Studies on the occurrence of ethylene in soil and, its effects on root growth.

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ABSTRACT OF THESIS

Soil samples were collectd from different depths of several selected soil types under both arable and grassland situations. The production of ethylene in the laboratory under anaerobic conditions by these soils, in fresh, air dried and oven dried states were compared. Drying was found to stimulate ethylene production, oven drying having most effect on initial ethylene production.

Ethylene formation was related to organic matter content, ethylene concentrations increasing with increased organic matter levels. Ethylene concentrations in arable soils were also related to the acidity, low pH favouring ethylene production. Grassland soils did not show this relationship.

High levels of added nitrate were found to reduce ethylene formation but even at 2000 ppm production was not inhibited completely. Low levels had a transient inhibitory effect but the maximum level of ethylene produced was unaffected.

Ethylene formation was stimulated in air dried soil by the addition of wheat and barley straw and by the addition of caesin, pepsin, ethanol, lactic acid and pyruvic acid. The exact nature of this stimulation is not known. Ethylene production in undried soil depleted of microbial substrates was promoted by all the three organic substrates supplied: ethanol, glucose and butyric acid.

Pea plants treated with 1.1 vpm ethylene showed a 50% reduction in root extension, 4.2 and 10 vpm treatments inhibited root extension completely.

Exposure of pea and clover plants to air containing 10 vpm ethylene resulted in a reduction in nodulation and in the nitrogen fixing capacity of those nodules present. Fresh and dry weight yields of pea pods and clover stems were reduced.

I hereby declare that this thesis was composed by myself and the work described was carried out entirely by myself except where indicated.

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INTRODUCTION

INTRODUCTION

In the past farmers concentrated on the nutritional status of their soils relying for good yields on fertiliser applications. However following the very wet autumn and winter of 1968-1969 attention was focussed on the physical stress modern farming systems impose on the soil. This stress is due partly to the increasing use of continuous arable cropping systems which have gradually led to a decrease in soil organic matter content and partly to the use of larger machinery. Soils low in organic matter are less well able to withstand the passage of heavy machinery and loss of soil structure is the result. Poorly structured soils adversely affect plant growth by impeding root development, by reducing the aeration status and by reducing the drainage capacity of the soil leading to waterlogged conditions.

Efficient crop production requires the optimum conditions of good soil structure and an adequate supply of nutrients and moisture. Various methods have been used to evaluate aeration conditions in the soil but correlation of these with plant growth also involves a consideration of the moisture content, temperature and soil strength relations which also influence root activity and plant growth.

Under aerobic conditions microbial activity in the soil produces primarily carbon dioxide and water as a result of the breakdown of organic matter. In areas of high microbial activity such as the rhizosphere there

will therefore be marked changes in oxygen consumption and carbon dioxide evolution. Root exudates, root respiration and in some plants, diffusion of oxygen from the shoots to the roots, together with microbial activity, will produce changes in the soil atmosphere which will be opposed by diffusion between the soil and the atmosphere.

However if for some reason diffusion is prevented, oxygen levels will fall to zero and anaerobic microbial processes will begin. Dramatic changes in the normal chemical and biological reactions in the soil occur; facultative anaerobic and true anaerobic organisms utilize oxidised soil components such as manganic oxide, ferric oxide, sulphate and breakdown products of organic matter, converting them to more reduced forms. Nitrate will be lost from the soil as a result of denitrification, leading to the release of gaseous nitrogen into the atmosphere. In addition other compounds may be formed which are toxic to plants, such as hydrogen sulphide, butyric acid and dimethyl sulphide. Methane and other hydrocarbons may be formed; of these ethylene is of particular interest as it is known to affect plant growth and development even at very low levels. Consequently much work has been done in this field and the emphasis of soil atmosphere studies has widened to include the occurrence of ethylene and its effects on crop growth. These studies were greatly facilitated by the development of gas chromatography which enabled trace amounts

of ethylene to be detected. Geochemical studies revealed that ethylene was present in the soil and subsequent work revealed that ethylene was produced by the soil under anaerobic conditions. In this work it was hoped to study some of the factors involved in the production of ethylene in the soil and how these were affected by soil type and soil management; also to look at some aspects of the effect of ethylene in the soil on root growth and development in legumes.

Factors affecting the evolution of ethylene by soil, and associated changes in soil atmosphere composition, were investigated by two experimental procedures. The first involved laboratory incubation of soils in sealed tubes; the second involved the monitoring of the soil atmosphere in undisturbed soil monoliths taken from the field into the greenhouse. Plant growth experiments were also carried out in the greenhouse, with two leguminous species, to investigate the effects of ethylene on root growth and on nodulation.

LITERATURE REVIEW

I. FACTORS AFFECTING SOIL ATMOSPHERE

The composition of the soil atmosphere is primarily influenced a) by the production and utilization of the soil gases and b) by the diffusion of these gases. Moisture and pore-space primarily affect the diffusion of gases, while it is microbial activity and energy sources which influence the production and utilization of gases (Epstein and Kohnke, 1957). These factors are discussed more fully below.

I.1. Diffusion

It is accepted that gas diffusion is the principal mechanism involved in the interchange of gases between plant roots or organisms in the soil and atmospheric air. Penman (1940) using columns of porous granular materials and prepared columns of soil crumbs, showed that

$$D/D_o = 0.66S$$

where D is the rate of diffusion in the material, D_o that in free air and S the proportion of the column volume occupied by air. Van Bavel (1952) obtained a slightly lower value, 0.6, for the coefficient, and found that if Penman's data was recalculated according to Van Bavel's model it agreed very closely.

Blake and Page (1948) were the first to examine successfully the diffusion process as it occurs in the soil in situ. Using the rate of evaporation of carbon disulphide from porous cups in the soil as a measure of diffusion they showed that there was a linear relationship between diffusion rates and porosity which varied

with soil type and cultivation history. The variation with soil type appeared to be due to structural differences, rates also varied according to cropping systems and the method of seed bed preparation. Later Currie (1960, 1961a) showed that this relationship could be represented as

$$D/D_o = \propto S$$

where $\propto$ depends on both the soil structure (tortuosity and shape of pores) and on the moisture content. Experimental data by Currie (1970) shows that at high moisture content when there are only a few air-filled pores $\propto$ can be as low as 0.1 or 0.2, while it is 0.6 for a dry soil. The relationship between porosity and $\propto$ for a particular soil depends on the structure and in a wet soil the value of $\propto$ will be higher for a well structured than a puddled soil (Bakker and Hidding, 1970).

Gaseous diffusion rates in a soil therefore depend on several factors:- the concentration gradient in the air-filled pores, the length of the diffusion path and the diffusion coefficient of the gas concerned (Russell, 1973), the latter being a function of air-filled porosity, soil structure and soil water content (Bakker and Hidding, 1970). By looking at the diffusion coefficients for oxygen and carbon dioxide in water and in air at 25°C (Table 1.) it is seen that movement of gases in the soil will be greatly reduced if water is present; hence a reduction in air-filled porosity due to the addition of water is more effective in reducing diffusion of air than a reduction due to compaction (Grable and Seimer, 1968).

Table 1. Diffusion coefficients of carbon dioxide and oxygen in air and water (after Grable, 1966).

	CO_2 cm^2/s	O_2 cm^2/s
Air	0.181	0.226
Water	2.04×10^{-5}	2.60×10^{-5}

According to Grable (1971) an important aspect of diffusion in soil is the non-uniformity of the distribution of the air-filled porosity with depth. Whereas the air-filled porosity in a soil sample 10 cm long can be uniform under a tension of 50 cm or more it increases sharply from top to bottom at zero tension. In a non-compacted sample the lower part of the sample may have a D/D_o ratio of zero, while the average air-filled porosity of the sample as a whole could be 20%.

Using figures for diffusion coefficients determined by Wesseling and van Wijk (1957), Currie (1961a), and Bakker and Hidding (1970), Greenwood (1975) has suggested that in general wherever the gas filled pore space exceeds 0.1 ml/ml the gas filled pores are continous. The diffusion coefficient within soil crumbs must also be considered, and Currie (1965) found that it was only about a fifth of that for the pore space around the soil crumb, due to the increased tortuosity of pores within the soil crumbs. The diffusion coefficient also decreased as the crumb size decreased due to the reduced porosity of the crumb.

As previously stated, the diffusion rate is greatly reduced by the presence of water. In addition the presence of micro-organisms in the soil water will also affect oxygen diffusion. If a soil aggregate is spherical the maximum radius r which it can possess without having an oxygen-free centre is given by

$$r^2=\frac{6DC}{M}$$

where D is the diffusion coefficient of oxygen in the crumb, C is the concentration of oxygen in the water at the outer surface of the crumb and M is the rate of oxygen uptake by micro-organisms in the crumb (Currie, 1961b, 1962; Greenwood and Berry, 1962; Greenwood and Goodman, 1964, 1967). Greenwood (1975) has suggested that in conditions such as those found in the United Kingdom oxygen-free zones in the soil are unlikely to occur unless they are separated from a gas phase by more than 0.9 cm of water, as in a saturated soil, but oxygen can rapidly reach very low levels in such soils (Greenwood and Goodman, 1967).

Other wokers (Avery, 1964; Thomasson and Robson, 1967) have shown that in clay soils aggregates may remain waterlogged after the pores between them have drained as indicated by the presence of greyish and ochreous internal mottling, also the smell of hydrogen sulphide may be detected on breaking open aggregates. This suggests that anaerobic conditions are not confined to water saturated soils but can occur within the soil aggregates.

I.2. Air-filled porosity

Air-filled porosity is defined as the total bulk volume not occupied by solids and water, and as outlined above greatly affects gaseous diffusion in the soil and thus soil aeration.

Much work has been done to determine the minimum air-filled porosity necessary for adequate soil aeration. Ten percent has been defined as the limiting condition for most crops (Grable, 1966; O'Connell, 1975). However there are exceptions, and air-filled porosities between 0 and 30% have all been suggested (Wesseling and van Wijk, 1957; Flocker et al, 1959; Baver, 1961; Vomocil and Flocker, 1961; Bateman, 1963; Brown et al, 1965; Grable and Siemer, 1968; Greenwood, 1969; Bakker and Hidding, 1970; Soane, 1970; Eavis, 1972; Shali, 1974).

Greenwood (1970) has suggested that it is probably only during temporary periods of waterlogging that soil aeration is insufficient for optimum growth. The minimum air-filled porosity necessary for optimum plant growth depends on many factors:- pore size (Bunt, 1961; Eavis, 1965; Eavis and Payne, 1969), soil texture (Hanks and Thorp, 1956; Boekel, 1963), while the minimum oxygen content necessary for normal root development will be affected by the water content of the soil (Ginrich and Russell, 1956, 1957) as well as by the bulk density (Tackett and Pearson, 1964; Archer and Smith, 1972; El Karouri, 1974) and the temperature (Cannon, 1925; Currie, 1975).

The wide variation observed in the minimum air-filled porosity required for plant growth provoked the following comment by Trouse (1971):

> "many different studies have been conducted to determine the requirements of a soil atmosphere for the development of specific plants but the results observed varied as much as the techniques and environments used."

As an alternative method of assessing the aeration requirements for plant growth attempts have been made to correlate plant growth with oxygen diffusion rate (ODR) (Phene et al, 1976). A value of 2×10^{-8} g/cm^2/min has been suggested (Letey and Stolzy, 1967) as a minimum value for root extension.

Bridge and Rixon (1976) have suggested that the respiratory quotient (RQ) should be used as an index of adequate aeration. These workers measured the oxygen uptake and RQ for three different textured soils and found a critical value of air-filled pore space above which RQ was always near unity. This corresponded to the transition from anaerobic to aerobic respiration and is in general agreement with the suggested minimum values of air-filled pore space required for plant growth.

I.3. Moisture

Numerous experiments have been carried out which illustrate that soil moisture content is one of the factors affecting the composition of the soil atmosphere, and that aeration is restricted by a high moisture content.

Bertrand and Kohnke (1957), by controlling the water table in soil columns and thus the moisture content, reported that high moisture content restricted the diffusion of oxygen in dense subsoils. Kristenson and Enoch (1964) also studied the composition of soil air in soil columns at different heights above a water table. They found that the soil air composition changed considerably as the water table was approached, the carbon dioxide content increasing as the oxygen content decreased. Wood and Greenwood (1971) have found that this inverse relationship between oxygen and carbon dioxide is linear until very low partial pressures of oxygen are reached. Kristenson and Enoch also found that the oxygen diffusion rate (ODR) decreased rapidly with increased soil depth and reached almost zero above the water table. It is possible to develop mathematical models to relate water table depth to moisture content and ODR in different soil types (Young Jr., 1969).

Changes in oxygen and carbon dioxide concentrations with altered moisture content were also found by Yamaguchi and coworkers (1967). They also studied the influence of soil temperature and found that carbon dioxide production in soil columns reached a peak and then gradually declined; this peak was reached more quickly at the higher moisture tensions and higher temperatures. However, the height of the peak was greater at higher temperatures and lower moisture tensions. In this experiment the moisture content of the soil was not maintained, so the soil was gradually drying out over

the period of the experiment. The oxygen concentration was inversely related to carbon dioxide concentration, while at the higher moisture level the nitrogen concentration rose above that present in air at all temperatures. As the soil gradually dried out the nitrogen content dropped to a value nearer that present in air.

Vadyunina and Borovinskay (1967) studied the composition of soil air in semi-arid regions, and found that the oxygen content was 20% except in soils above a high water table, where it varied between 13 and 16%. The high levels of carbon dioxide found in the profile appeared to be distributed in three layers. The carbon dioxide content varied sharply with time at the surface, was higher and more stable in the middle and was least at the bottom. The thickness of these layers varied with time. In laboratory experiments carbon dioxide production occured mainly in the humus horizon where it increased with moisture.

Giedrodj and Kowalinski (1968) reported that the dynamics of carbon dioxide and oxygen in soil were more affected by rainfall than by ground-water. They studied the atmosphere of a sandy soil with a ground-water level of 1 m and found that carbon dioxide concentrations varied from 0.1 to 15% at various depths in the profile and those of oxygen between 16 and 20%. The air content of the deeper horizons did not exceed 25% of the total porosity but that of the upper horizons was nearly 60%.

In laboratory experiments designed to calculate the evolution of carbon dioxide in soils maintained at

different moisture and temperature conditions over a given time period, it was found that optimum soil moisture was 40-80% of the maximum water holding capacity. Carbon dioxide evolution became almost constant after about 20 days (Ino and Mansi, 1969).

Oucharenko (1970), working on krasnozemic soils, showed that oxygen concentrations did not usually fall below 19-20% and carbon dioxide concentrations rarely exceeded 1-2% under normal moisture conditions. However, oxygen concentrations decreased and carbon dioxide concentrations increased after heavy and prolonged rain.

Dasberg and Bakker (1970) tried not only to correlate changes in soil aeration with fluctuating moisture content caused by irrigation but also to evaluate the usefulness of these measurements by trying to correlate them with plant growth. They found that after irrigation oxygen concentrations decreased and carbon dioxide concentrations increased but never to more than 6.5% due to the high solubility of carbon dioxide in the irrigation water. They also measured the oxygen diffusion rate (ODR) and air content of the soil and it was found that the latter gave the best correlation with plant growth.

Gavande (1969) also studied the effects of soil moisture on ODR and its relationship to plant growth; in this case the plant involved was cacao. He found that ODR, water use rate, soil temperature and growth of cacao were affected by soil moisture content in all soils studied.

Other workers have also found that the concentrations of gases such as carbon dioxide and oxygen may be affected by moisture content (Russell and Appleyard, 1915; Ginrich and Russell, 1956; Epstein and Kohnke, 1957; Miller and Johnson, 1964; Yamaguchi et al, 1964; Gawlik, 1968; Grechin and Ignat'en, 1969; Abrosinova and Bevat, 1970; Enoch and Dasberg, 1971; Audenko, 1972; Lees, 1972; Smith (K.A.) and Dowdell, 1974).

In general increased moisture content results in an increase in the carbon dioxide content and a corresponding decrease in oxygen content. When soil temperatures are low microbial activity is also low and changes in the soil atmosphere are slight, however if soil temperatures are high microbial activity will cause oxygen levels to fall. These effects are greatest in the top soil which contains more organic matter and a larger microbial population than the lower horizons.

The relationship between moisture content and moisture tension for soil horizons indicates the probable distribution of water and air in pores (Rixon, 1968). Soil horizons having a higher moisture content at a given moisture tension will have the greater proportion of capillary or water holding pores. It has been shown (Franken, 1969) that in fact air permeability of a soil and the carbon dioxide and oxygen concentration in the soil atmosphere are more affected by pore size distribution than by water-free pore space. The importance of individual pore size increased with their equivalent

diameter; soils with a high proportion of pores greater than 50 μ possessed good permeability and low carbon dioxide concentrations, whereas the reverse was true for soils with a high proportion of pores less than 50 μ. Amounts of carbon dioxide in the soil profile were closely related to air permeability of the A_p horizon.

If the moisture content of the soil is increased to the extent that the soil becomes saturated, then the percentage air space is almost zero. This prevents adequate diffusion and in fact it has been shown (Sides and Bardlen, 1970) that even in only partly saturated soils air-water equilibrium will be achieved much more slowly than generally believed. The rate of oxygen diffusion in a flooded soil through the water filled pores is about 10^{-4} times the rate of diffusion through air filled pores in a well drained soil. As a result of this slow rate of replenishment, oxygen is consumed by the soil micro-organisms and the soil rapidly becomes anaerobic. It is these short periods of anaerobic conditions that are likely to restrict plant growth (Greenwood, 1969) and reduce crop yields (Stolzy et al, 1975). A single day of waterlogging can restrict final yields considerably (Van't Woudt and Hagan, 1957; Erickson and Van Doren, 1960).

II. EFFECTS OF RESTRICTED AERATION ON PLANT GROWTH

II.1. Carbon dioxide

High carbon dioxide levels are believed to cause a reduction in water uptake (Kramer, 1940) and ion uptake

(Chang and Loomis, 1945). However, it is thought that adverse concentrations of carbon dioxide are not likely to be found often in agricultural soils (Russell, 1952; Carr, 1961; Currie, 1961b; Grable and Danielson, 1965a,b; Harrison, 1965a,b; Unger and Danielson, 1965; Greenwood, 1970). Greenwood (1975) stated that carbon dioxide partial pressures are unlikely to be appreciable unless a substantial proportion of the soil contains no oxygen.

II.2. Oxygen

Plant growth under low oxygen conditions shows certain characteristic symptoms. According to Van't Woudt and Hagan (1957) these include reduction in transpiration rate, yellowing and/or reddening of the leaves, epinasty, twisting of the plant tissue, widening of the leaf angle and the development of new adventitious roots near the soil surface, as well as the premature abscission of leaves and flowers. The chemical composition of the plant is altered by poor aeration. The concentration of many ions such as potassium, calcium and phosphate is decreased while that of sodium is increased (Stolzy and Letey, 1964; Labanauskas *et al*, 1966). The Krebs citric acid cycle is inhibited in flooded roots and oxygen stress causes amino butyric acid to accumulate (Fulton *et al*, 1964); toxic substances such as cyanide (Smith (E.), 1969) and ethanol may also accumulate (Erickson and Van Doren, 1960; Fulton and Erickson, 1964).

The growth of the plant itself may also be affected by the soil aeration, the level below which root growth

is restricted varying with different plant species (Vlamis and Davis, 1944; Hopkin et al, 1950; Kramer, 1951). However the reduction in the rate of elongation of the roots can be attributed to either mechanical resistance or aeration or both (Eavis and Payne, 1969; Taylor and Ratcliffe, 1969; Gooderham, 1973; El Karouri, 1974). Moisture availability may also affect elongation (Ginrich and Russell, 1957; Peters, 1957), and Eavis (1972) has shown that all three factors, moisture availability, mechanical impedance and aeration are involved in restricting root growth. Lack of oxygen causes a reduction in root respiration (Grable, 1966) and this is believed to be one of the main causes of the growth limiting effects of poor aeration (Harris and Van Bavel, 1957), another aspect is increased resistance to water movement through roots under these conditions (Kramer and Jackson, 1954; Kramer, 1965).

As a result of low oxygen concentrations the uptake and effective utilization of plant nutrients may be affected (Iljin, 1954; Williamson, 1964; Brouwer, 1965; Labanauskas et al, 1971, 1972). Some nutrients may become unavailable to the plant due to restricted root growth, increased resistance to transport of water and nutrients through the roots (Wesseling, 1974) and reduction to forms unavailable to the plant. For example nitrate is reduced by denitrification under anaerobic conditions to nitrous oxide and nitrogen, which are lost by diffusion to the atmosphere (Cady and Bartholomew, 1960;

Shaw, 1962; Broadbent and Clarke, 1965; Burford and Millington, 1968; Dowdell et al, 1972; Burford and Stefanson, 1973; Smith (K.A.) and Dowdell, 1973; Dowdell and Smith (K.A.), 1974).

Many workers (Brouwer, 1955; Butijn, 1961; Fulton and Erickson, 1964; Woolley, 1965; Greenwood, 1969) believe that restricted plant growth under anaerobic conditions is partly due to the formation of compounds in plants and soils that are toxic to the plant. Some of the compounds formed in the soil that are toxic to plants include hydrogen sulphide (Van't Woudt and Hagen, 1957; Ford and Calvert, 1966; Starkey, 1966; Connell and Patrick, 1968) and organic acids (Chandrasekaran and Yoshida, 1973) in particular butyric acid (Takijima, 1964; Hollis et al, 1967) and acetic acid (Lynch et al, 1976; Lynch, 1977; Lynch and Gunn, 1977; Niranjan Rao and Mikkelson, 1977). Methane and other hydrocarbons may also be formed (Davis and Squires, 1954; Barker, 1956; Alexander, 1961; Bell, 1969; Parr, 1969; Smith (K.A.) and Restall, 1971; Smith (K.A.) and Dowdell, 1973) in particular ethylene (Davis and Squires, 1954; Smith (K.A.) and Russell, 1969; Smith (K.A.) and Restall, 1971; Sheard and Leyshon, 1976) which is of interest due to its known physiological effects on plant growth.

III. ETHYLENE

III.1. Historical background

Ethylene has been known to affect plant growth since the beginning of the century. It was found that leaking

illuminating gas from gas mains caused defoliation of nearby trees (Girardin, 1864). However it was not until 1901 that Neljubow showed that it was ethylene that was the active constituent. Other workers then repeated his work and confirmed that it was the ethylene in illuminating gas and smoke that caused the unusual growth in seedlings. It was also found that ethylene increased the rate of senescence. Since that time a great deal of work has been carried out on the physiological effects of ethylene on plant growth and development, fruit ripening and the rate of senescence, and once it was established that ethylene was in fact produced by the plant it became accepted as one of the plant hormones. Although Cousins (1910) first suggested that ethylene might be produced by plants themselves when he showed that oranges produced a gas that promoted the ripening of bananas, it was more than 20 years before it was proved by chemical methods that ethylene was produced in the plant (Gane, 1934).

III.2. Effects of ethylene on plant growth

Ethylene affects many aspects of plant growth and development including seed germination, bud dormancy, flowering, sex expression, fruit ripening, abscission and senescence, response to gravity, hook opening and hypertrophy. These have been well documented in the reviews by Pratt and Goeschl (1969), Abeles (1972) and also the book "Ethylene in Plant Biology" by Abeles (1973). It is not intended to discuss them further here but to concentrate on those aspects of plant growth which are

more nearly related to ethylene in the soil, such as effects on root and shoot development.

III.2.i. Root and shoot elongation The effect of ethylene on shoot elongation is variable. In general stem elongation is inhibited but the growth of some monocotyledons is increased. Ethylene inhibits coleoptile growth in wheat (Roberts, 1951), and oat (Marinos, 1960; Meheriuk and Spencer, 1964), but stimulates growth of intact and excised rice coleoptiles (Imaseki and Pjon, 1970; Ku et al, 1970; Suge, 1971a,b). Elongation and auxin transport in the petiole of the aquatic plant Ranunculus sclerata are stimulated by ethylene, but in other plant systems the reverse is true (Musgrave and Watson, 1973). Stem elongation in another aquatic plant Callitriche intermedia is also promoted (Musgrave et al, 1972). It is believed that ethylene may be part of the general mechanism whereby aquatic plants adjust to the depth of the water. Other work relating flooding of plants to ethylene concentrations in the plant showed that ethylene levels were increased after submersion (Kawase, 1972).

In dicotyledons applied ethylene inhibits cell elongation, for example elongation of pea epicotyls is restricted. Similarly if pea epicotyls are subjected to stress this results in increased evolution of endogenous ethylene and this causes a reduction in length and increased diameter of both internodes and cells of internodes (Goeschl et al, 1966).

Ethylene also inhibits root elongation (Smith (K.A.) and Russell, 1969; Crossett and Campbell, 1975; Konings, 1975), although the response of different species varies (Smith (K.A.) and Robertson, 1971; Konings and Jackson, 1974) and may be counteracted by carbon dioxide (Radin and Loomis, 1969) although Smith (K.A.) and Robertson (1971) found that variations in carbon dioxide up to levels that were themselves inhibitory to root extension did not affect the response of roots to ethylene. The inhibition of root elongation is a reversible process and roots show a return to normal growth on removal of ethylene (Smith (K.A.) and Robertson, 1971).

It is believed the effects of ethylene on root growth are due to interaction between auxin and ethylene (Chadwick and Burg, 1967, 1970; Andreae et al, 1968). Other workers (Konings and Jackson, 1974) have shown that root elongation can be stimulated in a variety of plants by low levels of ethylene and that the responses of roots to ethylene are related to endogenous ethylene levels and thus responses may not be controlled by auxin in all plants.

III.2.ii. Stem and root thickness In conjunction with restricted elongation, thickening or swelling of stem tissue also occurs (Chadwick and Burg, 1970; Goeschl et al, 1966). Thickening of roots may also occur (Harvey and Rose, 1915; Smith (K.A.) and Robertson, 1971; Crossett and Campbell, 1975).

III.2.iii. Lateral root initiation Ethylene may promote the initiation of root primordia and root hairs (Zimmerman and Hitchcock, 1933; Borgstrom, 1939). Lateral root production is increased resulting in a greater branching of the root system in barley plants (Crossett and Campbell, 1975). These workers found that on removal of ethylene the extension of laterals formed during the ethylene treatment was greatly stimulated but the growth of those formed after treatment was inhibited.

III.2.iv. Nodulation and nitrogen fixation Ethylene at 0.4 ppm and above was found to be extremely inhibitory to nodulation and also inhibited nitrogen fixation of existing nodules in isolated roots of Phaseolus vulgaris (Grobbelaar et al, 1971b). Day et al, (1972, 1975) found that removal of ethylene by adding activated charcoal or by aeration with sterile air improved growth and nitrogen fixation by Trifolium subterraneum plants grown in plugged tubes, to rates close to those in plants grown in open pots. Drennan and Norton (1972) showed that ethrel (an ethylene releasing compound) inhibited nodulation of intact pea plants (Pisum sativum) when applied to the roots and also to the foliage. Using a split root system they showed that nodulation on roots on the untreated half of the plant was not inhibited and in fact increased nodulation compensated for the lack of nodulation on the treated half of the root system.

III.2.v. Stress ethylene As well as its normal regulatory production, ethylene is now also known to be produced as a result of stress conditions in the plant, whether these be due to mechanical damage, disease, climatic extremes or insect damage.

Drought causes a sharp increase in ethylene production in petioles and may lead to abscission (McMichael et al, 1972). Greatly increased levels of internal ethylene were found in Vicia faba due to drought conditions (El Beltagy and Hall, 1974); waterlogging had the same effect. Kawase (1972) also found that flooding caused an increase in internal ethylene levels in both intact and detached plant organs. Increased ethylene levels were also found in tomato plants together with an increased epinastic response (Jackson and Campbell, 1976).

Increased internal ethylene levels have also been found in some varieties of soya bean as a result of temperature changes; endogenous ethylene levels in these plants were enhanced at 25°C but were normal above and below this temperature (Saminy, 1970).

Other workers have shown that mechanical stress affects ethylene production by the plant, both in the shoots (Goeschl et al, 1966) and in the root (Kays, 1971; Kays et al, 1974). The presence of an inert granular medium appears to stimulate the production of ethylene within the inter-cellular spaces, but when a stress is applied, although there is an increase in ethylene in the shoots, the endogenous levels in the roots are lower in both barley and rye (Goss, 1974).

It has also been suggested (Hillman and Catchpole, 1970, 1975; Catchpole, 1971) that the production of ethylene by potato sprouts possibly due to mechanical stress may be one of the factors involved in the coiled sprout disorder of potatoes.

Wounding of the plant causes an increase in ethylene production in both leaf and petiole tissue (McAffee and Morgan, 1971).

III.3. Ethylene production by micro-organisms

As well as its production by, and effects on plants, ethylene is now known to be produced by, and to affect, both bacterial and fungal populations. There is evidence to show that ethylene in the soil is a result of microbial activity. The effect of temperature on the rate of ethylene production is compatible with microbial origin and the ability of a soil to evolve ethylene depends on the availability of microbial substrates and the activity of microbial populations (Smith (K.A.) and Jackson, 1974). It has been suggested that ethylene may be produced by a facultative anaerobe because of the similarity in its pattern of production to that of nitrous oxide, production beginning under anaerobic or near-anaerobic conditions and falling off as severe reducing conditions are produced (Smith (K.A.) and Restall, 1971). Other workers however have suggested that ethylene is formed aerobically both by bacteria (Primrose, 1976b; Primrose and Dilworth, 1976) and by fungi, anaerobic conditions being required to mobilize substrate (Lynch and Harper, 1974a).

Ethylene is known to be produced by Penicillium digitatum, a citrus fruit fungus (Biale, 1940; Miller et al, 1940; Young et al, 1951; Fergus, 1954; Jacobsen and Wang, 1959; Phan, 1962; Gibson, 1967; Chou and Yang, 1973), the bacteria Pseudomonas solanacearum (Freebairn and Buddenhagan, 1964; Swanson et al, 1972), Escherichia coli and other soil bacteria (Primrose, 1976a,b; Primrose and Dilworth, 1976) and many soil fungi (Ilag and Curtis, 1968).

Much work has been done to determine the metabolic pathways involved in the formation of ethylene by these micro-organisms. Lynch (1972), by amending a soil with glucose and methionine, isolated a fungus (Mucor hiemalis) and two yeasts (later identified as Candida vartivaari (13G) and Trichosporon cutaneum (5H)) (Lynch, 1975a) which produced ethylene when grown in pure culture. Mucor hiemalis is a common soil organism and it was found (Lynch and Harper,1974a) that methionine acts as a substrate for ethylene production, but maximum levels were only obtained when glucose was present. Production was also promoted by oxygen and it was suggested that the apparent need for anaerobic conditions in the soil was due to the fact that anaerobiosis released the substrates needed for ethylene production. In pure culture part of the process is believed to be extra-cellular (Lynch, 1974) and favoured by low fungal growth rates which are likely to occur in the soil (Lynch and Harper, 1974b).

Not all workers agree that Mucor hiemalis is the major ethylene producer in the soil; Smith (A.M.) and Cook (1974) found that heating the soil to 80°C prior to incubation promoted ethylene production, but M. hiemalis is killed at 60°C. Lynch (1975a) believes this is because the fungus produces an intermediate which can breakdown non-enzymically to ethylene, a process enhanced by high temperatures.

Primrose (1976b) and Primrose and Dilworth (1976) have shown that methionine can act as a substrate for ethylene formation in many bacteria. Furthermore, Primrose (1976a, 1977) has shown that in Escherichia coli ethylene formation occurs via an intermediate, 2 keto-4 methyl thiobutyric acid, which can be converted aerobically by peroxidase to ethylene. In cell free extracts this substrate can be converted aerobically or anaerobically to ethylene in the presence of flavin and light or in the presence of Fe^{3+}, Mn^{++} and Ca^{++}.

Considine and Patching (1975) have isolated a Penicillium sp. from blanket peat which produces ethylene when grown on a medium with phenolic acids as the sole source of carbon. Ethylene production appears to be by a process similar to that in Penicillium digitatum but differing from that observed in Mucor hiemalis and Escherichia coli, methionine failing to enhance production. However, as with these latter organisms, ethylene production by the Penicillium sp. is favoured by the presence of oxygen, suggesting that, under laboratory conditions, production of ethylene by fungi and bacteria

is an aerobic process. Phenolic acids in the soil are formed as the result of the decomposition of lignin and humic acid (Henderson, 1953; Ishikawa et al, 1963; Hurst and Burgess, 1967) and may affect germination and root growth in cereals (Stevenson, 1967). They are also believed to have a fungistatic effect. It now appears that these effects may be related to production of ethylene as a result of the break down of these phenolic acids by micro-organisms.

The possible effects ethylene in the soil can have on other micro-organisms has been shown by Smith (A.M.) (1973) who found that there was a correlation between the ability of a soil to produce ethylene and its ability to inhibit fungal germination (fungistasis). That a volatile unsaturated hydrocarbon might be involved has been suggested by other workers (Balis and Kouyeas, 1968; Watson and Ford, 1972). In later work, Balis (1976), although agreeing with the observation that ethylene causes fungistasis points out that it is not ethylene but an ethylene induced compound which may be allyl alcohol, which is the main inhibitor. Romine and Baker (1973) have shown that the inhibitory action of a volatile fungistatic factor (Hora and Baker, 1970) can be annulled with appropriate nutrients. Smith (A.M.) (1976b) has suggested that this is the reason other workers (Cornforth, 1975; Lynch, 1975a; Primrose, 1976b) have not observed inhibition of fungal colonies in the presence of ethylene.

