idfl,2)=flo(1,idfl,1)/intv(micr,sgf,1,N)
      flo(1,idfl,3)=flo(1,idfl,1)*parmfl(2,idfl)/intv(micr,sgf,1,P)

      go to 300
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
  11  continue
c...flow of Po from microbes to soil solution Poas a function of Po flow
c...from microbes  minus the P flow from microbes to labPo
      flo(1,idfl,3)=flo(1,idfl-1,1)/intv(micr,sgf,1,P)-flo(1,idfl-1,3)

      go to 300
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
  12  continue
c...C,N and P stabilization of labile pool
      flo(1,idfl,1)=flo(1,idfl-2,1)*(parmfl(1,idfl)*parmfl(2,idfl)+
     1 0.007)
      flo(1,idfl,2)=flo(1,idfl,1)/intv(micr,sgf,1,N)
      flo(1,idfl,3)=flo(1,idfl-2,3)*0.7

      go to 300
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
  13  continue
c...Microbial Ni and Pi uptake from the inorganic pool
      flo(1,idfl,2)=parmfl(1,idfl)*sv(1,inor,sgf,1,2)*
     1 sv(1,micr,sgt,1,1)/
     1  (parmfl(2,idfl)+sv(1,inor,sgf,1,2))
      if(intv(micr,sgf,1,N).le.parmfl(4,idfl))then
         enc = (intv(micr,sgf,1,N)-parmfl(3,idfl))/
     1       (parmfl(4,idfl)-parmfl(3,idfl))
         if(enc.lt.0.)enc=0.
         if(enc.gt.1.)enc=1.
      flo(1,idfl,2)= enc *flo(1,idfl,2)
                                          endif
      flo(1,idfl,3)=parmfl(7,idfl)*(sv(1,inor,sgf,1,3)- parmfl(9,idfl))
     1         *sv(1,micr,sgt,1,1)/ (parmfl(8,idfl) - parmfl(9,idfl)
     1                  +sv(1,inor,sgf,1,3) - parmfl(9,idfl))
```

```
      if(flo(1,idfl,3).lt.0.)flo(1,idfl,3)=0

      go to 300
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
   14 continue
c...Po release from strP to solPo as a function of specific rate of rele
c...release, plPo and microbial C
      flo(1,idfl,3)=parmfl(1,idfl)*(sv(1,str,sgf,1,3)-parmfl(2,idfl))
     1            *sv(1,micr,sgf,1,1)

      if(flo(1,idfl,3).lt.0.)flo(1,idfl,3)=0

      go to 300

c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
   15 continue
c...stabilization of strPo as a function of the flow strPo TO solPo mul
c...tiplied by a stabilization coefficient
      flo(1,idfl,3)=flo(1,idfl-1,3)*
     1      (parmfl(1,idfl)*parmfl(2,idfl)+0.05)

      go to 300
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
   16 continue
c...P flow from strPo to soil solution Pi as a function of mineralization

c...rate (r1), strPo, diesterase and a red. factor according swc
      if(sgf.eq.1)drv=driver(2)
      if(sgf.eq.2)drv=driver(4)
      flo(1,idfl,3)=parmfl(1,idfl)*sv(1,str,sgf,1,3)*drv*EW
      if(flo(1,idfl,3).lt.0.)flo(1,idfl,3)=0

