THESIS

STUDIES OF THE MECHANISMS OF STABILIZATION OF ORGANIC MATTER IN SEMIARID SOILS

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY Bryce F. Payne Jr.

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ABSTRACT OF THESIS

STUDIES OF THE MECHANISMS OF STABILIZATION OF ORGANIC MATTER IN SEMIARID SOILS

The effectiveness of three mechanisms of stabilization of soil organic matter (OM) were studied. These mechanisms are (1) chemical recalcitrance, (2) physical stabilization by adsorption onto soil particles, and (3) physical stabilization by occlusion within microaggregates. Mechanisms 1 and 2 were studied by incubation (mineralization) experiments using soil particle size fractions as sources of native soil OM of different chemical quality. These experiments examined microbial utilization and transformations of the C and P associated with the particle size fractions.

The characteristics of particle size fractions and the effects of ultrasonic dispersion on them were examined during fractionations using different combinations of sedimentation and ultrasonic dispersion of aqueous soil suspensions. The chemical compositions (C, N, and P) were consistently different for the ultrasonically dispersible and readily water-dispersible fine soil particle-size fractions. Carbon contents of the fine fractions ultrasonically dispersed from water-stable aggregates of >50 μm equivalent spherical diameter (ESD) were greater than those from smaller (<50 μm ESD) water-stable aggregates. Evidence was obtained that ultrasonic dispersion causes a redistribution of the organic matter associated with different particle size fractions. This has important

implications for interpretations of information obtained using ultrasonic dispersion techniques.

Several observations indicated the existence of anaerobic microsites inside the larger (>50 μm ESD) water-stable aggregates. A light blue-gray clay sediment indicating reduced forms of Fe and/or Mn appeared whenever ultrasonic dispersion was used. The fine silt from water-stable aggregates had DTPA-extractable Mn levels 53 times higher than the water-dispersible fine silt. The consistently higher C contents and different qualitative chemical character of the fractions from water-stable aggregates indicated that the decomposition of organic matter in these aggregates is limited by oxygen stress. Occlusion of organic matter in microaggregates is an important mechanism of organic matter stabilization.

The concepts of chemical recalcitrance and clay adsorption as mechanisms of organic matter stabilization were studied using incubations of three particle size fractions obtained from two semiarid grassland soils. Microbial transformations of C and P were assessed as indices of relative availability of native forms of these elements. Chemical recalcitrance was hypothesized to increase with increasing particle size. Stabilization by clay adsorption was hypothesized to increase with decreasing particle size. Three levels of cellobiose-C amendment were used to establish different levels of microbial activity. No utilization of soil organic C occurred without the cellobiose amendment. Net mineralization of P to plant-available forms was found after 124 hours at the lowest C amendment level for all particle size fractions. Net utilization of unextractable P was apparent during the period of increasing biomass. Among the fractions

equivalent microbial P was not held in equivalent extractable forms. Native soil C was extensively utilized for all fractions and at all C amendment levels. Although sorptive phenomena were active for all three fractions, there was no greater stabilization of soil organics by clay-sized than by fine-silt-sized particles. It was concluded that chemical recalcitrance was of relatively little importance in stabilizing native soil organic matter. The utilization of native organic nutrients was most closely related to the amount of readily available energy (cellobiose C) present.

From the results of the fractionation and incubation studies it was concluded that occlusion within microaggregates is the dominant mechanism of stabilization of soil OM.

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CHAPTER 1

INTRODUCTION

Microbial interconversions of organic and inorganic forms of nutrients in soils are key parts of nutrient cycles and influence plant uptake and soil quality. The rates and magnitudes of these nutrient transformations are related to biotic and abiotic factors in the soil environment. Among these factors are levels of microbial activity, chemical character of organic nutrient forms present, and soil physical qualities. Recent developments in soil particle size fractionation techniques have provided a means of obtaining different organic forms of nutrients in a chemically relatively undisturbed but physically distinct state associated with specific soil particle size fractions. The work presented in this thesis continues the search for a better understanding of the factors affecting the stability of soil organic matter.

A review of the literature (Chapter 2) showed soil organic matter is stabilized by three mechanisms: (1) chemical recalcitrance, a quality of the organic matter itself, and physical stabilization by (2) adsorption onto clay particles and (3) occlusions within soil microaggregates. Chemical recalcitrance is presumed to increase with increasing aromaticity and molecular weight of the organic matter. Clay adsorption has been assumed to be an important stabilization

process. Results of studies examining the importance of occlusion have been inconclusive.

My research involved development of soil particle size fractionation and incubation procedures and mineralization studies. The development of fractionation procedures involved expansion of a procedure developed for use on an analytical scale to one useful on a preparative scale (Chapter 3). Data were collected to evaluate the artifacts of ultrasonic dispersion of soils and quantify the chemical description of the soil particle size fractions. Information thus obtained allowed an evaluation of the relative importance of occlusion in microaggregates in the stabilization of organic matter.

The other two mechanisms of stabilization, chemical recalcitrance and adsorption on soil particles, were evaluated using an incubation technique (Chapter 4) in a mineralization study of the carbon (Chapter 5) and phosphorus (Chapter 6) associated with the fine silt, coarse clay, fine clay fractions from a aridic Argiustoll. The results of the fractionation and mineralization work are synthesized into an overall picture of the relative effectiveness of the three mechanisms of soil organic matter stabilization (Chapter 7). The potential importance of the findings and some of their implications to our understanding of soil/plant systems are discussed.

CHAPTER 2

LITERATURE REVIEW

Introduction

Our initial interest in working with the mineralization rates of fine soil separates was a better understanding of natural soil organic matter (OM). There has long been speculation regarding the importance of clay adsorption as a factor in stabilization of soil OM. D. W. Anderson et al. (1974a) developed a method using ultrasound for extraction and fractionation of soil OM. The method improved recoveries of extractable OM and indicated differences in aromaticity and molecular weight (mol wt) distributions of the alkali-extractable and ultrasonically extractable, clay-associated OM fractions. In a subsequent paper Anderson et al. (1981) found similar differences in aromaticity and mol wt distribution of the OM associated with the different particle size fractions obtained by fractionating an Argiboroll and Haploboroll from southeastern Saskatchewan using ultrasound as a dispersing agent. Other work has used various particle size fractionations to show differences in the character of the C, OM, N, etc., of particle size fractions for different soils (Anderson 1974b, Ladd et al. 1977, Travnikova and Titova 1978, Turchenek and Oades 1979, Young and Spycher 1979).

Based on information regarding the differences in the aromaticity and molecular weight of the OM associated with each particle size it

seemed appropriate to use particle size fractions as sources of OM to test the effects of clay association and aromaticity on mineralization rates. More definitive information on the effects of ultrasonic dispersion, especially as related to initial aggregate status of undisturbed soils and cations as they affect formation and stability of soil microaggregates, was necessary to understand and interpret experimental results. The clay mineralology of a soil affects the stability and character of OM (Martel and Paul 1974). A basic understanding of the probable effective mechanisms stabilizing OM against decomposition and causing occurrence of particular characteristics in particular particle size fractions is needed.

The principal mechanisms appeared to be chemical recalcitrance, physical stabilization by adsorption, and physical stabilization by occlusion/inclusion.

Chemical recalcitrance is often thought to be directly related to aromaticity (high C:H ratios). There are very few substrates that are not available to a well-nourished general soil biota, and minor relative differences in aromaticity might not in fact give measurably different mineralization rates. Since lignin is only very slowly degraded, compared with other substrates, and humic compounds have structures similar to lignin, humic compounds would be similarly recalcitrant. However, humic compounds do not have the nutrient-poor, homogeneous character of lignin and therefore such comparisons are probably of only limited usefulness. Aromaticity, then, is undoubtedly an oversimplistic and therefore less than totally reliable indicator of chemical recalcitrance. Of similar, if not equal importance, are N, P, S, and other nutrient element constituents

involved both structurally or as exchangeable or otherwise associated components of OM molecules. These elements, especially when structural components, can have important effects on kinds and distribution of groups capable of carrying a charge and thus acting as ion-exchange sites. They can also have important effects on the ability to form hydrogen bonds and OM molecular geometry. These characteristics will affect differences in kinds of adsorption reactions the OM molecules will undergo.

Physical stabilization by adsorption is a phenomenon associated essentially with the surfaces of single crystals of soil minerals, especially clays, or of soil OM molecules. That is, inorganic clays may adsorb OM; OM may adsorb other OM; OM or clay already adsorbed on some adsorbent may adsorb still other OM or clay. The "laminar" or three-dimensional buildup of OM/clay microaggregates by mutual adsorption seems a very likely event. This leads to the third principal mechanisms of stabilization, physcial stabilization by occlusion. Stabilization by adsorption is believed to change OM diffusibility and molecular geometry and thus liability to enzyme attack, while stabilization by occlusion/inclusion either eliminates or dimininshes enzyme diffusion to OM molecules so that the rate of their degradation is further reduced. It seemed important to consider as well the possibility of occlusion of OM by precipitation of secondary mineral coatings on surfaces of OM macromolecules or microaggregates. It was convenient to organize the literature obtained into six groups:

1. Clay minerals as adsorbent stabilizing agents.

2. OM, cations, oxides as stabilizing agents.

3. Enzymes and their occurrence in soil fractions (as examples of organic macromolecules and their activities associated with particle size fractions).

4. Aggregates as stabilizing agents.

5. The effects of ultrasound on microaggregates and the characteristics of particle size fractions.

6. Mineralization studies on particle size fractions.

Clay Minerals As Adsorbent Stabilizing Agents

Clays in soils preferentially adsorb certain organic compounds. Preferential adsorption has its possible corollary of preferential catalysis. Preferential adsorption also implies that adsorption reactions are probably equilibrium reactions, where the relative amounts of each species adsorbed is a function of the relative amounts in unadsorbed phases.

Wada and Inoue (1967) showed differences in the character, amount, and mineralization of humic substances from rotted clover leaves adsorbed onto two soils of different clay mineralogy that had been pretreated to destroy OM. One soil had montmorillonite, the other allophane as its principal clay mineral. The montmorillonitic soil showed an adsorption saturation with humic substances at about 0.65% C, while the allophanic soil showed no such saturation level up to 1.4% C. The authors suggested different mechanisms of adsorption for the two soils. Cation (calcium) bridging apparently is the important mechanism of adsorption in the montmorillonitic soil, while it apparently is not in the allophanic. Apparent humification (polymerization) was rapid in the first month of incubation with

essentially no changes in the next eight months. The degree of apparent humification was greater in the montmorillonitic than in the allophanic soil. There was an apparent equilibrium between 0.1 M NaOH-extractable and nonextractable C fractions. The nonextractable:extractable ratios were approximately 3:1 for the montmorillonitic soil and approximately 2:1 for the allophanic soil. The montmorillonitic soil initially adsorbed less C than the allophanic and its adsorbed C was less stable to leaching and mineralization. The authors mention that adsorption observed in this experiment would occur in very thin (millimeter) thicknesses around decomposing plant residues under field conditions.

The effects of adsorption of similar humic substances onto some allophane, montmorillonite, and kaolinite clays in the Ca and Al forms were later investigated by Inoue and Wada (1968). Allophanic and montmorillonitic minerals showed preferential adsorption of darker-colored (higher molecular wt) humic substances. The preference was more marked for allophanic minerals. Allophanic minerals were thought to adsorb humic substances initially onto anion-exchange sites with subsequent van der Waals interactions. Layer silicates, on the other hand, adsorbed by cation bridging thus giving stronger adsorption on crystal edges because of relatively high edge interlayer concentrations of clay-sorbed cations. Ca and Al clays did not differ in adsorption capacities. It was noted that the system used to test layer silicates gave results for the two polyvalent cation clays similar to those obtained for K clays, whereas in soil systems presence of polyvalent cations on sesquioxides are known to markedly increase adsorption of soil humic components.

Fulvic acids (FA) containing proteins/peptides were found to be preferentially adsorbed on external surfaces of the <0.3-μm fractions of a Wyoming bentonite in the Na form (Schnitzer et al. 1980). They concluded that the adsorption/desorption behavior of the proteinaceous material was governed by the FA that contained it. The adsorbed-FA extracts showed marked decreases in rates of decomposition compared with the FA extracts, which had not been sorbed onto clay. A role for the preferential adsorption of proteinaceous FA by montmorillonitic clays in the nitrogen enrichment of fine soil fractions was suggested.

Manos and Tsai (1980) compared adsorption by kaolin and activated carbon of a humic extract obtained from sewage sludge. They suggested that cation bridging and charge compatability are not the sole mechanisms of adsorption. Kaolin was found to selectively adsorb the high-mol wt, more colored components of the extract used.

A review by Rao et al. (1980) cites some of the work to date that has shown selective low-temperature abiotic catalysis and inhibition of organic synthesis and polymerization reactions of possible biological relevance. Clays may have similar catalytic roles in soil organic matter reactions, though the effects might be expected to be insignificant in comparison with biologically affected ones.

Organic Matter, Cations, Oxides As Stabilizing Agents

Stationary phases of high-molecular-weight organics have for some time been known to separate the components of mixtures of organic compounds because of differences in preferential adsorptions of each component--as in gas liquid chromatography. Though the organic components of soils are by no means comparable in their purity to

components used in GLC, there seems no reason to expect that soil OM molecules would not have some adsorptive affinity for other soil organics. Assuming that such adsorption occurs, it follows that some stabilization of less stable organic components of the adsorption complex might be expected.

Nelson et al. (1979) tested the stabilization of ^{14}C-labelled microbial cell wall and cytoplasmic fractions by various kinds of mixing with "model humic acid-type phenolic polymers." Among other findings, intimate mixing of cell wall components with model polymers was found to reduce decomposition by 14-28%, depending on the polymer. Decomposition of cytoplasmic components was reduced an average of 11%. Also interesting was that neither added whole cells nor cell components enhanced decomposition of native soil OM.

In an outstanding review of inorganic stabilizing agents by Duchaufour (1976), the effects of $CaCO_3$, Ca^{++}, and free Fe in stabilizing organic matter are discussed. In order of rapidity of effect and reverse order of strength of stabilization are Ca^{++}, $CaCO_3$, free Fe. The Ca^{++} probably acts as a bridge between negatively charged sites of separate organic molecules or OM and clay exchange sites. Duchaufour remarks that soils high in Ca^{++} are definitely the most complicated and difficult soil OM systems to study. $CaCO_3$ acts as a "sequestering agent." Organic matter in calcareous soils is sequestered with a coating of $CaCO_3$ and protected from decomposition until the coating is removed. Fe is effective only in acid soils, since amorphous and crystalline clay minerals, Ca^{++}, and $CaCO_3$ are much more rapid in associating with OM.

Enzymes and Their Occurrence in Soil Fractions

Evidence to date is not conclusive about selective association of specific enzymes with specific kinds of soil OM. Batistic et al. (1980) concluded that extractable enzymes occur "partly in soil as a carbohydrate-enzyme complex and partly as a humo-carbohydrate complex." However, no quantification of the kinds or amounts of enzymes in each type of complex was offered.

Nannipieri et al. (1980) found poor extraction by 0.14 M pyrophosphate of urease compared with phosphatase and casein-hydrolyzing protease, suggesting the possibility that these enzymes are associated with OM of different extractability. The proteases, enzymes highly susceptible to decomposition when in solution, are remarkably stable but often unextractable and showing reduced activity in soils. This is thought to be the effect of adsorption to soil colloids. It was concluded that sodium pyrophosphate is a good extractant of soil enzymes because it is a good extractant of soil OM.

Galstyan et al. (1968) added an enzyme preparation of known activity to a leached chernozem soil and a reclaimed saline alkaline soil. The soils were then allowed to equilibrate for 20 days with periodic additions of toluene as an antiseptic. After equilibrating for 20 days, the soils were ground and fractionated. The enzyme activities of the resulting particle size fractions were then determined. Enzyme activity was generally absent from fractions coarser than medium silt (5-10 μ). Each enzyme showed preference for particular size fractions; invertase for the 1- to 5-μ fraction, amylase for the <10-μ fractions, urease for the 5- to 10-μ fraction,

amylase for the <1-μ fractions, urease for the 5- to 10-μ fraction, and dehydrogenase in the <1-μ fraction. The authors suggest that although microbes adsorb to almost all soil particle size fractions, enzymes adsorb only to the fine fractions because of the OM and clay minerals present essentially only in the fines. It was also stated that enzymes are most strongly bound to soil particle at pHs optimum for their activities. Also, since adsorbed enzymes apparently do not lose their activities, adsorption must not involve the enzymes' active catalytic sites. Thus, some enzymes at least are probably selectively adsorbed and stabilized with retention of activity but loss of solubility and therefore ability to diffuse to substrates.

Aggregate Stabilization of OM

Most soils occur in some aggregated form. Though "good" aggregate status gives better soil aeration, water infiltration rates, etc., the effect of inclusion of soil OM within soil aggregates is often not directly considered. It was necessary to seek some understanding of the mechanisms of aggregate formation and conditions inside the aggregates, especially as these might affect enzyme/microbial degradation of OM.

Edwards and Bremner (1967b), in a paper on the effectiveness of ultrasound as a dispersing agent, proposed a theory of microaggregate (<250-μ) formation. They suggested that microaggregates are built up continuously by clay/clay, clay/OM, OM/OM adsorptions by polyvalent cation bridging. They found that almost all soils are completely dispersed when monovalent cations are substituted for polyvalent ones in soil samples. The effect is even greater if the polyvalents are

complexed or otherwise prevented from forming cation bridges. Mechanical disruption was discussed as an alternative to these ionic dispersions of soil aggregates: The greater the mechanical force, the greater the dispersion achieved.

Some mineralization data for two Saskatchewan soils were given (Edwards and Bremner 1967b). Especially noteworthy was that grinding the soils to pass a 50-μ sieve markedly increased N mineralization, while the same <50-μ ground soils when allowed to reaggregate by wetting and drying showed a marked decrease in N mineralized. This supported the idea that microaggregate formation has a stabilizing effect on organic matter in soils. Since finer-textured soils are more able to form microaggregates than coarser soils, greater N and OM accumulations occur in finer-textured soil--which seems consistent with field data.

The effects of aggregate formation on the decomposition of ^{14}C-labelled glucose and starch were investigated by Adu and Oades (1978). The substrates were thoroughly mixed into slurried soils and dried to finally yield aggregates. Starch within aggregates was protected from microbial decomposition, while the more soluble glucose apparently diffused out of the aggregates and was decomposed. Either subsequent grinding or drying to disrupt aggregates increased decomposition of previously unexposed starch.

Young and Spycher (1979) obtained water-dispersible particle size fractions from seven soils, including two inceptisols, an aridisol, a mollisol, a vertisol, an ultisol, and a spodosol. (The better structured soils gave some silt- and sand-sized water-stable aggregates composed of clay-sized particles.) On fractionation of

clay size fractions by density, light fractions were found to have high levels of C, higher C:N ratios, and lower alkali extractability of C than heavier fractions. Light fractions were found higher in amorphous minerals than the heavier fractions. They then offer a model of microaggregate structure, suggesting that the internal portions of microaggregates are shielded from the soil biosphere, while microaggregate surfaces are not so shielded.

The effects of aerobic and anaerobic incubation with water, glucose, peptone, or peptone and glucose added as substrates were studied by Skinner (1979). Dry original soil controls were run for comparison (wetted 24 hours before analysis). The incubation was at 24°C and carried out for 14 days. Soils initially contained 1- to 3-mm aggregates only. The amount of water-stable >1-mm aggregates from Ap horizons increased in all soils aerobically incubated, though the amount of increase varied among soils for different amendments. Anaerobic incubation gave more varied results, even though general increases in stability occurred. Up to 93.7% of the dry control soils disintegrated into aggregates <1 mm in diameter on contact with water. However, moist incubation for even one hour markedly increased stability, even if soils were previously sterilized. Soils sterilized by gamma radiation showed trends to increase in aggregate stability during incubation, while autoclaved soils behaved in a less predictable fashion. The role of polysaccharides as the cementing agents stabilizing aggregates was discussed. Perhaps the most important point to be drawn from the paper was the speed with which either biotic or abiotic factors can stabilize soil aggregates. Formation and stabilization of aggregates in times as short as one

hour support the possibility of entrapment of biomass, expecially microbial cells and residues, inside soil aggregates.

Smith (1980) developed a model to offer predictions of "extent of anerobic zones in aggregated soils." Variations in gaseous diffusion coefficients, respiration rates, and aggregate size are checked for effect on proportion of soil volume expected to be anaerobic. Model results indicated that gaseous diffusion coefficients were more important controlling factors than aggregate size. However, aggregates are apparently assumed to be of uniform mineral/OM composition regardless of size.

Effects of Ultrasound on Microaggregates and the Characteristics of Particle Size Fractions

Edwards and Bremner (1967a) endorsed the use of ultrasonic vibrations as a dispersing agent for mechanical analysis of soil particle size. After six years of use in their work, they found it to achieve as good a dispersion of <2-μ particles as the peroxide/calgon treatment, with the added benefit of much decreased effects on other soil qualities. Compared with chemical treatments, sonication gave suspensions with less tendency to floculate, dissolution of only trace amounts of organic or inorganic material, and no significant changes in pH or conductivity (compared with suspension of untreated soil). They recommend sonication as the method of choice in research dealing with "studies of the mechanisms of soil aggregation and dispersion and . . . on the nature and properties of soil minerals and of clay-organic matter complexes in soils."

Significant dissolution of Fe, Al, Si, and C occurred during ultrasonic dispersion of five gleysols from British Columbia (Hinds

and Lowe 1980a). Fe and Al dissolution were inversely related to C dissolution. These effects were greatly decreased by sonicating in 0.01 M $CaCl_2$ or $BaCl_2$ or 0.1 M NaCl aqueous solutions instead of H_2O. However, these soils may have been of a poor base status before sonication. The soils often showed less readily exchangeable cations (K, Ca, and/or Mg) in the sonicated than in untreated soil suspensions. The authors concluded that for maximum recoveries with minimum alteration, 0.01 M $CaCl_2$ solution should be used to suspend the soils. This may hold for similar soils, but it seems likely that the role of the $CaCl_2$ here is to saturate the soil with Ca with some reaggregation and floculation giving reduced dissolution. In soils with adequate Ca before treatment, such effects apparently have not been reported.

Anderson et al. (1981) fractionated a cultivated Argiboroll and a cultivated Haploboroll from Saskatchewan using ultrasonic dispersion. They found that 55-58% of the organic C was in the clay fraction and C:N ratios decrease with particle size. The fine silt and coarse clay fractions had higher humic acid contents with less extractable C or hydrolyzable N than the fine clay, where fulvic acids were dominant. He suggested use of size fractions as sources of native OM of different degrees of recalcitrance.

Broersma and Lavkulich (1980) found the weight of OM per unit of surface area generally increased with increasing particle size for some ultrasonically dispersed Vancouver Island Brunisols and Podzols. The sand, coarse silt, and fine silt were found to contain 5, 12, and 49%, respectively, of the total soil OM.

In another note, Hinds and Lowe (1980b) related data from five Gleysolic soils from British Columbia. C, N, S, and organic P (P_o) content of the fractions generally increased with decreasing particle size. C:N and C:S ratios decreased with decreasing particle size. In all except one soil (a saline humic Gleysol), C:P_o ratios were smaller in the clay fractions.

Some questions remain concerning how ultrasound causes dispersion of soil particles. Edwards' and Bremner's (1967a,b) suggestions regarding the importance cation bridge linkages and the disruption of these in soil dispersion seem reasonable. However, there is the possibility that desorption and resorption of OM and other processes may be occurring during ultrasonic treatments, as the data of Hinds and Lowe (1980a) could be indicating. Also, for all the disruptive power ultrasound has (used to prepare emulsions to 0.01 μ), it apparently does not achieve quantitative dispersion of soil aggregates/particles <20 μm ESD with common power settings and treatment times. It appears nevertheless to be the best option available at this time--far better than chemical methods.

Mineralization Studies on Particle Size Fractions

Mineralization studies by Ladd et al. (1977a,b) on soil particle size fractions obtained by fractionation with ultrasonic dispersion of a calcareous sandy and a calcareous clay soil into which ^{15}N-labelled NO_3 had been immobilized during 160-day incubations. Wheat straw and glucose were used as C amendments. Mineralization of organic ^{15}N was more rapid in sandy than in clay soil. Glucose as a C source amendment gave more rapid mineralization than straw. Wet/dry cycles

during incubation increased mineralization of native and labelled organic N. Newly immobilized (labelled) organic N was consistently more available than native organic N. Ratios of availability of labeled and unlabeled N did not vary with soil type but did with C amendments, wet/dry cycles, and incubation period.

In straw-amended soils a low-density fraction (<1.59 g/cm^3) and the fine-clay fraction showed the more marked decreases in organic ^{15}N. In glucose-amended soils the main decreases were in the silt and fine-clay fractions.

Chloroform treatment to release biomass organic N in the same soils used for the above-mentioned mineralization indicated that, on incubation, significant amounts of microbial biomass are probably associated with the light fractions from straw-amended soils and the silt fraction of all soils. In both sandy and clay soils, lighter fractions were more important in the silt-sized fraction. However, the yield of light fraction (nitrogen) was increased in the clay soil when the whole soil was sonicated and fractionated before the density separations. This was not true for the sandy soil. This series of papers (Ladd et al. 1977a,b; Amato and Ladd 1980) was informative but only indirectly so with respect to mineralization rate differences for the particular fraction sizes, since incubations were done on whole soils subsequently fractionated for analysis, not on particular fractions separately.

Cameron and Posner (1979) did mineralization studies on particle size fractions separately. However, their fractionation used only extended shaking in water to disperse soil aggregates. Electron micrographs indicated that the coarse clay and silt fractions were

free of undispersed aggregates. Subsequent sonication of the fractions to allow separation of a light fraction (<2.00 g/cm^3) yielded only small amounts of such material from the coarse silt and none from the finer fractions. The relative ease of dispersion of their kaolinitic soil probably should not be expected and apparently has not been found for calcareous, montmorilonitic soils.

Although the fine silt and coarse clay fractions were richest, the OM was fairly evenly distributed among the size fractions. Cameron and Posner suggested that this enrichment may have been because most microbes would sediment with these fractions during size fractionations.

Mineralizable N varied inversely with particle size. Aerobic incubation yielded almost pure NO_3, while anaerobic almost 100% NH_4^+-N. High C:N materials yielded more mineralized N under anaerobic than under aerobic incubation. Such differences decreased as C:N decreased, until in the fine clays there was no difference between aerobic and anaerobic incubations.

Cameron and Posner (1979) noted that anaerobically mineralizable N increased above 210-μ particle size. In similar, earlier work by Chichester (1969), such an increase was encountered at 20-μ particle size. Chichester, however, ultrasonically dispersed his soils before fractionation. Edwards and Bremner (1967b) cited 250 μ as the upper boundary for definition of microaggregates. If the relationship between high C:N ratios and higher anaerobic mineralization holds, then some of the >210-μ, low-density fractions might be high C:N OM particles and aggregates whose contents on sonication are shifted to the fine (<2-μ) fractions.

No literature has yet been encountered for P mineralization in particle size fractions. However, one study that has some usefulness here is that of Hedley, Stewart, and Chauhan (1982), which used a sequential fractionation designed to remove in order (1) biologically available inorganic P (P_i), (2) labile P_i and organic P (P_o) associated with soil surfaces (and a small amount of biomass), (3) P_i and P_o in biomass, (4) P_i and P_o chemisorbed to Fe and Al on soil surfaces and similarly in microbial cell debris, (5) labile P_i and P_o internal to soil aggregates, more stable (6) P_i (apatites) and (7) P_o.

Comparison of a soil under wheat-fallow cropping and permanent pasture showed that cropping diminished more of the organic P forms (75% of total P lost) than the inorganic. The organic P most affected was the more stable P_o (7). Though a sonication treatment was used to extract (5) labile P_i and P_o internal to soil aggregates, it was a mild treatment, and probably (6) the more stable P_o, reflects the organic P one might expect to be stabilized in microaggregates.

Periodic mixing during a 9-month incubation of two other soils (one P rich, one P poor) with no C amendment caused immobilization, especially of biologically available P mostly into (4), the chemisorbed P_i and P_o, and (7), the more stable P_o. Use of cellulose as a C amendment when adequate levels of P_i were present maintained the level of (5), P internal to soil aggregates, but decreased it when the C amendment was made and inadequate P_i was present. The indication was that the material (5) internal to soil aggregates may act as a C source or a P source.

The incubation with cellulose added increased the more labile forms of P. This was thought to have occurred because of the development of mostly fungal biomass with this treatment.

Microbial biomass-P levels were related to soil solution P_i levels. The soil with higher available P gave higher microbial biomass P and immobilization. Apparently, when available-P levels are adequate, immobilization is expected; however, when available P is inadequate, reutilization and recycling of the more labile P forms will occur.

CHAPTER 3

FRACTIONATION STUDIES

Introduction

The fractionation studies had three principal objectives:

1. To develop a soil particle size fractionation procedure of a preparative scale that did not require harsh chemical treatments for soil dispersion.
2. To prepare soil particle size fractions of qualities and in quantities appropriate for mineralization studies.
3. To provide information on the artifacts of such fractionation methods to improve interpretation of fractionation and mineralization study results.

This chapter, The Fractionation Studies, is in two sections: materials and methods, and results and discussion. The materials and methods section discusses the development of, and differences between, the fractionation procedures used. Three soil fractionations were carried out. The first allowed evaluation of the basic fractionation procedure and preparation of fractions for preliminary incubation studies. The second was principally a developmental modification of the first to improve volume and time efficiencies. The second fractionation also increased the resolution by separating the soil into fractions dispersed from larger equivalent spherical diameter (ESD), >50 μm, and smaller ESD, <50 μm, water-stable aggregates. This separation was accomplished by ultrasonically dispersing the larger

(>50-μm ESD) water-stable aggregates after they had been separated from the smaller by sedimentation. The third fractionation was designed specifically to prepare the fine silt (ESD 2 to 5 μm), coarse (ESD 0.2 to 2 μm) and fine (ESD <0.2 μm) clay fractions for mineralization studies. The third fractionation was executed with and without ultrasonic dispersion to allow evaluation of differences between water-dispersible soil fractions and those affected by water-stable aggregates.

Artifacts of ultrasonic soil dispersion and the role of microaggregates in soil organic matter stabilization are emphasized in the results and discussion section. The fractionation procedures, especially the second and third, provided fractions associated with specific structural components of the soils used. The composition of the fractions and certain qualities of suspensions of these fractions indicated the importance of occlusion in microaggregates in stabilizing OM and of an artifact of ultrasonic dispersion. Occlusion in microaggregates renders occluded substrates inaccessible to non-occluded microorganisms and limits oxygen availability to any occluded microroganisms, restricting their (decomposing) activites. The most important artifact of ultrasonic dispersion of soils is an apparent redistribution of organic material among the fine particle size fractions.

The Soils

Two soils, a Typic and an Aridic Argiustoll, were fractionated. Both soils were taken from sites not believed to have ever been cultivated. The Typic Argiustoll was of the Harney series from

eastern Kansas and used in the first and second fractionations. The Aridic Argiustoll was of the Ascalon series from northeastern Colorado and used in the third fractionation. Both soils were air dried. The Harney soil was ground to pass a 2-mm sieve. The Ascalon soil sample was not ground, since it readily broke into fine crumbs during sampling.

