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THE EFFECT OF TILLAGE ON PHOSPHORUS TRANSFORMATIONS IN SOILS

By

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ABSTRACT

THE EFFECT OF TILLAGE ON PHOSPHORUS TRANSFORMATIONS IN SOILS

By

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Conservation tillage is becoming more widely accepted as an alternative tillage system in crop production. Organic matter and microbial activity may increase with no-tillage (NT) compared to conventional tillage (CT). Accumulation of organic matter and phosphorus (P) near the surface of soil occurs in NT soils which may affect the distribution of P pools in soils. The effect of NT and CT on soil P fractions was investigated, and the turnover rates of ^{33}P added in soybean residues to soils from three experimental sites in Michigan was determined. The role of microorganisms in the turnover of ^{33}P added in residues was also studied in a never tilled soil. The effect of microorganisms was separated by sterilizing the soil with gamma irradiation before adding the residues. Phosphorus transformations were compared in the sterilized and non-sterilized soil.

Soybean plants labeled with ^{33}P were added to soils, incubated at field capacity, extracted periodically (0, 6, 12, 18, 26, and 34 days), and P analyzed in

the different P fractions. The fractions extracted were resin, $NaHCO_3$, microbial biomass, NaOH, NaOH after sonication, HCl, and residue P fractions.

With NT inorganic P accumulated in the calcareous soil which received P fertilizers. NaOH extractable organic P (Po) concentrations were higher in the NT treatments in the two non-calcareous soils. There was no effect of tillage on the labile Po pool.

The largest fraction of the applied ^{33}P was found in the inorganic labile form. This fraction decreased with time with an increase in the NaOH fraction in all three soils. An increase in the HCl fraction occurred in the calcareous soil. Phosphorus cycling through the microbial pool was evident before more of the ^{33}P ended up in the NaOH fraction. The effect of tillage on the ^{33}P turnover was minimal in all the P fractions extracted including the microbial fraction.

Twenty percent of the ^{33}P was found in the microbial pool at day 12 in the non-sterilized never tilled soil. About 6% to 11% of the ^{33}P found in the NaOH fraction is suspected to be organic and have cycled through the microbial pool.

TO MY PARENTS, BROTHER, AND SISTERS

FOR ALL THEIR LOVE

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INTRODUCTION

Soil Phosphorus (P) primarily originated from the mineral apatite. As weathering progresses P that is solubilized is utilized by microorganisms and plants or reprecipitated as secondary P minerals. The organic P is returned to the soil as compounds that have a range of resistance to microbial attack. These various fractions are shown in Figure 1.

In the natural ecosystem, soils that develop under forest vegetation contain a thin surface layer very high in organic matter. The P fractions in this layer are dominated by the organic and biological fractions shown on the right side of Figure 1. Soil horizons deeper in the profile, for example a Bt horizon, are dominated by inorganic reactions involving precipitation and adsorption of P compounds shown in the left side of Figure 1.

The development of agricultural systems utilizing the plow, referred to as conventional tillage (CT), mixes the surface soil layer with the mineral layers of soil giving a plow layer that is uniform but a different environment as far as soil P is concerned. The plow layer is generally dominated by the inorganic soil P reactions.

Conservation tillage is becoming more widely used to reduce erosion in crop production systems (Sharpley and Smith, 1989). Conservation tillage can effectively reduce soil erosion, can conserve soil water for greater crop production, and can reduce inputs of petroleum fuel and labor for agriculture production (Doran, 1980). But increases pesticide use.

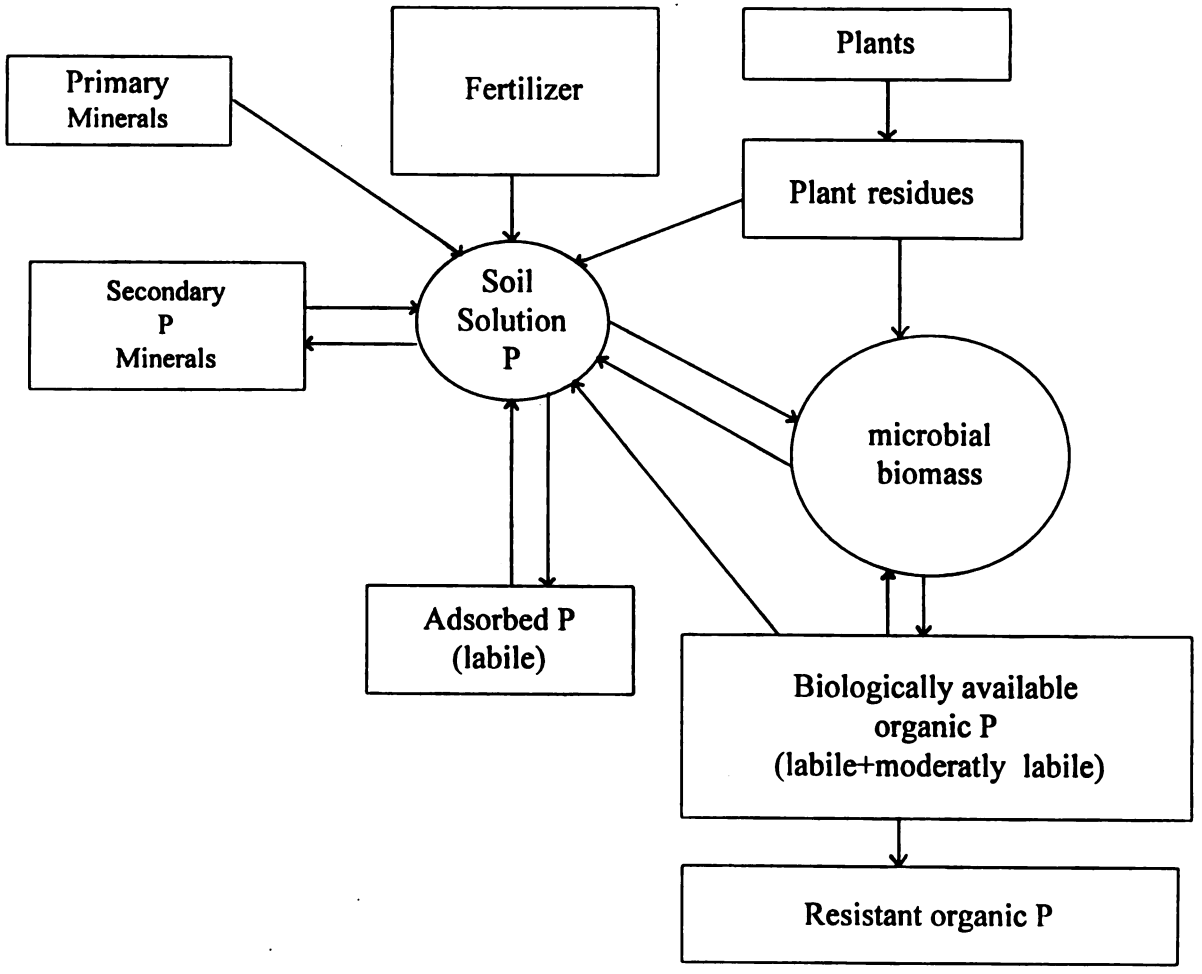

Figure1. The soil phosphorus cycle.

No-tillage (NT) is a type of conservation tillage in which the soil is left undisturbed prior to planting into a narrow seedbed. No-tillage promotes the accumulation of organic matter and nutrients at the surface layer of the soil, and often produces a decrease in the pH in the surface layers, especially when high rates of N fertilizers are applied.

Soil P is affected by tillage. Conventional tillage distributes the organic matter throughout the plow layer. On the other hand, with NT first P fertilizer applications are made to the surface or in a band close to the surface. Secondly, organic matter is deposited on the soil surface and not incorporated into the plow layer. Accumulation of soil P occurs in these surface layers. It has been reported that both inorganic and organic P levels increase in the surface layers of NT soils (Unger 1991, and Weil et al. 1988). This accumulation is believed to result in improved P availability due to less soil contact with soluble P and hence less soil fixation of P. It has been hypothesized that P cycling may be increased substantially in the surface layers of NT soils due to the increase in the microbiological activities in that layer. Higher microbiological activities were attributed to higher organic matter levels in NT soils (Weil et al. 1988).

The objectives of this study were to:

1. Determine if the adoption of no-tillage management practices altered the distribution of P fractions compared to conventional tillage in three sites in Michigan varying in chemical and physical properties.
2. Determine if the rate of transformation of P from ^{33}P labeled soybean residues added to soils is more rapid for no-till soils than for conventionally tilled soils
3. Determine if microbial activity enhanced the rate of transformation of added P into less labile P pools.

REVIEW OF LITERATURE

PHOSPHORUS CYCLE:

Phosphorus exists in nature in a variety of organic and inorganic forms. The concentration of P found in the soil solution may range from < 0.01 mg L^{-1} to 7-8 mg L^{-1} (Ellis, 1985). Inorganic forms of P occur in combination with Fe, Al, Ca, F, or other elements that are insoluble or very poorly soluble. Phosphorus solubility is complicated by common ion association and pH effects, and by the amount of P adsorbed on the surfaces of clay minerals (Paul and Clark, 1989). Octacalcium phosphate or ß-tricalcium phosphate form within two to three months of soluble P addition to calcareous soils and have low solubility. Iron phosphates are the more stable phase in strongly acid soils. Phosphorus is also adsorbed on clays and/or aluminum oxides/hydroxides and calcium carbonates. Adsorbed P is believed to be an intermediate phase in the P cycle and the crystallization of insoluble P compounds will remove P from the adsorbed phase (Ellis, 1985).

The very low level of P in soil solution and the necessity for its frequent renewal suggests that the transfer of soil organic matter P to inorganic P is primarily, but not exclusively, microbially mediated (Paul and Clark, 1989). Microorganisms are believed to play a major role in P cycling by both mineralizing and immobilizing P in the system, thereby affecting its availability for plant nutrition (Halstead and McKercher, 1975), e.g. the flux of P through the microbial biomass ranged from 13 to 26 kg P $ha^{-1}yr^{-1}$ in a dry tropical environment in India, indicating significant contribution to the P requirements of higher plants (Srivastava and Singh, 1991).

Buchanan and King (1992) found that microbial biomass P was generally greatest in the spring months followed by a significant decline in late spring and summer in a continuous maize and 2-year maize-wheat-soybean rotation agroecosystems under no-till and reduced chemical input management. A large proportion of the P from ^{33}P labeled medic residues was held in the microbial biomass even after 40 days of the residue application to a solonized brown loam soil containing ^{32}P labeled fertilizer and actively growing wheat plants (McLaughlin and Alston, 1986). A considerable proportion of the applied ^{32}P from fertilizer was incorporated into the biomass even in the absence of applied residues (McLaughlin and Alston, 1986). In a field experiment on a solonized brown loan soil with a pH of 8.3, McLaughlin et al. (1988b) found that the amounts of P in the microbial biomass in the soil were linearly related to soil water content. They also found that banding of P fertilizer decreases the amount of P from the fertilizer entering the microbial biomass. Most of the P taken up by the microbial biomass was derived from native soil P (i.e. not added that season).

McLaughlin et al.(1988c) conclude from a radio tracer study done on wheat pasture rotations on the same solonized brown soil that most of the fertilizer applied each year entered the soil inorganic P pool and only a small proportion of that year's application entered the wheat plants. Phosphorus held in the microbial biomass was four times that held in the crop and both plants and micro-organisms obtained the bulk of their P from the soil pool, emphasizing the importance of residual P (both organic and inorganic P) in crop nutrition (McLaughlin et al., 1988c).

CHEMICAL NATURE OF SOIL ORGANIC PHOSPHORUS:

The organic P content of soils varies considerably. This fraction may constitute from 20 to 80 % of the total P in the surface layer of soil (Dalal, 1977).

Despite extensive investigation, the chemical nature of organic P remains largely unidentified because soil organic phosphates are not discrete compounds and lengthy and complex analytical procedures are involved in characterizing organic P. Only about 50% to 70% of the organic P in soil has been identified (Stewart and McKercher, 1982). The compounds so far identified are the inositol phosphates, phospholipids, and nucleic acids (Dalal, 1977; Stevenson, 1982).

Inositol phosphates are esters of hexahydroxy benzene. The hexa phosphate ester, phytic acid, is the most common. Among the compounds identified, inositol phosphates constitute a large proportion of the soil organic P because they become stabilized through the formation of insoluble complexes with metal ions and other organic substances (Stevenson, 1982). Phospholipids are organic phosphate esters which are soluble in fat solvents. Their content in soils has a mean value of 1% (Anderson and Malcolm, 1974). Phosphoglycerides possibly form the dominant fraction of the soil phospholipids (Dalal, 1977). The phospholipids in soil may be contributed by plant debris, animal wastes, and microbial biomass and their synthesis and degradation is fairly rapid in soils. Nucleic acids such as ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) are produced in the soil during the decomposition of plant and animal residues by microorganisms (Stevenson, 1982).

TRANSFORMATION STUDIES:

Radio tracer techniques fall into two general categories involving either the addition of ^{32}P or ^{33}P-labeled organic material or compounds to the soil or the soil plant system (Blair and Boland, 1978; Dalal, 1979; Harrison, 1982a; McLaughlin et al. 1986,1988a) or isotope dilution of ^{32}P-labeled inorganic P (Till and Blair, 1978; Stewart and Hedley, 1980; Walbridge and Vitousek, 1987). The first allows accurate measurement of the mineralization of specific organic

substrates; the second can potentially be used to estimate mineralization of native soil organic P (Walbridge and Vitousek, 1987).

Isotopic Dilution Method

Walbridge and Vitousek (1987) used the isotope dilution technique to measure the mineralization of native soil organic P in acid organic soils from the North Carolina coastal plain. In this technique, $^{32}PO_4$ is used to label isotopically exchangeable soil P fractions. The release of unlabeled PO_4^{3-} from organic matter (the only P fraction assumed not labeled) dilutes the activity of $^{32}PO_4^{3-}$ in exchangeable forms and provides an estimate of the gross P mineralization. Regardless of the method used to label soils (adding an aliquot of carrier free $H_3{}^{32}PO_4$ and incubating at 25 ^oC or subjecting the soil to wetting-drying-mixing treatments after the $^{32}PO_4^{3-}$ addition) or the length of the equilibration period (up to 24 days), isotopic equilibrium was not achieved between the acid fluoride (AF) extractable and the $CHCl_3$-labile microbial PO_4-P (Pi) fractions. The $CHCl_3$-labile microbial inorganic P in their study was defined as the increase in AF-extractable Pi following an 18 hour $CHCl_3$ fumigation treatment. The net transfer of unlabeled PO_4 from soil organic matter through both extractable and microbial pools was estimated by using the isotope dilution in the sum of AF-extractable and $CHCl_3$ labile microbial Pi as this sum did not change significantly with time (Walbridge and Vitousek, 1987). With this technique they were able to detect a five-fold difference in P mineralization rates between two soils known to differ in P availability.

Addition of Labeled Organic Compounds:

Harrison (1982a) added ^{32}P labeled ribonucleic acid (RNA) to woodland soils and the amount of mineralization was determined, following the recovery of

^{32}P from soils and its partitioning into organic and inorganic forms. The rates of net mineralization of the [^{32}P]RNA in the 50 lake District woodland soils incubated for 24 hours at 13 ^{o}C ranged from -29 to 190 ng P cm^{-3} soil day^{-1} (equivalent to -0.75 to 5.7 μg P cm^{-3} soil $month^{-1}$). Negative values are attributable to a net microbial immobilization of the inorganic P present in the [^{32}P] RNA preparation. The rate of mineralization of labile organic P as indicated by the behavior of [^{32}P] RNA in the Lake District woodland soils is largely a function of soil pH and extractable Ca content, which increased 10 fold over the pH range of 3.1 to 7.9 (Harrison 1982b). There was also a strong interaction with the time of year. Mineralization was five times faster in spring than in autumn.

Addition of Labeled Organic Residues:

Phosphorus release from plant residues applied to the soil seems to depend on tissue type, age, and P nutritional status of plants (Chisholm et al. 1981), or perhaps more importantly on differences in the preparation and application of residues to the soil (Friesen and Blair, 1988). Friesen and Blair (1988) found 50 % of residue-derived P recovered from inorganic pools 11 days after incorporation while Blair and Boland (1978) recovered less than 1 % of the P in the soil inorganic fraction from plant material added 12 days earlier. Till and Blair (1978) and Dalal (1979) recovered about 5 % of the residue P at 14 days after addition. Blair and Boland (1978) and Till and Blair (1978) prepared their residues for application by cutting the labeled plant tissues into pieces less than 1 cm long, whereas Friesen and Blair (1988) finely ground the residues in a hammer mill prior to addition, an action which is likely to macerate cell walls and facilitate rapid release of the soluble P components to the soil solution.

The P content of plant material is an important factor for the mineralization - immobilization of soil P. Blair and Boland (1978) could not ascribe the greater

mineralization in the high P soil to either the P content of the soil or the P content of the added plant material alone. Fuller et al. (1956) reported that the P was more readily available from residues high in total P than from residues low in total P. Fuller et al (1956) also found that immobilization of soil P occurred if the added plant residues contained less than 0.2 % P. Arsjad and Giddens (1960) reported that under wetting and drying cycles the addition of soybean leaves resulted in a higher release of carbon dioxide from soil than when soybean stems were added. The reason being that the stem material, being a supporting material, would be high in structural carbohydrate (high in C) and low in protein (low in N and P). This would result in a wide C to P ratio in the stems, and as a result the mineralization rate would be lower.

Blair and Boland (1978) studied the release of ^{32}P from white clover plant residues in the presence and absence of growing oat plants in both low and high P status soils. Net reutilization of P from the added plant material after 48 days was highest in the high P system in the presence of plants (29.3%) and least in the low P system in the absence of plants (0.6%). The presence of plants did not significantly change the soil organic P levels, but there was a significant decline in the soil inorganic P in both soils when plants were present. Evidence from the soil inorganic P data suggests that the addition of plant material resulted in a significant immobilization of soil P only in the low P soil in the absence of plants. This is in contrast to the results of Enwezor (1976) who found that the degree of P immobilization increased after addition of organic matter to a soil with higher available P.

A close linear relationship was found between the ^{32}P recovery in the plant material and the plant P uptake (Blair and Boland, 1978). They concluded that the higher the P uptake by plants, the less inorganic P there will be in solution that will be subject to chemical fixation or microbial immobilization.

Friesen and Blair (1988) found, however, that cropping had no effect on the rates of release of P from crop residues. Simultaneous use was made of ^{32}P-labeled plant residues and ^{33}P-labeled soils to separate the effect of mineralization and immobilization in soils. Movement of ^{32}P and ^{33}P phosphate between various pools in the soil was determined as a function of time in the presence and absence of growing plants. The total activity of ^{32}P in the organic pool in the presence of plants closely followed that in their absence, indicating that plants had no significant effect on the rate of mineralization. In the same experiment, about 47 % of the added ^{32}P in plant residues was found in the organic pool, while 68 % was found in the four inorganic fractions (soluble P, Al-P, Fe-P, and Ca-P) according to Chang and Jackson (1957) fractionation scheme. Recovery was 115% suggesting inclusion of some organic P in the NaOH extracts. The Al-P (extracted with NH_4F solution) was more labile and available for absorption by plant roots while Fe-P (extracted with NaOH solution) was non labile, that is, not available for plants absorption. The presence of growing plants caused the amount of ^{32}P in the Al-P pool to decline markedly indicating that this is a labile pool, while ^{32}P entering the Fe-P was marginally reduced.

The relative contribution of plant residues and fertilizer to the P nutrition of wheat in a pasture/cereal system was examined by McLaughlin and Alston (1986) in a growth chamber and by McLaughlin et al. (1988a) in the field. In both studies ^{33}P labeled medic residues and ^{32}P labeled fertilizer were added to a solonized brown soil of pH 8.3 and wheat plants were grown. In the growth chamber experiment, 18.1% and 19.1% of the ^{32}P and the ^{33}P applied had entered the wheat plants after 34 days growth, while in the field experiment 11.6% and 5.4%, respectively, of the applied ^{32}P and ^{33}P had entered the wheat plants. This was related to lower soil temperatures and less moisture in the field.

Addition of ^{33}P labeled residues to soils depressed wheat dry weight, ^{31}P and ^{32}P (from fertilizer) uptake by the plant, while simultaneously increasing amounts of ^{31}P and ^{32}P incorporated into the microbial biomass (McLaughlin and Alston, 1986). Contribution of medic residues to the P nutrition of wheat plants was about one-fifth of that contributed by the fertilizer in the growth chamber experiment while the uptake of ^{33}P by wheat from plant residues in the fields was less than one third of that found in the growth chamber, despite the P concentration of the residues used in the field experiment being almost three times greater.