III.4. Ethylene in the soil

Neilson Jones (1935) found that when plants were treated with vapour from heated waterlogged soil the plant growth was affected in a way similar to that caused by ethylene. Plants grown under waterlogged conditions also show symptoms similar to ethylene treated plants (Kramer, 1951; Jackson, 1955, 1956). That ethylene as well as other gaseous hydrocarbons occurs in the soil was shown by Juranek (1959) and Smith (G.H.) and Ellis (1963). In later studies on ethylene in the soil (Smith (K.A.) and Russell, 1969) it was found that ethylene could reach levels which were known to affect root growth. Levels of up to 10 ppm were observed in some soils and it was shown that in barley 1 ppm levels were sufficient to reduce root elongation by about 50%, while 10 ppm inhibited it completely. Other species were less sensitive to ethylene, 10 ppm causing only a 25% reduction in root elongation in rice and 40% in rye, while concentrations up to 1 ppm actually caused an increase with both species (Smith (K.A.) and Robertson, 1971).

Further work on the factors which effect ethylene formation in the soil has suggested that ethylene is the result of enzyme activity and the rate of production can be related to organic matter content (Smith (K.A.) and Restall, 1971). These workers also found that drying and rewetting of soil affects ethylene production, as does the type of crop grown or additions of different forms of organic matter, ethylene production being

stimulated by growth of wheat (Smith (K.A.) and Restall, 1971), additions of barley residues (Lynch et al, 1975), hay and to a lesser extent farmyard manure and peat (El Karouri, 1974). The addition of glucose or peptone increases the rate of production, as does raising the temperature of the soil, but phosphate and sulphate have little effect (Smith (K.A.) and Restall, 1971). Production is depressed by high concentrations of nitrate (Smith (K.A.) and Restall, 1971; Sheard and Leyshon, 1976; Smith (A.M.), 1974; 1976a,b). Nitrate is also known to depress the formation of another hydrocarbon, methane (Takai et al, 1956; Yamane, 1957; Laskowski and Moraghan, 1967; Bell, 1969; Bollag and Czlonkowski, 1973).

Gas samples taken in the field with specially designed sampling probes (Dowdell et al, 1972) showed that ethylene production was very variable both with time and between neighbouring sites. But the levels found, between 0.5 and 5 ppm, were sufficient to effect plant growth in laboratory experiments. Smith (A.M.) (1976) also found that ethylene concentrations varied with time and from probe to probe, concentrations of 0.03-0.6 ppm were observed in the topsoil of both cultivated and uncultivated sites. Field studies by Bell (1975) over a three year period using a probe similar to that of Dowdell et al (1972) gave lower levels of ethylene, the highest being 1.05 ppm while the level most generally observed was 0.1 ppm. Low temperatures during the winter period and the generally low moisture content in the warmer months due to very dry summers are

thought to have prevented the formation of anaerobic conditions. On the few occasions when ethylene was detected over a period of several weeks, soil temperatures and soil moisture content were high.

Primrose and Dilworth (1976) examined water obtained from waterlogged soil in fields in which the wheat crop showed possible ethylene symptoms. Analysis showed that the water contained ethylene concentrations ranging from 0.2 to 2 ppm. Lill and McWha (1976) have shown that on incubation the litter layer from forest soils under Pinus radiata produces ethylene and suggested that this may be partially responsible for the sparse undergrowth in P. radiata forests.

Further studies by Smith (K.A.) and Dowdell (1974) showed a relationship between high moisture content and ethylene production in a sandy loam. In two clay soils ethylene levels increased with temperature during the spring. They have suggested that the factors which have greatest influence on ethylene concentrations in the field are temperature, depression of oxygen concentration, availability of substrates for microbial activity and the effect of moisture content on the air-filled porosity of the soil. Thus ethylene levels sufficient to affect root growth are most likely to occur in heavy soils during the spring and early summer and in light soils if they are particularly wet.

Another factor which may affect the concentration of ethylene in soil is its decomposition by soil micro-organisms. Breakdown of ethylene by micro-organisms

is not fully understood but co-oxidation of ethylene occurs in two bacteria, one methane utilizing (de Bont and Mulder, 1974), one ethane utilizing (Davis et al, 1956). It has been shown that the soil can absorb low concentrations of ethylene but this ability is lost after sterilization (Abeles et al, 1971; Smith (K.A.) et al, 1973). Yoshida and Suzuki (1975) have also shown that ethylene is degraded in the soil; lowland rice soil which has been continuously submerged showed greatest activity. The micro-organisms of the rhizosphere were thought to be responsible for this activity. Similarly Cornforth (1975) has shown that ethylene is decomposed in soil, but only in aerobic conditions. A small group of aerobic micro-organisms are involved and their activity is slowed at concentrations of carbon dioxide greater than 5%. An aerobic heterotrophic bacterium probably of the genus Mycobacteria which is capable of growing on ethylene exclusively has been isolated by de Bont (1975). As in the breakdown of higher unsaturated hydrocarbons (Klug and Markovetz, 1971) ethylene oxide appears to be a possible intermediate.

The occurrence of ethylene in the soil is therefore a complex process being affected by many factors, including rates of production and utilization, availability of substrates, and the physical condition of the soil (temperature, moisture content and structure), which will affect not only microbial activity but movement through the soil of those gases produced.

EXPERIMENTAL

Section 1.

Laboratory Incubation Experiments

Introduction

In earlier studies of the occurence of ethylene in soil (pp 27-30), a relationship with organic matter had been established, and added organic residues had been found to have a stimulatory effect. However relatively few soils had been studied. In the present work a much wider range of soil samples were studied in an attempt to find relationships between ethylene production and organic matter content. The effect of cultivation and depth on these relationships was also investigated. Different forms of organic matter were added and the effects studied, as were the effects of individual substrates.

Another factor known to affect ethylene evolution is the presence of high levels of nitrate (Smith (K.A.) and Restall, 1971; Smith (A.M.) and Cook, 1974; Smith (A.M.), 1976). Nitrate levels in the soils used were therefore determined and compared with ethylene production. Amendments with nitrate were made at concentrations higher than those found naturally in the soil.

As a result of these studies it was hoped to elucidate some of the factors influencing ethylene in the soil.

Materials and Methods

Sampling Soil samples were collected from 17 sites in Eastern Scotland chosen so as to cover a wide range of organic matter contents. Where possible, samples of

each soil type were taken from an arable and a grass site. Descriptions of the soils are given in Table 2. A more detailed description can be obtained from the Soil Survey Memoirs for: The country around Kilmarnock (Mitchell and Jarvis, 1956), The country around Haddington and Eyemouth (Ragg and Futty, 1967), The country around Perth, Arbroath and Dundee (Laing, 1976). Soil No. 6 although mapped as Darlieth was found to be Sauchieburn series, similarly soil No. 9 was part of the Queensferry complex (Duncan, 1977).

In preliminary experiments samples were collected in winter (February), sampling for the main experiment, incorporating additional sites, was then carried out in the following spring (May). Samples were taken from three depths:- top soil samples (0-10 and 10-20 cm) and a subsoil sample (30+ cm). The exact depth of the subsoil sample varied, being taken from below the plough depth in cultivated soils and from the B horizon in undisturbed soils. All soils were stored at 5°C until required.

Incubation Procedure Incubation experimants were carried out in polypropylene centrifuge tubes sealed with size 57 Suba Seal (turnover type) vaccine closures using fresh, air dried (at 30°C for 24 hours) and oven dried (at 105°C for 24 hours) samples of each soil. All incubations were carried out at 20°C and in duplicate, except in the nitrate addition experimant which was performed in triplicate. Before incubation the dried soils (<2 mm) were rewetted with distilled water to about 25% moisture content, a sample then being taken for an accurate determination of

Table 2. Soils used in the study of ethylene formation

* See Text

Soil	Description	Association	Series	Land Use
1	Freely drained brown earth	Eckford	Eckford	grass upland (240 m)
2	Freely drained brown earth	Sourhope	Sourhope	grass upland (240 m)
3	Freely drained brown earth	Sourhope	Sourhope	arable (150 m)
4	Imperfectly drained brown earth	Sourhope	Bellshill	arable (105 m)
5	Freely drained brown earth	Darlieth	Darlieth	grass upland (240 m)
6	Freely drained brown earth	Darlieth	Darlieth*	arable (340 m)
7	Freely drained brown earth	Darvel	Darvel	grass permanent pasture (45 m)
8	Freely drained brown earth	Darvel	Darvel	arable (60 m)
9	Imperfectly drained noncalcareous gley	Stirling	Cauldside*	grass permanent pasture (30 m)
10	Imperfectly drained noncalcareous gley	Stirling	Cauldside	arable (45 m)
11	Poorly drained noncalcareous gley	Rowanhill	Rowanhill	grass permanent pasture (210 m)
12	Poorly drained noncalcareous gley	Rowanhill	Rowanhill	arable (195 m)
13	Imperfectly drained noncalcareous gley	Rowanhill	Winton	grass ley (165 m)
14	Imperfectly drained noncalcareous gley	Rowanhill	Winton	arable (150 m)
15	Imperfectly drained brown earth	Rowanhill	Macmerry	grass ley (180 m)
16	Imperfectly drained brown earth	Rowanhill	Macmerry	arable (165 m)
17	Basin Peat			grass permanent pasture (150 m)

the percentage water in the sample, this figure being required in the interpretation of the gas analysis results. The weight of soil, the particle density and the moisture content being used to calculate the volume of gas in each tube and thus the concentration of both gaseous and dissolved ethylene produced by each soil.

After filling and sealing, each polypropylene tube was flushed with oxygen-free nitrogen to remove the oxygen and incubated at 20°C. Various gases have been used to achieve anaerobiosis: helium (Agundis, 1966; Parr et al, 1970), hydrogen (Braunberg and Beck, 1968; Smith (A.M.) and Cook, 1974), argon (Connell and Patrick, 1968; Parr et al, 1970; Smith (A.M.) and Cook, 1974), nitrogen (Hill and McCarty, 1967; Parr et al, 1970; Smith (K.A.) and Restall, 1971; Smith (A.M.) and Cook, 1974), and carbon dioxide (Stotzky and Goos, 1965). Parr and coworkers (1970) found that there was a greater respiratory activity in soils incubated in nitrogen compared with those incubated in helium and argon. Smith (A.M.) and Cook (1974), however, found that for ethylene production there was little difference between nitrogen, hydrogen and argon. Carbon dioxide was found at high levels to have an inhibitory effect on microbial activity (Stotzky and Goos, 1965). Hence nitrogen, being readily available and least expensive, was used through out in all experiments requiring anaerobiosis.

Gas samples were taken at intervals from each tube and analysed by gas chromatography (see Appendix) for hydrocarbons; oxygen levels were also measured to ensure

that anaerobic conditions were maintained. Kavanagh and Postgate (1970) found that ethylene was absorbed by rubber closures and could then be released in further experiments. In the present work, therefore, Suba Seals were discarded after use. Tests were carried out to see how much ethylene was absorbed from the polypropylene tube/rubber closure system. Sealed tubes were flushed with a standard gas mixture containing 10 vpm of ethylene and sampled after 1, 4 and 10 days. The results obtained are shown in Table 3. Slight absorption of ethylene did occur, an average of 1 vpm being lost from the system after 10 days; no correction for this loss has been used in any of the following results.

Table 3. Absorption of ethylene in polypropylene tubes sealed with rubber Suba Seals

Day	C_2H_4 (vpm)
1	9.98
4	9.12
10	9.02

Addition of Inorganic Forms of Nitrogen and Organic Compounds

Using the same incubation procedure the following were added as aqueous solutions (10 mls) to 40 g air dried soil No. 16 (0-10 cm) :-

Potassium nitrate	50, 100, 500, 1000, 2000 µg NO_3-N/g soil
Ammonium sulphate	250, 1000, 2500 µg NH_4-N/g soil
Glucose)	
Ethanol)	
Casein)	
Pepsin)	2.5 and 25 mg/g soil
Lactic acid)	
Pyruvic acid)	
Butyric acid)	

Additions of 10 mg of air dried, finely ground hay, wheat straw and barley straw per g of soil were also made using the same incubation procedure.

Additions of ammonium sulphate, glucose, ethanol and butyric acid were also made to undisturbed soil cores, but these will be described in detail in Section 2.

Analytical Methods Analysis of the soils used for pH texture and organic matter and nitrate nitrogen was carried out in the laboratory using the following methods:-

a) pH This was measured in a 1:2.5 suspension of soil and water on an Electronic Instruments Ltd. pH meter 7020 using a standard combination glass electrode.

b) Texture Particle size analysis was carried out on air dried soil using the pipette method (Kilmer and Alexander, 1949), and the particles separated according to U.S.D.A. size limits:

Sand: 2 - 0.05 mm

Silt: 0.05 - 0.002 mm

Clay: less than 0.002 mm

The texture was then determined by reference to the appropriate soil texture diagram.

c) Organic matter content Organic carbon was determined by the modified Tinsley method (Bremner and Jenkinson, 1960) using 0.5 g air dried soil ground to pass through a 0.2 mm sieve. The obtained values were converted by multiplying by the factor 1.74, to organic matter content expressed as a percentage of oven dry soil.

d) Nitrate nitrogen Nitrate nitrogen (NO_3-N) was determined on fresh soil by the phenoldisulphonic acid method (Bremner, 1965) using 20 g soil. Chu and Hance (1939) found that air drying and oven drying had little effect on nitrate nitrogen except in two soils, where increases were observed with oven drying which were found to be due to the acceleration of the natural process of nitrification. In this work the analysis was therefore also carried out on 8 samples of air dried and oven dried soil.

Results and Discussion

Soil Analysis Results of laboratory analysis for pH, organic matter and texture are shown in Table 4. The results of particle size analysis for soil Nos. 5 and 6, and 9 and 10 clearly show that samples of soils Nos. 6 and 9 were taken from pockets of other series known to be intermingled with the soil series shown on the soil maps.

Table 4. pH, organic matter content and texture of soils used in incubation experiments

Soil	Depth cm	pH	OM%	Texture
1	0-10	5.8	6.1	SL
	10-20	5.6	3.8	SL
	30+	5.8	2.0	SCL
2	0-10	4.8	10.4	SL
	10-20	4.8	1.3	SL
	30+	5.2	0.4	SL
3	0-10	6.5	3.2	L
	10-20	6.5	1.0	L
	30+	6.5	0.8	ZL
4	0-10	5.9	3.4	SCL
	10-20	5.4	3.4	SCL
	30+	6.2	0.5	SCL
5	0-10	5.7	8.7	L
	10-20	5.8	3.6	SCL
	30+	6.0	1.9	SCL
6	0-10	5.0	7.4	L
	10-20	4.2	2.3	ZCL
	30+	4.6	1.8	ZCL
7	0-10	6.6	6.7	SL
	10-20	5.8	6.0	SL
	30+	6.1	1.7	SL
8	0-10	5.5	6.4	SL
	10-20	5.4	6.2	SL
	30+	5.0	5.9	SL
9	0-10	6.9	4.1	SL
	10-20	6.4	4.2	SL
	30+	6.6	2.0	SCL
10	0-10	7.0	5.1	L
	10-20	7.2	5.1	ZCL
	30+	7.3	2.3	ZC
11	0-10	6.1	10.3	SCL
	10-20	5.4	5.6	SCL
	30+	5.2	5.7	SCL
12	0-10	6.8	2.5	SCL
	10-20	6.7	2.0	SCL
	30+	6.3	1.7	SCL
13	0-10	6.2	5.3	SCL
	10-20	6.2	5.1	SCL
	30+	6.5	1.3	SCL
14	0-10	6.3	6.6	SL
	10-20	6.2	3.3	SCL
	30+	5.9	1.0	SCL
15	0-10	6.0	6.0	SL
	10-20	6.3	5.0	SL
	30+	6.2	0.8	SL
16	0-10	6.4	4.8	SL
	10-20	6.0	4.8	SL
	30+	6.3	1.2	SL
17	0-10	6.4	84.2	-

S = Sand; Z = Silt; C = Clay; L = Loam

Soil Ethylene Production Preliminary experiments taking several samples from a given site showed some variability in ethylene production (Table 5.), however it was decided that samples for further studies would be taken from a single point at each site..

Table 5. Variability of ethylene production in air-dried soils with sampling point

Soil	C_2H_4 (ng/g)	
	individual samples	mean
3	13.4 7.4	10.4
13	6.5 3.6 7.7 8.4	6.6
14	8.8 8.7 10.3	9.3
15	14.7 15.9	15.3
16	8.5 9.3 13.7 16.5	12.0

As the experimental data in this work took the form of a skew distribution it was necessary to transform the data to a normal distribution by using the logarithms of the ethylene values before statistical analysis could be carried out.

Comparisons were made between the ethylene levels observed in the nine soils sampled in February and those

observed in soils collected in May from sites as near as possible to the first (within 2-3 sq m) (Table 6.) The differences between the ethylene levels observed were very variable.

Table 6. Total ethylene evolved after 10 days of incubation in 9 fresh soils sampled in February and May

Soil	C_2H_4 (ng/g soil)	
	February	May
1	0.01	< 0.01
2	29.4	7.9
3	1.8	0.82
4	0.21	0.60
12	0.02	< 0.01
13	0.02	< 0.01
14	2.5	1.9
15	5.8	5.4
16	0.37	2.8

Ethylene levels observed in the February soil samples on incubation after collection and after storage for 10 weeks at 5°C are shown in Table 7. There was no significant difference between the two treatments ($p>0.9$). Thus soil samples could be stored at 5°C until required without affecting the capacity of the soil to produce ethylene.

Table 7. Total ethylene evolved after 10 days of incubation in fresh soils and soils stored in moist conditions at 5°C

Soil	C_2H_4 (ng/g soil)	
	Fresh	Stored
1	0.01	0.40
2	29.4	26.4
3	1.8	1.0
4	0.21	0.31
12	0.02	0.01
13	0.02	0.08
14	2.5	2.0
15	5.8	7.3
16	0.37	1.1

The quantities of ethylene evolved by all soils after 1 and 10 days of incubation are shown in Tables 8 and 9. Both air and oven drying caused a significant increase in ethylene production. There was no significant difference ($p = 0.2$) between the two drying treatments in total ethylene production over the ten day period but the initial rate of evolution (0-1 day) was increased by oven drying, significantly higher levels ($p<0.1$) being obtained on the first day of the incubation in oven dried soils than in the air dried soils (Table 8), oven dried soils achieving levels of ethylene production similar to their 10-day value after only 1 day.

Table 8. Ethylene evolved from fresh, air and oven-dried soils after 1 day of anaerobic incubation

Soil	Ethylene (ng/g soil)								
	Fresh			Air Dried			Oven Dried		
	depth (cm)			depth (cm)			depth (cm)		
	0-10	10-20	30+	0-10	10-20	30+	0-10	10-20	30+
1	0.01	0.01	0.01	2.1	0.6	0.3	9.1	5.2	1.9
2	0.8	0.15	0.01	4.5	0.9	0.1	12.6	4.1	0.4
3	0.1	0.02	0.01	0.2	0.1	0.5	2.9	2.1	0.7
4	0.2	0.01	0.01	0.6	0.4	0.2	5.0	5.1	0.4
5	0.2	0.1	<0.01	5.4	1.1	0.07	5.8	3.7	0.9
6	2.6	0.02	0.01	11.0	0.2	0.2	5.8	0.6	2.4
7	2.1	0.01	<0.01	9.9	3.5	0.3	12.0	9.8	2.3
8	0.02	0.3	0.2	2.5	2.5	2.1	8.9	11.0	8.1
9	0.1	0.01	<0.01	1.8	3.1	0.08	9.6	3.2	0.6
10	<0.01	<0.01	<0.01	0.4	0.07	0.02	2.1	1.5	0.4
11	0.02	0.04	0.01	12.1	2.0	0.6	32.7	9.7	2.2
12	0.01	0.01	<0.01	0.9	1.0	0.01	6.5	4.8	0.7
13	0.05	0.07	<0.01	1.4	1.0	<0.01	5.1	3.3	0.8
14	1.15	0.35	0.01	0.8	0.4	0.09	4.5	3.3	1.1
15	1.9	1.0	0.01	1.5	1.4	0.2	3.2	3.3	0.7
16	0.2	0.3	0.01	1.1	1.6	0.09	11.8	6.0	0.6
17	<0.01	n.d	n.d	1.0	n.d	n.d	19.3	n.d	n.d

Table 9. Total ethylene evolved from fresh, air and oven-dried soils after 10 days of anaerobic incubation

Soil	Ethylene (ng/g soil)								
	Fresh			Air dried			Oven dried		
	depth (cm)			depth (cm)			depth (cm)		
	0-10	10-20	30+	0-10	10-20	30+	0-10	10-20	30+
1	0.01	0.01	0.01	10.1	4.0	0.9	9.8	5.8	1.7
2	7.9	1.5	0.01	29.5	4.0	1.2	13.5	4.5	0.6
3	0.8	0.01	< 0.01	4.3	0.5	2.4	3.3	2.6	0.8
4	0.6	< 0.01	< 0.01	7.5	7.3	0.7	5.4	5.5	0.4
5	< 0.01	< 0.01	< 0.01	12.8	3.7	0.6	10.5	4.3	1.2
6	1.6	0.01	0.01	30.0	0.8	0.6	7.7	1.0	3.4
7	1.5	0.9	< 0.01	17.8	13.4	1.5	13.2	11.4	3.2
8	2.1	0.5	1.5	17.6	17.7	15.2	10.5	12.1	8.9
9	0.5	0.9	0.01	5.1	8.4	0.4	5.6	4.5	1.0
10	< 0.01	0.01	< 0.01	1.9	1.7	1.0	3.2	2.2	1.0
11	0.01	0.01	< 0.01	24.2	3.3	1.3	37.2	10.6	3.3
12	< 0.01	< 0.01	< 0.01	2.2	2.8	0.3	7.6	6.8	1.2
13	< 0.01	< 0.01	< 0.01	6.5	2.2	0.5	6.3	4.6	1.3
14	1.9	2.4	0.01	2.2	2.1	0.2	5.9	4.3	1.9
15	5.4	3.9	< 0.01	13.9	13.1	2.3	4.7	4.7	1.0
16	2.8	0.5	< 0.01	11.9	13.6	1.4	6.9	7.3	0.9
17	1.0	n.d	n.d	8.9	n.d	n.d	19.8	n.d	n.d

Birch (1958) found that oven-drying resulted in much greater microbial activity of rewetting than air-drying. He found that drying did not affect the inherent decomposability of humus so that the effect of oven drying was due mainly to microbial changes. In a second paper (1959) Birch concluded that the differences were due mainly to liberation of water-soluble organic material, and also perhaps to the high metabolic activity associated with the physiological growth of the micro-flora, which on wetting begin to germinate and multiply. The activity declines as the culture ages and passes into the resting stage. It seems probable that the rapid evolution of ethylene observed in oven dried soils in the present work is the result of a similar cycle of microbial activity.

It has been suggested (Curran and Evans, 1947; Funke and Harris, 1968) that heat shock stimulates spores to germinate rapidly, and this results in a greatly increased respiratory activity. Smith (K.A.) and Restall (1971) studied the evolution of ethylene from the soil under different drying conditions (20°C and 35°C) and found that the pattern of ethylene evolution appeared to be compatible with the changes observed under similar conditions by Birch (1958, 1959). Other workers (Smith (A.M.) and Cook, 1974) found that heat treatment with aerated steam at 60°C prior to aerobic incubation promoted ethylene production but aerated steam at 82°C caused a reduction. Heat treatment of soil slurries at 80°C in a water bath before incubation enhanced production

compared with non-treated slurries. They suggested that these results showed that ethylene in the soil was not produced primarily by Mucor hiemalis as postulated by Lynch and Harper (1974a), because this fungus is killed by aerated steam at temperatures of 60°C and above. Lynch (1975a) confirmed that heat treatment of the soil enhanced ethylene production and also showed that ethylene production was enhanced by heat (100°C) in pure cultures of M. hiemalis even though the fungus itself was killed. Observations from his earlier work (Lynch, 1974) led him to suggest that a possible explanation was that M. hiemalis formed an intermediate in the pathway to ethylene which passed into the extracellular solution and was broken down non-enzymically, this process being promoted by heat at 100°C. Lynch and Harper (1974b) had shown that ethylene production was greatest during the periods of low fungal growth rates; lysis of fungal cells also caused an increase in ethylene production (Lynch and Harper, 1974a). Lynch (1975a) suggested that both these factors might be involved in the observed enhancement of ethylene production by heat, heat decreasing the fungal growth rate followed by cell lysis. The present work shows that in the range of soils studied initial enhancement of ethylene production as a result of oven drying is a general phenomenon, however, this high rate of production was not maintained throughout the incubation, unlike the situation in pure culture observed by Lynch (1975a). In the peat soil (No.17) and also the humic topsoil of soil No. 11, oven drying had a much greater effect on total ethylene

production than air drying. This may be due to the presence of different organic compounds in peat soils and associated alteration in the microbial population. Considine and Patching (1975) have isolated a Penicillium sp. from blanket peat which is capable of utilizing phenolic acids and producing ethylene. This ethylene production appears to be by a physiologically different process from that observed by Lynch (1972) and Lynch and Harper (1974a), and Considine and Patching (1975) suggested different roles for the two fungi M. hiemalis and Penicillium sp. in soil ethylene production.

Smith (K.A.) and Restall (1971) showed that the quantity of ethylene evolved after 10 days was approximately proportional to the organic matter content of the soil. The relationship between ethylene production and organic matter content in the present work was investigated by calculating the regression equations and correlation coefficients for the following sets of data:-

fresh soils - 0-10 cm arable, 10-20 cm arable, 30+ cm arable
- 0-10 cm grass, 10-20 cm grass, 30+ cm grass
- all depths arable
- all depths grass
- all depths arable + grass

air dried soils - as above

oven dried soils - as above

Calculation of the correlation coefficients for total ethylene production up to days 1, 2, 4, 7 and 10

respectively showed that there was a linear relationship with organic matter which increased in statistical significance up to day 7, which was similar to the relationship for day 10 because there was little change in ethylene production during the final three days. Figure 1. shows a typical change in ethylene concentrations during anaerobic incubation of a soil. Analysis showed that all soils behaved similarly in this respect. Hence only the comparisons and relationships relating to results for total ethylene production after 10 days are discussed below.

The relationship between total ethylene concentrations on day 10 and organic matter content is shown in Figures 2, 3 and 4 (mineral soils only), the significance of these relationships is shown in Table 10. Statistical comparison of the regression equations for fresh, air and oven dried soils showed that in fresh soils the relationship between ethylene production and organic matter was different to that in the dried soils. The total ethylene production had been increased by drying the soil prior to incubation, air drying at 30°C and oven drying at 105°C having similar effects, no significant difference between regressions for the dried soils being found.

In general examination of a soil profile with depth reveals three main layers, the A, B and C horizons. The C horizon is the parent material of the soil and has little or no biological activity, unlike the two upper horizons. The A horizon, the surface soil, is the layer of greatest biological activity containing an abundance

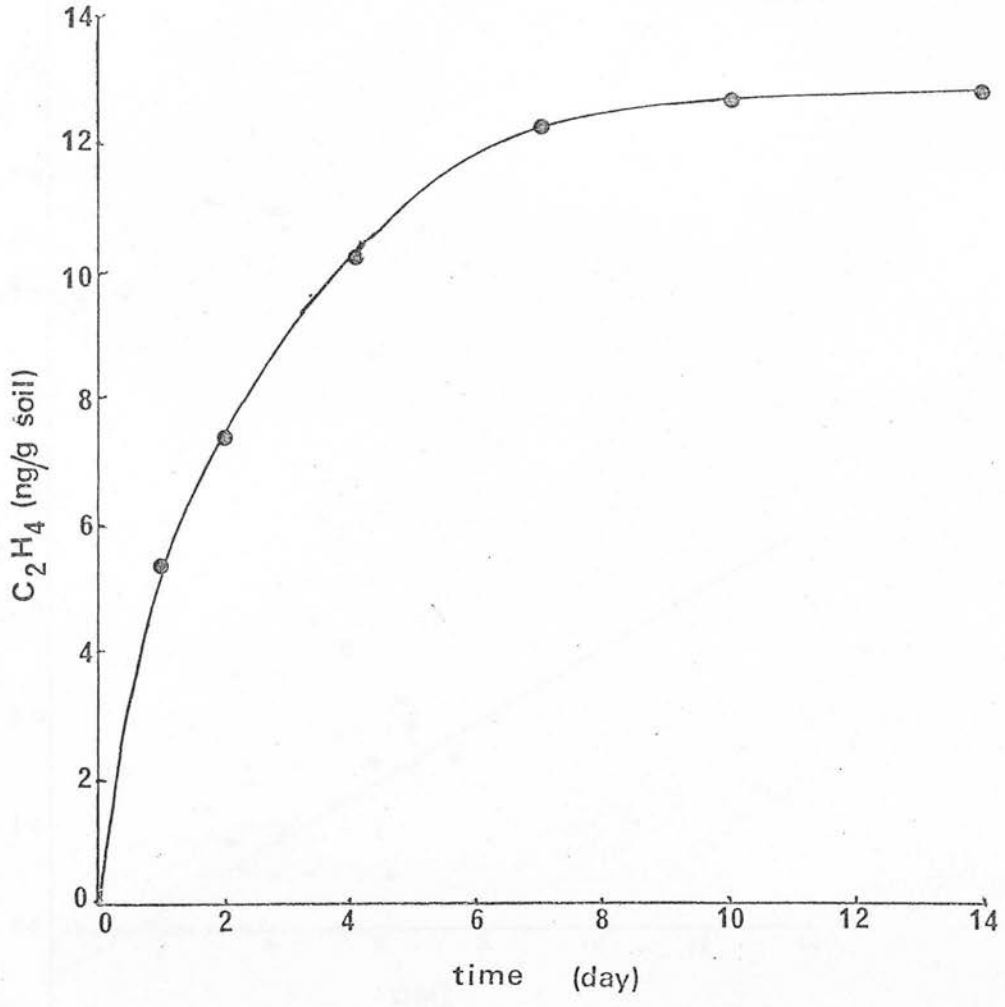

Figure 1. Typical change in ethylene concentration with time during anaerobic incubation

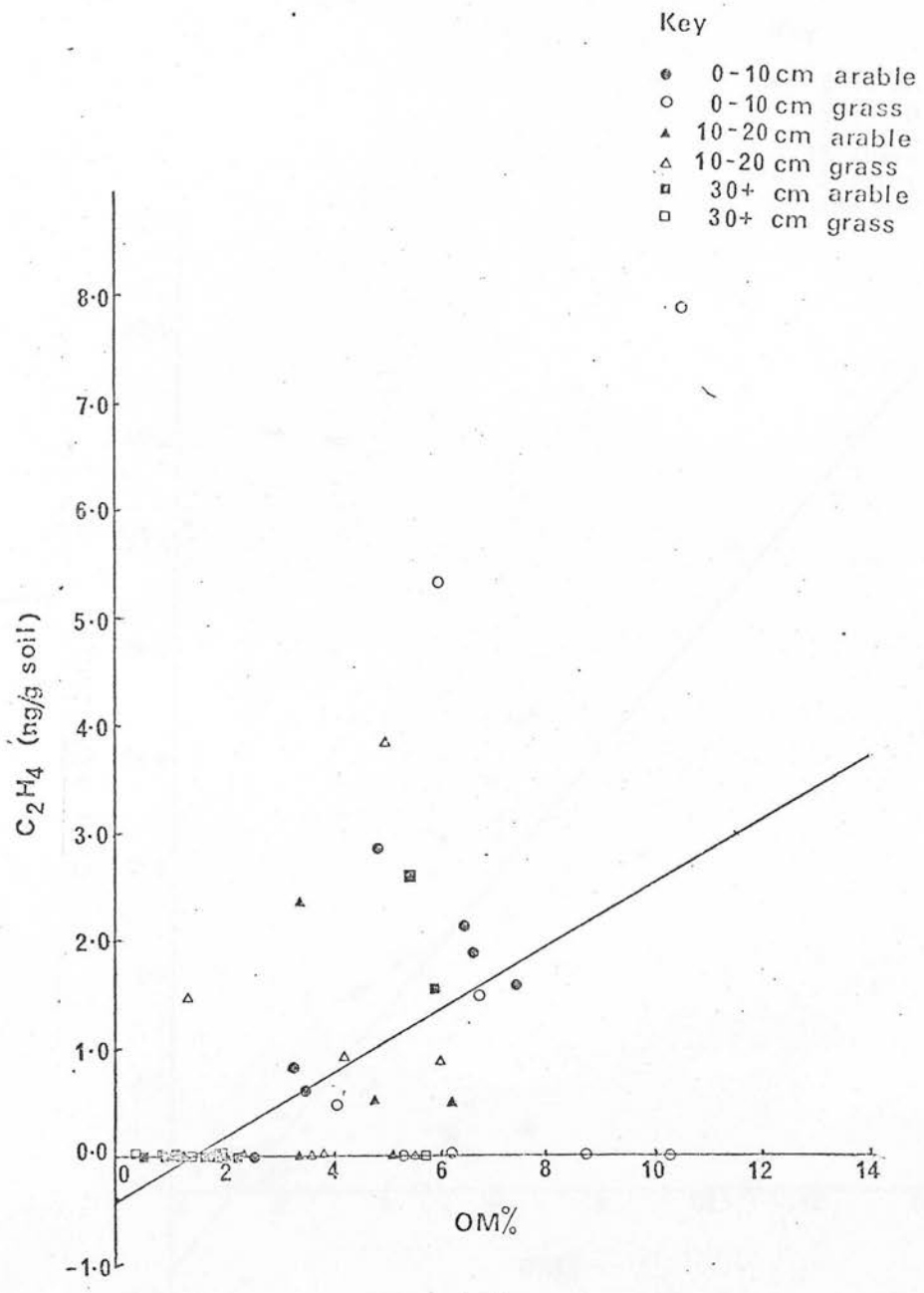

Figure 2. Relationship between organic matter content and ethylene production in fresh soil incubated anaerobically for 10 days