Equipment

Only nonstandard laboratory equipment used in the fractionations is mentioned here. Each fractionation, as a new step in a developmental process, used a different piece of equipment than its predecessors. The first fractionation used no unusual laboratory equipment.

A Heat Systems-Ultrasonics W-375 Sonicator was used to carry out ultrasonic dispersions for all three fractionations. The sonicator was equipped with a high-gain Q horn, with a 3/4-inch tip. During the second and third fractionations, the sonicator was calibrated using an unpublished procedure developed by L. Turnquist.

Three different centrifuges were used. An IEC Model K Size 2 centrifuge was used in the first fractionation. During the second fractionation, an IEC CRU-5000, with a greater volume capacity than the Model K was used. The third fractionation used a Sorvall SS-1A high-speed angle centrifuge equipped with a Szent-Gyorgyi-and-Blum-type continuous flow system (Sorvall KSB-1 system), which further increased volume efficiencies.

Analyses

Total N and total P were determined colorimetrically on digests obtained from a $K_2S_2O_8/H_2SO_4$ wet combustion of each fraction obtained from all three fractionations and the whole soils. Carbon was determined using the method of Nelson and Sommers (1975).

A greater depth of information on the composition of the fine silt and coarse and fine clay fractions from the third fractionation was sought. Organic and inorganic P was determined by the method described by Saunders and Williams (1955). The NH_4HCO_3-DTPA-extractable iron, manganese, copper, and zinc contents were determined by the CSU Soil Testing Laboratory.

Stoke's Law: Particle Sizes and Equivalent Spherical Diameter

Soil sedimentations based on Stoke's Law provide no direct information on size, shape, quality, or physical associations of different types of soil particles in the same size fraction. This is especially true for organic residues and particulate OM in fractions composed mainly of mineral particles. Soil organic materials vary in shape and density and as a group differ in these characteristics from soil mineral particles. The following is a brief discussion of how these differences limit the information obtainable from application of Stoke's Law in soil sedimentations.

Stoke's Law relates the velocity of fall (V) of a spherical particle to that particle's density (d_p) and diameter (radius $\underline{r}$), the density (d_m), and viscosity (μ) of the suspending medium, and the force (g) causing the particle's fall:

$$V = \frac{2}{9} \frac{(d_p - d_m)gr^2}{\mu} \quad .$$

The application of Stoke's law in sedimentations to separate particles solely on the basis of size (radius r) requires that all particles be spherical and that d_p, d_m, μ, and g be known. In practice, d_m, μ, and g are known or controlable, but for soils, d_p is not. Furthermore, soil particles are not spherical. Soil sedimentations, therefore, do not separate soil particles solely on the basis of size but on the basis of equivalent spherical diameter (ESD).

A particle has a given ESD when its shape, size, and density are related in such a way that it sediments at the same rate as a spherical particle of specified size and density. As a simple example, consider the following case in which shape has no effect on ESD. A mixture of spherical particles of different densities and sizes are separated by sedimentation based on Stoke's Law. The sedimentation procedure controls d_m, μ, and g. Under these conditions, $V \propto d_p r^2$, and all particles for which $d_p r^2$ has the same value will fall with the same velocity, sediment together, and have the same ESD. The ESD in this case can be defined by ESD = $d_p r^2$.

The ESD of soil particles cannot be so precisely defined. Densities and shapes vary greatly among soil particles depending on their composition and structure. Individual mineral crystals with relatively high densities but open or flat shapes (which cause them to flutter as they fall) may sediment at the same rate as a piece of fungal hyphae of much lower density but whose long, narrow shape allows it to become oriented in the direction of fall. Within a

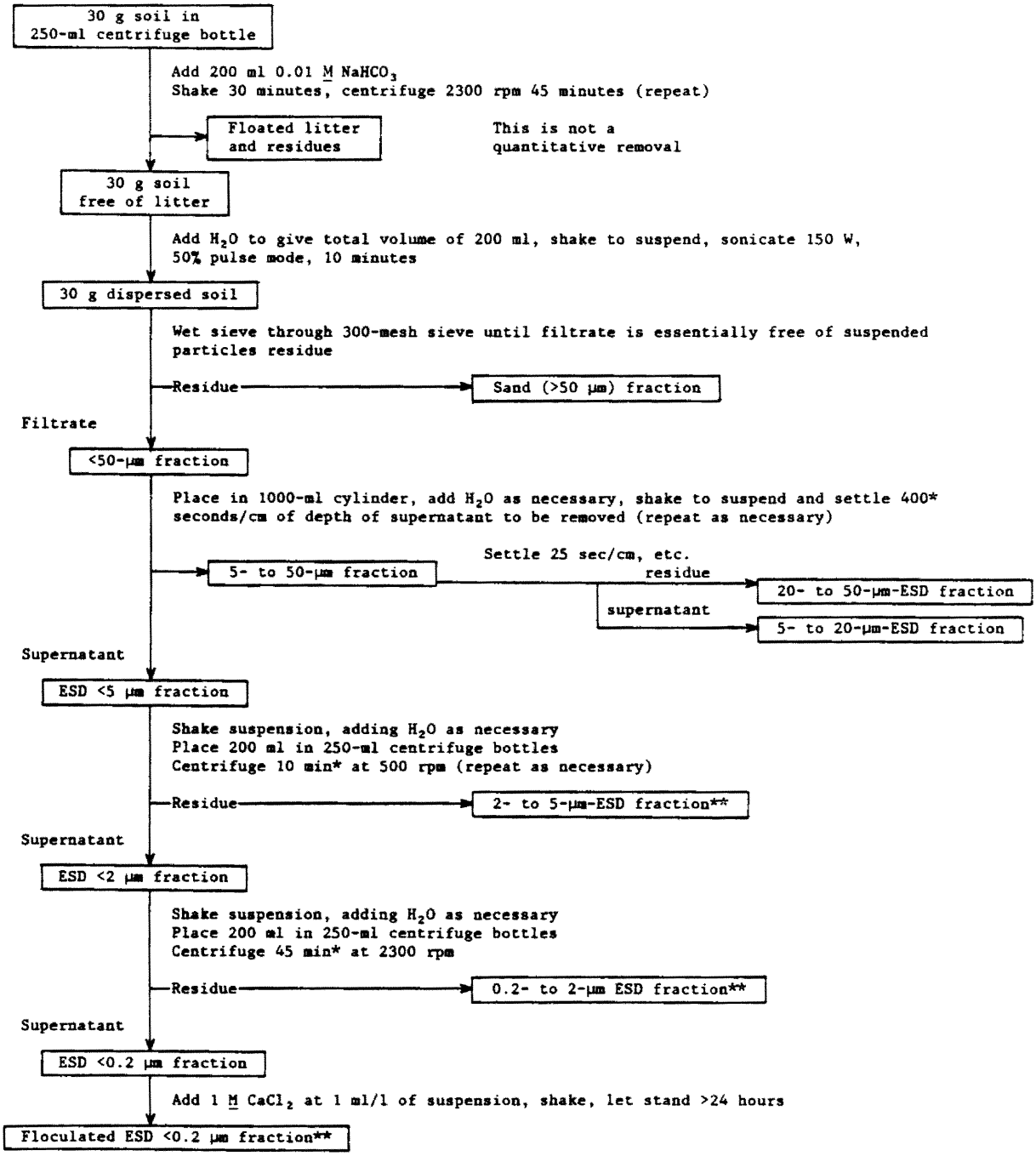
sedimented soil fraction, the particles' sizes, shapes, and densities vary widely. Stoke's Law tells us only that they are related by similar equivalent spherical diameters--i.e., sedimentation rates--and provides only an extremely coarse determination of the actual physical size of sedimented soil particles.

In this research, soil sedimentations based on Stoke's Law were used as a practical means of consistently fractionating soils. The desired particle size fractions were then examined and used. All fractionations were based on an assumed (mineral) particle density of 2.65 g/cm^3. For gravity sedimentation, settling times were taken from a nomograph of particle-settling time under gravity (Tanner and Jackson 1947). For centrifugations, settling times were calculated from Stoke's Law.

The First Fractionation Procedure

The first fractionation procedure was a direct modification of that of D. W. Anderson (personal communication, based on Ph.D. dissertation, 1972). The only major modification was increasing sample size from 20 g to 30 g. A flow diagram of the procedure is given in Fig. 3.1. No attempt was made to verify the function of the sonicator. It was later found to be malfunctioning and thus not dispersing the soil to the extent expected during the first fractionation. The fractions were freeze dried by placing them in a refrigerator freezer overnight and holding them under a vacuum in a desiccator jar until dry.

Fig. 3.1. Flow diagram of the first fractionation



*Times given are estimates. Each time was corrected for variations in temperature.

**These fractions transferred and concentrated into minimum volumes in 50-ml centrifuge tubes and frozen for drying.

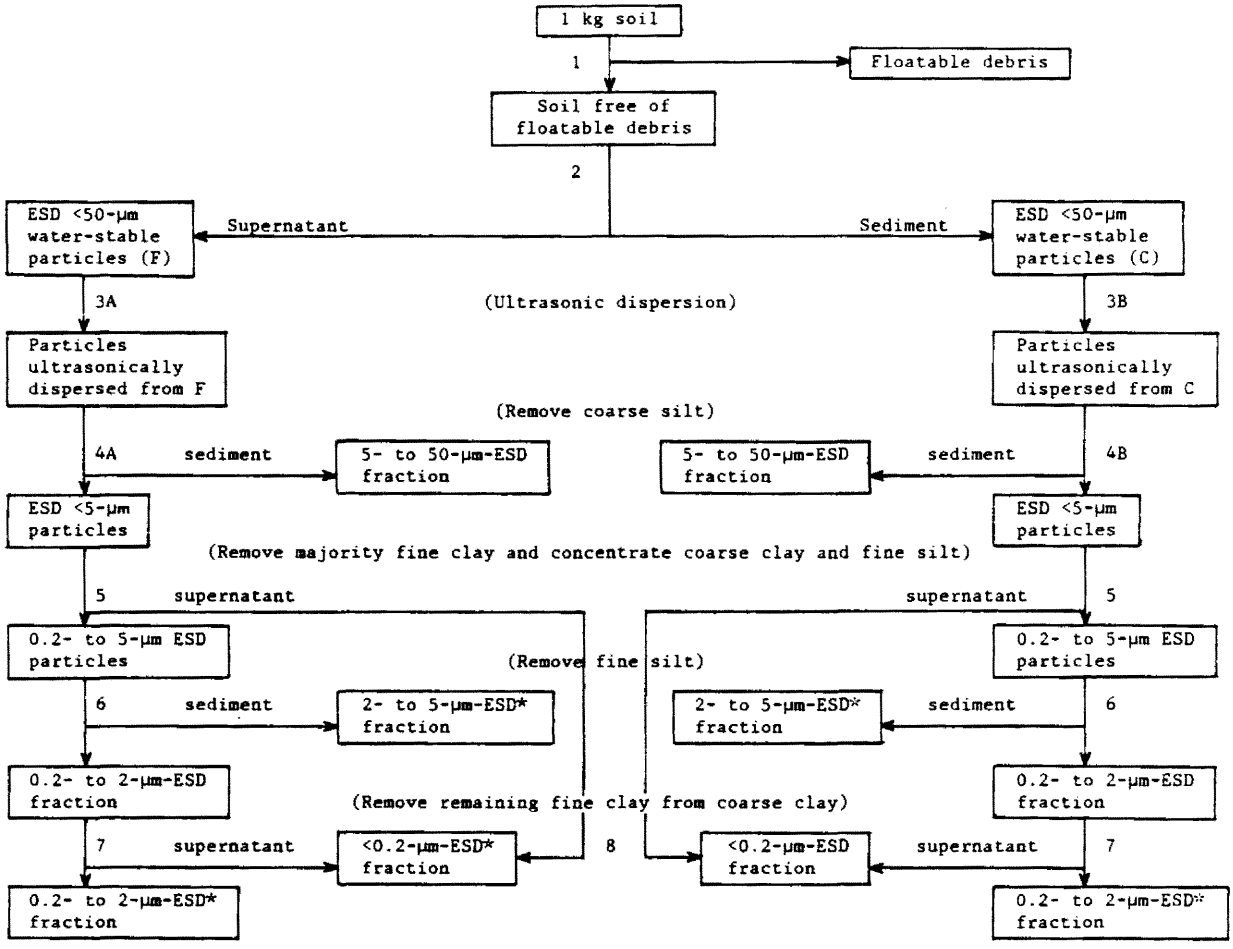
The Second Fractionation Procedure

The procedure used in the second fractionation is given in Fig. 3.2. The procedure allowed differentiation between larger (ESD >50 μm) and smaller (ESD <50 μm) water-stable aggregates on the basis of quantity and composition of the fractions ultrasonically dispersed from each aggregate size range. This differentiation was achieved by separating the >50-μm-ESD particles from the <50-μm-ESD particles before ultrasonic dispersion. Volume and time efficiencies were improved by changing the order of separation of the <5-μm-ESD particle size fractions. The fully separated fractions were freeze dried by shell freezing suspensions in a dry ice/ethanol bath and connecting to a freeze drier, which maintained a vacuum and a -60°C cooling coil temperature. Freeze drying in this manner prevented an unacceptable amount of reaggregation of separated soil particles, which occurred during freeze drying in the first fractionation. More detailed information on procedural differences can be obtained by comparing the flow diagrams of the three fractionations.

The Third Fractionation Procedure

The third fractionation was specifically designed to prepare approximately 100 g of each fraction of ESD <5 μm and to examine the differences between aggregate-affected and water-dispersible soil materials in these fractions. All dispersed particles of ESD >5 μm were separated and discarded. Ultrasonic dispersion was applied in the preparative fractionation, causing dispersion of water-stable and water-dispersible soil materials alike. A parallel fractionation used

Fig. 3.2. Flow diagram of the second fractionation



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[1]Float off debris in 5-l bucket (~2:1 H_2O:soil suspension)

[2]Suspension from 1 introduced on top of, and settled through, 110-cm (~16-l) column of distilled H_2O.

[3](A) Sonicate suspension from 2 in 2-l batches for 15 minutes at 120 w with auxiliary stirring.
(B) Sonicate sediment from 2 for 30 minutes at ~120 w with auxiliary stirring.

[4](A) Settle 400 sec/cm in 100-cm (~15-l) column of H_2O, resuspend and repeat, collecting all supernatants.
(B) As (A) except in 50-cm (~7-l) column.

[5]Centrifuge supernatant from 4 (in portions, removing supernatants from accumulating sediments) in 1-l centrifuge bottles for 45 minutes at 2300 rpm until 0.2- to 5-µm-ESD particles concentrate in 3 l, then resuspend in distilled water and sediment three times to wash, collecting all supernatants.

[6]As 5 except supernatant from 5 for 10 minutes at 500 rpm until 2- to 5-µm-ESD particles concentrate in 1 l.

[7]As 6 except supernatant from 6 until 0.2- to 5-µm-ESD particles concentrate in 1 l and wash four times.

[8]Combine supernatants (containing fine clay, ESD <0.2-µm particles) from 5 and 7, floculate by adding 1 ml 1 M $CaCl_2$ per liter of suspension and letting stand >24 hours, collect floculate.

*Subsequently freeze dried, using procedure described in text.

General Notes: Settling rates are approximate; actual rates must be determined for the actual temperature of the sedimentation.

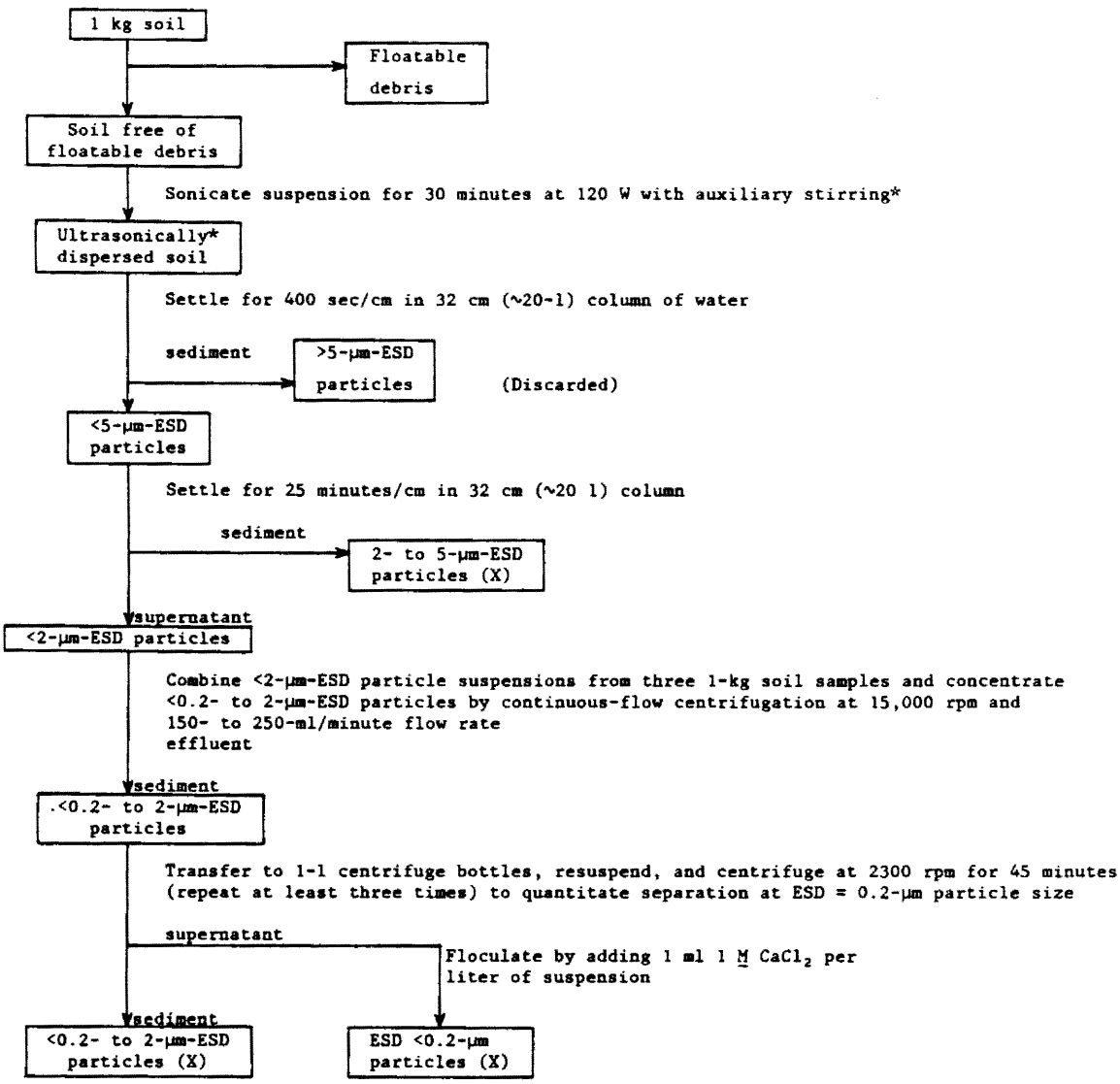
for dispersion only the mechanical agitation resulting from manipulating the soil suspensions. The particles dispersed in the parallel fractionation were those unaffected by waterstable soil aggregates and are referred to in this text as water dispersible. Time and volume efficiencies were improved by use of continuous-flow centrifugation and larger batch sedimentations than those used in the preceding two fractionations. The fully separated fractions were freeze dried as in the second fractionation. A detailed flow diagram of this procedure is given in Fig. 3.3. A comparative summary of the three fractionations is given in Table 3.1.

Occlusion in Microaggregates--Anaerobic Microsites

The evidence indicating the effectiveness of occlusion in microaggregates was both qualitative and quantitative. The qualitative evidence involved observation of differences in behavior and quality among certain sediments and suspensions during the fractionation procedures and microscopic observations of the fractions obtained. The quantitative evidence was from yield and chemical composition data of the three fractionations.

Ultrasonic dispersion of the larger (ESD >50 μm) water-stable aggregates (see second fractionation procedure) released a strong, distinctly sewerlike odor remarkably similar to that of a saturated histosol. The smaller aggregates (ESD <50 μm), on ultrasonic dispersion, developed the "earthy" odor normally associated with upland soils. Both the larger and smaller water-stable aggregates had been in water for the same length of time. That length of time (<2

Fig. 3.3. Flow diagram of the third fractionation



*This step was omitted in the parallel fractionation and ultrasonically; therefore, should read "water."

X subsequently freeze dried.

Note: Settling rates are approximate; actual rates must be determined for the temperature of each sedimentation.

Table 3.1. Comparative summary of the three fractionation procedures applied to two soils

	Harney			Ascalon	
	Fractionation				
	1	2a	2b	3a	3b
Principal dispersion technique	Ultrasonic (low power)	←	Ultrasonic (high power)	→	Minimal mechanical
Applied to	Whole soil	ESD <50 μm, water-stable soil materials	ESD >50 μm, water-stable soil materials	Whole soil	Whole soil
Fine soil particles collected	All water-dispersible and some in water-stable aggregates	All water-dispersible and most in smaller (ESD <50μm) water-stable aggregates	Most in the larger (ESD >50 μm) water-stable aggregates; essentially no water-dispersible	All water-dispersible and most in water-stable aggregates	All water-dispersible but none in water-stable aggregates
Comparisons enabled	Low to high levels of ultrasonic power (fractionation 1 vs fractionation 2)	Water-dispersible and the smaller water-stable aggregates to the larger water-stable aggregates		Directly: Fine fractions from whole soil to water-dispersible fine fractions. Indirectly: Aggregated fine fractions to water-dispersible fine fractions (by the difference 3a - 3b)	

hours) was not adequate to allow development of sufficient anaerobiosis to explain the odor observed from the larger aggregates.

Differences in the behavior of the accumulated supernatants and the fine clays they contained indicated organic matter and possibly mineralogical differences between the larger (ESD >50 μm) and smaller (ESD <50 μm) water-stable microaggretates. The suspension of fine clay from the <50-μm-ESD particles required a $CaCl_2$ concentration approximately double that required by the fine clay from the <50-μm ESD particles to achieve the same apparent degree of floculation. The supernatant left after floculation of the fine clays obtained from the <50-μm ESD particles showed no turbidity but was relatively strongly yellow colored. The same supernatant (same amount of added $CaCl_2$) for the fine clays from >50-μm ESD particles was slightly turbid but nearly colorless. After the floculation of the fine clays, the odors that appeared following dispersion of the two aggregate size groups were now found to be associated with the fine clay fraction from each group. There were also differences in the appearance of the two freeze-dried fine-clay fractions. The one from the <50-μm ESD aggregates was lighter in color and more crystalline in appearance than that from the >50-μm-ESD aggregates.

Three distinctly colored bands, presumed to represent iron minerals of different oxidation states, were observed in sedimented fractions. Two of these bands were observed in all fractionations, while a third appeared only during certain fractionations. One always occurred in the coarse silt fraction and was a layer of dense, black, crystalline-appearing particles that were attracted to magnets. These particles were presumed to be magnetite-like mineral particles. The

second band was distinctly orange-rust colored and always was observed beneath the sedimented coarse clay. These particles were presumed to be hematite-like minerals. The third colored band, when observed, was atop the sedimented coarse clay fraction and of a light blue-gray color, similar to that of mottles in gleyed soils. It was presumed to be relatively reduced iron/manganese minerals. The third band appeared only when larger water-stable aggregates were dispersed with adequately intense ultrasonic vibration (see fractionations 2b and 3a in Table 3.1) and never when less effective dispersions were used (Table 3.1, fractionations 1 and 3b), nor when larger water-stable aggregates had been removed (Table 3.1, fractionation 2a).

Presentation of quantitative evidence begins with the yield data of the fractionations. During the first fractionation, sonicator malfunctions caused poor dispersion and lower than expected yields of fine fractions (Table 3.2). This indicated that important quantities of fine soil particles, especially clays, are in stable aggregates.

The yields of the second and third fractionations confirmed the presence of important quantities of clays in water-stable aggregates (Tables 3.3 and 3.4). In the Harney soil, 52% of the fine clay, 38% of the coarse clay, and perhaps 20% of the fine silt were in the larger (>50-μm ESD) water-stable aggregates. If, as a conservatively high estimate, 50% of the >50-μm ESD waterstable soil components were water-stable aggregates before dispersion, then by mass those aggregates would be 37% clay. This shows that the clays are almost three times more important in aggretates than in the bulk soil. In the Ascalon soil, 54% of the fine clay, 71% of the coarse clay, and 22% of the fine silt are in aggregates not dispersed by mild agitation

Table 3.2. Yields of particle size fractions from the first fractionation of the Harney soil

Fraction	Yield (g)	Yield (% of soil)
Fine clay[1] (ESD <0.2 μm)	4.1	2.0
Coarse clay[1] (ESD 0.2-2 μm)	14.8	7.0
Fine silt[1] (ESD 2-5 μm)	12.1	5.5
Medium silt[2] (ESD 5-20 μm)	50	24
Coarse silt[2] (ESD 20-50 μm)	100	48
Sand[2] (ESD >50 μm)	40	19
Total	221	105.5

[1]Actual dry weight fraction.

[2]Estimated weight by hydrometer-measured density of suspension.

in water. Thus, the clays must be important components of water-stable aggregates. As a result of this concentration of clays, the aggregates must offer a microenvironment distinctly different from that of the bulk soil. The chemical character of aggregated soil particles should be particularly subject to the influence of the aggregate-defined microenvironment.

The ability of aggregates to affect the chemical character of aggregated particles was shown by the chemical characterization of the fractions obtained during the second and third fractionations. The chemical characterizations of the fractions obtained from the larger (ESD >50 μm) and smaller (ESD <50 μm) water-stable aggregates and water-dispersible particles are given in Table 3.5. The results show that in the Harney soil the fractions in the larger water-stable aggregates have markedly higher C and N contents than those in the smaller aggregates and water-disperisble aggregates. The aggregates

Table 3.3. Yields of particle size fractions from the second fractionation of the Harney soil

Fraction	>50-μm ESD water-stable soil components	<50-μm ESD water-stable soil components and water-dispersible* particles	Total fraction mass (Σ >50-μm ESD and <50-μm ESD results)[1]
Fine clay (ESD <0.2 μm)	28.6	26.5	55.1
Coarse clay (ESD 0.2-2 μm)	30.5	50.6	81.1
Fine silt (ESD 2-5 μm)	9.9	39.0	48.9
Coarse silt (ESD 20-50 μm)	48.9	529.0	578.0
Sand (ESD >50 μm)	202.0	--	202.0
Floated residue	--	--	8.0
Total	331.9	645.1	973.1[1]

[1]Total recovery (grams) from 1000 g of whole soil.

Table 3.4. Effects of dispersion techniques on yields of particle size fractions from the Ascalon soil (third fractionation)

Fraction	Yield (g) from 1000 g whole soil		
	Dispersed with		
	Ultrasonic vibration	Mild manual agitation	Aggregate affected[1]
Fine clay (ESD <0.2 μm)	14	6.5	7.5
Coarse clay (ESD 0.2-2 μm)	42	12	30
Fine silt (ESD 2-5 μm)	18	14	4
ESD >5 μm	926	967.5	41.5

[1]Calculated as difference between ultrasonically and easily water-dispersible particles:

$$\text{All dispersible particles} - \text{easily dispersible particles} = \text{aggregated particles}$$

in the Ascalon soil have the same effect on the carbon content of the aggregated coarse clay and the carbon and nitrogen contents of the fine-silt particles relative to the same water-dispersible fractions (Table 3.6). (The anomalous behavior, lower C content, of the fine-clay fraction is an artifact that is discussed later.) These results also suggest that stable aggregates are richer in carbon, nitrogen, and phosphorus than soil not in stable aggregates.

Determination of the NH_4HCO_3-DTPA-extractable elements in the fine Ascalon fractions showed that aggregation affects other elements besides C, N, and P (Table 3.7). The fine silt and coarse clay fractions from water-stable aggregates had higher Fe and Mn and lower Zn and Cu contents than the same water-dispersible fractions. Iron

Table 3.5. Effects of size of water-stable aggregates on the carbon, nitrogen, and phosphorus contents of aggregated fine particles in the Harney soil

Fraction	C:N:P contents (mg/g)	C:N ratio	C:P ratio	Enrichment ratios		
				C	N	P_T
Whole soil	25:2.1:0.47	11.9	53			
Aggregates of ESD >50 μm						
Fine silt	>70:6.6:0.80	10.6	88	2.8	3.1	1.7
Coarse clay	65:7.8:1.3	8.3	50	2.6	3.7	2.8
Fine clay	33:4.0:0.88	8.3	38	1.3	1.9	1.9
Aggregates of ESD <50 μm						
Fine silt	39:4.7;0.97	8.3	40	1.6	2.2	2.1
Coarse clay	42:5.1:1.2	8.2	35	1.7	2.4	2.6
Fine clay	24:3.4;0.82	7.1	29	0.96	1.6	1.7

Table 3.6. Effects of water-stable aggregates on the carbon, nitrogen, and phosphorus contents of the aggregated fine particles in the Ascalon soil

Fraction	C and P contents			Ratios			Enrichment ratios		
	C (mg/g)	N (ppm)	P_T (ppm)	C:N	$C:P_t$	$C:P_o$[1]	C	P_t	N
Ultrasonically dispersible									
Fine clay	23	2900	1250	7.9	18.4	35	2.1	4.6	3.7
Coarse clay	34	4000	1180	8.5	28.8	44	3.3	4.3	5.2
Fine silt	44	4300	960	10.2	45.8	70	4.4	3.6	5.5
Water dispersible									
Fine clay	27	3600	1230	7.5	22.0	41	2.8	4.6	4.6
Coarse clay	30	4000	1080	7.5	27.8	39	3.0	4.0	5.2
Fine silt	32	3400	870	9.4	36.8	53	3.2	3.2	4.4
Aggregate affected[2]									
Fine clay	20	2300	1270	8.7	15.7	31	1.4	4.1	2.9
Coarse clay	36	4000	1220	9.0	29.5	46	3.3	4.3	5.2
Fine silt	86	7500	1280	11.5	67.2	116	8.5	4.8	9.6
Whole soil	10	780	270	12.8	37.0	83	--	--	--

[1] P_o data are not presented in this text.