In the field experiment (McLaughlin et al. 1988a), native soil P (i.e. P not added that season) was the major contributor to the P nutrition of the plants. It is unlikely that P derived from pasture residues will contribute significantly to the nutrition of the first succeeding wheat crop if the Pi status of a soil is moderate to high. The ^{32}P data in the same experiment demonstrated that the fertilizer made a significant contribution to the P uptake of the wheat plants, even though it was added to a localized layer near the soil surface.

In summary, research has shown the transformation of P in added residues is affected by soil chemical properties, type of organic residues, and the presence of growing plants. Although microorganisms are presumed to affect residue decomposition and P transformations, little direct evidence exist to support this conclusion.

CONSERVATION vs CONVENTIONAL TILLAGE CROPPING SYSTEMS:

The conservation tillage information system center defines conservation tillage as any tillage and planting system that maintains at least 30% of the soil surface covered by residue after planting. No tillage is a type of conservation tillage in which the soil is left undisturbed prior to planting in a narrow seedbed, weed control being accomplished primarily with herbicides. Conventional tillage

refers to the combined primary and secondary tillage operations normally performed in preparing a seedbed, having essentially no plant residue left on the soil surface (Mannering et al., 1987). Reduced tillage is a means of reducing soil erosion losses, conserving soil water for greater crop production and reducing inputs of petroleum fuel and labor for agriculture production (Doran, 1980). No-till management systems reduce the risk of wind and water erosion thus reducing the risk of P movement from soils to surface waters (Harrison, 1985, Sharpley et al. 1993). Dissolved P concentration of runoff from no-till practices may be greater, however, than from conventional practices as a result of P accumulation on the surface (Sharpley et al. 1993, Ellis et al. 1985). But total P losses will be reduced by no-till.

Changes in the Soil Environment:

The physical, chemical, and biological soil environment for reduced or no-till farming differs greatly from that of conventional tillage (Doran, 1980). Eliminating plowing and therefore minimizing disturbance to soil organisms should lead to nutrient conservation through enhanced microbial immobilization of nutrients during decomposition resulting in more gradual nutrient release to provide long term fertility of the soil (Stinner et al. 1984).

No-till cropping often promotes the development of stratified pH and other nutrients (Eckert, 1991). Organic matter may accumulate in soils under no-till management, but it also may stratify, yielding high organic levels at the soil surface. Phosphorus tends to be higher in the surface layers of no-tilled soils. This stratification is believed to result in improved P availability due to less soil contact with soluble P and hence less soil fixation of P.

No-till treatment accumulated NH_4HCO_3-DTPA extractable P in the surface relative to stubble mulch and plow treatments (Follet and Peterson, 1988). Bray-

Kurtz P1 concentrations with moldboard plowing were more uniform throughout the 20 cm tillage management zone than for no-tillage (Karlen et al. 1991). Triplett and Van Doren(1969) found that most of the P fertilizer applied to the soil surface of no-tillage treatments remained in the surface 2.5 cm of soil. Equal annual applications of P resulted in more available P accumulation in the upper 5 cm of the untilled soil compared to the conventionally tilled soil (Shear and Moschler, 1969). Direct drilling resulted in an increased concentration of extractable P in the surface 0 to 5 cm of soil compared with moldboard plowing due both to the addition of fertilizer to the surface and to the decomposition of plant residues on the soil surface (Ellis and Howse, 1980/1981). Organic matter and acid soluble P were higher in the 0-15 cm soil layer of no tillage plots than on conventionally tilled plots after 9 years of continuous corn (Moschler et al. 1972). Greater concentrations of organic C and Bray-Kurtz P1 extractable P were found in the surface 5 cm than deeper in the sampled profile of a no-till soil (Eckert, 1991). No-tillage increased soil organic matter and $NaHCO_3$ extractable P concentrations in the 0 to 2 cm surface layer in a no-till soil relative to that in a conventional tillage soil (Unger, 1991). Organic C was significantly higher for NT soils in the 0 to 2 cm layer than in CT soils and generally higher in the upper 8 cm of soil (Weil et al. 1988). Total and dilute acid extractable P were higher in the 0 to 2 cm layer of NT plots; however, P levels dropped sharply under NT with depth compared to the more uniform distribution of CT profiles while organic P showed no pattern of stratification (Weil et al. 1988).

The rates of organic matter turnover and P cycling in the surface soil may be increased substantially in NT soils compared to plowed soils due to an increase in the microbiological activity in NT soils (Harrison, 1985). Surface soils from long-term NT and CT plots were characterized for microbial and biochemical components by Doran (1980). The counts of aerobic microorganisms, facultative

anaerobes, and denitrifiers in the 0-7.5 cm layer of no till were higher than in the same layer of plowed soil. Phosphatase, water, organic C, and N contents in the surface of no-till soil were significantly higher than in soils from conventional tillage; however, at lower depths the trends were reversed probably due to the burying of plant residues with plowing (Doran, 1980). There was a highly significant correlation between phosphatase enzyme activity and organic matter content, and between the enzyme activity and soil moisture content (Klein and Koths, 1980). Surface applied fertilizer N can be rapidly taken up by the microbes and immobilized into organic matter in no-till due to the higher activity of soil microbes (Blevins et al. 1983). Higher cumulative CO_2 evolution was found for NT compared to CT cores suggesting higher microbial activity which was attributed to the higher OM levels in NT soils (Weil et al. 1988). Moisture content was reported to be higher under no-tillage (Klein and Koths, 1980; Blevins et al., 1983; Elliot et al., 1984).

Greater P uptake and crop yield may occur in NT soils due to increased P mineralization rates and an improvement in the efficiency of P fertilizer utilization. Increased P fertilizer efficiency in the 0 to 20 cm depth of no-tillage soil was apparent, as evidenced by more acid-extractable P found in the soil after residual cropping (Moschler et al. 1975). Adoption of no-till compared to plow tillage maintained fertility status of top soil nearer to that of native prairie soil and higher yields were observed with no-till compared to plow treatments (Follett and Peterson, 1988). No reduction in crop yield was observed after 12 years of adoption of no-tillage (Karlen et al. 1991).

The beneficial effects of no-tillage are not always apparent. Spring wheat grown under direct drilled systems (no-till) were deficient in both N and P during early development while wheat grown under conventional tillage did not show the deficiency (Gates et al. 1981). Blevins et al. (1983) observed rapid acidification of

the soil surface after 10 years of continuous no-till corn production especially when higher N fertilizer rates were used resulting in increased levels of exchangeable Al and Mn, reduced levels of exchangeable Ca, and reduced yields.

EXTRACTION METHODS OF PHOSPHORUS:

Organic P (Po)

Organic P is determined indirectly by either the ignition or the extraction method. In the ignition method, organic P is determined by measuring the differences in the acid-extractable P in soil samples before and after ignition at 550 ^{o}C (Saunders and Williams, 1955). Soil organic P is generally overestimated by this method due to increased solubility of native soil inorganic P upon ignition especially at higher temperatures. Incomplete recovery of the released organic P may lead to low values of organic P. The extraction method employs successive extractions with HCl and NaOH; organic P being determined as the difference in the content of inorganic and total P in the extracts (Mehta et al., 1954). This method usually underestimates the content of organic P due to incomplete extraction and hydrolysis of some of the organic P. Organic P values are obtained in both methods by difference and may be subject to large percentage errors especially if the difference is between larger figures (Saunders and Williams, 1955).

Fractionation of Inorganic P (Pi):

Chang and Jackson (1957) presented a system for fractionation of inorganic soil P into the total amount of several discrete chemical forms by sequential extraction. The soil is extracted first with 1 N NH_4Cl to remove water soluble and loosely bound P and the exchangeable Ca. Aluminum phosphates (Al-P) are then

extracted with 1N NH_4F; iron phosphates (Fe-P) with 0.1N NaOH; calcium phosphates (Ca-P) with 0.5N H_2SO_4; and reductant soluble iron phosphates (iron oxide occluded) by $Na_2S_2O_4$-citrate solution. For soils high in iron oxides, the residue is extracted with neutral NH_4F to remove occluded aluminum phosphates. Alternatively, the residue is extracted with 0.1N NaOH to remove occluded aluminum-iron phosphates. The extractants used in this scheme do not separate each P fraction completely for example, 0.1 N NaOH, extracts Al-P, Fe-P, and organic P, while H_2SO_4 extracts Ca-P as well as considerable amounts of Al and Fe-P. Rinkenberger (1966) found that dicalcium phosphate was not completely removed from the calcareous Wisner silty clay loam by one extraction with NH_4Cl. The added $CaHPO_4$ to the soil appeared in the Al-P fraction, unless it was removed by successive extractions with the NH_4Cl first

Total P fractionation Schemes:

Total P (PT) fractionation schemes distinguish between inorganic P into fractions (labile, secondary, occluded, and primary minerals) that have been commonly described in the identification of P compounds in soil, while simultaneously providing information on labile and stable organic P forms and microbial P (Stewart and McKercher, 1982). The P fractions are divided into an anion exchange resin extractable which includes the most biologically available Pi (Amer et al., 1955). Resin extractable P approximates the total plant uptake of P and serves as a good biological measure of total plant available P in the soil (Bowman et al., 1978). Labile Pi and Po sorbed on the soil surface plus a small amount of microbial P are removed by 0.5M $NaHCO_3$ (Bowman et al. 1978). NaOH removes Pi and Po compounds held more strongly by chemisorption to Fe and Al components of soil surfaces while ultrasonification of the soil residue for 2 minutes at 75 watts in 0.1N NaOH enables extraction of Pi and Po held at the

internal surfaces of soil aggregates (Hedley et al., 1982; Tiessen et al. 1984). An acid extractant (1M HCl) removes mainly apatite-type minerals and then the more chemically stable Po forms and relatively insoluble Pi forms are dissolved by oxidation and acid digestion in H_2SO_4 and H_2O_2 (Hedley et al. 1982, and Tiessen et al. 1984). This fractionation can include microbial P which is calculated as the difference between $NaHCO_3$ extractable P before and after fumigation by chloroform (Hedley et al., 1982). These extractions require long shaking periods to allow the extractant to penetrate into the clay minerals. This coupled with strong reagents could cause organic P mineralization during the course of the extraction. No specific compounds are identified in each fraction and no correlation is available between each fraction and immediate or long term availability to plants except for the resin fraction.

Microbial P:

Direct measurement of the P content of the soil biomass is essential for the accurate assessment of the importance of the microbial biomass in P cycling and in crop nutrition (Brookes et al. 1982). Biomass P is determined as the difference between the amount of P extracted by 0.5 M $NaHCO_3$ (pH 8.5) from soil fumigated with $CHCl_3$ and the amount extracted from unfumigated soils (Brookes at al., 1982; Hedley and Stewart, 1982; McLaughlin et al., 1986). Chloroform was used in the vapor form on fresh soils by Brookes et al.(1982), because replication tended to be poorer with $CHCl_3$ liquid and Pi was determined after 0.5 hr of shaking. Hedley and Stewart (1982) on the other hand used ground and sieved soils (< 500 μm) that have been incubated at 60% field moisture capacity at 24 ^oC for 21 days and total P was measured after an extraction time of 16 h. Another difference between the two methods is the removal of resin extractable P from the

soil before lysing microbial cells with liquid $CHCl_3$ and extraction with $NaHCO_3$ in the Hedley and Stewart scheme.

McLaughlin et al. (1986) tested a range of gaseous, liquid and vapor biocides in combination with seven extractants for their ability to release P from soil microorganisms in situ. Chloroform and hexanol were found to be the most effective biocides with no significant differences between the liquid and the vapor form while the best extractant was 0.5M $NaHCO_3$ (pH 8.5).

Since micro flora differ from soil to soil, as do the amounts and forms of P released, calibration is necessary for each soil by adding organisms containing known amounts of P and the soil immediately fumigated. The amount of 0.5M $NaHCO_3$ extractable Pi and PT in fumigated soil with added micro-organisms, less that in fumigated soil without micro-organisms, gives the recovery of added microbial P (Kp) (Brookes et al. 1982). This could be time consuming and the microorganisms added may not reflect the status of the flora found in the soil which can add uncertainty in the estimates of microbial biomass P in soils. A Kp factor of 0.4 was found by Brookes et al. (1982) upon testing this procedure on eight soils. Hedley and Stewart (1982) found a similar Kp factor (0.37). Currently, a Kp factor of 0.4 is often used to correct for the low recovery of microbial P (Clarholm, 1993; and Srivastava and Singh, 1991), or no correction factor is used (Buchanan and King, 1992).

CHAPTER II

EFFECT OF NO-TILLAGE AND CONVENTIONAL TILLAGE ON P TRANSFORMATIONS IN SOILS

INTRODUCTION

Conservation tillage is becoming more widely accepted as an alternative system of crop production (Sharpley and Smith, 1989). The conservation tillage information system center defines conservation tillage as any tillage and planting system that maintains at least 30 percent of the soil surface covered by residue after planting. No-tillage (NT) is a type of conservation tillage where the soil is left undisturbed prior to planting into a narrow seedbed approximately 2-8 cm wide; weed control being accomplished primarily with herbicides. Conventional tillage (CT) refers to the combined primary and secondary tillage operation normally performed in preparing a seedbed, having essentially no plant residue left on the soil surface (Mannering et al. 1987).

Conservation tillage can help to reduce soil erosion, can conserve soil water for greater crop production, and can reduce fuel use (Doran, 1980; Phillips and Phillips 1984). No-till reduces the risk of wind and water erosion, thus reducing P movement from soils to surface waters (Harrison, 1985; Sharpley et. al 1993). Dissolved P concentration in runoff from no-till practices may be greater, however, than from conventional practices as a result of P accumulation on the surface (Ellis et al. 1985; Sharpley et al. 1993). But total P loss is reduced by no-till. Soil temperatures under conservation tillage can run 2 to 10 ^{o}C lower than the

same soil under conventional tillage (Phillips and Phillips, 1984). Cooler soil temperatures may be a disadvantage in temperate regions as planting date or plant emergence may be delayed especially for warm season crops. Lower soil temperature is an advantage in the tropics, where soil temperatures are too high for optimum plant growth and development (Phillips and Phillips, 1984). Rapid acidification of the soil surface in no-till soil may occur especially when high N fertilizer rates are used with a simultaneous increase in exchangeable Al and Mn and a decrease in exchangeable Ca^{2+} (Blevins et al., 1983).

No-till cropping often promotes the development of stratified pH, P and other nutrients (Eckert, 1991). Organic matter tends to be higher in the surface layers of NT soils (Moschler et al., 1972; Weil et al., 1988; Eckert, 1991; and Unger, 1991). Phosphorus tends to accumulate in the surface layers of no-till soils but levels decline sharply with depth compared to the more uniform distribution in conventionally tilled soils (Shear and Moschler, 1969; Triplett and Van Doren, 1969; Moschler et al. 1972; Ellis and Howse, 1980/1981; Follet and Peterson 1988; Weil et. al 1988; Eckert, 1991; Karlen et al., 1991; Unger, 1991). This stratification is believed to result in improved P availability due to less soil contact with soluble P and hence less soil fixation of P.

Organic matter turnover and P cycling may be increased substantially in no-till soils compared to plowed soils due to an increase in the microbiological activities of NT soils (Harrison, 1985). Higher microbiological activity was attributed to higher organic matter levels in NT soils (Weil et al. 1988). Although it is well established that P accumulates in the surface layers of NT soils, little work has been done on the distribution of P among the different inorganic and organic pools in NT soils compared to CT soils, especially within the microbial pool.

The objectives of this study were to

1. Determine if the adoption of no-tillage compared to conventional tillage altered the P fractions distribution in soils from three experimental sites in Michigan.
2. Determine if the rate of transformation of P from ^{33}P labeled soybean residues added to soils is more rapid for no-till soils than for to conventionally tilled soils.

MATERIALS AND METHODS:

Soils

Soils under NT and CT management practices from three different experimental sites in Michigan were sampled. The location of the soils and the past history are presented in Table 1. The conventional tillage operation of the Capac soil consisted of fall moldboard plowing to a depth of 0.2 m with secondary tillage in the spring consisting of one pass of a disk followed by one pass of a spring toothed harrow (Pierce et al., 1994). The Conventional tillage operation of the Kalamazoo soil consisted of spring moldboard plowing to a depth of 0.2 m followed by secondary tillage operation of disking and field cultivating operation. The conventional tillage operation of the Misteguay soil consisted of a fall plow followed by a field cultivating operation in the spring. The no-tillage treatments in all sites were planted with a no-till slot planter. Phosphorus fertilizers are applied annually to the Misteguay soil. However, the Capac soil has not had any P fertilizers applied since 1988 and the Kalamazoo soil since 1989. A composite of four samples per replication and a total of four replications were sampled from each site at a depth of 0 to 2 cm. The soils were sieved while moist, equal proportions of the replicates were mixed to give one sample per tillage treatment per soil series and then stored at 4 °C if not used within two weeks. Stored soils were left at room temperature for two weeks before the start of each experiment in

Table 1. Location and past history of the soils investigated.

	Soil Series		
	Capac loam[1]	Misteguay silty clay[2]	Kalamazoo loam[3]
Location	MSU research farm, E.Lansing	Bean and beet farm, Saginaw	KBS, Kalamazoo
Start of no-tillage	1980	1985	1989
Sampling date	May, 1993	Sep., 1993	Oct., 1993
Current Crop	Corn	Corn	Corn
Crop rotation	Corn/soybean	Corn/soybean/ sugarbeet	Corn/soybean

[1] Capac loam (Fine-loamy, mixed mesic Aeric Ochraqualf)

[2] Misteguay silty clay (Fine, mixed, calcareous, mesic Aeric Haplaquept)

[3] Kalamzoo loam (Fine-loamy, mixed, mesic, typic Hapludalf)

order for microorganisms to restore normal activity. Selected soil properties were measured by the standard methods and are presented in Table 2. The pH was measured of a 1:1 soil to water suspension using a glass electrode. Texture was determined by the pipette method after treatment to remove organic matter and calcium carbonate. Organic C was measured by the total combustion. The Misteguay soil was treated with acid to remove carbonates before organic C determination by total combustion. Organic C was also determined in the Misteguay soil by the Walkley-Black method. The cation exchange capacity (CEC) was measured using NH_4^+ as the saturating ion and Na^+ as the replacing ion (Page et al., 1982).

PREPARATION OF THE ^{33}P LABELED PLANT MATERIAL:

Soybean seeds were germinated in sand flats that had been rinsed with dilute acid solution and distilled water. The seedlings were transplanted into pots containing a modified Hoagland nutrient solution (B. Knezek, personal communication) that had $1/5^{th}$ the recommended concentration of P. Three plants were transplanted per pot and grown in a growth chamber. The growth conditions in the chamber were: day temperature of 27 °C, night temperature of 21 °C with 16 hours of light. After the plants were grown for two weeks, ^{33}P was added as orthophosphoric acid solution to the nutrient solution. The plants were grown for 7-8 days then harvested. Leaves and roots were separated and dried at 60 °C for 24 to 48 h. Roots were washed with a solution of ^{31}P to remove any ^{33}P that resided on the surface of the roots then rinsed with distilled water before drying. The plant material was ground to less than 4 mm size and the ^{31}P and ^{33}P concentrations in the plant material determined (Table 3).

Table 2. Selected chemical properties of the soils investigated.

		pH	Clay	Sand	Silt	Bray P	Org.C	CEC
			----------%----------			mg Kg^{-1}	%	$cmol_c kg^{-1}$
Capac	NT	5.45	13.3	55.8	30.9	107	2.11	16.6
	CT	6.24	13.7	54.1	32.2	94	1.67	16.2
Misteg.	NT	7.86	53.3	4.9	41.8	66	1.73†	29.4
	CT	8.06	55.5	4.7	39.8	32	1.25	25.8
Kalam.	NT	5.53	13.5	42.2	44.3	44	1.02	10.0
	CT	6.32	14.7	42.1	43.1	42	0.70	9.0

† Values by the Walkely-Black method were 1.68% and 1.19% for the NT and CT, respectively.

Table 3. Phosphorus concentration in soybean residues added to soils.

