STABILIZATION OF SOIL AGGREGATES BY PLANT ROOTS

BY

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SUMMARY

Red-brown earths are used extensively for agriculture in Australia including horticulture in the Goulburn Valley Irrigation Area. Problems of structural stability are widespread. Thus the effects of plant roots and crop rotation on the stability of aggregates of red-brown earths were studied.

The root system of ryegrass was more efficient than that of white clover in stabilizing aggregates of Lemnos loam because the root system of ryegrass was longer and supported a larger population of vesicular-arbuscular (VA) mycorrhizal hyphae in the soil. Electron micrographs show that the hyphae were covered with a layer of amorphous material, probably polysaccharide, to which clay particles appear firmly attached.

The effect of management of the ryegrass on the stability of aggregates was related to the growth of these hyphae in soil. The quickest way to stabilize aggregates was to grow the ryegrass with ample water and to clip the tops at monthly intervals. Stressing the plants by allowing them to wilt reduced the growth of the plants and the stability of aggregates. The results show that provided there has been sufficient growth of roots, VA mycorrhizal hyphae can persist in soil for at least several months after the plants die, although the hyphae may no longer be viable.

The effect of crop rotation on the stability of aggregates was measured in another red-brown earth, the Urrbrae fine sandy loam. Fifty years of crop rotations have decreased the stability of macroaggregates (>250 μm diameter) and simultaneously decreased the lengths of roots and hyphae and the % total organic matter in the soil. Regardless of the rotation, particles 50-250 μm diameter were very stable due to organic matter.

The results also show that several binding agents, both organic and inorganic, are responsible for stabilizing aggregates of various sizes. Organic matter is the main agent responsible for binding clay-sized particles (<2 μm diameter) into stable aggregates >50 μm diameter, and that organic matter also binds fine particles <0.2 μm diameter into aggregates <2 μm diameter. Cementing due to amorphous oxides, crystalline oxides and highly disordered alumino-silicates was also responsible for some of the binding of particles <0.2 μm diameter into aggregates. From these results, a model of an aggregate for a red-brown earth is proposed.

STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and this thesis contains no material published previously or written by any other person, except where due reference is made in the text of the thesis.

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CHAPTER 1

LITERATURE REVIEW

1.1 Definition of Soil Structure

Soil structure as defined by Marshall (1962) "is the arrangement of the soil particles and of the pore space between them. It includes the size, shape, and arrangement of the aggregates formed when primary particles are clustered together into larger separable units. According to this definition there are no "structureless" soils and the structure is altered if a soil is deformed in any way". In most soils the structure is dynamic and can change from time to time, responding to changes in climate, biological activity and management of the soil.

The best structure for plant growth is one where aggregates are stable to water, i.e. hold their shape and integrity when wetted, and have a diameter of 1-5 mm (Russell 1973). Stable aggregates of 1-5 mm diameter will maintain sufficient pores (at least 10% of total volume) of diameter >50 μm to allow water and air to move freely through the soil (Greenland 1979) and to allow excess water to drain through the subsoil (Bakker *et al.* 1973) preventing roots from dying in water-logged soil.

1.2 Collapse of Aggregates in Water

When air-dried aggregates are wetted rapidly, they may or may not slake into smaller aggregates, and dispersion (where particles of clay are released) may or may not follow slaking. Moist aggregates may disperse directly without slaking (Arnold 1978 Plate 5.1).

1.2.1 Slaking

Rapidly wetted aggregates slake because they are not strong enough to withstand forces set up by the rapid intake of water (Collis-George and Lal 1971; Emerson 1977). Cements or binding agents, either inorganic or organic (Russell 1973) may prevent aggregates from slaking either by

strengthening bonds between the aggregates (Quirk and Panabokke 1962) or by water-proofing the aggregates (Emerson 1977). In the field slaking occurs mainly in aggregates in the surface layer since those below the surface may never become air-dry, and are usually wetted slowly during irrigation or rainfall (Marshall 1959). When slaking is severe, a crust of low permeability forms which when full of water leads to poor aeration (McIntyre 1955; Millington 1959) and when dry becomes hard (Arndt 1965); such a crust may prevent emergence of seedlings.

1.2.2 Dispersion

When aggregates are immersed in water, spontaneous dispersion occurs if the clay swells to such an extent that attractive forces between the particles are no longer strong enough to keep them together (Emerson 1977). Clay-sized particles are released slowly and appear as a spreading cloud around the aggregates (Arnold 1978 Plate 5.1). In the field the dispersed clay may move and block pores which transmit or store water. Soils with a high proportion of exchangeable sodium or magnesium tend to disperse easily (Emerson 1977). Organic matter tends to oppose dispersion when the organic matter increases the attractive forces between particles of clay (Emerson 1962; 1967). However when a stable wet aggregate is sheared organic bonds may be broken easily and the organic matter may increase the repulsive forces between particles so that the aggregate collapses (Emerson and Dettman 1959; Emerson and Smith 1970). This may happen in the field when a wet soil is cultivated or is trampled by animals (Emerson 1977), or when raindrops disrupt unprotected aggregates at the surface (McCalla 1944). There is some evidence that small amounts of organic anions may disperse clay (Bloomfield 1963), probably by blocking the positive sites on the clay and by complexing the polyvalent cations which flocculate the clay (Greenland 1965), and that organic anions may mobilize fine clay down the profile (Thorp *et al.* 1957).

1.3 Nature of Binding Agents which Stabilize Aggregates

1.3.1 Inorganic binding agents

Clay itself may bind particles together into large aggregates (>100 µm diameter) especially in soils with high contents of clay (>30%) (Chesters *et al*. 1957; Krishna Murti *et al*.1977) even when levels of organic matter are low. However if the clay is coated with layers of hydrous oxides (Greenland 1971) or highly disordered alumino-silicates (Greenland and Wilkinson 1969) the surface reactions will be those of the hydrous oxides or alumino-silicates and not of the clay. Hydrous oxides of iron and aluminium do bind particles together into large (>100 µm diameter) highly stable aggregates especially in soils which contain large amounts of hydrous oxides (>10%) (Kroth and Page 1946; Chesters *et al*. 1957; Kuznetsova 1966; Krishna Murti *et al*. 1977).

In some soils, calcium carbonate may be precipitated as films on the surface of particles of clay and bind particles together (Russell 1973). However part of the binding by calcium carbonate is because it maintains a sufficient concentration of calcium ions in the soil solution to prevent the clay from swelling and dispersing (Rimmer and Greenland 1976).

Even in soils with low contents of hydrous oxides or calcium carbonate, inorganic binding agents are responsible for stabilizing small aggregates (<250 µm diameter) (Hamblin 1977; Turchenek and Oades 1978)) especially associated with organic materials.

1.3.2 Organic binding agents

Stable aggregates are destroyed in many soils when treated with hydrogen peroxide to remove the organic matter (Kuznetsova 1966; Edwards and Bremner 1967) indicating that organic matter is involved in aggregation. Three main groups of binding agents in soil are described in the literature.

The three main groups are:

a) Polysaccharides

Polysaccharides are organic binding agents which are metabolized readily by microorganisms. They include (i) microbial polysaccharides associated with added organic residues e.g. simple sugars, straw (Harris *et al.* 1966), and (ii) polysaccharides associated with the microbial biomass in the rhizosphere (Russell 1973) or with roots (Oades 1978). These materials are produced rapidly, but do not persist in soil, and are associated with very young, large (>250 μm diameter) aggregates (Guckert *et al.* 1975).

b) Microbial filaments

Microbial filaments have been shown in some soils to be associated with the stable aggregates especially after readily decomposable organic matter has been added to the soil (Russell 1973). The filaments form a fine network which appears to entangle particles of soil into stable aggregates. The hyphae are mixed intimately with inorganic particles although they are probably removed with the light fraction <2.0 SG (Greenland and Ford 1964; D. J. Carter and J. M. Oades unpublished results; Oades 1967).

c) Organo-mineral complexes

Organo-mineral complexes are old, highly degraded, highly aromatic organic matter mixed intimately with inorganic particles (Turchenek and Oades 1978). These bonds take a long time to build up in soil but persist for many years and probably form part of the humus which carbon dating has shown to be over 1000 years old (Russell 1971). These bonds are not affected by management of the soil, are associated with microaggregates (<250 μm diameter) (Edwards and Bremner 1967) and are probably included in the skeleton grains of Bal (1973).

Each of these groups of materials will be considered in turn.

a) Polysaccharides

i) Microbial polysaccharides

Many experiments have shown that when simple sugars or plant residues were incubated in soil, which was often inoculated with pure cultures of microorganisms (Harris *et al.* 1966; Aspiras *et al.* 1971), the stability of aggregates increased rapidly presumably due to microbial activity. Many extracellular polysaccharides isolated from pure cultures when added in low concentrations (0.5% or less) to soil or clay stabilize aggregates immediately (Martin 1945; Harris *et al.* 1963) and are often more effective than plant residues or polysaccharides extracted from plants (Geoghegan and Brian 1948; Martin and Richards 1963). The effect of polysaccharides lasted for a few days or weeks only (Harris *et al.* 1966; Aspiras *et al.* 1971) presumably because microorganisms or their enzymes were able to decompose the binding agents which had been produced. Those polysaccharides which persist for more than a few days may be protected by sorption on clay (especially when soil and organic matter dry together), by complexing with metal cations or phenolic compounds (Oades and Swincer 1968; Martin, J. P. 1971; Griffiths and Burns 1972).

When aggregates which had been stabilized by microorganisms in the presence of readily decomposable organic materials were treated with sodium periodate, the aggregates often became less stable (Harris *et al.* 1963; Watson and Stojanovic 1965; Aspiras *et al.* 1971) suggesting that polysaccharides were the binding agents (Hepper 1975).

However Allison (1968) criticized experiments with artifically produced aggregates and readily decomposable organic material because the organic bonds produced persist for a few days or weeks only and the bonds do not form aggregates, they simply hold particles together. In such experiments the soil generally remained moist yet localized drying which occurs near roots in natural soil may increase the adsorption of organic bonds to particles of clay (Greenland 1971) and thereby increase the persistence of

the polysaccharides and their efficiency to stabilize aggregates of soil (Oades and Swincer 1968). The soils were usually incubated at 25°C to 28°C (e.g. Martin 1945; Swaby 1949; Harris *et al.* 1964; Aspiras *et al.* 1971) which is at least several degrees higher than temperatures usually found in soils in the field (Cockroft and Hughan 1964; Waite Inst. Biennial Report 1974-5); yet temperature affects the microbial production and persistence of stable aggregates (Harris *et al.* 1966).

The organisms used to inoculate soil or to collect polysaccharides were saprophytic, and generally Gram negative rods or spore-forming bacteria or spore-forming fungi; they were selected because they grew profusely on artificial media where they often produced copious quantities of polysaccharides either as extracellular or capsular gums, or as gums in the cell wall. Such organisms may not have represented those in natural soil (Warcup 1967; Casida 1968; Mosse 1973) nor may they have produced polysaccharides in soil (Aspiras *et al.* 1971) . Extracellular microbial polysaccharides produced on artificial media have been characterized physically and chemically (Geoghegan and Brian 1948; Clapp *et al.* 1962; Clapp and Davis 1970) but there may or may not be a definite relationship between these properties and aggregation of soil.

Because it is easy to isolate soil organisms on artificial media rich in nutrients, and to collect polysaccharides produced by the organisms, an excessive number of experiments has been done on the effect of microbial polysaccharides on aggregation. But it is not always valid to extrapolate to the natural soil, results of experiments with organisms on artificial media (Bowen and Theodorou 1979).

However microbial polysaccharides probably do play a role in aggregation in natural soil. Electron micrographs of soil or thin sections of soil in the rhizosphere (Jackson *et al.* 1946; Marshall 1976; Foster and Rovira 1976; Foster 1978) have shown individual living bacteria or colonies of bacteria surrounded by a capsule, composed of polysaccharides, to which partides

of fine clay appeared to be attached firmly.These cells and polysaccharides which bind the particles of clay together are probably young, since only about 2% of the total organic carbon in soil consists of biomass (Jenkinson and Rayner 1977) and are probably precursors of less temporary binding materials.

ii) Plant polysaccharides - mucilage

As mentioned above, polysaccharides extracted from plants and added to soil can increase stable aggregation but generally not to the same extent as microbial polysaccharides. However mucilage, a slimy secretion consisting of polysaccharides, and which coats the epidermal surfaces of roots may be involved in aggregation (Oades 1978). The mucilage produced by roots of maize is rich in fucose (Oades 1978) which contains methyl groups which may enable the polysaccharides to sorb strongly onto clays (Greenland 1956).

Soil often remains on the surface of roots after they have been washed (Troughton 1957; Nambiar 1976), and it has been suggested that mucilage produced by the root (or by bacteria in the rhizosphere) may bind particles of soil together and to the root (Sprent 1975; Foster and Rovira 1976; Nambiar 1976). Electron micrographs showed that mucilage on barley roots sometimes filled the space between the root and surrounding bentonite or permutite (Jenny and Grossenbacher 1963), and inorganic particles of various sizes were seen adhering to the external surface of, or embedded in, the mucilage adhering to roots of several plant species (Greaves and Darbyshire 1972; Campbell and Rovira 1973; Rovira and Campbell 1974; Sprent 1975; Foster and Rovira 1976). That is,inorganic particles were sorbed onto organic mucilages rather than by the widely accepted mechanism of organic polymers sorbed onto inorganic particles (Oades 1978). Particles of clay were often oriented tangentially to the root. Different species of plants may produce different amounts of mucilage, e.g. ryegrass roots appeared to produce more mucilage than clover roots did (Campbell and Rovira

1973); such differences may explain some of the differences in the stabilization of soil by different plant species (Russell 1973).

The quantity of inorganic particles adhering to the surface of roots increased, especially near the root cap, the distal regions of the root tip and on the root hairs, when plants were stressed for water (Sprent 1975); mucilage appeared to stick the inorganic particles to the root tip. Nambiar (1976) and Martin (1977a) suggested that water-stressed roots produced more mucilage (when compared with unstressed roots), so that more particles of soil adhered to the root. However drying probably increases the contact between inorganic particles and the mucilage and so makes the bonds between them stronger (Greenland 1971).

It appears that plant mucilage does not persist on the surface of roots in soil, but is replaced by microbial mucilage which remains in close contact with particles (Oades 1978). Therefore plant mucilage may stabilize aggregates indirectly, rather than directly, by providing energy for micro-organisms which produce further stabilizing substances.

iii) Soil polysaccharides

Some polysaccharides, when extracted from soil, can stabilize clay or soil (Swaby 1949; Rennie *et al*. 1954; Whistler and Kirby 1956), but are generally less effective than microbial polysaccharides. This may be because the fraction of polysaccharide which is the most difficult to remove from soil may be the main fraction which stabilizes aggregates in natural soils (Oades and Swincer 1968). Soil polysaccharides which were capable of stabilizing aggregates contained a wide range of sugars but contained a low proportion of D-xylose which suggests that they probably originated from microorganisms (Whistler and Kirby 1956).

Many studies have shown that polysaccharides generally account for 5 to 25% of the total organic carbon (Swincer *et al*. 1969; Martin, J.P. 1971). The amount of carbohydrate may change but the proportion of the total organic carbon present as carbohydrate was affected little by agricultural practices

Oades 1967); however soil from pasture contained 2-3 times more polysaccharide with a molecular weight >100 000 than soil from a fallow-wheat rotation.

The polysaccharide content of soils has been correlated with stable aggregation (Rennie *et al.* 1954; Chesters *et al.* 1957; Acton *et al.* 1963; Oades 1967; Chatterjee and Jain 1970), although not always (Salomon 1962). The correlations were generally not good. For example the correlation coefficient between stable aggregation in two soils and their % polysaccharide was only 0.35 (Acton *et al.* 1963). The low correlations may have been because methods used to estimate polysaccharides were crude (Griffiths 1965), or only certain polysaccharides which were not a constant proportion of total polysaccharides stabilize aggregates (Stefanson 1971) and may be those most difficult to remove from soil (Oades and Swincer 1968). Polysaccharides isolated from some soils had widely differing molecular weights probably reaching several million (Swincer *et al.* 1968) so one may expect that they may vary in their efficiency to stabilize aggregates. Good correlations between light fraction or free organic materials and stable aggregation (Oades 1967) suggest that if polysaccharides do stabilize aggregates in the field, then it is those which microorganisms have produced recently which are important.

When some soils are treated with periodate, the stable aggregation decreases, especially in those soils which contain low contents of organic matter (and few inorganic bonds), that is those soils which are cropped annually or soils under young pastures (three years and less) (Greenland *et al.* 1962; Clapp and Emerson 1965; Stefanson 1971). The periodate-sensitive materials which stabilize aggregates under young pastures are probably polysaccharides and probably have a molecular weight of more than 100,000 and consist of large flexible polymers (Swincer *et al.* 1968). However recent results suggest that organic compounds other than polysaccharides are more important in stabilizing aggregates in some soils (Hamblin and Greenland 1977).

b) Microbial filaments and cells

i) Fungal hyphae

Fungal hyphae have been shown in some soils to be associated with stable aggregates (Hubbell and Chapman 1946; Bond and Harris 1964). Hyphae, which were not necessarily viable, were sticky and encrusted with fine particles of clay (Hubbell and Chapman 1946) and retained their strength when stable wet aggregates from the field were dissected (Bond and Harris 1964). Stable aggregates in sand-dune soils were also held together by fungal hyphae (Koske *et al.* 1975; Forster 1979). Although individual hyphae are not strong the combined strength of all hyphae especially in a three-dimensional network, would hold particles more or less equally in all directions so that aggregates would not form planes of weakness when wetted rapidly, that is the aggregates would not be susceptible to incipient failure (Quirk and Panabokke 1962).

It appears that fungi stabilize aggregates >2000 μm (Harris *et al.* 1966) or >560 μm diameter (Hubbell and Chapman 1946). This is probably because fungal hyphae are large and because they can grow in drier soil than bacteria can (Jackson 1975), they can grow across large pores in soil (Marshall 1976) which, in well-drained soils, are likely to be empty of water except during irrigation or heavy rainfall. Fungi have been shown to grow mainly in the outer parts of aggregates (Hattori 1973).

Many authors (e.g. Martin *et al.* 1955; Low and Stuart 1974) believe that stabilization of aggregates by fungi in the field would be limited to periods when easily decomposable material had been added to the soil in large amounts leading to a flush of hyphal growth (McGill *et al.* 1973). This may be true of the fungal species which most workers have studied. Such species produce characteristic spores, are isolated easily from soil and grow readily on dilution plates. However fungal hyphae were shown in the field always to be associated with water-stable aggregates of a red-brown earth, with little seasonal variation; unstable aggregates contained few hyphae (Bond and Harris 1964).

Saprophytic fungi which remain sterile in culture and which are rare or absent from dilution plates may have been responsible for some of the observed stability in the field, since some of these sterile species could be isolated from soil in the field throughout the year (Warcup 1967). This group includes dark coloured fungi which tend to persist in soil for longer periods than hyaline fungi (Martin *et al.* 1959; Hurst and Wagner 1969). Many dark coloured fungi are sterile forms or forms which in some species produce pycnida, sclerotia or ascocarps. The resistance of these fungi to microbial lysis is related to the presence of the dark pigments, melanins, in the fungal wall (Wagner 1975). These melanic fungi occur widely in soils (Warcup 1967) but tend to be less conspicuous than sporing fungi so that their importance in aggregation may have been overlooked; yet Martin *et al.* (1959) showed that some melanic fungi stabilized aggregates as effectively as hyaline fungi did.

Several pigments have been isolated from such fungi and have been characterized as being similar to humic compounds (Kang and Felbeck 1965; Haider and Martin 1967). Martin *et al.* (1967) showed that the fungus *Epicoccum nigrum* synthesized as "humic acid" (when cultured on artificial media) which persisted in soil and which stabilized aggregates.

ii) Other microbial filaments

In desert soils filaments of blue-green algae formed a solid and mechanically strong net which bound particles of soil or sand into a tough layer on the surface of the soil (Bond and Harris 1964; Went and Stark 1968). This layer may become leathery in water and even then may be difficult to break. Algae and lichens or algae and fungal hyphae may also form crusts in desert soils which stabilize the soils against erosion (Fletcher and Martin 1948; Shields *et al.* 1957).

iii) Bacterial cells

Although fungi constitute more than 50% of the microbial biomass in soil (Wagner 1975) and probably contribute more than bacteria do to the organic matter in soil (Paul and van Veen 1978), some organic bonds probably develop also from degraded bacterial cells in the rhizosphere or around decaying organic residues (Marshall 1976; Foster 1978).

c) Organo-mineral complexes

Highly degraded, highly aromatic humic material associated with amorphous iron, aluminium and aluminosilicates (Hamblin 1977; Tate and Churchman 1978; Turchenek and Oades 1978) form part of the large organo-mineral fraction of soil which constitutes 52-98% of the total organic matter in soils (Greenland 1965). The organo-mineral complexes include those bonds which link complexes of clay-polyvalent metal-organic matter, C-P-OM and $(C\text{-}P\text{-}OM)_x$, both of which are <2 μm diameter, into stable micro-aggregates $((C\text{-}P\text{-}OM)_x)_y$ which are <250 μm diameter as described by Edwards and Bremner (1967).

The organic matter in organo-mineral complexes has a narrow C/N and is extracted more easily than plant material by pyrophosphate; because the organic matter is highly degraded it tends to be in the dense fractions of soil (densities up to 2.30 g cm^{-3}) (Turchenek and Oades 1978). The organo-mineral bonds probably include the POM (physically stabilized organic matter) with a half-life of 49.5 years, and the COM (chemically stabilized organic matter) with a half-life of 1980 years (Jenkinson and Rayner 1977). However the extent to which the organic matter is protected because it is inaccessible to enzymes or sorbed onto the surface of clay, or because suitable enzymes are not present is yet to be determined (Oades and Ladd 1977).

Organo-mineral complexes are probably derived from the resistant fragments of hyphae, bacterial cells and colonies developed in the rhizosphere (Marshall 1976; Foster 1978; Turchenek and Oades 1978). The

organic matter is believed to be the centre of the aggregate with particles of fine clay sorbed onto it (Turchenek and Oades 1978) rather than the organic matter sorbed onto clay surfaces (Emerson 1959; Greenland 1965). However the organo-mineral complexes have not yet been defined chemically. It is likely that a precise chemical definition is not possible in the same way that a formula for humic acid cannot be written. Although some of the organo-mineral bonds can be broken with ultrasonic vibration (Edwards and Bremner 1967), in some soils, especially those with high % total carbon, organo-mineral complexes within particles 1-20 μm diameter resist ultrasonic vibration for up to 5 min (Hamblin 1977; Tate and Churchman 1978; Turchenek and Oades 1978).

1.4 The Effect of Plant Roots and Management on Water-Stable Aggregation

1.4.1 Soil management and aggregation

In soils where organic materials are the main binding agents, macroaggregates in cultivated soils are less stable than macroaggregates in corresponding virgin soil or soil under old pasture (Low 1954; Malik *et al.* 1965; Juo and Lal 1975). The stable aggregation (>2 mm diameter) to a depth of 50 mm in an old arable red-brown earth was about 2% of that in the corresponding virgin soil (Greacen 1958). The breakdown due to frequent cultivation is less where fallow is excluded from the rotation or where crop residues are not removed (Ramig and Mazurak 1964; Ridley and Hedlin 1968; Emmond 1971; Juo and Lal 1975). However the stable aggregation decreases gradually and residual effects of pasture on aggregation were still evident up to 4 years after ploughing a perennial pasture for cropping, the residual effect being related to the number of years of pasture before ploughing (Low 1954; Low *et al.* 1963; Mazurak and Ramig 1963). Some recovery is possible when an arable soil is sown to pasture; the stable aggregation to a depth of 15 mm of an old arable soil was doubled after three years of pasture (Clement and Williams 1958). However recovery may be slow and in some old English arable soils 50-100 years of pasture were

needed for the stability to reach that of old pasture (Low 1955).

Because the increase in stable aggregation under young pasture is related to the length of root (Barley 1953 ; Clement 1961) and because organic materials accumulate at the surface, most of the increase in aggregation is in the top layers of soil (Clement and Williams 1958). This reflects the depth of the cultivated layers, the depth to which phosphorus has been applied, and the volume of soil where most of the roots are. In pastures up to six years old, stable aggregation to a depth of 25 mm was up to eight times greater than that below a depth of 50 mm; in contrast in a virgin sample from the same soil, aggregation was highly stable and uniform with depth (Greacen 1958).

Stable aggregation under pasture varies seasonally, generally following periods of growth and death of roots and rapid microbial activity (Rennie *et al.* 1954). In non-irrigated soils in a temperate climate the stability reaches a peak in summer, possibly at the time when sorption between soil and organic bonds is maximum (Oades and Swincer 1968; Stefanson 1968), and declines slowly during autumn and winter (Wilson *et al.* 1947; Clement and Williams 1958). However the seasonal variation in stability of aggregates under pasture was only about 10% (Clement and Williams 1958). Periodate-resistant, pyrophosphate-resistant materials, possibly fungal hyphae (D. J. Carter and J. M. Oades unpublished results), were responsible for seasonal variation in a red-brown earth (Stefanson 1971).

However in cropped soils which are irrigated during summer, i.e. are subjected to repeated cycles of wetting and drying, the stable aggregation decreases over summer, especially when the soil is cultivated frequently (Cockroft and Hughan 1964). Severe restriction of microbial activity by dryness between irrigations increases the deleterious effect of physical disruption of aggregates, but if the soil is kept moist by frequent

irrigations microorganisms may produce binding agents which compensate partially for those broken physically (Tisdall *et al.* 1978).

There is some evidence that the stability of aggregates may be increased without organic matter, by being exposed to cycles of wetting and drying or freezing and thawing (Sillanpaa and Webber 1961; Russell 1973). On drying the particles of clay may become oriented (Brewer and Haldane 1957) lowering the entropy of the system.

1.4.2 Organic carbon

The stable aggregation of some soils is related to % total organic carbon (Low 1972; Clement 1961; Kutnetsova 1966). However correlations are not always good (Chesters *et al.* 1957; Oades 1967) because (a) only part of the organic matter is responsible for stable aggregation (Stefanson 1971), (b) in a given soil there is a % organic carbon above which aggregation does not increase further (Heinonen 1955; Kemper and Koch 1966; Grierson *et al.* 1972), (c) in some soils organic materials are not the main binding agents (Greenland 1971) and (d) because some of the stability is related to non-cultivation (Malik *et al.* 1965; Low 1972). The stability is sometimes related better to the free organic materials or to the light fraction (<2.0 SG) (Oades 1967) probably because this fraction acts as a substrate for the production of bonds, or because this fraction is a measure of the growth of roots and hyphae.

Residues released into the soil by roots are in the form of fine lateral roots, root hairs, sloughed-off cells from the root-cap, dead cells, lysates and volatile and water-soluble materials (Soper 1959; Rovira and McDougall 1967; Shamoot *et al.* 1968; Dickinson 1974). Martin (1977b; 1977c) showed that roots released into soil 5-15% of photosynthetically fixed carbon although part of it was carbon dioxide; in an experiment with wheat, 6.6% of the fixed carbon was found in the soil and 9.2% respired in the rhizosphere. The amount of carbon released by roots is related to the total length of root; regardless of species, plants grown in an atmosphere

of $^{14}CO_2$ from seedling stage to near maturity released 20-49 g organic material per 100 g harvested root (Shamoot *et al.* 1968).

Management of a soil can change the % organic matter two- or three-fold (Turchenek and Oades 1978). Organic matter usually accumulates under pasture (Clement 1961) since pasture plants produce 1.2 kg phytomass $m^{-2}y^{-1}$, 60% of which consists of root residues; 25-50% of roots decompose annually (Oades and Ladd 1977). Growing plants also appear to retard decomposition of organic carbon (Fuhr and Sauerbeck 1968); five years after ^{14}C-labelled ryegrass roots were added to soil,47% more ^{14}C remained in soil under grass than in bare soil (Jenkinson 1977).

Organic matter is lost from soils which are cultivated frequently, especially if fallow is included in the rotation or crop residues are removed (Ridley and Hedlin 1968; Martel and Paul 1974; Juo and Lal 1977). Cereal crops produce only 0.1 to 1.0 kg phytomass $m^{-2}y^{-1}$ (Oades and Ladd 1977) and organic residues are not supplied continuously; minimal amounts are added to soil during fallow. In cropped rotations, because the soil is cultivated frequently and is often without vegetative cover to protect it from physical disruption due to rapid wetting or the impact of raindrops (Low 1954; Clement and Williams 1958;McCalla 1959), previously inaccessible organic matter is exposed and oxidized by microorganisms (Rovira and Greacen 1957; Martel and Paul 1974; Adu and Oades 1978).

The root systems of pasture plants are extensive, and at a given depth the residues are distributed evenly through the soil. In the upper layers the soil is probably all rhizosphere (Thornton 1958);for example the half-distance between roots of *Lolium multiflorum* was 1.5 mm at a depth of 20 mm (Barley 1970), and abundant root hairs would reduce the distance even further. Pasture soils supply more energy than cultivated soils, so pasture soils support higher biological activity, as measured by mineralization of carbon and nitrogen, plate counts, and as observed by light and electron

microscopy (Clement and Williams 1962; Rovira 1978). This intense activity and localized drying in the rhizosphere under pasture plants provide intimate contact between particles of clay and organic matter, and lead to formation and stabilization of soil aggregates (Allison 1968; Oades 1978).

Although management of the soil changes the % total organic matter, certain forms of chemically defined fractions appear to be degraded at the same rate (Martel and Paul 1974; Oades and Turchenek 1978). Sixty years of cultivation decreased the total % organic carbon to 40% of that of virgin soil yet the distribution of hydrolyzable and non-hydrolyzable materials did not change. However humic materials from cultivated soils tend to contain fewer aliphatic groups e.g. CH, NH_2 and more carboxyl groups than humic materials from non-cultivated soils (Kimber and Searle 1970; Dormaar 1979) which may alter sorption between the organic matter and particles of clay.

Part of the increase in stable aggregation under pasture is probably related to the distribution of organic bonds within an aggregate. Turchenek and Oades (1978) showed that in a red-brown earth the 0.4 µm to 20 µm sized fraction obtained ultrasonically contained most of the organic material and that about 66% of that fraction was organic material. They showed also that in the same soil under pasture, 8% of the silt-sized fraction (2-20 µm diameter) consisted of particles less than 2 µm diameter. Whereas in unstable soil under wheat-fallow, none of the silt-sized fraction consisted of particles less than 2 µm diameter. The clay-sized particles (<2 µm diameter) appeared to have been bound into silt-sized aggregates. Aromatic humic materials (organo-mineral bonds) and microbial polysaccharides were possibly responsible for binding the clay particles into silt-sized aggregates under pasture. Scanning electron micrographs also suggested that organic colloids and inorganic particles were associated more intimately in soil from old pastures than from corresponding old arable soils (Low and Stuart 1974; Hamblin 1977). Plants may distribute substrate into coarse pores (2 µm to 50 µm diameter) which are large enough to accommodate micro-

organisms. This pore-size may be important since natural organic materials which lined the coarse pores (50 μm diameter) led to the greatest strength of aggregates (Greenland 1965).

1.4.3 Management of plants

In the absence of inorganic binding agents, aggregation is related to the % organic carbon. Therefore to maintain stable aggregates, plants should be managed so that they rapidly produce a large network of roots and maintain the % organic carbon at a predetermined optimal level determined by one of the various models which describe the turnover of organic matter (see Jenkinson and Rayner 1977; Paul and van Veen 1978).

Only small differences in aggregation arise from the use of different species of grasses (Pringle and Coutts 1956; Clement and Williams 1958) but the stability tends to increase more rapidly under grass than under legumes (Robinson and Jacques 1958). The differences are mainly because grasses tend to produce a more extensive fibrous root system than legumes (Jacques 1943; Barley 1953; Barber 1959). Roots of grasses may also release greater amounts of water-soluble material (Martin J. K. 1971) and mucilage (Campbell and Rovira 1973) than legumes.

Generally within 1-2 days of plants being clipped, roots stop growing for up to 2-3 weeks and many of the old roots die (Crider 1955; Butler *et al.* 1959; Oswalt *et al.* 1959). Hence recurrent clipping leads to cycles of decay and regrowth (Butler *et al.* 1959) and to additional release of water-soluble materials (Martin J.K. 1971).

Dry soil may lead to the release of additional organic materials by roots (Katznelson *et al.* 1955; Sprent 1975) and increases the contact between soil and roots (Nambiar 1976); therefore it is possible that recurrent wilting of plants may also increase the stable aggregation.

1.4.4 Localized drying around roots

Part of the effect of plants on stable aggregation is due to localized drying around roots (Allison 1968). Electron micrographs of the rhizosphere show that particles of clay close to roots tend to be oriented almost parallel to the axis of the root; the % of oriented particles increases with the age of the root and with decreasing radial distance from the root (Blevins *et al.* 1970; Greaves and Darbyshire 1972; Foster and Rovira 1976). The particles of clay were probably reoriented by the expanding roots and by localized drying around the roots from randomly dispersed positions to positions of minimal potential energy (Aylmore and Quirk 1959).

1.4.5 Indirect effect of plants

Plants may also improve the structure of soils indirectly by providing food for small animals, such as earthworms, enabling large populations to build up. Soil under three-year-old pasture had few earthworms but after eight years there were more than 1.5×10^{6} ha^{-1} (Low 1955). Earthworm casts generally contain more organic matter than the surrounding soil (Swaby 1950). The earthworm may stabilize structure by ingesting soil and mixing it intimately with humified organic materials in its gut (Swaby 1950; Barley 1959; Greenland 1965) or by lining its tunnels with mucilage. In a non-cultivated peach orchard where adequate food and water were present throughout the year, earthworm populations increased in three years to 2000 m^{-2} compared with 150 m^{-2} where food and water were scarce, and infiltration of water was increased 80-fold (Tisdall 1978).

CHAPTER 2

EXPERIMENTAL

2.1 Description of Soils

Two red-brown earths, Lemnos loam (Skene and Poutsma 1962) and Urrbrae fine sandy loam (Litchfield 1951) were used in the experiments. The surface horizon of each soil consists of a fine sandy loam which is free from calcium carbonate.

The surface horizon (0-10 cm) of Lemnos loam, was collected from land which had grown tomatoes recently under irrigation for two successive years resulting in poor water-stable aggregation. The particle-size analysis of soil, dispersed by the method described in Loveday (1974), was 33% clay, 34% silt, 29% fine sand and 3% coarse sand. Total organic carbon was 1.7% and the pH was 5.5 (1:2 soil:water).

Samples of soil were taken during winter from the surface horizon (0-10 cm) of Urrbrae fine sandy loam, from the C1 permanent rotation trial at the Waite Agricultural Research Institute. The particle-size analysis was 20% clay, 33% silt, 41% fine sand and 3% coarse sand; the pH was 5.7. The histories of the sites sampled varied from an old pasture to an alternate wheat-fallow rotation. Total carbon contents varied from 1.1% to 2.3% (Table 8). A sample was also taken from an adjacent area, which has never been cultivated but was top-dressed once more than 50 years ago, and is now grazed and supports introduced plant species. This soil contained 2.5% total organic carbon. All samples were composites of 20 cores (50 mm diameter). Live shoots were removed from each sample, and while still moist and friable, the soil was passed through a 10 mm sieve.

2.2 Chemical Pre-treatment of Aggregates

Sodium chloride, sodium periodate or sodium pyrophosphate

Air-dried soil (<10 mm; 20 g) was immersed for 6 h in 120 cm^3 0.02 M NaCl, 0.02 M $NaIO_4$ or 0.1 M $Na_4P_2O_7$, pH 10.0 (Stefanson 1971). All solutions contained a crystal of thymol to inhibit biological activity. The soil was then drained for 2 h or 16 h, and the size-distribution of water-stable particles determined (see section 3.3).

Hydrogen peroxide

Air-dried soil (<10 mm; 20 g) was moistened with distilled water, then small increments of 30% H_2O_2 were added until the suspension no longer effervesced. The suspension was boiled for 10 min to destroy excess H_2O_2, then cooled.

