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THE USE OF  $^{14}\text{C}$  IN STUDIES OF MICROBIAL ACTIVITIES

IN SOIL AGGREGATES

A Thesis

submitted by

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### SUMMARY

The following topics were surveyed in the literature review:

- (a) studies of soil organic materials using  $^{14}\text{C}$ ,
- (b) methods of determination of  $^{14}\text{C}$ ,
- (c) influence of aggregates on microbial activities,
- (d) drying and wetting cycles in soils.

A method was developed to determine  $^{14}\text{C}$  in finely ground soil samples, stabilized by CAB-O-SIL as suspensions, in toluene-PPO-dimethyl POPOP scintillant. Addition of internal standards to samples yielded efficiencies which allowed 100% recoveries of activity provided (a) samples were ground to  $<53\ \mu\text{m}$  diameter and (b) weights of samples were such that the optical density of the gel remained below 0.9 at 450 nm in a 1 cm cell.

The method was applied successfully to counting of  $^{14}\text{C}$  in soils, plant materials and freeze-dried, coloured extracts of soils.

Incubation of  $^{14}\text{C}$ -labelled glucose and starch incorporated into soil from which aggregates were prepared show that several factors are involved in the metabolism of organic substrates in soil aggregates. Two peaks of  $^{14}\text{CO}_2$  release occurred, the first between the 2nd and 5th days of incubation, and the second on the 8th or 9th days of incubation. In a fine sandy loam the first peak was shown to be due to a dominantly fungal population which utilized all  $^{14}\text{C}$ -glucose releasing about 40% of the  $^{14}\text{C}$  present as  $^{14}\text{CO}_2$ . In clay soils studies with single organisms suggested that bacteria were dominant during incubations of  $^{14}\text{C}$ -labelled carbohydrates in aggregates.

With the odd exception, all soils amended with either  $^{14}\text{C}$ -glucose or starch showed greater release of  $^{14}\text{CO}_2$  from control samples (substrates present in macropores only) than from aggregate samples (substrates in micropores and macropores). The rate of release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -glucose was inversely related to the size of aggregates in a fine sandy loam. For samples amended with starch release of  $^{14}\text{CO}_2$  was slightly higher from the larger aggregates.

A self-mulching clay showed the opposite trend with the release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -glucose being proportional to the size of aggregates. When starch was the substrate the rate of release of  $^{14}\text{CO}_2$  was again higher from the larger aggregates.

Physical disturbance by either drying and wetting cycles or mechanical disturbance of aggregate samples pre-incubated with a  $^{14}\text{C}$ -labelled substrate caused a flush of microbial activity based on  $^{14}\text{CO}_2$  evolution. The results showed that physical factors should be considered together with biological and chemical factors in interpretation of the flush of activity caused by a drying and wetting cycle.

In a fine sandy loam fungi were more active than bacteria when substrates were present in micropores and macropores of soil aggregates but both groups of organisms were active when substrates were within macropores only (controls). On the other hand, in a clay both groups of organisms competed well throughout the incubations probably due to the pore size distribution and more favourable pH for bacteria.

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and, to the best of the author's knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

JOSEPH KWASI ADU

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CHAPTER I. LITERATURE REVIEWUSE OF <sup>14</sup>C IN STUDYING MICROBIAL DECOMPOSITION  
OF SOIL ORGANIC MATTERIntroduction

It has long been established that under favourable conditions soil organic carbon is the major substrate for heterotrophic organisms.

To study the decomposition of organic carbon by the soil microflora, a known weight of the organic carbon has been added to the soil, incubated for a specific time with periodic determination of the CO<sub>2</sub> absorbed in a suitable trapping agent. Unamended samples of soils served as controls.

Limitations to such procedures were lack of reliable methods for determination of the CO<sub>2</sub> released, and uncertainty concerning the origin of the CO<sub>2</sub>. This was particularly important in demonstrating an effect designated as the "priming action" and defined as an increase or decrease in the rate of decomposition of native soil organic carbon as a result of introduction of fresh organic material (Jenkinson, 1966b). The priming action was one of the major interests in soil organic matter studies in addition to investigations on soil structure and fertility. The residual organic carbon not released as CO<sub>2</sub> during incubation was either used to build up cell structure or remained intact, depending on the nature of the organic source. Whatever the form of the residual carbon, some of it finally contributed to the soil organic matter.

Studies of the transformation of organic material added to soil were difficult until the advent of isotopic techniques (Broadbent and Norman, 1946). Broadbent and Norman used  $^{13}\text{C}$ -labelled plants to demonstrate "priming action". This was replaced by  $^{14}\text{C}$  as soon as this isotope became available (Bingeman et al., 1953). Because  $^{14}\text{C}$  has a half-life of 5730 years it needs no calculations to correct for decay rate, and sample size can be much smaller. As pointed out by Jenkinson (1971), isotopes have proved indispensable in studying turnover processes and rates in animal metabolism and can be used successfully in analogous soil organic matter problems. More than 100 papers have been published involving the use of  $^{14}\text{C}$  in soil studies (Jenkinson, 1971).

#### A. METHODS

##### 1. Methods of determining $^{14}\text{C}$

###### (i) Measurement of $^{14}\text{CO}_2$ during incubation

The usual method of determining  $^{14}\text{CO}_2$  involves the trapping of  $^{14}\text{CO}_2$  released during decomposition of  $^{14}\text{C}$ -labelled substrates.

Absorption has been carried out in different ways depending on which system of incubation was used.

One of the most important factors is that the trapping agent should be efficient. In a continuous flow system, the absorbent is separate from the soil sample and the trapping agent does not interfere with the incubation. In incubation studies with Warburg flasks or other static systems (Bartha and Pramer, 1965), the absorbing solution is close to the soil sample, and volatile agents (e.g. phenylethylamine) may affect

the incubation. A range of absorbents have been employed but caustic solutions [NaOH, KOH or Ba(OH)<sub>2</sub>] have been most often used in soil studies.

Some organic solutions, such as ethanalamine (Woeller, 1961; Jeffay and Alvarez, 1961), phenylethylamine and Hyamine (Passmann et al., 1956; Hempel, 1964) have been found to be efficient absorbents, and have been of use in biochemical studies. The advantage of these organic agents over inorganic absorbents is that they can be mixed directly with scintillation fluors.

The merits and limitations of various trapping solutions has been reviewed by Rapkin (1962). Duncombe and Rising (1969) compared the efficiency of CO<sub>2</sub> trapping by four different agents and combined them with different scintillation fluors. Phenylethylamine, Hyamine and KOH were found to be the best trapping agents but aqueous KOH gave low and variable recoveries when counted in water-miscible scintillators. However, counting in the presence of 4% w/v finely divided silica (aerosil) gave very high recoveries.

Despite the fact that the organic agents compared favourably with the KOH, further investigation showed that in studies using Warburg flask phenylethylamine caused almost complete inhibition (93 to 94.7%) of metabolic activities whilst Hyamine caused 18 to 42% inhibition. Thus, in a closed system, such as a Warburg flask, caustic solution is better used as a trapping agent, but a gelling agent should be added to the scintillation fluor.

Addition of gelling agent may not be necessary if the caustic mixes well with scintillation fluor. Such mixing has been achieved by the use of emulsifying agents such as Triton X-100 (Patterson and Green, 1965; Turner, 1968). Triton X-100 scintillant prepared by mixing 2:1 v/v of toluene (containing 0.4% PPO and 0.01% POPOP), and Triton X-100 (a detergent) is most satisfactory for aqueous samples (Turner, 1968). The system is capable of incorporating up to 15% by volume of aqueous sample giving a clear solution and good counting efficiency. However, above 15% two distinct phases appear which are unstable and poor counting results. The scintillation mixture is stable at low temperatures or at room temperature and the components do not deteriorate on storage.

There is a limit to the concentration of caustic solution or carbonate that is miscible with the scintillant. NaOH concentrations up to 0.2N or 0.06N sodium carbonate have been found to be miscible with the scintillant (Turner, 1968). Scintillant, however, becomes very sensitive to chemiluminescence in the presence of alkaline solution. This necessitates counting in the cold, after a long period of equilibration of the samples at the temperature of the spectrometer.

Before the advent of the liquid scintillation counting system the method often used in organic matter studies was the counting of the  $^{14}\text{C}$  in the form of  $\text{Ba}^{14}\text{CO}_3$  (this is discussed in the next Section).

(ii) Determination of  $^{14}\text{C}$  in soil

The determination of  $^{14}\text{C}$  in soil has generally involved oxidation of the  $^{14}\text{C}$ -labelled organic materials to carbon dioxide using either wet (Jenkinson, 1965; Oberlander and Roth, 1968; Sorensen, 1963,

1967) or dry combustion (Little, 1963; Mutatkar and Wagner, 1967).

The quantitative determination of the  $^{14}\text{C}$  after the initial treatment has however varied. Oberlander and Roth (1968), Sorensen (1963, 1967) and Little (1966) trapped the  $^{14}\text{CO}_2$  in sodium hydroxide solution, precipitated the carbonate as  $\text{Ba}^{14}\text{CO}_3$  or  $\text{Ca}^{14}\text{CO}_3$  (Little, 1966) and after filtration and drying counted the precipitate on an end-window Geiger Müller counter. Alternatively, aliquots of the alkaline solution have been rendered miscible with scintillation fluors and the  $^{14}\text{C}$  counted by liquid scintillation (Sorensen and Paul, 1971; Sorensen, 1972; Shields, 1972).

Jenkinson (1965), using a modification of Shaw's method (Shaw, 1959) trapped the  $^{14}\text{CO}_2$  in tetraethylammonium hydroxide and after adding a dioxan-based phosphor counted the homogeneous mixture by liquid scintillation. On the other hand, Mutatkar and Wagner (1967) used the liquid scintillation method but counted their precipitated  $\text{Ba}^{14}\text{CO}_3$  after drying and grinding by suspending in a thixotropic gel (CAB-O-SIL).

The difficulties encountered in obtaining a quantitative conversion of the  $^{14}\text{C}$ -labelled organic materials to  $^{14}\text{CO}_2$  and the subsequent treatments have rendered the above methods tedious and quantitation on the semi-micro scale is questionable.

The simplicity of construction of the Geiger Müller besides the fact that in some cases low level activity samples could be handled better made this system more suitable in the early stages of radioisotope studies. Its use in recent years has been suppressed by the introduction of the liquid scintillation system (Krebs, 1955), though it is

still employed in some fields.

There are a number of disadvantages with Geiger Müller counting:

(a) An elaborate sampling train is required to purify the gas and ensure the correct fill of the counting device.

(b) Great care is needed in sample preparation since trace contaminants and even moisture cause serious interference.

(c) The amount of sample which can be measured at one time is severely limited and routine low activity measurements are difficult.

(d) Because of self-absorption, Ba<sup>14</sup>CO<sub>3</sub> planchets for counting have to be made up to 'infinite thickness', which renders the method quite laborious and time consuming, particularly when dealing with large samples (Jenkinson, 1966c).

(e) Efficiency is low.

and (f) Contamination of the counting chamber is an ever-present possibility.

The advantages of the combustion method where a liquid scintillation spectrometer is employed for counting are dependent on (a) high counting efficiency and (b) availability of relatively large samples. The disadvantages, however, far outweigh the advantages.

(a) The use of liquid scintillation requires the use of samples with high activity since the <sup>14</sup>CO<sub>2</sub> is usually absorbed in relatively large volumes and to obtain homogeneous sample preparation only small aliquots can be taken for counting.

(b) Quantitative conversion of organic materials to CO<sub>2</sub> is difficult particularly on the semimicro scale.

There are now available on the market automated oxidisers (Packard Instrument Company Inc. and Beckman Instrument Co. Ltd.) which have partly overcome some of these problems. However, these are sophisticated devices which require special attention and also create other problems; e.g. the rate of oxygen flow should be within narrow limits to avoid explosion or formation of smoke.

The above account indicates the need for a better method of counting  $^{14}\text{C}$  in soil when many samples of low activity are involved.

## 2. Method of incubation

It has always been an assumption that substrates incorporated into soil for incubation studies have been well distributed within the soil. Thus during incubation of organic materials in the soil, a known weight of the substrate has been added to the soil and the mixture thoroughly stirred. Such a treatment has been intended to give an even distribution of both the substrate and micro-organisms within the soil matrix. The soil aggregates were usually not affected resulting in incomplete distribution of the substrate through the soil. Both the substrate and micro-organisms were distributed on the surfaces of, but not within, the aggregate. Thus an artificial situation was created.

The ultimate result from such a procedure is easy accessibility of the organic material to microbial attack. If this was the case under natural conditions then very little easily decomposable organic matter would be left in the soil.

In cultivated soil the organic matter eventually becomes well mixed with the soil, and some is locked up in aggregates. This explains

the increase in microbial activity after tillage (Rovira and Greacen, 1957) since after tillage more organic matter becomes available to micro-organisms.

If meaningful results are to be obtained from laboratory incubation experiments then a different approach from normal should be used. A method of incubation which would take into account the fact that organic materials could be found both within and outside aggregates would give results more meaningful in soil organic matter studies.

## B. INCUBATION OF $^{14}\text{C}$ -LABELLED COMPOUNDS AND MATERIALS IN SOIL

### 1. Laboratory studies

#### (i) Glucose

$^{14}\text{C}$ -glucose has often been used as the simplest form of carbohydrate to study both kinetics of  $\text{CO}_2$  release and turnover rates of soil organic matter (Bingeman et al., 1953; Simonart and Mayaudon, 1958; Jansson, 1960; Chahal and Wagner, 1965; Mutatkar and Wagner, 1967; Chahal, 1968; Persson, 1968; Cheshire et al., 1969; Oades and Wagner, 1971; Oades, 1974; Ladd and Paul, 1973). Glucose is a small water-soluble molecule vulnerable to attack by micro-organisms and is metabolised by most soil organisms within a few days of incorporation into soil.

Some results of  $^{14}\text{C}$ -glucose decomposition in different soils are presented in Table 1. The results do not allow any specific generalization to be made with respect to the soil characteristics, as results have differed from one incubation to another. There are however

Table 1. Laboratory studies on <sup>14</sup>C-labelled glucose decomposition in soil

| Source of Amendment | Soil Properties          |             |      | Amount added mg/100 g | Period of Incubation | <sup>14</sup> CO <sub>2</sub> released % Initial Value | Reference                     |
|---------------------|--------------------------|-------------|------|-----------------------|----------------------|--|-------------------------------|
|                     | Texture                  | Org. C (%)  | pH   |                       |                      |  |                               |
| (1) Glucose         | Peat (top 6")            | 44.5        | 4.9  | 240                   | 46 days              | 79.10  | Bingeman <i>et al.</i> (1953) |
|                     |                          |             |      | 480                   | 46 days              | 81.50  |                               |
| (2) Glucose         | Sandy loam               | 3.0         | 6.5  | 250                   | 60 days              | 88.96  | Simonart and Mayaudon (1958)  |
| (3) Glucose         | Sandy loam               | 1.79        | 6.9  | 62.5                  | 112 days             | 79.84  | Jansson (1960)                |
|                     |                          |             |      | 62.5                  | 105 days             | 76.80  |                               |
| Glucose             | Sandy clay loam          | 2.34        | 5.1  | 62.5                  | 98 days              | 72.16  | Jansson (1960)                |
|                     |                          |             |      | 62.5                  | 91 days              | 69.20  |                               |
|                     |                          |             |      | 62.5                  | 112 days             | 63.36  |                               |
|                     |                          |             |      | 62.5                  | 105 days             | 64.96  |                               |
|                     |                          |             |      | 62.5                  | 98 days              | 61.92  |                               |
|                     |                          |             |      | 62.5                  | 91 days              | 60.00  |                               |
| (4) Glucose         | Silt loam                | (a) 0.788   | 4.50 | 250                   | 90 days              | 75.00  | Chahal and Wagner (1965)      |
|                     |                          |             |      | 250                   | 90 days              |  |                               |
|                     |                          |             |      | 250                   | 90 days              |  |                               |
|                     |                          |             |      | 250                   | 90 days              |  |                               |
|                     |                          |             |      | 250                   | 90 days              |  |                               |
| Glucose             | Silt loam (soil columns) | same as (d) | (d)  | 250                   | 90 days              | 85.00  |                               |
|                     |                          |             |      | 250                   | 90 days              | 77.00  |                               |
| (5) Glucose         | Sandy loam               | 1.9         | 7.0  | 250                   | 36 months            | 82.00  | Persson (1968)                |
| Glucose             | Sandy clay loam          | 3.7         | 5.7  | 250                   | 36 months            | 86.60  | Persson (1968)                |
| (6) Glucose         | Loam sandy soil          |             |      | 250                   | 45 days              | 55.30  | Chahal (1968)                 |
|                     |                          |             |      | 250                   | 45 days              | 63.95  |                               |
|                     |                          |             |      | 250                   | 45 days              | 63.80  |                               |
| (7) Glucose         | Loam                     |             | 4.6  | 1000                  | 14 days              | 84.00  | Cheshire <i>et al.</i> (1969) |
| (8) Glucose         | Silt loam                | 1.24        | 6.00 | 128                   | 28 days              | 75.00  | Oades and Wagner (1971)       |
| (9) Glucose         | Fine sandy loam          | -           | -    | 300                   | 50 days              | 66.00  | Ladd and Paul (1973)          |
| (10) Glucose        | Fine sandy loam          | 2.8         | 6.5  | 80                    | 15 days              | 85.00  | Oades (1974)                  |

extremes of decomposition with release of  $^{14}\text{CO}_2$  varying from about 55 to 90% of the initial isotope incorporated. On the other hand, release of  $^{14}\text{CO}_2$  does not depend on the days of incubation since samples incubated for only 14 days (Cheshire et al., 1969; Oades, 1974) and those incubated for 60 days or up to three years (Simonart and Mayaudon, 1958; Chahal and Wagner, 1965; Persson, 1968) have all lost over 80% of initial activity added to the soil as  $^{14}\text{CO}_2$ .

Whilst conditions that prevail in laboratory incubations may differ from those in the field, attempted simulation of field conditions have not shown much difference between the two. Chahal and Wagner (1965) showed this by studying the decomposition of  $^{14}\text{C}$ -labelled glucose in flasks with 100 g of dried soil, and also in soil columns (simulating a soil profile) in plastic bags.

(ii) Decomposition of polysaccharides

Other  $^{14}\text{C}$ -labelled carbohydrates which have been used in decomposition studies include dextran (Oades and Wagner, 1971), starch (Cheshire et al., 1969), hemicellulose (Mayaudon and Simonart, 1959a; Sorensen, 1963, 1966, 1967) and cellulose (Mayaudon and Simonart, 1959b; Sorensen, 1963, 1967; Igel, 1969). All have been found to decompose in a manner similar to glucose, though the rate of decomposition may differ. Thus, despite the fact that, over 80% of all substrates were decomposed in 60 days during incubation of glucose, hemicellulose and cellulose (Simonart and Mayaudon, 1958) only 18% and 50% of both cellulose and hemicellulose respectively had decomposed in 10 days as opposed to 75% of glucose in the same period.

Cellulosic fractions prepared from two different sources decomposed differently (Mayaudon and Simonart, 1959a). The difference was attributed to the level of lignin in the two fractions. The differences in decomposition for the two fractions was reflected in the decomposition of the two components (cellulose and lignin) since 80% of cellulose alone was decomposed in contrast to 30.0% of lignin in the same period.

(iii) Incubation of plant components

Not only is there a difference in extent of decomposition of labelled components of the same plant prepared in a different way but also the same component from different plants decompose differently (cf. Mayaudon and Simonart, 1958, 1959a, and Sorensen, 1963). Sorensen's (1963) comparative figures for decomposition of hemicellulose, cellulose, straw, compost and lignin (prepared from barley plant) were 65%, 61%, 43%, 22% and 4% respectively in 100 days. All these figures were lower than those obtained by Mayaudon and Simonart (1958) for cellulose, hemicellulose (prepared from rye grass), and Mayaudon and Simonart (1959a) for lignin from the rice plant. The very low level of decomposition of lignin (Sorensen, 1963) has been attributed to the drastic treatment used to obtain it. However, this would not account for the difference between the two plants since the same drastic conditions had been used to prepare the two sources of lignin.

(iv) Incubation of other <sup>14</sup>C-labelled materials

The decomposition of a variety of <sup>14</sup>C-labelled compounds other than carbohydrates have been studied. The smallest compound used was <sup>14</sup>C-acetate (Stevenson and Ivarson, 1964; Ivarson and Stevenson,

1964; Sorensen and Paul, 1971; McGill, 1971; Shields, 1972).

From 80 to 89% was decomposed after 10 weeks incubation (McGill, 1971; Sorensen and Paul, 1971; Shields, 1972).

Stevenson and Ivarson (1964) and Ivarson and Stevenson (1964), however, showed that only about 25 to 30% of the acetate was released as  $\text{CO}_2$  in 6 to 9 hours of incubation, the rest was apparently assimilated into microbial tissue. This shows that it is even more rapidly utilized than glucose.

An initial lag phase, obtained by Sorensen and Paul (1971), McGill (1971), and Shields (1972) might be due to adjustment of the microbes to the soil pH prevailing since this lag phase was not obtained in the short-term studies of Stevenson and Ivarson (1964) and Ivarson and Stevenson (1964). That this might be the case is supported by Alexander's (1961) statement that "the absence of both ethanol and acetate in the soil may be due to the fact that they are easily metabolised. It seems likely then that the autochthonous population is adapted to the two compounds and therefore they are utilized without any lag phase".

(v) Incubation of  $^{14}\text{C}$ -labelled plant extracts

Work done on the decomposition of other  $^{14}\text{C}$ -labelled substrates, especially extracts of plant origin (Simonart et al., 1959, 1961; Mayaudon and Simonart, 1959b) show the same trend of decomposition as that of the carbohydrates, in that optimum metabolic rate was obtained during the first week of incubation. Thereafter, the rate slows down dramatically. However, the rate of decomposition was extremely slow in some extracts

resulting in very low total decomposition.

In an incubation of proteins (foliar globulin), hydrolysates of the proteins (foliar globulin), chlorophyll  $\beta$ , and  $\beta$ -carotene it was found that 45%, 71%, 11.00% and 10.00% respectively were released as  $\text{CO}_2$  in 30 days. In the same experiment, three phenolic aldehydes - syring-aldehyde, vanillin and p-hydroxy-benzaldehyde, decomposed and released  $\text{CO}_2$  to the extent of 83%, 82% and 84% respectively of the  $^{14}\text{C}$  added as substrate. Lower percentage decomposition of foliar globulin was attributed to the fixation of the protein by the organic matter.

(vi) Incubation of  $^{14}\text{C}$ -labelled plant material

The use of growth chambers (Andersen et al., 1961; Hayes and Mortensen, 1963; Jenkinson, 1960; Kuzin and Tokaskya, 1959; Sauerbeck, 1960; Scharpenseel, 1961; Scully et al., 1955; Sauerbeck and Führ, 1966; Smith et al., 1962, 1963; Zeller et al., 1966) which range from industrial scale (Scully et al., 1955) to laboratory scale (Jenkinson, 1960), have made possible the production of labelled plant materials. These have to be prepared uniformly-labelled otherwise results become difficult to interpret. Jenkinson (1966c) has cautioned on the use of non-uniformly labelled plant material in soil organic matter studies.

Decomposition of plant materials differs from simple compounds like glucose in that the rate of decomposition is slower. This is not unexpected of a material which consists of different forms of macromolecules with varying resistance to microbial attack. Thus, about 76 and 38% of glucose and rye grass had been released as  $\text{CO}_2$  during the first ten days of incubation (Simonart and Mayaudon, 1958a). However,

after 60 days all fractions had released 80 to 89% of  $^{14}\text{C}$  as  $^{14}\text{CO}_2$ .

In a similar experiment, Sorensen (1963) found that between 35 and 43% of  $^{14}\text{C}$ -barley straw had decomposed in 100 days as opposed to 65% of hemicellulose. Jenkinson (1960) used either rye grass roots or tops and found that about one-third of the labelled carbon remained in the soil after 156 days of incubation.

Different laboratories have obtained different results. This is evident from a summary of decomposition of  $^{14}\text{C}$ -labelled materials (Simonart and Mayaudon, 1966) in both laboratory and field. There are extreme figures obtained for other plants, e.g. alfalfa plant tissue (Keefer and Mortensen, 1963) decomposed to the extent of 50% in 160 days. Barley straw was decomposed more than rape (Sauerbeck, 1966), the extent of decomposition being related to the nitrogen content. Rape had a C/N ratio of 8.3 as against 58.3 for straw.

Decomposition of various components of maize, wheat and soybean plants was studied by Smith (1966). Figures obtained showed that there was decomposition to the extent of 68 to 96% in 28 days in a soil which was not pre-incubated. In 59 days, as much as 76 to 100% of the plant materials had decomposed. Incubation performed with pre-incubated soil samples gave losses from 60 to 100% in 28 days. The release of 100% of the  $^{14}\text{C}$  added, in the form of  $^{14}\text{CO}_2$  indicates experimental errors. Samples ground to less than 60 mesh-sieve released less  $^{14}\text{CO}_2$  than similar samples which were ground to less than 20 mesh. This difference was probably due to the fact that materials in the finer particles were rendered physically inaccessible to attack by microbes (Rovira and Greacen, 1957) or their enzymes, after mixing with the soil. Roots

were found to be more resistant to decomposition than other parts of the plant (Smith, 1966). Field studies have shown that, though decomposition of roots may initially be slow, as compared to above-ground phytomass, the overall rates of decomposition were similar for both roots and tops (Dahlmann and Kucera, 1965; Dahlmann, 1968; Ignatenko *et al.*, 1968; Jenkinson, 1960, 1971).

(vii) Incubation of  $^{14}\text{C}$ -labelled microbial tissue

The addition of  $^{14}\text{C}$ -glucose to soil is a means of labelling the biomass and recent metabolites (Oades and Ladd, 1974). Generally, in incubation studies involving  $^{14}\text{C}$ -glucose it may be assumed that after 3 to 5 days decomposition of labelled micro-organisms and their metabolic products rather than the original substrate is being followed.

It has recently been suggested (Ladd and Paul, 1973; Shields, 1972) that the period of depletion of  $^{14}\text{C}$ -glucose is as short as 1.5 days. About 96% of the glucose carbon had been metabolised at this stage (Ladd and Paul, 1973) with only 31% of the original glucose evolved as  $^{14}\text{CO}_2$ , the rest, about 65% probably in the soil biomass or metabolites.

Kinetics of decomposition after the initial period has revealed that metabolic activity is relatively low and it has therefore been assumed that microbial cells are comparatively resistant to decomposition (Mutatkar and Wagner, 1967; Wagner, 1968).

Whilst the in situ labelling of microbes has provided a lot of information in soil organic matter studies, the source of released  $^{14}\text{CO}_2$  has been unknown. The addition of labelled microbes directly to the

soil with the subsequent incubation therefore has allowed comparative studies of utilization of individual organisms (Mayaudon and Simonart, 1963) and different components of the organisms e.g. cell walls of a particular organism (Hurst and Wagner, 1969).

Incubation of uniformly labelled Azotobacter vinelandii (bacteria) and Aspergillus niger (fungus) in soil showed that 60% of the  $^{14}\text{C}$  of Azotobacter vinelandii and 45%  $^{14}\text{C}$  in the Aspergillus niger had been released as  $^{14}\text{CO}_2$  in 60 days (Mayaudon and Simonart, 1963). The low release of  $^{14}\text{CO}_2$  for Aspergillus niger was supported by Zeller *et al.* (1966) who obtained 55% and 48% for  $^{14}\text{CO}_2$  released in two different incubation studies lasting three months each.

A study on the decomposition of cytoplasmic and cell wall fractions of two different fungi - hyaline and melanic fungi - showed that the cell walls of hyaline fungi decomposed more readily than their cytoplasmic fractions (Wagner, 1968; Hurst and Wagner, 1969). In contrast, the cell wall of melanic fungi was comparatively more resistant to decomposition than the cytoplasmic fraction. The  $^{14}\text{CO}_2$  evolved by using cell wall of hyaline fungus was 60% more than that from the melanic fungus.

Thus, the assumption that cell walls of micro-organisms may accumulate in soil organic matter does not necessarily apply to hyaline fungi (Wagner, 1968; Hurst and Wagner, 1969).

Decomposition of Nitrosomonas europaea was approached in a different manner (Jenkinson, 1962). The idea was to demonstrate that  $^{14}\text{C}$  left in the soil after incubation actually ended up in the soil biomass.

Uniformly labelled Nitrosomonas europaea was incorporated into a calcareous soil, and subjected to the following treatments: chloroform vapour, oven drying or no treatment. Remoistening and incubation for ten days showed that the oven-dried soil evolved 37% of the microbial carbon as  $^{14}\text{CO}_2$  as opposed to 28% and 20% for the chloroform-treated and the untreated soil respectively. Since the sequence of decomposition was the same as had been previously obtained for a similarly treated labelled soil (pre-incubated with labelled plant material for a year in the field), it confirmed the hypothesis that the biomass was the fraction of the organic matter influenced by the treatment. The in situ labelling of the micro-organisms therefore, really took place.

## 2. Field studies

Except for the recent work by Shields, Paul, Lowe and Parkinson (1973), all studies with  $^{14}\text{C}$  in the field have been concerned mainly with labelled plant material. In the field Shields et al. (1973) used galvanised cylinders embedded in the soil to the plow depth of 12 cm. About 61% of the  $^{14}\text{C}$ -glucose was released as  $^{14}\text{CO}_2$  after 104 days of incubation.

On the whole, the proportion of added plant material retained in the soil as a result of decomposition under different climatic conditions has been similar (Jenkinson, 1971; Shields, 1972). With the exception of very acid soils, about one-third of the added plant carbon remained after a year and this fell to about one-fifth after five years. In acid soils, though release of  $^{14}\text{CO}_2$  might be retarded in the initial period, the effect appears transient (Oades and Ladd, 1974). This sequence of decomposition was obtained even if fresh green plant

materials were used. This contrasts with the general view that fresh plant materials rapidly decompose in the soil (Jenkinson, 1971).

However, decomposition within the first six months depended on the type of plant used. With rye grass (Jenkinson, 1964, 1965, 1968), the same amount (about one-third) was left in the soil after six months and one year. Matured wheat straw (Führ and Sauerbeck, 1968) decomposed to the extent of 40 to 45% in the first six months, but after one year 59 to 65% had been released as  $^{14}\text{CO}_2$  depending on whether the soil was bare or cropped. Similarly, 42 to 58% of immature maize had decomposed after six months, whilst the decomposition was 55 to 73% by the end of one year. Sauerbeck (1968) compared the turnover of uniformly labelled mature wheat straw, green rape tissues and tagged animal manure (rat dung). After 13 weeks in soil, 50% of the added carbon remained, with carbon from the straw being just as stable as that from the animal dung.

Initial decomposition of plant material in soil was retarded by growing plants (Jenkinson, 1971), and in plots under small grains as compared with those under root crops or fallow (Führ and Sauerbeck, 1968). Retardation of decomposition under growing plants has been attributed to drier conditions obtained in the presence of growing crops (Shields, 1972).

Even though the preliminary stages of decomposition of roots was slower compared with above-ground phytomass, the overall rate was similar for both tops and roots (Dahlmann and Kucera, 1965; Dahlmann, 1968; Ignatenko et al., 1968). Provided the carbon addition did not exceed 1.5% of the dry weight of the soil, and decomposition was allowed to continue for at least 3 to 6 months, the  $^{14}\text{CO}_2$  released above that of

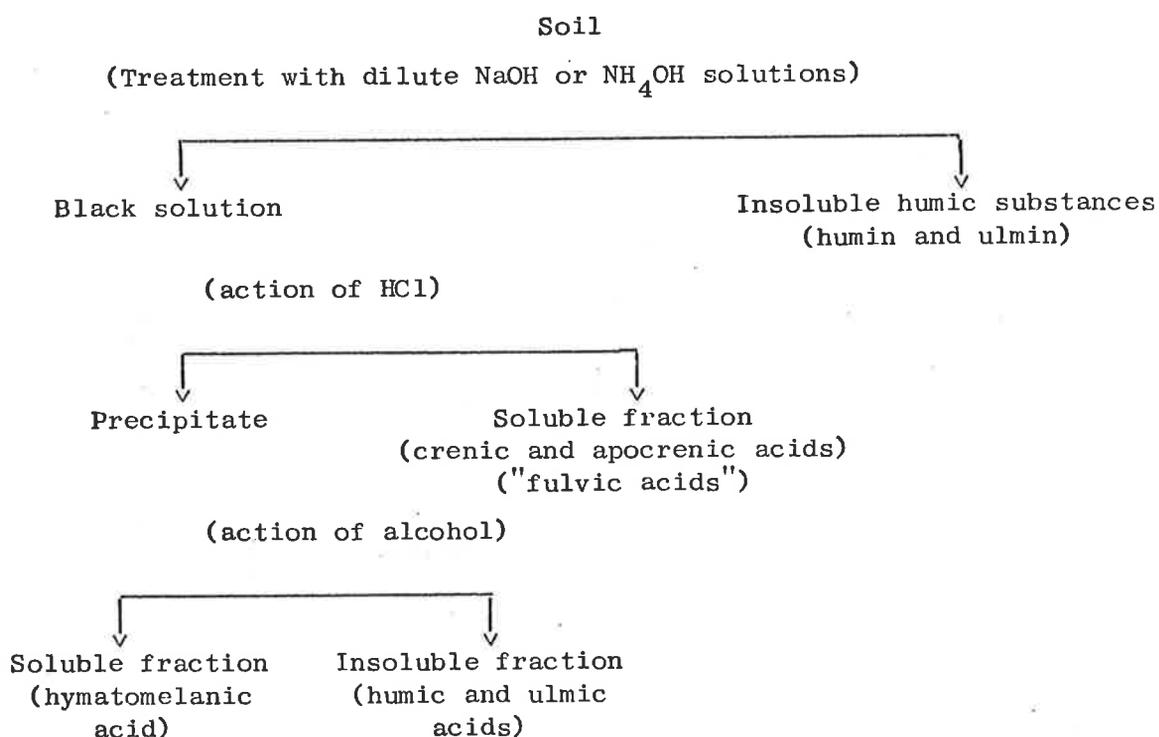
the control sample was always independent of quantity added (Jenkinson, 1971). This is in agreement with earlier work on non-isotopic materials (Pinck and Allison, 1951).

### 3. Fate of residual $^{14}\text{C}$ in the soil

#### (i) Fractionation procedure

Whilst decomposition of  $^{14}\text{C}$ -labelled material in soil has been determined by the release of  $^{14}\text{CO}_2$  or based on the activity of  $^{14}\text{C}$  left in the soil, the nature of the residual  $^{14}\text{C}$  remains unknown.

The distribution of  $^{14}\text{C}$  between various chemical fractions of soil organic matter has been determined after different periods of incubation. A number of different extraction and fractionation procedures have been used (Persson, 1968). In most cases however, they have been derived from a main type, illustrated as follows (Persson, 1968):



Individual investigators have modified this basic procedure to suit the purpose of the investigation. Simonart and Mayaudon (1966) and Ivarson and Stevenson (1964) have combined this with the proximate analysis of Waksman (1939).

(ii) Distribution of  $^{14}\text{C}$  in classical fractions of organic matter

Jenkinson (1971) has discussed in detail the limitations of classical fractionation of organic matter. Often the labelled material added to the soil is distributed through different fractions of soil organic matter before decomposition even starts. This was the case when labelled microbial tissue (Mayaudon and Simonart, 1965a) or plant proteins (Simonart and Mayaudon, 1961) were used.

The most serious disadvantage of the method was that rearrangements occurred during fractionation (Sauerbeck and Führ, 1968). More  $^{14}\text{C}$  labelled humic acid and less labelled fulvic acid was obtained when fresh labelled plant material was extracted with alkali in the presence of soil than when the plant material was extracted alone (Sauerbeck and Führ, 1968). It was concluded that certain acid soluble fulvic plant constituents were converted to the acid insoluble humic form during fractionation, presumably by sorption on soil colloids.

Generally, addition of  $^{14}\text{C}$  labelled protein (foliar globulin),  $\beta$ -carotene, lignin and the phenolic aldehydes (vanillin, syringaldehyde, p-hydroxy-benzaldehyde) to soil resulted in considerable  $^{14}\text{C}$  appearing in the humic acid fraction (Simonart and Mayaudon, 1966). On the other hand, incubation of  $^{14}\text{C}$  labelled barley, hydrolysates of globulin, hemicellulose, cellulose, chlorophyll and glucose in soils resulted in

more of the  $^{14}\text{C}$  in the insoluble humic fraction (Mayaudon and Simonart, 1958, 1959a, 1959b; Simonart and Mayaudon, 1958a, 1961; Simonart *et al.*, 1959. In most cases, there was very little incorporation of  $^{14}\text{C}$  into the fulvic acid fraction. Mayaudon and Simonart (1966) reported that when  $^{14}\text{C}$ -labelled glucose, cellulose or hemicellulose decomposed, the  $^{14}\text{C}$  residue was distributed in different fractions in proportions similar to that of the organic nitrogen, rather than the soil organic carbon.

It has also been demonstrated (Mayaudon and Simonart, 1963) that the classical chemical fractionation scheme fractionates microbial tissue and that the presence of soil alters this fractionation (Oades and Ladd, 1974).

|                            | <u>Soluble in 80%<br/>alcohol</u> | <u>Fulvic<br/>fraction</u> | <u>Humic<br/>fraction</u> | <u>Humin<br/>fraction</u> |
|----------------------------|-----------------------------------|----------------------------|---------------------------|---------------------------|
| <u>Aspergillus</u>         | 48.7                              | 9.8                        | 6.8                       | 34.7                      |
| <u>Aspergillus</u> in soil | 33.4                              | 8.5                        | 13.5                      | 44.6                      |
| <u>Azotobacter</u>         | 64.5                              |                            | 35.5                      | 0                         |
| <u>Azotobacter</u> in soil | 21.5                              |                            | 60.7                      | 7.6                       |

There was a marked shift towards insoluble fractions when the fractionation was done after addition of the labelled organisms to soil. Subsequent incubation revealed that  $^{14}\text{C}$  was lost from all fractions, particularly from the soluble and fulvic fractions, whilst the humin fraction eventually contained an increased proportion of the label. The above figures show that bacterial components contribute more to the soluble fractions whilst fungal components contribute more to the humin.

The evidence so far gathered shows that fungal components are less soluble and thus contribute to the humin fraction. However, Wagner (1968) and Hurst and Wagner (1969) pointed out that both cell wall and cytoplasm of hyaline fungi were readily utilized when incubated in soil, but cell walls of a melanic fungus showed considerable resistance to decomposition. High amounts of humin components in a number of soils, including acidic soils, may therefore be due in part to resistant, insoluble fungal materials (Oades and Ladd, 1974).

That this is the case has been demonstrated by a number of workers. Wagner (1968) quoted work by Kirkham in which fractionation of Aspergillus niger showed 54% of the microbial tissue in the humin fraction. Zeller et al., (1966) showed that after 30 days of incubation in soil of  $^{14}\text{C}$ -labelled Aspergillus niger, more than 60% of the  $^{14}\text{C}$  was incorporated in the humin fraction.

Carter and Oades (unpublished data) grew fungi for 10 days on an agar containing  $^{14}\text{C}$ -labelled glucose and allowed the hyphae to grow through a bed of sterilized soil aggregates, placed immediately above but not in contact with the agar. Fractionation of the aggregates plus labelled hyphae gave the following distribution of  $^{14}\text{C}$ .

| Fraction        | <u>Rhizoctonia<br/>solani</u> | <u>Stachybotrys<br/>atra</u> | <u>Ceratobasidium<br/>cornigerum</u> |
|-----------------|-------------------------------|------------------------------|--------------------------------------|
| Light fraction  | 46.7                          | 25.0                         | 20.9                                 |
| Acid extract    | 12.4                          | 13.5                         | 32.7                                 |
| Fulvic fraction | 3.7                           | 9.6                          | 7.8                                  |
| Humic fraction  | 8.6                           | 14.4                         | 8.8                                  |
| Humin fraction  | 28.5                          | 37.5                         | 29.7                                 |

Thus, if a soil is subjected to density fractionation before chemical treatment, a large part of the  $^{14}\text{C}$  is recovered in the light fraction which contained a lot of hyphae (Oades, 1974). Most of the hyphal material either floated as recognizable hyphal fragments at density 2 or was insoluble. Highest specific activities were found in the light fraction and the acid extracts. High specific activities have been found in an acid fraction (Oades, 1974), and also in extracts from mild acid treatments (Jenkinson, 1965).

(iii) Distribution of  $^{14}\text{C}$  in identifiable compounds during decomposition of  $^{14}\text{C}$ -labelled substrates

The report by Mayaudon and Simonart (1966) that when  $^{14}\text{C}$ -labelled glucose, cellulose or hemicellulose were decomposed the  $^{14}\text{C}$  residues were distributed in proportions approximating that of the soil organic nitrogen rather than the soil organic carbon, has demonstrated that most of the residual  $^{14}\text{C}$  was in either amino acids or proteins. That the amino acids are those normally encountered in microbial cell walls has been shown by Wagner and Mutatkar (1968) and Wagner (1968). Carbon-14 has been found in a wide range of amino acids (Oades and Ladd, 1974) but alanine, glycine, glutamic acid and lysine, which are abundant in microbial cell walls, have been found to possess the highest specific activities. Results obtained during incubation of either  $^{14}\text{C}$ -labelled glycine or acetate have shown the same trend (Ivarson and Stevenson, 1964; Mayaudon and Simonart, 1965b; Sorensen and Paul, 1971).

Distribution of  $^{14}\text{C}$  in individual monosaccharides has also been studied. There was a rapid incorporation of  $^{14}\text{C}$  into the hexoses -

glucose, galactose and mannose - which accounted for about 80% of the  $^{14}\text{C}$  in aldoses during decomposition of carbohydrates (Keefer and Mortensen, 1963; Cheshire *et al.*, 1969, 1971; Oades and Wagner, 1970, 1971; Oades, 1974; Oades and Ladd, 1974). There was only slight labelling of xylose and arabinose whilst labelling of rhamnose and fucose was not consistent. This demonstrates that most of the pentoses in soils come from plant materials (Oades and Ladd, 1974), whilst the hexoses arise mainly from microbial cells.

Oades (1974) has shown that a microbial glucan could be isolated in a light fraction (< 2.0 S.G.) and that most highly labelled sugars extracted by cold N-HCl were markedly concentrated in low molecular weight fractions according to gel chromatography on Sephadex G.10. There has also been incorporation of  $^{14}\text{C}$  into phenolics (Mayaudon, 1968; Sinha, 1972a and b) and probably other larger molecules of microbial origin would be labelled (Oades and Ladd, 1974).

(iv) Stability of  $^{14}\text{C}$ -labelled organic residues in the soil

The initial rate of microbial activity following introduction of  $^{14}\text{C}$ -labelled substrates has always been short-lived. Once a portion of the  $^{14}\text{C}$ -labelled substrates has been assimilated into microbial structures it becomes relatively inactive (Persson, 1968) in terms of decomposition. Evidence to support this has already been discussed [Section B.3.(iii)].

However, the biological stability of the  $^{14}\text{C}$  residues in soil cannot be attributed entirely to their chemical structures (Oades and Ladd, 1974). Rather the stability has been attributed to the existence

of conditions within the soil microenvironment which limit the opportunity for reaction between enzyme and substrate.

Residual  $^{14}\text{C}$  could be stabilized for a time in the soil biomass (Jenkinson, 1966d) and probably sorbed on soil colloids. Sorption on soil colloids was demonstrated by Wagner (1968) and Mutatkar and Wagner (1967), who were able to release about 30% more of the  $^{14}\text{C}$  residue after treating their humin fraction (obtained from a soil incubated with  $^{14}\text{C}$ -glucose) with hydrofluoric acid (HF) to destroy clay minerals.

Evidence has been given above [Section B.3.(iii)] that amino acids in microbial cells are heavily labelled during incubation of  $^{14}\text{C}$ -labelled substrates and sorption of the residual  $^{14}\text{C}$  on the inorganic fraction of the soil would therefore involve the organic nitrogen fraction of the residue.

Reports relating to theories that soil organic-N is stabilized by adsorption to clay minerals (Oades and Ladd, 1974) or reaction with resistant organic constituents (lignin, polyphenolic heterocondensates, tannins) has been reviewed by Estermann and McLaren (1959), Bremner (1965, 1967, 1968), Greenland (1965), Mayaudon (1968) and Swaby (1968).

Studies which support the hypothesis that clay minerals stabilize soil organic matter have been undertaken by Durand (1964), Sorensen (1965, 1967, 1969, 1972) and Colom and Wolcott (1967). Sorensen (1969) produced evidence to show that the metabolites fixed by addition of montmorillonite during incubation of  $^{14}\text{C}$ -glucose or  $^{14}\text{C}$ -cellulose were enzyme proteins excreted during the incubation.

Stability which involves reaction with the inorganic fraction of the soil may not involve only proteins or amino acids. Greenland (1965a and b) suggested that sorption of high molecular weight carbohydrates and oligosaccharides (and even monosaccharides present at high concentrations) could take place. Substrate was therefore rendered inaccessible to the enzyme unless there were other factors to change the situation. Such factors could be physical, such as wetting and drying cycles, or mechanical disturbance of the system to incorporate the substrate.

Stability of residual  $^{14}\text{C}$  in soil may also involve physical protection in soil micro-aggregates (Rovira and Greacen, 1957). Not much work has been done with labelled materials and work involving unlabelled substrates is discussed later.

#### 4. Factors affecting results obtained during decomposition studies in soil

Factors which affect the results in decomposition studies are not different from factors which have been encountered using unlabelled substrates. These factors include organic carbon and nitrogen contents of the soil, pH, oxygen supply, temperature, type of soil which also reflects the type of clay, and amount of organic material added. All these factors are encountered whether studies are done in the field or in the laboratory, and whether a simple compound like glucose or complex material such as whole plant material is used.

Oxygen supply during the early stages of decomposition or available surface area in the soil may influence the decomposition rate of a

compound such as glucose (Oades and Ladd, 1974). Both C/N ratio and pH may also play an important part in the initial stages of decomposition. Chahal and Wagner (1965) found that the level of  $^{14}\text{CO}_2$  released in the first week of incubation of  $^{14}\text{C}$ -glucose in a soil with different previous history was related more to the nitrogen content. However, all samples had released  $^{14}\text{CO}_2$  to the same extent by 90 days.

On the other hand, Simonart's (1964) earlier work showed that more  $^{14}\text{CO}_2$  was liberated during decomposition of  $^{14}\text{C}$ -glucose alone than that to which supplementary nitrogen was added.

Persson (1968) demonstrated an early effect of pH and the persistence of the pH effect was shown by Chahal (1968) who found that during incubation for 45 days a loamy soil of pH 4.75 lost only 55% of the incorporated  $^{14}\text{C}$ -glucose as  $^{14}\text{CO}_2$  as opposed to 64% from the same soil which had been adjusted to pH 7.4 or 8.9 with  $\text{Ca}(\text{OH})_2$  solution. Such differences in decomposition imposed by the pH of the soil has been attributed to the different microbial populations developing. Soils with a lower pH would presumably have a greater relative population of fungi (Wagner, 1968).

The nature of the clay present in the soil is important inasmuch as it affects the stability of residual  $^{14}\text{C}$  in the soil [this has been discussed fully elsewhere - Section B.3.(iv)].

Even though Broadbent and Bartholomew (1948) found that small amounts of  $^{13}\text{C}$ -labelled plant materials decomposed faster than large quantities, this finding has not been wholly supported by work with  $^{14}\text{C}$ -labelled plants (Hallam and Bartholomew, 1953; Sauerbeck, 1966, 1968). For

carbon additions of up to 2 per cent of the soil, the proportions of plant carbon lost and retained in the soil were little affected by the amount added (Stotzky and Mortensen, 1958; Mortensen, 1963; Sorensen, 1963; Jenkinson, 1965; Oberlander and Roth, 1968). This finding agrees with work with unlabelled substrates (Pinck and Allison, 1951).

In the field, decomposition was initially retarded in soils growing plants (Führ and Sauerbeck, 1968; Shields, 1972) and also retarded in soils supporting grain crops compared with those growing root crops, or bare fallow (Führ and Sauerbeck, 1968). Other factors such as physical accessibility have been discussed elsewhere (Section C.).

Problems in estimation of  $^{14}\text{C}$  activity either in the soil or released as  $^{14}\text{CO}_2$  may affect results. The high values obtained by Smith (1966) might have been due to chemiluminescence caused by the scintillation fluor used. This would be a problem unless the counting vials were kept in the counter long enough for this factor to decay. (Counting of  $^{14}\text{C}$  had been discussed in Section A.).

#### C. MICROBIAL UTILIZATION OF ORGANIC MATERIALS IN SOIL AGGREGATES

In soils which are tilled annually most of the organic materials (in the form of disintegrated leaves and stubble) will have been incorporated into aggregates. The rate of decomposition in such situations would depend on how the organic materials become accessible to the soil organisms or their enzymes.

Distribution of both organic matter and micro-organisms occur in a randomised manner (Rovira and Sands, 1971; Gray et al., 1968), though

Seifert (1962) found no difference in numbers of bacteria in aggregates of different sizes. The individual organisms and the organic materials may be regarded as occupying separate ecological niches. Any disturbance of the soil which would lead to the reshuffling of the whole system would therefore lead to either exposure of the organic materials in close proximity to the organisms or their enzymes in such a way as to increase decomposition or such a change may tend to seclude both the organic material and the organisms in such a way that decomposition of the organic matter is suppressed.

That the former is normally the case has been demonstrated by several workers. After 25 or more years of cropping, the mean organic matter content of 28 soils in Georgia had decreased from 3.29 to 1.43% (Alexander, 1961). After a rapid decline in the first few years, the decrease became more gradual with further disturbance through cultivation. Thus, a sandy loam (a virgin forest soil) contained 2.30% of organic matter but the concentration had fallen to 1.59% after 3 years of cultivation (Giddens, 1957). A similar trend of results was obtained for the Urrbrae fine sandy loam (red brown earth) on which long term rotation trials have been established at the Waite Institute. The nitrogen content declined from 0.222% to 0.135% after 20 years of cultivation (Clarke and Marshall, 1947). This decline was correlated with a decline in aggregation. The C/N ratio of the organic matter was 12 over this period and therefore the results represent changes in the soil organic matter content due to annual cultivation.

The sudden decline in organic matter followed by a more gradual loss could be explained on the basis of physical accessibility. The

initial disintegration of the soil aggregates exposed considerable organic material to attack but successive cultivations did not cause such severe aggregate breakdown. This is possible because besides the fact that the amount of organic material left in the soil would be smaller after several cultivations, the residual organic materials might be protected in micro-aggregates which are relatively stable to the mechanical disturbance of cultivation.

Though this decline in soil organic matter due to cultivation has been observed for some time, the explanation has always been biological. Rovira and Greacen (1957) were the first to demonstrate categorically that tillage destroys aggregates and exposes the organic materials hitherto hidden in the aggregates to microbial attack. This was done by laboratory simulation of tillage practices.

Subsequently, Waring and Bremner (1964), Craswell et al. (1970), and Craswell and Waring (1972a and b) have shown that mineralization of organic nitrogen is more rapid in smaller aggregates but that the effect is more pronounced in heavier soils (Craswell and Waring, 1972a and b). Similarly, Seifert (1962, 1964) and Keresteny et al. (1963) found that nitrification occurred faster in smaller aggregates than bigger ones but such a trend was attributed to differences in aeration of the aggregates of different sizes (Seifert, 1964). On the other hand, Greenwood (1961) did not find any differences in rate of microbial respiration in 2 mm and 2 to 4 mm aggregates. Lack of differences was attributed to uniform distribution of micro-organisms throughout all the aggregates.

Much more work has been done on mineralization of organic nitrogen than organic carbon. The sensitivity of isotopic methods would be of help in demonstrating the effect of aggregate sizes on microbial decomposition of soil organic carbon.

D. EFFECT OF WETTING AND DRYING CYCLES ON DECOMPOSITION OF ORGANIC MATTER

Wetting and drying cycles have been found to increase decomposition of organic matter in soil (Lebedjantev, 1924; Corbet, 1934; Gooding and McCalla, 1945; Soulides and Allison, 1961; Broadbent et al., 1964; Stevenson, 1956; Birch, 1958, 1959, 1960; Agarwal et al., 1971). The process is not as yet understood and a number of interpretations have been proposed.

Birch and Friend (1956) and Birch (1958, 1959, 1960) made comprehensive studies on this process on East African soils. On the basis of their work Birch (1960) reached the following conclusions:

(a) More water-soluble organic material could be extracted after a long period of air-drying and this effect is enhanced with soils dried at 100°C.

(b) Organic materials which appear on moistening a dry soil rapidly decompose.

(c) Drying brings about changes in the organic gels which continue during ageing and are enhanced by heat. The changes are probably due to increased porosity of the soil on drying and heating, each of which lead to increased surface area. These changes are reversible after moistening, thus leading to a rapid decline following the flush in decomposition.

(d) High rate of decomposition and nitrogen mineralization may also be due to the high microbial activity associated with the youthful phase of a developing microbial population. This activity declines as the microbial population ages and thus the decline does not involve lack of substrate.

Recent investigations (Agarwal et al., 1971) support the above interpretation.

Though the changes in physical characteristic of the soil have been ruled out as possible explanations of this process, it may be a contributory factor. This is supported by the finding (Birch, 1959a and b) that respiration rates of soils previously treated with different soil solutions were dependent on the type of cation present. Since the type of cation is known to influence structural stability it might be expected to influence the amount of organic matter exposed to microbial attack (Greenwood, 1968).

The use of isotopic techniques could help to elucidate such a problem.

CHAPTER II. AIMS OF THE PROJECT

Some important points emerge from the literature reviewed and these can be summarized as follows:

(a) Despite the attention that has been given to microbial utilization of soil organic matter relatively little work has been done on the influence of soil structure on microbial activity in soil.

(b) Extensive studies have been undertaken using  $^{14}\text{C}$ -labelled materials in soil, but no attempt has been made to utilize  $^{14}\text{C}$  as a tool to study microbial activity in soil aggregates.

(c) The relative importance of different soil microbial populations in metabolising organic materials in soil aggregates is not understood.

(d) Most studies on microbial activities in soil aggregates have been based mostly on mineralization of organic nitrogen and little attention has been given to the metabolism of soil carbohydrates in the aggregates.

(e) The question of the effect of wetting and drying cycles on release of  $\text{CO}_2$  from soils has not been fully elucidated and usually biological interpretations are used to explain the phenomenon, and

(f) Methods of determining  $^{14}\text{C}$  in soil have been tedious and time-consuming.

Initially, the aims of this project were to evaluate

(a) the effect of soil structure on microbial activity, with particular reference to metabolism of carbohydrates,

(b) to determine the importance of physical treatments such as wetting and drying and disruption of aggregates on the metabolism of  $^{14}\text{C}$  compounds present in these aggregates, and

(c) to determine which of the major groups of organisms (particularly fungi and bacteria) are more important during utilization of carbohydrates in soil aggregates.

#### Assessment of microbial activity

Microbial activity in the soil has been measured by the level of enzymes present, oxygen consumption or  $\text{CO}_2$  evolution. However, as pointed out by Stotzky (1960) both non-biological consumption of oxygen and evolution of carbon dioxide (Bunt and Rovira, 1955) can occur to give erroneous interpretation of microbial activities based on such methods. Microbial activities in soil or soil aggregates have also been measured by the mineralisation of organic nitrogen together with the evolution of carbon dioxide (Waring and Bremner, 1964; Craswell et al., 1970; Craswell and Waring, 1972a and 1972b). Whilst none of these indices is entirely appropriate they serve as a guide to biological activity in the soil.

To study the accessibility of organic materials in aggregates to micro-organisms it was necessary to incorporate  $^{14}\text{C}$ -labelled substrates into the soil from which aggregates were subsequently prepared, and then to determine the  $^{14}\text{CO}_2$  evolved during incubation by liquid scintillation spectrometry.

In preliminary studies, problems were encountered with conventional methods of determining  $^{14}\text{C}$  in soil. Because the experimental program

required extensive use of  $^{14}\text{C}$  it was considered that suspension counting of  $^{14}\text{C}$  in soils offered good prospects for a routine method.

Suspension counting by liquid scintillation spectrometry has been applied to  $\text{Ba}^{14}\text{CO}_3$  stabilized by gelling agents such as aluminium stearate (Funt, 1956), thixcin - a castor oil derivative (White and Helf, 1956) and CAB-O-SIL - a finely divided silica (Ott et al., 1959).

Page et al. (1964) applied the method of suspension counting in a thixotropic gel to wet and dry samples of  $^{14}\text{C}$ -labelled clay suspensions. Subsequently Königler and Süb (1968) applied the method to counting  $^{14}\text{C}$  in soil. During the work reported in this thesis similar accounts appeared on suspension counting of  $^{14}\text{C}$  in soil, in papers by Cheshire et al. (1972) and Helweg and Sorensen (1973). However, there are more complications involved than have been suggested by these workers. The main problems anticipated were colour quenching, self absorption and the effects of large quantities of heavy metals e.g. iron. These important factors have been overlooked by the previous workers using this method for counting  $^{14}\text{C}$ -labelled soil particles.

Since the reliability of the results obtained in this thesis depend on the accuracy of the method considerable time and effort was expended in establishing a reliable quantitative method for the determination of  $^{14}\text{C}$  in soil in semimicro amounts.

CHAPTER III. AN EXAMINATION OF A METHOD OF SUSPENSION COUNTING  
OF  $^{14}\text{C}$  IN SOIL, SOIL EXTRACTS AND PLANT MATERIALS  
BY LIQUID SCINTILLATION

1. Introduction

The requirement for a reliable routine method for the determination of  $^{14}\text{C}$  in soil has been mentioned and previous attempts to produce such a method described (Chapter II). Basically, the method was developed for soil particles tagged with  $^{14}\text{C}$ , but to broaden the use of the method coloured samples such as plant materials, and acid and alkaline extracts of soils were also examined.

Direct counting of soil particles tagged with  $^{14}\text{C}$  has several advantages. The necessity for quantitative combustion to  $\text{CO}_2$  is eliminated. The method is simple and can be applied to small samples with low activity.

2. Materials and methods

2.1. Preparation of standards

2.1.1. Materials

2.1.1.1. Chemicals

Benzoic acid- $^{14}\text{C}$  with two levels of activity was used. Benzoic acid- $^{14}\text{C}$  (specific activity  $5.32 \times 10^6$  d.p.m./g) obtained from Packard Instrument Corporation was used as a primary standard. Benzoic acid- $^{14}\text{C}$  (specific activity 453  $\mu\text{Ci}/\text{mg}$ ) from Radiochemical Centre, Amersham, England, was used as a secondary standard. PPO (2,5-Diphenyl-oxazole) (Packard Instrument Corporation Inc.) was used as a primary

solute whilst dimethyl POPOP (1,4-bis[2(4-methyl-5-phenyloxazolyl)]-benzene) also obtained from Packard Instrument Corporation Inc. was used as a secondary solute. Both the primary and secondary solutes were obtained as scintillation grade. Toluene (analytical grade) was used as the solvent. Triton X-100 (Rohm and Haas) technical grade was from Robert Bryce and Co., Australia. This product was centrifuged and used as an emulsifying agent in the preparation of scintillation fluor for counting  $^{14}\text{CO}_2$  absorbed in alkaline solution. The centrifuged technical grade Triton X-100 behaved in the same manner as "purified" Triton X-100 from Packard Instrument Corporation. Instagel, a commercially prepared scintillation fluor, was obtained from Packard Instrument Corporation Inc. and was used as such. CAB-O-SIL (Packard Instrument Corporation Inc.) is a finely divided silica, which served as a thixotropic gel.

#### 2.1.1.2. Mineral and soil samples

Five different samples were used to establish counting techniques. All samples were ground in a Siebtechnik mill to pass through a 300-mesh sieve ( $< 53 \mu\text{m}$ ) and their colours were compared with a Munsell Colour Chart.

The samples were:

- (a) Acid-washed sand (greyish-white N 8/0)
- (b) Red brown earth (dull brown 7.5 YR 5/4). This was a representative fraction of the top soil of Urrbrae fine sandy loam, which is under pasture at the Waite Research Institute.
- (c) Ground-water rendzina (grey N 5/0)

(d) A commercially available iron hydroxide (Goethite,  $\alpha$ -FeOOH) described as "natural yellow" (2.5Y 7/8).

(e) A commercially available iron oxide (Haematite,  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>) which is described as "natural red".

### 2.1.2. Methods

#### 2.1.2.1. Preparation of a secondary standard of <sup>14</sup>C-benzoic acid

The <sup>14</sup>C-benzoic acid which was obtained in a glass capsule was dissolved with five 2 ml portions of redistilled methanol and transferred to a 250 ml beaker. The solution was evaporated to dryness in vacuo over P<sub>2</sub>O<sub>5</sub> and NaOH mixture at room temperature.

To the <sup>14</sup>C-benzoic acid in the beaker, 2.0 g of recrystallized unlabelled benzoic acid was added. 100 ml of hot distilled water was added to dissolve the mixture. 50 ml of hot distilled water was used to rinse the weighing vial of the unlabelled benzoic acid and added to the mixture. The solution was stirred to effect even distribution of the <sup>14</sup>C.

The hot solution was allowed to crystallize at room temperature for six hours. It was then transferred to a refrigerator at 2°C for 24 hours to complete the crystallization. Excess water was decanted off with a Pasteur pipette, the tip of which had been tapered to exclude any crystals.

Decanted solution was placed in the refrigerator to allow further crystallization. The decantation process was repeated and the solution obtained from this (60 ml) was concentrated to about 10 ml and placed in the refrigerator at 2° for further crystallization. The yield of

crystals obtained from the supernatant of the first decantation was negligible and the second crop was not added to the bulk. The wet crystals were dried over  $P_2O_5$  in vacuo to a constant weight.

2.1.2.2. Standardization of the secondary  $^{14}C$ -benzoic acid standard

Both the primary and secondary standards of  $^{14}C$ -benzoic acid were dried in vacuo over  $P_2O_5$  at room temperature for 24 hours. 5 mg and 10 mg portions of each were weighed in triplicate into scintillation vials. 10 ml of toluene-PPO-dimethyl POPOP scintillant (0.5% PPO plus 0.03% dimethyl POPOP w/v in toluene) was added to each vial. The vials were shaken vigorously and counted in a Packard Tri-Carb scintillation spectrometer Model 3375 operating at  $4^\circ C$ , after allowing for the equilibration of the vials with the temperature of the counter. Vials were counted for 5 min to give a standard deviation of 0.1% for the secondary standard and 0.5% for the primary standard. The settings used were 20 to 1000 with 6% gain as the wider window and 20 to 100 with 6% gain as the narrow window. The efficiency of counting was based on the recovery of counts obtained for the primary standard. The diluted  $^{14}C$ -benzoic acid which contained 20,000 d.p.m./mg was referred to as the secondary standard.

2.1.2.3. Preparation of  $^{14}C$ -tagged soil samples

Samples (4.9 g < 53  $\mu m$ ) were mixed with 100 mg of the  $^{14}C$ -benzoic acid secondary standard. The mixture was ground for two minutes with a Siebtechnik mill to ensure uniformity of the  $^{14}C$  in the sample. The samples then contained 400 d.p.m./mg. The mill was thoroughly cleaned by grinding five lots of acid washed sand with acetone

rinsing between each grinding.

## 2.2. Determination of balance point of counting

### 2.2.1. Introduction

Preliminary studies showed that it was not appropriate to use the same setting of the counter for suspension counting as had been applied for counting liquid samples. Low efficiencies were obtained when percentage gain amplifications of 6 were used. This is due to different optical characteristics involved in homogeneous and heterogeneous systems. Thus to find the percentage amplification that would give the maximum counting efficiency but relatively low background counts, a "balance point of counting" (Arnold, 1954) was obtained.

The balance point is a position in which the beta spectrum under consideration is symmetrical in the analyser window and is generally at the gain which gives the maximum efficiency in the particular discriminator window selected (Packard, 1958; Kobayashi and Maudsley, 1969). This position can accommodate any small shift (in the spectrum of an isotope) due either to slight fluctuations in line voltage, or to the performance of electronic components (Kobayashi and Maudsley, 1969) or factors due to the sample itself (Packard, 1958). It is therefore regarded as the most favourable operating voltage from the point of view of sensitivity (Arnold, 1954).

### 2.2.2. Materials

<sup>14</sup>C-tagged solid samples of the red brown earth and goethite were used.

### 2.2.3. Method

5, 10 and 50 mg of  $^{14}\text{C}$ -tagged solid samples were weighed into duplicate counting vials which contained 10 ml toluene-PPO-dimethyl POPOP scintillant (0.5% w/v PPO and 0.03% w/v dimethyl POPOP in toluene) and 500  $\pm$  10 mg of CAB-O-SIL. Vials were capped tightly and shaken for 2 minutes on a Vortex mixer using maximum speed. Samples were allowed to equilibrate with the temperature of the counter, which was 4°C, for one hour. The counter window was then set at 20 to 1000 and percent gain amplification varied from 0 to 60%. Counts after 60% gain provided no further information.

The percentage gain that gave the maximum counting efficiency but minimum background counts was then chosen as the balance point. This setting was used all through the studies.

### 2.3. Counting of $^{14}\text{C}$ -tagged solid samples

Duplicate samples (5 to 100 mg) were weighed into scintillation vials. 500  $\pm$  10 mg of CAB-O-SIL were added, along with 10 ml of toluene-PPO-dimethyl POPOP scintillant (0.5% w/v PPO and 0.03% w/v dimethyl POPOP in toluene). Caps with polyseal cones were screwed tightly onto the vials, which were shaken for 2 minutes on a Vortex mixer using maximum speed. This treatment yielded a transparent or an opaque gel (depending on the colour of sample) containing a good dispersion of the samples. Vials prepared in this way were either stored at 4°C or allowed to equilibrate with the temperature of the counter (4°C) for one hour before counting. Window settings were 20 to 1000 with 20% gain in the wider channel (red) and 20 to 100 with 20% gain in the narrow (green) channel.

### 2.3.1. Background counts of vials

Initially the background counts of vials were determined by counting a representative number of the vials and then using the average background counts. Later, the 500 mg  $\pm$  10 mg of CAB-O-SIL was added to the scintillant and the vial plus gel counted before addition of weighed samples.

### 2.4. Application of method to samples containing a range of $^{14}\text{C}$ -labelled compounds

#### 2.4.1. Preparation of soils containing a range of $^{14}\text{C}$ -labelled compounds

To ensure that  $^{14}\text{C}$  present in compounds other than benzoic acid could be counted in soil suspensions, an incubation study was carried out.

##### 2.4.1.1. Materials

###### 2.4.1.1.1. Soils

An air-dry representative sample of 0 to 10 cm of Urrbrae fine sandy loam (red brown earth) (see Appendix) under grass legume pasture was used.

###### 2.4.1.1.2. Chemicals

Scintillation chemicals were as previously described (Section 2.1.1.1.). 1 N NaOH was used as an absorbent for released  $^{14}\text{CO}_2$ .

#### 2.4.1.2. Methods

##### 2.4.1.2.1. Incubation

100 mg of glucose containing 6.25  $\mu\text{Ci}$  of uniformly labelled  $^{14}\text{C}$ -glucose was added to 50 g of the air-dry topsoil of a red brown earth in a 250 ml pyrex conical flask. The soil was brought to approximately 70% of field capacity and thoroughly mixed to effect an even distribution of the substrates. A rubber-bung to which was attached a spiral steel wire made in such a way as to hold an "absorbing" vial (1.8 cm x 5 cm in size) was used as a stopper for the flask. A vial containing 8 ml of 1 N NaOH was suspended in the wire. Incubation was allowed to proceed for ten days at  $28 \pm 2^\circ\text{C}$  in an incubator. Absorbing vials were changed on the 1st, 3rd, 4th, 6th, 7th, 9th and 10th days respectively.