Soil	Total P concn. %	Total ^{33}P activity KBq g^{-1}	Specific ^{33}P activity MBq $g^{-1}P$
Capac	0.39	276	70
Misteguay	0.33	416	126
Kalamazoo	0.74	631	85

Activity in each fraction (% of total).†

Resin	70
$NaHCO_3$	8.6
NaOH 1	12.6
NaOH 2	1.7
HCl	1.85
H_2SO_4	5.2

† Plant tissue extracted by fractionation procedure

TREATMENTS

One hundred gram of field moist soil was weighed into a glass jar, 0.2 g of the ^{33}P labeled plant material (0.15 g leaves and 0.05 g roots) was added to the soil and thoroughly mixed, the soil moisture adjusted to field capacity, then incubated at 25 °C. Three replications were established per soil per extraction date. Incubation times were at 0, 6, 12, 18, 26, and 34 days.

EXTRACTION PROCEDURE:

The fractionation scheme used in this study was a modification of the procedures proposed by Hedley et al. (1982) and Tiessen et al. (1984):

1. Two sets (A & B) of 5 g of soil each were weighed into 250 ml centrifuge bottles. Four g of a strong anion exchange resin (Dowex 1x8-50, 20-50 mesh) in the bicarbonate form in a nylon mesh bag (< 53μm) and 200 ml of distilled water was added to the centrifuge bottle. The anion exchange capacity of the resin was 3.5 meq g^{-1} with a total capacity of 14 meq. The bottles were shaken for 16 to 18 h. The resin bag was removed and rinsed free of soil back into the centrifuge bottle in order to minimize loss of soil. The P in the resin was extracted by shaking the bag for 24 h with 0.5 N HCl. Both ^{33}P and ^{31}P were determined in this fraction. The P measured is inorganic labile P. Labile P is the most biologically available form of P to the plants (Amer et al., 1955). No organic P is found in this fraction. The soil remaining in the bottle was centrifuged at 5000 rpm for 20 minutes and the supernatant discarded as it contained no P.

2. After extraction with the resin, set B was extracted with 100 ml of 0.5 M $NaHCO_3$ (pH 8.5) for 1 h. Set A was fumigated with 2 ml of chloroform for 18 to 20 h. The chloroform was then allowed to evaporate for 18 to 20 h and extracted

with $NaHCO_3$ with the same procedure as set B. The solution was centrifuged at 5000 rpm for 20 minutes and inorganic ^{31}P (Pi), total ^{31}P (PT), and ^{33}P were determined in this fraction. The difference in Pi extracted between set A and set B is Pi in the microbial biomass. Organic ^{31}P (Po) was calculated as the difference between PT and Pi in the $NaHCO_3$ extracts before fumigation. The difference between Po in the fumigated and unfumigated samples is Po in the microbial biomass. No correction factor was employed in the calculation.

3. About 1.0 g of the wet soil was then sub-sampled from set A into a 40 ml centrifuge tube, dried overnight at 65 °C to determine dry weight of the soil. Thirty ml of 0.1 N NaOH was added, the tube shaken for 16 h, then centrifuged at 9000 rpm for 10 minutes and the supernatant collected. Analysis was done to determine ^{31}Pi, ^{31}PT, and ^{33}P. NaOH extractable Pi is P found in the secondary minerals and is considered to cycle slowly while NaOH Po is moderately labile P (Tiessen et al. 1984). Organic P was calculated as the difference between PT and Pi.

4. Twenty ml of 0.1 N NaOH was added to the tube, the sample sonicated for 2 minutes at 75 watts in an ice bath and then the volume made to 30 ml. The sample was shaken for 16 h, centrifuged at 9000 rpm for 10 min and the solution collected. Inorganic P, PT, and ^{33}P were determined. Organic P was calculated as the difference between PT and Pi. Inorganic P extracted in this fraction is occluded P and the organic P in this fraction is chemically and physically protected (Tiessen et al. 1984).

5. Thirty ml of 0.1 N HCl was then added to the sample, shaken for 16 h, centrifuged, and the solution collected. Inorganic ^{31}P and ^{33}P were determined. Acid extractable P is mainly calcium phosphates (Ca-P) and does not contain Po.

6. Finally the soil was digested with H_2SO_4 and H_2O_2 to determine residual P which may contain occluded Pi and chemically and physically protected Po (Tiessen et al., 1984).

Counting of the ^{33}P was done in a liquid scintillation counter with an open channel (0 to 2000 KeV) by adding 1 ml of sample to 10 ml of cocktail mix. All counts were corrected for background and decay. Phosphorus was determined by the method of Murphy and Riley (1962) using an automated flow injection analyzer. The pH of the $NaHCO_3$, NaOH, and NaOH after sonication extracted samples was adjusted to 2 with 0.5 N HCl, and the pH of the HCl extracted and H_2SO_4 digested samples was adjusted to a pH of 3 to 4 with 0.5 N NaOH before analysis of ^{31}P. Total P was determined in the $NaHCO_3$ and NaOH extracts by digesting the samples with H_2SO_4 and ammonium persulfate on a hot plate (USEPA methods for analysis of water 1978). The pH of the samples was adjusted to 3 before analysis of total P by the same method described above.

PARTITIONING OF INORGANIC AND ORGANIC P.

The $NaHCO_3$ extracts were also partitioned into inorganic and organic fractions using acidified molybdate and isobutanol according to the method of Jayachandran et al. (1992). A five ml aliquot of the $NaHCO_3$ extract was added to a 125 ml separatory funnel followed by five ml of acidified molybdate, 10 ml of isobutanol saturated with distilled water, and 10 ml of distilled water saturated with isobutanol. The separatory funnel was shaken for 2 min and allowed to settle.

In this process, molybdenum is complexed with Pi ions, and the phosphomolybdate complex is extracted into the isobutanol phase. After phase separation was complete, the aqueous phase was drained from the bottom of the funnel and Po was counted in this fraction. The isobutanol phase was washed by shaking for 1 min with 10 ml of 0.5 M H_2SO_4 saturated with isobutanol. The aqueous phase was discarded and ^{33}Pi was counted in the isobutanol phase.

Due to quenching problems, counting of ^{33}Pi in the isobutanol phase was done by taking 1 ml of the extract into a scintillation vial, allowing it to evaporate under the hood, and then dissolving the residue with 1 ml of 0.1 N HCl and counting as described above.

PRELIMINARY EXPERIMENT

A preliminary experiment was conducted to establish the distribution of P when applied in an inorganic form to the Kalamazoo soil. One ml containing 940 KBq of ^{32}P was added to 100 g of soil, the soil incubated, and extracted periodically. Three replications were established per soil per extraction date. incubation times were 0, 6, 12, 18, 26, and 34. The experimental conditions and the extraction procedure were the same as described before.

RESULTS

EFFECT OF TILLAGE AND SOIL TYPE ON SOIL P FRACTIONS

The soils used in this experiment have different chemical and physical properties and have been under NT for different number of years (Tables 1 and 2). Both the Capac and Kalamazoo soils are loam soils with pH lower than 6.5. The pH of NT samples is about one unit lower than for CT. The Misteguay is a calcareous silty clay soil with a pH of about 8 and little difference in pH due to tillage. Organic C concentration is the highest in the Capac soil, followed by the Misteguay and then the Kalamazoo soil. Organic C is higher in the NT compared to the CT treatments in all soils. This is expected because organic matter tends to accumulate in the surface layer of NT soils due to less mixing of organic matter with the soil.

Bray-Kurtz P1 levels are similar between the NT and CT treatments in both the Capac and Kalamazoo soils. This is due to the fact that P fertilizers have not been applied for several years to either soil and therefore no P accumulation is occurring in the surface layers of NT treatments. The NT Misteguay soil, however, has more than double the concentration of the Bray-Kurtz P1 levels than the CT soil. Phosphorus is accumulating on the surface layer of the NT Misteguay as P fertilizers are applied annually to this soil. The P accumulation in the surface layer of the Misteguay soil is also reflected in the labile ^{31}P extractable fraction (Table 4), where the difference between the NT and CT Misteguay was significant at the 1% level. The ^{31}P labile fraction was slightly higher in the CT Kalamazoo soil, however, significant at the 5% level. No differences were observed in the Capac soil. Again, this might be explained by the lack of addition of P fertilizer in both Capac and Kalamazoo soils in recent years. If P fertilizers were not applied, accumulation of inorganic P (Pi) should not occur in the surface layer of NT soils

Table 4. Labile ^{31}Pi as affected by tillage, incubation time, and soil series.

Time of incub.	Trt.	Capac	Misteguay	Kalamazoo	Mean
Days		---------- mg kg-1 ----------			
0	NT	52.7	59.9	25.4	46.0
	CT	48	34.5	31.3	37.9
6	NT	48.7	57.1	20.8	42.2
	CT	50	29.9	26.8	35.6
12	NT	45.7	57.3	20.3	41.1
	CT	47.0	31.2	25.6	34.6
18	NT	45.5	57.7	21.7	41.6
	CT	45.5	30.9	26.2	34.2
26	NT	48.2	60.6	20.8	43.2
	CT	46.3	33.3	23.3	34.3
34	NT	46.8	57.3	26.8	43.6
	CT	49.3	28.7	27.3	35.1
Mean	NT	47.9	58.3	22.6	
	CT	47.7	31.4	26.7	
Significant by "t" test:		n.s.	0.01	0.05	

and no differences would be expected in the Pi concentrations between the NT and CT treatments. The ^{31}Pi labile pool was fairly stable in the three soils with incubation time which indicated that the soils were at equilibrium with respect to the inorganic labile P fraction during incubation and the addition of plant labeled material.

The difference between PT and Pi extracted by 0.5 M $NaHCO_3$ is labile organic P (Po) (Table 5). This fraction is considered easily mineralizable and available to plants. Bowman and Cole (1978) found that 0.5 M $NaHCO_3$ (pH 8.5) extracted labile P compounds, like ribonucleic acid and glycerophosphates but not Na-phytate, a relatively resistant compound. All three soils had a larger labile ^{31}Po pool than the ^{31}Pi pool. Both Capac and Misteguay soils have higher organic C than the Kalamazoo soil which was reflected in slightly higher levels of labile ^{31}Po in the Capac and Misteguay soils compared to the Kalamazoo soil. There were no significant differences in the labile ^{31}Po between the NT and CT treatments in any of the three soils. The labile ^{31}Po levels fluctuated during incubation in Capac and Misteguay soils which may indicate that mineralization and immobilization reactions were occurring throughout the incubation period. The levels in Kalamazoo soil were stable after a initial drop between days 0 and 6.

The microbial ^{31}Pi data is presented in Table 6. Some of the $NaHCO_3$ inorganic P data for the Capac soil could not be analyzed due to fungal growth in some of the vials although they were acidified in order to eliminate this problem. Some of the values for this fraction in the Capac soil are an average of two replications only or just one value. This also true for the labile ^{31}Po and biomass ^{31}Po calculations in the Capac soil. The complete data for the three soils is presented in Tables 1 to 3 in the appendix. The microbial Pi levels were significantly higher in the NT compared to the CT Misteguay soil. The concentrations in the Misteguay soil were on average two times higher than the

Table 5. Labile ^{31}Po as affected by tillage, incubation time, and soil series.

Time of incub.	Trt.	Capac	Misteguay	Kalamazoo	Mean
Days		---------- mg kg-1 ----------			
0	NT	101	87	102	97
	CT	141	84	116	114
6	NT	64	61	50	58
	CT	86	109	37	77
12	NT	101	94	44	80
	CT	73	77	53	68
18	NT	96	85	50	77
	CT	108	90	51	83
26	NT	--	83	66	50
	CT	132	96	57	95
34	NT	118	103	61	94
	CT	140	105	67	104
Mean	NT	96	86	62	
	CT	113	94	63	
Significance by "t" test		n.s.	n.s.	n.s.	

Table 6. Microbial biomass ^{31}Pi as affected by tillage, incubation time, and soil series.

Time of incub.	Trt.	Capac	Misteguay	Kalamazoo	Mean
Days		----------mg kg-1 ----------			
0	NT	3.3	12.1	5.3	6.9
	CT	2.1	8.7	6.4	5.7
6	NT	6.5	20.6	9.0	12.0
	CT	9.0	8.0	5.5	7.5
12	NT	7.5	17.3	6.1	10.3
	CT	5.9	7.9	6.2	6.7
18	NT	3.3	14.1	5.0	7.5
	CT	7.5	7.2	6.9	7.2
26	NT	--	17.6	6.2	11.9
	CT	4.6	9.4	6.6	6.9
34	NT	11.4	16.7	5.3	11.1
	CT	4.7	6.2	6.3	5.7
Mean	NT	6.4	16.4	6.2	
	CT	5.6	7.9	6.3	
Significance by "t" test		n.s.	0.01	n.s.	

Kalamazoo or Capac soils. The Misteguay soil has been under NT for 9 years compared to only 4 years for the Kalamazoo soil. Higher ^{31}Pi in the biomass in the NT Misteguay is probably due to the accumulation of Pi in NT soils due to the addition of P fertilizers.

The ^{31}Po in the biomass was almost undetectable in both Misteguay and Kalamazoo soil (Table 7). Of the three soils, Capac has the highest organic C percentage and has been under NT for the longest period of time. Concentrations of microbial biomass ^{31}P ranged from 7 to 67 mg kg^{-1} in the NT soil during incubation. The mean of the microbial biomass ^{31}P in the Capac soil was 42 mg kg^{-1} for the NT and 12 mg kg^{-1} for the CT. This also may indicate a higher activity of the microorganisms in immobilizing and subsequently mineralizing P with the increase in the organic matter content of soils. The negative numbers encountered are due to large percentage errors as the values obtained are differences between two much larger values (Organic P in the $NaHCO_3$ extracts before and after fumigation). Normal variations and errors in extraction and determination give rise to considerable absolute differences in organic P values (Saunders and Williams, 1955).

The accumulation of P is again reflected in the NaOH extractable Pi in the Misteguay soil (Table 8) where there was a higher concentration in the NT treatment at all extraction dates (significant at the 1% level). This pool was stable with incubation in the Misteguay soil. No significant differences were found in the NaOH extractable ^{31}Pi between the NT and CT treatments in both Capac and Kalamazoo soils. There were some fluctuations in the NaOH ^{31}Pi pool for the Capac soil during the course of the incubation which may indicate that some P is moving in and out of this pool. The size of this pool was larger in both Capac and Kalamazoo soils compared to the Misteguay soil. The NaOH extractable Po (resistant or moderately labile Po) constitutes a larger pool in all the three soils

Table 7. Microbial biomass ^{31}Po as affected by tillage, incubation time, and soil series.

Time of incub.	Trt.	Capac	Misteguay	Kalamazoo
Days		---------- mg kg-1 ----------		
0	NT	67	13	- 24
	CT	11	- 24	- 48
6	NT	55	51	1
	CT	17	- 8	13
12	NT	7	- 13	20
	CT	37	15	10
18	NT	40	- 10	10
	CT	17	- 6	5
26	NT	--	17	12
	CT	8	22	10
34	NT	50	- 3	23
	CT	-21	-17	- 2
Mean	NT	42	9	7
	CT	12	- 3	- 2

Table 8. NaOH extractable ^{31}Pi as affected by tillage, incubation time, and soil series.

Time of incub.	Trt.	Capac	Misteguay	Kalamazoo	Mean
Days		---------- mg kg-1 ----------			
0	NT	120	85	134	113
	CT	108	50	142	100
6	NT	85	98	144	109
	CT	62	62	145	90
12	NT	119	77	137	111
	CT	155	50	116	107
18	NT	93	110	146	116**
	CT	51	57	107	72
26	NT	145	85	117	116
	CT	83	48	141	91
34	NT	183	85	146	138
	CT	148	55	137	113
Mean	NT	124	90	137	
	CT	101	54	131	
Significant by "t" test		n.s.	0.01	n.s	

**Significant at the 1% level

than the NaOH extractable Pi (Table 9). A higher concentration of NaOH Po was found in the NT treatment in all the three soils compared to the CT treatment and it is significantly higher in both Capac and Kalamazoo. This pool seemed to be stable in the Misteguay soil until day 26 when levels dropped dramatically; where as this pool was not stable in the Capac soil.

Occluded ^{31}Pi pool (extracted with NaOH after sonication) is small in all soils compared to the other pools and is relatively stable during the incubation period (Table 10). There was little effect of tillage on this pool except for the Misteguay soil where there was a significantly higher level in the NT treatment. The sonicated NaOH Po pool is, however, larger (Table 11). Tillage had little effect on this fraction. The size of this pool however is considerably larger in both Capac and Misteguay which have the higher organic C percentage.

Calcium phosphates (extracted by 1 N HCl) represent a large P pool for the Misteguay soil, which is expected as it is a calcareous soil (Table 12). There was no difference in the size of this P pool, however, between the two tillage treatments in the Misteguay soil. The CT treatment in the Kalamazoo soil had a significantly higher concentration of Ca-P than the NT treatment. Higher concentration of P in the Ca-P pool was also found in the CT treatment of the Capac soil although it was not significantly higher than the NT treatment. Less P in the Ca-P pool in the NT treatments of both Kalamazoo and Capac soils occurs because of the lower pH due to NT. In the pH range of 6 to 6.5 (the range of Capac and Kalamazoo CT), several P mineral can coexist including varscite ($AlPO_4$), strengite ($FePO_4$), dicalcium phosphate dihydrate, dicalcium phosphate, octa calcium phosphate, and ß-tricalcium phosphate (Lindsay, 1979). At soil pH lower than 6 (pH of Capac and Kalamazoo NT), Fe-P and Al-P are more dominant and less Ca-P is found. Aluminum P and Fe-P forms are extracted by NaOH. This is why the NaOH extractable P pool was higher in both Kalamazoo and

Table 9. NaOH extractable ^{31}Po as affected by tillage, incubation time, and soil series

Time of incub.	Trt.	Capac	Misteguay	Kalamazoo	Mean
Days		----------mg kg-1 ----------			
0	NT	512	614	377	501
	CT	437	668	271	459
6	NT	280	608	367	418
	CT	200	534	333	356
12	NT	582	552	341	492
	CT	613	661	318	531
18	NT	263	692	324	426
	CT	194	452	283	310
26	NT	399	378	324	367
	CT	288	332	284	301
34	NT	461	403	370	411
	CT	401	308	241	317
Mean	NT	416	541	350	
	CT	355	492	288	
Significance by "t" test		0.05	n.s.	0.05	

Table 10. NaOH extractable ^{31}Pi after sonication as affected by tillage, incubation time, and soil series.

Time of incub.	Trt.	Capac	Misteguay	Kalamazoo	Mean
Days		---------- mg kg-1 ----------			
0	NT	14.2	24.0	15.5	17.9
	CT	15.5	17.0	21.2	17.9
6	NT	5.9	12.9	14.1	11.0
	CT	8.7	11.9	16.5	12.4
12	NT	18.8	10.8	18.0	15.9
	CT	32.5	8.0	18.1	19.5
18	NT	14.7	17.4	20.2	17.4
	CT	9.8	8.2	15.5	11.2
26	NT	17.8	13.0	21.0	17.3
	CT	11.2	9.4	32.0	17.5
34	NT	30.4	11.2	18.8	20.1
	CT	27.2	9.3	20.8	19.1
Mean	NT	17.0	14.9	17.9	
	CT	17.5	10.6	20.7	
Significant by "t" test		n.s	0.05	n.s.	

Table 11. NaOH extractable ^{31}Po after sonication as affected by tillage, incubation time, and soil series.

Time of incub.	Trt.	Capac	Misteguay	Kalamazoo	Mean
Days		---------- mg kg-1 ----------			
0	NT	376	516	264	385
	CT	340	634	280	418
6	NT	240	531	277	349
	CT	184	498	274	319
12	NT	451	427	265	381
	CT	661	528	298	496
18	NT	234	512	252	333
	CT	173	420	253	282
26	NT	216	228	236	227
	CT	189	276	198	221
34	NT	279	282	230	264
	CT	281	276	206	254
Mean	NT	299	416	254	
	CT	305	439	252	
Significance by "t" test		n.s.	n.s.	n.s.	

Table 12. HCl extractable ^{31}Pi as affected by tillage, incubation time, and soil series.

Time of incub.	Trt.	Capac	Misteguay	Kalamazoo	Mean
Days		---------- mg kg-1 ----------			
0	NT	132	439	54	208
	CT	131	432	74	212
6	NT	118	467	58	214
	CT	109	479	69	219
12	NT	106	458	61	208
	CT	147	447	89	228
18	NT	122	614	70	269
	CT	113	498	62	224
26	NT	145	446	55	215
	CT	290	399	80	256
34	NT	148	487	57	231
	CT	157	422	73	217
Mean	NT	128	485	59	
	CT	158	446	74	
Significant "t" test		n.s.	n.s.	0.05	

Capac soils compared to Misteguay soil and was higher in the NT than the CT Capac.

