AN ABSTRACT OF THE THESIS OF

Peter T. Claycomb for the degree of Master of Science in Soil Science presented on April 21,1992.

Title: Measurement of Microbial Biomass Phosphorus in Oregon Soils

Redacted for Privacy

Abstract approved: _____
Peter J. Bottomley

Microbial mineralization of phosphorus (P) from complex soil forms is thought to be an important process in the P nutrition of plants. Furthermore, the microbial biomass itself has been implicated as a potential source of P that can become available to plants either upon biomass death and subsequent mineralization, or through predation, digestion, and excretion by soil fauna.

A study was conducted to determine the quantity of P held in the microbial biomass in five western Oregon soils. The soils were located in pastureland, forests, and transition zones between the two. Microbial biomass was determined on soil samples incubated in the laboratory at 75% (wt/wt) of water holding capacity at 15°C for ten days. The method was also tested on soils immediately after their recovery from the field.

To evaluate the P concentration in the biomass, soil

microbial biomass carbon (C) was determined by the chloroform fumigation-incubation method (CFIM) on soils preincubated as described for P determinations. Kinetic profiles of CO_2 evolution were followed in each soil to determine the appropriate control to use for estimating the contribution of nonbiomass C to CO_2 evolved.

Biomass P values were determined by measuring the difference between sodium bicarbonate ($NaHCO_3$) extractable P (organic plus inorganic) of fumigated and unfumigated soil samples. Inefficiency of P recovery from the soil samples was accounted for by using a recovery factor (k_p) determined for each soil. Biomass P values ranged between 19.1 and 37.6 mg P/kg soil and were within the range of values that have been reported in the literature. Simple linear regression showed a significant relationship ($r^2=0.78$, $p=0.05$) between biomass P and the Olsen test for extractable inorganic P. Despite the apparent success of the biomass P method on soils equilibrated in the laboratory, problems were encountered on some of the field samples. In some cases equivalent levels of P were extracted in both fumigated and unfumigated soil samples, and on one sampling date which followed a 1.3 cm rain event, the differences between fumigated and unfumigated samples were too great to be attributable to biomass P.

The kinetics of CO_2 evolution from fumigated and unfumigated samples of the five soils illustrated the full spectrum of problems that other researchers have encountered

in accounting for the contribution of nonbiomass C to the CO_2 evolved. As a result, biomass C was calculated by four methods that differ in the way the contribution of nonbiomass C to CO_2 released is determined. Values of biomass C ranged from 433 to 734 mg C/kg soil (most conservative method of calculation) and from 961 to 1102 mg C/kg soil (most liberal method of calculation). Using these biomass estimates, the percentage of P in the microbial biomass of the five soils ranged between either 2.4 and 3.7% or 0.9 to 1.9%. These P concentration values span the range reported in the literature for both soil biomass and laboratory-grown cultures.

Measurement of Microbial Biomass Phosphorus in Oregon Soils

by

Peter T.Claycomb

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed April 21, 1992

Commencement June 1992

APPROVED:

Redacted for Privacy

Professor of Soil Science in Charge of Major

Redacted for Privacy

Head of Department of Crop and Soil Science

Redacted for Privacy

Dean of Graduate School

Date Thesis is presented April 21, 1992

Typed for Peter T. Claycomb by Peter T. Claycomb

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

MEASUREMENT OF MICROBIAL BIOMASS PHOSPHORUS IN OREGON SOILS

INTRODUCTION

Phosphorus (P) is an essential element for living organisms, having an important role in many metabolic processes (Westheimer 1987). The nucleic acids, DNA and RNA, as well as many coenzymes are esters of phosphoric or pyrophosphoric acids. Phosphate esters are the principle forms of biochemical energy in organisms, and are essential intermediates in biochemical syntheses and degradations.

Unlike the carbon and nitrogen cycles, in which biological oxidations and reductions flux the elements from and to the atmosphere via the soil, soil P is derived almost solely from parent materials, and is cycled between organic and inorganic forms of the same oxidation state. (Walker and Adams 1958,1959, Walker 1964). Although ecosystems may receive inputs of P via surface erosion of soil materials (Smeck and Runge 1971, Smeck 1973, Sharpley 1980), these are relatively small and usually confined to local influence (i.e. within drainages). Because of its important biological functions and these limited inputs, the P content of parent material may be the limiting factor for the accumulation of carbon, nitrogen and sulfur in soil organic matter and thereby control the productivity in many ecosystems (Walker and Syers 1976).

The P content of soils varies considerably, depending

on the nature of the parent material and the degree of weathering. Most values for P content of surface soils of the United States fall within the range of 200 to 900 mg/kg soil. Some of the highest concentrations are found in the Pacific Northwest, ranging from 1000 to 1300 mg P/kg soil (Stevenson 1986). Soil P exists in a variety of inorganic and organic forms. The inorganic P (P_i) exists mainly as insoluble or poorly soluble minerals such as apatite and in complexes with iron and aluminum oxides, and much of the organic P (P_o) is insoluble and may be bound in organic-mineral complexes.

Because of its strong interaction with soil minerals and organic matter, the amount of total soil P does not necessarily reflect the amount of P in plant-available forms. Only a small fraction (about 1.5 uM, 0.045 ug/ml) of the total P is found as orthophosphate in the soil solution (Tate 1984). Greater than 90% of the P in soils is present as insoluble or chemically sorbed forms and in humus (Stevenson 1986). Due to the heterogeneity of soil materials, and the complexity of the association between the organic and inorganic P constituents, the role of this large fraction in plant nutrition has been difficult to assess. However, since plant roots require that P be in the solution phase (Barber 1962), the ability of a soil to supply plant available P will depend on factors affecting its release from those forms not readily available to plants.

Chemical fractionation of soil P.

The identification of individual P compounds in soils has met with limited success. To, date only about 50 to 70% of the P_o in soil has been chemically identified (Stewart and McKercher 1982). Furthermore, the difficulty in separating and quantifying organic and inorganic components of soil P has been noted (Anderson 1975, Dalal 1977). As a compromise, researchers have developed extraction procedures to chemically fractionate P and made attempts to relate these fractions to bioavailable P pools (Bowman and Cole 1978b, Hedley and Stewart 1982). The most biologically available or labile P_i is removable from soil by anion exchange resin extraction (Bowman et al. 1978a) and is thought to represent exchangeable P. Bowman and Cole (1978b) found that extraction of soil with 0.5M $NaHCO_3$ removed labile P_t and P_i, plus a small amount of P released from the microbial biomass. Hedley and Stewart (1982) found that the amount of P held in the microbial biomass could be estimated by treating soil with $CHCl_3$ prior to extraction with $NaHCO_3$. Subsequent extraction with 0.1M NaOH was found to release P_i associated with iron and aluminum and a significant portion of labile P_o (Ryden et al. 1977, McLaughlin et al. 1977). Treatment with 1M HCl extracts a fraction of P_i thought to be associated with apatite-like minerals (Williams et al. 1971). Following the sequential extraction steps, residual P is determined by oxidation and strong-acid digestion of the soil remains (Hedley and

Stewart 1982). This fraction is thought to consist of chemically stable P_o forms and insoluble P_i, and may comprise up to 90% of total soil P (Stevenson 1986).

The use of chemical P fractionation techniques in P cycling studies.

The chemical fractionation of soil P has been employed in studies which have elucidated components of the P cycle in soils. Hedley and Stewart (1982) used a sequential extraction procedure to demonstrate changes in the distribution of soil P as influenced by the use of various tillage practices. Sixty five years of cultivation of soil (wheat-wheat-fallow rotation, Udic Haploboroll) resulted in a 29% decrease in the total P content when compared to an adjacent permanent pasture. Of the total P lost, 52% was from the residual P fraction and 22% was from the extractable P_o (primarily that extractable in 0.1M NaOH). The remaining 26% of the reduction in total P was lost from the labile P_i fractions. The loss of total P was assumed to be related to cropping practices and illustrates the redistribution of P between residual and labile fractions. Microbial biomass P decreased (37%) by a similar proportion to the total P, suggesting that losses of labile P have an effect on the amount of P held in the soil microbial biomass.

Evidence for the role of the microbial biomass in soil P dynamics has been presented by Condron and Goh (1990).

These authors reported decreases in extractable P_o with concomitant increases in biomass P after liming a permanent pasture. In a highly weathered ultisol, Lee et al. (1990) showed that increases in microbial activity and biomass P were correlated with a decrease in the amounts of sorbed P_i and P_o while pools of soluble and labile P remained constant.

Hedley and Stewart (1982), in laboratory incubations simulating fallow soil conditions, obtained evidence for the seasonal redistribution of soil P among chemical fractions. The changes were attributed to microbial activity. Seasonal fluxes of soil P have likewise been reported in field studies. In a two year study of permanent pastures, Dormaar (1972) found that the magnitude of the NaOH-soluble P fraction increased by as much as 32% between August to October. In a survey of seven New Zealand soils Saunders et al. (1971) noted seasonal variations in P distribution. They hypothesized that winter buildup of labile P fractions could account for higher levels of available P in the spring when plant demand is highest. Furthermore, they suggested that available P arose in the spring from net mineralization of microbial biomass P following net immobilization of P by microorganisms during the fall and winter when plant demand was low.

The role of the microbial biomass in nutrient cycling.

The soil microbial biomass has long been known to be a reservoir of mineral nutrients (Jenkinson and Ladd 1981). Soil microorganisms have been shown to affect the supply of nitrogen, sulfur, and phosphorus to plants through cycles of mineralization and immobilization. Thus, the microbial biomass can behave as a source or a sink in the supply of mineral nutrients to plants. A number of mechanisms for the transfer of mineral nutrients from the microbial biomass to plants have been proposed. Seasonal variations in levels of labile P have been noted by a number of authors. Some researchers have suggested that, in some systems, net immobilization occurs at times of low plant demand, followed by net mineralization when plant growth is high (Dormaar 1972, Saunders et al. 1971). Protozoa and other microbial predators have been implicated in the mineralization of microbial biomass by their excretion of excess P and N (Darbyshire 1975, Coleman et al. 1983). It has been difficult to predict conditions under which the microbial biomass might act as a net source or sink for mineral nutrients, as the processes of mineralization and immobilization can occur simultaneously. Nonetheless, estimation of the size of the microbial biomass and the amount of potential plant available nutrients held within it remain important pursuits if the role of organically bound nutrients in the mineral nutrition of plants is to be understood.

C in the microbial biomass.

Indirect methods for the determination of microbial biomass C were first introduced by Jenkinson and Powlson (1976), who showed that the amount of CO_2 evolved from a soil over a ten day period following fumigation with $CHCl_3$, minus the amount of CO_2 evolved from a concurrently incubated unfumigated soil, was correlated with estimates of the size of the microbial biomass obtained by direct microscopic count. The principle of the chloroform fumigation-incubation method (CFIM) is that the majority of microbial cells are lysed upon fumigation. During the subsequent incubation period there is flush of respiration due to a recolonizing population mineralizing a fraction of C associated with the killed biomass. The unfumigated sample serves as a correction for any respiration occuring independent of fumigation, i.e. CO_2 derived from non-biomass C. Soil microbial biomass values ranging from 330 to 1060 mg biomass C/kg soil have been reported in the literature (Jenkinson and Ladd 1981).

The relationship of the size of the microbial biomass C pool to rates of organic matter cycling is poorly understood. Anderson and Domsch (1989) reported that the ratio of biomass C to soil organic C ranged from 0.62 to 4.04% in 26 long term experimental plots representing a number of temperate zone soil types. These data suggest that soils may vary widely in terms of the amounts of C available as a microbial substrate. Because the

decomposition of organic matter can be accompanied by the release of plant available N and P (Marumoto et al. 1982, Brookes et al. 1984), a number of studies have been undertaken to determine the amount of available C in soils (Davidson et al. 1987, Sikora and McCoy 1990). Voroney et al. (1981) proposed that soil organic matter is comprised of three fractions varying in their rates of decomposition. These are (a) a readily available fraction which microorganisms decompose quickly, (b) a fraction which is more slowly degradable due to substrate quality and (c) a fraction which is highly resistant to degradation because of structural complexity and other factors leading to humification.

While the ability to assess the availability of mineral nutrients by measuring the size of the microbial biomass would be useful, available methods have not provided results accurate enough for this application. Inaccuracy in CFIM is primarily a matter of choosing the correct control value to account for basal respiration of non-biomass C. Jenkinson (1976) reported that basal respiration was equal in both fumigated and unfumigated soils, justifying the subtraction of the CO_2 respired in an unfumigated sample from that respired concurrently in the fumigated soil. However, in other cases, the kinetics of CO_2 evolution from the fumigated and unfumigated soils are sufficiently different to suggest that the amount of basal respiration in fumigated soils may not accurately estimate the respiration in

unfumigated controls. Although a number of authors have addressed this issue (Jenkinson and Powlson 1986, Chausodd and Nicolardot 1982, Paul and Voroney 1980, Shen et al. 1987), there has been no concensus on suitable methodology for all soils and in all situations.

P in the microbial biomass.

In further attempts to elucidate the role of the microbial biomass in nutrient cycling, the CFIM has been modified by replacing the incubation period with a direct chemical extraction procedure to estimate the amount of various mineral nutrients held in the microbial biomass. In this manner, methods for the estimation of biomass sulfur (Sagaar et al. 1981), nitrogen (Voroney and Paul 1984, Brookes et al. 1985) and phosphorus (Hedley and Stewart 1982, Brookes et al. 1982) have been presented.

Although pathways of P transformation in soils are poorly understood, biomass P can represent a significant portion of of the actively cycling P. Simulation studies by Cole et al. (1977) predicted that microbial P uptake was 3 to 5 times greater than plant uptake. Estimates by Hahm et al. (1972) suggested that the microbial biomass of cool native grasslands could contain more P than the plant biomass. These findings suggest that, in addition to representing a source of labile P, the microbial biomass may act as a competitive sink for P in soils of low P status.

Estimates reported by Brookes et al. (1984) for the

size of the biomass P pool for a number of soils range from 5.3 to 106.0 mg P/kg soil, with a mean value of 40.7 mg P/kg soil. Biomass P accounted for 3.3 to 21.9% of total soil P with a mean of 19.2%, and P comprises between 1.4 and 4.7% of the microbial biomass, with a mean of 3.3%. Hedley and Stewart (1982), using biomass C values and microbial C:P ratios reported by Anderson and Domsch (1980) and Chuahan et al. (1981), calculated biomass P and reported that values should be expected to range from 1.5 to 50 mg P/kg soil. From work with a single soil receiving four organic amendments, they reported a range of 9.7 to 27.8 mg biomass P/kg soil (mean = 23.5), accounting for 3.3 to 4.0% (mean = 3.6) of the total soil P. In a survey of six Indian ultisols under different management regimes, Srivastava and Singh (1990) reported biomass P ranging from 17 to 35 mg P/kg soil (mean = 23.7 mg P/kg soil) which comprised 9 to 19 % (mean = 12 %) of the total soil P. The concentration of P in the microbial biomass varied from 2.1 to 5.5 % (mean = 3.5 %).