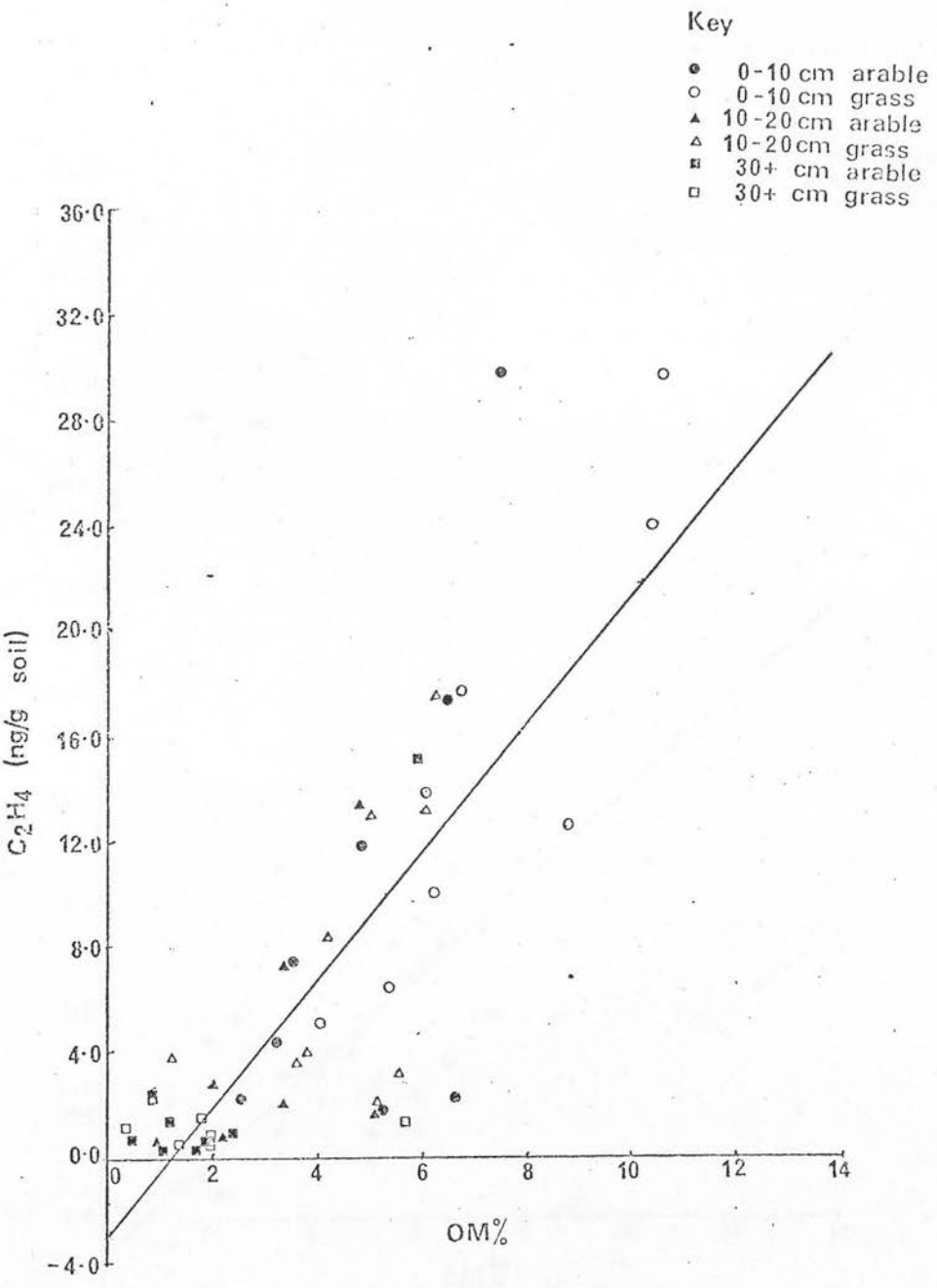

Figure 3. Relationship between organic matter content and ethylene production in air dried soil incubated anaerobically for 10 days

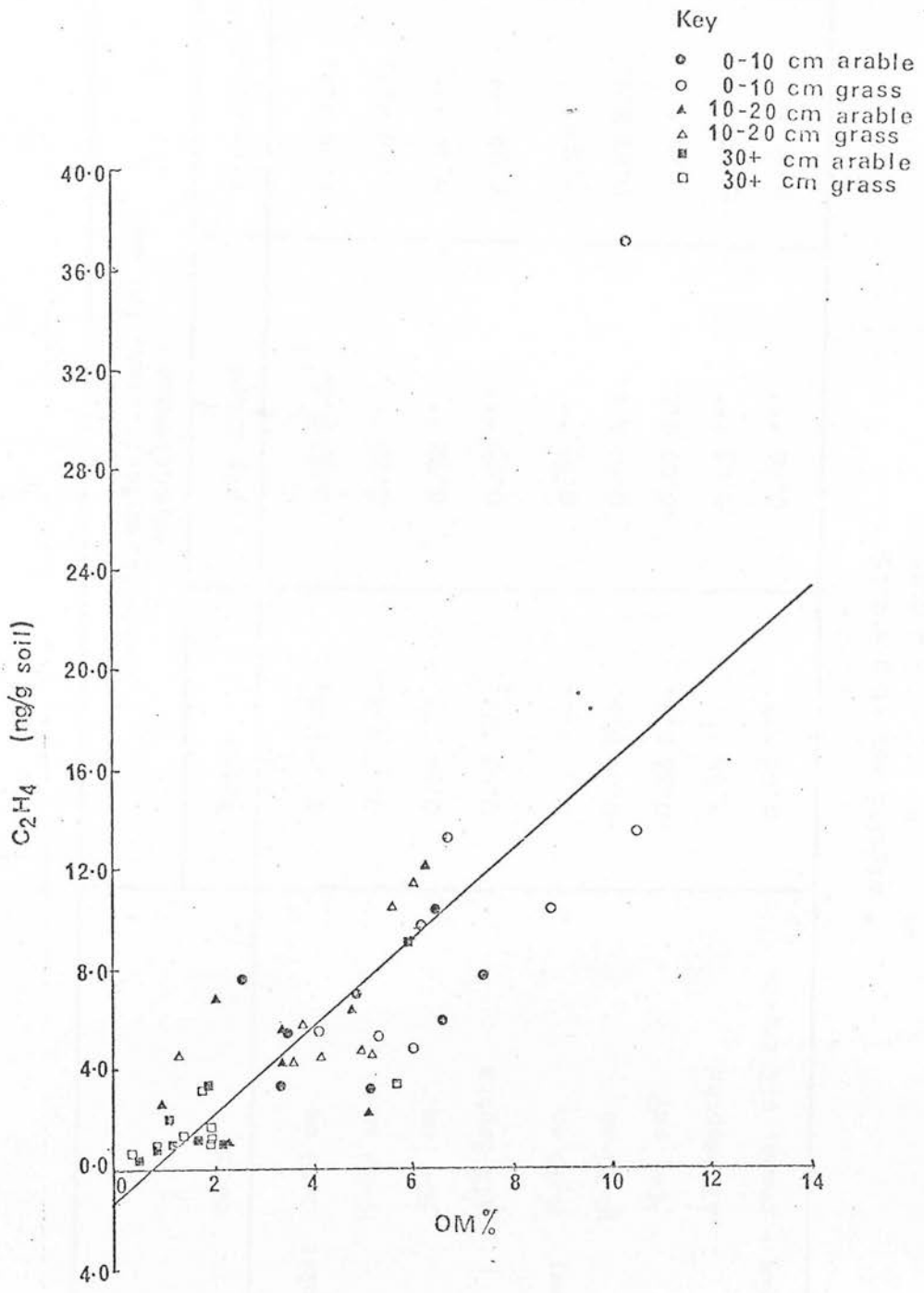

Figure 4. Relationship between organic matter content and ethylene production in oven dried soil incubated anaerobically for 10 days

Table 10. Relationship between ethylene evolved by soils after anaerobic incubation for 10 days, and the organic matter content

Soil	Correlation coefficient (r) and significance		
	Fresh	Air dried	Oven-dried
Arable: 0-10 cm	0.58 N.S.	0.63 N.S.	0.38 N.S.
10-20 cm	0.16 N.S.	0.75 *	0.59 N.S.
30+ cm	0.94 ***	0.92 **	0.94 ***
All depths	0.63 **	0.75 ***	0.74 ***
Grass: 0-10 cm	0.34 N.S.	0.89 **	0.70*
10-20 cm	-0.04 N.S.	0.40 N.S.	0.61 N.S.
30+ cm9	-0.28 N.S.	-0.07 N.S.	0.74 *
All depths	0.44 *	0.85 ***	0.77 ***
Arable + grass, all depths	0.48 ***	0.80 ***	0.75 ***

* significant at p = 0.05
** " " p = 0.01
*** " " p = 0.001

of roots, micro-organisms and even small animals. Plant and animal remains in the soil act as a source of nutrients for microbial activity and the resultant organic matter content is greatest is this layer. The B horizon, the subsoil underlying the A horizon, has little organic matter, few plant roots and a low level of microbial activity. To examine the effect of these changes on ethylene evolution at different depths in the soil, a comparison was made between the total ethylene produced, after incubation for 10 days, in the 0-10 cm samples and the 30+ cm samples of the mineral soils. Less ethylene was evolved by the subsoil samples (Table 9.) and this difference was found to be significant at the $p = 0.02$ level in fresh soils. Air drying enhanced the difference between the two depths so that the statistical significance increased ($p < 0.001$).

Comparisons were then made between the ethylene-organic matter regressions at each depth. It was found that depth had no effect on the relationship with organic matter, the difference between the regression line for 0-10 cm and the subsoil being stastically insignificant. The difference between the ethylene evolved from the two depths is therefore not due to a difference in the type or effectiveness of the organic matter but due to the reduced level of organic matter in the subsoil.

Organic matter in the soil is the result of both degradation and synthesis by micro-organisms and is being continually transformed. The rate of formation can be related to the structure and fertility of the soil and

also the level of biological activity. In undisturbed soil an equilibrium is established and the organic matter content remains relatively constant (Stevenson, 1964). Cultivation may destroy this equilibrium by altering the rate of carbon addition by crop removal and the rate of its mineralisation to carbon dioxide due to changes in the aeration status (Alexander, 1961). Addition of fertiliser may also affect microbial activity, in particular the micro-organisms involved in the evolution of ethylene are believed to be adversely affected by high levels of nitrate (Smith (A.M.) and Cook, 1974). Micro-organisms also differ in their sensitivity and tolerance to variations in pH, hence liming may alter the composition of the microbial population. In the soils used there were some differences in pH and nitrate content between arable and grass sites of the same soil type. The organic matter contents appeared to be unaffected by cultivation except between soils Nos. 2 and 3 and Nos. 11 and 12, in which the two grass sites had higher organic matter contents in the 0-10 cm depth samples.

A comparison was made between the ethylene concentration after 10 days of incubation for arable sites and grass sites of the same soil type. Contrary to the observations of Smith (A.M.) and Cook (1974), there was found to be no significant difference. Similarly a comparison of ethylene/organic matter regressions for arable and grass sites showed no significant difference. In the soils studied, therefore, cultivation has had no effect on the evolution of ethylene by directly affecting

the microbial population or by affecting the relationship between ethylene evolution and organic matter content.

It was thought that the pH of the soil might affect ethylene production in view of its effects on microbial populations, fungal growth being favoured by low pH compared with bacterial growth (Griffin, 1972). Similar calculations of correlation coefficients and regression equations were carried out for ethylene concentrations after 10 days and pH as described for the ethylene/organic matter relationship (p 42). These showed that for fresh soils there was a negative relationship between ethylene concentration and the soil pH but this was not significant (Table 11.). In some cases calculation of partial correlation coefficients, where the effect of organic matter was eliminated, resulted in a decrease in the value of the correlation coefficient; in other cases there was no change. With air dried soils there was a highly significant negative linear relationship between ethylene concentration and pH at the 0-10 cm depth for arable soils ($p = 0.01$), however elimination of the effect of organic matter reduced this to a less significant level ($p = 0.05$). There was no significant relationship at either the 10-20 cm depth or in the subsoil (30+ cm). However, omission of the values for soil No. 6 (10-20 cm depth) which had a pH of only 4.2, 0.4 pH units lower than any other sample, and which evolved very little ethylene, increased the value of the correlation coefficient at that depth; elimination of the effect of

Table 11. Relationship between ethylene evolved by soils after anaerobic incubation for 10 days, and pH

Soil	Fresh		Air Dried		Oven Dried	
	Correlation coefficient	Partial correlation coefficient	Correlation coefficient	Partial correlation coefficient	Correlation coefficient	Partial correlation coefficient
Arable 0-10 cm	-0.48	+0.17	-0.90**	-0.83*	-0.60	-0.50
10-20 cm	+0.065	+0.06	-0.22	-0.35	-0.03	-0.05
10-20 cm (omitting pH 4.2)	-0.14	-0.002	-0.73	-0.77*	-0.68	-0.64
30+ cm	-0.48	-0.25	-0.43	-0.10	-0.67	-0.86*
all dpeths	-0.15	-0.39	-0.42*	-0.42*	-0.29	-0.21
all depths (omitting pH4.2)	-0.26	-0.26	-0.57**	-0.97***	-0.49*	-0.60**
Grass 0-10 cm	-0.62	-0.57	-0.63	-0.11	-0.10	+0.79*
10-20 cm	+0.22	+0.30	+0.42	+0.25	-0.22	-0.90**
30+ cm	-0.13	-0.27	-0.23	-0.28	-0.27	+0.02
all depths	-0.27	-0.24	-0.12	-0.02	-0.05	+0.09
Arable + grass) all depths	-0.20	-0.13	-0.30*	-0.27	-0.13	-0.007
) all depths (omitting pH 4.2)	-0.25	-0.16	-0.37**	-0.33**	-0.19	-0.04

* signficant at p = 0.05
** " " p = 0.01
*** " " p = 0.001

organic matter from the correlation increased the value of the correlation coefficient. Similar omission of this low pH value from the correlation for arable soils over all depths resulted in a highly significant relationship and calculation of the partial correlation coefficient further increased the significance (Table 11). The quantity of ethylene evolved per unit of organic carbon increased more than 5-fold as the pH fell from neutrality to pH 5.0 (Figure 5). However for the air dried grass soils there were no significant relationships between ethylene concentrations and pH.

A comparable difference in the relationship between ethylene evolution and soil pH was found for the oven dried samples. For the arable soils the relationship was insignificant, but elimination of the effect of organic matter at the 30+ cm depth increased the correlation to a significant level; omission of the sample with pH 4.2 from the correlation for all depths also increased the correlation to a significant level. For the grass soils a similar non-significant relationship was found, but on calculation of the partial correlation coefficients the relationship became significant and positive at the 0-10 cm depth, and highly significant and negative at the 10-20 cm depth (Table 11).

Thus, these results showed a clear difference between the arable and grass soils in the relationship between ethylene and pH. A comparison of the regressions obtained for arable soils at all depths (omitting the sample with

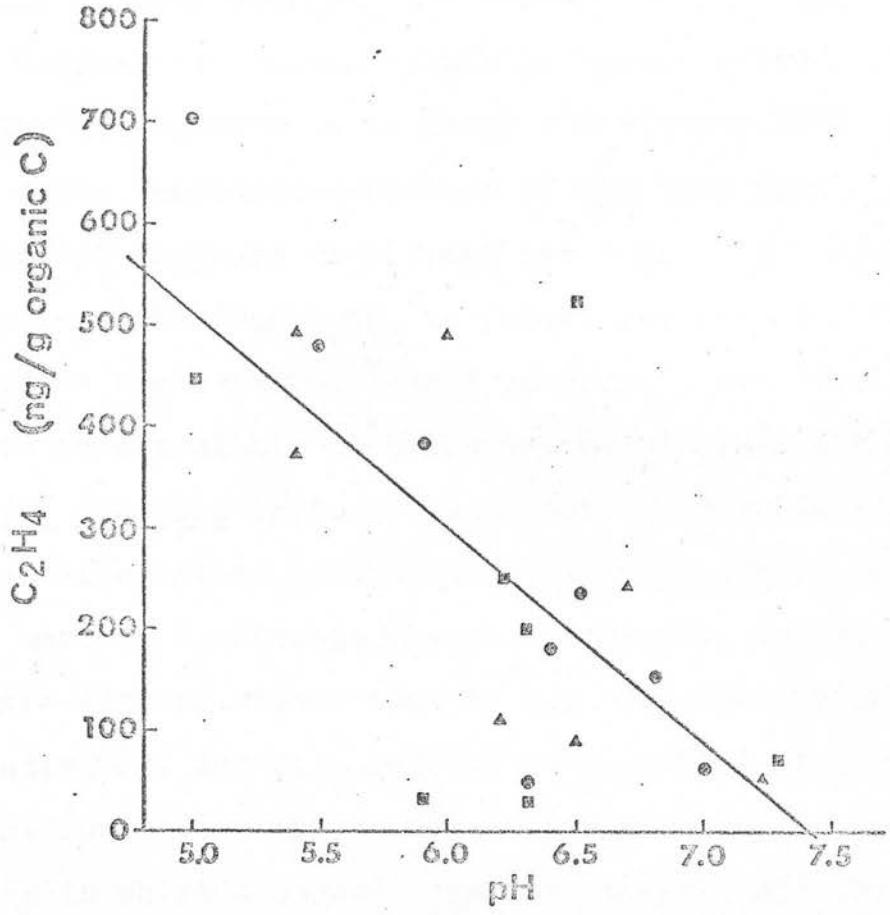

Figure 5. Relationship between ethylene evolved per unit organic carbon content, and pH, for air dried arable soils

●: 0-10 cm; ▲: 10-20 cm; ■: 30+ cm depth

pH 4.2) and grass soils at all depths showed that there was a significant difference between the regression lines irrespective of whether the soils were fresh, air or oven dried. This was in contrast to the comparison of regressions between ethylene and organic matter, which showed no difference between arable and grass sites. No explanation can be offered at present for these differences.

The increased evolution of ethylene from soils with a low pH suggests that fungi are a more likely source of ethylene than bacteria, as fungal numbers increase relative to bacteria under acid conditions (Griffin, 1972). This is compatible with the theory of Lynch (1972) that Mucor hiemalis is involved in soil production of ethylene. However ethylene production by M. hiemalis is favoured by aerobic conditions (Lynch and Harper, 1974a), although these workers showed that it can also occur under anaerobic conditions, but at a reduced rate. Thus the possibility that fungi were the source of ethylene in the present work in which anaerobic conditions were employed cannot be ruled out. A possibility for future work would be an examination of the effect of selective elimination of bacteria or fungi from a mixed population on the rate of evolution of ethylene.

The decrease in ethylene evolution with increased pH (Figure 5) suggests that well limed arable soils should be less liable to produce ethylene in concentrations sufficient to inhibit plant growth than unlimed soils. Hence apart from the direct beneficial effects of increasing

the pH of acid soils on crop growth and yields, liming may also have an indirect benefit for the crop if it inhibits the activity of ethylene producing micro-organisms, thus reducing one of the adverse effects on plant growth under anaerobic conditions. In soils under grassland this effect of pH is less well defined; this may be due to the inclusion of soils taken from peaty sites, it having already been suggested that these may behave differently compared with other soils (p 42).

Another factor of agronomic interest which was studied in detail was the addition of nitrate fertilisers. As already mentioned, other workers (Smith (A.M.) and Cook, 1974; Smith (A.M.), 1976ab) have reported that 20-200 ppm nitrate nitrogen inhibited ethylene production until denitrification was complete, and Smith (A.M.) (1976ab) has suggested that this may have wider implications in agriculture. He believes that there is a self-regulating oxygen-ethylene cycle in the soil which operates as follows: in areas of high microbial activity oxygen levels are reduced, leading to the development of anaerobic microsites in which ethylene will be formed by anaerobic micro-organisms. The diffusion of ethylene through the soil will result in the inhibition of aerobic micro-organisms and thus in a reduced oxygen demand. Oxygen will therefore diffuse back into the anaerobic microsites preventing anaerobic ethylene production; this permits the resumption of aerobic growth. Large inputs of nutrients temporarily annul the inhibitory

effects of ethylene enabling the decompostion of seasonal inputs of organic matter to proceed. Smith (A.M.) (1976b) has suggested that it is growth on nutrient rich media or lack of other microbial competition which has led other workers (Cornforth, 1975; Lynch, 1975a; Primrose, 1976b) to dispute the significance of ethylene in soil fungistasis. He further postulated that the inhibition of ethylene formation by nitrate fertilisers will result in an unbalancing of the oxygen-ethylene cycle. He also suggested that high nitrification rates may interfere with the availability of nutrients in reduced microsites, and consequently that conventional fertiliser practices should be replaced by minimum tillage, use of organic amendments and chemical inhibitors in order to improve the efficiency of crop production.

Contradictory evidence with regard to the effect of nitrate was provided by earlier work by Smith (K.A.) and Restall (1971), who had shown that ethylene was evolved throughout the period of anaerobic incubation (although at a reduced rate) in the presence of a much higher level of nitrate than would be found in the field (2000 ppm). In an attempt to resolve this question the production of ethylene was compared with the nitrate nitrogen present in the soils studied.

Low temperatures have long been used for the storage of fresh soil samples for inorganic nitrogen analysis. Gasser (1958) found that $-10^{o}C$ adequate to prevent change in ammonium and nitrate contents; Allen and Grimshaw (1962),

however, suggested that this was not satisfactory. In the present work changes in the nitrate contents of eight samples were studied. A temperature of 5°C appears to have been low enough to prevent changes in the nitrate content after storage for 8 weeks (Table 12). Similarly neither of the drying treatments caused increased nitrate concentrations due to the accelerated nitrification reported by Chu and Hance (1939). There were some changes but these were variable and relatively small consequently it was considered that for the purposes of studying the effect of nitrate content on ethylene evolution, the concentration of nitrate nitrogen could be assumed to be the same in fresh, air and oven dried treatments. So the values obtained for fresh unstored samples were used throughout.

Ethylene levels on the first day of the incubation (Table 8) were used for studies of nitrate effects, as nitrate might be expected to have most effect on ethylene evolutions at the beginning of the period of anaerobic incubation, before loss of nitrate by denitrification has occured.

In some individual soils it was possible to see a decrease in the ethylene evolution associated with high nitrate concentration. For example 2.14 and 0.02 ng ethylene/g soil were evolved from soils Nos. 7 and 8, which had nitrate contents of 12.8 and 29.0 µg/g respectively, showing that at the higher nitrate level ethylene production had been inhibited. However statistical analysis showed that for all soils there was no

Table 12. Nitrate-nitrogen content of fresh soils at all depths, and soils from 0-10cm stored moist, air dried and oven dried

NO_3-N (µg/g/soil)						NO_3-N (µg/g soil)					
Soil	Depth cm	Fresh	Stored Moist 8 weeks at 5°C	Air Dried	Oven Dried	Soil	Depth cm	Fresh	Stored Moist 8 weeks at 5°C	Air Dried	Oven Dried
1	0-10 10-20 30+	8.6 7.5 9.1				9	0-10 10-20 30+	4.1 2.4 2.9			
2	0-10 10-20 30+	4.2 3.6 1.3				10	0-10 10-20 30+	12.5 2.5 0.2	12.9	10.8	11.8
3	0-10 10-20 30+	22.3 7.0 6.0	22.6	16.4	20.3	11	0-10 10-20 30+	7.8 2.5 2.1			
4	0-10 10-20 30+	21.6 3.9 2.8	21.8	18.5	20.0	12	0-10 10-20 30+	2.1 1.2 1.6	2.4	2.6	2.5
5	0-10 10-20 30+	0.4 0.1 0.3				13	0-10 10-20 30+	4.2 5.8 5.5			
6	0-10 10-20 30+	8.3 1.6 1.3	8.0	7.4	6.4	14	0-10 10-20 30+	7.8 7.5 4.9	10.0	9.8	6.9
7	0-10 10-20 30+	12.8 1.9 2.5				15	0-10 10-20 30+	8.3 5.1 1.9			
8	0-10 10-20 30+	29.0 7.4 9.9	25.0	23.5	15.5	16	0-10 10-20 30+	8.6 11.5 2.8	9.6	8.6	7.8

significantly inhibitory effect. Regression equations and correlation coefficients between ethylene evolved after 1 day and nitrate nitrogen concentrations were calculated as described for organic matter content (page 42). The results obtained were very variable; both negative and positive relationships were obtained and only two relationships were statistically significant. These were the "fresh, grass, all depths" and "air dried, arable, subsoil" categories both of which gave positive linear correlations ($p = 0.01$) and not negative ones as expected from the work of Smith (A.M.) and Cook (1974). It was thought that the strong positive correlation with organic matter might be over-riding the expected negative effect of nitrate. A partial correlation was therefore calculated in which the effect of organic matter was eliminated. In most cases where the correlation between ethylene production and nitrate had previously been positive, elimination of organic matter effects decreased the correlation coefficient and in some cases the relationship did become negative. In other cases an already negative relationship had an increase in the value of the correlation coefficient but only in one instance to a significant level:- the correlation coefficient for air dried arable soils over all depths, increased from -0.27 (insignificant) to -0.76 (highly significant $p = 0.01$). In some cases elimination of the effect of organic matter had no effect at all. The relationship between ethylene production and nitrate

content in the soils studied appears therefore to be more complicated than suggested by Smith (A.M.) and Cook (1974), and Smith (A.M.) (1976ab)

Additions of Inorganic Forms of Nitrogen In further studies on the effect of nitrate on ethylene evolution nitrate nitrogen in the form of potassium nitrate was added in a range of concentrations extending to values much greater than those found in the field.

The effect of this added nitrate nitrogen on ethylene evolution is shown in Figure 6. At concentrations of 50 and 100 µg added nitrate nitrogen, only a transient effect on the evolution of ethylene occurred (6b, 6c), the achievement of the maximum levels being delayed by 1 and 2 days respectively. These maximum levels were not significantly different from that of the control. The addition of 500, 1000 and 2000 µg of nitrate nitrogen had a much greater effect (6d, e and f), reducing the total ethylene evolved after 10 days by 69, 77 and 85% respectively. The relationship between ethylene production on the first day of incubation and nitrate content is shown in Figure 7. Contrary to the findings of Smith (A.M.) and Cook (1974) and Smith (A.M.) (1976ab) ethylene evolution was not inhibited until denitrification was completed even at high levels of nitrate addition. Ethylene was evolved from the beginning of the incubation while denitrification was taking place (as indicated by the presence of nitrous oxide). High levels of nitrous oxide persisted in the treatments with the three highest

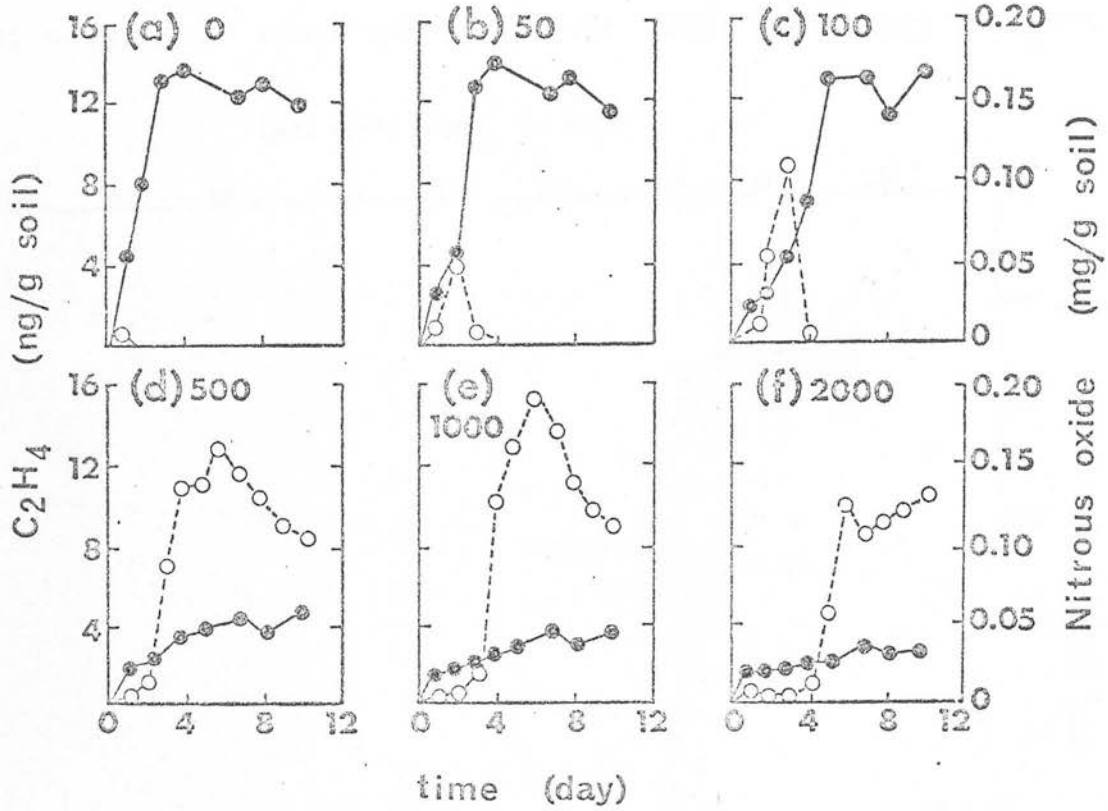

Figure 6. Effect of added nitrate on ethylene and nitrous oxide concentrations during anaerobic incubation

●: ethylene; ○: nitrous oxide. The numbers shown in (a)-(f) are initial nitrate-N concentrations (μg/g soil)

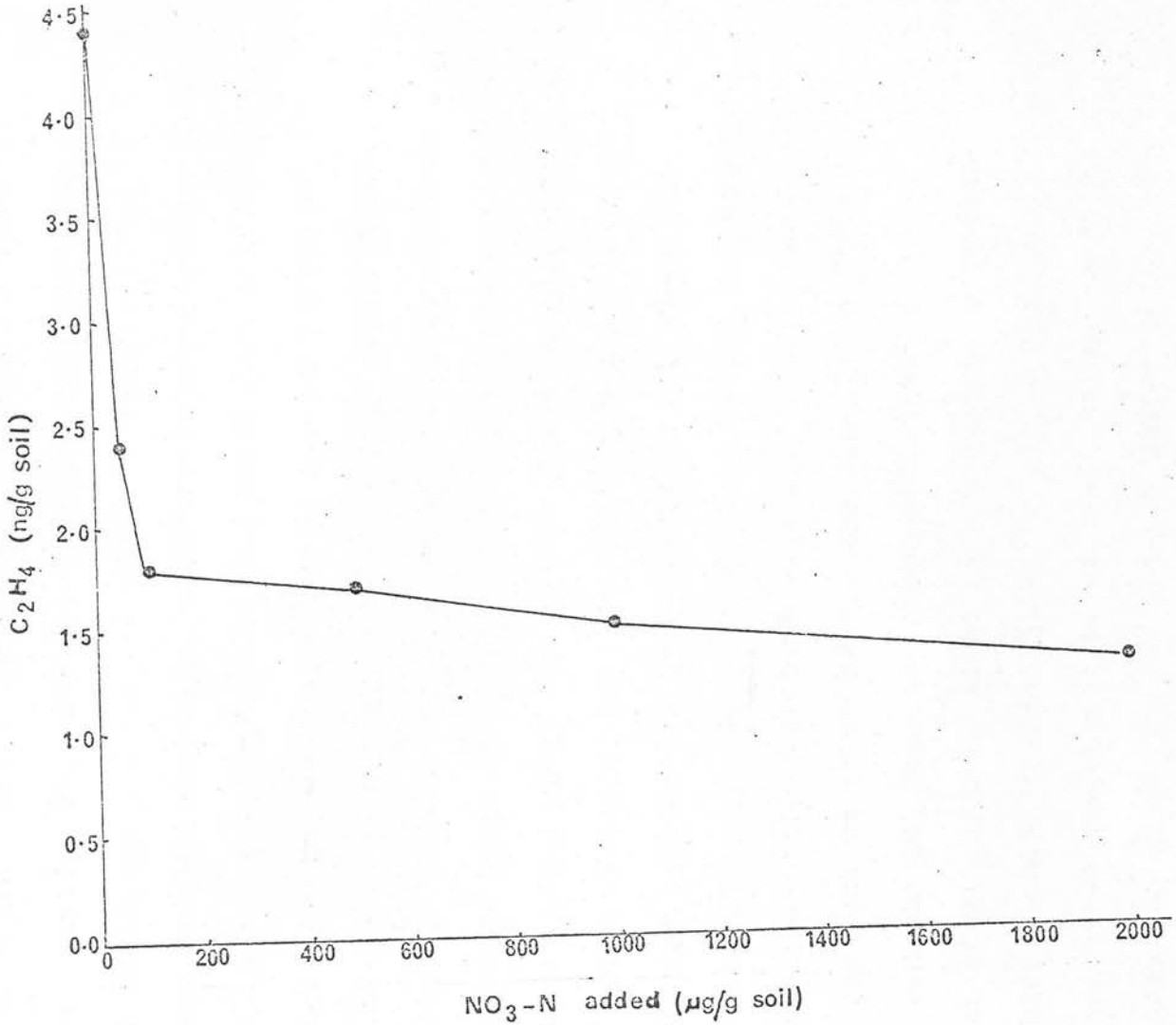

Figure 7. Effect of added nitrate on ethylene production in air dried soil incubated anaerobically for 1 day

levels of nitrate, remaining static after 13, 20 and 22 days respectively. This was thought to be due to the exhaustion of the carbon substrate, however, Bremner (1977) has suggested that the presence of nitrate nitrogen inhibits the reduction of nitrous oxide to nitrogen. A decline in nitrous oxide and ethylene levels was observed after 5-6 weeks. This was due to leaks developing in the Suba Seals, as a result of repeated sampling.