##### 2.4.1.2.2. Determination of $^{14}\text{CO}_2$

###### 2.4.1.2.2.1. Materials

Triton X-100 scintillant (Turner, 1968) was used as the scintillation fluor. This was prepared by dissolving 0.4% of PPO and 0.01% of dimethyl-POPOP in two volumes of Toluene (analytical grade) and adding one volume of Triton X-100 (this serves as an emulsifier). The scintillant prepared in this way could be mixed with 1.2 ml of 0.1 or 0.2 M NaOH or 0.06 M  $\text{Na}_2\text{CO}_3$ .

###### 2.4.1.2.2.2. Method

To ensure a mixing of the NaOH and the Triton X-100 scintillant 0.1 ml aliquots of the absorbent plus 0.9 ml  $\text{H}_2\text{O}$  were thoroughly mixed with 10 ml of the scintillant. The samples were counted

at 4°C after 24 hr equilibration with the counter temperature to eliminate chemiluminescence.

Settings used were 20 to 1000 at 6% gain for the wider window and 20 to 100 at 6% gain for the narrower window.

#### 2.4.1.2.3. Preparation of $^{14}\text{C}$ -labelled soil

After incubation, the soil was dried at 70°C overnight. It was then ground in such a way as to avoid a complete breakdown of all aggregates. Ground sample was passed through a range of sieves (stacked up together) with mesh sizes of 36, 44, 60, 120 and 300.

The 300 mesh sample (< 53  $\mu\text{m}$ ) was used to determine the  $^{14}\text{C}$  in the sample, whilst the other sizes were reserved for other studies. It was assumed that the  $^{14}\text{C}$  in the incubated soil sample was present in microbial tissue and metabolic products; that is in a wide variety of biological compounds.

#### 2.4.2. Counting of samples containing a range of $^{14}\text{C}$ -labelled compounds

The method of counting using the < 53  $\mu\text{m}$  diameter particles was the same as described (Section 2.3.) for counting solid standard samples.

#### 2.4.2.1. Determination of percentage efficiency for quench correction

The percentage efficiency for quench correction was done by internal standardization. A known weight of the secondary  $^{14}\text{C}$ -benzoic acid was dissolved in redistilled methanol in a small glass tube.

The top was covered with a rubber septum. 10  $\mu$ l of this standard containing 10,000 d.p.m. was withdrawn into each vial which had already been counted to obtain the counts/min in the samples. Vials were covered with their tops, shaken, and counted again. A smaller range of weights (10, 20, 40 and 100 mg) were used in this case.

## 2.5. Counting of $^{14}\text{C}$ -labelled plant material

### 2.5.1. Materials

Uniformly labelled wheat straw (specific activity 171  $\mu\text{Ci/g}$  C) was obtained from Landwirtschaftlich-Chemische, Bundesversuchsanstalt, Vienna. This was diluted with unlabelled wheat straw (3 parts labelled to 7 parts unlabelled) and ground in a Siebtechnik mill for 1 minute. The ground sample was then sieved through 60, 120 and 300 mesh ( $< 53 \mu\text{m}$ ) sieves.

### 2.5.2. Method

A sample of the diluted wheat straw ( $< 53 \mu\text{m}$ ) yielded 43,000 c.p.m./mg. 100 mg of this material was mixed with 4.9 g of  $< 53 \mu\text{m}$  acid-washed sand to give 860 c.p.m./mg. This was counted as described for standard solid samples (Section 2.3.).

## 3. Factors affecting counts

### 3.1. Effect of particle size on suspension counting

#### 3.1.1. Materials

##### 3.1.1.1. $^{14}\text{C}$ -tagged soil samples

Aggregates obtained after sieving the dried incubated soil sample (mentioned above) were used in this study.

### 3.1.1.2. $^{14}\text{C}$ -tagged plant material

The samples were the same as prepared under counting of plant material. 100 mg of each size fraction of the diluted  $^{14}\text{C}$ -labelled wheat straw was mixed with 4.9 g of  $< 53 \mu\text{m}$  acid-washed sand. The mixture was thoroughly stirred to effect an even distribution of the  $^{14}\text{C}$ .

### 3.1.2. Method

Counting of both  $^{14}\text{C}$ -labelled soil samples and  $^{14}\text{C}$ -labelled plant material was done in the same way as described for standard samples (Section 2.3.).

Efficiency of counting was done on the soil samples using internal standardization as described above (Section 2.4.2.1.).

Samples of the 124 to 250  $\mu\text{m}$  and 53 to 125  $\mu\text{m}$  of the soil were ground to  $< 53 \mu\text{m}$  and counted to check on the validity of results obtained for the first sample of  $< 53$ .

## 3.2. Stability of suspension

### 3.2.1. Materials

Samples used in studying the effect of particle sizes on counting were used.

### 3.2.2. Method

Samples counted after one hour equilibration with the counter temperature were kept in the refrigerator at  $4^{\circ}\text{C}$  for three weeks. These samples were counted again after the third week, using the same settings as in the other experiments.

### 3.3. Effect of colour

#### 3.3.1. Materials

Unlabelled fractions of mineral and soil samples (< 53  $\mu\text{m}$ ) used in preparing  $^{14}\text{C}$ -labelled standard solid samples were used. Scintillating materials and reagents were the same as before (Section 2.1.1.1.).

#### 3.3.2. Method

Red brown earth (25 mg, 35 mg and 50 mg), rendzina (25, 35, 45 and 50 mg), acid-washed sand (100 mg), haematite (5 mg and 10 mg) and goethite (5 mg and 10 mg) were weighed into scintillation vials. About 500 mg of CAB-O-SIL was added to each vial. A vial with CAB-O-SIL alone served as control. 10 ml of Toluene-PPO-dimethyl POPOP scintillant was added to each vial. The vials were shaken for two minutes on a Vortex mixer using the maximum speed.

Using a pasteur pipette, aliquots of prepared gel samples were withdrawn into 1 cm photo cells. Toluene-PPO-dimethyl POPOP scintillant alone which served as a blank was withdrawn into another cell. Cells were placed in the cell compartment close to the photomultiplier in a Unicam ultraviolet spectrophotometer, Model SP 800. This compartment is used to collect radiation over a large solid angle. It is therefore normally used with samples which diffuse the incident light. Thus, scattering of light due to the turbidity of the samples was eliminated. Spectral readings were then traced from 600 to 400 nm.

### 3.4. Effect of iron content of samples on counting

#### 3.4.1. Introduction

It was anticipated that one of the factors that would be limiting to the method was the content of iron in the soil. Even though the effect of this may initially manifest itself through the colour of the samples it was envisaged that the actual content (disregarding the colour) would hinder the efficiency of counting (probably through absorption of  $\beta$ -particles). It therefore became necessary to look into this assumption further.

#### 3.4.2. Materials

To avoid any complication due to colour, the choice of the source of iron was limited to weakly coloured salts, that is white. Basic ferric sulphate,  $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$  (Monsel's salt) was chosen since it was the only iron compound available which was not coloured.

The  $^{14}\text{C}$ -tagged acid-washed sand prepared for counting as a standard sample was used as the source for  $^{14}\text{C}$ .

#### 3.4.3. Methods

##### 3.4.3.1. Determination of percent iron in ferric salt

Iron was determined using thioglycollic acid method (Sandell, 1959), as described in the Appendix. The percentage of iron was 20.15.

### 3.4.3.2. Effect of iron on counting

#### 3.4.3.2.1. Use of toluene-PPO-dimethyl POPOP scintillant

The effect of the iron on counting efficiency was looked at on three different levels with respect to size of particles of the ferric sulphate. These were done in succession.

(i) 25 mg of the  $^{14}\text{C}$ -tagged acid-washed sand ( $< 53 \mu\text{m}$ ) containing 10,000 c.p.m. was weighed into duplicate 20 ml scintillation vials. 10 to 1000 mg of unground ferric sulphate (dried at  $70^\circ\text{C}$  overnight in an oven) were added to each vial.  $500 \pm 10$  mg of CAB-O-SIL was added, followed by 10 ml of toluene-PPO-dimethyl POPOP scintillant. Vials were capped and then shaken in a Vortex mixer at the maximum speed for 2 min. Samples were counted as already described (Section 2.3.).

(ii) Ferric sulphate was ground to  $< 250 \mu\text{m}$  and the procedure as in (i) followed.

(iii) Ferric sulphate ground to pass 350 mesh ( $45 \mu\text{m}$ ) sieve and procedure of (i) followed.

#### 3.4.3.2.2. Use of Triton X-100 scintillant

During the course of the above experiment with toluene-PPO-dimethyl POPOP scintillant, it was realised that the scintillant reacted with the ferric sulphate to yield a yellow colour. This complicated interpretations of results. Preliminary studies had shown that the use of Triton X-100 scintillant maintained the white colour of the ferric sulphate.

Experiments (i) and (ii) above were therefore repeated with the addition of Triton X-100.

### 3.5. Effect of entrapped air on counts

#### 3.5.1. Materials

Samples used were ground ( $< 53 \mu\text{m}$ ) samples of sterilized soil aggregates used in an incubation study of microbial activities in different sizes of aggregates.

#### 3.5.2. Method

Samples (5 mg and 10 mg) were weighed on a piece of aluminium foil into duplicate scintillation vials containing CAB-O-SIL gel (i.e. 500 mg CAB-O-SIL plus 10 ml toluene-PPO-dimethyl POPOP scintillant). Treatment and counting procedure followed the method described for standard solid samples. After the initial counting, samples were taken from the counter. Air bubbles were removed by subjecting the vials to a vacuum.

After removal of entrapped air vials were capped and recounted after allowing 1 hr equilibration with the counter temperature.

The experiment was repeated after addition of internal standard to determine efficiency of counting of the samples.

### 3.6. Effect of temperature of counting

#### 3.6.1. Materials

Twelve selected samples (vials) used in the previous experiment after addition of internal standard.

#### 3.6.2. Method

Samples were counted in a Packard Liquid Scintillation Spectrometer, Model 3375 operating at  $4^{\circ}\text{C}$  for two minutes. They were

then transferred to a similar counter operating at ambient temperature (20°C) and after 30 minutes equilibration with this temperature counted for two minutes.

Packard standards of the cold counter were counted on both counters to serve as a check on the efficiency of counting of both counters.

#### 4. Application of methods to counting of $^{14}\text{C}$ -labelled soil extracts and fractions

##### 4.1. Introduction

After establishing the reliability of the method it was examined for counting acid and alkaline extracts of a soil which had been incubated with uniformly labelled  $^{14}\text{C}$ -glucose.

##### 4.2. Methods

###### 4.2.1. Incubation

Incubation and extraction of fractions were as described by Oades (1974).

###### 4.2.2. Preparation of dry extracts and fractions

The extracted samples and fractions of the soil were freeze-dried. Dry samples were ground to  $< 53 \mu\text{m}$ .

###### 4.2.3. Counting of dry extracts and fractions

Ground samples ( $< 53 \mu\text{m}$ ) were treated and counted in the same way as  $^{14}\text{C}$ -tagged standard solid samples (Section 2.3.).

## 5. Results and discussion

### 5.1. Standardization of (recrystallized) $^{14}\text{C}$ -benzoic acid

Preliminary attempts to standardize recrystallized  $^{14}\text{C}$ -benzoic acid using a gas flow counter were not successful. The reason for preparing a secondary  $^{14}\text{C}$ -benzoic acid standard was to obtain a stock of the labelled  $^{14}\text{C}$ -benzoic acid with high activity. The standard benzoic acid from Packard Industries Incorporated did not contain sufficient  $^{14}\text{C}$  activity to be used in preparation of solid  $^{14}\text{C}$ -tagged samples.

Results (Table 2) obtained after counting the  $^{14}\text{C}$ -benzoic acid secondary standard showed that not all the activity was recovered from the preparation. If it is assumed that the quotation on the original stock was correct, then the counts should have shown 111,000 d.p.m./mg. Only about 20,000 d.p.m./mg was obtained which showed that about 80% of original counts was lost. Efficiency of counting based on the Packard Industries standard  $^{14}\text{C}$ -benzoic acid was 96%. This means that the  $^{14}\text{C}$ -benzoic acid was soluble in the toluene-PPO-dimethyl POPOP scintillant since 96% was the highest efficiency that could be obtained on the liquid scintillation spectrometer used.

Since benzoic acid is steam volatile, some of the  $^{14}\text{C}$  was probably lost during the dissolution in the hot water. However, the remaining activity was just enough to give reasonable counts for the  $^{14}\text{C}$ -tagged solid samples.

### 5.2. Balance point of counting

There was not much difference in counts between 15 to 20% gain amplification for 5 mg and 10 mg samples for both the red brown

Table 2. Standardization of secondary  $^{14}\text{C}$ -benzoic acid

| <u>Packard standard <math>^{14}\text{C}</math>-benzoic acid</u> |                                   |                                   |                 |                         |
|---|-----------------------------------|-----------------------------------|-----------------|-------------------------|
| Wt. of sample<br>per vial<br>in mg                              | Expected<br>counts/mg<br>(d.p.m.) | Observed<br>counts/mg<br>(c.p.m.) | %<br>Efficiency | Mean<br>%<br>Efficiency |
| 5.00  | 5320                              | 5131.4 $\pm$ 20.5                 | 96.5            |                         |
| 5.01  | 5320                              | 5149.0 $\pm$ 20.6                 | 96.8            |                         |
| 4.90  | 5320                              | 5135.00 $\pm$ 20.5                | 96.3            | 96.5<br>$\pm$           |
| 10.05   | 5320                              | 5117.4 $\pm$ 20.5                 | 96.2            | 0.11%                   |
| 10.15   | 5320                              | 5146.3 $\pm$ 20.6                 | 96.7            |                         |
| 10.00   | 5320                              | 5118.1 $\pm$ 20.5                 | 96.2            |                         |

| <u>Secondary <math>^{14}\text{C}</math>-benzoic acid standard</u> |                           |                     |   |
|---|---------------------------|---------------------|---|
| Wt. of sample<br>per vial<br>in mg                                | Observed counts<br>per mg | Counts in<br>d.p.m. | Mean counts in<br>d.p.m. with<br>std. error |
| 5.05  | 19535.6 $\pm$ 39.1*       | 20244.1 $\pm$ 40.5* |   |
| 5.00  | 19530.0 $\pm$ 39.1        | 20238.3 $\pm$ 40.5  |   |
| 5.10  | 19502.9 $\pm$ 39.0        | 20210.3 $\pm$ 40.4  | 20182.2<br>$\pm$                            |
| 10.10   | 18871.0 $\pm$ 37.7        | 19555.4 $\pm$ 39.1  | 159.2                                       |
| 10.05   | 20044.1 $\pm$ 40.0        | 10771.1 $\pm$ 41.5  |   |
| 10.00   | 19371.6 $\pm$ 38.7        | 20074.2 $\pm$ 40.1  |   |

\* Statistics of counting at 95% confidence limit.

earth and the goethite (Fig. 1). The use of greater amounts of sample weight per vial (50 mg) showed that the similarity of maximum count rate between gain amplifications extended further from 15 to 45% in the red brown earth and 15 to 50% for the goethite.

The results were not unexpected. The samples were acting as quenching agents and both samples were severe colour quenchers. Besides the reduction of the maximum efficiency there was a greater spectral spread and therefore a shift of the curves to the right in Fig. 1. Thus, the light pulses had been degraded and higher voltage or amplifications had to be applied to create equivalent effects in all samples (Packard, 1958). Again, because of the highly quenched nature of the samples, the  $^{14}\text{C}$ -spectrum was compressed towards the lower pulse height end (to such an extent as to approach that of tritium) for a sample weight of 50 mg. A situation might therefore have been created such that an increase in amplification could not change the count rate much since the number of photons caused by each  $^{14}\text{C}$  disintegration was so small that most of the pulses recorded in the photomultipliers probably started from single photoelectrons from the photocathodes, i.e. it was not possible to get smaller pulses through the photomultipliers. As the original light pulses were absorbed by the increasing colour intensity fewer pulses were detected at the photocathodes. A similar situation occurs in the case of tritium (Packard, 1958).

Recent computer simulation of colour quenching has shown that most colour-quenched photons travel shorter distances in the vial and therefore do not even reach the periphery of the vial (Malcolm and Stanley, 1973). Thus most of these photons do not escape the vial to reach the

Fig. 1. Balance point for counting of quenched samples of suspensions of a soil and goethite.

(a) 5 mg sample/vial

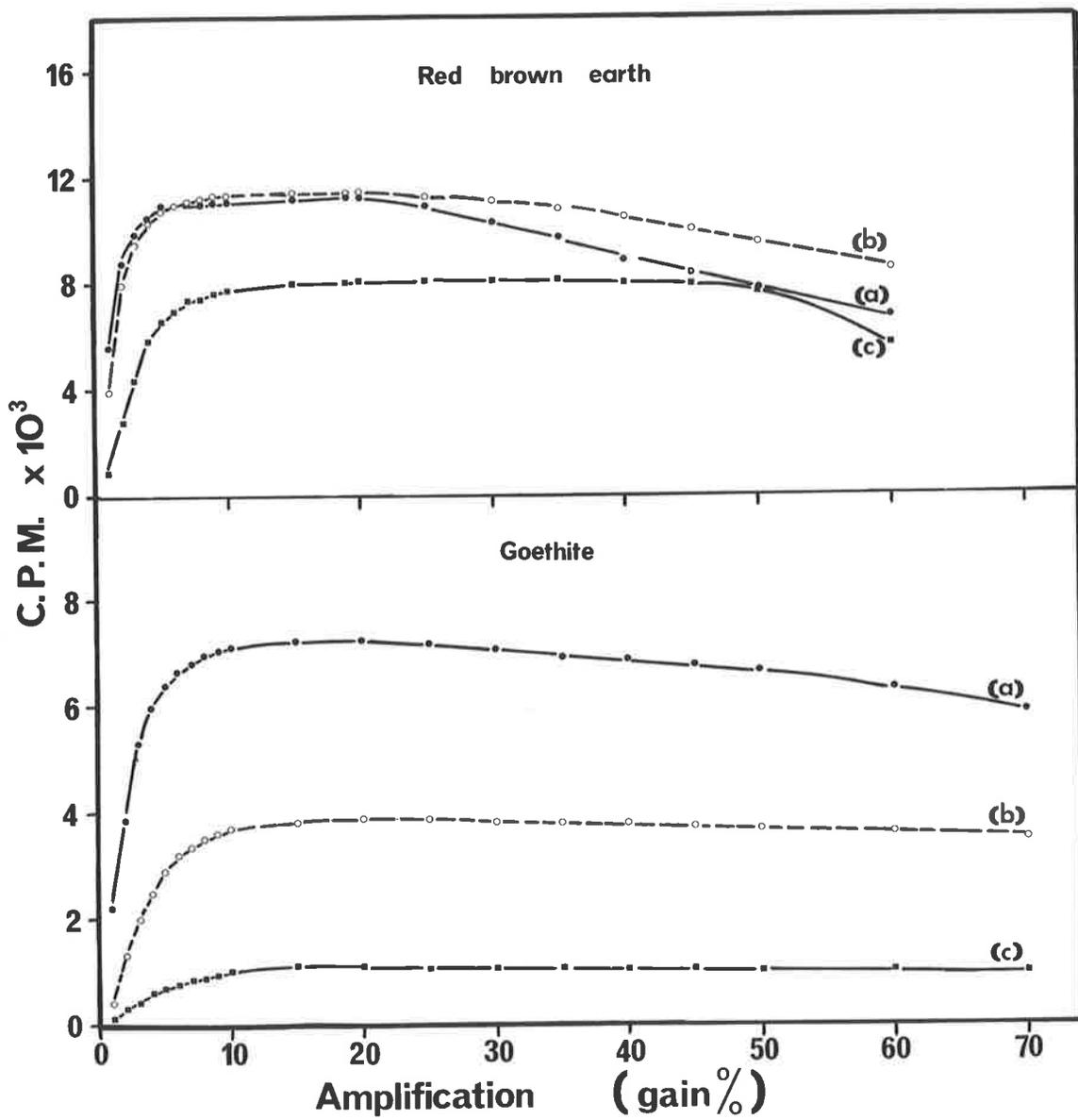
(b) 10 mg sample/vial

(c) 50 mg sample/vial

Background counts of vials were too low to fit the scale.

Scintillant used: Toluene-PPO-dimethyl POPOP plus CAB-O-SIL.

FIG. 1



photocathodes.

The difficulties involved in counting greater amounts of coloured samples will be described later.

### 5.3. Counting of $^{14}\text{C}$ -tagged solid samples

#### 5.3.1. Effect of sample size on recovery of activity

The recoveries of activity have been expressed as a percentage of the expected disintegrations per minute (d.p.m.) based on the weight of "standard sample" added. The linear parts of the curves (Fig. 2) were reproducible provided the particle size range of the sample was the same. Below a critical weight of the sample in the vial, the recoveries of activity could be used as percentage efficiencies of counting (Fig. 3). When sample weights exceeded the critical value, aggregation of particles occurred resulting in self-absorption of  $^{14}\text{C}$ - $\beta$  particles (Table 7, Section 5.8.2.). The gels also became obviously optically dense.

The best recoveries of activity were obtained for the standard acid washed sand. There was a linear relationship between recovery of activity of sample from 5 to 100 mg. This relationship could be represented by the equation  $Y = 103.112537 - 0.001936x$ . There was a correlation coefficient of 0.9371<sup>\*\*\*</sup> with a multiple correlation coefficient of 0.9441. The relationship between weight and percentage recovery over the range 5 to 100 mg for the rendzina could be represented by the equation  $Y = 103.984089 - 1.54177x + 0.006259x^2$ . For this

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\*\*\* significance at  $P < 0.001$

Fig. 2. Effect of weight of sample counted on recovery of  $^{14}\text{C}$  activity (disintegrations per minute)

Carbon-14 tagged solid samples ("Standard samples")

- (a) Acid-washed sand
- (b) Rendzina
- (c) Red brown earth
- (d) Goethite,  $\alpha\text{-FeOOH}$
- (e) Haematite,  $\alpha\text{-Fe}_2\text{O}_3$

FIG. 2

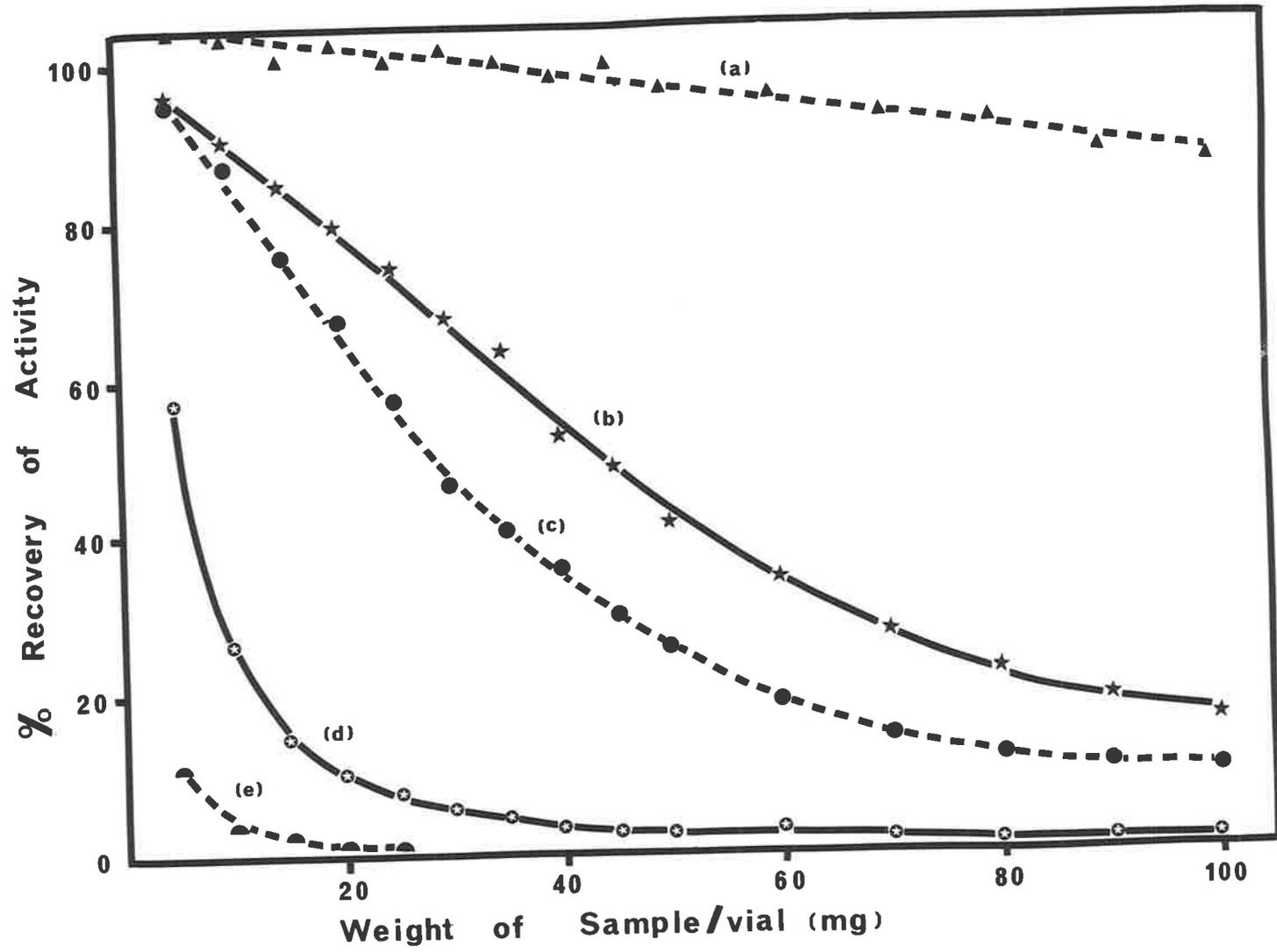
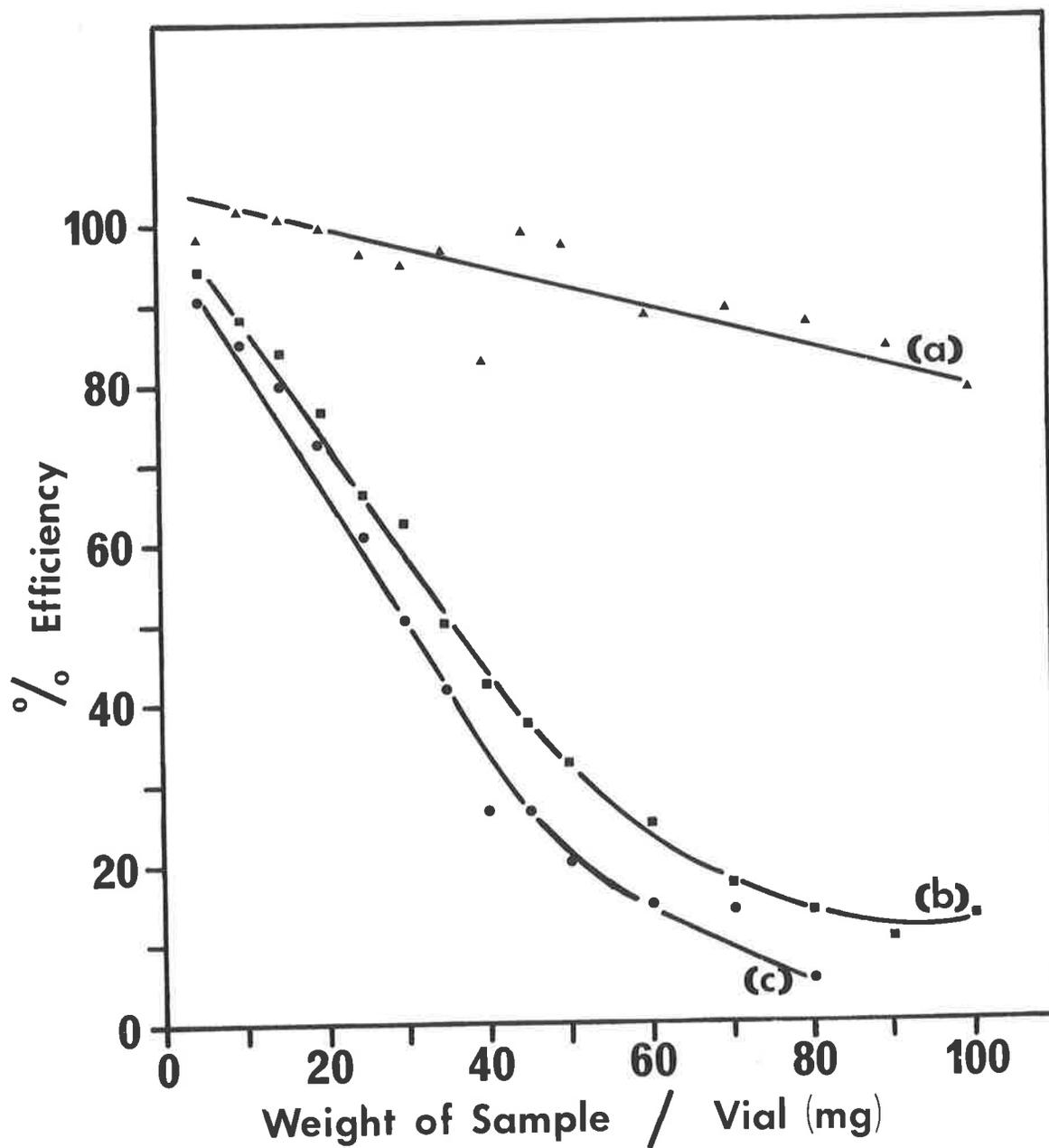


Fig. 3. Effect of weight of sample on efficiency of counting of  $^{14}\text{C}$ -tagged solid samples.

- (a) Acid-washed sand
- (b) Rendzina
- (c) Red brown earth

FIG. 3



sample, 95.84% of the variability was accounted for by the linear term. There was a correlation coefficient of 0.97945<sup>\*\*\*</sup> and a multiple correlation coefficient of 0.99615. The relationship in case of the red brown earth could be represented by the equation  $Y = 109.528386 - 2.414808x + 0.014685x^2$ . 83.577% of the variability could be accounted for by the linear term. The correlation coefficient was 0.9142<sup>\*\*\*</sup> and the multiple correlation coefficient was 0.9956. For practical purposes a straight line graph could have been drawn to represent a plot of weight against d.p.m. for the rendzina. The quadratic term was not as significant for the rendzina as it was for the red brown earth. This showed that it was necessary to construct separate graphs for each different sample. The most difficult samples were the two oxide samples. For the goethite 41.1% of the variability could be accounted for by the linear term and the relationship of counts to weight was represented by the equation  $Y = 43.427659 - 1.423994x + 0.010703x^2$ . Correlation coefficient was 0.64060<sup>\*\*\*</sup> with multiple correlation coefficient of 0.8530. The curve for the haematite was represented by the equation  $Y = 18.8804 - 1.696134x + 0.40667x^2$  with a correlation coefficient of 0.8700 and a multiple correlation coefficient of 0.9748. However, these values were not significant due to the smaller number of treatments.

Despite the high degree of variability accounted for by the linear terms of the equations for the rendzina and red brown earth, reasonable recoveries of activity could be obtained only up to 45 and 25 mg sample per vial for each sample respectively. These critical weights represent

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\*\*\* significance at  $P < 0.001$

the limits of the linear term for the rendzina and red brown earth. With 10 mg of sample per vial of the haematite, it was not possible to obtain meaningful counts. With more than 25 mg of this sample, the activity was lost, such that counts obtained were below background. As shown by the straight line relationship, up to 100 mg per vial of the acid washed sand could be counted.

The 25 mg per vial limit for the red brown earth agrees with the recent limit of 20 mg quoted by Helweg and Sorensen (1973) and 30 mg quoted by Cheshire et al. (1972) though different sizes of particles were used. The higher levels obtained for the acid washed sand and the weight limit for the rendzina as opposed to the mineral samples, however, points to the fact that there are complications involved in the use of the method.

The high recoveries obtained for the acid washed sand in contrast to the coloured samples were not unexpected. Similar results were obtained by Hayes et al. (1956) in their work with  $^{14}\text{C}$ -phenylamine and liver tissue. While in the greyish-white acid washed sand suspension, photons would be reflected when striking a particle, such photons would be absorbed by coloured suspensions. Such an absorption was not a major problem when small sample weights were used, but as the amount of material in the vial increased the effect of this factor became severe.

### 5.3.2. Percentage efficiencies for quench correction

To confirm the claim that percentage recoveries could have served as efficiencies of counting, internal standard was added to the contents of each vial for three samples - acid washed sand, rendzina and red brown earth.

Similar results were obtained for the percentage efficiencies as determined by internal standardization (Fig. 3) as were obtained on the basis of percentage recoveries (Fig. 2). The relationship between sample weight per vial and counts for the acid washed sand was not however, as good as that obtained by using percentage recoveries (Fig. 2). There was a correlation coefficient of 0.8844<sup>\*\*\*</sup> and a multiple correlation coefficient of 0.8872, so that the graph could be represented by a simple linear equation as follows  $Y = 101.98 - 0.204x$ .

This compares favourably with that ( $Y = 103.112537 - 0.001936x$ ) obtained for the percentage recoveries. The similarity between the two sources of curves (efficiencies by internal standardization and percentage recoveries respectively) for both the rendzina and the red brown earth was more pronounced. For the rendzina 90.63% of the variability was accounted for by the linear term with a correlation coefficient of 0.9517<sup>\*\*\*</sup> and a multiple correlation coefficient of 0.9912. The significance of the quadratic term was not all that high and the curve was represented by the equation  $Y = 108.88 - 2.0086x + 0.01034x^2$ . In the same manner for the red brown earth 90.06% of the variability was accounted for by the linear term. There was a correlation coefficient of 0.9486<sup>\*\*\*</sup> and a multiple correlation coefficient of 0.9804. The representative equation for the curve describing the relationship between efficiency and weight (5 to 80 mg) was therefore  $Y = 109.421 - 2.45491x + 0.01465x^2$  which was the same as that obtained for the percentage recoveries. Counts were below background beyond 80 mg due to severe quenching.

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\*\*\* significant at  $P < 0.001$

As previously mentioned, for practical purposes the curves can be represented by straight lines assuming separate curves are drawn for each soil. The slight difference between the relationship for the acid washed sand was probably due to the reflection of photons in the scintillation process due to the greyish-white colour of the sample (particles). Such reflection causes scattering of the light particles which might have given irregular counts from one vial to the other.

From the results obtained for the other samples however, it can be concluded that there was no need to determine efficiency of counting separately from the percentage recoveries in these particular experiments.

### 5.3.3. Background counts

Initially it was not necessary to determine the background of each vial especially when new vials were being used. These vials were high grade with low background counts. Counts ranged from 40 to 52 c.p.m. during the period of study. This range was brought about by differences in batches of vials.

The incorporation of the CAB-O-SIL into the vials before taking the background counts had negligible effect on the value.

### 5.4. Counting of samples containing a range of $^{14}\text{C}$ -labelled compounds

After 10 days of incubation of soil to which uniformly labelled  $^{14}\text{C}$ -glucose was added release of  $^{14}\text{CO}_2$  accounted for about 70% of the activity (Table 3) of the original labelled glucose incorporated. The activity obtained (after quench correction) using up to about 25 mg of  $< 53 \mu\text{m}$  accounted for about 30% of the  $^{14}\text{C}$  added before incubation (Fig. 4).

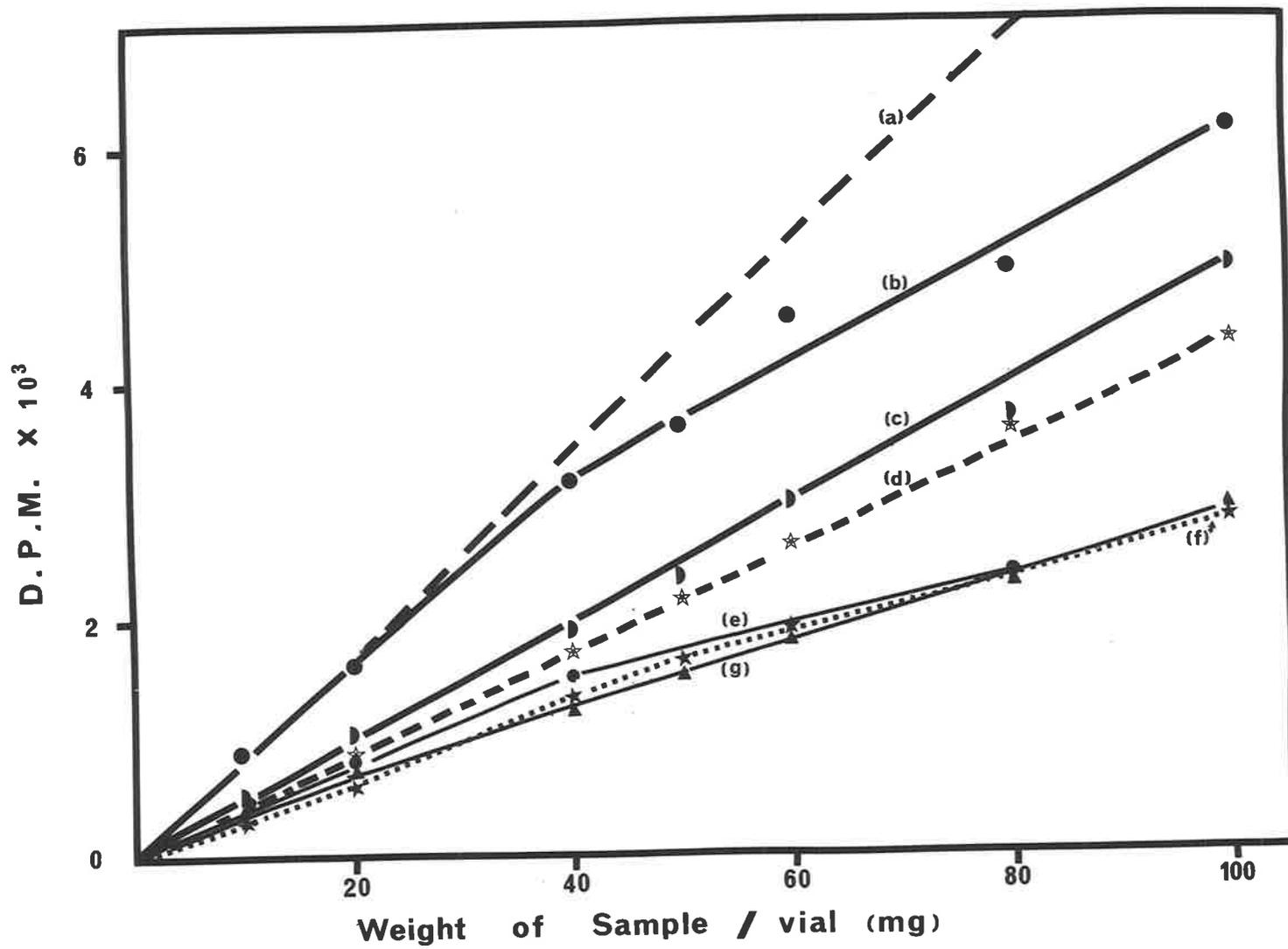
Table 3.     Suspension counting of soil

| Percentage release of $^{14}\text{CO}_2$ during incubation of<br>uniformly labelled $^{14}\text{C}$ -glucose |  |
|--|--|
| Day of harvest   | $^{14}\text{CO}_2$ released as % of<br>initial value incorporated<br>into the soil |
| 1  | 14.05  |
| 3  | 30.89  |
| 4  | 14.03  |
| 6  | 1.32   |
| 7  | 4.71   |
| 9  | 2.66   |
| 10   | 1.49   |
| Total $^{14}\text{CO}_2$ released  | 69.15  |

Fig. 4. Effect of particle size on recovery of activity (d.p.m.) of  $^{14}\text{C}$ -labelled microbial tissue in soil (after incubation of  $^{14}\text{C}$ -glucose in a fine sandy loam - red brown earth).

- (a) 100% recovery of activity remaining in soil
  - (b) < 53  $\mu\text{m}$
  - (c) 53 to 124  $\mu\text{m}$
  - (d) 124 to 250  $\mu\text{m}$
  - (e) 422 to 353  $\mu\text{m}$
  - (f) 353 to 422  $\mu\text{m}$
  - (g) 250 to 353  $\mu\text{m}$
-

FIG. 4



As shown for the standard samples above the critical limit of 25 mg, recovery of total activity (d.p.m.) becomes difficult. There was a deviation from the linear relationship (Fig. 4). With 100 mg per vial, the recovery was 70%, the rest of the activity having been lost through either self-absorption, colour quenching or other factors such as change in optical characteristics of the scintillant.

The results indicate that for a particular soil, provided the sample was ground to  $< 53 \mu\text{m}$  and the critical weight was not exceeded, the  $^{14}\text{C}$  activity could be determined. Thus, the chemistry of the  $^{14}\text{C}$ -labelled compounds in the soil is not important as the method worked equally well with  $^{14}\text{C}$ -labelled benzoic acid or microbial tissue and microbial products.

#### 5.4.1. Percentage efficiency for quench correction

The percentage efficiency versus weight curve (Fig. 5) is similar to the corresponding curve for standard samples (Fig. 2 or 3). It was offset due to a difference in the particle size distribution within the  $< 53 \mu\text{m}$  range caused by a second grinding of the standard samples after addition of the labelled benzoic acid.

The similarity of the curves demonstrates the reproducibility of the method. It also stresses the importance of the treatment given to a particular sample before the determination of the  $^{14}\text{C}$  activity. Samples of the same soil ground to different particle sizes ( $< 53 \mu\text{m}$ ) give different results. The  $< 53 \mu\text{m}$  particle size fraction is considerably smaller than the  $< 300 \mu\text{m}$  size suggested by Cheshire *et al.* (1972) and the  $< 100 \mu\text{m}$  quoted by Helweg and Sorensen (1973).

Fig. 5. Effect of particle size on efficiency of counting of  $^{14}\text{C}$ -labelled microbial tissue in soil (after incubation of  $^{14}\text{C}$ -glucose in a fine sandy loam - a red brown earth).

(a) 422 to 500  $\mu\text{m}$

(b) 353 to 422  $\mu\text{m}$

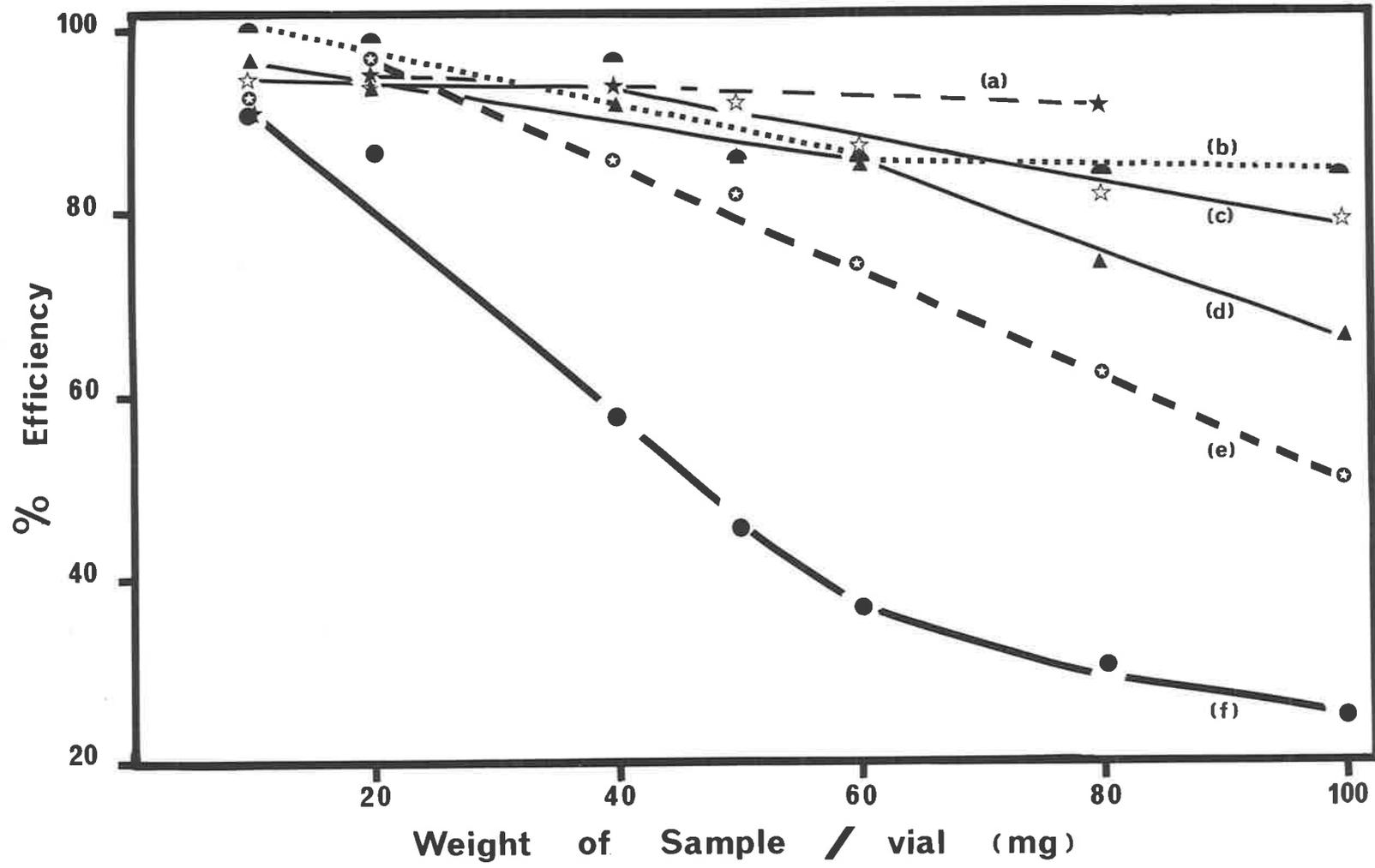
(c) 250 to 353  $\mu\text{m}$

(d) 124 to 250  $\mu\text{m}$

(e) 53 to 124  $\mu\text{m}$

(f) < 53  $\mu\text{m}$

FIG. 5



The importance of particle size in suspension counting is discussed later.

#### 5.5. Determination of $^{14}\text{C}$ -activity in soil by combustion

##### 5.5.1. Comparison of results obtained from combustion and suspension counting

During the initial stages of the development of the method of suspension counting it was thought necessary to compare results obtained by this method with those obtained using conventional ignition methods. Attempts were therefore made to determine  $^{14}\text{C}$  in the samples to which  $^{14}\text{C}$ -benzoic acid was added and also in soils before and after incubation with  $^{14}\text{C}$ -glucose.

Recoveries obtained from combustion in oxygen in a conventional tube furnace, in a Fisher carbon induction furnace and in a Packard automatic sample oxidiser varied from 70 to 90% and were never quantitative.

Samples sent to be combusted on a Beckman biological material oxidiser gave results which were in accordance with some of the results obtained in a conventional tube furnace.

Some results obtained with the conventional tube furnace using  $^{14}\text{C}$ -benzoic acid of different activity from the one used in the suspension counting are shown in Table 4a. Table 4b shows results obtained for soil mixed with  $^{14}\text{C}$ -glucose, and freeze-dried prior to incubation. Table 4c are the results obtained on a Beckman biological material oxidiser using the same  $^{14}\text{C}$ -benzoic acid plus soil mixture as used in the suspension counting.

Table 4. Results obtained using combustion method

- Results obtained using tube furnace

Table 4a. Benzoic acid-<sup>14</sup>C in red brown earth

Expected d.p.m. - 80,000/g

| Wt. of sample<br>used in mg | c.p.m./sample | % Efficiency<br>of counting | d.p.m./sample | d.p.m./g  |
|-----------------------------|---------------|-----------------------------|---------------|-----------|
| 639.00                      | 41,176.6      | 80.7                        | 51,024.30     | 79,850.2  |
| 644.25                      | 47,303.6      | 82.3                        | 57,477.00     | 89,215.4  |
| 640.15                      | 55,076.6      | 82.00                       | 67,166.6      | 104,923.2 |

Table 4b. Soil tagged with glucose-U-<sup>14</sup>C in red brown earth

Expected d.p.m. - 83,000/g

|       |         |      |         |           |
|-------|---------|------|---------|-----------|
| 52.65 | 3,287.5 | 76.0 | 4,325.7 | 82,159.5  |
| 53.8  | 3,420.0 | 76.0 | 4,500.0 | 83,643.1  |
| 50.0  | 3,685.0 | 80.5 | 4,577.6 | 91,552.0  |
| 54.30 | 4,432.5 | 78.0 | 5,682.7 | 104,653.8 |

Values are means of duplicate determinations.

Table 4c. Results obtained using Beckman biological material oxidiser

Haematite. Expected d.p.m. = 40,000/100 mg

| mg     | c.p.m. | S*    | Efficiency | d.p.m./sample | d.p.m./100 mg |
|--------|--------|-------|------------|---------------|---------------|
| 24,324 | 5'899  | 0,183 | 66,6       | 8'888         | 36'516        |
| 25,068 | 5'357  | 0,164 | 64,5       | 8'332         | 33'238        |
| 24,267 | 5'467  | 0,190 | 67,2       | 8'150         | 33'585        |

Average value: 34'446  $\pm$  980.4 d.p.m./100 mg

Red brown earth. Expected d.p.m. = 40,000/100 mg

| mg     | c.p.m. | S*    | Efficiency | d.p.m./sample | d.p.m./100 mg |
|--------|--------|-------|------------|---------------|---------------|
| 24,418 | 6'686  | 0,227 | 69,6       | 9'626         | 39'422        |
| 23,804 | 5'589  | 0,213 | 68,9       | 9'603         | 40'412        |
| 24,486 | 6'724  | 0,204 | 68,3       | 9'865         | 40'288        |
| 23,580 | 6'817  | 0,222 | 69,4       | 9'844         | 41'747        |

Average value: 40'450  $\pm$  417.1 d.p.m./100 mg

Goethite. Expected d.p.m. = 40,000/100 mg

| mg     | c.p.m. | S*    | Efficiency | d.p.m./sample | d.p.m./100 mg |
|--------|--------|-------|------------|---------------|---------------|
| 21,199 | 6'740  | 0,195 | 67,7       | 9'972         | 47'040        |
| 23,634 | 7'549  | 0,207 | 68,4       | 11'054        | 46'772        |
| 22,309 | 6'982  | 0,185 | 66,8       | 10'495        | 47'044        |
| 18,803 | 6'290  | 0,219 | 69,3       | 9'217         | 49'019        |

Average value: 47'469  $\pm$  520.71 d.p.m./100 mg

S\* - external standard

The sequence of results in the three tables shows an increase in d.p.m. (disintegrations per minute) down the table which corresponds to the sequence in which samples were combusted. There was a high percentage carry-over in all cases which did not depend on the source of the  $^{14}\text{C}$ . The counts expected from the samples combusted in the Beckman biological material oxidiser were 40,000 d.p.m./100 mg in each of the three tagged samples.

The results show increasing recoveries of activity beginning with the haematite sample and then red brown earth and goethite. The recoveries for haematite were low, for the red brown earth they were close to 100 per cent and for the goethite recoveries were high. Thus, counts lost from the haematite samples have been recovered in the goethite samples.

The following interpretation is considered to account for these results. There was incomplete combustion due to sublimation of the compound in the combustion tube. Higher counts were therefore obtained in subsequent determinations as a result of part of the sublimate from a previous sample combusting in addition to the recently introduced sample. There was further sublimation from the second sample and this continued as long as the combustions were carried out. The ideal situation was to combust blank samples after every single determination but this meant high background counts and also low recoveries. The high memory effect was even worse when a Packard automatic sample oxidiser was used. This, coupled with other problems such as quenching due to colouring of scintillant by smoke, rendered this equipment of limited value.

Probably this sort of anomaly in the combustion system could have been acceptable when  $^{14}\text{C}$ -benzoic acid was used as this compound sublimes at about  $122^\circ\text{C}$ . However, since the same sequence of results were obtained for  $^{14}\text{C}$ -glucose tagged soil, the ignition system was considered unreliable. A more sophisticated system could probably be devised to counteract some of these problems but it may not be worth the work and time involved. A second heating section in the furnace with copper or platinum catalysts to complete the oxidation together with finely controlled gentle heating during the initial stages would probably improve the performance of the automatic oxidisers for use with soil samples.

#### 5.6. Examination of soil extracts and fractions

The results (Table 5) obtained by counting various freeze-dried extracts and fractions of the red brown earth incubated with uniformly labelled  $^{14}\text{C}$ -glucose established confidence in the procedure and its applicability to recalcitrant coloured materials.

The 97.6% recovery of activity does not include some  $^{14}\text{C}$  known to be present in the heavy liquid (1,2-dibromo-3-chloro-propane) used to float the light fraction. Various attempts to count  $^{14}\text{C}$  in this organic liquid indicated that at least a further 1% of the  $^{14}\text{C}$  was present in compounds dissolved in the heavy liquid.

Attempts were also made to count  $^{14}\text{C}$  in materials behaving as high molecular weight compounds on Sephadex G-10 from both acid extract and fulvic acid extract. Aqueous aliquots of these samples were mixed with scintillant using either Instagel or Triton X-100. The percentage of  $^{14}\text{C}$  in these fractions determined using either of these emulsifiers

Table 5. Distribution of  $^{14}\text{C}$  in soil fractions and soil extracts determined by suspension counting and by homogeneous counting using Instagel or Triton X-100 scintillant

| Fraction              | % $^{14}\text{C}$ in sample |                       |
|-----------------------|-----------------------------|-----------------------|
|                       | Suspension                  | Instagel/Triton X-100 |
| Soil after incubation | 100                         |                       |
| Light fraction        | 17.5                        |                       |
| Acid extract > G-10   | 33.0                        | 12.3                  |
| < G-10                | 8.1                         |                       |
| Fulvic acid > G-10    | 20.7                        | 6.3                   |
| < G-10                | 2.0                         |                       |
| Humic acid            | 3.4                         |                       |
| Acetylation extract   |                             |                       |
| Chloroform            | 2.8                         |                       |
| Aqueous layer         | 2.5                         |                       |
| Final residue         | 7.6                         |                       |
| Total recovery        | 97.6                        |                       |

was about a third of the figure obtained by suspension counting.

The low recovery obtained using either the Instagel or the Triton X-100 scintillant could be explained by the fact that, even though the system appeared to be homogeneous, it was virtually heterogeneous, due to the formation of microphases. The internal standard added to the system presumably existed in a different phase from the sample and was counted at considerably higher efficiencies than the samples. Suspension counting of the freeze-dried fractions must have yielded quantitative results, otherwise the total recovery of  $^{14}\text{C}$  in the various fractions would not have been close to 100%.

#### 5.7. Counting of plant material

The top line of Fig. 6 shows the counts per minute obtained for  $< 53 \mu\text{m}$  size  $^{14}\text{C}$ -labelled wheat straw mixed with acid washed sand. The result was based on counts per minute obtained for counting a sample of  $< 53 \mu\text{m}$  of the  $^{14}\text{C}$ -labelled wheat straw diluted with unlabelled wheat straw (3 parts labelled to 7 parts unlabelled). This yielded 43,000 c.p.m./mg, and 100 mg of this material mixed with 4.9 g of  $< 53 \mu\text{m}$  acid washed sand therefore gave 860 c.p.m./mg. Recovery of counts has been based on c.p.m. since there was no reliable method of determining the original activity of the straw besides using the quotation from the source of purchase.

There was a linear relationship between weight and c.p.m. for 5 to 100 mg as was shown for the standard  $^{14}\text{C}$  labelled acid washed sand (Fig. 2). The critical weight per vial was definitely more than 100 mg since the counts obtained for 100 mg sample per vial was about twenty times the value obtained for 5 mg per vial (87,000 c.p.m. and 4,300

Fig. 6. Effect of particle size on counts in a  $^{14}\text{C}$ -labelled wheat straw/  
acid-washed sand mixture.

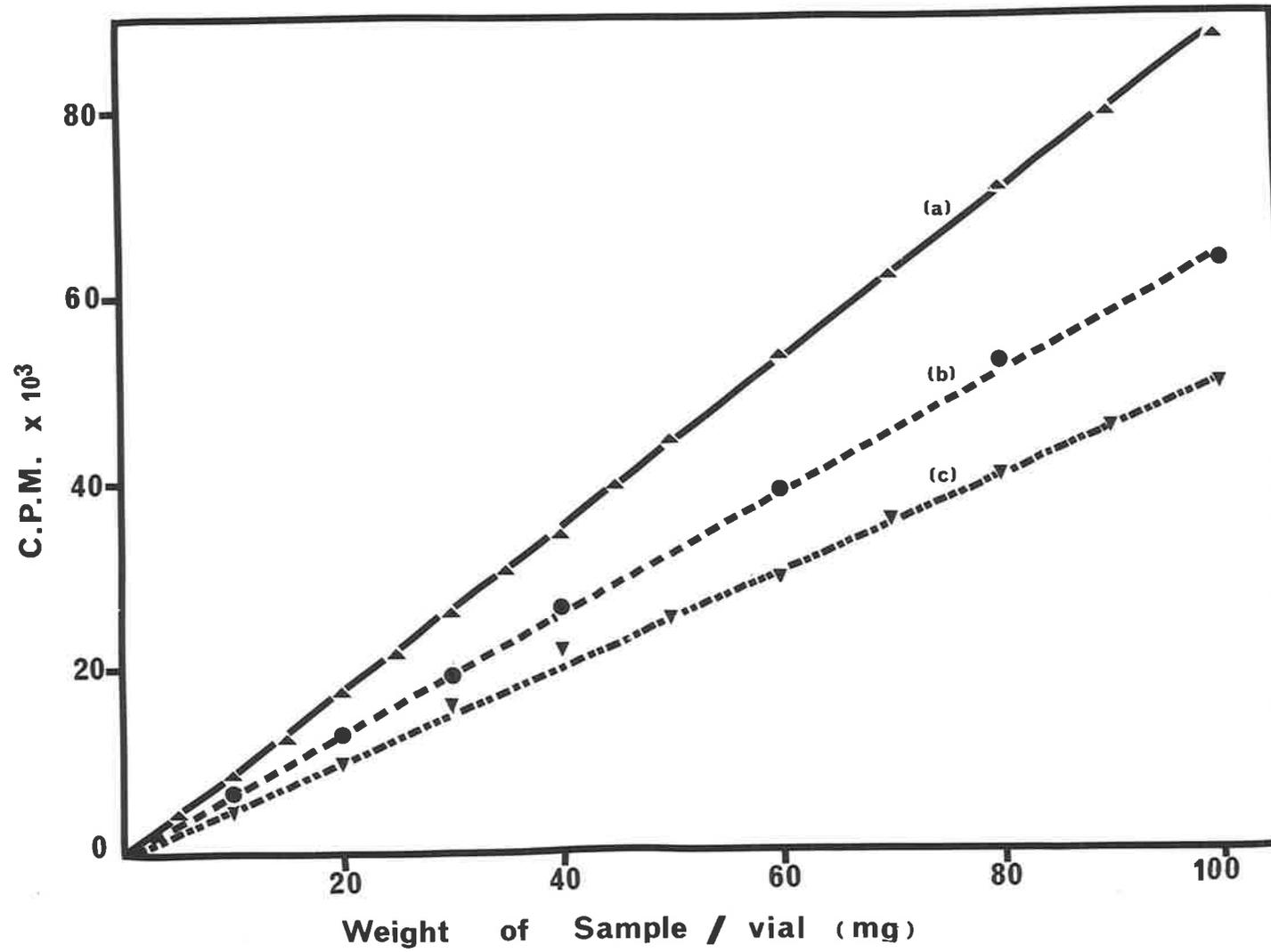
(a) < 53  $\mu\text{m}$

(b) 53 to 124  $\mu\text{m}$

(c) 124 to 250  $\mu\text{m}$

---

FIG. 6



c.p.m. respectively). 250 mg and 500 mg of the sample per vial gave recoveries of 80% and 70% respectively.

The direct relationship between weight and counts was based mainly on the weak colour present in the gel. The plant material together with the acid washed sand was very lightly coloured. Over 100 mg was therefore needed to produce factors which prevented recovery of total counts. The 80% and 70% recoveries obtained for the 250 mg and 500 mg respectively was probably due to increase in opacity of the gel and scattering of the light particles (Hayes et al., 1956).

#### 5.8. Factors affecting counts

##### 5.8.1. Effect of particle size

The average density of soil particles is  $2.6 \text{ g/cm}^3$ . It was therefore expected that variations in particle size would be reflected by changes in counting efficiency due to self-absorption of the  $^{14}\text{C}$   $\beta$ -particles (White and Helf, 1956). Using the weight of material ( $\text{Ba}^{14}\text{CO}_3$ ) which yields a layer of infinite thickness (about  $30 \text{ mg/cm}^2$ ) on an aluminium planchet (Calvin et al., 1949) it can be calculated that the critical diameter of soil particles is in the range 60 to 120  $\mu\text{m}$  to cover the density range 2.5 to  $5.0 \text{ g/cm}^3$ . The 300  $\mu\text{m}$  diameter particles used by Cheshire et al. (1972) were larger than this critical size range.

The percentage efficiency of counting with respect to particle size is shown in Fig. 5, and Fig. 4 shows the recovery of activity after correcting for efficiency. Even though there was a decrease in percentage counting efficiency with reduction in particle size (Fig. 5), there was an increase in recovery of activity (Fig. 4). Similar results

were recently reported by Helweg and Sorensen (1973) who found an increase in percentage recovery of activity with decrease in particle size.

The lower efficiencies of counting with increasing weight per vial obtained for the  $< 53 \mu\text{m}$  particles were probably due to changes in the optical properties as a result of increase in colour intensity, in addition to self-absorption due to the formation of microaggregates within the gel.

High percentage efficiencies were obtained for the larger particles because they were scattered sparingly in the suspension. When particles  $> 53 \mu\text{m}$  were present the gel was not significantly coloured even with increasing weight of the sample. However, the efficiencies given, using  $^{14}\text{C}$ -benzoic acid as an internal standard were too high and resulted in poor recoveries of activity (Fig. 4). Only particles  $< 53 \mu\text{m}$  yielded quantitative recoveries of activity and then only when the critical weight of about 25 mg sample per vial was not exceeded.

Activities for up to 25 mg of sample per vial for the 53 to 124  $\mu\text{m}$  and the 124 to 250  $\mu\text{m}$  sizes were about 60% of the activities obtained for the equivalent amount of  $< 53 \mu\text{m}$  sample per vial. Of the biggest particles (353 to 422  $\mu\text{m}$  and 422 to 500  $\mu\text{m}$ ) the differences ranged between 40 to 50%. The differences were due to increased self-absorption with increasing particle size. This cannot be corrected for.

On the other hand, with weights of sample greater than 25 mg, counts obtained for the bigger particles became higher than for the same weight of the  $< 53 \mu\text{m}$  samples. This situation was created by the relatively high level of quenching due to increased colour intensity

with increase in weight of the < 53  $\mu\text{m}$  particles. The comparative increase in colour intensity with the bigger particles was not as great and hence quenching was relatively less pronounced.

It was thought that the result of the effect of particle size might have been affected by segregation during sieving. The  $^{14}\text{C}$  might have been concentrated in the clay fraction (< 2  $\mu\text{m}$ ) resulting in high counts for the < 53  $\mu\text{m}$  samples.

Results (Table 6) obtained after recounting part of the 53 to 124  $\mu\text{m}$  and the 124 to 250  $\mu\text{m}$  particles ground to < 53  $\mu\text{m}$  showed that this was not the case. The recovery of activity increased and matched that of the original < 53  $\mu\text{m}$  fraction. This means that the 60% recovery given by the larger particle size fractions was real and not due to segregation of particles differing in activity during the sieving process.

The importance of particle size was also demonstrated with the plant material (Fig. 6). The c.p.m. for the same weight of sample (of the 53 to 124  $\mu\text{m}$  and 124 to 250  $\mu\text{m}$  particles) were 71 and 56% respectively of those given by the < 53  $\mu\text{m}$  fractions. This stresses the importance of grinding to a finer particle size in suspension counting of soil and plant materials.

These results are however, in contrast to those obtained by White and Helf (1956), who found almost constant efficiency over a range of mesh sizes of  $\text{Ba}^{14}\text{CO}_3$ , the finest particles being counted least efficiently. This was attributed to light scattering by the finer particles. In the same manner Cluley (1962) concluded in his work that sifting in counting  $^{14}\text{BaCO}_3$  by suspension was unnecessary. One obvious difference between these two works and the present method is colour.

Table 6. Effect of particle size on counting

Counts for particles reduced from 53 to 124  $\mu\text{m}$  and  
125 to 250  $\mu\text{m}$  to < 53  $\mu\text{m}$

| Weight per<br>vial<br>in mg | a  | b                                  | c  |
|-----------------------------|--|------------------------------------|--|
|                             | Original particle<br>size<br>53 to 124 $\mu\text{m}$<br>c.p.m. | Fresh < 53 $\mu\text{m}$<br>c.p.m. | Original<br>< 53 $\mu\text{m}$<br>c.p.m. |
| 10                          | 561.6 $\pm$ 42.4   | 935.6 $\pm$ 1.4                    | 875.4 $\pm$ 11                           |
| 20                          | 1,257.3 $\pm$ 11.6   | 1,550.7 $\pm$ 76.2                 | 1,385.4 $\pm$ 82.2                       |
| 40                          | 1,774.4 $\pm$ 73.3   | 1,623.8 $\pm$ 11.2                 | 1,610.3 $\pm$ 57.6                       |
| 100                         | 2,217.7 $\pm$ 82.5   | 1,464.8 $\pm$ 61.5                 | 1,383.2 $\pm$ 21.0                       |
| 124 to 250 $\mu\text{m}$    |  |                                    |  |
| 10                          | 797.2 $\pm$ 36.3   | 977.9 $\pm$ 96.0                   | 997.6 $\pm$ 71.6                         |
| 20                          | 1,028.5 $\pm$ 111.2  | 1,674.0 $\pm$ 103.7                | 1,509.2 $\pm$ 53.6                       |
| 40                          | 1,759.4 $\pm$ 86.9   | 1,782.2 $\pm$ 50.0                 | 1,646.8 $\pm$ 14.2                       |
| 100                         | 2,622.6 $\pm$ 108.4  | 143.7 $\pm$ 42.3                   | 1,465.7 $\pm$ 67.4                       |

Counts are means of triplicate samples with standard errors.

Probably, the magnitude of the effect of size would vary from one sample to the other (depending on other factors such as colour). Generally, however, one should expect a serious effect of size on recovery of counts with every soil.

#### 5.8.2. Self-absorption

The ratio of suspension counting efficiency to homogeneous internal standard efficiency has been denoted by the factor "f", and this deviates from unity by an amount that depends only on self-absorption of the  $^{14}\text{C}$   $\beta$ -particles (Hayes et al., 1956).

Results in Table 7 have been calculated using recoveries obtained on the basis of counts for 10 mg sample ( $< 53 \mu\text{m}$ ) containing a range of  $^{14}\text{C}$ -labelled compounds and homogeneous internal standard efficiencies (Fig. 3) obtained for the different size particles. Those for the rendzina were obtained by using percentage recoveries (obtained by counting part of the  $^{14}\text{C}$ -tagged standard samples) and internal standard percentage efficiencies.

The results explain why there were low recoveries from the larger particles of the incubated soil (Fig. 4) and to some extent why quantitative recoveries of activities for the smallest particles could be obtained for only up to 25 mg of the red brown earth and less than 45 mg of the rendzina (Fig. 2). The table also shows that self-absorption increased with increasing weight of sample while it decreased with particle size.