2.3 Measurement of Root Length

The total length of root or length of root infected with VA mycorrhizal fungi was measured in one sub-sample per replicate in a) water-stable particles separated by wet-sieving, or b) whole soil.

a) Water-stable particles

Discrete macroorganic particles were removed from the water-stable particles by flotation using a solution of sucrose (1.4 g cm^{-3}). The soil particles were washed with distilled water on Whatman No. 50 filter paper until the filtrate was free from sucrose (i.e. the filtrate did not react with anthrone reagent; usually 1 dm^3 water was needed). The oven-dried mass (105°C) of the material on each filter paper was determined. Aggregates were disrupted in water by a high-speed mixer and the roots were separated from the disrupted aggregates by flotation using distilled water (Barley 1955). The roots were cut into small segments and spread evenly on a 10 x 10 mm square grid or

a 5 x 5 mm square grid, depending on the size of sample. The length of root in each sample was determined either by $R = \frac{\pi NA}{2H}$ (Newman 1966), or by $R = \frac{11}{14} NG$ (Tennant 1975). Root length (R) was measured by counting the number of intercepts (N) of roots in a regular area (A) with randomly oriented lines of total length (H) or lines of a grid with grid unit (G).

b) Whole soil (<10 mm; 5 or 10 g)

Roots were separated from the whole soil by flotation using distilled water in as (a) above. In some samples of the whole soil, the length of root infected with VA mycorrhizal fungi was determined on roots which had been cleared with 10% KOH and stained with trypan blue (Phillips and Hayman 1970).

2.4 Measurement of Hyphal Length

The total length of hyphae was measured in one sub-sample per replicate in a) water-stable particles separated by wet-sieving, or b) whole soil.

a) Water-stable particles

Discrete macroorganic particles were removed from the water-stable particles by flotation using a solution of sucrose (1.4 g cm^{-3}). Aggregates were disrupted in water by a high-speed mixer and the hyphae were separated from the disrupted aggregates by flotation using distilled water. An aliquot (0.5 cm^3 or 1.0 cm^3) of a suspension of the hyphae (the size of aliquot depending on the length of hyphae) was stained with trypan blue, collected on a membrane filter within a circular area of diameter 15 mm (Hanssen *et al.* 1974) and the length of hyphae measured using a grid unit of 1.5 mm (Tennant 1975). The suspension of soil from which the hyphae had been removed was centrifuged, decanted and re-suspended several times until the

supernatant was free from sucrose. The oven-dried mass (105°C) of the soil was then determined.

b) Whole soil (<10 mm; 5 g or 10 g)

Hyphae were separated from the whole soil by flotation using distilled water as in (a) above.

The measurement of hyphal growth in soil is difficult partly because fungal hyphae are difficult to isolate from soil. The length of hyphae has been calculated from measurements of the mass of hyphae assuming a certain diameter and specific gravity of hyphae (Sanders and Tinker 1973). However hyphae from the one mycelium may differ in diameter (Mosse 1959) and it is also possible that the diameter may differ in different environments. Another method was to wash and agitate the soil in water repeatedly so that fungal hyphae floated free from soil particles, the fresh or dried mass of hyphae was used to indicate hyphal growth. However although adhering soil may have been cleaned off the hyphae under the microscope (Sanders *et al.* 1977), hyphae which were grown in a sand which contained little clay, were shown to have fine particles of clay (<0.2 μm diameter) attached firmly to the surface (Fig.10 and Fig.11). Hence the "mass of hyphae" (Sanders *et al.* 1977) probably included contaminating mineral soil and foreign organic material.

Most methods of measuring the total length of hyphae in soil involve observation through a microscope and are tedious especially when many samples need to be analyzed; at best they give only a rough measure of the length of hyphae. Errors arise partly because soil is heterogenerous making it difficult to obtain a representative sample, and partly because the hyphae are difficult to see and to measure. Jones and Mollison (1948) stained and mounted agar films of soil suspensions on slides and measured total length of hyphae in a defined area. Nicholas *et al.* (1965) made thin sections of soil and assessed seasonal

changes in the fungal population by measuring the length of hyphae per unit amount of soil. In a simplified method, Hanssen *et al.* (1974) stained and filtered dilute homogenized suspensions of soil through a membrane filter and, using a drawing apparatus, measured the length of hyphae per filter.

The method used in the experiments described in this thesis is similar to that of Hanssen *et al.* (1974) except that hyphae were measured more easily by the method of line intercepts. Errors arose in the method because (a) it was difficult to homogenize suspensions of soil and hyphae, (b) fragments of hyphae possibly adhered to the surfaces of glass when suspensions were diluted, and (c) not all hyphae may have been stained and been visible during counting. The method does not differentiate between dead and viable hyphae. However, the results on lengths of hyphae presented in this thesis show large and significant differences between soils from different treatments or with different histories.

CHAPTER 3

MEASUREMENT OF STABILITY

3.1 Introduction

There are many arbitrary methods for measuring the stability of aggregates. The methods include wet-sieving, permeability, infiltration, sedimentation of suspensions of aggregates in water, pore-size distribution, or visual assessment of aggregates after wetting and remoulding (Williams *et al.*1966; Collis-George and Laryea 1972). Results from different methods may rank the stability of soils in the same order but 'spacing' between the soils may depend on the method used (Collis-George and Laryea 1972); or results from one method may detect differences in stability between soils whereas another method may not (Williams *et al.* 1966). Results may vary considerably when the method is carried out by different people (Low 1954).

Therefore a method for measuring absolute stability was tested.

3.2 Absolute Measurement of Stability

North (1976) introduced a method of ultrasonic dispersion whereby an absolute value for the stability of a single soil could be determined. North used an ultrasonic probe to apply a range of known dispersive energies to a sample of soil to determine a dispersion characteristic for the test soil. This method was criticized by Koenigs (1978) and defended by North (1978).

The aim of the work described in this chapter was to use North's method to determine absolute stabilities of soils of different types and histories, and to determine the energies by which particles were bonded together into aggregates of different sizes.

3.2.1 Theoretical

North (1976) determined the power output P of the probe (system A) by measuring the increase in temperature $T_A(t)$ when a known mass of distilled water m_w^A in a Dewar vessel (heat capacity w_v) was irradiated for some time with heat losses H(t) at time t (system A)

$$P = (m_w^A c_w + w_v)\frac{dT_A}{dt} + H(t) \qquad (1)$$

where c_w = the specific heat of water.

Then assuming the same power output of the probe, the same viscosity of liquid and the same heat losses as that when water alone was used, North (1976) measured the increase in temperature $T_B(t)$ when a soil suspension in the same Dewar vessel and at the same initial temperature as above, was irradiated at power output P for time t (system B)

$$P = (m_w^B c_w + w_v)\frac{dT_B}{dt} + m_{sa} c_s \frac{dT_B}{dt} + L(t) + H(t) \qquad (2)$$

where m_w^B is mass water ($\simeq m_w^A$), L(t) is total energy rate involved in dispersion of soil present at time t, and c_s = the specific heat of soil.

In general L(t) will depend on the system, i.e. the amount of soil, amount of water, type of soil, power input and elapsed time.

Integrate equations (1) and (2) and put

$$H(t) = \int_0^t H(t)dt$$

$$\Delta T_A(t) = T_A(t) - T_A(0)$$

$$\Delta T_B(t) = T_B(t) - T_B(0)$$

where $T_A(0)$ and $T_B(0)$ are the initial temperatures in the systems A and B respectively.

$$L(t) = \int_0^t L(t)dt$$

Then

$$Pt - H(t) = (m_w^A c_w + w_v)\Delta T_A(t) = \lambda_A \Delta T_A(t) \quad (3)$$

$$Pt - H(t) = (m_w^B c_w + w_v)\Delta T_B(t) + m_{sa} c_s \Delta T_B(t) + L(t)$$

$$= \lambda_B \Delta T_B(t) + m_{sa} c_s \Delta T_B(t) + L(t) \quad (4)$$

Eliminate $Pt - H(t)$

$$L(t) = \lambda_A \Delta T_A - \lambda_B \Delta T_B - m_{sa} c_s \Delta T_B$$

$$= \lambda_A \Delta T_A \left[1 - \frac{\lambda_B \Delta T_B}{\lambda_A \Delta T_A}\left(1 + \frac{m_{sa} c_s}{\lambda_B}\right)\right]$$

$$= \left[Pt - H(t)\right]\left[1 - \frac{\lambda_B \Delta T_B}{\lambda_A \Delta T_A}\left(1 + \frac{m_{sa} c_s}{\lambda_B}\right)\right] \quad (5)$$

Generally $\lambda_A = \lambda_B$.

3.2.2 Comments on North's paper

It is not evident that $\Delta T_B / \Delta T_A$ will be constant in all cases, as North assumed. For small amounts of soil all dispersion may occur rapidly so that $\Delta T_A - \Delta T_B$ is approximately constant at longer times. Therefore a linear regression of ΔT_A against ΔT_B is now meaningless (see below).

Writing $L(t) = m_{sa} L$, where L is the dispersive energy, per g oven-dried soil, used in dispersing the soil may not necessarily be correct. The dispersive energy used in dispersing the soil may not necessarily be constant, independent of the mass of soil present. These comments are similar to those of Koenigs (1978).

It is reasonable to assume that heat losses (H(t)) will be the same in the presence or absence of soil. However the viscosity of the liquid in both systems will differ; this may or may not be important. It is also not known whether the power output of the probe is constant during irradiation, or in the presence and absence of soil.

3.2.3 Experimental

The ultrasonic instrument used in these experiments was a Branson Sonifier model B-12 with the standard 12.5 mm disruptor horn or probe. This instrument is tuned automatically. The vessels used were either a Dewar flask (100 mm x 67 mm diameter), or a glass tube (100 x 37 mm diameter) which was insulated by polystyrene foam contained in a beaker (175 x 140 mm diameter). Each vessel was fitted with a lid of polystyrene foam with two holes through which the probe and a thermistor could be inserted. Changes in temperature were measured with the pre-calibrated ITT thermistor (Type G 22-B) connected to a digital multi-meter (Marconi Instruments Ltd Model TF 2670), and could be measured to within 0.02 ^{o}C. The soils used were Urrbrae fine sandy loam which had been under pasture for 20 years, Strathalbyn sodic soil which had been used in a rotation of wheat and subterranean clover for several years, and Lemnos loam which had been used for growing ryegrass in pots for 30 weeks (see Chapter 6). Table 1 lists properties of the soils used.

Heat capacity of each vessel

The heat capacity of each vessel, fitted with its lid, and with the probe and thermistor inserted in the liquid, was measured by the heat of neutralization of 2.0 M NaOH and 2.0 M HCl. The total mass of liquid was either 210.04 g (Dewar flask) or 62.69 g (glass tube) to give volumes of liquid similar to those used in the later experiments. The cooling curve was obtained by plotting $\log_e(T - T_{surr})$ against t, where T was the temperature (^{o}C) of the liquid, T_{surr} was the temperature of the surrounding air, and t was the time in min. The regression equation for the curve was determined, and the time constant, τ, was equal to the inverse of the slope of the curve.

The heat capacity, w_v, is given by the equation

$$w_v = \frac{\Delta H}{T_1 - T_2} - c_w m_w \quad (J\ ^{o}C^{-1}),$$

where ΔH = the heat of neutralization ($J\ cm^{-3}$)

T_1 = the initial temperature of the mixture (^{o}C)

T_2 = the initial temperature of the NaOH (^{o}C)

c_w = the specific heat of water ($J\ g^{-1}\ ^{o}C^{-1}$)

m_w = total mass of liquid (g).

The heat capacity of the Dewar flask with 210 g water = 1054 $J\ ^{o}C^{-1}$ and for the glass tube with 63 g water = 365 $J\ ^{o}C^{-1}$.

Power output and dispersive energy of probe in Dewar vessel

The tip of the probe was immersed to a depth of exactly 16 mm in 210.00 g distilled water, or in 210.00 g distilled water + 6.00 g (or 25.00 g) air-dried aggregates in the Dewar flask fitted with its lid and thermistor. (When 25.00 g soil were used, it was necessary to raise the position of the probe by 2 mm so that the tip was still immersed to a depth of 16 mm.) Once the temperature of the water, or water + soil, had remained steady for 10 to 15 min, the system was sonified for up to 420 s with the temperature measured every 15 s. The probe was used at each of several power settings of the instrument (Table 2); each experiment was replicated five or six times.

Dispersive energy of probe in insulated glass tube

Air-dried aggregates (25.00 g) of Urrbrae fine sandy loam were wetted slowly under vacuum with 52.41 g de-aerated water in the glass tube. The tip of the probe was immersed to a depth of 16 mm in this system, or in 60.51 g de-aerated water, the total volume with or without soil being the same. The tube was fitted with its lid and thermistor. Once the temperature had remained steady for 10 min, the system was irradiated for 300 s with the temperature measured every 15 s. The probe was used at one power setting (101 watt) only, and it was necessary to tune the instrument continually by hand to keep the power setting constant since the automatic tuning device was not adequate.

Table 1. Properties of the soils used.

Property	Soil		
	Urrbrae fine sandy loam	Strathalbyn	Lemnos loam
% moisture	3.7	2.6	1.3
% organic matter	5.4	3.2	3.5
% mineral	90.9	94.2	95.2
c_s (J g^{-1} $^oC^{-1}$)	0.9225	0.8581	0.8177

Table 2. Power output of the probe at various power settings.

Power setting	α	S	Power output 90% C.I.
Water 88 W	0.02933	0.06782	30.91 ± 1.28
" 96 W	0.03112	0.07807	32.80 ± 1.48
" 102 W	0.03888	0.03708	40.98 ± 0.70
" 106 W	0.04618	0.05369	48.67 ± 1.02
" 118 W	0.06895	0.06470	72.68 ± 1.23
" 102 W(Repeat)	0.03924	0.04651	41.36 ± 0.88
" 101 W(Glass tube)	0.1483	0.5983	54.80 ± 3.97

If the soil and water were not de-aerated, the level of the water in the glass tube after irradiation would be lower than before irradiation; thus errors may have arisen because the depth of the tip of the probe in the water was variable, and differed from that when water alone was used.

3.2.4 Results

Calibration of the probe

Because the heat losses were large (see below) calibration of the probe in heating the water is done best by using the values for temperature over the first six time intervals (i.e. up to 90 s). A best fit

$$\Delta T_A(t) = \alpha t$$

$$\alpha = \sum_{i=1}^{6} \Delta T_A(t_i) t_i / \sum_{i=1}^{6} t_i^2$$

is performed and values of the power output are found by multiplying α by the heat capacity of the vessel. Confidence intervals of 90% are taken as

$$\pm t_5^{0.05} S \left(\sum_{i=1}^{6} t_i^2\right)^{-\frac{1}{2}} \times \text{heat capacity}$$

where

$$S^2 = \frac{1}{5} \left(\sum_{i=1}^{6} \Delta T_A^2(t_i) - \alpha^2 \sum_{i=1}^{6} t_i^2\right)$$

where $t_5^{0.05}$ is student's t-distribution.

Table 2 shows values for α, S and power output at various power settings.

Theoretical temperatures after water has been irradiated are calculated from the values of α; the heat losses are deduced from the differences between the theoretical and actual temperatures (Table 3). The heat losses were particularly large when the glass tube was used.

Where 6 g soil was used, $L(t)$ had the largest increase during 0-15 s, and increased to about 100 J for the Urrbrae and Strathalbyn soils, and 200 J for the Lemnos soil. Then $L(t)$ could fluctuate by about 20 J during following intervals of 15 s. With Urrbrae (set at 118 W) and Strathalbyn

Table 3. Actual and theoretical temperatures at various power settings.

Power setting	Ultimate time	Temperature (actual)	Temperature (theor.) ± 90% C.I.
88 W	420 s	10.92	12.32 ± 0.51
96 W	420 s	11.55	13.07 ± 0.59
102 W	240 s	9.16	9.33 ± 0.16
106 W	240 s	10.93	11.08 ± 0.23
118 W	240 s	15.95	16.55 ± 0.28
102 W(repeat)	360 s	13.66	14.13 ± 0.30
101 W(glass tube)	300 s	35.62	44.49 ± 3.22

Table 4. Dispersive energy in 0-15 s with various power settings and various soils.

Power setting	Soil	$L(t)$ Joule
88 W	6 g Lemnos	200.0
96 W	"	240.7
102 W	6 g Urrbrae	94.5
106 W	"	81.4
118 W	"	111.5
102 W	25 g Urrbrae	34.8
101 W	25 g Urrbrae (glass tube)	111.0
102 W	6 g Strathalbyn	122.2
106 W	"	90.05
118 W	"	126.6

(102 W), $L(t)$ increased in the interval of 15-240 s to about twice the value at 15 s (e.g. Fig.1c), whereas with Lemnos (set at 88 W or 96 W) $L(t)$ decreased in the interval 15-240 s (Fig.1a). With the other settings for Urrbrae or Strathalbyn there was no real change in $L(t)$ (e.g. Fig.1b). Because the heat losses were so large (Table 3) the fluctuations of $L(t)$ could have been due to fluctuations in heat loss; the typical differences in ΔT_A and ΔT_B were $0.1^{o}C$ to $0.2^{o}C$.

In the two cases where 25 g of Urrbrae were used, there was an initial jump in $L(t)$ at 15 s, and an almost steady increase at about half this rate (Fig. 1d and Fig. 1e) (Note change of scale in Fig. 1e).

It appears any energy used to dissipate the smaller quantities of soil (6 g) was absorbed in 0-15 s, whereas the 25 g samples absorbed decreasing amounts of energy with time.

The relationship between dissipation time and mass of soil present appears to be non-linear. The value of $L(t)$ after the first 15 s value varied with soil type rather than with power output (Table 4). Lemnos had high values (200 J at 88 W and 240 J at 96 W) and values for Urrbrae and Strathalbyn were both about 100 J. When 25 g Urrbrae was used, $L(t)$ at 15 s was 34.8 J whereas when 6 g Urrbrae was used $L(t)$ was 94.5 J.

3.2.5 Conclusions

In theory North's concept of dispersive energy is not valid. In practice the fluctuations in the heat loss are so large that real differences in $L(t)$ are difficult to detect. Therefore this method of an absolute measurement of soil structural stability was not used for experiments described in this thesis.

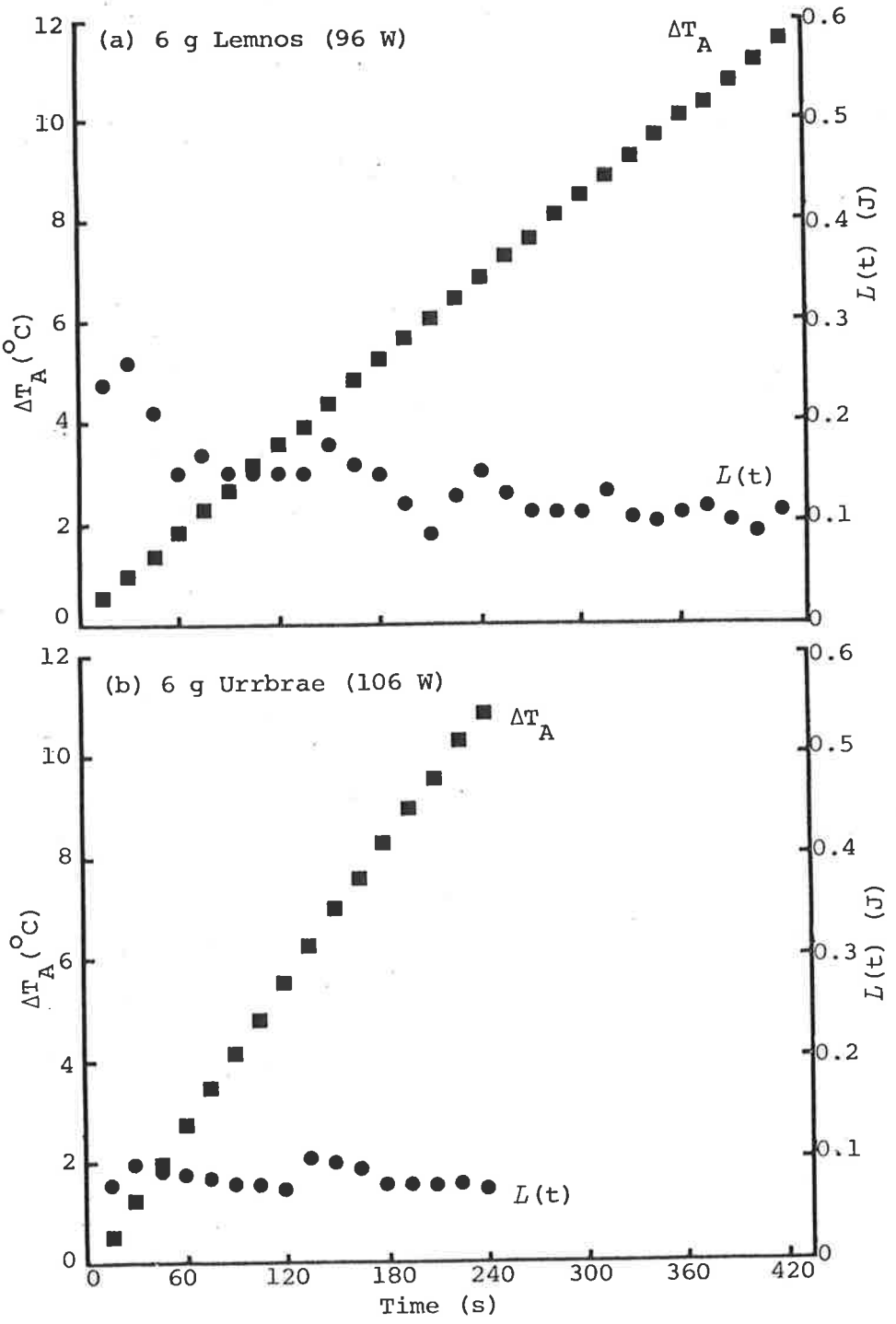
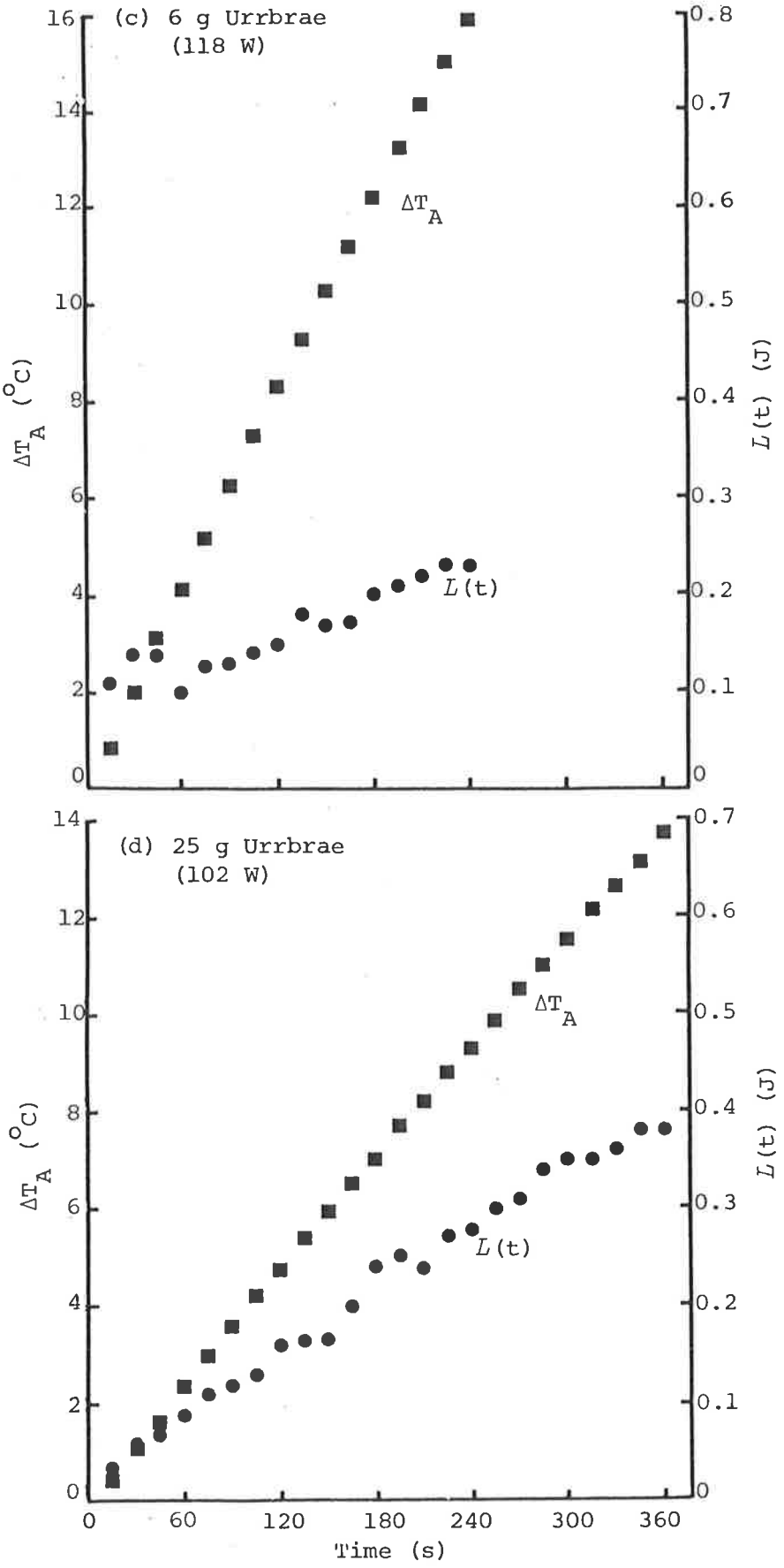
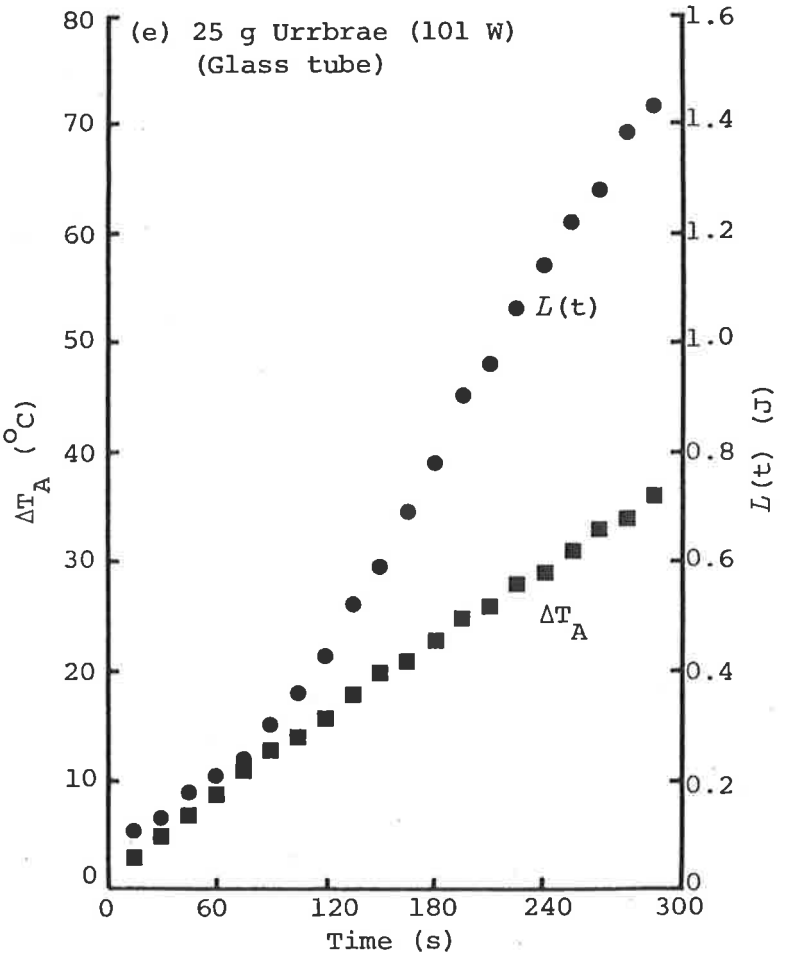


Fig. 1 Increase in temperature, ΔT_A, and changes in total dispersive energy, $L(t)$, with time.


(c) 6 g Urrbrae
(118 W)
ΔT_A
$L(t)$
ΔT_A (°C)
$L(t)$ (J)
(d) 25 g Urrbrae
(102 W)
ΔT_A
$L(t)$
ΔT_A (°C)
$L(t)$ (J)
Time (s)


(e) 25 g Urrbrae (101 W)
(Glass tube)
L(t)
ΔT_A
ΔT_A (°C)
L(t) (J)
Time (s)
0
10
20
30
40
50
60
70
80
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
0
60
120
180
240
300

3.3 Size-distribution of Water-stable Particles

The size-distribution of water-stable particles was obtained from wet-sieving and from sedimentation on the same sample.

3.3.1 Experimental

Air-dried or pre-wetted aggregates were wet-sieved after the method of Kemper (1965). The wet-sieving machine raised and lowered four nests of sieves simultaneously 37 times per min through 20 mm of water. The four sieves (50 x 80 mm diameter) of each nest had square holes of 2000 µm, 1000 µm, 500 µm and 250 µm respectively.

The method was tested on aggregates of Lemnos loam taken from unplanted pots or pots where ryegrass had grown for 8 weeks (see Chapter 4).

Air-dried soil (<10 mm; 20 g) was wet-sieved in 3.6 dm^3 distilled water in a cylinder (300 x 145 mm diameter) for 5 min (or with a few samples for 20 min). With some samples, the material which had passed through the sieves was re-suspended in the 3.6 dm^3 water by shaking the cylinder gently end-over-end four times in 15 s, and the size-distribution of water-stable particles was determined by sedimentation under gravity (equivalent spherical diameters <50 µm, <20 µm, <10 µm). With some samples, the material in the cylinder was re-suspended, and a 1 dm^3 aliquot of particles <10 µm was transferred to a sedimentation cylinder (1250 cm^3); the size-distribution of water-stable particles was determined by sedimentation under gravity or centrifugation (equivalent spherical diameters <2 µm, <0.2 µm respectively). The % of particles 50-250 µm diameter was obtained by difference, so the values contain accumulated errors. There are also errors in the size-distributions because the parts of the summation curve obtained from sieving and from sedimentation do not connect smoothly (Wilkinson 1975).

Pre-wetting of soil

Most air-dried samples were immersed directly in distilled water on wet-sieving, but some samples were pre-wetted under vacuum. For pre-wetting, air-dried soil (<10 mm; 20 g) was spread evenly on a mesh basket (80 mm diameter), and the basket placed on a filter paper in a desiccator which contained two beakers each with 15 cm^3 distilled water. The desiccator was evacuated for 3 min to deaerate and boil the water in the beakers; the system was kept under vacuum for 7 min to allow the atmosphere in the dessicator to become saturated. Deaerated distilled water was then allowed to flow into the evacuated desiccator at 5 cm^3 min^{-1} to flood the aggregates. Air was then allowed to flow back slowly to restore the pressure to atmospheric pressure. The sieves to be used for wet-sieving were inverted over the basket of saturated aggregates and the sieves inverted carefully to allow the aggregates to come into contact with the mesh of the top sieve (2 mm holes).

3.3.2 Results and discussion

Effect of pre-wetting

Wet-sieving showed that aggregates were disrupted less if they had been pre-wetted slowly under vacuum than if they had been wetted rapidly by direct immersion in water (Fig.2)*; hence aggregates >250 μm of this soil slake (i.e. break up into fragments) to particles most of which are 50-250 μm and 20-50 μm diameter, they do not disperse, i.e. release particles <2 μm. However the disruptive effect of rapid wetting was greater in the unplanted control than in the ryegrass treatment.

These results suggest that gypsum, which flocculates clay and thereby stabilizes small particles, is unimportant in the stability of Lemnos loam.

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*SE means less than 0.5 not shown on any figures in this thesis.
Fig. 2 and subsequent figures presented in this thesis show results for wet-sieving for 5 min, since 5 min separated soils as well as 20 min did.

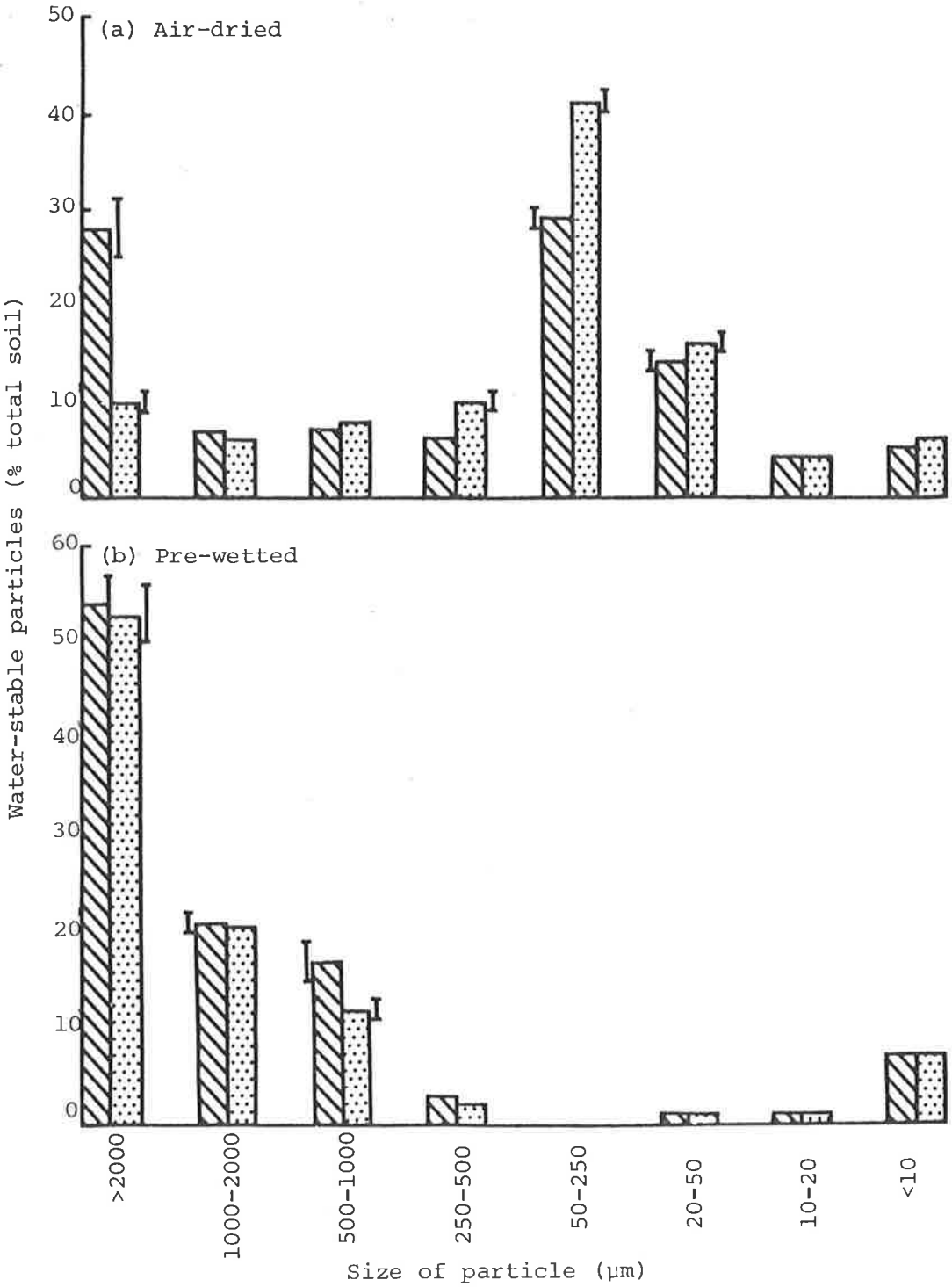


Fig. 2 The size-distribution of water-stable particles in air-dried or pre-wetted soil. ▨ Ryegrass; ⁘ Unplanted control. Vertical bar, 2 x SE mean.

The results of this experiment agree with those of others who worked with soils from the field and (a) who compared cultivated soil with either virgin soil or soil under grasses (Low 1954; Panabokke and Quirk 1957; Kemper and Koch 1966), or (b) who compared soil under continuous wheat fallow rotation with soil which grew wheat every year (Williams *et al.* 1966). Each of these groups of workers found that, when using the method of wet-sieving, use of air-dried aggregates separated the soils best. In the present experiment, pre-wetting of aggregates followed by wet-sieving was not severe enough to detect differences between the ryegrass treatment and the unplanted control. Therefore in the remainder of experiments reported in this thesis, the size-distribution of water-stable particles was measured on aggregates which had been wetted rapidly.

Lemnos loam has a high proportion (63%) of fine sand and silt, and, unless managed carefully when irrigated in the field, tends to slake and form a crust at the surface with a low infiltration rate and permeability (Cockroft and Tisdall 1978). At the surface, some of the fragments of slaked soil (50-250 μm and 20-50 μm diameter), along with any fine material already present, must move into many of the transmission pores (>50 μm diameter) (Greenland 1977) and prevent water moving freely through the soil. (For water to move freely, at least 10% of the soil volume should be in pores >50 μm diameter.)

During an irrigation season, Lemnos loam may be irrigated up to 20 times with the surface of the soil drying to air-dry between irrigations. The air-dried surface is usually wetted rapidly when irrigated by flood; therefore wet-sieving of rapidly wetted aggregates measures the stability of aggregates more appropriately than does wet-sieving of pre-wetted aggregates.