[2] Calculated using fraction masses (yields) from Table 3.4:

$$\frac{\text{(Ultrasonically dispersible fraction mass} \times \text{content)} - \text{(water-dispersible fraction mass} \times \text{content)}}{\text{aggregate-affected fraction mass}} = \text{aggregate-affected fraction content}$$

Table 3.7. (a) Effects of aggregation on NH_4HCO_3-DTPA-extractable elements in fine soil particle size fractions from the Ascalon soil

	Content of NH_4HCO_3-DTPA-extractable elements (ppm)				
Fraction	Zn	Cu	Fe	Mn	Ni
Ultrasonically dispersible					
Fine clay	18	17	470	36	13
Coarse clay	13	22	400	110	23
Fine silt	11	22	330	410	15
Water dispersible					
Fine clay	54	38	480	60	10
Coarse clay	36	45	280	85	9.7
Fine silt	14	42	200	32	2.9

(b) Enrichment (in extractable elements) of fractions from water-stable aggregates relative to water-dispersible fractions[1]

	Content of NH_4HCO_3-DTPA-extractable elements (ppm)				
Fraction	Zn	Cu	Fe	Mn	Ni
Fine clay	0.0	0.0	0.9	0.25	1.6
Coarse clay	0.1	0.3	1.6	1.4	2.9
Fine silt	0.04	0.0	4.0	53.0	35.0

[1]Calculated using fraction masses (Table 3.4) and the following relationship:

$$\text{Mass fraction in whole soil} = \text{mass fraction in water-soluble aggregates} + \text{mass fraction in water-dispersible fraction}$$

and Mn are two elements whose solubility in soils is often affected by redox potential, while Zn and Cu are not (Lindsay 1979). Iron and Mn solubility increase as the chemical environment becomes more reduced. (The reason for this anomalous behavior of the fine clay in Table 3.7 will become apparent later in this chapter.)

The existence of anaerobic microsites inside water-stable microaggregates of ESD >50 μm was suggested by five pieces of information. When ultrasonically dispersed >50-μm ESD water-stable aggregates released a distinct odor of anaerobiosis, which those of ESD <50 μm did not. When the larger water-stable aggretates (ESD >50 μm) were adequately dispersed (ultrasonically), a colored band of sediment, presumed to be reduced mineral forms of Fe and/or Mn, appeared, while no such band appeared from dispersion of the smaller aggregates or inadequate dispersion of any size aggregate. The Ascalon soil had NH_4HCO_3-DTPA-extractable bands of Fe and Mn that were higher for the fine silt and coarse clay fractions from water-stable aggregates than from the same water-dispersible fractions. The difference in color and behavior of the accumulated supernatants and fine clays from separate fractionations of the <50-μm-ESD and >50-μm-ESD Harney soil components suggested a qualitative difference in their associated organic compounds. The carbon contents of the fractions from aggregates were higher than those of water-dispersible fractions. The high levels of clays in water-stable aggreates suggest that diffusion into and out of such aggregates is much slower than in the bulk soil solution.

It was concluded that the interiors of water-stable microaggregates of ESD >50 μm are anaerobic microenvironments.

Oxygen-stressed conditions in the aggregates reduce decomposition rates and maintain higher carbon contents. Inhibited diffusion rates and incomplete decomposition cause a relative concentration of certain nutrient elements inside the aggregates. Occlusion in microaggregates is an important mechanism of organic matter stabilization.

Distribution of Nutrients Among Ultrasonically Dispersed Fine Soil Particle Size Fractions Is an Artifact

Ultrasonic dispersion was selected for this study for two reasons: (1) It had been shown to be effective in dispersing soils without use of supplemental chemical treatments (Edwards and Bremner 1967b and others). The residues of such chemical treatments are confounding contaminants in mineralization studies. (2) This dispersion method yields fine particle size fractions among which consistent differences in C:N ratios, humic:fulvic acid ratios, and nutrient levels exist (Anderson 1974a,b). Such fractions are sources of organic matter with predictably different qualities.

Despite all the accumulated empirical information on the use of ultrasonic dispersion, there was no information on any artifacts this technique produces. Lack of information on possible artifacts was a concern from the outset of my experimentation. Designing the fractionations to specifically examine artifacts of the use of ultrasound was not attempted. Indeed, it would have been extremely difficult to design experiments to specifically study artifacts. Instead, it was hoped that a thorough examination of the data accumulated during the fractionations might indicate the nature of any unknown artifacts.

The first indication of an artifact appeared from a comparison of the chemical characterization of the Harney soil fractions. Table 3.8 lists the C, N, and P contents of the fractions obtained in the first, lower-power sonication and the second, higher-power sonication fractionations. The fine silt fraction C, N, and P contents did not appear to be affected by increasing the ultrasonic energy applied. The C content of the coarse clay was clearly increased, while that of the fine clay decreased, with application of more ultrasonic energy.

Table 3.8. Effects of levels of ultrasonic energy used in dispersion on the distribution of carbon, nitrogen, and phosphorus among fine soil particle size fractions from the Harney soil

Fraction	Elemental (C:N:P_T) content (ppm)	C:N ratio	C:P_T ratio
Whole soil	25:2.1:0.47	11.9	53.2
	Dispersed with less ultrasonic energy[1]		
Fine silt	45:4.6:0.91	9.8	49.5
Coarse clay	43:4.1:0.99	10.5	43.4
Fine clay	33:2.4:0.72	13.8	45.8
	Dispersed with more ultrasonic energy[2,3]		
Fine silt	46:5.1:0.94	9.0	48.9
Coarse clay	51:6.1:1.2	8.4	42.5
Fine clay	27:3.6:0.84	7.5	32.1

[1]Less ultrasonic energy was applied during the first fractionation because of an instrument malfunction.

[2]Normal instrument function allowed application of more ultrasonic energy during the second fractionation.

[3]These results are weighted means of the <50-μm ESD and >50-μm ESD results in Tables 3.3 and 3.5 and are valid as whole-soil fractionation values, since all soil materials were accounted for.

The C:N ratios decreased and N and P contents of both clay fractions increased with more intense sonication. The $C:P_T$ ratio changed for the fine clay only. A redistribution of C, N, and P with a selective association of N-rich materials with the coarse clay and N- and P-rich materials with the fine clay fraction was apparent.

The effects of sonication on the C, N, and P distribution among the fine fractions from the Ascalon soil can be seen in Table 3.9. In this soil, sonication increased the C, N, and P content of the fine silt and the C and P of the coarse clay, while decreasing the C, N, and P content of the fine clay fraction. Sonication did not markedly affect the C:N ratios of the fractions. The C:P ratio increased with sonication for the fine silt and decreased for the fine clay, apparently as a result of a disproportionate loss of C from the fine clay and gain of C by the fine silt.

In both of these soils, ultrasonic dispersion caused a reduction in fine clay C content and an increase in the C content of the coarse clay and fine silt. The N and P contents were not so consistently affected in both soils. The consistent carbon behavior under the influence of ultrasonic vibration suggested that it is fraction-associated organics that are affected.

Redistribution of organics of specific character to specific fractions was indicated by the changes in C:N and C:P ratios that resulted from sonication. Without effective sonication, only the fine silt fractions of the two soils had comparable C:N ratios. When effectively sonicated, the same particle size fraction from both soils had similar C:N ratios and the ratios decreased with decreasing particle size. The C:P ratios were similarly affected, both soils

Table 3.9. Effects of ultrasonic dispersion on the carbon, nitrogen, and phosphorus contents of the fine soil particle size fractions from the Ascalon soil

Fraction	Elemental content (mg/g)				C:N ratio	$C:P_T$ ratio	$C:P_o$ ratio
	C	N	P_T	P_o			
Whole soil	10	0.78	0.27	0.12	12.8	37.0	
Dispersed by mild agitation (water dispersible)							
Fine silt	32	3.4	0.87	0.60	9.4	36.8	53
Coarse clay	30	4.0	1.08	0.77	7.5	27.8	39
Fine clay	27	3.6	1.23	0.66	7.5	22.0	41
Ultrasonically dispersible							
Fine silt	44	4.3	0.96	0.63	10.2	45.8	70
Coarse clay	34	4.0	1.18	0.78	8.5	28.8	44
Fine clay	23	2.9	1.25	0.65	7.9	18.4	35

showing a distinct decrease with decreasing particle size after an effective sonication.

The redistribution of Fe and Mn caused by ultrasonic dispersion of the Ascalon soil is related to the redistribution of carbon, while that of Zn and Cu is not (Table 3.10). The consistent relative reduction in Cu and Zn contents, independent of C enrichment, across all three fractions suggest that extractable forms of these elements are similar for all three fractions and may be inorganic. The parallel changes in the enrichment ratios of C and extractable Fe and Mn were interpreted as indicating that organics hold extractable forms of Fe and Mn. If this interpretation is correct, then the remarkable enrichment of the fine silt with Mn indicates that the different organics become associated with this fraction as a result of sonication.

Calculation of the C-to-extractable-Fe and C-to-extractable-Mn ratios suggest that the organics associated with all three fractions are modified by ultrasonic vibration (Table 3.11). The C:extractable-Fe

Table 3.10. Effects of ultrasonic dispersion on the distribution of carbon and certain NH_4HCO_3-DTPA-extractable elements among the fine soil particle size fractions of the Ascalon soil

	Enrichment ratios of ultrasonically dispersed fractions to water-dispersed fractions[1]				
Fraction	C	Fe	Mn	Cu	Zn
Fine clay	0.85	0.98	0.60	0.45	0.33
Coarse clay	1.13	1.43	1.29	0.49	0.36
Fine silt	1.38	1.65	12.81	0.52	0.79

[1]Calculated from data in Tables 3.4 and 3.7.

Table 3.11. Effects of ultrasonic dispersion on the C:extractable-Fe and C:extractable-Mn ratios of the fine soil particle size fractions of the Ascalon soil

Fraction	C:extractable-Fe ratio	C:extractable-Mn ratio
Fractions dispersed with mild agitation in water		
Fine clay	56	450
Coarse clay	107	353
Fine silt	160	1000
Fractions ultrasonically dispersed		
Fine clay	49	639
Coarse clay	85	309
Fine silt	133	107

ratios decrease with decreasing particle size with or without sonication but are consistently decreased across all three fractions by sonication. After sonication, there is a clear decrease in the C:extractable-Mn ratio with increasing particle size.

The C:extractable-Mn ratios are distinctly changed by ultrasonic vibration. Sonication increased the C:extractable-Mn ratio of the fine clay, while inducing a tenfold decrease in this ratio for the fine silt. Without sonication, C:extractable-Mn appear to increase with increasing particle size. With sonication, the C:extractable-Mn ratios clearly decrease with increasing particle size.

Results of the three fractionations indicated that ultrasonic dispersion causes a redistribution of certain nutrient elements among particle size fractions. Sonication consistently lowered C contents

in the fine clays, while it consistently increased C contents for the coarse clay and fine silt. The C:N, C:P, C:extractable-Fe, and C:extractable-Mn ratios of fractions dispersed without effective sonication did not indicate consistent differences in associated organic matter across the three fraction sizes. In contrast, the C:N, C:P, and C:extractable-Fe ratios decreased, while the C:extractable-Mn ratios increased, with decreasing particle size among ultrasonically dispersed fractions.

Microscopic examinations indicated that the organic-C forms present in the fractions differed among the particle size fractions. Two general classes of organic-C forms were observed: (1) C forms apparently adsorbed onto mineral particles, and (2) those not so adsorbed but adsorbed by organic particles or present as free bacterial, fungal, or root residues. Ultrasonic dispersion and the size of water-stable aggregates dispersed affected the distribution of the classes of C forms among the fractions.

Free bacterial, fungal, and root residues were absent from all three size fractions prepared without ultrasonic dispersion (Ascalon soil fractionation). The water-dispersible fine clay fraction was observed to contain only mineral-particle-adsorbed organic-C forms, whereas the water-dispersible fine silt contained all observable organic C in brown to black, nearly spherical particles of lower density. The density of these particles was calculated using the observed size and shape and Stoke's Law to be as low as 1.25 g/cm^3. The coarse clay fraction contained both these two organic-C forms. When ultrasonic dispersion was applied to the smaller (<50-μm ESD), water-stable aggregates from the Harney soil, the fractions obtained

were not, under microscopic examination, distinguishably different from those water-dispersible aggregates from the Ascalon whole soil.

Each fraction ultrasonically dispersed from the Ascalon soil contained more C forms than the same water-dispersible fractions. The ultrasonically dispersed fine clays contained some of the lower-density, darker, spherical particles, as well as large amounts of clay-adsorbed C. The proportion of the lower density, darker particles increased in the ultrasonically dispersed compared with the water-dispersible, coarse clay fraction. Free fungal and root residues, along with the dark, low-density particles, were the C forms present in the ultrasonically dispersed fine silt fraction. The fractions ultrasonically dispersed from the larger (>50-μm ESD) water-stable aggregates were similar to those ultrasonically dispersed from the Ascalon whole soil, except that more dark, low-density particles were present in both clay fractions and more free fungal and root residues were present in the fine silt.

The microscopic observations (and preceding examinations of the effect of ultrasonic dispersion on the chemical composition of the fractions) indicated that much of the carbon in the particle size fractions was ultrasonically dissociable from, or occurred as distinct particles within, each fraction. Most of the carbon in the ultrasonically dispersed particle size fractions had no discernable relationship to a specific particle size range in the intact soils, except that large amounts of dark, low-density, nearly spherical particles and fungal and root residues were ultrasonically dispersible from the larger (ESD >50 μm, up to about 2000 μm actual diameter) water-stable microaggregates. (Bacterial residues were probably

dispersed from the larger microaggregates as well but were beyond the resolution of the microscope used.)

The different carbon forms had become associated with each particle size fraction during soil fractionation. The association of specific carbon forms with specific fractions probably resulted from ultrasonic dispersion and sedimentation. Ultrasonic vibration dispersed presumably all soil microstructural units. The components were water-soluble and -insoluble organics and inorganics. Most of the soluble components were resorbed by the most compatible organic or inorganic particles. (These adsorbent particles then were fractionated by sedimentation according to their ESDs.)

In the fine clay fraction, clay minerals were the predominant sorbing agents. The highly charged, hydrophilic character of these surfaces made them more compatible with relatively hydrophilic (less aromatic and lower molecular weight) organics richer in ionizable moieties. In contrast, the sorbing agents in the fine silt fractions were insoluble organic particles. These organic particles likely have complex structures and are composed of relatively hydrophobic (more aromatic and higher molecular weight) organics or are fungal or root residues composed of biological polymers. The organics compatible with sorption to these organic particles were presumably different from those sorbed by clay minerals.

The various insoluble particles, bearing the ultrasonically dispersed organics they had adsorbed, were sedimented into the fractions according to their sizes, shapes, and densities. The carbon in each fraction is therefore referred to as "fraction-associated

carbon" to draw attention to its presence as an artifact of the fractionation procedure.

Conclusions

Three conclusions were drawn from interpretations of the information acquired from the fractions:

1. Anaerobic microsites exist in the interiors of water-stable microaggregates of ESD >50 μm.

2. Occlusion in microaggregates is an important mechanism of stabilization of soil organic matter.

3. The distribution of nutrient elements among ultrasonically dispersed soil particle size fractions is an artifact and does not reflect the distribution of these elements among these fractions in intact soils.

CHAPTER 4

METHODS AND MATERIALS OF THE INCUBATION STUDIES

Introduction

The incubation studies were undertaken to gain insight into the effectiveness of two mechanisms of stabilization: chemical recalcitrance and stabilization by adsorption onto clay particle surfaces. The fractionation studies allowed an evaluation of the relative effectiveness of stabilization of organic matter by occlusion in microaggregates while providing soil particle size fractions for the incubation studies. Each fraction's associated organic matter was, based on the literature, of different chemical character and affected to different extents by adsorption to clay surfaces. This chapter presents some details and considerations of the methodology used in the incubation studies.

Materials

Soil particle size fractions

The soil particle size fractions studied were the fine silt, coarse clay, and fine clay fractions prepared from the Ascalon soil.

Support matrix: A fine silica sand

The incubation method, based on 10-g microcosms, was tested in a preliminary experiment and found satisfactory. Ten grams of Martin

Marietta EI-70 silica sand treated with 2 M HCl to remove contaminant P provided inert bulk needed for adequate aeration of the simulated soil medium in the microcosms. Analyses indicated that this prepared sand contained insignificant extractable P or readily soluble salts. This sand had a bulk density (1.44 g/cm^3) and field capacity moisture content (15% w/w) reasonably similar to those of many coarse-textured soils.

Powdered $CaCO_3$ was added and thoroughly mixed into the sand to a concentration of 0.3% by weight. This amendment was to buffer any pH changes that might occur during incubation. (If acidity were neutralized during the incubation by the $CaCO_3$, a falsely high CO_2 reading would result. To examine this possibility, the total carbonates in two spare microcosms were determined at the end of the experiment. No change in carbonates was detected.)

Microcosms

The microcosms were prepared by weighing 10.00 ±0.01 g of dried silica sand, 0.185 g of fine clay, or 0.313 g of coarse clay, or 0.417 g of fine silt fraction into a 1-oz scintillation vial. These amounts of the fractions provided approximately 50 µg of 0.5 M $NaHCO_3$-extractable inorganic P per microcosm. Each loaded vial was capped and shaken to thoroughly mix the sand and fraction.

Nutrient amendments were prepared by adding appropriate quantities of cellobiose and $(NH_4)_2SO_4$ to volumes of a basal nutrient solution known as RSS (see Herzberg et al. 1978). The "appropriate quantities" were determined by calculating the concentration of the added nutrient in the RSS to give the desired amendment level when the

simulated soil in each microcosm was brought to field capacity with RSS. The cellobiose and $(NH_4)_2SO_4$ were always added to give an amendment C:N ratio of 5:1.

A soil inoculum was prepared. A 100-g portion of the original soil sample was brought to field capacity and incubated under aerobic conditions for 5 days at 25°C. To inoculate the microcosms, a 10:1 (water:soil by weight) suspension of this incubated soil was prepared.

The simulated soil in each microcosm was brought to near field capacity with the appropriately amended RSS. Each microcosm was then inoculated with two drops of the suspension from the incubated soil. Incubation was at 25°C.

Analytical Methods

Respiration measurements were made by determining the amount of CO_2 absorbed in a 1 N NaOH solution in the same closed atmosphere with the microcosms (see Coleman et al. 1978). The NaOH traps were prepared by measuring a known volume of 1.00 N NaOH into 1-oz scintillation vials. Each trap was then placed in a one-pint Mason jar with four microcosms (all of the same experimental treatment) and the jar was sealed with its screw-on lid. There were three replicates and therefore three Mason jars prepared for each treatment. The amount of CO_2 absorbed was determined by precipitating carbonates with $BaCl_2$ and back-titrating the unreacted NaOH with 0.01 N HCl. Respiration data were corrected to a per-microcosm basis by calculation. The volume of NaOH used in each trap and time between respiration measurements were adjusted for rates of CO_2 evolution.

Fungal and bacterial biomass were estimated on the four P sampling dates by a modified Jones and Mollison (1948) and the fluorescein isothiocyanate (Babiuk and Paul 1970) methods, respectively.

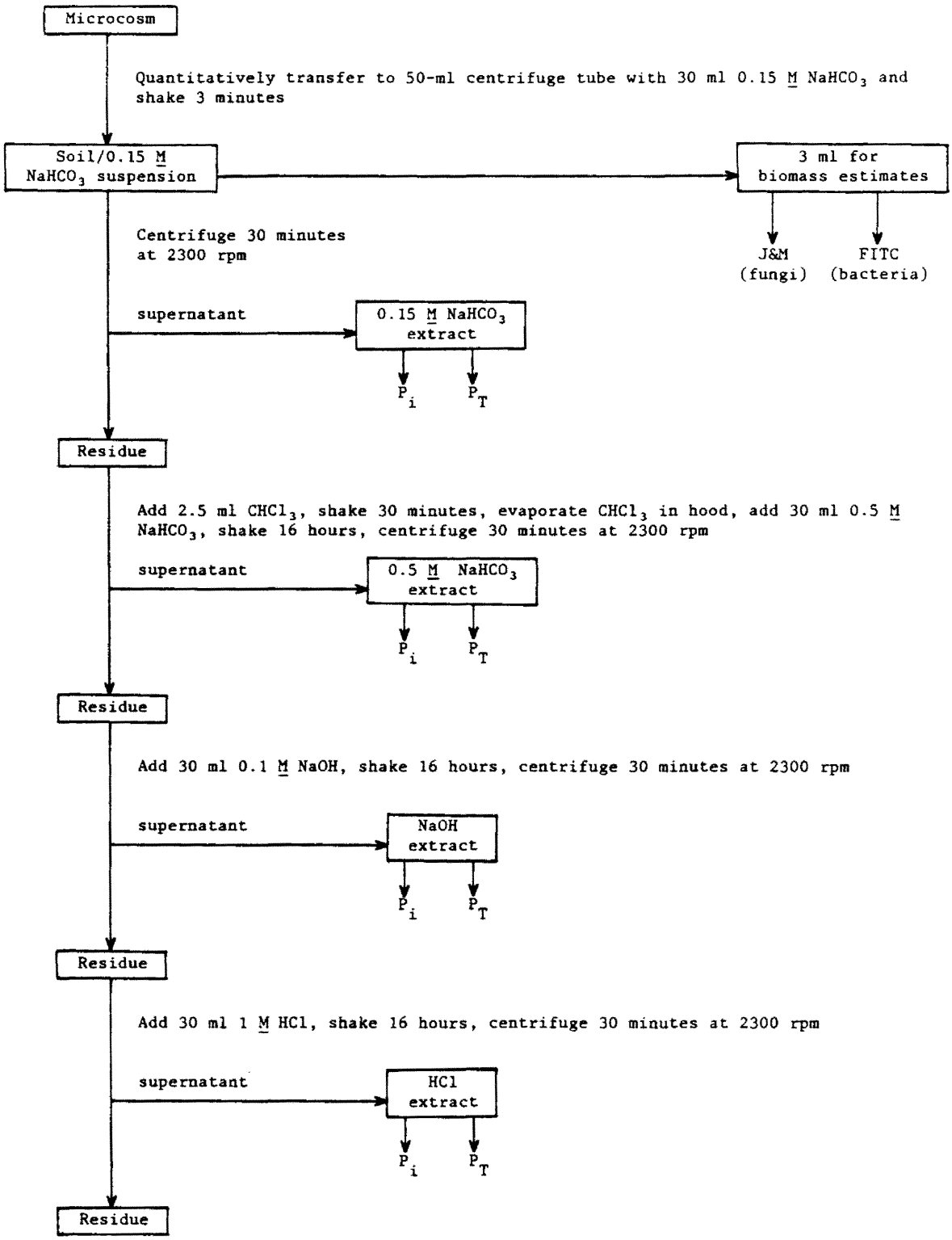
The P-extraction procedure used in this experiment was relatively complex. A flow diagram is given in Fig. 4.1. The quantitative transfer of the soils of each whole microcosm, the 30-ml extractant volume, and use of centrifugation to separate the soil from the extract had proven to be practical in the extraction procedure used in a preliminary incubation experiment and were retained in the extraction procedure in this experiment.

The extraction sequence was initiated by quantitatively transferring the simulated soil from each microcosm into a 50-ml Nalgene centrifuge tube, using 30 ml of 0.15 $\underline{M}$ $NaHCO_3$. The tubes were stoppered and shaken for three minutes. Each tube was then briefly hand-agitated, quickly opened, and a 3-ml aliquot was removed for use in the biomass estimates. The tubes were then centrifuged for 30 min at 2300 rpm in the IEC Model K size 2 or CRU-5000 centrifuge. Supernatant extracts were decanted and stored for P_i and total phosphorus (P_T) determinations.

Each tube then received 2.5 ml of chloroform, was stoppered, and mechanically shaken for 30 minutes. The tubes were opened and the chloroform evaporated by placing the tubes in a 55°C water bath inside a fume hood for not less than 4 hours.

Following the chloroform treatment, each tube received 30 ml of 0.5 $\underline{M}$ $NaHCO_3$ adjusted to pH 8.5 with NaOH. The tubes were then stoppered and shaken for 16 hours. After opening, the tubes were

Fig. 4.1. Flow diagram of phosphorus extraction procedure


Microcosm
Quantitatively transfer to 50-ml centrifuge tube with 30 ml 0.15 M $NaHCO_3$ and shake 3 minutes
Soil/0.15 M $NaHCO_3$ suspension
3 ml for biomass estimates
J&M (fungi)
FITC (bacteria)
Centrifuge 30 minutes at 2300 rpm
supernatant
0.15 M $NaHCO_3$ extract
P_i
P_T
Residue
Add 2.5 ml $CHCl_3$, shake 30 minutes, evaporate $CHCl_3$ in hood, add 30 ml 0.5 M $NaHCO_3$, shake 16 hours, centrifuge 30 minutes at 2300 rpm
supernatant
0.5 M $NaHCO_3$ extract
P_i
P_T
Residue
Add 30 ml 0.1 M NaOH, shake 16 hours, centrifuge 30 minutes at 2300 rpm
supernatant
NaOH extract
P_i
P_T
Residue
Add 30 ml 1 M HCl, shake 16 hours, centrifuge 30 minutes at 2300 rpm
supernatant
HCl extract
P_i
P_T
Residue

centrifuged at 2300 rpm for 30 minutes. The supernatants were decanted and collected for P_i and P_T determinations.

Extraction with 30 ml of 0.1 M NaOH followed, using the same procedure described for the prior $NaHCO_3$ extraction. Aliquots of the $NaHCO_3$ and NaOH extracts to be analyzed for P_i were pretreated to remove the interfering acid-insoluble organics. Pretreatment involved acidification to pH 1.5 with H_2SO_4, centrifugation at 10,000 rpm for 10 minutes at 0°C in a Sorvall RC-2B centrifuge, and decanting and collection of supernatant. Finally, extraction with 30 ml of 1 N HCl, using the same procedure, completed the sequence. Unextractable P was calculated by subtracting the total extractable P from the total P determined earlier (see Table 3.9).

Lysis with chloroform was used for two reasons. First, it should make the bicarbonate-extractable pool function as a sink for nearly all the P_i in microbial cells and most of the P_o. Second, such lysis was expected to lower the inherent variability, since the uncontrolled (probably inconsistent) maceration effects of a 16-hour shake of a 3:1 extractant:soil suspension would be much less important for previously lysed cells. Inconsistent maceration would otherwise cause variable distribution of the microbial P in the various extracts.

The distribution of biomass P among the extracts from this procedure has important differences from that of the more classical soil-P extraction procedures that do not entail intentional controlled lysis of microbial cells. Some release of biomass P should occur during the 0.15 M $NaHCO_3$ extraction, but the bulk is released during the chloroform treatment. The biomass P_o released on lysis with chloroform appears in the subsequent extracts, extractability

determined by adsorptive qualities of the soil and chemical qualities of the freed biomass P_o.

The 3-minute 0.15 M $NaHCO_3$ extract removed readily soluble low molecular weight and low-aromaticity organics bearing P_o and readily exchangeable and water-soluble P_i. There was a minimum of water-soluble P_i and P_o initially present in the fractions, since it was washed out during the fractionation procedure.

The chloroform lysis should have caused all the biomass P (less that extracted by the 0.15 M $NaHCO_3$) to be distributed among the other extracts and unextractables. Hedley, Stewart, and Chauhan (1982) described the other extractable forms as follows. The 16-hour 0.5 M $NaHCO_3$ (pH = 8.5) extraction removed the bulk of the exchangeable P_i and organics of moderate molecular weight, while the 0.1 M NaOH (hereafter NaOH) extracted P_i chemisorbed to Fe and Al components of soil surfaces and organics of moderate molecular weight and higher aromaticity and iron phosphates. The extraction of reduced iron phosphates--if present in significant amounts, as expected from the dispersion of anaerobic microsites occurring during the soil fractionations--by NaOH was expected to make this extract especially informative. The 1 N HCl (hereafter HCl) extracts primary mineral P, mainly calcium phosphates, some secondary mineral phosphate and polyphosphates. P_o in acid-soluble organics such as sugar and phospholipid esters and phosphorylated coenzymes will also be included in this extract. The unextractable P is probably in organics of high molecular weight and high aromaticity or in organics of variable character strongly adsorbed to more complex organics or to inorganic

surfaces, in insoluble microbial residues, and unknown inorganic forms.

The total C in some of the extracts was determined by oxidizing the C with permanganate in acid, trapping the carbon dioxide produced, and titrating to determine the amount of C extracted. This analysis was carried out when adequate supplies of extracts were available to supplement the other data.

Statistical Methods

Statistical analyses of the data were performed using t-tests, the SPSS program package multivariate analyses of variance (AOV) with a fully nested experiment design, and Tukey's Q-test for differences between means. The t-tests were used for analysis of C and P treatment means. The AOVs were performed for fraction, C level, and time variables over the entire experiment and for fraction and C level variables over certain time intervals. Tukey's Q-test was used to identify significant differences among the particle size fractions in the changes in P extractability over certain time intervals.

Experimental Design

An experiment to test the hypotheses was designed. Three amendment levels of readily available C were used to impose different levels of microbial activity. The fine silt and coarse and fine clay fractions were used to supply P_o in OM of different qualities (presumably with different molecular weights and aromaticities).

The amount of each fraction to be used in each microcosm was chosen as that which would provide 50 μg of available P_i per

microcosm. The amounts actually used were 0.185 g of fine clay, 0.313 g of coarse clay, and 0.417 g of fine silt. This amount, fifty micrograms, of available P_i was chosen to provide an available C:P nutrient-balanced system in each microcsom with an amendment of 5000 µg of available C (as cellobiose) per microcosm.

Two other C-amendment levels were chosen, one to provide adequate available C for significant microbial activity without an available P limitation and the other to provide high levels of microbial activity under conditions with relatively limited available P. The two levels were 1250 µ C per microcosm and 10,000 µg C per microcosm, respectively. Cellobiose was used as the C source for all C amendments and ammonium sulfate as the N source. Nitrogen was added at 20% of the added C level. Table 4.1 provides a summary of considerations and design of the experiment.

There were three replicates of each treatment, four destructive sampling times, and twelve respiration (nondestructive) sampling dates. The first sampling was immediately after preparation and inoculation of the microcosms to evaluate the P status before any microbial activity. The second sampling was as soon as possible after the initial respiration burst to evaluate the P status when microbial biomass was at or near maximum. The third sampling was well beyond the initial respiration peak but while respiration rates were still detectable to evaluate the P status after reduction of the earlier accumulated biomass to near a final minimum. The fourth sampling was to determine whether the fractions differed in how long detectable levels of respiration would be sustained with the same C amendments.

Table 4.1. Summary and design of the incubation experiment

Microcosm components common to all treatments

10.00 g silica sand (acid washed, distilled-water rinsed, dried and amended with 0.3 weight % $CaCO_3$, to provide a minimum buffering capacity)

1.5 ml of reference soil solution (RSS) amended with $(NH_4)_2SO_4$, to provide N at a level equal to 20% of the added cellobiose-C level

20 µl of inoculum, prepared by suspending 10 g of whole Ascalon soil (after incubating at field moisture capacity and 25°C for five days) in 100 ml RSS

Treatments

Soil fractions (in quantities to provide approximately 50 µg of bicarbonate-extractable inorganic P per microcosm)

Fine clay: 0.185 g containing 4260 µg C, 540 µg N, 230 µg P_T

Coarse clay: 0.313 g containing 10,6000 µg C, 1250 µg N, 370 µg P_T

Fine silt: 0.417 g containing 18,300 µg C, 1790 µg N, 400 µg P_T

Amendment carbon levels

1250, 5000, and 10,000 µg cellobiose C per microcosm to set available C:P conditons providing, respectively, low (no P stress), moderate (balanced C:P status), and high (P-stressed) levels of microbial activity

Experimental design

For carbon (nondestructive samplings)

3 soil particle size fractions	×	3 carbon amendment levels	×	12 sampling times	×	3 replicates	×	1 (CO_2) analytical measurement

For phosphorus (destructive samplings)

3 soil particle size fractions	×	3 carbon amendment levels	×	4 sampling times	×	3 replicates	×	10 analytical measurements*

*The 10 analytical measurements were four inorganic P, four total P, and biomass determinations.