The low values of "f" obtained for particles of  $> 53 \mu\text{m}$  are evidence of the expected self-absorption of the weak  $\beta$ -spectrum of the  $^{14}\text{C}$  by the

Table 7. Self-absorption  
f\* values for red brown earth and rendzina

| Weight<br>in mg | Red brown earth                     |               |               |               |              |      | Rendzina |
|-----------------|-------------------------------------|---------------|---------------|---------------|--------------|------|----------|
|                 | Particle diameter ( $\mu\text{m}$ ) |               |               |               |              |      |          |
|                 | 422 to<br>500                       | 353 to<br>422 | 250 to<br>353 | 124 to<br>250 | 53 to<br>124 | < 53 | < 53     |
| 10              | 0.64                                | 0.46          | 0.42          | 0.59          | 0.65         | 0.98 | 1.01     |
| 20              | 0.47                                | 0.36          | 0.43          | 0.55          | 0.60         | 0.98 | 1.02     |
| 40              | 0.44                                | 0.38          | 0.37          | 0.51          | 0.57         | 0.92 | 1.20     |
| 50              |                                     | 0.38          | 0.35          | 0.52          | 0.55         | 0.84 | 0.84     |
| 60              |                                     | 0.36          | 0.35          | 0.51          | 0.57         | 0.89 | 0.93     |
| 80              | 0.31                                | 0.33          | 0.33          | 0.53          | 0.51         | 0.71 | 0.93     |
| 100             |                                     | 0.33          | 0.33          | 0.50          | 0.57         | 0.71 | 0.83     |

$$f^* \text{ value} = \frac{\% \text{ suspension counting efficiency}}{\% \text{ homogeneous counting efficiency}}$$

particles of the suspension because of their relatively large size compared with the range of  $^{14}\text{C}$   $\beta$ -particles (Hayes et al., 1956). Self-absorption in the rendzina was not as strong as in the red brown earth. This could be part of the reason why efficiencies for this sample were higher than for the red brown earth. The rendzina contains more clay than the red brown earth, which would tend to make the density of particles lower, and also more of the particles were very small.

It is quite clear that the self-absorption is a significant factor unless particles of  $< 53 \mu\text{m}$  diameter are used. This is close to the predicted critical size of particles as calculated from a layer of infinite thickness (i.e. 60 to 120  $\mu\text{m}$ ).

### 5.8.3. Stability of suspension

The results obtained from counting suspensions formed using a range of weights and particle sizes of the red brown earth (Table 8) showed no significant difference between the counts obtained 1 hour after placing in the liquid scintillation spectrometer and those obtained after three weeks storage at  $4^{\circ}\text{C}$ . Any differences in counts were due to errors inherent in the counting system but not to decrease in counts as a result of the settling of the suspension.

The 500 mg of CAB-O-SIL formed a gel which was rigid enough to hold even the largest particles as found by Ott et al. (1959) and Lloyd-Jones (1970). However, evaporation of the scintillant did occur in occasional vials (White and Helf, 1956; Angel, 1968).

Table 8. Stability of suspension. Comparison between counts at 1 hour and after 21 days

| Weight<br>per vial<br>in mg | Counts after 1 hour at 4°C (c.p.m.) |                    |                    |                      |                    |                    | Counts after 21 days at 4°C (c.p.m.) |                    |                    |                     |                    |                    |
|-----------------------------|-------------------------------------|--------------------|--------------------|----------------------|--------------------|--------------------|--------------------------------------|--------------------|--------------------|---------------------|--------------------|--------------------|
|                             | 422 to<br>500 μm                    | 353 to<br>422 μm   | 250 to<br>353 μm   | 124 to<br>250 μm     | 53 to<br>124 μm    | < 53 μm            | 422 to<br>500 μm                     | 353 to<br>422 μm   | 250 to<br>353 μm   | 124 to<br>250 μm    | 53 to<br>124 μm    | < 53 μm            |
| 10                          | 244.0 ±<br>14.2                     | 357.4 ±<br>74.4    | 354.00 ±<br>0.8    | 532.1 ±<br>50.1      | 504.7 ±<br>7.7     | 1,247.7 ±<br>163.7 | 259.9 ±<br>26.55                     | 379.1 ±<br>74.5    | 379.7 ±<br>1.6     | 559.8 ±<br>49.9     | 539.3 ±<br>10.2    | 1,122.6 ±<br>45.3  |
| 20                          | 755.8 ±<br>78.4                     | 808.7 ±<br>11.70   | 801.6 ±<br>124.0   | 844.8 ±<br>63.6      | 1,613.0 ±<br>559.6 | 1,387.0 ±<br>35.00 | 808.1 ±<br>85.1                      | 828.9 ±<br>13.3    | 802.9 ±<br>101.9   | 878.4 ±<br>55.0     | 1,447.3 ±<br>406.2 | 1,317.2 ±<br>58.6  |
| 40                          | 1,298.3 ±<br>277.1                  | 1,417.4 ±<br>163.6 | 1,190.10 ±<br>98.1 | 2,410.0 ±<br>643.2   | 1,766.7 ±<br>142.9 | 1,828.0 ±<br>61.2  | 1,330.2 ±<br>293.1                   | 1,409.4 ±<br>73.9  | 1,224.9 ±<br>91.0  | 2,021.35 ±<br>252.8 | 1,731.9 ±<br>158.0 | 1,741.4 ±<br>18.3  |
| 50                          | 1,692.1 ±<br>181.7                  | 1,645.4 ±<br>29.60 | 1,451.8 ±<br>1.40  | 2,017.7 ±<br>53.1    | 1,981.0 ±<br>25.6  | 1,772.2 ±<br>93.00 | 1,522.3 ±<br>23.8                    | 1,679.5 ±<br>11.5  | 1,465.2 ±<br>18.4  | 2,026.5 ±<br>34.8   | 1,939.4 ±<br>0.5   | 1,734.4 ±<br>111.7 |
| 60                          | 1,641.8 ±<br>121.0                  | 1,751.0 ±<br>254.6 | 2,532.5 ±<br>310.1 | 2,699.0 ±<br>143.6   | 2,662.4 ±<br>359.6 | 1,436.3 ±<br>7.5   | 1,858.3 ±<br>65.2                    | 1,510.2<br>*       | 1,844.5 ±<br>378.5 | 2,303.00 ±<br>99.7  | 2,172.4 ±<br>23.6  | 1,690.5 ±<br>36.8  |
| 80                          | 2,145.2 ±<br>47.6                   | 2,118.6 ±<br>139.4 | 2,060.7 ±<br>68.7  | 2,699.0 ±<br>143.6   | 2,662.4 ±<br>359.6 | 1,436.3 ±<br>7.5   | 2,197.0 ±<br>66.3                    | 2,159.6 ±<br>107.3 | 2,090.8 ±<br>58.0  | 2,605.6 ±<br>38.6   | 2,485.0 ±<br>226.3 | 1,449.0 ±<br>34.7  |
| 100                         | 2,439.10 ±<br>168.9                 | 2,352.8 ±<br>18.20 | 2,213.9 ±<br>11.1  | 2,847.70 ±<br>122.50 | 2,135.5 ±<br>1.9   | 1,589.3 ±<br>79.5  | 2,469.3 ±<br>149.5                   | 2,469.3 ±<br>149.2 | 2,469.3 ±<br>149.2 | 2,832.0 ±<br>96.1   | 2,111.2 ±<br>47.8  | 1,573.2 ±<br>60.1  |

\* Only one vial used - the other vial was too dry to count.

Counts are with standard errors.

#### 5.8.4. Effect of colour

Colour quenching is one of the serious problems in liquid scintillation counting. With soils and soil extracts it is a major problem and is mainly responsible for the low recoveries of activity and low efficiencies shown in Figs. 2, 3 and 5.

This effect can be overcome in suspension counting up to a point by the use of internal standards, to determine efficiencies. The c.p.m. measured and corrected for these efficiencies will give 100% recoveries of activity until the critical amount of sample is exceeded when recoveries are not quantitative. The critical weights of sample for the rendzina, red brown earth, goethite and haematite samples were 45 mg, 25 mg, 10 mg and 5 mg respectively.

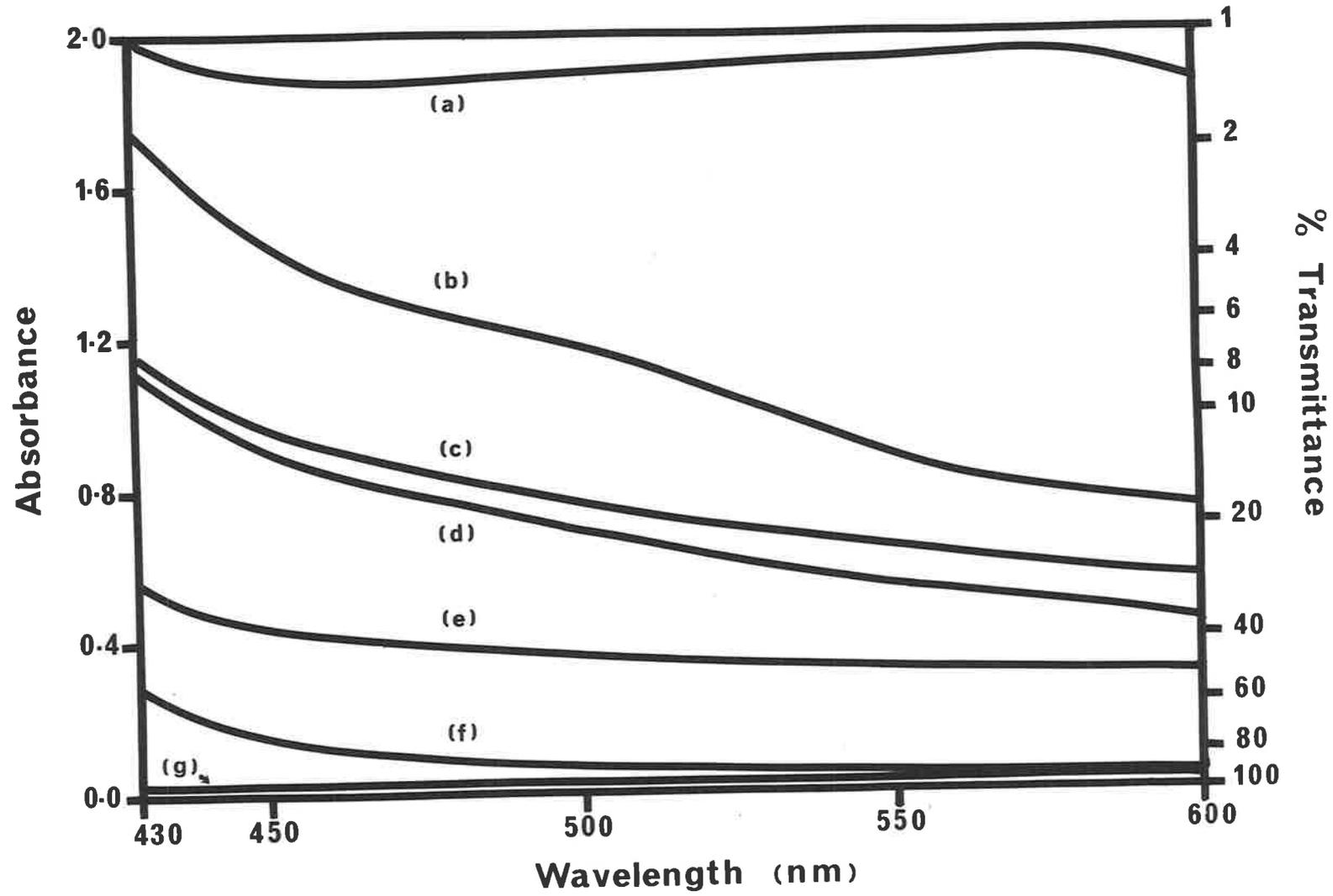
Fig. 7 shows that these weights were really the weights of sample responsible for increasing the optical density of the CAB-O-SIL scintillant mixture to a critical level. The spectra show that with 25 mg of the red brown earth and 45 mg of the rendzina in the vial a similar optical density (O.D.) was obtained, e.g. O.D. 0.9 at 450 nm (corresponding to about 13% transmittance). 100 mg of acid-washed sand gave an O.D. of 0.42 at 450 nm (i.e. about 45% transmittance). Quantitative recoveries of activity in the acid-washed sand were obtained using sample weights from 5 to 100 mg. On the other hand 5 mg of goethite or haematite both yielded optical densities well above 0.9 which appears to be the maximum if quantitative recoveries of activities are expected.

When the critical O.D. was exceeded, counting was non-coincident so that many events were not recorded. In addition the phototubes - bialkali (K-Cs) 9635B used in the Tri-Carb liquid scintillation

Fig. 7. Spectra for suspensions of  $^{14}\text{C}$ -labelled samples in toluene-PPO-dimethyl POPOP scintillant plus CAB-O-SIL, determined on a Unicam SP800 spectrophotometer in 1 cm cells

- (a) 5 mg Haematite
- (b) 5 mg Goethite
- (c) 45 mg Rendzina
- (d) 25 mg Red brown earth
- (e) 100 mg Acid-washed sand
- (f) CAB-O-SIL alone
- (g) Toluene-PPO-dimethyl POPOP scintillant alone

FIG. 7



spectrometer (Packard, Model 3375) shows maximum quantum efficiency (25%) with photons of wavelength near 400 nm, but this falls rapidly as the wavelength increases. Thus, increased absorbance in the 400 to 500 nm region eliminates the most efficient portion of quantum efficiency curve (Fig. 8). This means that problems of non-coincidence are compounded with quantum efficiency percentages of less than 10%.

To eliminate self-absorption, it is necessary to reduce particles to  $< 53 \mu\text{m}$ . This leads to lower counting efficiencies due to the increased colour or O.D. at 450 nm given to the gel by the dispersion of the finer particles. However, quantitative recoveries can be obtained using the internal standard until the optical density in a 1 cm cell exceeds 0.9 at 450 nm.

#### 5.8.5. Effect of iron

Fig. 9 shows the effect of iron on counting efficiency. The use of toluene-PPO-dimethyl POPOP scintillant gave a yellow colour with the basic ferric sulphate  $[\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}]$ . It was therefore thought that reduction in efficiency of counting might have been due to colour quenching. That this was not the case is shown in the different curves in Fig. 9.

Grinding of the ferric sulphate to  $< 250 \mu\text{m}$  reduced recoveries by about 15% of that obtained for the unground sample, while a second grinding to  $< 45 \mu\text{m}$  again reduced the recovery by a further 13%. Thus, while the colour was kept constant with respect to the weight of sample used, the effect of iron was increased due to decrease in particle size. On the other hand, reduction in particle size has the effect of light scattering due to increase in total surface area (Cluley, 1962). This

Fig. 8. Spectral response of phototubes [bialkali (K-Cs) 9635B] used in Tri-Carb liquid scintillation spectrometer (Packard, Model 3375).

FIG. 8

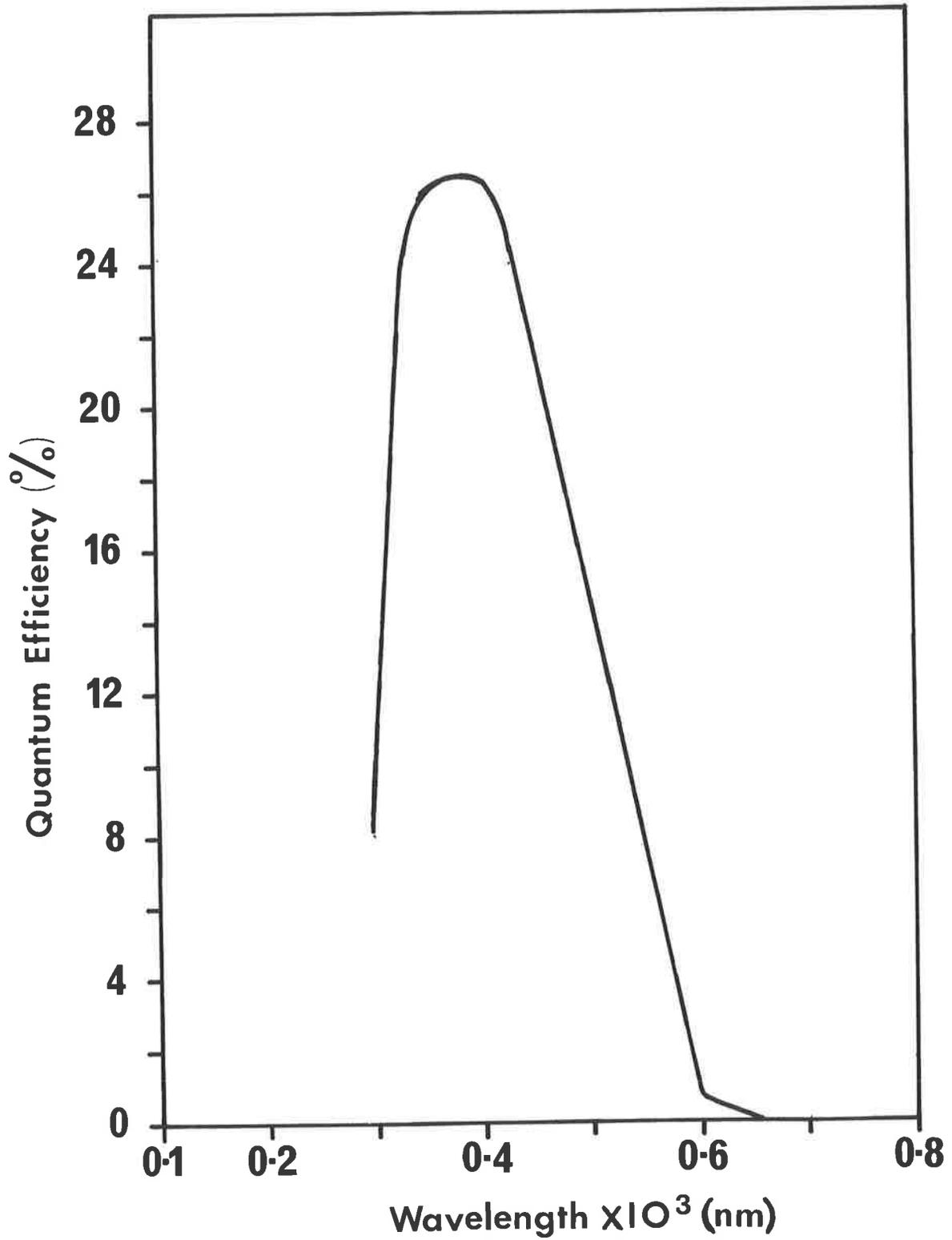


Fig. 9. Effect of iron in  $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$  on the recovery of activity (d.p.m.) in a standard  $^{14}\text{C}$ -benzoic acid-acid washed sand mixture.

(a) Unground

(b) < 250  $\mu\text{m}$

(c) < 45  $\mu\text{m}$

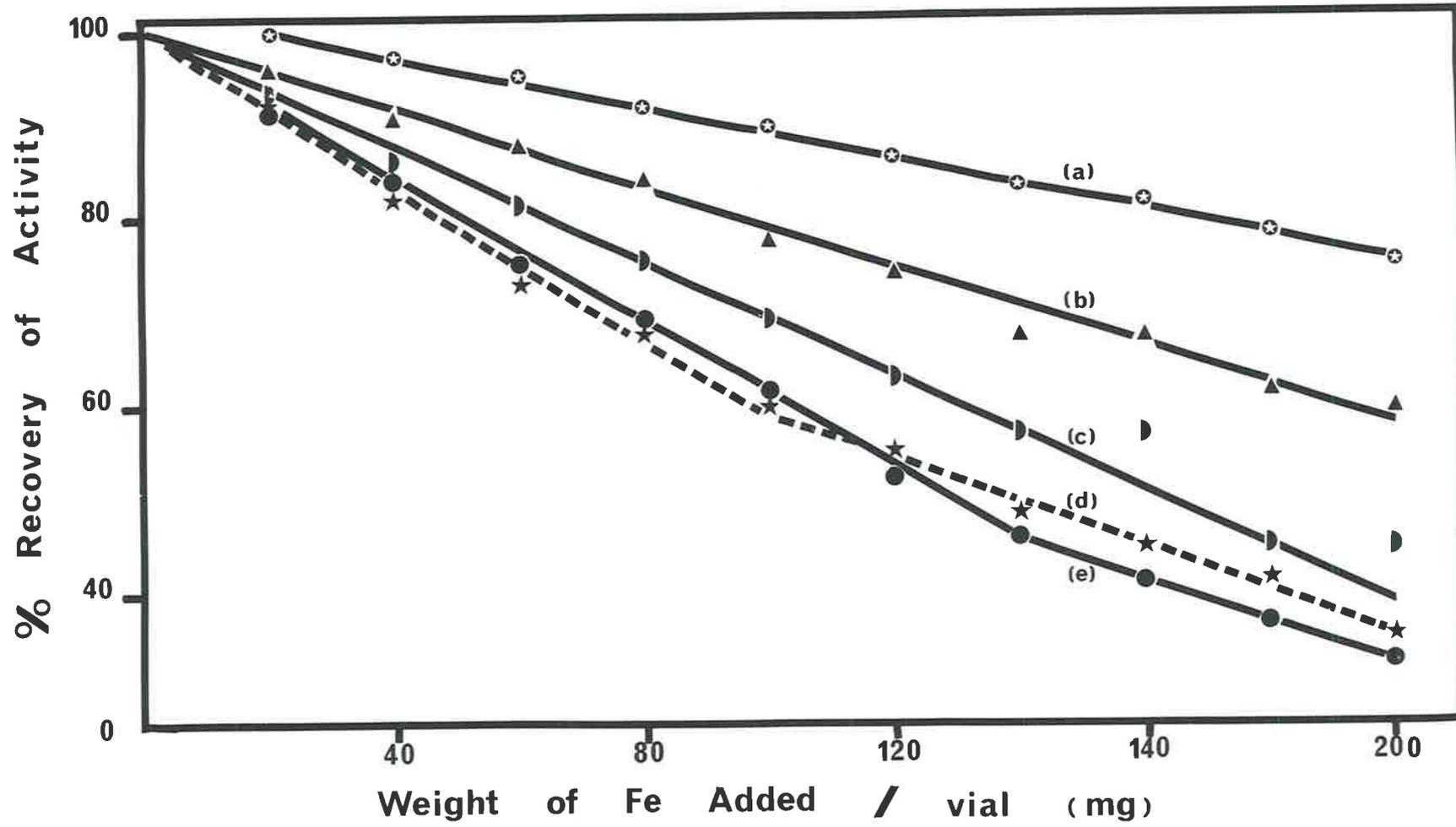
(d) < 250  $\mu\text{m}$

(e) < 45  $\mu\text{m}$

a, b, and c, were counted using toluene-PPO-dimethyl POPOP scintillant with CAB-O-SIL.

d and e were counted using toluene-PPO-dimethyl POPOP with Triton X-100 scintillant mixture.

FIG. 9



may account for the more drastic effect of Triton X-100 scintillant on counting efficiency than the toluene-PPO-dimethyl POPOP scintillant. Though it maintained the greyish-white colour of the ferric sulphate, Triton X-100 (an emulsifier) dispersed the ferric sulphate better in the scintillant. The better dispersion increased the opacity of the gel and as the amount of the ferric sulphate was increased, scattering of the light emitted increased (Cluley, 1962; Helf and White, 1957) resulting in lower counts.

With soil samples, however, this factor will not be of great importance unless samples very rich in iron oxides are studied, since the amount of iron required to reduce counts by 10% was about 20 mg of elemental iron per vial. Heavy metals such as iron are more likely to influence  $^{14}\text{C}$  counting through colour quenching.

#### 5.8.6. Effect of entrapped air

There was no difference between counts obtained for samples with air bubbles and the same samples counted with air bubbles removed (Tables 9a and 9b). In some isolated cases there was even reduction in counts whilst there was a small increase in others.

This was probably due to changes in sample characteristics such as evaporation of scintillant during the process of removal of the air bubbles. The counting statistics of the counter might also have been involved.

The lack of any significant improved effect on counts after de-aeration showed that the air bubbles did not affect the energy-transfer process in the scintillating gel. Thus the air did not serve as a quencher in this experiment.

Table 9a. Effect of entrapped air on counting efficiency

|     | Counts with air bubbles<br>(c.p.m.) | Counts without air bubbles<br>(c.p.m.) |
|-----|-------------------------------------|--|
| 1.  | 1,038.0 $\pm$ 20.8 *                | 1,044.6 $\pm$ 31.3 *                   |
| 2.  | 1,095.6 $\pm$ 21.9                  | 1,076.4 $\pm$ 32.3                     |
| 3.  | 1,028.2 $\pm$ 20.6                  | 1,050.8 $\pm$ 31.5                     |
| 4.  | 254.0 $\pm$ 12.7                    | 261.4 $\pm$ 13.1                       |
| 5.  | 240.1 $\pm$ 12.0                    | 233.8 $\pm$ 11.7                       |
| 6.  | 245.7 $\pm$ 12.3                    | 265.6 $\pm$ 13.3                       |
| 7.  | 232.6 $\pm$ 11.6                    | 239.0 $\pm$ 12.00                      |
| 8.  | 276.9 $\pm$ 13.8                    | 278.6 $\pm$ 13.9                       |
| 9.  | 280.3 $\pm$ 14.0                    | 287.8 $\pm$ 14.4                       |
| 10. | 320.9 $\pm$ 16.0                    | 319.5 $\pm$ 16.0                       |
| 11. | 297.5 $\pm$ 14.9                    | 307.4 $\pm$ 15.4                       |
| 12. | 816.9 $\pm$ 24.5                    | 796.5 $\pm$ 39.8                       |
| 13. | 463.3 $\pm$ 13.9                    | 476.2 $\pm$ 23.8                       |
| 14. | 657.2 $\pm$ 19.7                    | 655.1 $\pm$ 32.8                       |
| 15. | 1,255.9 $\pm$ 25.1                  | 1,242.0 $\pm$ 37.3                     |
| 16. | 420.7 $\pm$ 12.6                    | 418.9 $\pm$ 20.9                       |
| 17. | 847.0 $\pm$ 25.4                    | 836.1 $\pm$ 25.1                       |
| 18. | 703.1 $\pm$ 21.1                    | 721.1 $\pm$ 21.6                       |
| 19. | 1,347.1 $\pm$ 26.9                  | 1,338.1 $\pm$ 26.8                     |
| 20. | 190.5 $\pm$ 9.5                     | 188.8 $\pm$ 9.4                        |

\* Statistics of counting at 95% confidence limit.

Table 9b. Effect of entrapped air on counting efficiency  
(After internal standardization)

|     | Counts with air bubbles<br>(c.p.m.) | Counts without air bubbles<br>(c.p.m.) |
|-----|-------------------------------------|--|
|     | *                                   | *                                      |
| 1.  | 14,834.5 $\pm$ 207.7                | 14,463.0 $\pm$ 202.5                   |
| 2.  | 15,119.0 $\pm$ 211.7                | 15,135.5 $\pm$ 211.9                   |
| 3.  | 14,964.0 $\pm$ 209.5                | 14,962.0 $\pm$ 209.5                   |
| 4.  | 14,391.0 $\pm$ 201.5                | 14,420.0 $\pm$ 201.9                   |
| 5.  | 14,030.5 $\pm$ 196.4                | 14,081.5 $\pm$ 197.1                   |
| 6.  | 14,339.5 $\pm$ 200.8                | 14,568.0 $\pm$ 204.00                  |
| 7.  | 14,438.0 $\pm$ 202.1                | 14,436.0 $\pm$ 202.1                   |
| 8.  | 15,198.5 $\pm$ 212.8                | 15,107.5 $\pm$ 211.5                   |
| 9.  | 14,530.5 $\pm$ 203.4                | 14,520.5 $\pm$ 203.3                   |
| 10. | 14,931.5 $\pm$ 209.0                | 14,940.0 $\pm$ 209.2                   |
| 11. | 16,262.5 $\pm$ 227.7                | 16,354.0 $\pm$ 229.0                   |
| 12. | 14,599.5 $\pm$ 204.4                | 14,705.5 $\pm$ 205.9                   |
| 13. | 17,024.5 $\pm$ 238.3                | 16,884.0 $\pm$ 236.4                   |
| 14. | 16,075.5 $\pm$ 225.1                | 16,160.0 $\pm$ 226.2                   |
| 15. | 16,083.0 $\pm$ 225.1                | 16,175.0 $\pm$ 226.5                   |
| 16. | 17,524.0 $\pm$ 245.3                | 17,351.5 $\pm$ 242.9                   |
| 17. | 17,288.5 $\pm$ 242.0                | 17,321.0 $\pm$ 242.5                   |
| 18. | 16,520.5 $\pm$ 231.3                | 16,343.0 $\pm$ 228.8                   |
| 19. | 15,396.0 $\pm$ 215.5                | 15,537.0 $\pm$ 217.5                   |
| 10. | 16,482.0 $\pm$ 230.7                | 16,438.5 $\pm$ 230.1                   |

\* Statistics of counting at 95% confidence limit.

The magnitude of the quenching factor depends on oxygen solubility in the solvent which is determined by the external pressure and the temperature, and on the effect of oxygen quenching on the solvent and solute molecules (Birks, 1964). According to Ziegler et al. (1955, 1956) the quenching by oxygen involves both the concentration of the solute and the temperature of the solution. The higher the concentration of the solute, the greater is the effect of oxygen. On the other hand, Seliger (1958) has indicated that there is relatively large increase in fluorescence yield (increase in light output) upon reduction of temperature. Possible explanations for this are a reduction of internal quenching and self-quenching which is a property of concentration of the solute. The increase in viscosity could make for much better energy transfer and there is a possible change in the spectral emission of the solute to match even better the photocathode sensitivity.

Since the samples were counted at low temperature (4°C) all these possible factors might have operated. The reduction in efficiency of counting by dissolved oxygen might have been nullified by the increase light output due to the low temperature. In this situation removal of air would not affect the efficiency of counting significantly. Again solubility of the air was probably reduced in the presence of the gel (CAB-O-SIL) and hence quenching was reduced, since there would be no reaction between the air and either solvent or solute which would tend to produce compounds such as peroxides (Schram and Lombaert, 1963) and thus cause chemical quenching (lack of chemical quenching is one of the advantages of suspension counting).

Considering the time involved in the process of de-aeration and possible loss of counts, it was concluded that it was not necessary to

remove air bubbles.

#### 5.8.7. Effect of temperature of counting

The temperature at which suspension samples were counted had no effect on activity (number of counts observed) (Table 10).

The difference in counts between the two counters operating at the two temperatures (4° and 20°C) was brought about by factors inherent in the counters themselves and not differences in temperature. The counts obtained depended on the efficiencies of the two machines. There was a mean percentage difference of  $9.15 \pm 0.48\%$ . This was unexpected because of the differences in the phototubes in the two machines. The phototubes in both machines were the same [EM1, bialkali (K-Cs) 9635B] but there was a difference of 10% in efficiencies of the two. The machine operating at the colder temperature (2 to 4°C) has tubes with operating efficiency of 54% whilst the one at ambient temperature (20°) has efficiency of 64%.

Thus with the scintillant (toluene-PPO-dimethyl POPOP) used, samples could be counted at any temperature. The gels remained more viscous at the lower temperature. This is essential to cut down settling time of the particles. Suspensions kept at 4°C were stable for up to 3 weeks. Samples kept at 20°C may not be stable for such a long period.

If a scintillant including Triton X-100 is used and the samples being counted are alkaline, then a lower operating temperature is better to avoid chemiluminescence. It is also necessary not to leave samples on benches for a long time to avoid high levels of counts caused by phosphorescence.

Table 10. Effect of temperature of counting

|     | Counts at 4°C (c.p.m.) | Counts at 20°C (c.p.m.) |
|-----|------------------------|-------------------------|
|     | *                      | *                       |
| 1.  | 16,126.5 $\pm$ 225.8   | 17,492.5 $\pm$ 244.9    |
| 2.  | 16,584.5 $\pm$ 232.2   | 17,686.5 $\pm$ 247.6    |
| 3.  | 15,612.5 $\pm$ 218.6   | 17,112.5 $\pm$ 239.6    |
| 4.  | 15,281.0 $\pm$ 213.9   | 16,725.0 $\pm$ 234.2    |
| 5.  | 14,834.5 $\pm$ 207.7   | 16,353.0 $\pm$ 228.9    |
| 6.  | 15,119.0 $\pm$ 211.7   | 16,486.0 $\pm$ 230.8    |
| 7.  | 14,964.0 $\pm$ 209.5   | 16,673.0 $\pm$ 233.4    |
| 8.  | 14,391.0 $\pm$ 201.5   | 15,768.0 $\pm$ 220.8    |
| 9.  | 14,030.5 $\pm$ 196.4   | 15,233.0 $\pm$ 213.3    |
| 10. | 14,339.5 $\pm$ 200.8   | 15,601.5 $\pm$ 218.4    |
| 11. | 14,438.0 $\pm$ 202.1   | 15,547.5 $\pm$ 217.7    |
| 12. | 15,198.5 $\pm$ 212.8   | 16,174.0 $\pm$ 226.4    |

\* Statistics of counting at 95% confidence limit

CHAPTER IV. USE OF UNSTERILIZED SOIL AGGREGATES  
TO STUDY MICROBIAL ACTIVITIES

1. Introduction

It was pointed out in the literature review that the conventional method of incubation in the laboratory provides artificial results because the organic substrates are distributed in the soil in such a manner that the soil aggregates are virtually empty.

The influence of soil aggregates on microbial activity has been studied by a number of workers (Hattori, 1967, 1969; Nishio et al., 1968; Seifert, 1962, 1964; Greenwood and Berry, 1962; Craswell et al., 1970; Craswell and Waring, 1972a and b). Of the indices used to measure microbial activities in soil (Chapter II) release of CO<sub>2</sub> has often been adopted particularly during incubation of carbohydrates.

Where carbon dioxide evolution was used as the index, it was trapped in sodium hydroxide and titrated using acid. Such a method is not sufficiently sensitive to reveal small changes in carbon dioxide release unless large amounts of carbon dioxide are released in the studies.

Even though the use of <sup>14</sup>C in soil organic matter studies has been going on for about two decades, this tool has not been employed in studies involving microbial activities in soil aggregates. The contribution of <sup>14</sup>C to soil organic matter studies has been significant. The introduction of liquid scintillation spectrometry (Hiebert and Watts, 1953) made the use of isotopes, especially weak β-emitters such as <sup>14</sup>C,

more feasible. The advantage of liquid scintillation in such work is its sensitivity.

As mentioned above (Chapter II), the evolution of  $^{14}\text{CO}_2$  may not be a total measure of microbial activity but it will give an idea of the activity and also the extent of disappearance of  $^{14}\text{C}$ -labelled substrates introduced into a soil. It was thus decided to incubate  $^{14}\text{C}$ -labelled substrates in soil so that the evolved  $^{14}\text{CO}_2$  could be accurately determined by liquid scintillation spectrometry. This would allow slight differences to be revealed even when low activity samples were used. Incorporation of the  $^{14}\text{C}$  source into the soil followed by building of the aggregates from the amended soil was considered to simulate natural conditions where organic materials are found within the aggregates.

It was also decided to use two substrates; a small, water soluble compound which is mobile and a larger compound to represent a relatively immobile fraction of soil organic matter.

The initial studies involved use of unsterilized soils.

One problem envisaged in the Urrbrae fine sandy loam was the breakdown of aggregates on wetting. Therefore, unlabelled starch was used as a binding material in the  $^{14}\text{C}$ -glucose amended samples.

## 2. Materials and methods

### 2.1. Preparation of $^{14}\text{C}$ -labelled soil

#### 2.1.1. Materials

##### 2.1.1.1. Soils

Soils used in this and subsequent studies are described in the Appendix.

Soils obtained from two plots (0 to 10 cm) of the Urrbrae fine sandy loam (a red brown earth) were used. One of the plots had been under grass-legume pasture for over 25 years, and is hitherto referred to as permanent pasture (PP) sample. The other had been subjected to a wheat fallow rotation for over 40 years and is hitherto referred to as wheat fallow (WF) sample.

#### 2.1.1.2. Reagents

Unlabelled starch was used to stabilize aggregates. Uniformly labelled  $^{14}\text{C}$ -glucose (specific activity 10 mC/mM) and uniformly labelled  $^{14}\text{C}$ -starch (specific activity 1.2 mC/mg) were used as substrates and sources of  $^{14}\text{C}$  for labelling the soil.

#### 2.1.2. Methods

##### 2.1.2.1. Incorporation of $^{14}\text{C}$ -glucose into soil

Unlabelled starch (0.45 g) was dissolved in 10 ml of hot distilled water in a 100 ml beaker (see Appendix). 25 ml of cold distilled water was added to cool the solution. To the solution 29.55 g of soil (< 250  $\mu\text{m}$  particles) was added followed by another 25 ml of cold distilled water so that water to soil ratio was about 2:1. 1 ml of glucose solution containing 0.25  $\mu\text{Ci}$  of uniformly labelled  $^{14}\text{C}$ -glucose in 50 mg of unlabelled glucose was added to the mixture. The sample was then stirred with a glass rod. The beaker was placed in a bigger beaker containing ice (which acted as a cooling jacket) to avoid a rise in temperature during sonification.

The mixture was sonified for three minutes using a Branson Sonifier [Model LS75 (Branson Instruments Inc.)] with a power output of 75 watts and a vibration frequency at the probe tip of 20 Kc/sec. The tip of the probe was about 3 mm from the bottom of the beaker. Particles

stuck to the probe were washed into the sample with more distilled water.

About 10 samples were prepared each time. Samples to be used as controls were prepared in the same manner but without incorporation of  $^{14}\text{C}$ -labelled glucose.

#### 2.1.2.2. Drying of $^{14}\text{C}$ -tagged wet soil

Wet labelled soil samples prepared as above were transferred quantitatively into either 500 ml freeze-drying flasks or 1 litre pyrex round-bottomed flasks. Samples were then freeze-dried overnight using a dry ice-alcohol mixture in a freeze-drier operated by a vacuum pump.

The freeze-drying cabinet could handle only five flasks at a time. The rest of the wet samples were therefore stored at  $-15^{\circ}\text{C}$ . These were thawed prior to freeze-drying to avoid cracking of the flasks.

Freeze-dried  $^{14}\text{C}$ -tagged soils were put in sealed perspex containers and kept in a cold ( $-15^{\circ}\text{C}$ ) room until needed.

#### 2.1.2.3. Incorporation of $^{14}\text{C}$ -starch into soil

The procedure was essentially the same as described above for glucose except that 400 mg of unlabelled starch was used for stabilization instead of the 450 mg used for glucose amended samples.

1 ml of starch solution containing 0.125  $\mu\text{Ci}$  uniformly labelled  $^{14}\text{C}$ -starch in 50 mg starch was added as the source of  $^{14}\text{C}$ -labelled substrate.

## 2.2. Preparation of aggregates

### 2.2.1. Methods

#### 2.2.1.1. Aggregates > 1 mm

Freeze-dried soil into which either  $^{14}\text{C}$ -labelled glucose or  $^{14}\text{C}$ -labelled starch was incorporated was wetted to about 60% of the field capacity in a porcelain boat (or a pyrex bowl 7.5 cm in diameter by 5 cm deep). The sample was well mixed with a spatula and the thick paste pressed through a perspex block with cylindrical holes designed in such a way that the diameter was the same as the length of the cylinder. The top was levelled with spatula. Aggregates were dried at  $45^{\circ} \pm 2^{\circ}\text{C}$  (whilst still in the block) in a forced draught oven overnight. Dried aggregates were removed from the perspex block by pressing them down with a glass rod or a piece of steel wire (depending on the size of aggregate). Aggregates not for immediate use were stored in a cold ( $-15^{\circ}\text{C}$ ) room in sealed containers.

#### 2.2.1.2. Aggregates < 1 mm

For  $< 250 \mu\text{m}$  aggregates and control samples, freeze-dried samples were wetted to about 60% of field capacity and dried at  $45 \pm 2^{\circ}\text{C}$  in a forced draught oven for 24 hours. Samples were then gradually ground in such a way that the particles passed through a 60 mesh ( $< 250 \mu\text{m}$ ) sieve but not 300 mesh ( $< 53 \mu\text{m}$ ) sieve. For  $< 53 \mu\text{m}$  particles, prepared 7 mm aggregates were ground to pass a 300 mesh sieve.

## 2.3. Incubation studies

### 2.3.1. Materials

Samples used were artificially prepared aggregates of the

two soils containing the two substrates (glucose or starch). Aggregate sizes of 5 mm, 3 mm, < 250  $\mu\text{m}$  and < 53  $\mu\text{m}$  were used.

Controls were samples to which the  $^{14}\text{C}$ -labelled substrates were added in a conventional way after the aggregates were prepared. Substrates were introduced into the soil and mixed with a glass rod so that the substrates were distributed through the macropores. This is in contrast to the situation in the aggregate samples where the  $^{14}\text{C}$ -substrates were mixed with the soil before the aggregates were prepared and therefore substrates were assumed to be distributed within the micropores of the aggregates.

Incubating flasks were 250 ml pyrex conical flasks.

0.2 N and 0.1 N NaOH solution was used as absorbing agent for released  $^{14}\text{CO}_2$ .

### 2.3.2. Methods

#### 2.3.2.1. Wetting of aggregates

##### 2.3.2.1.1. Samples > 250 $\mu\text{m}$

About 20 g of aggregates of each size were weighed in duplicate. A double sheet of filter paper was cut to fit snugly into a petri dish. This was wetted and the aggregates placed carefully on the soaked filter paper. Samples were allowed to stand for about 20 minutes within which time the aggregates gradually absorbed water. This could be seen by the capillary rise of the water up the aggregates which took 5 minutes. Fifteen min was allowed for equilibration.

Wet aggregates were placed on a dry filter paper for a few seconds to remove excess water from the outside of the aggregates before being

transferred into pre-weighed incubation flasks. The flask and contents were then weighed to determine water content of the aggregates. If this was more than 80% of field capacity (see Appendix) the aggregates were allowed to stay on a dry filter paper for a longer period and if less than 80%, they were again transferred to the moisture-soaked filter paper in the petri dish to allow further wetting.

#### 2.3.2.1.2. Samples < 250 $\mu\text{m}$

Finer samples (< 250 and < 53  $\mu\text{m}$ ) were weighed into incubation flasks and the required amount of water added (about 8 ml for the permanent pasture soil and about 6 ml for the wheat fallow soil) to bring the samples to 80% of field capacity. Samples were then stirred to distribute the water. Wetting of all samples was done in a cold (3°C) room.

#### 2.3.2.1.3. Control samples

Twenty g (< 250  $\mu\text{m}$  aggregates) of both soils were weighed. Eight ml of glucose solution containing 0.5  $\mu\text{Ci}$  of glucose- $^{14}\text{C}$  in 50 mg of unlabelled glucose and another 8 ml of solution of starch containing 0.15  $\mu\text{Ci}$  of starch- $^{14}\text{C}$  in 50 mg of unlabelled soluble starch were used to bring samples of the permanent pasture to 80% of field capacity.

Six ml of both glucose and starch solutions with the same levels of  $^{14}\text{C}$  activity as used for the permanent pasture samples were used to bring the samples of the wheat fallow soil to 80% field capacity. Treatment was the same as for samples of < 250  $\mu\text{m}$ .

### 2.3.2.2. Incubation

Wet aggregates were transferred into weighed duplicate 250 ml pyrex conical flasks (unless otherwise stated these flasks will be referred to as "incubating flasks" throughout this thesis).

The weight of the flask and contents was noted. A rubber bung with steel coil was fitted into the top of the flask, and an absorbing vial (1.8 cm internal diameter x 5 cm internal depth) containing 8 ml of 0.2 N NaOH was suspended in the coil. All flasks were kept in the cold room after the above treatment. They were later re-inoculated with 1 ml of a 1% suspension of fresh soil from the respective plots. Flasks were transferred to an incubator set at  $28 \pm 2^{\circ}\text{C}$ . A beaker containing distilled water was placed in the incubator to provide humidity. Incubation was allowed to go on in the dark for 28 days.

#### 2.3.2.2.1. Change of absorbing vials

Vials were changed every half-day for the first week of incubation and thereafter changed daily for the rest of incubation period. 0.2 N NaOH was used as the absorbent in the first three days. This was changed to 0.1 N thereafter since the 0.2 N NaOH did not mix well with the scintillant.

#### 2.3.2.2.2. Determination of $^{14}\text{CO}_2$

1 ml aliquots of absorbent were added to 10 ml of Triton X-100 scintillant (as described in Chapter III, Section 2.4.1.2.2.1.) in vials the background counts of which had been pre-determined. The vial was shaken vigorously to blend the mixture and obtain a clear solution. Vials were kept in the counter (Packard liquid scintillation

spectrometer, Model 3375) for over 24 hours at 4°C to eliminate chemiluminescence. Samples were later counted using 20 to 1000 at 6% gain in the wide window and 20 to 100 at 6% gain in the narrow window.

#### 2.3.2.2.3. Determination of efficiency of counting for quench correction

Efficiency of counting was determined to correct for quenching in the samples. This was done by internal standardization.

Since there were too many samples to deal with throughout the incubation period, samples for each harvest were counted, using the external standard of the counter to obtain an external standard ratio. The value of these ratios is such that it provided a direct relative quenching characteristic in each sample. Vials were thus grouped according to their external standard ratio. Internal standard was added to three samples of each group. 10  $\mu$ l of  $^{14}\text{C}$ -toluene containing  $2.06 \times 10^4$  d.p.m. was added as the internal standard. Vials were shaken vigorously and recounted, using the same settings.

#### 2.4. Determination of initial $^{14}\text{C}$ activity in aggregate samples

A fraction of each sample of aggregates was not used for the incubation. These samples were ground with the Siebtechnik mill to pass a 300 mesh sieve ( $< 53 \mu\text{m}$ ).  $^{14}\text{C}$  counting of samples was done by suspension counting (Adu and Oades, 1973).

10 mg fractions were weighed into duplicate vials and the procedure then followed as described in Chapter III, Section 2.3.

Quench correction (efficiency) was determined by internal standardization as described above. A fraction of the secondary standard

benzoic acid- $^{14}\text{C}$  (specific activity 40,000 d.p.m./mg) dissolved in redistilled methanol was added.

### 3. Results and discussion

#### 3.1. Release of $^{14}\text{CO}_2$ during incubation

##### 3.1.1. $^{14}\text{C}$ -glucose amended samples

The results for the samples from the permanent pasture soils were similar but there were some minor differences (Figs. 10A, 10B, 11A and 11B).

The rate of release of  $^{14}\text{CO}_2$  from all the aggregates prepared from permanent pasture plot was high and reached a maximum during the 1st day of incubation (Fig. 10A). On the other hand, with the wheat fallow samples only the  $< 250 \mu\text{m}$  and the 3 mm aggregates showed maximum release of  $^{14}\text{CO}_2$  on the 1st day of incubation (Fig. 11A). All other samples showed maximum release of  $^{14}\text{CO}_2$  on the 2nd day of incubation. However, there was a lag phase of 0.5 days for all the samples of the wheat fallow except for the  $< 250 \mu\text{m}$  and the control sample whilst with the permanent pasture samples only the  $< 53 \mu\text{m}$  sample showed a lag period for 0.5 day.

A sharp decline in the rate of release of  $^{14}\text{CO}_2$  from all samples followed the initial peak and by the 4th day of incubation the rate of release of  $^{14}\text{CO}_2$  as percentage of initial  $^{14}\text{C}$  from all aggregate samples had dropped to about 1 to 3% per day. The rate of release of  $^{14}\text{CO}_2$  from the control sample of the wheat fallow soil was higher (ca 8% of initial  $^{14}\text{C}$  at the 4th day). There was a minor flush of  $^{14}\text{CO}_2$  from all samples on the 8th day of incubation (Figs. 10A, 11A) followed

Fig. 10. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -glucose in unsterilized, artificially-prepared aggregates of different sizes.

Soil: Urrbrae fine sandy loam

Plot: Permanent pasture

Aggregate sizes:

1. 5 mm
2. 3 mm
3. 1.5 mm
4. < 250  $\mu\text{m}$
5. < 53  $\mu\text{m}$
6. Control

For Figs. 10 through to 21  $\bar{I}$  represents L.S.D. (0.05) (the least significant difference at the 5% level). This was calculated using the standard error of the means for the curves.

FIG. 10

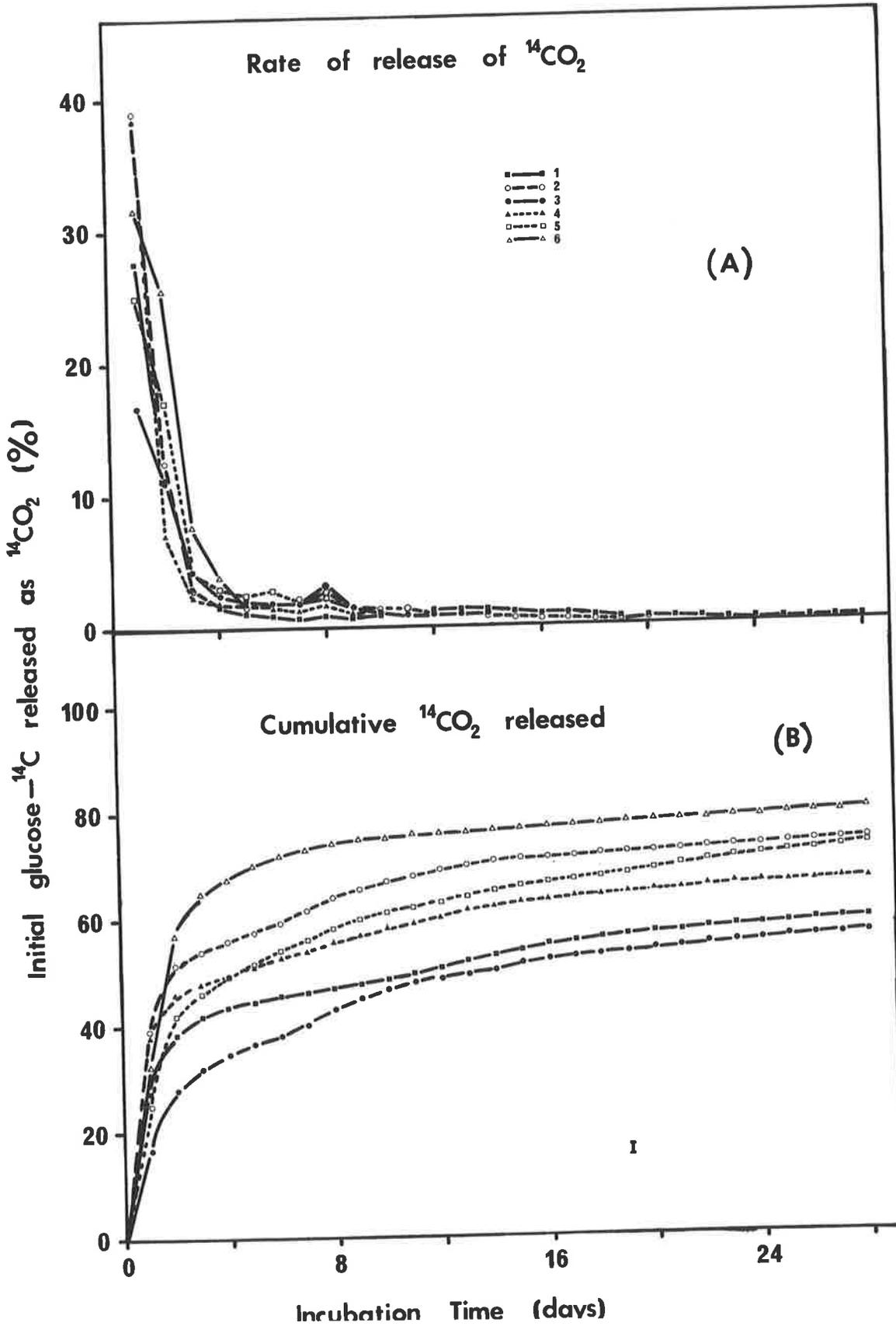


Fig. 11. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -glucose in unsterilized, artificially-prepared aggregates of different sizes.

Soil: Urrbrae fine sandy loam

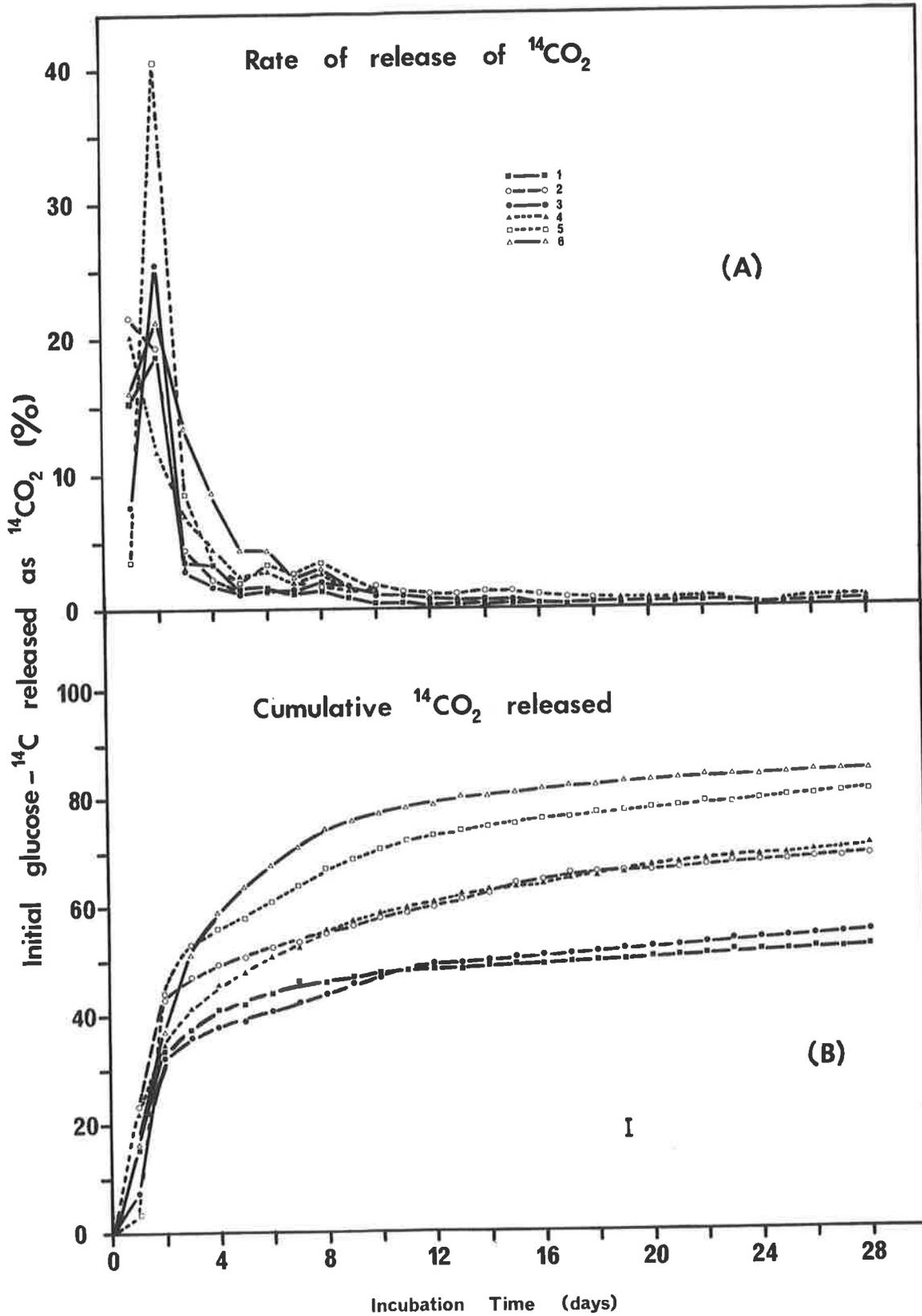
Plot: Wheat fallow

Aggregate sizes:

1. 5 mm
2. 3 mm
3. 1.5 mm
4. < 250  $\mu\text{m}$
5. < 53  $\mu\text{m}$
6. Control

I = L.S.D.(0.05)

FIG. 11



by a sharp decline in the rate of release of  $^{14}\text{CO}_2$ . After the 10th day of incubation all samples (including controls) showed a low rate of  $^{14}\text{CO}_2$  release.

Except for the 1.5 mm sample, between 39 and 57% of the initial  $^{14}\text{C}$  had been released as  $^{14}\text{CO}_2$  from the permanent pasture sample by the 2nd day of incubation (i.e. at the time of maximum rate of release of  $^{14}\text{CO}_2$  from samples of both soils) whilst the corresponding percentage values of  $^{14}\text{C}$  released as  $^{14}\text{CO}_2$  from the wheat fallow samples were between 33 and 44%. The residual  $^{14}\text{C}$  in most of the samples (particularly all samples of the permanent pasture except the 1.5 mm aggregates) was probably in microbial cell structures or metabolites. More than 40% of initial  $^{14}\text{C}$  had been released as  $^{14}\text{CO}_2$  from all samples by the 7th day of incubation (Table 11). The resistance of the residual  $^{14}\text{C}$  to attack by microbial enzymes after the 7th day is shown by the low release of  $^{14}\text{CO}_2$  (Table 11). This slow release caused flattening off of the  $^{14}\text{CO}_2$  evolution curves (Figs. 10B and 11B).

### 3.1.2. Analysis of results for statistical significance

Before detailed comparison of results were attempted it was necessary to assess the reliability of the data and the Biometry Section of the Institute was consulted resulting in the following approach to evaluating the results.

Analysis of results was done by fitting quadratic regression curves to the cumulative  $^{14}\text{CO}_2$  values (Y) on days of incubation (X) and comparing to see if there was any difference between curves or positions (intercepts) of the individual sizes of aggregates. Combined regression coefficients were used when there were no differences, and the equation

Table 11. Cumulative  $^{14}\text{CO}_2$  released expressed as percentage of initial  $^{14}\text{C}$  at different periods during incubation of  $^{14}\text{C}$ -glucose in soil aggregates

|   | Period of incubation (days) | % initial $^{14}\text{C}$ released as $^{14}\text{CO}_2$ |      |        |                   |                    | Control |
|---|-----------------------------|--|------|--------|-------------------|--------------------|---------|
|   |                             | 5 mm   | 3 mm | 1.5 mm | 250 $\mu\text{m}$ | < 53 $\mu\text{m}$ |         |
| Permanent pasture   | 7                           | 46.2   | 61.6 | 40.2   | 54.0              | 56.3               | 73.0    |
|   | 14                          | 53.00  | 70.4 | 50.3   | 62.3              | 65.1               | 76.7    |
|   | 21                          | 57.4   | 72.7 | 53.8   | 65.3              | 69.8               | 78.2    |
|   | 28                          | 59.1   | 74.0 | 56.6   | 66.6              | 73.2               | 79.1    |
| Mean of cumulative $^{14}\text{C}$ released as $^{14}\text{CO}_2$ |                             | 51.3   | 66.8 | 46.9   | 59.4              | 62.0               | 73.6    |
| L.S.D. (0.05)   |                             | 1.58   |      |        |                   |                    |         |
| Wheat fallow  | 7                           | 44.9   | 53.8 | 42.5   | 52.8              | 63.8               | 70.8    |
|   | 14                          | 48.9   | 62.7 | 50.0   | 62.6              | 74.6               | 80.3    |
|   | 21                          | 50.8   | 66.9 | 52.8   | 67.5              | 78.5               | 83.6    |
|   | 28                          | 52.3   | 68.9 | 55.1   | 70.9              | 81.1               | 85.1    |
| Mean of cumulative $^{14}\text{C}$ released as $^{14}\text{CO}_2$ |                             | 46.6   | 59.6 | 46.9   | 59.1              | 69.2               | 73.6    |
| L.S.D. (0.05)   |                             | 2.63   |      |        |                   |                    |         |

then followed the model

$$Y = \bar{Y}_i + b_{1c} (X - \bar{X}_i) + b_{2c} (X^2 - \bar{X}_i^2)$$

where Y is the percentage cumulative (total)  $^{14}\text{CO}_2$  released over a particular period of time,  $\bar{Y}_i$  is the mean cumulative value for aggregate size i;  $b_{1c}$  is the combined regression coefficient for the linear term; X is the number of days of incubation,  $\bar{X}_i$  mean of X; and  $b_{2c}$  the combined regression coefficient for the quadratic term. However, if there was a difference between regressions, the equation followed the model

$$Y = \bar{Y}_i + b_{1i} (X - \bar{X}_i) + b_{2i} (X^2 - \bar{X}_i^2)$$

where all terms are the same as in the first model except that  $b_{1i}$  and  $b_{2i}$  are regression coefficients for the linear and quadratic terms respectively for aggregates of size i.

When there was no difference between regressions or positions, the equation used for all sizes followed the model

$$Y = \bar{Y} + b_{1c} (X - \bar{X}) + b_{2c} (X^2 - \bar{X}^2)$$

where all terms are the same as in first model except that  $\bar{Y}$  and  $\bar{X}$  represent means for Y and X for combined data of all sizes. The mean of cumulative percentages of  $^{14}\text{C}$  released as  $^{14}\text{CO}_2$  for the aggregates in each soil were then compared to see if there were differences between values of two individual sizes (see Appendix for regression equations).

### 3.1.3. Comparison of aggregate sizes with respect to $^{14}\text{CO}_2$ release

Mean values between two individual sizes were significantly different for the samples from the pasture soil (Table 11). The control

sample was significantly different from any of the aggregate sizes ( $P < 0.001$ ). Similarly the difference between means of any of the other aggregates and the 3 mm sample was significant ( $P < 0.001$ ). Despite the fact that the total  $^{14}\text{C}$  released as  $^{14}\text{CO}_2$  (at the end of the incubation period) was about the same for the  $< 53 \mu\text{m}$  and the 3 mm samples (73.20% and 74.02% respectively) the difference between mean values of the two sizes was significant ( $P < 0.001$ ). This difference was caused by the relatively low release of  $^{14}\text{CO}_2$  in the  $< 53 \mu\text{m}$  (0.25%) as opposed to 12.4% for the 3 mm aggregates on the 1st day of incubation.

In the wheat fallow samples differences between the mean values of the various aggregate sizes were significant ( $P < 0.001$ ) from the mean value of the control (Table 11). The differences between means for the 3 mm and the 5 mm or 1.5 mm were also significant ( $P < 0.001$ ). However, means of  $^{14}\text{CO}_2$  released for the 3 mm and  $< 250 \mu\text{m}$  aggregates were not significantly different, and such was the case for the means of 5 mm and 1.5 mm aggregates.

Even though there were differences between results obtained from samples of different aggregate size in the two soils, these differences were smaller than expected. For the smaller aggregates and the control the differences were not large although they were statistically significant. The range for percentage  $^{14}\text{C}$  released as  $^{14}\text{CO}_2$  for the 5 mm aggregates was 55.7 to 62.7% and 50.6 to 54.1% for the pasture and the wheat fallow samples respectively, and for the 3 mm aggregates the ranges for the two soils were 72.2 to 75.8% and 70.4 to 67.5% respectively. A reliable comparison could not be made between the corresponding values for the  $< 250 \mu\text{m}$  and the  $< 53 \mu\text{m}$  samples from the two soils

because of the breakdown of incubation flasks in each of the wheat fallow samples.

Although the differences in  $^{14}\text{CO}_2$  released between aggregates of different sizes do not follow a simple relationship they showed that aggregates do affect the release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -glucose. Since the soils used were of fine sandy loam texture, the results are in contrast to the claim by Craswell and Waring (1972a and b) that the effect of aggregates on release of  $\text{CO}_2$  during incubation only occurs in heavy clay soils.

Glucose is easily metabolised by micro-organisms during incubation. It has been suggested that  $^{14}\text{C}$ -glucose is all utilized during incubation in the soil in as short a period as 1.5 days (Ladd and Paul, 1973). The results presented above support this claim. The high release of  $^{14}\text{CO}_2$  in the first two days of incubation in both two soils followed by a sharp drop on the 3rd day showed that after the 2nd day the substrate was probably exhausted and the remaining  $^{14}\text{C}$  was in a form relatively resistant to microbial attack (Mutatkar and Wagner, 1967; Wagner, 1968). This was probably in the biomass and microbial metabolites (Ladd and Paul, 1973). The results also show that the micro-organisms had immediate access to the substrate as might be expected of a small water soluble carbon compound. However, the differences in the relative release of  $^{14}\text{CO}_2$  between the aggregates demonstrated that there were differences either in the continued availability of the  $^{14}\text{C}$ -glucose or in the ability of the microbial populations to multiply. The cumulative release of  $^{14}\text{CO}_2$  was highest for the control samples since most of the  $^{14}\text{C}$ -glucose was within the macro-pores. This means the organisms had

direct access to it, in contrast to the situation occurring in the aggregates where the  $^{14}\text{C}$  was well distributed through the pore system, some no doubt in discontinuous pores.

The availability of the substrate in the smaller aggregates (except for the 1.5 mm size) was greater than in some of the bigger aggregates because the smaller aggregates provided bigger surface area for the organisms or their enzymes to explore. The relatively low release of  $^{14}\text{CO}_2$  from the 5 mm aggregates can be explained by assuming that a significant proportion of the labelled glucose was trapped in discontinuous pores. Alternatively, the possibility of anaerobic respiration in this sample cannot be ruled out since the radius (2.5 mm) of the aggregates was nearer the 3.0 mm size limit for aerobic respiration predicted by Greenwood (1968). The existence of anaerobic pockets within soil aggregates would no doubt differ from soil to soil. The results for the 1.5 mm size in both soils were somewhat out of place. This anomaly may have been caused by the method of preparation of the aggregates which might have rendered this size less porous. Because of the small size of these aggregates extra mechanical force was needed to get the soil paste through the holes in the perspex building block. This might have caused it to be more dense and less porous which might have affected both diffusion of oxygen into the aggregates and  $^{14}\text{CO}_2$  out of the aggregates.

#### 3.1.4. Interpretation of the $^{14}\text{CO}_2$ release curves

A detailed interpretation of the first and second peaks of  $^{14}\text{CO}_2$  release is given in Chapter VIII. The first peak is considered to be due to a primary predominantly fungal population which utilized

all available substrate. The second peak is thought to be the result of a secondary predominantly bacterial population utilizing metabolic and mycolytic products of the primary population.

However, other factors may have been involved. The control should give the highest peak for release of  $^{14}\text{CO}_2$  in the initial stages of incubation. The fact that the peak for release of  $^{14}\text{CO}_2$  from the control samples was more diffuse than those from the aggregates suggests that the method of incorporation of the  $^{14}\text{C}$  into the controls versus the samples, and subsequent treatments could be involved.

Since the  $^{14}\text{C}$ -glucose was incorporated into the aggregates which were subjected to wetting and drying before the beginning of the actual incubation, it is thought that the samples were pre-incubated and also partly sterilized due to the drying treatment. This sterilization followed by incubation would give a sudden peak for release of  $^{14}\text{CO}_2$  as a result of attack by micro-organisms on the dead fraction of the population which initially utilized part of the  $^{14}\text{C}$ -glucose (Birch, 1958). However, such an effect would be short-lived (Greenwood, 1968). This could explain the sudden sharp drop in  $^{14}\text{CO}_2$  released after the first peak of  $^{14}\text{CO}_2$  release. The drop in the release of  $^{14}\text{CO}_2$  in the control was not relatively as sharp because the  $^{14}\text{C}$ -glucose was incorporated prior to the incubation period and was not therefore pre-incubated. The pre-incubation also meant that some of the  $^{14}\text{C}$  remaining in the aggregates was in the cell structures or metabolites.