On the other hand, most aggregates in the field lie beneath the immediate surface, so that when irrigated, these buried aggregates are

usually wetted by capillarity and tend not to slake. Hence pre-wetting is more appropriate than direct immersion for aggregates beneath the immediate surface.

CHAPTER 4

SHORT-TERM STABILIZATION OF AGGREGATES OF LEMNOS LOAM BY THE ROOT SYSTEMS OF PLANTS

4.1 Introduction

When many arable soils are sown to pasture, the stability of aggregates of soil increases with a concomittant increase in the levels of organic matter in soil (see section 1.4). These increases, which tend to be more rapid under grass than under legumes, are attributed mainly to the organic matter supplied by living, decomposing or dead roots. Several authors have tried to identify the fractions of the organic matter in soil which are related to the stability of aggregates of soil by selectively removing complexes of organic and inorganic materials and by measuring the subsequent decrease in stability.

The pot experiments described in this chapter were designed to determine the effect, up to 20 weeks, of roots of ryegrass or of white clover on the stability of aggregates of Lemnos loam. The experiment aimed also to relate changes in stability to total length of roots, to total length of hyphae, and to periodate-sensitive materials and pyrophosphate-sensitive materials.

4.2 Experimental

4.2.1 Preparation of soil in pots

Air-dried (3% water g/g) soil (<6 mm) was placed over an 8 mm layer of black plastic beads (3 x 3 mm) in individual pots (165 x 155 mm diameter). A solution of inorganic nutrients was added to the soil via a PVC tube (180 x 30 mm) placed vertically in the centre of each pot with one end in the layer of beads. The solution was added over 24 h to wet each sample from below to a matric suction equivalent to -10 kPa and added 388 mg N, 97 mg P and 97 mg K per pot. Once the surface of each sample was wet,

seeds of New Zealand perennial ryegrass (*Lolium perenne* L.) or of white clover (*Trifolium repens*) were sown and a polythene film (12.5 μm thick) covered each pot to reduce loss of water. When the seeds had germinated the polythene film was removed and the seedlings were thinned. The aim was to get the roots to develop as rapidly as possible, and close enough together so that all the soil between the roots was rhizosphere (Thornton 1958; Barley 1970). The planted pots and unplanted controls were incubated in a water-bath at 18°C in a glasshouse. Whenever necessary, water was added to the samples and controls to restore each pot to a given mass; the aim was to restore the matric suction to -10 kPa. Treatments were replicated four times, and sufficient samples were set up to allow destructive sampling after each period of incubation. At each sampling the size-distribution of water-stable particles was determined. Water was added to saturate the soil in each pot to be sampled. The saturated soil was removed carefully from the pot and dried until the soil was moist and friable. The soil was sieved (10 mm) and air-dried.

Experiment 1

Stabilization of aggregates by the root systems of ryegrass

Eighteen ryegrass plants were grown in 2 kg soil in a pot for up to 20 weeks. The nutrient solution contained NH_4NO_3, $Ca(H_2PO_4)_2$ and K_2SO_4. During weeks 11 and 12 after sowing, 194 mg N was added to each pot containing plants, and at week 13 and weekly thereafter 388 mg N was added. The mean maximum and mean minimum air-temperatures were 28°C and 18°C respectively. In this experiment the soil was not wetted evenly; once the polythene film was removed from the pots after germination, the top 15 mm of soil became air-dry and remained air-dry until the end of the experiment.

The particle-size distribution of water-stable particles, with or without pre-treatment with chloride, periodate or pyrophosphate was measured. The length of roots combined with water-stable aggregates was measured on separate sub-samples.

Experiment 2

Seventy-five plants of ryegrass or 40 white clover plants were grown in 3 kg soil per pot for up to 14 weeks. The nutrient solution contained

$NH_4H_2PO_4$, $Ca(NO_3)_2$, NH_4NO_3 and K_2SO_4. Three weeks and 6 weeks after sowing, 194 mg N was added to each pot containing plants. To ensure that the soil in each pot was wetted evenly, the PVC tube in the centre of each pot was perforated so that the soil could be wetted radially from the centre as well as from below. The soil in each pot was covered with an 8 mm mulch of black plastic beads (3 x 3 mm) to reduce loss of water from the surface of the soil during incubation. The mean maximum and mean minimum air-temperatures were 20°C and 14°C respectively. The total length of root and length of infected root in the whole soil, and the length of hyphae in the whole soil and that combined with water-stable aggregates were each measured on separate sub-samples.

4.2.2 Identification of the fungi

Ten individual water-stable aggregates >2000 μm from each of four ryegrass treatments from experiment 2 were dispersed in 10% sodium hexa-metaphosphate, and the organic matter separated by sieving (53 μm aperture). The organic matter was stained with Congo red and examined under high power (x 500) with an optical microscope. Ten fields per separate were examined, and in each field intercepts of two hair-lines with non-septate hyaline hyphae with characteristic shape of VA mycorrhizal fungi (Mosse 1959) were counted. Intercepts of the hair-lines with septate or coloured hyphae were also counted. The two hair-lines, in one eyepiece of the microscope, were perpendicular to each other and were rotated after the intercepts in each field had been counted.

4.3 Results and Discussion

4.3.1 Stability

After the growth of ryegrass for 8 weeks, but not for 6 weeks, water-stable particles 50-250 μm diameter were bound up into water-stable aggregates >2000 μm diameter (Fig. 3 and Fig. 4). The ryegrass increased progressively the % water-stable particles >2000 μm diameter from two-fold

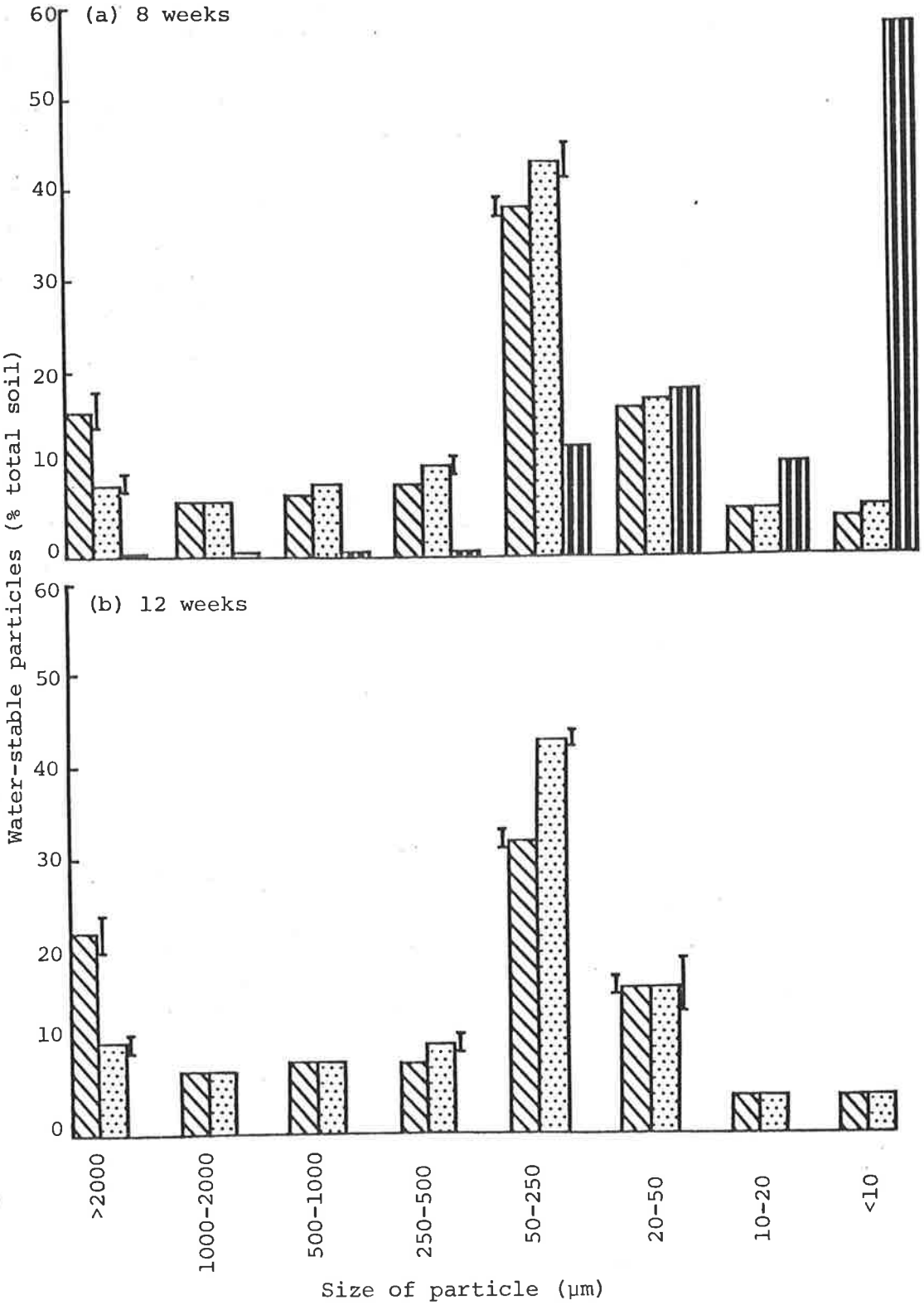
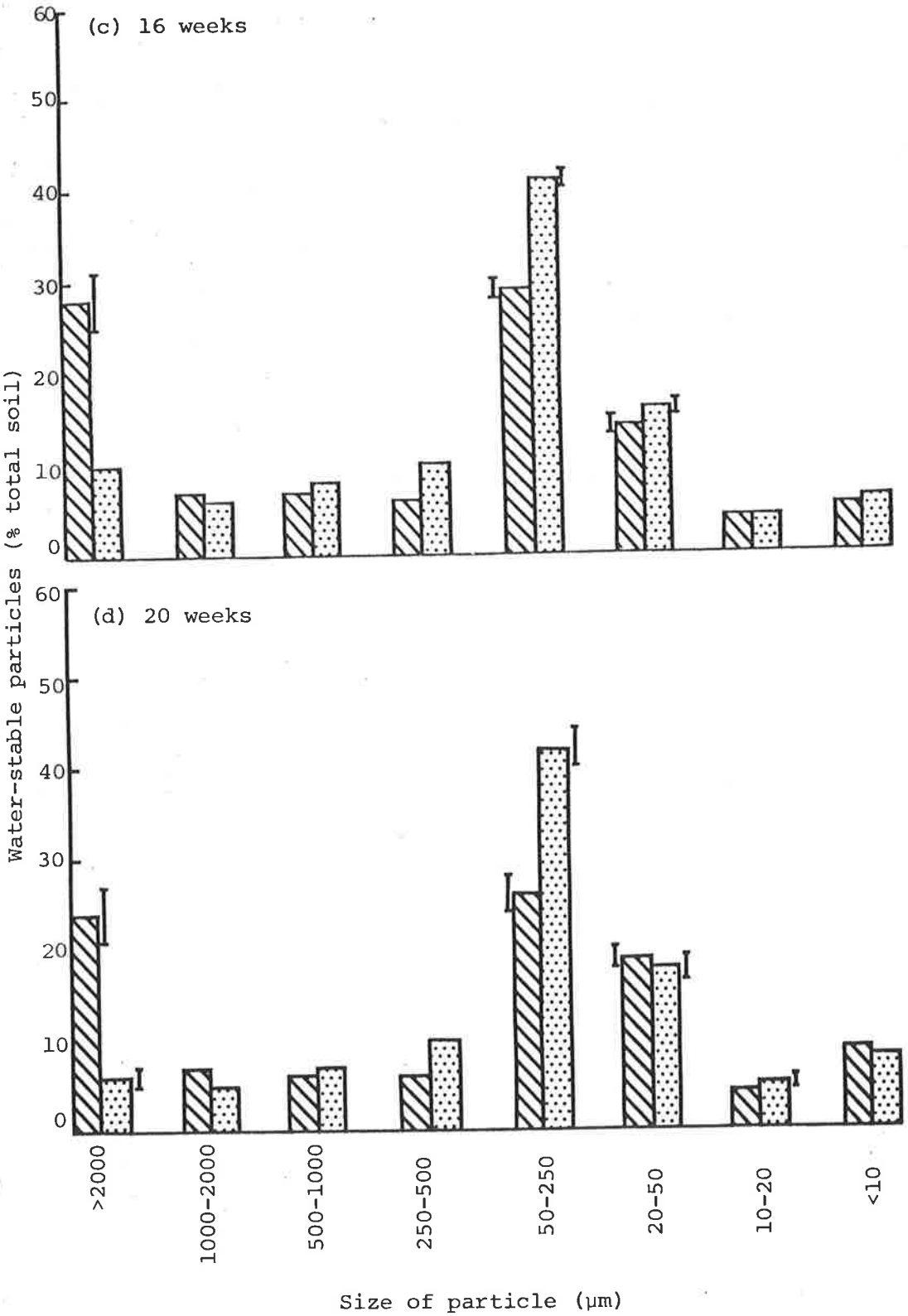


Fig.3 The effect of ryegrass on the size-distribution of water-stable particles in soil. ▨ Ryegrass; ⁘ Unplanted control; ▥ Original soil, fully dispersed. Vertical bar, 2 x SE mean.


(c) 16 weeks
(d) 20 weeks
Water-stable particles (% total soil)
0
10
20
30
40
50
60
>2000
1000-2000
500-1000
250-500
50-250
20-50
10-20
<10
Size of particle (µm)

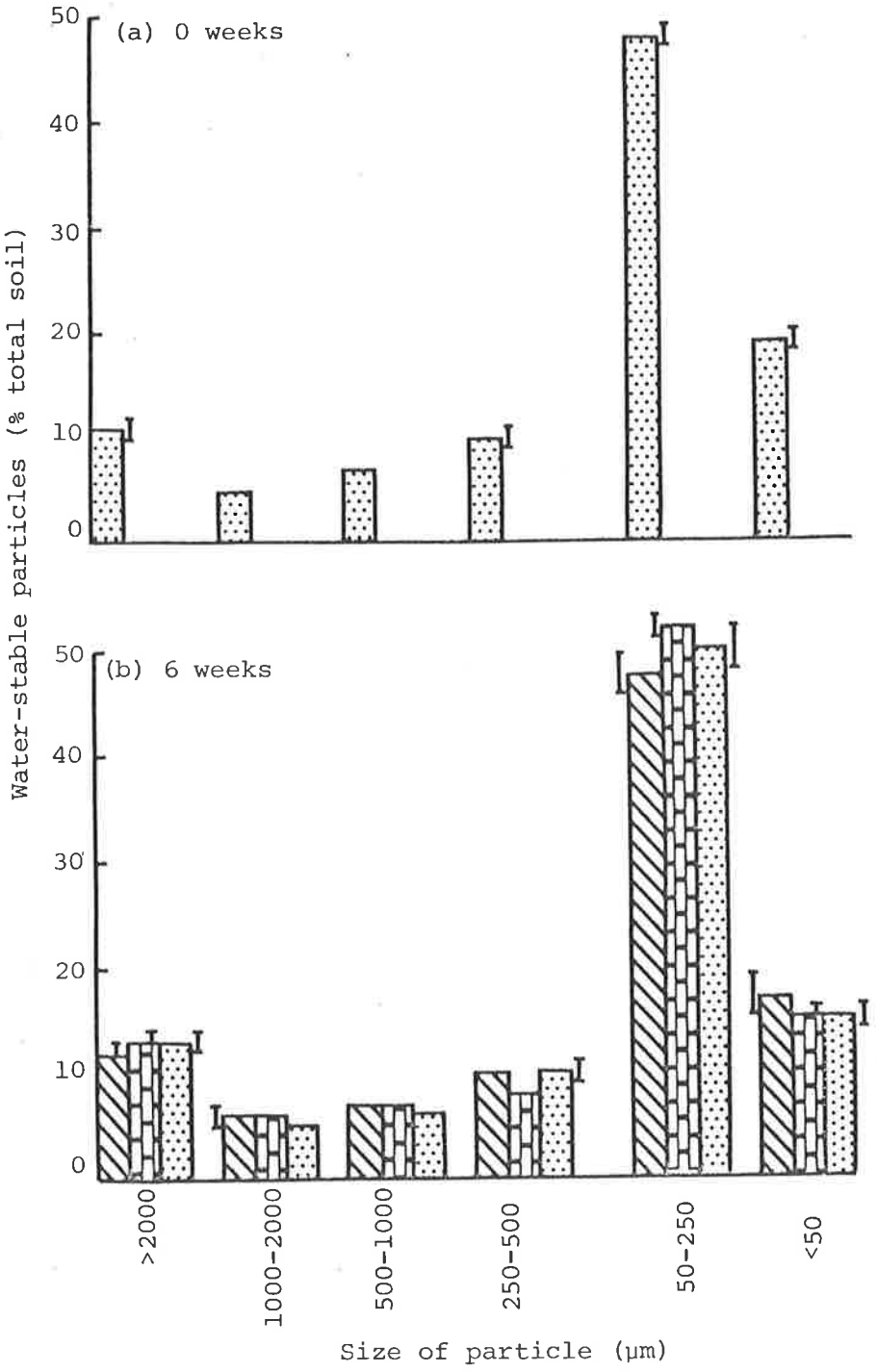
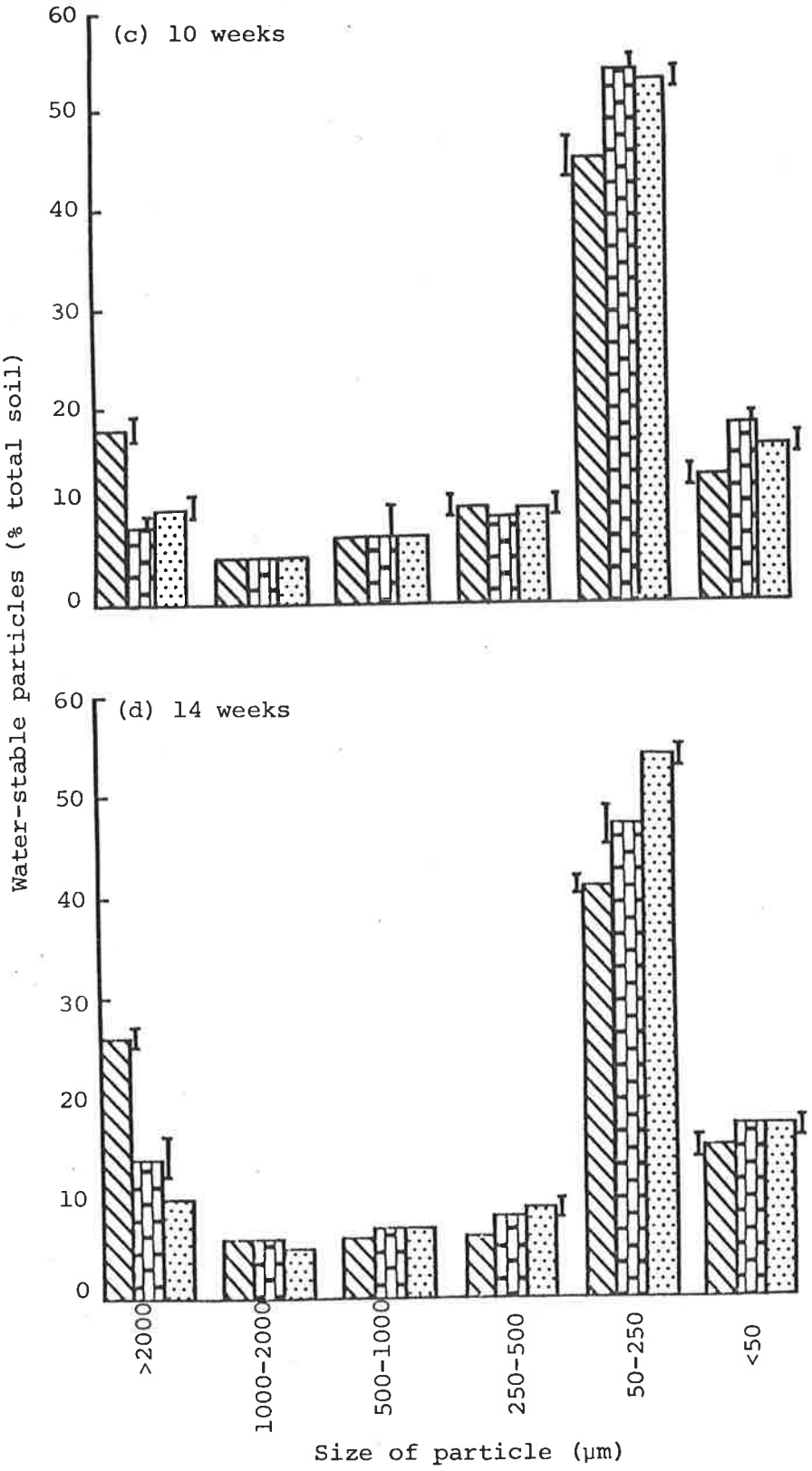


Fig. 4 The effect of ryegrass and white clover on the size-distribution of water-stable particles in soil.
Ryegrass; White clover; Unplanted control.
Vertical bar, 2 x SE mean.


(c) 10 weeks
(d) 14 weeks
Water-stable particles (% total soil)
60
50
40
30
20
10
0
>2000
1000-2000
500-1000
250-500
50-250
<50
Size of particle (µm)

(8 weeks) to four-fold (20 weeks). After 20 weeks growth the % water-stable particles 1000-2000 µm diameter was increased by one-third.

White clover had a slight effect and unplanted control had no detectable effect on the stability of aggregates from 0 week up to 14 weeks (Fig.4).

After growth for 14 weeks, the length of roots in the ryegrass treatment was eight times that of white clover (Table 5), i.e. the stabilization of aggregates was related to the length of root in the total soil. These results agree with those of other workers (e.g. Robinson and Jacques 1958).

Table 5. The effect of ryegrass and white clover on total root length, length of infected root and hyphal length in total soil.

Treatment	Root length mm g^{-1} total OD soil		% Infection	Hyphal length mm g^{-1} total OD soil
	Total	Infected		
Ryegrass	1100 ±73	144 ±39	13.3	19.6 ±1.1
White clover	134 ±16	68 ±10	50.8	8.8 ±1.0
Unplanted control	-	-	-	5.7 ±0.7

± values are ± SE mean.

4.3.2 Length of roots combined with stable aggregates

After the growth of ryegrass for 8 weeks and for 12 weeks (experiment 1) stable aggregates >2000 µm diameter contained a greater length of root per g than stable aggregates 1000-2000 µm, 500-1000 µm or 250-500 µm diameter (Fig.5). Length of root per g soil increased in all stable aggregates >250 µm diameter at 12 weeks compared with 8 weeks.

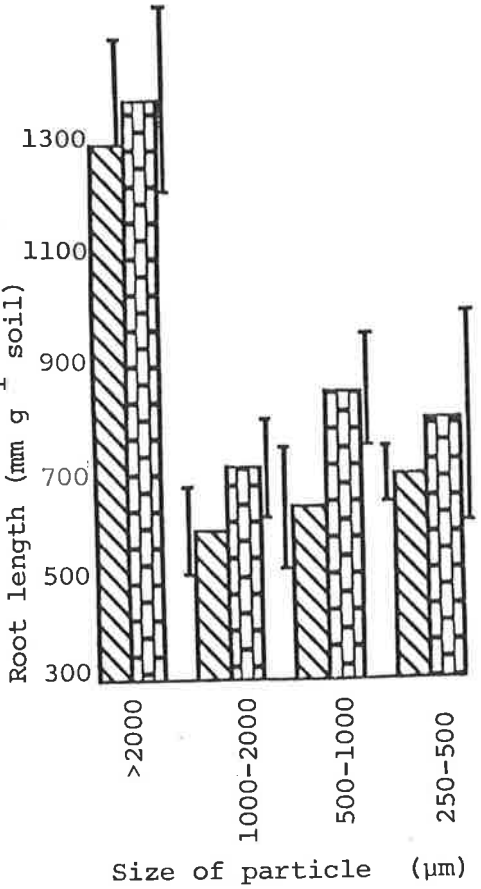


Fig.5 Length of ryegrass roots combined with water-stable particles in soil.
▨ 8 weeks; ▦ 12 weeks;
Vertical bar, 2 x SE mean.

In this method, the roots were oven-dried before their length was measured. However previous results, not reported here, had shown that the length of root of ryegrass plants did not change on oven-drying (105^{o}C).

These water-stable particles contained more roots than are found in soils under actively growing pasture in the field so presumably all the soil in each ryegrass treatment was rhizosphere soil. Barley (1970) showed that for grasses the length of root per cm^{3} in the top 10-15 cm was 300 mm to 500 mm, and the half-distance between roots of *Lolium multiflorum* was 1.5 mm at a depth of 2 cm.

4.3.3 Chemical pre-treatments

After the growth of ryegrass for 12 weeks (experiment 1), sodium periodate (when compared with sodium chloride) reduced by 33% the % water-stable particles >2000 μm diameter and increased concomittantly the % water-stable particles 50-250 μm and 20-50 μm diameter (Fig.6). Sodium periodate did not change the stability of aggregates from the unplanted control.

After the growth of ryegrass for 16 weeks, sodium periodate reduced by 25% the % water-stable particles >2000 μm diameter, and sodium pyrophosphate reduced the % stable particles >250 μm diameter from both the ryegrass and the unplanted control (Fig.7). (Because the ionic strength of sodium pyrophosphate (0.1 M) differed from that of sodium chloride (0.02 M) the effects of these treatments cannot be compared (Clapp and Emerson 1965).) However neither treatment with sodium periodate nor with sodium pyrophosphate reduced the stability of aggregates >2000 μm diameter from the ryegrass treatment to the stability of the unplanted control (>2000 μm diameter), which had been treated with sodium chloride, sodium periodate or sodium pyrophosphate. Therefore the ryegrass treatment contained some bonding substances not contained by the unplanted control. The periodate-resistant and pyrophosphate-resistant materials responsible for part of the stability due to the growth of roots of ryegrass were

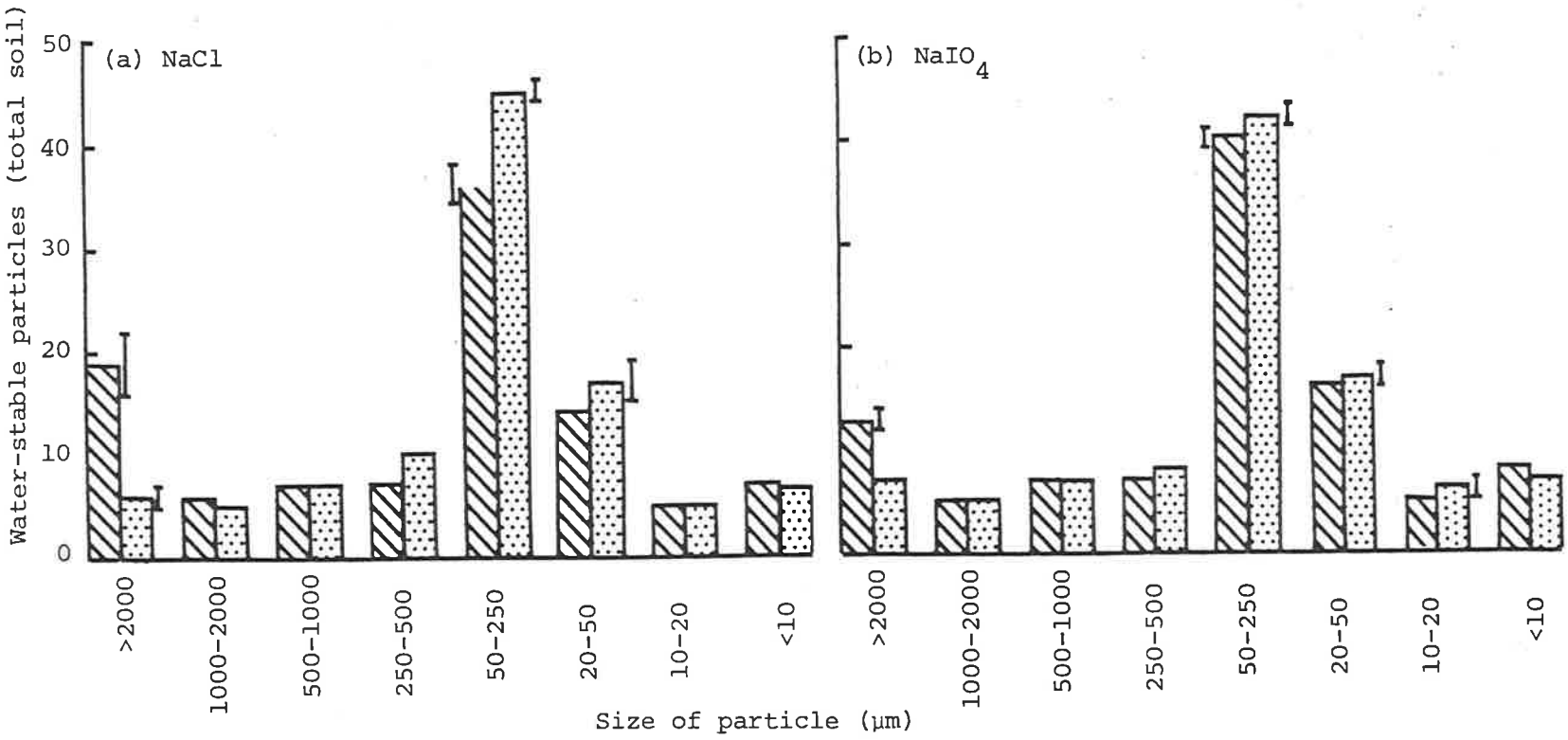


Fig. 6 The size-distribution of water-stable particles after chemical treatment (sampled at 12 weeks).
▨ Ryegrass; ⊡ Unplanted control. Vertical bar, 2 x SE Mean.

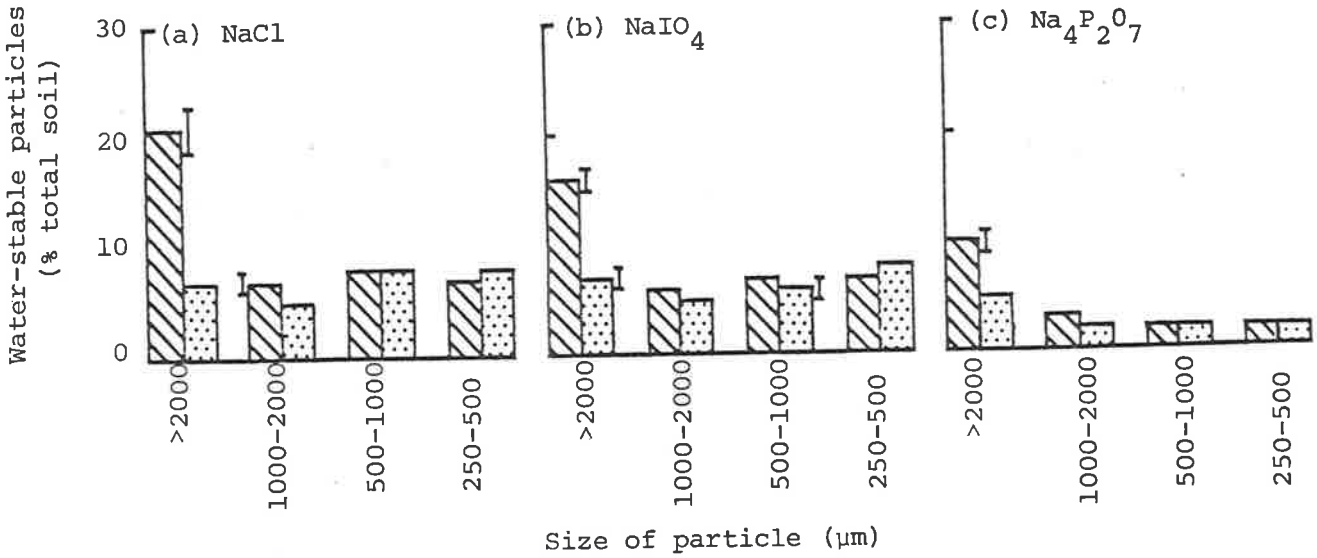


Fig. 7 The size-distribution of water-stable particles after chemical treatment (sampled at 16 weeks). ▨ Ryegrass; ⊡ Unplanted control. Vertical bar, 2 x SE mean.

probably associated with fungal hyphae (D.J. Carter and J.M. Oades unpublished results).

By treating aggregates of a red-brown earth with either sodium periodate or sodium pyrophosphate, Stefanson (1971) also removed some of the stability of the aggregates; the longer the soil had been under pasture the more important were the pyrophosphate-sensitive materials in stable aggregation. Periodate-sensitive materials in several English soils which contained high proportions (17-55%) of silt were also shown to be relatively less important in stable aggregation than pyrophosphate-sensitive materials (Hamblin and Greenland 1977).

Sodium periodate readily breaks the bond between two adjacent carbon atoms if both carry a hydroxyl group. Hence Mehta *et al.*(1960) suggested that a dilute solution of sodium periodate and sodium tetraborate when added to aggregates of soil would oxidize glycol groups of polysaccharides in the aggregates and degrade the resulting polyaldehydes to fragments of low molecular weight. Periodate treatment without post-treatment with borate has the same effect (Clapp and Emerson 1965). Stefanson (1971) used 0.1 M sodium pyrophosphate at pH 10.0 to disrupt clay particles bound together by organic polymers bridged to the clay surface by polyvalent cations. However it is not understood precisely which fraction of organic matter in soil is removed or influenced by solutions of either periodate or pyrophosphate.

4.3.4 Length of fungal hyphae combined with stable aggregates

After the growth of plants for 14 weeks (experiment 2), stable aggregates from ryegrass contained greater lengths of hyphae per g than stable aggregates from the white clover treatment and from the unplanted control (Fig. 8). In the ryegrass treatment, stable aggregates >2000 µm diameter contained greater hyphal lengths per g than stable aggregates <2000 µm diameter.

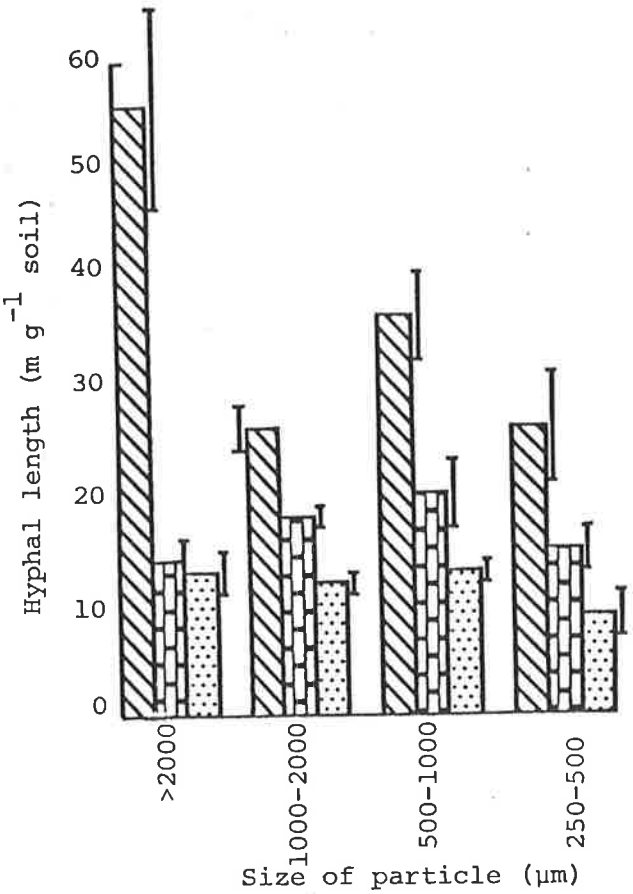


Fig. 8 Length of hyphae combined with water-stable particles in soil (sampled at 14 weeks).
Ryegrass; White clover; Unplanted control.
Vertical bar, 2 x SE mean.

These water-stable aggregates contained less hyphae than soils from the field which were shown elsewhere to contain over 1000 m hyphae per g total soil (Hanssen *et al.*1974; Sundman and Sivela 1978).

The present results suggest that hyphae in the rhizosphere of ryegrass may be involved in the binding of aggregates <2000 μm diameter into aggregates >2000 μm diameter. These results tend to agree with those of Harris *et al.* (1966), who suggested that fungi stabilized artifical aggregates <500 μm diameter into aggregates >2000 μm diameter and that the stability was related closely to the onset and subsequent development of macroscopic hyphae on and in the aggregates.