Due to the variable reactivity of P in different soil types, conclusions concerning the comparisions of biomass P values across soils are difficult to make. The majority of biomass P investigations, and most notably those in which the procedures were developed, were conducted with neutral to slightly alkaline soils of low clay content (Cole et al. 1978, Hedley and Stewart 1982, Brookes et al. 1982), and may not give valid estimates with all soils. McLaughlin et al.

(1986) have suggested the need to calibrate individual soils for biomass P estimation. However the focus on the standardization of methods has resulted in the sacrifice of intensive work involving soils of different character.

Hedley and Stewart (1982) discussed the possibility that in some soils, the methods to extract biomass P may also measure some amount of native P_o. They further suggested that, in some cases, P released by $CHCl_3$ fumigation could be immobilized by microbial uptake or by sorption by soil surfaces, resulting in varying efficiency in the recovery of extracted biomass P. The efficiency factor (k_p) is determined by measuring the recovery of known amounts of P added to soil before fumigation and extraction, and is used to correct for biomass P released by fumigation but not recovered in the extraction procedure.

A value of k_p = 0.40 (Brookes et al. 1982) has been widely used by many authors in estimating biomass P values (Srivastava and Singh 1990, Condron and Goh 1990). However a k_p value of 0.37 was reported by Hedley and Stewart (1982) and McLaughlin and Alston (1986) reported k_p values ranging from 0.33 to 0.57 in three different soils. In studies involving the response of microbial P to soil amendments, uncorrected values were used to calculate biomass P values (Sparling et al. 1985; and Lee et al. 1990).

Another issue in the methodolgy for determining biomass P is that of measuring P_t versus P_i released by chloroform. Brookes et al. (1982) found that P_i accounted for 90.2 %

(65.5 to 129.0 %) of the P released by $CHCl_3$ fumigation in the soils they tested. Consequently, they concluded that biomass P could be reliably estimated by the flush of P_i released by fumigation. This permits the omission of the acid digestion step which converts P_o to P_i. However, Hedley and Stewart (1982) found no relationship between P_i and P_t extracted by $CHCl_3$ fumigation and $NaHCO_3$ extraction. They stated that the rate of hydrolysis of P_o to P_i changes once the cell membrane is lysed by $CHCl_3$ and varies with soil components, cell composition, and treatment.

Differences in soil handling and treatment may also contribute to problems in comparing biomass P values across soils. Experimental objectives may determine the way in which soils are sampled and pretreated. Hedley and Stewart (1982) developed their method using air-dried, sieved soil that was rewet to 60% of field moisture capacity and incubated for 21 days prior to biomass P determination. This treatment was intended to provide steady state conditions optimal for the establishment of the microbial biomass. The air-drying treatment was objected to by Brookes et al. (1982), who preincubated field moist soil for ten days prior to use. No preincubation treatment was employed by McLaughlin and Alston (1986), who proposed that immediate biomass P determination following sampling provided better information about the field status of the microbial biomass.

Sparling et al. (1985) reported that air-drying soil

could render significant amounts of microbial P extractable in $NaHCO_3$. By releasing P of microbial origin into the unfumigated controls, this would contribute to an underestimate of biomass P. West et al. (1986) concluded that sampling date and preincubation treatment had inconsistent effects on the size of the biomass P and that these were unrelated to changes in biomass C and N. These results illustrate the importance of determining k_p values and the proportion of microbial P released as P_i and P_o from soils under different conditions. Estimation of biomass P therefore must include assessments of the effects of soil type and preconditioning treatment in order to both standardize and calibrate methods to individual soils and experimental conditions.

Relationship between microbial biomass P and plant available P.

The capacity of a soil to supply plant available P has been assessed by a number of methods developed to estimate the size of a labile P pool. Olsen et al. (1954) introduced the use of sodium bicarbonate extracts to estimate levels of plant available P, suggesting that P_i extracted by 0.5M $NaHCO_3$ represented a labile pool which is in equilibrium with soil solution P. The Olsen P test has been positively correlated with plant response to P fertilization and is widely used in soil testing laboratories (Olsen and Sommers 1982). Extraction with 0.5M $NaHCO_3$ was originally thought

to solubilize calcium phosphates by precipitating $CaCO_3$ in calcareous soils Olsen et al. 1954), and to increase the solubility of iron and aluminum phosphates in acid soils by raising the pH optimum for P solubility (Lindsay and Moreno 1960). However, acid digestion of $NaHCO_3$ extracts has shown that the procedure also extracts some P_o, and it has been suggested that a portion of the P_i measured in the Olsen test arises from hydrolysis of P_o during the test procedure (Bowman and Cole 1978, Sparling et al. 1985).

Soil is typically air-dried and sieved in preparation for soil testing procedures. Sparling et al. (1985) found that increases in Olsen P upon air-drying 15 soils was related to biomass P levels estimated by $CHCl_3$ fumigation of moist soils. The microbial contribution to the 0.5M $NaHCO_3$ extractable P_i ranged from 4 to 76%. Air-drying of soils decreases levels of biomass C (Powlson and Jenkinson 1976) and biomass P (Brookes at al. 1982, Hedley and Stewart 1982), further suggesting that some of the Olsen P may be derived from microbial cells killed by air-drying (Bowman and Cole 1978). For soils of xeric moisture regimes with distinct wetting and drying periods, these observations may suggest mechanisms for the turnover of P through the microbial biomass on a seasonal basis.

OBJECTIVES

A study was conducted to characterize the phosphorus status of five western Oregon soils, and to assess the distribution and magnitude of various chemical fractions of soil P.

Objective 1: To determine the magnitudes of various chemical fractions of soil P in five western Oregon soils.

Objective 2: To estimate the amount of P held in the microbial biomass in these soils.

Objective 3: To determine the magnitude of the microbial biomass in these soils and a a consequence to estimate the concentration of P in the microbial biomass.

MATERIALS AND METHODS

Site descriptions.

The study site is located on the Oregon State University Cattle Research Facility and in Paul Dunn State Forest in northeast Benton County near Corvallis, Oregon, SE1/4 sec 23. and SW1/4 sec. 24, T. 10 S, R. 5 W (Soil Survey of Benton County Area, USDA, SCS, 1975). A transect of Soap Creek Valley was drawn with the easternmost end located on Soap Creek Road approximately one half mile from the junction of Soap Creek Road and Tampico Road. The transect runs perpendicular to Soap Creek and is 3/4 miles long, and crosses the Luckimute, Dolph and Looney geomorphic surfaces (Balster and Parsons 1968).

Site descriptions for soils A through E are presented in Table 1. The soils are derived from alluvium and colluvium weathered from basic igneous rock. Sites were chosen to represent the transition from valley bottom to steep upland, and the transition from permanent pasture to mature forest. Sites A through D were located along the main transect. Because a mature forest site could not be located along the main transect, site E was located approximately 0.5 miles south of site D.

Sampling Methods.

Within each site, two sampling transects (100 m) were situated perpendicular to the main transect. Soil samples

were taken at 1 meter intervals with an Oakfield soil corer (diameter 1.5 cm) to a depth of ten centimeters from the soil surface. Samples were composited within each site in the field and transported to the laboratory, where they were stored at 4°C until used (less than seven days). All soils were sieved through a number ten sieve (2.00 mm mesh size), and all visible plant material was removed prior to analyses.

Soils were analyzed for pH, total organic C, total N, and extractable P by the Olsen and Bray methods, following the procedures of the Soil Testing Laboratory at Oregon State University (Horneck et al. 1989). Sub-samples of soil were used for the fractionation of soil P and for the determination of microbial C and P.

Pre-equilibration of soil for microbial biomass determinations and chemical P fractionation.

Air-dried composite samples from each site were sub-sampled (3 replicates) after sieving, adjusted to 75% (wt/wt) water holding capacity and incubated for 21 days in large test tubes (3x25 cm) suspended in a thermostatted water bath at 15°C. This treatment has been recommended (Brookes et al. 1982, Hedley and Stewart 1982) to re-establish equilibrium conditions after soil sampling, drying and rewetting.

Chemical fractionation of soil P.

Soil P was chemically fractionated following a modification of the method of Hedley and Stewart (1982). Two steps were omitted from the original fractionation scheme. Resin extractable P was not included because preliminary work showed this quantity to be very small and the efficiency of extraction was low (data not shown). The sonication treatment followed by extraction with 0.1 M NaOH was also omitted because earlier studies by Leung and Bottomley (1987) showed the magnitude of this fraction for these soils to be very small.

After the incubation period, triplicate subsamples were removed from each replicate (equivalent of 1.0 gram oven dry weight) and subjected to the P fractionation scheme. Soil samples were placed in 50-ml polyallomer centrifuge tubes in a desiccator lined with damp filter paper. A beaker containing 50 ml of chloroform ($CHCl_3$) was placed in the desiccator and soils were fumigated as follows. The $CHCl_3$ was instigated to boil by evacuating the desiccator with a vacuum pump for five minutes. The soils were fumigated for 24 hours, after which the desiccator was repeatedly evacuated and purged with air until all traces of $CHCl_3$ were removed.

Twenty-five ml of 0.5M $NaHCO_3$ were added to each of the centrifuge tubes which were shaken on a reciprocal shaker for 16 hours at 24°C. The extracts were centrifuged (10 minutes, 10,000 rpm, 4°C). Supernatants were filtered

through 25 mm, 0.45 µm pore size filters, and analyzed for P_i by the molybdate-blue method (Murphy and Riley 1962) as follows: A 2.5 ml aliquot of the extract was mixed with 2.0 ml of deionized water and 1.5 ml of combined reagents prepared by mixing 25 ml 2.5M H_2SO_4, 2.5 ml of an antimony potassium tartrate stock solution (2.743 g $K(SbO)C_4H_4O_6.1/2$ H_2O/liter), 7.5 ml ammonium molybdate stock solution (40 g $(NH_4)_6Mo_7O_{24}.4H_2O$/ liter) and 15.0 ml of freshly made 0.1M ascorbic acid solution. Samples were mixed with a vortex stirrer and incubated at room temperature for 30 minutes while color developed.

A second aliquot of the original supernatant was subjected to a sulfuric acid-ammonium persulfate digest as follows. 2.5 ml of the extract were placed in a 50 ml conical flask. 1.0 ml 5.5M H_2SO_4 was added, followed by 0.4 g ammonium persulfate. Approximately 25 ml deionized H_2O was added, and the samples were autoclaved for 1 hour at 121°C, 20 psi. After autoclaving, the samples were neutralized with 5M NaOH and brought to a volume of 100 ml with deionized water. The digested samples were then analyzed for phosphate as described above to give the total (inorganic plus organic) P in the extract. Microbial P was estimated from the difference between the total P extractable in 0.5 M $NaHCO_3$ after $CHCl_3$ fumigation, minus the $NaHCO_3$ extractable P in an unfumigated sample (Brookes et al. 1982, Hedley and Stewart 1982). The fumigation and extraction procedures are outlined in the description of the

fractionation scheme above. The soil residue remaining in the centrifuge tube was subsequently extracted with 25.0 ml of 0.1 M NaOH, followed by 25.0 ml of 1.0 M HCl. Total P and P_i presented above were determined on each subsequent extract as described above, with the exception that the 1.0 M HCl extract was not digested for determination of total P. Finally, the soil residue was transfered to a 75 ml Kjeldahl tube and heated to 350°C for a total of five hours (including one hour after digest cleared) in 5 ml concentrated H_2SO_4, Three ml of 35% H_2O_2 were added after the digests began to boil.

Estimating the efficiency of recovering P from the microbial biomass (k_p).

The efficiency of extracting P with 0.5 M $NaHCO_3$ was determined for each soil. Phosphorus (25 µg P/gram soil) was added to soil prior to fumigation either as P_i (KH_2PO_4) or as lyophilized bacterial cells of known P content (1.0±0.05%, determined by sulfuric acid-persulfate digestion). A duplicate sample of soil was fumigated and extracted with 25.0 ml of 0.5M $NaHCO_3$ concomitant with the P spiked sample. The proportion of P recovered from the spike was calculated as follows:

$$K_p = (P_s - P_c)/P_a$$

where K_p = the proportion of added P recovered, P_s = the amount of P measured in the spiked sample, P_c = the amount of P measured in the unspiked control and P_a = the amount of

P added to the spiked sample. In all subsequent samples, microbial biomass P was calculated with the formula

$$P_b = (P_f - P_u)/K_p$$

where P_b = the amount of P held in the microbial biomass (mg P/kg oven dry soil), P_f = the total P extracted by 0.5 M $NaHCO_3$ after $CHCl_3$ fumigation, P_u = the total P extracted by 0.5 M $NaHCO_3$ in an unfumigated control soil and K_p is defined as above.

Determination of microbial C.

Microbial biomass C was determined by the chloroform fumigation-incubation technique (Jenkinson and Powlson 1976), using soil sampled and pre-incubated in the same manner as described for the P fractionation scheme. After the pre-equilibration, six replicate ten gram samples of each soil (oven dry weight) were placed in 150 ml glass serum bottles. Three replicates of each soil were fumigated as described above, and the remaining three were placed in desiccators without $CHCl_3$ to serve as controls. After the 24 hour fumigation period, the bottles were flushed with air, fitted with rubber septa, and incubated in the dark at 24°C for 20 days. CO_2 was measured in portions (0.5 ml) of the headspace of the bottles sampled after 7, 10 and 20 days of incubation. CO_2 was analyzed by gas chromatography using a Carle Series 100 gas chromatograph equipped with a thermal conductivity detector.

Biomass C was calculated using the following equation:

$$C_b = (C_f - C_u)/K_c$$

where C_b = biomass C, C_f = the cumulative amount of carbon respired as CO_2 in the fumigated soil over a ten day period, C_u = the amount of carbon respired during a specific time interval in the unfumigated soil. Four values of biomass C were calculated using four different values of C_u. The latter represented cumulative CO_2 respired during different time intervals in the unfumigated control (see table 5). K_c = 0.45, and represents the proportion of biomass killed by fumigation that is mineralized during the incubation period (Jenkinson and Powlson 1976).

RESULTS

Soil test data for the five soils (all silty clay loams) are presented in Table 2. The soils showed little variation in the range of pH, (5.8-6.3), organic matter (5.4%-6.4%) or total N (0.19%-0.29%). Both Olsen and Bray extractable levels of P_i were very low, giving values which placed all soils in the category of the highest recommended rates of P fertilization (Fertilizer Guide 4, Oregon State University Cooperative Extension Service, 1983).

Fractionation of soil P.

The results of the P fractionation scheme are shown in Table 3. Total P varied among soils, ranging from 628-1444 mg P/kg soil. Soils C and D showed substantially lower values than soils A, B and E, making possible the comparison of groups of soils of high and low total P status. Residual P ranged from 176-669 mg P/kg soil, and comprised between 16.4-49.5% of the total P. In the case of soils A, B, C, and D, the percentages of P in different fractions were not distributed differently despite the differences in the total amounts in each fraction. For example, although the quantities of P extractable in 1.0 M HCl varied between 81 and 266 mg P/g soil, they represented a similar percentages of the total P (6.9-19.7%) in each soil. In contrast, the P extractable in 0.1 M NaOH

ranged from 37.3-49.9%, with soil E having a high value of 70%.

Determination of microbial biomass P.