That the pre-incubation (drying) caused transformation of the  $^{14}\text{C}$  into complex compounds was demonstrated by results (Table 12a and b) obtained for the determination of  $^{14}\text{C}$  activity (disintegration per minute)

Table 12a. Initial  $^{14}\text{C}$  activity in dry aggregates

Permanent pasture samples

| Size of<br>Aggregates | $^{14}\text{C}$ -glucose incorporated<br>aggregates |           | $^{14}\text{C}$ -starch incorporated<br>aggregates |           |
|-----------------------|---|-----------|--|-----------|
|                       | activity in d.p.m.*                                 |           | activity in d.p.m.*                                |           |
| 5 mm                  | a   | 351,260.4 | 59.1   | 180,735.6 |
|                       | b   | 338,240.2 |  | 260,324.8 |
| 3 mm                  | a   | 274,387.2 | 74.0   | 147,602.6 |
|                       | b   | 266,642.0 |  | 115,304.7 |
| 1.5 mm                | a   | 207,261.4 | 56.6   | 185,878.8 |
|                       | b   | 192,500.3 |  | 149,211.9 |
| 250 $\mu\text{m}$     | a   | 222,732.3 | 66.6   | 188,963.2 |
|                       | b   | 237,900.9 |  | 175,262.3 |
| < 53 $\mu\text{m}$    | a   | 192,668.6 | 73.2   | 166,798.2 |
|                       | b   | 192,668.6 |  | 161,915.1 |

\* Mean of duplicate determinations

Table 12b. Initial <sup>14</sup>C activity in dry aggregates

Wheat fallow samples

| Size of<br>Aggregates |   | <sup>14</sup> C-glucose incorporated<br>aggregates | <sup>14</sup> C-starch incorporated<br>aggregates |
|-----------------------|---|--|---|
|                       |   | activity in d.p.m.*                                | activity in d.p.m.*                               |
| 5 mm                  | a | 240,579.1  | 233,003.43  |
|                       | b | 289,954.8  | 209,907.41  |
| 3 mm                  | a | 176,594.0  | 212,976.6   |
|                       | b | 232,856.7  | 167,147.3   |
| 1.5 mm                | a | 245,945.4  | 152,916.01  |
|                       | b | 159,227.8  | 86,691.7  |
| 250 μm                | a | 145,829.9  | 74,715.1  |
|                       | b | n.d.   | 251,003.2   |
| < 53 μm               | a | 150,315.7  | 140,489.0   |
|                       | b | n.d.   | 107,999.5   |

\* Mean of duplicate determinations

n.d. not determined; flask broke

in the different sizes of aggregates, by the suspension method developed in Chapter III. The percentage recovery of  $^{14}\text{C}$ , based on the activities incorporated prior to freeze-drying ranged from 24 to 63%. Many predictable factors were involved in this loss but the loss as  $^{14}\text{CO}_2$  and utilization of the  $^{14}\text{C}$ -glucose by existing organisms through the various stages of preparation of the dried aggregates might have played an important part. During the incubation the rate of  $^{14}\text{CO}_2$  release was probably affected by the source of the existing  $^{14}\text{C}$  in the aggregates.

### 3.2. Release of $^{14}\text{CO}_2$ during incubation

#### 3.2.1. $^{14}\text{C}$ -starch amended samples

Because starch is less soluble and more likely to be sorbed than glucose, it was expected that the rate of release of  $^{14}\text{CO}_2$  brought about by microbial activity during incubation would be less and the effects of structure would be more obvious.

Results (Figs. 12 and 13) showing the rate of release of  $^{14}\text{CO}_2$  from aggregates of both the permanent pasture (Fig. 12) and the wheat fallow soils (Fig. 13) indicate that these assumptions were substantially correct. A first peak of release of  $^{14}\text{CO}_2$  appeared on the 1st day of incubation for the permanent pasture samples (Fig. 12A) as was obtained for the glucose amended samples (Fig. 10A). The wheat fallow samples (Fig. 13A) also showed similar trend to the glucose amended samples (Fig. 11A) with maximum rate of release of  $^{14}\text{CO}_2$  appearing on the 1st and 2nd day of incubation.

There was again a sharp decline in the rate of release of  $^{14}\text{CO}_2$  from all samples following the initial peak as was obtained for the

Fig. 12. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -starch in unsterilized, artificially-prepared aggregates of different sizes.

Soil: Urrbrae fine sandy loam

Plot: Permanent pasture

Aggregate sizes:

1. 5 mm
2. 3 mm
3. 1.5 mm
4. < 250  $\mu\text{m}$
5. < 53  $\mu\text{m}$
6. Control

I = L.S.D. (0.05)

FIG. 12

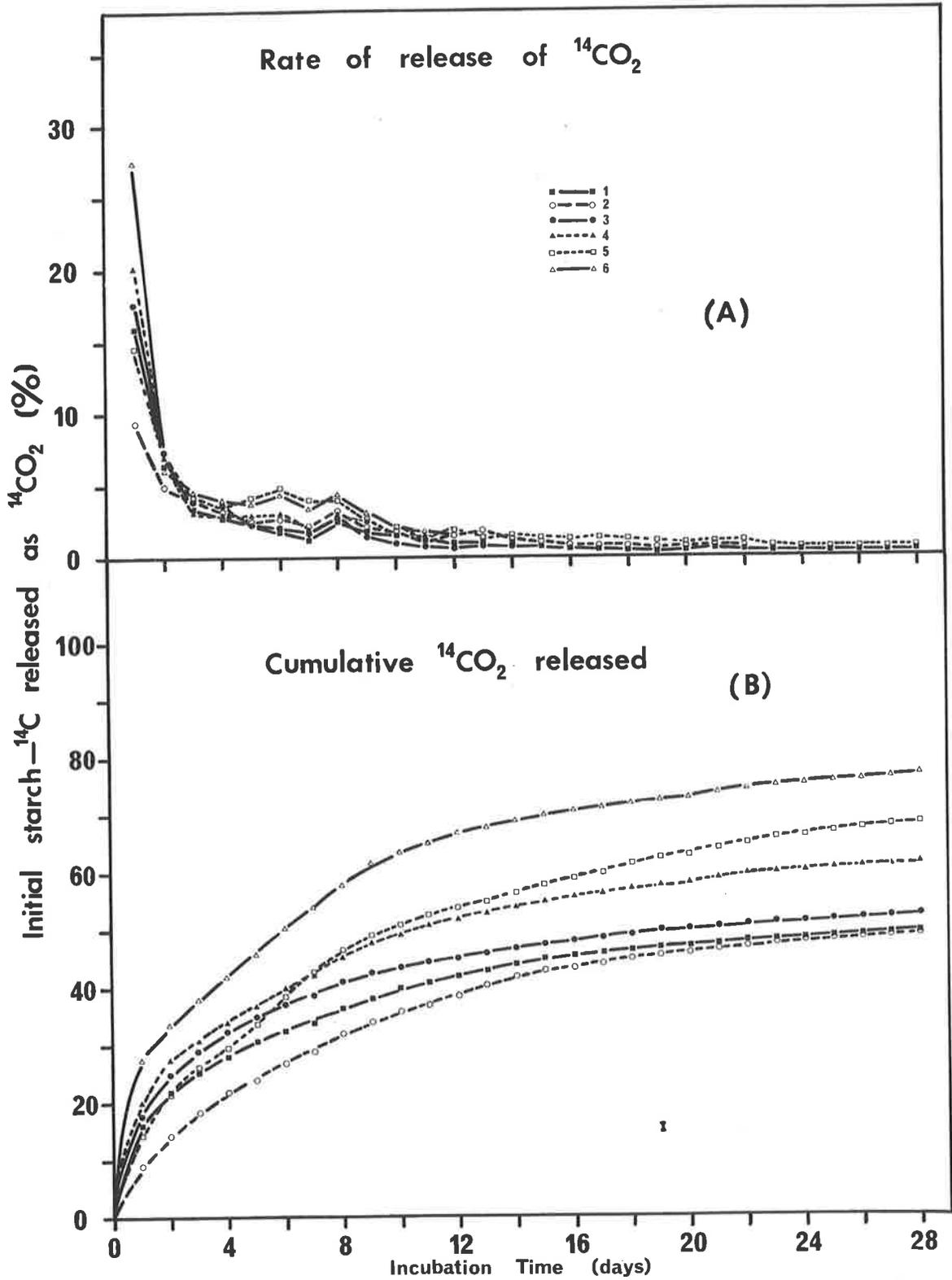


Fig. 13. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -starch in unsterilized, artificially-prepared aggregates of different sizes.

Soil: Urrbrae fine sandy loam

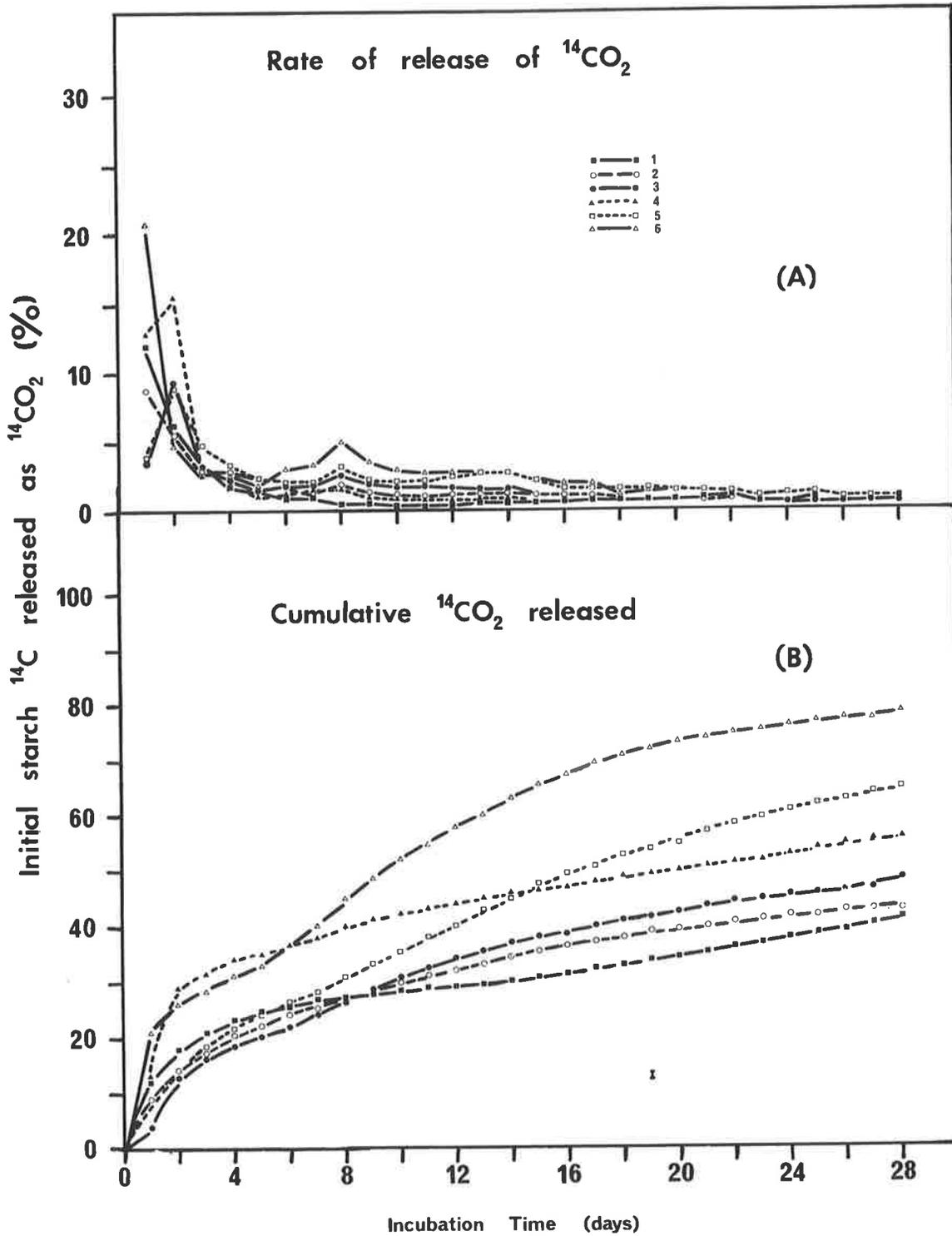
Plot: Wheat fallow

Aggregate sizes:

1. 5 mm
2. 3 mm
3. 1.5 mm
4. < 250  $\mu\text{m}$
5. < 53  $\mu\text{m}$
6. Control

$\bar{I}$  = L.S.D. (0.05)

FIG. 13



glucose amended samples and the rate of release was between 2 and 4% from the 4th day until the 7th day of incubation. A rise in the rate of release of  $^{14}\text{CO}_2$  was again observed on the 8th day of incubation but was followed by a sharp decline. The rate of release of  $^{14}\text{CO}_2$  then remained between 1 and 3% of the initial  $^{14}\text{C}$  from the 9th day until the 14th day of incubation when it dropped to 0.2 to 1% per day for the remainder of the incubation period (Figs. 12A and 13A).

The percentage of the initial  $^{14}\text{C}$  which was released as  $^{14}\text{CO}_2$  at the time of the first peak of  $^{14}\text{CO}_2$  release was 28% for the control sample of the permanent pasture and was similar to the 32% release obtained for the glucose amended control sample. Similarly about 22% of the initial  $^{14}\text{C}$  was released as  $^{14}\text{CO}_2$  from control samples of the wheat fallow in both glucose and starch amended samples. Thus the initial availability was similar for both substrates in the control samples (where substrates were distributed between macropores) showing that the chemistry of the two substrates had little effect on the rate at which they were metabolised by micro-organisms.

On the other hand, results for the aggregates show protection of the starch. Only about half as much of the initial  $^{14}\text{C}$  released as  $^{14}\text{CO}_2$  from the glucose amended samples was obtained for the corresponding starch amended samples of the permanent pasture during the 1st day of incubation (except for the 1.5 mm sample which was about equal - 17% from both samples). For most of the wheat fallow samples and the 3 mm aggregates of the permanent pasture, the release of  $^{14}\text{CO}_2$  was about one-quarter that obtained for the corresponding glucose amended sample.

The cumulative  $^{14}\text{CO}_2$  released as percentage of initial  $^{14}\text{C}$  in samples during the first week of incubation (Table 13), particularly from the wheat fallow samples, did not follow the expected trend. However it still showed that  $^{14}\text{CO}_2$  release from the control samples was higher than release from any of the aggregate samples. The results show that starch in the control samples was more easily accessible than in aggregates through which it was uniformly distributed. Similarly amongst the aggregates the results show that starch was more protected from attack by microbial enzymes in the larger aggregates of both soils. This was evident particularly in the permanent pasture samples. More than 40% of initial  $^{14}\text{C}$  was released as  $^{14}\text{CO}_2$  from the smaller aggregates of the permanent pasture soil but less than this value from the larger aggregates during the 1st week of incubation (Table 13). The mean values of  $^{14}\text{CO}_2$  released by the end of incubation (i.e. 28 days) showed that the amount of  $^{14}\text{CO}_2$  released was inversely related to the size of aggregates (except for the 5 mm aggregates from the permanent pasture soil).

### 3.3. Comparison of $^{14}\text{CO}_2$ values released from the different sizes of aggregates

Analysis of results were carried out in the same manner as described for the glucose incorporated samples.

In the permanent pasture samples, the difference between means of two individual sizes was significant ( $P < 0.001$ ). The mean value of one aggregate size was higher than the mean for immediate bigger sizes (Table 13).

Table 13. Cumulative  $^{14}\text{C}$  released expressed as percentage of initial  $^{14}\text{C}$  at different periods during incubation of  $^{14}\text{C}$ -starch in soil aggregates

| Soil type   | Period of incubation (days) | % initial $^{14}\text{C}$ released as $^{14}\text{CO}_2$ |      |        |                   |                    | Control |
|---|-----------------------------|--|------|--------|-------------------|--------------------|---------|
|   |                             | 5 mm   | 3 mm | 1.5 mm | 250 $\mu\text{m}$ | < 53 $\mu\text{m}$ |         |
| Permanent pasture   | 7                           | 34.0   | 28.9 | 38.7   | 42.2              | 42.6               | 53.8    |
|   | 14                          | 44.2   | 41.8 | 46.8   | 54.3              | 56.5               | 69.0    |
|   | 21                          | 47.6   | 46.7 | 50.7   | 59.1              | 64.4               | 73.7    |
|   | 28                          | 49.6   | 49.0 | 52.6   | 61.7              | 68.5               | 76.8    |
| Mean of cumulative $^{14}\text{C}$ released as $^{14}\text{CO}_2$ |                             | 40.7   | 37.6 | 44.2   | 50.3              | 52.4               | 63.5    |
| L.S.D. (0.05)   |                             | 0.84   |      |        |                   |                    |         |
| Wheat fallow  | 7                           | 26.7   | 25.4 | 24.2   | 38.2              | 28.0               | 40.1    |
|   | 14                          | 30.2   | 34.5 | 36.8   | 45.7              | 45.6               | 63.4    |
|   | 21                          | 35.6   | 40.2 | 43.6   | 51.7              | 58.7               | 75.2    |
|   | 28                          | 41.5   | 43.2 | 48.9   | 56.2              | 65.1               | 78.9    |
| Mean of cumulative $^{14}\text{C}$ released as $^{14}\text{CO}_2$ |                             | 30.6   | 32.5 | 33.6   | 44.2              | 42.9               | 58.3    |
| L.S.D. (0.05)   |                             | 0.88   |      |        |                   |                    |         |

There were significant differences ( $P < 0.001$ ) between the curves for the wheat fallow samples as evidenced by regression analysis. Mean values for  $^{14}\text{CO}_2$  released were all significantly different from each other. However, even though there was significant difference between values for the  $< 250 \mu\text{m}$  and the  $< 53 \mu\text{m}$  samples, and the cumulative totals showed that more  $^{14}\text{CO}_2$  was released from the  $< 53 \mu\text{m}$  samples than from the  $< 250 \mu\text{m}$  samples, the mean of the latter was higher (44.21% compared with 42.89%). The difference was caused by large differences in release of  $^{14}\text{CO}_2$  during the first two days of incubation. This difference persisted up to the 14th day when the values of the two sizes of aggregates levelled off (45.72% for the  $< 250 \mu\text{m}$  and 45.61% for the  $< 53 \mu\text{m}$ ).

Total  $^{14}\text{CO}_2$  released as a percentage of initial  $^{14}\text{C}$  activity in all aggregates (except the control samples) were lower than their corresponding values in the permanent pasture samples (Table 13).

#### 3.4. Comparison with glucose amended samples

The results for the  $^{14}\text{C}$ -starch amended samples were similar to those obtained with the  $^{14}\text{C}$ -glucose amended samples. The differences in percentage  $^{14}\text{C}$  released as  $^{14}\text{CO}_2$  in all the aggregate sizes compared to the 77 to 79% released in the controls showed evidence of factors which prevented the attainment of maximum release of  $^{14}\text{CO}_2$ . The low values for  $^{14}\text{CO}_2$  release obtained for the 5 mm aggregate sizes in both soils could be partly explained by anaerobic respiration caused by the large size of the aggregates, since the maximum limit for aerobic respiration in soil aggregates has been predicted to be 3.0 mm in radius (Greenwood, 1968). However, this would vary with the soil, and 2.5 mm radius was near enough to the 3 mm limit. On the other hand, the low

values obtained for the 3 mm samples in both soils indicated that the labelled substrates were to some extent protected by existing within aggregates. The smaller particles present a larger surface area so more substrate would be accessible and release of  $^{14}\text{CO}_2$  would tend to be higher. Surface area is considered an important factor in microbial utilization of organic materials in soils during incubation (Oades and Ladd, 1974).

Two peaks in the rate of  $^{14}\text{CO}_2$  release curves were again evident. The occurrence of the first peak of release of  $^{14}\text{CO}_2$  in the permanent pasture soil on the same day as for the glucose-treated samples was similar to the observation by Cheshire et al. (1969) who obtained a peak of  $^{14}\text{CO}_2$  production on the 5th day of incubation for both glucose and starch samples. Differences in elapsed time at which the second flush of  $\text{CO}_2$  occurred could be due to differences in the soils used. The rate of release of  $^{14}\text{CO}_2$  would be slower in a loamy soil of pH 4.6 than in a fine sandy loam of pH 6.0 (Persson, 1968; Chahal, 1968; Oades and Ladd, 1974).

### 3.5. Problems of interpretation

Complications in interpretation of the above results were brought about by the procedure used in preparing the aggregates (as observed for the glucose samples). That is, samples could be considered to be partly pre-incubated due to the wetting and drying procedure used during preparation. It was expected that compared with glucose a larger part of the original  $^{14}\text{C}$ -starch might have remained intact before the main incubation was started since it is less mobile than glucose. The wide range of  $^{14}\text{C}$  activity determined as initial values for the

aggregates (Table 12a and b), however, demonstrated that some of the  $^{14}\text{C}$  was lost during the preparation of the aggregates. This loss was probably partly due to utilization by the native microflora during the preparation and subsequent drying of the aggregates. The  $45^\circ\text{C}$  used for drying was chosen to suppress activity of most mesophiles whilst at the same time providing a minimum operating temperature for thermophilic organisms. The maximum time taken for drying of the aggregates was six hours. This meant the organisms did not have much chance to utilize much of the substrate. On the other hand, since recoveries of  $^{14}\text{C}$  originally incorporated into the soil (before aggregates were prepared) ranged from 31 to 94% it could be assumed that some of the  $^{14}\text{C}$  in the aggregates before incubation was in a wide range of macromolecules. Thus, the release of  $^{14}\text{CO}_2$  would have depended on the nature of these complex compounds.

The differences in  $^{14}\text{CO}_2$  released in the two soils of different cultural background was expected. Activities of enzymes hydrolysing starch is most positively significantly correlated with both amounts of soil organic carbon and vegetation on the soil (Ross, 1966). Soils under legume pasture were more active in starch hydrolysing enzymes than soils under grass. The soil under permanent pasture had organic carbon of 2.65% as opposed to 1.04% of the wheat fallow. Thus, the activity of the enzyme and the organisms producing it was probably higher in the permanent pasture samples.

### 3.6. Conclusions

The results obtained for both glucose and starch treated samples have shown that release of  $^{14}\text{CO}_2$  was affected by aggregate size.

The effects were not as large as anticipated and other factors were also involved. Even with glucose as a substrate uniform distribution throughout aggregates appeared to render some of the glucose inaccessible to organisms presumably in discontinuous pores. There was not a simple inverse relationship between total  $^{14}\text{CO}_2$  release and aggregate size for reasons which are not clear, but although the interpretation is made difficult because of the method of preparation of the aggregates it is obvious that over a 28 day incubation period release of  $^{14}\text{CO}_2$  is less from the aggregates than from the control.

The differences in release of  $^{14}\text{CO}_2$  between control and aggregates was much greater with starch as a substrate. The percentage  $^{14}\text{CO}_2$  released from both glucose and starch controls was similar indicating that the availability of the two substrates to organisms is similar. However, with one exception, the release of  $^{14}\text{CO}_2$  from glucose during the initial two days was twice that or more than the release from starch in the aggregate samples, indicating a significant physical factor in the utilization of the macromolecule compared with glucose.

The use of unsterilized soil meant attack of substrates from both within and outside aggregates which may have minimized the differences between aggregate sizes expected. It was also possible that the influence of aggregates in protecting organic material from microbial attack might not be well demonstrated in the one soil type studied. Thus, the problem warranted further investigation, using a wider range of soils.

CHAPTER V. USE OF STERILIZED SOIL AGGREGATES  
TO STUDY MICROBIAL ACTIVITIES

1. Introduction

In using unsterilized, artificially prepared aggregates in the previous section, it was assumed that micro-organisms were distributed both within and outside the aggregates. Some of these organisms might have been killed by drying but some of the mesophiles as well as the thermophiles would no doubt survive the treatment and would be active after wetting.

Thus, after inoculation the  $^{14}\text{C}$ -substrates might have been attacked from both inside and outside the aggregates. There would not be much difference in released  $\text{CO}_2$  between the aggregate sizes but a difference would be expected between the aggregates and the controls. In control samples, substrates were supposed to be only within the macropores and the introduced organisms which would be the more active fraction of the population would also be within the macropores. Hence, they would have easier access to the substrates.

The results in the last section revealed that amounts of  $^{14}\text{CO}_2$  released from aggregates of different sizes were different, but the differences between aggregates and controls were not as large as expected.

It was thought that the way the aggregates were built was too artificial because of the mechanical factors involved.

To look at the problem in detail, it was decided to use sterilized soil aggregates so that after inoculation with fresh soil solution,

attack of the substrates within the aggregates by the organisms or their enzymes would be assumed to come from outside. The manner of preparing the aggregates was also changed in order to produce aggregates under more natural conditions.

With the report by Craswell and Waring (1972a and b) that differences in microbial activity between aggregate sizes only occurred in heavier soils, it was decided to use two heavy clay soils in addition to the fine sandy loam used in the previous section.

## 2. Materials and methods

### 2.1. Preparation of $^{14}\text{C}$ -labelled soil samples and aggregates

#### 2.1.1. Materials

##### 2.1.1.1. Soils

Two heavy clay soils were employed in addition to the Urrbrae fine sandy loam.

Urrbrae B horizon - a red clay soil, and a grey clay (a heavy textured self-mulching soil) were used. Characteristics of these soils are given in the Appendix.

##### 2.1.1.2. $^{14}\text{C}$ source

Uniformly labelled  $^{14}\text{C}$ -glucose (Glucose-U- $^{14}\text{C}$ ) and uniformly labelled  $^{14}\text{C}$ -starch (Starch-U- $^{14}\text{C}$ ) both with the same specific activities as given in the previous section were used.

Polyvinyl alcohol (PVA) Shin Etsu, grade P-20S was prepared in 1% concentration and used to stabilize the aggregates.

## 2.1.2. Methods

### 2.1.2.1. Labelling with Glucose-U-<sup>14</sup>C

To 300 g of soil (< 250  $\mu\text{m}$ ) in a 500 ml graduated beaker, 50 ml solution of glucose containing 1.2 g of unlabelled glucose with 20  $\mu\text{Ci}$  of glucose-U-<sup>14</sup>C was added. 100 ml of distilled water was used to wash the beaker containing the glucose onto the soil. Chloroform (4 ml) was added to suppress microbial activities (particularly during the drying period). The mixture was thoroughly stirred with a glass rod to give an even distribution of the <sup>14</sup>C within the soil. The sample was dried in a forced draught oven at  $45 \pm 2^\circ\text{C}$ .

All four samples were prepared in the same manner.

### 2.1.2.2. Preparation of control samples

Controls were initially treated in the same way as labelled samples. About 100 g of representative samples were wetted without addition of any source of <sup>14</sup>C. Samples were dried, ground to pass 60 mesh (< 250  $\mu\text{m}$ ) sieves, and weighed into polythene bags ready for sterilization.

### 2.1.2.3. Labelling with starch-U-<sup>14</sup>C

Samples were prepared by the same procedure as described for labelling with <sup>14</sup>C-glucose.

250 ml of starch solution containing 10  $\mu\text{Ci}$  of <sup>14</sup>C in 600 mg of soluble starch was added to 300 g of soil.

### 2.1.2.4. Preparation of aggregates

Dried soils were ground to pass 8, 16, 60, 120 and 300 mesh sieves. Sizes of 1 to 2 mm, 124 to 250  $\mu\text{m}$  and < 53  $\mu\text{m}$  aggregates

were thus obtained.

#### 2.1.2.5. Stabilization of aggregates

Aggregates were spread in small amounts in petri dishes which were arranged on a bench. A chromatography spray (150 ml) filled with 1% solution of PVA [poly(vinyl alcohol)] was suspended on a stand at a height of 24". An air jet was connected to the spray so that the PVA solution was sprayed in fine droplets on to the aggregates. Aggregates were dried at  $45 \pm 2^\circ\text{C}$  and the process repeated. The bigger aggregates were easily separable from each other after drying but the finer samples had to be ground and sieved again.

Samples were kept in a cold ( $-15^\circ\text{C}$ ) room until further treatment.

#### 2.1.2.6. Sterilization of aggregates

Aggregates were sterilized by gamma-radiation in excess of 2.5 Mrad by Westminster Carpets, Dandenong, Vic. Australia.

Aggregates were weighed into duplicate self-sealing plastic bags. They were packed in an insulated box (to avoid breakage of aggregates) and the package sent for sterilization.

### 2.2. Incubation of sterilized aggregates

Sterilized samples were kept in the cold room until needed.

#### 2.2.1. Methods

##### 2.2.1.1. Wetting of aggregates

Samples were brought from the cold room to the laboratory just prior to the beginning of experiment.

Part of each sample was weighed into duplicate incubation flasks (250 ml pyrex conical flasks). The remaining fraction was left in the polythene bags and this was used for determining initial activity of the sample.

Wetting was done with a chromatography spray as described above with distilled water in place of PVA solution. The water was sprayed directly onto the aggregates in the incubation flasks. All samples were wetted to 80% of the field capacity, but were not stirred, except for the controls.

#### 2.2.1.2. Preparation of inoculant (fresh soil suspension)

Fresh soil samples from the Urrbrae permanent pasture plot and wheat fallow rotation plot were used. Only these two samples were available in the fresh form.

1.5 g and 1.3 g of fresh soil of the permanent pasture and wheat fallow samples respectively were weighed into 100 ml graduated cylinders. Distilled water was added up to the mark. Cylinder was well shaken to mix. The suspension prepared this way represented 1% of the fresh sample on oven-dry weight basis.

#### 2.2.1.3. Incubation

Flasks with wet aggregates were corked with rubber bung and its attached coiled steel wire. Absorbing vial with 8 ml of 0.1 N NaOH was suspended in the flask. 1 ml of 1% fresh soil suspension was used to inoculate the samples.

All samples except those of wheat fallow rotation were inoculated with fresh soil from the permanent pasture plot. This had to be done

since there were no fresh samples for the Urrbrae B horizon and the grey clay.

Samples were incubated at  $28^{\circ} \pm 2^{\circ}\text{C}$ .

#### 2.2.1.3.1. Controls

Samples were treated in the same way except that substrates were added during the wetting of samples. 200 mg of glucose containing 1  $\mu\text{Ci}$  of glucose-U- $^{14}\text{C}$  was added to duplicate 50 g samples. 100 mg of soluble starch containing 0.625  $\mu\text{Ci}$  of starch-U- $^{14}\text{C}$  was added in solution to 50 g ( $< 250 \mu\text{m}$ ) samples and stirred to distribute the substrates.

#### 2.2.1.3.2. Change of absorbing vials during incubation

This was done daily for the first 14 days. They were then changed once in two days and then two consecutive times of 4 days duration each.

Samples were thus incubated for a period of 24 days.

#### 2.2.1.3.3. Determination of $^{14}\text{CO}_2$

This was done as has been described already (Chapter III, Section 2.4.1.2.2.2.) using the same scintillant.

#### 2.2.1.3.4. Quench correction for samples

This was done by determining efficiency of counting by internal standardization using  $^{14}\text{C}$ -toluene standard as described already (Chapter IV, Section 2.3.2.2.3.).

2.2.1.4. Determination of initial  $^{14}\text{C}$  activity in aggregate samples

Initial  $^{14}\text{C}$  activity in aggregate samples was determined as described in Chapter IV, Section 2.4.

3. Results and discussion

3.1. Release of  $^{14}\text{CO}_2$  from  $^{14}\text{C}$ -glucose amended samples

3.1.1. Fine sandy loam samples

Samples from the two fine sandy loam soils behaved similarly. The results were similar to those obtained with the non-sterile samples. An initial flush of  $^{14}\text{CO}_2$  occurred showing maximum release on the 2nd, 3rd or 4th day of incubation (Figs. 14 and 15). A gradual (compared with non-sterile samples) decline in release of  $^{14}\text{CO}_2$  followed, leading to minimum rate of release on the 7th day of incubation (Figs. 14A and 15A).

A second flush of  $^{14}\text{CO}_2$  appeared in all samples on the 9th day of incubation. This flush was followed by a sharp decline in the rate of release of  $^{14}\text{CO}_2$  and by the 14th day of incubation release of  $^{14}\text{CO}_2$  had declined to a constantly low level which was maintained until the end of the incubation. This suggested that after 14 days of incubation the source of  $^{14}\text{C}$  was resistant to attack by microbial enzymes. The cumulative  $^{14}\text{CO}_2$  released at this time (Table 14) indicated that all the  $^{14}\text{C}$ -glucose had been utilized and the residual  $^{14}\text{C}$  was in microbial tissues or metabolites.

Fig. 14. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -glucose in sterilized, artificially-prepared aggregates of different sizes.

Soil: Urrbrae fine sandy loam

Plot: Permanent pasture

Aggregate sizes:

1. 1 to 2 mm
2. 124 to 250  $\mu\text{m}$
3. < 53  $\mu\text{m}$
4. Control

$\bar{\quad}$  = L.S.D. (0.05)

FIG 14

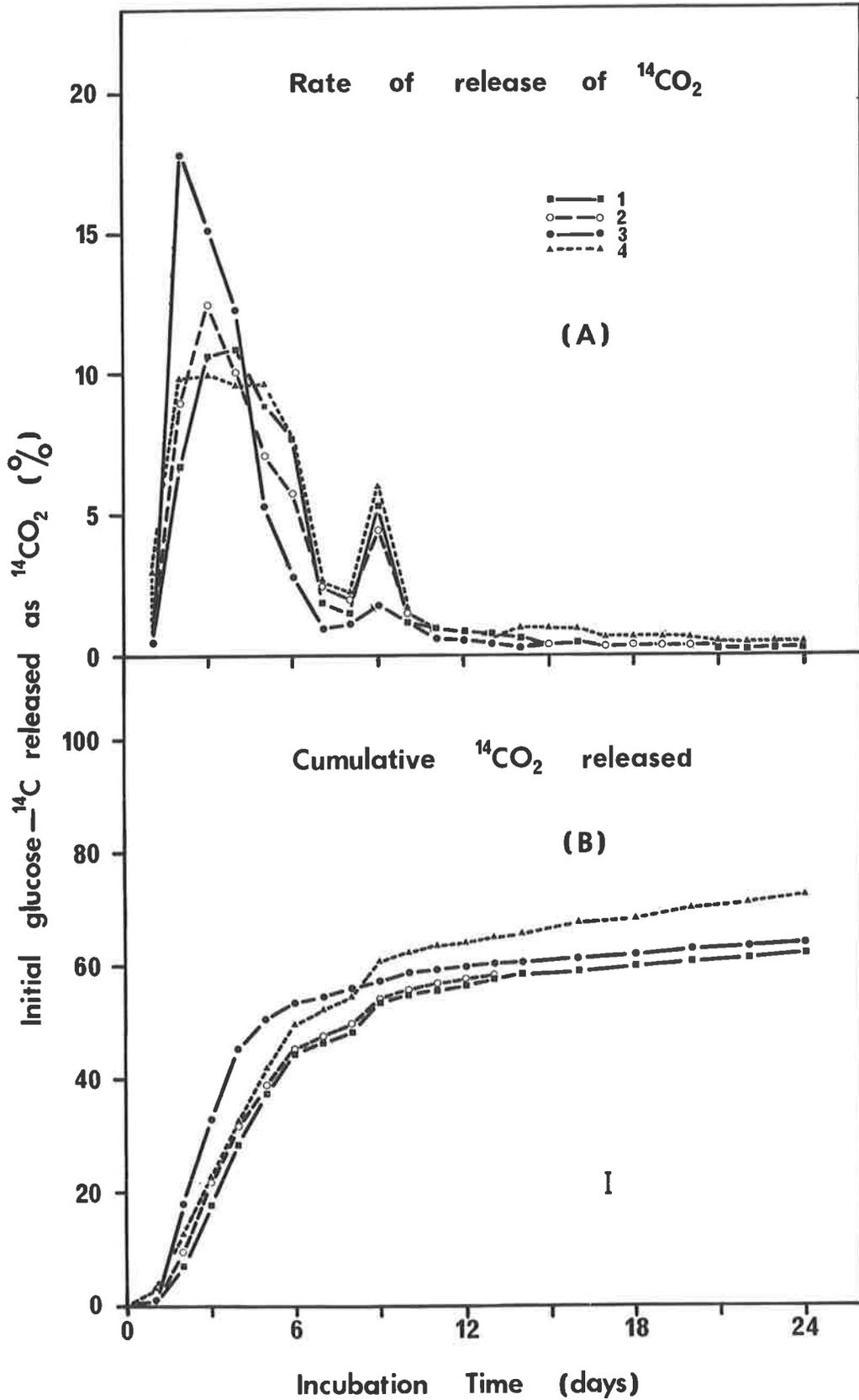


Fig. 15. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -glucose in sterilized, artificially-prepared aggregates of different sizes.

Soil: Urrbrae fine sandy loam

Plot: Wheat fallow

Aggregate sizes:

1. 1 to 2 mm
2. 124 to 250  $\mu\text{m}$
3. < 53  $\mu\text{m}$
4. Control

$\bar{I} = \text{L.S.D. (0.05)}$

FIG. 15

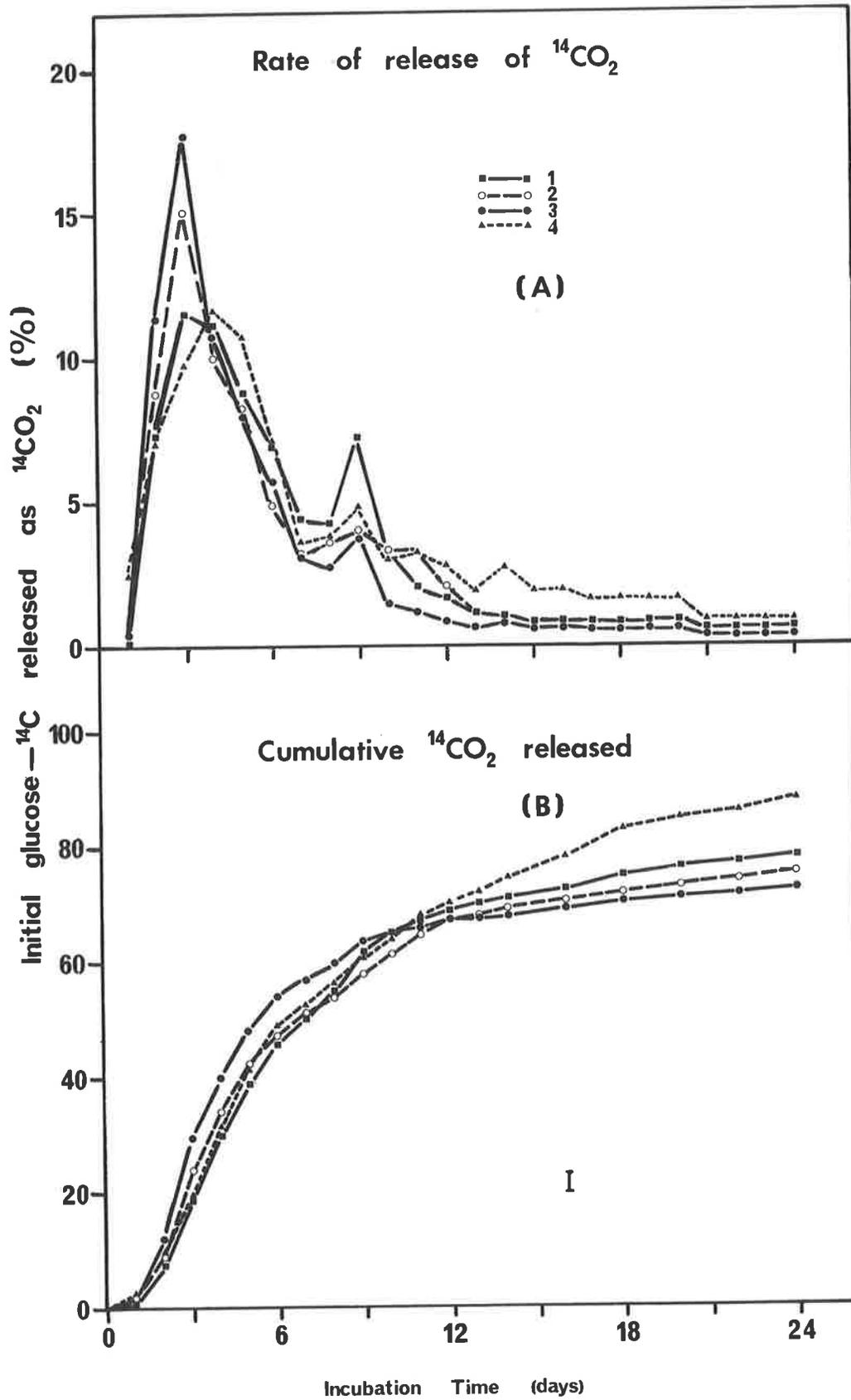


Table 14. Cumulative release of  $^{14}\text{C}\text{O}_2$  expressed as percentage of initial  $^{14}\text{C}$  at different periods during incubation of  $^{14}\text{C}$ -glucose in artificial aggregates of different soils

|                   | Days | % initial $^{14}\text{C}$ released as cumulative $^{14}\text{C}\text{O}_2$ |                          |                    |         |
|-------------------|------|--|--------------------------|--------------------|---------|
|                   |      | 1 to 2 mm  | 124 to 250 $\mu\text{m}$ | < 53 $\mu\text{m}$ | Control |
| Permanent pasture | 7    | 47.2   | 47.7                     | 54.7               | 52.5    |
|                   | 14   | 58.5   | 59.6                     | 60.7               | 65.9    |
|                   | 24   | 62.4   | 62.4                     | 64.2               | 72.5    |
| Mean values       |      | 49.1   | 49.7                     | 54.0               | 56.4    |
| L.S.D. (0.05)     |      | 3.04   |                          |                    |         |
| Wheat fallow      | 7    | 50.4   | 50.4                     | 57.0               | 52.5    |
|                   | 14   | 70.9   | 68.9                     | 68.3               | 74.6    |
|                   | 24   | 78.2   | 75.2                     | 72.5               | 87.7    |
| Mean values       |      | 58.7   | 57.3                     | 58.6               | 62.6    |
| L.S.D. (0.05)     |      | 2.60   |                          |                    |         |
| Red clay          | 7    | 32.9   | 45.2                     | 39.7               | 46.6    |
|                   | 14   | 71.2   | 72.5                     | 64.2               | 68.2    |
|                   | 24   | 81.4   | 84.8                     | 76.6               | 84.1    |
| Mean values       |      | 53.2   | 57.8                     | 50.7               | 58.1    |
| L.S.D. (0.05)     |      | 2.26   |                          |                    |         |
| Grey clay         | 7    | 70.6   | 59.3                     | 47.5               | 54.3    |
|                   | 14   | 80.2   | 67.3                     | 64.9               | 76.8    |
|                   | 28   | 83.5   | 71.3                     | 70.6               | 83.0    |
| Mean values       |      | 71.2   | 59.6                     | 53.7               | 63.3    |
| L.S.D. (0.05)     |      | 2.98   |                          |                    |         |

The results therefore support the suggestion that cumulative measurements made after 1 to 2 weeks of incubation are adequate for comparison between soils in studies of the effects of physical disruption on organic matter decomposition (Craswell and Waring, 1972a).

The release of  $^{14}\text{CO}_2$  from both control samples was slow and reached a maximum on the 3rd and 4th day of incubation for the pasture and wheat fallow soils respectively. There was a negligible lag phase in the control samples followed by a constantly high level of  $^{14}\text{CO}_2$  release for 4 days. This suggested an even supply of energy ( $^{14}\text{C}$ -glucose) for the developing microbial population.

The cumulative release of  $^{14}\text{CO}_2$  after different incubation periods (Table 14) shows that more than 45% of the initial  $^{14}\text{C}$  had been released from all samples by the 7th day of incubation. More  $^{14}\text{CO}_2$  (52 and 57%) was released from the  $< 53 \mu\text{m}$  and control samples indicating a limited availability of the  $^{14}\text{C}$ -glucose to attack by micro-organisms in the larger aggregates.

The cumulative  $^{14}\text{CO}_2$  released (as percentage of initial  $^{14}\text{C}$ ) at the end of the incubation (Table 14) was similar for all aggregate sizes. However, the mean values (Table 14) show that there were significant differences between the control and the  $< 53 \mu\text{m}$  sample and the other aggregate samples. Although more  $^{14}\text{CO}_2$  was released from the  $< 53 \mu\text{m}$  sample than from the other aggregate samples of the wheat fallow soil by the 7th day of incubation (Table 14) this was offset by greater  $^{14}\text{CO}_2$  release after this period from the larger aggregates. There were no significant differences between mean values for the  $^{14}\text{CO}_2$  released from

aggregates at the end of the incubation but the control was significantly different from all the aggregate samples. Thus, in both soils the availability of glucose was less in aggregated samples, presumably due to limited accessibility of substrate to organisms.

### 3.1.2. Clay samples

Results obtained for the two clay soils (Figs. 16 and 17) were different from those obtained for the fine sandy loam samples (Figs. 14 and 15) and the clay soils differed from each other. There was a period of adjustment of the micro-organisms to the new environment in the red clay aggregates (Fig. 16A). Consequently, there was a lag period in the rate of release of  $^{14}\text{CO}_2$  for three days which probably delayed maximum release of  $^{14}\text{CO}_2$  until the 5th and 6th day of incubation for aggregates and the 4th day for the control sample.

There was no lag phase for samples of the grey clay (Fig. 17A) and maximum release of  $^{14}\text{CO}_2$  was reached by the 2nd day of incubation for all samples except the 53  $\mu\text{m}$  sample, which attained maximum  $^{14}\text{CO}_2$  release on the 4th day of incubation.

In spite of the late maximum rate of release of  $^{14}\text{CO}_2$  from the red clay, all samples of both clay soils showed the relatively inactive phase (between the 7th to 8th day) followed by a flush of  $^{14}\text{CO}_2$  on the 9th day of incubation. This rise was quite pronounced for the 1 to 2 mm aggregates of the red clay (Fig. 16A) whilst for the grey clay it was highest in the control sample.

The rate of release of  $^{14}\text{CO}_2$  after the 9th day of incubation from both clays was similar to that observed for other soils with relatively

Fig. 16. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -glucose in sterilized, artificially-prepared aggregates of different sizes.

Soil: Red clay

Aggregate sizes:

1. 1 to 2 mm
2. 124 to 250  $\mu\text{m}$
3. < 53  $\mu\text{m}$
4. Control

I = L.S.D. (0.05)

FIG.16

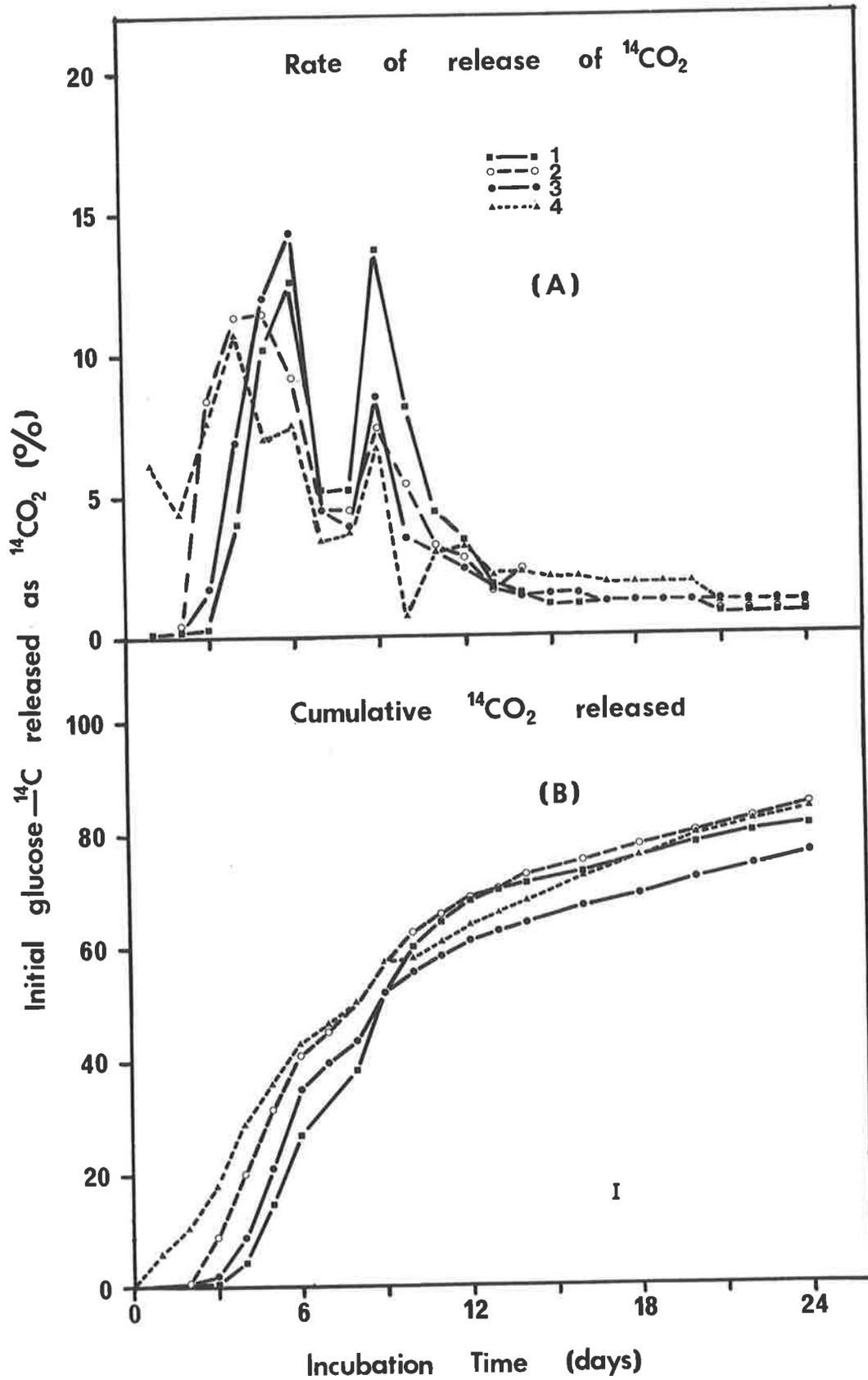


Fig. 17. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -glucose in sterilized, artificially-prepared aggregates of different sizes.

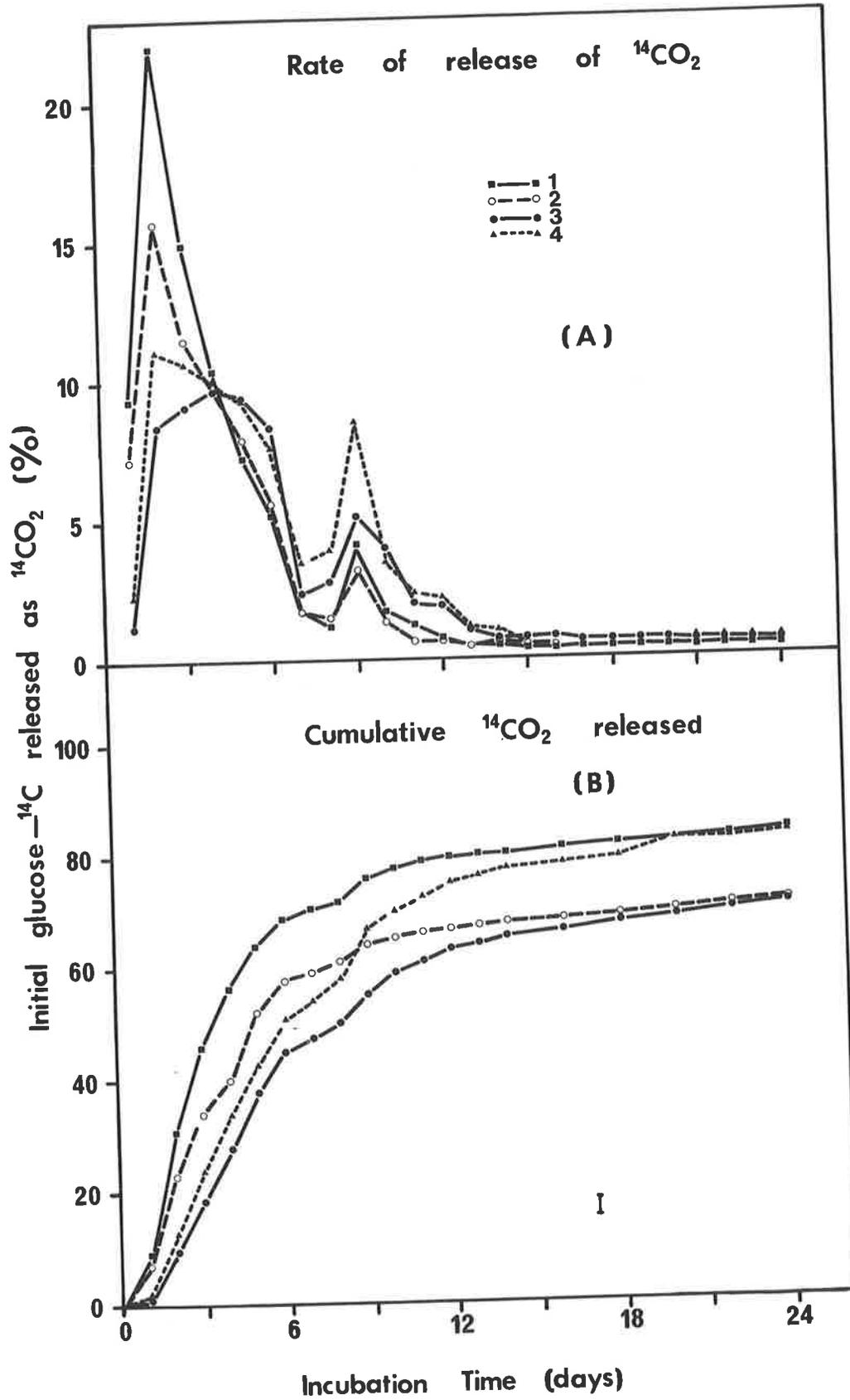
Soil: Grey clay

Aggregate sizes:

1. 1 to 2 mm
2. 124 to 250  $\mu\text{m}$
3. < 53  $\mu\text{m}$
4. Control

$\bar{I}$  = L.S.D. (0.05)

FIG. 17



low microbial activities after the 14th day of incubation. However, there were differences between values of released  $^{14}\text{CO}_2$  after the 14th day of incubation (Table 14) in both clays.

Values of cumulative  $^{14}\text{CO}_2$  released during the first week of incubation (Table 14) were highest for the larger aggregates in contrast to the situation in the fine sandy loam samples where the reverse occurred. With the red clay the mean values of  $^{14}\text{CO}_2$  released from the control and the 124 to 250  $\mu\text{m}$  samples were significantly different from the values obtained for other aggregates. For the grey clay, the initial differences between the values of released  $^{14}\text{CO}_2$  (Table 14) persisted and therefore significant differences existed between all samples.

A common aspect of the two clay soils was that less  $^{14}\text{CO}_2$  was released from the  $< 53 \mu\text{m}$  samples (in contrast to the fine sandy loam). The main difference between the clays was that higher mean values for release of  $^{14}\text{CO}_2$  were obtained for the larger aggregates from the grey clay whilst in the red clay samples the mean value for the 1 to 2 mm sample was lower than the mean value for the control and the 124 to 250  $\mu\text{m}$  aggregates (Table 14).

### 3.1.3. Interpretation of results

#### 3.1.3.1. $^{14}\text{CO}_2$ release curves

The occurrence of a second peak in plot of rate of release of  $^{14}\text{CO}_2$  was not in itself significant, but the appearance of the peaks at the same time from different samples (9th day of incubation) was surprising. In Chapter IV, the second peak of  $^{14}\text{CO}_2$

release appeared on the 8th day of incubation, but there was no initial lag phase in the release of  $^{14}\text{CO}_2$ . Presumably, the factors contributing to the appearance of the second peak in both studies were the same, but, in the present studies the initial lag phase (of 1 day for the fine sandy loam) had extended the period before occurrence of the second peak by one day.

Detailed interpretation of the appearance of the second peak is given in Chapter VIII.

All studies on irradiation of soils have shown that, apart from sterilization, effects on the physical and chemical properties have been mild and often negligible (Skujins, 1967). However, phytotoxic effects in irradiated soils have been observed during growth of subterranean clover in Urrbrae fine sandy loam (Bowen and Rovira, 1961). The long lag period in the red clay samples was probably due to adjustment of the organisms to their new environment as the period of lag phase depends on many factors (Lichstein, 1959).

#### 3.1.3.2. Behaviour of the grey clay

The initial high rate of release of  $^{14}\text{CO}_2$  from the larger aggregates of the grey clay was probably due to the high affinity of this type of soil for water. The clay content was more than 50% and the minerals were mainly illitic and randomly interstratified material with little kaolinite. The soil is a self-mulching clay so that aggregates are destroyed and created during drying and wetting cycles.

Wetting the clay caused extensive swelling and structural reorganization of aggregates so that porosity and water movement within the

aggregates was increased. At the same time diffusion of the glucose into macropores was probably faster since solute diffusion depends on the volumetric water content of the soil (Griffin, 1972). Thus, the  $^{14}\text{C}$ -glucose was rendered more accessible to enzyme attack by extensive structural reorganization on wetting.

On the other hand, the swelling of the  $< 53 \mu\text{m}$  particles on absorption of water decreased interaggregate porosity and gaseous exchange because all pores were water filled (Stotzky, 1966). Hence, the lower rate of release of  $^{14}\text{CO}_2$  from an anaerobic situation.

The red clay behaved differently from the grey clay because, even though it swells on wetting it has not got the characteristics of a self-mulching soil.

Compared with the fine sandy loam aggregates more  $^{14}\text{CO}_2$  was released from aggregates of the clay soils. However, release of  $^{14}\text{CO}_2$  from the control samples of the wheat fallow soil was about the same as that released from the controls of the two clay soils.

### 3.2. Release of $^{14}\text{CO}_2$ from $^{14}\text{C}$ -starch amended samples

#### 3.2.1. Fine sandy loam samples

The rates of release of  $^{14}\text{CO}_2$  from samples amended with starch are shown in Figs. 18 and 19. Again as shown for the glucose-amended samples, similarity existed between samples from the two fine sandy loam soils (Figs. 18A and 19A). Maximum release of  $^{14}\text{CO}_2$  from the aggregates and control samples of both soils occurred on the 2nd day of incubation for all samples except for the 1 to 2 mm aggregates of the wheat fallow. The control samples showed the highest percentage

Fig. 18. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -starch in sterilized, artificially-prepared aggregates of different sizes.

Soil: Urrbrae fine sandy loam

Plot: Permanent pasture

Aggregate sizes:

1. 1 to 2 mm
2. 124 to 250  $\mu\text{m}$
3. < 53  $\mu\text{m}$
4. Control

$\bar{I}$  = L.S.D. (0.05)

FIG.18

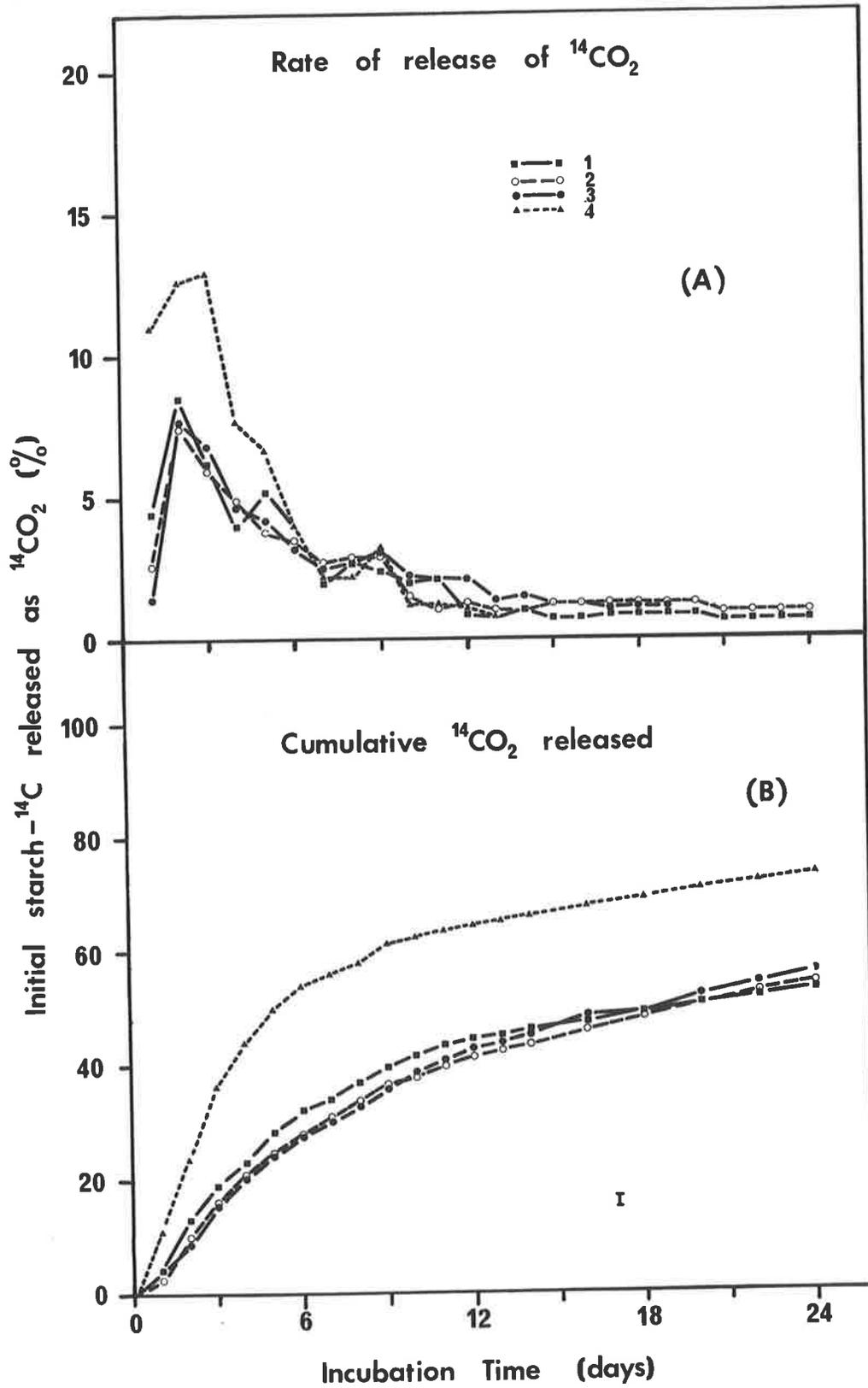


Fig. 19. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -starch in sterilized, artificially-prepared aggregates of different sizes.

Soil: Urrbrae fine sandy loam

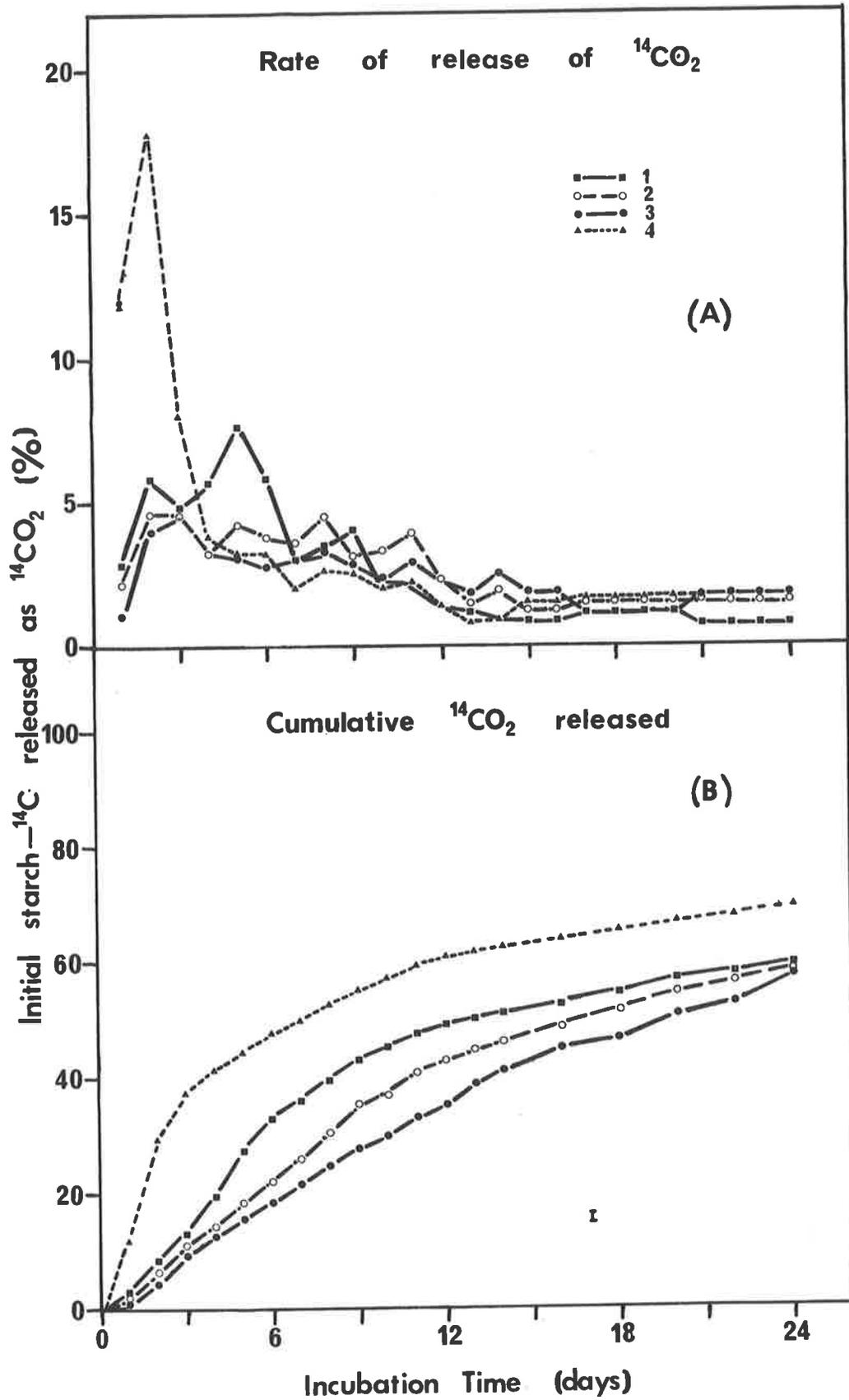
Plot: Wheat fallow

Aggregate sizes:

1. 1 to 2 mm
2. 124 to 250  $\mu\text{m}$
3. < 53  $\mu\text{m}$
4. Control

$\bar{I} = \text{L.S.D. (0.05)}$

FIG. 19



release (13% for permanent pasture and 18% for wheat fallow) at the time of maximum  $^{14}\text{CO}_2$  release.

More than 40% of the initial  $^{14}\text{C}$  introduced into the control samples had been released as  $^{14}\text{CO}_2$  by the 4th day of incubation in both soils. Over the same period the corresponding value was about 20% for all the aggregates of the permanent pasture and the larger aggregates (1 to 2 mm) of the wheat fallow. It was not until after 8 days of incubation that the cumulative  $^{14}\text{CO}_2$  released from any of the aggregate samples reached 40% (Table 15).

Instead of the sharp decline in the rate of evolution of  $^{14}\text{CO}_2$  after the initial flush (as noted for glucose amended samples) all samples showed a gradual stepwise decline in release of  $^{14}\text{CO}_2$  with several minor peaks up to the 14th day of incubation. At this time 41 to 66% of the initial  $^{14}\text{C}$  had been released as  $^{14}\text{CO}_2$  from all samples with more than 60% having been released from the control (Table 15). The table also shows that there were only minor differences between the aggregates of both fine sandy loam soils with respect to cumulative  $^{14}\text{CO}_2$  released by the 14th day of incubation. The occurrence of flushes of  $^{14}\text{CO}_2$  up to the 14th day of incubation demonstrates that metabolism of starch in aggregates was more complex than that of glucose.