      go to 300

c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
   17 continue
c...P flo from solPo to soil solution Pi as a function of mineralization
c...rate (r2), sol Po, diesterase and swc factor
      if(sgf.eq.1)drv=driver(2)
      if(sgf.eq.2)drv=driver(4)
      flo(1,idfl,3)=parmfl(1,idfl)*sv(1,solPo,sgf,1,3)*drv*EW
      if(flo(1,idfl,3).lt.0.)flo(1,idfl,3)=0
c       if(flo(1,idfl,3).lt.0.01*sv(1,inor,sgf,1,3))flo(1,idfl,3)=0.
      if(sv(1,solPo,sgf,1,3).lt.0.001)flo(1,idfl,3)=0.
```

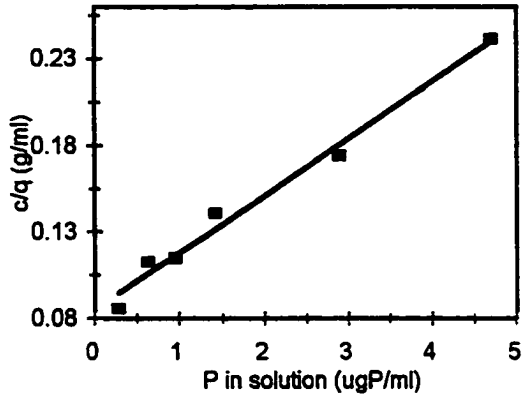
```
      go to 300
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
   18 continue
c...N and P mineralization of labile organic pool
c...N mineralization of lab No as a function of N mineralization rate,
c...labNo and a red. factor according to swc
      flo(1,idfl,2)=parmfl(1,idfl)*sv(1,lab,sgf,1,2)*EW
c...P mineralization of labPo as a function of P mineralization (r3),
c...labPo and a reduction factor according to swc
      if(sgf.eq.1)drv=driver(2)
      if(sgf.eq.2)drv=driver(4)
      flo(1,idfl,3)=parmfl(2,idfl)*sv(1,lab,sgf,1,3)*drv*EW
      if(flo(1,idfl,3).lt.0.)flo(1,idfl,3)=0

      go to 300
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
   19 continue
c...P mineralization of stabPo as a function of P mineralizatio rate (r4)
c...stabPo, monoesterase and a reduction factor according swc
      if(sgf.eq.1)drv=driver(1)
      if(sgf.eq.2)drv=driver(3)
      flo(1,idfl,3)=parmfl(1,idfl)*sv(1,stab,sgf,1,3)*drv*EW

      go to 300
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
   20 continue
c...P flow from solution Pi to labile Pi
      flo(1,idfl,3)=sv(1,inor,sgf,1,3)*parmfl(1,idfl)
      if(flo(1,idfl,3).lt.0.)flo(1,idfl,3)=0

      go to 300
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
   21 continue
c...P flow from labile Pi to solution Pi
      flo(1,idfl,3)=parmfl(3,idfl)*sv(1,labPi,sgf,1,3)
      if(flo(1,idfl,3).lt.0.)flo(1,idfl,3)=0

      go to 300
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
   22 continue
c...P flow from labPi to sadPi
      flo(1,idfl,3)=sv(1,labPi,sgf,1,3)*parmfl(1,idfl)

      go to 300
```

```
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/

  23 continue
c... P flow from strongly adsorbed Pi to lab Pi
      flo(1,idfl,3)=sv(1,sadPi,sgf,1,3)*parmfl(1,idfl)

      go to 300
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
  24 continue
c...Pi flow from metabolic pool to the soil solution Pi
      flo(1,idfl,3)=sv(1,met,sgf,1,3)*parmfl(1,idfl)
      if(flo(1,idfl,3).lt.0.)flo(1,idfl,3)=0

      go to 300
c/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/\/
 300  continue

      do 301 jj=1,nelm
 301  call dxdt(fgf,sgf,plf,fgt,sgt,plt,idfl,jj)