Residual P, constituting chemically stable Po forms and relatively insoluble Pi forms dissolved by oxidation and acid digestion, is presented in Table 13. No significant differences were found between the NT and CT treatment in all the three soils. Residual ^{31}P constituted a larger pool in the Misteguay soil than in both the Capac and Kalamazoo soils. This pool was fairly stable with incubation time in all the three soils except for day 12 in the CT Capac soil and day 18 in the NT Misteguay soil where levels were higher with no apparent reason.

Table 13. Residual ^{31}P as affected by tillage, incubation time, and soil series.

Time of incub.	Trt.	Capac	Misteguay	Kalamazoo	Mean
Days		---------- mg kg-1 ----------			
0	NT	189	523	213	308
	CT	219	491	308	340
6	NT	135	464	259	286
	CT	110	451	344	302
12	NT	234	478	240	317
	CT	407	544	290	414
18	NT	153	759	274	395
	CT	122	487	254	288
26	NT	169	487	212	289
	CT	162	441	316	306
34	NT	211	379	275	288
	CT	240	411	308	320
Mean	NT	182	515	246	
	CT	214	471	303	

PHOSPHORUS TRANSFORMATIONS FROM APPLIED RESIDUES.

The largest fraction of the applied ^{33}P was initially in the inorganic, labile pool in all the three soils (Figures 1, 2, and 3). It ranged from 72.3 to 81.9% in the Capac soil, 56.6 to 56.5% in the Misteguay soil, and 68.2 to 65.3% in the Kalamazoo soil at day 0 in the NT and CT treatments, respectively. The second largest fraction was the NaOH extractable P fraction where the concentrations in the Capac soil were 12.2 and 7.5%, the Misteguay soil 20.8 and 17.9%, and the Kalamazoo soil 20.5 and 23.2% at day 0 in the NT and CT, respectively. The labeled residues contained a high percentage of the ^{33}P as inorganic P at the time of addition to soil. The labile ^{33}P fraction decreased rapidly during the first week of incubation in all the three soils. After the first week, the labile ^{33}P fraction decreased slowly throughout the incubation period in all the three soils. At the end of the experiment there was from 20 to 35% of the ^{33}P remaining in the labile fraction. There were no significant differences in the labile P fraction between the NT and CT treatments except on day 18 where there was a significantly higher concentration (at the 5% level) of labile ^{33}P in the CT treatment of the three soils. The decrease in the ^{33}P labile concentration was significant in both the NT and CT soils between day 0 and 34 (Table 14). This decrease was greatest in both the Capac and Kalamazoo soils. The drop was 43.6 and 49.5% for the NT, and 47.9 and 42.4% for the CT in the Capac and the Kalamazoo soil respectively. The decrease in the Misteguay soil on the other hand was less (28.5 and 33.6% for the NT and CT treatments, respectively).

There was a gradual increase in the NaOH extractable P in the Capac soil between days 0 and 18 then a sharper increase after day 18 (Figure 1). The P concentration in the NaOH fraction was slightly higher in the NT Capac soil at all extraction dates except on day 12. This point, however, is doubted to be a real increase and can not be explained. There was also a sharp increase in the NaOH

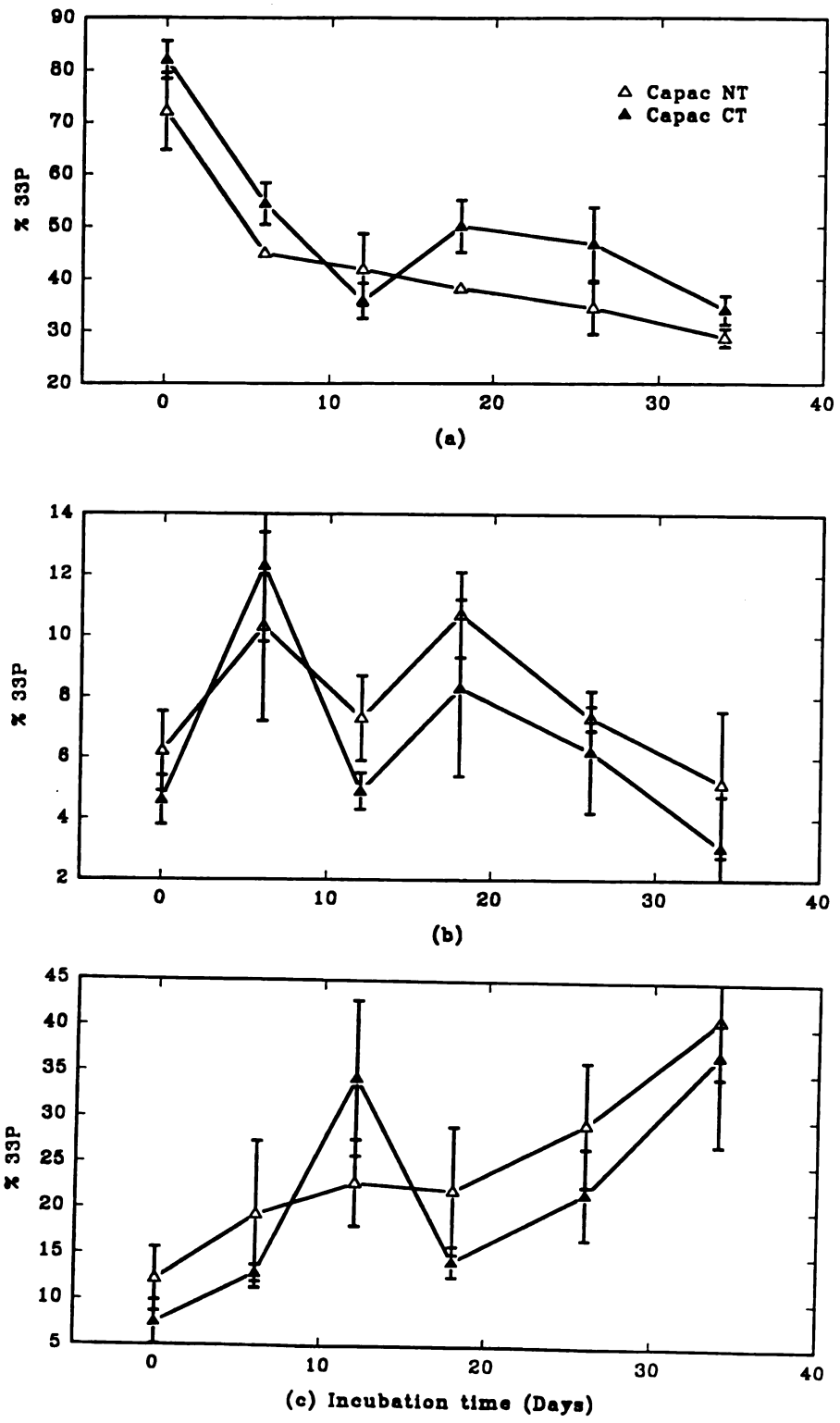

Figure 1. Transformation of ^{33}P in the (a) labile (b) microbial, and (c) NaOH fractions in the Capac soil.

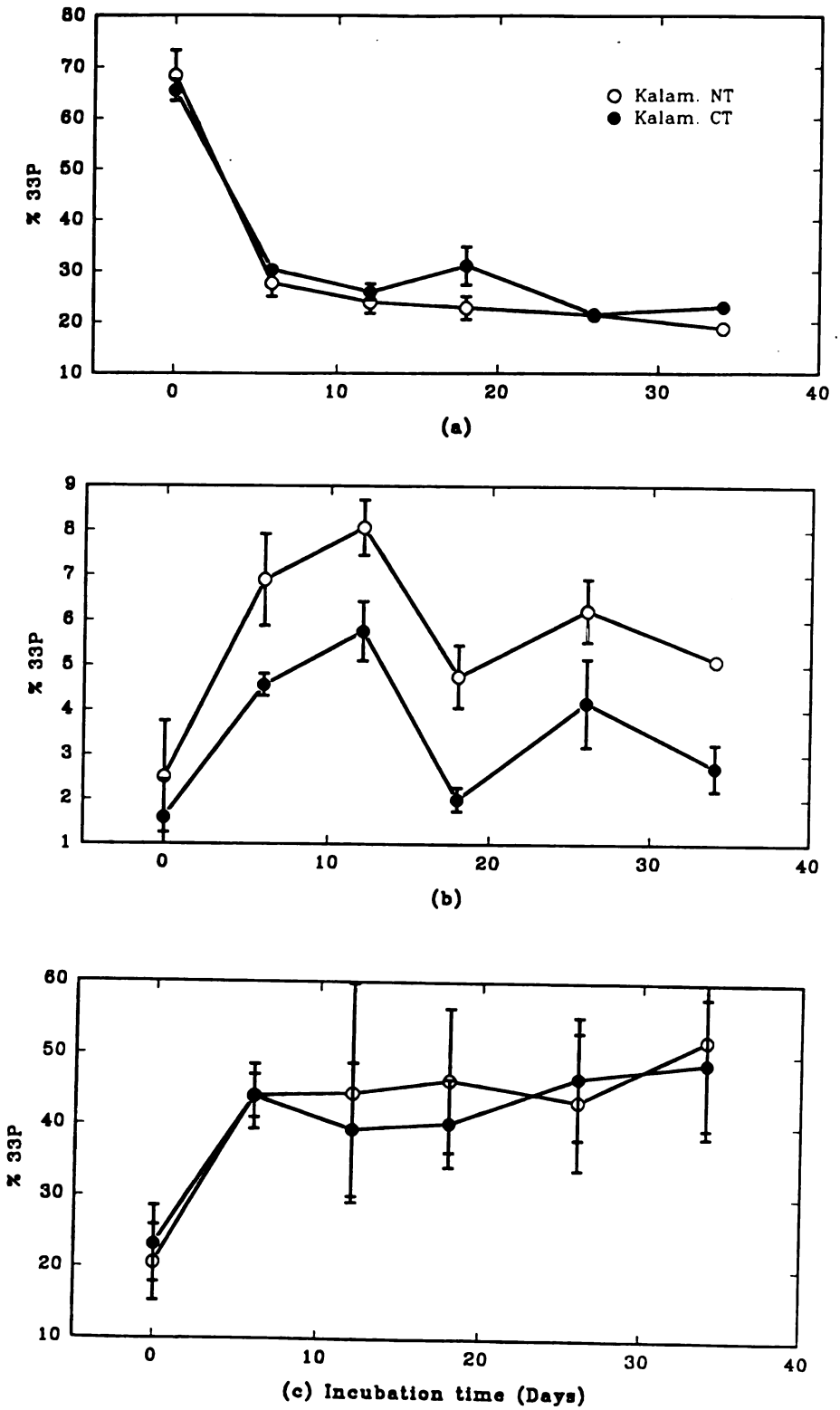

Figure 2. Transformations of ^{33}P in the (a) labile (b) microbial, and (c) NaOH fractions in the Kalamazoo soil.

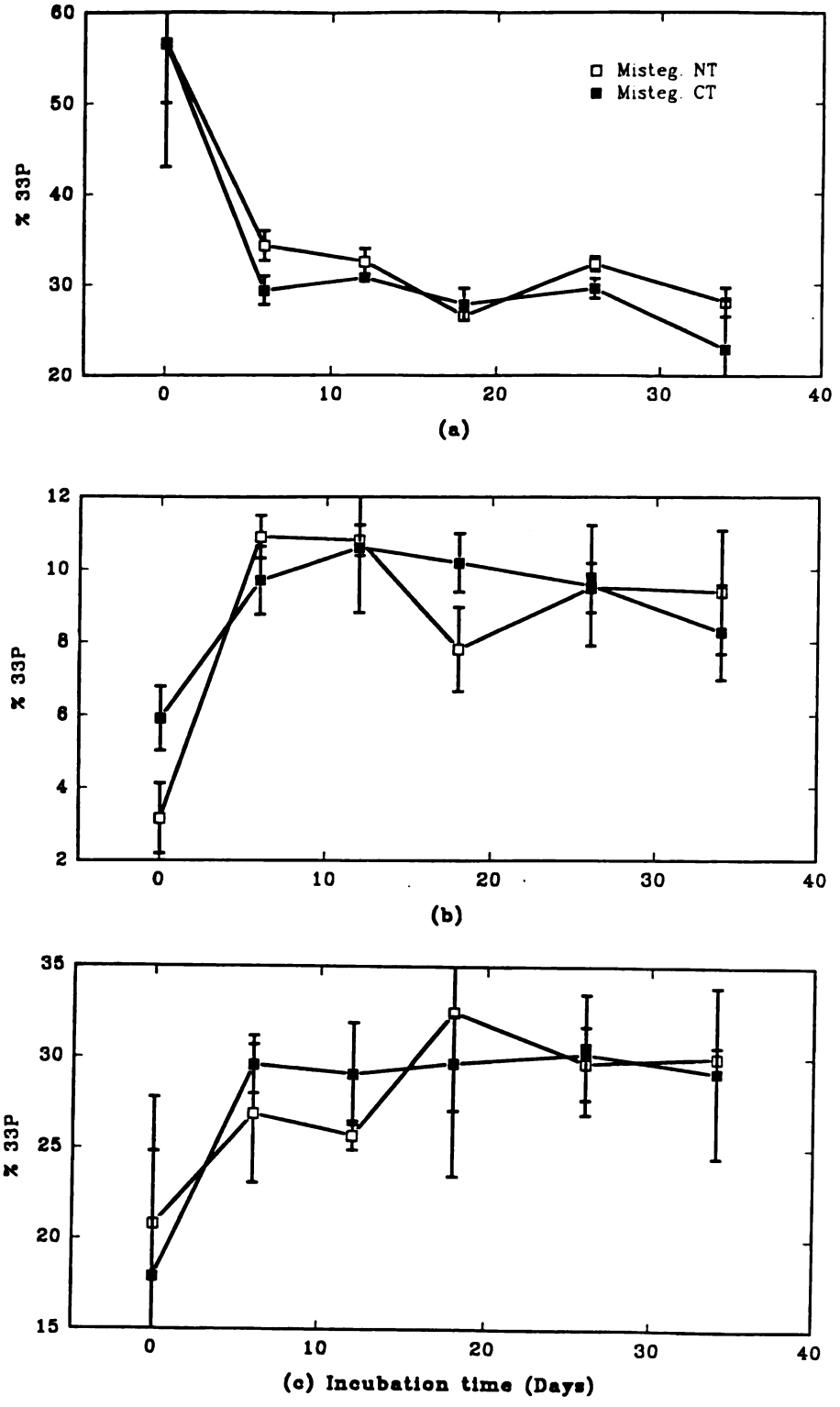

Figure 3. Transformation of ^{33}P in the (a) labile (b) microbial, and (c) NaOH fractions in the Misteguay soil.

Table 14. Labile ^{33}P change between the beginning and the end of the incubation period.

	NT			CT		
Soil	Day	Day 34	Diff	Day 0	Day 34	Diff
Capac	72.3	28.7	43.6	81.9	34.0	47.9
Misteguay	56.6	28.1	28.5	56.5	22.9	33.6
Kalamazoo	68.2	18.7	49.5	65.3	22.9	42.4
Mean	65.7	25.2		67.9	26.6	
Significance by "t" test			0.01			0.01

extractable P in the Kalamazoo soils between days 0 and 6 in both NT and CT treatments (Figure 2). The concentration of ^{33}P at day 6 in the Kalamazoo soil in the NaOH fraction was about double that of day 0 in both the NT and CT treatments. Levels in Kalamazoo soil fluctuated slightly after day 6 with higher percentages in the NT treatment in three out of the last four extraction dates. The increase was small in the NT treatment of the Misteguay soil in the first 6 days where it was about 6% (Figure 3). There was another increase between days 12 and 18 before levels stabilized. The increase in the CT Misteguay soil between days 0 and 6 was much greater than for NT (about 12%), but levels did not change much after 6 days. The difference in the NaOH extractable P between the NT and CT treatments in all the three soils was not significant. A higher percentage of the ^{33}P was also transformed into the NaOH fraction in both Capac and Kalamazoo soils relative to that in the Misteguay soil (Table 15). The increase was only 9.2% in the NT Misteguay soil while it was 28.7% and 31.4% for the NT Capac and Kalamazoo soils respectively. Similar percentages were found for the CT soils.

There was an increase in the microbial biomass ^{33}P in all the three soils from day 0 to day 6. In both Capac and Kalamazoo soils, there appeared to be cycling of the ^{33}P in and out of this pool throughout the incubation period. The NT Misteguay fits this pattern also. Levels are higher (significant at the 1% level) in the NT Kalamazoo soil compared to the CT at all extraction dates, while levels in the Capac soil are higher in five out of the six extraction dates. The CT Misteguay had an initial increase in the microbial ^{33}P between days 0 and 6. Levels in the CT Misteguay leveled off and started to decrease slowly afterwards.

The NaOH extractable ^{33}P following sonication (occluded ^{33}P) comprised a very small fraction of the total P extracted in all the three soils with no

Table 15. NaOH extractable ^{33}P change between the beginning and the end of the incubation period.

	NT			CT		
Soil	Day 0	Day 34	Diff	Day 0	Day 34	Diff
Capac	12.2	40.9	28.7	7.5	37.0	29.5
Misteguay	20.8	30.0	9.2	17.9	29.2	11.3
Kalamazoo	20.5	51.9	31.4	23.2	48.6	25.4
Mean	17.8	40.9		23.2	48.6	
Sig. by "t" test			n.s			n.s.

differences between tillage treatments (Table 16). The ^{33}P extracted in the Ca-P pool (table 17) represented a small fraction in both the Capac and Kalamazoo soils, but a much higher fraction in the Misteguay soils (Figure 4). The Ca-P fraction increased in the NT and CT Misteguay soils between days 0 and 18 and then the concentrations leveled off. This increase is related primarily to the differences in pH between the soils. Both Capac and Kalamazoo soils have pH ranging from 5 to 6.5 while the Misteguay soil has a pH near 8. While labile ^{33}P levels started high in all the soils, ^{33}P was distributed differently. A larger percentage was extracted in the NaOH fraction in both Kalamazoo and Capac soils while a lower percentage was extracted from the Misteguay soil. Calcium phosphates comprised a good proportion in the Misteguay soil but not in the other two soils. This is expected as the dominant P forms in low pH soils are Fe and Al phosphates which are extracted by NaOH, but it does show immediate competition of the inorganic Ca-P for labeled P added to the soils. There were no significant differences in the ^{33}P concentration in this fraction between the tillage treatments in all the three soils. Residual P (Table 18) comprised a very small fraction in the Capac soil (an average of 1.8 and 2.9% in the NT and CT, respectively) while a slightly higher percentage was found in the Kalamazoo soil (an average of 6.8 and 7.9% in the NT and CT respectively). The residual P in the Misteguay soil comprised a higher fraction than the other two soils (an average of 8.7% in both the NT and CT treatments).

Partitioning of inorganic and organic ^{33}P

The ^{33}Pi and ^{33}Po in the $NaHCO_3$ solutions extracted before and after fumigation, were partitioned into physically separate solutions before radiation counting. The ^{33}Pi was counted in the isobutanol phase. But the ^{33}Po could not be directly counted in the aqueous phase. Labeled polymeric material is often

Table 16. NaOH extractable ^{33}P after sonication as affected by tillage, incubation time, and soil series.

Time of incub.	Trt.	Capac	Misteguay	Kalamazoo	Mean
Days		---------- mg kg-1----------			
0	NT	1.3	3.8	1.3	2.1
	CT	0.3	3.8	2.0	2.0
6	NT	0.2	2.1	2.5	1.6
	CT	0.8	2.7	3.1	2.2
12	NT	5.0	2.5	4.5	4.0
	CT	7.7	3.1	4.0	4.9
18	NT	3.8	3.5	4.6	3.9
	CT	3.6	2.3	3.8	3.2
26	NT	0.5	4.8	5.4	3.6
	CT	0.7	3.7	6.6	3.7
34	NT	4.7	3.1	4.6	4.1
	CT	3.8	3.4	5.6	4.3
Mean	NT	2.6	3.3	3.8	
	CT	2.8	3.4	4.2	

Table 17. HCl extractable ^{33}P as affected by tillage, incubation time, and soil series.