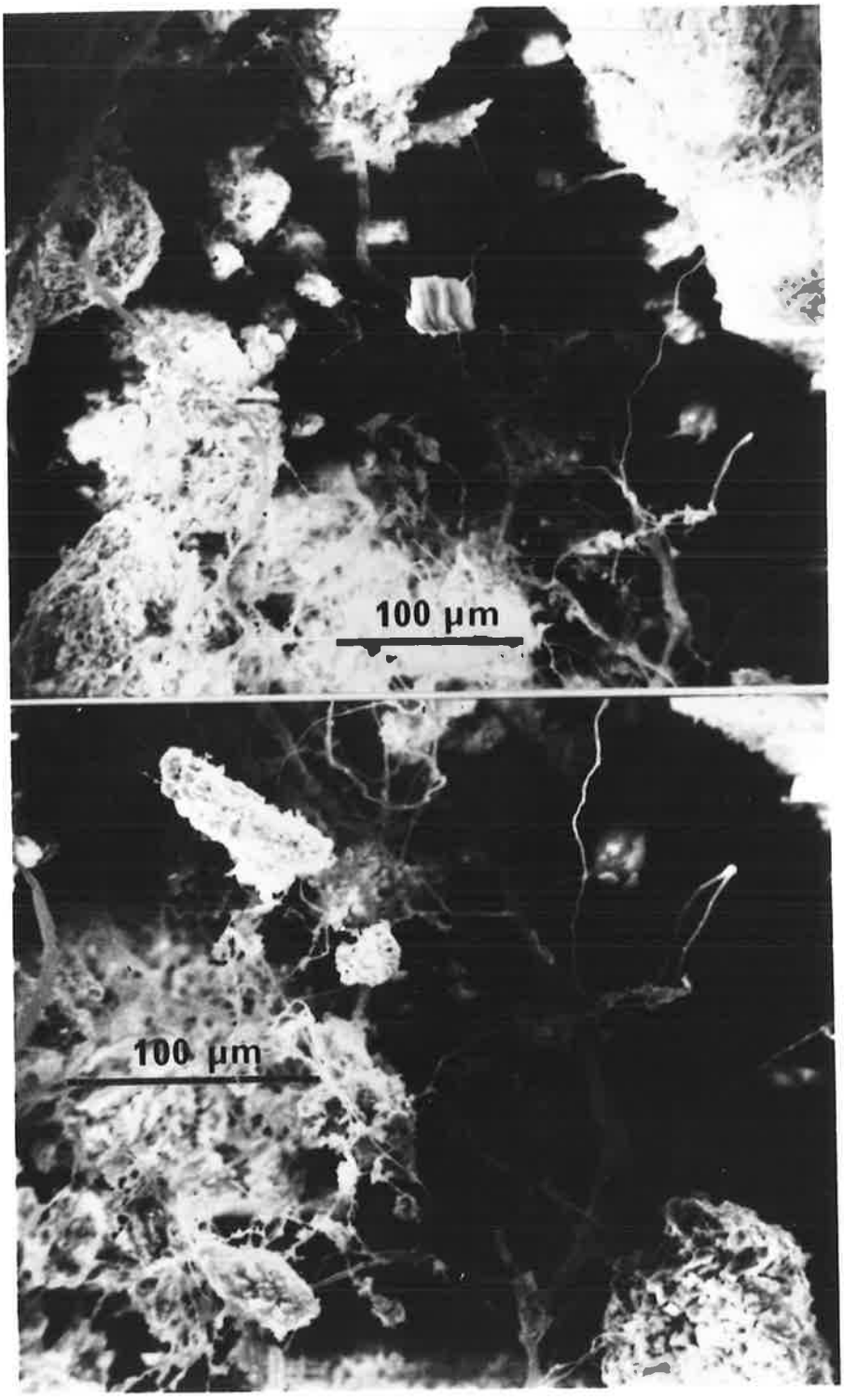
Identification of the fungi in stable aggregates

Most of the hyphae present in the aggregates stabilized by ryegrass belonged to two types of VA mycorrhizal fungi. Of the 2642 intercepts of hyphae with the cross-hairs counted, 2607 were of hyphae which belonged to this group of fungi. The translucent hyphae formed an extensive network within each aggregate and soil particles adhered firmly to them (Fig.9).

4.3.5 Mycorrhizal infection of roots from the total soil

After growth for 14 weeks the percentage infection of white clover roots (experiment 2) was about four times that of ryegrass roots (Table 5). Crush (1975) in a field survey also found that white clover was more highly infected than ryegrass roots. However, in this experiment, because the ryegrass had eight times the length of root of white clover, the total length of infected root of ryegrass was twice that of white clover. The total length of infected root was related to the hyphal length in the total soil. Part of the greater stabilization of aggregates by ryegrass than white clover (Fig.4) appears to be related to the greater length of root infected by VA mycorrhizal fungi in the ryegrass.

Fig. 9 Scanning electron micrographs of hyphae of VA mycorrhizal fungi binding soil particles into water-stable aggregates.


100 µm
100 µm

4.4 Conclusion

The results of the two experiments described in Chapter 4 show that the root system of ryegrass was more efficient than that of white clover in stabilizing aggregates of Lemnos loam. The increase in stability by ryegrass was related to the length of root, length of root infected with VA mycorrhizal fungi and to the hyphal length of these fungi in the soil. These results lead to the hypothesis that much of the stabilization of soil aggregates by the root systems of ryegrass is due to VA mycorrhizal hyphae associated with the roots.

CHAPTER 5

STABILIZATION OF SOIL AGGREGATES BY VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI

5.1 Introduction

VA mycorrhizal fungi, which belong to the family Endogonaceae, are found in almost all soil types associated with plants of different taxonomic groups (Mosse 1973) with little, if any, host specificity (Mosse 1975). Although they abound in soils these fungi have not yet been cultured on artificial media and so are generally not recorded by soil microbiologists. However these fungi can be demonstrated by direct observation of soil (Nicolson 1967) or of whole roots (Phillips and Hayman 1970), or by wet-sieving and decanting soil (Sanders and Tinker 1973). Although the plant does not usually depend on mycorrhizas, the fungus is an obligate symbiont. The mycorrhizas enable the plant to extend its root system and to take up larger amounts of nutrients, especially phosphorus, than non-mycorrhizal plants (Sanders and Tinker 1971).

The morphology of the mycorrhizas has been described in detail (Mosse 1959; Nicholson 1967). The fungus exists in two phases with a mycelium in the root cortex connected to an external mycelium in the rhizosphere and soil; as much of the fungus can be outside as inside the roots (Nicolson 1967). The hyaline hyphae which form the external mycelium vary in their diameter (up to 27 μm) and in the thickness of their walls (up to 4 μm) (Mosse 1959; Nicolson 1959). The large thick-walled hyphae, which are non-septate, are long-lived ; the fine thin-walled hyphae, which are non-septate when growing actively but become septate as they age, are short-lived and distintegrate gradually, leaving angular projections on the thick-walled hyphae where the fine hyphae were attached formerly. Spores (or external vesicles) which vary in size (20 μm to 150 μm diameter) and shape, are usually terminal. Inside the cortex of the root, the hyphae form vesicles which are probably organs for storage, and arbuscules which may release material into the cortical

cells (Nicolson 1967).

The external hyphae can extend up to 80 mm from the surface of a root (Rhodes and Gerdemann 1975) and frequently form a loose network to which fine particles of soil and organic material are attached firmly and are difficult to wash off (Mosse 1959; Went and Stark 1968).

The aim of the experiment described in this chapter was to test the hypothesis that much of the stabilization of soil aggregates by the root system of ryegrass (see Chapter 4) is due to VA mycorrhizal hyphae associated with the roots.

5.2 Experimental

5.2.1 Preparation of soil in pots

Lemnos loam (2 kg) was set up in pots as described in Chapter 4. Four treatments were imposed:

T_1 Fumigated soil, no inoculum;

T_2 Non-fumigated soil, no inoculum;

T_3 Fumigated soil, with E3 inoculum;

T_4 Fumigated soil, with washings of roots infected with E3.

Each pot containing air-dried soil was sealed in a plastic bag, with or without a beaker containing 100 cm^3 chloroform. After 24 h, soils were exposed to the air for 48 h or until chloroform could not be detected. The nutrient solution which contained $NH_4H_2PO_4$, $Ca(NO_3)_2$, NH_4NO_3 and K_2SO_4 was then added, and the pots left for 1 week before the seeds were sown, to eliminate any toxic effects of the fumigant (Mulder 1979). The surfaces of 50 ryegrass seeds were sterilized by shaking the seeds for 15 min in 7% sodium hypochlorite, and then rinsing five times in sterile distilled water. Seeds in some treatments were not inoculated; others were inoculated with pieces of onion root which were infected with the vesicular-arbuscular (VA) mycorrhizal fungus E3 (similar to spore type E3 described by Gilmore (1968)), or inoculated with washings from infected

onion roots to ensure that inoculated mycorrhizal and inoculated non-mycorrhizal treatments received the same contaminating organisms (Sanders *et al.* 1975). The soil in each pot was covered with an 8 mm layer of autoclaved (120°/15 min) black plastic beads. The plants were grown for 10 weeks. The mean maximum and mean minimum air temperatures were 26°C and 15°C respectively. Eight weeks after sowing 194 mg nitrogen was added to each pot. Treatments were replicated four times. The size-distribution of water-stable particles, the total length of root, the length of infected root and the hyphal length in the total soil were measured on separate sub-samples.

5.2.2 Examination of VA mycorrhizal hyphae, and the amorphous material on their surface

Ryegrass was grown for 3 weeks in a mixture of 90% autoclaved coarse sand (120°C/30 min) and 10% Caliph sand which contained yellow-vacuolate spores of Endogonaceae (G. D. Bowen personal communication). The roots were washed and agitated in water to remove large particles of adhering soil, and mycorrhizal hyphae were picked off the roots under the microscope. The hyphae were then either examined under an electron microscope or used to collect the amorphous material on their surface.

Electron microscopy

Scanning EM (SEM)

Hyphae were fixed in 3% gluteraldehyde in phosphate buffer (pH 7), post-fixed in 1% osmium tetroxide, dehydrated in graded solutions of ethanol in water (R.C. Foster personal communication), then graded solutions of amyl acetate in ethanol followed by critical-point drying (Manual, E3000 Critical point drier, Polaron Equipment Ltd UK). The sample was coated with carbon and platinum-palladium, and examined with a Joel JEM-100 CX electron microscope with a scanning electron image device (ASID-4D).

Transmission EM (TEM)

Hyphae were embedded in 2% agar, treated with 3% glutaraldehyde in phosphate buffer (pH 7), post-treated in 1% osmium tetroxide, and dehydrated in graded solutions of ethanol (in water (R.C. Foster personal communication)). The specimens were then embedded in low viscosity resin. Ultra-thin sections (90 nm) were cut with a glass knife, placed on Formvar-coated copper grids (200 mesh), coated with carbon and examined with a Joel JEM-100 CX electron microscope. Soil polysaccharides which do not normally react with osmium tetroxide were rendered electron-dense in some sections by pre-treatment with ruthenium red (Cagle *et al.*1972). Some sections were stained with lead citrate so that the cell wall became electron-dense.

Analysis of amorphous material on the surface of hyphae

The hyphae were shaken gently in 1.5 cm^3 0.1 M sodium bicarbonate for 18 h to extract the amorphous material from the surface of the hyphae (J.M. Oades personal communication). The suspension was centrifuged at 3000 rpm for 5 min and the hyphae removed. The extract was neutralized with dilute hydrochloric acid and (a) tested for sugars by the anthrone agent, (b) scanned with ultraviolet light to detect absorbance, and (c) sugar components of the hydrolyzed extract were separated by paper chromatography.

a) Test for hexoses

The hyphal extract was made up to a known volume and the concentration of hexoses (neutral sugars) was determined by a colorimetric method (Oades 1967) using solutions of D-glucose as the standard. The aliquot (0.1 cm^3) was mixed with 0.5 cm^3 anthrone reagent (anthrone 0.2 g in 100 cm^3 Merck H_2SO_4 91% cm^3/cm^3) in a test-tube in an ice bath. To develop the colour in the solutions the tubes were placed in a boiling water-bath for 10 min, and then cooled in tap water. Within 1 h, extinction was measured at 630 µm in 1 cm cells in a Unicam recording spectrophotometer.

b) Ultraviolet scan

The hyphal extract was diluted 1:6 with distilled water and scanned with a Varian UV spectroscan.

c) Hydrolyzed extract

The extract was made 1 M with respect to sulphuric acid and hydrolyzed by refluxing for 2 h, and neutralized with barium carbonate while still hot. The extract was evaporated to dryness and extracted with redistilled methanol several times to discard the salt and to recover the sugars. The resultant monosaccharides were separated on two Whatman No.1 chromatography papers by descending chromatography for 3 h at 26°C using ethyl acetate:pyridine:water (10:4:3) v/v. (M. Tate personal communication) The extract and standard solutions were each added in several 0.2 µl lots to one spot, and dried with a hair-drier between each lot so that the final spot was 2-3 mm diameter. About 0.5 µg or 1.5 µg sugar was added to each spot, and the spots were located with dips of silver nitrate-sodium hydroxide or p-anisidine respectively.

5.3 Results and Discussion

5.3.1 Stability and hyphal length

Fumigation with chloroform halved the mycorrhizal infection (Table 6). Other workers have found that fumigants may eliminate or reduce the formation of VA mycorrhizas (Nesheim and Linn 1969; Kleinschmidt and Gerdemann 1972) but sufficient inocolum may survive fumigation - possibly being protected in fragments of roots - so that VA mycorrhizas may develop rapidly (Filer and Toole 1968).

Fumigation with chloroform reduced slightly the stability of aggregates (Table 6). After growth of ryegrass for 10 weeks all treatments increased the % water-stable aggregates >2000 µm diameter. Although the plants were the same size, the effect on stability was greater with the fumigated, E_3-inoculated plants (Treatment T_3) and the plants with

Table 6. The effect of fumigation and infection of roots of ryegrass by VA mycorrhizal fungi on the % water-stable particles >2000 µm diameter.

Treatment	Root lengths mm g^{-1} total soil		% infection	Hyphal length m g^{-1} total soil	OD shoots per pot ($80^{o}C$)	% stable aggregates > 2000 µm	
	Total	Infected				0 week	10 week
T_1 fumigated no inoculum	812±46	67±12	8.2±1.3	5.9±0.7	17.8±0.4	7.0±0.6	10.2±1.0
T_2 non-fumigated no inoculum	785±18	161±8	20.5±0.8	11.3±0.5	18.1±0.4	8.9±0.6	13.5±0.5
T_3 fumigated with E_3 inoculum	730±24	159±12	21.7±1.1	13.7±0.8	17.8±0.4	nd	15.9±1.2
T_4 fumigated with washings	736±36	69±5	9.5±0.8	6.6±0.4	17.8±0.6	nd	10.3±0.6

naturally occurring mycorrhizal fungi (Treatment T_2); however the effect of T_2 was only just significantly greater than fumigated non-inoculated plants (Treatment T_1).

When all of the data from all treatments were considered together there was a relationship between length of hyphae in total soil and the water-stable particles >2000 µm diameter (Fig.10). The regression equation was $y = 0.67x + 6.16$, $r^2 = 0.63$.

These results suggest that most of the increase in the % water-stable particles >2000 µm diameter was due to the binding of small aggregates into large aggregates by VA mycorrhizal hyphae.

5.3.2 Electron microscopy

Ultra-thin sections of the hyphae were difficult to cut because quartz in the sample often shattered the glass knife and ripped holes in the film. However TE micrographs of ultra-thin sections show that the hyphae grown in sand were covered with a layer of amorphous material (M), possibly polysaccharide, to which clay particles (C) appear attached firmly (Fig.11), and sometimes oriented in an edge-to-face manner at the hyphal wall. The amorphous material was rendered electron-dense by pre-treatment with ruthenium red, which suggests that it contains polysaccharides (Cagle *et al.* 1972). The hyphae in the micrographs were probably dead before treatment because the cytoplasm has not been fixed. An SE micrograph of the same fungus also shows particles of oriented clay attached firmly to the surface of the hyphae (Fig.12).

5.3.3 Sugar components of amorphous material on the surface of the hyphae

The TE micrograph (Fig.11) suggests that the amorphous material may be polysaccharide. The unhydrolyzed crude extract of the amorphous material collected from the surface of 88 m hyphae contained 12.7 µg hexoses as detected by the anthrone reagent. A scan with ultraviolet

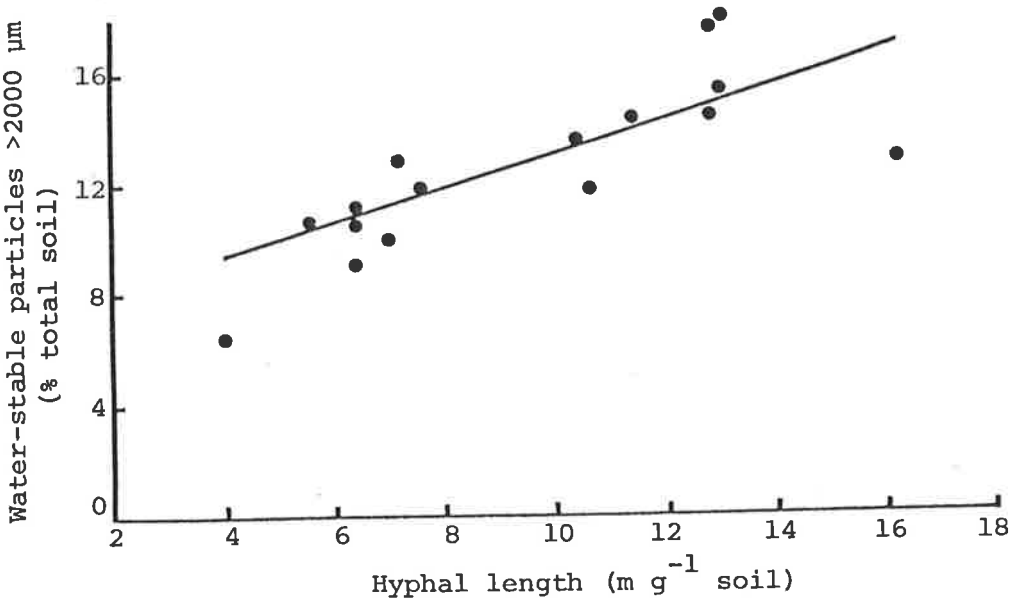


Fig.10 Relationship between hyphal length in the whole soil and % water-stable particles >2000 µm diameter.
$y = 0.67x + 6.16$ $r^2 = 0.63$.

Fig. 11 Transmission electron micrographs of sections of hyphae of VA mycorrhizal fungi with particles of clay attached firmly.
a) cell wall, W, unstained,
b) cell wall, W, stained with lead citrate and amorphous material, M, stained with ruthenium red. Clay particles, C.

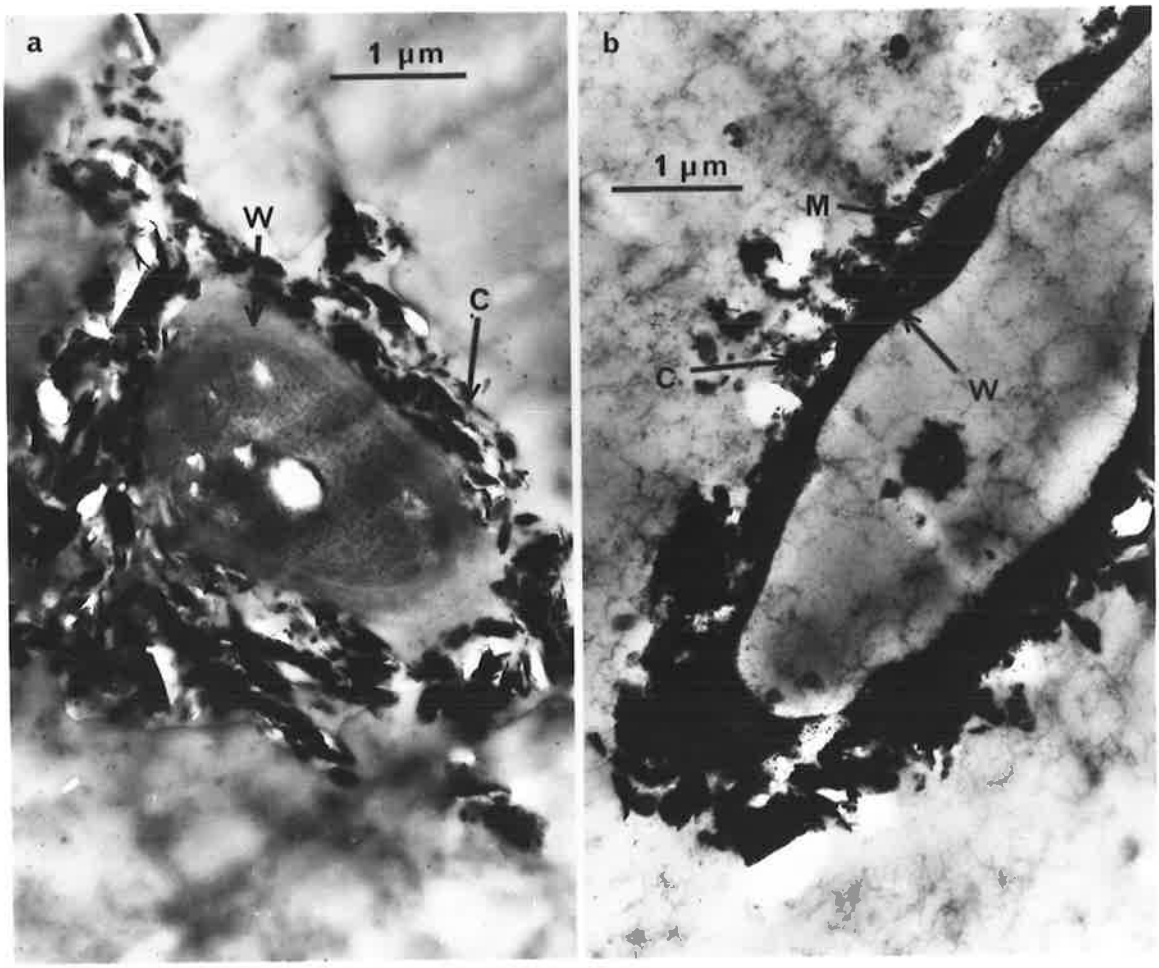
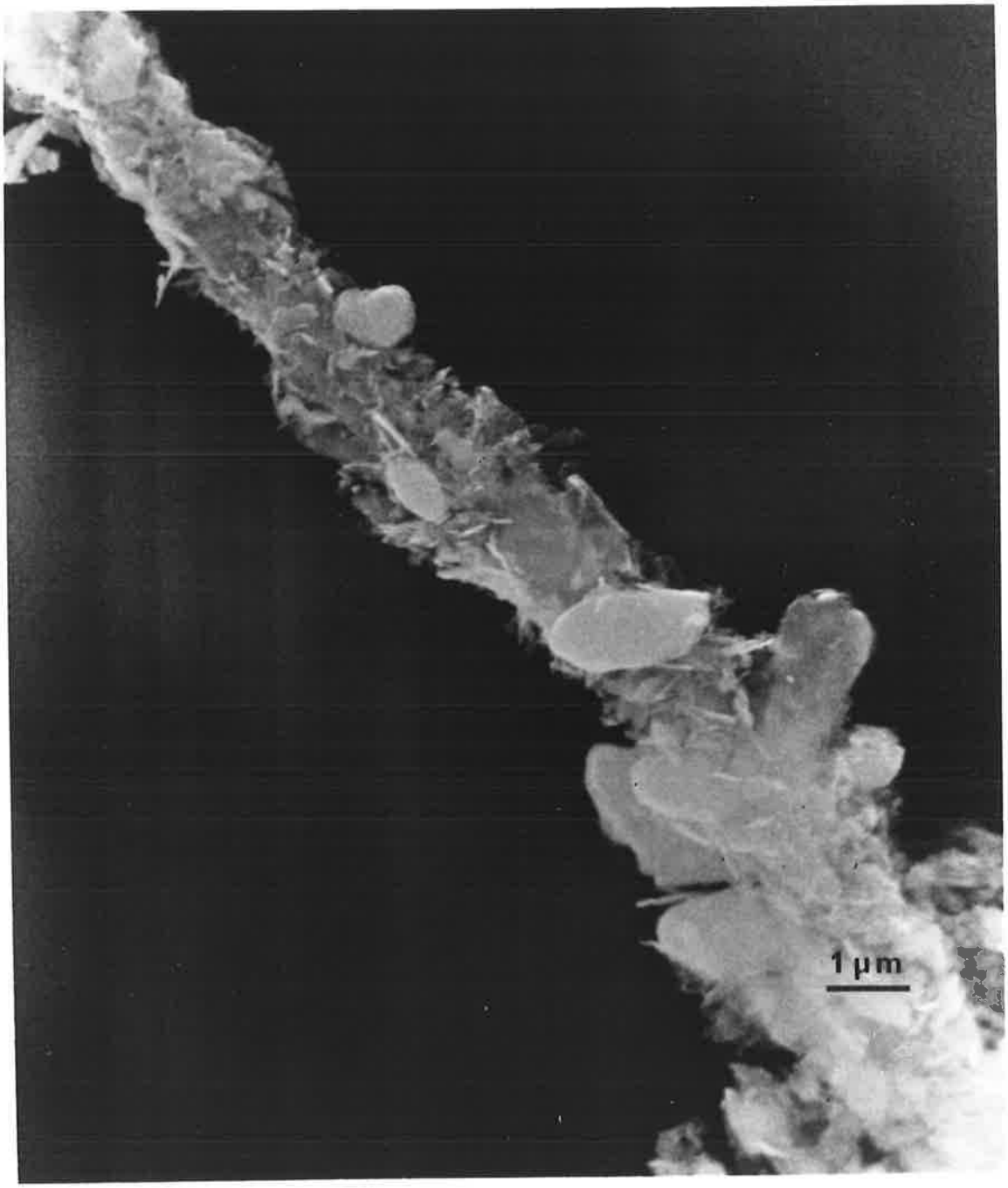

a
1 µm
W
C
b
1 µm
M
C
W

Fig. 12 Scanning electron micrograph of a hypha of a VA mycorrhizal fungus with particles of clay attached firmly.


1 µm

light on the unhydrolyzed extract showed a small peak, representing absorbance, at 270 nm which did not change with pH. This peak suggests that the extract did not contain phenolic compounds, but possibly contained traces of aromatic compounds, proteins or nucleotides; at the end of the scan were smaller peaks due possibly to α and β unsaturated acids or uronic acids.

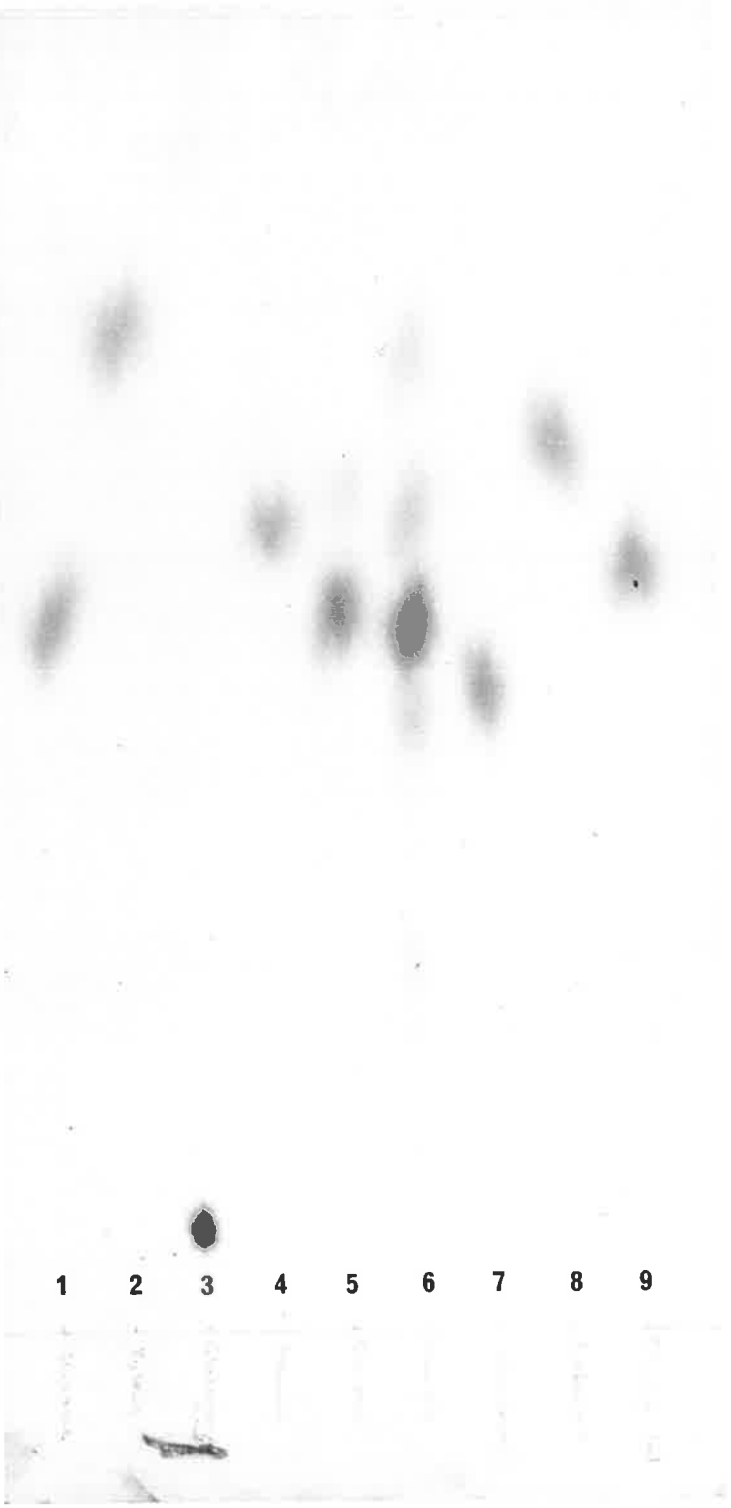
Paper chromatography of the sugars from the hydrolysate showed that the material contained mainly glucose with lesser amounts of galactose and xylose (Fig.13). Glucose and glactose in soil are considered to originate in part from microorganisms, but xylose is generally considered to originate from plants and not from microorganisms since xylose is not labelled strongly when soils are incubated with ^{14}C (Oades and Wagner 1971). Another sugar detected on the chromatograph was thought to be possibly mannose although it did not fluoresce with p-anisidine (Hough *et al*.1950).

5.4 Conclusions

The results presented in this chapter show that VA mycorrhizal hyphae associated with the roots of ryegrass are capable of stabilizing aggregates of Lemnos loam. The hyphae appear to be covered with a layer of amorphous material, probably polysaccharides, to which clay particles are attached firmly.

Fig. 13 Paper chromatography of hydrolyzed extract of the amorphous material from the surface of hyphae, in solvent system ethyl acetate:pyridine:water (10:4:3 v/v).

1, 5 and 9 = standard glucose
2 = standard xylose
3 = standard glucuronic acid
4 = standard mannose
6 = hydrolyzed extract
7 = standard galactose
8 = standard arabinose.


1
2
3
4
5
6
7
8
9

CHAPTER 6

THE MANAGEMENT OF RYEGRASS TO STABILIZE AGGREGATES OF A RED-BROWN EARTH

6.1 Introduction

The results described in Chapter 5 show that after the growth of ryegrass for 8 weeks, but not for 6 weeks, water-stable particles 50-250 µm diameter of Lemnos loam were bound into water-stable aggregates >2000 µm diameter; at least part of the stability was due to VA mycorrhizal hyphae supported by the root system. This work was extended for up to 52 weeks in pots to study the effect of management of the plants on roots, hyphae and size-distribution of water-stable particles in Lemnos loam.

Plants under different systems of management have been shown to release organic materials into soil, either in frequent small amounts from living plants (Katznelson *et al.*1955, Butler *et al.* 1959), or in one large amount when the plants die. Since dead roots or other decomposable organic materials, when added to an unstable soil, stabilize aggregates (Browning and Milan 1941; Robinson and Jacques 1958), it should be possible to define the management of plants which will lead to maximal stability of aggregates.

Dry soil limits the growth of roots (Russell1977) and root hairs (Kramer and Kozlowski 1960) and may lead to the release of additional water-soluble materials or mucilage (see section 1.4.3). Drying may also lead to better contact between soil and roots, which results in the binding of particles of soil into stable aggregates in the rhizosphere (Campbell and Rovira 1973).

It is not known whether aggregates become more stable with frequent small additions of organic materials (produced by recurrent stresses such as clipping or wilting) or with one large addition (killing of plants).

Generally within 1-2 days of clipping of leaves, roots stop growing for up to 2-3 weeks and many of the old roots die (see section 1.4.3). Hence recurrent clipping leads to cycles of root decay and regrowth and to

additional release of water-soluble materials.

6.2 Experimental

Sixty plants of ryegrass (*Lolium perenne*) were grown in 3 kg of Lemnos loam in an enamel pot. Nutrients were added to all pots containing ryegrass as described in section 4.2.1 (Experiment 1). Whenever necessary, each pot was wetted to restore the matric suction to -10kPa. Pots were maintained in a water-bath at 18°C in a glasshouse, the mean maximum and mean minimum air temperatures were 28°C and 18°C respectively. Treatments were replicated four times and sufficient samples were prepared to allow destructive sampling.

In the field Lemnos loam is used for irrigated horticultural crops for about 20 weeks each year, which allows about 30 weeks during which ryegrass could be used solely to increase the stable aggregation of the soil. Therefore, treatments were chosen to fit in with current practices in the field leading to the following alternatives. Either (a) the growth and most of the decomposition of the ryegrass roots should be completed within 30 weeks, i.e. equivalent to most of the stabilization being completed by the beginning of the next cropping season, or (b) the growth should be completed within 30 weeks and most of the decomposition continued for a further 22 weeks, i.e. equivalent to much of the stabilization occurring during the next cropping season.

There were 8 treatments including an unplanted control:

Unplanted control. Pots with no plants were watered every 3 to 4 weeks. The soil was sampled from separate pots at 0 weeks and 20 weeks.

Ryegrass control. Ryegrass was grown under optimal conditions for 20, 30 or 52 weeks, and watered every 1 to 3 days.

Wilted ryegrass. Eight weeks from sowing the plants were allowed to wilt before each watering. The soil was sampled at 20 weeks.

Clipped ryegrass (2 week). Eight weeks from sowing and every 2 weeks thereafter, the ryegrass leaves were clipped 30 mm from the surface of the soil and the soil was watered every 1 to 3 days. The soil was sampled at 20 weeks.

Clipped ryegrass (4 week). Ten weeks from sowing and every 4 weeks thereafter, the ryegrass leaves were clipped to 30 mm from the surface of the soil and the soil was watered every 1 to 3 days. The soil was sampled at 30 weeks.

Killed ryegrass (10 week). Ten weeks from sowing the ryegrass leaves were sprayed with Paraquat (1,1-dimethyl-4,4-bipyridylium dimethyl sulphate) and the dead plants were watered every 3 to 4 weeks. The soil was sampled at 30 weeks.

Killed ryegrass (16 week). Sixteen weeks from sowing, the ryegrass leaves were sprayed with Paraquat and then watered at 18 weeks. The soil was sampled at 20 weeks.

Killed ryegrass (30 week). Thirty weeks from sowing, the ryegrass leaves were sprayed with Paraquat; the soil was watered every 3 to 4 weeks. The soil was sampled at 52 weeks.

The stability of aggregates, the length of roots and length of hyphae combined with stable aggregates, and the effect of pre-treatment with sodium periodate or sodium pyrophosphate (after samples were drained for 16 h) were each measured on separate sub-samples from some experimental treatments.

6.3 Results and Discussion

6.3.1 Management of ryegrass and aggregate stability

Optimal growth of ryegrass

The growth of ryegrass bound particles 50 to 500 μm diameter into water-stable aggregates >1000 μm diameter most of which were >2000 μm diameter (Fig. 14). This agrees with previous results (see section 4.3).

There was almost a linear increase of % water-stable particles

>2000 µm diameter with the time for which ryegrass was grown without wilting. As the stabilization increased with time, the root length and the hyphal length per g stable particle >2000 µm diameter increased also (Table 7). Most of the hyphae in the stable aggregates >2000 µm diameter were VA mycorrhizal fungi.

Table 7. The effect of management of ryegrass on water-stable particles >2000 µm diameter.