The experimental design was three soil particle size fractions × three amendment levels × three replicates × four sample dates and is summarized in Table 4.1.

Methods of Presentation of Results

The large amount of data collected requires that only tables and plots of means and comparisons of means be presented in the following chapters. Carbon results were analyzed for significance using only a t-test. Phosphorus results were analyzed using two different analyses of variance (AOVs) and Tukey's Q-test for differences between means. The first AOV examined the effects of C-amendment level, fractions, and time (Table 4.2). The high consistency of the effects of time allowed a second AOV to be performed to examine the effects of C-amendment levels and fractions over specific time intervals (Table 6.1). Tukey's Q-test was used in the second AOV. A 95% confidence level was used unless otherwise noted. Separate discussions of the carbon and phosphorus results follow.

Table 4.2. Summary of statistical analysis of variance of incubation experiment treatment effects on extractable forms of phosphorus

Source	Degrees freedom	Significance of F-value							
		0.15 M $NaHCO_3$		0.5 M $NaHCO_3$		NaOH		HCl	
		P_i	P_o	P_i	P_o	P_i	P_o	P_i	P_o
Fractions	2	<0.001	<0.001	0.051	<0.001	<0.001	<0.001	<0.001	<0.001
(Added) C level	2	<0.001	NS	0.003	0.005	NS	0.016	NS	NS
Fractions × C level	4	0.049	NS	NS	0.027	NS	NS	NS	NS
Time	3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Time × fractions	6	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	NS	NS
Time × C levels	6	<0.001	<0.001	<0.001	<0.001	NS	0.004	NS	NS
Time × fractions × C levels	12	<0.001	NS	NS	0.002	NS	NS	NS	NS

CHAPTER 5

MICROBIAL UTILIZATION OF FRACTION-ASSOCIATED ORGANIC CARBON

Introduction

This chapter presents the carbon transformations results from the incubation experiment. The microbial transformations of fraction-associated P were the principal subject of this study. It was necessary to ascertain whether equivalent amounts of microbial activity occurred for all fractions at each carbon level before comparability of P transformation conditions could be assumed. Respiration measurements, based on the evolution of CO_2, were the most convenient means of monitoring microbial activity. Microbial biomass was determined to establish growth patterns. The combined C data were examined to test the hypothesis regarding the utilization of soil organic matter C by soil microbes. The development of the hypotheses was based on the character of the particle size fractions and their associated organic carbon as indicated by the literature and my own fractionation work (see chapter 3).

Various researchers report that the aromaticity and molecular weight of the organic matter associated with ultrasonically dispersed fine soil particle size fractions consistently increase with increasing particle size. There is a widely held assumption that the availability of organic matter to soil microbes decreases as

aromaticity and molecular weight increase (see the introduction of chapter 2). Based on this information, Anderson et al. (1981) hypothesized that the fine clays in soils function as a storehouse of readily available organic nutrients.

The data presented in chapter 3 indicated that relationships among the fractions with respect to their associated organic matter were artifacts of ultrasonic dispersion. The association of a particular quality of organic matter with a particular ultrasonically dispersed fraction could no longer be interpreted to mean that such specific OM quality-particle size associations are important in intact soil. Anderson et al.'s (1981) hypothesis therefore required modification.

It must be made clear that even though ultrasonically dispersed particle size fractions do not directly indicate the location in intact soils of specific kinds of organic matter, they nevertheless allow the separation of different kinds of organic matter from soils without the use of chemical extractants or dispersing agents. The data reported in chapter 3 did not conflict with the literature reports of consistent qualitative differences in the fine soil fraction's associated organic matter. The microscopic examinations of the fractions indicated that aromaticity and molecular weight of the fraction's associated organic C should increase with increasing particle size. These two qualities, however, were not measured in this study but on the basis of the literature and observations were assumed to increase with increasing particle size.

Stabilization of O.M. by clay adsorption could be important only in the two clay fractions. Since adsorption capacity is related to

surface area, the fine clay fraction must have had the greatest clay adsorption among the three fractions. In contrast, the fine silt fraction had essentially no clay particles and therefore no clay adsorption. For reasons discussed in chapter 3, it was concluded that the organics in the fine silt fraction must function as adsorbing agents. The coarse clay fraction must have had both clay and organic adsorbing agents.

Two relationships among the fractions arose as the bases of the development of the hypothesis. First, aromaticity and molecular weight were believed to increase with increasing particle size. Second, the amount of (stabilization of O.M. by) clay adsorption must have decreased with increasing particle size. Further, the preparation of the fractions had eliminated stabilization of O.M. by occlusion within microaggregates from the fractions.

It was desired to test three hypotheses (H_a-H_c), which may be expressed as follows:

> Under similar conditions of microbial activity, the extent of utilization of the nutrients contained in soil organic matter is related to (H_a) the aromaticity and molecular weight of that organic matter, (H_b) the extent to which the organic matter is adsorbed onto particle surfaces, and (H_c) the character of the adsorbent surface and the mechanism of adsorption.

The fact that all three of the mechanisms, which were the bases of the above hypotheses, were effective to various extents in each of the fractions made formal testing of each of the three hypotheses impossible.

To develop a hypothesis that could be practically tested, it was assumed that stabilization of organic matter by adsorption was equivalent across all three fractions, whether the adsorbent was clay surfaces, as in the fine clay, or organics, as in the fine silt fraction. This assumption had two advantages. First, the data would indicate if it was grossly unreasonable. Second, it made hypothesis H_a subject to practical test using the fractions. Hypothesis H_a is hereafter referred to as H_1.

Since the extent of utilization of fraction-associated C could not be foreseen, it was necessary to design the experiment to provide treatments with different levels of microbial activity. This design allowed hypothesis H_2 to be tested:

> The extent of utilization of nutrients in soil organic matter increases as the level of microbial activity increases.

Microbial activity was expected to occur in three stages during the incubation. Stage I was a period of rapid utilization of substrate, bacterial growth, and CO_2 evolution. Stage II was a period of slow, relatively steady microbial activity and CO_2 evolution. Stage III was the period after the cessation of significant CO_2 evolution. Phosphorus sampling times were designed to be (1) before microbial activity (zero hours incubation), (2) when microbial biomass and net P immobilization were at a maximum (at the end of stage I), (3) when microbial biomass had decreased and P mineralization approached a maximum (at the end of stage II), and (4) after an extended period of no microbial activity (at the end of stage III). During the incubation, the P sampling times were chosen using the

respiration rates of the 5000 μg cellobiose-C treatment (Fig. 5.1) to estimate the end of each of the stages of microbial activity. Stage I was estimated to end at 124 hours, when the end of the expected respiration peak became apparent. Stage II was estimated to end at 814 hours incubation, the first respiration sampling when any treatment's respiration rate became not significantly different from zero (by t-test). Stage III was terminated at 1584 hours, when for two consecutive sampling dates the majority of treatments had mean respiration rates not significantly different from zero. (Data were collected for stage III but no attempt to analyze them is presented in this thesis.)

Cumulative Respiration--Verification of Microbial Utilization of Fraction-Associated Carbon

The cumulative respiration results (Fig. 5.2) showed the microbial activity to be comparable among the three fractions at each C level. The only significant differences occurred at the lowest C level (1250 μg cellobiose per microcosm) but were not large. It was concluded that microbial activities and therefore strictly microbial P transformations were comparable for all three fractions at each C level.

Microbial utilization of fraction-associated carbon was shown by the cumulative respirations (total over stages I and II). For all fractions at the 1250-μg cellobiose-C level and the fine silt at the 5000-μg cellobiose-C level, cumulative respiration exceeded the amounts of cellobiose C added (see Table 5.1). In these treatments, fraction-associated carbon must have been utilized in quantities at

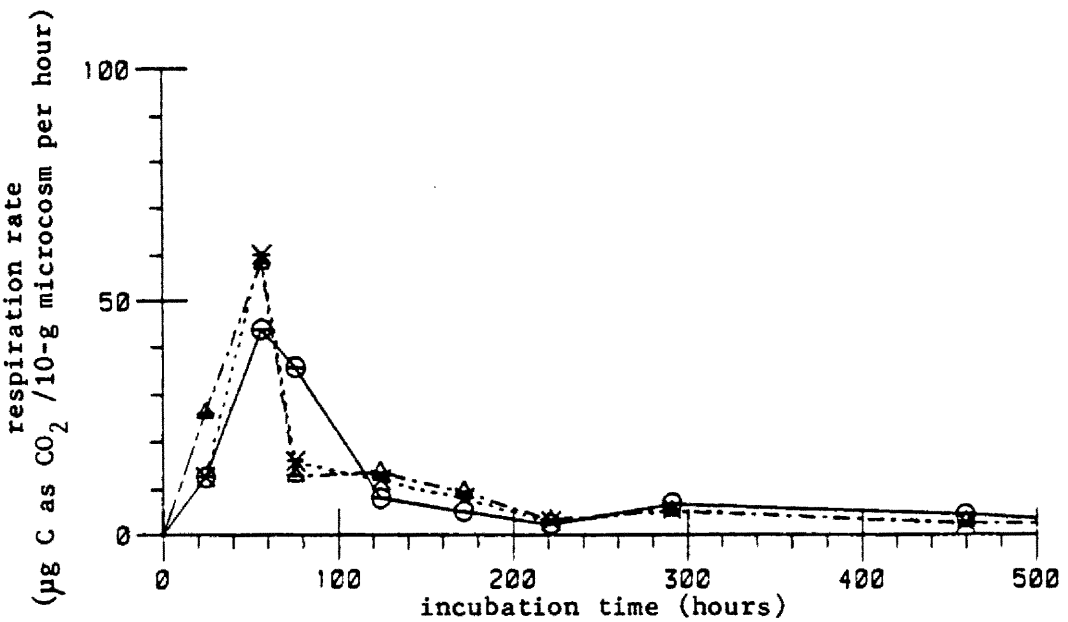
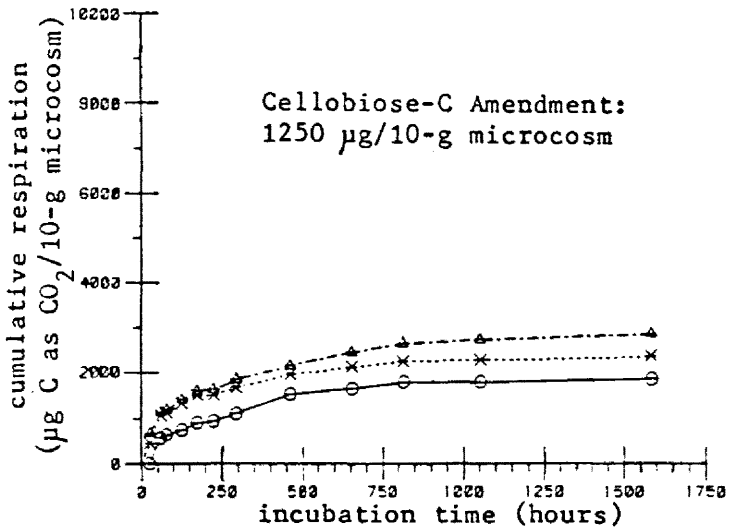
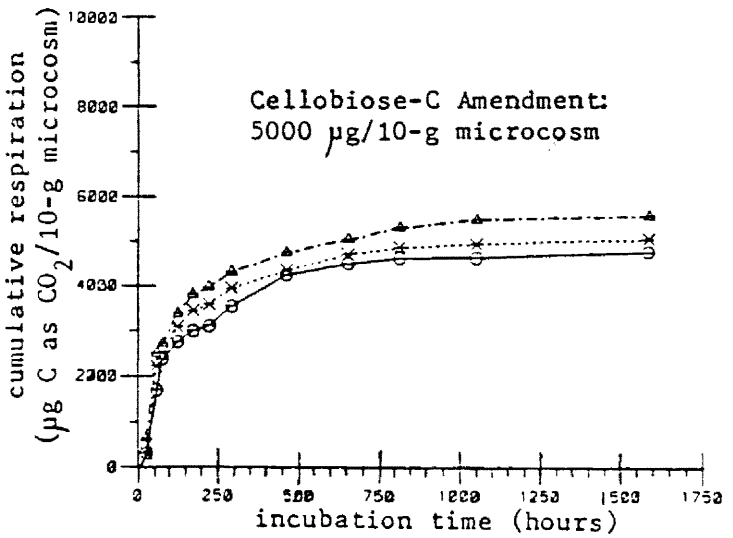


Fig. 5.1. Effects of incubation time and fraction treatment [0.185 g fine clay (o), 0.313 g coarse clay (x), and 0.41 g fine silt (Δ) per 10-g microcosm] on respiration rate at the 5000-µg (cellobiose-C per 10-g microcosm) carbon amendment level

Fig. 5.2. Effects of the fraction [0.185 g fine clay (o), 0.313 g coarse clay (x), or 0.417 g fine silt (Δ) per 10-g microcosm] and carbon amendment (1250, 5000, or 10000 μg cellobiose-C per 10-g microcosm) treatments, and incubation time on cumulative respiration


Cellobiose-C Amendment:
1250 µg/10-g microcosm
cumulative respiration
(µg C as CO_2/10-g microcosm)
incubation time (hours)


Cellobiose-C Amendment:
5000 µg/10-g microcosm
cumulative respiration
(µg C as CO_2/10-g microcosm)
incubation time (hours)

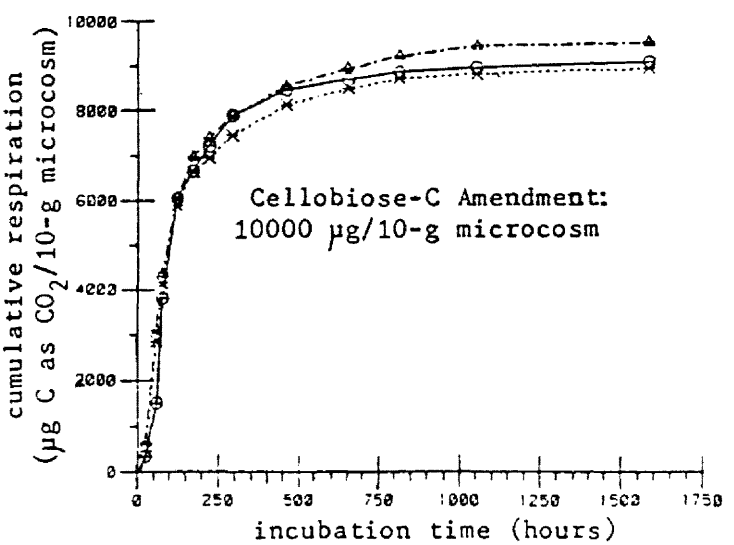

Cellobiose-C Amendment:
10000 µg/10-g microcosm
cumulative respiration
(µg C as CO_2/10-g microcosm)
incubation time (hours)

Table 5.1. Treatment effects on microbial activity as indicated by cumulative respiration

Fraction	Cellobiose-C amendment level	Cumulative respiration over entire period of microbial activity	Minimum fraction-associated carbon utilized
	----- (μg C per 10-g microcosm) -----		
Fine clay	1250	2080	830
	5000	4630	--
	10000	8880	--
Coarse clay	1250	2190	940
	5000	4870	--
	10000	8800	--
Fine silt	1250	2580	1330
	5000	5363	360
	10000	9250	--

least as large as the difference between cumulative respiration and amendment carbon. This simple analysis of the carbon data did not recognize that a considerable portion of the microbially utilized carbon was incorporated into biomass and metabolites. Therefore, the following analysis of the carbon data was carried out.

Stage I: Respiration, Estimated Biomass, and Quantification of Microbial Utilization of Fraction-associated Carbon

The biomass data were collected as cell numbers and sizes by C. Morley and J. Frey. The results were converted to microbial biomass-C estimates. Raw data were converted to biovolume. Biovolume was converted to biomass C, assuming a cell density of 1.2 g/cm^3 and 70% water content. These values are compromises between the classical laboratory values of 1.1 and 80% and those suggested for some soil

conditions by Van Veen and Paul (1979) of 1.3 and 50%. C was assumed to be 40% of the dry weight of the cells.

The converted biomass data are shown in Table 5.2. The biomass estimates confirmed that CO_2 evolution was a valid index of microbial activity under the conditions of this experiment. The biomass peak was at the end of stage I (124 hours incubation).

Growth yield efficiencies for stage I were calculated from the cumulative respiration and biomass C data as follows:

$$\frac{\text{Biomass-C}}{\text{carbon evolved as } CO_2 + \text{biomass C}} \times 100 = \text{growth yield efficiency (\%).}$$

The efficiencies (Table 5.3) ranged from 57% to 75%, with a mean of 66%. This mean is reasonably close to published values (Payne and Wiebe 1978) for growth efficiencies (60%) of rapidly growing bacterial populations. Because of the inherent variability of biomass determinations, a 60% efficiency was applied to the more accurate respiration data to calculate estimated biomass C in all subsequent discussions of Stage I C transformations. The biomass calculated using the 60% efficiency are also shown in Table 5.3.

The cumulative respiration and biomass C present for each treatment at the end of Stage I are presented in Table 5.3. The quantity of carbon utilized by the microbes during stage I is given by

$$\begin{array}{lcl} \text{Total carbon utilized} & = & \text{carbon evolved as } CO_2 + \text{carbon assimilated into biomass} + \text{carbon in metabolites.} \end{array}$$

Microbial metabolites were presumed negligible during Stage I, the period of rapid bacterial growth. Therefore, the total carbon

Table 5.2. Effects of C-amendment level and fraction on carbon in microscopically estimated biomass on destructive sampling dates*

Treatment†	Sample time: 124 (stage I)	814 (stage II)	1584 (stage III)
	- - - (μg biomass C per 10-g microcosm) - - -		
FC × 1250	8930	4880	2000
FC × 5000	14000	6090	4660
FC × 10000	21700	6860	5880
CC × 1250	10200	5250	4830
CC × 5000	12300	5710	3080
CC × 10000	20300	8720	4520
FS × 1250	11000	3325	1300
FS × 5000	11700	4520	4520
FS × 10000	16100	6510	2910

*All treatments were estimated to have 7350 μg biomass C/microcosm at initiation of incubation.

†FC, CC, FS designate fine clay, coarse clay, fine silt treatments, respectively, while numbers indicate C-amendment level in μg/10-g microcosm.

utilized was estimated as the sum of carbon evolved as CO_2 and carbon assimilated into the biomass (see Table 5.4).

There were only two sources of carbon in the microcosms, the cellobiose and the fraction-associated organic carbon. Table 5.4 shows that for all nine treatments the total carbon utilized in stage I exceeded the cellobiose carbon. The carbon utilized in excess of the cellobiose C was fraction associated. The estimates of fraction-associated C utilized are shown in the fifth column of Table 5.4.

Table 5.3. Effects of various treatments on respired C (as $CO_2\uparrow$) and estimated biomass C after 124 hours incubation

Fraction†	Cellobiose-C amendment level	Cumulative respiration	Calculated increase in biomass*	Microscopically estimated increase in biomass	Estimated carbon assimilation rate x§
	- - - - - - - - (µg C per 10-g microcosm) - - - - - - - -				
Fine clay (4260 µg associated carbon)	1250	1050	1580	1580	0.60
	5000	2740	4100	6650	0.71
	10000	6050	9080	14400	0.75
Coarse clay (10600 µg associated carbon)	1250	1280	1920	2800	0.69
	5000	3080	4620	4900	0.61
	10000	5900	8850	13000	0.69
Fine silt (18300 µg) associated carbon)	1250	1350	2030	3680	0.73
	5000	3410	5120	4380	0.57
	10000	6090	9140	8750	0.59

†10-g microcosm amended with 0.185 g fine clay (FC) or 0.313 g coarse clay (CC) or 0.417 g fine silt (FS) and 1250 or 5000 or 10000 µg cellobiose C.

*Assuming 60% of substrate C is assimilated into bacterial biomass.

§$\underline{x}$ is the ratio of C assimilated into biomass to C utilized, calculated as

$$\frac{\text{Increase in biomass C}}{\text{increase in biomass C + C evolved as } CO_2}.$$

Table 5.4. Carbon transformations during Stage I. Estimates of the utilization of fraction-associated carbon

Fraction	Cellobiose-C amendment level	Stage I cumulative respiration	Stage I calculated biomass C	Stage I total C utilized	Fraction-associated carbon utilized	Ratio of cellobiose C to fraction-associated C	Portion of fraction-associated carbon utilized
	- - - - - - - - - - - - (μg C per 10-g microcosm) - - - - - - - - - - - -						
Fine clay (4260 μg	1250	1050	1580	2630	1380	0.29	0.32
associated carbon)	5000	2740	4100	6840	1840	1.17	0.43
	10000	6050	9080	15130	5130	2.35	1.20
Coarse clay (10600 μg	1250	1280	1920	3200	1950	0.12	0.18
associated carbon)	5000	3080	4620	7700	2700	0.47	0.25
	10000	5900	8850	14800	4800	0.94	0.45
Fine silt (18300 μg	1250	1350	2030	3380	2130	0.07	0.12
associated carbon)	5000	3400	5120	8420	3520	0.27	0.19
	10000	6090	9140	15230	5230	0.55	0.29

The estimates of utilization of fraction-associated carbon indicated that

1. Fraction-associated carbon was utilized for all fractions at all carbon amendment levels.
2. Utilization of fraction-associated carbon increased with increasing carbon amendment (level of microbial activity).
3. These increases were not proportional; increasing the carbon amendment by four and eight times increased utilization of fraction-associated C by averages of only 1.4 and 2.6 times, respectively.
4. The proportion of fraction-associated carbon utilized increased with decreasing particle size.

The fourth observation listed above indicates that the fine-clay-associated carbon was more available to microbes. However, utilization of fraction-associated carbon increased as the quantity of fraction-associated carbon present in the microcosms increased (FS > CC > FC). Therefore, it was not clear whether possible differences among the fractions in microbial availability of C had been a factor in determining utilization or only the quantity of carbon present. Further, preliminary experimentation had shown that the fraction's associated carbon was not utilized if a readily available substrate was not added. To restate, the fraction-associated carbon had to be "primed."

Priming and Availability of Fraction-Associated C

In view of the above, the use of the term availability had to be reconsidered. Availability as initially used in this thesis referred only to the time rate at which microorganisms utilize a given carbon

substrate. By this definition, under similar conditions glucose is more available than cellobiose, and cellobiose is more than cellulose. More available substrates are expected to be utilized more rapidly than those that are less available. This is important with respect to the fraction's associated carbon. The fraction-associated carbon that is more available would "turn over" and/or decompose more rapidly in the field.

However, the fraction-associated carbon was both "available" and "unavailable." When present as the sole substrate, fraction-associated carbon was not utilized--when not "primed," it was unavailable. When a relatively small amount (125 ppm C) of readily available substrate ("priming") was added, the fraction-associated C became relatively available, providing more than half of all the carbon utilized. When the readily available C level increased, the fraction-associated C provided one-third or less of the utilized C. This decrease in relative contribution occurred even in the fine silt and coarse clay treatments, in which each microcosm contained more fraction-associated than cellobiose carbon. These results indicated that the availability of fraction-associated carbon was not time dependent but dependent on the amount of readily available carbon ("priming") initially present. Availability was redefined for substrates that require priming as "the extent of utilization of the more recalcitrant substrate for a given priming ratio." Priming ratio for this presentation is defined as the ratio of the quantity of readily available carbon to the quantity of fraction-associated carbon present at the initiation of microbial activity.

A plot of the availabilities (as redefined above) of the three fractions' associated-C forms is shown in Fig. 5.3. The plot suggests that when the quantity of cellobiose C present is less than about one-fifth the amount of fraction-associated carbon, the availabilities of the different fractions' carbon are similar. When the readily available carbon is one-fifth to three-fourths the fraction-associated carbon, the carbon associated with the fine clay is more available than that associated with either of the other two fractions. Data were insufficient to compare carbon availabilities among the fractions beyond a priming ratio of about 0.75.

Stage II: Mineralization of Biomass Carbon Without Utilization of Fraction-Associated Carbon

The respiration peak of Stage I was assumed to end as a result of the exhaustion of readily available carbon substrates. The Stage I carbon data indicated that both cellobiose and fraction-associated carbon were being readily utilized.

The increase in biomass over Stage I (Table 5.2) indicated that this was a period of net immobilization. The decrease in biomass over Stage II (Table 5.5) indicated that Stage II was a period of mineralization of nutrients immobilized into the biomass developed during Stage I. For such mineralization to occur, autolysis of, and predation on, bacterial cells must have been the predominant activities during Stage II. If this were the case, then the microbial biomass present at the end of Stage I must have been the source of C respired to CO_2 and therefore Stage II cumulative respiration should not exceed the decrease in microbial biomass C that occurred during

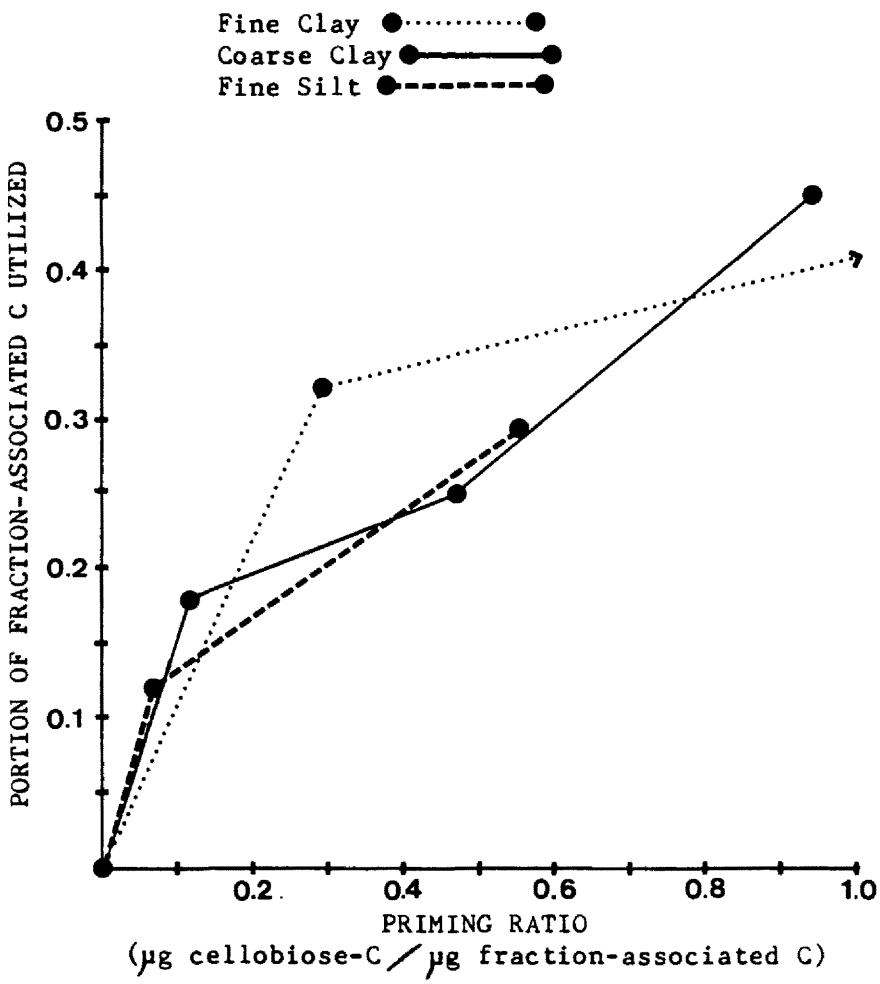


Fig. 5.3. Effects of the priming ratio on the availability (utilization) of fraction-associated carbon

Table 5.5. Carbon transformations during Stage II

Fraction	Cellobiose carbon amendment level	Biomass C present after		Decrease in biomass C during Stage II	Stage II cumulative respiration (CR)	Stage II CR as proportion of decrease in biomass C
		Stage I (calculated)	Stage II (determined)			
		(μg C per 10-g microcosm)				
Fine clay	1250	8930	4880	4050	1030	0.25
	5000	11450	6090	5360	1880	0.35
	10000	16430	6860	9570	2840	0.30
Coarse clay	1250	9270	5250	4020	900	0.22
	5000	11970	5710	6260	1790	0.29
	10000	16200	8720	7480	2900	0.39
Fine silt	1250	9380	3330	6050	1230	0.20
	5000	12470	4520	7950	1950	0.25
	10000	16490	6510	9980	3160	0.32

Stage II. The Stage II carbon results presented in Table 5.5 show that the cumulative respiration during Stage II accounted for 20-39% of the decrease in microbial biomass C. Therefore, it was concluded that Stage II was a "mineralization" period during which most microbial respiration was at the expense of the bacterial biomass produced during Stage I.

Conclusions

1. Microbial utilization of fraction-associated C occurred for all fractions.

2. Microbial utilization of fraction-associated C occurred for all levels of microbial activity. No utilization occurred without initiation of microbial activity by use of a cellobiose carbon amendment.

3. The availability of fraction-associated C is more closely related to the amount of cellobiose C initially added than to the quality of the O.M. or of the particles with which it is associated (H_1 was not well supported but H_2 was).

4. Within the range of conditons tested, the fine clay's associated C is probably slightly more available than that of the coarse clay and fine silt fractions.

5. All utilization of fraction-associated C occurred during Stage I, the period of bacterial growth.

6. The principal C transformation during Stage II was "mineralization" (release) of nutrients immobilized (assimilated) into the bacterial biomass during Stage I.

7. At each C-amendment level all three fractions were subjected to comparable levels of microbial activity.

8. The data did not indicate that either chemical recalcitrance or adsorption of O.M. on soil particles caused important stabilization of organic matter by reducing its biochemical availability to soil microbes.

CHAPTER 6

MICROBIAL TRANSFORMATIONS OF FRACTION-ASSOCIATED PHOSPHORUS

Introduction

The objective of this study was to compare the three soil fractions with respect to the transformations of P measurable after similar amounts of microbial activity (as indicated by respiration and biomass data). In order to make comparisons as efficiently as possible, it was decided to compare the changes (increases or decreases, ΔP) seen in each extraction pool (using the statistical methods described in chapter 4) over Stage I (ΔT_I), Stage II (ΔT_{II}), and after both Stages I and II (ΔT_{I+II}) among the nine fraction × C treatments. This is in fact an analysis of differential effects, differentiating phosphorus with respect to time, $\Delta P/\Delta T$ (interval). The changes during the 0- to 124-hour initial peak respiration Stage (Stage I) reflect changes due to microbial P uptake, which was the dominant process during this time interval. Changes occurring during the 124- to 814-hour secondary, relatively steady respiration stage (Stage II) indicate mineralization of that P immobilized into the the biomass during Stage I. The 0- to 814-hour changes indicated net effects after a full immobilization/mineralization cycle had been completed. (Means of the inorganic (P_i) and total phosphorus (P_T) results for each extraction for each treatment and sample date are shown in Appendix B.) No statistical analysis of the 814- to 1584-hour

incubation period (Stage III) data was attempted since detectable respiration had ceased at 814 hours and it would be difficult even to speculate whether the changes seen after this were induced by low-level biotic or abiotic factors or some unknown combination of the two.