Time of incub.	Trt	Capac	Misteguay	Kalamazoo	Mean
Days		---------- mg kg-1 ----------			
0	NT	0.12	3.8	0.3	1.4
	CT	0.12	4.8	0.7	1.9
6	NT	1.5	9.0	2.7	4.4
	CT	1.4	10.4	2.4	4.7
12	NT	0.6	10.5	2.1	4.4
	CT	1.4	14.8	7.1	7.8
18	NT	1.75	14.1	3.1	6.3
	CT	1.14	14.1	3.1	6.1
26	NT	2.63	13.1	3.2	6.3
	CT	3.23	13.8	3.9	7.0
34	NT	0.74	14.5	3.7	6.3
	CT	2.34	14.6	3.7	6.9
Mean	NT	1.22	10.8	2.5	
	CT	1.61	12.1	3.5	

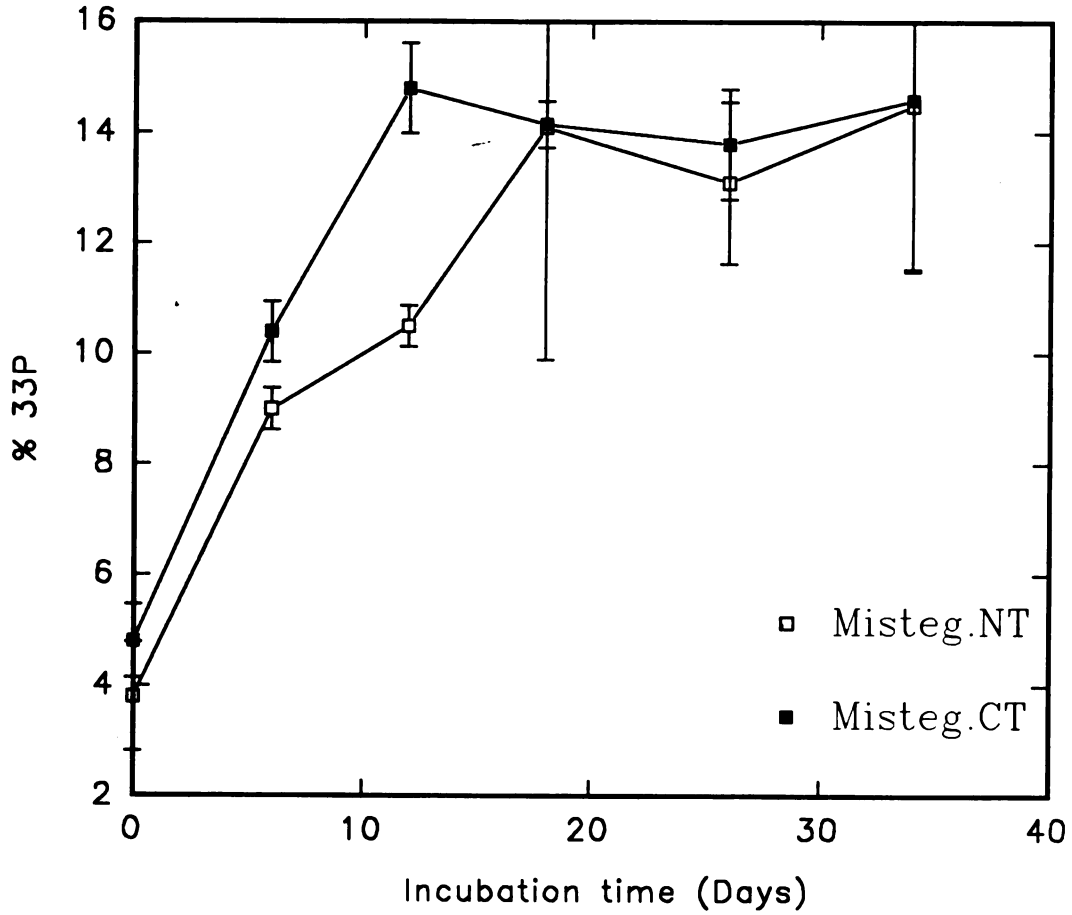

Figure 4. HCl extractable ^{33}P in the Misteguay soil with incubation time.

Table 18. Residual ^{33}P as affected by tillage, incubation time, and soil series.

Time of incub.	Trt.	Capac	Misteguay	Kalamazoo	Mean
Days		---------- mg kg-1 ----------			
0	NT	1.5	7.2	2.2	3.6
	CT	0.25	8.3	3.1	3.9
6	NT	0.2	9.6	5.6	5.1
	CT	0.8	9.3	8.5	6.2
12	NT	1.4	11.6	7.3	6.8
	CT	3.3	3.9	9.4	5.5
18	NT	0.19	10.7	7.8	6.2
	CT	2.2	9.7	7.9	6.6
26	NT	4.6	4.1	8.8	5.8
	CT	4.8	5.3	9.3	6.5
34	NT	3.0	9.0	8.4	6.8
	CT	5.85	15.7	9.1	10.2
Mean	NT	1.8	8.7	6.8	
	CT	2.9	8.7	7.9	

adsorbed onto glass (Gordon, 1973). In the separation process, ^{33}Po was probably adsorbed onto the separatory funnel. Harrison (1982a) could not count ^{32}P remaining in the organic form [^{32}P]RNA applied to soils due to the adsorption onto glass. During the separation procedure, ^{32}Pi transferred to the isobutanol phase with an efficiency of >99.5%. Jayachandran et al. (1992) tested this partitioning procedure using KH_2PO_4 and several organic compounds including glycerophosphate, sodium phytate, ribonucleic acids and derivatives. Inorganic P was completely recovered in the isobutanol phase with acid molybdate. Organic P remained in the aqueous phase during separation. Although Jayachandran et al. (1992) recommended using this procedure to quantify P mineralization in soil using isotope dilution, they did not test it on labeled P compounds.

The ^{33}Pi found in the microbial biomass was calculated from the difference in the counts in samples before and after fumigation in the isobutanol phase. In the Capac soil, the ^{33}Pi percentage increased between the first 2 extraction dates in the NT and CT treatments (Figure 5). This indicated that ^{33}Pi from the labile pool was assimilated into the microbial pool. The ^{33}Pi seemed to have the same cycling pattern as the total ^{33}P in the biomass.

In the Kalamazoo soils, the ^{33}Pi percentages increased between day 0 and day 6, decreased at day 12, and remained constant afterwards (Figure 6).

In the Misteguay soil, the ^{33}Pi percentage was constant throughout the incubation period, except for an initial increase in the NT treatment between day 0 and day 6 (Figure 7).

PRELIMINARY EXPERIMENT

The data from the preliminary experiment with inorganic ^{32}P application to the Kalamazoo soil is presented in the appendix. About 75% of the ^{32}P was

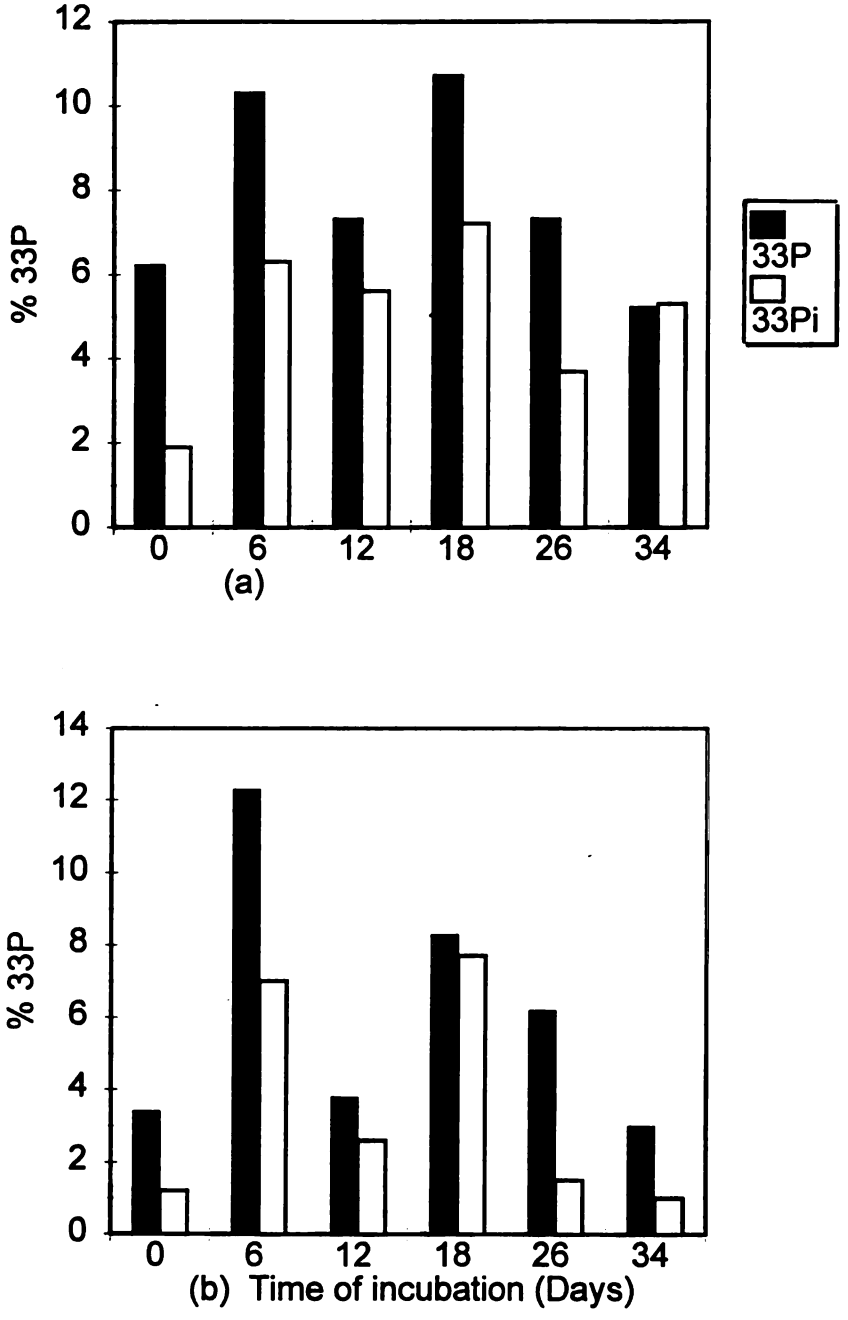

Figure 5. Inorganic and total ^{33}P in the microbial fraction in (a) NT and (b) CT Capac soil.

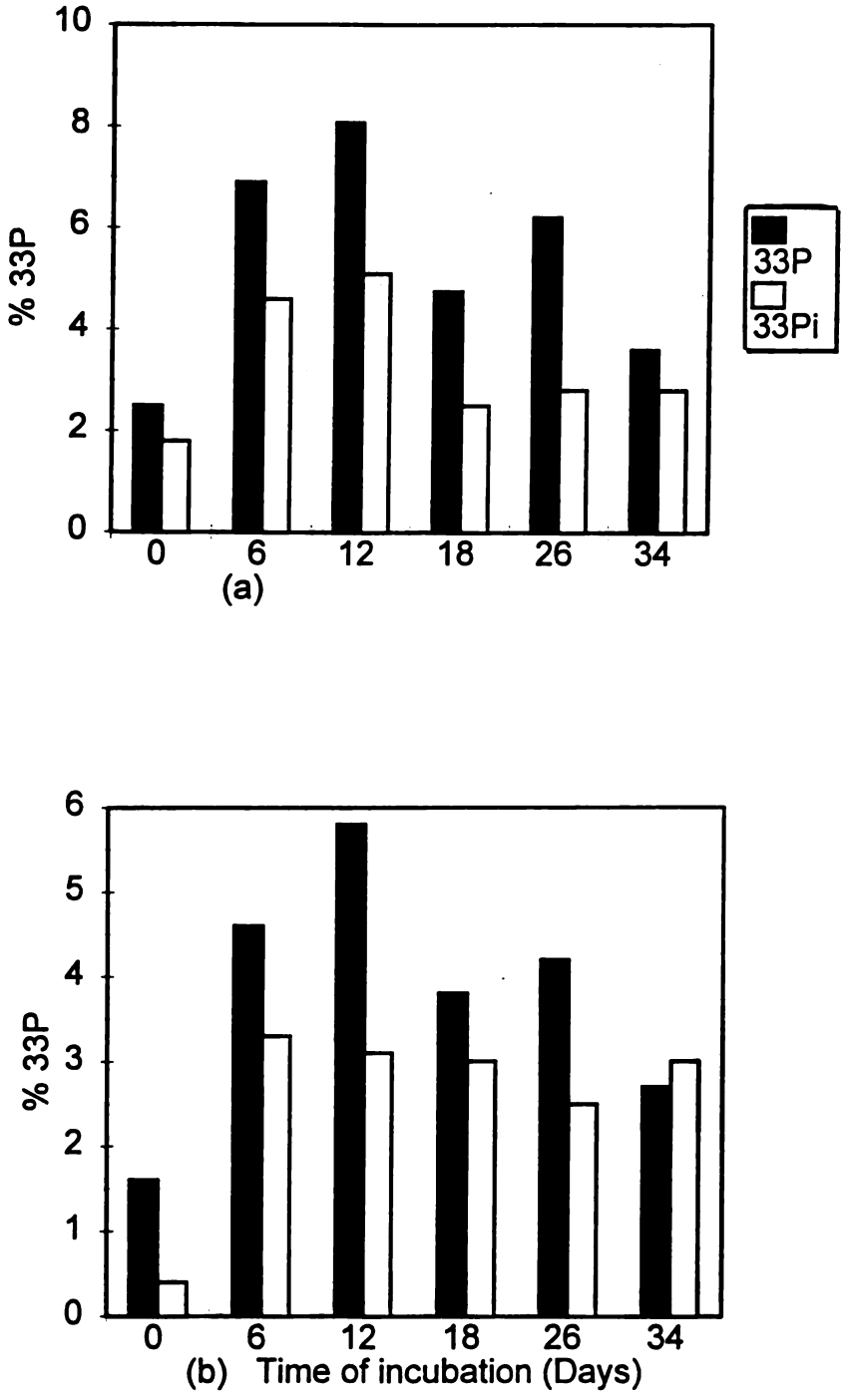

Figure 6. Inorganic and total ^{33}P in the microbial fraction in (a) NT and (b) CT Kalamazoo soil.

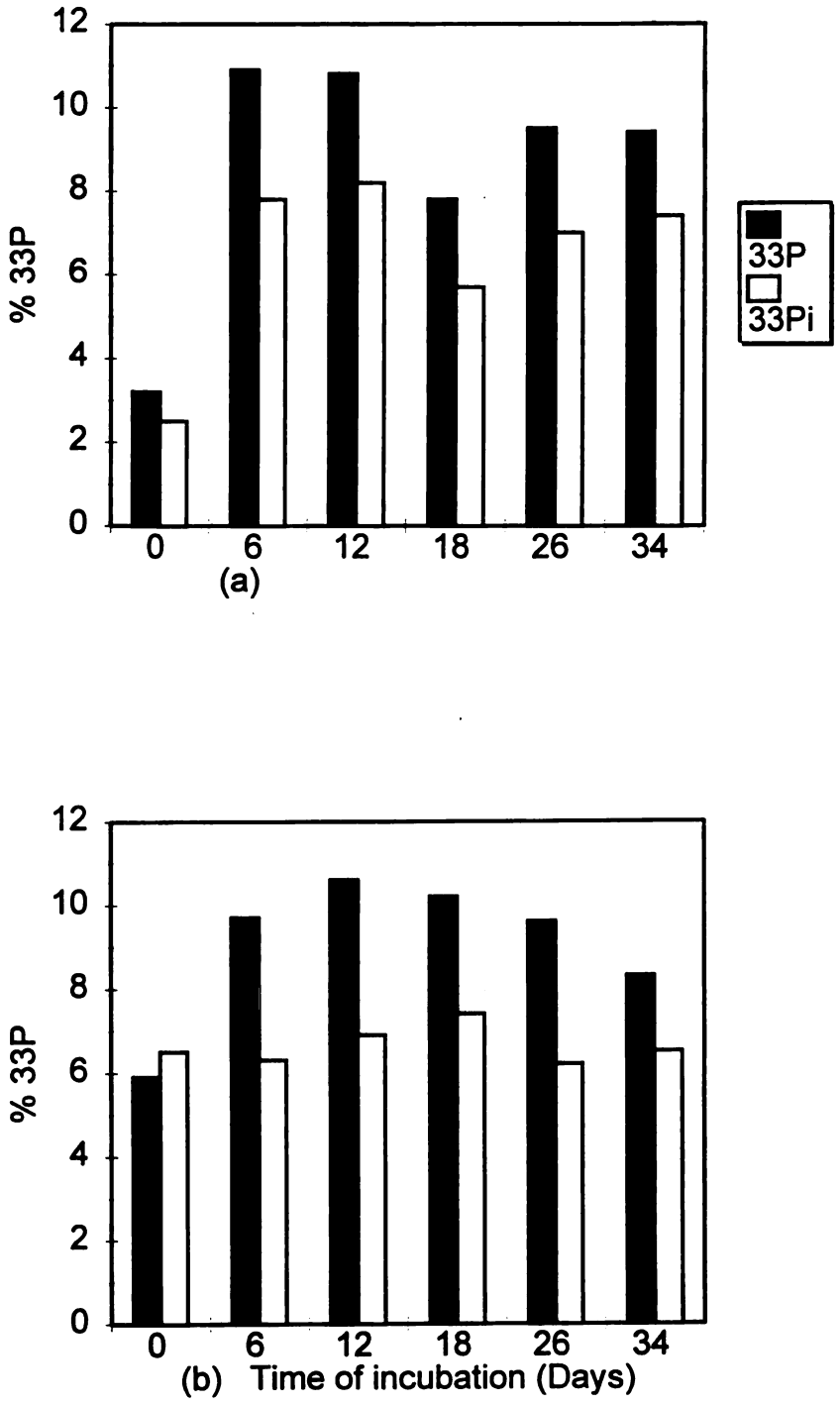

Figure 7. Inorganic and total ^{33}P in the microbial fraction in (a) NT and (b) CT Misteguay soil.

extracted in the resin fraction at day 0 (Appendix, Figure 1). Levels decreased rapidly between days 0 and 6 with an increase in the microbial pool (Appendix, Figure 2), and the NaOH pool (Appendix, Figure 3). The decline of the ^{32}P in the microbial pool occurred at day 12 for the CT soil, and at day 26 for the NT soil. This is an indication of higher microbial activity in the NT Kalamazoo soil. This decrease was coupled with an increase in the NaOH pool.

DISCUSSION

Accumulation of organic matter and chemical nutrients in the surface layers of soils is a common outcome in soils under no-till management practices. The soils in this study were sampled at a depth of 0 to 2 cm and all had a higher organic C content in the NT treatments compared to the CT treatments. Phosphorus fertilizers have not been applied in recent years to either the Capac or the Kalamazoo soils. This explains the lack of inorganic P accumulation in the 0 to 2 cm sampled surface layer of the NT treatments in these two soils. In both the Capac and Kalamazoo NT soils, CaP and residual P pools are smaller compared to the CT soils (Figures 8 & 9). Due to the lower pH of the NT soils, Residual P and CaP are transforming into other forms of P mainly NaOH extractable Pi and Po. Higher levels of NaOH Pi were found in the NT Capac soil, but were not significantly different from that in the CT soil.

Organic P is expected to be higher in the NT soils compared to CT soils due to the addition of large amounts of plant residues and accumulation of organic matter in these surface layers. It is hypothesized that due to the increase in the amounts of residues left on the soil surface of NT soils, an increased proportion of P would be incorporated into the microbial biomass and cycled into labile and resistant organic P forms. This is expected to result in greater accumulation of organic P in NT soils compared to CT soils.