Treatment	% stable particles >2000 µm	Total OD leaves per pot 80°C (g)	Root length m g^{-1} stable particles >2000 µm	Hyphal length m g^{-1} stable particles >2000 µm
Ryegrass control (sampled 20 week)	23.7±3.0	31.7±1.1	nd	nd
Ryegrass control (sampled 30 week)	33.5±1.4	54.9±3.1	2.1±0.2	15.6±0.6
Ryegrass control (sampled 52 week)	45.4±3.2	75.7±1.2	4.9±0.6	22.4±2.7
Wilted ryegrass (sampled 20 week)	17.4±1.4	25.1±3.1	nd	nd
Clipped ryegrass(2 week) (sampled 20 week)	26.5±2.1	25.0±1.6[A]	nd	nd
Clipped ryegrass(4 week) (sampled 30 week)	42.0±2.8	34.5±0.9[A]	2.4±0.3	18.0±2.7
Killed ryegrass(10 week) (sampled 30 week)	5.3±0.4	14.9±0.8	0.8±0.1	6.9±0.2
Killed ryegrass(16 week) (sampled 20 week)	26.6±2.8	34.2±0.9	nd	nd
Killed ryegrass(30 week) (sampled 52 week)	34.1±2.3	64.7±1.0	2.5±0.3	12.7±2.5

[A] includes mass OD (oven-dried) leaves removed at all clippings and at harvest.

nd not determined because of lack of time.

Absence of plants

In pots without plants (*Unplanted control*), bonds were destroyed in aggregates >2000 µm and 50-250 µm diameter leading to an increase in particles 20-50 µm and <10 µm diameter (Fig.14). In an earlier study, intermittent wetting and drying decreased the stability of another red-brown

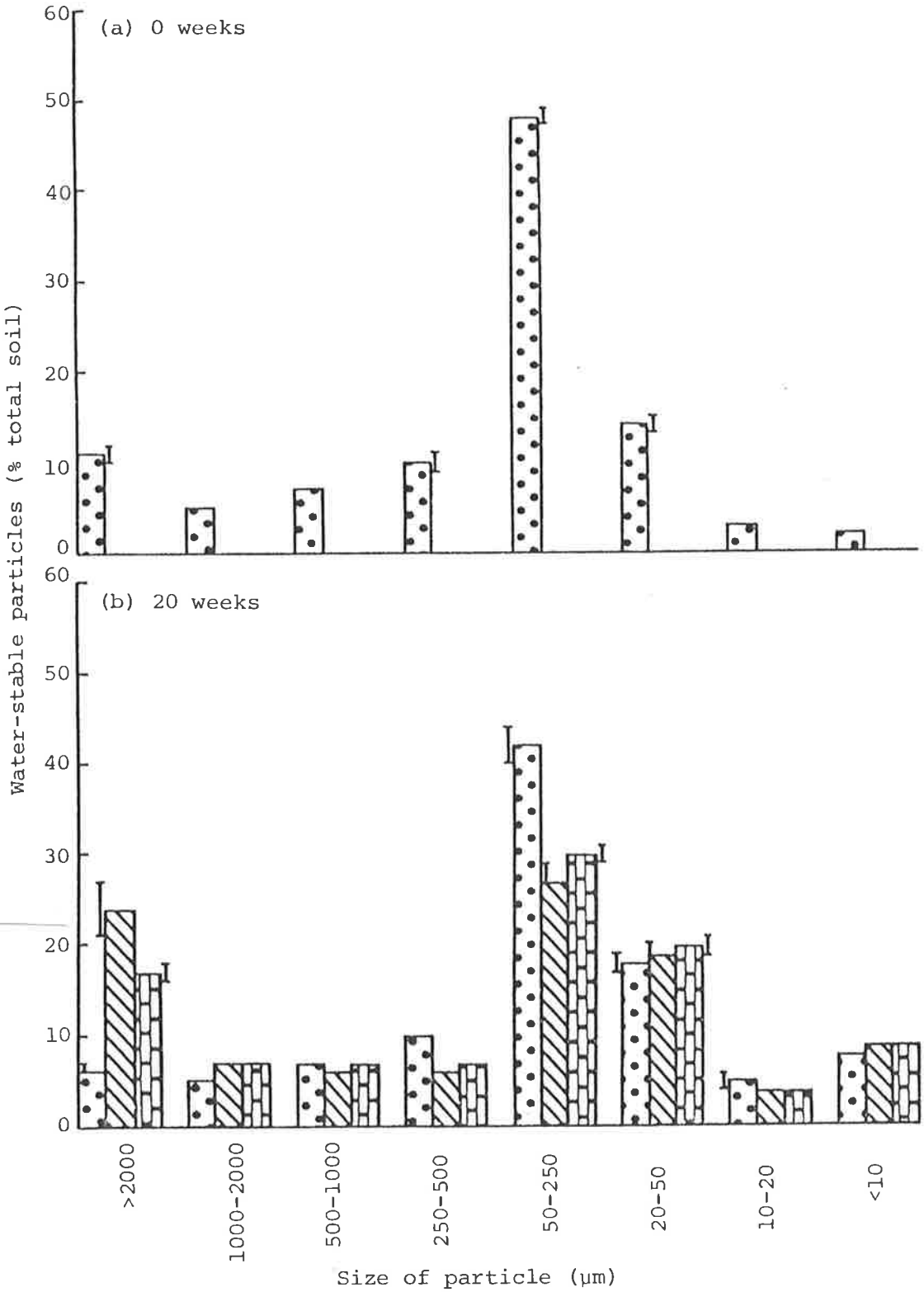
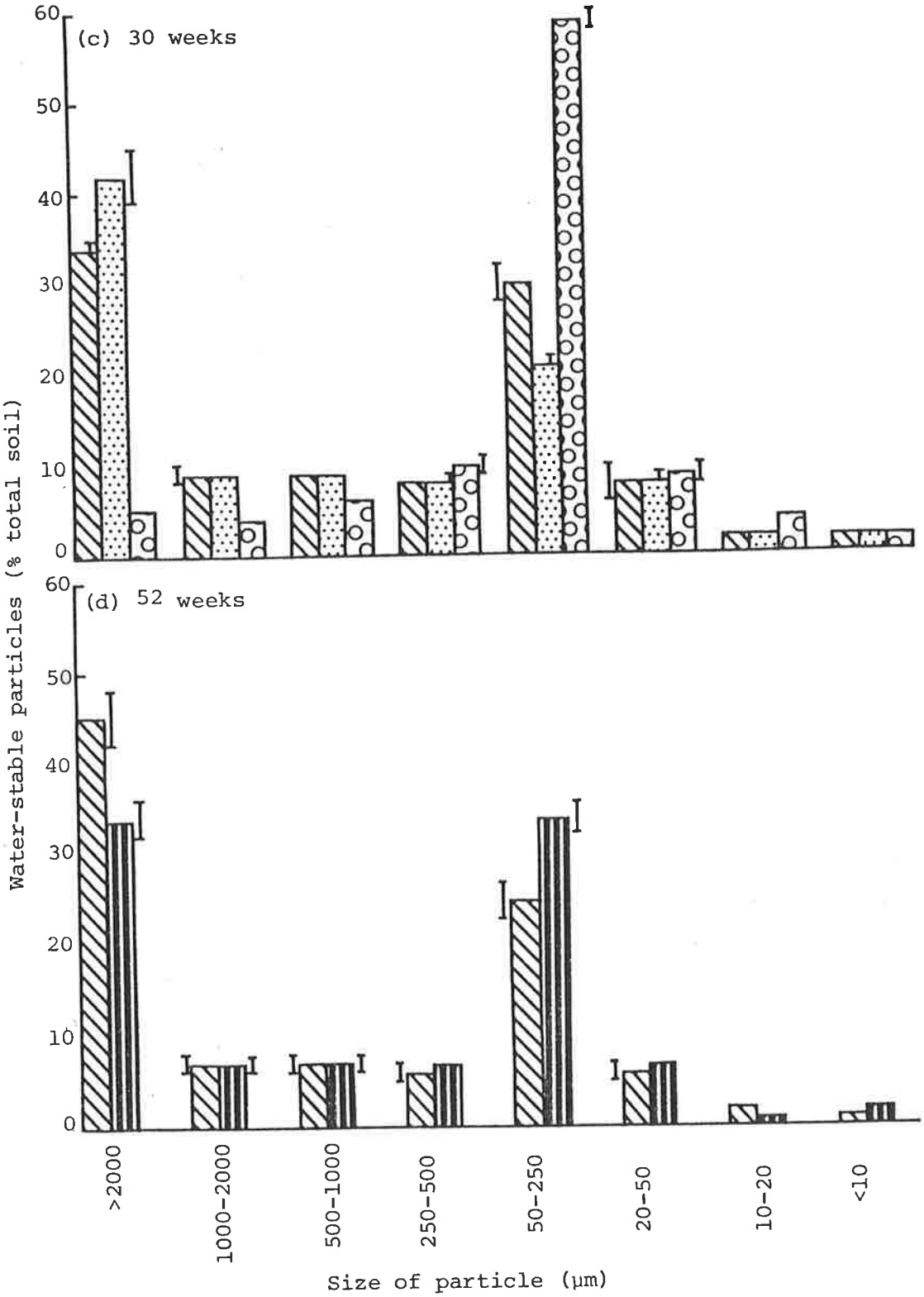


Fig.14 The effect of management of ryegrass on the size-distribution of water-stable particles in soil.
Unplanted control; Ryegrass control;
Wilted ryegrass; Clipped ryegrass (4 week);
Killed ryegrass (10 week); Killed ryegrass (30 week). Vertical bar, 2 x SE mean.


(c) 30 weeks
(d) 52 weeks
Water-stable particles (% total soil)
60
50
40
30
20
10
0
>2000
1000-2000
500-1000
250-500
50-250
20-50
10-20
<10
Size of particle (µm)

earth, Shepparton fine sandy loam, probably because microorganisms degraded organic bonds and because aggregates were disrupted physically when wetted rapidly (Tisdall *et al.* 1978). The size of aggregates has been shown to decrease in other soils during cycles of wetting and drying (Willis 1955; Rovira and Greacen 1957; Soulides and Allison 1961).

Stress treatments

Wilting. The only treatment of the ryegrass which significantly affected the stability of aggregates from 0-20 weeks was recurrent wilting *(Wilted ryegrass)*, which reduced the growth of the plants (Table 7) and reduced the build-up of water-stable aggregates (Fig.14). Based on this result wilting was no longer considered useful for increasing the stability of aggregates.

Clipping. When the plants were clipped every 4 weeks (*Clipped ryegrass (4 week)*), more particles 50-250 µm diameter were bound into water-stable particles >2000 µm diameter at 30 weeks (Fig.14), than in the ryegrass control after 30 weeks. The % water-stable particles >2000 µm diameter stabilized by clipping of ryegrass (sampled at 30 weeks) did not differ significantly from that when ryegrass was grown under optimal conditions for 52 weeks. That is, clipping led to the same effect as the ryegrass control in 58% of the time, The increased stable aggregation was not related directly to the root length per g stable aggregate >2000 µm diameter (Table 7), but there was evidence of greater hyphal length in stable aggregates >2000 µm diameter although this effect was not statistically significant. Most of the hyphae were VA mycorrhizal fungi. It is possible that clipping released organic materials which stimulated the growth of the external hyphae of these fungi, either directly, or indirectly by an effect on other organisms in the soil. Although there is little evidence that VA mycorrhizal hyphae can use carbohydrate derived from outside a living root, these fungi have been observed inside dead or

dying roots (in the presence of living roots) in soils in the field (Hirrel *et al.* 1978), and several workers have obtained limited hyphal growth from surface-sterilized mycorrhizal roots (Gerdemann 1968). It is not known whether saprophytic organisms (themselves influenced by materials released on clipping) stimulate VA mycorrhizal fungi, although there is some evidence of interaction between bacteria and ectomycorrhizal fungi (Bowen and Theodorou 1979). Clipping the plants every two weeks (*Clipped ryegrass (2 week)*) gave a particle-size distribution which was not significantly different at 20 weeks from that given by the ryegrass control which was watered frequently. This was to be expected because clipping every 2 weeks may not have allowed the plants sufficient time to recover fully from the stress imposed (Crider 1955; Oswalt *et al.* 1959).

Decomposition of killed roots and associated hyphae

When the plants were killed at 10 weeks (*Killed ryegrass (10 week)*) and the pots watered every 3 to 6 weeks for a further 20 weeks to enable the roots to decompose, particles > 500 µm diameter disintegrated to particles 50-250 µm diameter (Fig.14). Intermittent wetting and drying also probably disrupted aggregates. The decrease in stability was accompanied by a decrease in hyphal length and root length per g stable aggregates >2000 µm diameter, when compared with the ryegrass control sampled at 30 weeks (Table 4).

It is important to compare *Unplanted control* at 20 weeks with *Killed ryegrass (10 week)* sampled at 30 weeks (Fig.14), both of which had been subjected to intermittent wetting and drying for 20 weeks. In the soil which contained decomposing roots (*Killed ryegrass (10 week)*) there was a build-up of water-stable particles 50-250 µm diameter from particles < 50 µm diameter. Bacteria rather than fungi may have stabilized these particles 50-250 µm diameter while the roots were decomposing; it has been suggested that bacteria stabilize particles < 2000 µm (Harris *et al.* 1966) or < 560 µm diameter (Hubbell and Chapman 1946).

When the ryegrass control sampled at 30 weeks is compared with ryegrass killed at 30 weeks and allowed to decompose until 52 weeks, it is seen that the stability of particles >2000 µm diameter changed little although 22 weeks of decomposition led to a slight, but statistically insignificant, increase in the stability of particles 50-250 µm diameter.

The root lengths and hyphal lengths per g stable particles >2000 µm diameter of these treatments were also similar. Therefore the resistant parts of the roots or hyphae do not appear to have decomposed when plants were killed at 30 weeks and sampled at 52 weeks. Most of the hyphae were VA mycorrhizal fungi, although the hyphae were not necessarily viable. Presumably 3 to 4 weeks after the plants were killed (in *Killed ryegrass (10 week)* and *Killed ryegrass (30 week)*) there would have been a flush of growth of non-mycorrhizal fungi, but at 20 or 22 weeks after the plants were killed (i.e. sampled at 30 weeks or 52 weeks respectively) most non-mycorrhizal hyaline fungi would have been decomposed and only the hyphae with the most resistant cell walls would have remained undecomposed (Kimura and Egawa 1956; Bloomfield and Alexander 1967; J. Warcup personal communication). Bond and Harris (1964) found that hyphae associated with stable aggregates in the field appeared to be old and probably not viable.

When the ryegrass was killed after 16 weeks growth (*Killed ryegrass (16 week)*) there was no detectable difference in stability at 20 weeks from *Ryegrass control*.

6.3.2 Chemical pre-treatments

Sodium periodate

Additional sub-samples from all experimental treatments were pre-treated with periodate or pyrophosphate before examination of aggregate stability.

After the growth of ryegrass control for 30 weeks, sodium periodate, which probably destroys polysaccharides, just significantly decreased the percentage of water-stable particles >2000 µm and 1000-2000 µm diameter

(Fig.15). Sodium periodate had almost no effect on the stability of particles from the ryegrass control at 52 weeks (Fig.16), so there was a slight increase in particles >2000 μm diameter which were resistant to periodate when compared with ryegrass control at 30 weeks. Stefanson (1971) reported that the older a pasture, the less important periodate-sensitive materials were in the stabilization of a red-brown earth.

Sodium periodate had almost no effect on the stability of aggregates from *Killed ryegrass (10 week)* sampled at 30 weeks (Fig.15). However, when plants were killed at 30 weeks and the roots allowed to decompose for a further 22 weeks (Fig.16) sodium periodate reduced the % water-stable particles 50-250 μm and 20-50 μm diameter when compared with the ryegrass control sampled at 30 weeks (Fig.15). There was little change in the size-distribution of water-stable particles between *Ryegrass control* sampled at 30 weeks and *Killed ryegrass (30 week)* sampled at 52 weeks (Fig.14), but periodate-sensitive materials were important where ryegrass had been killed and the roots allowed to decompose where there was an increase mainly in % stable particles 20-250 μm diameter (Fig.15; Fig.16). The implications are that the decomposition of roots resulted in stabilization of these small aggregates by microbial mucilages (periodate-sensitive materials).

Sodium pyrophosphate

After the growth of ryegrass for 30 weeks, sodium pyrophosphate, which probably complexes polyvalent cations responsible for binding organic materials to particles of clay, significantly decreased the % water-stable particles >50 μm diameter and increased the % particles <50 μm (Fig.15). The effect of sodium pyrophosphate showed similar trends in % water-stable particles in the ryegrass control at 30 weeks and at 52 weeks,but there was an increase in pyrophosphate-resistant materials in particles >2000 μm diameter in the ryegrass control sampled at 52 weeks (Fig.16).

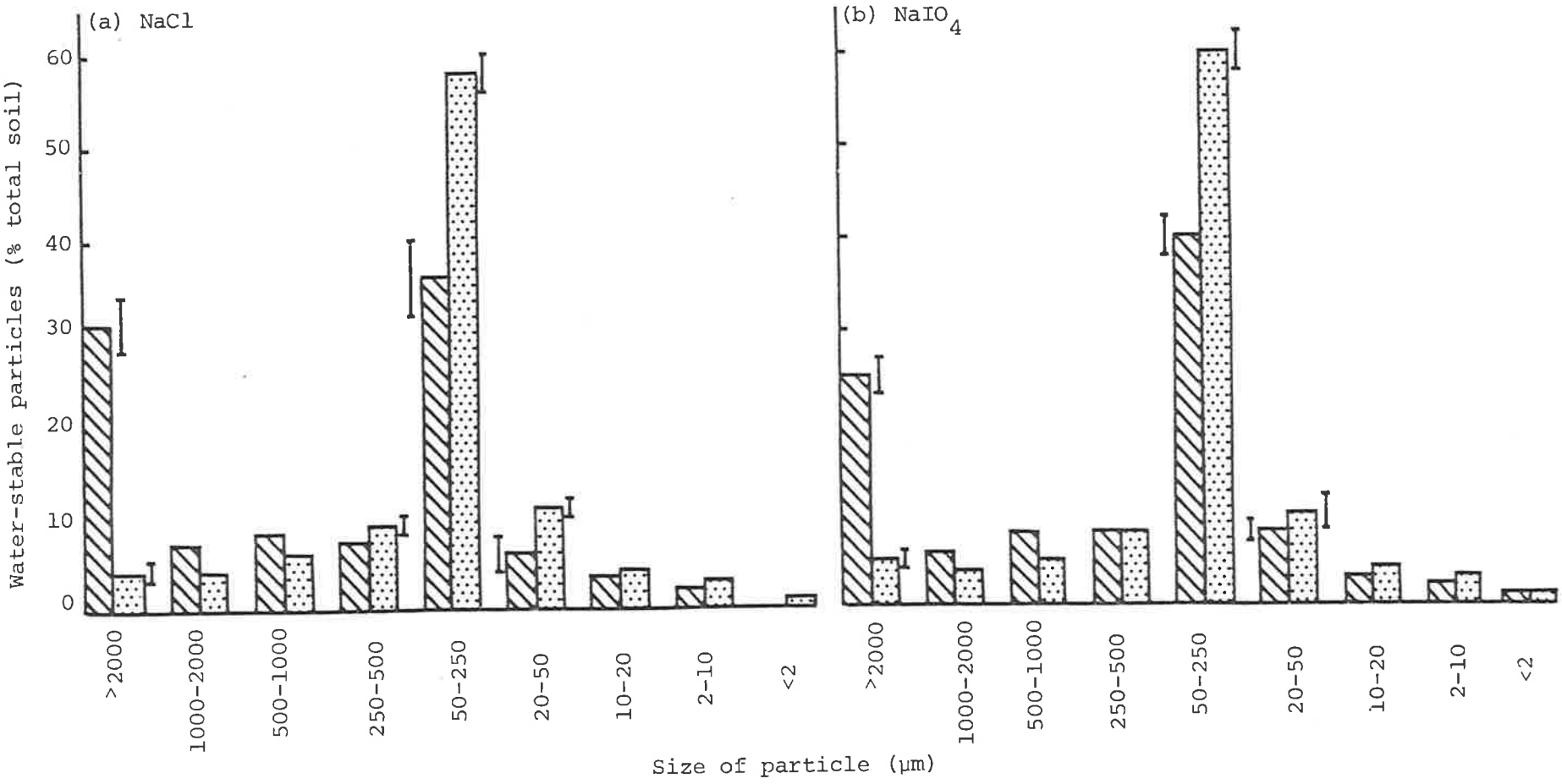
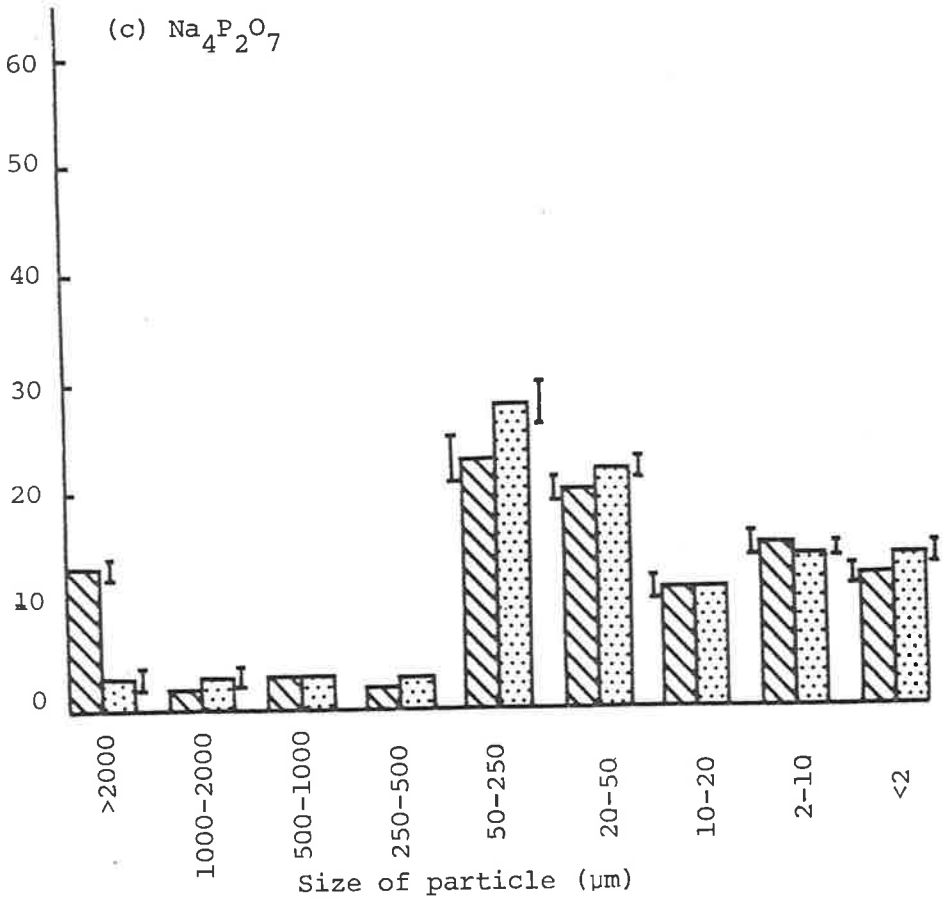


Fig.15 The size-distribution of water-stable particles after chemical treatment (sampled at 30 weeks)
▨ Ryegrass control; ⁘ Killed ryegrass (10 week). Vertical bar, 2 x SE mean.


(c) $Na_4P_2O_7$
60
50
40
30
20
10
0
>2000
1000-2000
500-1000
250-500
50-250
20-50
10-20
2-10
<2
Size of particle (μm)

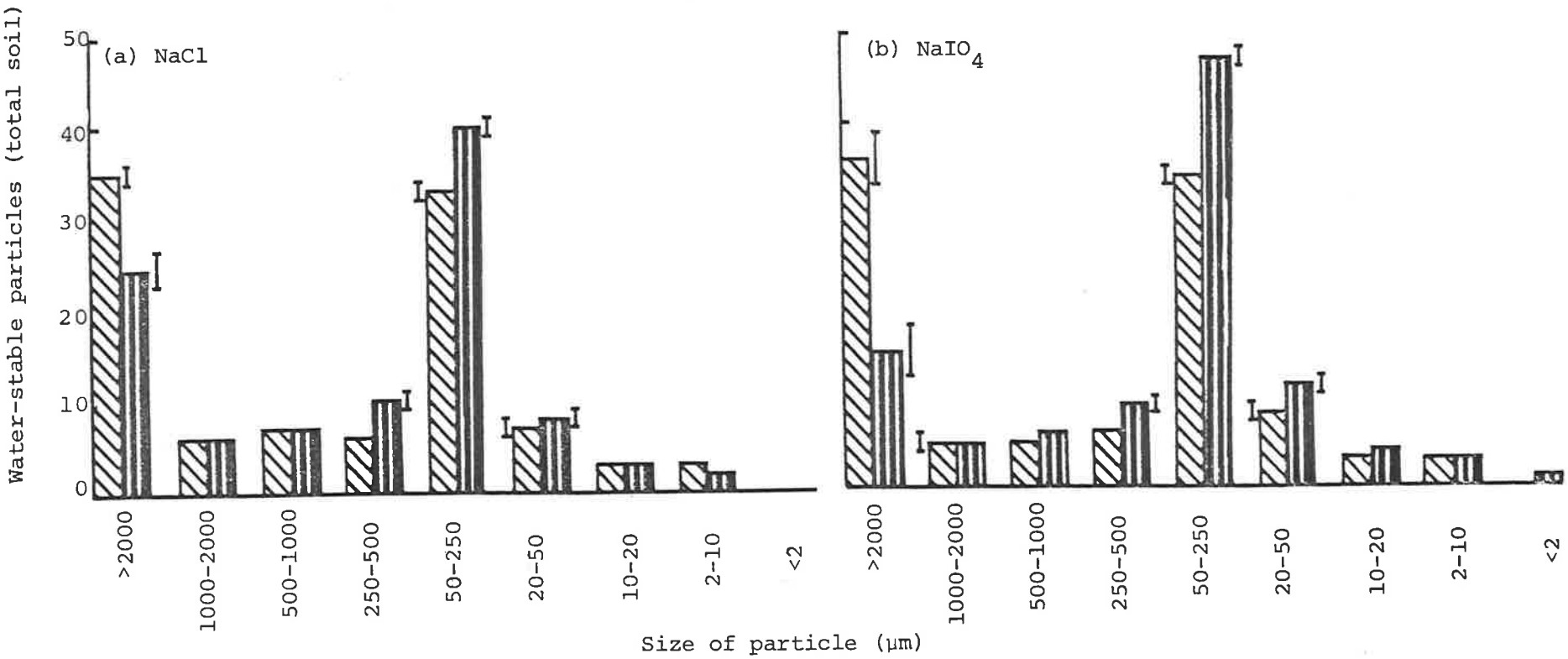
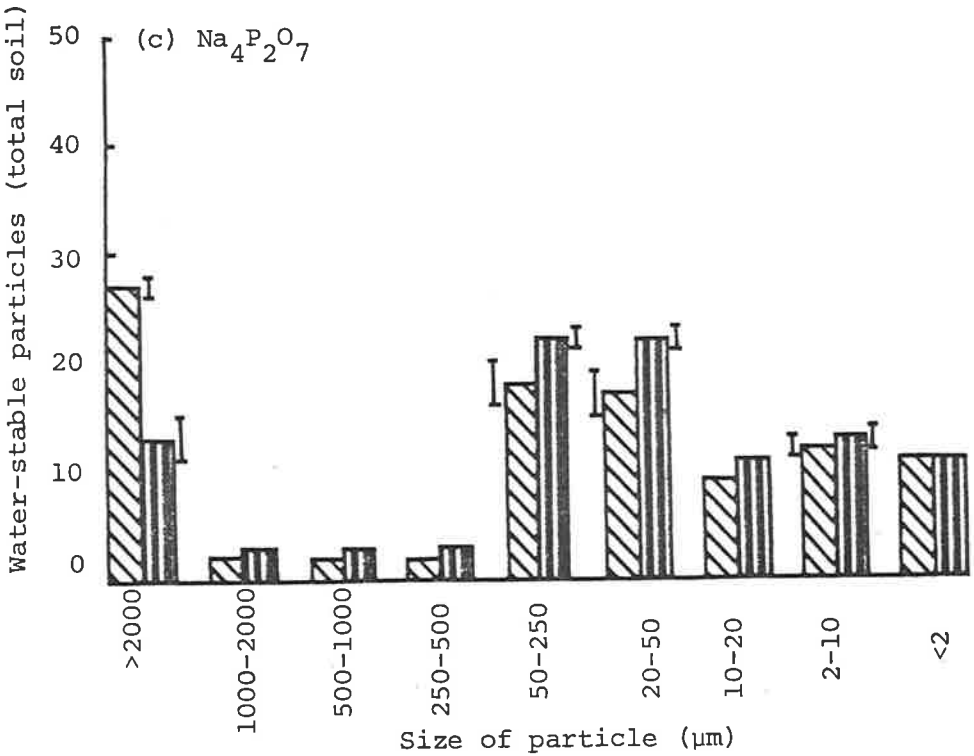


Fig.16 The size-distribution of water-stable particles after chemical treatment (sampled at 52 weeks).
Ryegrass control; Killed ryegrass (30 week). Vertical bar, 2 x SE mean.


(c) $Na_4P_2O_7$
Water-stable particles (total soil)
50
40
30
20
10
0
>2000
1000-2000
500-1000
250-500
50-250
20-50
10-20
2-10
<2
Size of particle (µm)

Stefanson (1971) also found that the surface soil of a red-brown earth under pasture contained additional pyrophosphate-resistant materials than the same soil under a range of pasture-cropping rotations. Such an increase is compatible with the build-up of periodate-resistant and pyrophosphate-resistant materials in the form of fungal hyphae (D. J. Carter and J. M. Oades unpublished results) and roots.

6.4 Conclusions

The management of ryegrass over the first 20 weeks had little effect on the stability of aggregates, except that recurrent wilting retarded the build-up of water-stable particles >2000 μm diameter.

Ryegrass grown for 30 weeks bound particles 50-250 μm diameter into water-stable particles >2000 μm diameter, the benefits were cumulative for up to 52 weeks, apparently because there was an increase in the growth of roots and VA mycorrhizal hyphae. Periodate-resistant materials became unimportant in the stabilization of aggregates as the plants grew.

The quickest way to reach maximum stability was to clip plants every 4 weeks between 10 and 30 weeks, possibly partly because clipping increased slightly the growth of mycorrhizal hyphae.

When ryegrass plants were killed at 10 weeks and the roots allowed to decompose for a further 20 weeks there was insufficient growth of roots and hyphae to persist and prevent disruption of aggregates by intermittent wetting and drying. However there was a build-up of particles 50-250 μm diameter from particles <50 μm diameter. A similar but insignificant trend was seen when plants were killed at 30 weeks and allowed to decompose for a further 22 weeks. As roots and hyphae decomposed there was a build-up of pyrophosphate-resistant materials which stabilised particles <50 μm diameter.

The results described in this chapter show that, provided there has been sufficient growth of roots, VA mycorrhizal hyphae can persist in soil for at least several months after the plants die, although the hyphae may

no longer be viable. Therefore in the field, part of the stability produced during the pasture phase of a rotation persists for at least a few months during the cropping phase (Low *et al*. 1963).

The practical implications of the results described in this chapter are that, to obtain maximal stable aggregation, plants should be grown with an ample supply of water, and clipped monthly (e.g. cut for hay), so that the plants rapidly produce a large network of roots and hyphae; bare ground, such as fallow or burning the stubble, should be avoided.

CHAPTER 7

THE EFFECT OF CROP ROTATION ON AGGREGATION IN URRBRAE FINE SANDY LOAM

7.1 Introduction

The results presented in Chapters 4, 5 and 6 show that ryegrass, grown in pots for up to 1 year, stabilized large aggregates (>1000 µm diameter) in Lemnos loam; part of the stability appeared to be due to roots and hyphae. The effect of management was related to the effect on the growth of VA mycorrhizal hyphae which persisted for at least several months after the plants had died, although the hyphae may no longer have been viable.

This work was extended to the field to study the effect of 50 years of crop rotations on roots, hyphae and % organic matter and water-stable aggregates in Urrbrae fine sandy loam (see section 2.1). The relative effects of management on the stability of macroaggregates (>250 µm diameter) and microaggregates (<250 µm diameter) (Edwards and Bremner 1967) were determined. This aspect was not studied in earlier work on the structural stability of red-brown earths (Greacen 1958; Greenland *et al.* 1962; Cockroft and Hughan 1964).

7.2 Experimental

The histories of the sites of Urrbrae fine sandy loam which were sampled (see section 2.1) are shown in Table 8. The size-distribution of water-stable particles was measured on two sub-samples per plot, before and after various physical and chemical treatments described below. The particle-size distribution was also measured on fully dispersed samples of soil from the plot which grew wheat every year. The length of roots and of hyphae were each measured in separate duplicate samples of the whole soil. Results of duplicate samples were averaged.

Table 8. The crop rotation, and carbon and nitrogen contents of soils examined.

Soil	Rotation	Phase sampled	Total organic carbon % total soil	Organic carbon (LF removed) % total soil	Total nitrogen[A] % total soil
Virgin	-	-	2.5	1.6	not determined
PP	Old pasture	Pasture	2.3	1.8	0.20
PPPWWP	2 years wheat-4 years pasture	Pasture	2.0	1.9	0.15
WPPPPW	" " " " " "	Wheat	1.9	1.6	0.14
PPW	Pasture-pasture-wheat	Wheat	1.9	1.3	0.14
WW	Wheat every year	Wheat	1.7	1.0	0.11
WPF	Wheat-pasture-fallow	Fallow	1.6	1.2	0.12
PFW	" " "	Wheat	1.2	0.8	0.08
WF	Wheat-fallow	Fallow	1.2	1.0	0.08
FW	" "	Wheat	1.1	0.9	0.07

[A] J. S. Russell and J. M. Oades (personal communication).

7.2.1 Physical pre-treatment

Ultrasonic probe

Duplicates of air-dried soil (<10 mm; 20 g) were suspended in 50 cm^3 distilled water in 150 cm^3 tall-form beakers. The tip of a Branson B-12 (150 W, 50/60 Hz) ultrasonic probe was immersed 15 mm below the surface of the suspension and to the side of the beaker to avoid a vortex produced by stirring. The suspension was treated ultrasonically for 5 min with the meter set at 100 W.

End-over-end shaking

Duplicates of air-dried soil (<10 mm; 20 g) were shaken end-over-end in 1000 cm^3 distilled water in a sedimentation cylinder (1250 cm^3) for 30 min, and left for 16 h.

7.2.2 Chemical pre-treatment

Hydrogen peroxide

Six of the soils were selected to give a range of total organic carbon from 1.1% to 2.5%. Duplicates of air-dried soil were treated with H_2O_2 to remove the organic carbon (see section 2.2).

Chloride, periodate or pyrophosphate

Duplicates of air-dried soil were treated with NaCl, $NaIO_4$ or $Na_4P_2O_7$ (see section 2.2).

7.2.3 Fractionation of soil

Six of the soils were selected to give a range of total organic carbon from 1.1% to 2.5%. Air-dried soil (<10 mm; 20 g) was wet-sieved to separate particles >2000 µm, 1000-2000 µm, 500-1000 µm and 250-200 µm. The material which had passed through the sieves during wet-sieving was then passed through a 53 µm sieve to separate particles 50-250 µm. The soil which was collected on each sieve was air-dried. The remaining soil

was fractionated by sedimentation under gravity (equivalent spherical diameters 20-50 μm, 10-20 μm and <10 μm). The fractions 20-50 μm and 10-20 μm were air-dried. The suspensions of fractions <10 μm were made 0.01 M with respect to $AlCl_3$. Most of the supernatant was discarded and the remaining liquid removed by freeze-drying. The samples were dialysed against distilled water and freeze-dried.

7.2.4 Removal of light fraction from soils

Total soil

Duplicates of air-dried soil (<10 mm; 5 g) were shaken in 10 cm^3 of a solution of $ZnBr_2$ (density 1.8 g cm^{-3}) on a Spex Mill for 3 min. The samples were transferred to 100 cm^3 centrifuge tubes and made up to 80 cm^3 with further $ZnBr_2$. The tubes were stoppered, shaken by hand and allowed to stand for 30 min before being centrifuged.

The supernatant plus the light fraction were decanted onto a Millipore pre-filter under suction on a water-pump. The filter and light fraction were washed with a few cm^3 of acetone and left under suction until dry. The light fraction was collected and air-dried.

The residues of soil were washed and centrifuged three times with acetone.