Values for the percent recovery of P (K_p) added to soils before the fumigation and extraction of biomass P are presented in Table 4. The recovery of P added as bacterial cells (P_m) ranged from 23.0 to 32.0%. In a separate experiment, P_i was added at the same rate as P_m before the fumigation and extraction procedure. The recoveries of added P_i ranged from 11.0 to 33.0% and , with the exception of soil E (11%), are similar in magnitude to the efficiencies for recovery of added P_m. Considering the relatively high P adsorbing capacities of the soils in the present study, and the similarity of recoveries of P_m and P_i, it can be concluded that the efficiency of recovering biomass P in these soils is influenced primarily by their strong P sorption characteristics.

Bicarbonate extractable P increased after $CHCl_3$ fumigation in all of the soils tested (Figure 2a-c). The increases in $NaHCO_3$ extractable P after fumigation were relatively small (Figure 1a), ranging from 15.5-25.0 percent of the $NaHCO_3$ pool, indicating that the soils contained a large amount of background P upon which microbial P was determined. The level of $NaHCO_3$ extractable P_i (Figure 2b) after $CHCl_3$ fumigation showed a slightly broader range, with increases ranging from 15.5-38.8 percent of the $NaHCO_3$

extractable P_i pool. Soils C and D showed the largest relative increases in P_i released by fumigation.

Figure 1c shows a comparison of $NaHCO_3$ extractable P_t and P_i caused by the fumigation treatment. While the changes in P_t and P_i were almost equivalent in soils C and D, the proportion of P_t released as P_i ranged between 24.4 and 65.0%. The variable proportion that P_i contributes to the total P released by fumigation indicates that biomass P values should be calculated from P_t released by $CHCl_3$ fumigation.

The results of the biomass P method applied to samples of soils immediately after recovery from the field are presented in Figure 2a-e. Soils at each site were sampled four times during the summer and fall of 1989. The sampling scheme consisted of a midsummer date (July 24) in which microbial activity, measured as respiration, was zero (data not shown) followed by three weekly sampling dates (Sept. 20-Oct. 9) during the onset of fall rain. The study sites received 1.3 cm rain on September 23. Changes in gravimetric water content during the sampling period are shown in figure 3.

In soils A through D, 12 of 16 values of P_t released after $CHCl_3$ fumigation were greater than values of P_t from the unfumigated soils. However, on some sampling dates the $NaHCO_3$ extractable values also increased so that in four cases fumigated values were less than, or not substantially different from the unfumigated values. This situation was

especially prevalent in soil E, but was also observed in soils A and B. In the cases of soils A and B, the values of $NaHCO_3$-extractable P_t recovered from unfumigated soils in July and September were as high as values obtained in preequilibrated fumigated samples of the same soils (see Fig. 1a).

Three of the five soils (A,C and D) showed a pattern of slightly decreasing values of $CHCl_3/NaHCO_3$-P_t and $NaHCO_3$-P_t between July and September. This was followed in all three cases by large increases in $CHCl_3$-P measured on October 2, eight days after the rain event, with both fractions returning to pre-rain levels by the October 9 sampling date. None of the $CHCl_3$ induced increases in $NaHCO_3$-P_t observed in soils A,C and D were accompanied by concomitant decreases in the $NaHCO_3$-extractable P_t fractions, suggesting that the source of the P was some other fraction. Neither soil B nor soil E followed this pattern. Soil B showed a decrease in $NaHCO_3$ P following the rain event, but no other trend was evident. Soil E showed a trend of increasing $CHCl_3/NaHCO_3$-P_t and $NaHCO_3$-P_t during the period following the rain, but likewise did not show the marked increases seen in soils A, C, and D.

The magnitudes of the $CHCl_3$ induced increases that occured in soils A, C, and D between September 20 and October 2 cannot be solely attributable to increases in the size of the microbial biomass. Using the results of the October 2 sampling and the K_p values established for each

soil (Table 4), biomass values of 146.9, 172.4, and 221.7 mg P/kg soil are obtained for soils A,C and D respectively. These numbers greatly exceed those reported in the literature (23.7-39.4 mg P/kg soil, see Table 7). It is likely that the $CHCl_3$ induced increases in $NaHCO_3$-extractable P_t included non-microbially derived P.

Determination of microbial biomass C.

The characteristics of CO_2 evolution from fumigated and unfumigated samples of the five soils over a twenty day period are presented in Figure 4a-e. In all cases, the fumigated soils rapidly produced CO_2 with 90 to 100% of the twenty day total being produced by the seventh day of the incubation.

Although all five soils showed similar kinetics of CO_2 evolution during the first ten days of incubation, their behavior beyond ten days followed two different trends. Over the ten to twenty day period, fumigated samples of soils B and C produced CO_2 at rates comparable with those measured in the unfumigated samples. In contrast, CO_2 production had ceased after ten days in fumigated samples of soils A,D and E, but not in their unfumigated counterparts.

There has been a longstanding controversy over the proper use of respiratory controls for the estimation of biomass carbon. The selection of a proper control should be related to the kinetics of CO_2 evolution that are observed in the fumigated sample. The unfumigated sample is intended

to provide an estimate of the basal respiration occuring independent of fumigation. If CO_2 is not produced in the fumigated sample following the flush, as in the case of soils A, D, and E, then it follows that all CO_2 evolved is the product of $CHCl_3$-killed biomass and an unfumigated control should not be subtracted. If, on the other hand, CO_2 production continues in the fumigated sample at rates comparable to those in the unfumigated sample, then it could be argued that some amount of non-biomass organic carbon contributes to the CO_2 observed during the flush. This would justify the use of an unfumigated control. While these examples represent the extremes of the responses to $CHCl_3$ fumigation, the true ability of the recolonizing microbial populations to degrade non-biomass organic carbon is unknown, resulting in the proposal of a number of different control values to be used in the estimation of biomass carbon (Shen et al. 1987).

Because the response of the soils used in this study fit both of the above cases, biomass carbon was calculated by four different methods (Table 5). If no control value is subtracted (method 1, Voroney and Paul 1984), the estimates for each soil are similar (961.3-1102.4 mg C/kg soil) since the amounts of CO_2 released from the fumigated soils were almost equivalent. This method may be the most appropriate for soils A,D and E in which CO_2 evolution in the fumigated samples had ceased by day ten. Method 2, originally proposed by Jenkinson and Powlson (1976), gave the lowest

estimates of biomass C (432.8-733.9 mg C/kg soil). The limitation of method 2 stems from the concern that the CO_2 accumulated in the control sample between zero and ten days might be artificially high due to the mineralization of biomass killed during the processing of samples. However, the preincubation step should reduce this problem. Nonetheless, this issue is addressed by method 3 (Jenkinson and Powlson 1976), on the assumption that the artifacts introduced by sampling and handling are complete at ten days.

Chaussod and Nicolardot (1982) proposed that the CO_2 released from the fumigated sample during the post-flush (10-20 day) period represents the true rate at which the recolonizing population can degrade non-biomass carbon. Biomass carbon values for soils A,D and E calculated by method 4 (1020.1-1045.6 mg C/kg soil) were similar to those of method 1, since the amounts of CO_2 evolved after ten days were virtually zero. Values for soils B and C were similar for methods 3 and 4 since the quantities of CO_2 accumulated in the ten to twenty day period were similar in fumigated and unfumigated soils. The values obtained for soils A, D, and E by method 4 were substantially higher than those for soils B and C (720.7-776.6 mg C/kg soil) because measurable CO_2 accumulated in the fumigated samples of the latter after ten days.

Comparison of microbial biomass C and P estimates.

Values of biomass C and P in preincubated soils are presented in Table 6. Biomass P values ranged from 19.1-36.7 mg P/kg soil, with soil C having the lowest value. Microbial biomass C values were determined by methods 1 and 2, since they provide both the upper and lower limits of the range.

As a consequence, the percentage of P in the microbial biomass is presented as two ranges of values. Soil C gave both the highest and the lowest values: 3.7% and 0.9% for methods 1 and 2, respectively. The mean of the percentage P in the microbial biomass for all soils was 2.8% for method 1, and 1.5% for method 2, with the mean for all soils and both methods at 2.2%.

DISCUSSION

Attempts to measure microbial biomass P and C illustrated a variety of problems, some of which have been encountered by researchers for the past fifteen years. For example, the k_p values determined for the soils used in this study (k_p = 0.22 to 0.32) were lower than other values reported in the literature. This is not surprizing since many of the upland pasture and forest soils of western Oregon are high in organic matter and clay content and have high P adsorbing capacities (Almendras and Bottomley 1987, Gardener and Christensen 1991). Soils used in previously reported studies varied significantly from those in the present study in terms of pH, texture and organic matter content (Hedley and Stewart 1982, Brookes et al. 1982, Mclaughlin and Alston 1986, Srivastava and Singh 1989). In addition, soil P was distributed quite differently among chemical fractions (Hedley and Stewart 1982, O'Halloran et al. 1987, Lee et al. 1990). Therefore, it is somewhat unfortunate that several researchers who have reported estimates of biomass P in microbial biomass have not determined a k_p value for their own soils and have used the estimates of others (Sparling et al. 1985, Srivastava and Singh 1990).

Another issue of methodolgy relates to the variable contribution of P_i to the total P released by fumigation in the soils used in the present study. Although the reason

for the variability in the proportion of P_i is unclear, it would be inappropriate to assume that biomass P could be estimated by the P_i released by fumigation. It would be necessary to first establish for a particular soil what proportion of the total P released was composed of P_i, and whether or not that proportion was consistent over time and treatment.

Despite the low k_p values, it was concluded that biomass P was successfully measured in soils equilibrated under constant environmental conditions. The ranges of values reported here are well within the range reported by others (Brookes and Powlson 1982, Hedley and Stewart 1982). It should be pointed out, however, that values for biomass P for soils similar to those of the present study are scarce. Srivastava and Singh (1988) reported a mean of 23.6 mg biomass P/kg soil for six ultisols in India. While this is slightly lower than the mean of biomass P reported here (31.5 mg P/kg soil), and those reported by Hedley and Stewart (1982) or Brookes and Powlson (1982) (29.2 and 39.4 mg P/kg soil respectively), the values of Srivastava and Singh (1988) were calculated assuming a K_p = 0.40 (Brookes and Powlson 1982). Because differences in texture and pH of these soils could be expected to result in a relatively high P-fixing capacity, a k_p value of 0.40 may result in an underestimate of biomass P. Recalculating the value reported by Srivastava and Singh (1988) using a K_p value of 0.27 (i.e. the mean value of K_p determined for soils A-E)

gives a value of 35.1 mg biomass P/kg soil, which brings it into the range of values reported by others.

Attempts to validate the biomass P method on samples recovered directly from the field were disappointing. Two problems were recurrent. First, the values of the $NaHCO_3$-extractable P recovered from unfumigated soils changed substantially with sampling date, and often were not significantly less than the values obtained from fumigated soils. The small differences at the July and September dates may reflect a reduction in the size of the microbial biomass brought on by continued summer drought. Sparling et al. (1985, 1986) have shown that air-drying of soils can decrease the size of the microbial biomass while increasing levels of $NaHCO_3$-extractable P. It is therefore conceivable that $NaHCO_3$-extractable P increased as a result of the mineralization of biomass P. However, calculation of biomass values using these small differences should be interpreted cautiously. The field trials of the biomass P method were conducted on single samples composited by site and were not replicated. However, based on the results of the biomass P determination on equilibrated soils (standard error of the mean of the differences ranged from 4.5 to 5.0), calculation of biomass P values from the majority of the July and September samples would give unreliable estimates, as six of the ten show differences of less than 5.0 mg P/kg soil. As a result of this imprecision, conjecture concerning the dynamics of the microbial biomass

based on microbial P values in this range would seem imprudent.

Secondly, on the sampling date after the soils were rewet by the rainfall event, the increases in $NaHCO_3$-extractable P caused by fumigation were so large as to exceed realistic estimates of biomass P. An explanation for the large increases may be that the physical-chemical changes that accompany the wetting of soils made some P fraction extractable with chloroform. An alternate explanation is that the efficiency of recovery of extracted P (K_p) may be higher in rewetted soils. It has been demonstrated that the P adsorption capacity of a soil increases as the soil dries (Haynes and Swift 1985), and this could result in variation in K_p values. However, this seems an unlikely explanation because although the uncorrected biomass P values returned to pre-rain levels one week after the rain, soil water content remained relatively high. Although its nature is unknown, the rapid increases and decreases in $CHCl_3/NaHCO_3$ P suggest it to be a potential source or sink in terms of P availability.

Measurements of microbial biomass carbon were made so that the quantity of microbial P could be compared as a percentage of the microbial biomass. However, uncertainties were placed on the biomass C values due to the problem of deciding on an appropriate value for the amount of CO_2 respired from nonbiomass carbon. As pointed out in the results section, the five soils used in this study gave a

cross-spectrum of the kinds of responses that other workers have used to justify different kinds of control values (Shen et al. 1989). Nonetheless, while the magnitudes of biomass P (mean = 31.5 mg P/kg soil) were similar to values reported in the literature,the mean values of microbial P as a percentage of the microbial biomass varied from 1.5 to 2.8%. These values depend on whether biomass C was calculated by method 1 or method 2, respectively. The concentration of P in microorganisms grown under laboratory conditions with nonlimiting quantities of P ranges from about 1 to 3% (Hedley and Stewart 1982, Brookes et al. 1982). P concentrations of soil grown organisms ranging from 1.4 to 4.7% have been reported by Brookes et al. (1982). Given the wide range in possible P content of microorganisms, it is quite possible that microbial biomass with a low P content can be an effective sink for labile P, and that microorganisms of high P content could be a potential mineralizable source of P. Indeed, Mclaughlin et al. (1987) have shown that rhizosphere and microorganisms can immobilize a large portion of P_i added to soil.

Linear regression analyses of biomass P on various other P fractions are presented in Table 8. The only significant correlation observed was between biomass P and Olsen P (r^2=0.78). This is interesting, in that it suggests a

relationship between biomass P and an index of plant-available P. Air-drying of soils has been shown to decrease

levels of biomass P (Brookes et al. 1982). Sparling et al. (1985, 1987) reported that 4-76 percent of the P in 0.5 M $NaHCO_3$ extracts of dried soils was due to the release of P from soil microbes killed by desiccation, and that soils high in organic matter and low in Olsen P were most likely to show the effects of microbial contribution to available P. These observations may provide an explanation for the correlation between biomass P and Olsen P in the present study, suggesting that biomass P is related to the plant-available P pool.

A non-significant correlation (r^2=0.72) between biomass P and total P was found, supporting the earlier suggestion that the size of the microbial biomass is related to the amount of total P in the soil.

Table 1. Site descriptions, Soap Creek Valley.