The levels of NaOH extractable ^{31}Po were significantly higher in the NT Capac and Kalamazoo soils compared to the CT soils (Figures 8 and 9). But there were no significant differences in the labile Po or the biomass Po concentrations between the NT and CT treatments in either of these two soils. This indicates that organic P is accumulating into more resistant fractions (NaOH extractable) in the NT soils and not in the more labile fractions ($NaHCO_3$ extractable). This is also shown in the ^{33}P data. In the Capac soil, labile ^{33}P decreased rapidly during the

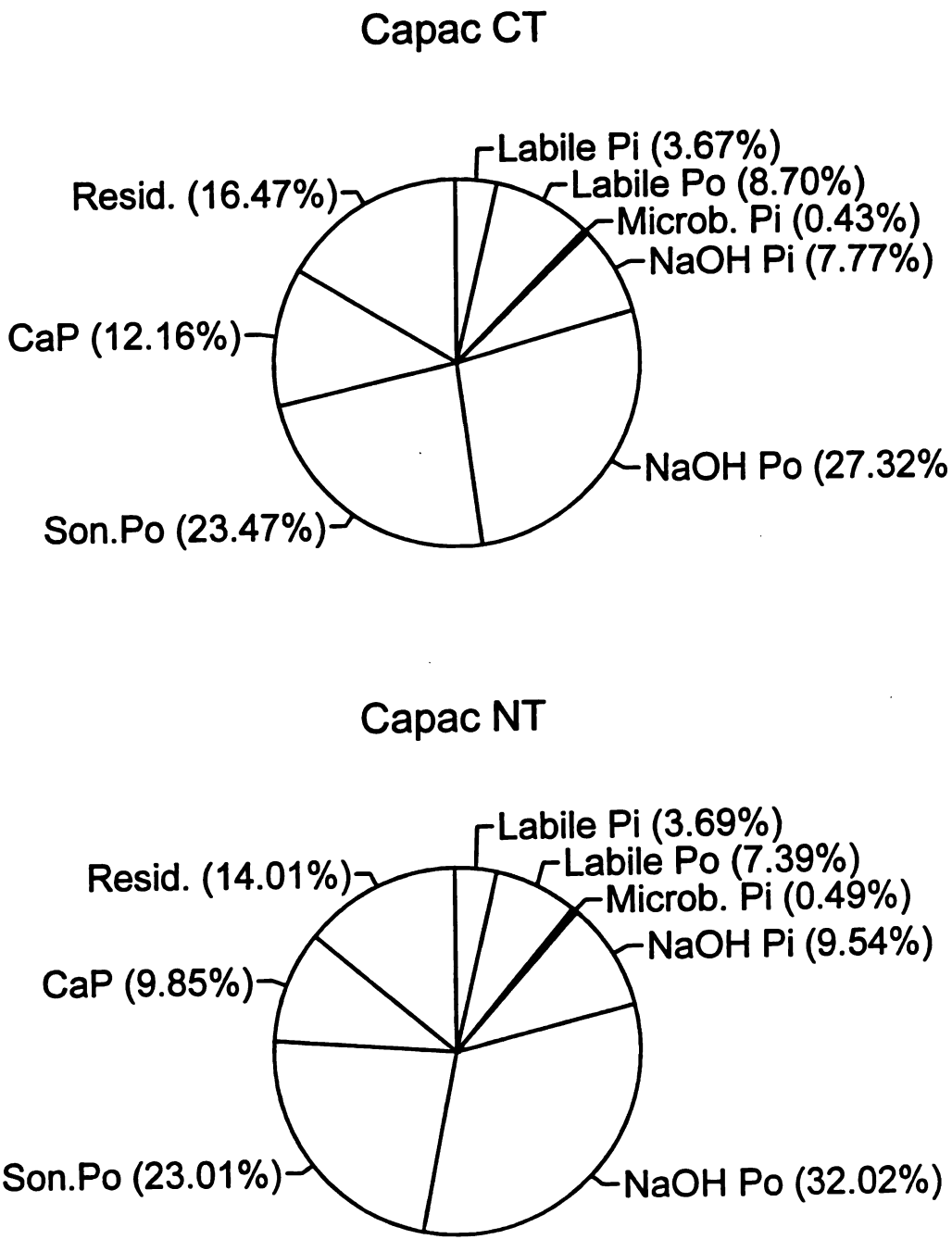

Figure 8. Inorganic and organic ^{31}P fractions in the NT and CT Capac soil

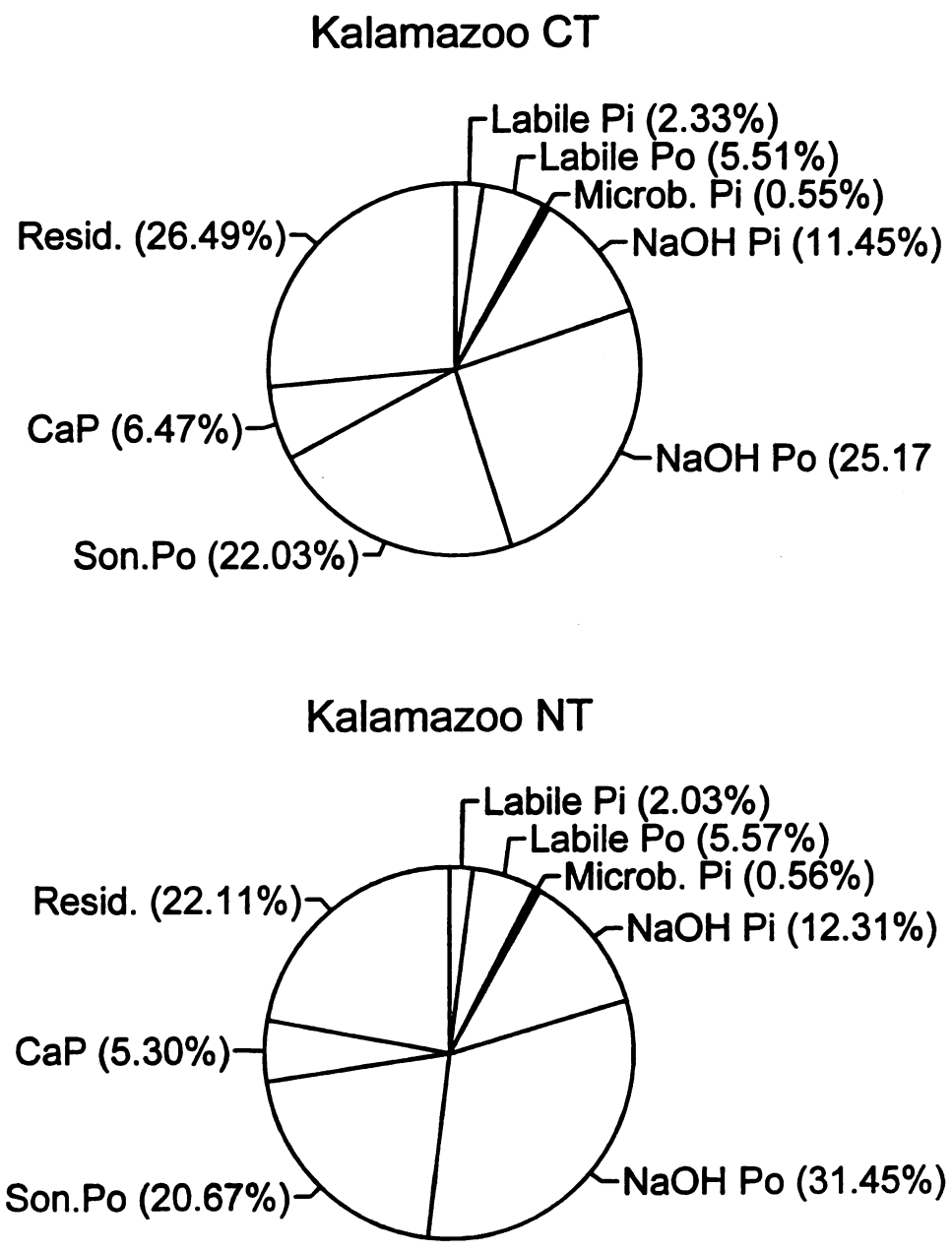

Figure 9. Inorganic and organic ^{31}P fractions in the NT and CT Kalamazoo soil

first week of incubation coupled with increases in the microbial biomass P and NaOH extractable P. Microbial biomass ^{33}P decreased at day 18 with a simultaneous increase in the NaOH extractable P. This indicates that a portion of the labile ^{33}P cycled through the microbial pool before ending up in the more resistant NaOH extractable fraction in the organic form. But a large proportion of the labile ^{33}P ended up directly into the NaOH pool without cycling through the microbial pool and is believed to be inorganic in nature. Phosphorus is fixed as AlP and FeP in this pH range of soils and the reaction is fast.

In five out of the six extraction dates, the NT Capac soil had a higher concentration of microbial ^{33}P compared to the CT Capac soil. This indicates higher microbial activity in the NT Capac soil than the CT Capac soil.

In the Kalamazoo soil, a similar pattern followed with some differences. Phosphorus in the microbial biomass was higher in the NT Kalamazoo soil than in the CT soil at all extraction dates with the cycling effect being more broad with time. Levels declined at day 26 with a corresponding increase in the NaOH extractable ^{33}P. This again indicates that a portion of the labile ^{33}P went through the microbial pool before ending up in the NaOH pool. McLaughlin et al. (1988) found that most of the plant residue ^{33}P was present as inorganic P at the time it was added to the soil, but only 7 days later almost 40 % had been incorporated into organic fractions of the soil which is similar to what we found in our study.

Inorganic and organic P fractions in the Misteguay soil is presented in Figure 10. Accumulation of Pi in the surface layer of the NT soils was evident in the NT Misteguay soil. Higher levels of labile ^{31}Pi, microbial ^{31}Pi, andNaOH ^{31}Pi were found in the NT Misteguay soil compared to the CT Misteguay soil. Phosphorus fertilizers are applied annually to the Misteguay soil. Due to the lack of incorporation of P fertilizers in soils under no-tillage, an accumulation of Pi is expected in the surface layers. Follett and Peterson (1988) found that undisturbed

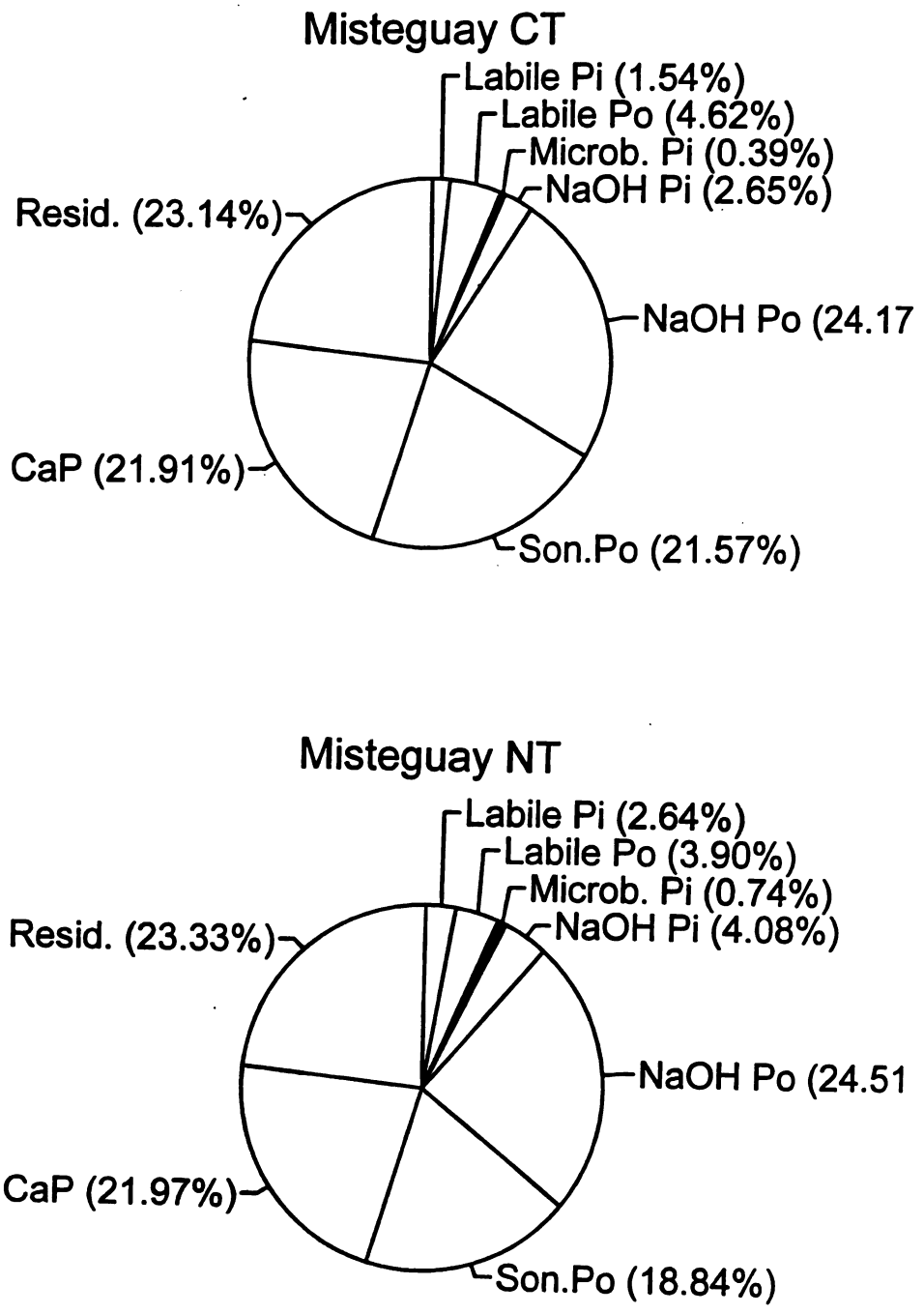

Figure 10. Inorganic and organic ^{31}P fractions in the NT and CT Misteguay soil.

NT soils accumulated NH_4HCO_3-DTPA extractable P in the surface relative to stubble mulch and plow treatments when P fertilizers have been applied. Weil et al. (1988) noted marked stratification of inorganic P at the 0 to 2 cm layer of NT plots. As P fertilizer application rates increased from 0 to 20 and 78 kg ha^{-1}, the stratification was even more dramatic.

Although an accumulation of inorganic P was found in the different P fractions extracted in the NT Misteguay soil, this was not the case for organic P. There were no significant differences in the Misteguay soil in either the labile Po or the resistant Po concentrations between the NT and CT treatments. This could be due to the fact that the Misteguay soil is a calcareous soil and there is a competition between the CaP pool and the organic pools for the P released from decomposition of plant residues. This conclusion is supported by the ^{33}P data that shows although part of the labile ^{33}P goes into the NaOH fraction, about 14% of the ^{33}P is fixed into the CaP pool in the Misteguay soil. The percentage of the ^{33}P going into the NaOH fraction in the Misteguay soil is less than that found in both the Capac and Kalamazoo soils. The cycling pattern in the microbial biomass was evident into the NT Misteguay soil, but not in the CT Misteguay soil. Microbial biomass ^{33}P levels increased in the first week in the CT Misteguay soil, then stabilized and started declining in the last two extraction dates. The decline was coupled with increases in the CaP and the residual P fractions.

The NaOH extractable Po after sonication pool is larger in the Misteguay than the Capac and Kalamazoo soils, however there is no difference between the NT and CT in all the three soils.

The fact that a high percentage of the ^{33}P appeared at day 0 in the three soils in the inorganic labile fraction is probably due the fact that a high percentage of the P applied from the ^{33}P labeled soybean residues was in the inorganic labile fraction (Table 3). The phosphate reserve in vegetative tissue is the inorganic

phosphate of the vacuoles and in P deficient plants it is especially the level of inorganic phosphate of stems and leaves that are decreased (Mengel and Kirkby, 1987). The manner in which the residues were handled in terms of drying and grinding before being applied to the soil may also have been a factor. Friesen and Blair (1988) attributed the rapid appearance of plant residue P in the soil inorganic P, 11 days after incorporation in the soil, to the presence of soluble inorganic P in the residues which entered the soil solution directly. The plants were finely ground in a hammer mill prior to addition to the soil, an action which is likely to macerate cell walls and facilitate rapid release of the soluble P components to the soil solution (Friesen and Blair, 1988). Blair and Boland (1978) prepared the plant material by crushing the plant material into pieces less than 1 cm long and found less than 1% of the ^{32}P from white clover plant residues in the inorganic fraction 12 days after addition. In our experiment, the plant material was dried and ground to a coarse fraction which may explain the high percentage of the ^{33}P appearing in the inorganic fractions in the soil at day 0 of incubation.

Labile P was fixed into other P forms in different proportions in the soil within the first week of application depending on the pH of the soil. In the Capac and Kalamazoo soils which have a low pH, a high proportion of the P was NaOH extractable which includes FeP, AlP, and resistant organic P forms. In the Misteguay soil, labile P was fixed into mainly three fractions, the NaOH extractable fraction which includes the P fractions mentioned above, the HCl extractable fraction which is mainly Ca-P minerals, and the residual fraction extracted by H_2SO_4 digestion. The increase in the Ca-P fraction in the Misteguay soil occurred between days 0 and 6 and levels stabilized afterwards which indicates that it is probably in the form of dicalcium phosphate. Residual P is mainly resistant inorganic and organic P forms which are very insoluble and unavailable to the plants. The fact that this fraction was higher in the Misteguay

soil and not in the Capac and Kalamazoo soils means that pH is the major factor in affecting the solubility and availability of P in soils.

CONCLUSION

The effect of tillage on ^{31}P pools distribution in soils was minimal. When no P fertilizer was applied, there was little effect on the quantity of inorganic P in each fraction even though the NT system has resulted in an accumulation of organic matter in the soil surface layer and lower pH. But there was an increase in resistant organic P fraction due to NT reflecting the greater soil organic matter content. Less CaP and residual P was also found in the NT systems probably due to the decrease in soil pH

When P fertilizer was added yearly in the management system, NT resulted in much higher levels of Pi in the surface layer. This occurs because fertilizer P is added to a much smaller volume of soil.

Tillage had little effect on residue P transformation. Labile P transformed into more resistant fractions in both tillage systems. In low pH soils, labile P was fixed into the NaOH pool in inorganic and organic forms. In the high pH soil, Labile P was fixed into the NaOH and HCl pools. Calcium phosphates extracted with HCl represent a large pool in high pH soils. It is concluded that inorganic P chemistry dominates the system.

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CHAPTER III

TRANSFORMATION OF P IN A SOIL STERILIZED BY GAMMA IRRADIATION

INTRODUCTION

Although the contribution of the microbial population to the turnover of the P fraction of the soil organic matter is not clearly understood, there is little doubt that the microbial population both mineralizes and immobilizes P in the system, therefore affecting its availability for plant nutrition (Halstead and McKercher, 1975). Approximately 0.0025% or 25 ppm organic P could be associated with the soil microorganisms. This is approximately 5 to 10% of the total organic P found in many soils.

Phosphate-containing organic matter is almost certainly accumulated in soil as a result of microbial activity (Cosgrove, 1967). The implication is that organic P in plant and animal remains is mineralized fairly rapidly and is then used for the synthesis of microbial organic phosphates. More evidence is needed, however, to establish that stable organic phosphates in soil originates by microbial synthesis rather than the accumulation of resistant fractions of plant and animal residues (Cosgrove, 1977).

Of the minor constituents in soils, the phospholipids are likely to be of plant and animal origin, but their base components, choline and ethanolamine, are known to also occur in microorganisms (Cosgrove, 1977). Nucleic acids are apparently of microbial origin (Halstead and McKercher, 1975). The

quantitatively most important identified group of phosphate esters in soil is the inositol phosphate mixture. Its origin in soils is still uncertain; although myo-inositol hexaphosphate is a common plant constituent, especially in mature seeds, phosphates of other inositols are unknown to plants. The inositol phosphates is therefore assumed to be of microbial origin, but as yet no organisms capable of producing inositol polyphosphates have been isolated (Cosgrove, 1977).

For research purposes treatment of the soil with Gamma irradiation provides a useful means of partial or complete sterilization, according to the dose applied. The process produces very little rise in the temperature of the sample (Cawse, 1975). Increases in the extractability of several elements following irradiation have been reported. Lensi et al. (1991) report very small variations in pH and exchangeable cation concentrations after sterilizing the soil with 25 kGy (2.5 Mrad) dose. But there was an increase in soluble organic C attributed to the lysis of killed cells and to the release of organic acid.

In a previous study, it was found out that ^{33}P released from plant residues applied to soils transformed rapidly into other resistant fractions mainly the NaOH fraction. It is not known if the P transformations that occurred were mainly microbially mediated or were inorganic chemical fixation of P. The objective of this experiment was to determine if microbial activity altered the rate and quantity of added P as it is transformed into less labile P fractions.

MATERIALS AND METHODS

Soil

The soil used was a never-tilled soil with native vegetation (unplanted reference, rep. # 23) from the Kellogg Biological Station. The soil is a Kalamazoo loam (Fine-loamy, mixed, mesic Typic Hapludalf). The sampling was done on September, 1993. Ten samples were taken from the across the field at a depth of 15 cm and mixed to make one sample. The sample was sieved while moist, and stored at 4° C till use. Before the start of the experiment, the soil was kept at room temperature for two weeks in order for microorganisms to restore normal activity. Relevant soil properties were measured and are reported in Table 1. Texture was measured by the pipette method after treatment with H_2O_2 to remove the organic matter. The pH was measured in a 1:1 soil to solution ratio using a glass electrode. Organic C was measured by the total combustion method.