After the 14th day of incubation the rate of release of  $^{14}\text{CO}_2$  declined to a low constant level (about 1% per day) until the end of incubation and showed the more resistant nature of the  $^{14}\text{C}$  source to enzyme attack after this period. The mean values of cumulative  $^{14}\text{CO}_2$  released, expressed as percentage of initial  $^{14}\text{C}$  (Table 15) showed that the early differences between the samples probably contributed to the

Table 15. Cumulative release of  $^{14}\text{CO}_2$  expressed as percentage of initial  $^{14}\text{C}$  at different periods during incubation of  $^{14}\text{C}$ -starch in artificial aggregates of different soils

|                   | Days | % initial $^{14}\text{C}$ released as cumulative $^{14}\text{CO}_2$ |                          |                    |         |
|-------------------|------|---|--------------------------|--------------------|---------|
|                   |      | 1 to 2 mm   | 124 to 250 $\mu\text{m}$ | < 53 $\mu\text{m}$ | Control |
| Permanent pasture | 7    | 34.4  | 30.9                     | 30.4               | 56.0    |
|                   | 14   | 46.2  | 43.4                     | 45.5               | 66.4    |
|                   | 24   | 53.1  | 53.9                     | 55.9               | 73.1    |
| Mean values       |      | 39.7  | 38.0                     | 38.5               | 59.2    |
| L.S.D. (0.05)     |      | 1.67  |                          |                    |         |
| Wheat fallow      | 7    | 36.1  | 25.9                     | 21.6               | 50.2    |
|                   | 14   | 51.0  | 46.4                     | 41.4               | 62.5    |
|                   | 24   | 59.6  | 58.9                     | 53.4               | 69.5    |
| Mean values       |      | 42.8  | 38.0                     | 33.3               | 56.8    |
| L.S.D. (0.05)     |      | 1.28  |                          |                    |         |
| Red clay          | 7    | 30.5  | 41.2                     | 18.0               | 26.8    |
|                   | 14   | 45.0  | 58.0                     | 34.5               | 39.7    |
|                   | 24   | 58.4  | 70.6                     | 47.9               | 46.6    |
| Mean values       |      | 38.3  | 49.3                     | 28.6               | 32.5    |
| L.S.D. (0.05)     |      | 1.39  |                          |                    |         |
| Grey clay         | 7    | 50.6  | 53.4                     | 47.1               | 39.6    |
|                   | 14   | 64.4  | 65.3                     | 60.8               | 52.9    |
|                   | 24   | 72.6  | 75.2                     | 68.3               | 60.3    |
| Mean values       |      | 56.7  | 57.9                     | 52.3               | 45.2    |
| L.S.D. (0.05)     |      | 2.15  |                          |                    |         |

significant differences between pairs of samples within the wheat fallow soil. These differences existed in spite of the fact that about 60% of the initial  $^{14}\text{C}$  in all aggregate samples was released as  $^{14}\text{CO}_2$  in 24 days.

A similar situation occurred with the permanent pasture soil in which the mean value of the cumulative  $^{14}\text{CO}_2$  released from the larger aggregates (1 to 2 mm) was significantly different from the value for the 124 to 250  $\mu\text{m}$ , though total  $^{14}\text{CO}_2$  released as a percentage of initial  $^{14}\text{C}$  was about the same (Table 15). All controls were significantly different from aggregate samples.

The results show that the  $^{14}\text{C}$ -starch was most accessible to attack by enzyme in the bigger aggregates but only marginally so.

### 3.2.2. Clay soils

The two clay soils behaved similarly to the glucose amended samples (Figs. 20 and 21). A one day lag phase was observed for the red clay (Fig. 20A). Subsequently the rate of release of  $^{14}\text{CO}_2$  reached a maximum by the 3rd day of incubation. The differences between samples was evident by this time with the rate of release of  $^{14}\text{CO}_2$  being highest in the 124 to 250  $\mu\text{m}$  aggregates.

There was no lag period in rate of release of  $^{14}\text{CO}_2$  from the grey clay samples (Fig. 21A). A high release of  $^{14}\text{CO}_2$  occurred on the 1st day of incubation from all the samples with the rate of release of  $^{14}\text{CO}_2$  from the 1 to 2 mm aggregates being highest. The maximum rate of release of  $^{14}\text{CO}_2$  was reached on the 2nd day of incubation for all samples with the release from control being low (12% of initial  $^{14}\text{C}$  released as  $^{14}\text{CO}_2$ ).

Fig. 20. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -starch in sterilized, artificially-prepared aggregates of different sizes.

Soil: Red clay

Aggregate sizes:

1. 1 to 2 mm
2. 124 to 250  $\mu\text{m}$
3. < 53  $\mu\text{m}$
4. Control

I = L.S.D. (0.05)

FIG. 20

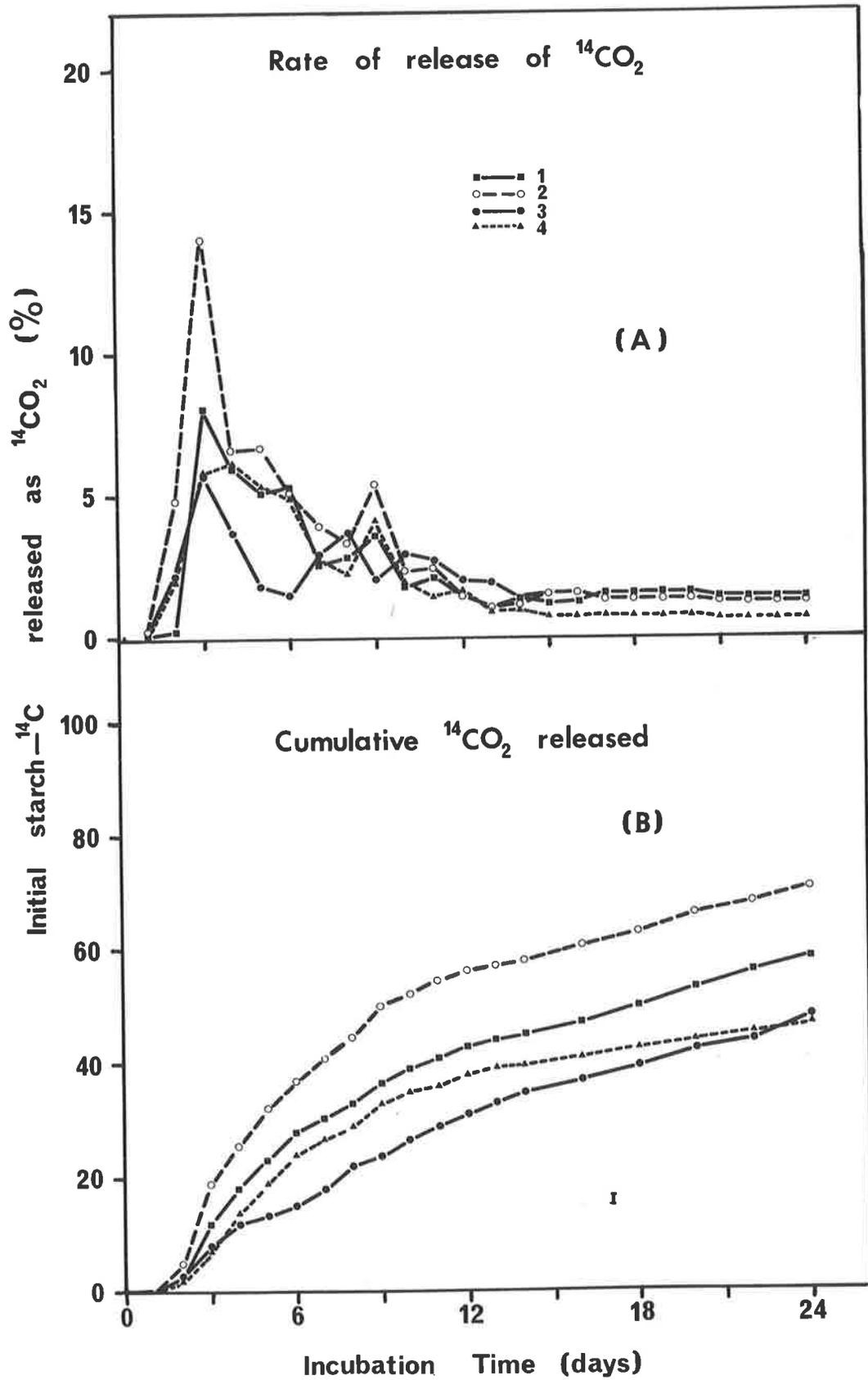


Fig. 21. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -starch in sterilized, artificially-prepared aggregates of different sizes.

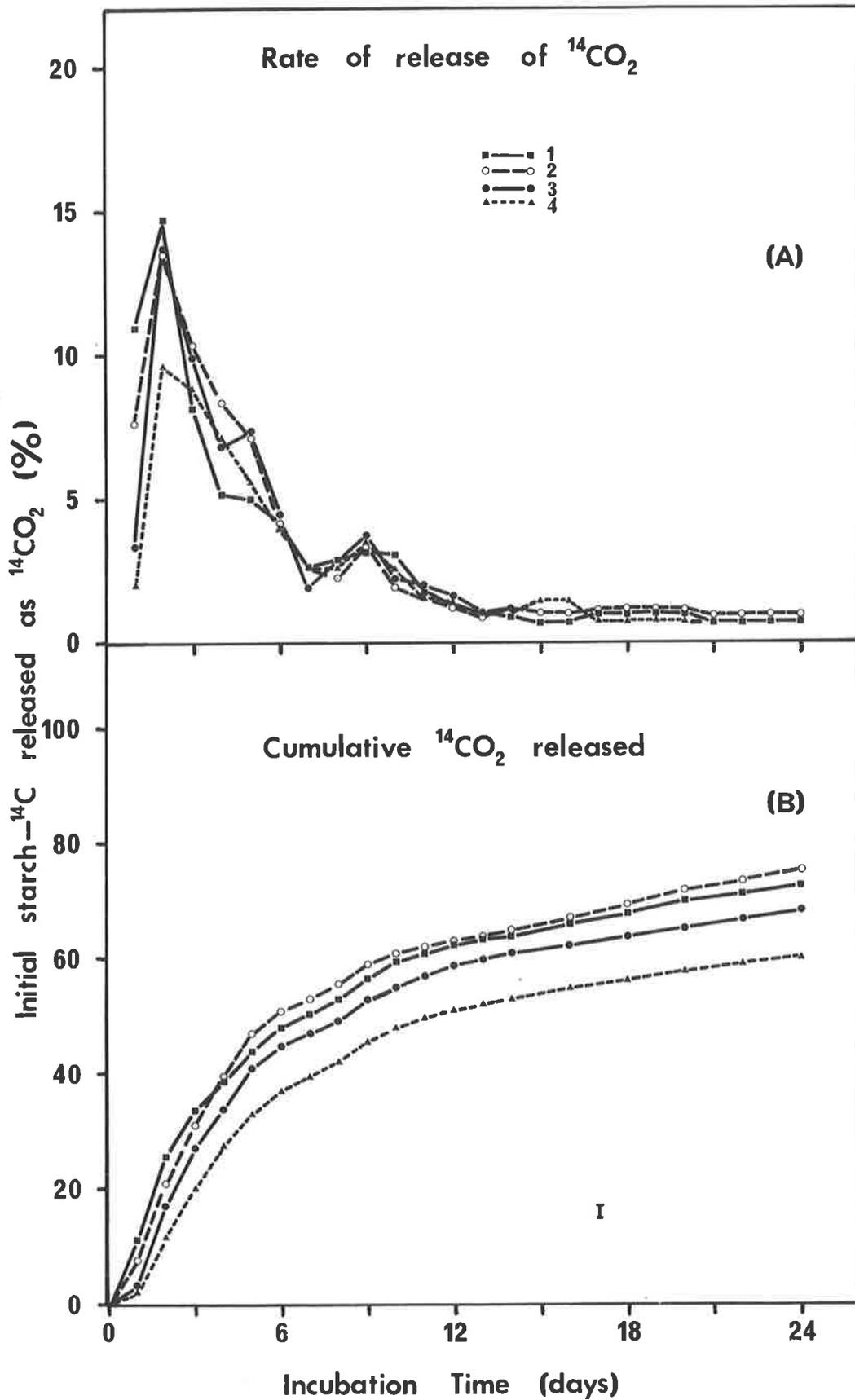
Soil: Grey clay

Aggregate sizes:

1. 1 to 2 mm
2. 124 to 250  $\mu\text{m}$
3. < 53  $\mu\text{m}$
4. Control

$\bar{I}$  = L.S.D. (0.05)

FIG. 21



Although about 40% of the initial  $^{14}\text{C}$  had been released as  $^{14}\text{CO}_2$  by the 4th day of incubation from the larger aggregates of the grey clay only 28% and 35% had been released from the control and the  $< 53 \mu\text{m}$  samples respectively within the same period. The corresponding values of  $^{14}\text{CO}_2$  released from the red clay samples was between 14 to 26%. Here again values of  $^{14}\text{CO}_2$  released from the  $< 53 \mu\text{m}$  and the control were lowest (12 to 14% of initial  $^{14}\text{C}$ ), and the value for the  $124 \mu\text{m}$  to  $250 \mu\text{m}$  was the highest (about 26% of initial  $^{14}\text{C}$ ). Only the  $124 \mu\text{m}$  to  $250 \mu\text{m}$  aggregates from the red clay showed 40% release of the initial  $^{14}\text{C}$  by the 7th day of incubation (Table 15). This was in contrast to the high values of cumulative  $^{14}\text{CO}_2$  released from the grey clay. These results show that though some similarities existed between the two clays, they behaved differently in many aspects.

After the initial flush of  $^{14}\text{CO}_2$  the trend of events was similar (in both clay soils) to that observed for the fine sandy loam with a gradual decline as well as minor flushes in release of  $^{14}\text{CO}_2$ . Cumulative  $^{14}\text{CO}_2$  expressed as percentage of initial  $^{14}\text{C}$  released from aggregate samples of the grey clay was over 60% by the 14th day of incubation (Table 15). Only in the  $124 \mu\text{m}$  to  $250 \mu\text{m}$  aggregates of the red clay was a value approaching 60% reached.

Whilst with the red clay differences existed between mean values of cumulative  $^{14}\text{CO}_2$  released from the different samples (Table 15), with the grey clay, there was no significant difference between the values of the large aggregates. The values for the large aggregates were, however, different from the smaller aggregates and the control sample.

### 3.3. Comparison of the four soils

An examination of the results obtained from the four soils showed that the loams behaved quite differently from the clays and that the two clays showed interesting differences. For all the soils, mean values for the cumulative release of  $^{14}\text{CO}_2$  from the 1 to 2 mm samples was always the second highest (Table 15). In the fine sandy loam samples, release of  $^{14}\text{CO}_2$  from the controls was highest in both soils, followed by the 1 to 2 mm samples, whilst in the clays, release of  $^{14}\text{CO}_2$  from the 124 to 250  $\mu\text{m}$  samples was highest in both samples. The mean cumulative release of  $^{14}\text{CO}_2$  from the 1 to 2 mm was the next highest.

All other factors being equal except size, the release of  $^{14}\text{CO}_2$  might have been expected to be lowest in the bigger aggregate samples and highest from the control samples. Thus, the high release of  $^{14}\text{CO}_2$  from the control samples of the fine sandy loam soils was as expected.

The slightly higher release from the bigger aggregates of the fine sandy loam soil, particularly in early stages of incubation, suggested either that the  $^{14}\text{C}$ -starch was more accessible to enzyme attack or conditions were more favourable for microbial growth. Both factors could have operated.

In the first instance, the breakdown of the aggregates was possible. The 1% solution PVA [poly(vinyl alcohol)] used to stabilize the aggregates should have given the aggregates some resistance against sudden breakdown. Visual examination did not reveal any serious damage to the aggregates as caused by transportation and gamma irradiation. However, it was possible that friction between aggregates during transportation created some fractures which, on wetting, caused failure of the

aggregates by serving as weak points. On the other hand, the micro-organisms may have found the aggregate samples a better medium for growth.

Comparing all soils it was observed that even within the first week of incubation the release of  $^{14}\text{CO}_2$  from the  $< 53 \mu\text{m}$  (except for the grey clay) was quite low (Table 15). This meant that factors controlling release of  $^{14}\text{CO}_2$  from  $< 53 \mu\text{m}$  samples were different from those in the other samples. The low release of  $^{14}\text{CO}_2$  from the  $< 53 \mu\text{m}$  samples particularly for the clays was probably due to poor exchange of gases and anaerobiosis caused by swelling of the clays. The two clays had to be ground with a Siebtechnik mill to pass a 300 mesh sieve ( $< 53 \mu\text{m}$ ). The result of such treatment was the production of a vast range of particle sizes, many probably as fine as  $2 \mu\text{m}$  to  $50 \mu\text{m}$ . The wetting of such a sample results in a minimum of air-filled pores and poor gas diffusion. Oxygen is only 4% as soluble as  $\text{CO}_2$  and its diffusion coefficient in water is of the order of 0.01% of its diffusion coefficient in air (Hillel, 1971). A similar explanation is offered for the relatively low release of  $^{14}\text{CO}_2$  from the controls of the clay sample.

#### 3.4. Comparison of results with those obtained by other workers

The results obtained, particularly for the aggregates of the fine sandy loam, are in contrast to the claim that the effect of physical accessibility of organic materials in soil to microbial attack is best observed in heavier soils (Craswell and Waring, 1972a and 1972b).

The results discussed in this Chapter show that there was a higher release of  $^{14}\text{CO}_2$  from control samples than from aggregate samples though

differences between aggregates were small. The organic materials present in micropores of aggregates would be protected from attack by enzymes in both loamy and clay soils, and the protection would differ from soil to soil, even amongst the clays.

The results obtained in this experiment cannot be fairly compared with results obtained from studies on effect of aggregates on either release of CO<sub>2</sub> or oxygen consumption (Craswell and Waring, 1972a and 1972b). Neither can the results be compared with those obtained from studies of aggregate size effects on nitrogen mineralization in soil (Waring and Bremner, 1964; Seifert, 1962, 1964; Craswell et al., 1970).

In the studies by these workers results obtained were not based on the initial content of the organic matter in the individual aggregate samples. Neither the amount of organic matter nor the chemical nature of the organic fraction in the different aggregate sizes was determined. Hence the results obtained for different sizes of aggregates were not compared on the same basis, and cannot be compared with the present work.

The rate of decomposition of different groups of <sup>14</sup>C-labelled micro-organisms in soil is different (Mayaudon and Simonart, 1963). Similarly, <sup>14</sup>C-labelled micro-organisms of the same group but different species (e.g. fungi) decompose at different rates. In addition various microbial components decompose at different rates and to different extents in soil (Wagner, 1968; Hurst and Wagner, 1969). It is with this information to hand that one must consider the work of Chichester (1969, 1970) who observed that the mineralization capacity of different particle size fractions of soil were different, the smaller particle

fractions yielding most mineral nitrogen. The distribution of both different species of micro-organisms as well as different components of organisms into different size fractions could alter the rate of either consumption of oxygen or release of  $\text{CO}_2$  as well as mineralization of organic nitrogen during decomposition studies of organic matter in soil aggregates. As suggested by Robinson (1967), increased nitrogen mineralization in size fractions of soils subjected to grinding and sieving is "simple mechanical sorting of the soil particles relative to the carrier of the organic matter".

The C/N ratio of soil has been found to decrease with decrease in particle size, and decomposition of organic matter in soil is influenced by C/N ratio (Oades and Ladd, 1974).

To study the influence of particle size or soil structure on decomposition of organic materials in the soil, both the chemistry and the content of organic matter in different size fractions should be known. Results should therefore be expressed on the basis of the content of the organic matter in the fractions and not just on a weight basis.

The results obtained in these experiments have been compared on a sound basis. The chemical nature of substrates was known and therefore at least one factor which was likely to complicate interpretation of results has been eliminated. At the same time, the use of a labelled substrate and expression of results as percentages allowed a fair comparison to be made between the release of  $\text{CO}_2$  from the different aggregate sizes.

The largest size of aggregates used (1 to 2 mm) was a convenient size for (optimum microbial activities) aerobic respiration studies in soil. The rate of release was therefore more important than cumulative release. However, unless the organic source was relatively mobile (e.g. glucose) the rate of release would be the same up to a certain limit of aggregate size. With soluble organic materials diffusion is an important factor and the rate of release of  $^{14}\text{CO}_2$  initially would be inversely proportional to the size of the aggregates (as observed for the glucose-treated samples of the fine sandy loam soils).

For a more complex and less mobile carbohydrate (or compound) however, diffusion of the substrate would play a minor part and diffusion of enzymes to the substrates would be a major factor. In this case factors involved in preventing interaction between organic matter and microbial enzymes (such as sorption of the enzymes by the soil colloids) would be the same for all sizes of aggregates (Greenwood, 1968).

Disruption of the aggregates to effect disposition of the components of the aggregates including the trapped organic source would then change the situation.

CHAPTER VI. THE INFLUENCE OF PHYSICAL TREATMENTS ON RELEASE OF $^{14}\text{CO}_2$  FROM PRE-INCUBATED SAMPLES1. Introduction

Results from the two previous sections indicated that there was some protection accorded to the  $^{14}\text{C}$ -labelled substrates incorporated into aggregates. This effect was not as great as expected and the protection was revealed more in the rate of release of  $^{14}\text{CO}_2$  than in the cumulative release. The nature of the residual  $^{14}\text{C}$  not accounted for in the form of  $^{14}\text{CO}_2$  especially in samples where  $^{14}\text{C}$ -starch was incorporated, was not known. The residual  $^{14}\text{C}$  might have been in the form of  $^{14}\text{C}$ -starch as incorporated or in tissues and metabolites of the organisms.

If aggregate sizes influence the protection of  $^{14}\text{C}$ -labelled substrates incorporated into them, then physical treatments which lead to the disruption of the aggregates should lead to an increased release of  $^{14}\text{CO}_2$  during incubation. The assumption was that if the residual  $^{14}\text{C}$  in  $^{14}\text{C}$ -glucose and  $^{14}\text{C}$ -starch amended samples was incorporated into microbial structures, which means present in complex compounds, then, depending on the severity of the treatment, the resulting effect should be almost the same for both  $^{14}\text{C}$ -glucose and  $^{14}\text{C}$ -starch treated samples. If however, residual  $^{14}\text{C}$ -starch is present in aggregates a treatment which disrupts aggregates should cause a greater release of  $^{14}\text{CO}_2$  than from aggregates after incubation with glucose.

Physical factors involved in this study were (a) drying and wetting cycles and (b) shaking of flasks to break down the aggregates.

The literature on drying and wetting effects has been discussed, whilst shaking would be similar to the tillage effect as carried out by Rovira and Greacen (1957). As pointed out in the Literature Review, application of  $^{14}\text{C}$  to such studies has so far been lacking.

## 2. Materials and methods

### 2.1. Materials

Incubated samples from previous studies (Chapter V) were used. All even numbered samples from each pair of duplicate samples were used for drying and wetting studies and the odd numbers used for the shaking experiments.

### 2.2. Methods

#### 2.2.1. Drying and wetting cycles

##### 2.2.1.1. Drying

Drying was carried out in a forced draught oven at  $60^{\circ}\text{C}$  overnight. Samples were dried in the incubation flasks.

##### 2.2.1.2. Incubation

Dried samples were wetted by spraying with water to 80% of field capacity as determined by weighing.

Absorbing vials containing 8 ml of 0.1 N NaOH were suspended in the flasks and samples incubated for 4 days, then dried again overnight. This process of drying and wetting followed by 4 days of incubation was repeated 7 times.

#### 2.2.1.3. Extra treatment during incubation

After repeating the initial drying and wetting cycles three times samples were not dried before the 4th period. This step was taken on the assumption that the drying might have killed or suppressed the activity of the micro-organisms. Thus incubation was allowed to go for 8 days rather than 4 days. This treatment was repeated twice.

Samples were also reinoculated with 1 ml of 1% fresh soil solution (described already) after wetting. Incubation was continued for 4 days and the treatment repeated twice for periods of 6 days after drying and wetting.

#### 2.2.1.4. Change of vials

This was done every four days for seven consecutive times and for two consecutive periods lasting six days each.

#### 2.2.1.5. Determination of $^{14}\text{CO}_2$

This was done by liquid scintillation spectrometry, using the same scintillation fluor and settings as described previously (Chapter III, Section 2.4.1.2.2.).

#### 2.2.1.6. Quench correction for samples

Efficiency was determined by internal standardization, using  $^{14}\text{C}$ -toluene standard as described already. The efficiency obtained was used to correct for any quenching in the samples.

### 2.3. Effect of breakdown of aggregates

#### 2.3.1. Method

Breakdown of aggregates was carried out by swirling of flasks on a vortex shaker using the maximum speed.

### 2.3.2. Incubation and other treatments

Incubation, and any other treatments during incubation, was the same as described for drying and wetting cycles except that shaking the samples was used in place of drying and wetting.

Determination of  $^{14}\text{CO}_2$  and correction for quenching followed the same procedure as described already.

## 3. Results and discussion

This section was basically a continuation of the incubation experiments carried out in the previous Chapter (Chapter V). Because each of the duplicate samples from the incubation studies was used for separate experiments, there were no replicates in the present studies. The assessment of the effects of drying and wetting and of shaking on the release of  $^{14}\text{CO}_2$  at 4 day intervals during incubation was based on the quantity of  $^{14}\text{CO}_2$  released expressed as a percentage of the initial  $^{14}\text{C}$  added to the samples at the beginning of the incubation described in Chapter V.

It was assumed in Chapter V that since release of  $^{14}\text{CO}_2$  from all the samples was low and uniform after the 14th day of incubation, the residual  $^{14}\text{C}$  in the samples was either inert or physically protected from microbial attack. It was also possible that toxic substances were present limiting further microbial activities. Thus, a change in the rate of release of  $^{14}\text{CO}_2$  from the samples should directly or indirectly be attributed to the physical treatment imposed upon the samples.

For convenience, an increase in the release of  $^{14}\text{CO}_2$  during incubation after the treatment is referred to as a "positive effect", whilst a

decline in released  $^{14}\text{CO}_2$  in relation to the previous value is termed a "negative" effect. This was done because there were no conventional forms of control samples. The rate of release of  $^{14}\text{CO}_2$  at the end of preceding experiment was used as a basal rate for comparison of  $^{14}\text{CO}_2$  release in the present experiments. Each treatment therefore, had as a control the value obtained for the preceding 4 days.

### 3.1. Drying and wetting treatments

#### 3.1.1. General effect

The first drying and wetting cycle followed by incubation caused a rise in release of  $^{14}\text{CO}_2$  from most of the samples of all soils whether  $^{14}\text{C}$ -glucose amended or  $^{14}\text{C}$ -starch amended (Figs. 22, 23, 24, 25). Except for the red clay (which showed about a 40% decline in the starch amended samples) all control samples showed a rise in the rate of release of  $^{14}\text{CO}_2$  which ranged from 20 to 90%.

Further drying and wetting cycles did not show any substantial effect on release of  $^{14}\text{CO}_2$  from the samples. There were occasional rises in  $^{14}\text{CO}_2$  production from some soils, particularly the clay soils and  $^{14}\text{C}$ -starch treated samples of the wheat fallow soils but they were not consistent.

Inoculating samples after the drying and wetting treatment with fresh soil suspension before incubation gave a rise in release of  $^{14}\text{CO}_2$  in some samples, but again it was inconsistent and further introduction of fresh soil did not affect  $^{14}\text{CO}_2$  production. A rise in  $^{14}\text{CO}_2$  production following inoculation indicated that the drying and wetting treatment had some injurious effect on the organisms. The effect of inoculation was small, inconsistent and of doubtful significance. When

Fig. 22. Rate of release of  $^{14}\text{CO}_2$  during drying and wetting cycles (followed by incubation) from samples pre-incubated with a  $^{14}\text{C}$ -labelled substrate.

Soil: Urrbrae fine sandy loam

Plot: Permanent pasture

Aggregate sizes:

1. 1 to 2 mm
2. 124 to 250  $\mu\text{m}$
3. < 53  $\mu\text{m}$
4. Control



No drying and wetting treatment



Re-inoculated with 1 ml 1% fresh soil suspension

FIG. 22

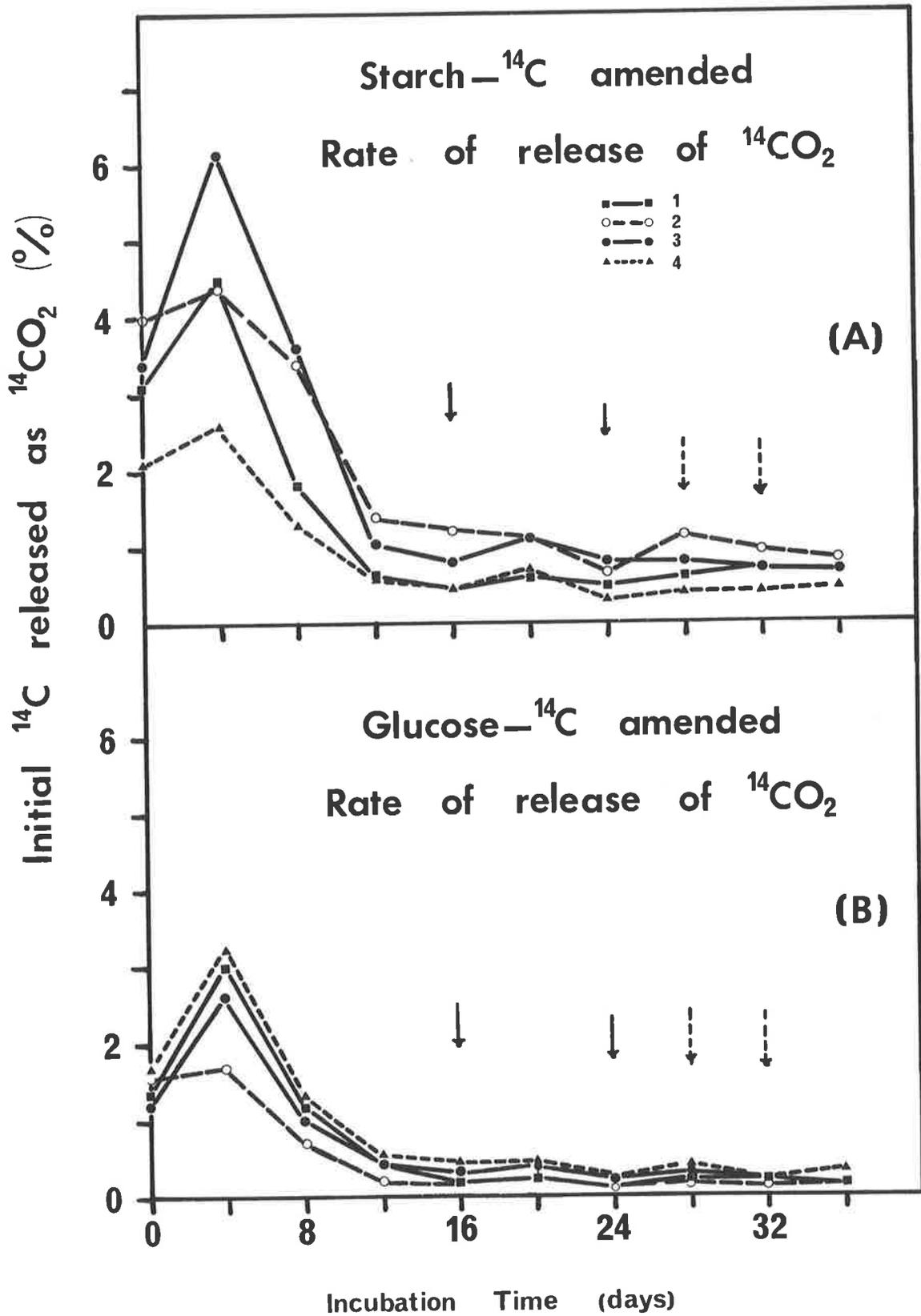


Fig. 23. Rate of release of  $^{14}\text{CO}_2$  during drying and wetting cycles (followed by incubation) from samples pre-incubated with a  $^{14}\text{C}$ -labelled substrate.

Soil: Urrbrae fine sandy loam

Plot: Wheat fallow

Aggregate sizes:

1. 1 to 2 mm
2. 124 to 250  $\mu\text{m}$
3. < 53  $\mu\text{m}$
4. Control

↓ No drying and wetting treatment

↓ Re-inoculated with 1 ml 1% fresh soil suspension

FIG. 23

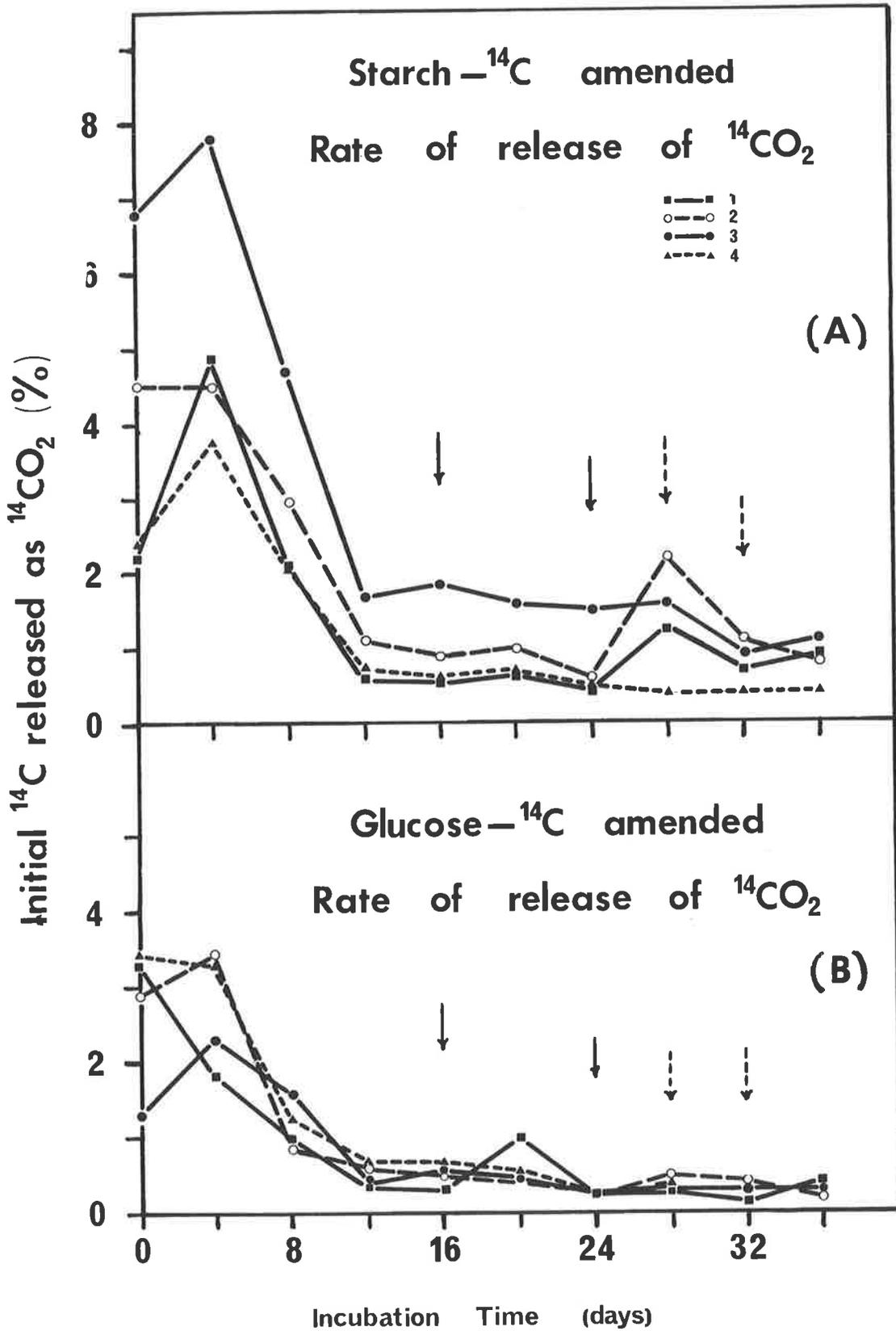


Fig. 24. Rate of release of  $^{14}\text{CO}_2$  during drying and wetting cycles (followed by incubation) from samples pre-incubated with a  $^{14}\text{C}$ -labelled substrate.

Soil: Red clay

Aggregate sizes:

1. 1 to 2 mm
2. 124 to 250  $\mu\text{m}$
3. < 53  $\mu\text{m}$
4. Control

↓ No drying and wetting treatment

↓ Re-inoculated with 1 ml 1% fresh soil suspension

FIG. 24

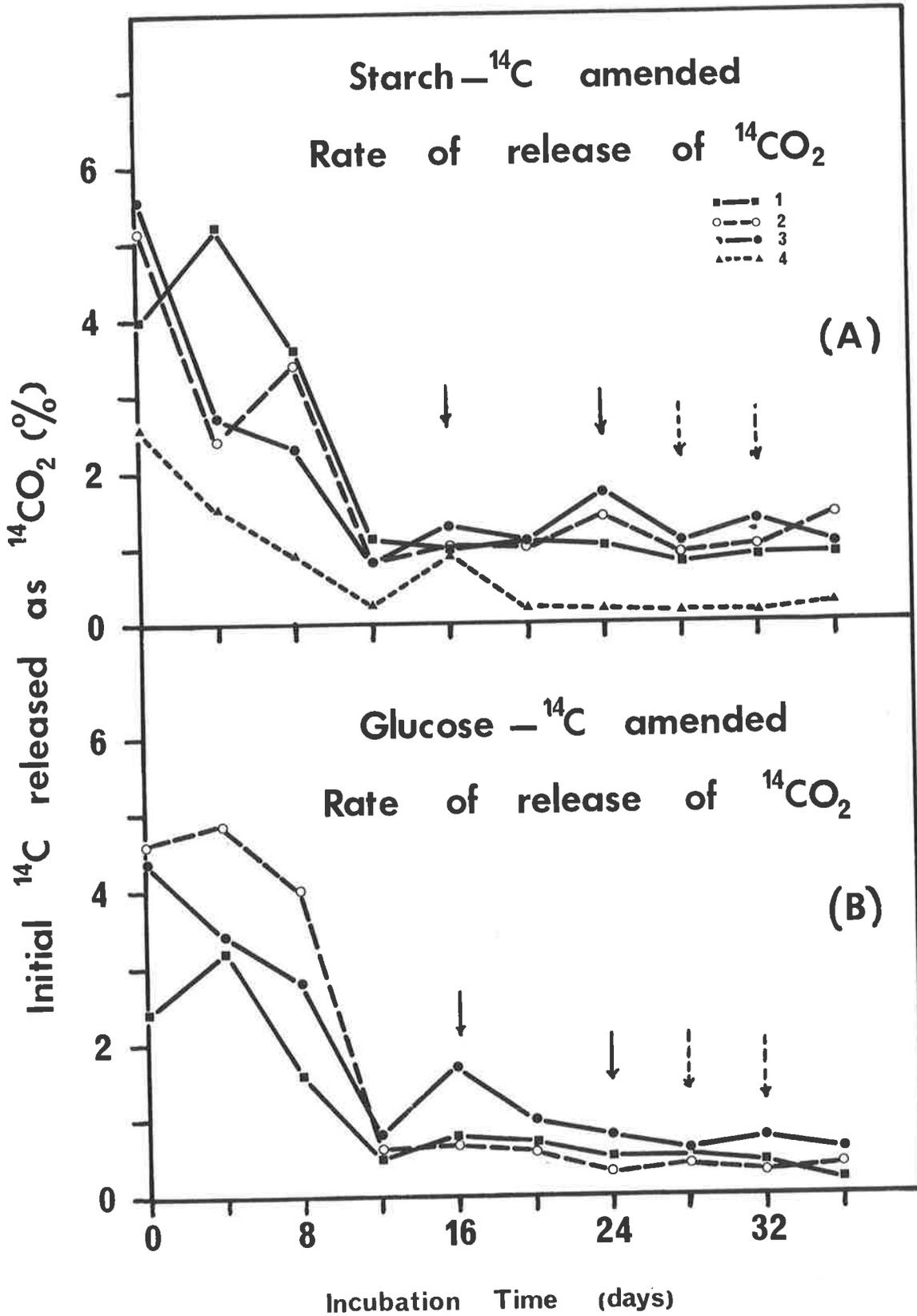


Fig. 25. Rate of release of  $^{14}\text{CO}_2$  during drying and wetting cycles (followed by incubation) from samples pre-incubated with a  $^{14}\text{C}$ -labelled substrate.

Soil: Grey clay

Aggregate sizes:

1. 1 to 2 mm
2. 124 to 250  $\mu\text{m}$
3. < 53  $\mu\text{m}$
4. Control

↓ No drying and wetting treatment

↓ Re-inoculated with 1 ml 1% fresh soil suspension



incubation was allowed to proceed for two consecutive 4-day periods before a further treatment, a rise in released  $^{14}\text{CO}_2$  was obtained for the second 4-day period for the clay soils. This suggested that the microflora in the clays needed more than 4 days to recover from the drying treatment.

### 3.1.2. Residual $^{14}\text{C}$ content

Total cumulative release of  $^{14}\text{CO}_2$  expressed as a percentage of the residual  $^{14}\text{C}$  in each sample before the wetting and drying treatments showed that there was a definite relationship between  $^{14}\text{CO}_2$  released and the level of residual  $^{14}\text{C}$  in the samples (Table 16). All soils except the red clay showed poor correlations between residual  $^{14}\text{C}$  and  $^{14}\text{CO}_2$  released by the drying and wetting treatments when glucose and starch amended samples were not separated. However, there was a high negative correlation ( $r = 0.8380 \pm 0.0769^{***}$ ) between  $^{14}\text{CO}_2$  released as percentage of residual  $^{14}\text{C}$  and the level of residual  $^{14}\text{C}$  in all  $^{14}\text{C}$ -glucose amended samples. On the other hand, there was a poor correlation ( $r = -0.2389$ ) between the two variables ( $^{14}\text{CO}_2$  released and residual  $^{14}\text{C}$  in samples) for the  $^{14}\text{C}$ -starch amended samples.

The difference between starch and glucose amended samples was obvious in the fine sandy loam soils where the correlation between release of  $^{14}\text{CO}_2$  and residual  $^{14}\text{C}$  was negative for the  $^{14}\text{C}$ -glucose amended samples but the two variables were positively correlated in the  $^{14}\text{C}$ -starch amended samples. This positive correlation for the starch amended samples for the sandy loam compared with the negative correlation

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\*\*\* Significance level  $P < 0.001$

Table 16. Cumulative release of  $^{14}\text{CO}_2$  during several drying and wetting cycles  
in relation to residual  $^{14}\text{C}$  in aggregate samples of different soils

| Soil type  | Sample                   | Residual $^{14}\text{C}$ as % of original |                | Released $^{14}\text{CO}_2$ as % of residual $^{14}\text{C}$ |                |
|--|--------------------------|---|----------------|--|----------------|
|  |                          | Glucose amended                           | Starch amended | Glucose amended  | Starch amended |
| Urrbrae fine<br>sandy loam<br>- permanent<br>pasture | 1 to 2 mm                | 38.07                                     | 43.05          | 15.91  | 25.78          |
|  | 124 to 250 $\mu\text{m}$ | 46.76                                     | 47.27          | 8.14   | 33.64          |
|  | < 53 $\mu\text{m}$       | 37.70                                     | 38.63          | 15.73  | 42.60          |
|  | Control                  | 31.96                                     | 30.56          | 22.75  | 24.29          |
| Urrbrae fine<br>sandy loam<br>wheat fallow           | 1 to 2 mm                | 18.03                                     | 39.85          | 32.28  | 32.59          |
|  | 124 to 250 $\mu\text{m}$ | 22.87                                     | 39.72          | 39.13  | 40.52          |
|  | < 53 $\mu\text{m}$       | 33.26                                     | 42.26          | 19.98  | 56.47          |
|  | Control                  | 9.38                                      | 36.00          | 83.04  | 27.69          |
| Red clay   | 1 to 2 mm                | 15.60                                     | 37.94          | 56.49  | 43.61          |
|  | 124 to 250 $\mu\text{m}$ | 18.44                                     | 38.94          | 67.81  | 37.55          |
|  | < 53 $\mu\text{m}$       | 23.97                                     | 48.28          | 57.27  | 29.80          |
|  | Control                  | *   | 53.21          | *  | 9.26           |
| Grey clay  | 1 to 2 mm                | 20.05                                     | 25.49          | 25.15  | 31.35          |
|  | 124 to 250 $\mu\text{m}$ | 29.55                                     | 21.06          | 10.77  | 34.99          |
|  | < 53 $\mu\text{m}$       | 25.88                                     | 23.52          | 28.55  | 44.60          |
|  | Control                  | 12.45                                     | 39.18          | 40.41  | 22.58          |

\* Flask cracked

for glucose amended samples could be explained in terms of increased accessibility of residual  $^{14}\text{C}$ -starch due to structural rearrangements of aggregates during drying and wetting (Greenwood, 1968). The negative correlation between  $^{14}\text{CO}_2$  released and residual  $^{14}\text{C}$  in glucose amended samples is not understood but the factors involved are thought to be biological rather than physical factors involving protection of a non-diffusible substrate.

### 3.1.3. Aggregate size

The influence of aggregate size on  $^{14}\text{CO}_2$  release during drying and wetting cycles is not evident in the glucose amended samples (Figs. 22B, 23B, 24B, 25B). The control and aggregate samples behaved similarly in terms of  $^{14}\text{CO}_2$  release. This is consistent with conclusions drawn in the previous sections, which were that virtually all the  $^{14}\text{C}$ -glucose was utilized because it is soluble in water, not sorbed, and would thus diffuse towards points of low concentration i.e. points of utilization by the micro-organisms. The labelled organisms and metabolic products would exist on the outside of aggregates. Release of  $^{14}\text{CO}_2$  from such materials by drying and wetting would be mainly due to biological and/or chemical factors.

On the other hand, work in previous sections showed that some  $^{14}\text{C}$ -starch was protected inside aggregates except in samples of the grey clay. Where  $^{14}\text{C}$ -starch was present within aggregates a treatment causing aggregate disruption would expose the  $^{14}\text{C}$ -starch to microbial attack. Thus, a larger flush of  $^{14}\text{CO}_2$  release should occur from aggregate samples compared with control samples. The results (Figs. 22, 23, 24, 25) show that except for the grey clay this was the case, particularly for the

1 to 2 mm aggregates.

This difference between starch and glucose amended samples i.e. an increase in  $^{14}\text{CO}_2$  released caused by the drying and wetting treatment over and above that released from control samples can be interpreted as due to disruption of aggregates rendering further substrate accessible to microbial attack. Although this additional effect was not large it was of the same magnitude as the flush of  $^{14}\text{CO}_2$  released from control samples and indicates that in some soils physical factors are as important as biological and chemical factors in enhanced microbial activity after a drying and wetting cycle.

#### 3.1.4. Interpretation of the flush of microbial activity after a drying and wetting cycle

The interpretation of results obtained from studies concerning drying and wetting treatments is difficult. More often than not the interpretations have been biased towards biological and chemical processes resulting from the treatment (Stevenson, 1956; Soulides and Allison, 1961; Agarwal, 1971; Jagger, 1970; Birch, 1959). Physical factors have been given little consideration, though it has been observed that intermittent drying and wetting cycles during incubation had an adverse effect on the stability of soil aggregates (Soulides and Allison, 1961; Willis, 1955).

All factors emphasized by previous workers might have contributed to the results obtained in the present study. Thus, the drying and wetting cycles might have had either direct physical effects on structure or indirect effects on soil micro-organisms and organic materials resulting in increased biological availability of the  $^{14}\text{C}$  source.

Birch (1959) suggested that fragmentation and increased porosity of organic gels on drying and heating might bring about an increase in surface area of the organic substrates available to enzyme attack. An increase in the rate of release of  $^{14}\text{CO}_2$  could arise from abiotic processes as noted by Bunt and Rovira (1955) and Jagger (1970). However, such processes occur at a very slow rate and could not have contributed much to the results obtained in the present studies (Jagger, 1970).

Again, Jagger (1970) found that extraction of soluble organic substances immediately following drying gave no peak in  $\text{CO}_2$  evolution during subsequent incubation studies, but the addition of the extract to pure quartz-sand resulted in a flush of  $\text{CO}_2$ . This supports the hypothesis that the increase in release of  $\text{CO}_2$  after drying and rewetting followed by subsequent incubation was due to increase in water soluble organic compounds (Agarwal, 1971; Stevenson, 1956; Birch, 1959; Soulides and Allison, 1961).

Warcup (1957) reported that during summer (when the water content of the soil fell to 1.6 to 2.0% corresponding to a relative humidity of 30 to 50%) most fungi in a wheat fallow soil were killed. There was however, an increase in activity after commencement of rains. The drying at  $60^\circ\text{C}$  for 24 hours in the present study was drastic enough to kill some of the fungi and bacteria (Stevenson, 1956), particularly gram-negative strains present in macropores (Hattori, 1970). Those organisms which might have survived the treatment probably depended on the dead organisms for energy. This presumably contributed to the rise in  $^{14}\text{CO}_2$  released from samples (particularly  $^{14}\text{C}$ -glucose amended samples) from which high amounts of  $^{14}\text{CO}_2$  had been already released.

In the < 53  $\mu\text{m}$  samples various cracks (which might have resulted from shrinkage) appeared in the samples on drying. These cracks coupled with micro-cracks (created by disruptive action of the entrapped air on rewetting) provided an easy avenue for soil water containing enzymes to advance to the various parts of the soil to attack the  $^{14}\text{C}$ -labelled source. This explains the rise in released  $^{14}\text{CO}_2$  from the < 53  $\mu\text{m}$  samples particularly of the fine sandy loam soils. On the other hand a decline in released  $^{14}\text{CO}_2$  was noted in the clay soils despite the cracks resulting from the drying, because the highly pulverised nature of these samples presumably prevented water advancing deeply into various micropores as well as preventing gas exchange.

### 3.2. Shaking treatment

#### 3.2.1. General effects

Shaking of the flasks (containing samples) on a vortex swirler initially caused a flush of  $^{14}\text{CO}_2$  from some samples of the fine sandy loam (particularly those from the pasture soil) (Figs. 26, 27, 28, 29). This rise in the rate of release of  $^{14}\text{CO}_2$  was observed particularly for the 124 to 250  $\mu\text{m}$  and the 1 to 2 mm aggregates, whilst for the < 53  $\mu\text{m}$  aggregates, only the glucose amended wheat fallow soil showed a rise in released  $^{14}\text{CO}_2$ . There was, however, a decline in  $^{14}\text{CO}_2$  production in the control samples of the fine sandy loam. On the other hand, the clay samples (except for the control of the starch amended grey clay) showed a decline in the rate of release of  $^{14}\text{CO}_2$  from all samples.

Subsequent treatments showed occasional flushes of  $^{14}\text{CO}_2$  evolution from the big aggregates of the fine sandy loam (particularly the starch

Fig. 26. Rate of release of  $^{14}\text{CO}_2$  during intermittent shaking treatments (followed by incubation) from samples pre-incubated with a  $^{14}\text{C}$ -labelled substrate.

Soil: Urrbrae fine sandy loam

Plot: Permanent pasture

Aggregate sizes:

1. 1 to 2 mm
2. 124 to 250  $\mu\text{m}$
3. < 53  $\mu\text{m}$
4. Control

↓ No shaking treatment

↓ Re-inoculated with 1 ml 1% fresh soil suspension

FIG. 26

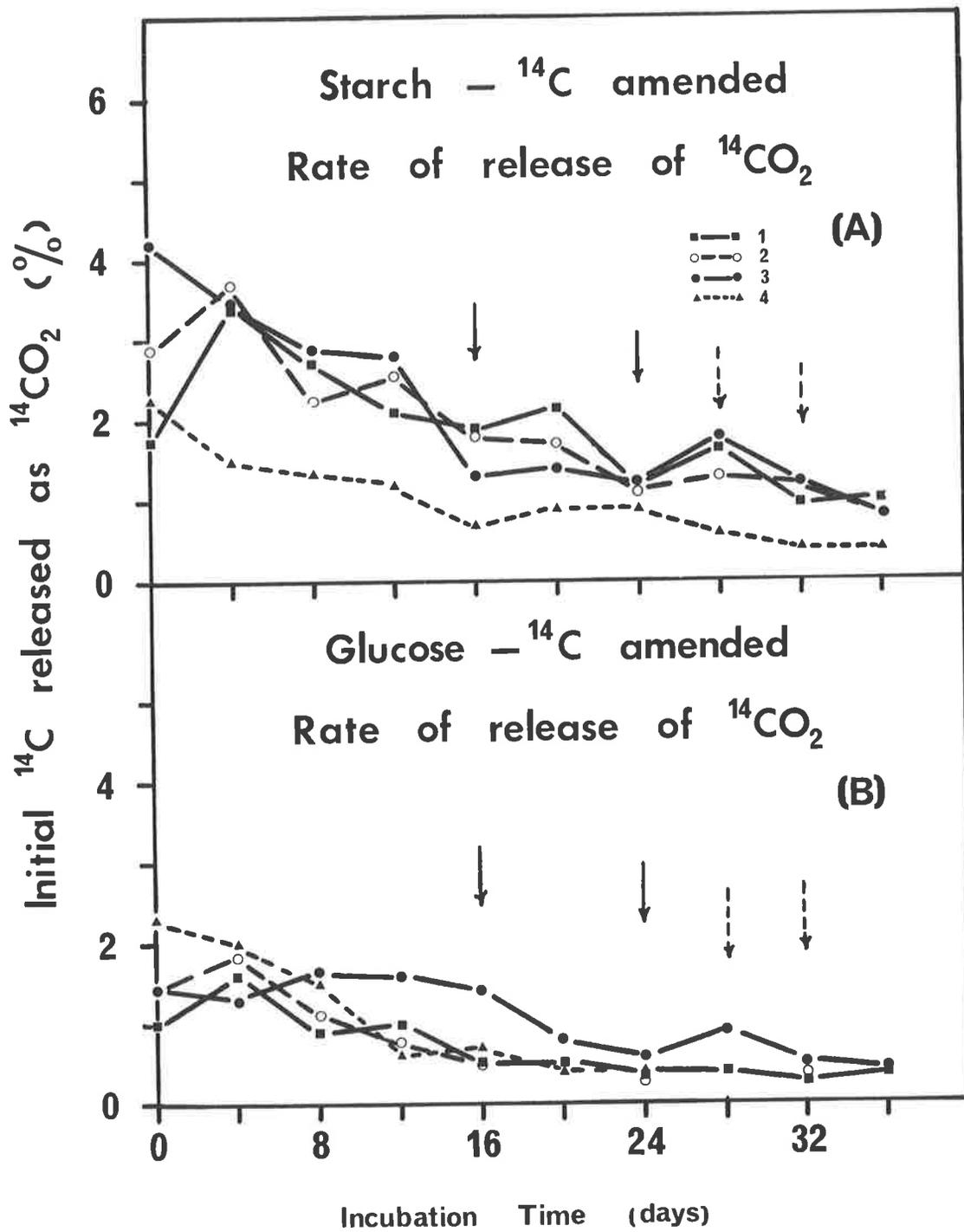


Fig. 27. Rate of release of  $^{14}\text{CO}_2$  during intermittent shaking treatments (followed by incubation) from samples pre-incubated with a  $^{14}\text{C}$ -labelled substrate.

Soil: Urrbrae fine sandy loam

Plot: Wheat fallow

Aggregate sizes:

1. 1 to 2 mm
2. 124 to 250  $\mu\text{m}$
3. < 53  $\mu\text{m}$
4. Control

↓ No shaking treatment

↓ Re-inoculated with 1 ml 1% fresh soil suspension

FIG. 27

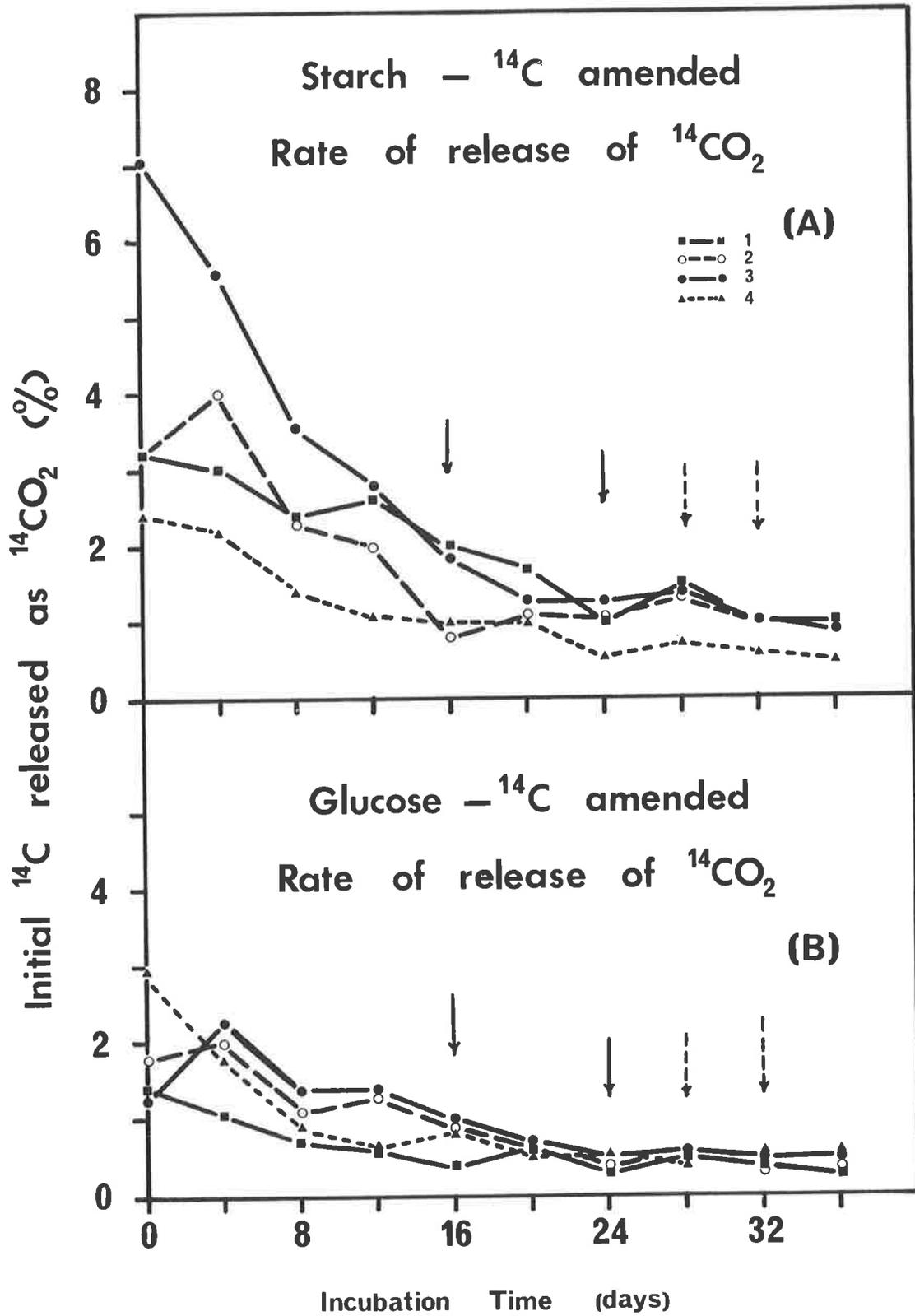


Fig. 28. Rate of release of  $^{14}\text{CO}_2$  during intermittent shaking treatments (followed by incubation) from samples pre-incubated with a  $^{14}\text{C}$ -labelled substrate.

Soil: Red clay

Aggregate sizes:

1. 1 to 2 mm
2. 124 to 250  $\mu\text{m}$
3. < 53  $\mu\text{m}$
4. Control

↓ No shaking treatment

↓ Re-inoculated with 1 ml 1% fresh soil suspension

FIG. 28

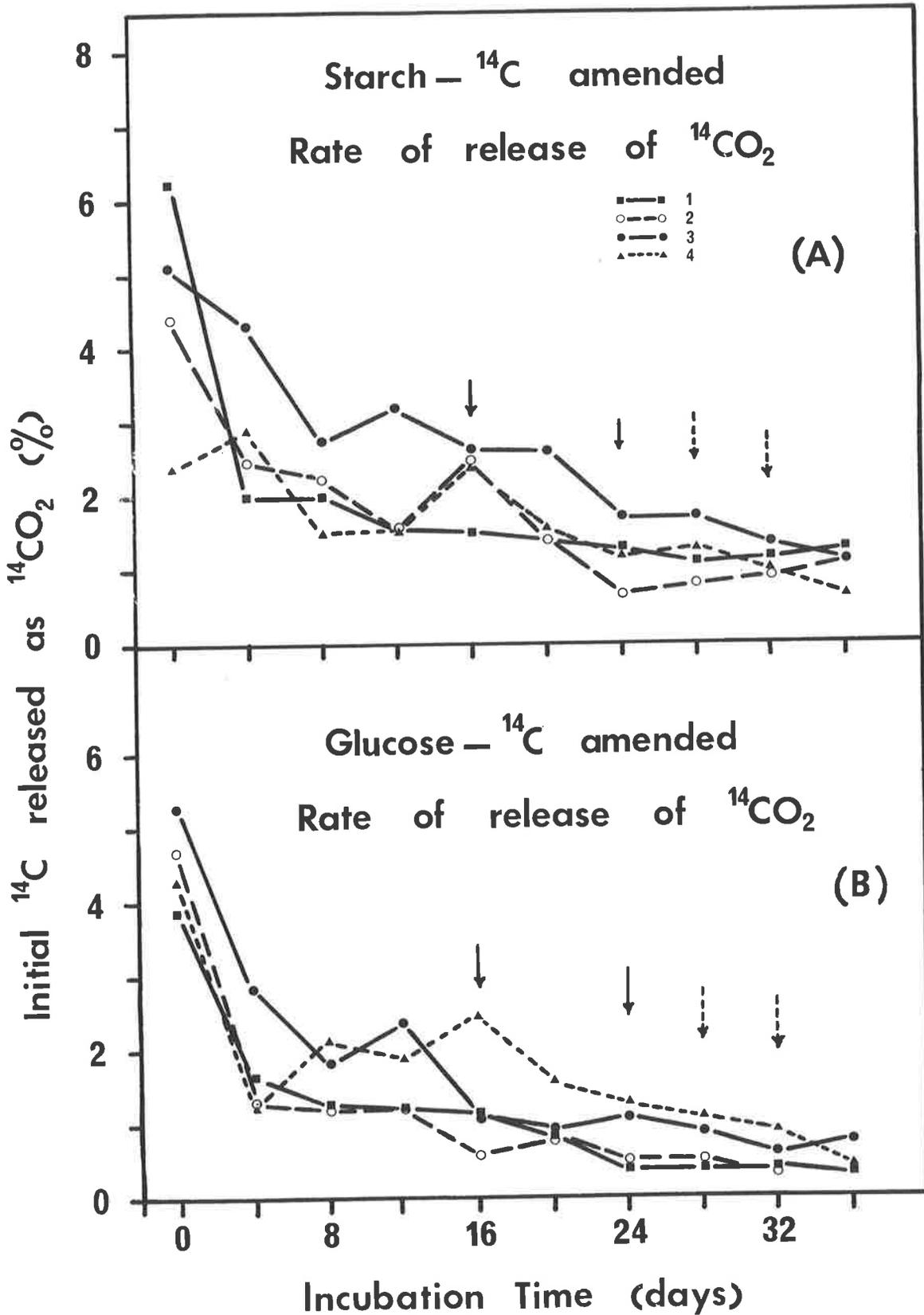


Fig. 29. Rate of release of  $^{14}\text{CO}_2$  during intermittent shaking treatments (followed by incubation) from samples pre-incubated with a  $^{14}\text{C}$ -labelled substrate

Soil: Grey clay

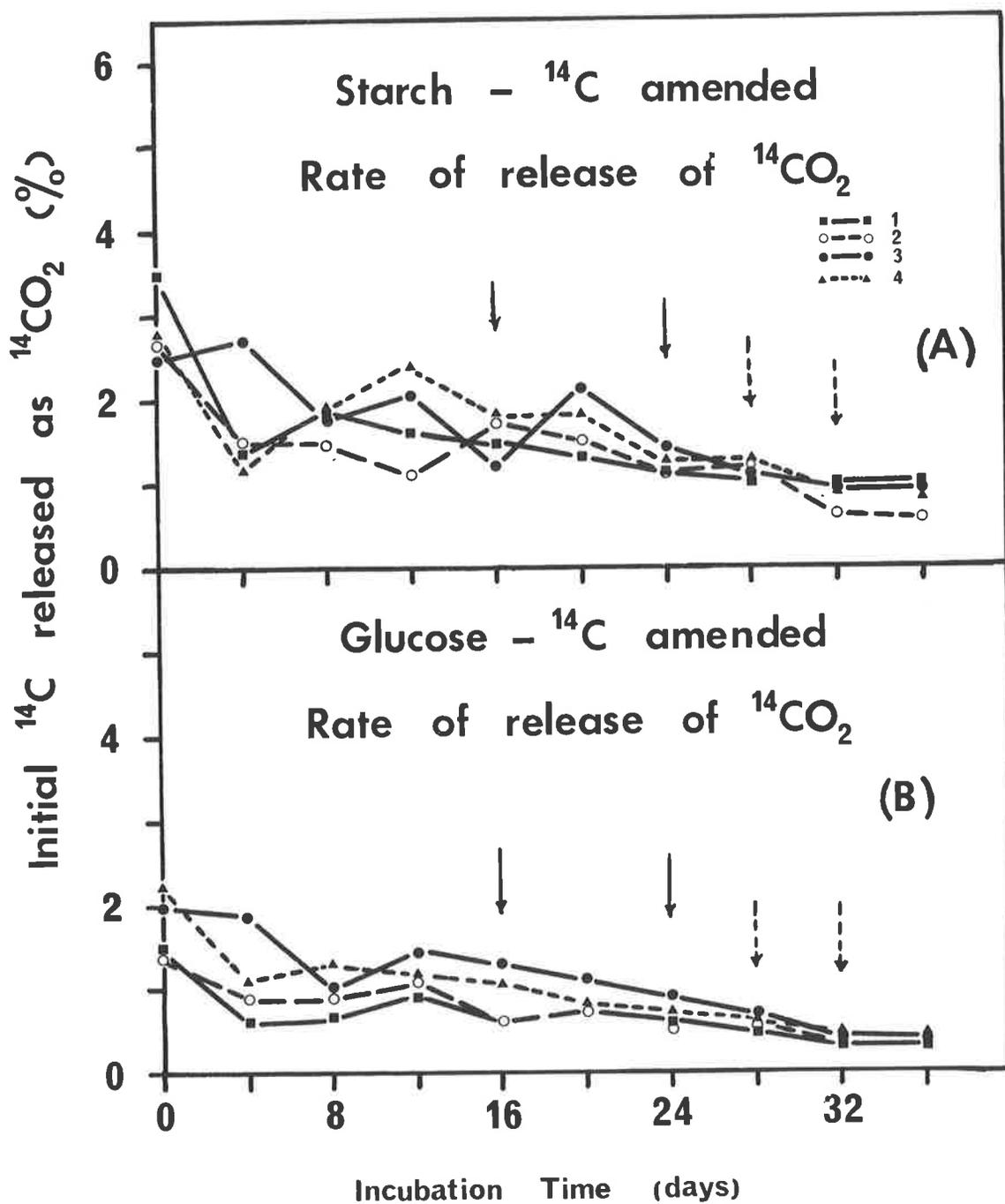
Aggregate sizes:

1. 1 to 2 mm
2. 124 to 250  $\mu\text{m}$
3. < 53  $\mu\text{m}$
4. Control

↓ No shaking treatment

↓ Re-inoculated with 1 ml 1% fresh soil suspension

FIG. 29



amended samples) and the < 53  $\mu\text{m}$  and control samples of the clay soil, but these were not consistent. Allowing incubation to proceed for two consecutive 4-day periods before further treatment had a positive effect on release of  $^{14}\text{CO}_2$  for the second 4-day period in the red clay samples only.