Soil A	
Series	Abiqua silty clay loam.
Classification	Fine, mixed, mesic, Cumulic Ultic Haploxeroll.
Landform	Alluvial fan, east side of valley.
Vegetation	Permanent pasture dominated by *Trifolium subterraneum* and annual and perennial grasses.
Soil B	
Series	Bashaw clay.
Classification	Very fine, montmorillonitic, mesic, Typic Pelloxerert.
Landform	Alluvial bottomland.
Vegetation	Permanent pasture dominated by Vicia sp., *T. subterraneum* and annual and perennial grasses.
Soil C	
Series	Dixonville silty clay loam (3 to 12 percent slopes).
Classification	Fine, mixed, mesic, Pachic Ultic Haploxeroll.
Landform	Toeslope, west side of valley.
Vegetation	Unimproved pasture, annual and perennial grasses, *Rhus diveraloba*, occasional *Quercus garriana*, *Pseudotsuga menzesii*, and *Prunus* sp.
Soil D	
Series	Dixonville silty clay loam (12 to 20 percent slope).
Classification	Fine, mixed, mesic, Pachic Ultic Haploxeroll.
Landform	Midslope, west side of valley.
Vegetation	Young forest, *Pseudotsuga menzesii*, *Quercus garriana*, and *Acer macrofolium*.
Soil E	
Series	Price silty clay loam
Classification	Fine, mixed, mesic, Dystric Xerocrept.
Landform	Hillside, approximately two miles west of transect, aspect northeast
Vegetation	Mature forest, *Pseudotsuga menzesii*, *Abies grandis* and Acer macrofolium.

Table 2. Some chemical characteristics of the soils.

Soil	pH[1]	O.M.[2] (g/kg)	Total N[3] (g/kg)	Olsen-P[4] (mg/kg)	Bray P[5] (mg/kg)
A	5.8	64	2.3	9.0	2.5
B	5.8	66	2.9	7.0	<0.1
C	6.3	60	2.2	3.0	<0.1
D	6.3	62	2.1	5.0	<0.1
E	6.2	54	1.9	6.0	4.0

1. pH measured in 1:2 soil/water suspensions.

2. Determined by the Walkley-Black digestion and titration method.

3. Determined by Kjeldahl digestion followed by ammonium analysis.

4. Determined by extraction of soil with 0.5M $NaHCO_3$.

5. Determined by extraction of soil with 0.03M NH_4F in 0.025M HCl.

Table 3. Quantities of P in different chemical fractions of the soils.

Soil	Total	P Fraction[1]			
		Residual	HCl	NaOH	$NaHCO_3$
		mg P/kg soil			
A	1444[2]	669(46.3)[3]	100(6.9)	635(44.0)	40.2(2.8)
B	1352	369(27.4)	266(19.7)	674(49.9)	43.0(3.0)
C	623	240(38.5)	81(13.1)	277(44.4)	24.9(4.0)
D	787	390(49.5)	85(10.8)	294(37.3)	18.1(2.3)
E	1073	176(16.4)	87(8.1)	751(70.0)	58.9(5.5)

[1] See materials and methods for working definitions of each fraction.

[2] Total P equals the sum of all fractions.

[3] Numbers in parentheses express the quantity of P in each fraction as a percentage of total P.

Table 4. Percent recovery of P added to soil[a].

	% Recovery	
Soil	Microbial P[b]	Inorganic P[c]
A	32.0 (3.7)[d]	33.0 (3.1)
B	22.0 (4.1)	32.0 (2.7)
C	29.0 (3.4)	27.0 (3.4)
D	23.0 (5.3)	33.0 (4.0)
E	25.0 (4.2)	11.0 (4.5)

[a] Added to soil prior to fumigation.

[b] 25 µg P/g soil added as lyophylized bacterial cells.

[c] 25 µg P/g soil added as K_2HPO_4.

[d] Numbers in parentheses are standard errors.

Table 5. Determination of soil microbial biomass C concentrations using different control values.

Soil	Calculation Method[2] I	II	III	IV
	mg C/kg Soil			
A	1102.4	733.9	938.2	1045.6
B	961.3	606.9	787.3	776.6
C	1098.5	432.8	690.9	720.7
D	1092.3	653.9	629.6	1023.9
E	1044.4	670.4	973.5	1020.1

1 Calculated as described in materials and methods using a $k_c = 0.45$.

2 I No control subtracted.

II Subtract the CO_2 evolved from the unfumigated soil during the 0-10 day incubation period.

III Subtract the CO_2 evolved from the unfumgated soil during the 10-20 day incubation period.

IV Subtract the CO_2 evolved from the fumiated soil during the 10-20 day incubation period.

Table 6. Comparison of P and C in the microbial biomass[1].

Soil	Biomass (mg/kg soil)			P concentration (g/kg dry wt.) of microbial biomass[4]	
	P[2]	C[3]			
		Method[5]			
		I	II		
A	36.7(14)[6]	731.9(31)	1102.4(29)	25	17
B	35.9(18)	606.9(31)	961.3(34)	30	19
C	19.1(17)	432.8(53)	1098.5(46)	37	9
D	31.7(10)	653.9(18)	1092.3(23)	24	15
E	34.3(20)	670.4(41)	1044.4(40)	26	16

1 Soil pre-incubated for 21 days at 75% WHC, 15°C.

2 Calculated with k_p determined for individual soils (see Table 3).

3 Determined by CFIM, k_c = 0.45.

4 Assume microbial biomass is 50% C, dry wt.

5 Control = CO_2 respired from 0-10 days, unfumigated soil.

6 Numbers in parentheses are standard errors.

Table 7. Interstudy comparisons of soil microbial biomass P values[1].

Reference[2]	Biomass C	Biomass P	P concentration (g/kg dry wt. microbial biomass)	P concentration (g/kg total soil P	K_p
	mg/kg soil				
This Work	619.5	31.5	25	28	0.27
1	594.5	39.4	33	40	0.40
2	ND[3]	29.2	ND	ND	0.37
3	385.5	23.7	31	71	0.40

1 Mean values of all soils tested.

2 1. Brookes et. al, 1984. n = 5.
2. Hedley and Stewart, 1982. n = 1.
3. Srivstava and Singh, 1988. n = 6.

3 Not determined.

Table 8. Simple linear regression of biomass P on various soil P chemical fractions[1].

P Fraction	Intercept	Slope	R^2
Olsen[2]	14.41	2.85	0.78*
Total	13.02	0.018	0.72
NaOH	18.65	0.025	0.32
$NaHCO_3$	22.77	0.24	0.28

1 Soils A through E, n = 5.

2 See materials and methods for descriptions of the chemical analyses.

* significant at p = 0.05.

Figure 1a. The influence of chloroform fumigation upon the concentration of P_t extractable in 0.5M $NaHCO_3$.

Figure 1a.

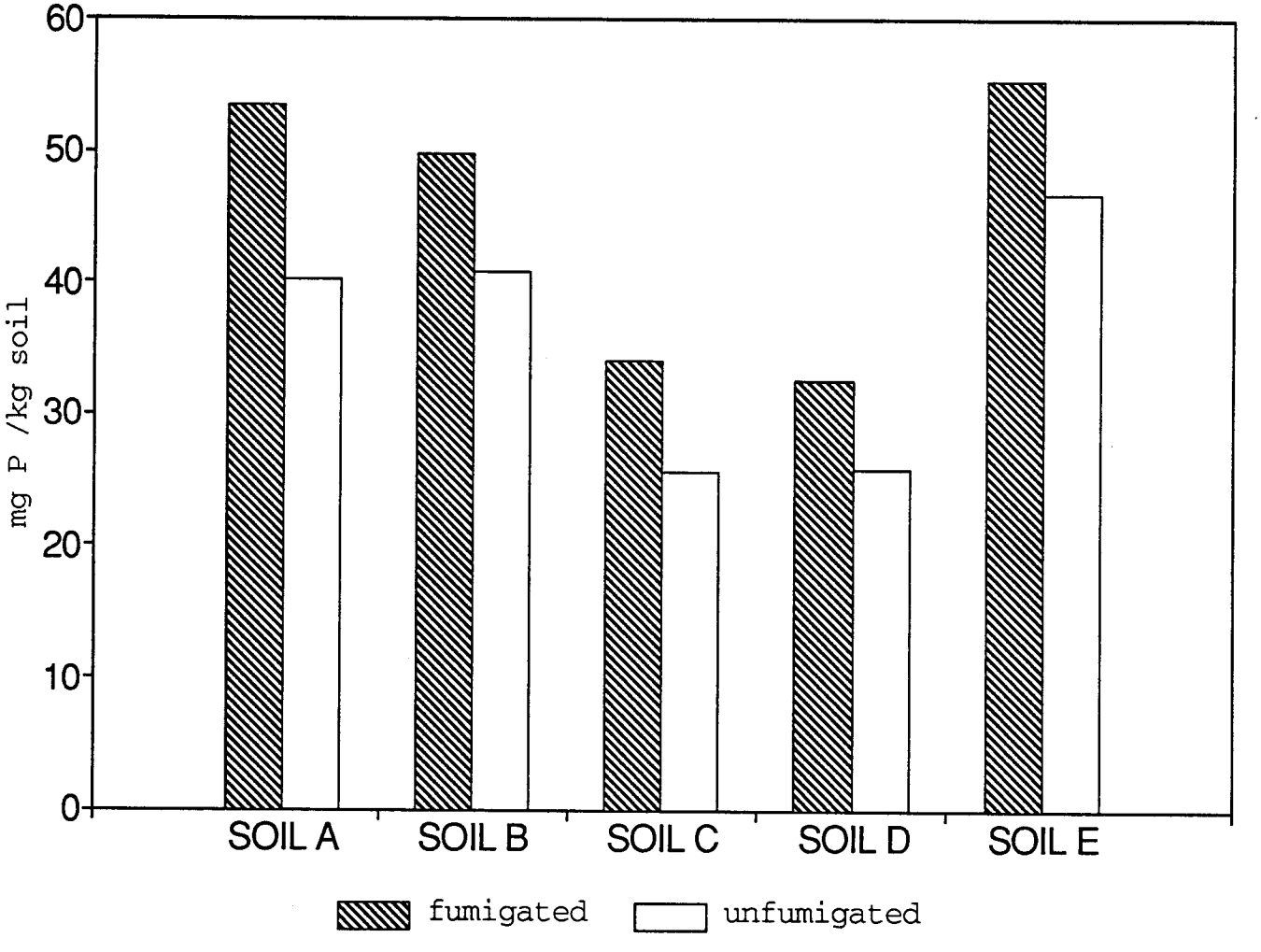

60
50
40
30
20
10
0
mg P /kg soil
SOIL A
SOIL B
SOIL C
SOIL D
SOIL E
fumigated
unfumigated

Figure 1b. The influence of chloroform fumigation upon the concentration of P_i extractable in 0.5M $NaHCO_3$.

Figure 1b.

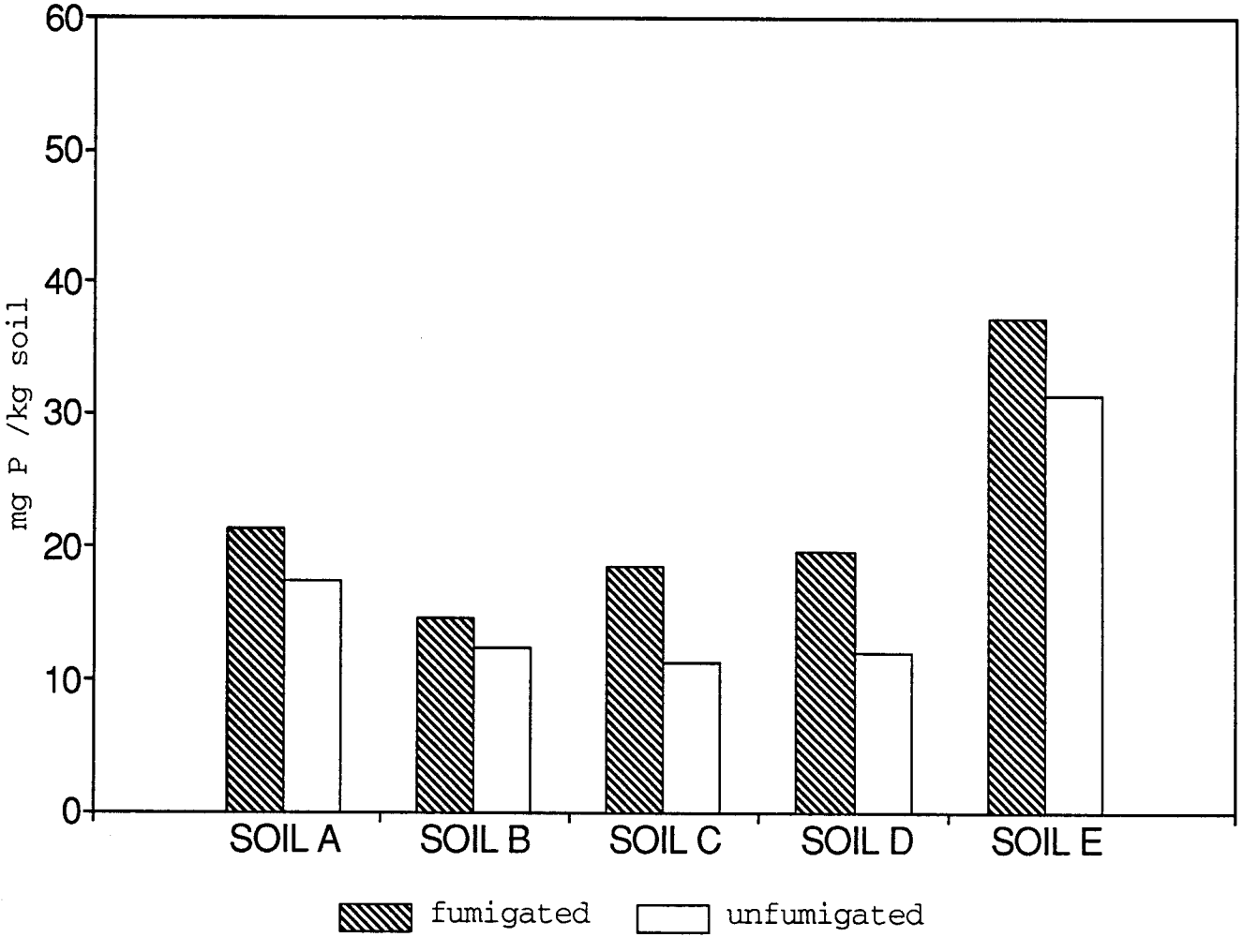

60
50
40
30
20
10
0
mg P /kg soil
SOIL A
SOIL B
SOIL C
SOIL D
SOIL E
fumigated
unfumigated

Figure 1c. A comparison of the differences between the concentration of $NaHCO_3$ extractable P_t and P_i recovered from fumigated and unfumigated soil samples.

Figure 1c.