These results show that, contrary to the findings of Smith (A.M.) and Cook (1974) and Smith (A.M.)(1976ab), any effect on ethylene levels due to natural levels of nitrate nitrogen is likely to be of little significance in the field. During a period of waterlogging ethylene concentrations would be likely to reach physiologically significant levels while the nitrate is being denitrified. In fine textured soils where anaerobic zones may persist for prolonged periods the short delay in achieving the maximum concentration is unlikely to be of any consequence.

The experiments with high levels of nitrate confirm the observations of Smith (K.A.) and Restall (1971) that nitrate reduces but does not completely inhibit the formation of ethylene. It is of interest to note that at the high levels of added nitrate the hydrocarbons butane and but-1-ene were also evolved, as indicated by large peaks (up to 60% f.s.d.) on the chromatogram, but these were not measured quantitatively. These hydrocarbons were not found in the control soils.

Smith (A.M.) and Cook (1974) and Smith (A.M.)(1976ab) also investigated the effects of inorganic nitrogen added as ammonium ion. They found ammonium nitrogen had a variable effect, in some soils stimulating ethylene production and in others having no effect. No details of the incubation procedures or concentrations used were given. In the present work three concentrations of ammonium nitrogen in the form of ammonium sulphate were added. The results of analysis for ethylene (Table 13) show that for the first four days of the incubation

Table 13. Ethylene production in soil amended with ammonium nitrogen

Day	C_2H_4 (ng/g soil)			
	Control	250 µg/g added NH_4-N	1000 µg/g added NH_4-N	2500 µg/g added NH_4-N
1	0.8	0.8	0.7	0.7
2	2.9	2.6	3.4	3.0
4	7.1	6.1	6.8	7.5
7	8.5	5.5	6.0	7.6
10	8.7	4.5	5.5	6.5
13	7.2	3.2	5.7	6.4

ammonium nitrogen had no effect on ethylene evolution. In the later stages on the incubation ethylene levels were depressed, particularly in the 250 µg/g treatment. In this treatment ethylene levels began to decrease after day 4 of the incubation, dropping to a level 58% of that

found in the control. The concentration of methane, ethane and carbon dioxide continued to rise and there was no leakage of oxygen. Hence the fall in ethylene concentration was probably due to decomposition by micro-organisms and not because of any loss from the system. Similar decomposition of ethylene was observed in the 25 mg/g glucose and 25 mg/g pyruvic acid additions (p 59) and also in the undisturbed soil monoliths (p 79).

Degradation of ethylene in the soil has been observed under anaerobic conditions (Yoshida and Ancajas, 1971; Yoshida and Suzuki, 1975) and aerobic conditions (Abeles et al, 1971; Smith (K.A.) et al, 1973; de Bont, 1975; Cornforth, 1975; Flett et al, 1975). Other workers (Davis et al, 1956; de Bont and Mulder, 1974) have isolated bacteria capable of degrading ethylene and it is possible that certain conditions favour the activity of these bacteria. Alternatively under the conditions described here the growth and activity of other microbial populations not yet isolated may be favoured resulting in the observed fall in ethylene levels.

Additions of Organic Compounds It has been found that the type of crop grown in a soil may affect its subsequent ethylene production (Smith (K.A.) and Restall, 1971). The addition of different sources of organic matter has also been found to affect this process, for example El Karouri (1974) found that hay gave a 10-fold increase in ethylene, and that additions of peat and farmyard manure doubled ethylene production. Stevens (1973) and Burford (1975) found that additions of slurry increased

ethylene evolution. Stevens (1973) believed that this was partly due to repeated applications of slurry which sealed soil pores and resulted in the development of anaerobic conditions. Lynch et al (1975) found that barley residues had a greater effect on ethylene evolution than bean and cabbage residues. The differences in ethylene evolved after addition of several forms of organic matter may be due to variations in the content of readily decomposable material. The differences may also be attributed to individual variations in products of decomposition. Different organic compounds may be produced only some of which may act as substrates for ethylene producing micro-organisms.

To investigate this further, additions of hay, barley straw and wheat straw were made. This experiment was carried out jointly with Miss W. Grant, a final year honours student. The levels of ethylene observed are shown in Table 14.

Table 14. Ethylene production in soil amended with different forms of organic matter

Day	C_2H_4 (ng/g soil)			
	Control	Hay	Wheat Straw	Barley Straw
1	2.1	5.0	8.2	5.0
2	8.1	9.9	26.9	18.1
5	12.2	13.5	44.3	31.4
7	13.5	14.8	47.1	35.0

These figures show that wheat straw has most effect on ethylene production. Smith (K.A.) and Restall (1971) had previously found that when wheat was grown in soil prior to incubation, ethylene production by this soil was 10 times that in an uncropped soil. This suggests that wheat as a crop favours ethylene production both as a result of microbial decomposition of the straw and as a result of the micro-organisms associated with the rhizosphere of the growing plant. Addition of barley straw also increased ethylene evolution but to a lesser extent. Lynch et al (1975) also found that barley residues increased ethylene production. Addition of hay had no effect, apart from accelerated production during the first day, this is in contrast to the results of El Karouri (1974). It was also found in the present work that the addition of hay stimulated the production of the longer chain hydrocarbons. It is possible that the lack of stimulation of ethylene by hay residues was due to the high production of the gas from the unamended soil compared with that from the soil used by El Karouri. Lynch (1975b) has shown that soils vary in their response to substrate additions but soils low in existing substrate show a much greater response.

In plants methionine is known to be a precursor of ethylene (Abeles, 1973; Yang, 1974), and Lynch (1972) has shown that it can also be a precursor in microbial synthesis of ethylene in the soil under anaerobic conditions. During the active growth of aerobic micro-organisms methionine does not accumulate, however it does do so under anaerobic conditions and will therefore

be available for ethylene production (Holding, 1977). Many bacteria can produce ethylene from methionine under aerobic conditions (Primrose and Dilworth, 1976; Primrose, 1976b). Under anaerobic conditions decomposition of organic matter is the result of fermentative processes, and metabolic products such as pyruvic, formic, acetic, lactic, propionic, butyric and succinic acids, ethanol, propanol, butanol, butylene glycol, acetone, methane and hydrogen are evolved (Alexander, 1961; Stevenson, 1964). It was thought therefore that some stimulation of ethylene production observed on the addition of straw might be due to one of these products of anaerobic fermentation. The effect of a variety of organic compounds, including some known products of anaerobic fermentation, on ethylene production from soil was therefore studied (Table 15).

Promotion of ethylene formation was most obvious in the first 1-2 days. Production of ethylene was enhanced by addition of 2.5 mg/g of casein, pepsin, glucose and ethanol, and by 25 mg/g of lactic and pyruvic acids. It was depressed by butyric acid and the higher rate of glucose. It would appear therefore that these compounds can act as substrates for the micro-organisms producing ethylene. They may be intermediates in the metabolic pathway leading to ethylene or they may be acting as an energy source to enable the micro-organisms to break down other compounds to ethylene. The picture is, however, far from clear and more work is needed,

Table 15. Ethylene production under anaerobic conditions by soils amended with organic substrates

Substrate mg/g	Ethylene (ng/g soil)					
	Day 1	Day 2	Day 4	Day 7	Day 10	Day 13
Control	0.8	2.9	7.1	8.5	8.7	7.2
Casein, 2.5	1.4	7.7	8.9	7.8	7.1	7.5
Casein, 25	1.1	5.4	3.4	3.2	3.5	4.6
Pepsin, 2.5	1.6	6.4	8.0	7.4	7.9	9.0
Pepsin, 25	0.5	3.5	4.9	5.5	5.6	8.6
Glucose, 2.5	1.3	6.5	7.5	8.2	7.6	9.0
Glucose, 25	0.9	2.9	5.7	1.8	1.4	1.2
Lactic acid, 2.5	0.8	1.6	2.6	3.4	4.3	5.9
Lactic acid, 25	2.1	4.8	6.4	7.0	7.1	7.8
Ethanol, 2.5	1.0	4.1	8.7	8.5	9.3	10.3
Ethanol, 25	0.9	1.6	2.2	2.6	2.4	2.5
Pyruvic acid, 2.5	1.6	2.6	3.2	5.6	6.4	8.3
Pyruvic acid, 25	7.8	10.3	9.1	8.9	8.2	7.3
n-Butyric acid, 2.5	0.6	1.1	1.4	1.6	2.4	2.6
n-Butyric acid, 25	1.9	2.8	4.1	5.2	5.7	5.6

including the isolation of micro-organisms specific to the utilisation of these particular compounds, before any conclusions can be drawn. It is of interest that some of the amendments had an effect on the production of other gaseous hydrocarbons. Initial methane production was greatly stimulated by treating with 25 mg/g soil casein and pyruvic acid, and by day 4 by similar treatment with pepsin and glucose. At the lower concentration stimulation was seen in the casein and pepsin treatments and later in the glucose and pyruvic acid treatments. Ethane levels were unaffected by any of the treatments but propane production was increased by treatment with 25 mg/g soil of butyric and lactic acid and propylene by both concentrations of butyric acid, and in later stages by glucose and casein at both concentrations, and pepsin at the lower. The longer chain hydrocarbons butane and but-1-ene were produced in small quantities in the later stages of the incubation with most treatments, but 25 mg/g ethanol in particular promoted but-1-ene considerably. Hydrogen was also evolved in the treatments with glucose, casein and pepsin at both concentrations.

The evolution of ethylene from the soil appears therefore to be dependent on the presence of suitable substrates. These substrates are made available by the breakdown of organic matter. Thus although it is often of benefit for the fertility of the soil to keep organic matter contents fairly high, the addition of undecomposed

organic matter by ploughing in straw or farmyard manure may lead to deleterious effects if anaerobic decomposition develops.

Organic acids such as acetic and butyric acids are formed under anaerobic conditions (Takijima, 1964; Hollis et al, 1967; Lynch, 1977; Niranjan Rao and Mikkelson, 1977) and these may effect root growth. Takijima (1964) found that the degree of root growth inhibition was higher than that estimated from the concentration of organic acid present and suggested some other factor might also be present. It now appears that under anaerobic conditions ethylene and organic acids may be present together, both having adverse effects on plant growth.

The effect of crop residues on ethylene formation (Smith (K.A.) and Restall, 1971; El Karouri, 1974; Lynch et al, 1975) and the effects of soil structure are likely to result in localised production of ethylene in the field. This will lead to wide variations in ethylene levels throughout the soil, and ethylene from anaerobic microsites may diffuse through the soil to aerobic zones. Other workers studying the production of ethylene in the field have found very variable levels of ethylene from 0.0 to 10 ppm (Dowdell et al, 1972), 0.01 to 1.05 ppm (Bell, 1975), 0.03 to 6.0 ppm (Smith (A.M.) and Cook, 1974) and 0.0 to 75 ppm (Smith (K.A.) and Dowdell, 1974). However it appears from the present work that in field soils ethylene levels are unlikely

to be high unless poor structure and high moisture content results in anaerobic conditions developing in the presence of undecomposed crop residues, conditions which under normal rainfall and good soil management ought to be rare.

High microbial activity leading to an increased oxygen demand and resulting in anaerobic conditions, is most likely to occur in the spring when temperatures rise but the soil still has a fairly high moisture content. However studies on soils collected in the spring did not result in high levels of ethylene being obtained. As the samples were collected following a mild winter (1975-76) it is possible that, in the arable soils at least, all suitable substrates for ethylene production had been exhausted. It is also possible that only in the presence of large quantities of substrate is the population of ethylene producing organisms able to multiply sufficiently to produce ethylene at a rate greater than that at which it is degraded. This proposal may also account for the increased ethylene formation brought about by drying; the ethylene forming organisms may germinate and multiply more rapidly on rewetting than the organisms responsible for degradation.

Griffin (1972) quoted published data to show that fungi attain maximum growth rates on glucose at lower concentrations than those which are optimal for bacteria. Thus, in the present work, stimulation of ethylene at the lower rate of glucose but not at the higher, is compatible with a mainly fungal origin. This coupled with the observed increase in ethylene evolution with decreasing pH suggests that fungi are perhaps the major ethylene producers in the soil.

Section 2.

Experiments with Undisturbed Soil Monoliths

Introduction

In addition to the incubation experiments in the laboratory, in which small samples of soil were subjected to anaerobic conditions and the subsequent products of anaerobic respiration measured, an attempt was made to measure the changes in soil atmospheric composition which take place throughout a column of undisturbed soil. The composition of the soil atmosphere represents a dynamic equilibrium between the processes of respiration in the soil and diffusion between the soil and the atmosphere. Under conditions of high biological activity or restricted gaseous exchange carbon dioxide levels in the soil might be expected to increase and oxygen levels decrease. Poor diffusion rates may be due to reduced air filled pore space as a result of compaction or high moisture content. The effects of heavy applications of water (simulating severe rainfall) on the development of anaerobiosis in three contrasting soils were compared. Of the soils chosen, two, Nos. 12 and 17, were known to produce ethylene under anaerobic conditions. The third, soil No. 16, due to its poor drainage status (Mitchell and Jarvis, 1956), was expected to show major changes in aeration status with depth. Kohnke *et al* (1940) stated that undisturbed soil blocks or monoliths gave a better representation of the soil in its natural state than

lysimeters filled with disturbed soils; triplicate replication was considered necessary by Kohnke, to compensate for the non-uniformity of the soil, and accordingly this recommendation was adopted in this work.

Materials and Methods

Collection of Monoliths Three monoliths of each soil were collected in rigid PVC tubing 22 cm i.d., wall thickness 1 cm, cut into 50 cm lengths. The monoliths were obtained by placing the tubing upright on the soil surface (cleared of any vegetation) and digging away the soil around the outside, pressure being exerted on the top of the PVC cylinder to force it into the ground (Overrein, 1968; Cannell et al, 1973). Once each cylinder was full it was carefully removed from the pit and sealed in polythene for transport back to the laboratory. In the laboratory excess soil at the bottom of the monoliths was carefully cut away to give a smooth surface and covered with nylon gauze. The monoliths were weighed and then lifted onto previously prepared sand tanks.

Sand Tanks These sand tanks which are similar in principle to the sand box apparatus designed by Harst and Stakman (1965), are described by Shali (1974), so only a brief description will be given here. The tanks are made of 3 mm aluminium plate, 1.2 x 0.9 m in area and 23 cm in depth and filled with 3 layers of graded sand particles and each tank fitted with a tensiometer (Webster, 1966). Each tank has 4 outflows which are connected outside the

tank by means of plastic "T" pieces and nylon tubing via a 3-way glass stopcock to the main drain, through which tension can be applied if required. For the purposes of this experiment, however, the tanks were used as a means of maintaining a constant water table. The tanks were filled with water to a depth of 1 cm and connected via the 3-way tap to a reservoir so that the water level was maintained.

Preparation of Monoliths for Soil Atmosphere Studies Once placed in position on the sand tanks holes were drilled in the side of each PVC cylinder to accomodate four 3 mm i.d. x 15 cm stainless steel sampling tubes at 10, 20, 30 and 40 cm depth, a thermometer at 25 cm depth and two tensiometers at 15 and 30 cm depth. Sampling was carried out through Suba Seals but because of the need to replace them frequently each sampling tube was fitted with a glass stopcock obtained by cutting down burette stopcock replacements. These were held in place by Araldite high stress adhesive and fitted with size 13h Suba Seal type rubber closures (Figure 8). The use of stopcocks enabled the rubber seals to be renewed without gas exchange taking place. All tubing, thermometers and tensiometers were sealed into the PVC cylinders with Silastic 734 RTV adhesive/sealant (a cold-curing silicone rubber). Plasticine was used to prevent diffusion down the walls of the cylinder due to shrinkage of the soil.

The soil monoliths were flooded at intervals with water equivalent to a 5 cm depth in the cylinder by means

of a tightly fitting plastic sleeve held in place by a rubber band (cut from tyre inner tubing). Then they were allowed to drain. Gas samples were taken for analysis for oxygen, carbon dioxide and hydrocarbons, in particular ethylene. The temperature and moisture tension were recorded throughout each cycle.

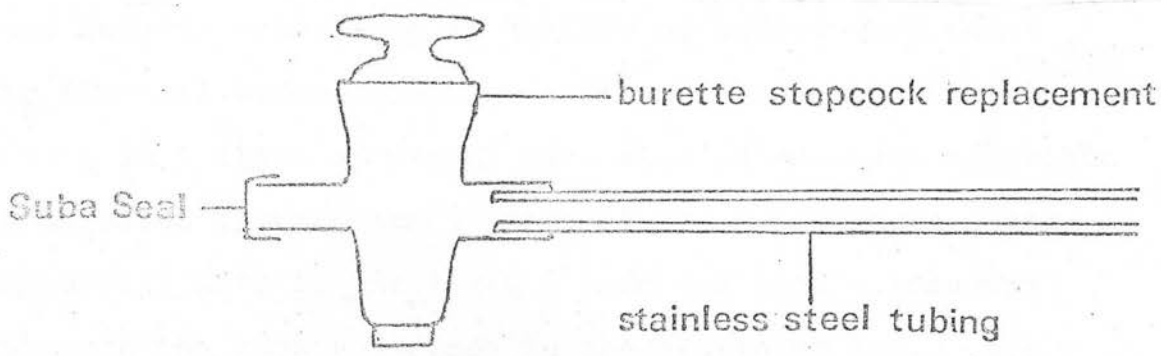

Figure 8. Gas sampling point using a glass stopcock

Sealed Cores The monoliths of soils Nos. 16 and 17 were completely sealed by cementing an 8 mm thick lid onto the PVC tubing with "Plumbers Mait", a pliable water-proof sealing compound. Each lid was fitted centrally with a B57 Suba Seal type rubber closure to enable gas samples to be taken from immediatly above the soil surface. The lids were removed and replaced at intervals so that the soil was subjected to alternating aerobic and anaerobic conditions. Gas analysis and the recording of temperature and moisture contents were carried out as before.

Substrate Addition Laboratory incubations in which a number of carbohydrate-containing materials and some of their known degradation products were added to soil showed that these had a variable effect on ethylene production (Table 15). Glucose and ethanol promoted

ethylene formation at low concentrations but depressed production at high rates, butyric acid depressed production at both rates. Thus when evolution of ethylene in soil No. 16 had been reduced to a very low level following depletion of substrates as a result of the sequence of aerobic/anaerobic cycles, solutions of glucose, ethanol and butyric acid (10 g in 1 litre of water) were added to the soil before sealing. Ethanol and ammonium sulphate (10 g in 1 litre of water) were also added under anaerobic conditions by allowing 1 litre of the solution to drain gradually into the soil via a tube and needle inserted through the rubber stopper in the aluminium lid. The sequence of additions is shown in Table 16, the addition of water only, acting as a control.

Transport of Gas Samples Samples for inorganic gases were taken with 1 ml polypropylene syringes and samples for hydrocarbon analysis with 1 ml glass syringes because the rubber diaphragms of disposable syringes absorb ethylene (Smith (K.A.) and Restall, 1971). Samples were stored for transport by pushing the needle into a rubber bung; samples could be stored in this way for 6 hours without deterioration of the sample (Table 17).

Table 17. Concentration of ethylene and carbon dioxide after storage for 6 hours

Time hours	C_2H_4 vpm	CO_2 %
0	10.0	1.0
6	9.8	1.0

Table 16. Sequence of substrate addition to soil monoliths

	Soil Monolith 1	Soil Monolith 2	Soil Monolith 3
Addition 1	10g glucose in 1000 ml water	1000 ml water	1000 ml water
Addition 2	10g glucose + 10g ethanol in 1000 ml water	1000 ml water	1000 ml water
Addition 3	10g glucose + 10g butyric acid in 1000 ml water	10g ethanol in 1000 ml water	1000 ml water
Addition 4	10g ammonium sulphate in 1000 ml water (anaerobic)	10g ethanol in 1000 ml water (anaerobic)	10g butyric acid in 1000 ml water

Results and Discussion

Atmosphere Studies in Unsealed Monoliths The results of gas analysis for oxygen, carbon dioxide and ethylene throughout the experiment are shown in Figures 9-11 and the data for soil moisture tension and temperature in Figures 12-14.

Soil No. 12, a sandy clay loam (Figure 9 a-d) showed a reduction in oxygen concentration from atmospheric particularly in response to addition of water, which was most marked at the 40 cm depth (Figure 9d). Carbon dioxide levels showed similar fluctuations, levels increasing after flooding. There was no clear evidence for a gradient of oxygen and carbon dioxide concentrations with depth.

The results for soil No. 16, a sandy loam, are shown in Figure 10 a-d. It can be seen that there was no indication of a gradient with depth through the uppermost 30 cm, although at the lowest depth, 40 cm (Figure 10d), oxygen levels were sometimes reduced to 12-14%. Similarly carbon dioxide levels remained low, being rarely higher than 2%.

In the peat soil, No. 17 (Figure 11 a-d), there was a much greater reduction in the oxygen level from atmospheric although once again there was no clear evidence that a gradient had been established down the cylinder. There was no gradient in the carbon dioxide levels at the various depths either, although concentrations were higher than in the sandy loam (soil No. 16) cores.

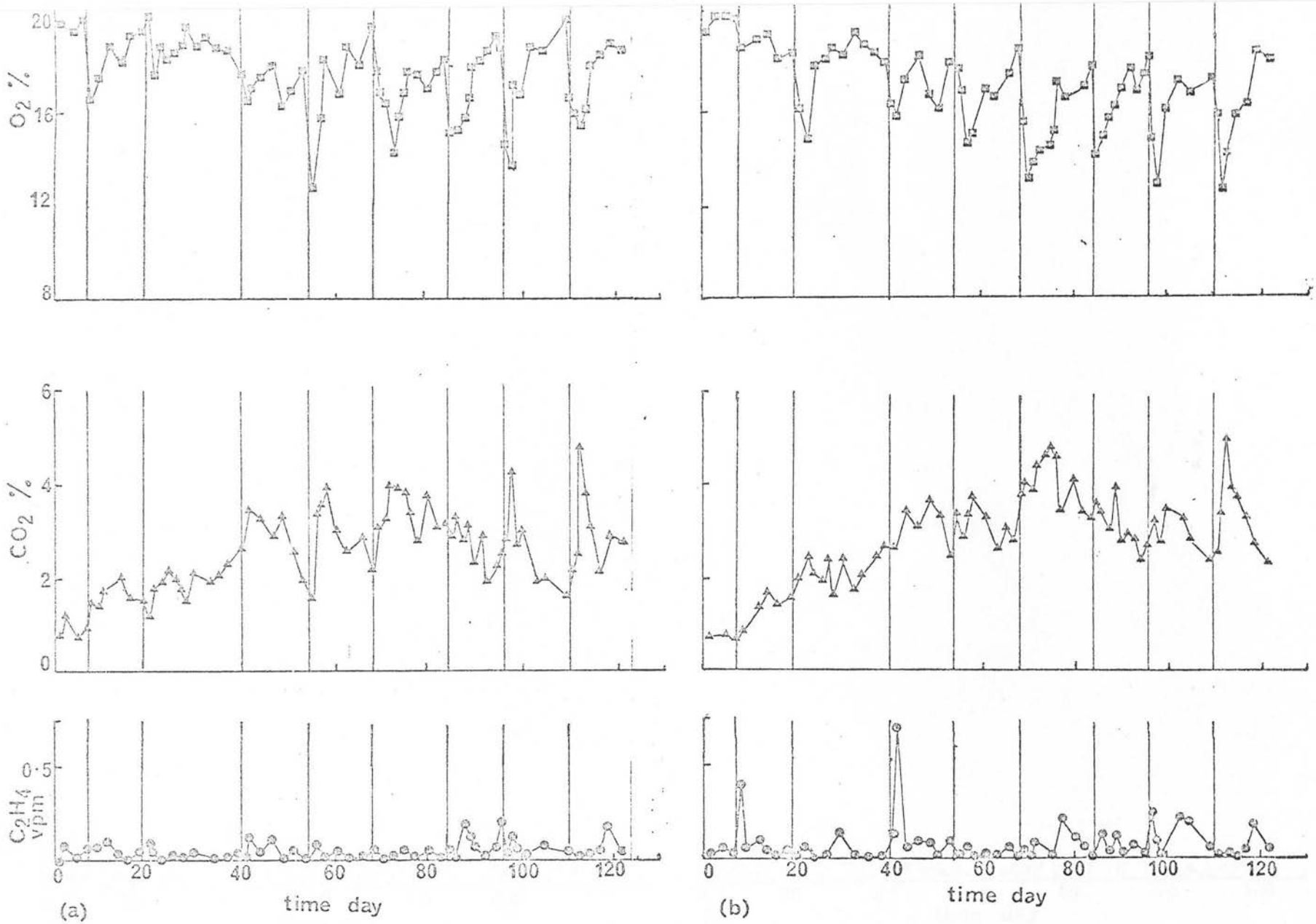

Figure 9 Oxygen, carbon dioxide and ethylene concentrations in a sandy clay loam soil (a) at 10 cm depth; (b) at 20 cm depth. Vertical lines indicate water additions

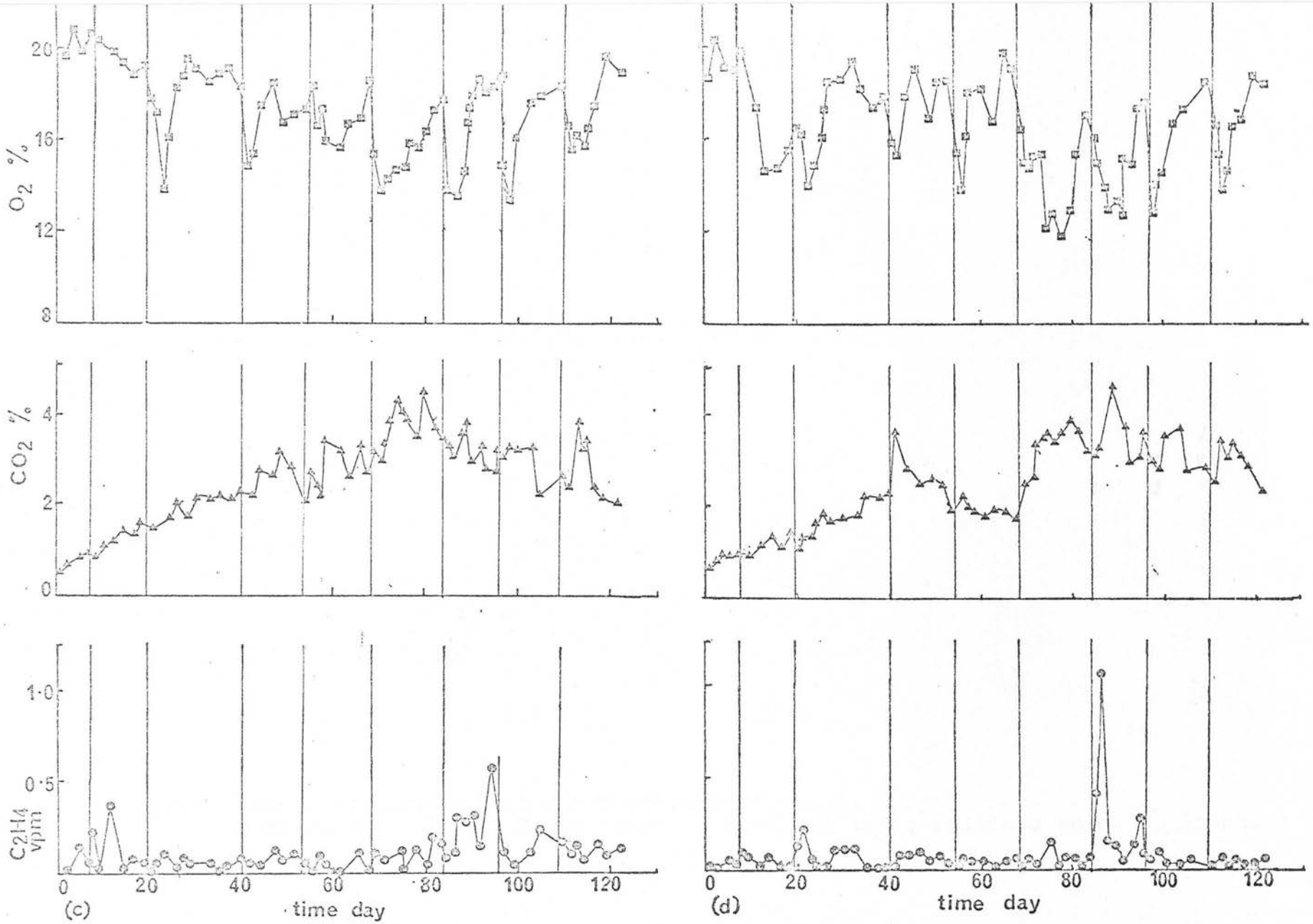

Figure 9 (c) at 30 cm depth; (d) at 40 cm depth.

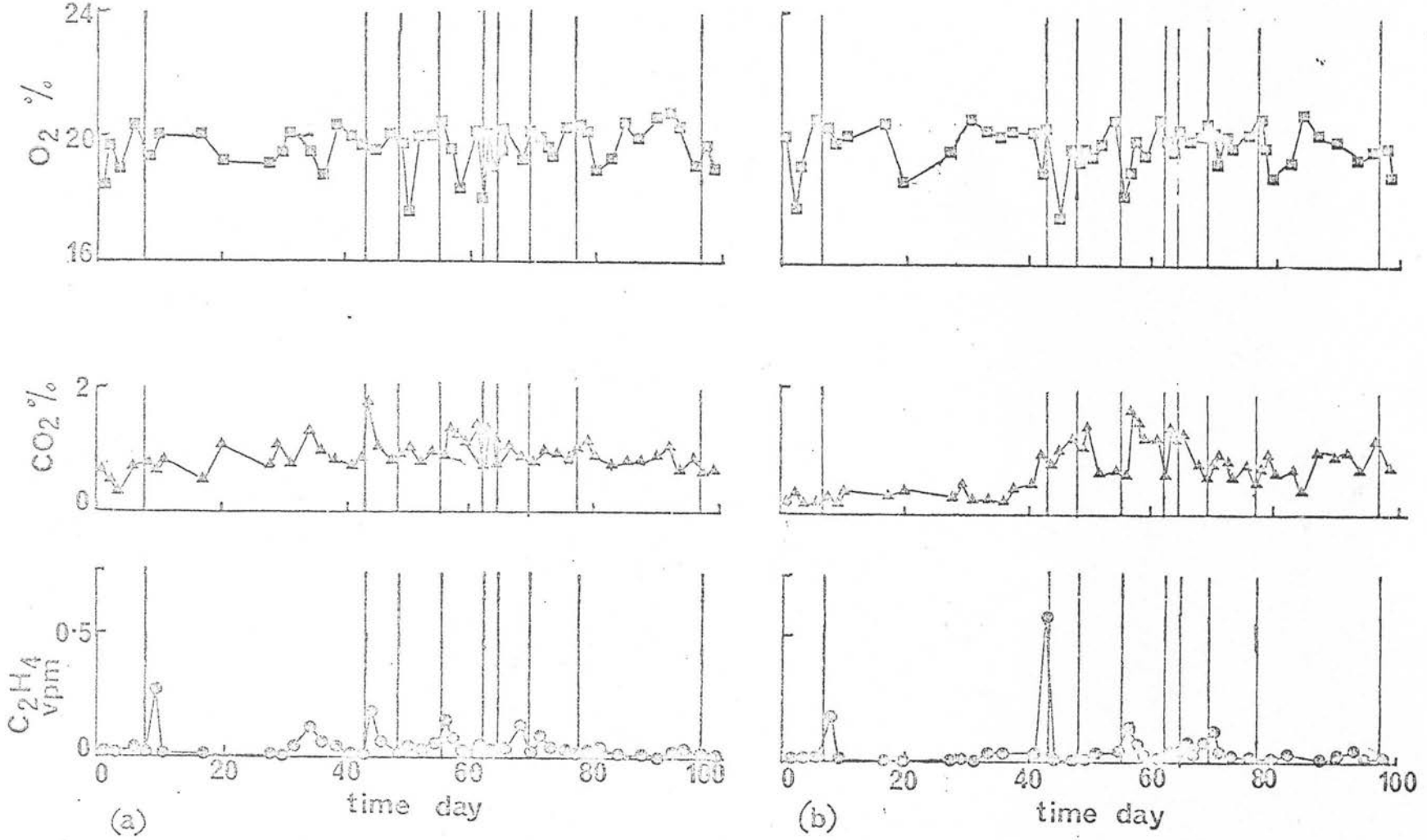

Figure 10 Oxygen, carbon dioxide and ethylene concentrations in a sandy loam soil. (a) at 10 cm depth; (b) at 20 cm depth. Vertical lines indicate water additions.