Irradiation of the soil was done by a Cobalt-60 irradiator at the nuclear reactor laboratory at the University of Michigan for the purpose of sterilization. The dose rate was measured with Reuter-Strokes ion chamber which is calibrated annually against a National Bureau of Standards source. The gamma dose rate was 637690 rad h^{-1} for 7.85 h for a total gamma dose of 5.01 Mrad (50 KGy). The irradiation was continuous except for an interruption time of 6 min, while the soil was being rotated 180 degrees half-way through the irradiation to achieve a uniform dose. Microbiological tests were done on the irradiated and non-irradiated soils and the results are presented in Table 2. Before the initiation of the experiment, aerobic bacteria plate counts were done on R2A medium, and fungal plate counts on Rose Bengal Agar. Anaerobes (vegetative cells) were tested by adding 0.001 g of the non-irradiated soil to fluid Thioglycollate broth tubes while 3 g were used for the irradiated soil. Anaerobes (spores) were tested by shocking

Table 1. Selected properties of the soil investigated.

Soil Series		Kalamazoo loam
pH		5.34
Sand	%	40.0
Silt	%	44.5
Clay	%	15.5
Organic C	%	3.04
CEC	$cmol_c\ kg^{-1}$	8.85
Bray-Kurtz P1	$mg\ kg^{-1}$	31.3

Table 2. Microbiological results on irradiated vs non-irradiated soil.

	Number of organisms per g of wet soil			
	Aerobic bacteria	Fungi	Anaerobes (vegetative cells)	Anaerobes (spores)
		Start of Experiment		
N.IRR.†	$2.3x10^8$	$>3.0x10^5$	+++ growth in 1 d	+++ growth in 5 d
IRR.‡	<10	<10	No growth after 21 d	No growth after 21 d
		End of Experiment		
IRR. + plants	$7.4x10^4$	$3.8x10^4$	$9.3x10^4$	ND§
IRRI. - plants	<10	<10	No growth after 21 d	ND

† Non-irradiated
‡ Irradiated
§ Not determined

another set of the fluid Thioglycollate Broth tubes at 80 ^{o}C for 10 min. All analysis were done in triplicates except the fungi count which was done in duplicates. After the end of the experiment, aerobic and fungal plate counts were done on R2A medium while anaerobes and facultative anaerobes were counted in a fluid thioglycollate broth incubated at 25 ^{o}C for 21 days.

Preparation of the ^{33}P labeled plant material:

Soybean seeds were germinated in sand flats that had been rinsed with dilute acid solution and distilled water. The seedlings were transplanted into pots containing a modified Hoagland nutrient solution (B. Knezek, personal communication) that had 1/5th of the recommended concentration of P. Three plants were transplanted per pot and grown in a growth chamber. The growth conditions in the chamber were: temperature of 27 ^{o}C day, and 21 ^{o}C night with 16 h of light. After the plants were grown for two weeks, ^{33}P as orthophosphoric acid solution was added to the nutrient solution. The plants were grown for eight days then harvested. Leaves and roots were separated and dried at 60 ^{o}C for 48 h. Roots were washed with a solution of ^{31}P to remove any ^{33}P residing on the surface of the roots, then rinsed with distilled water before drying. The plant material was ground to less than 4 mm size and the ^{31}P and ^{33}P concentrations in the plant material determined. The plants had a P content of 0.5%, a total activity of the ^{33}P of 316 KBq g^{-1}, and a specific activity of 58 MBq $g^{-1}P$. The plant material was not sterilized.

Treatments

One hundred gram of each of the irradiated and non-irradiated soils was weighed into a glass jar, 0.2 g of the ^{33}P labeled plant material (0.15 g leaves and 0.05 g roots) was added to the soil and thoroughly mixed, the soil moisture

adjusted to field capacity with sterilized distilled water, then incubated at 25 ^{o}C. Three replications were established per soil per extraction date. The incubation times were 0, 4, 8, 12, 18, and 24 days.

Extraction Procedure

The fractionation scheme used in this experiment was a modification of the procedures proposed by Hedley et al. (1982) and Tiessen et al. (1984) and was slightly different from the procedure in the previous experiments.

1. Two sets (A & B) of 5 g of soil each were weighed into a 250 ml centrifuge bottle. Four g of a strong anion exchange resin (Dowex 1x8-50), 20-50 mesh in the bicarbonate form in a nylon mesh bag (< 53μm) and 200 ml of distilled water was added to the centrifuge bottle. The capacity of the resin was 3.5 meq g^{-1} with a total capacity of 14 meq. The bottles were shaken for 16 to 18 h. The resin bag was removed and rinsed free of soil back into the centrifuge bottle in order to minimize loss of soil. The P in the resin was extracted by shaking the bag for 24 h with 0.5 N HCl. Both ^{33}P and ^{31}P were determined in this fraction. The P measured is inorganic labile P. The soil remaining in this bottle was centrifuged at 5000 rpm for 20 min and the supernatant discarded as it contained no P.

2. After extraction with the resin, set B was extracted with 100 ml of 0.5 M $NaHCO_3$ (pH 8.5) for 1 h. Set A was fumigated with 2 ml of chloroform for 18 to 20 h. The chloroform was then allowed to evaporate for 18 to 20 h and then extracted with the same procedures as set B. The solution was centrifuged at 5000 rpm for 20 min and inorganic ^{31}P (Pi), total ^{31}P (PT), and ^{33}P were determined. The difference in Pi extracted between set A and set B is Pi in the microbial

biomass. Organic ^{31}P (Po) in the microbial biomass was calculated as the difference between Po in the fumigated and unfumigated samples.

3. Next two 16 h extractions with 0.1 N NaOH were done. The bottles were centrifuged for 20 min at 5000 rpm. Analysis was done to determine ^{31}Pi, ^{31}PT, and ^{33}P. The data presented is the sum of the two extractions. Organic P was calculated as the difference between PT and Pi. NaOH extractable Pi is P found in the secondary minerals, and NaOH extractable Po is moderately labile P (Tiessen et al. 1984).

4. Residual P was determined by digesting the remaining soil with HNO_3 and $HClO_4$ acid mixture on a hot plate. Both ^{31}P and ^{33}P were determined.

Counting of the ^{33}P was done in a liquid scintillation counter with an open channel by adding 1 ml of sample to 10 ml of cocktail mix. All counts were corrected for background and decay. The ^{31}P was determined by the method of Murphy and Riley (1962) using an automated flow injection analyzer. The pH of the $NaHCO_3$ and NaOH extracted samples was adjusted to 3 or 4 with 0.5 N HCl, and the pH of the HNO_3 and $HClO_4$ digested samples was adjusted to the same pH with 0.1 N NaOH. Total P was determined in the $NaHCO_3$ and NaOH extracts by digesting the samples with H_2SO_4 and ammonium persulfate on a hot plate (USEPA methods for the analysis of water, 1978). Samples were analyzed for P by the same method described above after adjusting the pH.

RESULTS AND DISCUSSION

The microbiological tests done on the irradiated soil shows that sterilizing the soil with gamma irradiation was complete (Table 2). The labeled plant material added to the soil was not sterilized. The microbiological tests done on the soil after the end of the experiment shows that some microorganisms began to colonize the sterilized soil. But the counts for the bacteria were much less than was found in the non-irradiated soil. The counts for the fungi was ten fold less in the irradiated soil than in the non-irradiated soil. It is suspected that most of the growth was mold introduced from the plants. Tests done on irradiated soils that were incubated at the same conditions without added plants revealed no growth of microorganisms. It is believed that the microbial growth did not affect any P transformations that occurred during the course of the experiment. Very little ^{31}P or ^{33}P was found in the microbial biomass in the irradiated soil during the course of the experiment. This would indicate that microbial activity was low during the course of the experiment.

The apparent effect that irradiation had on the P fractions was a flush of P from microorganisms (Table 3). The levels of $NaHCO_3$ extractable P increased from 6 mg kg^{-1} in the non-irradiated soil to 29 mg kg^{-1} in the irradiated soil. This is expected as microorganisms were killed by the gamma irradiation, P was released from their cells. Increases in other P pools occurred. Small increases occurred in the resin extractable Pi, and NaOH extractable Pi. The $NaHCO_3$ Po increased from 93 to 166 mg kg^{-1}, and NaOH Po from 330 to 422 mg kg^{-1}. Organic P in the soil solution was reported to increase when subjected to a five Mrad dose, while P extracted by an ammonium acetate solution was increased by a dose of 0.6 Mrad (Cawse, 1975). Residual P decreased from 4617 to 4450 mg kg^{-1}. Phosphorus released from the solubilization of some of the residual P was

Table 3. Changes in pH and soil P fractions due to a 5 Mrad dose of gamma irradiation

	non-irradiated soil	irradiated soil
pH	5.34	5.47
Resin Pi mg kg^{-1}	23	41
$NaHCO_3$ Pi mg kg^{-1}	6.3	29.1
Microbial Pi mg kg^{-1}	22.0	2.8
$NaHCO_3$ P_o mg kg^{-1}	93	166
NaOH Pi mg kg^{-1}	140	154
NaOH Po mg kg^{-1}	330	421
Residual P mg kg^{-1}	4617	4450
Sum of fractions	5231	5264

converted into other labile ($NaHCO_3$ extractable) and moderately labile (NaOH extractable) organic fractions.

The ^{31}P pools were, in general, fairly stable with incubation. The labile and NaOH extractable ^{31}Pi fractions (Table 4) did not change much with incubation. The labile and NaOH extractable ^{31}Po were, however, variable during incubation especially the NaOH pool (Table 5). No significant changes are believed to be occurring, and the variability is due to the nature of organic P determinations.

The soil used had a high level of organic matter. The microbial activity is expected to be high. This is reflected in the concentration of the ^{31}P in the microbial biomass which was 22 mg kg^{-1} at day 0 and increased gradually to 30.5 mg kg^{-1} at day 12 then started to decrease after that (Table 4). The ^{33}P incorporated into the microbial biomass reached 20% of the applied ^{33}P at day 12 (Figure 3).

More than 70% of the ^{33}P at day 0 of incubation was found in the inorganic labile fraction (Figure 1). Levels dropped from 70 to 25% between days 0 and 4 in the non-irradiated soil, then decreased gradually until day 18. The decrease was more pronounced between days 18 and 24. This decrease was coupled with an increase in the microbial biomass fraction (Figure 2) and the NaOH extractable fraction (Figure 3). Levels in the NaOH fraction increased from 18 to 39% between days 0 and 4. Levels seemed to stabilize between days 4 and 12 and started to increase again between days 12 and 24. The ^{33}P incorporated in the microbial biomass in the non-irradiated soil increased sharply from 5 to 15% during the first four days of incubation and reached 20% at day 12 (Figure 2). Levels started to decrease slowly at the last two extraction dates.

The trend of the ^{33}P transformation was similar in the irradiated soil with two major differences. Very small concentrations of the ^{33}P were found in the

Table 4. Inorganic ^{31}Pi and residual fractions in the irradiated and non-irradiated never tilled soil with incubation time.

	Time of incubation (Days)					
	0	4	8	12	18	24
Labile	---------- mg kg^{-1} ----------					
N.IRR.	23	23	19	21	18	19
IRR.	41	45	42	41	39	40
Microbial						
N.IRR.	22.0	27.3	29.3	30.5	27.4	27.5
IRR.	2.8	6.1	4.3	5.5	4.9	5.6
NaOH						
N.IRR.	140	156	152	152	146	143
IRR.	154	156	160	159	159	148
Residual						
N.IRR.	4617	5700	5782	5531	4258	4722
IRR.	4450	5594	3925	4517	2935	4342

Table 5. Organic ^{31}P fractions in the irradiated and non-irradiated never tilled soil with incubation time.

	Time of incubation (Days)					
	0	4	8	12	18	24
Labile	----------mg kg^{-1}----------					
N.IRR.	93	100	113	121	113	130
IRR.	167	103	152	119	165	135
Microbial						
N.IRR.	15.2	-19.5	- 7.8	-14.7	11.2	-16.0
IRR.	25.9	2.4	-32.6	- 1.9	-42.0	- 3.9
NaOH						
N.IRR.	330	355	425	391	335	288
IRR.	421	339	362	458	347	282

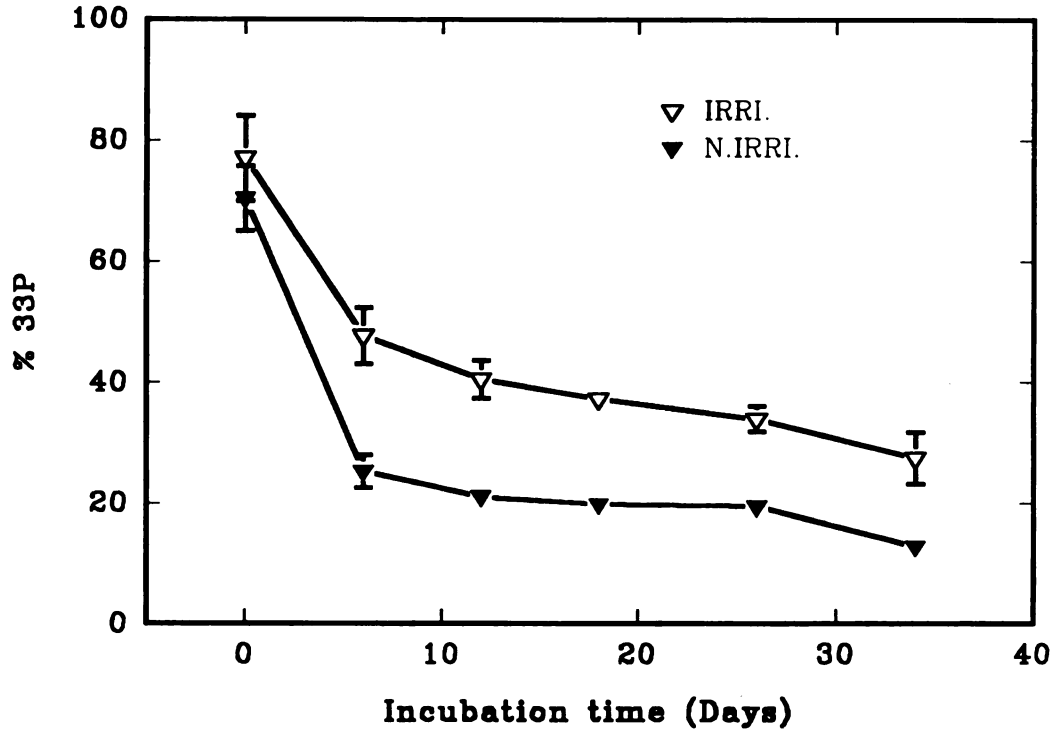

Figure 1. Labile ^{33}P in the irradiated and non-irradiated never-tilled soil.

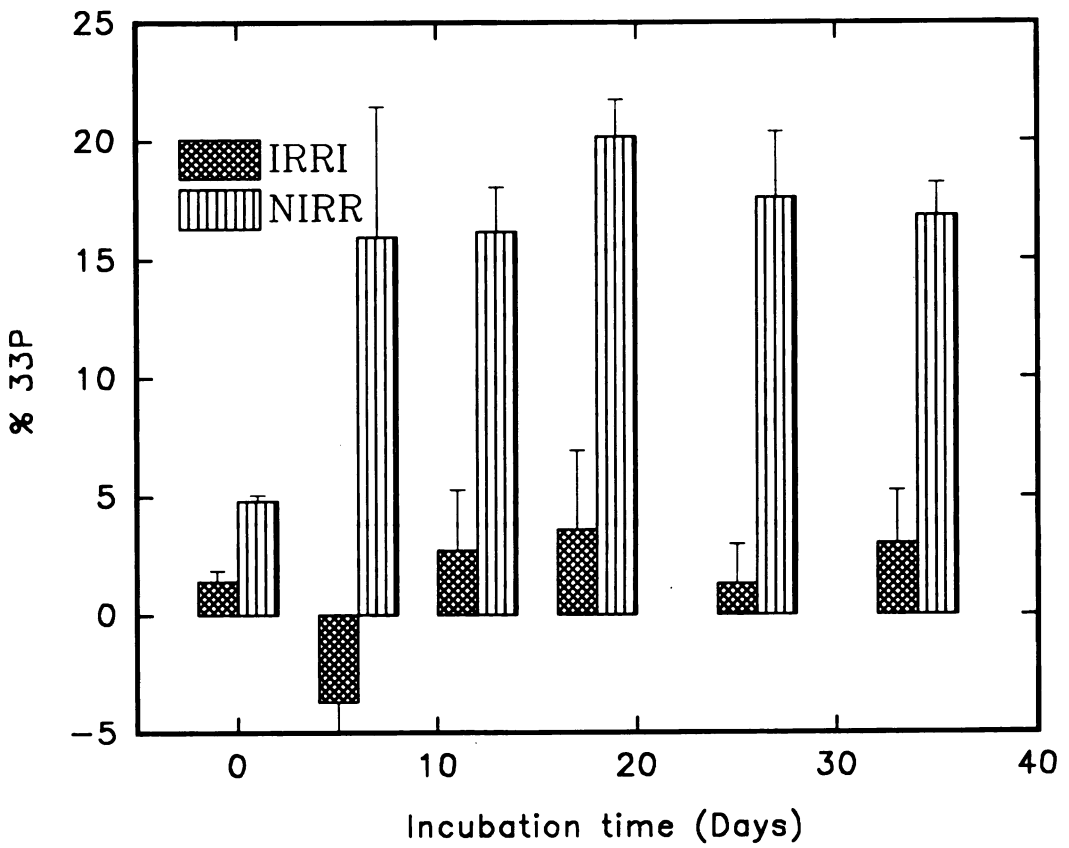

Figure 2. Microbial biomass ^{33}P in the irradiated and non-irradiated never-tilled soil.

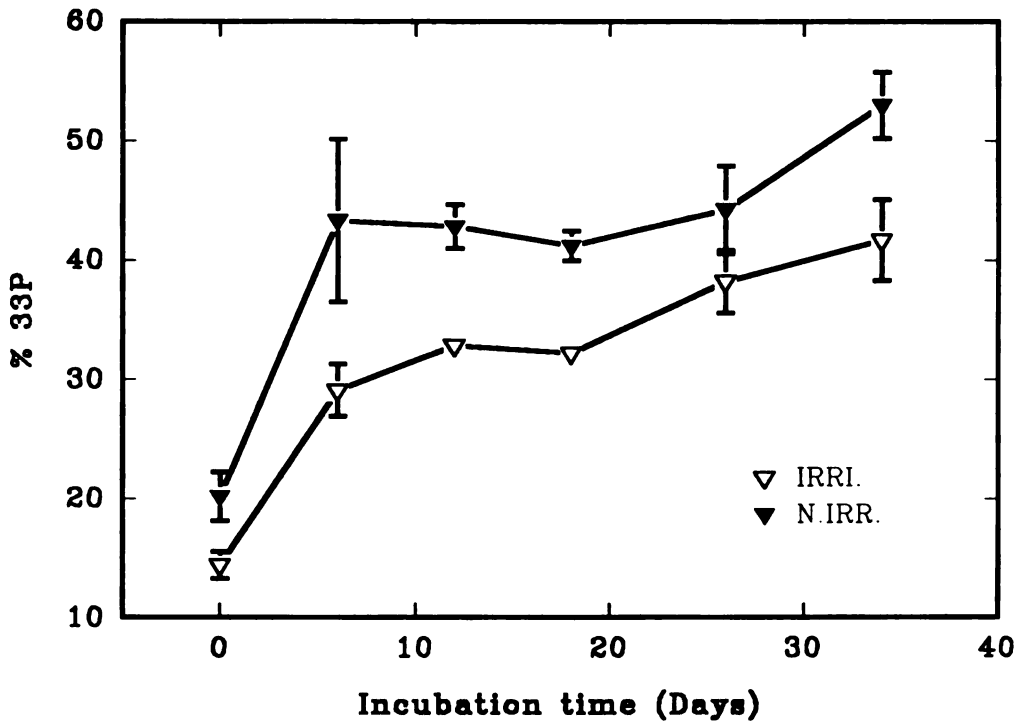

Figure 3. NaOH extractable ^{33}P in the irradiated and non-irradiated never-tilled soil.

microbial biomass throughout the experiment and the concentrations of the ^{33}P that transformed from the inorganic labile extractable fraction into the NaOH fractions were lower. The percentage of the ^{33}P in the inorganic labile fraction in the irradiated soil was about 15 to 22% higher than that in the non-irradiated soil throughout the incubation period. The ^{33}P in the $NaHCO_3$ extractable fraction before fumigation was 2.7 to 11% higher in the irradiated soil than in the non-irradiated soil (Figure 4). The levels in the NaOH fraction were 6 to 14% lower in the irradiated soil than the non-irradiated soil. It seems more of the ^{33}P was found in the labile P fraction and the $NaHCO_3$ extractable fraction before fumigation in the irradiated soil than in the non-irradiated soil.