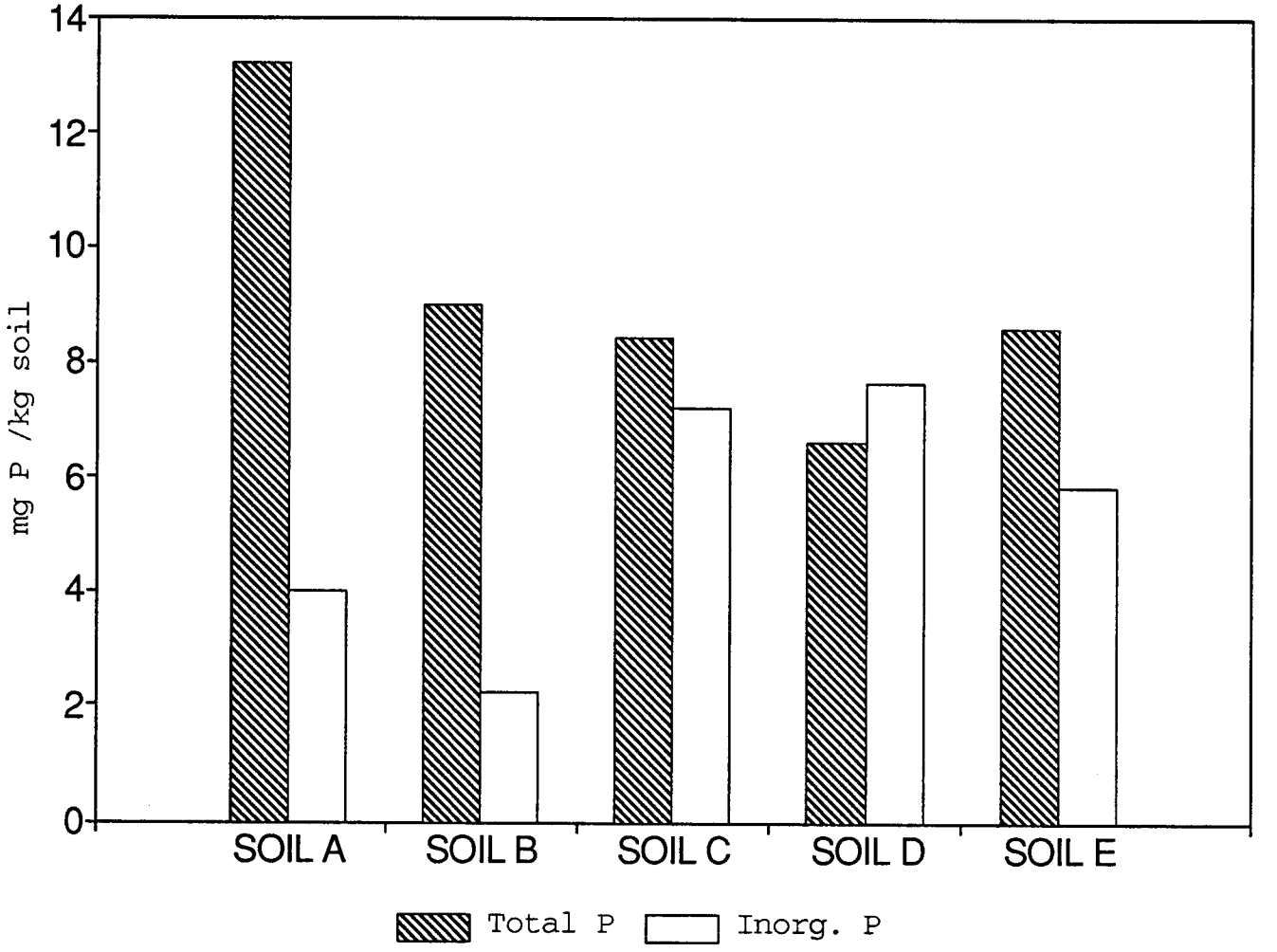

14
12
10
8
6
4
2
0
mg P /kg soil
SOIL A
SOIL B
SOIL C
SOIL D
SOIL E
Total P
Inorg. P

Figure 2a. Concentration of $NaHCO_3$-extractable P_t in fumigated and unfumigated field samples of soil A.

Figure 2a.

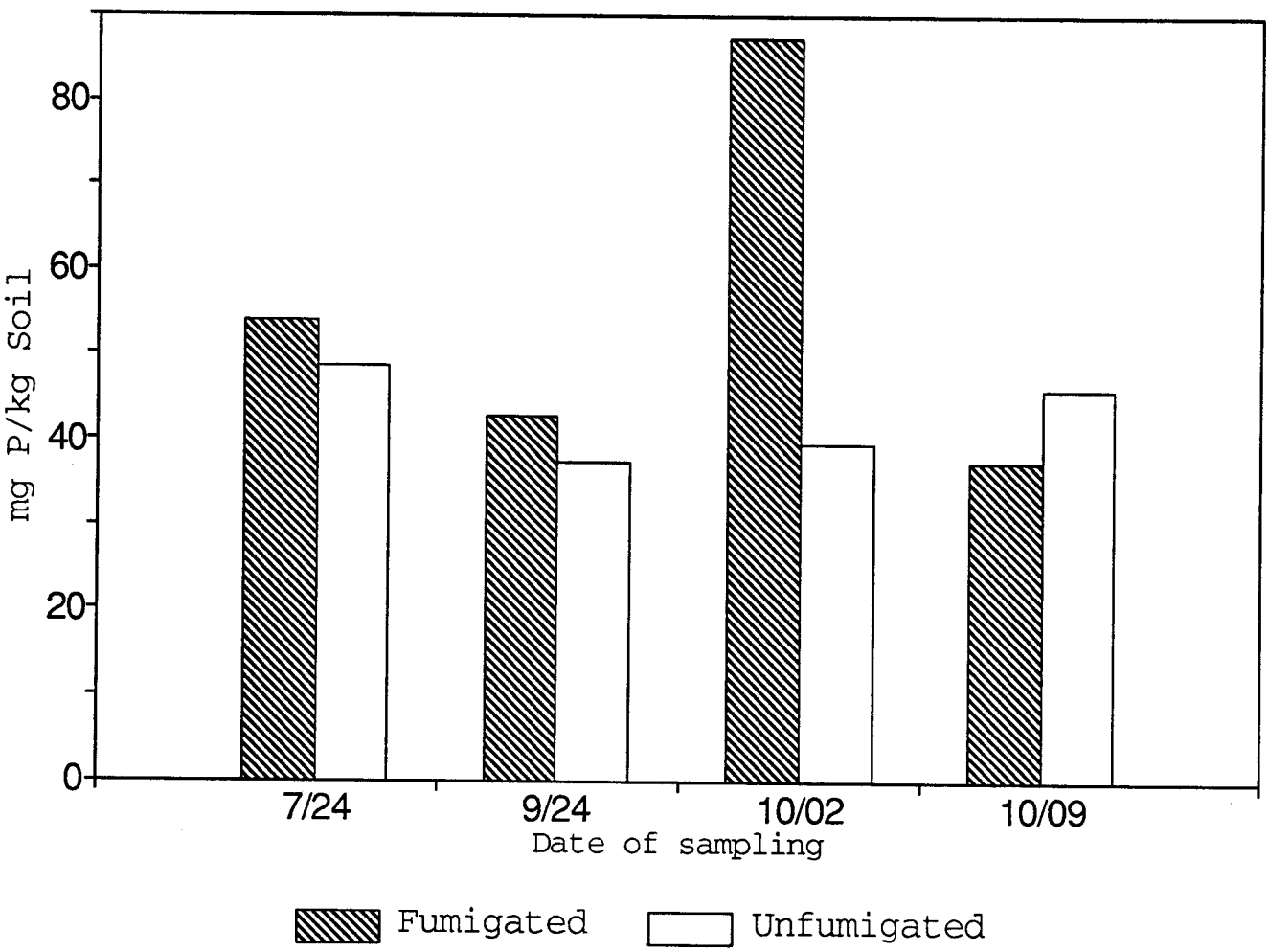

80
60
40
20
0
mg P/kg Soil
7/24
9/24
10/02
10/09
Date of sampling
Fumigated
Unfumigated

Figure 2b. Concentration of $NaHCO_3$-extractable P_t in fumigated and unfumigated field samples of soil B.

Figure 2b.

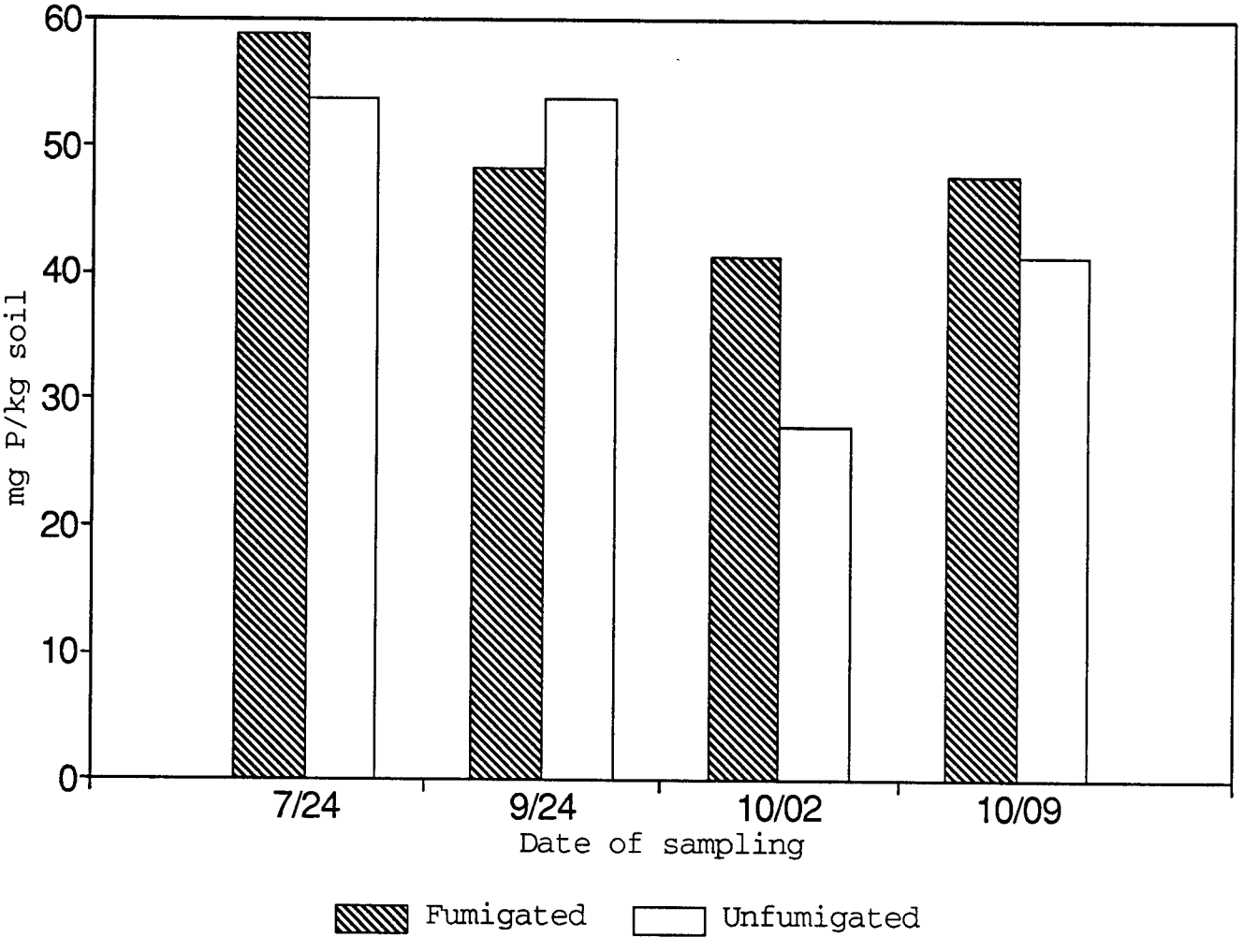

60
50
40
30
20
10
0
mg P/kg soil
7/24
9/24
10/02
10/09
Date of sampling
Fumigated
Unfumigated

Figure 2c. Concentration of $NaHCO_3$-extractable P_t in fumigated and unfumigated field samples of soil C.

Figure 2c.

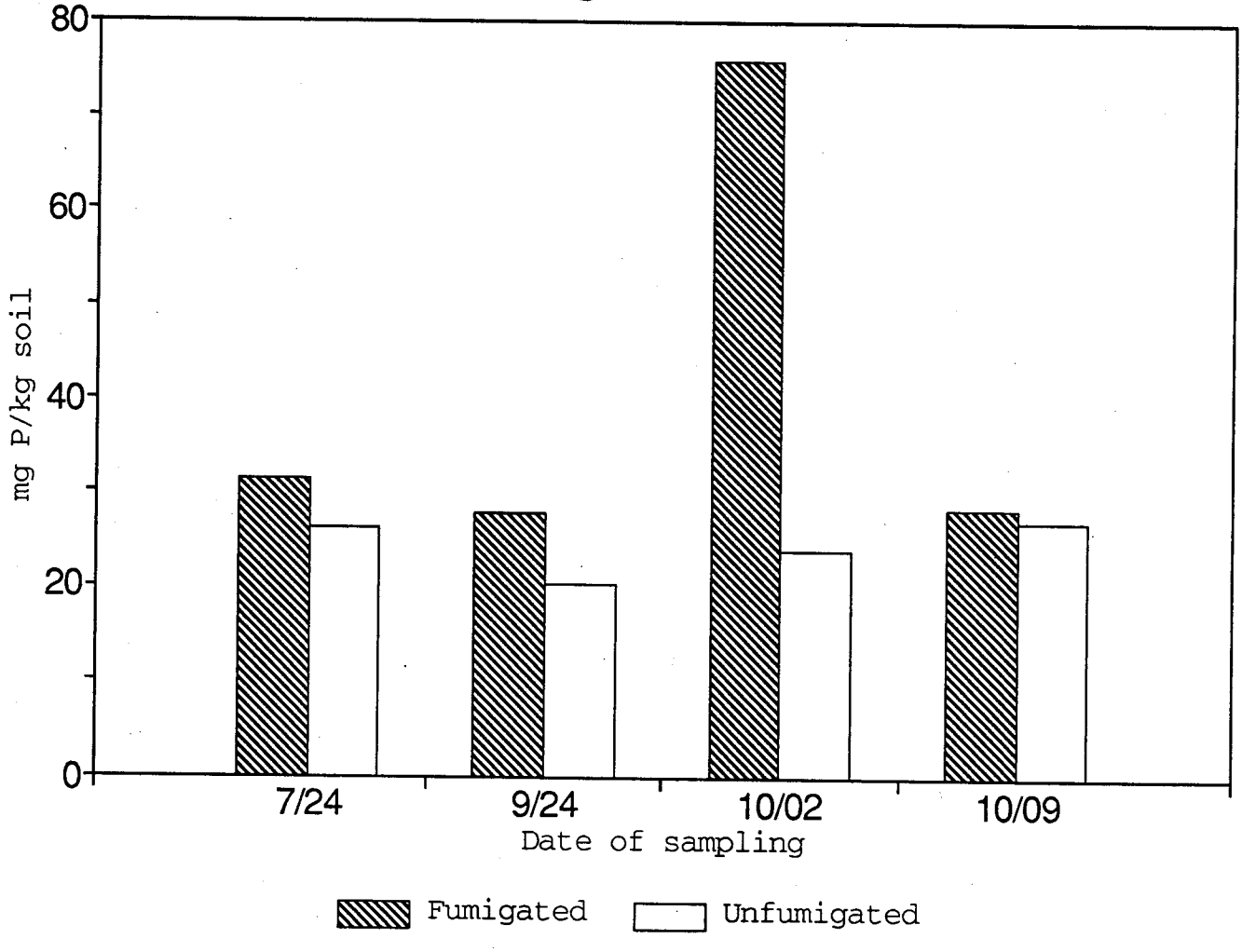

80
60
40
20
0
mg P/kg soil
7/24
9/24
10/02
10/09
Date of sampling
Fumigated
Unfumigated

Figure 2d. Concentrations of $NaHCO_3$-extractable P_t in fumigated and unfumigated field samples of soil D.