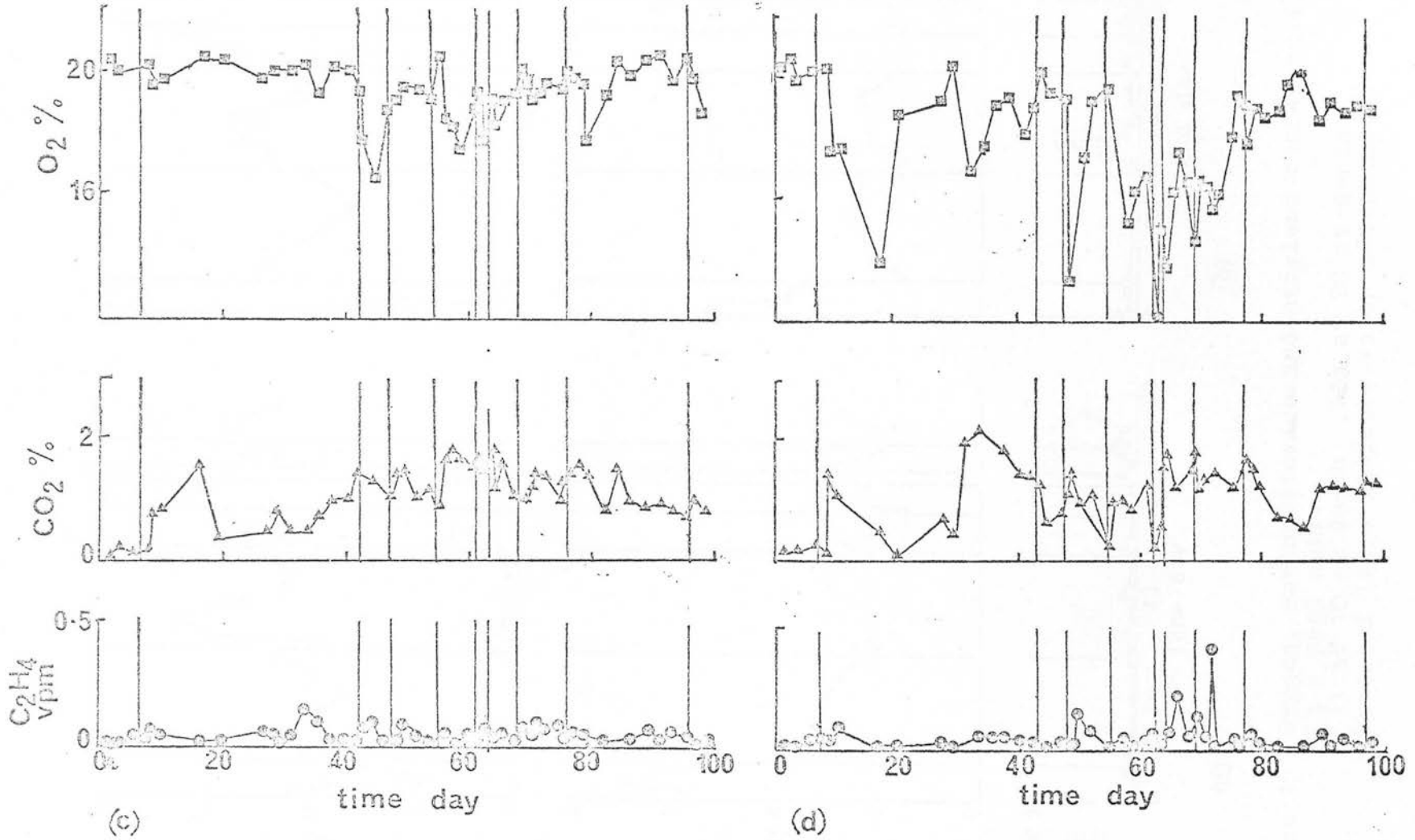

Figure 10 (c) at 30 cm depth; (d) at 40 cm depth

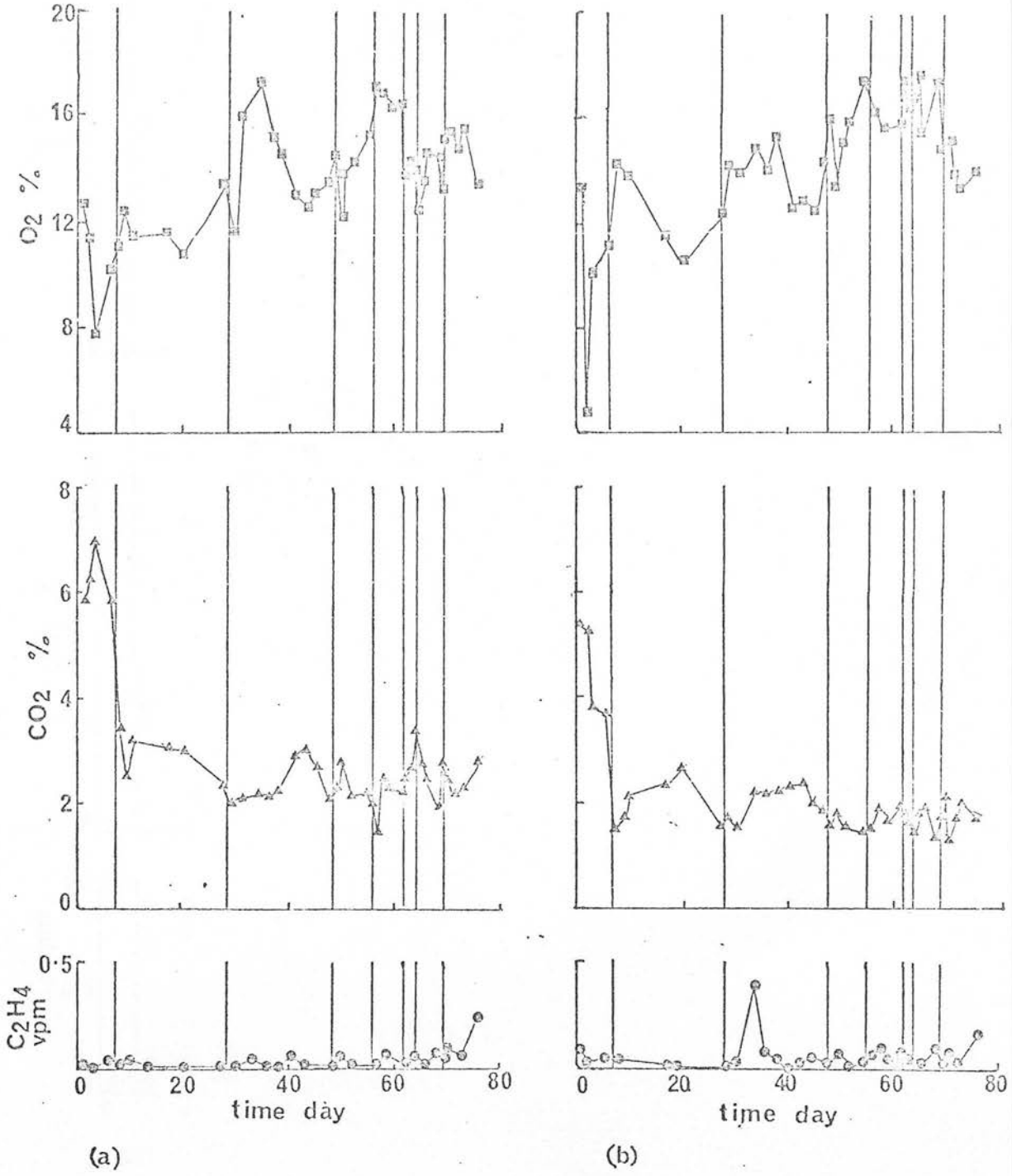

Figure 11 Oxygen, carbon dioxide and ethylene concentrations in a peat soil.
(a) at 10 cm depth; (b) at 20 cm depth
Vertical lines indicate water additions

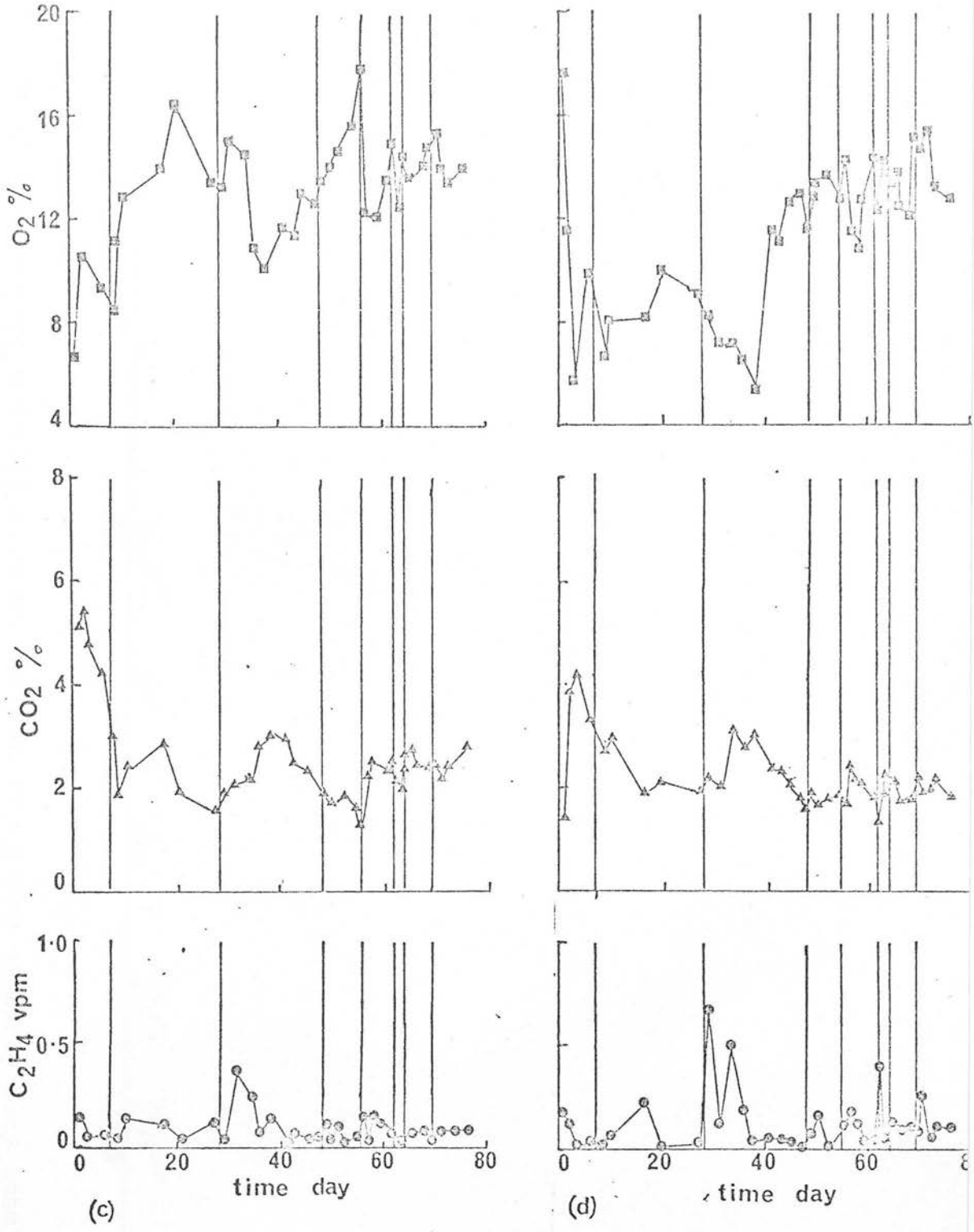

Figure 11 (c) at 30 cm depth; (d) at 40 cm depth.

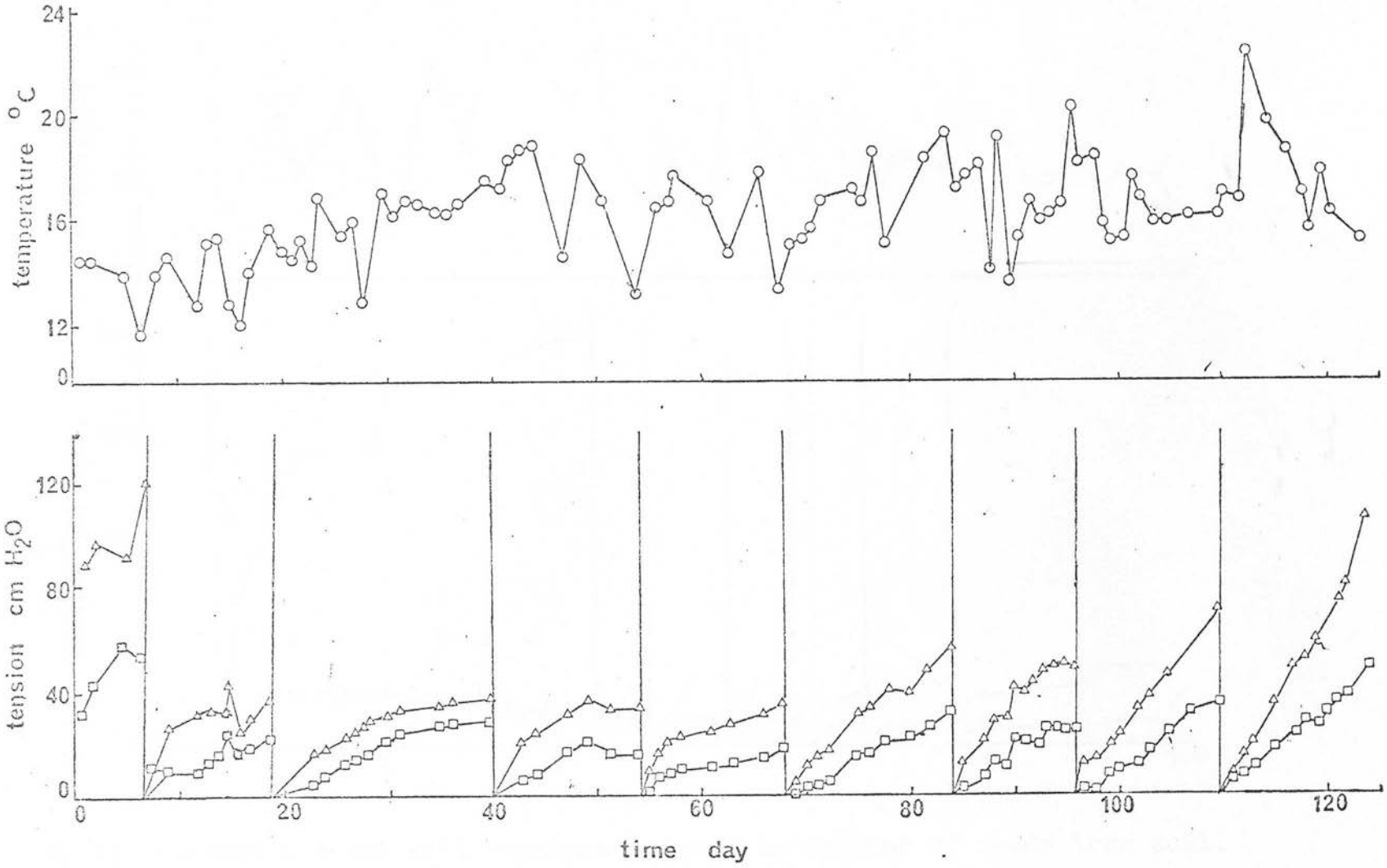

Figure 12 Temperature and soil moisture tension in columns of sandy clay loam soil. Vertical lines indicate water addition.
△: 15 cm depth; □: 30 cm depth.

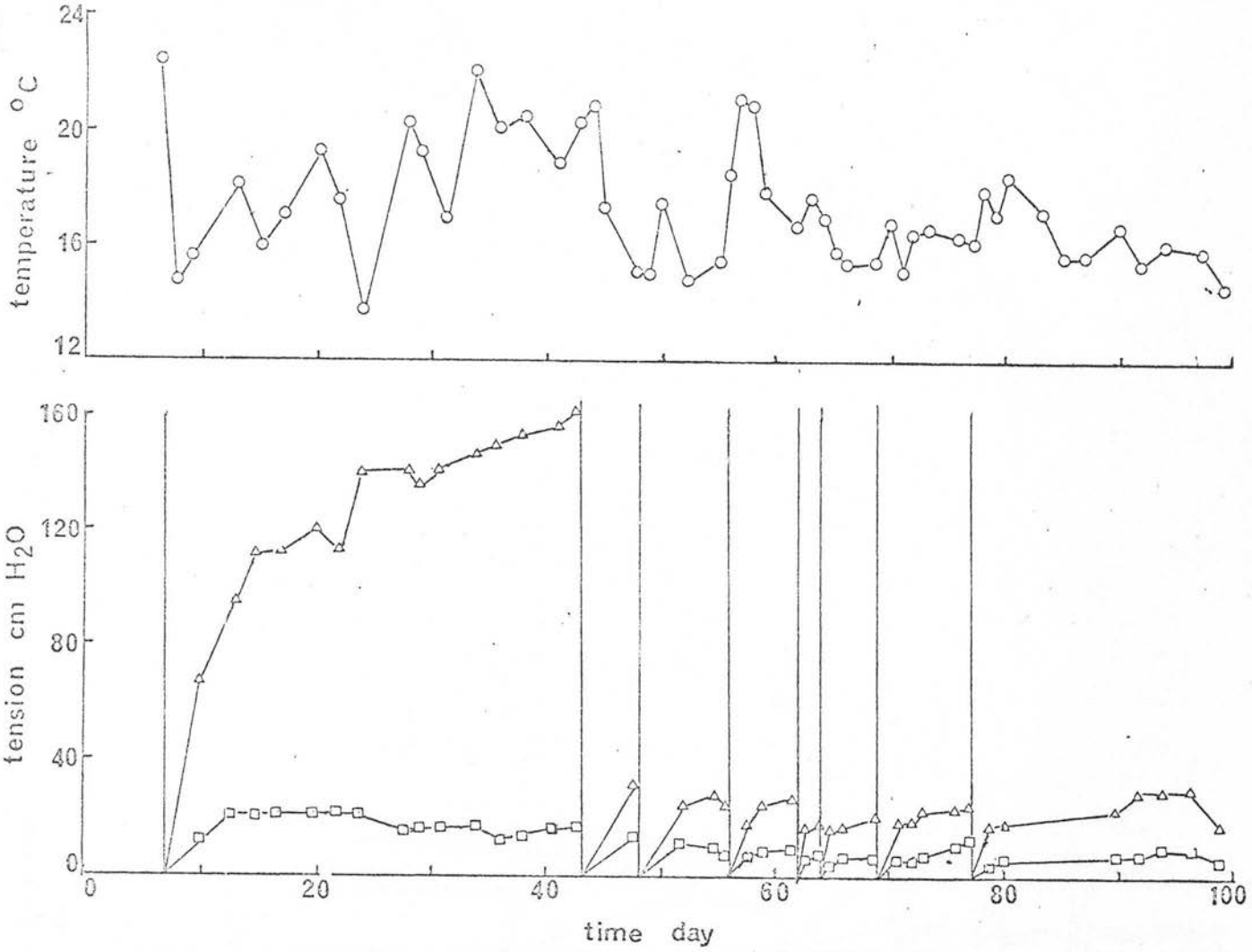

Figure 13 Temperature and soil moisture tension in columns of sandy loam soil. Vertical lines indicate water addition.
△: 15 cm depth; □: 30 cm depth.

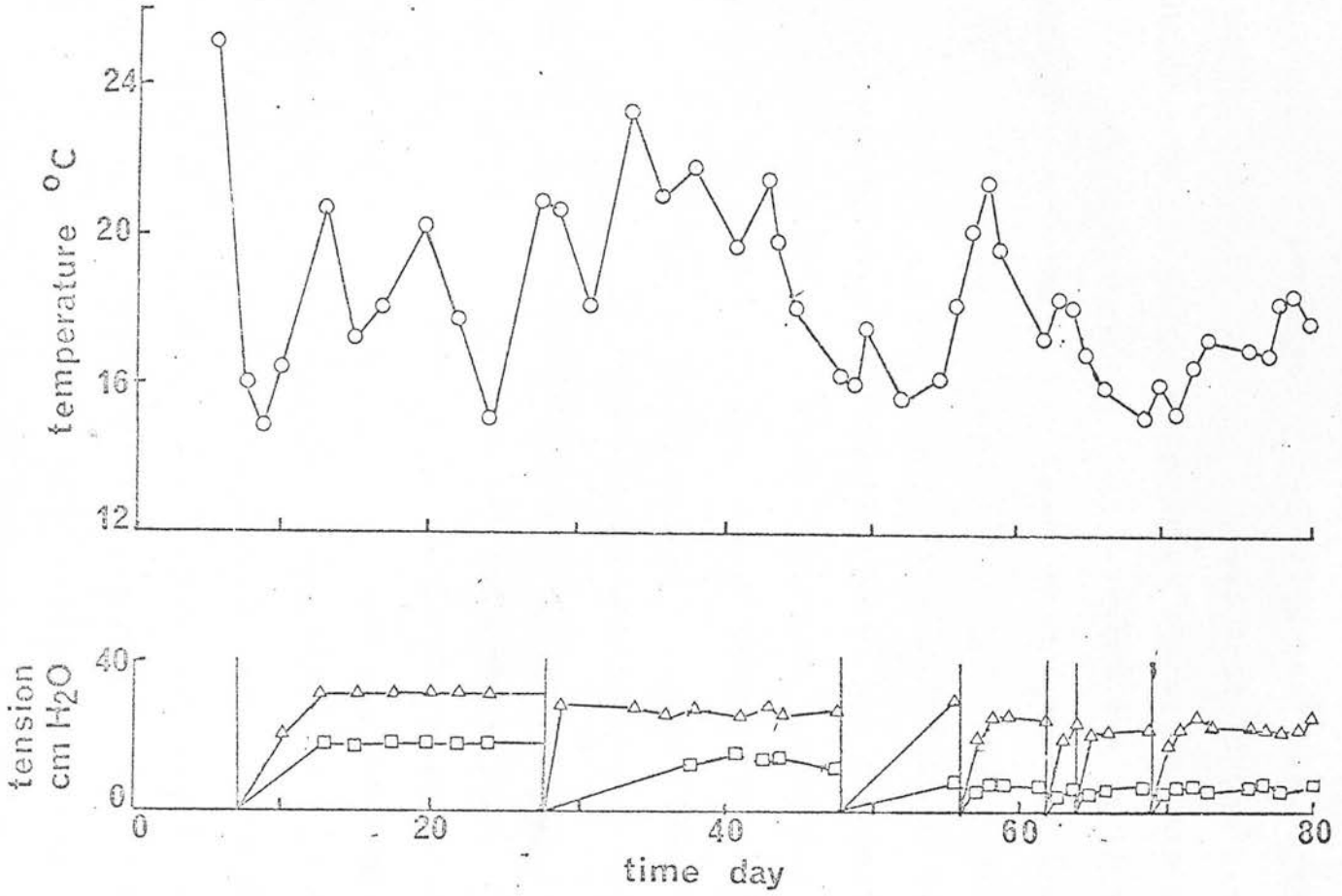

Figure 14 Temperature and soil moisture tension in columns of peat soil. Vertical lines indicate water addition.
△: 15 cm depth; □: 30 cm depth.

Figures 12-14 show variations in temperature during the course of the experiment. The data for soil moisture tension show the fluctuations in tension following each addition of water, a greater tension being observed at the 15 cm depth compared with the 30 cm depth.

When a soil is flooded water replaces the air in the soil pores and this causes a reduction in the oxygen content and in the capacity for gaseous exchange. Microbial activity then leads to the development of anaerobic conditions and the subsequent production of the gases associated with anaerobic conditions. These include carbon dioxide and hydrogen (Oginsky and Umbreit, 1959), methane (Barker, 1956), and other hydrocarbons including ethylene (Smith (K.A.) and Russell, 1969; Smith (K.A.) and Restall, 1971). Denitrifying bacteria may produce nitrogen and nitrous oxide (Cady and Bartholomew, 1960). Other substances including the gas hydrogen sulphide are also produced but these were not detected in the chromatographic separations used for this work.

Under the conditions existing in the soil cores, i.e. a permamently high moisture content with periodic flooding, it was expected that hydrocarbons and increased carbon dioxide levels would be detected and also perhaps hydrogen and nitrous oxide. However ethylene levels in all soils were low, although fluctuations occurred which could be related to the addition of water, ethylene concentrations increasing temporarily following water addition. The addition of water also caused fluctuations

in ethane, propane and propylene levels, in particular propane, but methane was not affected. This is probably because methane is formed predominantly under severely reducing conditions, and complete anaerobiosis did not occur in this experiment.

The decrease in oxygen levels and corresponding increase in carbon dioxide levels found in soils Nos. 12 and 17 are similar to those obtained by Yamaguchi et al (1967), who observed carbon dioxide levels of 2-5% and oxygen levels of 15% at 15-20°C and 35 cm depth, after 20 days in Yolo sandy loam. Ethylene was not measured in their work, but other workers using columns of soil under a variety of conditions, have measured ethylene levels (Smith (K.A.) and Russell, 1969; Smith (K.A.) and Restall, 1971; Smith (A.M.), 1973; El Karouri, 1974). Smith (A.M.) (1973) obtained very high levels of ethylene under what he claimed were aerobic conditions, but these results have since been disputed by other workers (Lynch and Harper, 1974c). Smith (K.A.) and Russell (1969) using cylinders 7.5 cm diameter and 75 cm high filled with soil at field capacity, showed that ethylene concentrations varied from 0.07 ppm at 15 cm depth to 0.14 ppm at 60 cm depth. Smith (K.A.) and Restall (1971) compacted the surface of columns of a clay soil (bulk density 1.08 g/cm^3); after the first compaction oxygen levels fell to 2%, after the second compaction (bulk density 1.35 g/cm^3) the oxygen concentrations decreased to less than 1% and ethylene was

produced. El Karouri (1974) found that compaction to a bulk density of 1.5 induced the accumulation of ethylene in a silt loam and in a clay soil. Neither the sandy clay loam (soil No. 12) nor the sandy loam (soil No. 16) used in the present work appeared to be well structured, particularly in the subsoil. The bulk densities of the three soil types used are shown in Table 18. This,

Table 18. Effect of depth on bulk density in soil monoliths

Soil	12			16			17		
Depth (cm)	14	35	50	5	20	45	10	30	50
Bulk density (g/cm^3)	1.28	1.65	1.55	1.24	1.42	1.55	0.49	0.48	0.54

together with the high water content maintained in the soil cores might have been expected to have resulted in anaerobiosis and subsequent evolution of ethylene. Possible reasons why this did not occur are the occurrence of cracks either within the soil or between the soil and the cylinder, and complete exhaustion of organic substrates necessary for microbial activity. Checks for cracks were made between water additions; a few were found and the soil around them pressed together to seal them. Exhaustion of substrates for microbial activity had certainly not occurred, as is shown in the next section where anaerobic conditions were induced by sealing the cores with lids. Failure to produce anaerobic condtions appears therefore to have been due to adequate aeration throughout the profile.

Atmosphere Studies in Sealed Monoliths The oxygen, carbon dioxide and ethylene concentrations in soil Nos. 16 and 17 are shown in Figure 15. Samples were taken from above the surface and the same four depths as before, but as the concentrations of the individual gases were very uniform with depth the results shown are the means for each gas. The speed with which this uniformity was achieved (within 24 hours) is probably indicative of good soil structure within the monoliths. As shown in Figure 15, sealing the soil monoliths resulted in the development of an-aerobic conditions due to microbial activity. In the peat soil (No. 17) the rate of achievement of anaerobiosis, within 4 days, was similar for each of four successive anaerobic/aerobic cycles (Table 19).

In the sandy loam (soil No. 16) the rate of oxygen consumption was not only slower (anaerobiosis being achieved after 8 days instead of only 4 days) but also decreased in the 3rd and 4th cycles such that oxygen levels did not fall below 6%. This may be due to the lower temperature or to a reduction in the amount of substrate available for microbial activity. As the lower temperature does not appear to have affected the rate of decrease of oxygen in the peat soil (No. 17) to the same extent it seems likely that it was lack of available substrate which had reduced the rate of oxygen consumption in soil No. 16.

It can be seen from Figure 15 that very little ethylene was produced in soil No. 16 but in the peat (No.17)

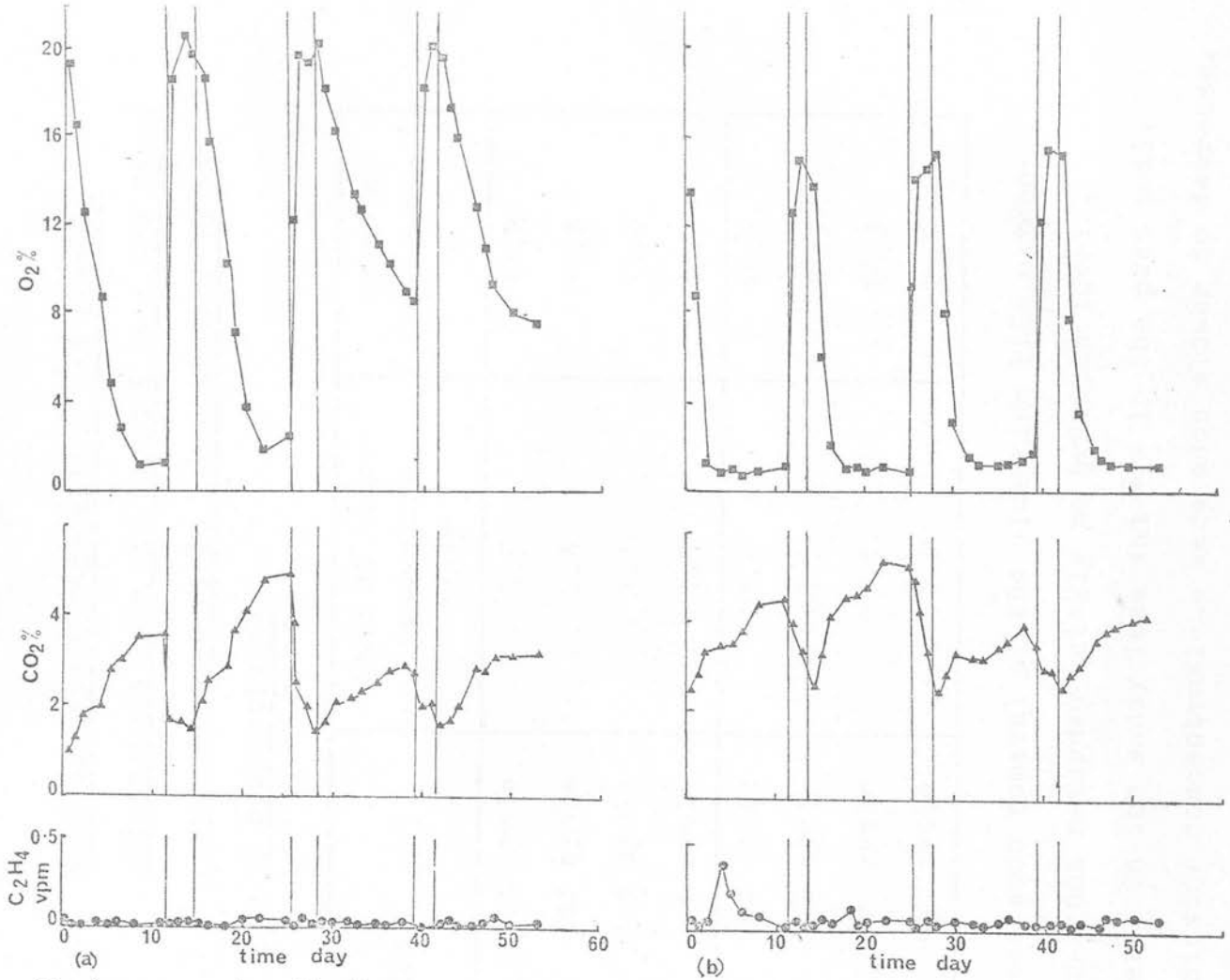

Figure 15 Oxygen, carbon dioxide and ethylene concentrations in (a) sandy loam soil and (b) peat soil during a series of aerobic/anaerobic cycles.

ethylene levels increased once the oxygen concentration had been reduced to a low level (less than 2%). This was particularly marked in the first period of anaerobiosis but was less in the subsequent cycles.

Table 19. Rate of oxygen consumption and average temperature over the first 8 days and first 4 days of each cycle for soil Nos. 16 and 17 respectively

Soil		Rate of oxygen consumption %/day	Average Temperature °C
16	1st cycle	2.3	14.8
	2nd cycle	2.3	17.8
	3rd cycle	1.3	12.0
	4th cycle	1.5	13.2
17	1st cycle	3.2	15.3
	2nd cycle	3.5	16.3
	3rd cycle	3.5	12.3
	4th cycle	3.3	13.7

After each removal of the aluminium lids oxygen concentrations returned quickly to previous levels, atmospheric in the sandy loam and 14% in the peat soil. Carbon dioxide concentrations were much slower to decrease. This may have been due to carbon dioxide dissolved in the soil water being slowly released or due to continued microbial activity producing carbon dioxide.

Substrate Additions The results of analysis for ethylene in samples taken from the monoliths (mean over all depths) following addition of the various substrates, are shown in Figures 16-18. From these it is seen that the addition of ammonium sulphate (Figure 16) had no effect on ethylene evolution, as was found in the test tube incubations (p 55).