A summary of the AOV of the $\Delta P/\Delta Ts$ is in Table 6.1. Appendix C contains the detailed results of the statistical analysis (AOV) of the effects of the different fraction by C amendment level treatments on the differential of phosphorus with respect to time interval. The results of this AOV ($\Delta P/\Delta Fs$) are discussed in conjunction with the presentation of the plots of data means for each P extraction pool. This chapter presents and discusses the quantities of P extracted in each step of the sequential extraction procedure (see chapter 4) for the various fraction × C-level treatments and sample times. The bicarbonate- and alkali-extractable P_i results are followed by the bicarbonate- and alkali-extractable P_o, the acid-extractable P, and the total extractable P results.

Inorganic Phosphorus in the Bicarbonate and Sodium Hydroxide Extracts

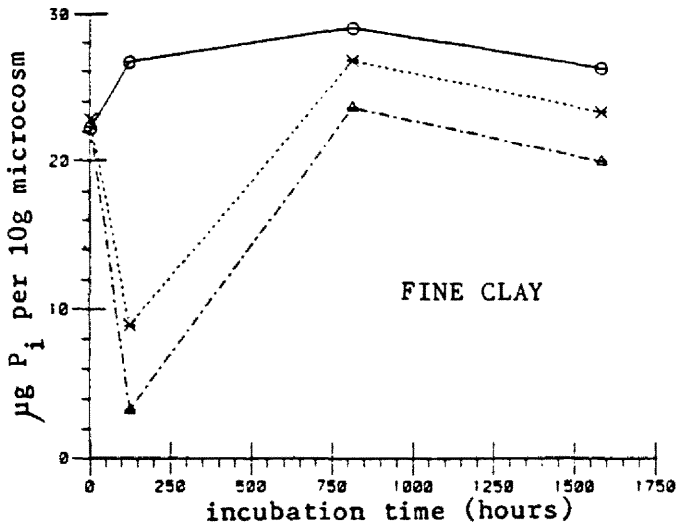
The 3-Minute 0.15 M $NaHCO_3$ P_i

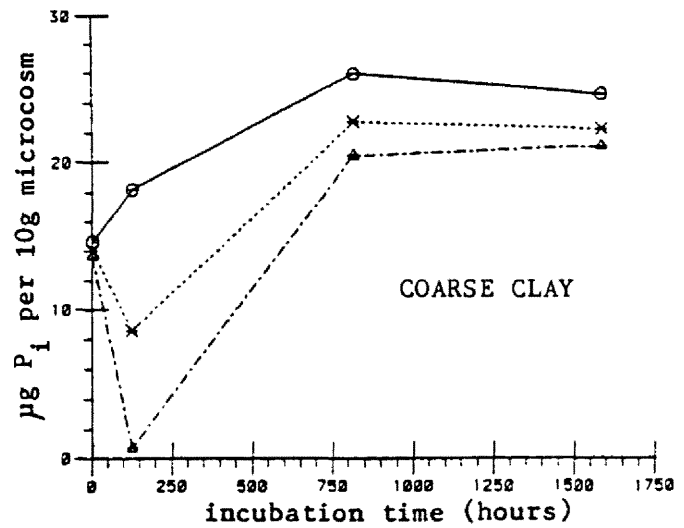
For Stages I and II the effects of fractions, C-amendment levels, and their interactions were all highly significant for the 0.15 M $NaHCO_3$ P_i (Table 6.1). Over the 814-hour period the net effects of fractions and C levels retained their high significance, while those of interaction became marginally significant. The plots of the data means suggest similar importance for fraction and C amendment level (Fig. 6.1).

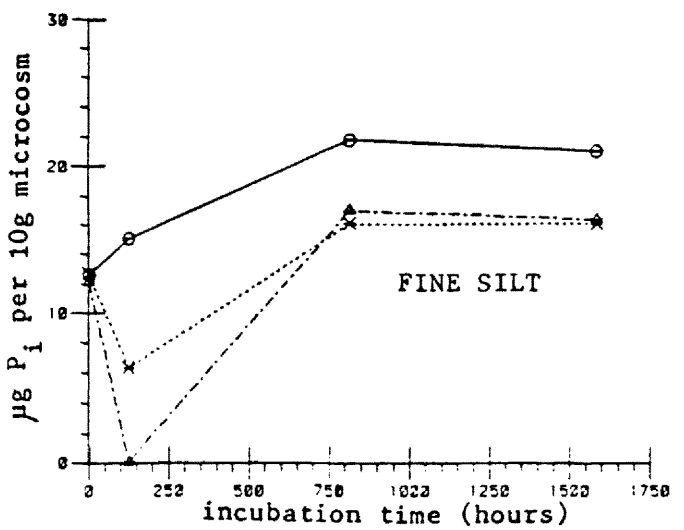
Table 6.1. Summary of statistical analyses of changes (over specific time intervals) in P extracted from various particle size fractions as affected by C amendment

Source	Significance of F-value								
	0.15 M $NaHCO_3$		0.5 M $NaHCO_3$		NaOH		HCl		
	P_i	P_o	P_i	P_o	P_i	P_o	P_i	P_o	Unextractable
Stage I (period of increasing biomass)									
Fractions	<0.001	<0.001	0.003	<0.001	NS	0.001	NS	NS	0.017
C level	<0.001	0.004	NS	0.058	NS	0.014	NS	NS	NS
Fractions × C level	<0.001	NS	NS	0.045	NS	NS	NS	NS	NS
Stage II (period of decreasing biomass)									
Fractions	<0.001	<0.001	NS	0.024	<0.001	0.030	NS	NS	NS
C level	<0.001	<0.001	0.001	0.058	0.104	NS	NS	NS	NS
Fractions × C level	<0.001	NS	NS	0.018	NS	NS	NS	NS	NS
Net effects (Σ Stage I + Stage II)									
Fractions	<0.001	0.017	0.007	NS	<0.001	0.111	NS	NS	NS
C level	<0.001	NS	0.007	0.007	NS	NS	NS	NS	NS
Fractions × C level	0.109	NS	NS	0.030	NS	NS	NS	NS	0.023

Fig. 6.1. The 0.15 M $NaHCO_3$ P_i results. The effects of carbon amendment level (o = 1250, x = 5000, Δ = 10000 μg cellobiose C per 10-g microcosm) on the inorganic phosphorus (P_i) extracted from each soil particle size fraction treatment (0.185 g fine clay, 0.313 g coarse clay, 0.417 g fine silt fraction per 10-g microcosm) by suspending each 10-g microcosm in 30 ml of 0.15 M $NaHCO_3$ and shaking for three minutes. Each data point shown indicates the mean over three microcosms for each treatment on each sampling date.


30
20
10
0
µg P_i per 10g microcosm
FINE CLAY
0
250
500
750
1000
1250
1500
1750
incubation time (hours)


30
20
10
0
µg P_i per 10g microcosm
COARSE CLAY
0
250
500
750
1000
1250
1500
1750
incubation time (hours)


30
20
10
0
µg P_i per 10g microcosm
FINE SILT
0
250
500
750
1000
1250
1500
1750
incubation time (hours)

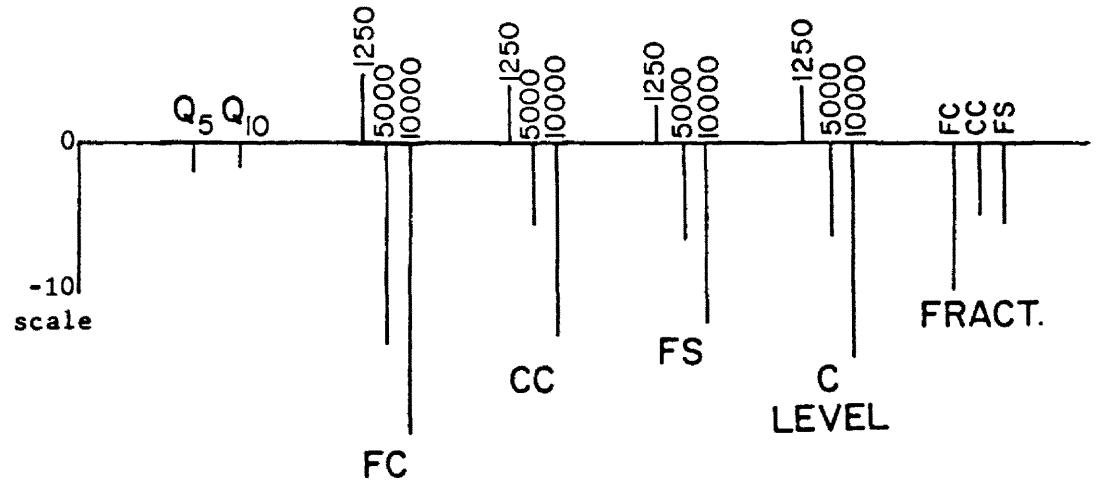
The lowest C amendment gave the highest 0.15 M $NaHCO_3$ P_i levels after microbial activity for all fractions. The upper plot is of the 0.15 M $NaHCO_3$-extractable P_i results for the FC with the indicated C amendment levels. A similar pattern was observed for all three fractions (Fig. 6.1). At the lowest C amendment level all three fractions showed increases in "water" P_i by 124 hours incubation. At the higher C levels a decrease was seen to 124 hours, followed by an increase to 814 hours.

An examination of the detailed results of the AOV (Fig. 6.2) indicated the significant differences among the fractions and C levels. (For convenience, the details of the AOV--where significant differences appear--are presented graphically in appropriate places in the text. As mentioned earlier, the complete details are tabulated in Appendix C.) During Stage I the FC showed an increase in 0.15 M $NaHCO_3$ P_i at the 1250 C level, significantly greater than that of the fine silt but not that of the coarse clay. Over all C levels the 0.15 M $NaHCO_3$ P_i in the FC decreased significantly more during the uptake period than it did in the FS or CC. There were significant differences among the C levels. 0.15 M $NaHCO_3$ P_i increased at the lowest C level while decreasing at the 5000 µg C and even more at the 10,000 µg C levels during Stage I.

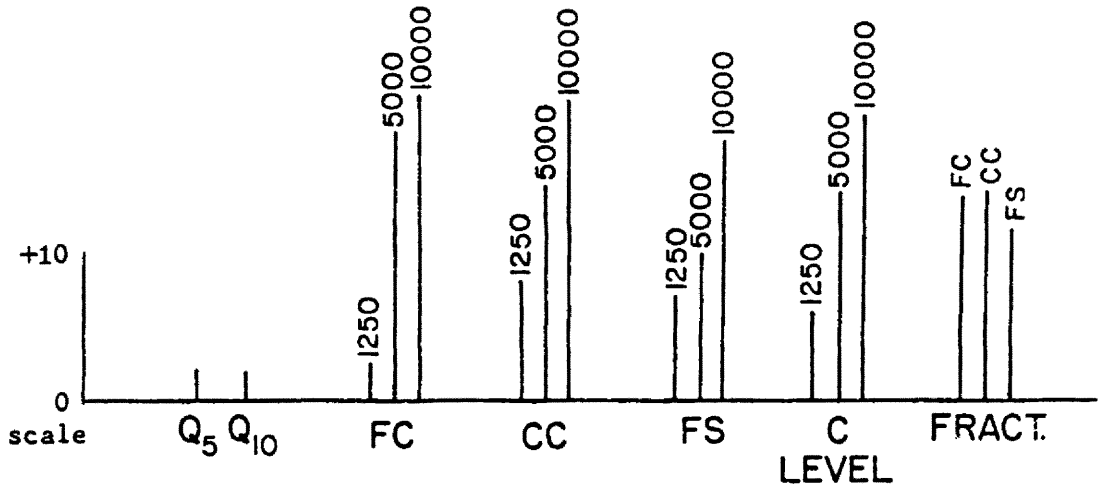
During the "remineralization" period, Stage II, 0.15 $NaHCO_3$ P_i increases significantly less for the FC at 1250 µg C level than for the CC and FS. However, at the 5000 µg C level in the FC this P_i form increased significantly more than in the CC, where it did more than in the fine silt. At the 10,000 µg C level the FC and CC had greater increases in 0.15 M $NaHCO_3$ P_i than the FS. During Stage II over all C

Fig. 6.2. Changes in the inorganic phosphorus extracted by 0.15 M $NaHCO_3$ occurring over respiration Stages I and II and total respiration. Vertical lines in a set represent the indicated treatment effects as modifiers of the effect of the treatment indicated below the set. All data are per 10-g microcosm. Vertical line on far left is a scaling line equivalent to 10 μg P/10-g microcosm. Q = Tukey's Q; FC = 0.185 g fine clay; CC = 0.313 g coarse clay; FS = 0.417 g fine silt; 1250, 5000, 10000 indicate C amendment levels in μg cellobiose C/10-g microcosm; C LEVEL = main effects of C amendment levels; FRACT = main effects of soil particle fractions. Results are presented only when at least two of the mean treatment effects were significantly different.

STAGE I -- Period of increasing biomass



STAGE II -- Period of decreasing biomass



Net changes after all respiration (ΣStages I & II)

levels the FC and CC had greater increases in 0.15 M $NaHCO_3$ P_i than did the FS, while over all fractions the 1250 µg C level had significantly less increase in this P_i form than the 5000 µg C level, which had less than the 10,000 µg C level, indicating that the amount of P returned into this pool during Stage II was directly related to the amount of biomass accumulated during Stage I. The generally greater changes in the 0.15 M $NaHCO_3$ inorganic P pool in the FC probably indicated a larger and more active exchange capacity than existed in the other fractions; that is, this pool had greater relative importance in the FC than in the other fractions.

The 0.15 M $NaHCO_3$ inorganic P results are of particular interest since, in the extraction procedure used, this extraction pool was the only one that assessed P status before lysis of the biomass. That is, it was the index of "plant available" solution inorganic P levels and directly measured net (but not total) mineralization. Examination of the results (Figs. 6.1 and 6.2) indicated that net mineralization occurred two ways. At the lowest C amendment level, but not at the higher levels, net mineralization occurred during Stage I and was sustained throughout the incubation. Net mineralization at the two higher C levels occurred by the end of Stage II. Apparently, considerable mineralization of soil-fraction-associated organic P occurred during Stage I for all C levels but at the higher C levels the rapidly increasing microbial biomass assimilated any available P. This assimilated P was released during Stage II, a portion of which appeared as net mineralization in the 0.15 M $NaHCO_3$ inorganic P pool. The final net mineralization (after Stage II) was significantly higher for the 1250 C level. Stage I net mineralization was higher for the

FC than the FS. By the end of Stage II, the CC showed a net mineralization significantly greater than the FC or FS.

The above information was adequate to allow consideration of the hypotheses: Under similar environmental conditions, the extent of microbial utilization of the nutrients in soil organic matter increases as (H_1) the molecular weight and aromaticity of the organic matter decreases, and (H_2) the level of microbial activity increases. Microbial utilization of organic P must be examined as microbially induced total mineralization of organic P. Total mineralization is the sum of net mineralization and uptake. The microbial biomass, among the soil fraction treatments at each C level, developed under similar nutritional and environmental conditions. It was therefore presumed that the biomass P levels were similar for all the soil fractions at each amendment C level. Further, over all the soil fraction treatments at the 5000 and 10000 μg C amendment levels, it was presumed that all available P was rapidly taken up, while at the 1250 μg level some "luxury consumption" by the microbes may have occurred. Thus, at each C level uptake can be considered equal for all three fraction treatments. Since uptake was a constant among the soil fraction treatments, total mineralization can be indexed by net mineralization.

The associated O.M. of the FC and CC fractions had lower molecular weight and aromaticity than that of the FS. The significantly higher net mineralization for the FC fraction treatment during Stage I and the CC at the end of Stage II indicated that the relative availability of organic P does increase with decreasing molecular weight and

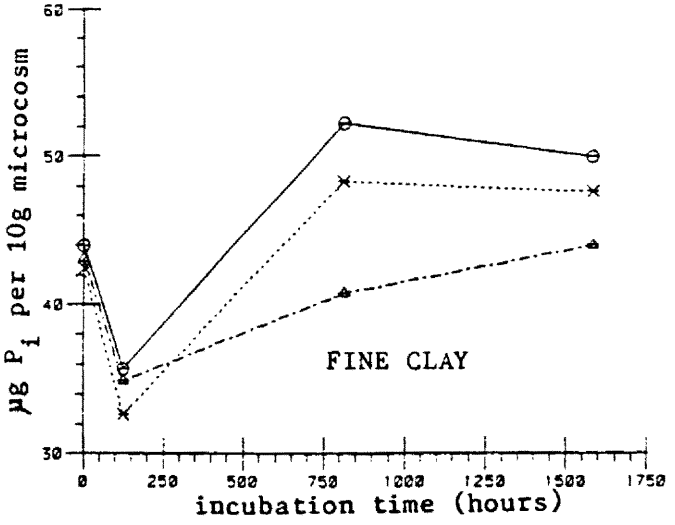
aromaticity. (Reasons for the Stage I predominance of the FC and the Stage II of the CC will become apparent later in the text.)

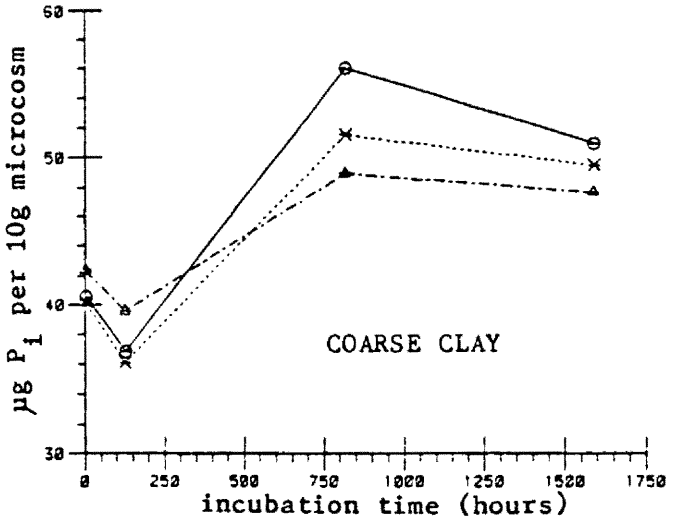
In a similar fashion, if total mineralization was proportional to microbial activity--that is, to C amendment levels--then net mineralization should be equal for all C level treatments. This was not the case. Further, as stated above, by the end of Stage I net mineralization had occurred at the 1250 C level, with net immobilization at the two higher C levels. Significantly less immobilization occurred at the 5000 than at the 10000 C level. Thus, although total organic P mineralization likely increased with increased microbial activity (P stress), it did not increase proportionately. An interesting relationship between C stress and organic P mineralization is suggested by these results. As C stress increases (microbial activity decreases), mineralization of organic P does not decrease proportionately. Apparently, there is an optimum soluble-C level to provide maximum net mineralization of organic P.

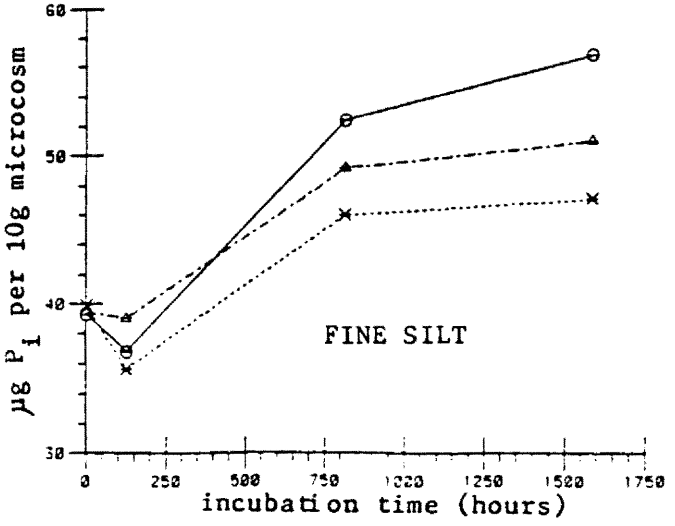
The 16-Hour 0.5 M $NaHCO_3$ P_i

The 0.5 M $NaHCO_3$ P_i decreased for all treatments during Stage I (see Fig. 6.3). The size of the decrease appeared to be related to both fraction and C treatment. It is important to recall that the biomass was lysed with chloroform before the bicarbonate extraction. The bicarbonate P_i pool was severely taxed by the high microbial activity (growth) in Stage I. Upon lysis the biomass P_i was released; almost all appeared as bicarbonate extract P_i. This makes the bicarbonate P_i results for the 124-hour sample time very interesting in that there were no differences between any treatments. One

Fig. 6.3. The 0.5 M $NaHCO_3$ P_i results. The effects of carbon amendment level (o = 1250, x = 5000, Δ = 10000 μg cellobiose C per 10-g microcosm) on the inorganic phosphorus (P_i) extracted from each soil particle size fraction treatment (0.185 g fine clay, 0.313 g coarse clay, 0.417 g fine silt fraction per 10-g microcosm) by suspending the previously extracted residue of each 10-g microcosm in 30 ml of 0.5 M $NaHCO_3$ and shaking for 16 hours. Each data point shown indicates the mean over three microcosms for each fraction × C level treatment on each sampling date.


µg P_i per 10g microcosm
60
50
40
30
0
250
500
750
1000
1250
1500
1750
incubation time (hours)
FINE CLAY


µg P_i per 10g microcosm
60
50
40
30
0
250
500
750
1000
1250
1500
1750
incubation time (hours)
COARSE CLAY


µg P_i per 10g microcosm
60
50
40
30
0
250
500
750
1000
1250
1500
1750
incubation time (hours)
FINE SILT

interpretation is that the exchangeable P_i pool was nearly exhausted with all treatments. The biomass P_i upon lysis restored the bicarbonate P_i to equal levels for all treatments, indicating lower concentration of P_i in the biomass as C level increased, a possible response to P stress.

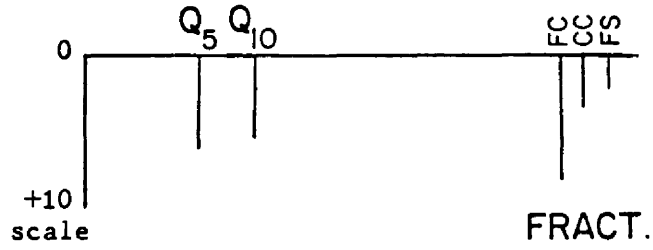
The analysis of variance (Table 6.1) indicated that fractions had significant effects only during Stage I, C levels only during Stage II, while both had significant net (over the 814 hours) effects. The details of the AOV (Fig. 6.4) showed that during Stage I the only significant difference was that the 0.5 $\underline{M}$ $NaHCO_3$ P_i of the FC over all C levels decreased more than did that of the CC or of the FS. During Stage II changes were correlated with C level but not fraction; the lower the C level the greater the increase in 0.5 $\underline{M}$ $NaHCO_3$ P_i during this period. Over the 0- to 814-hour period 0.5 $\underline{M}$ $NaHCO_3$ P_i, as did 0.15 $NaHCO_3$ P_i, increased most over all fractions at the lowest C level and over all C levels most in the CC.

The 16-Hour 0.1 $\underline{N}$ NaOH P_i

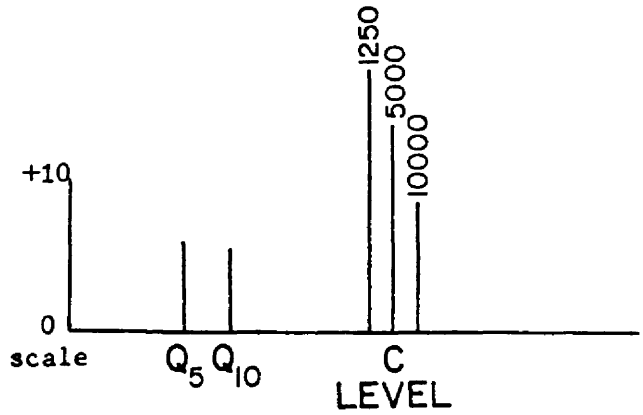
The alkali-extractable P_i (see plots in Fig. 6.5) showed marked decreases through 814 hours of incubation (the end of the detectable respiration). The AOV of data means (Table 4.2) indicated that the alkali P_i levels were correlated only to time and fraction. Thus, the AOV of the changes over time (Table 6.1) of alkali P_i indicated no correlation to any treatments during Stage I, even though there was a small decrease in this P_i form for all treatments. During Stage II, decreases in alkali P_i were strongly correlated with the fractions, the FC decreasing less than the CC or FS (Table 6.1, Fig. 6.6). The

Fig. 6.4. Changes in the inorganic phosphorus extracted by 0.5 M $NaHCO_3$ occurring over respiration Stages I and II and total respiration. Vertical lines in a set represent the indicated treatment effects as modifiers of the effect of the treatment indicated below the set. All data are per 10-g microcosm. Vertical line on far left is a scaling line equivalent to 10 μg P/10-g microcosm. Q = Tukey's Q; FC = 0.185 g fine clay; CC = 0.313 g coarse clay; FS = 0.417 g fine silt; 1250, 5000, 10000 indicate C amendment levels in μg cellobiose C/10-g microcosm; C LEVEL = main effects of C amendment levels; FRACT = main effects of soil particle fractions. Results are presented only when at least two of the mean treatment effects were significantly different.

STAGE I -- Period of increasing biomass



STAGE II -- Period of decreasing biomass



Net changes after all respiration (≤Stages I & II)

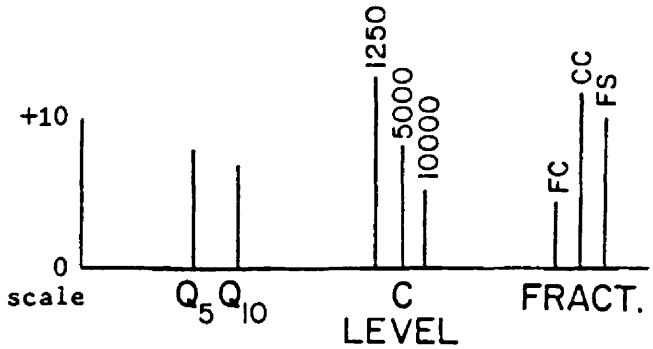
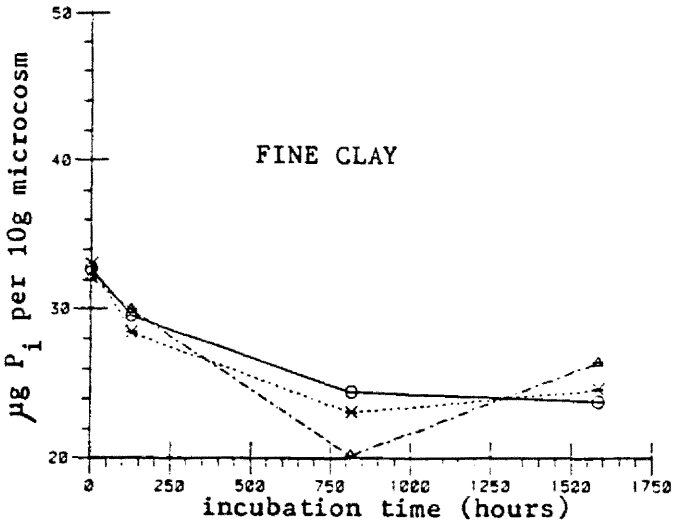
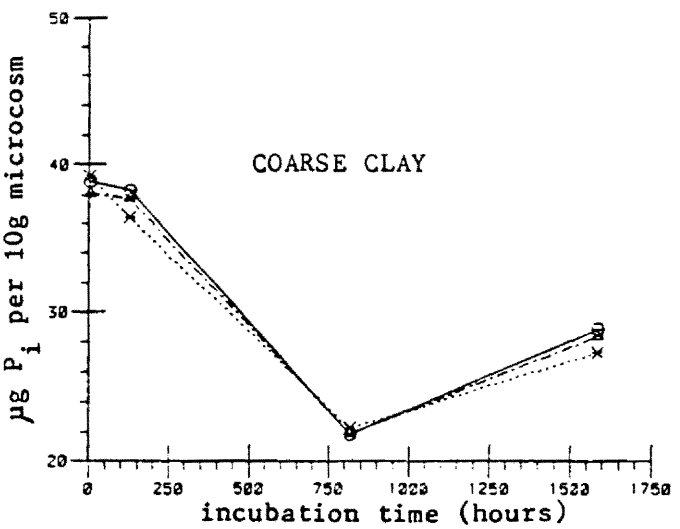


Fig. 6.5. The NaOH P_i results. The effects of carbon amendment level (o = 1250, x = 5000, Δ = 10000 μg cellobiose C per 10-g microcosm) on the inorganic phosphorus (P_i) extracted from each soil particle size fraction treatment (0.185 g fine clay, 0.313 g coarse clay, 0.417 g fine silt fraction per 10-g microcosm) by suspending the previously extracted residue of each 10-g microcosm in 30 ml of 0.1 N NaOH and shaking for 16 hours. Each data point shown indicates the mean over three microcosms for each fraction × C level treatment on each sampling date.


FINE CLAY
µg P_i per 10g microcosm
incubation time (hours)
50
40
30
20
0
250
500
750
1000
1250
1500
1750


COARSE CLAY
µg P_i per 10g microcosm
incubation time (hours)
50
40
30
20
0
250
500
750
1000
1250
1500
1750

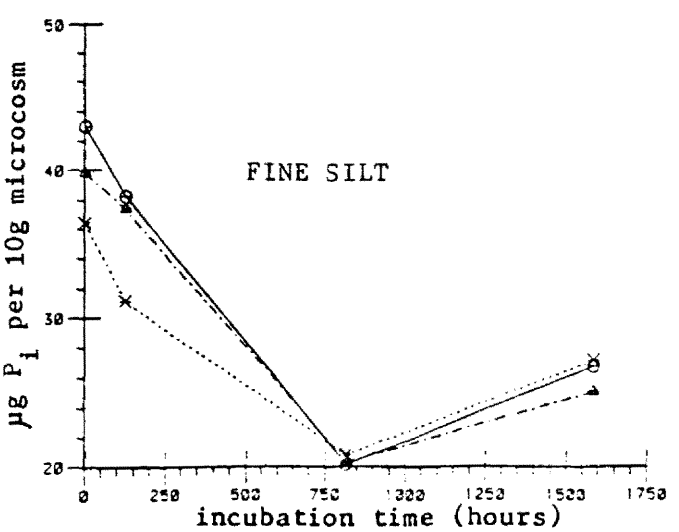
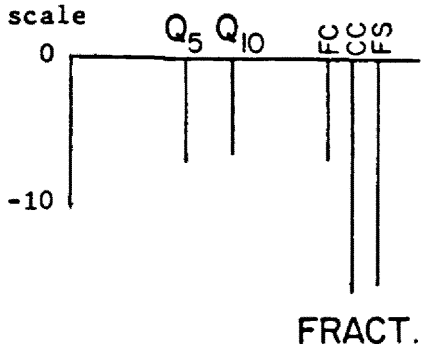

FINE SILT
µg P_i per 10g microcosm
incubation time (hours)
50
40
30
20
0
250
500
750
1000
1250
1500
1750

Fig. 6.6. Changes in the inorganic phosphorus extracted by 0.1 M NaOH occurring over respiration Stages I and II and total respiration. Vertical lines in a set represent the indicated treatment effects as modifiers of the effect of the treatment indicated below the set. All data are per 10-g microcosm. Vertical line on far left is a scaling line equivalent to 10 μg P/10-g microcosm. Q = Tukey's Q; FC = 0.185 g fine clay; CC = 0.313 g coarse clay; FS = 0.417 g fine silt; 1250, 5000, 10000 indicate C amendment levels in μg cellobiose C/10-g microcosm; C LEVEL = main effects of C amendment levels; FRACT = main effects of soil particle fractions. Results are presented only when at least two of the mean treatment effects were significantly different.