Fractions of soil

Up to 2 g of each particle-size fraction of the six selected soils was shaken in up to 4 cm^3 solution of $ZnBr_2$ (density 1.8 g cm^{-3}) in the ratio of 1:2 (mass:vol) on a Spex Mill for 3 min. The samples were transferred to 50 cm^3 centrifuge tubes and made up to 40 cm^3 with further $ZnBr_2$. The tubes were stoppered, shaken by hand and allowed to stand for 30 min before being centrifuged. The supernatant plus light fraction were decanted off and discarded since the fractions of soil collected on sieves (i.e. all particles >50 μm) were contaminated with 'free' light

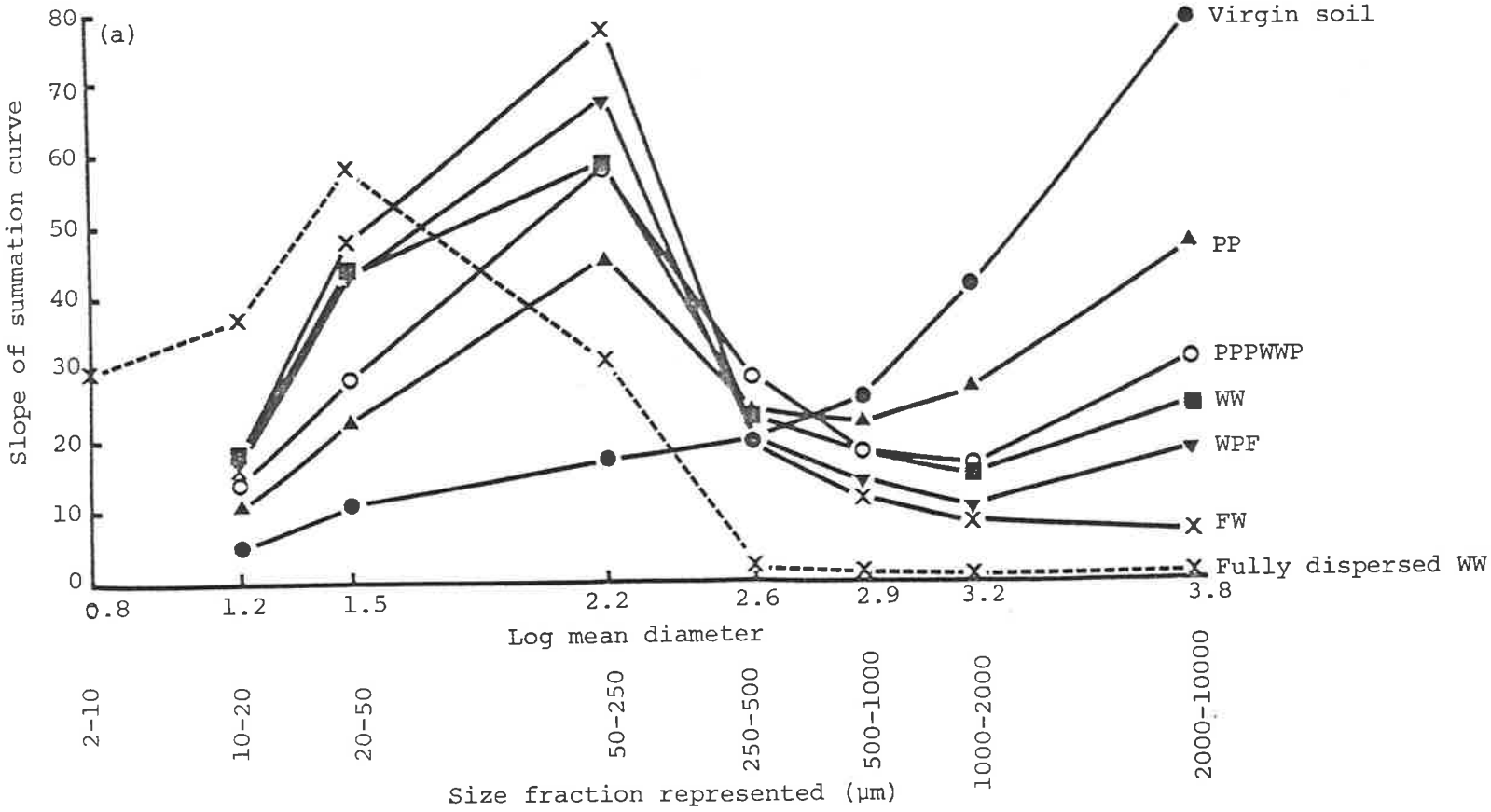
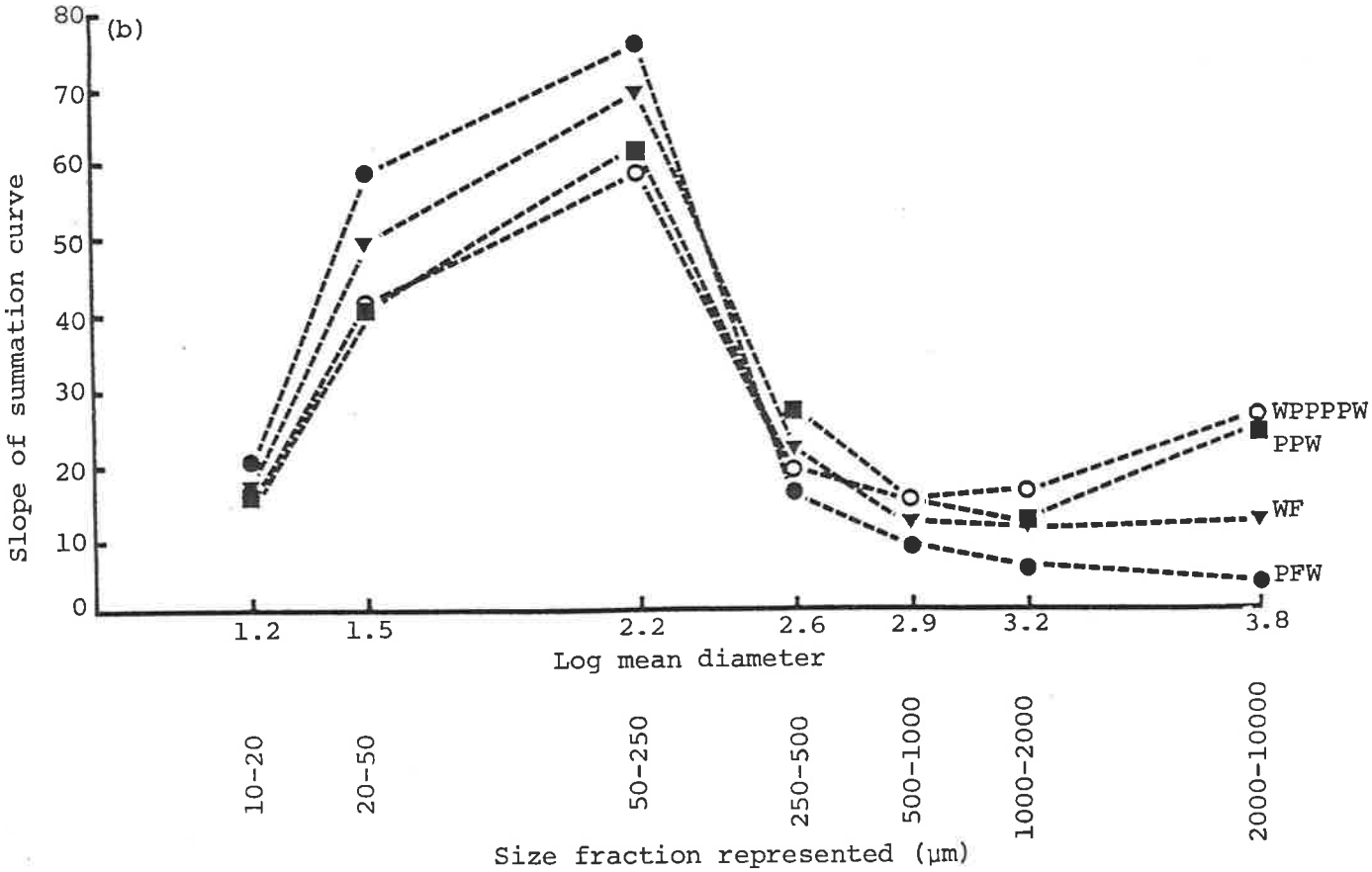


Fig.17 The effect of crop rotation on the size-distribution of water-stable particles from Urrbrae fine sandy loam.


(b)
Slope of summation curve
80
70
60
50
40
30
20
10
0
WPPPPW
PPW
WF
PFW
1.2
1.5
2.2
2.6
2.9
3.2
3.8
Log mean diameter
10-20
20-50
50-250
250-500
500-1000
1000-2000
2000-10000
Size fraction represented (µm)

fraction. The residues of soil were washed and centrifuged three times with acetone and air-dried.

7.2.5 Organic carbon

Organic carbon content (Young and Lindbeck 1964) was determined on the soil before and after the light fraction had been removed. The organic carbon in the light fraction (% total soil) was obtained by difference. The organic carbon content of each soil fraction was determined after the light fraction was removed.

7.2.6 Scanning electron microscopy

A fraction of soil of particle-size 50-250 µm diameter was collected (see section 7.2.2) from a sample of WF, and freeze-dried. A sample was coated with carbon and platinum-palladium and examined with a Joel JEM-100 CX electron microscope with a scanning electron image device (ASID-4D).

7.3 Results and Discussion

7.3.1 Stability

The slopes of the summation curve for each soil were determined and plotted against log mean particle-diameter (corrected to one decimal place) to give a distribution-diagram (Fig.17). The virgin soil yielded the highest % water-stable particles >1000 µm diameter, and the lowest % water-stable particles <250 µm diameter. Therefore 50 years of agricultural practices on Urrbrae fine sandy loam have broken down water-stable particles >1000 µm into microaggregates most of which are 20-50 µm and 50-250 µm diameter. In the non-cultivated old pasture the breakdown has been the least severe. The results show also that particles 50-250 µm were very stable in all of these soils, and that agricultural practices have released little material <10 µm diameter (Table 9). An SE micrograph (Fig.18) shows part of a typical water-stable particle 50-250 µm diameter consisting of particles 10-20 µm and <10 µm diameter. These results

Table 9. The effect of crop rotation on water-stable particles <10 μm (% total soil).

Soil	Water-stable particles <10 μm (% total soil)
Virgin soil	1.7
PP	2.5
PPPWWP	3.7
WPPPPW	4.6
PPW	4.4
WW	5.2
WPF	4.4
PFW	6.2
WF	4.6
FW	4.6

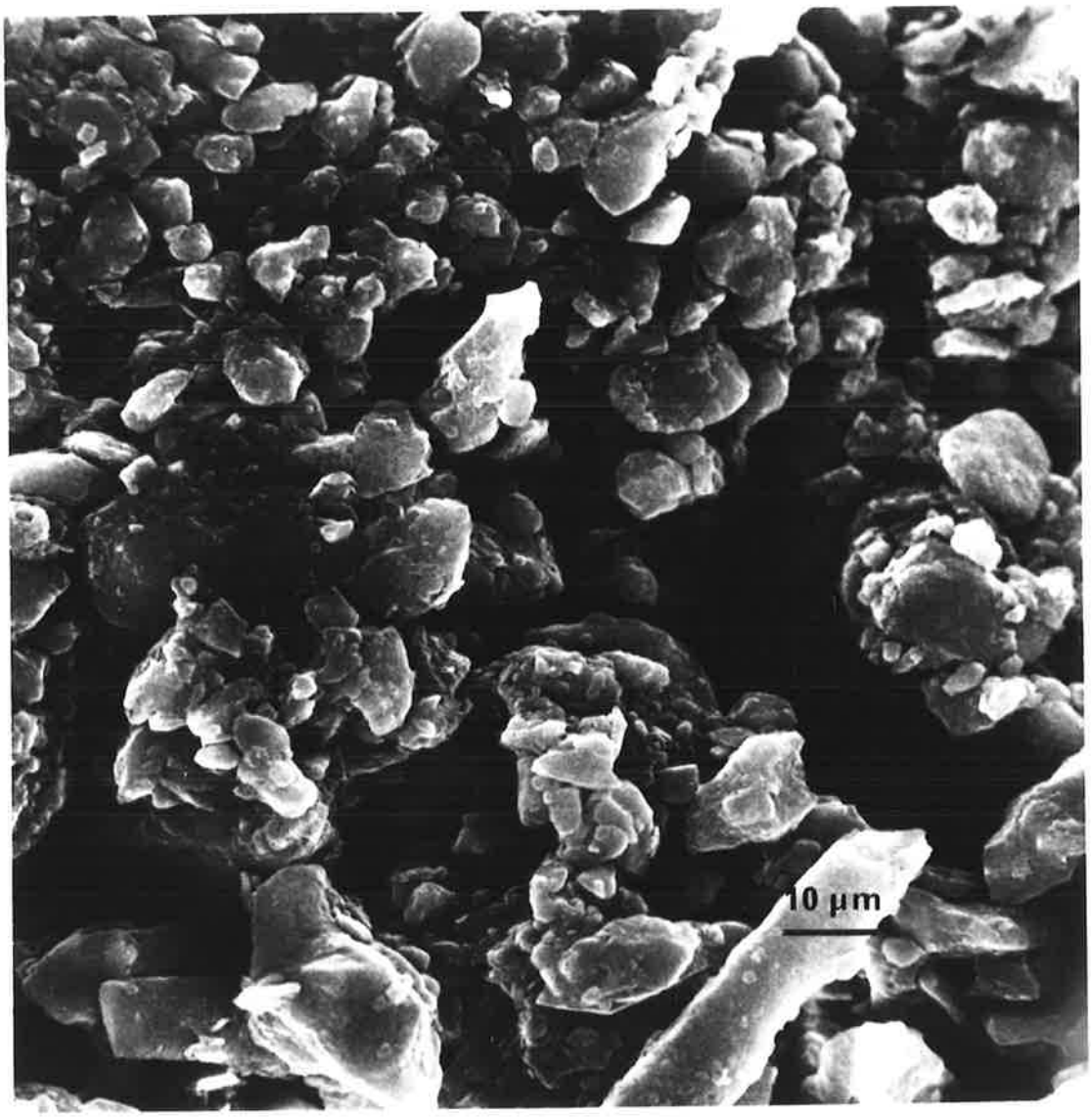
suggest that for the stability of microaggregates management is less important than the characteristics of the soil. Edwards and Bremner (1967) suggested that microaggregates were the only very stable particles in the soil (see section 1.3.2); in their model, macroaggregates (>250 μm diameter) were disrupted easily when an air-dried soil was wetted rapidly.

Before the soil in the rotation trial was sown to permanent pasture, it had been used as arable land. So the stability of aggregates after 30 years of pasture has not reached that of the virgin soil. Low (1965) also found that aggregation increased slowly under pasture (see section 1.4.1).

7.3.2 Influence of management on the % organic matter in the whole soils

As has been found elsewhere (Clement 1961; Grierson *et al.* 1972) the decrease in the amounts of water-stable macroaggregates was associated with a decrease in % total organic carbon in the soil. The rotations can be

Fig. 18 Scanning electron micrograph of part of a water-stable particle 50-250 μm diameter from the FW rotation of Urrbrae fine sandy loam.


10 µm

separated into three groups according to the stability of macroaggregates and % total organic carbon. The non-cultivated plots contained the greatest amount (>55%) of stable macroaggregates, and had the highest % total organic carbon (>2.3%). Those plots with fallow in the rotation contained the smallest amount (26%) of stable macroaggregates and contained the lowest % total organic carbon (<1.6%). The remaining cultivated plots fell between these two extremes with respect to stability and % organic carbon (Fig. 17 and Table 8).

In the non-cultivated soils plant materials are added continually to the soil, leading to high % total organic matter. Growing plants also appear to protect organic carbon in soil from some decomposition (Jenkinson 1977). In the rotations including crops less plant material is added annually (see section 1.4.2) which is reflected in a lower % total organic matter. Minimal amounts of plant residues are added during fallow and, because the soil is cultivated frequently, more organic materials are oxidized than in non-cultivated soil (Rovira and Greacen 1957; Adu and Oades 1978).

It may be that the extra stability not contributed by total carbon in the non-cultivated soils (i.e. virgin soil and old pasture) is due to the distribution and perhaps to the type of organic material in the soil (see section 1.4).

The % organic carbon is related also to the degree to which soil is bound into macroaggregates in the field. As the organic carbon in the whole soil increases, more of the fine particles are bound up into large aggregates.

7.3.3 Correlations between % organic carbon and aggregation

Correlations between % organic carbon and stability of particles were best with the macroaggregates, and only correlations between water-stable aggregates >2000 μm diameter are discussed here since they were generally

about the same as those between % organic carbon and water-stable aggregates >250 μm. Stability in the agricultural soils (i.e. all soils except the virgin soil) was related closely to the % total carbon (r^2=0.93) (Fig.19). The % water-stable particles >2000 μm diameter was also related to the % total nitrogen in the whole soil (r^2=0.93). Hence the stability was related to the % total organic matter. The virgin soil had a higher % water-stable particles >2000 μm diameter than expected from its organic carbon content (Fig.19). It is possible that one cultivation of the virgin soil would bring its stability into line in Fig.19 (Low 1972) by destroying the mulch of dead grass and leaves and exposing the soil to the weather.

The correlation between % total organic carbon and stability of particles <10 μm diameter was poor; with agricultural soils r^2 = 0.53, and with all soils r^2 = 0.68, thus showing further the limited effect of agricultural practices on dispersion (disruption of microaggregates) as opposed to slaking (disruption of macroaggregates).

7.3.4 Organic matter - fractions of soil

Water-stable particles >2000 μm, 1000-2000 μm, 500-1000 μm and 250-500 μm diameter in the six selected soils were stabilized mainly by organic matter since they were broken down by hydrogen peroxide into water-stable particles mainly <50 μm diameter (Fig.20). Microaggregates in all the agricultural soils were also stabilized by organic matter.

The results described in Chapters 5 and 6 suggest that the organic binding agents which stabilize macroaggregates are not fresh materials but decomposing roots and hyphae, associated intimately with inorganic material and not removed in the light fraction (SG <1.8 g cm^{-3}). The fine network of roots and hyphae does not persist in soils; cultivation speeds up the decomposition of the network so that the macroaggregates become unstable. Plants need to be grown to replenish the fine network of roots and hyphae.

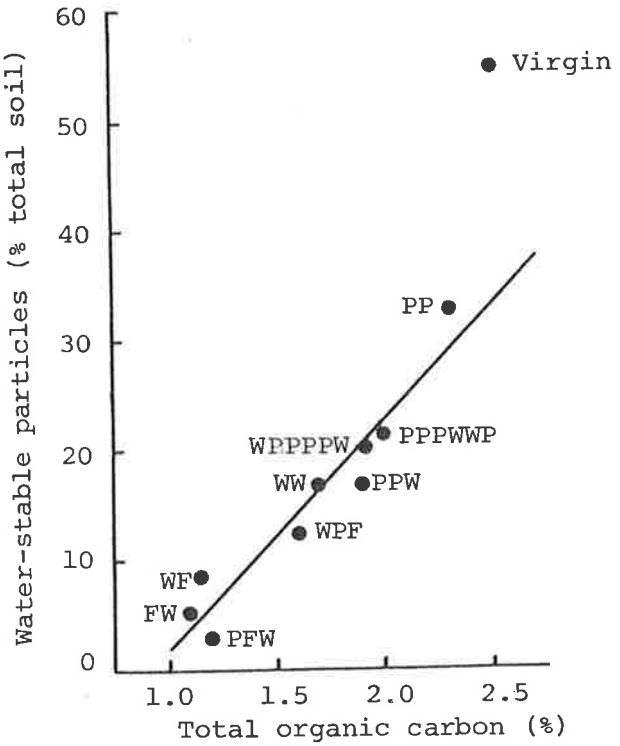


Fig. 19 The relationship between % total organic carbon and water-stable particles >2000 μm diameter.

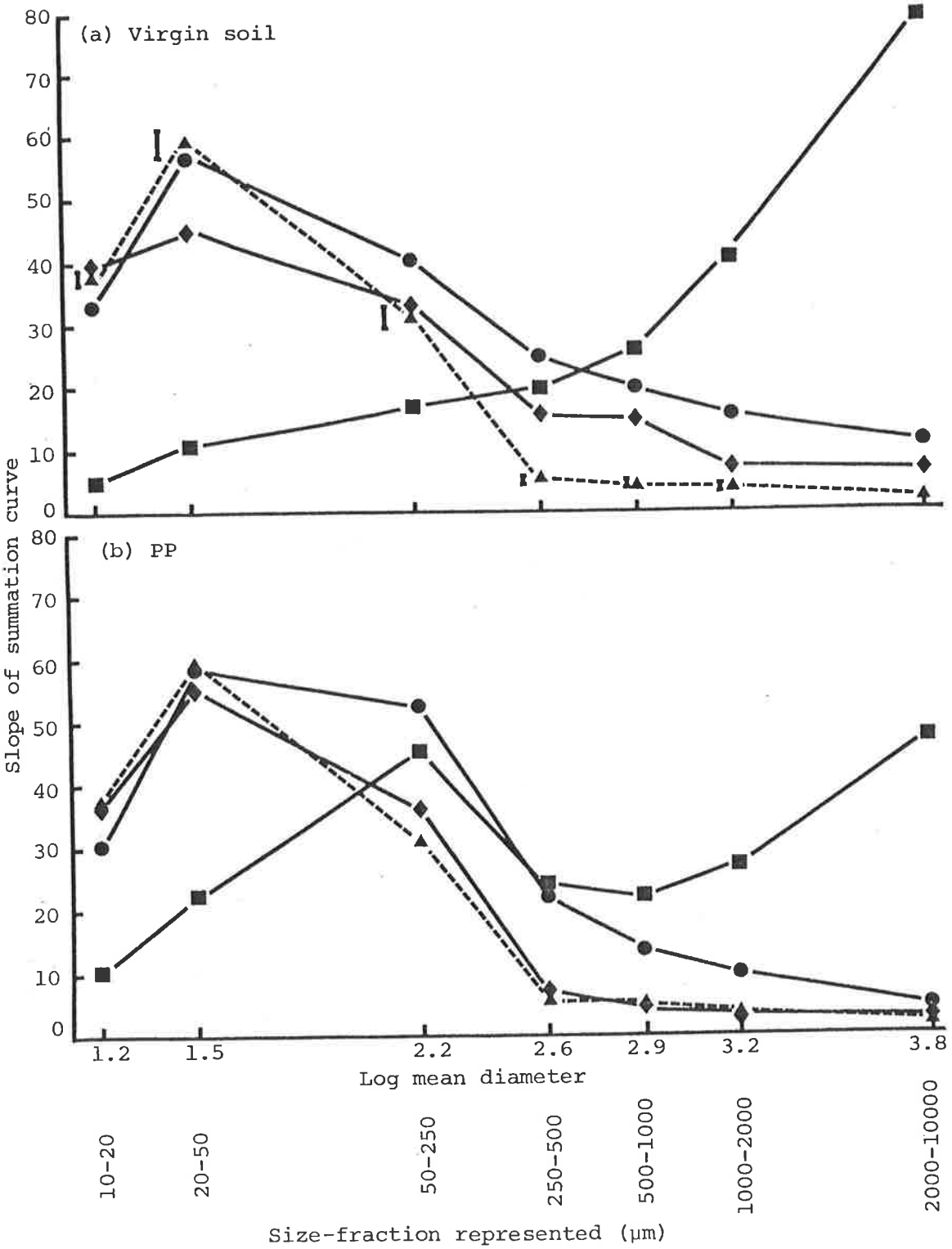
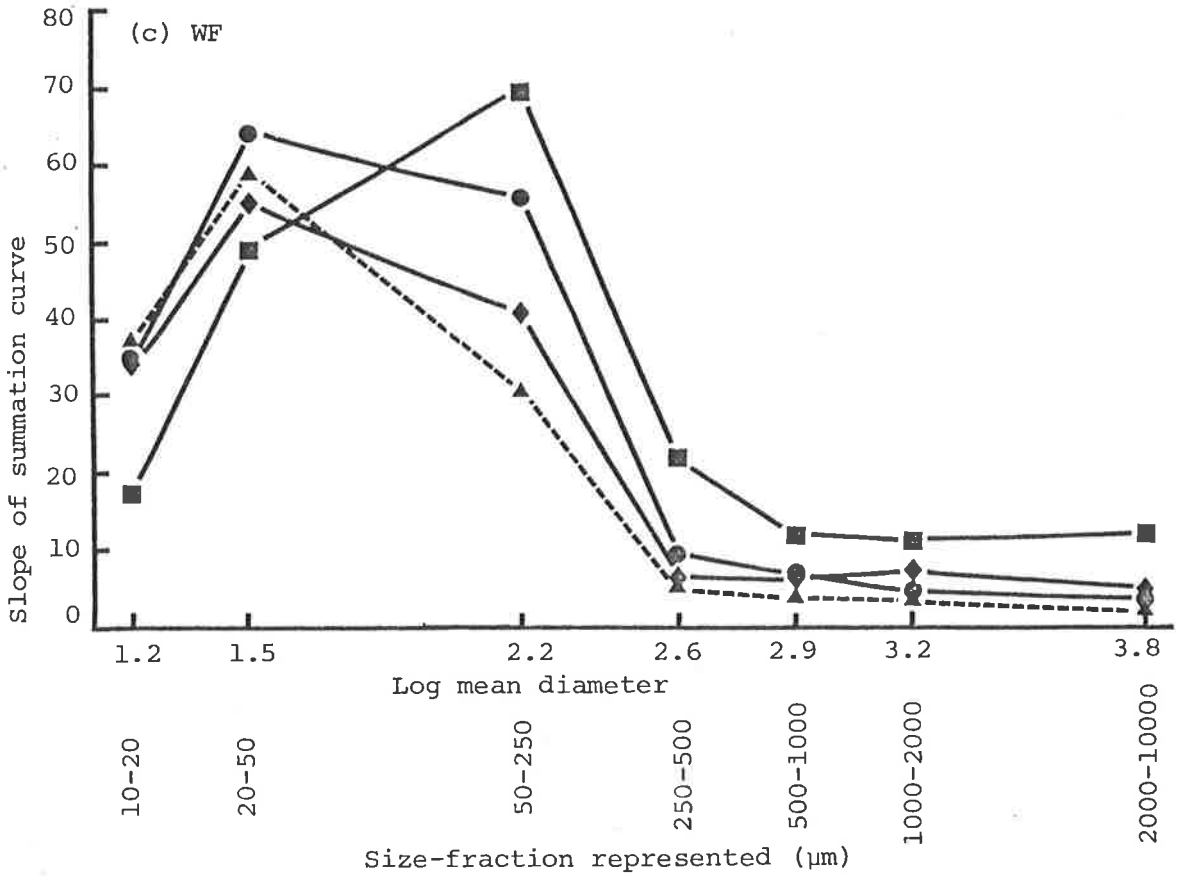


Fig.20 The size-distribution of water-stable particles after physical treatment compared with treatment with hydrogen peroxide. ■—■ Untreated; ●—● End-over-end shaking; ◆-◆ Probe; ▲--▲ H_2O_2 (mean of all treatments). Vertical bar, 2 x SE mean.


(c) WF
Slope of summation curve
80
70
60
50
40
30
20
10
0
1.2
1.5
2.2
2.6
2.9
3.2
3.8
Log mean diameter
10-20
20-50
50-250
250-500
500-1000
1000-2000
2000-10000
Size-fraction represented (µm)

In the microaggregates, the organic binding agents cannot be defined precisely but they must be efficient since these particles contain only one-third to one-half as much organic carbon (light fraction removed) as the macroaggregates (Fig.21) which are destroyed by agricultural practices. Presumably there are numerous bonding mechanisms responsible for stabilizing microaggregates, the effects of which are additive and lead to strong binding.

7.3.5 Resistance of particles to physical disruption

Generally, macroaggregates from the virgin soil and old pasture were more stable than those from other soils because they resisted vigorous physical disruption (see Fig.20 for selected results). Within experimental error, the resistance to physical disruption of all cultivated soils was similar to WF. Greacen (1958) also found in the same rotation trial over 20 years earlier that aggregates from virgin soil resisted physical disruption more than aggregates from short-term pasture (3-year-old) or cultivated soil.

The bonds which stabilize particles 20-250 μm diameter must be strong since in the field these particles are stable to cultivation; even 50% of the sample from the FW plot consisted of water-stable particles 50-250 μm diameter, and particles 20-50 μm diameter from FW have not been broken down to particles <20 μm diameter.

7.3.6 Organic binding agents

Results of the previous section show that agricultural practices decreased the stability of macroaggregates by affecting both the amount and nature of the organic binding agents present. Hence the effects of various organic materials were studied.

Roots and hyphae

In all soils where cultivation was included in the rotation the %

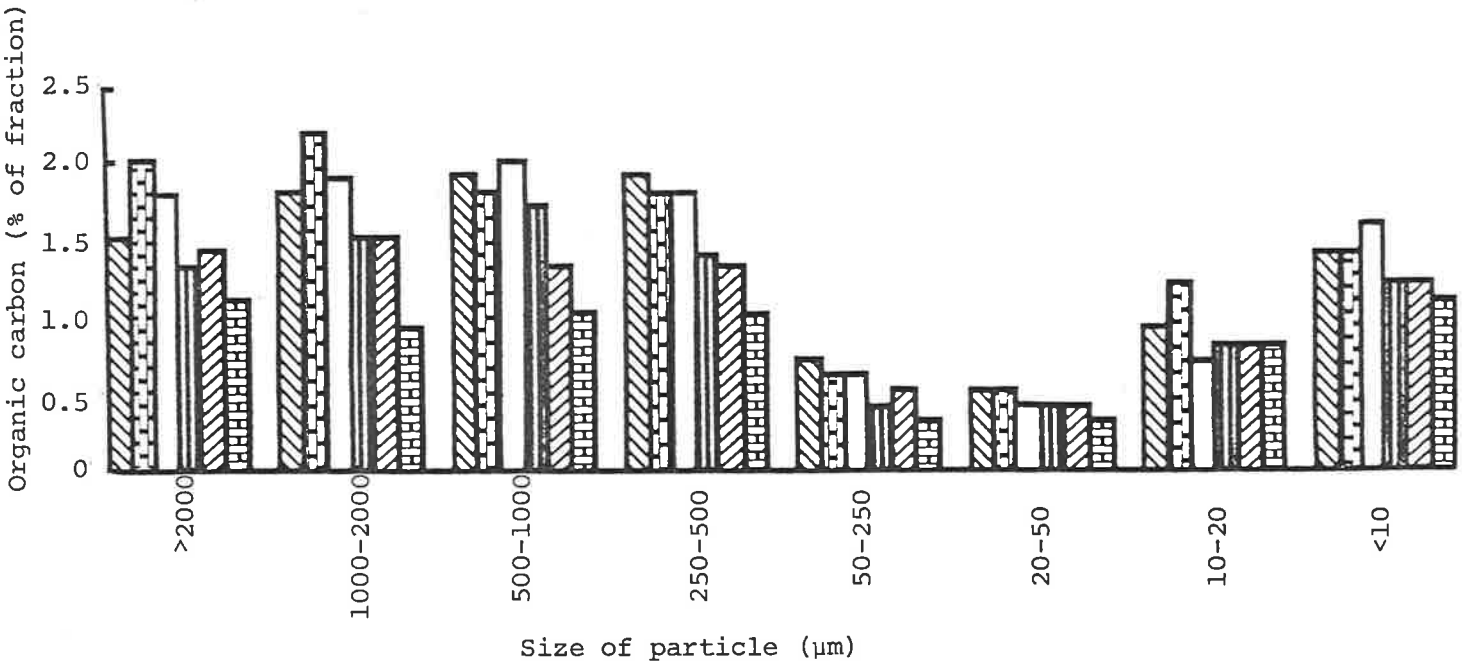


Fig.21 Organic carbon in water-stable particles (light fraction removed) as % of each fraction. Virgin soil; PP; PPPWWP ; WW; WPF; FW.

water-stable particles >2000 μm diameter was related directly to the total length of roots (Fig.22) in the whole soil (r^2= 0.81) and to the total length of hyphae in the whole soil (r^2= 0.77). (Similar correlations with particles >250 μm diameter were poorer than with particles >2000 μm diameter.) The length of roots and of hyphae in the cultivated soils are each related to the % total organic carbon (r^2= 0.95, r^2= 0.76 respectively). The virgin soil contained the greatest hyphal length (19 m g^{-1} soil), soils in rotations without fallow contained an average hyphal length of 13 m g^{-1} and soils with fallow contained an average of 5 m g^{-1} soil. These results imply that roots and hyphae are important for the stability of macro-aggregates in the field in the long-term; their importance in pot experiments in the short-term was described in Chapters 4, 5 and 6. In samples from both the virgin soil and old pasture, root length and hyphal length are above the regression line (Fig.22). It is inferred that in the two non-cultivated soils some factors other than living roots and hyphae are binding particles into stable macroaggregates.

Soils with fallow in the rotation contained few hyphae. Bond and Harris (1964) also found few water-stable aggregates and few hyphae in soil from the fallow plots collected 19 years previously. Bond and Harris (1964) found that up to 6 years of pasture were necessary for sufficient growth of hyphae in soil to produce any water-stable aggregates. The present results suggest that (in the absence of a fallow in the rotation) sufficient hyphae can grow beneath a wheat crop to bind particles into water-stable aggregates.

Organic compounds

The organic compounds which stabilized particles >250 μm diameter from all soils, and those 50-250 μm diameter from all soils except virgin soil, were largely resistant to pre-treatment with periodate (see Fig.23 for selected results). Therefore polysaccharides are not important in the

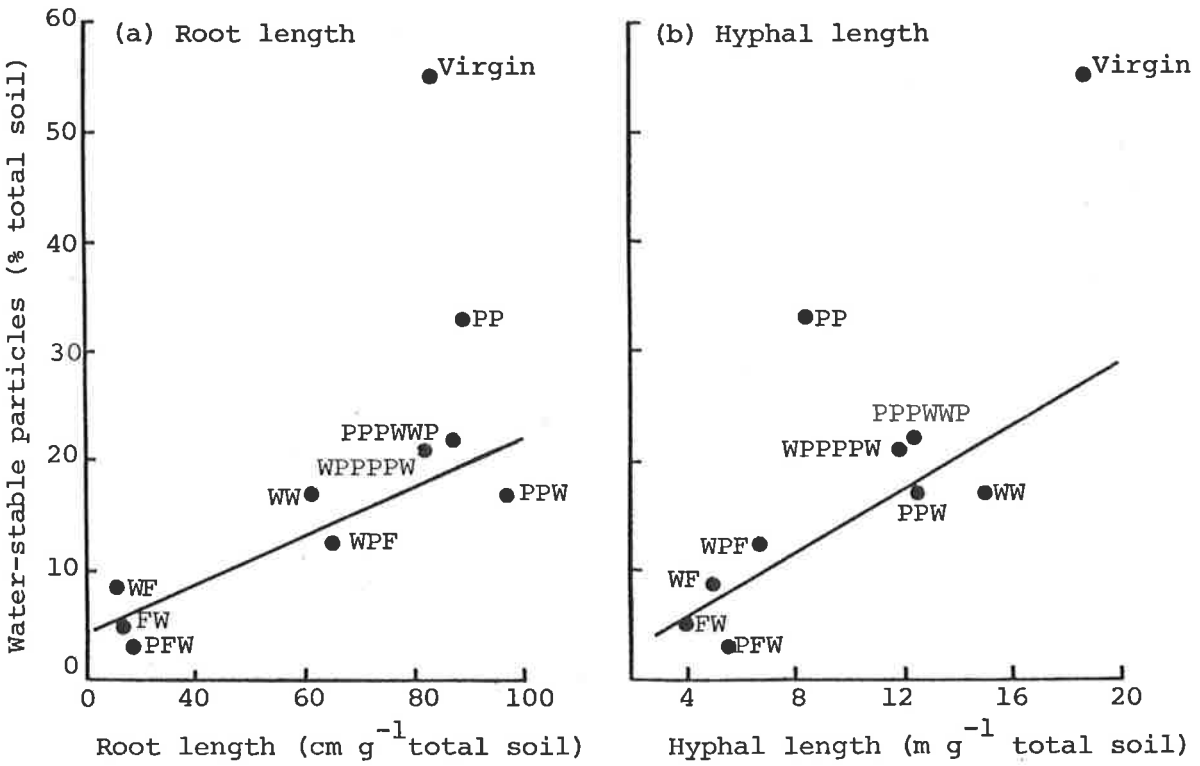


Fig.22 The relationship between root length or hyphal length in the total soil and % water-stable particles >2000 μm diameter.

(a) $y = 0.22x + 0.13$ $r^2 = 0.93$ (cultivated soils only);

(b) $y = 1.45x - 0.02$ $r^2 = 0.77$ (cultivated soils only).