Although there was an inconsistent rise in release of  $^{14}\text{CO}_2$  in samples of the fine sandy loam when they were re-inoculated with fresh soil suspension the treatment did not have any effect on release of  $^{14}\text{CO}_2$  in the clay soils. Further introduction of the fresh soil inoculant resulted in a decline of  $^{14}\text{CO}_2$  production in some samples. As pointed out in the preceding section the effect of the inoculation was of doubtful significance.

### 3.2.2. Residual $^{14}\text{C}$

Although there was some relationship between residual  $^{14}\text{C}$  present in the samples (before the treatment was applied) and values of cumulative  $^{14}\text{CO}_2$  released (Table 17) the correlation between the two variables was poor, and allows no conclusions to be drawn. Correlations between the two variables in the different soils were similar to those obtained in the drying and wetting treatments. It is worth mentioning that the correlation between  $^{14}\text{CO}_2$  released as percentage of residual  $^{14}\text{C}$  and the level of residual  $^{14}\text{C}$  in starch amended samples was better ( $-0.6310 \pm 0.1505$ ) than the corresponding value ( $-0.2389$ ) obtained during the drying and wetting treatments.

### 3.2.3. Aggregate size

The effect of aggregate size on the rate of  $^{14}\text{CO}_2$  release during shaking of the flasks followed by incubation was not evident in

Table 17. Cumulative release of  $^{14}\text{CO}_2$  during intermittent shaking treatments  
in relation to residual  $^{14}\text{C}$  in aggregate samples of different soils

| Soil type  | Sample                   | Residual $^{14}\text{C}$ as % of original |                | Released $^{14}\text{CO}_2$ as % of residual $^{14}\text{C}$ |                |
|--|--------------------------|---|----------------|--|----------------|
|  |                          | Glucose amended                           | Starch amended | Glucose amended  | Starch amended |
| Urrbrae fine<br>sandy loam<br>- permanent<br>pasture | 1 to 2 mm                | 37.26                                     | 50.91          | 17.12  | 33.70          |
|  | 124 to 250 $\mu\text{m}$ | 28.57                                     | 44.20          | 23.02  | 39.46          |
|  | < 53 $\mu\text{m}$       | 33.93                                     | 49.53          | 28.68  | 36.36          |
|  | Control                  | 23.02                                     | 23.34          | 30.42  | 35.90          |
| Urrbrae fine<br>sandy loam<br>wheat fallow           | 1 to 2 mm                | 25.04                                     | 41.08          | 20.75  | 41.92          |
|  | 124 to 250 $\mu\text{m}$ | 26.80                                     | 43.04          | 30.06  | 36.31          |
|  | < 53 $\mu\text{m}$       | 21.90                                     | 44.78          | 43.33  | 46.52          |
|  | Control                  | 15.14                                     | 25.08          | 52.11  | 39.24          |
| Red clay   | 1 to 2 mm                | 21.69                                     | 45.46          | 37.72  | 32.16          |
|  | 124 to 250 $\mu\text{m}$ | 12.01                                     | 20.08          | 61.94  | 73.31          |
|  | < 53 $\mu\text{m}$       | 23.05                                     | 55.94          | 56.17  | 40.37          |
|  | Control                  | 15.89                                     | 53.70          | 85.90  | 28.92          |
| Grey clay  | 1 to 2 mm                | 13.07                                     | 29.53          | 40.63  | 43.43          |
|  | 124 to 250 $\mu\text{m}$ | 27.68                                     | 28.54          | 23.09  | 39.87          |
|  | < 53 $\mu\text{m}$       | 33.00                                     | 39.91          | 29.27  | 37.85          |
|  | Control                  | 21.52                                     | 40.27          | 37.00  | 35.25          |

the glucose amended samples (Figs. 26B, 27B, 28B, 29B) but was shown in the starch amended samples (particularly in the big aggregates from the fine sandy loam) (Figs. 26A, 27A). The magnitude of the effect of shaking on the vortex swirler depended on the thickness of the incubating flasks. The flasks used for incubating the clay samples were thick-walled and heavy and were difficult to shake. Thus, whilst the swirling effectively caused the breakdown of the fine sandy loam aggregates the same treatment was not drastic enough to cause breakdown of the clay aggregates (particularly the 1 to 2 mm aggregates).

Cumulative  $^{14}\text{CO}_2$  released expressed as a percentage of the residual  $^{14}\text{C}$  (Table 17) showed that in the fine sandy loam where breakdown of the big aggregates occurred, the ratio of  $^{14}\text{CO}_2$  released from the starch amended samples to that of the corresponding value for the glucose amended sample was about 2 for the 1 to 2 mm aggregates and decreased to 1 with decrease in aggregate size. On the other hand with the clay samples where the big aggregates did not break down the ratio of the values of released  $^{14}\text{CO}_2$  from the two sources of  $^{14}\text{C}$  was about 1. This ratio decreased to about 0.3 for the control sample of the red clay, and was variable in the grey clay.

The increase in release of  $^{14}\text{CO}_2$  from the starch amended samples, over and above the corresponding value for the glucose amended samples (particularly for the big aggregates of the fine sandy loam) due to the shaking treatment could be attributed to two factors only: (a) that the species of micro-organisms in the glucose and starch amended samples were different. The shaking treatment may have affected the various organisms differently; or (b) the breakdown of the aggregates exposed

more of the  $^{14}\text{C}$ -starch to attack by enzyme. This effect was not evident for the glucose amended samples because all the  $^{14}\text{C}$ -glucose added had been utilized and the  $^{14}\text{C}$  was present in microbial tissue and metabolites. This labelled microbial tissue was probably more resistant to attack by enzymes than  $^{14}\text{C}$ -starch.

Factor (a) could not have been important otherwise the effect should have been the same in all the starch amended samples, and the values of cumulative  $^{14}\text{CO}_2$  released from all samples should have been the same. Thus, the disruption of the aggregate resulting from the shaking treatment [factor (b)] must have been responsible for the release of  $^{14}\text{CO}_2$  from the starch amended samples.

#### 3.2.4. Interpretation of rise in release of $^{14}\text{CO}_2$ after shaking followed by incubation

It was mentioned (under aggregate size, p. 145) that other effects due to the shaking or swirling of the flasks could cause a flush in release of  $^{14}\text{CO}_2$  during incubation. Besides breakdown of the aggregates, re-organization of the soil particles as well as redistribution of micro-organisms, enzymes and  $^{14}\text{C}$ -labelled source could occur. The result of this redistribution of the above components could result in interactions leading to a rise in release of  $^{14}\text{CO}_2$  (Greenwood, 1968). This probably accounts for the inconsistent flushes of release of  $^{14}\text{CO}_2$  particularly for the  $< 53 \mu\text{m}$  and control samples of the clay soils.

The decline in the rate of release of  $^{14}\text{CO}_2$  from the  $< 53 \mu\text{m}$  of the fine sandy loam was probably due to the production of an anaerobic environment as the soil was turned into a paste as particles dispersed on shaking.

The only effect other than physical breakdown of the aggregates, and rearrangement of the soil components, that could have caused a rise in rate of release of  $^{14}\text{CO}_2$  was death of micro-organisms which would provide an energy source for the fraction of the population that survived the treatment. The treatment could have caused disintegration of fungal hyphae but would not have killed the organisms. The contribution to the  $^{14}\text{CO}_2$  released resulting from utilization of dead organisms should therefore have been negligible. The occasional flushes of  $^{14}\text{CO}_2$  from the glucose amended samples after treatment were probably due to  $^{14}\text{C}$ -labelled microbial products which had been produced in pores. The shaking treatment exposed such materials to further microbial attack resulting in a small flush of  $^{14}\text{CO}_2$ .

### 3.2.5. Comparison of the shaking, and drying and wetting treatments

The values of cumulative  $^{14}\text{CO}_2$  released from samples due to the drying and wetting treatment (Table 16) and those due to the shaking treatment (Table 17) were similar. However, these totals were obtained in different ways. During the drying and wetting treatments most of the  $^{14}\text{CO}_2$  was released after the first treatment. On the other hand, although the first shaking treatment was not as effective as the first drying and wetting cycle, the release of  $^{14}\text{CO}_2$  during subsequent shaking treatments was greater than the release of  $^{14}\text{CO}_2$  in the later drying and wetting cycles. The shaking treatment was not as severe on the microbial population as the drying and wetting cycle, and unlike the drying and wetting treatment the effect of shaking should not involve significant chemical and biological factors. The effects of the shaking

treatment were expected to be due more to physical than to biological or chemical factors. The similarity of the cumulative  $^{14}\text{CO}_2$  released as a result of repeated drying and wetting and shaking treatments suggests that physical factors are important in both processes.

### 3.3. Conclusion

The validity of the results may be limited by lack of a conventional form of control but the rises in evolution of  $^{14}\text{CO}_2$  resulting from the treatments would not have occurred without the treatment. The release of  $^{14}\text{CO}_2$  from the samples during incubation in the previous chapter was uniformly low after 14 days of incubation (e.g. Figs. 17, 18). Physical treatments were necessary to stimulate microbial activity. It can be concluded that the first drying and wetting cycle had a positive effect on the release of  $^{14}\text{CO}_2$  during a subsequent incubation. This positive effect was followed by a series of negative effects. The following factors may be involved:

- (a) the killing of organisms to render microbial tissue available to a new population;
- (b) production of more water soluble organic materials;
- (c) increase in surface area of organic colloids;
- (d) disruption of aggregates to expose previously protected substrate to attack by enzymes; and
- (e) re-arrangement of the soil physical, chemical and biological components so that they may interact.

The results obtained from the shaking treatment, though initially not as effective as the drying and wetting treatments, are similar to

those obtained from studies on simulated tillage in the laboratory (Rovira and Greacen, 1957). The relative importance of the above factors in stimulating microbial activity is not known. The results in this chapter suggest that disruption of aggregates and re-arrangement of soil components could be as important as the chemical and biological factors in some soils.

CHAPTER VII. COMPARATIVE STUDIES OF ACTIVITIES OF SPECIFIC

MICRO-ORGANISMS IN SOIL AGGREGATES

1. Introduction

Despite the considerable effort spent on studies of soil organic matter with  $^{14}\text{C}$ -labelled materials, information on the importance of specific organisms is lacking. Work on microbial activities in soil aggregates has not so far involved the role of individual organisms involved in the metabolism of organic matter.

It has been shown in the previous experiments that two major peaks of release of  $^{14}\text{CO}_2$  were involved during incubation of  $^{14}\text{C}$ -labelled substrates especially where glucose-U- $^{14}\text{C}$  was used as substrate. It was suggested that these two peaks corresponded to development of two different groups of organisms, that is fungi and bacteria. Both groups contributed to the release of  $^{14}\text{CO}_2$  within these two peaks, with one group being more important than the other at a particular time during the incubation.

Recently, Anderson and Domsch (1973) have used selective inhibition techniques to evaluate the relative contribution of bacteria and fungi to soil respiration. Such a method seems however, to be more applicable to short term studies and the procedure needs to be applied to a wide range of organisms to make its use more reliable.

On the other hand, if pure cultures of individual organisms are introduced into a particular sample amended with  $^{14}\text{C}$ -substrate, then the release of  $^{14}\text{CO}_2$  is a result of the activity of this organism.

Hence comparative studies of individual organisms can be made.

It was not possible to use many organisms since even strains within the same species are manifold. The aim was to use few species of two major groups of microflora in the soil, that is, fungi and bacteria, and at the same time obtain some idea of the role of the groups involved in metabolising organic materials in soil aggregates.

Choice of different fungi was based on the work by Warcup (1957) which showed that though the fungi chosen occur in the soil of the wheat fallow plot (Urrbrae fine sandy loam) their role in utilizing the soil organic matter was not known.

Six fungi were selected on the basis of Warcup's findings and two bacteria which normally occur in soil were chosen.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Soils

Urrbrae fine sandy loam from the permanent pasture plot and the grey clay (all described in Appendix) were used. All samples were tagged with either  $^{14}\text{C}$ -glucose or  $^{14}\text{C}$ -starch.

#### 2.1.2. Micro-organisms

Pure cultures of the following organisms - six fungi and two bacteria - were used. These were kindly provided by Dr. Warcup of the Plant Pathology Department, Waite Research Institute.

Organisms used were: (a) Rhizoctonia solani; (b) Aureobasidio pullulans; (c) Mucor hiemalis; (d) Aspergillus flavus; (e) Trichoderma

sp.; (f) Penicillium sp., all representing the fungi and (g) Bacillus subtilis and (h) Pseudomonas putida representing the bacteria.

All organisms were provided on agar slants. Fungi were used as mycelial strands. Fractions of bacteria from agar slants were suspended in 10 ml sterile water and served as a stock suspension for inoculation.

## 2.2. Methods

### 2.2.1. Incorporation of glucose-U-<sup>14</sup>C into soil

1 kg of soil (< 250  $\mu\text{m}$ ) was wetted with 600 ml distilled water in a beaker. 200 ml glucose solution containing 4 g of unlabelled glucose and 100  $\mu\text{Ci}$  of uniformly labelled <sup>14</sup>C-glucose (glucose-U-<sup>14</sup>C) was added to the sample. 4 ml chloroform was added to suppress microbial activity and the mixture stirred thoroughly with a glass rod to ensure an even distribution of <sup>14</sup>C within the soil. The sample was dried in a forced draught oven at  $55 \pm 2^\circ\text{C}$ .

### 2.2.2. Incorporation of starch-U-<sup>14</sup>C into soil

Essentially the same procedure was used as for glucose except that 400 ml of water was added to the soil initially. 40 ml of starch solution containing 2 g soluble starch and 50  $\mu\text{Ci}$  uniformly labelled <sup>14</sup>C-starch (starch-U-<sup>14</sup>C) was used.

### 2.2.3. Preparation of <sup>14</sup>C-labelled aggregates

Aggregates used were 1 to 2 mm size. It was not possible to obtain sufficient aggregates of this size at one time. Hence after drying the sample was ground gradually and sieved to obtain the 1 to 2 mm fraction. Finer fractions of soil were re-wetted, dried and the process repeated until the required weight was obtained. Aggregates obtained

from each treatment were used separately since  $^{14}\text{C}$  activity differed from one batch to the other. Because controls were also aggregates, the samples would be referred to as "aggregates" to differentiate them from "controls".

#### 2.2.4. Preparation of control samples

Control samples were prepared in the same manner as for  $^{14}\text{C}$ -labelled aggregates, except that labelled substrates were not mixed with the soil before the preparation of the aggregates. Unlabelled soil was wetted, dried and ground to obtain 1 to 2 mm aggregates, which were stabilized and sterilized by the same procedure as used for the labelled aggregates.

Solutions containing 50 mg glucose with 1  $\mu\text{Ci}$  of  $^{14}\text{C}$ , or 50 mg starch with 1  $\mu\text{Ci}$  of  $^{14}\text{C}$  was introduced into the respective samples just before incubation. Thus, substrates were distributed within macropores in contrast to the distribution within both micropores and macropores in samples labelled before aggregates were prepared.

#### 2.2.5. Stabilization of aggregates and control samples

Aggregates were stabilized with 1% solution of PVA by spraying in the same manner as described earlier (Chapter V, Section 2.1.2.5.).

#### 2.2.6. Sterilization of aggregates and control samples

Sterilization was by gamma-irradiation (more than 2.5 M. rads) as described previously (Chapter V, Section 2.1.2.6.).

### 2.2.7. Preparation for incubation

Weighing of samples into incubation flasks was done under strictly sterile conditions in a quality control laminar flow chamber. Samples were weighed into duplicate sterile (sterilized by autoclaving) incubation flasks.

#### 2.2.7.1. Wetting of aggregates

Samples were wetted to 80% of field capacity in a sterilized cabinet (a perspex cabinet with suspended ultraviolet light), using a chromatography spray - as described already.

#### 2.2.7.2. Inoculation of samples with organisms

Introduction of individual organisms onto aggregates was carried out in the sterilized quality control laminar flow chamber. Fungi were added as mycelial strands by taking a portion of fungal hyphae onto the aggregates. The bacteria were added in suspension. 1 ml aliquots of the prepared suspension were introduced into each flask.

### 2.2.8. Incubation

This followed the same procedure as used in previous studies. 0.1 N NaOH used as the absorbent for released  $^{14}\text{CO}_2$  was prepared with sterile water.

Incubation was at  $28^\circ \pm 2^\circ\text{C}$  using the same incubator.

$^{14}\text{C}$ -glucose tagged samples were incubated for 16 days whilst  $^{14}\text{C}$ -starch tagged samples were incubated for 24 days.

Absorbing vials were changed daily for the initial 14 days, then on every second day thereafter.

#### 2.2.8.1. Determination of $^{14}\text{CO}_2$

$^{14}\text{CO}_2$  was determined as described in Chapter III, Section 2.4.1.2.2.).

#### 2.2.9. Determination of initial activity of $^{14}\text{C}$ in samples

The method of suspension counting of  $^{14}\text{C}$  in soil particles developed in the initial studies was used. The same method of determining efficiency for quench correction was followed.

### 3. Results and discussion

#### 3.1. Incubation of $^{14}\text{C}$ -glucose amended samples

##### 3.1.1. Release of $^{14}\text{CO}_2$ from aggregate samples inoculated with specific organisms

Release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -glucose in sterilized aggregates inoculated with specific organisms differed with the groups of organisms and type of soil (Figs. 30, 31).

Results of only two of the fungi (Rhizoctonia solani and Aspergillus flavus) have been shown, together with those of two bacteria (Pseudomonas putida and Bacillus subtilis). This was done because the various fungi behaved similarly. Carbon dioxide evolution results for the remaining fungi are presented in the Appendix.

##### 3.1.1.1. Fine sandy loam

For the fine sandy loam (permanent pasture) release of  $^{14}\text{CO}_2$  from aggregate samples was higher from samples inoculated with fungi than from samples inoculated with bacteria (Fig. 30). There was virtually no lag phase and from 2 to 7% of the initial  $^{14}\text{C}$  in aggregates

Fig. 30. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -glucose distributed within micropores and macropores of sterilized, artificial aggregates.

Samples were inoculated with different specific organisms.

Soil: Urrbrae fine sandy loam

Plot: Permanent pasture

Specific organisms:

1. Rhizoctonia solani
2. Aspergillus flavus
3. Bacillus subtilis
4. Pseudomonas putida

Aggregate size used: 1 to 2 mm

For Figs. 30 through to 37,  $\bar{I}$  = Least significant difference at the 5% level, calculated using standard errors of the means of the curves.

FIG. 30

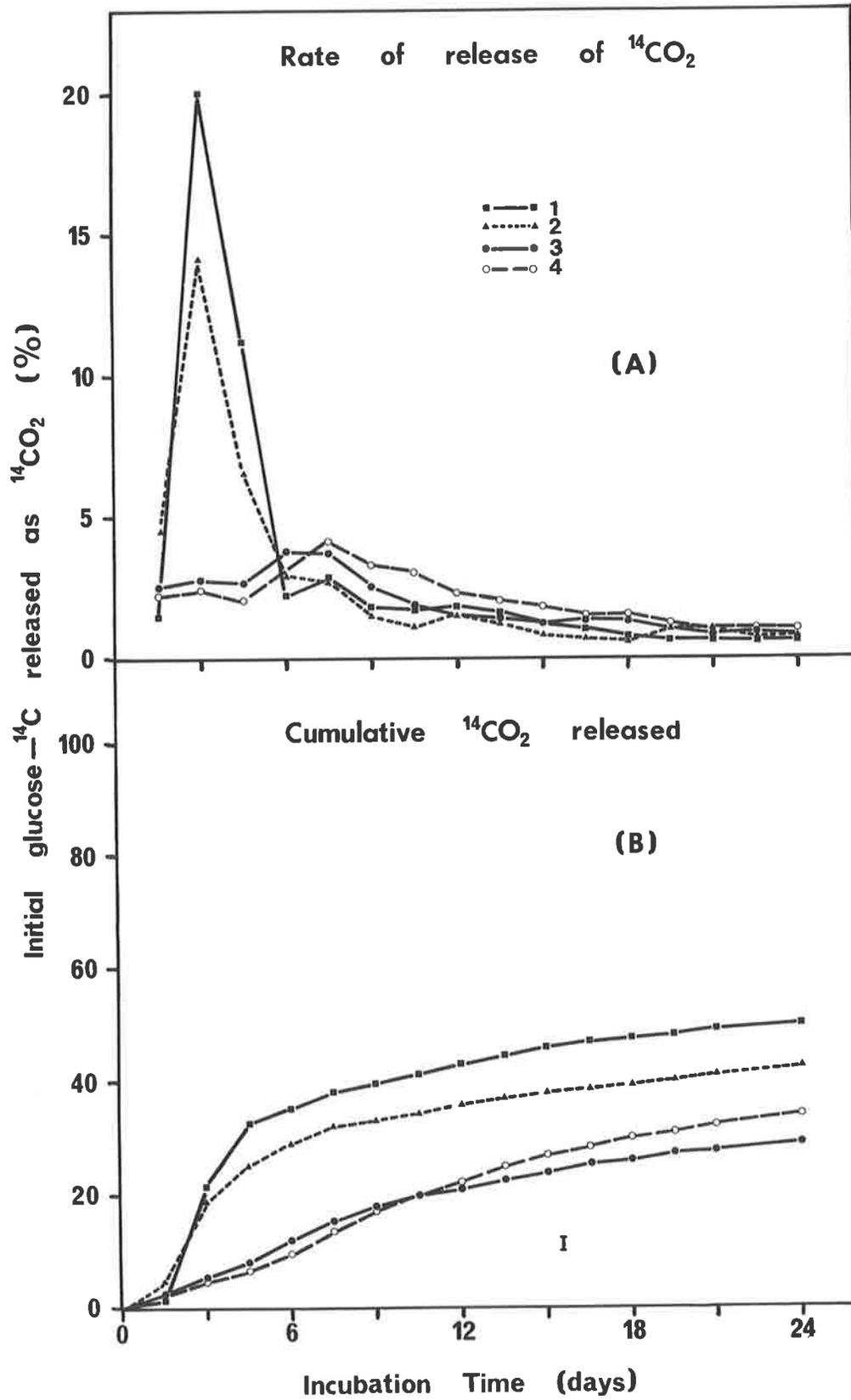


Fig. 31. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -glucose distributed within micropores and macropores of sterilized, artificial aggregates.

Samples were inoculated with different specific organisms.

Soil: Grey clay

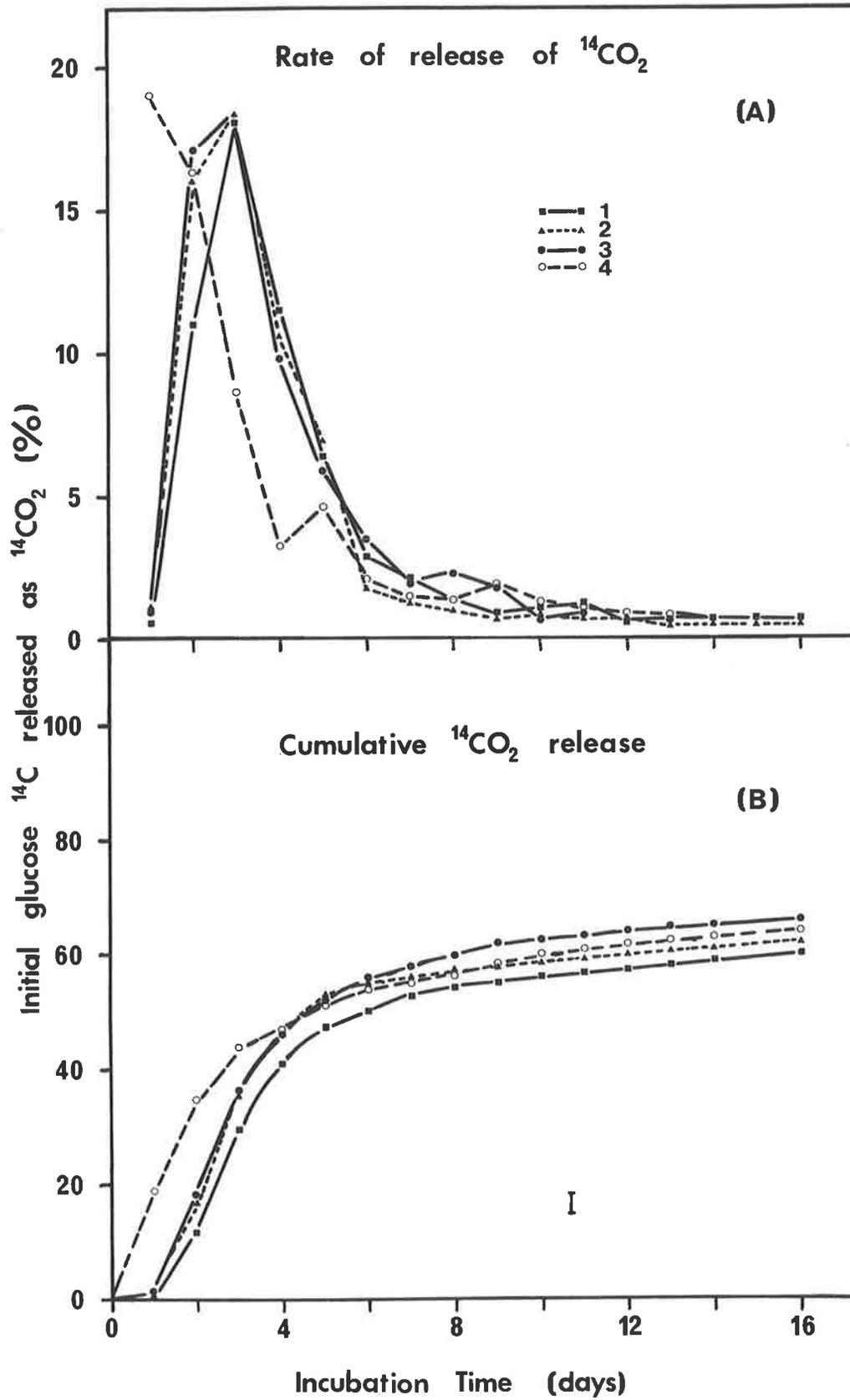
Specific organisms:

1. Rhizoctonia solani
2. Aspergillus flavus
3. Bacillus subtilis
4. Pseudomonas putida

Aggregate size used: 1 to 2 mm

I = L.S.D. (0.05)

FIG. 31



was released as  $^{14}\text{CO}_2$  from the aggregate samples inoculated with fungi on the first day of incubation whilst only 2 to 3% was released from the corresponding samples inoculated with bacteria. Release of  $^{14}\text{CO}_2$  reached a maximum on the second day of incubation for all samples inoculated with fungi (except Aureobasidio pullulans). By this time, 10 to 22% of the original  $^{14}\text{C}$  had been released as  $^{14}\text{CO}_2$  from samples inoculated with the fungi but only 5% from samples inoculated with bacteria. There was a sharp decline in release of  $^{14}\text{CO}_2$  from samples inoculated with fungi and  $^{14}\text{CO}_2$  release remained constantly low after the 7th day of incubation.

On the other hand, the rate of  $^{14}\text{CO}_2$  release from the samples inoculated with bacteria was slow, and spread evenly up to the 10th day of incubation, though maximum release was observed on the 5th day of incubation. Between 23 and 45% of the initial  $^{14}\text{C}$  had been released as  $^{14}\text{CO}_2$  from aggregates inoculated with fungi (Table 18) by the first week of incubation but only about 20% of the initial  $^{14}\text{C}$  had been released as  $^{14}\text{CO}_2$  from aggregates inoculated with bacteria. There was no flush of  $^{14}\text{CO}_2$  at day 8 or 9 which was characteristic of the  $^{14}\text{CO}_2$  release curves for mixed cultures as shown in the previous sections.

#### 3.1.1.2. Grey clay

The rate of  $^{14}\text{CO}_2$  release from the grey clay aggregates followed a different trend from the fine sandy loam (Fig. 31). The differences between the two soils were expected from the previous work with mixed cultures of organisms. The bacteria and fungi behaved similarly in the grey clay. Samples inoculated with Pseudomonas putida released 19% of the  $^{14}\text{C}$  added on the first day of incubation. The rate

Table 18. Release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -glucose distributed within micropores and macropores of sterilized artificial soil aggregates inoculated with different specific micro-organisms

| Fine Sandy Loam; Aggregates |  |                               |                       |                           |                        |                        |                           |                          |
|-----------------------------|--|-------------------------------|-----------------------|---------------------------|------------------------|------------------------|---------------------------|--------------------------|
| Days of incubation          | % initial $^{14}\text{C}$ released as $^{14}\text{CO}_2$ |                               |                       |                           |                        |                        |                           |                          |
|                             | <u>Rhizoctania solani</u>                                | <u>Aureobasidio pullulans</u> | <u>Mucor hiemalis</u> | <u>Aspergillus flavus</u> | <u>Trichoderma sp.</u> | <u>Penicillium sp.</u> | <u>Pseudomonas putida</u> | <u>Bacillus subtilis</u> |
| 7                           | 41.3   | 44.7                          | 23.2                  | 34.3                      | 37.3                   | 27.6                   | 19.9                      | 19.8                     |
| 16                          | 49.9   | 57.2                          | 34.5                  | 42.7                      | 51.0                   | 45.2                   | 34.1                      | 29.8                     |
| Mean of cumulative values   | 39.6   | 42.7                          | 23.7                  | 33.2                      | 37.4                   | 30.0                   | 21.0                      | 18.5                     |
| Combined means              |  |                               | 34.4                  |                           |                        |                        | 19.8                      |                          |
| L.S.D. (0.05)               |  |                               |                       | 1.90                      |                        |                        |                           |                          |
| Grey Clay; Aggregates       |  |                               |                       |                           |                        |                        |                           |                          |
| 7                           | 52.5   | 49.5                          | 53.0                  | 56.2                      | 57.2                   | 42.0                   | 55.3                      | 57.7                     |
| 16                          | 60.3   | 56.8                          | 59.0                  | 62.2                      | 65.6                   | 56.2                   | 64.0                      | 66.2                     |
| Mean of cumulative values   | 46.8   | 44.8                          | 47.7                  | 50.2                      | 51.8                   | 39.3                   | 53.6                      | 52.6                     |
| Combined means              |  |                               | 46.7                  |                           |                        |                        | 53.1                      |                          |
| L.S.D. (0.05)               |  |                               |                       | 3.38                      |                        |                        |                           |                          |

of release from samples inoculated with all the other micro-organisms was low on the 1st day of incubation (which indicated adaptation to the environment) but there was a rapid rise in release of  $^{14}\text{CO}_2$  on the 2nd day of incubation. Except for samples inoculated with Penicillium sp., the rate of release of  $^{14}\text{CO}_2$  from all samples reached a maximum by the 3rd day of incubation.

In contrast to the fine sandy loam, over 50% of the initial  $^{14}\text{C}$  was released as  $^{14}\text{CO}_2$  from all samples (except for those inoculated with Penicillium sp. - from which 42% was released) in 7 days of incubation (Table 18). The fungi released slightly more  $^{14}\text{CO}_2$  from the grey clay but for samples inoculated with bacteria the amounts released were about 3 times the corresponding values for the fine sandy loam.

The slow rate of release of  $^{14}\text{CO}_2$  from most of the samples of the fine sandy loam (particularly from samples inoculated with bacteria) indicated that most of the  $^{14}\text{C}$ -glucose was still not utilized after 7 days. However, only 9 to 17% of the original  $^{14}\text{C}$  was released as  $^{14}\text{CO}_2$  from all samples of both soils within the remaining 9 days of incubation (Table 18), which demonstrates limited microbial activity after the first week (Figs. 30, 31) of incubation. Table 18 again shows that release of  $^{14}\text{CO}_2$  (particularly from the fine sandy loam samples inoculated with bacteria) in some samples did not reach 40% of the initial  $^{14}\text{C}$  by the end of the incubation period. On the other hand, over 60% of initial  $^{14}\text{C}$  was released as  $^{14}\text{CO}_2$  from most samples of grey clay.

In the fine sandy loam the fungi were considerably more active in releasing  $^{14}\text{CO}_2$  than the bacteria (Table 18). In the grey clay the bacteria were more active in releasing  $^{14}\text{CO}_2$ , and except for aggregates

inoculated with the Trichoderma sp. and Aspergillus flavus sp. no fungus was as active as the bacteria, as shown by the means of cumulative  $^{14}\text{CO}_2$  released at the end of the incubation (Table 18).

### 3.1.2. Release of $^{14}\text{CO}_2$ from control samples

Compared with the aggregate samples the rate of release of  $^{14}\text{CO}_2$  from all the control samples of both soils was maintained at a relatively high level after a lag period of a day (Figs. 32, 33). There was no sharp peak, though the rate of  $^{14}\text{CO}_2$  release from most of the samples had reached a maximum by the 3rd day of incubation, by which time 10 to 33% of the initial  $^{14}\text{C}$  was released as  $^{14}\text{CO}_2$  from the fine sandy loam samples. Samples inoculated with Bacillus subtilis released more  $^{14}\text{CO}_2$  (33%) than any of the samples inoculated with a fungus. Similarly, 8 to 33% of the initial  $^{14}\text{C}$  was released as  $^{14}\text{CO}_2$  from the grey clay samples with the samples inoculated with both bacteria releasing more (> 30%) of the initial  $^{14}\text{C}$  as  $^{14}\text{CO}_2$  than any of the fungus-inoculated samples. This pattern of steady release of  $^{14}\text{CO}_2$  was similar to that noted for the control samples during incubation of  $^{14}\text{C}$ -glucose in sterilized aggregates inoculated with soil suspension. It was probably associated with the distribution of the  $^{14}\text{C}$ -glucose within the macropores.

With the fine sandy loam samples the rate of  $^{14}\text{CO}_2$  release (particularly from samples inoculated with bacteria) could only be regarded as being realistic for the first week of incubation, since after this period samples inoculated with bacteria were found to be contaminated with Trichoderma sp. This contamination may have been responsible for the flushes of  $^{14}\text{CO}_2$  observed for the bacteria on the 9th and 10th days

Fig. 32. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -glucose present in macropores only (control) of sterilized, artificial aggregates.

Samples were inoculated with different specific organisms.

Soil: Urrbrae fine sandy loam

Plot: Permanent pasture

Specific organisms:

1. Rhizoctonia solani
2. Aspergillus flavus
3. Bacillus subtilis
4. Pseudomonas putida

Aggregate size used: 1 to 2 mm

I = L.S.D. (0.05)

FIG. 32

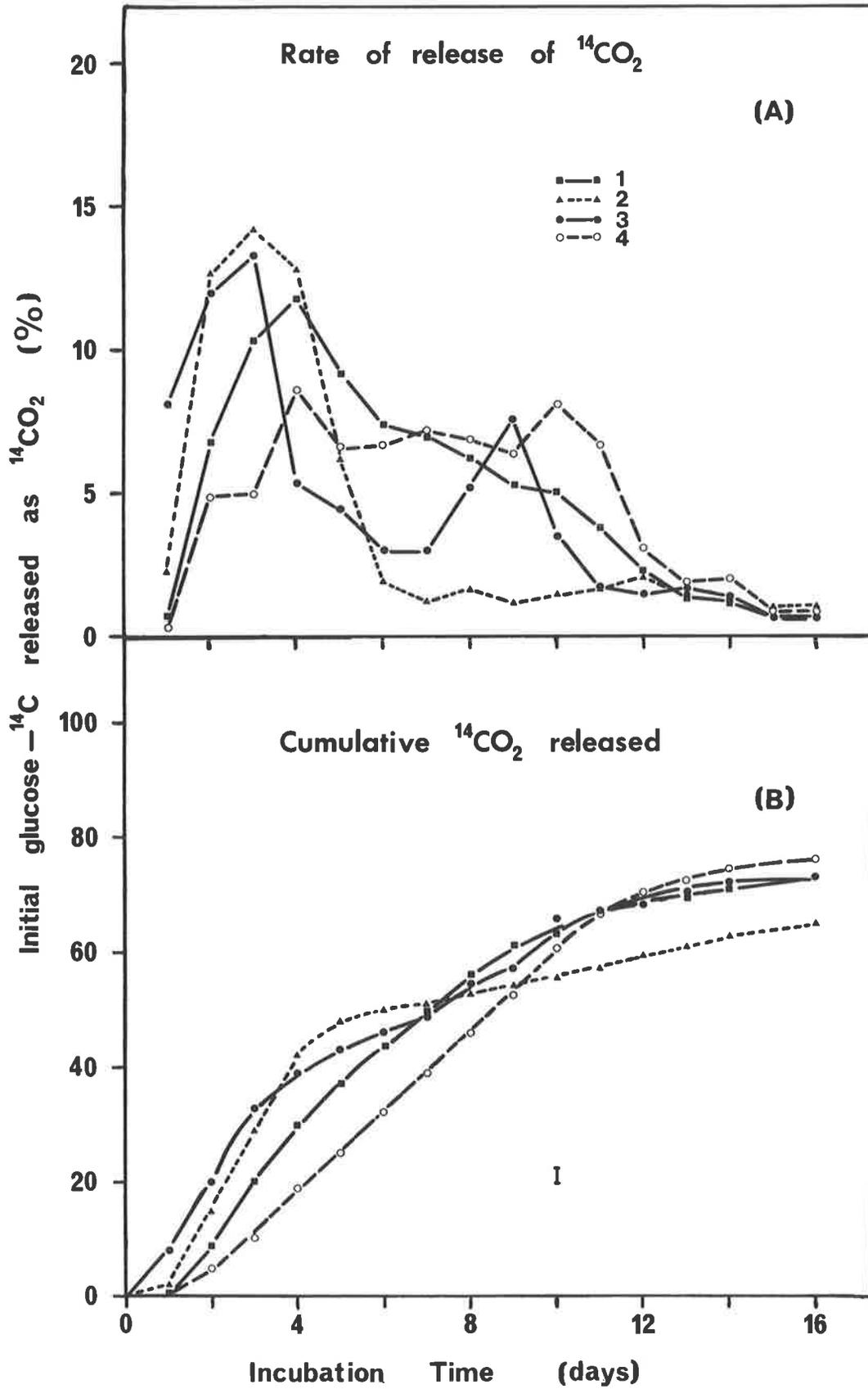


Fig. 33. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -glucose present in macropores only (control) of sterilized, artificial aggregates.

Samples were inoculated with different specific organisms.

Soil: Grey clay

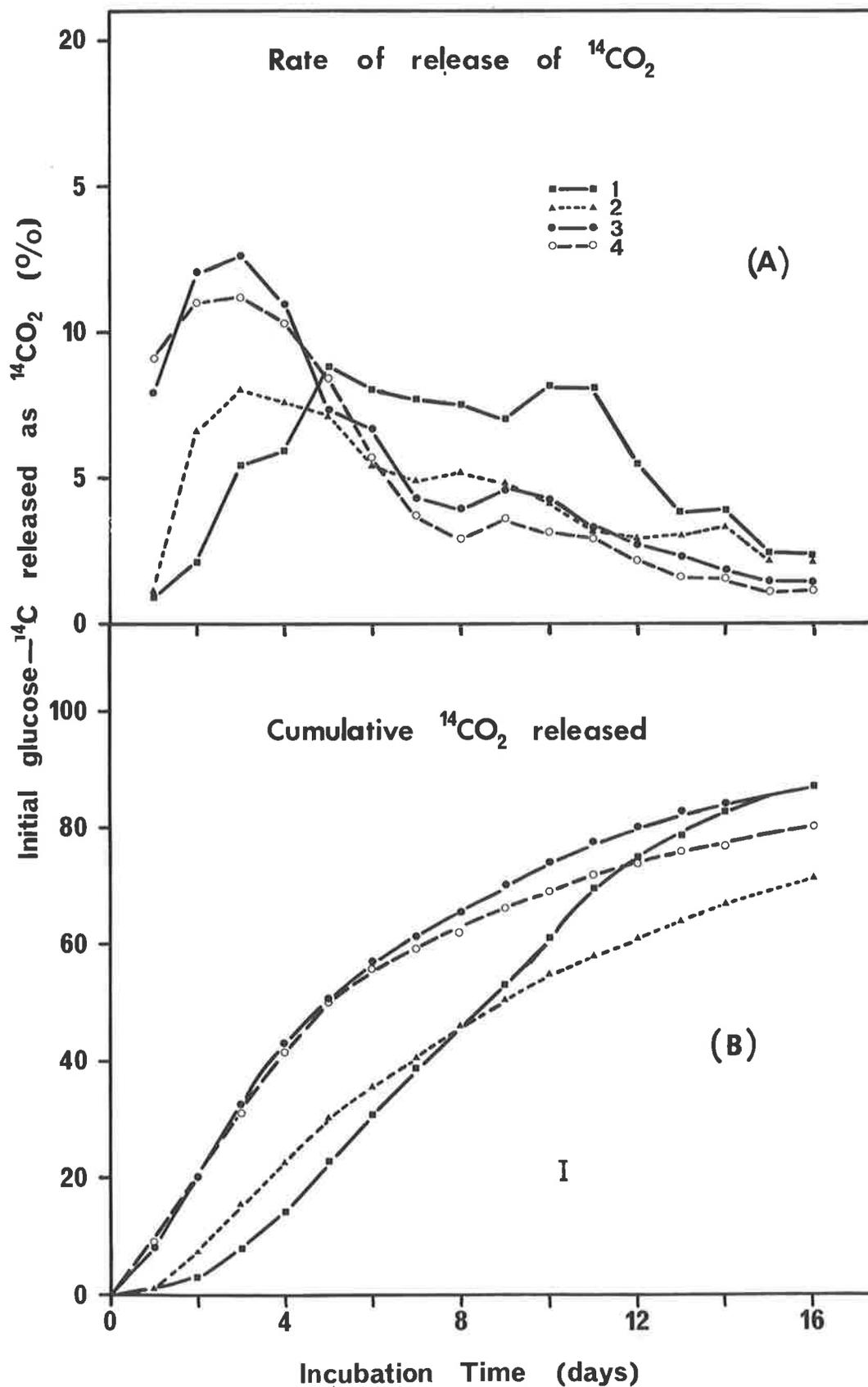
Specific organisms:

1. Rhizoctonia solani
2. Aspergillus flavus
3. Bacillus subtilis
4. Pseudomonas putida

Aggregate size used: 1 to 2 mm

$\bar{I} = \text{L.S.D.}(0.05)$

FIG. 33



of the incubation.

For the fine sandy loam about twice the amount of  $^{14}\text{CO}_2$  was released from controls inoculated with bacteria than was released from the corresponding aggregate samples within the first week of incubation (Table 19). Similarly, the percentages of  $^{14}\text{CO}_2$  released from control samples inoculated with fungi were higher than the corresponding values for  $^{14}\text{CO}_2$  released from aggregate samples (Table 18) within the same period.

With the grey clay, the rate of  $^{14}\text{CO}_2$  release was similar in all samples. Slightly more  $^{14}\text{CO}_2$  was released from samples inoculated with bacteria than from samples inoculated with fungi (Table 19) within the first week of incubation for both control and aggregate samples.

Table 19 shows that within the first week, release of  $^{14}\text{CO}_2$  from most of the samples was more than 40%, which suggested that probably the remaining  $^{14}\text{C}$  in the soil was in microbial tissue or metabolites [if 60 ± 5% efficiency of conversion of the  $^{14}\text{C}$  to microbial tissue is assumed (Payne, 1970)]. The rate of release of  $^{14}\text{CO}_2$  from samples inoculated with Penicillium sp., Aureobasidio pullulans and Rhizoctonia solani remained high after 7 days and a rise in the rate of release of  $^{14}\text{CO}_2$  from samples inoculated with the first two fungi was observed for both soils on the 9th day of incubation, but for samples inoculated with Rhizoctonia solani this rise was observed only in the grey clay. This flush of activity may indicate that these organisms were capable of reusing their own metabolic products when the original substrate was exhausted.

The rate of release of  $^{14}\text{CO}_2$  from all samples of both soils was at about the same level (1 to 3% per day) by the 14th day of incubation up

Table 19. Release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -glucose present in macropores (control)

after inoculated with different specific organisms

Fine Sandy Loam; Control

| Days of incubation        | % initial $^{14}\text{C}$ released as $^{14}\text{CO}_2$ |                               |                       |                           |                        |                        |                           |                          |
|---------------------------|--|-------------------------------|-----------------------|---------------------------|------------------------|------------------------|---------------------------|--------------------------|
|                           | <u>Rhizoctonia solani</u>                                | <u>Aureobasidio pullulans</u> | <u>Mucor hiemalis</u> | <u>Aspergillus flavus</u> | <u>Trichoderma sp.</u> | <u>Penicillium sp.</u> | <u>Pseudomonas putida</u> | <u>Bacillus subtilis</u> |
| 7                         | 53.2   | 49.6                          | 58.2                  | 51.3                      | 43.4                   | 38.5                   | 39.2                      | 49.3                     |
| 16                        | 79.8   | 77.9                          | 75.0                  | 65.2                      | 70.5                   | 70.0                   | 76.4                      | 73.3                     |
| Mean of cumulative values | 53.2   | 51.6                          | 52.1                  | 48.0                      | 46.6                   | 43.2                   | 45.1                      | 52.7                     |
| Combined mean             | 49.1   |                               |                       | 49.1                      |                        |                        |                           |                          |
| L.S.D. (0.05)             | 2.00   |                               |                       |                           |                        |                        |                           |                          |

Grey Clay; Control

|                           |      |      |      |      |      |      |      |      |
|---------------------------|------|------|------|------|------|------|------|------|
| 7                         | 38.7 | 39.8 | 41.1 | 40.6 | 53.0 | 46.9 | 59.4 | 61.6 |
| 16                        | 87.4 | 73.5 | 78.2 | 71.4 | 77.7 | 76.9 | 79.2 | 87.2 |
| Mean of cumulative values | 47.3 | 44.7 | 45.6 | 43.5 | 50.5 | 45.7 | 57.4 | 61.3 |
| Combined mean             | 44.6 |      |      | 59.4 |      |      |      |      |
| L.S.D. (0.05)             | 2.60 |      |      |      |      |      |      |      |

to the end of the incubation period. Cumulative values of  $^{14}\text{CO}_2$  released at the end of incubation (Table 19) show that amount of  $^{14}\text{CO}_2$  released during the period from 8th to the 16th day of incubation was high. With the fine sandy loam samples even though differences existed between specific organisms (Table 19) both groups of micro-organisms were equally active in releasing  $^{14}\text{CO}_2$  as depicted by the lack of difference between the combined means. On the other hand, in the grey clay the bacteria were more active than the fungi in releasing  $^{14}\text{CO}_2$  during the incubation of  $^{14}\text{C}$ -glucose.

### 3.1.3. Comparison between aggregates and control samples

The difference between the two soils with respect to release of  $^{14}\text{CO}_2$  during incubation was best observed when the controls were compared with the aggregate samples (i.e. samples with substrates within micropores and macropores and those with substrates within macropores only) (Table 20). Although in both soils total cumulative release of  $^{14}\text{CO}_2$  from control samples were higher than the corresponding values from aggregate samples (compare Tables 18 and 19) the mean values showed that this was not the case for all samples in the grey soil (lower part of Table 20).

In the fine sandy loam mean values of all control samples were significantly higher than the corresponding values for the aggregate samples. However, with the grey clay (except for those samples marked with asterisks in Table 20), all values for unmarked samples of both aggregates and controls were about equal.

Higher values for  $^{14}\text{CO}_2$  release were obtained from all control samples of both soils during the second week of incubation compared with

Table 20. Comparison of release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -glucose within micropores and macropores (aggregates) and  $^{14}\text{C}$ -glucose within macropores (control) after inoculated with different specific organisms

| Fine Sandy Loam |  |                               |                       |                           |                        |                        |                           |                          |
|-----------------|--|-------------------------------|-----------------------|---------------------------|------------------------|------------------------|---------------------------|--------------------------|
| Samples         | % initial $^{14}\text{C}$ released as $^{14}\text{CO}_2$ |                               |                       |                           |                        |                        |                           |                          |
|                 | <u>Rhizoctonia solani</u>                                | <u>Aureobasidio pullulans</u> | <u>Mucor hiemalis</u> | <u>Aspergillus flavus</u> | <u>Trichoderma sp.</u> | <u>Penicillium sp.</u> | <u>Pseudomonas putida</u> | <u>Bacillus subtilis</u> |
| Aggregates      | 39.6   | 42.7                          | 23.7                  | 33.2                      | 37.4                   | 30.00                  | 21.00                     | 18.5                     |
| Controls        | 53.2   | 51.6                          | 52.1                  | 48.00                     | 46.6                   | 43.2                   | 45.1                      | 52.7                     |
| L.S.D. (0.05)   | 2.47   | 1.00                          | 1.42                  | 3.18                      | 1.00                   | 1.04                   | 1.52                      | 1.36                     |
| Grey Clay       |  |                               |                       |                           |                        |                        |                           |                          |
| Aggregates      | 46.8   | 44.8                          | 47.7                  | 50.2                      | 51.8                   | 39.3                   | 53.6                      | 52.6                     |
| Controls        | 47.3   | 44.7                          | 45.6                  | 43.5                      | 50.5                   | 45.7                   | 57.4                      | 61.3                     |
| L.S.D. (0.05)   | 3.15   | 2.40                          | 2.52                  | 3.18                      | 3.22                   | 4.70                   | 2.20                      | 3.06                     |

\* Significance at  $P < 0.001$

the corresponding aggregate samples (Tables 18 and 19). If the amounts of  $^{14}\text{CO}_2$  released within this period are calculated on the basis of residual  $^{14}\text{C}$  as at the 7th day of incubation, then the release of  $^{14}\text{CO}_2$  was greater during this period than during the first week of incubation. From 30 to 60% of the residual  $^{14}\text{C}$  (at day 7) was released as  $^{14}\text{CO}_2$  from the fine sandy loam controls and only for samples inoculated with Mucor hiemalis and Aspergillus flavus was release of  $^{14}\text{CO}_2$  less in the second week compared with the first week of incubation.

With control samples for the grey clay,  $^{14}\text{CO}_2$  released in the second week calculated on the basis of residual  $^{14}\text{C}$  as at the 7th day of incubation showed that 50 to 80% of the residual  $^{14}\text{C}$  was released as  $^{14}\text{CO}_2$  which was higher than the corresponding values of  $^{14}\text{CO}_2$  released in the first week of incubation.

On the other hand, with the aggregates from both soils  $^{14}\text{CO}_2$  released during the second week was low. Again if calculations were done on the same basis, then with aggregates from both soils only 13 to 27% of residual  $^{14}\text{C}$  was released as  $^{14}\text{CO}_2$  from all samples during the second week, and all the values were lower than the values of  $^{14}\text{CO}_2$  released during the first week of incubation. Thus, the residual  $^{14}\text{C}$  within aggregates (in micropores) was more resistant to attack by enzymes than the residual  $^{14}\text{C}$  in the control samples.

Despite the higher values of cumulative  $^{14}\text{CO}_2$  released from the grey clay aggregates than the corresponding values for the fine sandy loam, the resistance of the residual  $^{14}\text{C}$  to attack by enzymes was similar in both soils. This means similar factors influenced the release of  $^{14}\text{CO}_2$  during the second week of incubation.

#### 3.1.4. Interpretation of results

Release of  $^{14}\text{CO}_2$  from the control samples showed that all the specific micro-organisms used in the present study could utilize  $^{14}\text{C}$ -glucose. The lack of significant difference between the combined values of released  $^{14}\text{CO}_2$  for the bacteria and the corresponding values of the fungi in the control samples of the fine sandy loam suggested that availability of the  $^{14}\text{C}$ -glucose to both groups of organisms in the control samples was similar. It was also shown in the previous chapter that because of the self-mulching characteristic of the grey clay  $^{14}\text{CO}_2$  release from the bigger aggregates (1 to 2 mm) was high which indicated an easy access of the glucose to microbial attack. In the present studies the same size of aggregates was used and the high release of  $^{14}\text{CO}_2$  from these samples in contrast to the release from the fine sandy loam aggregates was in accordance with the results obtained in the last chapter. Of particular interest, however, was the fact that the two species of bacteria used were more active in releasing  $^{14}\text{CO}_2$  than the fungal species from the grey clay aggregates, whilst it was vice versa in the fine sandy loam aggregates. In effect the aggregates of the grey clay behaved in about the same way as the two controls up to a point. Factors involved were probably similar.

The slower release of  $^{14}\text{CO}_2$  from the aggregates of the fine sandy loam inoculated with bacteria cannot be attributed to only physical environmental characteristics (e.g. pH, soil atmosphere, moisture) since these factors are similar in both controls and aggregates. The slow  $^{14}\text{CO}_2$  release must therefore be due to the factors controlling the availability of the  $^{14}\text{C}$ -glucose. The release of  $^{14}\text{CO}_2$  from the aggregates

depended on the ability of the particular organism to extract the substrate from the micropores of the aggregates, if it was assumed that rate of release of  $^{14}\text{CO}_2$  was proportional to the rate of utilization of the  $^{14}\text{C}$ -glucose (Behera and Wagner, 1974). The rate of substrate utilization will then depend on growth characteristics or movement of the different groups of organisms.

Griffin (1972) has related the growth of fungi and bacteria in soil to the growth on agar. According to his hypothesis, mycelium is able to spread rapidly over and beneath an agar surface (with about 20 molecules thickness of water film at -33 bar matric potential) because the older parts of the hypha provide an anchorage on the surface while growth is localized at the apex of a relatively rigid and approximately linear hypha. The substrate is thus rapidly explored and utilized. Bacteria, however, remain as small, localized colonies on such a surface. Reproduction leads to the formation of another simple cell that is usually pulled back within the boundary of the parent colony by surface tension. This last aspect would be the situation on an aggregate with micropores narrower than the size of the bacterium. Directed linear growth outwards is usually impossible because of the lack of a rigid, elongate form.

In soil, water is held within pores but the same basic considerations probably apply. Hyphae will be able to spread along the walls of drained pores or even across the pore from side to side, whereas bacterial spread will, at best, be extremely slow in the absence of continuous water pathways of the requisite dimensions (Griffin, 1972). If it is accepted that bacterial spread and hence efficient utilization

of a substrate is bound up with the existence of water-filled pores, then the following requisites are essential for bacterial movement in a soil:

(a) The smallest diameter along the length of the pore must be large enough to permit passage of the bacterium whether by Brownian or flagellar movement.

(b) Whether such a pore and those larger than it will be air or water-filled will depend upon the matric potential of the system, and

(c) To permit appreciable movement, there must be enough water-filled pores of the requisite diameter to provide a continuous pathway.

Differences between the two soils with respect to these factors were probably responsible for the contrasting results obtained for the aggregate samples of the two soils.

For a soil of the texture of the fine sandy loam, there is a fixed structural framework within which the swelling of the clay is internally accommodated on wetting. This was shown by lack of differences between pF-water content curve and pF-non-polar liquid content curve for a similar soil (Quirk and Panabokke, 1962). The size of micropores within the aggregates may change on swelling but probably not to the extent of providing a requisite diameter for the size of the particular bacterium under study. At pF 2, the pore radius of the greater portion of natural aggregates of the fine sandy loam is of the order of  $10^4 \text{ \AA}$  ( $1 \text{ \mu m}$ ) (Quirk and Panabokke, 1962). This means that in such a soil most organisms would be excluded from such pores. The pore radius of the aggregates used in this study was not measured but since the aggregates were prepared under as near natural conditions as possible,

the pores could be assumed to be of the same size or smaller than those present in natural aggregates. Both Pseudomonas putida and Bacillus subtilis are of the order of 0.5 to 0.7  $\mu\text{m}$  in diameter, which means they would be excluded from entering most of the micropores of the aggregates.

Moreover, because of the small volume change on wetting the complete displacement of air-filled pores by water in the aggregates of the fine sandy loam cannot be guaranteed. Such an incomplete displacement of air by water would mean a discontinuity of the pathway within which the bacterium would move along the pore assuming the size of the pore to be of a requisite size. In the fine sandy loam aggregates, the only means of utilization of glucose was by diffusion of the glucose to the bacterium. However, solute diffusion decreases linearly as a first approximation with volumetric water content so that it would be restricted by those factors (e.g. discontinuity in the pathway along the pores) that also restrict bacterial movement (Griffin, 1972).

The only factor to affect both bacterial movement and fungal spread was the sizes of the micropores within the aggregates. However, the fungi would be able to explore a larger surface area, so that even if the pores were too narrow for hyphal penetration the amount of substrate available would be greater. This would account for higher release of  $^{14}\text{C}$  as  $^{14}\text{CO}_2$  from aggregate samples inoculated with fungi than those inoculated with bacteria.

Even within the fungi (except Aureobasidio pullulans) the percentage release of  $^{14}\text{C}$  as  $^{14}\text{CO}_2$  from any of the samples of the fine sandy loam was less than the value (59%) obtained from the corresponding samples inoculated with mixed microbial population within the same period (Fig.

14, Chapter V). In the absence of competition higher values of  $^{14}\text{CO}_2$  should have been released than the values obtained here (Table 18), unless most of the  $^{14}\text{C}$ -glucose utilized was used in building up new cell tissue (Alexander, 1961). It is likely that this result was due to the greater utilization of microbial tissue and extracellular products by a succession of associated micro-organisms where mixed populations were used as compared to a system involving pure cultures as suggested by Parr and Norman (1964). The relatively high amounts of  $^{14}\text{CO}_2$  released from the control samples (Table 19) showed that this was not the case and it could be assumed that the fungi could not explore the aggregate fully. This might suggest that growth of the hyphae into the micropores was not successful and that diffusion of the substrates probably played a major role in the availability of glucose to the specific organisms. The differences in release of  $^{14}\text{CO}_2$  from aggregate samples of the fine sandy loam inoculated with the two groups of organisms was therefore due more to the differences in surface areas explored by a particular organism.

The high release of  $^{14}\text{CO}_2$  from all the grey clay aggregates irrespective of the organisms used, but particularly higher release from samples inoculated with bacteria, was probably due to the self-mulching characteristic of this soil. Since the soil swells without slaking a system of large pores were created, making the substrates readily available. Besides the creation of large pores the extensive absorption of water provided all the essential pre-requisites discussed above for movement of the bacteria. This, coupled with the favourable high pH (7.6) provided a better environment for optimum activity of the bacteria. The presence of expanding lattice minerals in this soil may

have provided a better buffering capacity to the soil to maintain the pH (Stotzky and Rem, 1966).

The release of  $^{14}\text{CO}_2$  from the samples after some time would depend on the ability of a particular micro-organism to utilize its own metabolites and the dead fraction of the population. The high release of  $^{14}\text{CO}_2$  from control samples during the second week of incubation (8th to 16th day) showed therefore that most of the organisms could either utilize their own metabolites or more  $^{14}\text{C}$ -glucose was still available to attack by the organisms. Since more than 40% of the initial  $^{14}\text{C}$  was released as  $^{14}\text{CO}_2$  from all these samples by the first week of incubation (Table 19), there would not be free  $^{14}\text{C}$ -glucose available (unless 60 ± 5% efficiency of conversion of carbon to cell tissue adopted was too high). This factor would probably differ from one group of organisms to another (Alexander, 1961).

### 3.2. Incubation of $^{14}\text{C}$ -starch amended samples

#### 3.2.1. Release of $^{14}\text{CO}_2$ from aggregate samples inoculated with specific organisms

The rate of release of  $^{14}\text{CO}_2$  from starch amended aggregate samples of both soils was slow (Figs. 34, 35).

##### 3.2.1.1. Fine sandy loam

With the fine sandy loam samples the trend of release of  $^{14}\text{CO}_2$  (Fig. 34) was similar to the trend in the glucose amended samples (Fig. 30). The rate of release of  $^{14}\text{CO}_2$  was higher from the samples inoculated with fungi (1 to 12% release on the first day) than from samples inoculated with bacteria (1 to 2% release on the first day).

Fig. 34. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -starch distributed within micropores and macropores of sterilized, artificial aggregates.

Samples were inoculated with specific organisms.

Soil: Urrbrae fine sandy loam

Plot: Permanent pasture

Specific organisms:

1. Rhizoctonia solani
2. Aspergillus flavus
3. Bacillus subtilis
4. Pseudomonas putida

Aggregate size used: 1 to 2 mm

$I = \text{L.S.D.}(0.05)$

FIG. 34

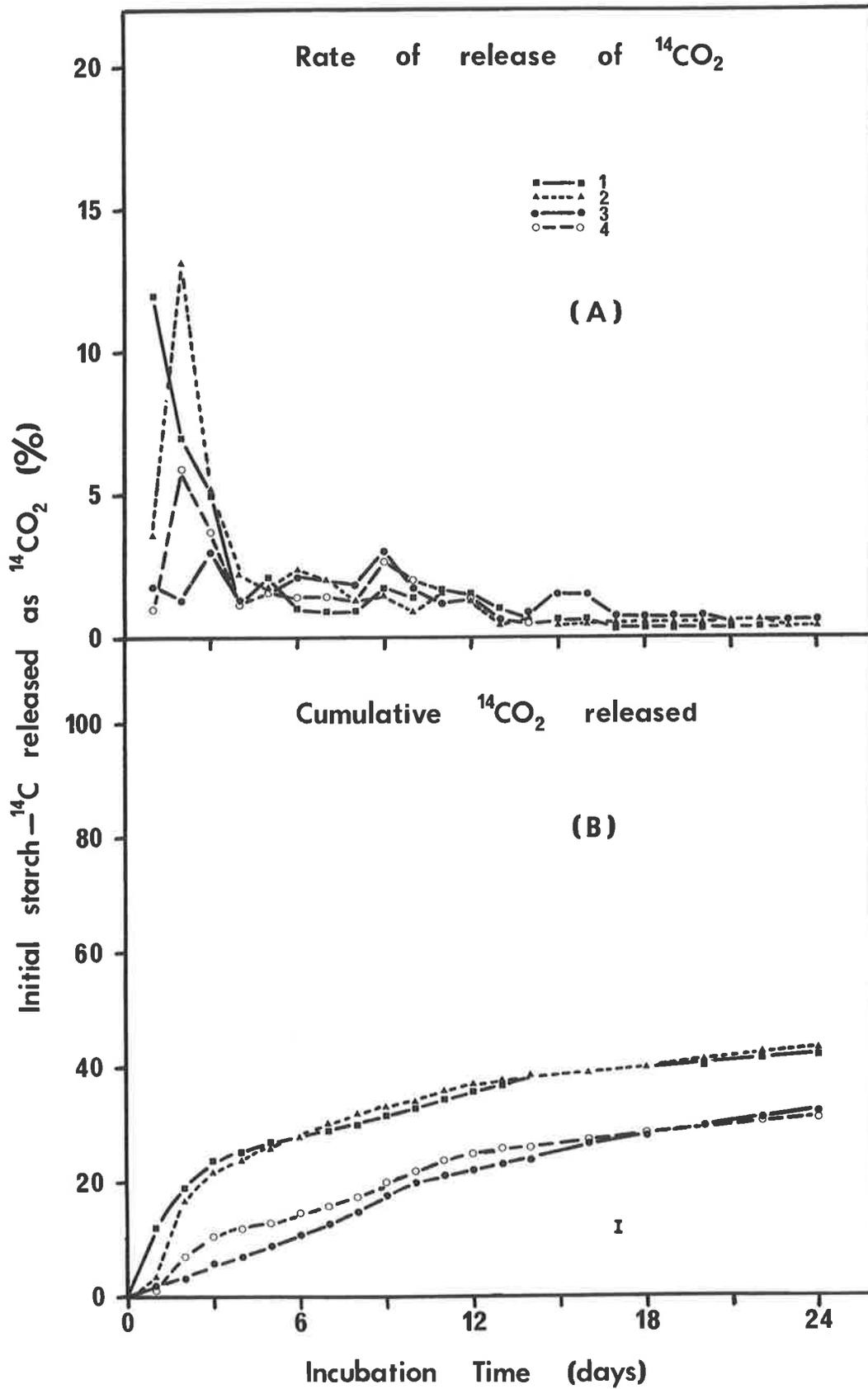


Fig. 35. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -starch distributed within micropores and macropores of sterilized, artificial aggregates.

Samples were inoculated with different specific organisms.

Soil: Grey clay

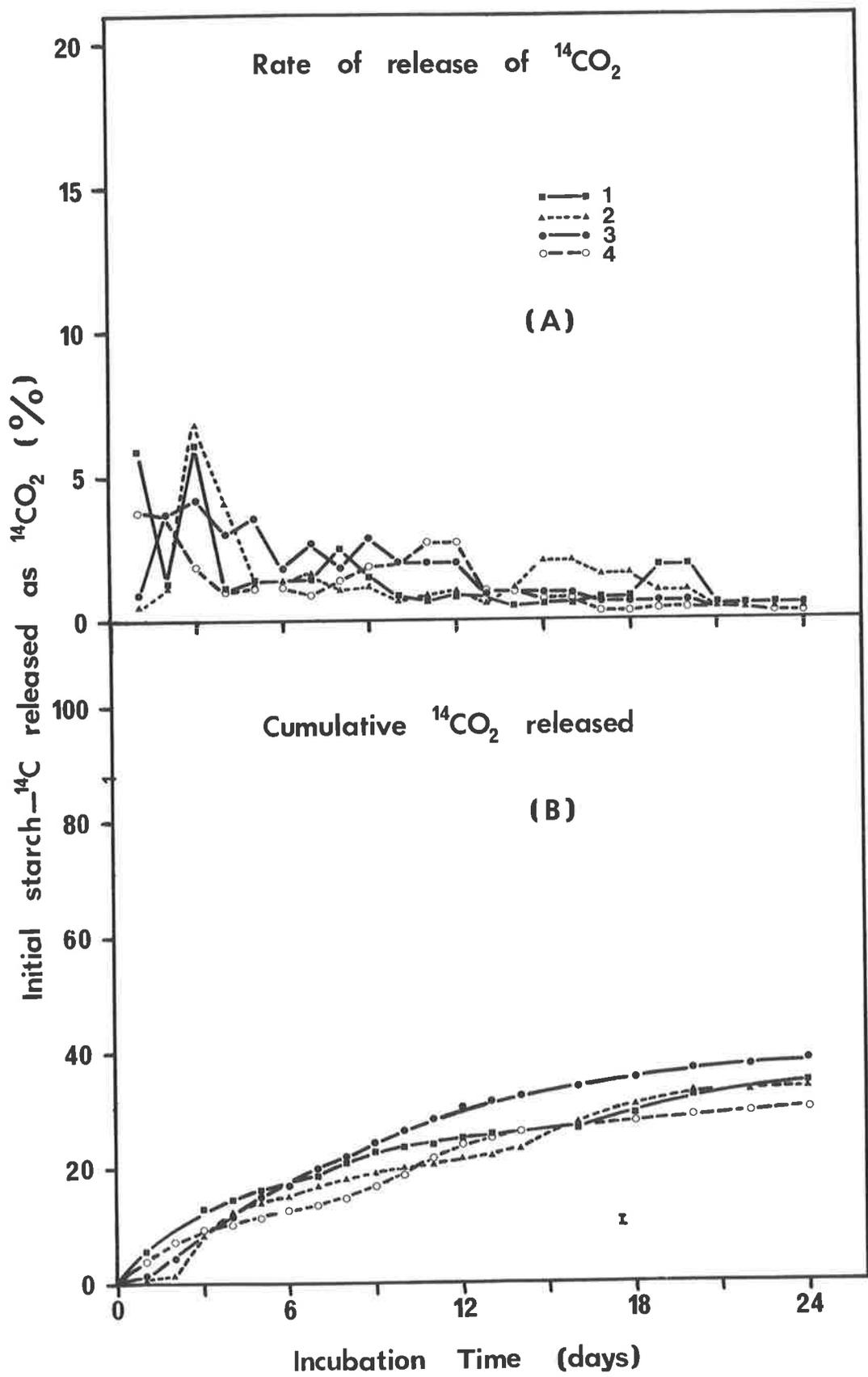
Specific organisms:

1. Rhizoctonia solani
2. Aspergillus flavus
3. Bacillus subtilis
4. Pseudomonas putida

Aggregate size used: 1 to 2 mm

$I = \text{L.S.D. (0.05)}$

FIG. 35



Maximum release of  $^{14}\text{CO}_2$  occurred on the 2nd day for samples inoculated with all organisms except Penicillium sp. and Bacillus subtilis which showed maximum release on the 3rd day of incubation. Between 6 and 20% of the initial  $^{14}\text{C}$  had been released as  $^{14}\text{CO}_2$  from samples inoculated with fungi at the time of maximum release of  $^{14}\text{CO}_2$ , but only about 6% from samples inoculated with bacteria.