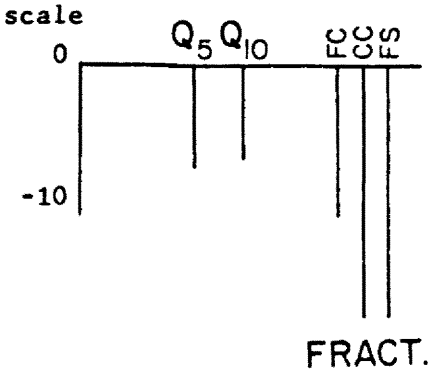
STAGE I -- Period of increasing biomass

(No treatment effects were significantly different from zero)

STAGE II -- Period of decreasing biomass



Net changes after all respiration (ΣStages I & II)



peculiar continuous decreases in this P_i form seem to indicate a dissolution process, presumably of reduced mineral phosphates exposed in the fractions during ultrasonic dispersion of soil microaggregates (reduced microsites; see chapter 3). The increases seen (Fig. 6.6) in Stage III (814-1584 hours) were probably the result of precipitation of P_i into alkali-extractable mineral forms during this apparently abiotic period.

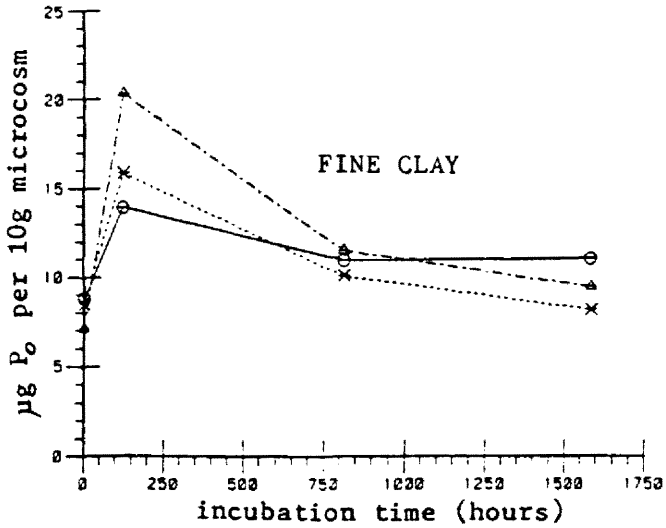
Organic Phosphorus in the Bicarbonate and Sodium Hydroxide Extracts

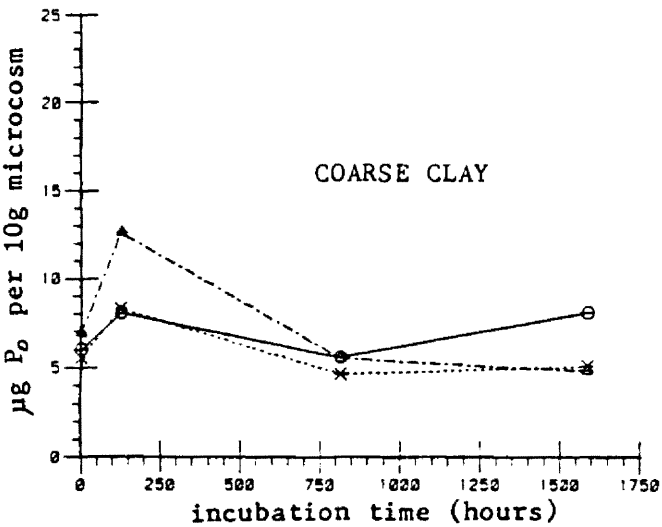
The 3-Minute 0.15 M $NaHCO_3$ P_o

There seemed to be a peak in this P_o pool at 124 hours for all fractions (see Fig. 6.7), the height of which might be related to the C amendment level and fraction. Beyond 124 hours, 0.15 $NaHCO_3$ P_o values seem to decline to similar levels for CC and FS and a somewhat higher level for the FC. Turning to the summary of the AOV (Table 6.1), the entries for the 0.15 M $NaHCO_3$ P_o pool indicate significant Stage I and Stage II effects for fractions and C levels. Net (814-hour) effects were significant only for the fractions.

The details of the AOV ($\Delta P/\Delta T$, Fig. 6.8) show that during Stage I the 0.15 M $NaHCO_3$ P_o increased more over all fractions at the highest C level and over all C levels in the FC. During Stage II similar differences but opposite in direction occurred. The net changes over the entire 814 hours were signficantly different only between the FC, which showed a small increase in, and the CC, which showed a small decrease in, 0.15 M $NaHCO_3$ P_o. Again, as with the 0.15 M $NaHCO_3$ P_i, the changes in the 0.15 M $NaHCO_3$ P_o pool during Stage I were greater

Fig. 6.7. The 0.15 M $NaHCO_3$ P_o results. The effects of carbon amendment level (o = 1250, x = 5000, Δ = 10000 μg cellobiose C per 10-g microcosm) on the organic phosphorus (P_o) extracted from each soil particle size fraction treatment (0.185 g fine clay, 0.313 g coarse clay, 0.417 g fine silt fraction per 10-g microcosm) by suspending each 10-g microcosm in 30 ml of 0.15 M $NaHCO_3$ and shaking for three minutes. Each data point shown indicates the mean over three microcosms for each treatment on each sampling date.


FINE CLAY
µg P_o per 10g microcosm
incubation time (hours)
0
5
10
15
20
25
0
250
500
750
1000
1250
1500
1750


COARSE CLAY
µg P_o per 10g microcosm
incubation time (hours)
0
5
10
15
20
25
0
250
500
750
1000
1250
1500
1750

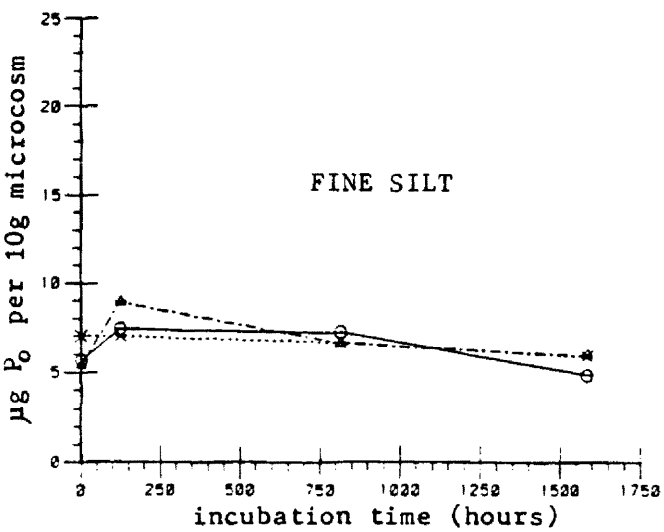
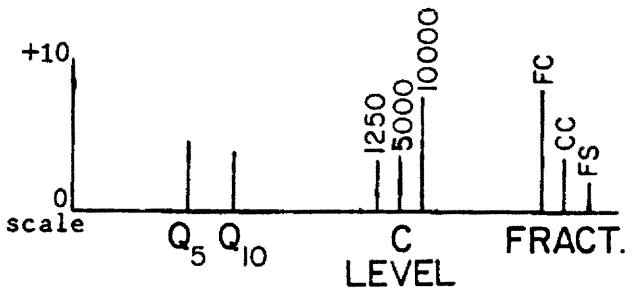
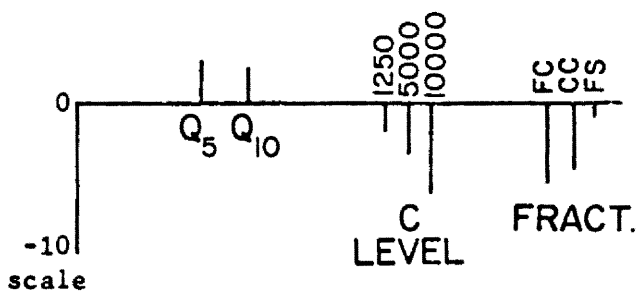

FINE SILT
µg P_o per 10g microcosm
incubation time (hours)
0
5
10
15
20
25
0
250
500
750
1000
1250
1500
1750

Fig. 6.8. Changes in the organic phosphorus extracted by 0.15 M $NaHCO_3$ occurring over respiration Stages I and II and total respiration. Vertical lines in a set represent the indicated treatment effects as modifiers of the effect of the treatment indicated below the set. All data are per 10-g microcosm. Vertical line on far left is a scaling line equivalent to 10 μg P/10-g microcosm. Q = Tukey's Q; FC = 0.185 g fine clay; CC = 0.313 g coarse clay; FS = 0.417 g fine silt; 1250, 5000, 10000 indicate C amendment levels in μg cellobiose C/10-g microcosm; C LEVEL = main effects of C amendment levels; FRACT = main effects of soil particle fractions. Results are presented only when at least two of the mean treatment effects were significantly different.

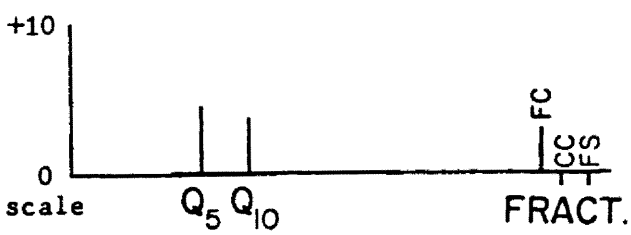
STAGE I -- Period of increasing biomass



STAGE II -- Period of decreasing biomass



Net changes after all respiration (ΣStages I & II)



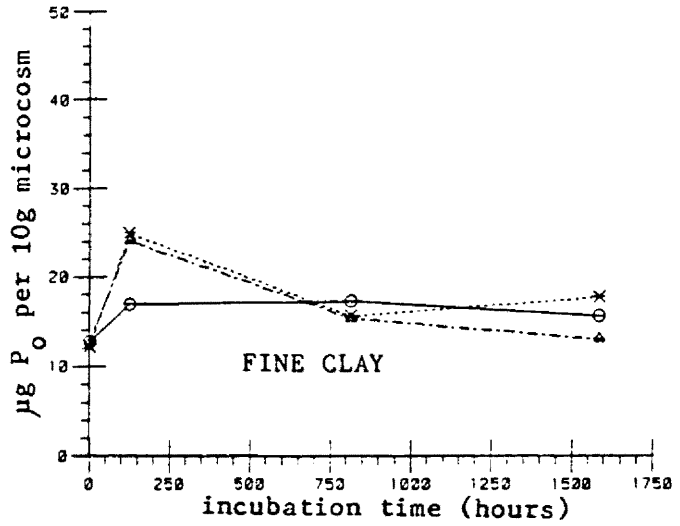
for the FC. This probably was due to a capacity of the FC to stabilize (adsorb) more P_o (probably released by mechanical disturbance of the biomass) into readily extractable forms than the CC or FS, which presumably took the same P_o into less extractable forms. This probably was a factor causing the FC to be the only fraction to show a net (814-hour) increase in this P form. The changes in P_o during stages I and II related to C level (biomass) as expected within the limitations of the techniques used.

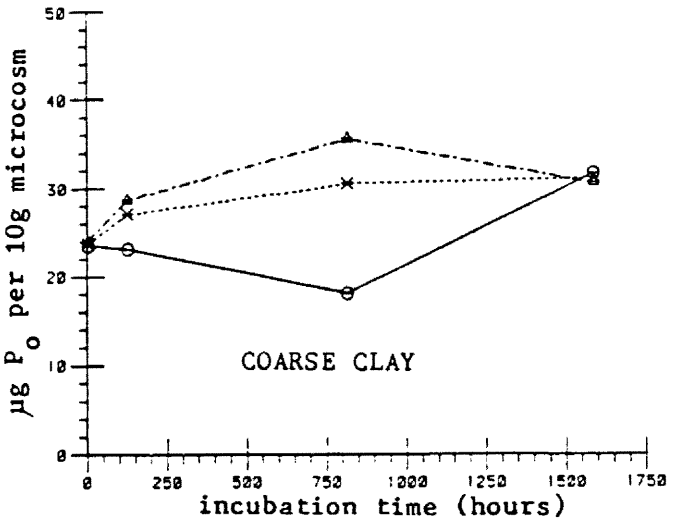
The 16-Hour 0.5 M $NaHCO_3$ P_o

The 0.5 M bicarbonate-extractable P_o means (Fig. 6.9) generally showed initial increases (to 124 hours), followed by later decreases. The FC seemed to show greater initial increases and more consistent (over all levels) subsequent behavior in this P pool than the CC or FS. Referring to the AOV (Table 6.1), the changes in this P_o pool occurring during Stage I were highly correlated to the fractions but only marginally to the C level or fraction by C interaction. During Stage II interaction becomes important along with the fractions. Net (over the 814 hours) changes, however, were most significantly related to C level.

Details of the AOV (Fig. 6.10) indicated that during Stage I the FC showed a greater increase in 0.5 M bicarbonate P_o than did the CC and FS. During Stage II at the highest C level the FC had a large decrease, while the other two fractions increased. The net (Σ over Stages I and II) changes showed at the highest C level, the FC and CC had smaller increases than did the FS and that over all fractions, the highest C level gave the largest increase in bicarbonate P_o.

Fig. 6.9. The 0.5 M $NaHCO_3$ P_o results. The effects of carbon amendment level (o = 1250, x = 5000, Δ = 10000 μg cellobiose C per 10-g microcosm) on the inorganic phosphorus (P_i) extracted from each soil particle size fraction treatment (0.185 g fine clay, 0.313 g coarse clay, 0.417 g fine silt fraction per 10-g microcosm) by suspending the previously extracted residue of each 10-g microcosm in 30 ml of 0.5 M $NaHCO_3$ and shaking for 16 hours. Each data point shown indicates the mean over three microcosms for each fraction × C level treatment on each sampling date.


µg P_o per 10g microcosm
FINE CLAY
incubation time (hours)


µg P_o per 10g microcosm
COARSE CLAY
incubation time (hours)

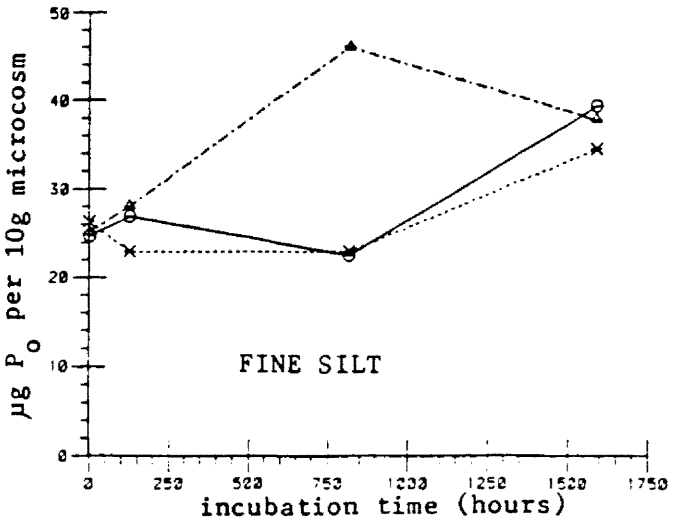
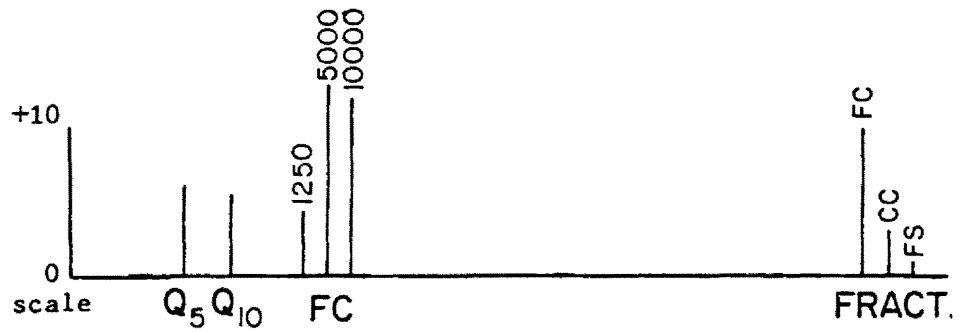
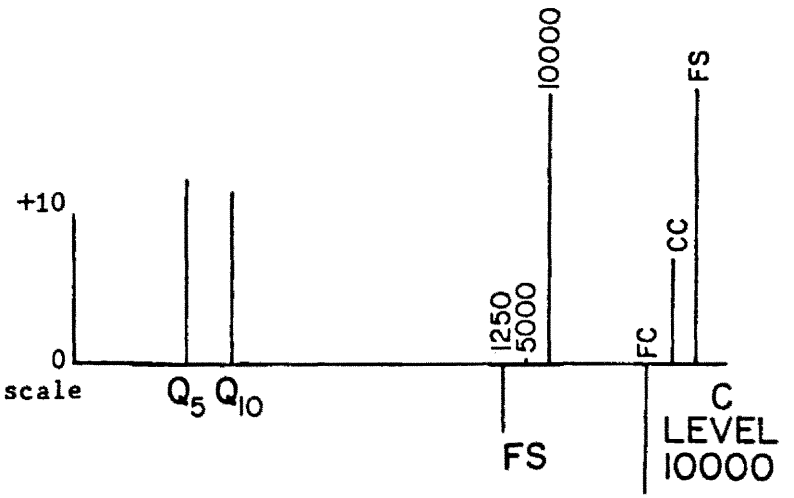

µg P_o per 10g microcosm
FINE SILT
incubation time (hours)

Fig. 6.10. Changes in the organic P extracted by 0.5 M $NaHCO_3$ occurring over respiration Stages I and II and total respiration. Vertical lines in a set represent the indicated treatment effects as modifiers of the effect of the treatment indicated below the set. All data are per 10-g microcosm. Vertical line on far left is a scaling line equivalent to 10 μg P/10-g microcosm. Q = Tukey's Q; FC = 0.185 g fine clay; CC = 0.313 g coarse clay; FS = 0.417 g fine silt; 1250, 5000, 10000 indicate C amendment levels in μg cellobiose C/10-g microcosm; C LEVEL = main effects of C amendment levels; FRACT = main effects of soil particle fractions. Results are presented only when at least two of the mean treatment effects were significantly different.

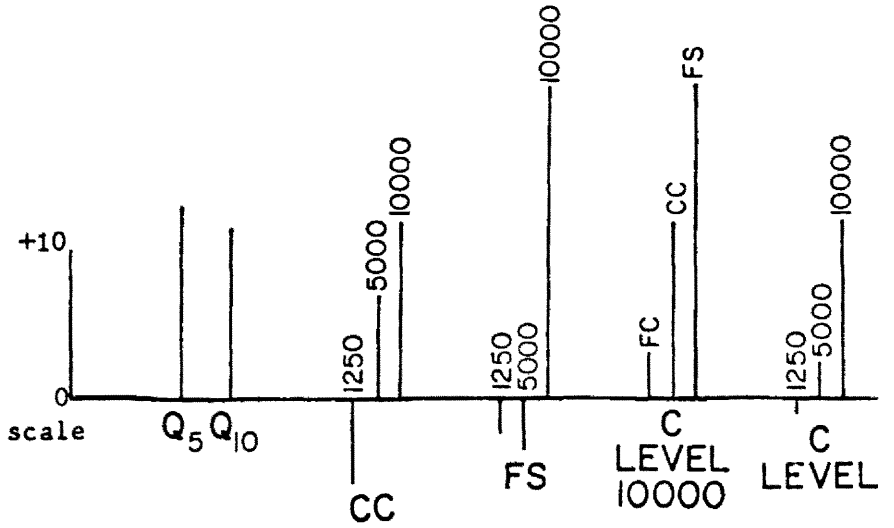
STAGE I -- Period of increasing biomass



STAGE II -- Period of decreasing biomass



Net changes after all respiration (Stages I & II)

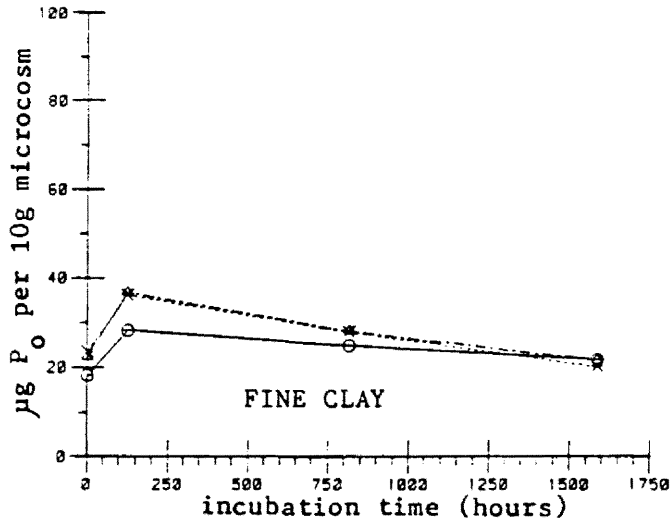


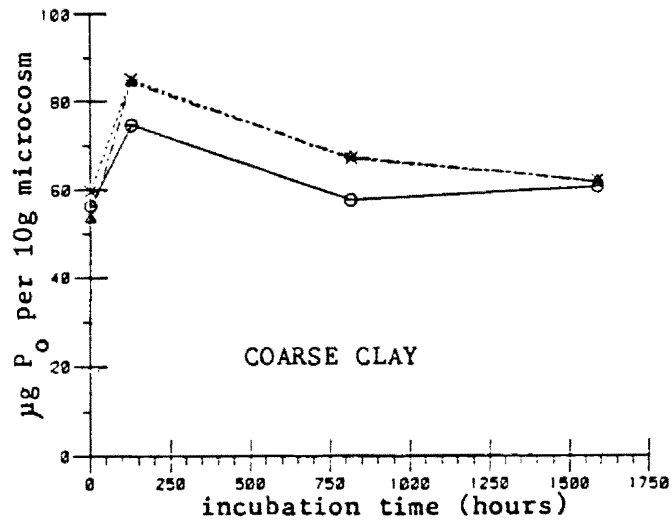
The FC was the fraction that appeared most dynamic with respect to the 0.5 M $NaHCO_3$ P_o pool as well as the 0.15 M $NaHCO_3$ P_o pool. At the end of Stage I, when equivalent biomass P_o was released (during chloroform lysis)into each fraction, more was retained in the bicarbonate P_o pool by the FC than by the CC or FS. Significant interaction of fractions and C levels complicated further interpretations.

The 16-Hour 0.1 M NaOH P_o

The alkali-extractable P_o (see plots Fig. 6.11) showed an increase during Stage I, decreasing to near initial levels during Stage II. The AOV ($\Delta P/\Delta Ts$) (Table 6.1) indicated that these changes were correlated with fraction and C level during Stage I but only to fraction during Stage II. The details of the AOV (Fig. 6.12) indicated that the changes in the NaOH P_o pool were significantly different for C levels and fractions, though not among individual treatments. During Stage I over all C levels the NaOH P_o in the CC increased significantly more than in the FC or FS and the 10,000 µg C level gave a significantly greater increase than did the 1250 µg C level. During Stage II the only significant difference was between the coarse clay, which decreased by 17.3 µg P_o, and the fine clay, which decreased by only 6.8 µg P_o, per microcosm. Over the whole 814 hours there were no significant differences among treatments and no correlation by the AOV. For this pool the CC was the most dynamic fraction, followed by FS and finally the FC. Apparently, the CC may have been adsorbing into the alkali-extractable pool some of the same P_o the FC was holding in the 0.15 M and/or 0.5 M bicarbonate-extractable

Fig. 6.11. The NaOH P_o results. The effects of carbon amendment level (o = 1250, x = 5000, Δ = 10000 μg cellobiose C per 10-g microcosm) on the inorganic phosphorus (P_i) extracted from each soil particle size fraction treatment (0.185 g fine clay, 0.313 g coarse clay, 0.417 g fine silt fraction per 10-g microcosm) by suspending the previously extracted residue of each 10-g microcosm in 30 ml of 0.01 N NaOH and shaking for 16 hours. Each data point shown indicates the mean over three microcosms for each fraction × C level treatment on each sampling date.


µg P_o per 10g microcosm
120
80
60
40
20
0
FINE CLAY
0
250
500
750
1000
1250
1500
1750
incubation time (hours)


µg P_o per 10g microcosm
100
80
60
40
20
0
COARSE CLAY
0
250
500
750
1000
1250
1500
1750
incubation time (hours)

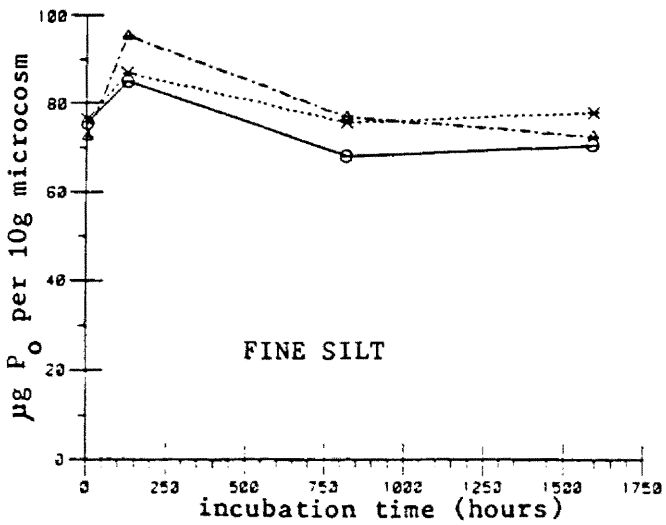
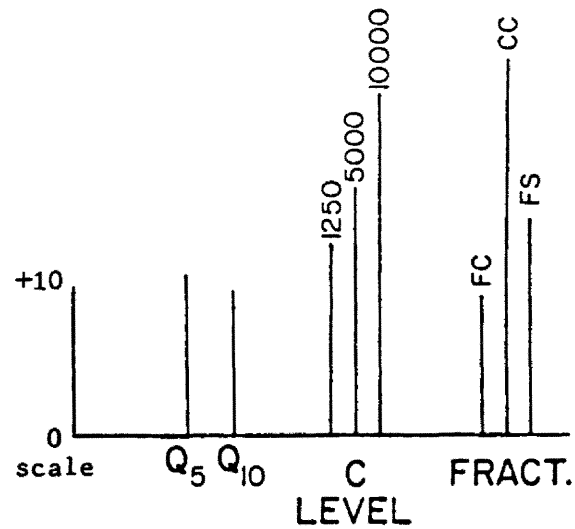
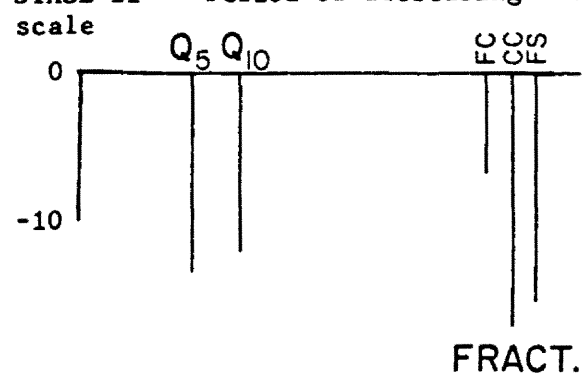

µg P_o per 10g microcosm
100
80
60
40
20
0
FINE SILT
0
250
500
750
1000
1250
1500
1750
incubation time (hours)

Fig. 6.12. Changes in the organic P extracted by 0.15 M NaOH occurring over respiration Stages I and II and total respiration. Vertical lines in a set represent the indicated treatment effects as modifiers of the effect of the treatment indicated below the set. All data are per 10-g microcosm. Vertical line on far left is a scaling line equivalent to 10 μg P/10-g microcosm. Q = Tukey's Q; FC = 0.185 g fine clay; CC = 0.313 g coarse clay; FS = 0.417 g fine silt; 1250, 5000, 10000 indicate C amendment levels in μg cellobiose C/10-g microcosm; C LEVEL = main effects of C amendment levels; FRACT = main effects of soil particle fractions. Results are presented only when at least two of the mean treatment effects were significantly different.

STAGE I -- Period of increasing biomass



STAGE II -- Period of decreasing biomass



Net changes after all respiration (ΣStages I & II)

(No treatment effects were
significantly different from zero)

pools. It follows that the FS apparently adsorbed the same P_o into the alkali-extractable and unextractable pools.

Comparison of the increases in total extractable P_o resulting from the increase in biomass P_o during Stage I illustrated the retention of the same P_o in different pools by the different fractions. Due to equivalent (e.g., nutritional) growth conditions for all fractions at each C level [with the exception of the FC × 1250 treatment, which had less total available C than the CC × 1250 or FS × 1250 treatments (see results and discussion, carbon, chapter 5) during Stage I] equivalent quantities and qualities of biomass P_o developed for all fractions. Upon lysis these equal amounts of biomass P_o were released and adsorbed by each fraction according to its particular adsorption capabilities. The amount of the biomass P_o appearing in each extract for each fraction was related to these adsorption capabilities. A certain portion of the biomass P_o was unextractable due to its chemical qualities but another portion may have been adsorbed into unextractable forms if the adsorbing fraction was capable of such strong adsorption. Table 6.2 shows the estimated Stage I extractable biomass P_o (increases in total extractable P_o during Stage I), which should be nearly equal for all fractions if their adsorption capabilities were the same. Indeed, the results for the two clay fractions are effectively equal. However, the fine silt had considerably smaller increases in extractable P_o, indicating an adsorption of more biomass P_o into unextractable forms by this fraction. Of the same P_o the FC apparently retained more in the 0.15 $\underline{M}$ $NaHCO_3$ and 0.5 $\underline{M}$ $NaHCO_3$ pools, the CC more in the NaOH pool, and the FS more in the unextractable pool.

Table 6.2. The effects of carbon amendment level on the increase in extractable organic phosphorus* for each fraction during Stage I

Fraction treatments†	Carbon amendment level		
	1250	5000	10000
FC	35	49	64
CC	39	49	64
FS	28	15	49

*A conservative estimate of the biomass P_o produced during Stage I.