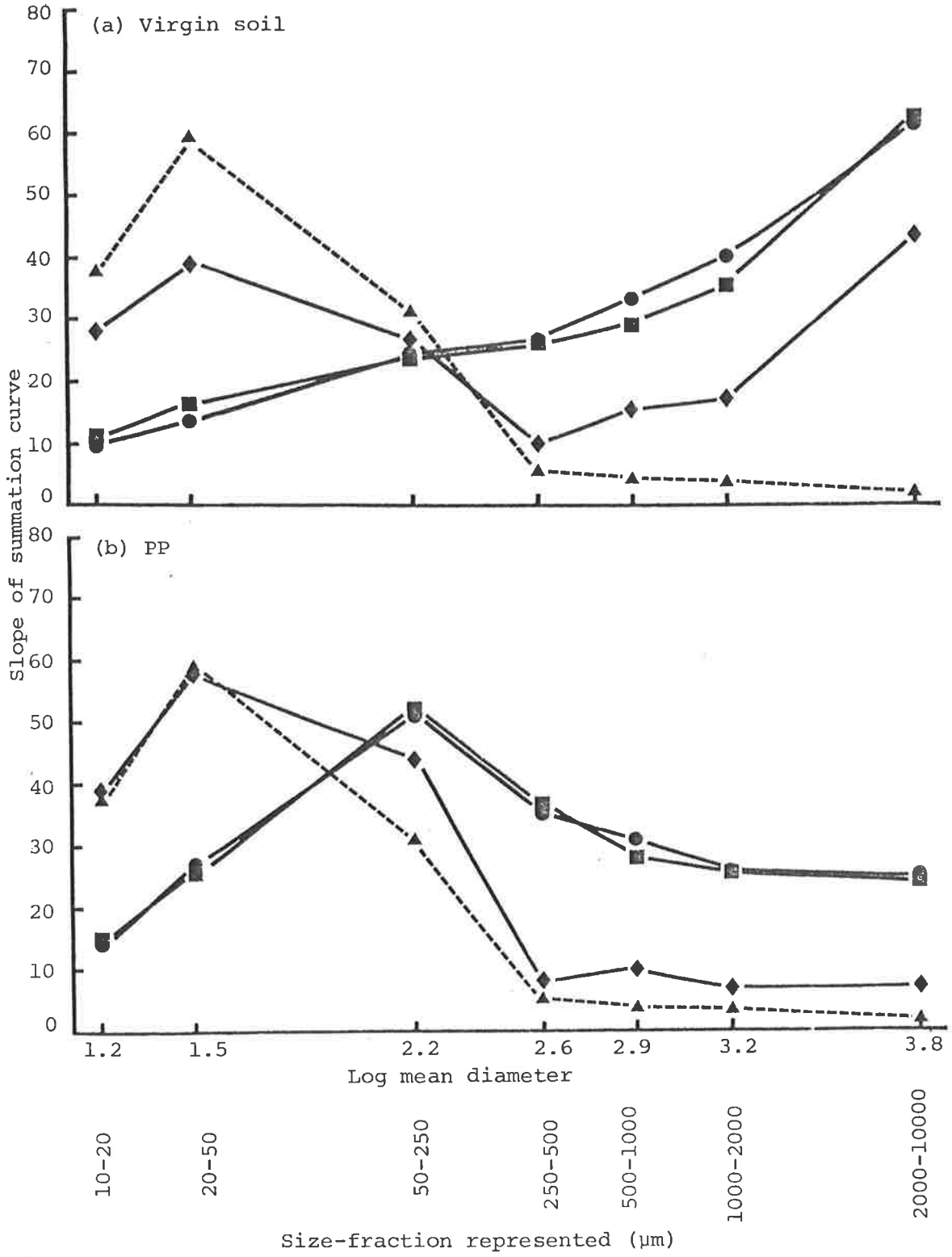
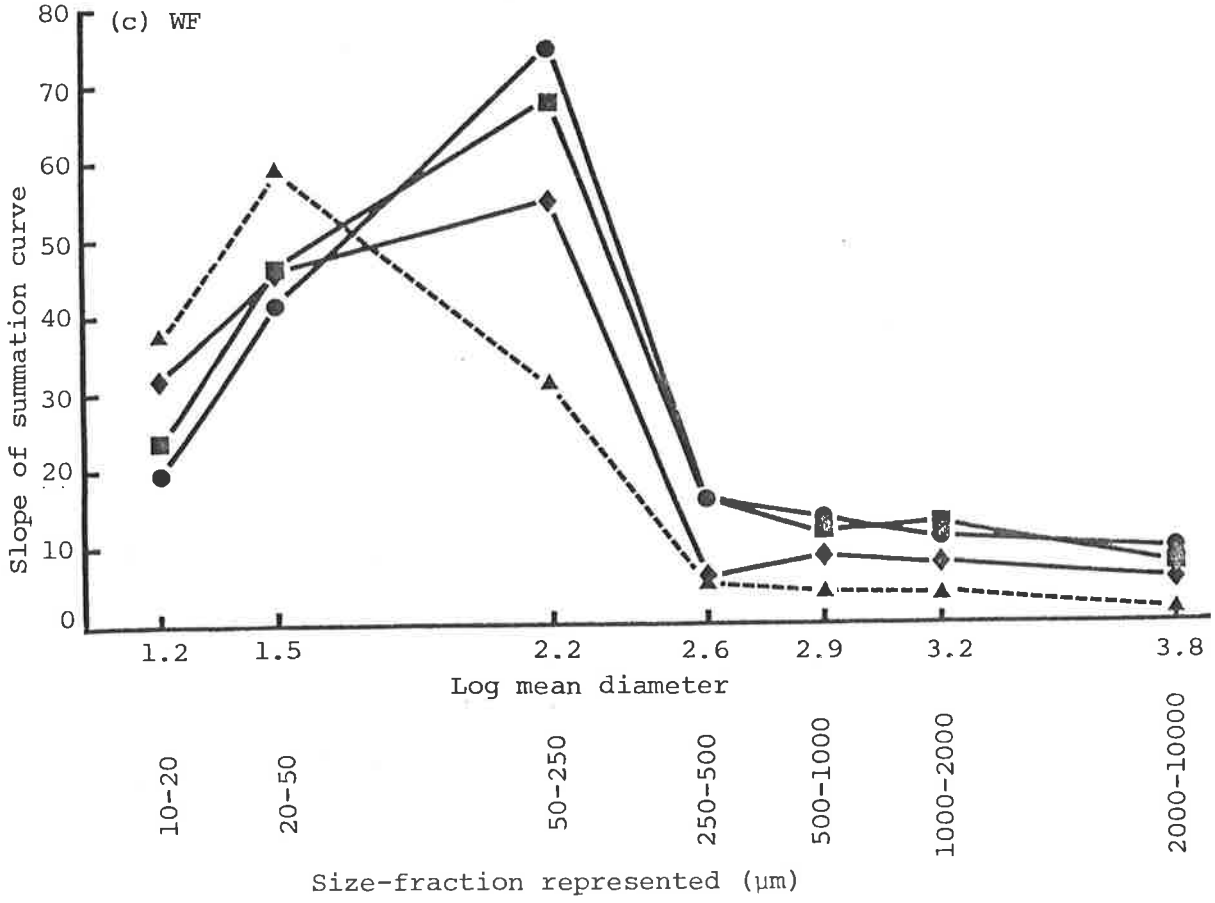


Fig.23 The size-distribution of water-stable particles from Urrbrae fine sandy loam after chemical treatment. ●—● NaCl; ■—■ $NaIO_4$; ◆—◆ $Na_4P_2O_7$; ▲--▲ H_2O_2 (mean of all treatments).


(c) WF
Slope of summation curve
80
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3.8
Log mean diameter
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50-250
250-500
500-1000
1000-2000
2000-10000
Size-fraction represented (µm)

stabilization of particles >50 µm in these soils (see section 1.3.2).

All plots contained particles >2000 µm diameter which were resistant to pyrophosphate; i.e. the particles probably contained fungal hyphae (D. J. Carter and J. M. Oades unpublished results); however a larger proportion of particles >2000 µm diameter were resistant to pyrophosphate in the virgin soil than in the other soils. It is important to compare the effect of pyrophosphate on macroaggregates (>250 µm diameter) from the virgin soil with the effect on particles 50-250 µm diameter from WF. Although macroaggregates had disintegrated due to management in WF, the particles 50-250 µm diameter which had been released contained about the same proportion of pyrophosphate-resistant materials as the virgin macroaggregates. Pyrophosphate-sensitive materials, which are possibly bound to soil by polyvalent cations (Stefanson 1971), stabilized particles >20 µm diameter in WF and particles >50 µm diameter in the other soils.

7.4 Conclusions

Fifty years of crop rotation decreased the macroaggregation (>250 µm diameter) but not microaggregation, and simultaneously decreased the lengths of roots and of hyphae in the soil. However macroaggregation was correlated with % total organic matter, and with lengths of roots and of hyphae.

Particles 50-250 µm diameter were stabilized by organic matter but were not affected by management. The organic binding agents in particles 50-250 µm diameter cannot be defined, although they included organic matter sensitive to pyrophosphate but not to periodate.

The practical implications of these results are that structural stability can be controlled by management including crop rotation. Rotations which include fallow and multiple cultivations decrease stability. Stability can be improved by growing plants with extensive root systems (or by adding large amounts of organic matter in some other way) with minimum cultivation.

CHAPTER 8

ORGANIC AND INORGANIC BINDING AGENTS

8.1 Hydrous Oxides

The main forms of hydrous oxides and disordered inorganic material in soils are those of iron, aluminium, manganese and silicon, either alone or mixed intimately, and are either residual from the parent material or products of weathering of primary minerals. The forms of hydrous oxides and disordered inorganic material present in a soil depend on the parent material,intensity of weathering and leaching,fluctuating water-tables, pH of the soil solution, and organic matter and biological activity in the soil (Mitchell *et al*. 1964; Greenland and Oades 1968; Habibullah and Greenland 1971). The hydrous oxides are precipitated from the soil solution (Greenland and Oades 1968). They may appear adsorbed on the surface of clay crystals as discrete particles as a highly irregular crust, or as a film (Greenland and Wilkinson 1969; Russell 1973; Rengasamy and Oades 1977; 1979). Initially the oxides may be disordered and amorphous to X-rays and electrons; with ageing under certain conditions they may crystallize as discrete particles (Greenland *et al*.1968); Schwertmann and Taylor 1977). Well-drained surface soils in temperate climates tend to contain mainly amorphous material (Mitchell *et al*. 1964) possibly partly because strongly adsorbed organic anions inhibit crystallization (Schwertmann 1966; Greenland and Oades 1968; Schwertmann *et al*. 1968). Some complexes of iron and aluminium with organic matter move in solution in soil (Mitchell *et al*. 1964); others appear to interact with clay minerals (McKeague *et al*. 1971; Stefanson 1971; Giovannini and Sequi 1976).

Chemical reagents are used to dissolve selectively, and to quantify, the various forms of hydrous oxides and disordered inorganic materials present. The methods vary in their efficiency and no method is specific. The techniques can usually be grouped under one or more of the following headings:

a) Disordered oxides and products of recent weathering, mainly iron oxides, are extracted in acid ammonium or sodium oxalates (Russell 1973).

b) Crystalline iron oxides are dissolved by reduction of ferric to ferrous iron, e.g. by sodium dithionite, and removed either in acid solution or with a chelating agent.

c) Disordered alumino-silicates and free silica and alumina are dissolved with alkaline reagents.

d) Organic forms of iron (less so of aluminium) together with various amounts of organic matter are extracted with acetylacetone in water (Martin and Reeve 1957) or with potassium (or sodium) pyrophosphate (pH 10) (McKeague *et al*. 1971). The pyrophosphate probably attacks clay minerals also.

e) Organic forms of iron and aluminium (without extraction of the organic matter) are probably extracted with acetylacetone in benzene (Giovannini and Sequi 1976).

Hydrous oxides of iron and aluminium associated with clay surfaces in strongly weathered and acidic soils are responsible for the well aggregated structure of such soils. The mechanism by which hydrous oxides stabilize aggregates of soil are not understood fully. McIntyre (1956) suggested that colloidal iron oxides were precipitated and dried irreversibly, and that complexes of iron-organic matter in solution inhibited deflocculation. Gillman (1974) suggested that intense weathering produced positive charges on clay surfaces leading to a low net charge and therefore little water-dispersible clay. When discussing mechanisms by which hydrous oxides aggregates particles of clay from suspension, Rengasamy and Oades (1977) differentiated between the terms coagulation, flocculation and cementation. According to their definition, coagulation, which is reversible, destabilizes a colloidal suspension by reducing the repulsive potential of the system. Flocculation, which is irreversible, destabilizes a colloidal suspension by forming chemical bridges between negatively charged clay particles, so that a three-dimensional network of flocs is formed.

Rengasamy and Oades (1977) found that the most efficient flocculants for clays were low molecular weight polycations which had a high positive charge. Cementation is an extension of flocculation where additional metal hydroxide is precipitated on the surface of the particles of clay so that the particles become enveloped in a matrix of metal hydroxide; the stability of laterites is due to cementation.

There has been much discussion in the literature as to the relative importance of iron and aluminium in aggregation of soils (Prebble and Stirk 1959; Deshpande *et al*. 1964; Tweneboah *et al*. 1967). However it is now clear that aluminium is substituted isomorphously in lattices of iron oxides and hydroxy species, and that copolycations of iron and aluminium can be used to flocculate clays close to the pH of natural soils (Rengasamy and Oades 1979).

Organic forms of iron and aluminium probably influence the stability of aggregates in soil since when such iron and aluminium are removed from soil, either alone or along with the organic matter, the stability decreases (Giovannini and Sequi 1976). These results add weight to the proposal of Edwards and Bremner (1967) that polyvalent metals bind organic matter to clays to form stable microaggregates which themselves are bound by polyvalent metal hydroxides into aggregates.

Disordered silica, alumina and alumino-silicates which have been shown to adhere strongly to the surfaces of clay particles of several red-brown earths (but not red earths rich in iron oxides) (Greenland and Wilkinson 1969) influence the surface chemistry and charge characteristics of the clay; this disordered material may also act as a cementing agent and change physical properties of the clay such as microaggregation, crushing strength, porosity and water-holding capacity (Yeoh 1979).

8.2 Experimental

The soil used (Lemnos loam) was taken from pots in which ryegrass had grown for 10 weeks, and then the soil kept moist for a further 20 weeks (see Chapter 6).

Samples were each treated with a sequence of reagents to dissolve or influence organic matter and various forms of hydrous oxides. The reagents were:

Reagent	Main material removed	Reference
0.02 M sodium chloride	-	Stefanson (1971)
0.02 M sodium periodate	polysaccharides	"
0.1 M sodium pyrophosphate	complexes of polyvalent cations and organic matter	"
30% hydrogen peroxide	organic matter	Loveday (1974)
0.2 M ammonium oxalate pH 3.0	disordered iron oxides	McKeague and Day (1966)
1.0 M sodium acetate-sodium dithionite pH 5.0	crystalline iron oxides	Bromfield (1965)
5% sodium carbonate	highly disordered silica, alumina and alumino-silicates	Follet *et al.* (1965)

Treatments, which were replicated four times, were:

Sodium chloride, sodium periodate or sodium pyrophosphate. (see section 2.2).

Hydrogen peroxide. Air-dried soil (<10 mm; 3 g) was moistened with water and then treated with 30% hydrogen peroxide until the suspension no longer effervesced. The suspension was boiled for 10 min to destroy excess hydrogen peroxide, and dialysed against distilled water. Some samples were evaporated to dryness on a rotary vacuum evaporator at 40°C; the size-distribution of water-stable particles was measured on the other samples.

Ammonium oxalate. Samples dried by rotary evaporation were re-suspended in 100 cm^3 ammonium oxalate at pH 3.0, and shaken end-over-end in the dark for 2 h. The suspension was centrifuged, the supernatant removed and the residues dialysed against distilled water before evaporation to dryness.

Sodium acetate-sodium dithionite. Dried samples were re-suspended in 1.0 M sodium acetate buffer, pH 5.0, heated to 60°C and reacted for 30 min at 60°C with 2.0 g of 1:1 mixture of sodium dithionite and sodium meta-bisulphate (Bromfield 1965). The suspension was centrifuged and the supernatant removed. The residues were re-suspended in 50 cm^3 sodium acetate buffer and kept at 60°C for 30 min, centrifuged and the supernatant removed. To ensure that iron oxides were removed completely, the whole process of treatment with dithionite and washing was repeated to give a total of three treatments. The residues were dialysed against distilled water.

Sodium carbonate. Suspensions of samples were diluted to 600 cm^3 so that they contained 5% sodium carbonate and were shaken end-over-end at 20°C for 16 h, centrifuged and the supernatant removed. The residues were then re-suspended in the 600 cm^3 of sodium carbonate at 100°C, and the suspension kept at 100°C for 2 h then centrifuged and the supernatant removed. The treatment in hot 5% sodium carbonate for 2 h and centrifuging were repeated and the supernatant removed.

The treatments were:

T_1 sodium chloride

T_2 sodium periodate

T_3 sodium pyrophosphate

T_4 hydrogen peroxide

T_5 hydrogen peroxide : ammonium oxalate:sodium acetate-sodium dithionite

T_6 hydrogen peroxide:ammonium oxalate:sodium acetate-sodium dithionite: sodium carbonate

T_7 hydrogen peroxide:sodium acetate:sodium dithionite

T_8 hydrogen peroxide:sodium acetate-sodium dithionite:sodium carbonate

T_9 hydrogen peroxide:sodium carbonate.

Measurement of stability

The size-distribution of water-stable particles was measured on the samples pre-treated with sodium chloride, periodate or pyrophosphate, by wet-sieving and sedimentation under gravity (see section 3.3). Two lots of each remaining treated soil were combined (total of 6 g air-dried original soil) and brought to 450 cm^3 with distilled water, except where samples had been treated with sodium carbonate where the volume was reduced to 450 cm^3 by rotary evaporation. The size-distribution of water-stable particles <50 μm diameter was determined by sedimentation under gravity or centrifugation (equivalent spherical diameters <50 μm, <20 μm, <10 μm, <2 μm and <0.2 μm). The remainder of each sample was passed through a nest of sieves to determine the distribution of water-stable particles >250 μm diameter.

Electron microscopy

Suspensions of each treated sample were spotted and dried on electron microscope grids by the method described by Greenland *et al.* (1968) except that the suspensions were not dispersed with an ultrasonic probe. Platinum-carbon replicas of each treated sample were also prepared (Greenland and Wilkinson 1969). The grids and replicas were examined with a Joel JEM-100X electron microscope.

8.3 Results and Discussion

The losses in mass of soil (as % of the original sample) due to the various treatments were as follows:

Hydrogen peroxide	3.4%
Ammonium oxalate	2.6%
Sodium acetate-sodium dithionite	3.4%
Sodium carbonate	4.1%

The slopes of the summation curve for each treatment were plotted against the log mean particle-diameter to give a distribution diagram

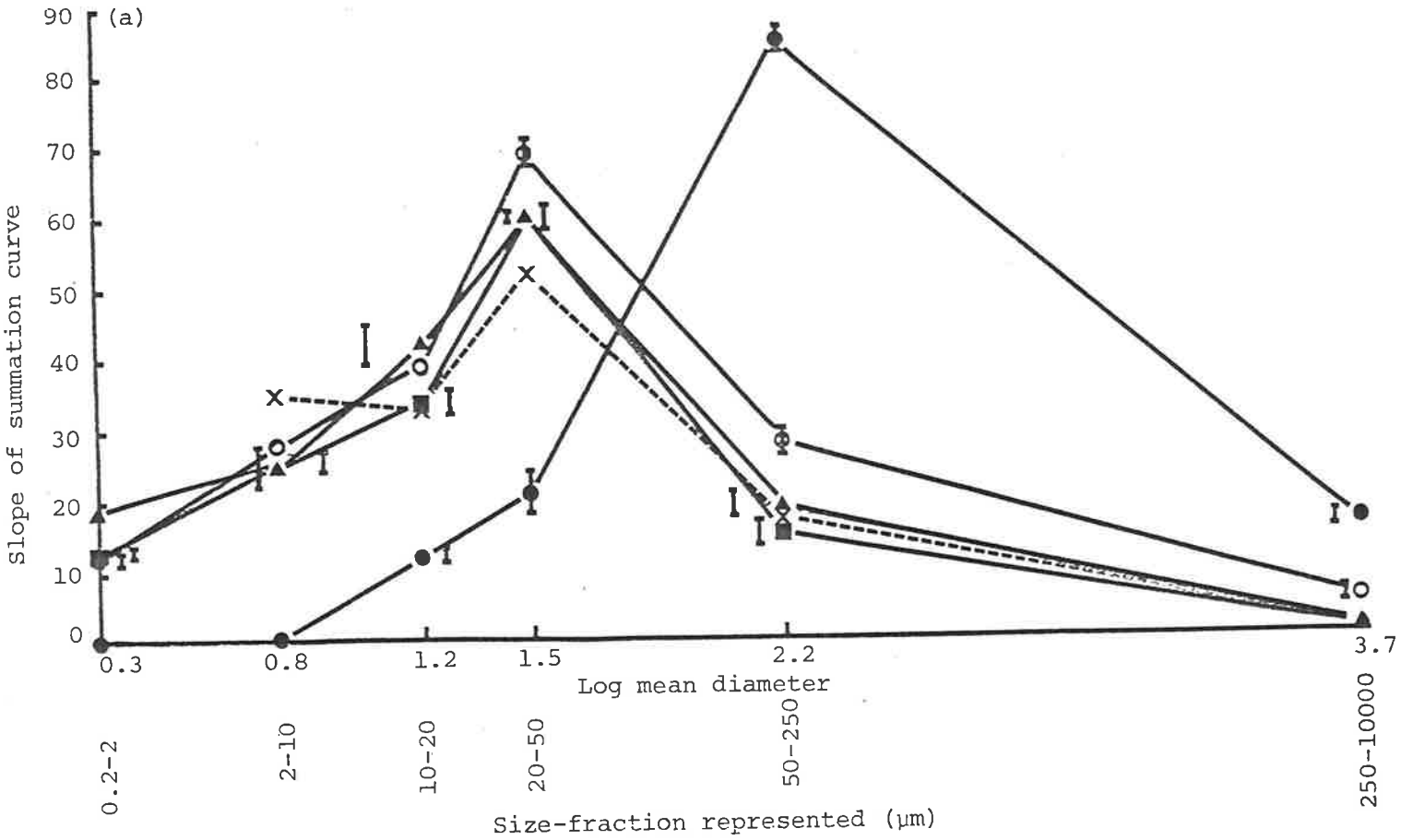
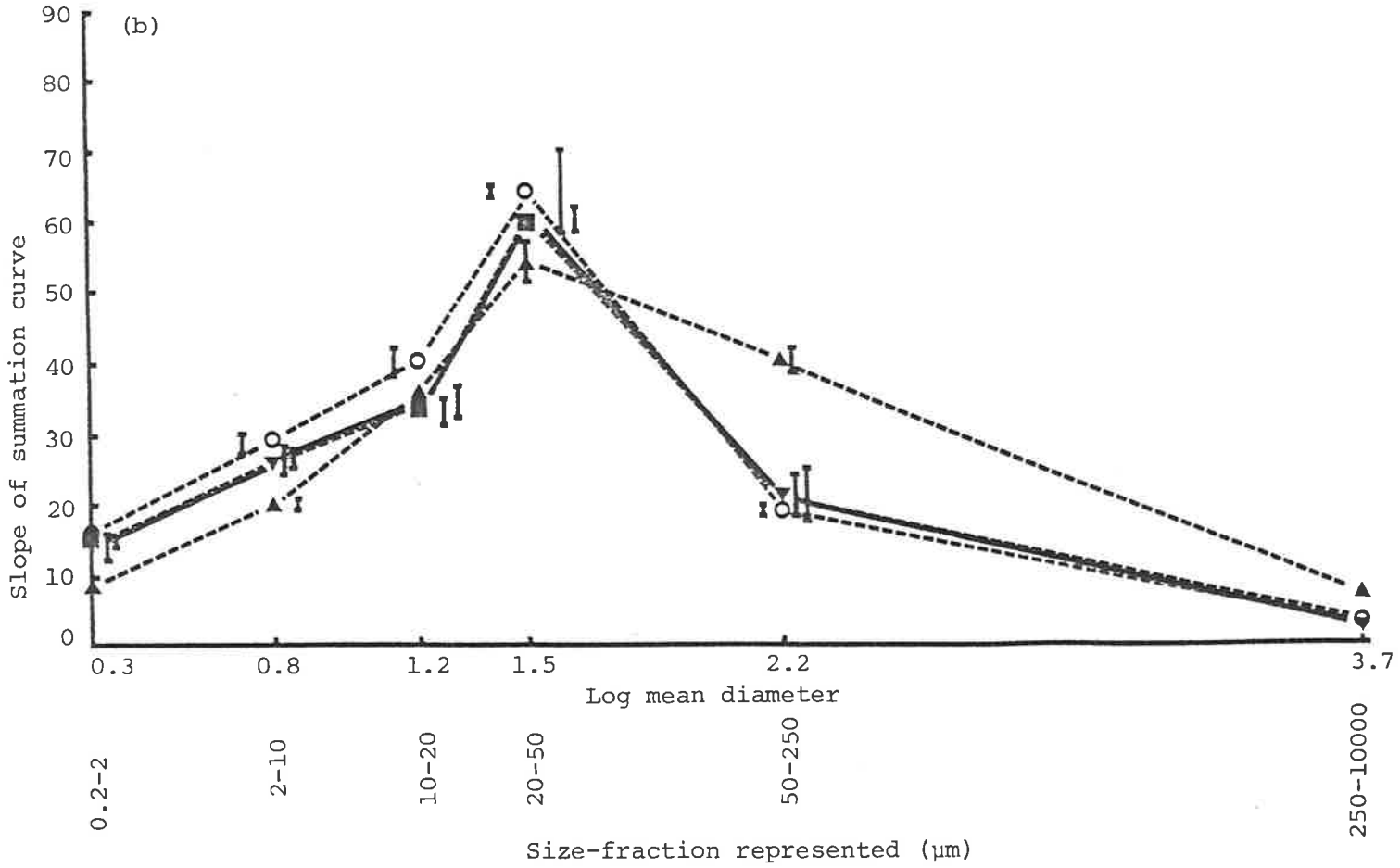


Fig.24 The size-distribution of water-stable particles after chemical treatment.
●—● Untreated; ▲--▲ T_3; o—o T_4; ▲—▲ T_5;
■—■ T_6; o--o T_7; ▼—▼ T_8; ■--■ T_9; x--x Fully dispersed.
Vertical bar, 2 x SE mean.


(b)
Slope of summation curve
90
80
70
60
50
40
30
20
10
0
0.3
0.8
1.2
1.5
2.2
3.7
Log mean diameter
0.2-2
2-10
10-20
20-50
50-250
250-10000
Size-fraction represented (µm)

(Fig.24). With peroxidized samples, results of the distribution of all particle sizes except 50-250 μm were expressed as % of original oven-dried sample; values for 50-250 μm were expressed as % of the total soil recovered after treatment, and as these values were obtained by difference they contain accumulated errors. With non-peroxidized samples, results of all particle sizes were expressed as % of the original oven-dried sample.

Organic matter. Results obtained from aggregates of Lemnos loam before and after the chemical treatments showed that various mechanisms are involved in binding particles into aggregates of different sizes. (Fig.24 and Table 10). Treatment of the soil with hydrogen peroxide (T_4), which removes part of the organic matter, released many particles 0.2 μm - 50 μm diameter (Fig.24) but few particles <0.2 μm diameter (Table 10).

TABLE 10 The effect of chemical pre-treatments on the water-stable particles (<0.2 μm diameter) in Lemnos loam as % total soil.

Treatment	Water-stable particles <0.2 μm (% total soil)
Untreated	0
T_1	0
T_2	0
T_3	4.9±0.1
T_4	0.5±0.1
T_5	3.9±0.2
T_6	6.2±0.4
T_7	1.6±0.2
T_8	4.0±0.5
T_9	5.8±0.3
Thoroughly dispersed soil	12.3

± values are ± SE mean.

TE micrographs (Fig.25) were similar to those of Turchenek and Oades (1978), and confirmed that peroxide released much material < 2 μm from aggregates. Electron micrographs of carbon replicas of soil show that peroxide treatment did not remove the encrustions from the surfaces of particles of clay (Fig.25b; 25d). These results show that organic matter is responsible for much of the binding of particles 0.2 μm - 50 μm diameter (but not <0.2 μm diameter) into water-stable aggregates > 50 μm diameter.

Crystalline oxides. Treatment of peroxidized soil with sodium acetate-sodium dithionite to remove crystalline hydrous oxides (T_7) changed the colour of the soil from red-brown to grey and released particles <2 μm diameter, including particles <0.2 μm diameter (Table 10), from particles 50-250 μm diameter, and just significantly from particles >250 μm diameter (Fig.24). As there was an increase in clay present, at least some of the removal of iron led to the removal of cement between clay particles. Electron micrographs showed that the treatment also removed discrete particles of iron oxide but had no noticeable effect on the surface of the clay particles (Fig. 26a; 26b). These results show that crystalline hydrous oxides are responsible for some of the stability of particles 50-250 μm diameter.

Disordered oxides. In samples from T_5 from which disordered hydrous oxides had been removed, particles <2 μm diameter (most of which were <0.2 μm diameter) were released (Fig. 26c; Table 10). Electron micrographs of carbon replicas of clay particles appeared to be similar whether the samples had been treated with sodium acetate-dithionite with (T_5) or without (T_7) ammonium oxalate (Fig. 26d; 26b). (TE micrographs of such samples treated with ammonium oxalate showed material which could not be explained, although it possibly consisted of crystals of oxalate (Fig.26c)). These results show that disordered hydrous oxides are responsible for some of the binding of particles <2 μm diameter (including particles <0.2 μm diameter) into water-stable aggregates.

Fig. 25 Transmission micrographs of clay-sized particles of Lemnos loam
a) untreated,
b) carbon replica of a),
c) peroxidized,
d) carbon replica of c).

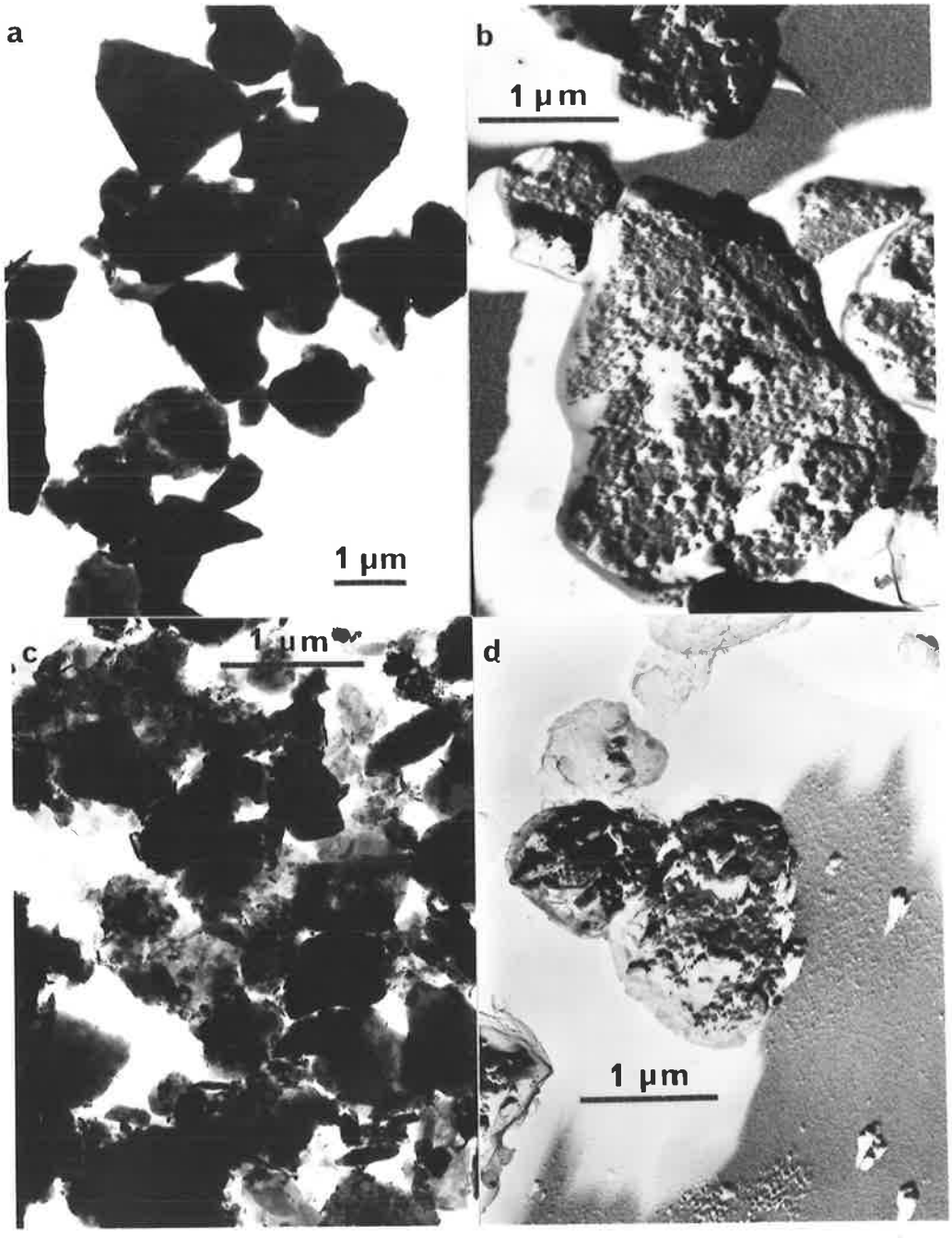

a
1 µm
b
1 µm
c
1 µm
d
1 µm

Fig. 26 Transmission electron micrographs of peroxidized clay-sized particles of Lemnos loam
a) treated with sodium acetate-sodium dithionite,
b) carbon replica of a),
c) treated with ammonium oxalate and sodium acetate-sodium dithionite,
d) carbon replica of c).