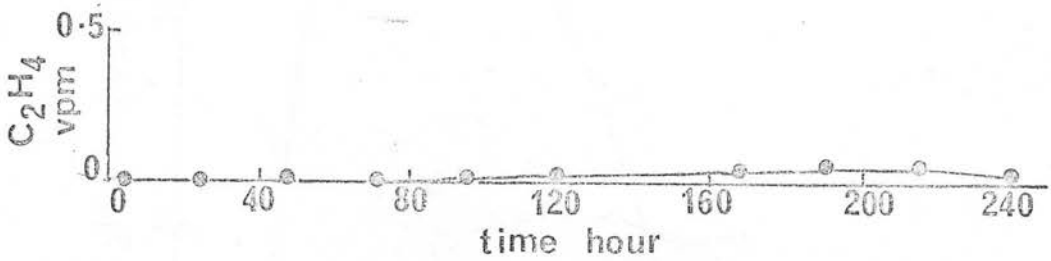

Figure 16. Effect of anaerobic addition of ammonium sulphate on ethylene production

Additions of glucose alone (Figure 17b) caused a transient increase in ethylene production, possibly indicating complete degradation of this added glucose by the soil micro-organisms coupled with breakdown of the ethylene produced by other organisms. Addition of a further litre of water containing 10 g glucose and 10 g ethanol gave a much larger increase in ethylene and a steady state was achieved (Figure 17c). Addition of ethanol alone (Figure 17d) produced much higher concentrations of ethylene, ethylene production occurring once oxygen levels had fallen below 4%, but once again levels declined after reaching a maximum. A subsequent addition

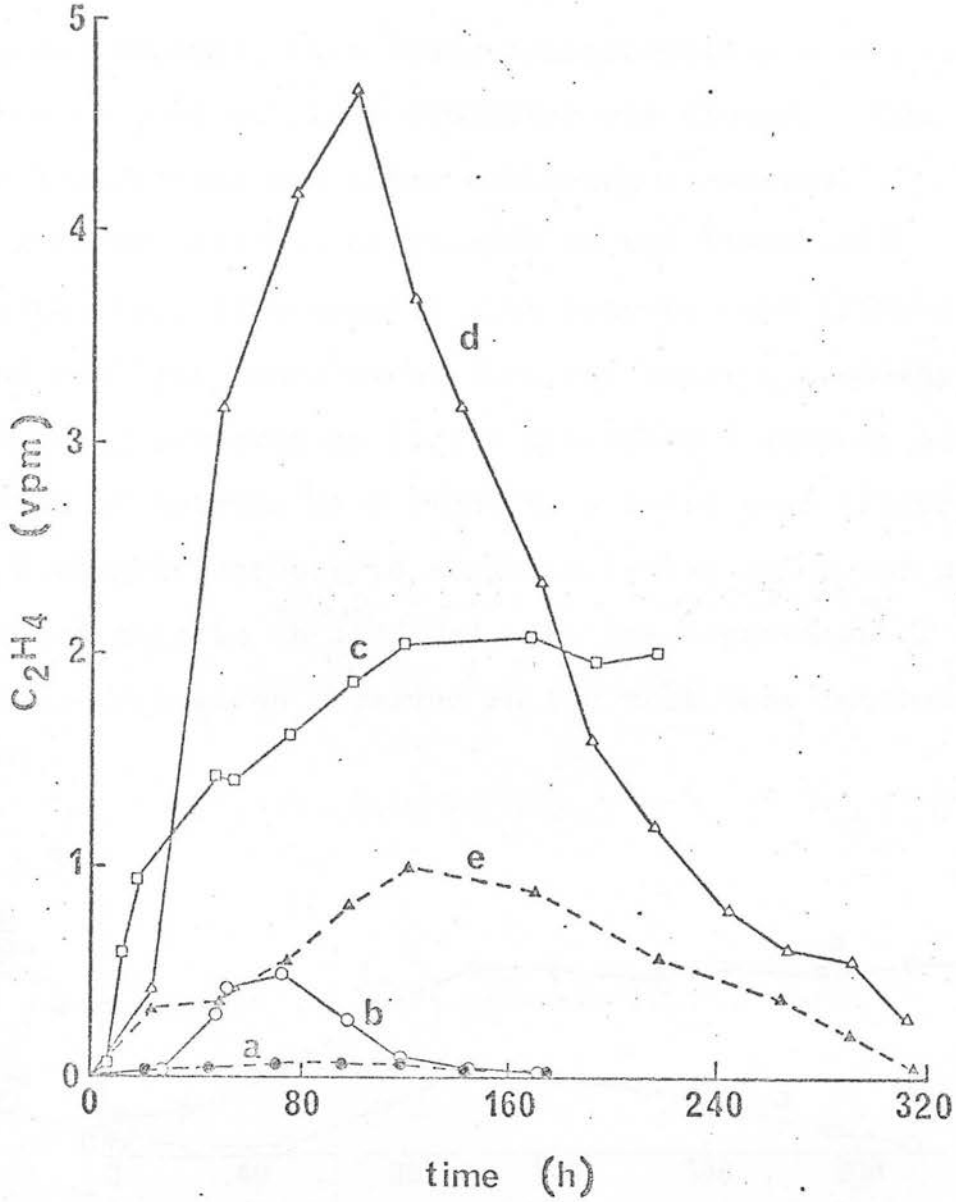

Figure 17. Effect of organic amendments on ethylene concentration in a sealed column of sandy loam soil

Amendments:-

a: control, b: glucose, c: glucose and ethanol, d: ethanol (all under initially aerobic conditions, e: ethanol, under anaerobic conditions

of ethanol to an already anaerobic monolith (Figure 17e) caused a second increase in ethylene levels. In this instance, however, much lower concentrations were produced and the rate of ethylene evolution was slower. Once again levels declined after achieving a maximum.

A third addition of glucose to the first soil monolith, this time coupled with butyric acid (Figure 18a), caused a slight increase in ethylene levels, a steady state being achieved as in the glucose and ethanol addition. Addition of butyric acid alone to a third core (Figure 18b) gave a slight increase in ethylene levels which was not sustained this is in conflict with the depression of ethylene production observed in the test tube incubations (p 59).

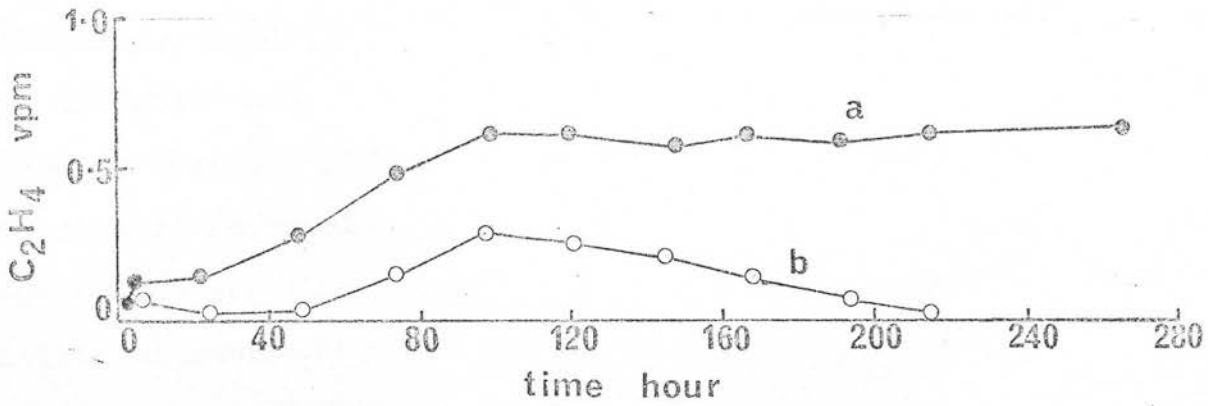

Figure 18. Effect of addition of a: glucose and butyric acid, b: butyric acid on ethylene concentration in a sealed column of sandy loam soil

The effects on the production of other hydrocarbons also differed in the soil monolith treatments compared

with the test tube incubations. Ethanol which in the tube incubations had little effect on the other hydrocarbons except but-1-ene, was found to have most effect on the production of methane (particularly the anaerobic addition), ethane and propane. Ethane production which had previously been unaffected by any of the added substrates was, in the monoliths, promoted by all amendments. Propylene production was slightly stimulated by all treatments but the combined addition of glucose and butyric acid was the most effective. This is similar to the tube incubations when it was promoted by the addition of butyric acid and by the addition of glucose although in the monoliths these treatments had only a slight effect. No explanation can be given for the differences between the test tube incubations and the additions to monoliths at present.

All additions of a single substrate to the monoliths caused an increase in ethylene production (with the exception of ammonium sulphate), butyric acid having least effect and aerobic addition of ethanol the greatest. Addition of glucose and ethanol, and glucose and butyric acid also resulted in enhanced ethylene production. The effectiveness of each substrate in stimulation of ethylene production was as follows: ethanol > glucose and ethanol > ethanol (anaerobic) > glucose and butyric acid > glucose > butyric acid.

Smith (A.M.) and Cook (1974) and Smith (A.M.) (1976b) have suggested that ethylene formation is the result of

activity by anaerobic spore-forming bacteria which depend in part on aerobic organisms or aerobic conditions to provide the substrates necessary for ethylene formation. Furthermore Smith (A.M.) (1976a) has postulated an oxygen-ethylene cycle in the soil which will result in the most prolonged and greatest production of ethylene when the soil contains a number of microsites with anaerobic/aerobic interfaces. However, he has not attempted to isolate the organisms involved in ethylene production. The validity of the oxygen-ethylene cycle which depends on the inhibiting effect of ethylene on micro-organisms has now been questioned by Smith (K.A.) (1978) who found that up to 50 ppm ethylene had no effect on microbial respiration. He concluded that the emanation of ethylene from anaerobic microsites was not resposible for the general regulation of soil microbial activity.

Isolation of soil micro-organisms capable of producing ethylene by other workers has shown that fungi (Ilag and Curtis, 1968; Lynch, 1972; Considine and Patching, 1975), yeasts (Lynch, 1972) and bacteria (Primrose and Dilworth, 1976; Primrose, 1976b) can all produce ethylene when grown in suitable media. Ilag and Curtis (1968) did not specify whether their incubations were aerobic or anaerobic, but other workers (Lynch and Harper, 1974a; Considine and Patching, 1975; Primrose and Dilworth, 1976; Primrose, 1976ab) have all found that ethylene production by microbial culture was greatest under aerobic conditions; this is in general agreement with studies on ethylene production in other biological systems

(Mapson, 1969). However in the soil ethylene appears only to be formed under anaerobic or near anaerobic conditions (Smith (K.A.) and Russell, 1969; Smith (K.A.) and Restall, 1971; Smith (K.A.) and Dowdell, 1974). Lynch and Harper (1974a) suggested that in the soil anaerobic conditions might be required to release necessary substrates, and Primrose and Dilworth (1976) observed that the slow production of ethylene by bacteria grown on artificial media might be enhanced by additional growth factors in the soil.

In the present work addition of each of the organic substrates to soil monoliths promoted ethylene production to some extent but not until oxygen concentrations had fallen below about 4%. However, ethanol, which gave the greatest enhancement of ethylene production when added to an aerobic soil, on addition to an already anaerobic soil column gave less enhancement (25%) and maximum production was achieved after 120 hours instead of 100 hours. This suggests that both anaerobic and aerobic pathways exist, but that maxumum production is favoured by the latter. Of course, the maximum equilibrium concentrations in the soil profile may still occur in anaerobic situations, because of the reduced losses due to gaseous diffusion. An alternative explanation is that aerobic conditions enhanced the breakdown of ethanol to some intermediate which was then utilised under anaerobic conditions for ethylene production. This idea is in agreement with the statement of Smith (A.M.)

(1976b) that: "anaerobes depend in part on aerobes or aerobic conditions for production of substrates for ethylene formation". Certainly it appears that a quite different pathway may be involved in the formation of ethylene in the presence of ethanol compared with that in the presence of methionine as outlined by Primrose (1976a; 1977) using Escherichia coli strain SPA O. Previous work had shown that Penicillium sp. could produce ethylene from substrates other than methionine such as ∝ ketoglutarate or glutarate (Chou and Yang, 1973) and phenolic acids (Considine and Patching, 1975). From the results of the present work it appears that in a soil depleted of nutrients for microbial production of ethylene, the addition of ethanol, and to a lesser extent glucose and butyric acid can promote hydrocarbon formation but the micro-organisms and the metabolic pathways involved are not understood.

In each of the additions of a single substrate ethylene levels decreased after about 5 days. As the soil monoliths were completely sealed and there was no decline in the carbon dioxide concentrations it was concluded that the ethylene had been lost as a result of microbial degradation at a rate greater than that at which it was being produced. In this particular case the most probable cause would appear to be lack of substrate. Where glucose was present in addition to butyric acid and to ethanol, ethylene concentrations reached a constant level after about 5 days, thus if

degradation of ethylene was taking place it was matched by the continuing production of ethylene. This suggests that the extra glucose provided additional substrate either directly or as one of its degradation products. Iniation of ethylene evolution from glucose alone occurred at a similar time to that from ethanol and butyric acid, that is as soon as oxygen concentrations fell below 2-4%. The formation of ethylene in the soil appears, therefore, to be a complex process, probably involving several micro-organisms and metabolic pathways.

Section 3.

Plant Experiments

Introduction

Legumes have a world wide distribution and include many major food, forage and pasture plants. The growth of legumes as an agricultural crop has two advantages, independence of soil nitrogen and potential improvement in nitrogen status of the soil. The nitrogen fixing ability of the legume depends on a symbiotic relationship between the plant and the appropriate strain of Rhizobium bacteria.

The active life of the nodule usually depends on the growth habit of the host plant, nodules functioning for a relatively short period during the plant's active vegetative growth. Adequate supplies of calcium, nitrogen, oxygen and carbon dioxide are required for optimum nodule development and activity. Removal of plant tops, lack of photosynthesis and attack by nematodes, insects or fungi can lead to disintegration or shedding of nodules. Other factors adversely affecting nodule development include: inter- and intra- species competition, the presence of heavy metals, pesticide chemicals, and combined forms of nitrogen, and also extremes of temperature, moisture conditions and pH (Lie, 1974; Vincent, 1974). Recent reports have shown that ethylene may affect nodule development. The inhibition of nodulation has been demonstrated in excised bean roots (Grobbelaar

et al, 1970; 1971b) and in clover grown in flasks on agar (Day et al, 1972; Day et al, 1975). Also, Ethrel, a compound which releases ethylene, has been shown to reduce nodulation in intact peas (Drennan and Norton, 1972). The effect of ethylene on other aspects of plant growth (particularly root development) has already been discussed. In this experiment the effect of ethylene on the growth and development of peas and white clover grown in soil was studied, in particular the effects on root extension (peas only) and nodulation, using concentrations of ethylene no higher than those known to occur under field conditions.

Materials and Methods

Two plant species were used: peas (Pisum sativum L. var. Feltham First) and white clover (Trifolium repens L. var. Huia). Three experimental systems were used to study the effects of ethylene on plant growth and these are described below.

All experiments were carried out in a greenhouse and plants were watered daily. Natural day length (about 14 hours) and temperatures (about 20°C) were used in systems (a) and (b). In the third system (c) an artificial 16 hour day was introduced using eight 2.4 m, 125 w white fluorescent strip lights; these gave a light intensity of 365 lux. Electric heating was used to keep the temperature at about 20°C.

Streams of air, with and without ethylene, were humidified by passing through water-filled filter flasks before entering the plant containers. The volume of gas flowing through the flasks was calibrated in terms of the number of bubbles per unit time, and the flow-rates were adjusted by means of screw clips acting on flexible tubing.

Experimental Systems

(a) Kilner-type preserving jars Pea seeds were soaked overnight in tap water prior to germination in seed boxes filled with potting compost. Once the seedlings had emerged they were transferred to specially prepared preserving jars (Figure 19). These each contained 50 ml of nutrient solution in which was placed a Perspex support covered with filter paper, marked in centimetres for visual estimation of root length (Smith and Robertson, 1971). Two holes were drilled in each lid. A stainless steel tube 3.0 mm i.d. x 9 cm was held in place by a rubber grommet which lined the outer hole. The tube and grommet were sealed in place by Silastic 734 RTV adhesive sealant. The tube was fitted with a size 13h Suba Seal rubber closure, through which a stream of air containing ethylene could be pumped via a hypodermic needle, a second needle acting as a gas outlet. The jars were wrapped in black polythene to exclude light.

A pea seedling was placed in the central hole so that the root was in contact with the filter paper inside the jar, and the shoot and the seed remained above the lid (Figure 19). Various techniques were used to hold the seedling in place before a satisfactory method was found that did not damage it. This involved placing a plasticine ring round the hole, then with the seedling in position the area around the pea seed was filled with a cold-curing silicone rubber, RTV 11 (setting agent RTV 9811) to give a gas-tight seal around the seedling. Stopcock grease and paraffin wax (m.pt. 45°C) were rejected as sealing agents as they failed to give adequate support to the seedling and a gas-tight seal. Silastic 734 RTV and RTV 162 silicone rubber adhesive/sealants were also rejected because they were found to be toxic to the peas seedlings.

After 2 days the seedlings were treated with 3.5 ml/min. air containing 1.1, 4.2 and 10 vpm of ethylene. Control plants were left open to the atmosphere by means of a gas outlet through the rubber closure. Treatment continued for 9 days and root growth was measured daily using the graduations on the filter paper.

(b) Perspex boxes These were constructed from 2 sheets of Perspex 45 cm x 59 cm (2 mm thickness) cemented to 14 mm x 14 mm strips of Perspex, which formed the side walls and bottom of the boxes. Each box

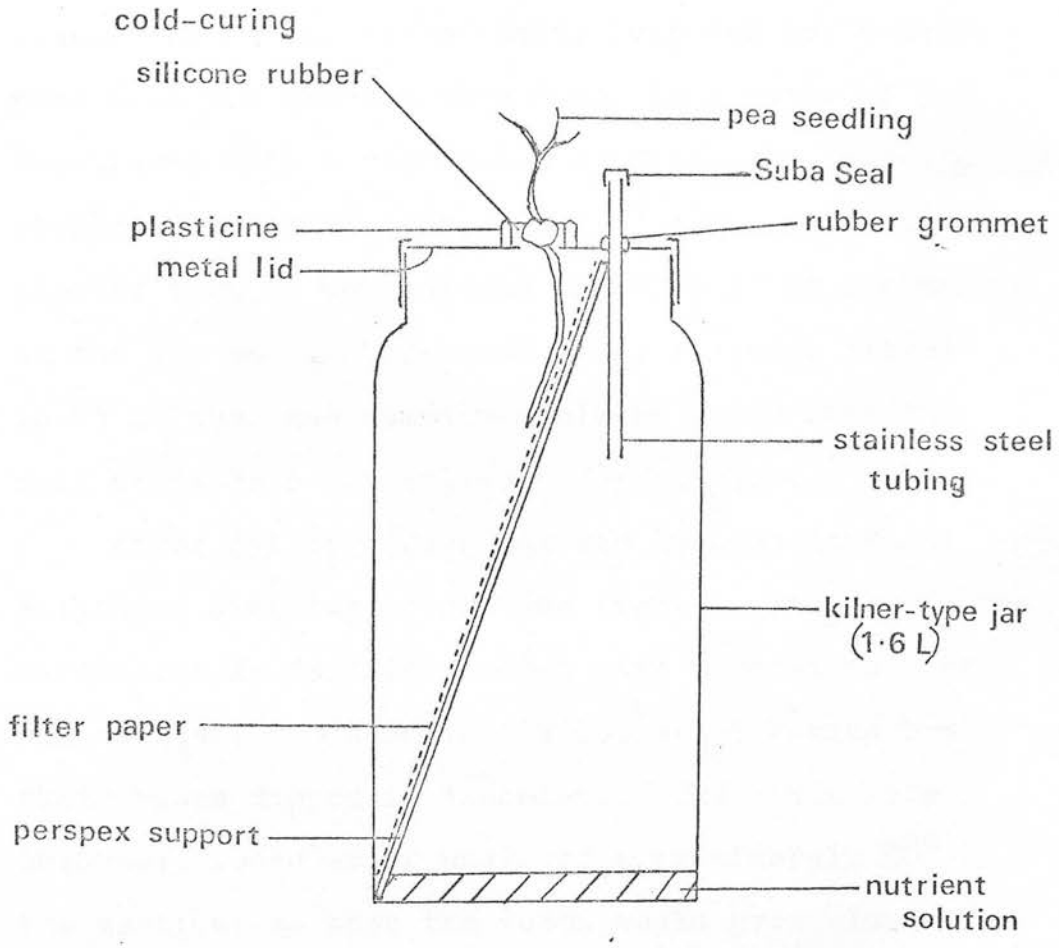

Figure 19. Kilner-type preserving jar system used to study root extension

had 3 holes, 5 mm diameter, 10.5 cm apart drilled in the bottom and fitted with stainless steel tubing (3.0 mm i.d.) to act as gas inlets, sealed in place with Silastic 734 RTV adhesive sealant. A layer of glass wool was placed in the bottom of the box prior to filling with soil. The soil used was a mixture of Darvel series sandy loam and No. 4 sand, particles not greater than 4 mm, in a ratio of 3:1 inoculated with a suspension of Rhizobium legiminosarum strains 9 and 1003 containing 10^9 organisms/ml. A plastic tube 20 cm long was postitioned in the centre of the box and a Pharmaseal 3-way stopcock fitted to it so that gas samples could be taken from the soil atmosphere for analysis for ethylene.

After filling, each box was covered in black polythene sheeting to exclude light. Four seedlings germinated as described above were planted in each box at equally spaced intervals, after having had their roots dipped in inoculum. The boxes were then positioned at an angle of approximately 30° to the vertical so that the roots would grow along the wall of the container and thus could be easily observed and daily measurement of root growth made. After 14 days, air containing 10 vpm of ethylene was passed at a rate of 3.5 ml/min. into one box. The second, which was to be the control, was treated with air only (3.5 ml/min.). Treatment continued for 14 days. Daily measurement of root length was

carried out for the first 4 days of treatment. Photographs were taken after 7 and 14 days.

(c) Plant pots (i) Peas were grown from seed (two plants per pot) in 12 cm diameter plastic plant pots, containing the same soil/sand mixture as system (b). Half the total of 12 pots acted as a control and were untreated and the other half were exposed to air containing 10 vpm of ethylene, which entered through a tube inserted in the base of the plant pot and sealed with Silastic 734 RTV. Ethylene treatment began 4 weeks after planting and continued until plants reached maturity 7 weeks later.

(ii) Clover was grown from seed in pots as described in (i) above. The soil was inoculated with a suspension of Rhizobium trifolii (10^9 organisms/ml). After germination the seedlings were thinned to give between 27 and 34 plants per pot. Exposure to air containing 10 vpm of ethylene began 4 weeks after planting and continued for 6 weeks after which time the plants were harvested.

After harvesting growth was assessed by measurements of root and shoot length, root and shoot dry weights, nodule number, node number (peas only) and stem number (clover only). Nodule efficiency was determined by measuring the reduction of acetylene to ethylene as an assay of nitrogenase activity in the nodules (Koch and Evans, 1966). This was done by placing an excised root in a 50 ml polypropylene

centrifuge tube sealed with a B57 Suba Seal type rubber closure. A 5 ml sample of acetylene was injected into each tube and the amount of ethylene produced by the nodules at 20°C measured by gas chromatography after 30 minutes.

Results and Discussion

Root Extension In the experiments using the kilner jar system, growth of pea roots was retarded at the 1.1 vpm concentration but not inhibited completely (Figure 20) and only very slight swelling of the tips was visible. At the two higher concentrations the plants' normal response to gravity was inhibited and the root tips were not only swollen, but were bent in a horizontal direction. The roots were also covered in root hairs. At the 4.2 vpm level growth was inhibited entirely after 4 days of treatment. At the 10 vpm level however, there was little further root extension vertically, the apparent increase in length of 0.5 cm being almost entirely accounted for by the curving and swelling of the root, the extent of curvature being much greater at the 10 vpm level than at the 4.2 vpm level. Hence at the 10 vpm level root extension in pea seedlings was almost completely inhibited, no further growth of the main root taking place after 3 days of treatment. The controls grew at an average rate of 0.96 cm/day and the plants treated with 1.1 vpm at a rate of 0.50 cm/day, over the 9 day period of treatment. Peas appear therefore to be similar in their sensitivity to ethylene to barley; in the

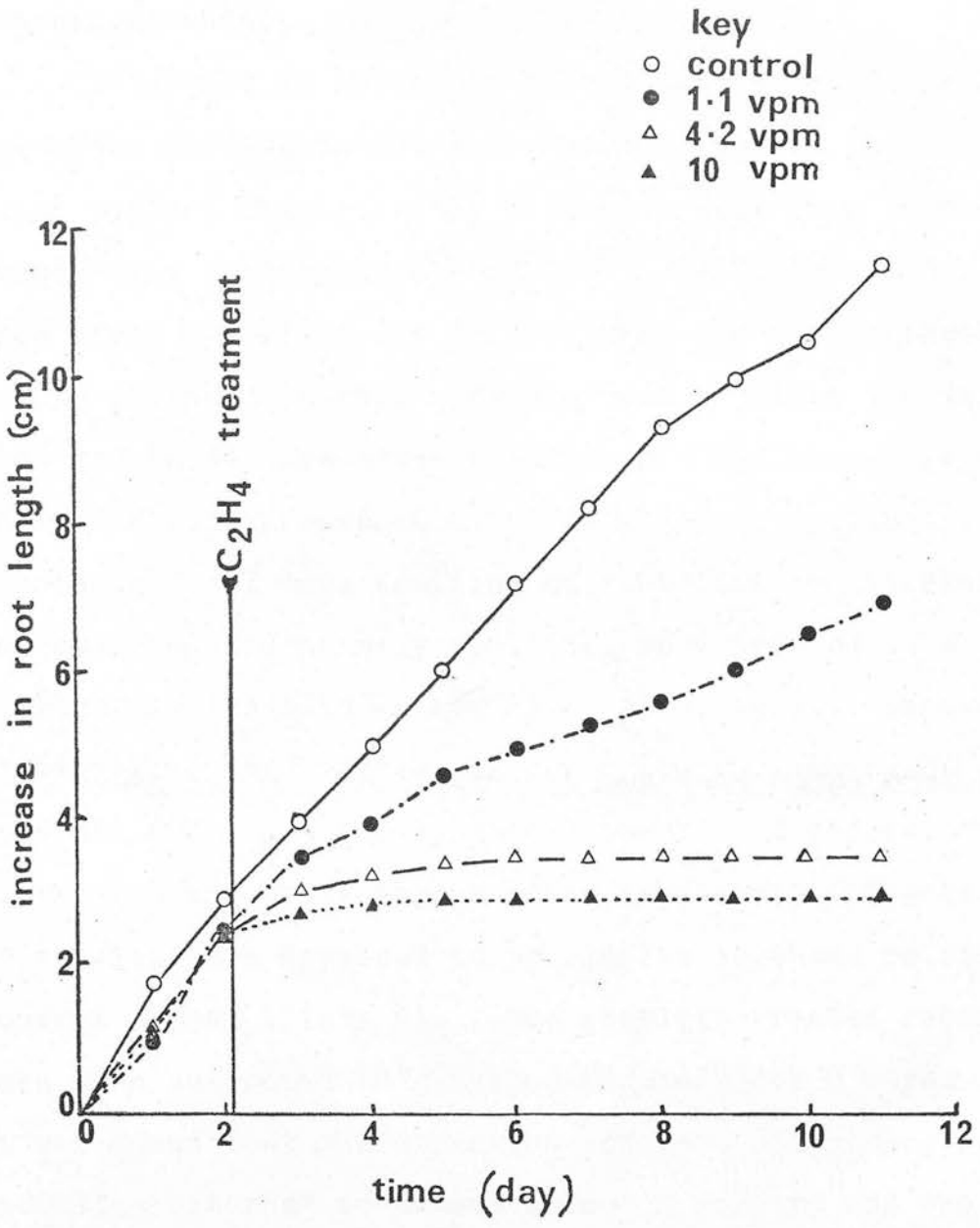

Figure 20. Effect of three concentrations of ethylene on root extension

latter species 10 vpm of ethylene has been found to cause root extension to cease by the end of 3 days, while 1 vpm reduced root extension by about 50% (Smith (K.A.) and Robertson, 1971).

The effect of treatment with 10 vpm of ethylene on pea roots growing in the soil-sand mixture in the Perspex boxes was not significantly different from that in the kilner-type preserving jar system. Swelling could be seen after the first day of treatment and little further growth of the main root axis occurred. Plate 1 shows root growth 14 days after planting the seedlings, immediatly before exposure to ethylene. After ethylene treatment for 7 days swelling of root tips and laterals had occurred and primary root tips were growing in a horizontal direction (Plate 2). After 14 days exposure to ethylene, the treated plants showed thickening of all laterals (Plates 3 and 4) both those formed before treatment began and those formed after treatment. The length of the laterals appeared to be similar to those in the control plants (Plate 5). The ethylene treated roots were then subjected to ethylene-free air for 8 days. It was found that the direction and rate of primary root extension returned to normal (about 1 cm/day) and the root had a normal unswollen appearance on removal of ethylene (Plate 6); black spots mark the point at which regrowth started. Lateral roots continued to grow but lost their swollen appearance. There was no difference between the rate of growth of laterals on the ethylene

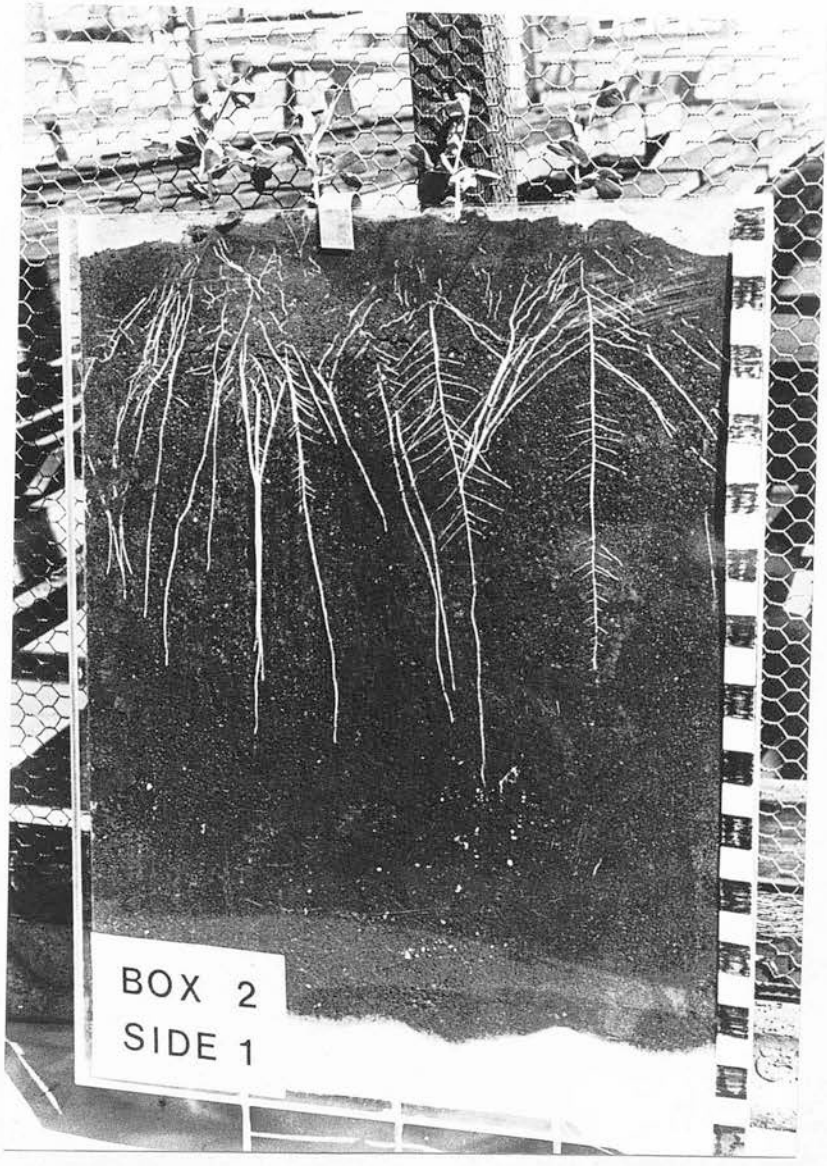

Plate 1. Root growth of pea plants after 14 days growth in Perspex boxes. (Scale marked in 2 cm intervals).