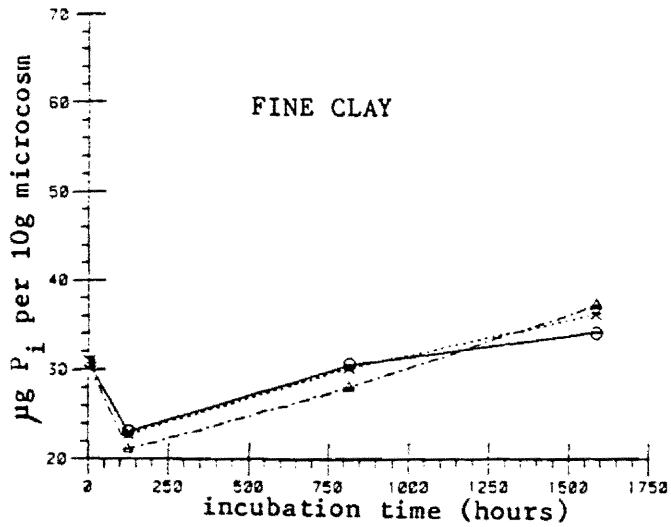
†FC = 0.185 g fine clay per 10-g microcosm; CC = 0.313 g coarse clay per 10-g microcosm; FS = 0.417 g fine silt per 10-g microcosm.

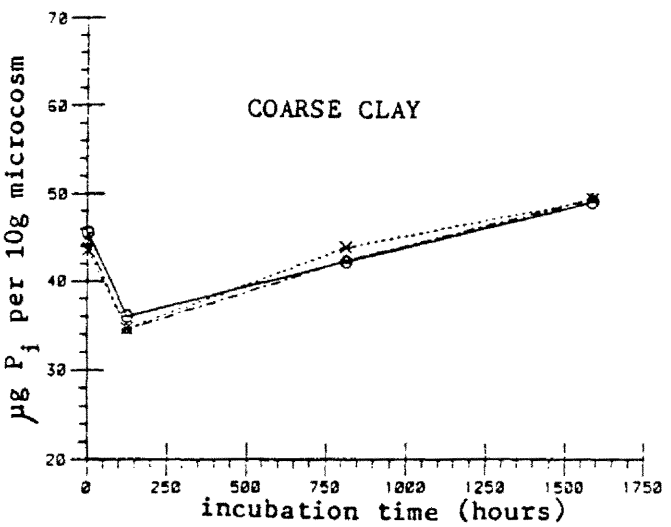
The Acid-extractable Phosphorus

The patterns of changes of P_i (Fig. 6.13) and P_o (Fig. 6.14) extracted by a 16-hour shake in 1 N HCl were consistently opposite. HCl P_i initially dropped apparently in relation to the amendment C level, while P_o increased. Beyond 124 hours, P_i increased while P_o dropped.

The HCl P_i pool decreased by a mean of 9.6 µg P_i per microcosm during Stage I with no correlation to treatment by the AOV (Tables 4.2 and 6/1). During Stage II a mean increase in HCl P_i of 7.8 µg per microcosm occured without correlation to treatments. Overall, the net change through 814 hours of incubation was zero. The HCl P_o showed a pattern very similar but opposite in direction to that of the P_i, an initial increase, followed by a decrease, with no overall change and no correlation with treatments. During Stage I, HCl-extractable P_i

Fig. 6.13. The HCl P_i results. The effects of carbon amendment level (o = 1250, x = 5000, Δ = 10000 μg cellobiose C per 10-g microcosm) on the inorganic phosphorus (P_i) extracted from each soil particle size fraction treatment (0.185 g fine clay, 0.313 g coarse clay, 0.417 g fine silt fraction per 10-g microcosm) by suspending the previously extracted residue of each 10-g microcosm in 30 ml of 1 M HCl and shaking for 16 hours. Each data point shown indicates the mean over three microcosms for each fraction × C level treatment on each sampling date.


FINE CLAY
µg P_i per 10g microcosm
70
60
50
40
30
20
0
250
500
750
1000
1250
1500
1750
incubation time (hours)


COARSE CLAY
µg P_i per 10g microcosm
70
60
50
40
30
20
0
250
500
750
1000
1250
1500
1750
incubation time (hours)

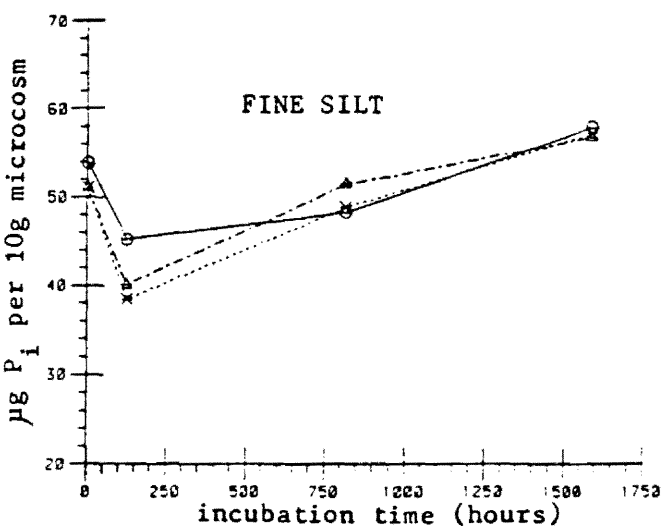
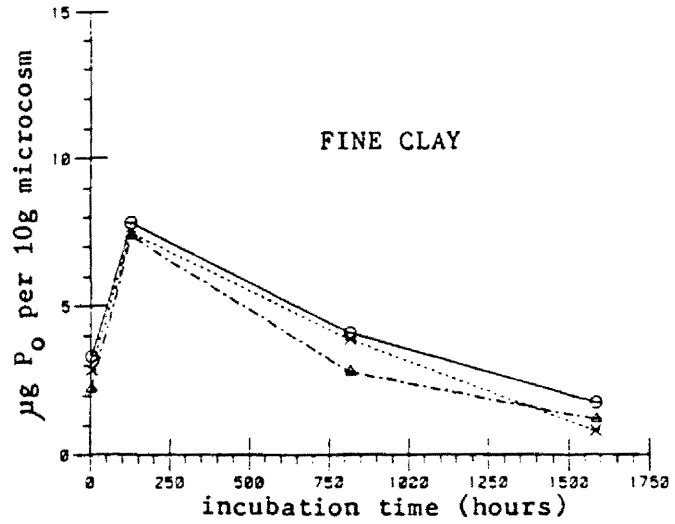
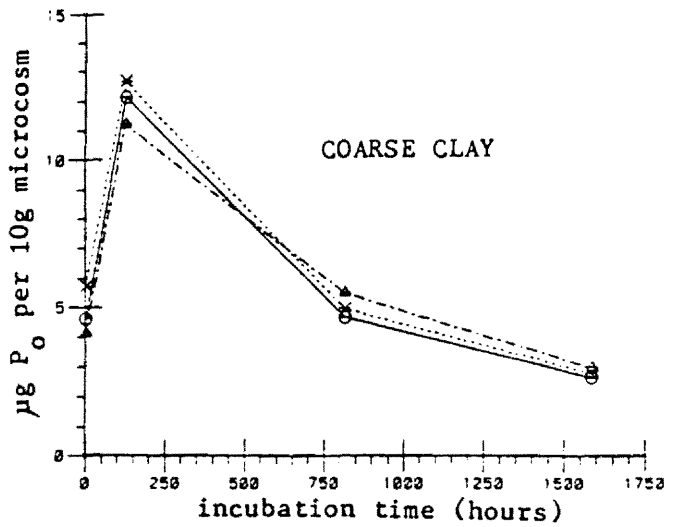
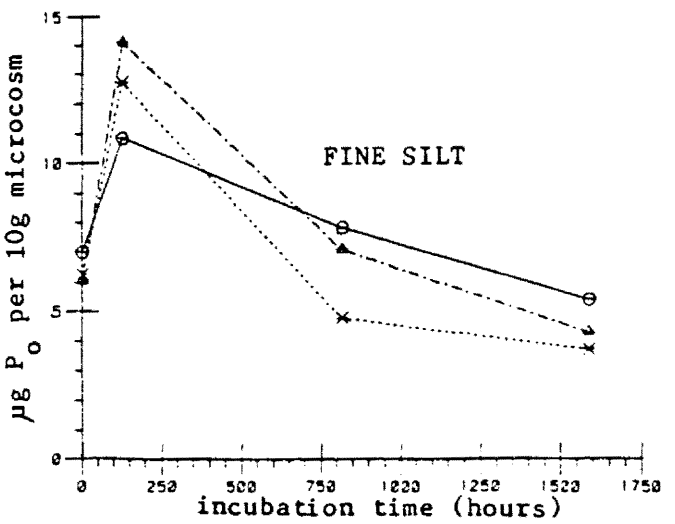

FINE SILT
µg P_i per 10g microcosm
70
60
50
40
30
20
0
250
500
750
1000
1250
1500
1750
incubation time (hours)

Fig. 6.14. The HCl P_o results. The effects of carbon amendment level (o = 1250, x = 5000, Δ = 10000 μg cellobiose C per 10-g microcosm) on the inorganic phosphorus (P_i) extracted from each soil particle size fraction treatment (0.185 g fine clay, 0.313 g coarse clay, 0.417 g fine silt fraction per 10-g microcosm) by suspending the previously extracted residue of each 10-g microcosm in 30 ml of 1 M HCl and shaking for 16 hours. Each data point shown indicates the mean over three microcosms for each fraction × C level treatment on each sampling date.


FINE CLAY
µg P_o per 10g microcosm
incubation time (hours)
0
5
10
15
0
250
500
750
1000
1250
1500
1750


COARSE CLAY
µg P_o per 10g microcosm
incubation time (hours)
0
5
10
15
0
250
500
750
1000
1250
1500
1750


FINE SILT
µg P_o per 10g microcosm
incubation time (hours)
0
5
10
15
0
250
500
750
1000
1250
1500
1750

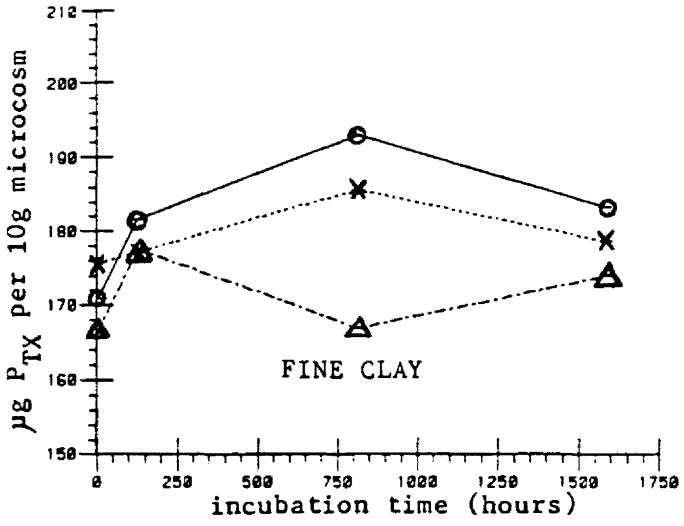
forms were utilized, while acid-extractable biomass P_o is generated and released by the chloroform lysis, giving the encountered peak in the P_o data. During Stage II, the acid-extractable biomass P_o is biologically released and mineralized. The acid-extractable P_i re-accumulated during Stage II to appear as an increase in this P_i form.

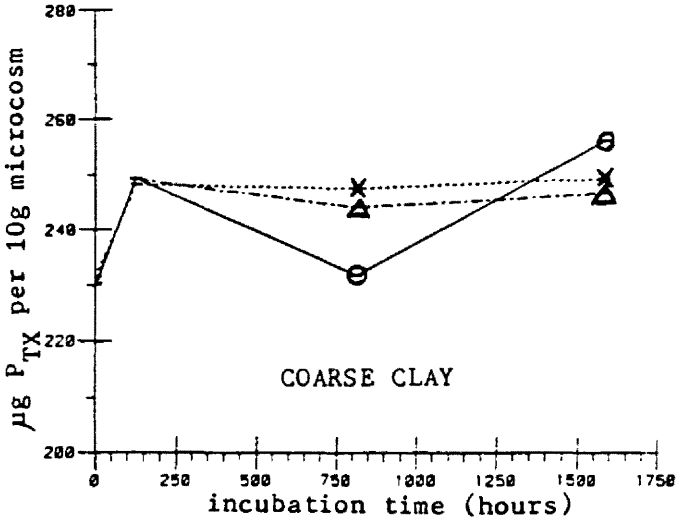
Total Extractable Phosphorus and Unextractable Phosphorus

Total extractable phosphorus (P_{Tx} Σ of P_T in all extracts) showed some correlation with treatments (see Table 6.1 and Figs. 6.15, 6.16). During Stage I the CC showed a significantly higher increase in P_{Tx} than did the FS. No correlation between P_{Tx} and treatments was found during Stage II. However, the net overall changes in P_{Tx} through the 814 hours showed that at the 1250 µg C level the FC that had increased was significantly different from the FS that had decreased. At the 5000 µg C level both the FC and CC had increased in P_{Tx} and had a change significantly different from that of the FS that had decreased. The FS showed a net increase over the 814 hours at the 10,000 µg C level significantly different from the decreases this fraction had shown for the two lower C levels. The P_{Tx} data was necessarily highly variable since it is the sum of four separate data points. Even with this limitation it is apparent that the unextractable-P forms were active in the microcosms and that most of this activity occurred during Stage I.

An important role for unextractable P as the source of P mineralized into available forms was suggested. During Stage I, P_{Tx} for CC increased significantly more than it did for the FC or FS (see

Fig. 6.15. The total extractable P results. The effects of carbon amendment level (o = 1250, x = 5000, Δ = 10000 μg cellobiose C per 10-g microcosm) on the inorganic phosphorus (P_i) extracted from each soil particle size fraction treatment (0.185 g fine clay, 0.313 g coarse clay, 0.417 g fine silt fraction per 10-g microcosm). Each data point shown indicates the mean over three microcosms for each fraction × C level treatment on each sampling date.


212
200
190
180
170
160
150
µg P_{TX} per 10g microcosm
FINE CLAY
0
250
500
750
1000
1250
1500
1750
incubation time (hours)


280
260
240
220
200
µg P_{TX} per 10g microcosm
COARSE CLAY
0
250
500
750
1000
1250
1500
1750
incubation time (hours)

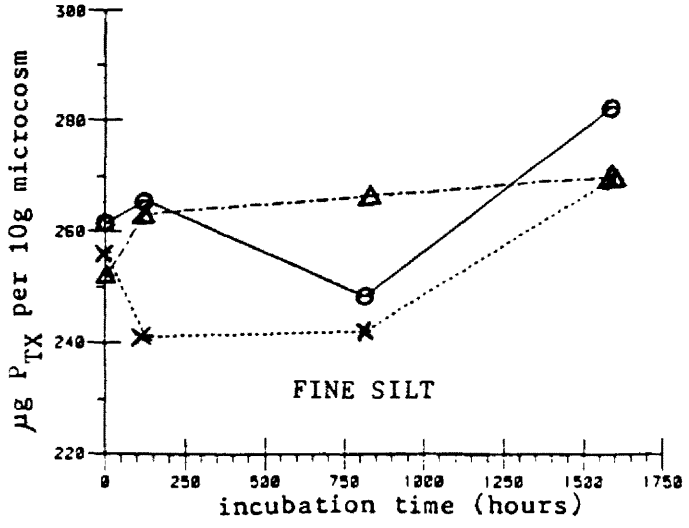
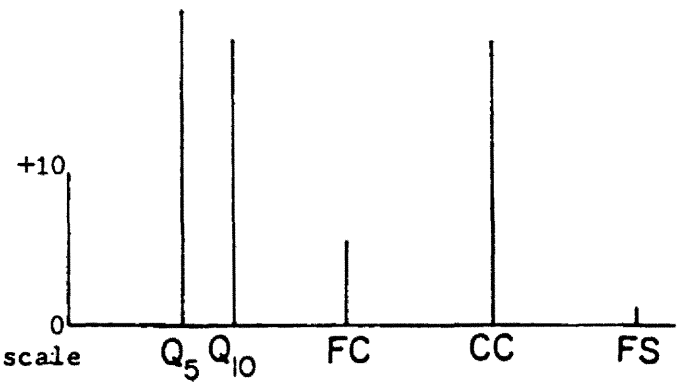

300
280
260
240
220
µg P_{TX} per 10g microcosm
FINE SILT
0
250
500
750
1000
1250
1500
1750
incubation time (hours)

Fig. 6.16. Changes in the total extractable P occurring over respiration Stages I and II and total respiration. Vertical lines in a set represent the indicated treatment effects as modifiers of the effect of the treatment indicated below the set. All data are per 10-g microcosm. Vertical line on far left is a scaling line equivalent to 10 μg P/10-g microcosm. Q = Tukey's Q; FC = 0.185 g fine clay; CC = 0.313 g coarse clay; FS = 0.417 g fine silt; 1250, 5000, 10000 indicate C amendment levels in μg cellobiose C/10-g microcosm; C LEVEL = main effects of C amendment levels; FRACT = main effects of soil particle fractions. Results are presented only when at least two of the mean treatment effects were significantly different.

STAGE I -- Period of increasing biomass



STAGE II -- Period of decreasing biomass

(No treatment effects were
significantly different from zero)

Net changes after all respiration (ΣStages I & II)

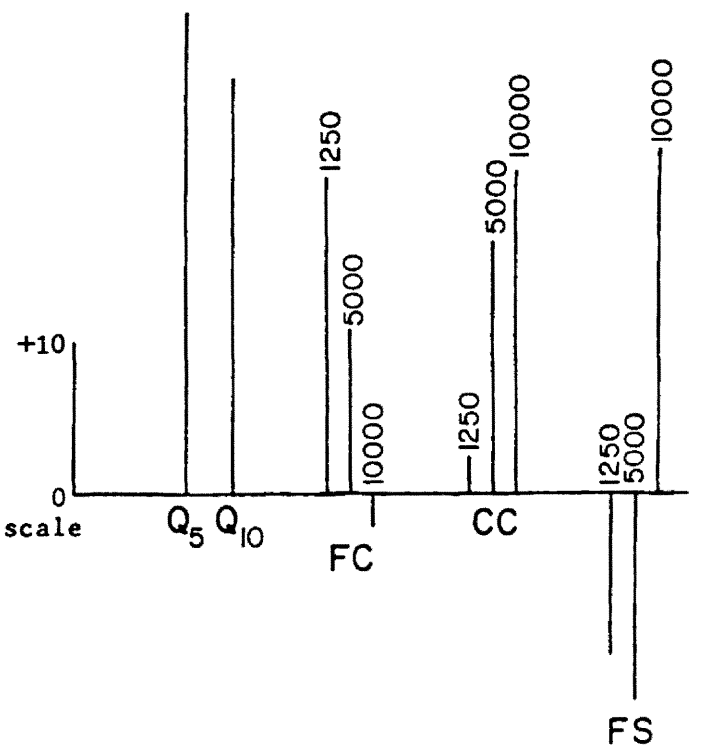


Fig. 6.16). The net (0- to 814-hour) increase in 0.15 M $NaHCO_3$ P_i was significantly greater for the CC than for the FC or FS (see Fig. 6.2). The net increase in (0.5 M) bicarbonate P_i was significantly greater for the CC than for the FC (see Fig. 6.4). That is, the fraction that had the most important increase in (mineralization into, after adjusting for inputs from NaOH P_i pool) available P_i forms was the fraction that had the most important Stage I decrease in unextractable P. Regardless of C level there were no consistent significant net changes in other P extraction pools to account for the P appearing in the available P_i (bicarbonate-extractable) pools.

The total extractable-P pool is, as is the 0.15 M $NaHCO_3$ inorganic P pool, of particular interest with respect to the hypotheses this experiment was to test. The 0.15 M $NaHCO_3$ inorganic P was a direct measure of net mineralization during Stage I. The total extractable was similarly a measure of the enhancement of P availability, but in a less classical manner. As discussed above, the unextractable P functions as the ultimate P source. If total extractable P increased, it must be at the expense of unextractable P and be presumed to increase at least the more readily mineralizable, if not the directly available P forms. To rephrase, where the 0.15 M $NaHCO_3$ extract measured the results after the last step of the mineralization process, the total extractable P indexes the first step (as assessed by the extraction procedure used in this experiment). The FC initially had no unextractable C (data not shown) and therefore no unextractable P_o. Thus, no utilization of unextractable P_o could have occurred in this fraction. The large increase in total extractable P for the CC over the FS during Stage I supports the

hypothesis that the availability of organic P increases with decreasing molecular weight and aromaticity. The consistency of activity of the P_{Tx} pool across C levels with a lack of significant effects of C levels does not support the hypothesis that P stress (level of microbial activity) increases microbial utilization of organic P. These conclusions regarding the hypotheses agreed with those reached using the 0.15 M $NaHCO_3$ inorganic P results. The strong interactions apparent in the net changes after all respiration (Fig. 6.16) were considered the result of the different adsorption capabilities and capacities (for similar organic P) among the fractions.

Conclusions

1. During the period of increasing biomass (Stage I) all P_o forms (except unextractable) increase, while all P_i forms (except the 0.15 M $NaHCO_3$ P_i at the lowest C amendment level) decrease.

2. During the period of decreasing biomass (Stage II), these effects are reversed except for the unextractable P, which is unaffected during this period, and NaOH P_i, which continues to decrease.

3. The continuous decrease in NaOH P_i was suggested to be an artifact of ultrasonic dispersion, which released reduced mineral P from soil microaggregates.

4. There was no net mineralization of any extractable P_o pool.

5. The increases in available P_i (0.15 and 0.5 M $NaHCO_3$ extractable P_i) must have been due to the net decreases in

the NaOH P_i and/or unextractable-P pools. For the CC the increases in available P_i were greater than the decreases in NaOH P_i, with the difference made up by a decrease in unextractable P (presumably P_o).

6. The unextractable P results indicate that significant net decreases occurred in this pool only during Stage I, the period of rapid increases in biomass and utilization of nutrients, including those in the native OM.

7. The unextractable P pool was not significantly affected during Stage II, when biomass was decreasing and all nutrients, except C, must have become more available.

8. The adsorption of P_o differed among the fractions. The fractions differed in retention of the same P_o against extraction. The fine silt fraction retained more of the same P_o against extraction than the clay fractions did.

9. From the 0.15 $\underline{M}$ $NaHCO_3$ inorganic P and total extractable P results, the hypothesis that "the extent of utilization of organic nutrients (P) is inversely related to the molecular weight and aromaticity of the organic matter containing those nutrients" should be tentatively accepted. The hypothesis that "microbial utilization of organic nutrients (P) increases as the level of microbial activity increases" is also correct. Even at the lowest (nonzero) level of microbial activity large amounts of organic P were utilized. These amounts did not increase proportionately with increasing microbial activity (P stress).

CHAPTER 7

SUMMARY: SIGNIFICANCE OF RESULTS AND INDICATIONS FOR FUTURE RESEARCH

Introduction

In this chapter, I would like to synthesize the results of my work into the broader understanding of soils. I hope that this synthesis will indicate some avenues for resolving some long-standing questions and perhaps generate some new directions for research.

A review of the literature indicated that three mechanisms are suspected to affect the stability of soil OM: (1) chemical recalcitrance, (2) physical stabilization by adsorption, or (3) physical stabilization by occlusion. Chemical recalcitrance is believed to be related to the chemical character of the OM itself. Chemical recalcitrance is generally considered to increase as molecular weight and aromaticity increase. Adsorption by clays has been shown under certain conditions to slow decomposition of certain types of extractable OM. Different soil minerals have been shown to preferentially adsorb organics of different chemical character. Occlusion of insoluble substrates in artificial aggregates has been shown to slow microbial utilization. However, information on experiments directly examining the existence and importance of these mechanisms still appear to be lacking in the literature. My work was an attempt to begin developing such information.

The work was designed to treat the three mechanisms as controllable variables. Control of chemical recalcitrance and physical stabilization by adsorption was achieved by use of ultrasonically dispersed soil particle size fractions. Such fractions differ predictably and consistently in the chemical character of their associated OM. Among the fine fractions (ESD <5 μm) molecular weight and aromaticity (considered indices of chemical recalcitrance) of the associated OM decrease with decreasing particle size. The relationship between particle size fraction and the chemical character of associated organic matter was suspected to arise from the selective adsorption of low molecular weight, less aromatic organics by clay particles. Thus, use of three fine soil particle size fractions provided OM with three levels of chemical recalcitrance and three levels of adsorption to soil particles. Physical stabilization by occlusion was minimized by dispersion of the soil particles during fractionation.

Stabilization of Organic Matter by Occlusion in Microaggregates

The effectiveness of physical stablization of soil OM by occlusion in microaggregates was suggested by the effects of ultrasonic dispersion on soil suspensions. Odors and colors characteristic of anaerobic environments were apparent upon dispersion of larger water-stable microaggregates ESD >50 μm but not when smaller aggregates ESD <50 μm were dispersed. Extractability of redox-affected mineral nutrients differed between aggregate-affected and -unaffected particle fractions of the same size. During an incubation experiment, NaOH P_i decreased in a manner suggestive of a dissolution of some

mineral-P form. The only such mineral P that could explain the observed NaOH P_i behavior was reduced iron phosphates, presumably released from microaggregates during dispersion. The existence of anaerobic, chemically reduced microsites in the interior of water-stable microaggregates was indicated. Anaerobic conditions greatly reduce decomposition rates. The C contents of the particle size fractions, especially the CC and FS, dispersed from water-stable microaggregates were consistently higher than those of the comparable water-dispersible fractions. A model of microaggregate formation will be presented later in this chapter.

Occlusion as a Controllable Variable in the Study of Soils

Dispersion of soil microaggregates during the particle size fractionations drastically reduced occlusion of soil OM. However, aggregates of ESD as small as 50 μm might have stabilizaing effects on OM by occluding it. It was observed that during drying soil particle size fractions re-associate into stable aggregates often with dimensions considerably greater than 50 μm. This re-aggregation phenomenon was controlled by use of an appropriate freeze-drying process that provided the fractions in a dry state with no aggregates greater than 50 μm. Lack of such control of re-aggregation or of initial aggregation status of soils--the OM occlusion effectiveness of which may vary considerably among different soils--may be a primary cause for the variability and contradictory results so common in previous OM decomposition and mineralization studies.

Ultrasonic Dispersion and the Character of Soil Particle Size Fractions

The adsorption of soil organics by soil particles apparently is a rapid, selective, but relatively weak association. This was indicated by the apparent alterations of the distribution patterns of C, N, P, and CEC by ultrasonic vibration of soil suspensions (and by the extractable-P results, which will be discussed later). Water-soluble organics and organics with relatively high N and P contents are preferentially adsorbed by the FC particles. C-rich organics are apparently the preferred adsorbate of the FS particles. Soil particle size fractions obtained without use of ultrasonic dispersion show similar C contents and CECs (CEC results not shown). Application of ultrasound reduces the C content without affecting the CEC of the FC, while increasing the C content and reducing the CEC of the FS. The CC is intermediate between the FC and FS in its response to ultrasonic vibration. A rapid, selective but weak adsorption of organics is indicated by the specificity and speed with which it occurs during the ultrasonic treatment. The soil particles, however, must retain adsorbed organics against extraction by water, since soluble C in the supernatant is not affected by ultrasonic vibration. This information has implications for the use and interpretation of results from ultrasonically prepared soil particle size fractions and for understanding adsorption as a mechanism for stabilizing soil OM. In intact soils it is doubtful that the specificity of each particle size fraction is ever fully expressed--spatial constraints probably cause adsorbates to adsorb on the first, not necessarily the most selective, adsorbent they encounter. The fractions are thus useful as agents of

selecting and stabilizing organics of different chemical character for research use, but their effectiveness in this role in intact soils has not been verified. Some component(s) of the FS fraction apparently function(s) as an adsorbent, just as clay particles do in the FC. The predominance of primary minerals and low surface area in the FS fraction and the preference of this fraction for C-rich adsorbates suggest that the adsorbing surfaces in this fraction are organic. When organics are functioning as sorbers, the process may involve considerable absorption as well as adsorption. These differences in relative importance of organic and inorganic sorbers and adsorption and absorption processes in turn suggest that susceptibility of adsorbed organics to extractants may be expected to differ among the fractions. The apparent relative ease and speed of desorption and resorption events suggest that stabilization of soil OM by adsorption may be relatively weak. The selectivity of the fractions for specific classes of organics might cause certain organics to be more effectively stabilized by adsorption than others. The suggested relative unimportance of the adsorption mechanism was fortunate, since no means of quantifying the adsorption of organics was encountered.

Ultrasonically Dispersed Fractions to Study Organic Matter Stabilization

The effects of different levels of microbial activity on the transformations of the C and P associated with the soil particle size fractions were studied in incubation experiments. These experiments were designed to use the expanded knowledge of the relative characteristics of ultrasonically dispersed soil particle size

fractions to control the relative importance of each of the three proposed mechanisms of soil OM stabilization. Stabilization by occlusion was essentially eliminated. Stabilization by adsorption was indicated to be a relatively weak mechanism whose effects should be apparent, if important, in differences among the fractions in response to similar adsorbates and extractants. The chemical recalcitrance of the associated OM was hypothesized to be directly related to the particle size fraction, increasing with increasing particle size. Different levels of microbial activity were provided by supplying different amendment levels of readily available C substrate.

The Microbial Availability of Fraction-Associated Carbon

Respiration and biomass data showed that two phases occurred during incubation, the first of increasing biomass and the second of decreasing biomass. Insignificant respiration was occurred without the addition of soluble-C substrate. The respiration data showed that C in the OM was used at the lowest C-amendment level, but not at higher C-amendment levels. Combining the respiration and biomass data to better account for C transformations it became apparent that C in the OM was utilized by the microbes at all C-amendment levels and that utilization of OM C increased with increasing C-amendment level. However, the differential effect of C-amendment level on OM C utilized generally decreased as C-amendment level increased. Increasing the C-amendment level by a factor of four increased utilization by only a factor of 1.3 to 2.6. Further examination of the differential effects among the particle size fractions suggested that the OM C in the FC

was more available than that in the two larger fractions. The ineffectiveness of adsorption as a stabilizing mechanism was indicated by the complete utilization of OM C at the highest C level in the FC fraction--where C stress was at a minimum and adsorptive surface area was at a maximum. The C data showed that the largest portion of native soil OM C was utilized during the period of increasing biomass and indicated little or no utilization during the period of decreasing biomass.

The Microbial Availability of Fraction-Associated Phosphorus

The 0.15 M $NaHCO_3$-extractable-phosphorus results indicated that net mineralization to plant-available inorganic P occurred by the end of the period of increasing biomass at the lowest C-amendment level for all fractions. The rate of P utilization outstripped the rate at which mineralization and dissolution processes could supply P at the two higher C levels during the period of increasing biomass. The decrease in biomass that followed was accompanied by net mineralization, as indicated by the two bicarbonate-extractable-P pools at all C levels. However, the net P mineralization in both these pools decreased with increasing C level, indicating a direct relationship between C stress and net P mineralization. Furthermore, the utilization of unextractable P was apparent and similar at all C levels for each fraction during Stage I, suggesting that the first step in the mineralization process is unrelated to C level (P stress). The 0.5 M $NaHCO_3$-extractable organic P was the only organic-P pool significantly affected by C level after the cessation of respiratory

activity. This organic-P pool increased with increasing C level, indicating that net mineralization of this pool was directly related to C stress (inversely related to P stress). Thus, as with utilization of OM C, it appears that utilization of organic P increases with increasing C level (increasing P stress) but not proportionately.