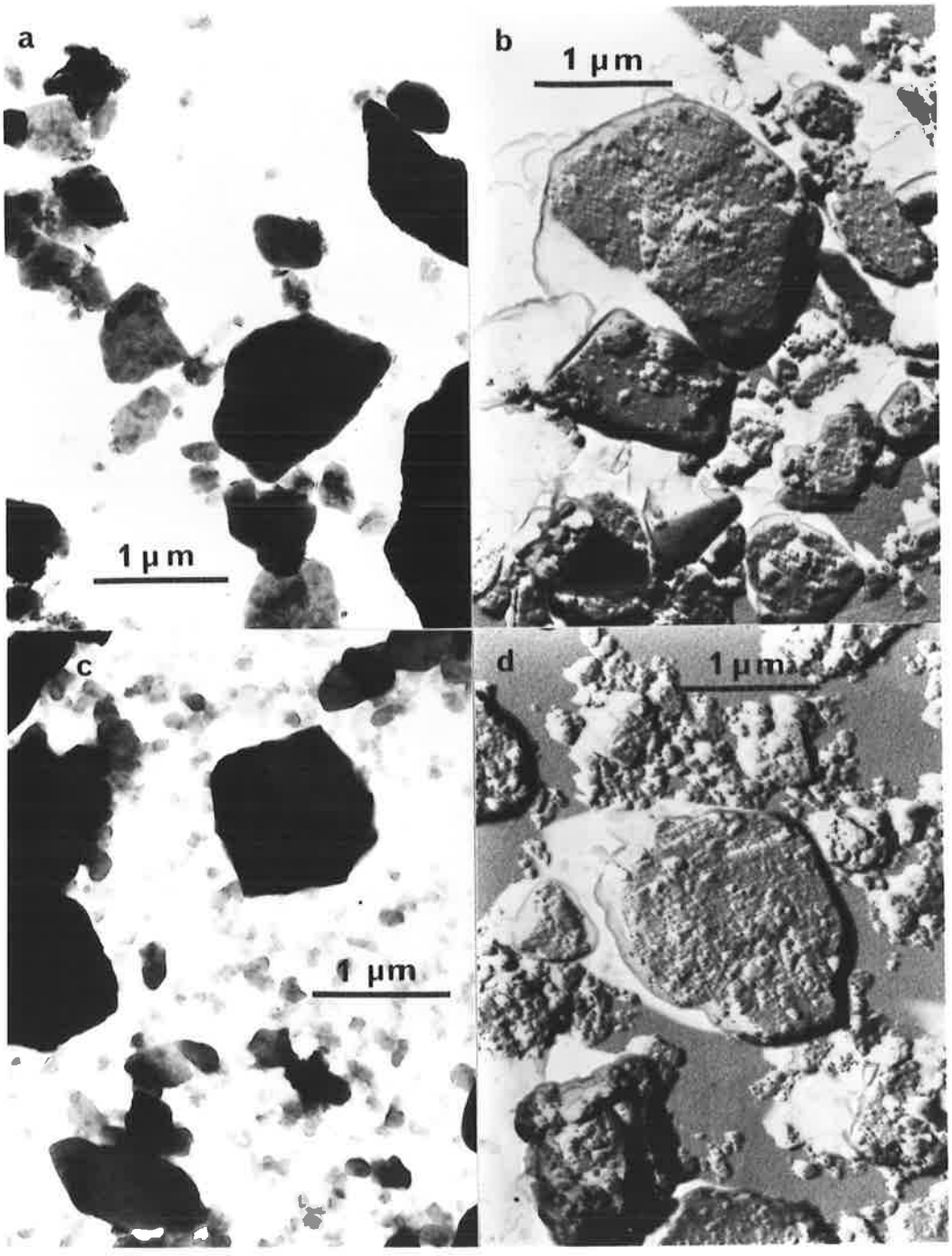
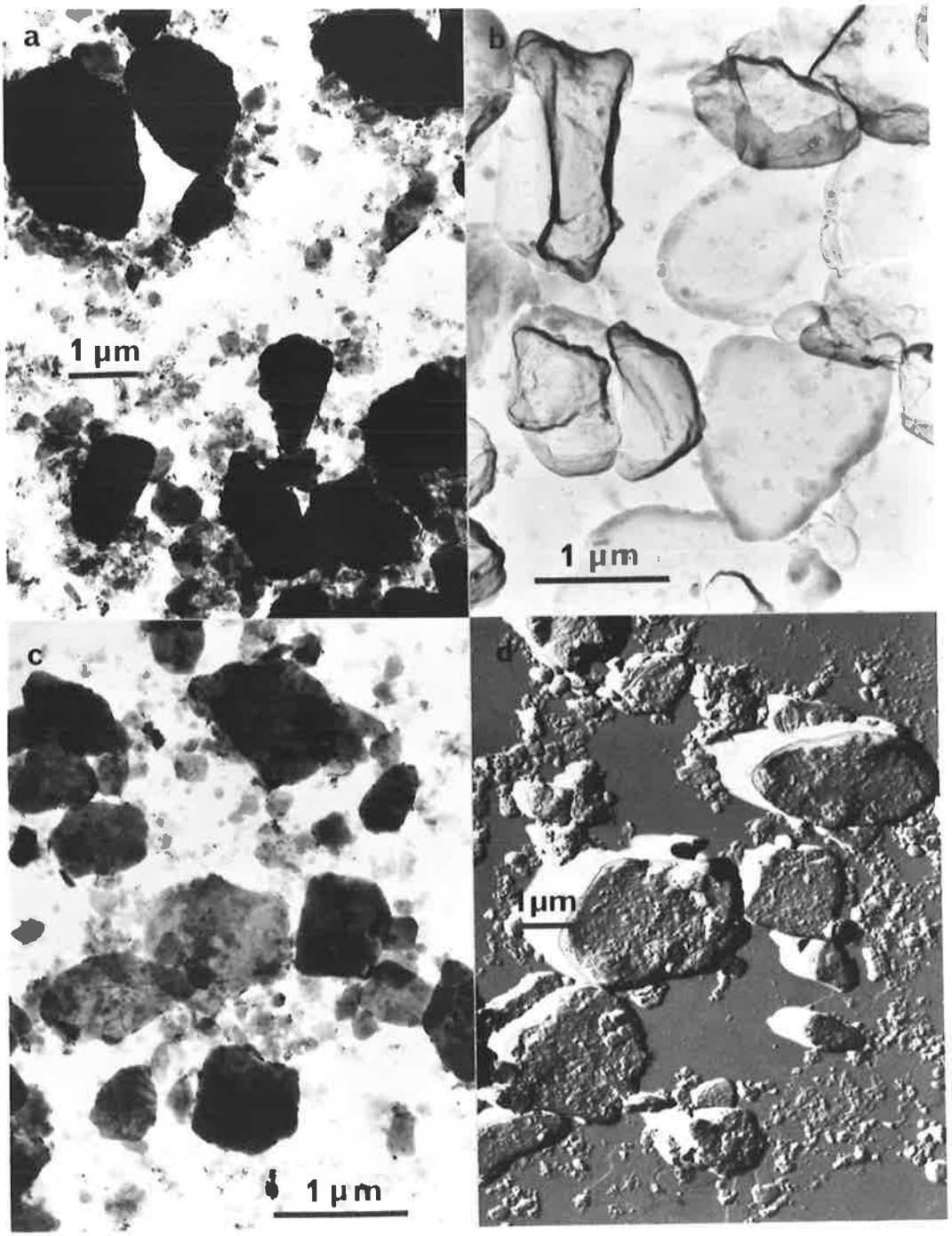

a
1 µm
b
1 µm
c
1 µm
d
1 µm

Fig. 27 Transmission electron micrographs of peroxidized clay-sized particles of Lemnos loam
a) treated with sodium carbonate,
b) carbon replica of a),
c) treated with sodium pyrophosphate,
d) carbon replica of c) .


a
1 µm
b
1 µm
c
1 µm
d
1 µm

Disordered alumino-silicates. Sodium carbonate which removed highly disordered alumino-silicates, alumina and silica, had the greatest effect on peroxidized samples by releasing particles <0.2 μm diameter from aggregates of 10-50 μm and >250 μm diameter (Fig.24; Table 10). Electron micrographs of carbon replicas of clay particles showed that the sodium carbonate removed a crust with a highly irregular surface to leave particles with predominantly smooth surfaces (Fig. 27b). Greenland and Wilkinson (1969) obtained similar micrographs with samples of Urrbrae fine sandy loam treated with sodium carbonate. TE micrographs of the peroxidized samples of Lemnos loam treated with sodium carbonate showed small discrete electron-dense crystalline iron oxides suggesting that sodium carbonate had dissolved material bound to iron oxide (Fig. 27a).

Polysaccharides. Treatment of the soil with sodium periodate had no significant effect on the size-distribution of water-stable particles. These results agree with those described in Chapter 4.

Polyvalent cations. Treatment of the soil with pyrophosphate, which destroys complexes of polyvalent cations and organic matter (Stefanson 1971) released many particles 0.2-50 μm diameter and <0.2 μm diameter (Fig.24; Table 10). Electron micrographs also show that sodium pyrophosphate dispersed fine clay (Fig.27c) but did not remove encrustions from the surface of particles of clay (Fig.27d). Hence organo-mineral complexes appear to be responsible for much of the stability of aggregates >50 μm diameter. These bonding mechanisms are probably similar to those found by Turchenek and Oades (1978) in Urrbrae fine sandy loam in the very stable aggregates 1-20 μm diameter obtained after ultrasonic treatment of a soil from under pasture (see section 1.3.2).

8.4 Conclusions

The results of this chapter show that several binding agents, both organic and inorganic, are responsible for stabilizing aggregates of various sizes in Lemnos loam. The methods used give no information on the

binding agents within stable aggregates 2-50 μm diameter. However the results show that the following binding agents, in decreasing order of importance, are responsible for binding particles into aggregates:

Binding agent	Particles bound (μm)	Aggregates formed (μm)
Organic matter	<2	>50
	<0.2	<2
Highly disordered alumino-silicates	<0.2	>250
Polyvalent metal-organic matter complexes	<0.2	>50
Disordered hydrous oxides	<0.2	>250
Crystalline hydrous oxides	<0.2	50-250

The results show that organic matter is the main binding agent within macroaggregates (>250 μm diameter) from Lemnos loam. On the other hand microaggregates 50-250 μm diameter are stabilized by more than one type of binding agent, that is by organic matter and complexes of polyvalent metal-organic matter, whose effects are additive; therefore particles 50-250 μm diameter from red-brown earths are highly stable (see section 7.3.5).

CHAPTER 9

GENERAL DISCUSSION

Using information from the literature and from results presented in this thesis, in this chapter I will describe three main groups of organic binding agents; then leading on from that description I will propose a model of an aggregate from a red-brown earth.

9.1 Organic Binding Agents

There are three main groups of organic binding agents in soil. The age and degree of degradation of the organic matter (not the proportion of chemically defined components) determine the groups, and determine also the age, size and degree of stability of the aggregate. The three main groups of binding agents are (a) short-term, (b) medium-term and (c) persistent. Each of these groups of binding agents will be considered in turn.

a) Short-term binding agents

Short-term binding agents are the microbial and plant polysaccharides described in section 1.3.2; they are produced rapidly but are degraded rapidly and do not persist in soils. They are associated with large (> 250 µm diameter) transient aggregates.

b) Medium-term binding agents

Intermediate binding agents consist of viable or slightly degraded microbial cells associated with a fine network of roots and hyphae in young aggregates (Hubbell and Chapman 1946; Bond and Harris 1964) mainly vesicular-arbuscular (VA) mycorrhizal hyphae (see section 4.3.4). The organic materials are mixed intimately with inorganic particles so that they are not removed with the light fraction <1.8 SG (see section 7.3.4) although they are probably removed with the light fraction < 2.0 SG (Greenland and Ford 1964; Oades 1967; D. J. Carter and J. M. Oades unpublished results). The inorganic particles probably become bound

closely to the organic matter during localized drying in the rhizosphere and become difficult to remove (Greenland 1971a). These materials build up in soils within a few weeks (see section 4.3.1) and persist for at least a few months or years (see section 6.3.1) and are affected by the management of the soil.

Medium-term binding agents stabilize macroaggregates >250 µm diameter (see section 7.4) probably because fine roots and fungal hyphae are relatively large and because they can grow across large pores in soil (see section 1.3.2). The stability of Lemnos loam and of Urrbrae fine sandy loam was related to the length of external hyphae of VA mycorrhizal fungi (see sections 5.3.1 and 7.3.6) and most of the hyphae which have been reported to stabilize aggregates in the field in the presence of plants were probably these fungi (see section 1.3.2).

Medium-term binding agents have not yet been defined chemically. However fungal hyphae have wide C/N ratios (Oades and Ladd 1977) and, because they are insoluble in NaOH, they are probably included in the humin fraction. Organic matter which was not extracted with HCl and NaOH was thought to be partly decomposed organic matter encrusted with inorganic materials; this hypothesis was supported by the fact that further light fraction (<2.0 SG) was obtained from the soils after they were treated chemically and dispersed with ultrasonic vibration (Oades 1972). The medium-term binding agents were periodate-resistant, and probably consisted of organic matter which was bound to particles of clay by polyvalent cations since the bonds were degraded partly by pyrophosphate (see sections 4.3.3 and 6.3.2).

Vesicular-arbuscular mycorrhizal fungi

Although these fungi are widespread in soils and do not appear to be associated with specific hosts (Mosse 1975) they are obligate symbionts and cannot be cultured on artificial media (Mosse 1973) so were not implicated until recently in the stability of aggregates of soil. It is

believed that VA mycorrhizal fungi tend to be most abundant in soils with low or unbalanced levels of nutrients (Mosse 1973); however some plants are mycorrhizal even in fertile soils (Sanders *et al.* 1975). It is not known how long these fungi persist in soil once the host has died, but hyphae were still present in soil several months after the plants were killed although the hyphae may not have been viable (see section 6.4).

Most work on these fungi has aimed to increase the growth of plants (Mosse 1973) through studies of factors which affect the infection of the host and the production of spores in soil, e.g. water content and temperature of the soil, light intensity and daylength (Furlan *et al.* 1973; Hayman 1974; Reid and Bowen 1979). Little is known of the factors which affect the growth of external hyphae in soil, yet the stability of macroaggregates depends on hyphal length. However the fungi produce extensive hyphae in soil (see section 4.3.4) and have been reported to extend 10 mm from the surface of the root (Mosse 1959; Sanders and Tinker 1973). They extended 30 mm (Hattingh *et al.* 1973) and 80 mm (Rhodes and Gerdemann 1975) from the root in soil in modified petri dishes; however the hyphae may have grown preferentially along the soil plane in the petri dishes so the distances quoted may not represent growth in natural soil. The length of hyphae may or may not be related to length of infected root (Sanders *et al.* 1973; Cooper 1975). Ryegrass and white clover roots after 14 weeks growth supported respectively 1360 mm and 1290 mm hyphae per 10 mm infected root although the ryegrass had eight times the total root length of the white clover (see section 4.3) . Although the fungi do not appear to have specific hosts (Mosse 1975) some spore types were shown to produce more hyphae than others on onions (Bevege and Bowen 1975).

Strains of these fungi have been selected because they stimulate the growth of plants (Mosse 1973); but strains which stabilize aggregates of soil most efficiently are yet to be selected. The strains selected

should (i) produce profuse external hyphae (covered with mucilage) under different species of plants during the cropping and pasture phases; (ii) produce spores which will persist under adverse conditions, and can then germinate and grow through the soil to reach the root system; (iii) become established in natural soils with indigenous VA mycorrhizal fungi (Mosse *et al.* 1969). It may not be necessary to have a strain which is highly infective to be plentiful in the rhizosphere (Cooper 1975).

However because these fungi cannot be cultured on artificial media and because it is difficult at present to produce an inoculum and get the fungi established (Mosse 1973; Crush and Pattison 1975), it may be more profitable to learn to manage indigenous VA mycorrhizal hyphae to get maximum stabilization of aggregates.

c) Persistent binding agents

Persistent binding agents give rise to the organo-mineral bonds described in section 1.3.2, i.e. highly degraded, highly aromatic humic material (probably derived from the resistant fragments of hyphae, bacterial cells and colonies) associated with amorphous iron, aluminium and alumino-silicates. Persistent bonds are strong, not affected by management of the soil and are associated with microaggregates (<250 µm diameter) (Edwards and Bremner 1967; see also section 7.3.1). Even in an old arable red-brown earth which contained only 1.1% total organic carbon, 54% of the soil consisted of water-stable particles 50-250 µm diameter (see section 7.3.5). Although some of the persistent bonds can be broken with ultrasonic vibration (Edwards and Bremner 1967), in some soils, especially those with high % total carbon, persistent bonds within particles 1-20 µm diameter resist ultrasonic vibration for up to 5 min (Hamblin 1977; Tate and Churchman 1978; Turchenek and Oades 1978).

9.2 Model of an Aggregate

9.2.1 Introduction

Several models have been proposed to describe the way in which individual mineral particles are held together to form water-stable aggregates of soil. Misono and Sudo (1958) and Sudo (1962) (both quoted in Hattori 1973) suggested that particles <20 μm diameter are bound into water-stable secondary particles 20-60 μm diameter, and that these secondary particles in turn form larger soil aggregates.

Emerson (1959) suggested that parallel clay crystals (about 5 μm diameter) are grouped together closely enough (about 0.1 μm to 1.3 μm apart) to behave in water as a unit which he called a domain. His model, which is not drawn to scale, shows that organic matter stabilizes the aggregate mainly by forming and strengthening bonds between clay domains and between quartz particles and domains, though the quartz particles may also be linked directly by organic matter.

Quirk and Aylmore (1971) use the term quasi-crystal to describe the regions of parallel alignment of individual alumino-silicate lamellae in montmorillonite, which exhibits intra-crystalline swelling; they use the term domain to describe the regions of parallel alignment of crystals for illite and other fixed lattice clays, which exhibit inter-crystalline swelling only.

Edwards and Bremner (1967) suggested that macroaggregates (>250 μm diameter) which contain sand grains are weak and are broken easily in the field. Their model shows that the only stable aggregates are micro-aggregates (<250 μm diameter) which consist of complexes of Clay-Polyvalent metal-Organic Matter (C-P-OM) where clay is bonded to humified organic matter through polyvalent metals. Particles of (C-P-OM) and $(C\text{-}P\text{-}OM)_x$ both of which are <2 μm diameter form microaggregates $((C\text{-}P\text{-}OM)_x)_y$ which are <250 μm diameter. Bonds of C-P-C and OM-P-OM, and even of aluminium or iron oxide, or H-bonds may occur also. Edwards and Bremner also

suggested that fragments of humified organic matter may be bonded to a single clay particle, and that a single fragment of humified organic matter may be bonded to more than one clay particle.

The interactions between organic polymers and mineral surfaces are complex but the mechanisms are known and have been reviewed by Greenland (1965; 1971a), Mortland (1970) and Theng (1979). The most important mechanism of interaction probably involves bridges of polyvalent cations between the surface of the clay particles or hydroxy polymers and the ligand groups of organic polymers, e.g. carboxyl groups.

9.2.2 Model of an aggregate from a red-brown earth

In this model, an aggregate is broken down in four stages:

>2000 μm → 20-250 μm → 2-20 μm → 0.2-2 μm → <0.2 μm

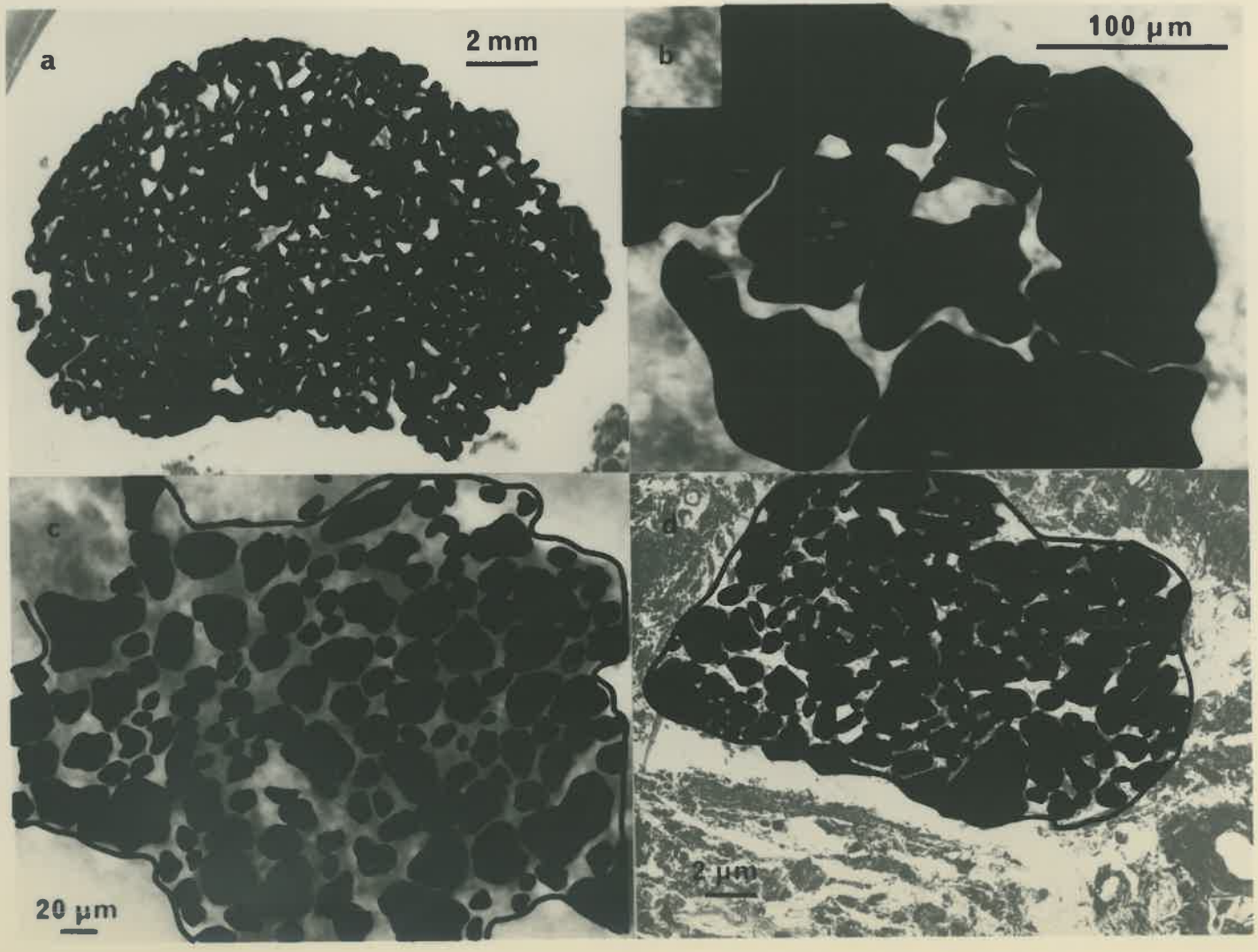
The evidence for these stages is as follows:

a) In a red-brown earth with a low content of organic carbon (1.1%), air-dried aggregates >2000 μm diameter when wetted rapidly broke down into water-stable aggregates and particles 20-250 μm diameter; whereas in a soil with a high content of organic carbon (2.5%) aggregates >2000 μm diameter did not slake (see section 7.3). A cross-section of a water-stable particle >2000 μm diameter impregnated with white Araldite (Ciba-Geigy Australia) shows that the particle is porous (Fig.28a) and consists dominantly of particles of about 20-250 μm diameter (Fig.28b).

b) Aggregates from a red-brown earth with a high content of organic carbon (2.7%), when treated with ultrasonic vibration for up to 5 min broke down into stable aggregates and particles 2-20 μm and <2 μm diameter (Turchenek and Oades 1978). A cross-section (Fig.28c) and a scanning electron micrograph (Fig.18) of a water-stable aggregate 20-250 μm diameter show that the aggregate consists dominantly of particles of about 2-20 μm diameter.

c) Aggregates from a red-brown earth with a low content of organic carbon

Fig. 28 a) A cross-section of a water-stable particle >2000 µm diameter from Lemnos loam impregnated with white Araldite,
b) and c) enlargements of a),
d) transmission electron micrograph of an ultra-thin section of rhizosphere soil.


a
2 mm
b
100 µm
c
20 µm
d
2 µm

(1%) when treated with ultrasonic vibration for 5 min broke down into particles <2 μm diameter (Turchenek and Oades 1978).

A transmission electron micrograph of a rhizosphere soil shows a water-stable particle 2-20 μm which consists of particles <2 μm diameter (Fig. 28d) bound closely together. Fordham and Norrish (1979) also describe particles which are bound together by strands of glutinous material, probably organic, to this level of aggregation. However some particles 2-20 μm diameter are probably simply large floccules (see below).

d) Some particles <2 μm diameter have been shown to be aggregates of fine material held together by organic matter and iron oxides (Burford *et al.* 1964; Turchenek and Oades 1978). Other particles <2 μm diameter are often floccules where individual clay plates come together to form a fluffy mass (Quirk 1978).

The model may also apply generally to soils where organic matter is the main binding agent, but the levels of aggregation may differ. For example in a Narrabrai black earth, aggregates 1000-2000 μm diameter broke down directly to water-stable particles of about 30 μm diameter (Collis-George and Lal 1970).

Water-stable aggregates at each stage will be considered in turn.

Aggregates >2000 μm diameter

In red-brown earths, water-stable aggregates >2000 μm diameter consist of aggregates and particles 20-250 μm diameter held together mainly by organic material (Fig.24) associated with a fine network of roots and hyphae (Fig.9). The stability of aggregates >2000 μm diameter is related to the length of root and of VA mycorrhizal hyphae. Ryegrass plants with 1.1 m roots and 19.6 m VA mycorrhizal hyphae per g soil, more than doubled the stable aggregation (>2000 μm diameter) of a red-brown earth, whereas clover plants with 0.13 m roots and 8.8 m hyphae per g soil increased the stable aggregation slightly (see section 4.3). The organic matter includes medium-term binding agents (see section 9.1) and, in soils which contain low levels

of organic matter, short-term binding agents. Clay is sorbed onto the surface of the organic matter (see Fig.11; Fig.12) rather than organic polymers sorbed onto the clay (Greenland 1965). Because the stability of particles >2000 µm diameter is related to the growth of roots and hyphae the stability is affected by agricultural practices (see Chapter 7).

Inorganic binding agents including highly disordered alumino-silicates and crystalline iron oxides also stabilize aggregates >2000 µm diameter but to a lesser extent than organic materials (see Chapter 8).

Aggregates 20-250 µm diameter

Aggregates 20-250 µm are stable to rapid wetting and are not destroyed by agricultural practices; even in an old arable Urrbrae fine sandy loam, over 70% of water stable particles were 20-250 µm diameter (see Fig.17). However aggregates 20-250 µm diameter can be destroyed by ultrasonic vibration (Turchenek and Oades 1978). Aggregates 20-250 µm diameter consist largely of particles 2-20 µm diameter bonded together by various cements including persistent organic materials and crystalline oxides and highly disordered alumino-silicates (Fig.24). The aggregates 20-250 µm diameter are very stable, partly because they are small, but also because they contain several different types of binding agents whose effects are additive. The individual organic bonds must be strong because particles 20-250 µm diameter contained only about 30-50% of the organic carbon in the much less stable particles >250 µm diameter (see section 7.3.4). It is not easy to define the site or size of the organic materials within stable particles 20-250 µm diameter because the soil is associated intimately with the organic material. Particles 20-250 µm diameter would be included in the stable microaggregates $((C\text{-}P\text{-}OM)_x)_y$ described by Edwards and Bremner (1967).

Aggregates 2-20 µm diameter

Water-stable aggregates 2-20 µm diameter consist of particles <2 µm diameter bonded together so strongly by persistent organic bonds that they

are not disrupted by agricultural practices; some particles 2-20 µm diameter from under old pasture resist ultrasonic vibration for 5 min (Turchenek and Oades 1978). Particles 2-20 µm diameter, obtained by ultrasonic dispersion or trituration from soils with high contents of clay and high base status, often have high contents of organic materials (Oades and Ladd 1977; Turchenek and Oades 1978). This organic-rich fraction (2-20 µm diameter) is often highly stable especially in chernozemic soils and in soils under old pasture (Pokotilo 1967; Turchenek and Oades 1978).

Development of stable particles 2-20 µm diameter

Electron micrographs of soils or thin sections of soils in the rhizosphere show individual bacteria or colonies of bacteria surrounded by a capsule, composed of carbohydrate, to which particles of fine clay appear firmly attached (see section 1.3.2). The clay particles which may enclose the bacteria completely, are sometimes oriented tangentially to the bacterial surface to a distance of 0.1 µm from the bacterial surface. The fact that such associations between live bacterial cells and clay particles appear to form aggregates 2-20 µm diameter, is supported by the results of Ladd *et al.* (1977), Yamagishi (1968, quoted by Hattori 1973), Ahmed (1980) and J. M. Oades (unpublished results) who found that a significant part of the microbial biomass was present in silt-sized fractions. However since only about 2% of the organic matter in soils consists of biomass (Jenkinson and Rayner 1977), silt-sized aggregates consisting of live bacteria must be newly formed aggregates. When the bacterial colonies have died and their contents have decayed, characteristic fibrous components of the bacterial capsule remain (Foster 1978) thus leading to an older aggregate. Presumably at a later stage of development of such an aggregate, the remains of the colony with its capsule could not be identified as such, but appears as a matrix of organic matter binding particles of clay. This organic matter is possibly part of the physically protected organic matter (POM) described by Jenkinson and Rayner (1977) and as seen in the transmission electron

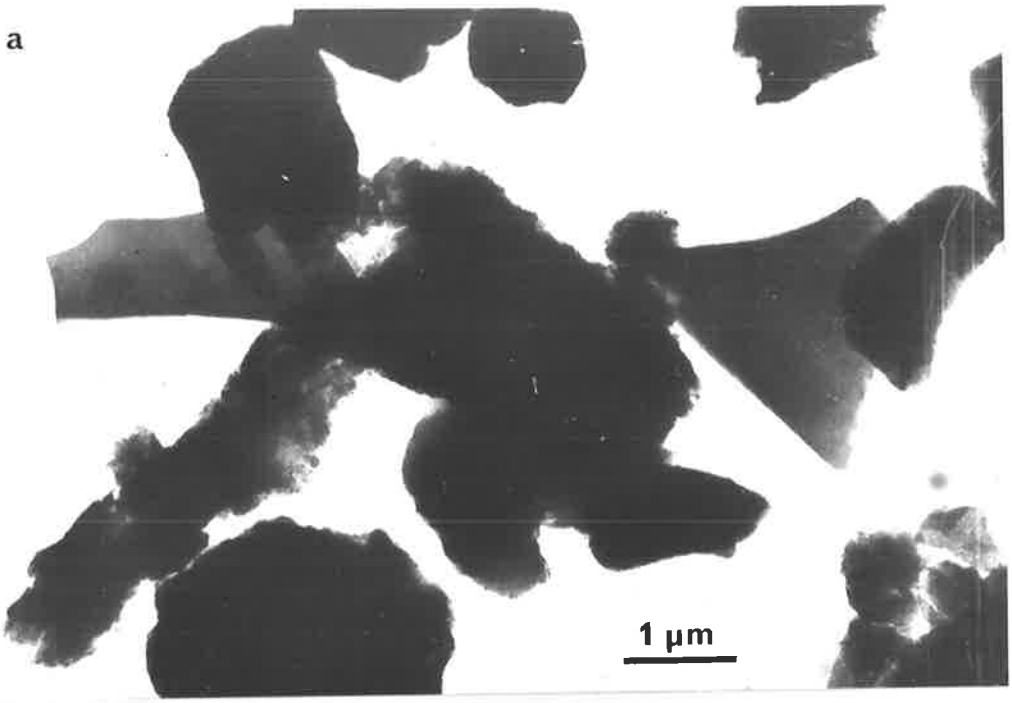
micrograph of a thin section of soil from the rhizosphere (Foster 1978, Plate 2). There is also chemical evidence that aggregates 1-5 µm diameter are old and protect organic matter, which consists mainly of humic acids, within the aggregates (Oades and Ladd 1977). However aggregates derived from bacterial colonies may represent only a small number of particles stabilized by microbial debris since fungi contributes more to soil organic matter than do bacteria (Paul and van Veen 1978). Fungal hyphae have been shown to produce a layer of amorphous material, probably polysaccharide to which particles of clay were attached firmly (see section 5.3). Fragmentation of the hyphae could lead to small aggregates stabilized by fungal debris as is shown in the electron micrograph (Fig. 29a) of particles 2-20 µm diameter where fine clay surrounds rod-shaped organic material and Fig. 29b of fine clay surrounding a rod-shaped bacterium. The hyphal fragments could be derived from VA mycorrhizal fungi which were associated with a living plant, or from saprophytic fungi which grew rapidly in the soil after the addition of readily decomposable material e.g. straw. As with the bacterial colonies, further decay of the hyphal fragment could lead to a matrix of physically protected organic matter at the centre of a stable aggregate.

Aggregates <2 µm diameter

Water-stable particles <2 µm diameter are often floccules where individual clay plates (which may consist of individual lamellae or groups of lamellae called sheets) come together to form a fluffy mass. Initially the plates are not parallel but are attracted edge-to-face to form an open card-house structure (Quirk 1978). However on drying the system tends to lower its entropy so that the plates are then parallel and if aligned perfectly will form a crystal 4 nm wide. The crystals may then be joined into larger units with slit-shaped pores 2.5 nm to 4.1 nm between the crystals (Murray and Quirk 1979). In surface soils perfect alignment of clay plates probably rarely occurs so that the arrangement within particles 2 µm diameter is probably somewhere between that of card-house structure

Fig. 29 Scanning electron micrographs of particles separated from a) Urrbrae fine sandy loam from under permanent pasture, b) Toje latosol.

a



b

and that of a crystal. The plates are held together by van der Waal's forces, H-bonding and coulombic attraction. However the charges of ions associated with the surface of clay are influenced by organic and inorganic materials (Greenland 1965; 1971a). For example, organic materials may increase or decrease the attraction between the particles (see Chapter 1).

Some particles <2 μm diameter have been shown to be aggregates of very fine material held together by organic matter and iron oxides (Burford *et al*. 1964; Turchenek and Oades 1978). In particles <2 μm diameter organic material is probably sorbed onto the surfaces of clays, i.e. polymers of negatively charged organic matter join particles of clay to form "strings of beads" whereas uncharged polymers form "coats of paint" which spread around groups of particles which are already close together (Greenland 1965).

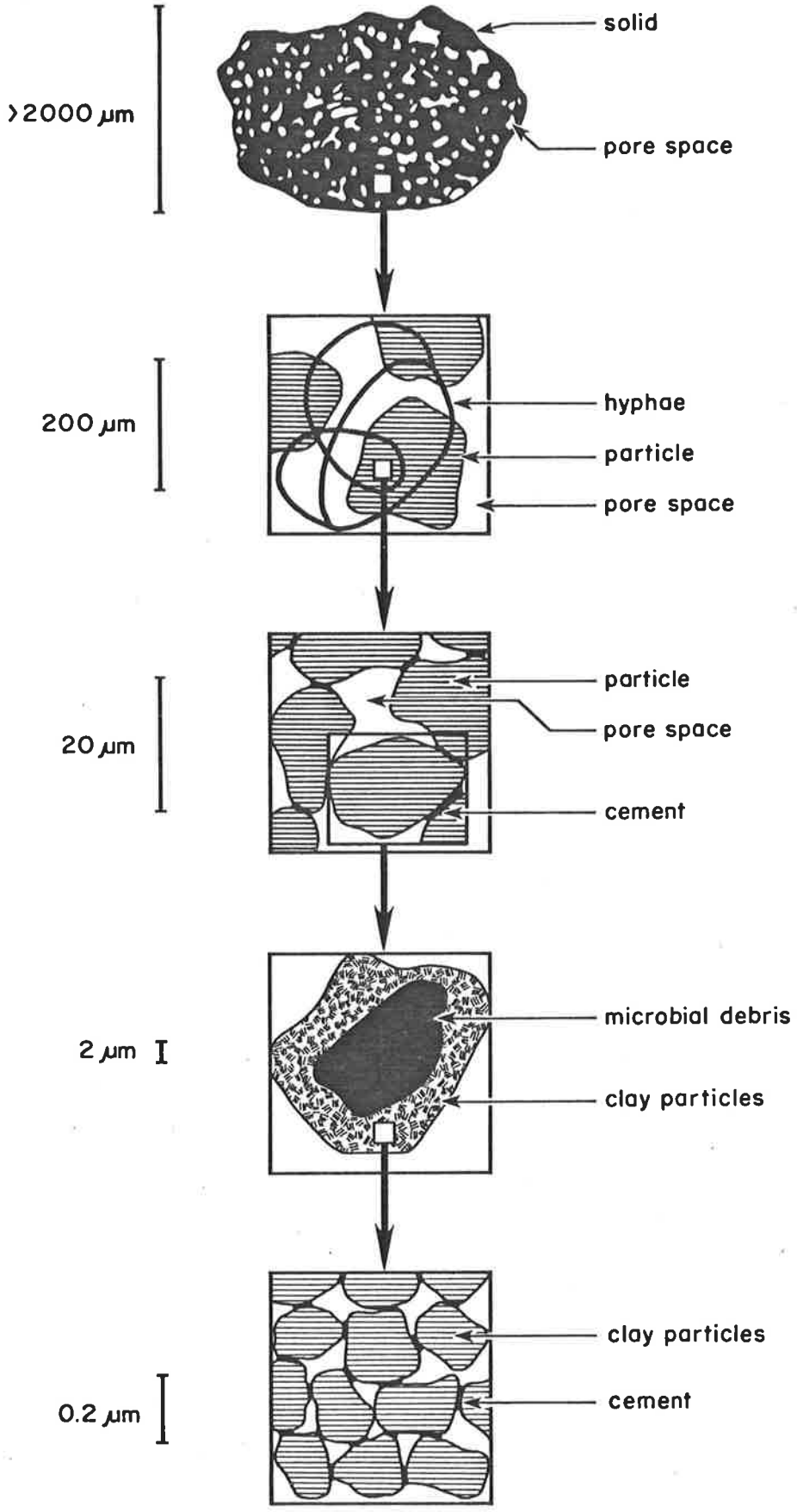
An idealized model can now be drawn to scale showing that an aggregate of soil is built up of structural units of various sizes held together by various bonding mechanisms (Fig.30).

In soil there is considerable overlap between the proposed stages leading to an aggregate with a diameter measured in mm, although there appears to be sufficient evidence to warrant the various stages proposed, particularly the larger stages. The evidence indicates that there is not a smooth continuum of particle-sizes of water-stable particles and that stability of the various stages is associated with a dominant binding agent.

It would be interesting to examine other soils with different textures to determine the size of particle produced by slaking and by ultrasonic dispersion.

AGGREGATE FORMED	MAJOR BINDING AGENT
50-250 μm → >2000 μm	Roots and hyphae (medium-term organic)
2-20 μm → 20-50 μm	Plant and fungal debris encrusted with inorganics (persistent organic)
<2 μm → 2-20 μm	Microbial and fungal debris encrusted with inorganics (persistent organic)
<0.2 μm → 0.2-2 μm	Amorphous alumino-silicates oxides and organic polymers sorbed on clay surfaces + Electro-static bonding, flocculation (permanent inorganic)

Fig.30 Model of an aggregate from a red-brown earth.


>2000 µm
solid
pore space
200 µm
hyphae
particle
pore space
20 µm
particle
pore space
cement
2 µm
microbial debris
clay particles
0.2 µm
clay particles
cement

APPENDIX

Stabilization of Soil Aggregates by the Root Systems of Ryegrass

by

J. M. Tisdall and J. M. Oades

Tisdale, J. M. & Oades, J. M. (1979). Stabilization of soil aggregates by the root systems of ryegrass. *Australian Journal of Soil Research, 17*(3), 429-441.

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