There was a sharp decline in  $^{14}\text{CO}_2$  release from most of the samples (particularly those inoculated with fungi) after the initial flush of  $^{14}\text{CO}_2$  and by the 4th day of incubation the rate of release of  $^{14}\text{CO}_2$  was from 1 to 2%/day. There were subsequently intermittent flushes of  $^{14}\text{CO}_2$ , the most prominent being on the 9th day of incubation from samples inoculated with bacteria. Evolution of  $^{14}\text{CO}_2$  from all samples after the 14th day of incubation had declined to about 1% per day and this was maintained until the end of incubation period.

The  $^{14}\text{CO}_2$  released during different periods of incubation (Table 21) shows that the fungi released more  $^{14}\text{CO}_2$  than bacteria. Even so, only four of the samples inoculated with fungi released 40% of the  $^{14}\text{C}$  added indicating that much of the  $^{14}\text{C}$ -starch was protected from attack by the micro-organisms.

#### 3.2.1.2. Grey clay

In contrast to the rapid release of  $^{14}\text{CO}_2$  from the grey clay aggregates amended with  $^{14}\text{C}$ -glucose, the rate of release of  $^{14}\text{CO}_2$  expressed as percentage of initial  $^{14}\text{C}$  from  $^{14}\text{C}$ -starch amended aggregates was quite low (Fig. 35). The rate of evolution of  $^{14}\text{CO}_2$  from all samples inoculated with fungi reached a maximum by the third day of incubation when 8 to 13% of the initial  $^{14}\text{C}$  had been released as  $^{14}\text{CO}_2$ .

Table 21. Release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -starch distributed within micropores and macropores of sterilized artificial soil aggregates inoculated with different specific micro-organisms

| Fine Sandy Loam           |  |                               |                       |                           |                        |                        |                           |                          |
|---------------------------|--|-------------------------------|-----------------------|---------------------------|------------------------|------------------------|---------------------------|--------------------------|
| Days of incubation        | % initial $^{14}\text{C}$ released as $^{14}\text{CO}_2$ |                               |                       |                           |                        |                        |                           |                          |
|                           | <u>Rhizoctonia solani</u>                                | <u>Aureobasidic pullulans</u> | <u>Mucor hiemalis</u> | <u>Aspergillus flavus</u> | <u>Trichoderma sp.</u> | <u>Penicillium sp.</u> | <u>Pseudomonas putida</u> | <u>Bacillus subtilis</u> |
| 7                         | 29.1   | 31.6                          | 28.6                  | 30.4                      | 16.1                   | 18.1                   | 16.2                      | 13.4                     |
| 16                        | 39.1   | 43.4                          | 39.8                  | 39.3                      | 30.3                   | 29.6                   | 27.5                      | 27.0                     |
| 24                        | 42.3   | 45.9                          | 43.1                  | 43.4                      | 38.0                   | 35.6                   | 31.3                      | 32.1                     |
| Mean of cumulative values | 33.7   | 36.6                          | 33.8                  | 33.8                      | 23.3                   | 23.9                   | 22.0                      | 20.3                     |
| Combined mean             |  | 32.5                          |                       |                           |                        |                        | 21.7                      |                          |
| L.S.D. (0.05)             |  |                               | 0.89                  |                           |                        |                        |                           |                          |
| Grey clay                 |  |                               |                       |                           |                        |                        |                           |                          |
| 7                         | 18.9   | 19.0                          | 14.4                  | 16.9                      | 14.2                   | 13.5                   | 13.6                      | 19.9                     |
| 16                        | 27.3   | 26.0                          | 22.0                  | 27.5                      | 23.1                   | 22.9                   | 27.5                      | 34.3                     |
| 24                        | 35.5   | 33.7                          | 31.2                  | 34.1                      | 29.6                   | 32.4                   | 30.3                      | 38.6                     |
| Mean of cumulative values | 23.7   | 21.8                          | 18.7                  | 21.9                      | 18.8                   | 19.1                   | 20.9                      | 26.5                     |
| Combined mean             |  | 20.7                          |                       |                           |                        |                        | 23.7                      |                          |
| L.S.D. (0.05)             |  |                               | 0.83                  |                           |                        |                        |                           |                          |

On the other hand, release of  $^{14}\text{CO}_2$  from samples inoculated with bacteria reached a maximum on the 1st and 2nd days of incubation and a high rate of release was maintained for several days in the sample inoculated with Bacillus subtilis. About 8% of the initial  $^{14}\text{C}$  had been released as  $^{14}\text{CO}_2$  by the 3rd day of incubation.

Most of the  $^{14}\text{C}$  was left in the aggregates at the end of incubation since  $^{14}\text{CO}_2$  released as percentage of original  $^{14}\text{C}$  from all samples at the end of incubation was low (Table 21). However, the bacteria (particularly Bacillus subtilis) were considerably more active in releasing  $^{14}\text{CO}_2$  than the fungi (Table 21).

### 3.2.2. Release of $^{14}\text{CO}_2$ from control samples

#### 3.2.2.1. Fine sandy loam

Evolution of  $^{14}\text{CO}_2$  from the control samples of the fine sandy loam (particularly from samples inoculated with bacteria) was high (Fig. 36). The rate of evolution of  $^{14}\text{CO}_2$  had reached a maximum by the 2nd day of incubation (except for Aureobasidio pullulans) when 11 to 26% of the initial  $^{14}\text{C}$  had been released as  $^{14}\text{CO}_2$  from samples inoculated with fungi (only 4% from Aureobasidio pullulans). The corresponding figures for samples inoculated with bacteria were 17 and 23%.

Release of  $^{14}\text{CO}_2$  declined to about 2% per day by the 7th day of incubation. Contamination of samples inoculated with bacteria and Rhizoctonia solani was observed after the 7th day of incubation. This contamination may have caused the flush of  $^{14}\text{CO}_2$  at days 8 and 9 for the sample inoculated with Rhizoctonia solani, but no such flush occurred with samples inoculated with bacteria.

Fig. 36. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -starch present in macropores only (control) of sterilized, artificial aggregates.

Samples were inoculated with different specific organisms.

Soil: Urrbrae fine sandy loam

Plot: Permanent pasture

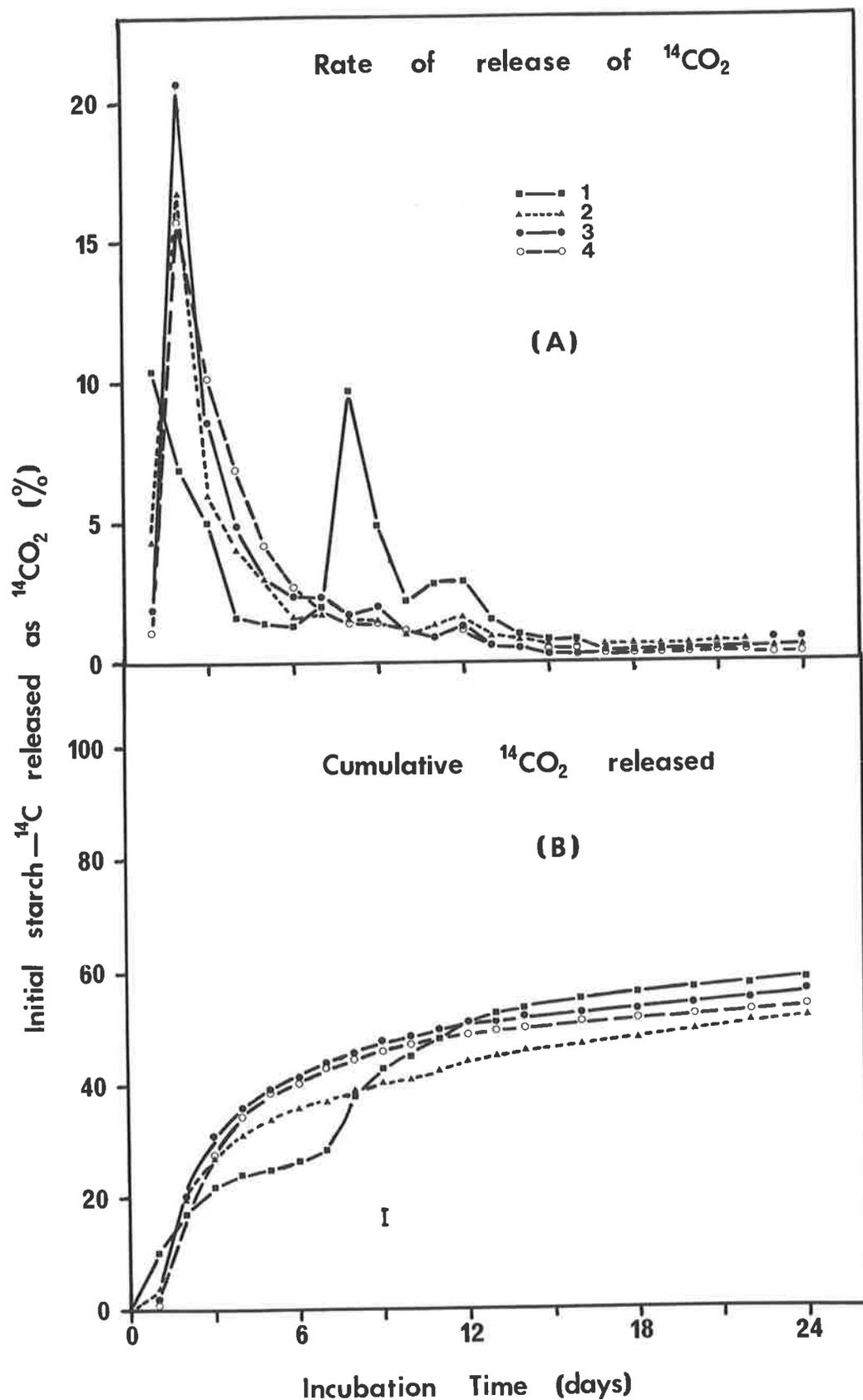
Specific organisms:

1. Rhizoctonia solani
2. Aspergillus flavus
3. Bacillus subtilis
4. Pseudomonas putida

Aggregate size used: 1 to 2 mm

$\bar{I}$  = L.S.D. (0.05)

FIG. 36



More than 50% of the initial  $^{14}\text{C}$  had been released from all samples at the end of incubation, but the bacteria were more active in releasing  $^{14}\text{CO}_2$  than the fungi (Table 22).

#### 3.2.2.2. Grey clay

The rate of release of  $^{14}\text{CO}_2$  from the grey clay control samples was higher from the samples inoculated with bacteria, which attained maximum rate of release on the first day of incubation than with fungi which showed maximum on the 2nd day of incubation (Fig. 37). Production of  $^{14}\text{CO}_2$  declined slowly with intermittent flushes of  $^{14}\text{CO}_2$  and the rate was maintained at about 1 to 3% of initial  $^{14}\text{C}$  per day until the end of incubation.

Values for  $^{14}\text{CO}_2$  released as a percentage of the initial  $^{14}\text{C}$  was lower than the corresponding values in the fine sandy loam during the first week of incubation (Table 22) and only with Aspergillus flavus and Bacillus subtilis was 40% of the initial  $^{14}\text{C}$  released. However, by the end of incubation release of  $^{14}\text{C}$  as  $^{14}\text{CO}_2$  from all samples was over 50% (Table 22). There were again differences between samples inoculated with specific organisms and the bacteria were again more active than fungi in releasing  $^{14}\text{CO}_2$  from controls of the grey clay.

#### 3.2.3. Comparison between release of $^{14}\text{CO}_2$ from aggregates and control samples

As in the  $^{14}\text{C}$ -glucose amended samples, release of  $^{14}\text{CO}_2$  from control samples compared to the aggregate samples in the fine sandy loam (Table 23) showed that all organisms used in this study could release more  $^{14}\text{CO}_2$  from control samples than from the aggregate samples.

Table 22. Release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -starch present in macropores (control) and inoculated with different specific organisms

| Fine Sandy Loam           |  |                               |                       |                           |                        |                        |                           |                          |
|---------------------------|--|-------------------------------|-----------------------|---------------------------|------------------------|------------------------|---------------------------|--------------------------|
| Days of incubation        | % initial $^{14}\text{C}$ released as $^{14}\text{CO}_2$ |                               |                       |                           |                        |                        |                           |                          |
|                           | <u>Rhizoctonia solani</u>                                | <u>Aureobasidio pullulans</u> | <u>Mucor hiemalis</u> | <u>Aspergillus flavus</u> | <u>Trichoderma sp.</u> | <u>Penicillium sp.</u> | <u>Pseudomonas putida</u> | <u>Bacillus subtilis</u> |
| 7                         | 28.6   | 44.1                          | 41.9                  | 37.4                      | 28.9                   | 36.4                   | 43.2                      | 43.8                     |
| 16                        | 55.2   | 55.5                          | 50.8                  | 47.2                      | 44.9                   | 52.9                   | 51.1                      | 52.5                     |
| 24                        | 58.6   | 59.5                          | 54.5                  | 51.7                      | 50.2                   | 58.0                   | 53.7                      | 56.5                     |
| Mean of cumulative values | 43.2   | 45.3                          | 44.9                  | 40.9                      | 35.6                   | 42.7                   | 44.1                      | 45.9                     |
| Combined Mean             |  | 42.1                          |                       |                           |                        |                        | 45.0                      |                          |
| L.S.D. (0.05)             |  |                               |                       | 2.10                      |                        |                        |                           |                          |
| Grey Clay                 |  |                               |                       |                           |                        |                        |                           |                          |
| 7                         | 36.2   | 36.5                          | 34.3                  | 40.6                      | 30.0                   | 30.2                   | 31.9                      | 43.0                     |
| 16                        | 55.4   | 47.3                          | 47.6                  | 52.6                      | 47.7                   | 45.6                   | 47.9                      | 52.6                     |
| 24                        | 62.9   | 52.8                          | 53.5                  | 56.5                      | 55.5                   | 53.0                   | 54.7                      | 56.6                     |
| Mean of cumulative values | 45.2   | 39.6                          | 39.6                  | 44.8                      | 37.3                   | 37.3                   | 39.0                      | 45.2                     |
| Combined mean             |  | 40.6                          |                       |                           |                        |                        | 42.1                      |                          |
| L.S.D. (0.05)             |  |                               |                       | 1.41                      |                        |                        |                           |                          |

Fig. 37. Release of  $^{14}\text{CO}_2$  during incubation of uniformly labelled  $^{14}\text{C}$ -starch present in macropores only (control) of sterilized, artificial aggregates.

Samples were inoculated with different specific organisms.

Soil: Grey clay

Specific organisms:

1. Rhizoctonia solani
2. Aspergillus flavus
3. Bacillus subtilis
4. Pseudomonas putida

Aggregate size used: 1 to 2 mm

$\bar{I}$  = L.S.D. (0.05)

FIG. 37

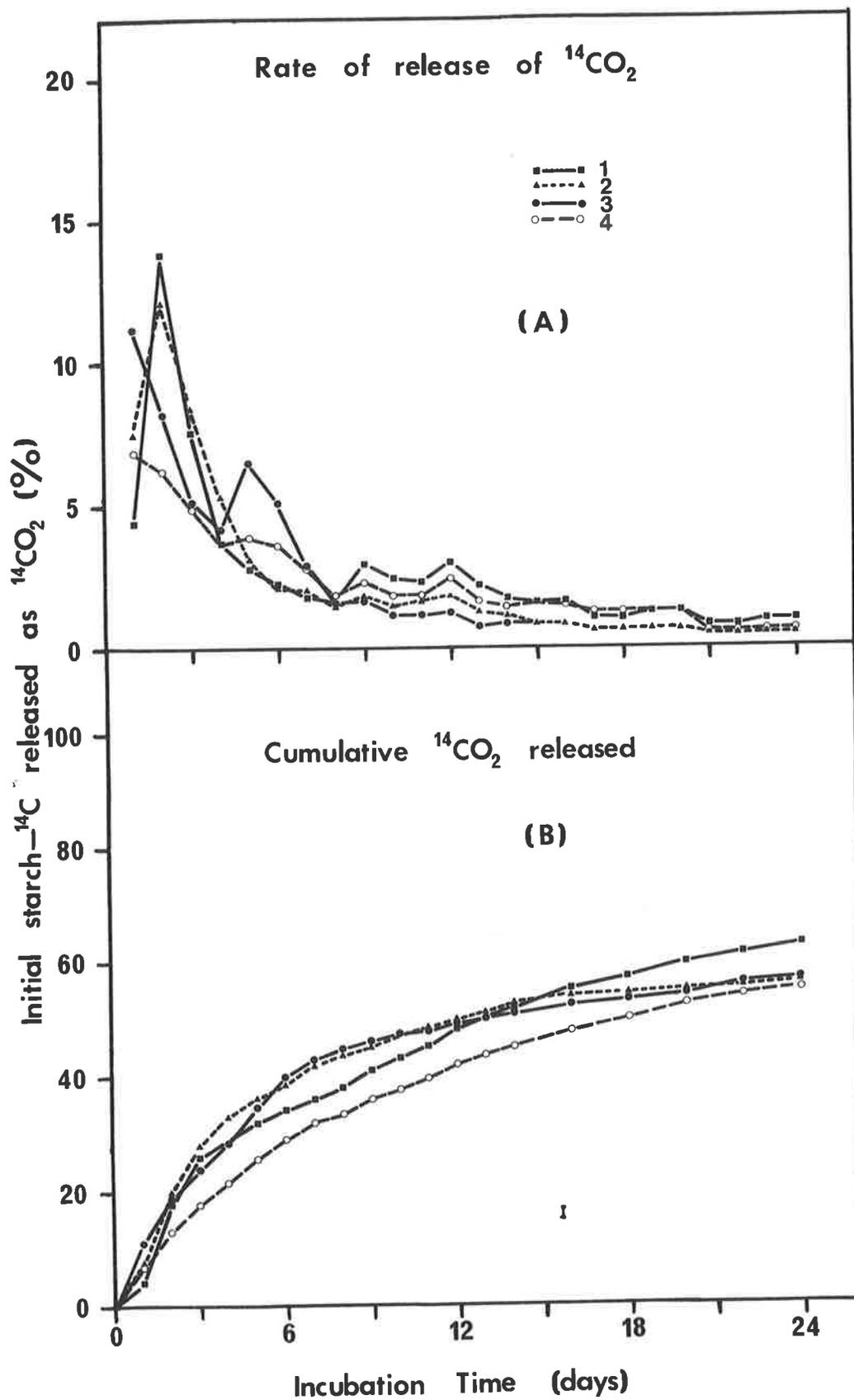


Table 23. Comparison of release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -starch within micropores and macropores (aggregates) and in macropores only (control) inoculated with different specific organisms (i.e. aggregates vs. control)

| Fine Sandy Loam |  |                               |                       |                           |                        |                        |                           |                          |
|-----------------|--|-------------------------------|-----------------------|---------------------------|------------------------|------------------------|---------------------------|--------------------------|
| Samples         | % initial $^{14}\text{C}$ released as $^{14}\text{CO}_2$ |                               |                       |                           |                        |                        |                           |                          |
|                 | <u>Rhizoctonia solani</u>                                | <u>Aureobasidio pullulans</u> | <u>Mucor hiemalis</u> | <u>Aspergillus flavus</u> | <u>Trichoderma sp.</u> | <u>Penicillium sp.</u> | <u>Pseudomonas putida</u> | <u>Bacillus subtilis</u> |
| Aggregates      | 33.7   | 36.6                          | 33.8                  | 33.8                      | 23.3                   | 23.9                   | 22.0                      | 20.3                     |
| Controls        | 43.2   | 45.2                          | 44.9                  | 40.9                      | 35.6                   | 42.7                   | 44.1                      | 45.9                     |
| L.S.D. (0.05)   | 1.12   | 2.03                          | 1.82                  | 1.82                      | 0.89                   | 0.99                   | 1.99                      | 1.97                     |
| Grey Clay       |  |                               |                       |                           |                        |                        |                           |                          |
| Aggregates      | 23.7   | 21.8                          | 18.7                  | 21.9                      | 18.8                   | 19.1                   | 20.9                      | 26.5                     |
| Controls        | 45.2   | 39.6                          | 39.6                  | 44.8                      | 37.3                   | 37.3                   | 39.0                      | 45.2                     |
| L.S.D. (0.05)   | 1.21   | 1.59                          | 1.31                  | 1.46                      | 0.79                   | 0.91                   | 0.66                      | 1.89                     |

Thus the low values for release of  $^{14}\text{CO}_2$  as percentage of initial  $^{14}\text{C}$  from the aggregate samples (Table 21) was not due to the inability of the organisms to utilize starch as a source of energy. It must therefore have been due to factors associated with the distribution or supply of the  $^{14}\text{C}$ -starch (i.e. the distribution of the  $^{14}\text{C}$ -starch in micropores or macropores). However, despite the fact that some mean values for  $^{14}\text{CO}_2$  released from control samples inoculated with some fungi (particularly Rhizoctonia solani, Aureobasidio pullulans, Mucor hiemalis and Penicillium sp.) compared favourably with release from samples inoculated with bacteria, the combined mean values were significantly different. This is in contrast to the situation in the glucose amended samples where there was no difference between the two groups of micro-organisms in the control samples of the fine sandy loam.

On the other hand, unlike the glucose amended samples where for samples of the grey clay inoculated with fungi no difference was observed between the means of  $^{14}\text{CO}_2$  released from control and the aggregate samples (except for Aspergillus flavus and Penicillium sp.) all means for controls were significantly higher than the corresponding values for the aggregates. The low release of  $^{14}\text{CO}_2$  from the aggregates did not reflect the self-mulching characteristics of the grey clay during utilization of the  $^{14}\text{C}$ -starch by the specific organisms, as has been noted for samples inoculated with mixed culture of unknown constitution.

#### 3.2.4. Interpretation of results

Release of  $^{14}\text{CO}_2$ , particularly from the aggregate samples was low, and with all soils used release of  $^{14}\text{CO}_2$  from control samples was higher than from aggregate samples. Determination of residual  $^{14}\text{C}$

(in the aggregates) by suspension counting of freeze-dry samples (Adu and Oades, 1973) after incubation (Appendix) showed that most of the  $^{14}\text{C}$  remained in the soil. This indicated that the residual  $^{14}\text{C}$ -starch was either intact or had been used to build microbial tissues.

It was shown in Chapters IV to VI, that starch was protected from attack by micro-organisms, when present in soil aggregates. The higher release of  $^{14}\text{CO}_2$  from the control samples in the present studies, as opposed to the aggregate samples confirms the findings obtained in the previous chapters.

The rate of release of  $^{14}\text{CO}_2$  from substrates within aggregates depends on the extensive growth of the organism to bring it in contact with as much substrate as possible. Thus the rate of release of  $^{14}\text{CO}_2$  from aggregate samples of the fine sandy loam inoculated with bacteria was slower than from samples inoculated with fungi because of the extensive growth of the latter. The relatively high surface to volume ratio of individual hyphae in the three dimensional branching system developed by the fungi (particularly filamentous fungi) presents a large interface to interact with outside conditions, e.g. for rapid exchange of materials between hyphae and soil (Waid, 1960). The extensive surfaces of the fungal hyphae distribute extracellular enzymes throughout the soil. Thus hydrolysis of substrates such as starch can occur giving rise to small water soluble moieties which may be assimilated by the cell if conditions are favourable (Hawker, 1957; Sussman, 1957).

Although soluble starch, which is mainly amylose, was used in these studies, hydrolysis by extracellular enzymes of the micro-organisms would be necessary before assimilation. This would require close

proximity of the organisms and starch. The importance of physical separation of substrates from potentially active micro-organisms in governing the rate of release of  $\text{CO}_2$  during incubation has recently been shown by studies of microbial decomposition of chitin as influenced by different sizes of glass beads (Ou and Alexander, 1974).

The low release of  $^{14}\text{CO}_2$  from aggregates of the grey clay was not consistent with the high release of  $^{14}\text{CO}_2$  from the same size of aggregates inoculated with soil suspension in Chapter V. The reason for this is not known. However, hydrolysis of starch by both bacterial and fungal enzymes is very complex (Greenwood and Milne, 1968). It is possible that a single organism produces a limited range of enzymes compared with a mixed population and utilizes starch less rapidly than a mixture of organisms. With the control samples where the substrate was within macropores release of  $^{14}\text{CO}_2$  was higher because of the proximity of the micro-organisms to the substrate, which excludes complications due to sorption and deactivation of the starch degrading enzymes.

In both soils release of  $^{14}\text{CO}_2$  from both aggregate samples inoculated with Trichoderma sp. and Penicillium sp. was comparatively low. Burges (1960) outlined a growth pattern of Penicillium as follows: "A small piece of substrate is densely colonized by the fungus. Spore production occurs heavily over the surface of the substrate and there is no extension of the mycelium into the surrounding soil". This sort of growth pattern was observed for both Penicillium sp. and Trichoderma sp. In the aggregates inoculated with these fungi spores appeared in sparse patches on the soil. This was in contrast to profuse sporulation of the Trichoderma sp. on aggregate samples amended with glucose from which

comparatively more  $^{14}\text{CO}_2$  was released (Table 18).

Although control samples of the fine sandy loam inoculated with bacteria were contaminated by Aspergillus flavus, a second peak release of  $^{14}\text{CO}_2$  from the contaminated samples was not observed as noted in the glucose amended samples. The flush of  $^{14}\text{CO}_2$  probably depended on the relative competitive interactions between the two organisms. In the glucose amended samples Trichoderma sp. was the invading organism. Presumably, the bacteria were able to counteract the antagonistic activity of the Aspergillus flavus but not the activity of Trichoderma sp. The high rise in the rate of release of  $^{14}\text{CO}_2$  from the control samples inoculated with Rhizoctonia solani after invasion by Aspergillus flavus was probably due to poor competition of Rhizoctonia solani compared with the invading Aspergillus flavus.

### 3.3. Conclusion

Release of  $^{14}\text{CO}_2$  from aggregate samples of the fine sandy loam amended with either  $^{14}\text{C}$ -glucose or  $^{14}\text{C}$ -starch was higher from samples inoculated with fungi than with bacteria. In control samples of the same soil, the situation was reversed, particularly with Bacillus subtilis.

In the grey clay samples release of  $^{14}\text{CO}_2$  from aggregate samples inoculated with Bacillus subtilis was more than from samples inoculated with fungi.

The results therefore show that when substrates are in macropores of the soil (i.e. easily accessible) bacteria and fungi play an equally important part in degradation of substrates introduced into the soil. Thus, release of  $\text{CO}_2$  from soil to which an organic substrate is added

would enhance the activities and growth of bacteria if the substrates are distributed in such a way as to be present in the macropores. This probably accounts for the high bacterial counts during the initial few days of incubation of glucose in a soil (Behera and Wagner, 1974; Shields et al., 1973; Ladd and Paul, 1973). On the other hand, in soils in the field a large proportion of organic matter is distributed through the aggregates. Depending on the nature of the soil (cf. fine sandy loam and grey soil above) the effective microbial population may either consist mainly of fungi or bacteria, or similar proportions of both groups of organisms. For a soil such as the fine sandy loam fungi would probably be more important than bacteria in metabolising the substrates whereas in soil such as the grey clay bacteria would be more significant. It has been observed that fungi such as Rhizoctonia solani are associated with decomposing wheat straw in the Urrbrae fine sandy loam (Warcup, 1957). Amongst the fungi the capacity to grow extensively into pores in aggregates (e.g. Rhizoctonia solani) would be an advantage over those fungi which sporulate rather than grow vegetatively.

However, the results obtained cannot be extrapolated too far since the specific organisms grown were not subjected to any competition or antagonistic effects from other organisms.

The high release of  $^{14}\text{CO}_2$  from control samples of the fine sandy loam and the grey clay samples inoculated with Bacillus subtilis indicated that a high proportion of the substrate available to the normal soil population could be utilized by this organism as Bacillus subtilis is ubiquitous in soil.

The results confirm the protection of starch within aggregates and indicate that when substrates are in macropores readily accessible to micro-organisms then bacteria should compete well with fungi, if antagonistic effects are neglected. However, when substrates are in micropores then the fungi utilize the substrate more efficiently than bacteria. These statements are made neglecting the effects of pH and water regime.

CHAPTER VIII. INTERPRETATION OF THE  $^{14}\text{CO}_2$  RELEASE CURVES  
DURING INCUBATION OF  $^{14}\text{C}$  LABELLED SUBSTRATES IN SCIL

1. Evidence for successive development of different microbial populations

Few attempts have been made to explain the factors responsible for the shape of the cumulative  $\text{CO}_2$  release curves during laboratory incubations. It has generally been assumed that such curves are smooth, showing a rapid release of  $\text{CO}_2$  over the first few days followed by a gradual decrease in  $\text{CO}_2$  production until a low basal rate is reached. Carbon-14 studies have shown that some of the recently added substrate carbon becomes resistant to attack by micro-organisms by mechanisms which defy precise definition.

The work described in the previous three chapters shows clearly, that if the  $\text{CO}_2$  release curve is examined carefully it is complex rather than smooth. When the rate of release of  $\text{CO}_2$  by a mixed microbial population was plotted on a daily basis, then in most samples two peaks of  $\text{CO}_2$  release were obtained. The major peak occurred between the 2nd and 5th days of incubation. The second, minor peak appeared consistently after 8 or 9 days incubation.

Ladd and Paul (1973) obtained three definite slopes in  $\text{CO}_2$  evolution curves during incubation of  $^{14}\text{C}$ -glucose in the laboratory. This suggested either changes in resistance of the  $^{14}\text{C}$  source to enzyme attack or change in microbial populations. Jensen (1936) and Shields (1972) observed that there was successive development of different

microflora during laboratory incubation studies of soil amended with substrates. The primary population, active during the period of rapid attack was dominated by fungi which were postulated to be subsequently degraded by a secondary population of bacteria and actinomycetes (Shields, 1972). On the other hand, it has recently been reported that during laboratory incubation of  $^{14}\text{C}$ -glucose, bacteria were the primary active population, and fungal growth was noted when the bacterial count declined after 3 to 5 days (Behera and Wagner, 1974).

Lysis of mycelial cell walls has been observed by various workers (Jones and Webley, 1967, 1968; Jones et al., 1968; Lockwood, 1960; Skujins et al., 1965; Mitchell and Alexander, 1963). The process has often been attributed to cell wall degrading enzymes produced by lytic micro-organisms (Eveleigh et al., 1968; Mitchell and Alexander, 1963; Skujins et al., 1965). Evidence has been produced to show that soil mycolysis was probably caused by autolysis (lysis of enzymes present in the mycelium itself) and that amendment of soil with glucose or peptone temporarily annulled soil mycolysis whereas chitin amendment enhanced mycolysis (Lloyd and Lockwood, 1966). Ko and Lockwood (1970) confirmed the previous results of Lloyd and Lockwood (1966) and showed that autolysis was induced by nutrient (energy source) deprivation with the activated enzymes dissolving cell constituents. Mycolysis can also involve only loss of protoplasm from live fungal hyphae and the protoplasm and empty cell walls can then be attacked by other organisms (Bumbieris and Lloyd, 1966). The nutrients released by lysing fungal cells into the surrounding soil stimulate growth of other micro-organisms in an orderly sequence with bacteria followed by protozoa developing in a wet soil (Bumbieris and Lloyd, 1966).

Such processes probably occurred in the present study and might partly explain the results obtained. The primary population that utilized the introduced  $^{14}\text{C}$ -glucose were probably predominantly fungal. After 40% of the initial  $^{14}\text{C}$  had been released as  $^{14}\text{CO}_2$  the remaining  $^{14}\text{C}$  in the soil was presumably mainly incorporated into the fungal cell walls or their metabolites. Utilization of all the glucose i.e. the energy source for heterotrophic organisms, induced autolysis of the existing fungal population. The protoplasm released stimulated growth of other organisms, and at the water content used in these studies (80% field capacity or 25% v/w), the growth of bacteria would be enhanced more than any other group of organisms (Bumbieris and Lloyd, 1966). These developing bacteria could then exist on the  $^{14}\text{C}$ -labelled protoplasm released by mycolysis and subsequently on more resistant components of the lysing hyphae.

If this hypothesis is correct, fungal numbers should decline drastically and bacterial numbers increase at the time of appearance of the second peak of release of  $^{14}\text{CO}_2$ . Because most of the bacteria would be in their logarithmic phase of growth, the presence of an easily-metabolised energy source such as protoplasm would be exhausted quickly, and if the release of  $^{14}\text{CO}_2$  was assumed to be proportional to substrate utilization (Behera and Wagner, 1974), there would be a sudden drop in  $\text{CO}_2$  release. This would partly explain the sudden rise of  $^{14}\text{CO}_2$  on the 9th day of incubation followed by a sudden drop on the 10th day. Differences in the amount of  $^{14}\text{CO}_2$  released in the second peak could be due to different species of bacteria utilizing the fungal products, and also to the variable resistance of fungal tissues.

1.1. Quantitative estimation of soil micro-organisms during incubation of glucose in the fine sandy loam

To determine whether the two peaks of  $^{14}\text{CO}_2$  release were due to different groups of micro-organisms quantitative estimations of bacteria and fungi were made at intervals during incubation of glucose in the fine sandy loam.

It was not possible to carry out this determination on the aggregate samples during incubation, since any withdrawal of part of the samples might have caused breakdown of the aggregates. However, since the appearance of the two peaks occurred in the control samples it was thought appropriate to use a control sample for the study. Glucose was chosen as a substrate since the second peak was more pronounced in samples with glucose incorporated.

1.1.1. Materials and methods

1.1.1.1. Materials

A representative sample of Urrbrae fine sandy loam (0 to 10 cm) from the permanent pasture plot (characteristics given in the Appendix) was used.

Samples were sieved to obtain a < 2 mm fraction.

1.1.1.2. Methods

1.1.1.2.1. Incubation

1 g of unlabelled glucose in 35 ml of water was added to 100 g (< 2 mm) of soil to bring the sample to about 80% of field capacity. Sample was incubated at  $28 \pm 2^\circ\text{C}$  in the same manner as described in other sections.

Incubation was allowed to go for 14 days and samples withdrawn for microbial estimation at days 0, 3, 6, 7, 10 and 14.

#### 1.1.1.2.2. Estimation of soil micro-organisms

Microbial numbers were estimated by the method of Jones and Mollison (1948) with slight modification, as described in the Appendix.

#### 1.1.2. Results and discussion

##### 1.1.2.1. Microbial growth during incubation of glucose in the fine sandy loam

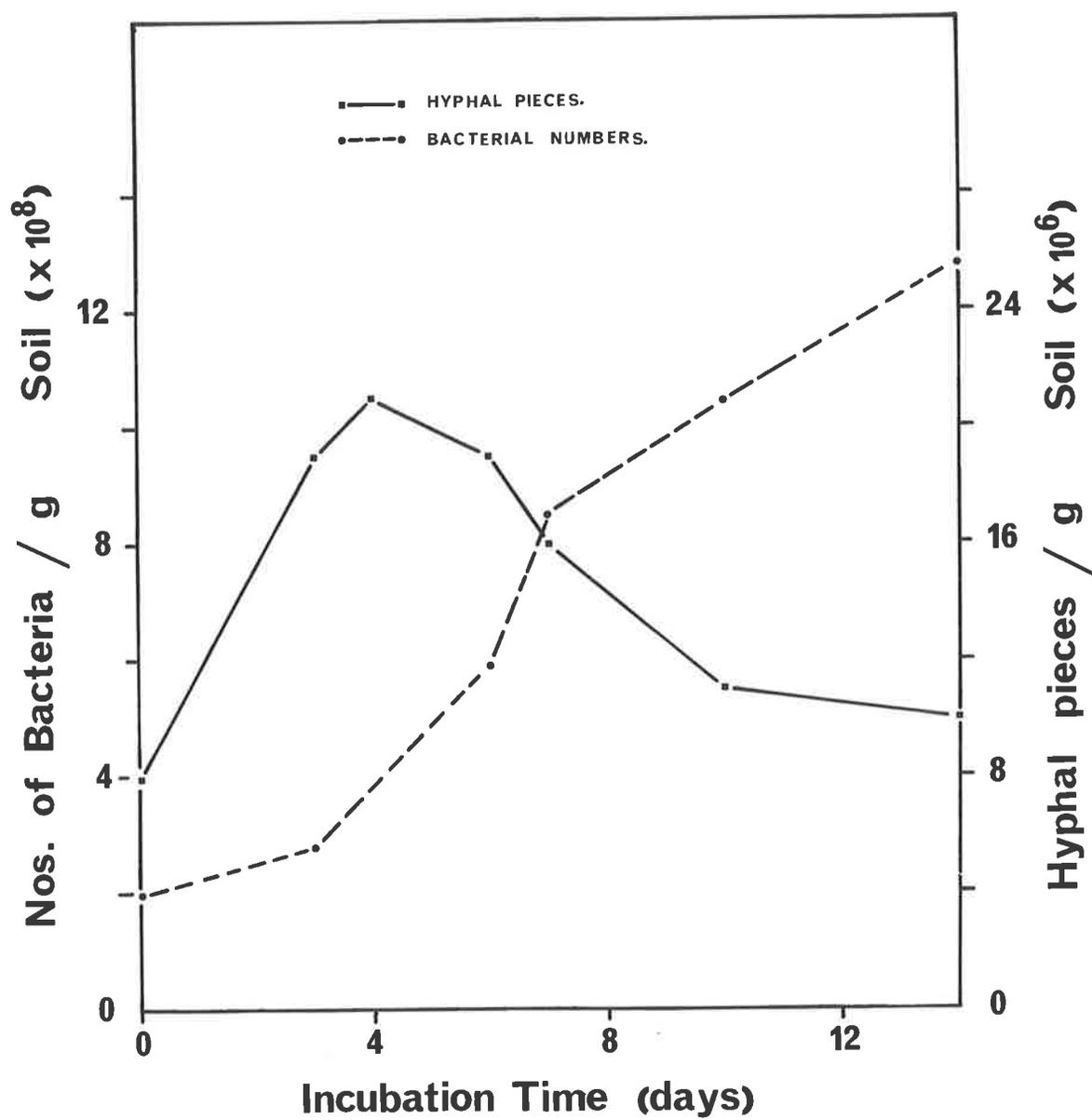
Changes in microbial populations during the incubation of glucose in the fine sandy loam are shown in Fig. 38. The numbers of fungi represent hyphal pieces. Some pieces of hypha extended over the field of view whilst others ranged from 2 to 60  $\mu\text{m}$  in length. Rather than introduce further errors in estimating fungal mass it was decided to present numbers of pieces of fungal hypha, irrespective of size.

At zero time the soil contained  $4 \times 10^6$  hyphal pieces per gram soil and  $2 \times 10^8$  bacteria per gram soil. Although both groups of population were growing steadily during the first few days of incubation, growth of fungi was faster than the bacteria. By the 4th day of incubation numbers of hyphal pieces had reached a maximum of  $21 \times 10^6/\text{g}$  of soil and thereafter started to decline. Thus, by the 4th day the fungal mass had increased five-fold whereas bacterial numbers had increased by a factor of two only ( $4 \times 10^8/\text{g}$  soil). Subsequently, bacterial numbers increased to  $8 \times 10^8$  by the 7th day of incubation by which time the number of hyphal fragments had decreased to  $16 \times 10^6$  per g of soil. From day 7 fungal mass (in the form of hyphal pieces) disappeared

Fig. 38. Microbial populations developing during incubation of uniformly labelled  $^{14}\text{C}$ -glucose.

Soil: Urrbrae fine sandy loam

FIG. 38



rapidly, and only  $10 \times 10^6$  hyphal fragments per gram of soil were present at the 11th day and 14th day of incubation.

By the 14th day of incubation numbers of bacteria had reached  $13 \times 10^8$ /g soil.

The period of maximum development of the fungi corresponded to the maximum release of  $^{14}\text{CO}_2$  from most of the samples studied (2nd to 6th day of incubation). The 7th day of incubation was a period of low release of  $^{14}\text{CO}_2$ , probably due to the disappearance of all glucose and intensive competition between bacteria and fungi.

The flush of  $^{14}\text{CO}_2$  on the 8th and 9th days of incubation is thought to be due to the utilization by bacteria of protoplasmic or other readily available components of fungal hyphae. Hyphal degradation was noticed at this stage of the incubation and it is considered that most of the hyphal fragments were dead or inactive due to lack of an energy source.

This pattern of successive development of micro-organisms during incubation has been observed for different forms of substrates (Jensen, 1936; Tribe, 1961; Shields, 1972). Thus, the same interpretation is thought to account for the second peak of  $^{14}\text{CO}_2$  release for both starch and glucose amended samples in sterile and non-sterile soils.

In the incubation studies using specific organisms in Chapter VII, it was shown that there was no second peak of  $^{14}\text{CO}_2$  release in samples except for some control samples which were contaminated with other organisms. For example, during incubation of  $^{14}\text{C}$ -glucose in the fine sandy loam, there was a rise in release of  $^{14}\text{CO}_2$  when samples inoculated with bacteria were contaminated with Trichoderma sp. This is further

evidence which indicates that the second peak of  $^{14}\text{CO}_2$  production is due to the metabolism of components of a primary population by a subsequent or secondary microbial population. This contamination showed that the second flush of  $\text{CO}_2$  is not necessarily due to bacterial decomposition of fungal products as the reverse situation may also occur. The work of Chapter VII showed that the bacteria were three times as active in the grey clay as in the fine sandy loam and were as efficient as the fungi in utilizing the glucose. Thus, the sequence of microbial populations is not necessarily fungi followed by bacteria but may well be one species of organism followed by another, depending on both the soil and the substrate.

It is clear from the incubations of glucose in the fine sandy loam with inoculation of specific organisms (Chapter VII) that the first peak of  $^{14}\text{CO}_2$  release was due to fungi, but only with a mixed population does a second peak of release occur.

## 2. Carbon assimilation by micro-organisms

The efficiency of conversion of carbon into microbial tissues (carbon assimilation) by a mixed population grown in culture on simple organic substrates has been found to be  $60 \pm 5\%$  (Payne, 1970). Shields et al. (1973) obtained a similar value for mixed populations in a soil amended with  $^{14}\text{C}$ -glucose in the field. Ladd and Paul (1973) reported that during laboratory incubation of  $^{14}\text{C}$ -glucose in soil 96% of the substrate was metabolised in 1.5 days with 31% of the organic glucose C evolved as  $^{14}\text{CO}_2$  and 65% was assumed to remain in microbial biomass or metabolites.

Similarly, an efficiency of conversion of organic carbon into cell components of aerobically growing organisms of  $60 \pm 5\%$  has been reported for soil micro-organisms utilizing a wide range of substrates (Mayberry et al., 1967; Mackechnie and Dawes, 1969). Even with complex substrates (e.g. skim milk powder, casein, lactose) the micro-organisms in activated sludge have been found to oxidize 32 to 43% of the carbon and the remainder was assumed to have been incorporated into cell components (Hoover et al., 1951). Thus, 60% efficiency of carbon conversion into microbial components could be justified for both glucose and starch. On the other hand, an efficiency of conversion of 39% was recently obtained during laboratory incubation of glucose in soil (Behera and Wagner, 1974). Since these workers (Behera and Wagner) reported that the glucose was utilized mainly by bacteria there may be differences due to the dominant organisms present.

One way of estimating the efficiency of conversion during incubation of organic substrates in soil in the laboratory is to base it on evolved  $\text{CO}_2$  and assume that at the point of greatest change in slope of the  $\text{CO}_2$  release curve all the residual C in the soil is in microbial tissue or their metabolites (Jansson, 1960; Persson, 1968; Wagner, 1968; Sorensen and Paul, 1971). On this basis the  $^{14}\text{CO}_2$  release curves for the control samples in the present studies could be used to evaluate the efficiency of conversion of the  $^{14}\text{C}$  into microbial tissue and metabolites (there is no means of differentiating between the two in soil). The mean value of  $^{14}\text{CO}_2$  released on the linear portion of the curves (Figs. 14B, 15B, 16B, 17B, control samples) was about 42% of the original  $^{14}\text{C}$  incorporated into the soil. This gives an efficiency close to 60%

which agrees with the values obtained by Shields et al. (1973) and Ladd and Paul (1973).

Ladd and Paul (1973) observed that 1.5 days was the time taken for utilization of glucose by micro-organisms, and about 65% was assumed to be converted into microbial tissues and metabolites, during incubation of  $^{14}\text{C}$ -glucose in soil. Similarly, conversion of glucose into microbial tissue was achieved within 2 days during recent incubation studies in the laboratory (Behera and Wagner, 1974).

The time taken for each sample to release 40% of the original  $^{14}\text{C}$ -glucose as  $^{14}\text{CO}_2$  has been summarized in Table 24. The figures are rounded off to the nearest day.

For samples containing glucose (except the 1 to 2 mm samples of the red clay) over 40% of the initial  $^{14}\text{C}$  as  $^{14}\text{CO}_2$  was released by the 7th day of incubation, and it seems reasonable to assume that release of  $^{14}\text{CO}_2$  after the 7th day was the result of utilization of microbial tissue or their metabolites. This fits well with the low release of  $^{14}\text{CO}_2$  on the 7th day of incubations in all cases, and the suggestion that  $^{14}\text{CO}_2$  released on the 8th and 9th day of the incubations and thereafter emanates from microbial products.

With starch as a substrate in aggregates 40% release of the  $^{14}\text{C}$  was not reached until well after 7 days of incubation. This indicates that physical factors influenced the rate of utilization of the starch and protected some of it from microbial attack. Under these conditions some  $^{14}\text{CO}_2$  was released from microbial products before all the starch was utilized. Some of the  $^{14}\text{C}$  released has probably been through two

Table 24. Time taken for release of 40% of initial  $^{14}\text{C}$  as  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -glucose

| Size of aggregates<br>in mm | Days              |              |            |           |
|-----------------------------|-------------------|--------------|------------|-----------|
|                             | Fine sandy loam   |              | Clay soils |           |
|                             | Permanent pasture | Wheat fallow | Red clay   | Grey clay |
| 1 to 2                      | 5                 | 5            | 8          | 3         |
| 0.124 to<br>0.250           | 5                 | 5            | 6          | 4         |
| < 0.053                     | 4                 | 4            | 7          | 5         |
| Control                     | 5                 | 5            | 6          | 5         |

populations of organisms before 40% of the  $^{14}\text{C}$  was released.

It can be concluded that in the fine sandy loam the release of  $^{14}\text{CO}_2$  was initially predominantly due to a fungal population which flourished for several days, until the energy source was exhausted. This first flush of  $^{14}\text{CO}_2$  represented about 40% of the initial  $^{14}\text{C}$  added in agreement with an efficiency of conversion of carbon close to 60%. The declining fungal population suffered mycolysis and the readily available components acted as a substrate for a predominantly bacterial population which released a second minor flush of  $^{14}\text{CO}_2$  before all the remaining materials became relatively resistant to further microbial attack.

CHAPTER IX. GENERAL DISCUSSION

The aims of the thesis were (a) to evaluate the influence of soil structure on microbial activity with particular reference to degradation of carbohydrates; (b) to determine the importance of physical treatments such as drying and wetting cycles and disruption of aggregates on the metabolism of carbohydrates present in aggregates, and (c) to assess the relative importance of bacteria and fungi in metabolism of carbohydrates present in soil aggregates.

The carbohydrates used as substrates were labelled with  $^{14}\text{C}$  and the most important analytical procedures used in the thesis were counting of  $^{14}\text{CO}_2$  in alkaline solution and the counting of  $^{14}\text{C}$  in soil.

Method of counting  $^{14}\text{C}$  in soil

The counting of  $^{14}\text{CO}_2$  in NaOH was done by rendering a small volume of the alkali miscible with the toluene-PPO-dimethyl POPOP scintillant using Triton X-100. However, problems were encountered with quantitative oxidation of carbon in soils and considerable effort was expended in developing the method for liquid scintillation counting of  $^{14}\text{C}$  in finely ground soil suspensions. The method developed in Chapter III should prove useful in future studies of soil organic matter.

The most important factors which affect the efficiency of the method are (a) self-absorption of the  $^{14}\text{C}$   $\beta$ -particles which is governed by the size of the soil particles and (b) colour quenching. It is essential that samples be ground to less than about  $< 53 \mu\text{m}$ . Using the

weight of material ( $\text{Ba}^{14}\text{CO}_3$ ) which yields a layer of infinite thickness, that is about  $30 \text{ mg/cm}^2$  on an aluminium planchet (Calvin et al., 1949) it was calculated that the critical diameter of soil particles is in the range of 60 to 120  $\mu\text{m}$  to cover the density range 2.5 to  $5 \text{ g/cm}^2$ . Activities for up to 25 mg of sample per vial of  $^{14}\text{C}$  tagged red brown earth of 53 to 250  $\mu\text{m}$  particle size was about 60% of the activities obtained for the same amount of  $< 53 \mu\text{m}$  sample per vial. The recovery of activity decreased with increase in particle size even with  $^{14}\text{C}$ -labelled plant material and shows the importance of self-absorption in suspension counting of  $^{14}\text{C}$ -labelled materials. Thus, for successful application of the method to counting any  $^{14}\text{C}$ -labelled solid material the particle size should first be considered.

Acid or alkaline extracts of soil must be neutralized before freeze-drying and grinding otherwise incomplete drying occurs. The grinding is then incomplete often resulting in flakes, which causes self-absorption which cannot be corrected for.

Colour of the sample is the other major factor since colour quenching is one of the most serious problems in liquid scintillation spectrometry. The colour also limits the weight of sample per vial that can be counted to achieve total recovery of activity. Thus, for weakly coloured samples such as acid washed sand tagged with  $^{14}\text{C}$  up to 100 mg of sample can be counted. The high quenching characteristics encountered with the goethite and haematite shows that when  $^{14}\text{C}$  is present in soils dominated by sesquioxides e.g. lateritic soils and krasnozems, counting is unlikely to yield quantitative recoveries. On the other hand,  $^{14}\text{C}$  present in soils such as the grey clay may be

counted quantitatively. The optical density (1 cm) of the gel at 450 nm must be less than 0.9 for quantitative recoveries of activity (Adu and Oades, 1973). This critical optical density determines what weight of sample per vial may be used if quantitative recoveries of counts are to be obtained.

Although the method of suspension counting of  $^{14}\text{C}$ -labelled solid particles has been used successfully in this thesis, and shows that liquid scintillation counting of  $^{14}\text{C}$  in insoluble solid samples or freeze-dried coloured extracts is possible, there is still room for improvement of the method. Neutralized acid and alkaline extracts of soils which are difficult to grind can be suspended in the scintillant and subjected to ultrasonic dispersion before addition of the thixotropic gel. The time needed to achieve complete dispersion of the particles must be determined for each sample. Highly coloured (e.g. red and yellow) samples can be diluted with weakly coloured unlabelled materials such as acid washed sand, but this lowers the activity.

It is possible that other scintillants such as toluene containing 10 g per litre of PBD [2-Phenyl-5-(4-biphenyl)-1,3,4-oxadiazole] may prove to be a better scintillant than PPO and dimethyl POPOP, particularly with samples containing impurity quenchers (Birks, 1973). The application of such a scintillant to counting  $^{14}\text{C}$ -labelled suspensions could prove useful.

#### Influence of soil aggregates on microbial activity

##### Distribution of substrates and micro-organisms in samples

The influence of soil structure on the metabolism of carbohydrates was more complex than expected. With the fine sandy loam the rate of

release of  $^{14}\text{CO}_2$  from unsterilized samples (the 1.5 mm aggregates excluded) was more rapid than from corresponding aggregate sizes of the sterilized samples. This difference was associated with the distribution of micro-organisms involved in metabolism of the introduced substrates.

One of the major factors influencing the degradation of organic materials in soil is the distribution of both the organic substrates and the soil micro-organisms. Alexander (1961) has estimated that, theoretically, a population of  $10^8$  bacteria per g soil would occupy only 0.01 per cent of the total volume of soil. On the assumption that an average bacterium measures  $1 \times 0.8 \mu$  the percentage of the surface area of sand grains colonized by  $1.85 \times 10^6$  bacteria/g of soil has been estimated to be only 0.02 per cent in an  $A_1$  horizon (Gray *et al.*, 1968). Sands and Rovira (1971) observed that there was a heterogeneous distribution of fluorescent pseudomonads through soil, and suggested that they probably occur as discrete colonies associated with particulate organic matter. These observations indicate that micro-organisms cover a very small proportion of the surfaces in soils.

Except for soluble organic substrates the greater proportion of organic material in soil is unevenly distributed. Thus, organic materials are normally inaccessible to attack, particularly by bacteria. Although fungi can grow through the soil (as opposed to bacteria) their growth is more often than not limited by the narrow diameters of micropores in the soil particularly in aggregates (Quirk and Panabokke, 1962) where most of the organic materials may be secluded.

The higher rate of release of  $^{14}\text{CO}_2$  from non-sterile fine sandy loam samples as opposed to the corresponding sterile samples shows the importance of distribution of micro-organisms within the soil aggregates. In the non-sterile samples micro-organisms would be distributed within micropores and macropores of the aggregates so that the organic substrates were attacked from both within and outside the aggregates. In sterile samples however, organisms were introduced into macropores only and would have to move towards the substrate or vice versa resulting in the possibilities of enzyme sorption (Skujins, 1967) and limitations in microbial movement because of narrow pore sizes (Griffin, 1972).

#### The influence of aggregates

With few exceptions the rate of release of  $^{14}\text{CO}_2$  from either glucose or starch amended samples was always higher from control samples than from aggregate samples. Control samples of both starch and glucose amended samples behaved similarly which indicated that the chemistry of the substrate was not a major factor controlling the metabolism of the two substrates used.

The main difference between the control samples and the aggregate samples was the distribution of the substrates. In the control samples substrates were within macropores into which organisms were introduced, allowing rapid and complete interaction between substrate and microbial enzymes. With aggregate samples on the other hand, substrates were distributed within micropores and macropores. Thus, some substrate was physically inaccessible in small pores, especially in larger aggregates.

Recent studies on microbial decomposition of chitin as influenced by glass beads, have shown that inorganic particles (and for that matter aggregates) in soil may serve as an effective barrier to microbial colonization of a particulate substrate (Ou and Alexander, 1974). The  $^{14}\text{C}$ -starch was protected from enzymes by a similar mechanism and both results show the necessity for proximity of an organic substrate to micro-organisms if the organism is to depend on such substrate for energy, growth and maintenance.

#### Type of soil

The microbial metabolism of carbohydrates as influenced by aggregate sizes differed in the soils studied and it is not possible to make a simple statement on the effect of aggregate size.

#### Fine sandy loam

The results obtained from studies with the fine sandy loam do not support the statement that influence of aggregate sizes on decomposition of soil organic matter is best observed in clay soils (Craswell and Waring, 1972a). With fine sandy loam samples (< 20% clay) more  $^{14}\text{CO}_2$  was released from control samples than from aggregate samples whether glucose or starch amended. With the odd exception, the release of  $^{14}\text{CO}_2$  from non-sterile samples (of the fine sandy loam) was inversely related to the size of aggregates.

The higher rate of release obtained from smaller aggregates of the fine sandy loam amended with glucose showed that aggregate size is an important factor in metabolism of soluble, easily decomposable samples. Diffusion becomes a major factor during metabolism of such simple mobile

substrates and the smaller the size of aggregates the shorter the path length for diffusion. With bigger aggregates the rate of release of CO<sub>2</sub> was slower because of tortuosity and increased path length for diffusion. This factor affects both the gas exchange as well as diffusion of the soluble substrates to points of low concentration.

The release of <sup>14</sup>CO<sub>2</sub> from starch amended sterile aggregates of the fine sandy loam (particularly the wheat fallow) was marginally higher from the larger aggregates than from smaller aggregates. The larger aggregates presumably favoured growth of micro-organisms (particularly fungi) more than the smaller aggregates.

The rate of release of CO<sub>2</sub> or rate of oxygen uptake during incubation of glucose in different sizes of glass microbeads (37 μm and 149 μm) was higher from the smaller size beads when samples were inoculated with either soil suspension or with only Bacillus subtilis (Parr and Norman, 1964: Parr et al., 1967). When samples were inoculated with either only Trichoderma viride or Aspergillus terreus, CO<sub>2</sub> released was higher from the larger microbeads.

Micro-organisms growing in the larger aggregates (particularly during the initial stages of incubation) in the present studies were probably dominated by fungi. This agrees well with the finding that fungi were the major component of microbial population which initially developed during incubation of glucose in the fine sandy loam (Chapter VIII). The dominance of the fungi may have been enhanced in larger aggregate samples.

### Clay soils

The clay soils behaved differently from the fine sandy loam and from each other. The differences between the two clays could not be attributed to their clay content. The red clay is mainly illite and kaolinite with little interstratified material. On the other hand the grey clay is mainly illite and randomly interstratified material with very little kaolinite. Both have over 50% of clay ( $< 2 \mu\text{m}$ ) but there is presence of calcium carbonate in the grey clay. However, the self-mulching characteristics of the grey clay is lacking in the red clay and may account for the differences in behaviour of the clays.

The results show that metabolism of glucose in the clay soils was similar. However, differences existed between the two clays in the starch amended aggregate samples.

With the red clay release of  $^{14}\text{CO}_2$  from starch amended aggregates was highest from 124 to 250  $\mu\text{m}$  and lowest for both 1 to 2 mm and  $< 53 \mu\text{m}$  samples. With aggregates 250  $\mu\text{m}$  in diameter and larger, substrates would be protected from attack by microbial enzymes whilst below 124  $\mu\text{m}$  the swelling of the clay particles on wetting decreased air-filled porosity and prevented efficient gas exchange. It is likely that macromolecules in aggregates of this type of soil will persist, unless the soil is subjected to physical disturbance.

On the other hand, the higher rate of release of  $^{14}\text{CO}_2$  from starch amended aggregates of the grey clay (even for the smallest aggregates compared with the fine sandy loam or the red clay) show that with a soil with a self-mulching characteristic most organic materials are easily

accessible. This may account for the low organic carbon content (1.6%) in this soil, a figure which is inconsistent with a calcareous soil of high clay content and reasonable rainfall (410 mm/annum).

#### Physical treatment

Carbohydrates can be protected in soil aggregates from attack by microbial enzymes and a treatment such as drying and wetting or disruption of the aggregates can expose such substrates to microbial attack. The results obtained from the drying and wetting treatments and shaking treatments showed that physical factors (such as disruption of aggregates) need to be considered in addition to chemical and biological factors in attempts to explain the flush of microbial activity caused by a drying and wetting cycle.

The low activities of fungi in the fine sandy loam during summer had been attributed to death of the fungi due to the high temperatures which reduce the water content to as low as 1.6 to 2.0% i.e. well below the wilting point (6.7%) of the soil (Warcup, 1957). The increase in activity at the onset of the seasonal rains has been related to the revival or germination of resting spores which survived the summer. The results obtained in this thesis show that the increase in activity could be due to exposure of substrates which were previously protected in soil aggregates.

In tropical areas with long dry periods alternating with two or more rainy seasons, the process of drying and wetting would be a major factor in decreasing the soil organic carbon content.

Bacteria versus fungi in metabolism of organic substrates in soil aggregates

Determination of the contribution of bacteria and fungi to the metabolism of organic substrates is difficult because of the wide range of organisms involved. Even within the same species differences exist between strains. In soil bacteria and fungi do not exist in isolation. Competition exists between the two groups of organisms and one group of organisms may be more active than the other at a particular time. Actinomycetes are also involved in degradation of organic materials in soil.

The results of Chapter VII show that some organic substrates may exist in inaccessible microsites. This effect would differ with the type of soil. Fungi were found to be more active in metabolising  $^{14}\text{C}$ -labelled substrates in aggregates of the fine sandy loam. However, with substrates present in macropores bacteria were equally active. In the grey clay bacteria were more active than fungi. The variability in  $^{14}\text{CO}_2$  released from samples inoculated with specific organisms, and the differences between soils, indicate that further studies using more organisms as well as soils with different characteristics could prove useful.

$^{14}\text{CO}_2$  release curves

The two main peaks obtained during the incubation of the  $^{14}\text{C}$ -labelled substrates with samples inoculated with soil suspension have been attributed to development of different groups of organisms. It is evident from the results in Chapter VIII, that this was the case

though the groups of organisms dominant at a particular period would again depend on the type of the soil. With the fine sandy loam fungi were the first group of organisms developing as evident from the results of Chapters VII and VIII. With the clay soils, bacteria presumably were the first to develop. Since no microbial counts were carried out on the clay samples the successive development of different microbial populations during the incubation can only be inferred. Results in Chapter VII indicate that with the grey clay, bacteria were probably the dominant organisms throughout the incubation.

Suggestions for further research on effect of structure on metabolism of carbohydrates

The preparation of aggregates, particularly for the unsterilized samples was too artificial and there was no control on the mechanical forces involved. This might have led to the production of aggregates with different porosities and pore sizes. It would be useful to prepare aggregates with controlled pore size and porosity as well as diameter. If this was possible then the results obtained would be more reliable.

More labelled substrates (e.g. particulate <sup>14</sup>C-labelled materials such as plant material) could be used instead of soluble materials such as glucose. The incorporation of such substrates into aggregates would give an idea of the persistence of litter which has been incorporated into soil aggregates.

Results obtained from laboratory studies are often criticised for being too artificial. The studies carried out in this thesis could be extended to the field. Incorporation of the <sup>14</sup>C-labelled substrates

(e.g. plant material) could be prepared as carried out in Chapter V, and the incubation carried out in microplots in the field. This would give an idea of the rate of disappearance of organic substrates in soil aggregates in the field.

An aspect of the project requiring further investigation is the effect of the physical treatments (i.e. drying and wetting and disruption of aggregates).

APPENDIX 1

SUSPENSION COUNTING OF  $^{14}\text{C}$  IN SOIL, SOIL EXTRACTS AND  
PLANT MATERIALS BY LIQUID SCINTILLATION

by J.K. Adu and J.M. Oades

Adu, J. K. & Oades, J. K. (1974). Suspension counting of  $^{14}\text{C}$  in soil, soil extracts and plant materials by liquid scintillation. In P. E. Stanley & B. A. Scoggins (Eds.) *Liquid scintillation counting: recent developments*, (pp. 207-221). New York, Academic Press.

NOTE:

This publication is included in the print copy  
of the thesis held in the University of Adelaide Library.



APPENDIX 2SOILS USED IN THIS PROJECT

Some relevant analytical data are given in Table 25.

| <u>SOILS</u>                                      | <u>DESCRIPTION</u>   |
|---|--|
| 1) Urrbrae fine sandy loam<br>(permanent pasture) | Red brown earth from a plot which has been under pasture for over 20 years (continuously under pasture since 1950) at the Waite Agricultural Research Institute.<br>Depth 0-10 cm.<br>Reference: Piper, 1938 and Stephens, 1962. |
| 2) Urrbrae fine sandy loam<br>(wheat-fallow)      | As above, but under cultivation in an alternate wheat-fallow rotation for over 45 years (maintained since 1926) at the Waite Agricultural Research Institute field trials.<br>Depth 0-10 cm.                                     |
| 3) Red brown earth<br>'B' horizon (red clay)      | Subsurface soil of Urrbrae fine sandy loam.<br>Depth 35-50 cm.   |
| 4) Grey clay                                      | This is a self-mulching clay with a little soft carbonate from Longerenong, Agricultural College, Horsham, Victoria.<br>Depth 0-10 cm.<br>Reference: Stace <u>et al.</u> , 1968)   |

Table 25. Analytical data of soils used

| Soil                                    | Depth<br>(cm) | pH 1.5<br>(H <sub>2</sub> O) | Inorg. C<br>% | Org. C<br>% | N<br>% | Particle size |         |         |         |
|---|---------------|------------------------------|---------------|-------------|--------|---------------|---------|---------|---------|
|   |               |                              |               |             |        | CS<br>%       | FS<br>% | Si<br>% | Cl<br>% |
| 1) Urrbrae<br>(permanent<br>pasture)    | 0-10          | 6.0                          | -             | 2.65        | 0.22   | 2             | 44      | 35      | 18      |
| 2) Urrbrae<br>(wheat-fallow)            | 0-10          | 5.8                          | -             | 1.04        | 0.08   | 19            | 34      | 13      | 17      |
| 3) Urrbrae<br>'B' horizon<br>(red clay) | 35-50         | 6.8                          | -             | 0.74        | 0.089  | 1             | 19      | 19      | 60      |
| 4) Grey clay                            | 0-10          | 7.6                          | 0.18          | 1.62        | 0.085  | 13            | 18      | 8       | 56      |

APPENDIX 3Stabilization of remoulded soil aggregates by  
incorporation of starch1) Introduction

To avoid breakdown of the artificially prepared aggregates during the wetting (in preparation for incubation) it was necessary to stabilize them by addition of a suitable macromolecule. Starch was chosen because it was one of the  $^{14}\text{C}$ -labelled substrates being used and there is good evidence to demonstrate the effectiveness of various polysaccharides as aggregate stabilizing agents (Martin et al., 1965; Harris et al., 1966; Swincer et al., 1968).

2) Preparation of aggregates

Soluble starch initially dissolved in hot water was added to <250  $\mu\text{m}$  portions of the soil to give samples containing from 0.25% to 2% by weight of starch. Aggregates (3 mm) were prepared from the amended samples and used for the following experiments.

3) Stability of aggregates

The water droplet method of McCalla (1944) to determine the stability of aggregates was found inappropriate since too many drops were required to disintegrate the aggregates. Thus, the wet sieving technique of Geoghegan and Brian (1948) as described by Jones and Griffith (1967) was employed.

Samples (10 g) of remoulded aggregates were placed in 150 ml of

water in a 250 ml cylinder. The cylinder was inverted once, allowed to stand for 30 min and then inverted 30 times within a period of one min. The contents were transferred to a 60 mesh sieve standing in a shallow bowl of water. The sieve was moved up and down 30 times and the water allowed to drain out completely each time. The contents of the sieve were then evaporated to dryness at 105°C and the weight of water stable aggregates (>0.25 mm) expressed as a percentage of original 10 g sample.

#### 4) Results

The results are presented with the average for duplicate samples (fig. 39). As expected, the stability of aggregates prepared from soils under permanent pasture without starch were higher than the corresponding samples from the wheat-fallow soils.

Because the stability of the untreated wheat-fallow aggregates was low there was a greater response to the addition of starch. Addition of 0.25% by weight of starch resulted in 60 to 80% of the aggregates remaining >0.25 mm after the sieving procedure. The addition of starch at this level was not so effective in aggregates from the soil under pasture and greater stability of aggregates from both soils was obtained with large additions of starch.

Although addition of 2% starch by weight gave higher percentages of stable aggregates, it was decided to add only 1.5% by weight to avoid addition of too much carbon to the samples.