Fine Soil Particles as Adsorbing Agents

The extract-defined P pools allowed evaluation of a portion of the biomass organic P and the adsorptive behavior of the soil fractions toward similar organic adsorbates. Because of similar biomasses developing for all the fractions at each C level during incubation, similar quantities and qualities must have developed, as shown by the extractable-organic-P results. The same extractable organic P is held in different extraction pools by the different soil particle size fractions. The FC held more in more readily extracted forms, while the CC and FS held it in progressively more difficultly extracted forms. Thus, the sorbent behavior of the three particle size fractions differs; but, as mentioned above, this has little effect on the stabilization of organics against utilization by microbes. Furthermore, the extractable organic P and C results (C results not shown) indicated that utilization of these elements by microbes rendered them ultimately more unextractable. However, because of the findings mentioned above, it is not expected that this can be translated to mean that utilization of nutrients by microbes renders them ultimately less available.

The Stabilization of Organic Matter, Its Availability, and the Priming Effect

The following conclusions were drawn regarding the effects and effectiveness of the three proposed mechanisms of stabilization of soil OM. Among the three proposed mechanisms, chemical recalcitrance is apparently the least important. A slight difference was indicated between the availability of the FC-associated OM and the CC- and FS-associated OM. Despite the slight difference in availability, the OM of all three fractions is readily utilized in the presence of a readily available energy source. Adsorption of soil OM onto soil particles probably is second in importance of the three mechanisms. Its most significant function in soils is apparently stabilization of organics against solubilization in the soil solution. In solution the organics are more subject to leaching and the probability of encountering an enzyme that may act upon them is increased. The reduction in the probability of encounter with an enzyme that results from adsorption of organics from solution is suggested to explain why no soil OM was used without a readily available energy source. It was apparent that the amount of soil OM utilized is related to the quantity of readily available substrate present. That is, for sorbed, water-insoluble organic matter associated with soil particle size fractions, a "priming effect" definitely exists. Biochemical "availability" of soil OM is thus affected little, if at all, by adsorption; but physical "availability" (accessibility) is reduced. The energy required to act across the distances imposed by this reduction in accessibility is apparent as the "priming effect." It should be mentioned that the quantity of energy required may be

expected to vary with the qualities and quantities of the soluble and adsorbed substrates. Physical stabilization by occlusion apparently is the most important mechanism for stabilization of soil OM against microbial degredation. Though not studied in my incubation experiments, the expression of its effectiveness was apparent during preparation of the soil particle size fractions. It is well suited to study by the same techniques used in the incubation experiments reported in this thesis.

The existence of a "priming effect" and the parallel relationship between utilization of soil OM C and organic P suggest that exoenzymes are probably the "tools" of the soil microbes in degrading soil OM. Apparently, C-oriented enzymes initiate the mineralization process. This may be achieved by affecting the OM molecules in a way that provides greater exposure of substrate sections--in the present case, linkages involving P--by shortening chain lengths, "unwrapping" OM complexes, or perhaps directly solubilizing small portions of the OM. Once the C-oriented enzymes have exposed the P-bearing moieties, the phosphatases proceed to remove the P as rapidly as environmental conditions permit. Furthermore, C-oriented degradation proceeds simultaneously with the P-oriented until all organic character is lost or organics are reduced to entities small enough to be taken up by the microbes. The "priming effect" is attributable not to the lack of "available" substrate but to the immobility of both the water-insoluble OM and the microbes capable of degrading it. Exoenzymes are the means by which the microbes "reach" substrate that cannot diffuse to them. Energy is required to produce these exoenzymes and, since these enzymes are themselves substrates, to maintain them in the soil

solution. As suggested above, the "priming effect" results from this energy requirment. As with occlusion as the principal mechanism of stabilizing OM, the role of exoenzymes in the decomposition of soil OM is subject to study by the techniques described in this thesis and by other means.

Implicit in the preceding discussion of the role of exoenzymes is the necessity for the substrate organic matter to be accessible to the enzymes. The effectiveness of occlusion in microaggregates in stabilizing soil OM might be due to its rendering the substrate OM inaccessible to enzymes. However, if there is readily available substrate inside the aggregate--as is probably most often the case--then the OM is subject to degradation by the enzymes generated within the aggregate itself and stabilization would not be expected. If occlusion could reduce the availability of energy despite the presence of considerable substrate C, then stabilization of the occluded OM would result. This is achieved by limiting the availability of oxygen. The anaerobic utilization of the readily available C inside the microaggregates leads to the development of chemically reduced microsites internal to these active microaggregates. The evidence for the existence and location of these microsites was discussed earlier.

A Model of Microaggregate Formation

The following is a model for the formation of microaggregates discussed above. Its development requires incorporating the effects of plants. The soil is the plant's principal source of mineral nutrients. The plant is the soil's principal source of energy and C. Interestingly, it is also often the soil's principal consumer of

oxygen. Thus, activities of plant roots are the soil's principal source of reducing potential. Plant roots are known to exude microbially available substrates into the soil. This increases the local demand for oxygen by stimulating the activity of microorganisms in the vicinity of the root. Simultaneously with this intense localized demand for oxygen the plant is taking up considerable amounts of water. The mass flow of this water carries clay particles to the surface of the root. As this process continues, two barriers to oxygen diffusion develop, a physical barrier of accumulated clay particles and a biological barrier of actively respiring microbes and roots. Eventually, the plant ceases to take up water through this particular portion of root, and the accumulation of clay particles decreases greatly or ceases. At some point, this section of root dies. However, the barriers to oxygen have been established. The dead root now lies encased in a shell of accumulated clay particles with intermingled bacteria and root exudates. Decomposition of the root residue is restricted by the availability of oxygen, whose entrance to the interior of the aggregate is blocked by a "wall" of clay complete with its own oxygen demand in the form of contained microbes and microbial substrate. A stable microaggregate is formed complete with chemically reduced interior and means of maintaining those reduced internal conditions.

Such aggregates in soils provide a means of explaining many otherwise puzzling soil phenomena. They provide a location for the existence of non-spore-forming strict anaerobes. The existence of significant amounts of the reduced forms of many nutrient elements in soils that have been well aerated for thousands of years could be an

expression of the presence of these aggregates. The occurrence of normally anaerobic processes in well-aerated soils could be accounted for--for example, denitrification.

Microaggregates and Agronomic Practices in Soil Development and Degradation

Finally, the existence of these microaggregates and the apparent lability of organic matter and its associated nutrients when not protected by them have clear agronomic implications. The incubation experiments showed the remarkable lability of organic nutrients in unprotected soil OM. A relationship between the investment of readily available C sources and the net mineralization of plant nutrients was apparent. There is an optimum investment level of readily available substrate to provide net mineralization and another for net immobilization. It is important that the level for net mineralization is the lower of the two. As a result, when net mineralization of plant nutrients occurs, it is accompanied by net decomposition of soil OM. Plants that are better at inducing mineralization of organic nutrients by controlling the quantity and quality of their root exudates are probably better producers. Unfortunately, the same plants are net destroyers of soil OM and thus soil quality. Crop plants probably represent this type of plant, having been bred over many generations for maximum yield with minimum input--i.e., the ability to "mine" the soil. Wild grasses may represent the opposite extreme, having evolved in conjunction with the developing soil. Grasses that could affect--by timely and appropriate investment of C along their roots, net mineralization in the vicinity of root uptake

of nutrients, and net immobilization of available nutrients along portions not actively taking up nutrients--would allow the development of soils with optimally high organic matter contents and fertility for the given environment. The utilization of organic nutrients and careful return of unused nutrients to organic forms maximized fertility with minimzed losses via denitrification, leaching, etc. The lability of organic nutrients and the likelihood of relatively direct control by plants of their utilization indicates that soil OM and fertility are ultimately the same. Soil OM is important not just for its effects on soil structure, tilth, CEC, etc., but also because it is directly involved in plant nutrition and soil fertility.

Indications for Future Research--Soil Fractionations

Soil particle size fractions have shown considerable potential for use in future research. All soil fractionations introduce artifacts, as do soil-dispersion techniques. Observations made during the fractionation work presented in this thesis, however, indicate that these artifacts can be controlled and used to advantage. By appropriately combining ultrasonic (or other) dispersion techniques with fractionations, it should be possible to verify the existence and characteristics of the soil microaggregates, whose interiors apparently are reduced microsites, as well as other soil phenomena. For example, by separating several subsamples of a soil, each at a different particle size, and then ultrasonically dispersing the resulting fractions, the particle size at which the first significant evidence of reduced conditions (Fe^{++}, Eh, dehydrogenase activity, etc.) appears is the minimum size aggregate in which such conditions develop. By

fractionating two samples of the same soil, one ultrasonically dispersed and the other not, the importance of microaggregates in the total soil mass could be verified. The effects of different treatments on soil microstructure could be similarly verified. Many other, more specific applications are possible in such areas as rhizosphere effects on microaggregate dynamics and vice versa; microaggregates as affected by cultivation techniques and cropping systems; effects of microaggregates on soil fertility, moisture capacity, and availability; implications of microaggretate status for soil management and conservation.

Indications for Future Research--Mineralization Studies

The incubation technique developed and applied in this work provides the capability to measure specific nutrient transformations occurring during the incubation of soil components or other substrates without unknown or uncontrolled nutrient sources. It does so in a simulated soil matrix that, as indicated by the results of my experimentation, allows microbial growth to proceed in a manner similar to that of true soils. Experiments similar to the one conducted to study P and C transformations could be performed for N or S transformations. Questions arising from the results of this first P and C work could be resolved. Is the C/P nutrient system C (energy) driven as it appears it might be? Are the differences among the soil fractions in their associated OM meaningful relative to intact soils or only an artifact of physical dispersion techniques limited to use as an aid to laboratory studies? Precisely how intense is the turnover of organic nutrients--apparently very intense--under these

incubation conditions? How intense in intact soil? Further experimentation using this incubation technique could answer these and similar questions. The application of isotope dilution techniques within such experiments should enhance the directness and quantitativeness of results.

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APPENDIX A

RESPIRATION DATA

This appendix lists the mean quantity of C respired (measured as CO_2 evolved and absorbed in NaOH trap, see Chapter 4) for each fraction × C level treatment over the indicated time intervals. The data listed are means of three replicates. The number in parentheses listed with each mean is the standard deviation of the samples (replicates) about that mean.

Appendix Table A-1. Mean cumulative respiration during each time interval in µg C per 10-g microcosm (over three replicates; number in parentheses is standard deviation of each sample mean)

Fraction	Cellobiose C amendment (µg C per microcosm)	Elapsed incubation time (in hours)											
		24	56	75	124	172	221	291	460	653	814	1053	1584
Fine clay	1250	332 (22)	556 (24)	44 (98)	116 (48)	147 (54)	36 (54)	171 (3)	420 (189)	117 (30)	141 (69)	12 (18)	66 (66)
	5000	294 (32)	1390 (124)	672 (72)	388 (44)	243 (117)	102 (81)	462 (81)	687 (186)	273 (156)	114 (24)	24 (18)	138 (66)
	10000	330 (100)	1172 (26)	2302 (52)	2242 (8)	612 (27)	564 (180)	678 (63)	579 (60)	231 (30)	174 (6)	108 (90)	144 (30)
Coarse clay	1250	428 (52)	624 (164)	28 (106)	204 (92)	159 (102)	24 (133)	168 (52)	273 (126)	159 (0)	123 (12)	18 (30)	78 (12)
	5000	292 (26)	1912 (134)	298 (68)	576 (14)	369 (51)	129 (87)	363 (45)	399 (66)	366 (147)	165 (24)	45 (36)	120 (18)
	10000	460 (136)	2390 (130)	1310 (64)	1742 (60)	744 (75)	312 (48)	495 (18)	681 (96)	360 (63)	246 (63)	57 (18)	138 (24)
Fine silt	1250	660 (54)	458 (86)	10 (110)	224 (18)	183 (99)	39 (48)	225 (21)	294 (48)	306 (45)	180 (12)	45 (--)	132 (18)
	5000	662 (228)	1856 (30)	240 (94)	650 (104)	435 (66)	153 (57)	351 (21)	444 (66)	312 (24)	252 (24)	198 (60)	72 (80)
	10000	638 (90)	2450 (192)	1292 (10)	1712 (22)	879 (9)	417 (48)	507 (15)	657 (27)	402 (27)	300 (9)	228 (48)	108 (72)

APPENDIX B

PHOSPHORUS EXTRACTIONS DATA

This appendix gives the mean quantity of P extracted in each step of the sequential P extraction procedure for each fraction × C level treatment and sampling time (see Chapter 4, Fig. 4.1). The data listed are means over three replicates. The number in parentheses listed with each mean is the standard deviation of the samples (replicates) about that mean.

Appendix Table B-1.

Fraction	Cellobiose-C amendment (µg C per microcosm)	Extract		Elapsed incubation time (hours) 0	124	814	1584
				Extracted P (µg/10 g microcosm)			
Fine clay	1250	0.15 M $NaHCO_3$ (3-minute)	P_i	22.2 (0.8)	26.7 (2.2)	29.0 (0.8)	26.3 (1.5)
			P_T	31.0 (1.1)	40.5 (3.2)	39.9 (3.4)	37.4 (3.8)
		0.5 M $NaHCO_3$ (16-hour)	P_i	43.9 (1.6)	34.1 (1.0)	52.2 (1.3)	50.0 (1.6)
			P_T	56.0 (1.9)	52.5 (1.7)	69.5 (1.8)	65.6 (1.7)
		1 N NaOH (16-hour)	P_i	32.7 (0.6)	29.5 (1.5)	24.3 (0.4)	23.8 (0.2)
			P_T	50.8 (2.8)	57.7 (5.0)	49.1 (1.9)	45.3 (0.8)
		1 N HCl (16-hour)	P_i	30.7 (0.6)	23.0 (0.9)	30.5 (1.1)	34.1 (0.8)
			P_T	34.0 (0.8)	30.8 (0.6)	34.6 (0.3)	35.1 (0.7)
	5000	0.15 M $NaHCO_3$ (3-minute)	P_i	22.8 (0.1)	8.9 (1.5)	26.8 (0.8)	23.3 (1.8)
			P_T	31.2 (3.8)	24.7 (2.5)	36.9 (1.3)	31.6 (2.0)
		0.5 M $NaHCO_3$ (16-hour)	P_i	42.4 (0.9)	32.6 (3.8)	48.3 (1.7)	47.6 (3.3)
			P_T	54.6 (0.4)	57.5 (6.8)	64.0 (3.4)	65.4 (6.2)
		1 N NaOH (16-hour)	P_i	32.9 (2.4)	28.4 (1.5)	23.0 (0.9)	24.6 (2.3)
			P_T	56.2 (1.6)	64.6 (2.7)	50.9 (1.7)	44.8 (1.1)
		1 N HCl (16-hour)	P_i	30.8 (1.9)	22.7 (1.0)	30.2 (1.1)	36.2 (0.9)
			P_T	33.7 (2.4)	30.2 (1.5)	34.1 (2.0)	37.0 (0.9)
	10000	0.15 M $NaHCO_3$ (3-minute)	P_i	22.5 (1.3)	3.2 (0.8)	23.6 (1.1)	20.0 (4.4)
			P_T	29.6 (1.6)	23.2 (3.4)	35.1 (3.4)	31.9 (6.2)
		0.5 M $NaHCO_3$ (16-hour)	P_i	42.8 (0.8)	34.9 (2.2)	40.6 (3.0)	44.0 (0.5)
			P_T	55.6 (1.0)	59.0 (2.0)	56.1 (3.9)	57.0 (1.7)
		1 N NaOH (16-hour)	P_i	32.1 (4.3)	29.9 (0.2)	20.2 (1.6)	26.4 (2.2)
			P_T	54.5 (3.2)	66.6 (4.7)	50.9 (1.7)	48.0 (1.5)
		1 N HCl (16-hour)	P_i	30.6 (4.1)	21.0 (0.5)	28.0 (1.9)	37.2 (1.0)
			P_T	32.8 (4.3)	28.4 (2.1)	30.7 (2.6)	38.4 (1.0)

Appendix Table B-1 (cont'd)

Fraction	Cellobiose-C amendment (μg C per microcosm)	Extract		Elapsed incubation time (hours) 0	124	814	1584
				Extracted P (μg/10 g microcosm)			
Coarse clay	1250	0.15 M $NaHCO_3$	P_i	14.6 (0.6)	18.2 (0.8)	25.9 (2.0)	24.6 (2.3)
		(3-minute)	P_T	20.6 (1.7)	25.6 (0.8)	31.5 (2.2)	32.6 (3.2)
		0.5 M $NaHCO_3$	P_i	40.5 (2.0)	36.8 (1.2)	56.0 (3.6)	51.0 (4.8)
		(16-hour)	P_T	64.0 (3.0)	59.8 (2.4)	74.2 (5.5)	82.7 (6.7)
		1 N NaOH	P_i	38.8 (4.5)	38.2 (1.2)	21.7 (0.6)	28.8 (1.3)
		(16-hour)	P_T	95.1 (10.9)	113.0 (8.0)	79.3 (2.4)	89.5 (6.3)
		1 N HCl	P_i	45.6 (3.7)	36.0 (2.6)	42.3 (2.6)	59.1 (1.7)
		(16-hour)	P_T	50.2 (4.2)	48.1 (3.5)	46.9 (3.7)	51.7 (1.9)
	5000	0.15 M $NaHCO_3$	P_i	14.0 (0.5)	8.6 90.5)	22.7 (0.1)	22.2 (0.8)
		(3-minute)	P_T	19.5 (1.8)	16.9 (1.0)	27.4 (0.8)	27.3 (1.2)
		0.5 M $NaHCO_3$	P_i	40.1 (1.7)	36.0 (2.6)	51.5 (1.8)	49.5 (4.8)
		(16-hour)	P_T	63.8 (2.2)	63.2 (1.4)	82.2 (8.9)	80.8 (8.9)
		1 N NaOH	P_i	39.2 (5.8)	36.4 (1.5)	22.2 (0.7)	27.2 (1.5)
		(16-hour)	P_T	98.8 (11.4)	121.4 (8.4)	89.3 (1.0)	89.4 (5.3)
		1 N HCl	P_i	43.5 (3.5)	34.6 (1.7)	44.0 (2.2)	49.4 (1.4)
		(16-hour)	P_T	49.2 (5.1)	47.2 (6.4)	48.9 (2.3)	52.2 (1.6)
	10000	0.15 M $NaHCO_3$	P_i	13.6 (0.5)	0.7 (0.4)	20.6 (0.4)	21.1 (0.6)
		(3-minute)	P_T	20.2 (1.3)	13.3 (2.6)	26.0 (0.5)	25.9 (1.3)
		0.5 M $NaHCO_3$	P_i	42.3 (1.8)	39.6 (2.5)	48.9 (2.5)	47.7 (2.9)
		(16-hour)	P_T	66.3 (3.2)	68.4 (2.7)	81.0 (7.1)	78.4 (5.3)
		1 N NaOH	P_i	38.0 (3.6)	37.5 (1.1)	21.8 (1.4)	28.4 (0.5)
		(16-hour)	P_T	91.4 (9.1)	122._ (6.5)	89.4 (6.9)	90.2 (2.8)
		1 N HCl	P_i	44.6 (2.2)	34.5 (2.0)	42.5 (1.4)	49.5 (1.3)
		(16-hour)	P_T	48.7 (2.1)	45.7 (3.2)	47.9 (1.3)	52.4 (1.5)

Appendix Table B-1 (cont'd)

Fraction	Cellobiose-C amendment (μg C per microcosm)	Extract		Elapsed incubation time (hours) 0	124	814	1584
				Extracted P (μg/10 g microcosm)			
Fine silt	1250	0.15 M $NaHCO_3$	P_i	12.7 (0.6)	15.1 (0.8)	21.8 (0.4)	21.0 (1.6)
		(3-minute)	P_T	18.2 (1.3)	22.4 (0.5)	29.0 (1.8)	25.8 (2.8)
		0.5 M $NaHCO_3$	P_i	39.2 (1.9)	36.7 (1.7)	55.4 (6.6)	56.9 (2.9)
		(16-hour)	P_T	64.0 (3.2)	63.7 (1.4)	74.9 (7.4)	96.2 (5.7)
		1 N NaOH	P_i	42.9 (3.3)	38.2 (1.0)	20.1 (2.2)	26.7 (1.5)
		(16-hour)	P_T	118._ (10.0)	123._ (5.7)	88.2 (8.9)	97.4 (7.6)
		1 N HCl	P_i	53.9 (2.7)	45.2 (0.7)	48.3 (6.0)	57.9 (1.0)
		(16-hour)	P_T	60.8 (3.9)	56.0 (7.0)	56.1 (7.8)	63.2 (3.1)
	5000	0.15 M $NaHCO_3$	P_i	12.7 (0.6)	6.3 (0.7)	16.1 (0.7)	16.1 (0.3)
		(3-minute)	P_T	19.9 (3.2)	13.3 (1.6)	22.7 (0.2)	21.9 (0.9)
		0.5 M $NaHCO_3$	P_i	39.2 (1.9)	35.6 (2.0)	46.0 (4.5)	47.1 (5.9)
		(16-hour)	P_T	66.3 (3.6)	58.5 (9.3)	68.9 (11.5)	81.8 (9.5)
		1 N NaOH	P_i	36.4 (5.2)	36.5 (1.2)	20.8 (2.3)	27.1 (1.6)
		(16-hour)	P_T	113._ (5.4)	118._ (14.4)	96.7 (8.5)	105._ (9.1)
		1 N HCl	P_i	51.1 (3.2)	43.5 (1.1)	48.8 (4.4)	57.2 (1.9)
		(16-hour)	P_T	57.4 (3.9)	51.1 (10.7)	53.6 (4.5)	60.9 (2.6)
	10000	0.15 M $NaHCO_3$	P_i	12.1 (0.8)	0.1 (0.1)	17.0 (0.3)	16.3 (1.9)
		(3-minute)	P_T	17.5 (1.2)	9.0 (0.8)	23.6 (0.7)	22.3 (0.2)
		0.5 M $NaHCO_3$	P_i	39.4 (3.0)	39.0 (1.0)	49.2 (2.7)	51.0 (2.6)
		(16-hour)	P_T	64.6 (4.1)	67.1 (2.7)	87.1 (9.1)	89.0 (6.6)
		1 N NaOH	P_i	39.8 (5.0)	37.4 (2.2)	20.3 (0.8)	25.0 (1.4)
		(16-hour)	P_T	112._ (13.7)	133._ (9.4)	97.3 (3.3)	97.8 (4.6)
		1 N HCl	P_i	51.2 (2.4)	40.1 (1.6)	51.5 (1.0)	56.8 (1.9)
		(16-hour)	P_T	57.2 (3.5)	54.2 (1.6)	58.5 (3.6)	61.1 (2.5)

APPENDIX C

ANALYSES OF VARIANCE OF THE CHANGES IN EXTRACTABLE FORMS OF PHOSPHORUS OVER TIME INTERVALS

By way of clarification, this table is subdivided into 27 subtables. The main table is organized into three columns, each labeled for a specific incubation time interval and nine rows each for a different P-extraction pool. Each subtable has four columns and four rows. The first three rows (from the top) and first three columns (from the left) show the mean change in the P-extraction pool during the time interval for the fraction × C level treatment indicated. The bottom row ($\bar{C}$) shows the mean change for each C level, while the rightmost column ($\bar{F}$) gives the mean for each fraction. The number in the lower right corner gives the mean change for all treatments for that time and extraction pool. The table entries are for treatments for which the analysis of variance (AOV) gave an F-value of ≤ 0.05 (unless otherwise indicated by superscripts described below). Letters to the right of, or just below, a table entry indicate significant differences. If two entries in the same row have the same letter below them, then they are not significantly different from each other. Similarly, two entries in the same column with the same letter to the right of each are not significantly different from each other. A letter accompanied by superscript 10 indicates that the means are significantly different at the 90% confidence level. Missing entries or entries in parentheses appear for the treatments

that the AOV indicated to have no or poor correlation ($F > 0.05$ or 0.10 as indicated by superscripts in the table).

Appendix Table C-1.

Fraction	ΔT_I (0-124 hours)				ΔT_{II} (124-814 hours)				ΔT_{814} (0-814 hours)			
	C amendment level (μg C/microcosm)											
	1250	5000	10000	$\bar{F}$	1250	5000	10000	$\bar{F}$	1250	5000	10000	$\bar{F}$
0.15 M $NaHCO_3$ P_i (3-minute)												
FC	4.5 a +a	-13.9 a b	-19.3 a c	-9.6 a	2.3 a +a	17.9 a +b	20.3 a c	13.5 a	6.8	4.0	1.1	4.0 a
CC	3.6 ab +a	-5.4 b a	-12.9 b b	-4.9 b	7.8 b a	14.1 b b	19.3 a c	13.9 a	11.3	8.7	6.7	8.9 b
FS	2.5 b +a	-6.4 b b	-12.0 b c	-5.3 b	6.7 b a	9.8 c b	17.0 b c	11.2 b	9.2	3.4	4.9	5.8 a
$\bar{cs}$	+3.5 a	-8.6 b	-14.7 c	-6.6	5.6 a	13.9 b	19.0 c	12.9	9.1 a	5.4 b	4.2 b	6.2
0.15 M $NaHCO_3$ P_o (3-minute)												
FC	5.2	7.5	13.2	8.6 a	-3.0	-5.8	-8.8	-5.9 a	2.2	1.7	4.4	2.8 a[10]
CC	2.1	2.8	5.7	3.5 b	-2.5	-3.6	-7.1	-4.4 a	-0.4	-0.9	-1.4	-0.9 b[10]
FS	1.8	0.0	3.6	1.8 b	-0.2	-0.4	-2.4	-0.9 b	1.6	-0.3	1.2	-0.8 ab
$\bar{cs}$	3.0 a	3.4 a	7.5 b	4.7	-1.9 a	-3.3 b	-6.1 b	-3.8	1.1	0.2	1.4	0.9
0.5 M $NaHCO_3$ P_i (16-hour)												
FC	-8.3	-9.8	-8.2	-8.8 a	16.6	15.7	5.8	(12.7)	8.3	5.9	-2.4	3.9 a
CC	-3.7	-4.1	-2.7	-3.5 b[10]	19.3	15.5	9.4	(14.7)	15.6	11.4	6.6	11.2 b[10]
FS	-2.5	-4.3	-0.4	-2.4 b[5]	15.7	10.4	10.2	(12.1)	13.2	6.1	9.8	9.7 a
$\bar{cs}$	(-4.8)	(-6.1)	(-3.8)	-4.9	17.2 a	13.9a[10]	8.5 b	13.2	12.4 a	7.8 ba	4.7 b	8.3

Appendix Table C-1 (cont'd)

Fraction	ΔT_I (0-124 hours) 1250	5000	10000	$\bar{F}$	ΔT_{II} (124-814 hours) 1250	5000	10000	$\bar{F}$	ΔT_{814} (0-814 hours) 1250	5000	10000	$\bar{F}$
					C amendment level (µg C/microcosm)							
0.5 M $NaHCO_3$ P_o (16-hour)												
FC	4.1	12.7	11.6		0.5	-0.3	-8.7 a		4.6	3.4	2.8 a	
	+a	b	b	9.5 a			↓	-5.8			↓	(3.6)
CC	-0.3	3.4	4.8		-5.0	3.5	6.9 b		-5.4	6.9	11.7 a	
	+			2.6 b				1.8	+a	b[10]	B	(4.4)
FS	2.2	-3.4	2.8		-4.5	0.0	18.0 b		-2.2	-3.4	20.9 b	
	+			0.5 b	+a	a	b	4.5	+a	a	b	(5.1)
cs	2.0	4.2	6.4	4.2	-3.0	-1.9	5.4	0.2	-2.0 a	2.3 ab	11.8 b	4.4
0.1 M NaOH P_i (16-hour)												
FC				(-3.3)	-5.1	-5.4	-9.8		-8.3	-9.9	-12.0	
								-6.8 a				-10.1 a
CC	ΔP_i = -2.9 (s = 1.8) N.S.D. zero				-16.4	-14.2	-15.9		-17.1	-17.0	-16.2	
				(-1.2)				-15.5 b				-16.8 b>[5]
FS					-18.1	-10.3	-17.0		-22.8	-15.6	-19.4	
				(-4.2)				-15.1 b				-19.3 b
cs	(-2.8)	(-4.3)	(1.7)	(-2.9)	(-13.2)	(-10.0)	(-14.2)	-12.5	(-16.1)	(-14.2)	(-15.9)	-15.4
0.1 M NaOH P_o (16-hour)												
FC	10.1	12.9	14.4		-3.5	-8.3	-8.6					
				9.1 a				-6.8 a>[10]				(5.6)
CC	18.6	25.4	30.9		-17.3	-17.9	-16.7		ΔP_o = 4.0 (s = 5.9) N.S.D. zero			
				25.0 b				-17.3 b				(7.6)
FS	9.7	10.5	22.9		-17.0	-11.0	-18.6					
				14.4 a[10]				-15.5 ab				(-1.2)
cs	12.8 a[10]	16.3 ba	22.7 b	17.3	(-12.6)	(-12.4)	(-14.6)	-13.2	(-0.2)	(3.8)	(8.1)	(4.0)

Appendix Table C-1 (cont'd)

Fraction	ΔT_I (0-124 hours) 1250	5000	10000	$\bar{F}$	ΔT_{II} (124-814 hours) 1250	5000	10000	$\bar{F}$	ΔT_{814} (0-814 hours) 1250	5000	10000	$\bar{F}$
					C amendment level (µg C/microcosm)							
1 M HCl P_i (16-hour)												
FC				(-8.5)				(7.3)				(1.1)
CC	ΔP_i = -9.6 (s = 1.5)				ΔP_i = 7.8 (s = 2.5)				ΔP_i N.S.D. zero			
FS				(-9.6)				(7.9)				(-1.7)
$\bar{cs}$				(-10.8)				(8.3)				(-2.5)
	(-8.7)	(-9.9)	(-10.3)	-9.6	(5.6)	(8.7)	(9.1)	(7.8)	(-3.0)	(-0.8)	(-1.5)	(1.8)
1 M HCl P_o (16-hour)												
FC				(4.8)				(-4.0)			(0.8)	
CC	ΔP_o = 6.0 (s = 1.5)				ΔP_o = -5.7 (s = 2.0)				ΔP_o N.S.D. zero			
FS				(7.2)				(-7.0)				(0.8)
$\bar{cs}$				(6.1)				(-6.1)				(0.1)
	(5.2)	(6.1)	(6.8)	6.0	(-4.8)	(-6.4)	(-5.8)	-5.7	(0.5)	(-0.4)	(1.0)	(0.4)
Total Extractable P												
FC	9.2	1.5	5.1	5.3 ba					20.7 a ↓	10.1 ba ↓	-2.4	
CC	17.5	17.3	22.5	19.1 a					2.0 ba	16.4 a	20.9	
FS	4.1	-15.2	11.4	0.1 ba>10					-13.2 b +a	-14.2 b>5 a	22.9 b	
$\bar{cs}$												