The NaOH extractable P fraction is believed to contain inorganic P (Al-P and Fe-P) and organic P. The ^{33}P incorporated into the NaOH fraction in the irradiated soil is probably inorganic P that is fixed as Al-P and Fe-P minerals. It is not suspected to contain organic P as P in the microbial biomass was very low. The additional percentages of ^{33}P that were found in the non-irradiated soil in the NaOH fraction (6 to 14%) is probably organic P that has been transformed through microbial activity. The ^{33}P going into the NaOH fraction in the non-irradiated soil was about 14% higher at day 4 than the levels in the irradiated soil. Phosphorus immobilization by the microorganisms into the NaOH fraction was very fast during the first four days of incubation. The ^{33}P levels in the NaOH fraction in the non-irradiated soil stabilized between days 4 and 12, and started increasing again afterwards. The ^{33}P levels in the microbial fraction, on the other hand, increased till day 12 and started decreasing afterwards. This may be an indication of the microbial activity working on the more resistant fraction of the ^{33}P labeled plants between days 4 and 12. The ^{33}P is cycling between the NaOH

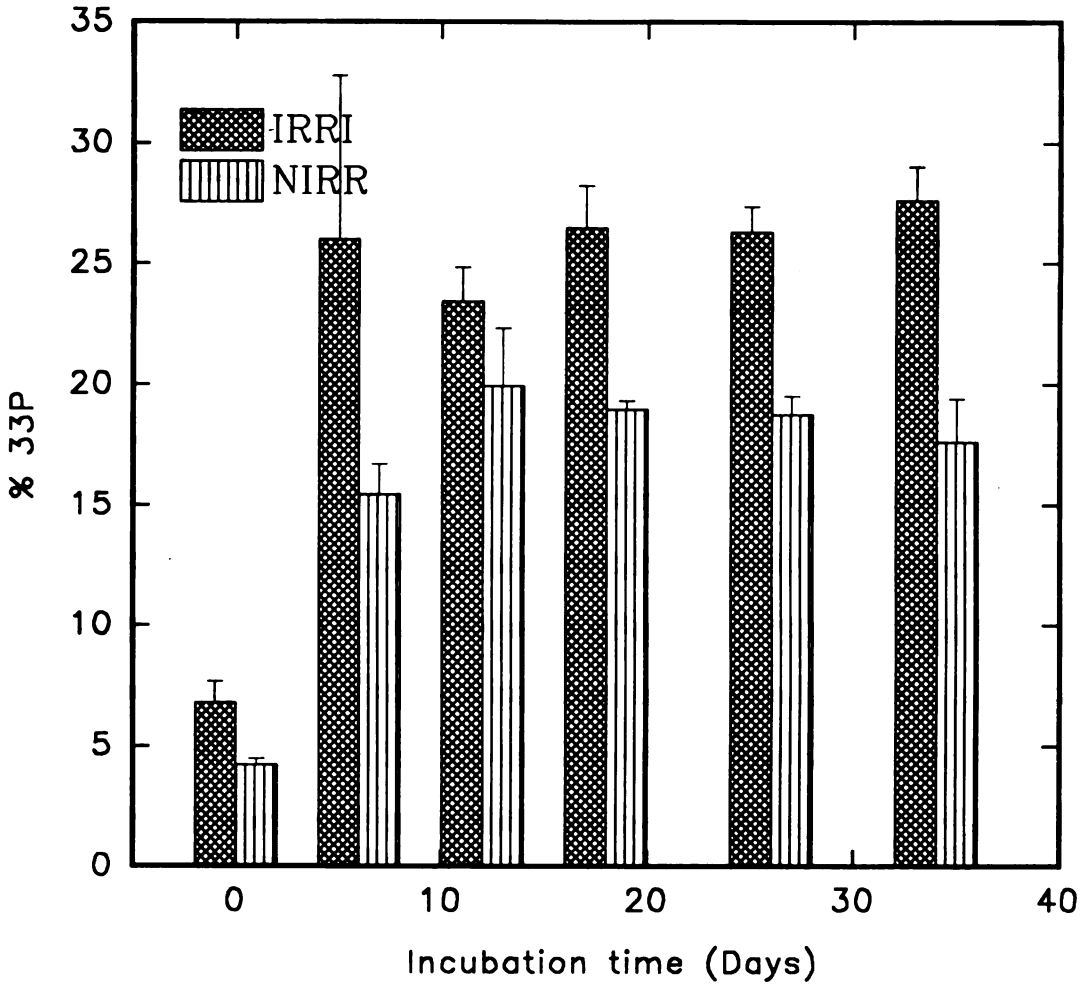

Figure 4. $NaHCO_3$ extractable ^{33}P befor fumigation in the irradiated and non-irradeated never-tilled soil.

and the microbial pool during that period. After day 12, the microbial activity started declining indicated by the gradual decrease in the ^{33}P in the microbial fraction and more ^{33}P remaining in the NaOH fraction.

Very little ^{33}P went into the residual fraction extracted by digestion with perchloric acid. Percentages did not exceed 2.5% and were not different between the irradiated and the non-irradiated soil. This is expected as this pool is a very stable one. It includes chemically stable Po forms and relatively insoluble Pi forms. Cycling of P in and out of this pool is very slow.

CONCLUSIONS

Sterilization of soils by Gamma irradiation is a useful tool that have been used in the microbiology field and has potential important applications in research. This study showed that P transformation of applied ^{33}P labeled residues into the soils happens very fast. The most important transformation is P going form labile inorganic fraction into moderately labile inorganic and organic fractions. About 53% of the ^{33}P was found in the NaOH extracted fraction at day 24 of incubation. It is hypothesized that about 11% is organic and have cycled through the microbial biomass before ending up in the NaOH fraction.

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CHAPTER IV

SUMMARY AND CONCLUSIONS

The effect of tillage on forms of soil P and on transformation of added P to soils was investigated. Soils under conventional and no-tillage management systems were sampled from three experimental sites in Michigan to obtain soils with different physical and chemical properties and years under no-till. The Capac soil is a loam (fine-loamy, mixed mesic Aeric Ochraqualf) with a pH of 6.2 for the CT, and 5.4 for the NT, and has been under NT for 13 years. The Kalamazoo soil is also a loam (fine-loamy mixed, mesic typic Hapludalf) with a pH of 6.3 for the CT, and 5.5 for the NT. Kalamazoo soil has been under NT for four years. The Misteguay soil (fine, mixed calcareous, mesic Aeric Haplaquept) has a pH of 8.0 for the CT and 7.9 for the NT. Misteguay has been under NT for 8 years. Soybean plant residues labeled with ^{33}P were added to the soils. The soils were then incubated at controlled laboratory conditions, and periodically extracted to determine the P content of the different P fractions .

The P fractions extracted were: resin extractable P; $NaHCO_3$ extractable P, P in the microbial biomass; NaOH extractable P; NaOH extractable P after sonication, HCl extractable P; and residue P after digestion with H_2SO_4 and H_2O_2. The ^{31}Pi and ^{33}P concentrations were determined in all fractions. In addition, total P was determined in the $NaHCO_3$, NaOH, and NaOH after sonication extracts in order to calculate organic P in these fractions.

To determine if microorganisms altered the rate of P transformation from residues added to soils, a never tilled soil with native vegetation was sampled from the Kellogg Biological station and sterilized by gamma irradiation and the fate of ^{33}P from labeled soybean plant residues added to sterilized and non-sterilized soil was determined. Phosphorus was extracted into the following fractions: resin, $NaHCO_3$, microbial, NaOH, and residual ($HClO_4$ acid digestion).

When no P fertilizer was added to soils, there was little effect of tillage on the quantity of inorganic P in each of the fractions even though the NT system had resulted in accumulation of organic matter in the surface layer of soil and a lower soil pH. But there was an increase in organic P due to NT reflecting the greater soil organic matter content.

When P fertilizer was added yearly in the management system, NT resulted in much higher levels of Pi. This occurs because the P fertilizer is added to a much smaller volume f soil.

There were no differences between the NT and the CT treatments in the labile Po in any of the three soils which indicates that this pool is probably a dynamic pool which cycles P through the organic matter rather than accumulating P. Measuring organic ^{31}P in the microbial biomass was not successful. Negative numbers were obtained due to large standard errors associated with such a measurement.

The Calcium ^{31}P pool in the Kalamazoo soil was smaller in the NT system. This was related to the decrease in pH of the soil due to no-tillage.

The ^{33}P added in the form of plant residues was distributed in the three soils in a very similar manner with few differences. The largest fraction of the ^{33}P was in the inorganic form at the time of application to soils. But after incubation, a large percentage of the ^{33}P ended up in the NaOH extractable pool in the Capac and the Kalamazoo soils, and in the NaOH and HCl extractable pools in the

Misteguay soil. This was due to the differences in the pH between the soils. Both Capac and Kalamazoo soils having low pH, the dominant inorganic P forms will be Fe-P and Al-P which are extracted by NaOH. The Misteguay soil is a calcareous soil with a pH of about 8. Calcium phosphates (extracted with HCl) represent a large pool in this soil. It is concluded that inorganic P chemistry dominates the system.

Tillage had little effect on the distribution of the ^{33}P into the various P fractions. The ^{33}P cycling through the microbial pool was, in general, a bit higher in the NT soils than in the CT soils, but differences were not significant. The ^{33}P found in the microbial pool went through one or two cycles in that pool before being converted into the more resistant NaOH fraction.

Sterilization of a soil by irradiation immediately released microbial biomass P to the labile inorganic P and labile organic P fractions. When ^{33}P labeled soybean residues were added, 15% of the ^{33}P was found in the microbial biomass within 4 days in the non-sterilized soil. The non-sterilized soil also removed more ^{33}P into the NaOH extractable fraction. About 10 percent more ^{33}P was found in this fraction for the non-sterilized soil showing that microorganisms were responsible for the transformation.

Sterilizing soils with gamma irradiation has important potential research applications. The process of sterilization involved minimal changes in pH and P fractions extracted in the soil.

APPENDIX

Table 1. Recovery of ^{31}Pi and ^{31}PT by extraction with 0.5 M $NaHCO_3$ (pH 8.5) and P flush caused by fumigation.

Time of incub. Days	Capac NT				Capac CT			
	Unfumigated		Fumigated		Unfumigated		Fumigated	
	^{31}Pi	^{31}PT	^{31}Pi	^{31}PT	^{31}Pi	^{31}PT	^{31}Pi	^{31}PT
	mg kg^{-1}							
0	43.4‡	144	46.7	214	29.3	170	31.4	183
6	38.7†	103	45.2	165	24†	110	33‡	136
12	36.9	138	44.4	153	22.6	96	28.5	139
18	45.2†	141	48.5	184	24.2‡	132	31.7	157
26	--	158	54.6	247	24.3	156	28.9	169
34	32.8†	151	44.2	212	21‡	161	25.7	145
Mean		139	47.3	196	24.2	137.5	29.9	155

Table 2. Recovery of ^{31}Pi and ^{31}PT by extraction with 0.5 M $NaHCO_3$ (pH 8.5) and P flush caused by fumigation.

Time of incub.	Kalamazoo NT				Kalamazoo CT			
	Unfumigated		Fumigated		Unfumigated		Fumigated	
Days	^{31}Pi	^{31}PT	^{31}Pi	^{31}PT	^{31}Pi	^{31}PT	^{31}Pi	^{31}PT
	mg kg^{-1}							
0	8.7	111	14	92	6.4	122	11.3	79
6	7.3	57	16.3	67	5.5	43	10.3	61
12	9.9	53.5	16	80	6.2	59	12.1	75
18	9.7	60	14.7	75	6.9	58	10.7	67
26	9.5	76	15.7	94	6.6	64	10.5	78
34	8.3	69	13.6	97	6.3	73	9.0	74
Mean	8.9	71	15.1	84	6.3	70	10.7	72

Table 3. Recovery of ^{31}Pi and ^{31}PT by extraction with 0.5 M $NaHCO_3$ (pH 8.5) and P flush caused by fumigation.

Time of incub.	Misteguay NT				Misteguay CT			
	Unfumigated		Fumigated		Unfumigated		Fumigated	
Days	^{31}Pi	^{31}PT	^{31}Pi	^{31}PT	^{31}Pi	^{31}PT	^{31}Pi	^{31}PT
	---------- mg kg^{-1} ----------							
0	10.3	97	22.4	122	5.7	90	14.3	220
6	8.7	231	29.3	142	6.9	116	15	116
12	8.3	102	25.7	106	5.2	82	13.2	105
18	9.3	94	23.4	98	5.7	96	12.9	96.7
26	7.1	90	24.7	125	4.2	100	13.6	131
34	6.5	110	23.2	124	6.5	112	12.7	101
Mean	8.4	121	24.8	120	5.7	99	13.6	128

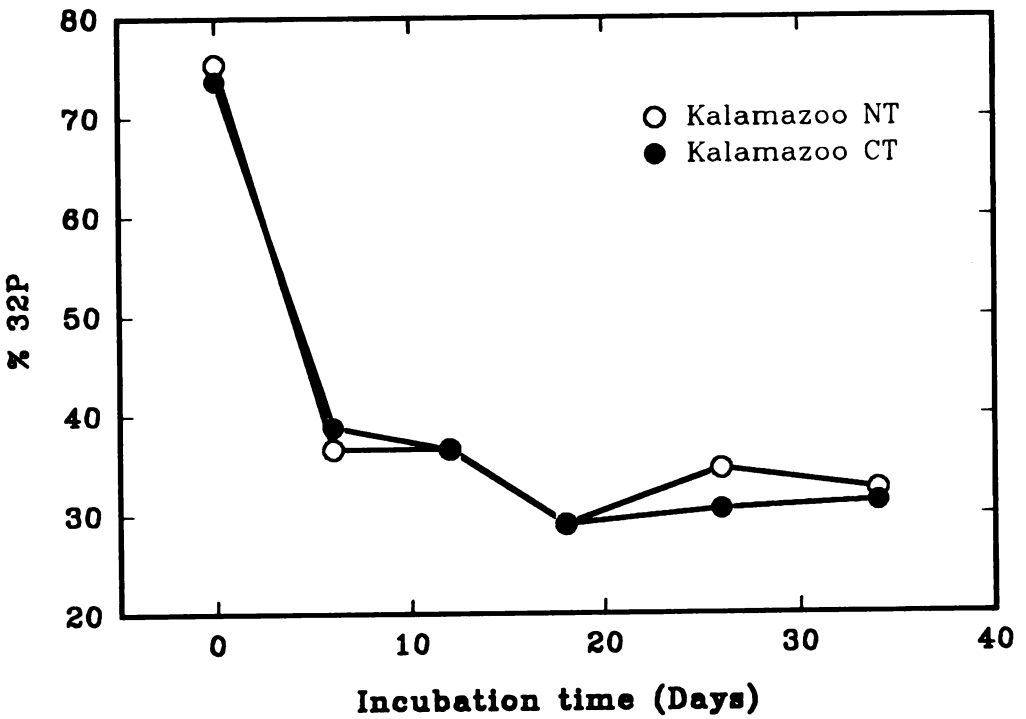

Figure 1. Labile ^{32}P in the Kalamazoo soil with incubation time.

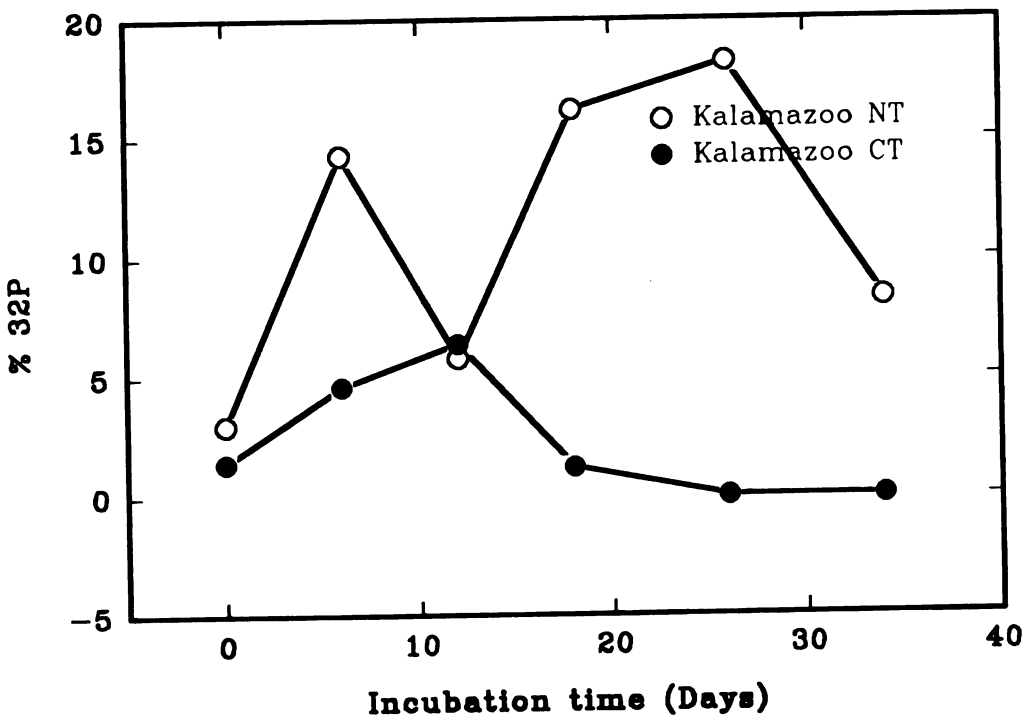

Figure 2. Microbial ^{32}P in the Klamazoo soil with incubation time.

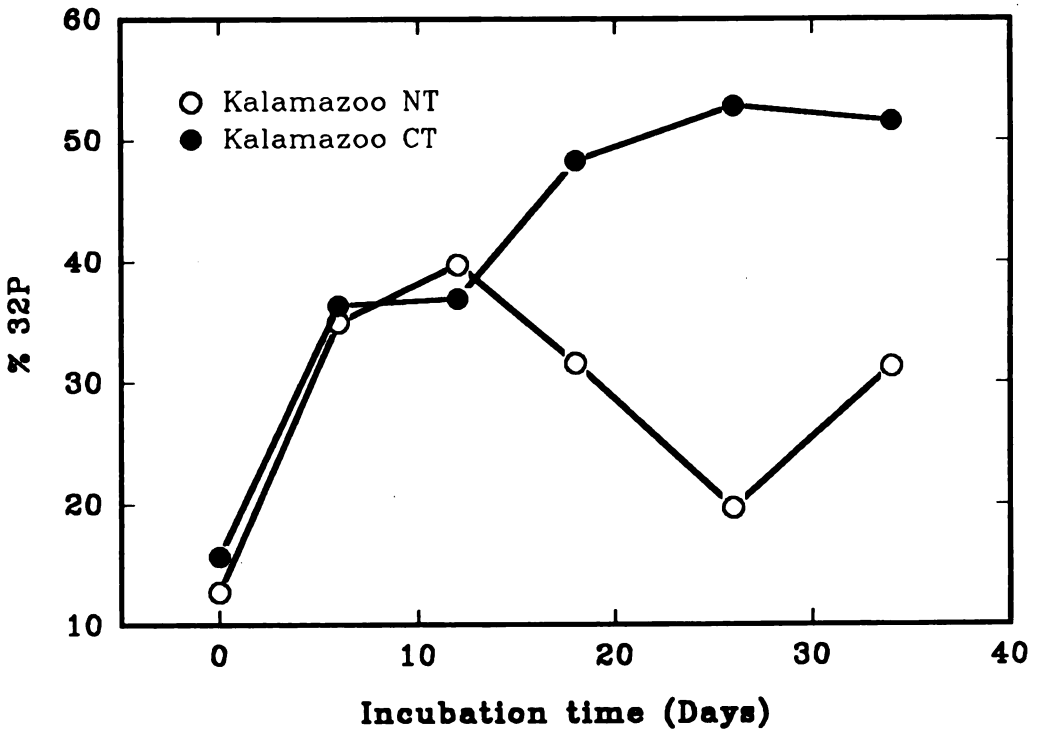

Figure 3. NaOH extractable ^{32}P in the Klamazoo soil with incubation time.

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