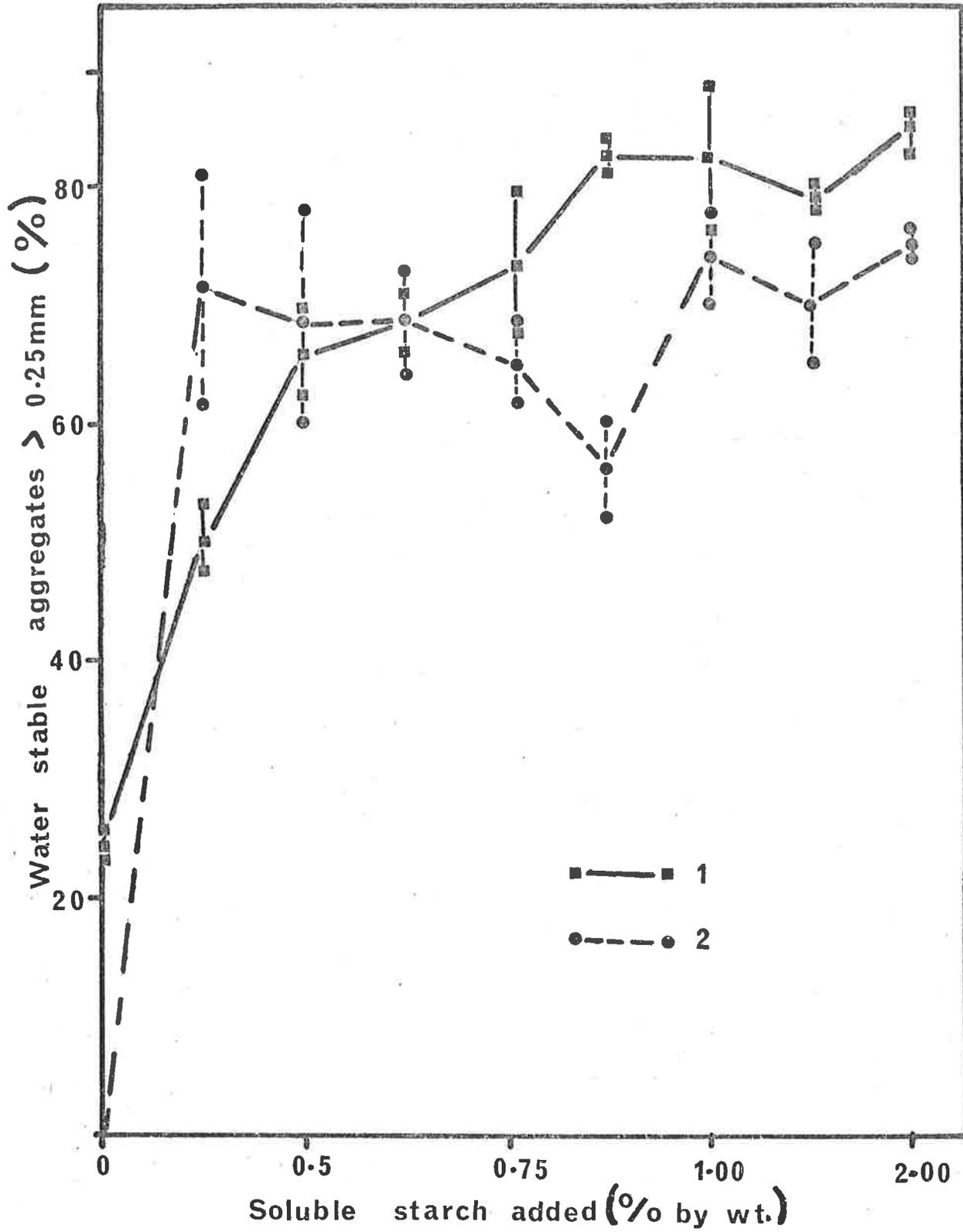
Fig. 39. Effect of soluble starch on stability of remoulded soil aggregates.

Soil: Urrbrae fine sandy loam

1. Permanent pasture samples
2. Wheat-fallow samples

┆ represents range of duplicate determinations

FIG 39



APPENDIX 4Effect of water content on release of  $^{14}\text{CO}_2$  during  
incubation of  $^{14}\text{C}$ -glucose in soils1) Introduction

Microbial activities in soil are governed by a number of factors and water content plays an important role. Adequate water contents give optimum microbial growth but excess reduces aerobic microbial activity by retarding gaseous diffusion, thus reducing the oxygen supply. The respiration of the soil microflora utilizing organic substrates is usually greatest when the water holding capacity of the soil between 60 and 80% (Alexander, 1961).

Although such water contents are recommended in the literature they vary from soil to soil and it was considered necessary to establish the optimum water content of the soils for incubation of glucose.

2) Materials

Representative samples (<250 $\mu\text{m}$ ) of air dry Urrbrae fine sandy loam (0-10 cm) collected from both permanent pasture and wheat-fallow plots were used.

Uniformly labelled  $^{14}\text{C}$ -glucose was used as substrates.

3) Method

Samples (30 g) were weighed into eight 250 ml incubation flasks. Glucose solution (50 mg with 0.2  $\mu\text{Ci}$   $^{14}\text{C}$ ) was added to each flask in a volume of water calculated to give duplicate samples with 40%, 60%, 80% and

100% of the water holding capacity of the soil respectively.

Samples were inoculated with 1 ml of a 1% suspension of fresh soil and incubated for 21 days in the dark at  $28 \pm 2^\circ\text{C}$ . NaOH (0.1 N) was used as an absorbing agent for  $^{14}\text{CO}_2$  released and  $^{14}\text{C}$  in  $\text{CO}_2$  released was determined by liquid scintillation spectrometry using Triton X-100 scintillant (Turner, 1968).

#### 4) Results

The results (figs. 40 and 41) show that maximum release of  $^{14}\text{CO}_2$  was obtained from the pasture soil wetted to 60 to 100% water holding capacity but with the wheat-fallow soil maximum release was obtained from the sample wetted to 100% of the water holding capacity.

In both soils, the release of  $^{14}\text{CO}_2$  from samples wetted to 40% of water holding capacity was low. The anomalous increase in the rate of release of  $^{14}\text{CO}_2$  from the wheat-fallow sample after 11 days incubation was due to the condensation on the walls of the flask which drained onto the soil.

The results show that for the permanent pasture sample, a water content of 60 to 100% of the water holding capacity could have been used to obtain optimum release of  $^{14}\text{CO}_2$ . With the wheat-fallow, wetting the soil to 100% water holding capacity gave the greatest release of  $^{14}\text{CO}_2$ . Similar release of  $^{14}\text{CO}_2$  was obtained from both soils wetted to 60 and 80% of the water holding capacity which agrees with the results obtained by Katznelson and Stevenson (1956).

It was thought that despite the high release of  $^{14}\text{CO}_2$  from the samples wetted to 100% water holding capacity in other soils, e.g.

Fig. 40. Effect of water content on release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -glucose in soils.

Soil: Urrbrae fine sandy loam

Plot: Permanent pasture

1. 100% water holding capacity

2. 80% " " "

3. 60% " " "

4. 40% " " "

FIG 40

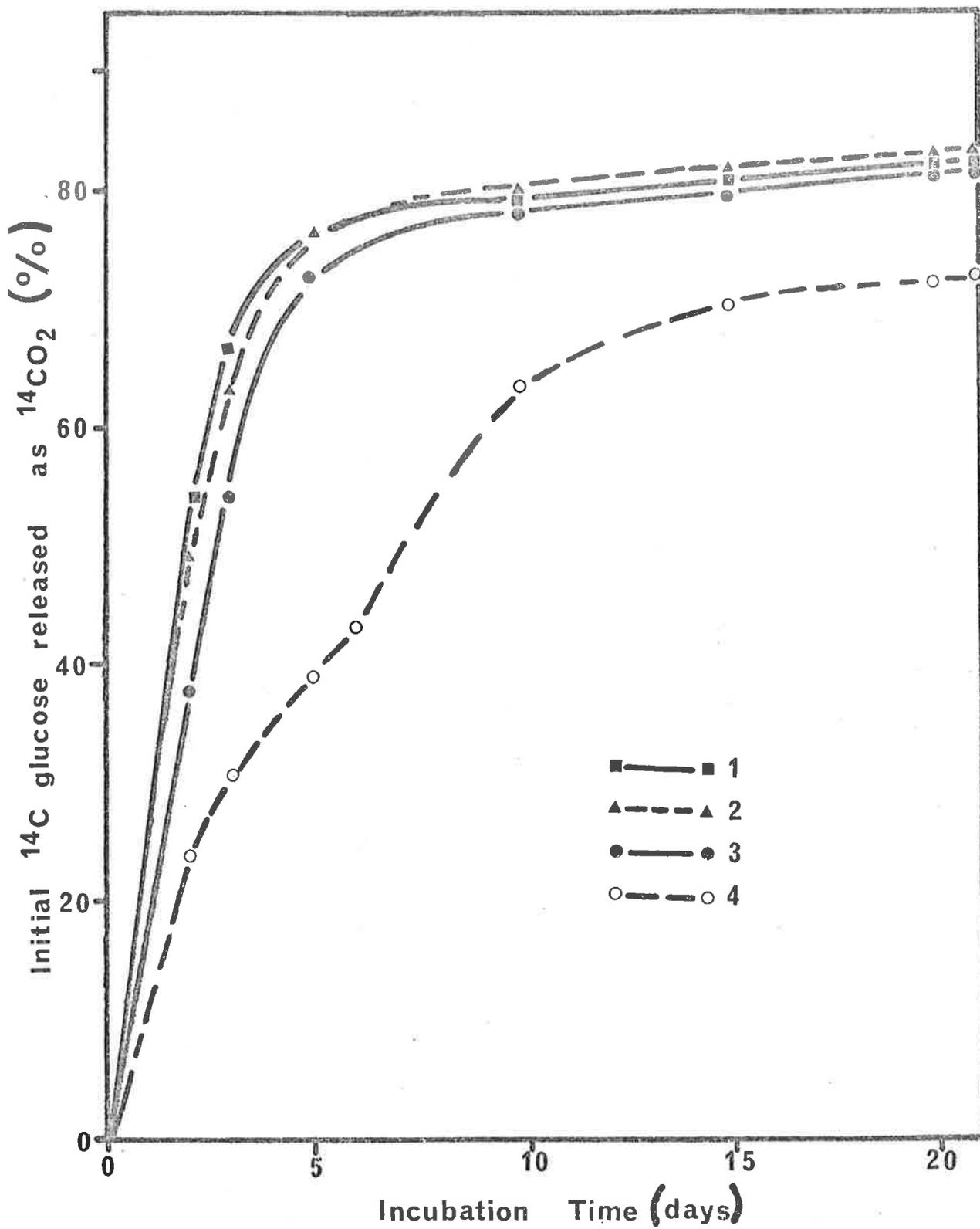


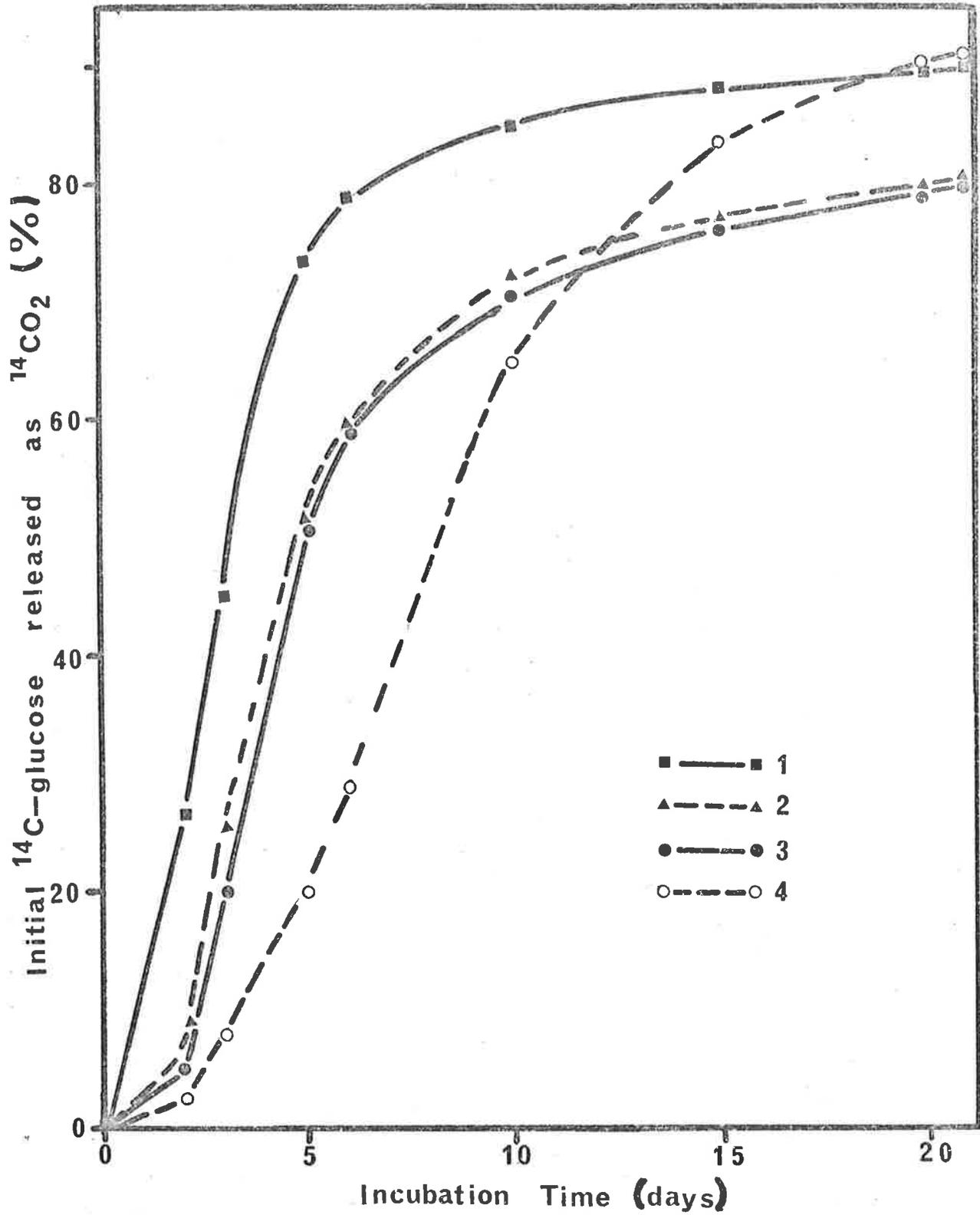
Fig. 41. Effect of water content on release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -glucose in soils.

Soil: Urrbrae fine sandy loam

Plot: Wheat-fallow

1. 100% water holding capacity
2. 80% " " "
3. 60% " " "
4. 40% " " "

FIG 41



heavy clay soils, such water contents would result in anaerobic respiration. Since soils wetted to 80% of the water holding capacity showed release of about 80% of the initial  $^{14}\text{C}$  by the 21st day of incubation it was decided to standardize on this water content for the incubation studies in this thesis.

APPENDIX 5Determination of iron in ferric sulphate (Monsel's salt)[ $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$ ] using the thioglycollic acid method

A known weight was dissolved in water and a convenient aliquot of the test solution was added to a 100 ml graduated flask. A piece of litmus paper was added to the solution and 2 N ammonia solution were added until the litmus turned blue. A few drops of 2 N HCl were then added until the litmus just turned red. The following reagents were added in succession:

5 ml 20% w/v citric acid,

2 ml aqueous solution of thioglycollic acid, and

10 ml 10% ammonia.

The solution was shaken and made up to 100 ml with distilled water.

A solution of ferrous ammonium sulphate was used for preparing a standard and then extinction of the solution measured at 540 nm.

APPENDIX 6Regression equations for  $^{14}\text{CO}_2$  evolution curves

The method of calculation has been presented in Chapter IV, Section 3.1.2.

Y = cumulative  $^{14}\text{CO}_2$  released (as percentage initial  $^{14}\text{C}$ ).

x = days of incubation.

Chapter IV

Equations have been arranged in the following order for the aggregate sizes:

- (a) 5 mm      (b) 3 mm      (c) 1.5 mm      (d) <250  $\mu\text{m}$   
 (e) <53  $\mu\text{m}$       (f) control.

(A)  $^{14}\text{C}$ -glucose amended samples(i) Permanent pasture samples

- (a)  $Y = 33.6450 + 1.95605x - 0.0382x^2$   
 (b)  $Y = 44.5217 + 2.73472x - 0.06322x^2$   
 (c)  $Y = 21.87730 + 2.94259x - 0.06418x^2$   
 (d)  $Y = 41.51852 + 2.10395x - 0.04594x^2$   
 (e)  $Y = 34.62883 + 3.17377x - 0.06799x^2$   
 (f)  $Y = 50.73821 + 3.08062x - 0.07937x^2$

(ii) Wheat-fallow samples

- (a)  $Y = 28.05118 + 2.36823x - 0.05775x^2$   
 (b)  $Y = 34.60719 + 2.95594x - 0.06489x^2$   
 (c)  $Y = 22.53201 + 2.908601x - 0.06489x^2$   
 (d)  $Y = 29.47059 + 3.48221x - 0.07602x^2$   
 (e)  $Y = 29.98878 + 5.00145x - 0.12108x^2$   
 (f)  $Y = 32.25866 + 5.44943x - 0.13668x^2$

(B) <sup>14</sup>C-starch amended samples(i) Permanent pasture samples

- (a)  $Y = 17.36846 + 2.72389x - 0.05868x^2$   
 (b)  $Y = 8.9726 + 3.26822x - 0.06805x^2$   
 (c)  $Y = 20.99316 + 2.75193x - 0.06076x^2$   
 (d)  $Y = 21.20065 + 3.37190x - 0.07181x^2$   
 (e)  $Y = 14.59871 + 4.25344x - 0.08664x^2$   
 (f)  $Y = 26.7107 + 4.35356x - 0.09590x^2$

(ii) Wheat-fallow samples

- (a)  $Y = 16.87311 + 1.19590x - 0.01320x^2$   
 (b)  $Y = 10.01578 + 2.38309x - 0.04402x^2$   
 (c)  $Y = 6.435175 + 2.86161x - 0.05193x^2$   
 (d)  $Y = 22.558015 + 2.26225x - 0.04054x^2$   
 (e)  $Y = 5.536475 + 3.61497x - 0.05477x^2$   
 (f)  $Y = 14.930785 + 4.50635x - 0.07990x^2$

Chapter V

Equations have been arranged in the following order for the aggregate sizes:

(a) 1 - 2 mm      (b) 124 -250  $\mu$ m      (c) <53  $\mu$ m      (d) control

(A)  $^{14}$ C-glucose amended samples(i) Permanent pasture samples

$$(a) \quad Y = 2.1124 + 7.02521x - 0.19991x^2$$

$$(b) \quad Y = 2.71365 + 7.02521x - 0.19991x^2$$

$$(c) \quad Y = 6.98511 + 7.02521x - 0.19991x^2$$

$$(d) \quad Y = 9.39865 + 7.02521x - 0.19991x^2$$

(ii) Wheat-fallow samples

$$(a) \quad Y = -2.85293 + 8.77839x - 0.23580x^2$$

$$(b) \quad Y = 0.971198 + 8.05590x - 0.21753x^2$$

$$(c) \quad Y = 5.957704 + 7.93736x - 0.22829x^2$$

$$(d) \quad Y = -0.908515 + 8.42376x - 0.20488x^2$$

(iii) Red clay samples

$$(a) \quad Y = -20.831885 + 9.75261x - 0.23449x^2$$

$$(b) \quad Y = -11.170296 + 9.24214x - 0.22787x^2$$

$$(c) \quad Y = -14.161643 + 8.62996x - 0.21088x^2$$

$$(d) \quad Y = 1.221015 + 7.16451x - 0.16024x^2$$

(iv) Grey clay samples

(a)  $Y = 21.87941 + 7.59349x - 0.22319x^2$

(b)  $Y = 15.26912 + 6.74345x - 0.19571x^2$

(c)  $Y = -2.704561 + 7.81346x - 0.21203x^2$

(d)  $Y = 0.251543 + 9.04311x - 0.24472x^2$

(B) <sup>14</sup>C-starch amended samples(i) Permanent pasture samples

(a)  $Y = 5.699787 + 4.60182x - 0.11525x^2$

(b)  $Y = 3.192615 + 4.41798x - 0.10009x^2$

(c)  $Y = 1.739705 + 4.63574x - 0.10356x^2$

(d)  $Y = 17.513507 + 5.97201x - 0.16169x^2$

(ii) Wheat-fallow samples

(a)  $Y = -0.13450 + 5.77343x - 0.14340x^2$

(b)  $Y = -2.62056 + 4.85038x - 0.09805x^2$

(c)  $Y = -1.66315 + 3.70538x - 0.05552x^2$

(d)  $Y = 20.54667 + 4.96966x - 0.12688x^2$

(iii) Red clay samples

(a)  $Y = -1.82922 + 5.01302x - 0.11051x^2$

(b)  $Y = -0.76303 + 6.65006x - 0.16211x^2$

(c)  $Y = -2.6722 + 3.47920x - 0.05999x^2$

(d)  $Y = -3.73771 + 4.88602x - 0.12185x^2$

(iv) Grey clay samples

(a)  $Y = 14.01951 + 5.89629x - 0.15190x^2$

(b)  $Y = 15.24121 + 5.89629x - 0.15190x^2$

(c)  $Y = 9.64580 + 5.89629x - 0.15190x^2$

(d)  $Y = 45.18812 + 5.89629x - 0.15190x^2$

Chapter VII

Equations have been arranged in the following order for the different organisms:

(a) Rhizoctonia solani(b) Aureobasidiopullulans(c) Mucor hiemalis(d) Aspergillus flavus(e) Trichoderma sp.(f) Penicillium sp.(g) Pseudomonas putida(h) Bacillus subtilis(A) <sup>14</sup>C-glucose amended samples(i) Permanent pasture: aggregate samples

(a)  $Y = 6.3899 + 7.03294x - 0.28420x^2$

(b)  $Y = 1.50193 + 8.39730x - 0.32283x^2$

(c)  $Y = 1.55052 + 3.95608x - 0.12247x^2$

(d)  $Y = 7.23963 + 5.31346x - 0.20509x^2$

(e)  $Y = 5.21423 + 6.08930x - 0.20936x^2$

(f)  $Y = 5.6337 + 3.68116x - 0.07452x^2$

(g)  $Y = -3.27226 + 3.99669x - 0.10372x^2$

(h)  $Y = -1.50538 + 3.57562x - 0.11065x^2$

(ii) Grey clay: aggregate samples

(a)  $Y = -2.52312 + 10.74766x - 0.44954x^2$

(b)  $Y = -1.23478 + 10.02198x - 0.41930x^2$

(c)  $Y = 2.520605 + 9.94852x - 0.42165x^2$

(d)  $Y = 1.37694 + 10.92304x - 0.47136x^2$

(e)  $Y = -0.99438 + 11.51398x - 0.48218x^2$

(f)  $Y = -8.99949 + 9.60580x - 0.35692x^2$

(g)  $Y = 21.26431 + 6.67615x - 0.26117x^2$

(h)  $Y = 0.3606 + 11.45635x - 0.48281x^2$

(iii) Permanent pasture: control samples

(a)  $Y = -13.86806 + 12.47377x - 0.41653x^2$

(b)  $Y = -11.39370 + 11.52036x - 0.37377x^2$

(c)  $Y = -14.28007 + 13.13748x - 0.48470x^2$

(d)  $Y = 0.47501 + 9.7054x - 0.37384x^2$

(e)  $Y = 4.03611 + 6.99468x - 0.18020x^2$

(f)  $Y = -10.38243 + 8.95228x - 0.24094x^2$

(g)  $Y = -13.59325 + 9.40839x - 0.22398x^2$

(h)  $Y = 3.59947 + 9.11711x - 0.30413x^2$

(iv) Grey clay: control samples

(a)  $Y = -13.88135 + 8.57105x - 0.12466x^2$

(b)  $Y = -4.50315 + 7.76495x - 0.17926x^2$

(c)  $Y = -10.97381 + 8.95281x - 0.20844x^2$

(d)  $Y = -7.19249 + 8.36475x - 0.21847x^2$

(e)  $Y = -16.89641 + 12.48720x - 0.41458x^2$

$$(f) \quad Y = -20.33918 + 13.84966x - 0.55328x^2$$

$$(g) \quad Y = 2.16028 + 10.64833x - 0.37689x^2$$

$$(h) \quad Y = 0.589920 + 11.27841x - 0.37664x^2$$

(v) Permanent pasture: comparison of aggregate and control samples

Equations have been arranged in the following order:

- (a) aggregate samples      (b) control samples

The order of the specific organisms is the same as has been set out previously.

(1) Rhizoctonia solani

$$(a) \quad Y = 6.3899 + 7.03294x - 0.28420x^2$$

$$(b) \quad Y = -13.86805 + 12.47377x - 0.41653x^2$$

(2) Aureobasidio pullulans

$$(a) \quad Y = 1.50193 + 8.39730x - 0.32283x^2$$

$$(b) \quad Y = -11.3937 + 11.52036x - 0.37377x^2$$

(3) Mucor hiemalis

$$(a) \quad Y = 1.55052 + 3.95608x - 0.12247x^2$$

$$(b) \quad Y = -14.28007 + 13.13748x - 0.48470x^2$$

(4) Aspergillus flavus

$$(a) \quad Y = 7.23963 + 5.31346x - 0.20509x^2$$

$$(b) \quad Y = 0.47501 + 9.7054x - 0.37384x^2$$

(5) Trichoderma sp.

$$(a) \quad Y = -11.78572 + 8.08930x - 0.20936x^2$$

$$(b) \quad Y = 4.03611 + 6.99468x - 0.18020x^2$$

(6) Penicillium sp.

$$(a) \quad Y = 5.63370 + 3.68116x - 0.07452x^2$$

$$(b) \quad Y = -10.38243 + 8.95228x - 0.24094x^2$$

(7) Pseudomonas putida

$$(a) \quad Y = -3.26686 + 3.99669x - 0.10372x^2$$

$$(b) \quad Y = -13.59325 + 9.40839x - 0.22393x^2$$

(8) Bacillus subtilis

$$(a) \quad Y = -1.51731 + 3.57562x - 0.11065x^2$$

$$(b) \quad Y = 3.60056 + 9.11711x - 0.30413x^2$$

(vi) Grey clay: comparison between aggregates and control samples

(1) Rhizoctonia solani

$$(a) \quad Y = -2.52312 + 10.74766x - 0.44954x^2$$

$$(b) \quad Y = -13.88135 + 8.57105x - 0.12466x^2$$

(2) Aureobasidio pullulans

$$(a) \quad Y = -1.23425 + 10.02198x - 0.41930x^2$$

$$(b) \quad Y = 17.94060 + 7.76495x - 0.41930x^2$$

(3) Mucor hiemalis

$$(a) \quad Y = 2.52061 + 9.94852x - 0.42165x^2$$

$$(b) \quad Y = -10.97381 + 8.95281x - 0.20844x^2$$

(4) Aspergillus falvus

(a)  $Y = 1.37694 + 10.92304x - 0.47136x^2$

(b)  $Y = -7.19249 + 8.36475x - 0.21847x^2$

(5) Trichoderma sp.

(a)  $Y = -0.99438 + 11.51398x - 0.48218x^2$

(b)  $Y = -16.89641 + 12.48720x - 0.41458x^2$

(6) Penicillium sp.

(a)  $Y = -17.8552 + 11.72763x - 0.45510x^2$

(b)  $Y = -11.48176 + 11.72763x - 0.45510x^2$

(7) Pseudomonas putida

(a)  $Y = 21.26431 + 6.67615x - 0.26117x^2$

(b)  $Y = 2.16024 + 10.64833x - 0.37689x^2$

(8) Bacillus subtilis

(a)  $Y = 0.36095 + 11.45635x - 0.48281x^2$

(b)  $Y = 0.61992 + 11.27941x - 0.37664x^2$

(B) <sup>14</sup>C-starch amended samples

Equations have been arranged in the following order:

(a) Rhizoctonia solani(b) Aureobasidio pullulans(c) Mucor hiemalis(d) Aspergillus flavus(e) Trichoderma sp.(f) Penicillium sp.(g) Pseudomonas putida(h) Bacillus subtilis

(i) Permanent pasture: aggregate samples

(a)  $Y = 14.31139 + 2.49194x - 0.05750x^2$

(b)  $Y = 11.91515 + 3.28700x - 0.08047x^2$

(c)  $Y = 13.05456 + 2.70648x - 0.06212x^2$

(d)  $Y = 9.69282 + 3.26377x - 0.08187x^2$

(e)  $Y = 2.15058 + 2.11881x - 0.02593x^2$

(f)  $Y = 2.43318 + 2.47961x - 0.04661x^2$

(g)  $Y = 1.38260 + 2.60253x - 0.05834x^2$

(h)  $Y = -1.42394 + 2.51653x - 0.04751x^2$

(ii) Grey clay: aggregate samples

(a)  $Y = 6.07289 + 1.98400x - 0.03526x^2$

(b)  $Y = 1.60606 + 2.42220x - 0.04915x^2$

(c)  $Y = 3.21826 + 1.52588x - 0.017481x^2$

(d)  $Y = 0.94109 + 2.29039x - 0.03772x^2$

(e)  $Y = 0.98105 + 1.98837x - 0.03459x^2$

(f)  $Y = 1.70513 + 1.74849x - 0.02190x^2$

(g)  $Y = 1.52010 + 2.30096x - 0.04585x^2$

(h)  $Y = -1.53772 + 3.59689x - 0.08279x^2$

(iii) Permanent pasture: control samples

(a)  $Y = 5.14433 + 4.98180x - 0.11851x^2$

(b)  $Y = -0.34969 + 6.60213x - 0.18070x^2$

(c)  $Y = 18.80012 + 3.66428x - 0.09644x^2$

(d)  $Y = 13.08111 + 3.81189x - 0.09722x^2$

(e)  $Y = 4.72715 + 3.8220x - 0.08274x^2$

$$(f) \quad Y = 5.08791 + 4.90578x - 0.11592x^2$$

$$(g) \quad Y = 10.62975 + 5.00114x - 0.14235x^2$$

$$(h) \quad Y = 13.41929 + 4.75997x - 0.13257x^2$$

(iv) Grey clay: control samples

$$(a) \quad Y = 9.19320 + 4.41622x - 0.09393x^2$$

$$(b) \quad Y = 4.87392 + 4.82430x - 0.12507x^2$$

$$(c) \quad Y = 7.05800 + 4.26522x - 0.10183x^2$$

$$(d) \quad Y = 13.10979 + 4.35619x - 0.11139x^2$$

$$(e) \quad Y = 0.24966 + 4.75659x - 0.10957x^2$$

$$(f) \quad Y = 3.74974 + 4.21392x - 0.09351x^2$$

$$(g) \quad Y = 6.06836 + 4.02217x - 0.08499x^2$$

$$(h) \quad Y = 12.38196 + 4.62148x - 0.12245x^2$$

(v) Permanent pasture: comparison of aggregate and control samples

Equations have been arranged in the following order.

(a) aggregate samples      (b) control samples

(1) Rhizoctonia solani

$$(a) \quad Y = 14.31139 + 2.49194x - 0.05750x^2$$

$$(b) \quad Y = 5.14437 + 4.98180x - 0.11851x^2$$

(2) Aureobasidio pullulans

$$(a) \quad Y = 11.91515 + 3.28700x - 0.08047x^2$$

$$(b) \quad Y = -0.34969 + 6.60213x - 0.18070x^2$$

(3) Mucor hiemalis

(a)  $Y = 21.28286 + 3.1854x - 0.07928x^2$

(b)  $Y = 15.0234 + 3.1854x - 0.07928x^2$

(4) Aspergillus flavus

(a)  $Y = 7.83507 + 3.53783x - 0.08955x^2$

(b)  $Y = 14.9409 + 3.53783x - 0.08955x^2$

(5) Trichoderma sp.

(a)  $Y = 2.15018 + 2.11881x - 0.02593x^2$

(b)  $Y = 4.72715 + 3.8220x - 0.08274x^2$

(6) Penicillium sp.

(a)  $Y = 2.43318 + 2.47961x - 0.04661x^2$

(b)  $Y = -6.84873 + 4.90578x - 0.11592x^2$

(7) Pseudomonas putida

(a)  $Y = 1.38260 + 2.60253x - 0.05843x^2$

(b)  $Y = 10.62973 + 5.00114x - 0.14235x^2$

(8) Bacillus subtilis

(a)  $Y = -1.40352 + 2.51653x - 0.04761x^2$

(b)  $Y = 13.41929 + 4.75997x - 0.013257x^2$

(vi) Grey clay: comparison of aggregates and control samples(1) Rhizoctonia solani

(a)  $Y = 6.07289 + 1.9840x - 0.03526x^2$

(b)  $Y = 9.19320 + 4.41622x - 0.09393x^2$

(2) Aureobasidio pullulans

(a)  $Y = 1.60606 + 2.42220x - 0.04915x^2$

(b)  $Y = 4.85767 + 4.82430x - 0.12507x^2$

(3) Mucor hiemalis

(a)  $Y = 3.21826 + 1.52588x - 0.01748x^2$

(b)  $Y = 7.05800 + 4.26522x - 0.10183x^2$

(4) Aspergillus flavus

(a)  $Y = 0.94109 + 2.29039x - 0.03772x^2$

(b)  $Y = 13.10979 + 4.35619x - 0.11139x^2$

(5) Trichoderma sp.

(a)  $Y = 0.98105 + 1.98837x - 0.03459x^2$

(b)  $Y = 0.24966 + 4.75659x - 0.10957x^2$

(6) Penicillium sp.

(a)  $Y = 1.70513 + 1.74849x - 0.02190x^2$

(b)  $Y = 3.74974 + 4.21392x - 0.09351x^2$

(7) Pseudomonas putida

(a)  $Y = 1.5201 + 2.30096x - 0.04585x^2$

(b)  $Y = 6.06836 + 4.02217x - 0.08499x^2$

(8) Bacillus subtilis

(a)  $Y = -5.3772 + 3.59689x - 0.08279x^2$

(b)  $Y = 12.38196 + 4.62148x - 0.12245x^2$

APPENDIX 7

COMPARATIVE STUDIES OF ACTIVITIES OF SPECIFIC  
MICROORGANISMS IN SOIL AGGREGATES

(Tables 26 to 33)

Table 26. Release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -glucose within micropores and macropores of sterilized soil aggregates inoculated with different specific organisms

Fine sandy loam (permanent pasture): aggregates

| Days of incubation | Initial $^{14}\text{C}$ as $^{14}\text{CO}_2$ (%) |            |                |            |                 |            |                 |            |
|--------------------|---|------------|----------------|------------|-----------------|------------|-----------------|------------|
|                    | Aureobasidio pullulans                            |            | Mucor hiemalis |            | Trichoderma sp. |            | Penicillium sp. |            |
|                    | Rate  | Cumulative | Rate           | Cumulative | Rate            | Cumulative | Rate            | Cumulative |
| 1                  | 2.41  | 2.41       | 3.25           | 3.25       | 5.03            | 5.03       | 7.41            | 7.41       |
| 2                  | 12.08   | 14.49      | 6.40           | 9.65       | 12.86           | 17.89      | 8.54            | 15.95      |
| 3                  | 12.53   | 27.02      | 3.15           | 12.80      | 5.62            | 23.51      | 0.65            | 16.60      |
| 4                  | 10.27   | 37.29      | 3.71           | 16.51      | 6.66            | 30.17      | 1.39            | 17.99      |
| 5                  | 3.82  | 41.11      | 2.97           | 19.48      | 3.44            | 33.61      | 2.81            | 20.80      |
| 6                  | 1.62  | 42.73      | 2.01           | 21.49      | 2.00            | 35.61      | 4.06            | 24.86      |
| 7                  | 2.01  | 44.74      | 1.73           | 23.22      | 1.72            | 37.33      | 2.78            | 27.64      |
| 8                  | 2.04  | 46.78      | 1.78           | 25.00      | 2.06            | 39.39      | 3.19            | 30.83      |
| 9                  | 1.58  | 48.36      | 1.58           | 26.58      | 1.87            | 41.26      | 2.07            | 32.90      |
| 10                 | 2.03  | 50.39      | 2.14           | 28.72      | 1.93            | 43.19      | 2.06            | 34.96      |
| 11                 | 1.55  | 51.94      | 0.99           | 29.71      | 1.96            | 45.15      | 1.98            | 36.94      |
| 12                 | 1.02  | 52.96      | 1.97           | 30.78      | 2.06            | 47.21      | 2.50            | 39.44      |
| 13                 | 1.99  | 54.15      | 1.05           | 31.83      | 1.37            | 48.58      | 2.11            | 41.55      |
| 14                 | 1.46  | 55.61      | 0.94           | 32.77      | 0.71            | 49.29      | 1.10            | 42.65      |
| 15                 | 0.79  | 56.40      | 0.85           | 33.62      | 0.86            | 50.15      | 1.29            | 43.94      |
| 16                 | 0.79  | 57.19      | 0.84           | 34.46      | 0.86            | 51.01      | 1.28            | 45.22      |

Table 27. Release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -glucose within micropores and macropores of sterilized soil aggregates inoculated with different specific organisms

Grey clay: aggregates

| Days of incubation | Initial $^{14}\text{C}$ as $^{14}\text{CO}_2$ (%) |            |                |            |                 |            |                 |            |
|--------------------|---|------------|----------------|------------|-----------------|------------|-----------------|------------|
|                    | Aureobasidio pullulans                            |            | Mucor hiemalis |            | Trichoderma sp. |            | Penicillium sp. |            |
|                    | Rate  | Cumulative | Rate           | Cumulative | Rate            | Cumulative | Rate            | Cumulative |
| 1                  | 0.64  | 0.64       | 2.44           | 2.44       | 1.12            | 1.12       | 0.77            | 0.77       |
| 2                  | 13.83   | 14.47      | 17.58          | 20.02      | 14.32           | 15.44      | 5.02            | 5.79       |
| 3                  | 14.39   | 28.86      | 13.53          | 33.55      | 18.04           | 33.48      | 6.72            | 12.51      |
| 4                  | 9.43  | 38.29      | 7.69           | 41.24      | 11.90           | 45.38      | 13.11           | 25.62      |
| 5                  | 6.42  | 44.71      | 5.92           | 47.16      | 7.94            | 53.32      | 9.66            | 35.28      |
| 6                  | 2.99  | 47.70      | 3.43           | 50.59      | 2.35            | 55.67      | 4.15            | 39.43      |
| 7                  | 1.82  | 49.52      | 2.42           | 53.01      | 1.56            | 57.23      | 2.54            | 41.97      |
| 8                  | 1.42  | 50.94      | 1.40           | 54.41      | 1.93            | 59.16      | 3.43            | 45.40      |
| 9                  | 1.13  | 52.07      | 0.88           | 55.29      | 1.27            | 60.43      | 2.59            | 47.99      |
| 10                 | 1.81  | 53.88      | 1.03           | 56.32      | 0.96            | 61.39      | 1.59            | 49.58      |
| 11                 | 0.90  | 54.78      | 0.90           | 57.22      | 0.80            | 62.19      | 1.43            | 51.01      |
| 12                 | 0.52  | 55.30      | 0.41           | 57.63      | 1.20            | 63.39      | 1.66            | 52.67      |
| 13                 | 0.47  | 55.77      | 0.36           | 57.99      | 1.03            | 64.42      | 1.50            | 54.27      |
| 14                 | 0.42  | 56.19      | 0.43           | 58.42      | 0.50            | 64.92      | 0.84            | 55.01      |
| 15                 | 0.29  | 56.48      | 0.29           | 58.71      | 0.34            | 65.26      | 0.58            | 55.59      |
| 16                 | 0.28  | 56.76      | 0.29           | 59.00      | 0.33            | 65.59      | 0.57            | 56.16      |

Table 28. Release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -glucose within macropores of sterilized soil aggregates inoculated with different specific microorganisms

Fine sandy loam (permanent pasture): control

| Days of incubation | Initial $^{14}\text{C}$ as $^{14}\text{CO}_2$ (%) |            |                |            |                 |            |                 |            |
|--------------------|---|------------|----------------|------------|-----------------|------------|-----------------|------------|
|                    | Aureobasidio pullulans                            |            | Mucor hiemalis |            | Trichoderma sp. |            | Penicillium sp. |            |
|                    | Rate  | Cumulative | Rate           | Cumulative | Rate            | Cumulative | Rate            | Cumulative |
| 1                  | 0.39  | 0.39       | 1.18           | 1.18       | 3.93            | 3.93       | 0.82            | 0.82       |
| 2                  | 8.45  | 8.84       | 5.98           | 7.16       | 13.03           | 16.96      | 5.40            | 6.22       |
| 3                  | 11.06   | 19.90      | 11.03          | 18.19      | 11.60           | 28.56      | 7.88            | 14.10      |
| 4                  | 10.04   | 29.94      | 10.49          | 28.68      | 6.23            | 34.79      | 7.34            | 21.44      |
| 5                  | 7.41  | 37.35      | 10.56          | 39.24      | 2.01            | 36.80      | 4.56            | 26.00      |
| 6                  | 6.38  | 43.73      | 10.04          | 49.28      | 4.03            | 40.83      | 6.95            | 32.95      |
| 7                  | 5.90  | 49.63      | 8.90           | 58.18      | 2.61            | 43.44      | 5.50            | 38.45      |
| 8                  | 6.51  | 56.14      | 4.95           | 63.13      | 2.92            | 46.36      | 6.81            | 45.26      |
| 9                  | 5.52  | 61.66      | 1.99           | 65.12      | 3.28            | 49.64      | 6.89            | 52.15      |
| 10                 | 6.77  | 68.43      | 2.78           | 67.90      | 3.32            | 52.96      | 5.34            | 57.49      |
| 11                 | 3.01  | 71.44      | 1.79           | 69.69      | 4.41            | 57.37      | 3.06            | 60.55      |
| 12                 | 1.35  | 72.79      | 1.32           | 71.01      | 4.43            | 61.80      | 2.94            | 63.49      |
| 13                 | 1.72  | 74.51      | 1.23           | 72.24      | 3.55            | 65.35      | 2.68            | 66.17      |
| 14                 | 1.41  | 75.92      | 1.10           | 73.34      | 2.46            | 67.81      | 1.26            | 67.43      |
| 15                 | 1.01  | 76.93      | 0.82           | 74.16      | 1.35            | 69.16      | 1.20            | 68.73      |
| 16                 | 1.01  | 77.94      | 0.82           | 74.98      | 1.35            | 70.51      | 1.29            | 70.02      |

Table 29. Release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -glucose within macropores of sterilized soil aggregates inoculated with different specific microorganisms

Grey clay: control

| Days of incubation | Initial $^{14}\text{C}$ as $^{14}\text{CO}_2$ (%) |            |                |            |                 |            |                 |            |
|--------------------|---|------------|----------------|------------|-----------------|------------|-----------------|------------|
|                    | Aureobasidio pullulans                            |            | Mucor hiemalis |            | Trichoderma sp. |            | Penicillium sp. |            |
|                    | Rate  | Cumulative | Rate           | Cumulative | Rate            | Cumulative | Rate            | Cumulative |
| 1                  | 0.84  | 0.94       | 0.56           | 0.56       | 0.91            | 0.91       | 1.09            | 1.09       |
| 2                  | 8.86  | 9.70       | 2.56           | 3.12       | 3.56            | 4.47       | 5.04            | 6.13       |
| 3                  | 9.69  | 19.39      | 9.35           | 12.47      | 7.68            | 12.15      | 7.75            | 13.88      |
| 4                  | 6.65  | 26.04      | 9.46           | 21.93      | 10.64           | 22.79      | 8.36            | 22.24      |
| 5                  | 5.50  | 31.54      | 8.38           | 30.31      | 11.05           | 33.84      | 8.97            | 31.21      |
| 6                  | 4.13  | 35.67      | 5.93           | 36.24      | 11.07           | 44.91      | 8.53            | 39.74      |
| 7                  | 4.08  | 39.75      | 4.88           | 41.12      | 8.05            | 52.96      | 7.20            | 46.94      |
| 8                  | 4.71  | 44.46      | 5.08           | 46.20      | 5.53            | 58.49      | 5.73            | 52.67      |
| 9                  | 4.29  | 48.75      | 4.53           | 50.73      | 4.53            | 63.02      | 5.01            | 57.68      |
| 10                 | 5.94  | 54.69      | 7.11           | 57.84      | 3.79            | 66.81      | 4.25            | 61.93      |
| 11                 | 5.19  | 59.88      | 5.77           | 63.61      | 3.31            | 70.12      | 4.55            | 66.48      |
| 12                 | 3.81  | 63.69      | 3.87           | 67.48      | 2.85            | 72.97      | 4.22            | 70.70      |
| 13                 | 2.96  | 66.65      | 3.23           | 70.71      | 1.79            | 74.76      | 2.64            | 73.34      |
| 14                 | 3.00  | 69.65      | 3.49           | 74.20      | 0.87            | 75.63      | 1.24            | 74.58      |
| 15                 | 1.94  | 71.59      | 1.98           | 76.18      | 1.02            | 76.65      | 1.14            | 75.72      |
| 16                 | 1.93  | 73.52      | 1.97           | 78.15      | 1.01            | 77.66      | 1.13            | 76.85      |

Table 30. Release of  $^{14}\text{C}$  during incubation of  $^{14}\text{C}$ -starch distributed within micropores and macropores of sterilized soil aggregates inoculated with different specific microorganisms

Fine sandy loam (permanent pasture): aggregates

| Days of incubation | Initial $^{14}\text{C}$ released as $^{14}\text{CO}_2$ (%) |            |                |            |                 |            |                 |            |
|--------------------|--|------------|----------------|------------|-----------------|------------|-----------------|------------|
|                    | Aureobasidio pullulans                                     |            | Mucor hiemalis |            | Trichoderma sp. |            | Penicillium sp. |            |
|                    | Rate   | Cumulative | Rate           | Cumulative | Rate            | Cumulative | Rate            | Cumulative |
| 1                  | 9.61   | 9.61       | 8.01           | 8.01       | 1.22            | 1.22       | 1.29            | 1.29       |
| 2                  | 10.43  | 20.04      | 11.81          | 19.82      | 5.09            | 6.31       | 4.88            | 6.17       |
| 3                  | 3.61   | 23.65      | 4.31           | 24.13      | 3.32            | 9.63       | 5.18            | 11.35      |
| 4                  | 1.57   | 25.22      | 0.77           | 24.90      | 2.70            | 12.33      | 2.57            | 13.92      |
| 5                  | 1.62   | 26.84      | 1.38           | 26.28      | 1.23            | 13.56      | 1.36            | 15.28      |
| 6                  | 2.16   | 29.00      | 1.09           | 27.37      | 1.10            | 14.66      | 1.21            | 16.49      |
| 7                  | 2.55   | 31.55      | 1.27           | 28.64      | 1.43            | 16.09      | 1.63            | 18.12      |
| 8                  | 1.59   | 33.14      | 0.67           | 29.31      | 1.22            | 17.31      | 1.28            | 19.40      |
| 9                  | 1.74   | 34.88      | 1.17           | 30.48      | 1.44            | 18.75      | 1.55            | 20.95      |
| 10                 | 1.53   | 36.41      | 1.86           | 32.34      | 0.97            | 19.72      | 1.22            | 22.17      |
| 11                 | 1.78   | 38.19      | 2.35           | 34.69      | 1.20            | 20.92      | 1.65            | 23.82      |
| 12                 | 2.03   | 40.22      | 2.24           | 36.93      | 2.29            | 23.21      | 1.89            | 25.71      |
| 13                 | 1.19   | 41.41      | 0.77           | 37.70      | 1.24            | 24.45      | 0.57            | 26.28      |
| 14                 | 0.91   | 42.32      | 0.76           | 38.46      | 1.73            | 26.18      | 0.69            | 26.97      |
| 15                 | 0.54   | 42.86      | 0.66           | 39.12      | 2.08            | 28.26      | 1.31            | 28.28      |
| 16                 | 0.54   | 43.40      | 0.66           | 39.78      | 2.08            | 30.34      | 1.31            | 29.59      |
| 17                 | 0.37   | 43.77      | 0.40           | 40.18      | 1.04            | 31.38      | 1.28            | 20.87      |
| 18                 | 0.36   | 44.13      | 0.40           | 40.58      | 1.03            | 32.41      | 1.27            | 32.14      |
| 19                 | 0.33   | 44.46      | 0.38           | 40.96      | 0.86            | 33.44      | 0.63            | 33.47      |
| 20                 | 0.32   | 44.78      | 0.38           | 41.34      | 0.86            | 34.13      | 0.63            | 34.40      |
| 21                 | 0.33   | 45.11      | 0.40           | 41.44      | 1.08            | 35.21      | 0.69            | 35.09      |
| 22                 | 0.32   | 45.43      | 0.40           | 42.14      | 1.07            | 36.28      | 0.69            | 35.78      |
| 23                 | 0.26   | 45.69      | 0.46           | 42.60      | 0.84            | 37.12      | 0.41            | 36.19      |
| 24                 | 0.25   | 45.94      | 0.46           | 43.06      | 0.84            | 37.96      | 0.40            | 36.59      |

Table 31. Release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -starch distributed within micropores and macropores of sterilized soil aggregates inoculated with different specific microorganisms

Grey clay: aggregates

| Days of incubation | Initial $^{14}\text{C}$ released as $^{14}\text{CO}_2$ (%) |            |                |            |                 |            |                 |            |
|--------------------|--|------------|----------------|------------|-----------------|------------|-----------------|------------|
|                    | Aureobasidio pullulans                                     |            | Mucor hiemalis |            | Trichoderma sp. |            | Penicillium sp. |            |
|                    | Rate   | Cumulative | Rate           | Cumulative | Rate            | Cumulative | Rate            | Cumulative |
| 1                  | 0.60   | 0.60       | 0.67           | 0.67       | 0.43            | 0.43       | 0.52            | 0.52       |
| 2                  | 0.62   | 1.22       | 3.98           | 4.65       | 1.10            | 1.53       | 3.34            | 3.86       |
| 3                  | 7.89   | 9.11       | 4.75           | 9.40       | 6.69            | 8.22       | 3.93            | 7.79       |
| 4                  | 3.89   | 13.00      | 1.72           | 11.12      | 2.64            | 10.86      | 2.34            | 10.13      |
| 5                  | 2.39   | 15.39      | 0.89           | 12.01      | 1.05            | 11.91      | 1.09            | 11.22      |
| 6                  | 2.15   | 17.54      | 0.84           | 12.85      | 0.99            | 12.90      | 0.70            | 11.92      |
| 7                  | 1.42   | 18.96      | 1.51           | 14.36      | 1.31            | 14.21      | 1.61            | 13.53      |
| 8                  | 0.96   | 19.92      | 0.81           | 15.17      | 1.24            | 15.45      | 1.21            | 14.74      |
| 9                  | 0.94   | 20.86      | 1.34           | 16.51      | 1.42            | 16.87      | 1.26            | 16.00      |
| 10                 | 0.61   | 21.47      | 0.82           | 17.33      | 0.77            | 17.64      | 1.09            | 17.09      |
| 11                 | 0.57   | 22.04      | 0.60           | 17.93      | 0.92            | 18.56      | 1.35            | 18.44      |
| 12                 | 0.88   | 22.92      | 1.04           | 18.97      | 0.98            | 19.54      | 2.37            | 20.81      |
| 13                 | 0.86   | 23.78      | 0.28           | 19.25      | 0.51            | 21.05      | 0.47            | 21.28      |
| 14                 | 0.46   | 24.24      | 0.39           | 19.64      | 0.89            | 20.94      | 0.65            | 21.93      |
| 15                 | 0.88   | 25.12      | 1.19           | 20.82      | 1.08            | 22.02      | 0.50            | 22.43      |
| 16                 | 0.87   | 25.99      | 1.19           | 22.02      | 1.08            | 23.10      | 0.49            | 22.92      |
| 17                 | 0.92   | 26.91      | 0.95           | 22.97      | 0.97            | 24.07      | 0.77            | 23.69      |
| 18                 | 0.91   | 27.82      | 0.94           | 23.91      | 0.96            | 25.03      | 0.76            | 24.45      |
| 19                 | 1.26   | 29.08      | 2.01           | 25.92      | 1.00            | 26.03      | 1.28            | 25.73      |
| 20                 | 1.25   | 30.33      | 2.01           | 27.93      | 0.99            | 27.02      | 1.28            | 27.01      |
| 21                 | 0.66   | 30.99      | 0.74           | 28.67      | 0.77            | 27.79      | 1.78            | 28.79      |
| 22                 | 0.65   | 31.64      | 0.74           | 29.41      | 0.76            | 28.55      | 1.78            | 30.57      |
| 23                 | 1.03   | 32.67      | 0.87           | 30.28      | 0.50            | 29.05      | 0.93            | 31.50      |
| 24                 | 1.02   | 33.69      | 0.87           | 31.15      | 0.50            | 29.55      | 0.92            | 32.42      |

Table 32. Release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -starch distributed within macropores of sterilized soil aggregates inoculated with different specific microorganisms  
 Fine sandy loam (permanent pasture): control

| Days of incubation | Initial $^{14}\text{C}$ released as $^{14}\text{CO}_2$ (%) |            |                |            |                 |            |                 |            |
|--------------------|--|------------|----------------|------------|-----------------|------------|-----------------|------------|
|                    | Aureobasidio pullulans                                     |            | Mucor hiemalis |            | Trichoderma sp. |            | Penicillium sp. |            |
|                    | Rate   | Cumulative | Rate           | Cumulative | Rate            | Cumulative | Rate            | Cumulative |
| 1                  | 1.28   | 1.28       | 8.66           | 8.66       | 3.06            | 3.06       | 4.38            | 4.38       |
| 2                  | 2.68   | 3.96       | 17.37          | 26.03      | 8.16            | 11.22      | 8.13            | 12.51      |
| 3                  | 9.38   | 13.34      | 8.17           | 34.20      | 5.16            | 16.38      | 6.56            | 19.07      |
| 4                  | 10.35  | 23.69      | 3.34           | 37.54      | 5.64            | 22.02      | 6.21            | 25.28      |
| 5                  | 13.16  | 36.85      | 1.68           | 39.22      | 2.97            | 24.99      | 4.99            | 30.27      |
| 6                  | 4.91   | 41.76      | 1.05           | 40.27      | 1.82            | 26.81      | 3.69            | 33.96      |
| 7                  | 2.31   | 44.07      | 1.61           | 41.58      | 2.11            | 28.92      | 2.47            | 36.43      |
| 8                  | 1.51   | 45.58      | 1.06           | 42.94      | 1.68            | 30.60      | 1.80            | 38.23      |
| 9                  | 1.47   | 47.05      | 1.38           | 44.32      | 1.77            | 32.37      | 1.96            | 40.19      |
| 10                 | 1.25   | 48.30      | 1.24           | 45.56      | 1.32            | 33.69      | 1.96            | 42.15      |
| 11                 | 1.48   | 49.78      | 1.43           | 46.99      | 1.44            | 35.13      | 1.91            | 44.06      |
| 12                 | 1.92   | 51.70      | 1.41           | 48.40      | 1.90            | 37.03      | 2.74            | 46.80      |
| 13                 | 1.31   | 53.01      | 0.63           | 49.03      | 1.95            | 38.98      | 1.22            | 48.02      |
| 14                 | 0.90   | 53.91      | 0.67           | 49.70      | 3.61            | 42.59      | 1.53            | 49.55      |
| 15                 | 0.79   | 54.70      | 0.55           | 50.25      | 1.15            | 43.74      | 1.67            | 51.22      |
| 16                 | 0.79   | 55.49      | 0.54           | 50.79      | 1.14            | 44.88      | 1.67            | 52.89      |
| 17                 | 0.52   | 56.01      | 0.47           | 51.26      | 0.62            | 45.50      | 1.13            | 54.02      |
| 18                 | 0.51   | 56.52      | 0.47           | 51.73      | 0.62            | 46.12      | 1.12            | 55.14      |
| 19                 | 0.69   | 57.21      | 0.45           | 52.18      | 0.52            | 46.64      | 0.59            | 55.73      |
| 20                 | 0.68   | 57.89      | 0.44           | 52.62      | 0.51            | 47.15      | 0.58            | 56.31      |
| 21                 | 0.39   | 58.28      | 0.41           | 53.03      | 1.02            | 48.07      | 0.58            | 56.89      |
| 22                 | 0.39   | 58.67      | 0.41           | 53.44      | 1.02            | 49.19      | 0.57            | 57.46      |
| 23                 | 0.44   | 59.11      | 0.55           | 53.99      | 0.50            | 49.69      | 0.27            | 57.73      |
| 24                 | 0.43   | 59.54      | 0.54           | 54.53      | 0.50            | 50.19      | 0.27            | 58.00      |

Table 33. Release of  $^{14}\text{CO}_2$  during incubation of  $^{14}\text{C}$ -starch distributed within macropores of sterilized soil aggregates inoculated with different specific microorganisms

Grey clay: control

| Days of incubation | Initial $^{14}\text{C}$ released as $^{14}\text{CO}_2$ (%) |            |                |            |                 |            |                 |            |
|--------------------|--|------------|----------------|------------|-----------------|------------|-----------------|------------|
|                    | Aureobasidio pullulans                                     |            | Mucor hiemalis |            | Trichoderma sp. |            | Penicillium sp. |            |
|                    | Rate   | Cumulative | Rate           | Cumulative | Rate            | Cumulative | Rate            | Cumulative |
| 1                  | 1.58   | 1.58       | 2.59           | 2.59       | 1.80            | 1.80       | 2.25            | 2.25       |
| 2                  | 8.74   | 10.32      | 10.25          | 12.84      | 5.76            | 7.56       | 8.47            | 10.72      |
| 3                  | 9.50   | 19.82      | 9.43           | 22.27      | 5.87            | 13.43      | 6.68            | 17.40      |
| 4                  | 5.44   | 25.26      | 4.80           | 27.07      | 5.79            | 19.22      | 4.21            | 21.61      |
| 5                  | 4.24   | 29.50      | 2.49           | 29.56      | 4.35            | 23.57      | 3.00            | 24.61      |
| 6                  | 3.99   | 33.49      | 1.95           | 31.51      | 3.27            | 26.84      | 2.47            | 27.08      |
| 7                  | 3.01   | 36.50      | 2.83           | 34.34      | 3.18            | 30.02      | 3.12            | 30.20      |
| 8                  | 1.70   | 38.20      | 1.67           | 36.01      | 2.31            | 32.33      | 2.50            | 32.70      |
| 9                  | 1.67   | 39.87      | 1.73           | 37.74      | 2.59            | 34.92      | 2.34            | 35.04      |
| 10                 | 1.43   | 41.30      | 1.48           | 39.22      | 1.99            | 36.91      | 1.69            | 36.73      |
| 11                 | 1.62   | 42.92      | 1.69           | 40.91      | 2.09            | 39.00      | 1.90            | 38.63      |
| 12                 | 1.60   | 44.52      | 2.40           | 43.31      | 2.49            | 41.49      | 2.46            | 41.09      |
| 13                 | 0.96   | 45.48      | 1.15           | 44.46      | 1.21            | 42.70      | 1.11            | 42.20      |
| 14                 | 0.67   | 46.15      | 1.25           | 45.71      | 1.87            | 44.57      | 1.39            | 43.59      |
| 15                 | 0.57   | 46.72      | 0.92           | 46.63      | 1.59            | 46.16      | 1.03            | 44.62      |
| 16                 | 0.56   | 47.28      | 0.92           | 47.55      | 1.58            | 47.74      | 1.02            | 45.64      |
| 17                 | 0.58   | 47.86      | 0.85           | 48.40      | 1.48            | 49.22      | 1.24            | 46.88      |
| 18                 | 0.58   | 48.44      | 0.84           | 49.24      | 1.47            | 50.69      | 1.23            | 48.11      |
| 19                 | 0.86   | 49.30      | 0.93           | 50.17      | 1.11            | 51.80      | 1.04            | 49.15      |
| 20                 | 0.85   | 50.15      | 0.93           | 51.10      | 1.10            | 52.90      | 1.03            | 50.18      |
| 21                 | 0.56   | 50.71      | 0.56           | 51.66      | 0.71            | 53.61      | 0.77            | 51.05      |
| 22                 | 0.56   | 51.27      | 0.55           | 52.21      | 0.70            | 54.31      | 0.77            | 51.72      |
| 23                 | 0.79   | 52.06      | 0.64           | 52.85      | 0.61            | 54.92      | 0.65            | 52.37      |
| 24                 | 0.78   | 52.84      | 0.64           | 53.49      | 0.60            | 55.52      | 0.64            | 53.01      |

APPENDIX 8Quantitative estimation of soil microorganisms during incubation  
using a modification of the method of Jones and Mollison (1948)

0.5 g of wet sample was ground up with a pestle in 5 ml of sterile distilled water. The slurry was allowed to stand for particles to settle and the supernatant decanted into a 100 ml sterilized flask. The process of grinding was repeated and supernatant added to the first decantation.

The volume was made up to 50 ml with 1.5% sterile agar and the flask shaken vigorously. After allowing about 5 sec for sedimentation of heavier grains an aliquot of the sample was immediately removed just below the surface and placed under a coverslip on a haemocytometer.

After allowing the agar to set, the coverslip was removed from the haemocytometer by sliding it off under sterile distilled water. Excess agar was removed from the grooves within the haemocytometer. By gentle agitation in the sterile distilled water the films gently floated off onto microscopic slides. These were slowly dried at room temperature overnight and then immersed in a stain containing 15 ml phenol (5% aqueous), 1 ml aniline blue (1% aqueous) and 4 ml glacial acetic acid filtered 1 hr after preparation. The stained film was rapidly washed and dehydrated in 95% ethanol.

100 random fields were counted under oil immersion for both bacteria and fungi respectively.

APPENDIX 9Determination of residual  $^{14}\text{C}$  activity in soil after incubation

Few, if any, workers have attempted a total balance of  $^{14}\text{C}$  during incubations of  $^{14}\text{C}$ -labelled substrates in soil. Because determination of  $\text{CO}_2$  is difficult and titration methods insensitive, it was considered that experiments based only on determination of  $^{14}\text{CO}_2$  released are open to considerable error. After development of the method for counting  $^{14}\text{C}$ -labelled soils as suspensions, it was decided to obtain some  $^{14}\text{C}$  recoveries for some of the incubated soils.

The samples used were those from the studies of specific organisms utilizing  $^{14}\text{C}$ -labelled starch in the Urrbrae fine sandy loam.

Method

Samples were freeze-dried at the end of the incubation, ground to  $<53\ \mu\text{m}$ , and a  $^{14}\text{C}$  activity determined by suspension counting of the soil particles (Chapter III).

Results

The results (Table 34) show that most of the initial  $^{14}\text{C}$  in the aggregates of the fine sandy loam (permanent pasture) amended with starch was left in the soil after incubation.

The nature of the residual  $^{14}\text{C}$  was not known but it was assumed that most of the initial  $^{14}\text{C}$  was present in starch protected from attack by the specific organisms (Chapter VII).

With few exceptions the total  $^{14}\text{C}$  recovered as  $^{14}\text{CO}_2$  and as residual  $^{14}\text{C}$  (column 3 of Table 34) was between 85 and 98%. If experimental errors are taken into account, it can be concluded that both the absorbing system and the method of counting the  $^{14}\text{C}$  (either as  $^{14}\text{CO}_2$  or as suspensions) were reliable.

The 15% losses of initial  $^{14}\text{C}$  could be due to several factors including losses of  $^{14}\text{CO}_2$  during change of absorbing vials. The cumulative total for  $^{14}\text{CO}_2$  released also contains the sum of errors for each individual determination.

Determinations of residual  $^{14}\text{C}$  after incubation were not made in all the studies in this thesis but the results obtained in this section indicate that the assumption made in the previous chapters that most of the initial  $^{14}\text{C}$  not recovered as  $^{14}\text{CO}_2$  during incubation was left in the soil was sound.

Table 34. Residual  $^{14}\text{C}$  in aggregates after incubation of  $^{14}\text{C}$ -starch  
in a fine sandy loam (permanent pasture) inoculated with  
specific organisms

|    | Initial $^{14}\text{C}$<br>released as<br>$^{14}\text{CO}_2$ (%)<br>(a) | Initial $^{14}\text{C}$<br>recovered as<br>residual $^{14}\text{C}$ (%)<br>(b) | Initial $^{14}\text{C}$<br>recovered as<br>$^{14}\text{CO}_2$ plus<br>residual $^{14}\text{C}$<br>(a + b) |
|----|---|--|---|
| 1  | 42.2  | 43.3   | 85.5  |
| 2  | 42.1  | 45.8   | 87.9  |
| 3  | 43.5  | 47.4   | 90.9  |
| 4  | 48.3  | 45.3   | 93.6  |
| 5  | 45.0  | 52.0   | 97.0  |
| 6  | 41.1  | 46.8   | 87.9  |
| 7  | 46.4  | 41.5   | 87.9  |
| 8  | 40.4  | 57.7   | 98.1  |
| 9  | 37.3  | 54.8   | 92.1  |
| 10 | 38.6  | 58.0   | 96.6  |
| 11 | 36.4  | 52.5   | 88.9  |
| 12 | 34.6  | 59.1   | 93.7  |
| 13 | 30.7  | 49.5   | 80.2  |
| 14 | 31.9  | 46.4   | 78.3  |
| 15 | 32.2  | 59.00  | 91.2  |
| 16 | 31.4  | 61.5   | 92.9  |

90.8 ± 5.6  
S.D.

APPENDIX 10Experimental errors

The standard errors used in calculating least significant differences (LSD) between measurements of cumulative release of  $^{14}\text{CO}_2$  included contributions from many factors. Factors included were (a) replicated differences, (b) volumetric errors in preparing samples for counting  $^{14}\text{C}$  from a known amount of absorbent, (c) losses of  $^{14}\text{CO}_2$  during incubation due to change of absorbing vials, (d) gravimetric errors arising from preparation of samples (soil) for suspension counting and (e) counting errors inherent in the liquid scintillation spectrometer.

Because duplicate samples were used in all incubation studies, errors arising from replicates might have contributed much to the standard errors. Thus the biological differences which occur during incubations were included in the standard error. In some cases there were wide differences between the initial rates of release of  $^{14}\text{CO}_2$  from duplicate samples but, despite the limitations of using duplicate samples only, the large number of samples involved did not permit the use of further replicates.

The errors introduced by pipetting of samples for counting would have contributed to the standard errors; since the chances of over-estimation and under-estimation were about the same no particular bias would result in the release curves for  $^{14}\text{CO}_2$ .

Errors arising from the suspension counting method were small and duplicate counts showed only 1% differences and where determination of

$^{14}\text{C}$  was done in triplicate the mean standard error obtained was about 2% ( $1.97 \pm 0.29\%$ ) for 10 determinations. The grinding of samples to  $<53 \mu\text{m}$  before suspension counting eliminated sampling errors due to poor distribution of  $^{14}\text{C}$  in aggregates or soils.

The original  $^{14}\text{C}$  activity in the starch amended samples was half that for the  $^{14}\text{C}$ -glucose amended samples. Determination of  $^{14}\text{C}$  (by suspension counting) in the starch amended samples always gave 50% of the values obtained for the  $^{14}\text{C}$ -glucose amended samples, which confirmed the reliability of the method of counting  $^{14}\text{C}$ .

Any counting errors inherent in the spectrometer (other than standard errors from counting time) were unavoidable. Throughout the studies counting was done for the longest time possible to reduce standard deviation of counting to a minimum, and errors from counting should not have contributed much to the standard error values.

Change of  $\text{CO}_2$  absorbing vials during incubation was done as quickly as possible so that large errors were unlikely but cannot be quantified.

Standard errors obtained for the  $^{14}\text{CO}_2$  evolution curves were low and therefore contributed to low values of least significant differences (LSD). This shows that the various errors described above were not high and enable the results to be presented and discussed with confidence.

Errors undoubtedly occurred in the counting of microbial populations in Chapter VIII. The method of Jones and Mollison (1948) does not allow differentiation between dead and living organisms. However, the results were obtained from a large number of fields and were adequate for the purpose for which they have been used.

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