Figure 2d.

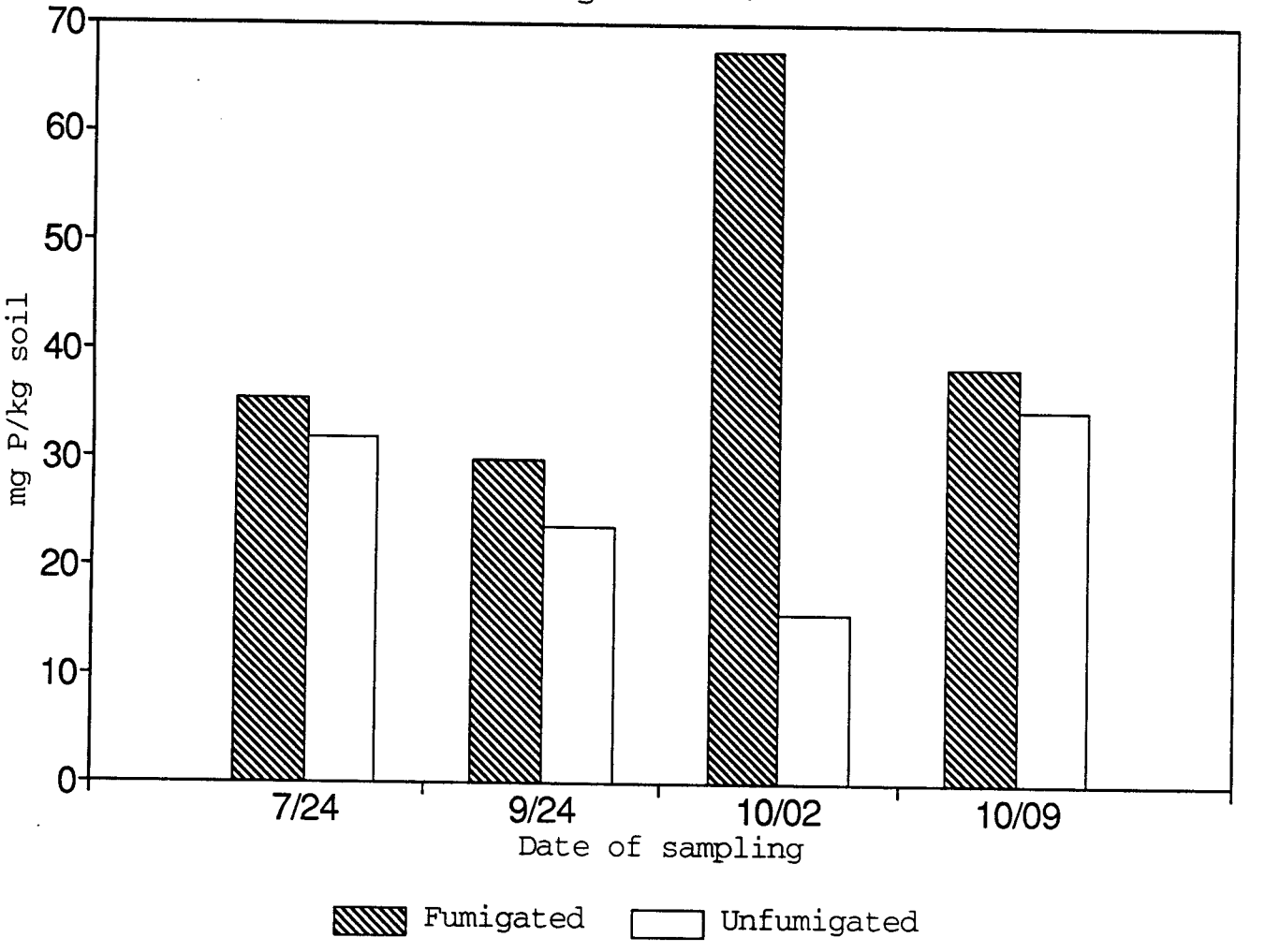

70
60
50
40
30
20
10
0
mg P/kg soil
7/24
9/24
10/02
10/09
Date of sampling
Fumigated
Unfumigated

Figure 2e. Concentrations of $NaHCO_3$-extractable P_t in fumigated and unfumigated field samples of soil E.

Figure 2e.

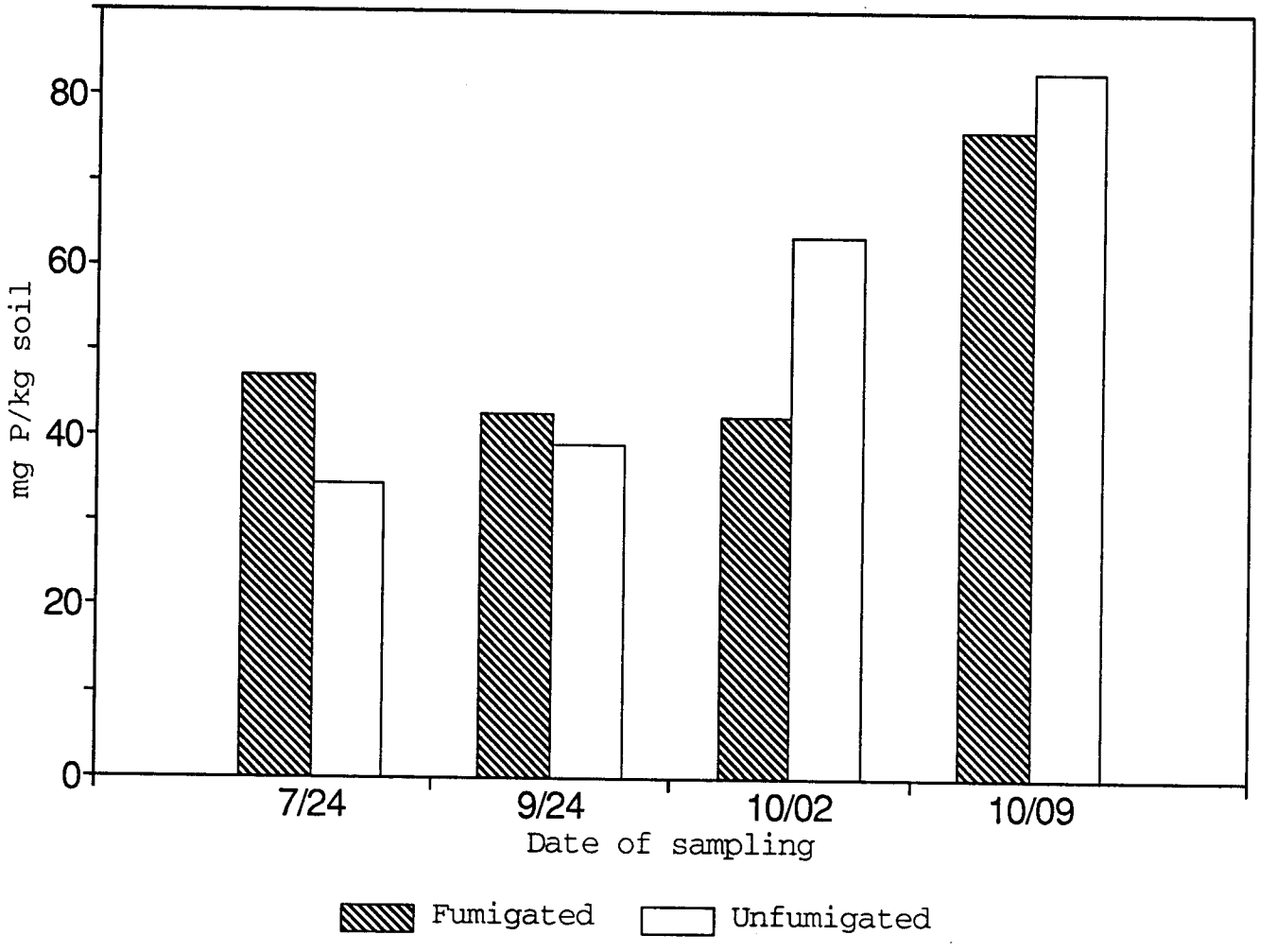

80
60
40
20
0
mg P/kg soil
7/24
9/24
10/02
10/09
Date of sampling
Fumigated
Unfumigated

Figure 3. Changes in gravimetric water content of field soils during the sampling period.

Figure 3.

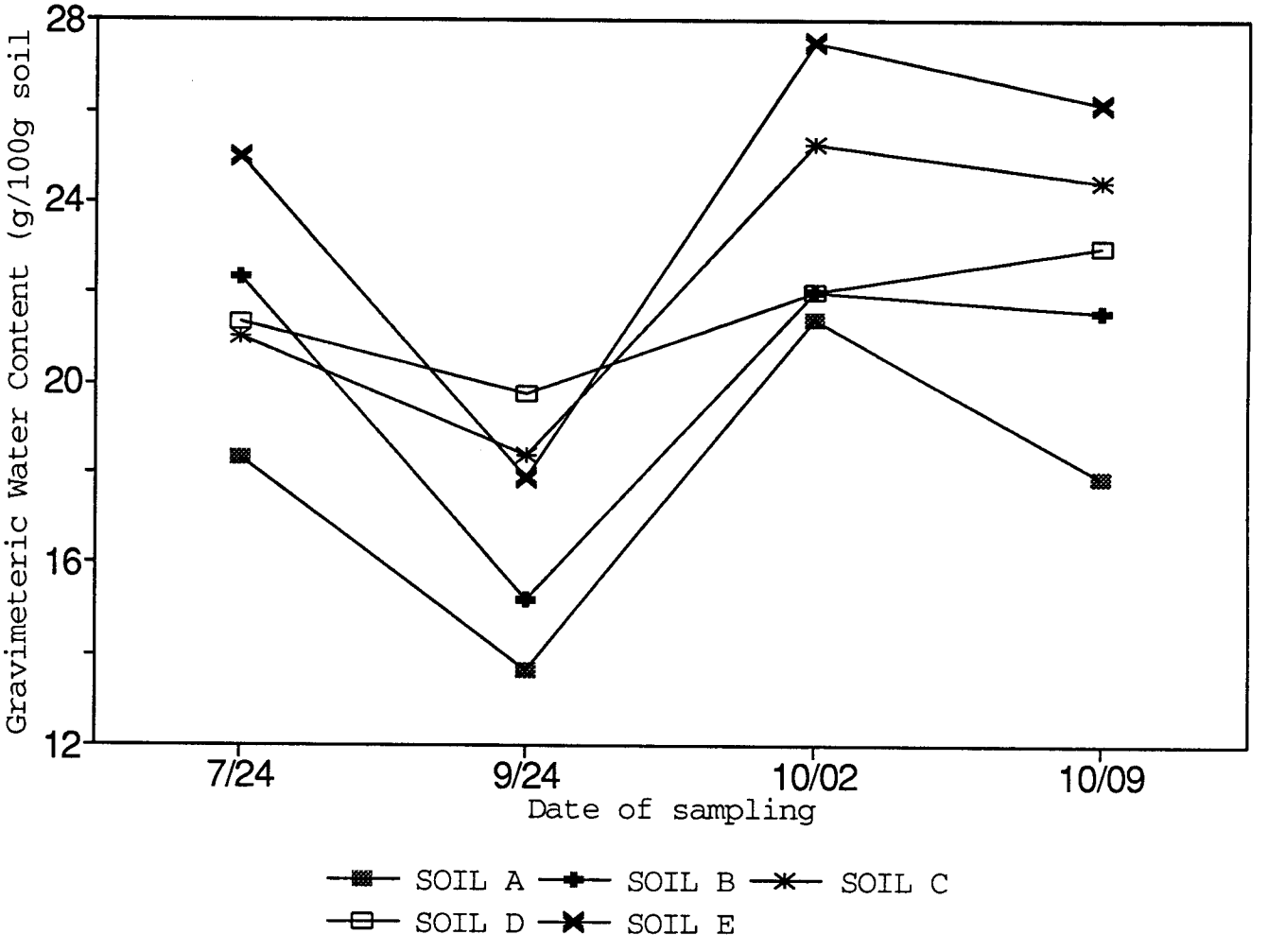

Gravimeteric Water Content (g/100g soil
28
24
20
16
12
7/24
9/24
10/02
10/09
Date of sampling
SOIL A
SOIL B
SOIL C
SOIL D
SOIL E

Figure 4a. Cumulative CO_2 evolution from equilibrated fumigated and unfumigated samples of soil A.

Figure 4a.

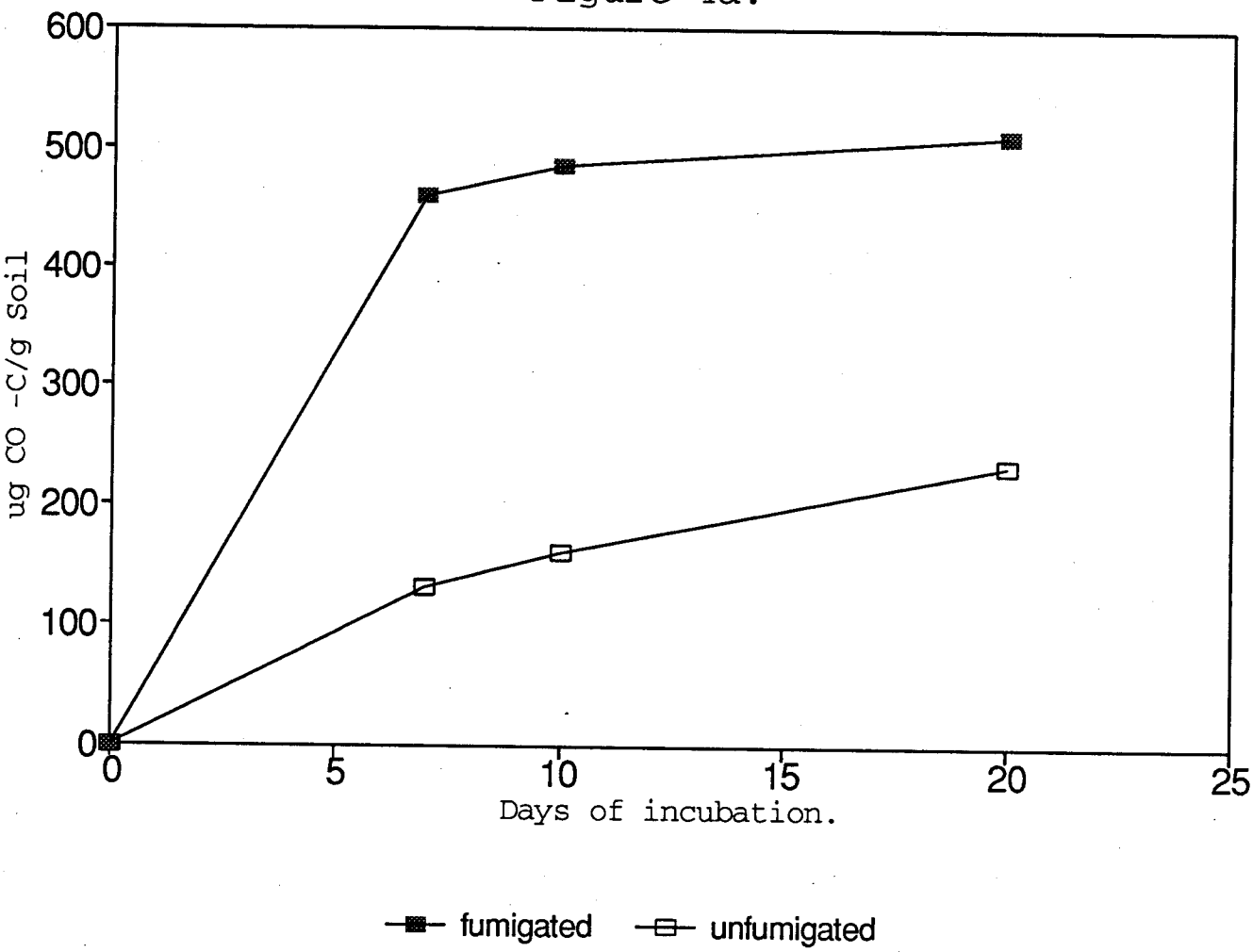

600
500
400
300
200
100
0
ug CO -C/g Soil
0
5
10
15
20
25
Days of incubation.
fumigated
unfumigated

Figure 4b. Cumulative CO_2 evolution from equilibrated fumigated and unfumigated samples of soil B.