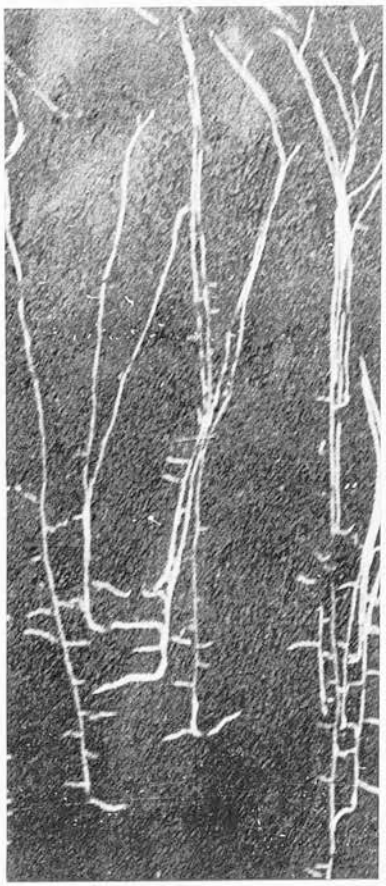

Plate 2. Root growth of pea plants after 7 days treatment with air containing 10 vpm ethylene

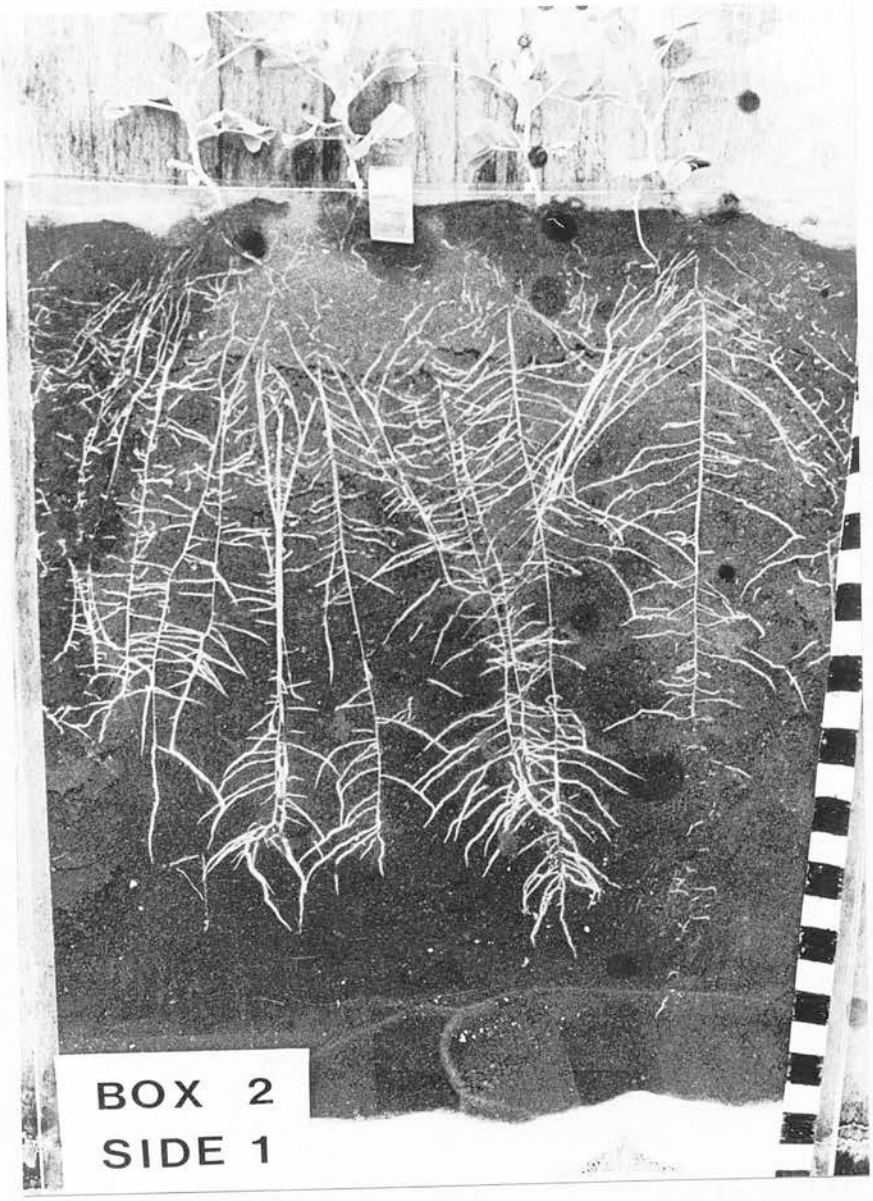

Plate 3. Root growth of pea plants after 14 days treatment with air containing 10 vpm ethylene

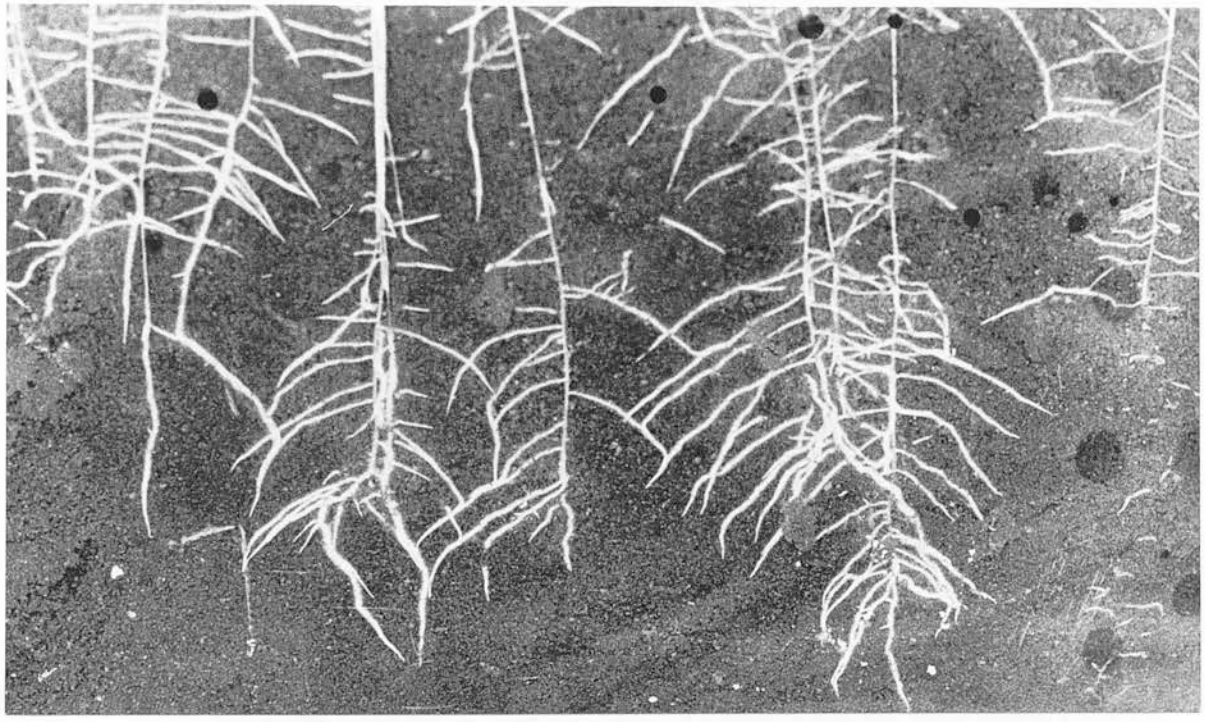

Plate 4. Effect of 14 days treatment with air containing 10 vpm ethylene on growth of root tips of pea plants

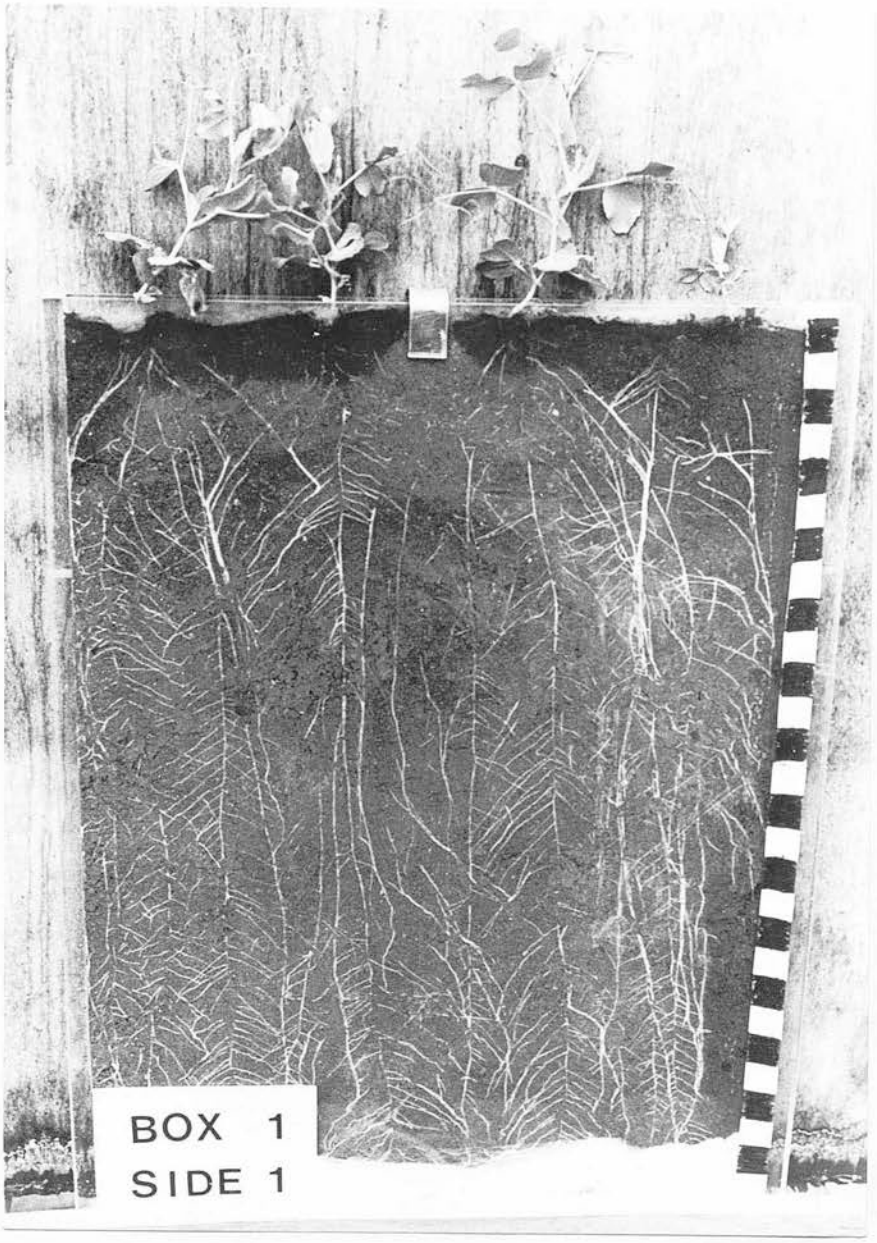

Plate 5. Root growth of control pea plants after 28 days growth

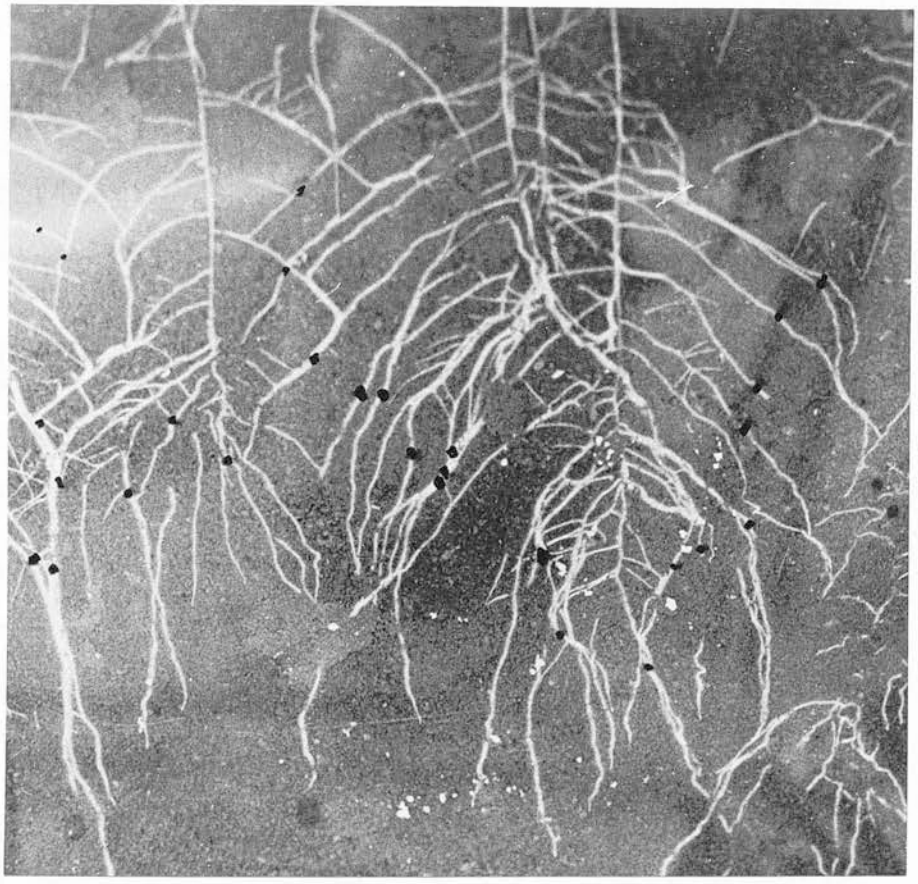

Plate 6. Root growth of ethylene treated pea plants on removal of ethylene. (Black spots mark the point at which regrowth started).

treated plants and those on control plants. This is in contrast to ethylene treated barley roots which, on transfer to an ethylene-free environment, had an extension rate in laterals initiated during the period of treatment five times that of laterals in the control plants (Crossett and Campbell, 1975).

Nodulation None of the roots of the plants grown in Perspex boxes, neither those of the control plants, nor those of plants treated with ethylene, developed nodules during the course of the experiment described above. However, pea seedlings grown in conventional plant pots at the same time, under the same temperature and light regime, were satisfactorily nodulated. These plants were taller and more healthy in appearance than those in the Perspex boxes. They were also upright whereas those in the boxes were bent, even though the variety used was a dwarf variety which did not need support. It was thought, therefore, that the absence of nodules might have been due to the plants being under stress, in view of the bending of the stems and the apparent weaker growth in the experimental plants. The experiment was therefore repeated with all the conditions remaining the same except that wiremesh was used to support the shoots of the plants in the boxes. However, no nodules were visible on completion of the experiment.

It was decided, therefore, to study the effects of ethylene on plants grown in pots, so the system described in (c) above was used:

(i) Peas It had been found in the course of previous work that nodules were sparse and poorly developed if plants were harvested before flowering. Therefore in this experiment ethylene treatment was continued for 7 weeks, by which time flowering was complete and pea pods had been formed, before separating the roots from the soil to investigate the effects on nodulation. Other workers (Schinghammer, 1960; Schinghammer et al, 1970) have found that nodulation in peas grown under fluorescent lights (18 hour day at 20-23°C) had taken place within 16 days after inoculation. Seedlings were grown in sterile vermiculite and contamination by foreign rhizobia and other micro-organisms was prevented. It is thought that the slower initiation of nodules in the present work may have been due to slow development of the introduced rhizobial population in the soil. Survival of an introduced rhizobial strain depends on other soil micro-organisms. It is difficult to introduce and establish a new strain in a soil containing the same rhizobial species because strains differ in their ability to establish themselves in competition with rhizobia already in the soil (Read, 1953; Baird, 1955; Jenkins et al, 1954; Ireland and Vincent, 1968). Other factors affecting the rhizobial population in the soil include attack by predators such as protozoa, myxobacteria and rhizophage; competition between rhizobia and other soil bacteria for nutrients and the presence of toxic metabolites of other micro-organisms in the rhizosphere inhibit the

infection process and nodule maturation (Lie, 1974; Vincent, 1974).

The effect of ethylene treatment on the growth of pea plants is shown in Table 20. It is seen from this table that although the ethylene treated roots were shorter than the controls (but not statistically significant), the dry weights were similar, indicating that increased lateral and/or radial growth had compensated for any loss in length of the main root.

Table 20. The effect of exposure to air containing 10 vpm ethylene on root and shoot growth in pea plants

	control	C_2H_4 10 vpm
Nodules/plant	38.4	11.4***
Main root axis mean length (cm)	18.5	16.3
Main shoot mean length (cm)	19.7	17.4
Number of nodes	10.4	10.4
Mean root fresh weight (g)	1.37	0.97
Mean root dry weight (g)	0.16	0.17
Mean shoot fresh weight, incl. pods (g)	3.86	2.55
Mean shoot dry weight, incl. pods (g)	0.64	0.31**
Mean shoot fresh weight, excl. pods (g)	1.08	1.34
Mean shoot dry weight, excl. pods (g)	0.37	0.23

** significant at p = 0.01

*** significant at p = 0.001

These effects on root growth are similar to those obtained by Crossett and Campbell (1975) for barley and by

Grobbelaar et al (1971b) for excised bean roots. Root fresh weight was reduced slightly in ethylene treated plants (but not significantly); similar observations were made by Drennan and Norton (1972) who found that root fresh weight was decreased in Ethrel-treated pea plants.

Observations during the course of the experiment showed no obvious differences in overall appearance and rate of maturation of the plants. Table 20 shows that shoots of both treated and control plants were of similar length, and had similar fresh and dry weights, but the control plants had heavier pods.

Ethylene treated plants had fewer and smaller nodules than control plants (Plates 7a, 7b). Those nodules present on ethylene treated plants tended to be on the same root and close together. It was thought that this was probably due to uneven diffusion of ethylene through the pot. Gas samples taken from the soil by means of plastic tubing attached to a syringe showed that the ethylene concentrations in the soil in the pots at the sampling points, were about 7-8 vpm. Crossett and Campbell (1975) have shown that the effects of ethylene on barley root growth are not translocated from treated roots to un-treated roots. Similarly Drennan and Norton (1972) have shown that only roots treated with Ethrel exhibit inhibition of nodulation, neighbouring un-treated roots actually formed more nodules than on control plants

Plate 7. Root growth of (a) control plants and (b) plants treated with air containing 10 vpm ethylene for 7 weeks.

(ii) Clover The effect of ethylene treatment on the growth of clover is shown in Table 21. These results show that, unlike the pea plant, stem length is reduced by ethylene treatment and stem fresh and dry weights are significantly reduced. Root length and root fresh and dry weights were reduced slightly and nodulation was significantly decreased, although the reduction in nodulation was less in ethylene treated clover plants (44%), than in similarly treated pea plants (70%).

Table 21. The effect of exposure to air containing 10 vpm ethylene on root and stem growth in clover plants

	control	C_2H_4 10 vpm
Nodules/plant	16.5	9.2**
Main root axis mean length (cm)	10.2	9.8
Longest stem mean length (cm)	10.0	8.7**
Number of stems, mean	4.7	4.3
Mean root fresh weight (g)	0.10	0.09
Mean root dry weight (g)	0.022	0.017
Mean stem fresh weight (g)	0.27	0.18*
Mean stem dry weight (g)	0.045	0.027**

* significant at p = 0.02

** significant at p = 0.01

Day et al (1972) and Day et al (1975) found that removal of ethylene from clover plants grown on agar

increased nodulation. The present work shows that the presence of ethylene in soil inhibits nodulation in clover plants.

Nitrogenase activity of nodules Acetylene reduction assays were carried out on roots of both control and ethylene treated pea and clover plants. The rates of ethylene formation are shown in Table 22, and indicate

Table 22. Acetylene reduction by excised roots of control and ethylene treated plants

	C_2H_4 (ng/plant/min)		C_2H_4 (ng/nodule/min)	
	Pea	Clover	Pea	Clover
Control Plants	1.44	6.29	0.036	0.44
Ethylene treated plants	0.04	1.77	0.003	0.13

that nitrogenase enzymes were present in the nodules in both plant treatments, but that the nitrogenase activity was significantly ($p = 0.01$) suppressed in the ethylene treated plants compared with the control plants. Nodulation and N-fixation in excised bean roots (Grobbelaar et al, 1970, 1971b) and in intact clover plants (Day et al, 1972, 1975) is inhibited by ethylene, and the ethylene releasing compound Ethrel inhibits nodulation in intact peas (Drennan and Norton, 1972). Grobbelaar et al used concentrations of ethylene of 0.4, 8 and 300 ppm. It was found that nodulation was inhibited completely by 8 and 300 ppm and was greatly reduced by the 0.4 ppm

treatment. These workers also found that nodulated roots in treatments from which ethylene was continuously removed, fixed more nitrogen than nodulated roots from which ethylene was not removed or to which 10 and 100 ppm of ethylene was added. This suggested that endogenous ethylene produced by the excised roots also inhibited nitrogen fixation. Drennan and Norton (1972) used Ethrel (2 chloroethane phosphoric acid). This is an ethylene-releasing chemical used in horticulture for the promotion of ripening in fruit and modification of growth characteristics (Sterry, 1975). Concentrations used in horticulture are usually referred to in terms of the "active ingredient", i.e. the ethylene-releasing compound, rather than in terms of the quantity of ethylene released. It is the concentration of the active ingredient in Ethrel which is referred to in the work of Drennan and Norton. These concentrations were 2, 4 and 8 ppm. The authors state (Drennan, 1977) that it would have been preferable to have used ethylene equivalents but it was not possible to judge how rapidly or over what time period the ethylene was being formed in the experimental system. It is therefore difficult to determine the actual significance of the concentrations these authors used. In the present work it has been shown that nodulation and nitrogenase activity are inhibited when roots are grown in soil exposed to ethylene concentrations which have previously been shown to occur under field conditions (Smith (K.A.) and Dowdell, 1974).

The most probable explanation of the failure of pea plants in the Perspex boxes to nodulate is inhibition due to light (Rudin, 1956; Grobbelaar *et al*, 1971a; Holstein *et al*, 1971). Although the boxes were covered with black polythene to keep out the light, the polythene was removed periodically to observe the root growth and to take photographs. Evidence to support this theory was obtained from a further experiment. On the successful completion of the plant pot experiment more seeds were sown in one Perspex box which was covered with black polythene as usual and left undisturbed until the plants reached maturity. On removal of the plants from the boxes it was found that nodulation had taken place (Plate 8).

One implication of the suppression of nitrogenase activity by ethylene is the possible error associated with the use of the acetylene-reduction assay as a quantitative measure of nitrogen fixation. This is because the accumulation of ethylene produced by the reduction of acetylene will have an inhibitory effect on further nitrogen fixation. Nitrogen fixation rates calculated from acetylene-reduction measurements could therefore be in error if the exposure of the roots and nodules to ethylene was sufficiently prolonged.

In the field situation the occurrence of ethylene in the soil may affect nodulation in the legume crop and thus the ability to fix nitrogen. Ethylene in the soil may be only of short duration and may not affect the

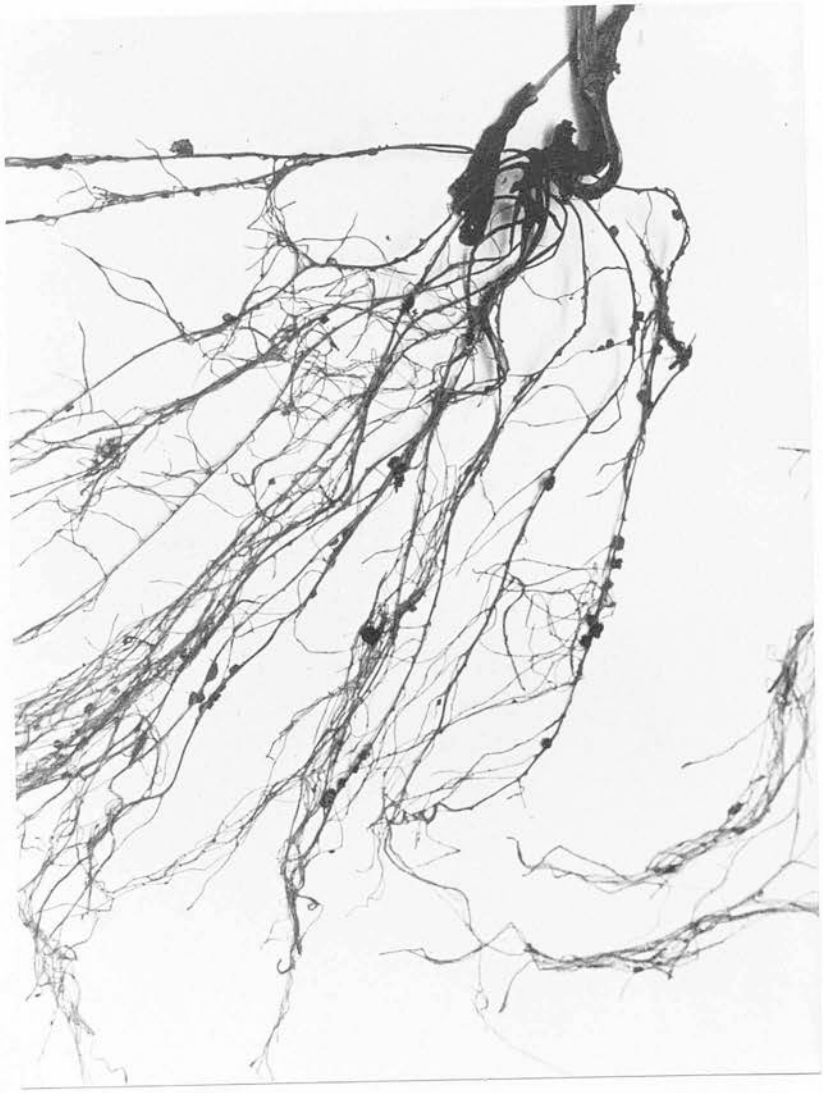

Plate 8. Nodule development in pea plants grown in Perspex boxes continously covered with black polythene

plant growth as a whole (plant roots recovered from ethylene treatment and grew normally). However, if ethylene is present for long periods the economic value of the crop may be reduced. For example, in the present work prolonged ethylene treatment (6-7 weeks) resulted in a 57% reduction in the fresh weight and a 70% reduction in the dry weight of pea pods; a similar reduction in a field crop would result in a major loss of income. Similarly, the fresh weight of clover stems was reduced by 33% and the dry weight by 40%. A comparable reduction in a field crop would mean that the quantity of fodder harvested from the crop would be reduced.

Comparisons between the experiments described here and field conditions are complicated by effects due to aeration. In the experiments ethylene treatment was given in air, but in the field ethylene production in soils is associated with high moisture contents and low oxygen levels. This does not mean, however, that the inhibitory effects of ethylene are likely to be cancelled out. Both waterlogging and lack of oxygen are known to adversely affect nodule formation and efficiency (Lie, 1974; Vincent, 1974), and a combination of waterlogged and anaerobic conditions and possible associated production of ethylene might well have extremely deleterious effects on both legume growth and successful symbiosis. The interactions between ethylene and aeration in this context have yet to be investigated.

GENERAL DISCUSSION

and

SUMMARY

GENERAL DISCUSSION and SUMMARY

Laboratory incubation studies have shown that the quantity of ethylene formed in soil is related to the organic matter content and the pH of the soil: production being promoted by increased soil organic matter content, and depressed by high soil pH. Cultivation appears to have no effect on the capacity of the soil to produce ethylene, however the relationship between ethylene and pH is stronger in arable soils than in grass soils. The reason for this difference is not understood and further work is needed to investigate possible differences in microbial populations and availability of substrates between arable and grassland soils.

Comparisons of ethylene production in fresh soils and air dried soils showed that drying had a marked effect. Generally fresh soils evolved little ethylene, but air drying increased this by factors up to 2000-fold. The value of the correlation coefficient between ethylene evolution and organic matter content was greater in dried soils than in fresh soils. This suggests that while organic matter is the ultimate source of substrates for microbial formation of ethylene, the quantities of these substrates is generally low in fresh soil, but is dramatically increased by drying. It is considered, therefore, that in studies of ethylene production in soils, results obtained with air dried soils should not be used as a direct indication of the levels of ethylene that might be found in fresh soil in the field.

The addition of straw to air dried soil stimulated ethylene production. The addition of hay had no effect. It is thought that some stimulation of ethylene production might have been observed on the addition of hay if a soil with a lower capacity for ethylene production had been used. Similarly in view of the lower ethylene production observed in fresh soils compared with air dried soils, greater stimulation might have occurred if hay had been added to a fresh soil. This suggestion is supported by the results obtained when glucose, ethanol and butyric acid were added to air dried soil in sealed tubes compared with addition of the same compounds to undisturbed soil cores taken from the field and maintained in a moist condition. The untreated soil monoliths produced no ethylene while addition of each of the three organic substrates produced maximum ethylene concentrations ranging from 0.28 to 4.65 vpm. In the test tube incubation studies with air dried soil, glucose and ethanol increased ethylene production but by a smaller proportion compared with the levels in the control than the undisturbed monoliths, while butyric acid inhibited ethylene formation.

Other organic compounds which enhanced ethylene production in dried soil include caesin, pepsin, lactic acid and pyruvic acid. The mechanisms involved in the enhancment of ethylene production and the micro-organisms involved is not known and further work is needed to isolate micro-organisms capable of producing ethylene

from these compounds in pure culture. It is not known from the present work whether these compounds are precursors of ethylene or whether they are merely promoting the growth of micro-organisms capable of breaking down other compounds present in the soil to ethylene.

The addition of ethanol to aerobic soil in an undisturbed monolith resulted in promotion of ethylene evolution once oxygen levels fell below 4%. However, a subsequent addition of ethanol to an already anaerobic soil gave less enhancement (25%) and maximum production was delayed by 20 hours. These studies suggest that both aerobic and anaerobic pathways exist in the formation of ethylene but that maximum production is favoured by initial aerobic conditions. More work is needed on the metabolic pathways involved in ethylene formation, on the conditions which favour production, and on the organisms involved. In the present work the enhanced formation of ethylene at low pH suggests that fungi may be responsible. The stimulation of ethylene formation at the lower rate of glucose addition but not at the higher rate also supports this (Griffin, 1972). Further investigation is needed on the relative importance of fungi and bacteria in soil production of ethylene, and how this is affected by the aeration status of the soil and by drying of the soil.

High nitrate levels were found to depress, but not completely inhibit, the formation of ethylene in contradiction to the findings of Smith (A.M.) (1974). Thus in the field situation it is likely that ethylene

concentrations could still reach physiologically significant levels, the presence of nitrate merely causing a delay in attainment of maximum concentrations.

Studies of the effect of ethylene on the growth of peas and clover showed that nodulation and nitrogen fixation were inhibited in both species by the presence of ethylene and led to possible doubts as to the suitability of the acetylene-reduction method as a quantitative assay for nitrogen fixation if exposure of roots and nodules to ethylene produced during the test is prolonged. Plant growth was not visibly altered in either species apart from the reduction in nodules, but fresh and dry weight measurements showed that production of pods (in peas) and foliage (in clover) were significantly reduced by ethylene treatment, root length was also reduced slightly, but fresh and dry weights of roots were not affected.

Degradation of ethylene has generally been reported to take place aerobically as ethylene diffuses to aerobic zones. In the present work a decrease in ethylene concentrations under anaerobic conditions was observed, indicating that degradation of ethylene can take plsce anaerobically at the site of production. Accumulation of ethylene will therefore only occur when the rate of production is greater than the rate of degradation, and it seems likely that the levels of ethylene observed in a soil profile represent the balance between on the one hand, evolution, and on the other hand, degradation processes (both aerobic and anaerobic) and losses due to gaseous diffusion.

The common agricultural practices of ploughing in stubble, adding farm yard manure, incorporation of chopped straw and minimum cultivations in which surface trash is mixed with the soil, may all be potential sources of ethylene production by soil micro-organisms if anaerobic conditions develop as a result of a reduced rate of oxygen diffusion caused by excessive wetness and/or soil compaction. Thus the risk of ethylene formation and subsequent damage to a crop is highest in the spring when, the incorporated organic matter may still be present, and high moisture levels coupled with rising soil temperatures result in rapid microbial activity, depletion of oxygen in the soil and subsequent ethylene production. Other compounds known to be toxic to plants such as organic acids including acetic and butyric acid are also formed under these conditions. This is also a time when the crop is also beginning to respond to the rising temperatures by more rapid growth than during the winter months, and is therefore more susceptible. Thus, effects on root growth under field conditions are complex, and no one factor is likely to be the universal cause of inhibited growth. However the results of this work, implicating the decomposition products of plant residues in ethylene production, indicate that the presence of ethylene must be considered to be a factor affecting plant growth, in addition to those others identified under these conditions.

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APPENDIX

Gas Chromatography

Principles

Chromatography is a means of separation of two or more substances in a mixture by partition between a stationary phase and a moving phase. In gas chromatography the mobile phase is a gas, and in recent years its use as an analytical technique has spread very rapidly due to the combination of easy resolution of related compounds and high sensitivity which it provides.

There are two types of gas chromatography. Firstly, gas-liquid chromatography, in which the stationary phase is a liquid held on an inert support and secondly, gas-solid chromatography in which the stationary phase is an active solid. The mixture under investigation is injected into a stream of carrier gas at the head of a column of separating medium, whose composition varies according to the components to be separated. The sample mixture is taken by the carrier gas onto the column where the various components are resolved; some may go straight through the column, others may be completely absorbed, while others, due to partition between the stationary phase and the carrier gas, will gradually be separated one from another during their passage along the column. As the gases or vapours pass a suitable detector, the output signal of the detector (converted to a recording chart trace) will then indicate the presence of the various components of the sample and their abundance. There

are a wide variety of detectors available and the signal from these detectors can be amplified if necessary before recording. In the present work two detectors were used, one based on changes in thermal conductivity and one on changes in flame ionisation characteristics. These are described in more detail below.

Katharometer This is a thermal conductivity cell in which changes of conductivity of the carrier gas stream due to the presence of the sample components are used to produce an electrical signal. Electrically heated filaments are mounted in each of two passages in a metal block; pure carrier gas is passed through one as a reference, and the fractionated components of the sample mixture carried in the carrier gas from the column go through the other. Changes in conductivity caused by gases other than the carrier gas passing over the filament result in temperature changes in the filament which lead to changes in resistance which can be detected and measured. The magnitude of the response depends on the thermal conductivity of the separated gas compared with that of the carrier gas, and the direction of the response upon whether this thermal conductivity is larger or smaller than that of the carrier gas. Gases commonly used as carriers are helium, hydrogen, nitrogen and argon, much larger responses being obtained with helium and hydrogen.

Flame ionisation detector This is based on the conduction of electricity by gases and is more sensitive than the

katharometer. It is very suitable for the determination of trace amounts of hydrocarbons in air or other inorganic gas mixtures. The organic components of the injected sample provide a source of ions by burning in the hydrogen flame. These ions are collected by an anode maintained at a suitable potential (usually 100-300 V) above that of the cathode (the flame jet) causing a change of current which can be recorded. Unlike the katharometer the flame detector is a selective detector, with little or no response to non-combustible gases.

Experimental

Analysis of experimental samples was carried out on a Pye "Series 104" chromatograph fitted with a flame ionisation detector and a heated Katharometer.

Hydrocarbon separation was achieved on a 2 m x 6.0 mm stainless steel column of alumina F1, 60-80 mesh, (Smith (K.A.) and Restall, 1971) partially deactivated with sodium iodide (Scott and Phillips, 1965). For the separation of inorganic gases two columns were prepared as follows:
a) a 2 m x 3.0 mm stainless steel column of molecular sieve 5A, 60-80 mesh, activated at 300°C, for the separation of oxygen and nitrogen (Smith (D.H) and Clark, 1960) and
b) a 2 m x 6.0 mm stainless steel column of Poropak Q (porous polymer beads), 50-80 mesh, activated at 200°C for the separation of carbon dioxide and nitrous oxide (Bell, 1968). All three columns were fitted into a single oven, an extra injection port being fitted to

accommodate the molecular sieve column which was connected in parallel to the Porapak Q column (Figure 21).

Carrier gases and auxiliary gases were connected to the chromatograph by 3 mm o.d. annealed copper tubing and the flow regulated by gas flow controllers.