      return
      end
```

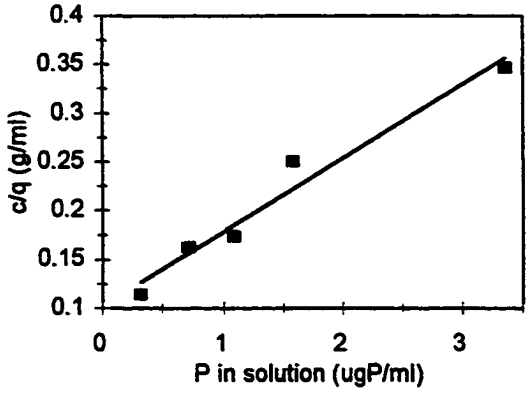
APPENDIX C



A
Maximum sorption capacity:
18.3 μgP g^{-1} soil
Sorption affinity: 0.37 ml g^{-1} soil
P sorbed at 0.2 μgP ml^{-1}:
1.3 μgP g^{-1} soil
r^2: 0.98

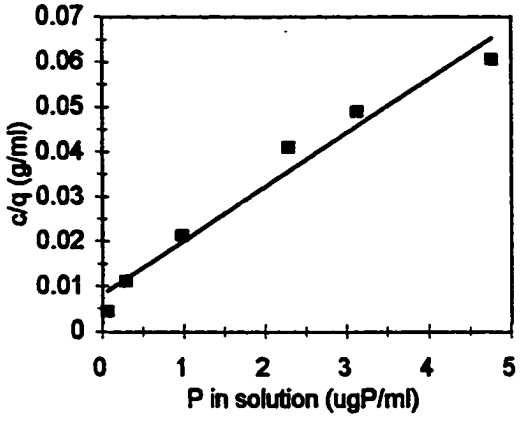


B
Maximum sorption capacity:
30.3 μgP g^{-1} soil
Sorption affinity: 0.38 ml g^{-1} soil
P sorbed at 0.2 μgP ml^{-1}:
2.1 μgP g^{-1} soil
r^2: 0.98

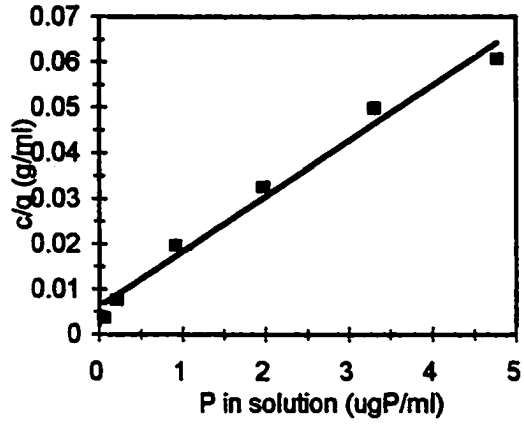


C
Maximum sorption capacity:
13.3 μgP g^{-1} soil
Sorption affinity 0.73 ml g^{-1} soil
P sorbed at 0.2 μgP ml^{-1}:
1.7 μgP g^{-1} soil
r^2: 0.96

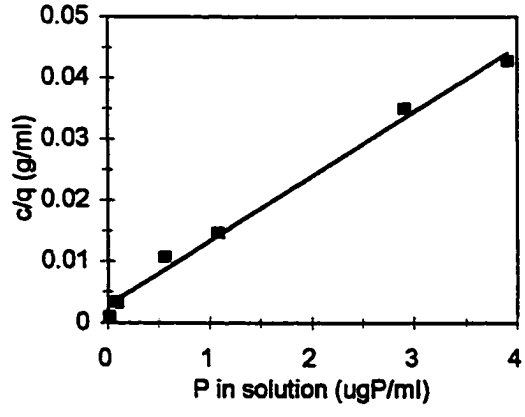
Langmuir isotherms for phosphate adsorption and soil P adsorption parameters obtained 60 days after incorporation of A) sorghum residue, B) crotalaria residue, and C) control (without residues) in the Ultisol.



A
Maximum sorption capacity:
83.2 μgP g^{-1} soil
Sorption affinity: 1.45 ml g^{-1} soil
P sorbed at 0.2 μgP ml^{-1}:
18.8 μgP g^{-1} soil
r^2: 0.97



B
Maximum sorption capacity:
82.1 μgP g^{-1} soil
Sorption affinity: 1.90 ml g^{-1} soil
P sorbed at 0.2 μgP ml^{-1}:
22.0 μgP g^{-1} soil
r^2: 0.98



C
Maximum sorption capacity:
94.1 μgP g^{-1} soil
Sorption affinity: 3.71 ml g^{-1} soil
P sorbed at 0.2 μgP ml^{-1}:
40.2 μgP g^{-1} soil
r^2: 0.99

Langmuir isotherms for phosphate adsorption and soil P adsorption parameters obtained 60 days after incorporation of A) sorghum residue, B) crotalaria residue and C) control (without residues) in the Alfisol.