Figure 4b.

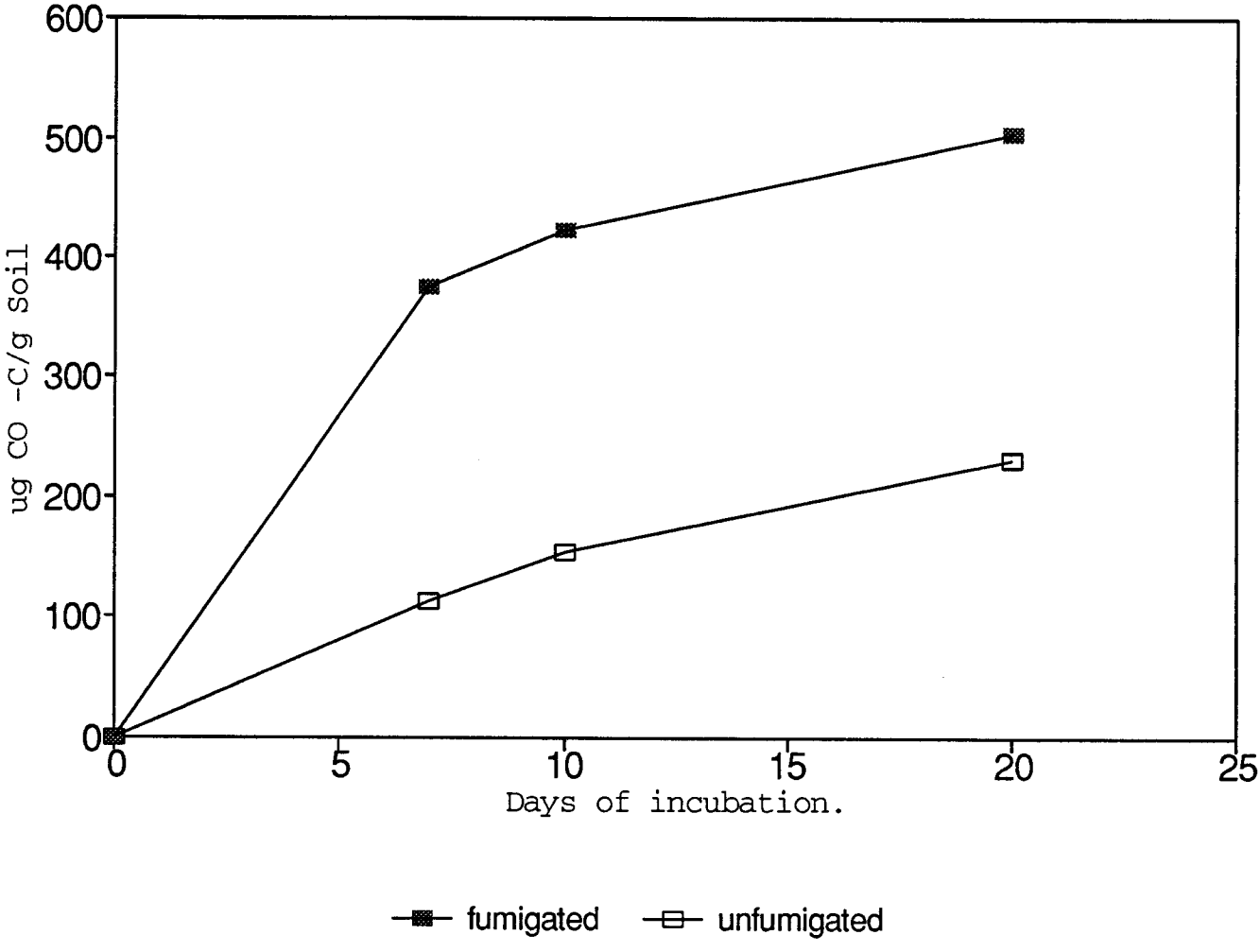

600
500
400
300
200
100
0
ug CO -C/g Soil
0
5
10
15
20
25
Days of incubation.
fumigated
unfumigated

Figure 4c. Cumulative CO_2 evolution from equilibrated fumigated and unfumigated samples of soil C.

Figure 4c.

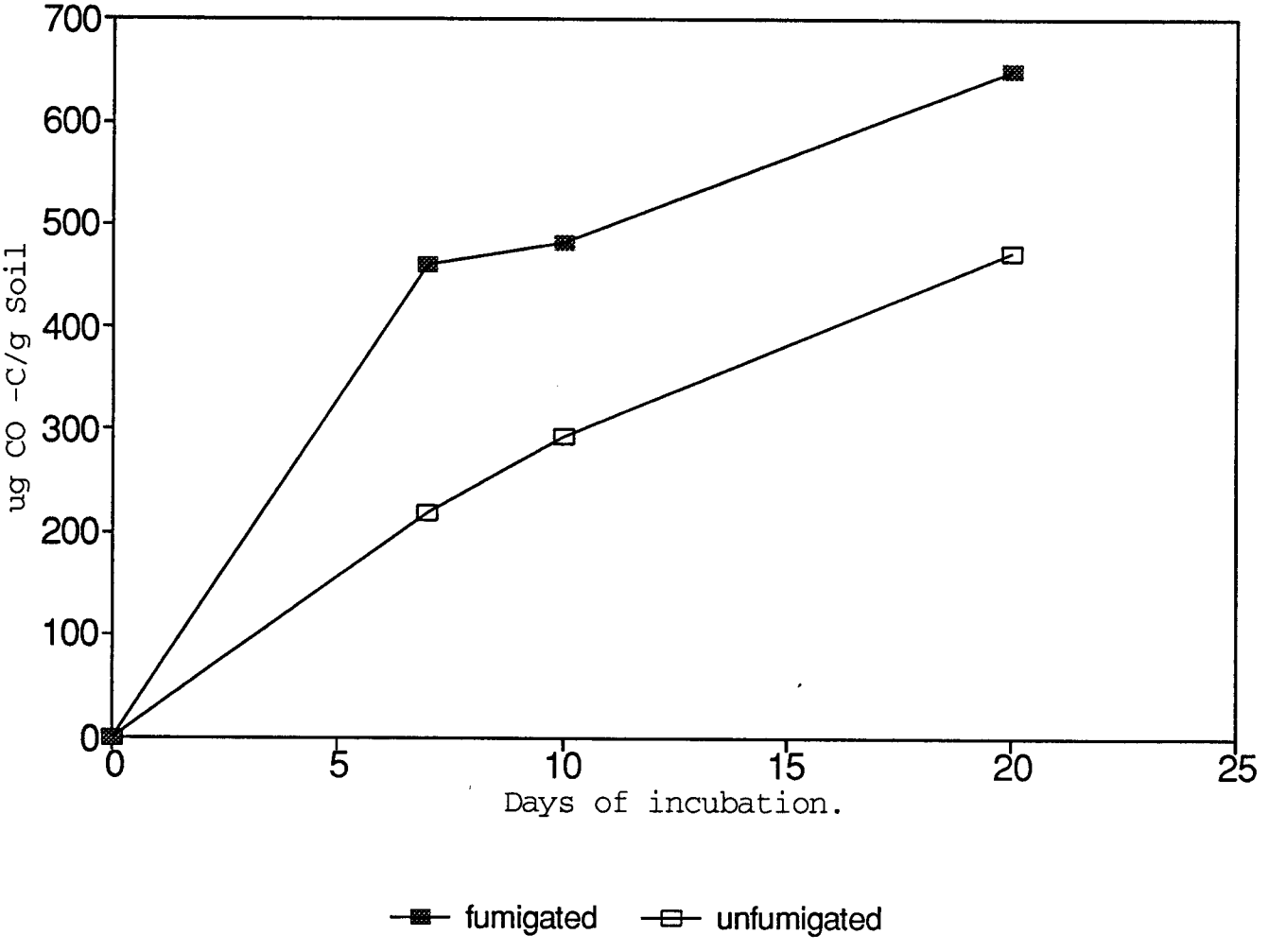

700
600
500
400
300
200
100
0
ug CO -C/g Soil
0
5
10
15
20
25
Days of incubation.
fumigated
unfumigated

Figure 4d. Cumulative CO_2 evolution from equilibrated fumigated and unfumigated samples of soil D.

Figure 1d.

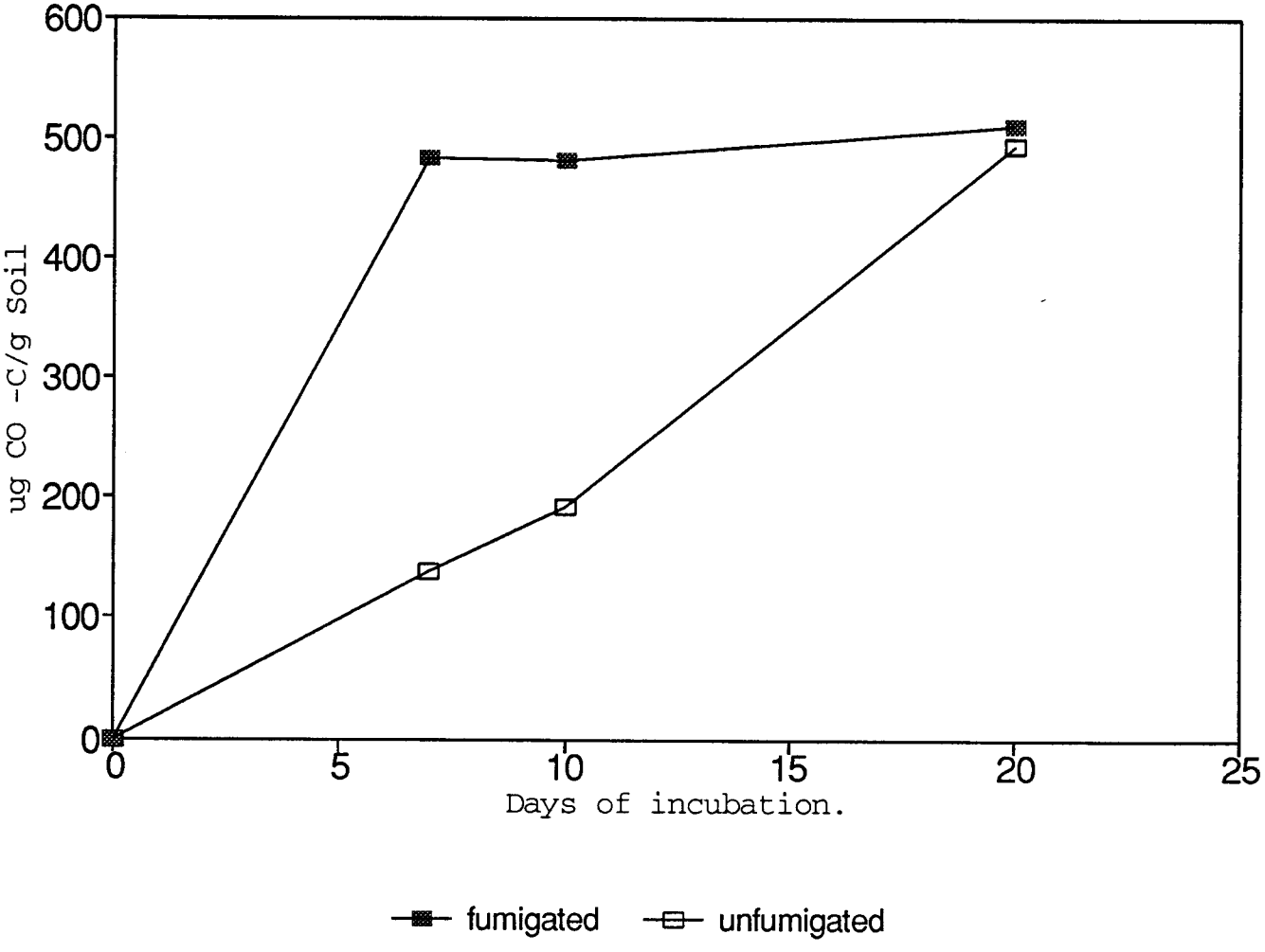
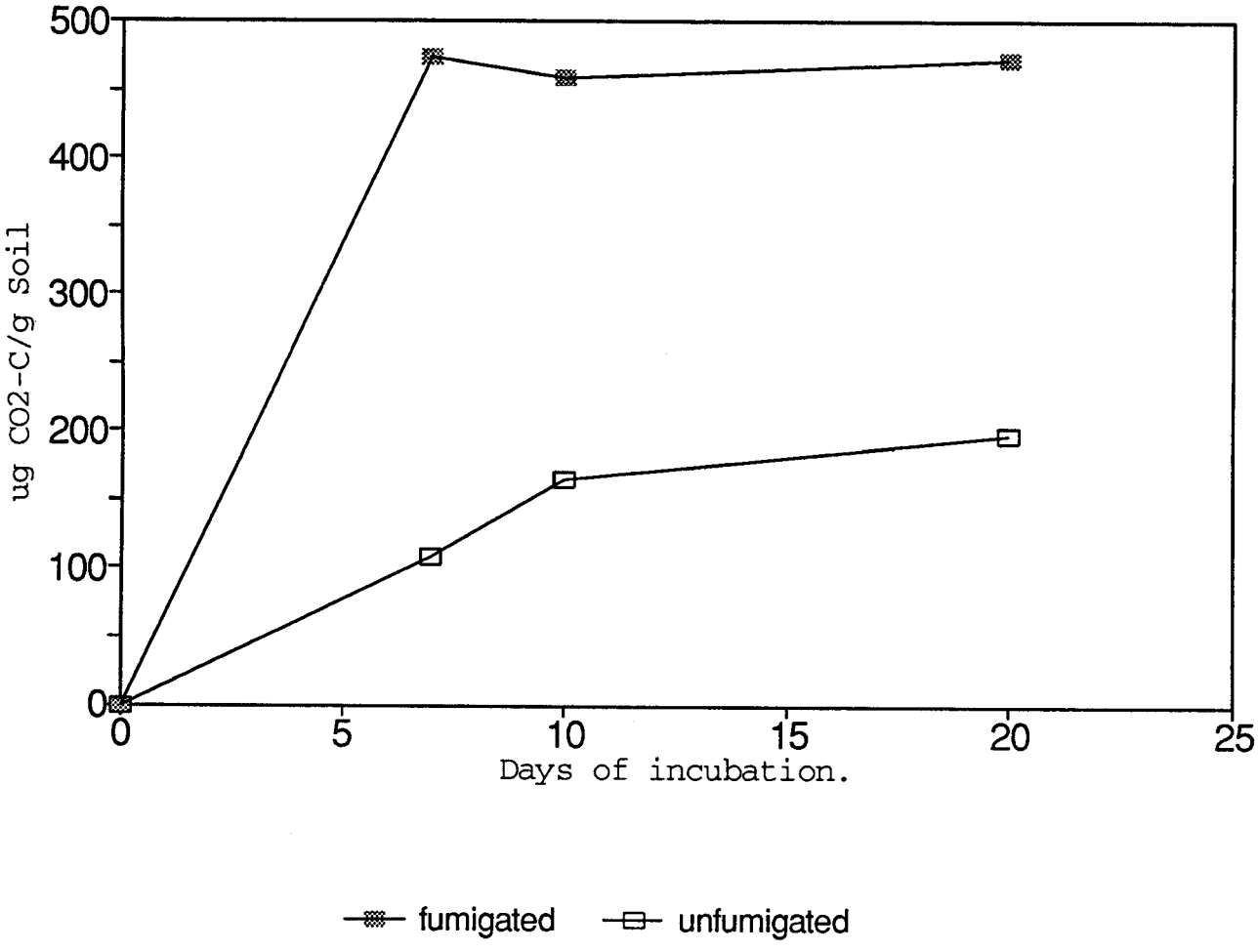

600
500
400
300
200
100
0
ug CO -C/g Soil
0
5
10
15
20
25
Days of incubation.
fumigated
unfumigated

Figure 4e. Cumulative CO_2 evolution from equilibrated fumigated and unfumigated samples of soil E.