A 13 cm x 6.0 mm glass pre-column packed with magnesium perchlorate (to absorb moisture from samples for inorganic gas analysis) was prepared and fitted below the injection port. A brass T piece and two lengths of 1.5 mm o.d. stainless steel capillary tube were used to split the sample between the Porapak Q and the molecular sieve 5A columns (Figure 21). The lengths of tubing were adjusted to 270 mm and 195 mm to give a sample split ratio of 9:1. The separated gases were recombined before passing through the katharometer. In this way a single injection could be used for simultaneous analysis of both carbon dioxide and oxygen and nitrogen, and the results recorded on a single chromatogram. Due to the 9:1 split ratio no attenuation adjustment was needed if the ratio of oxygen to carbon dioxide was in the range 6-60 approximately. The great majority of gas samples analysed were within these limits. Injection ports were also fitted immediatly above the two columns so that samples could be injected directly onto a specific column if required. The flow rate of the helium carrier gas was 55 ml/min. When analysis of nitrous oxide was not required, the column oven was maintained at 110°C and the detector oven at 160°C. For the analysis of nitrous

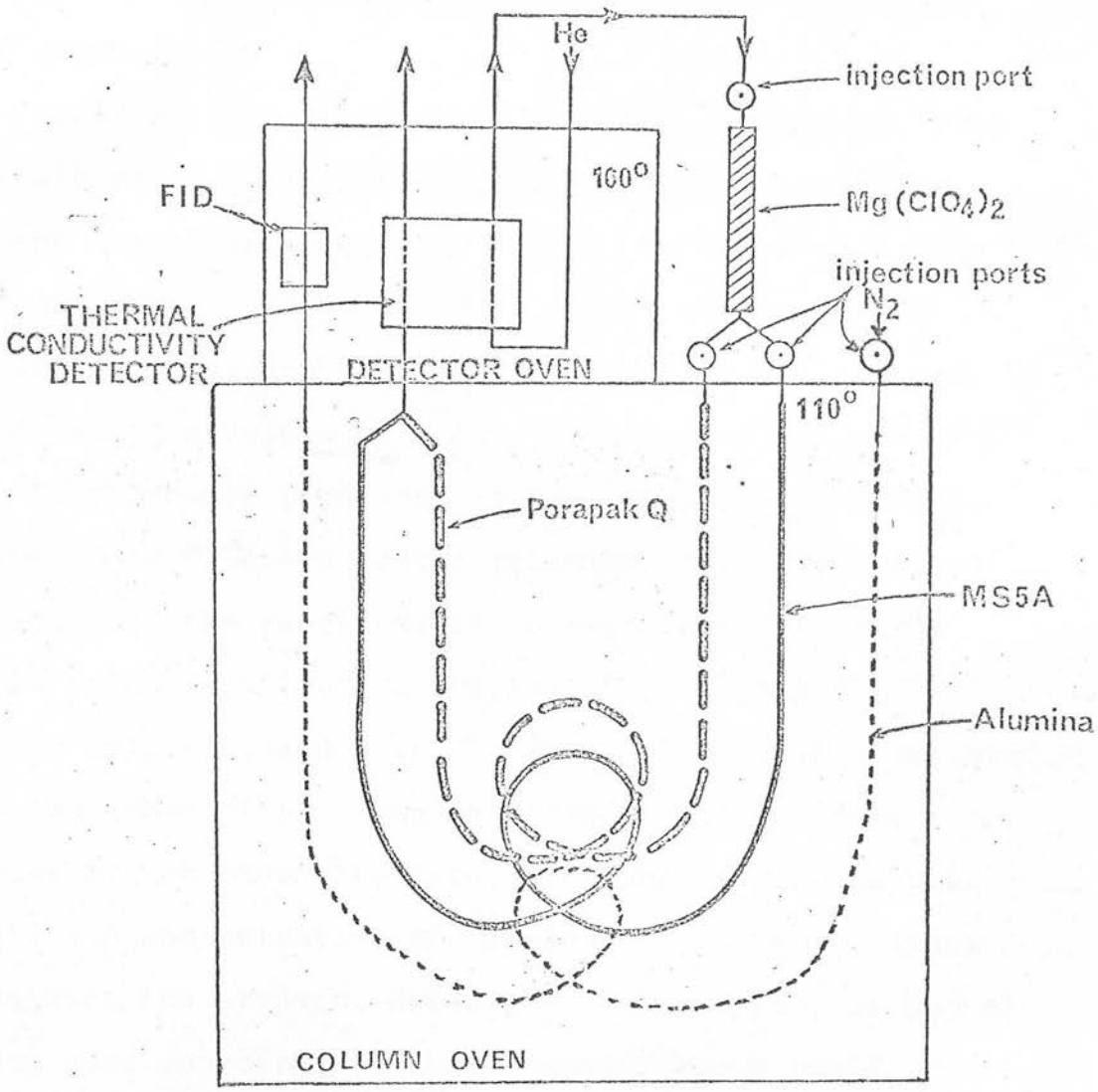

Figure 21. Gas chromatography system.

oxide the temperature of the column oven was reduced to 60°C; this gave adequate separation from carbon dioxide.

For analysis of hydrocarbons samples were injected straight onto the alumina column at 110°C. The flow rate of nitrogen carrier gas was 75 ml/min and the hydrogen and air flow rates for the flame were set at 75 ml/min and 480 ml/min respectively. Typical chromatograms for inorgainc gases and hydrocarbons are shown in Figures 22-24.

Analyses were carried out with gas samples of known composition to check the linearity of the relationship between sample size and peak height, as shown in Figures 25-28. For all gases, except nitrogen, the relationship between peak height and sample volume was linear up to a volume of 2 ml. Samples of greater than 2 ml gave broader peaks and it was necessary to measure peak area to obtain a linear relationship. With nitrogen, linearity of the relationship between peak height and sample volume was lost in samples greater than 1 ml (Figure 28), nitrogen being the only peak to show distortion from the symmetrical Gaussian shape. Using 0.5 ml samples it was shown that the relationship between peak height and concentration of nitrogen was linear (Figure 29). Throughout the project, where possible, samples of 0.5 ml volume were injected, so that concentrations could be calculated by direct comparison of peak heights of the injected sample with those obtained by injection of a standard mixture. When only smaller volumes could be

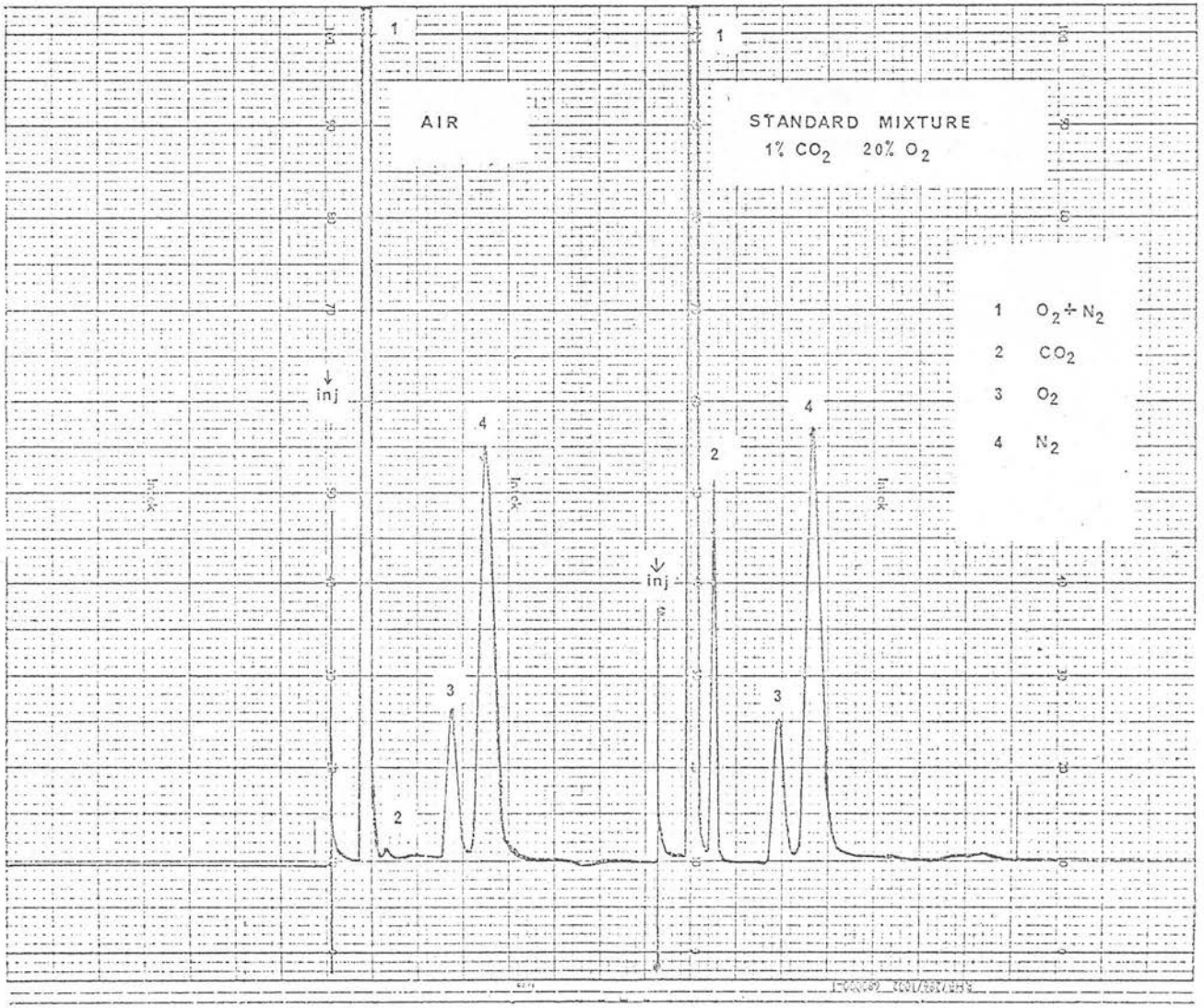

Figure 22. Chromatogram showing separation of inorganic gases in air and in standard mixture.

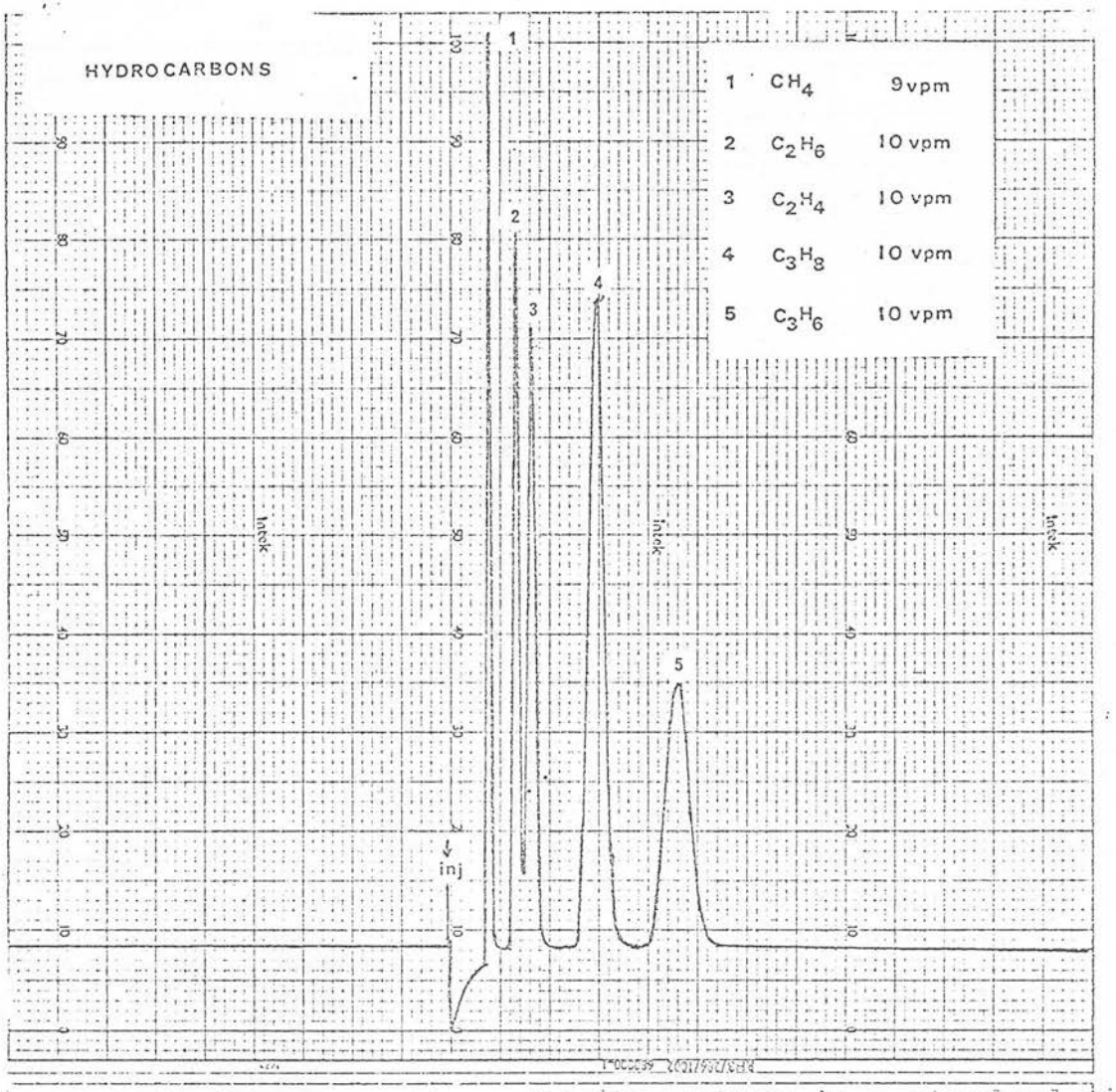

Figure 23. Chromatogram showing separation of hydrocarbons in a standard mixture.

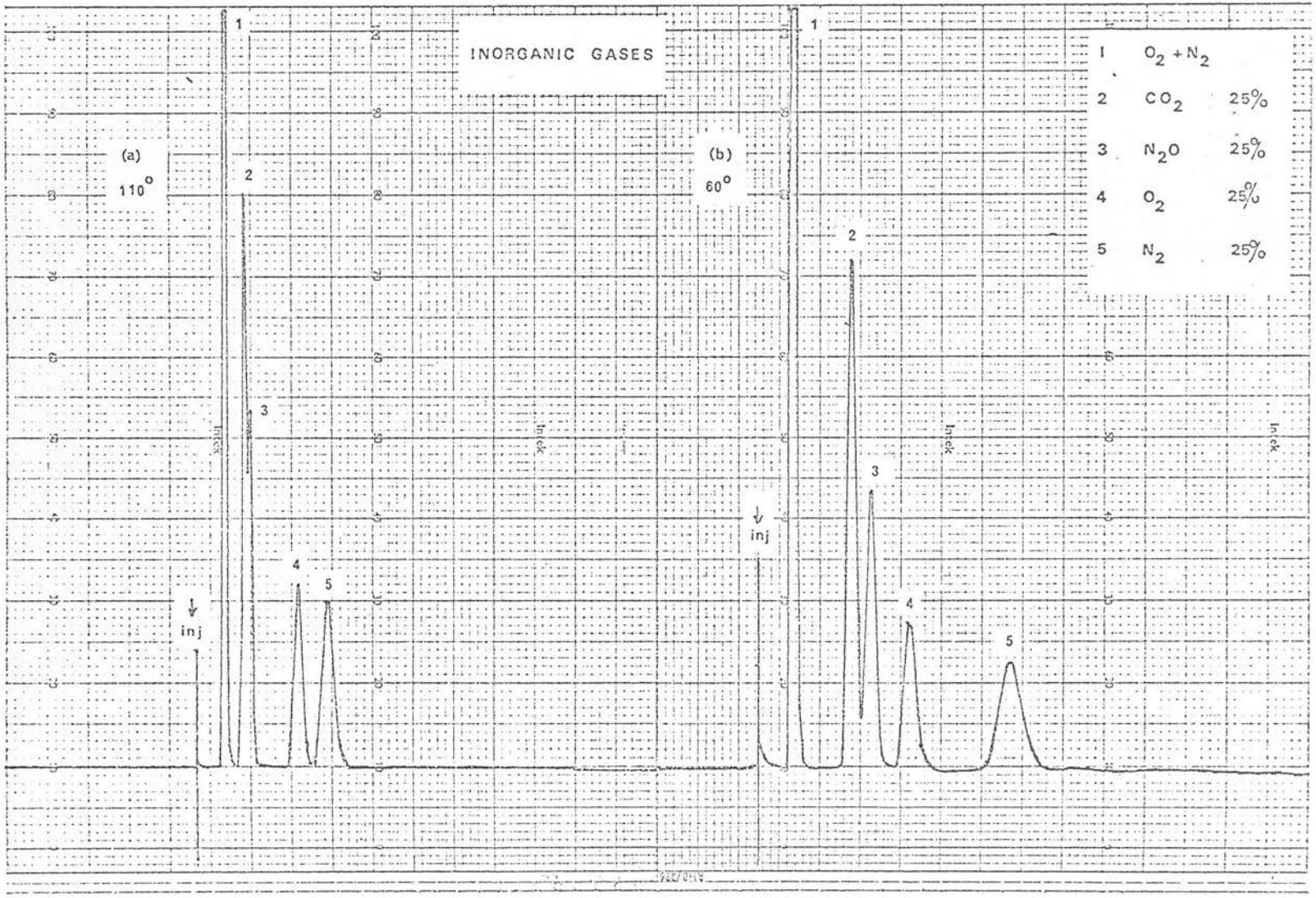

Figure 24. Chromatogram showing separation of nitrous oxide in a standard mixture.

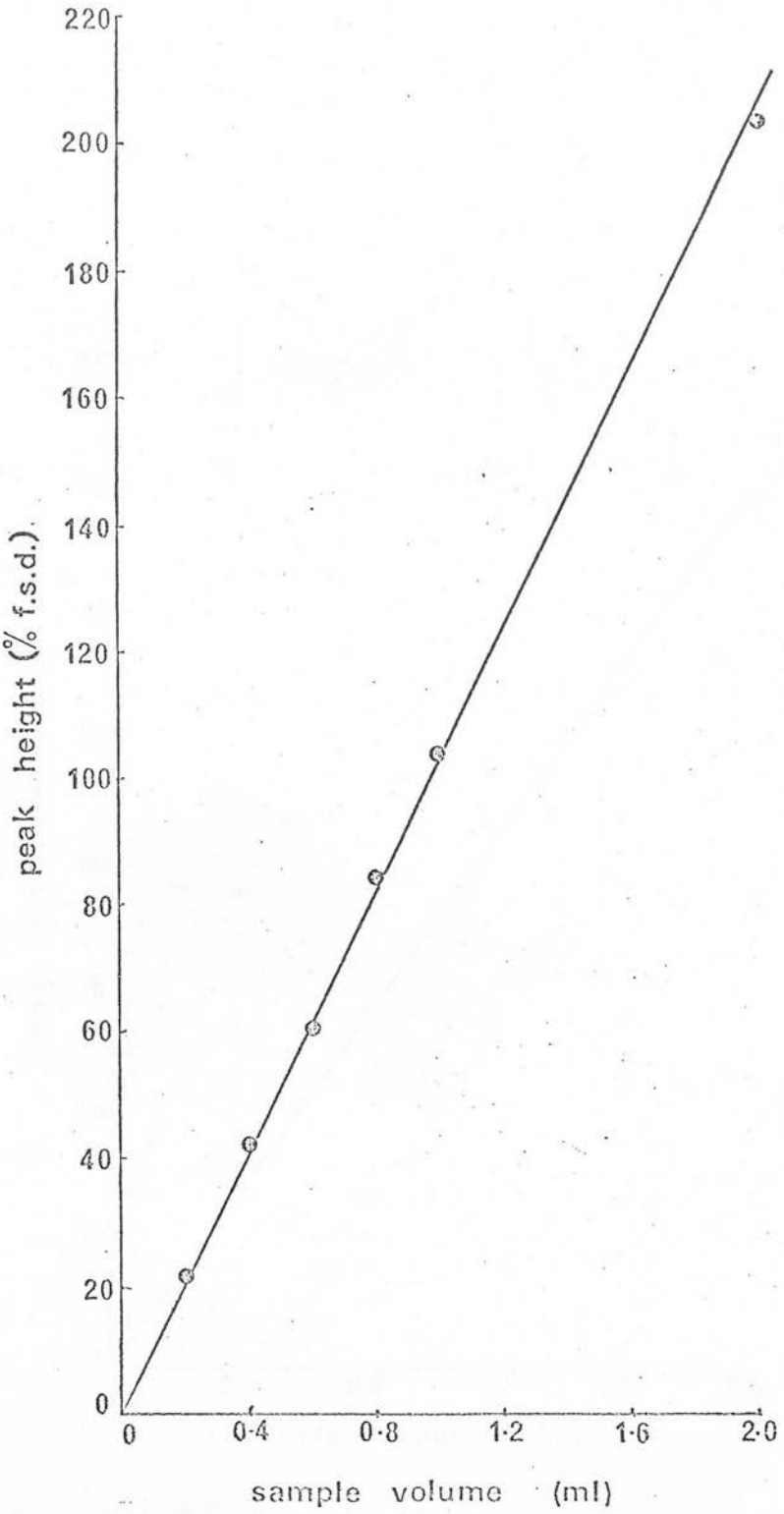

Figure 25. Relationship between detector response and sample volume for ethylene (response normalised to x 10 attenuation).

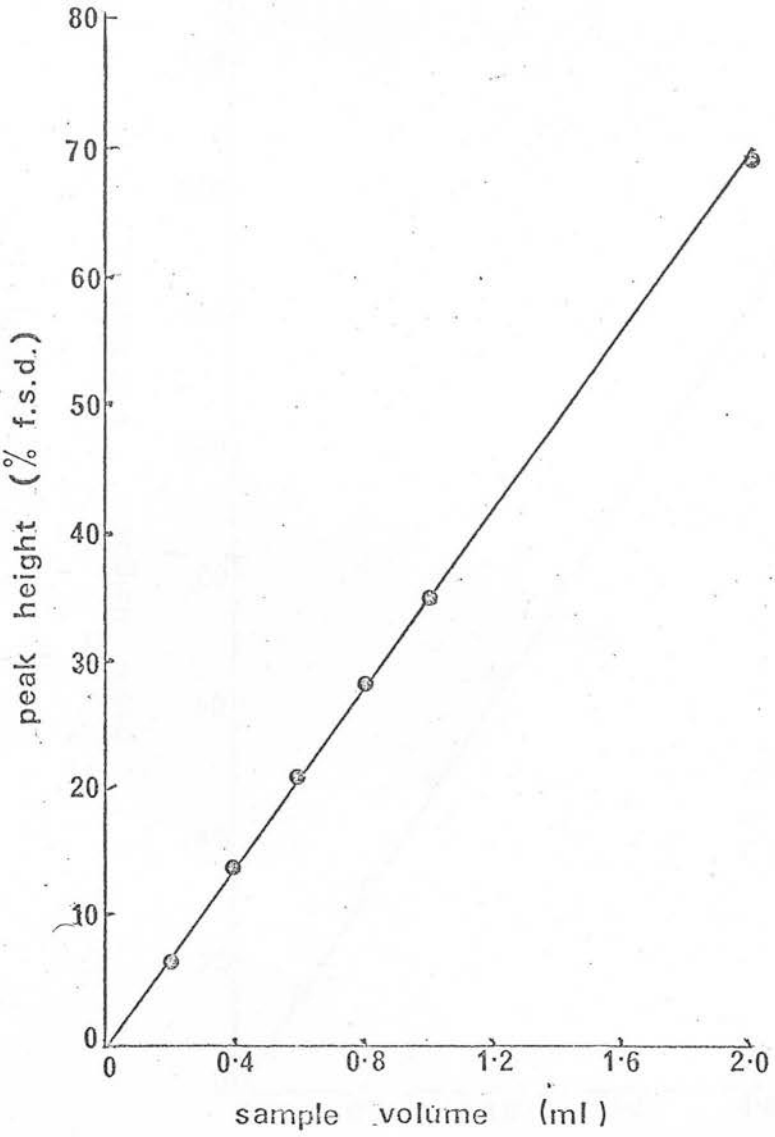

Figure 26. Relationship between detector response and sample volume for oxygen (response normalised to x 2 attentuation).

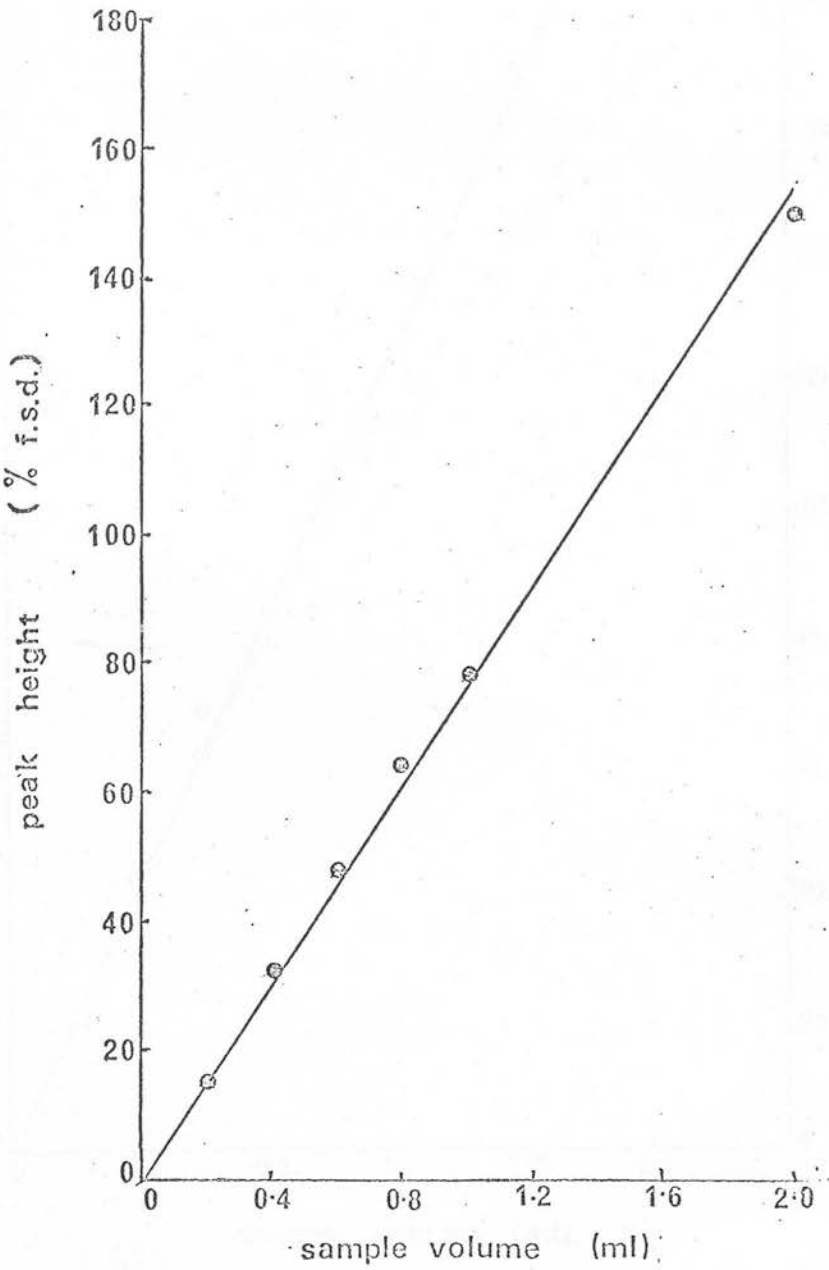

Figure 27. Relationship between detector response and sample volume for carbon dioxide (response normalised to x 2 attenuation).

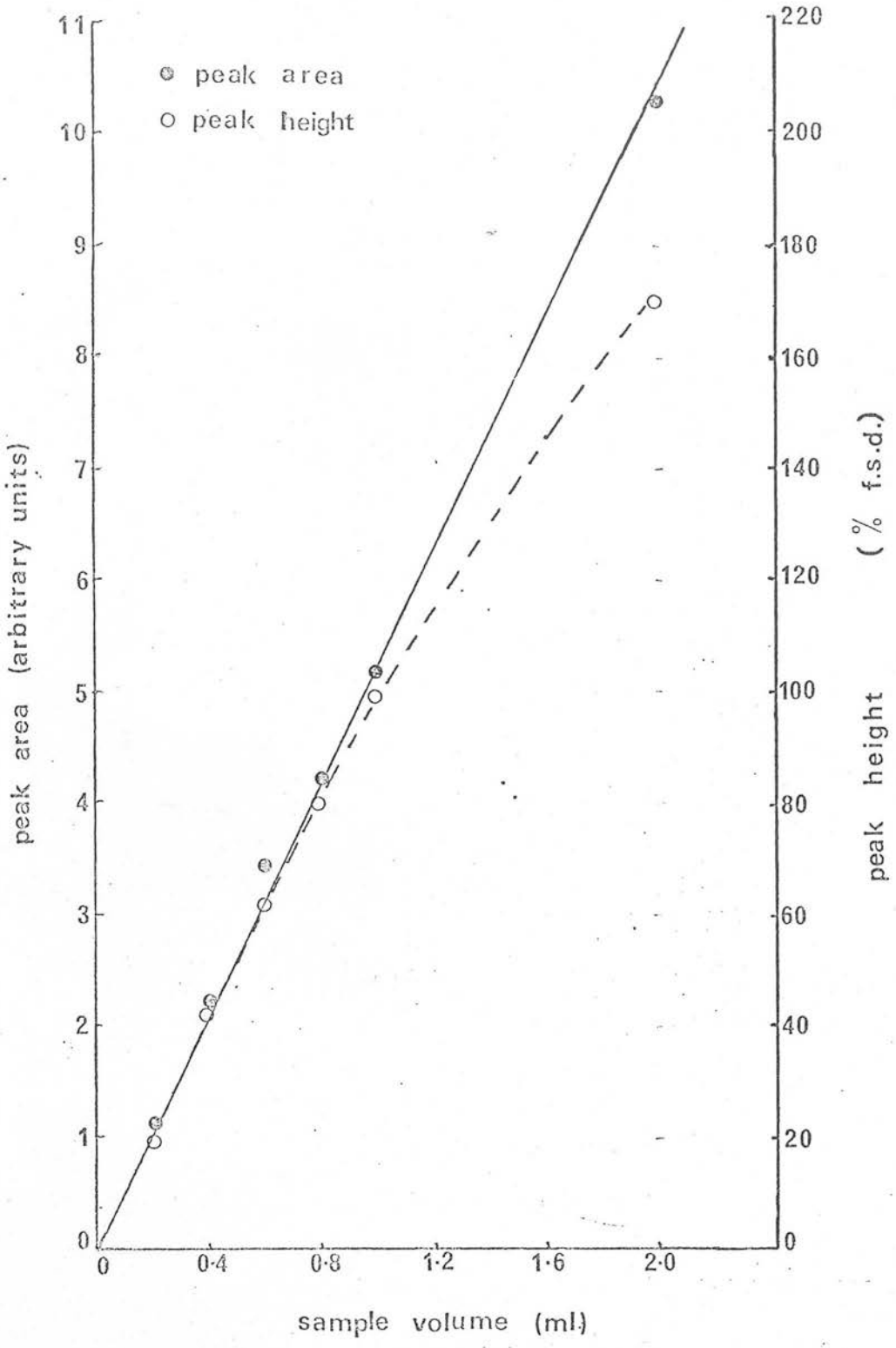

Figure 28. Relationship between detector response and sample volume for nitrogen (response normalised to x 2 attenuation).

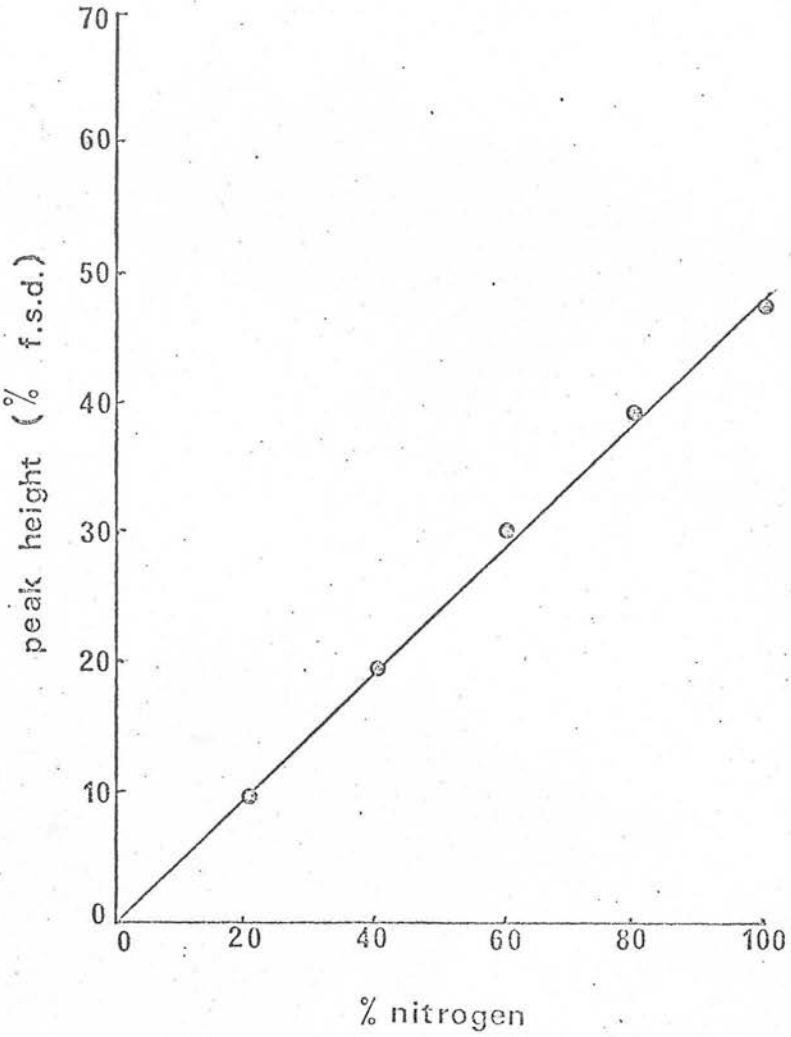

Figure 29. Relationship between detector response and nitrogen concentration in a gas mixture (response normalised to x 2 attenuation).

obtained, as during water addition to undisturbed monoliths (Section 2.), the concentration in a 0.5 ml volume could be calculated knowing that the response was linear over this range.