Figure 4e.


500
400
300
200
100
0
ug CO2-C/g Soil
0
5
10
15
20
25
Days of incubation.
fumigated
unfumigated

Literature Cited

Almendras, A.S., Bottomley, P.J. (1988) Cation and Phosphate influences on the nodulating characteristics of indigenous serogroups of *Rhizobium Trifolii* on soil grown *Trifolium Subterraneum*, L. Soil Biol. Biochem. 20: 345-351.

Anderson, G. (1975) Other phosphorus compounds. In: Soil Components. Vol. 1. Organic Components: 305-331.(J.E. Gieseking ed.). Springer-Verlag, Berlin

Anderson, J.P.E and Domsch, K.H. (1980) Quantities of plant nutrients in the microbial biomass of selected soils. Soil Science 130: 211-216.

Anderson, T. and Domsch, K.H. (1989) Ratios of microbial biomass carbon to total organic carbon in arable soils. Soil Biol. Biochem. 21(4): 471-479.

Balster, C.A. and Parsons, R.B. (1969) Late pleisocene stratigraghy, southern willamette valley, Oregon. Northwest Science 43:116-129.

Barber, S.A. (1962) A diffusion and mass-flow concept of soil nutrient availability. Soil Science 93: 39-49.

Bowman, R.A. and Cole, C.V. (1987a) Transformations of organic phosphorus substances in soil as evaluated by NaHCO3 extraction. Soil Science 125: 49-54.

Bowman, R.A. and Cole, C.V. (1978b) An exploratory method for fractionation of organic phosphorus from grassland soils. Soil Science 125: 95-101.

Brookes, P.C., Landman, A., Pruden, G. and Jenkinson, D.S. (1985) Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17(6): 837-842.

Brookes, P.C., Powlson, D.S. and Jenkinson, D.S. (1982) Measurement Of microbial phosphorus in soil. Soil Biol. Biochem. 14:319-329.

Brookes, P.C., Powlson, D.S. and Jenkinson, D.S. (1984) Phosphorus in the soil microbial biomass. Soil Biol. Biochem. 16:169-175.

Chuahan, B.S., Stewart, J.W.B. and Paul, E.A. (1981) Effect of labile inorganic phosphate status and organic carbon additions on the microbial uptake of phosphorus in soils. Can J. Soil Sci. 61:373-385.

Chausodd, R. and Nicolardot, B. (1982) Mesure de la biomasse microbienne dans les sols cultives. 1. Approach cinetique et estimation simplifee du carbone facilement mineralisable. Revue d'Ecologie et de Biologie du Sol. 19:501-512.

Cole, C.V., Elliot, E.T., Hunt, H.W. and Coleman, D.C. (1978) Trophic interactions in soils as they affect energy and nutrient dynamics. V. Phosphorus transformations. Microb. Ecol. 4: 381-387.

Cole, C.V., Innis, G.I. and Stewart, J.W.B. (1977) Simulation of phosphporus cycling in semi-arid grasslands. Ecology 58:1-15.

Coleman, D.C., Ried, C.P.P and Cole, C.V. (1983) Biological strategies of nutrient cycling in soil systems. In: Advances in Ecological Research 14: 1-56. (MacFayden, A. and E.O. Ford eds.). Academic Press, New York.

Condron, L.M. and Goh, K.M. (1990) Nature and availability of residual phosphorus in longterm fertilized pasture soils in New N. Z. J. of Agric. Sci., Cambridge 114: 1-9.

Dalal, R.C. (1979) Mineralization of carbon and phosphorus from carbon-14 and phosphorus-32 labelled plant material added to soil. Soil Sci. Soc. Am. J. 43:915-916.

Darbyshire, J. (1975) Soil Protozoa-Animalcule in the subterranean micro-environment. In: Soil Microbiology A Critical Review. N.Walker ed.: Butterworths, London.

Davidson, E.A., Galloway, L.F. and Strand, M.K. (1987) Assessing available carbon: Comparison of techniques across selected forest soils. Commun. Soil Sci. Plant Qual. 18:45-64.

Dormaar, J. (1972) Seasonal patterns of soil organic phosphorus. Can. J. of Soil Sci. 28:83-85.

Gardener, D.T. and Christensen, N.W. (1991) ear seedling response to phosphorus on a phosphate-fixing soil. Commun. Soil Sci. Plant Anal. 22:1597-1603.

Hahm, B.J., Stewart, J.W.B and Halstead, R.L. (1972) The Phosphorus cycle in native grassland ecosystems. pp. 571-586. In: Isotopes and Radiation in Soil-Plant Relationships Including Forestry. International Atomic Energy Agency, Vienna.

Haynes, R.J. and Swift, R.S. (1985) Effects of air-drying on the adsorption and desorption of phosphate and levels of extractable phosphate in a group of acid soils, New Zealand. Geoderma 35: 145-157.

Hedley, M.J., Stewart, J.W.B and Chauhan, B.S. (1982) Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci. Soc. Am. J. 46: 970-976.

Hedley, M.J. and Stewart, J.W.B. (1982) Method to Measure microbial phosphate in soils. Soil Biol. Biochem. 14:377-385.

Horneck, D.A., Hart, J.M., Toepper, K. and Koepsell, B. (1989) Methods of soil analysis used in the soil testing laboratory at Oregon State University. U.S. Agric. Exper. Sta., SM 89(4).

Jenkinson, D.S. (1976) The effects of biocidal Treatments of metabolism in soil--IV. The decomposition of fumigated organisms in soil. Soil Biol. Biochem. 8:203-208.

Jenkinson, D.S. and Ladd, J.N. (1985) Microbial biomass in soil: Measurement and turnover. In: Soil Biochemistry Vol.5 E.A. Paul and J.N. Ladd ed.: Marcel Dekker, Inc. New York.

Jenkinson, D.S. and Powlson, D.S. (1976) The effects of biocidal treatments on metabolism in soil--V. A method to measure the soil microbial biomass. Soil Biol. Biochem. 8:209-213.

Jenkinson, D.S. and Powlson, D.S. (1976) The effects of biocidal treatments on metabolism in soil--I. Fumigation with chloroform. Soil Biol. Biochem. 8(167-177).

Ladd, J.N. and Martin, J.K. (1985) Soil organic matter studies. pp. 67-98. In: Isotopes and Radiation in Agricultural Sciences. Vol. 1 (L'Annunziata, M.F. and Legg, J.O. eds.).

Lee, D., Han, X.G. and Jordan, C.F. (1990) Soil phosphorus fractions, aluminum, and water retention as affected by microbial activity in an ultisol. Plant and Soil 121: 125-136.

Leung, K. and Bottomley, P.J. (1987) Influence of phosphate on the growth and nodulation characteristics of *Rhizobium trifolii*. Appl. and Environ. Microbiol. 53:2098-2105.

Lindsay, W.L. and Moreco, E.C. (1960) Phosphate phase equilibria in soils. Soil Sci. Soc. Am. J. 24:177-182.

Marumoto, T., Anderson, J.P.E. and Domsch, K.H. (1982) Mineralization of nutrients from the soil microbial biomass. Soil Biol.Biochem., 1982, 14: 469-475.

McLaughlin, J.R.,Reyden, J.C. and Syers, G.K. (1977) Development and evaluation of a kinetic model to describe phosphate sorption by hydrous ferric oxide gels. Geoderma 18:295-307.

McLaughlin, M.J. and Alston, A.M. (1986) Measurement of phosphorus in the soil nicrobial biomass: A modified procedure for field soils. Soil Biol Biochem. 18: 437-443.

McLaughlin, M.J., Alston, A.M. and Martin, J.K. (1987) Transformation and movement of P in the rhizosphere. Plant and Soil 97:391-399.

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

O'Halloran, J.P., Stewart, J.W.B. and De Jong, E. (1987) Changes in P forms and availability as influenced by managenment practices. Plant and Soil 100:113-126.

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Dep. of Agric. Circ. 939.

Oregon State University Cooperative Extension Service. (1983) Fertilizer Guide 4, Subclover-grass pasture.

Paul, E.A. (1984) Dynamics of organic matter in soils. Plant and Soil 76: 275-285.

Paul, E.A. and Voroney, R.P. (1980) Nutrient and energy flows through the microbial biomass. In: Contemporary Microbial Ecology. pp.215-237. (D.C. Ellwood, J.C. Hedger, M.J. Latham, J.M Lynch and J.H Slater eds.) Academic Press, New York.

Powlson, D.S. and Jenkinson, D.S. (1976) The effects of biocidal treatments on metabloism in soils-II. Gamma irradiation, autoclaving, air-drying and fumigation. Soil Biol. Biochem. 8:179-188.

Ryden, J.C., McLaughlin, J.R. and Syers, J.K. (1977) Mechanisms of phosphate sorption by soil ferric anhydrous oxide gel. J. Soil Sci. 28:72-92.

Saggar, S., Bettany, J.R. and Stewart, J.W.B. (1981a) Measurement of microbial sulfur in soil. Soil Biol. Biochem. 13:493-498.

Saunders, W.M.H and Metson, A.J. (1970) Seasonal variation of phosphorus in soil and pasture. N.Z. J. Agric. Res. 14:307-328.

Sharpley, A.N. (1985) Phosphorus in fertilized and unfertilized agricultural soils. Soil Sci. Soc. Am. J. 49:905-911.

Shen-Min, Shen, Brookes, P.C. and Jenkinson, D.S. (1989) Soil respiration and the measurement of microbial biomass C by the fumigation technique in fresh and in air-dried soil. Soil Biol. Biochem. 19:153-158.

Sikora, L.J. and McCoy, J.L. (1990) Attempts to determine available carbon in soils. Biol. Fert. Soils 1990, 9:19-24.

Smeck, N.E. (1973) Phosphorus: An indicator of pedogenic weathering processes. Soil Science 115:199-206.

Sparling, G.P., Milne, J.G.D. and Vincent, K.W. (1987) Effect of soil moisture regime on the microbial contribution to olsen phosphorus values. N.Z. J. Agric. Res. 30:79-84.

Sparling, G.P., Speir, T.W. and Whale, K.N. (1986) Changes in microbial biomass C, ATP content, soil phospho-monoesterase and phosph-diesterase activity following air-drying of soils. Soil Biol. Biochem. 18:363-370.

Sparling, G.P., Whale, K.N. and Ramsay, A.J. (1985) Quantifying the contribution from the soil microbial biomass to the extractable P levels of fresh and

air-dried soils. Aust. J. Soil Res. 23:613-621.
Srivastava, S.C. and Singh, J.S. (1988) Carbon and phosphorus in the soil biomass of some tropical soils of India. Soil Biol. Biochem. 20:743-747.

Stevenson, F.J. (1986) Cycles of Soil: John Wiley and Sons, London.

Stewart, J.W.B. and McKercher, R.B. (1982) Phosphorus cycle. In: Experimental Microbial Ecology. pp. 221-228. Burns, R.G. and Slater, J.H. eds. Blackwells, Oxford.

Stewart, J.W.B. and Tiessen, H. (1987) Dynamics of soil organic phosphorus. Biogeochemistry 4:41-60.

Tate, K.R. (1984) The biological transformation of P in soil. Plant and Soil 76: 245-256.

Voroney, R.P., and Paul E.A. (1984) Determination of k_c and k_N *in situ* for calibration of the chloroform fumigation incubation method. Soil Biol. Biochem. 16:9-14.

Voroney, R.P., Van Veen, J.A. and Paul, E.A. (1981) Organic C dynamics in grassland soils. 2. Model validation and simulation of long term effects of cultivation and rainfall erosion. Can. J. Soil Sci. 61:211-224.

Walker, T.W. (1965) The significance of phosphorus in pedogenesis. In: Experimental Pedology. (Hallsworth, S.G. and Crawford, D.V., eds.) pp.295-315. Butterworths, London.

Walker, T.W. and Syers, J.K. (1976) The fate of phosphorus during pedogenesis. Geoderma 5:1-19.

Walker, T.W. and Adams, A.F.R. (1958) Studies on soil organic matter: 1. Influence of phosphorus content of parent materials on accumulation of carbon, nitrogen, sulfur and organic phosphorus in grassland soils. Soil Science 85:307-318.

Walker, T.W. and Adams, A.F.R. (1959) Studies on organic matter: 2. Influence of increased leaching at various stages of weathering on levels of carbon, nitrogen, sulfur and organic and inorganic phosphorus. Soil Science 87:1-10.

West, A.W., Ross, D.J. and Cowling, J.C. Changes in microbial C, N, P and ATP contents, numbers and respiration on storage of soils. Soil Biol. Biochem. 18:141-148.

Westheimer, F.H. (1987) Why nature chose phosphates. Science 235:1173-1178.

Williams, J.D.H., Mayers, T. and Nriagu, J.O. (1980) Extractability of phosphorus from phosphate minerals common in soil and sediments. Soil. Sci. Soc. Am. J. 87:135-140.

Williams, J.D.H., Syers, J.K., Harris, R.F. and Armstrong, D.E. (1971) Fractionation of inorganic phosphorus in calcareous lake sediments. Soil Sci. Soc. Am. J. 35:250-255.