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THE MICROBIOLOGY OF ARABLE SOIL SURFACES

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Abstract

Whilst much is known about the physics and erosion of soil surfaces on a millimetre scale, little is known about the associated microbiology, particularly in temperate arable systems. The vast majority of research regarding microbial interactions at soil surfaces has concerned microbiotic crusts. However, such surface crusts take many years to form and then only in relatively undisturbed soil systems. Arable soil surfaces are subject to relatively extreme environmental conditions, potentially undergoing rapid changes in relation to temperature, water status and solar radiation compared to deeper soil zones. These extreme environmental parameters are likely to have a large impact on the biota found at the arable soil surface when compared to that which occurs in deeper soil zones.

Phenotypic profiling using phospholipid fatty acid (PLFA) analysis, microbial biomass, and chlorophyll concentration were used to characterise soil microbial communities with the aim of quantifying differences within the surface layers of arable systems on a millimetre scale. This field work was supported with a series of microcosm-scale studies in which parameters such as length of time between disturbance events and the quality of light reaching the soil surface were controlled. Using microcosms subjected to simulated rainfall and imaged using X-ray computed tomography scanning, the effects of the soil surface microbiota on associated physical properties including structural integrity, porosity, erodibility and hydrological properties were investigated.

This research showed that given sufficient time between disturbance events, environmental parameters such as temperature and wet:dry cycling were sufficient to drive the formation of a distinct soil surface phenotype, which appeared to be consistently confined to an order of depth of *circa* 1 mm. It was notable that the PLFA 16:0 was consistently associated with discrimination between phenotypes between soil surface layers. Calculation of the ratio of fungal to bacterial PLFA biomarkers showed a consistently higher ratio of fungi to bacteria present in the soil surface layer to a depth of *circa* 1 mm, providing evidence that fungi grow preferentially over the soil surface compared to through the soil matrix.

Further investigation demonstrated that light, particularly at photosynthetically active wavelengths, was the main driving factor in the establishment of the distinct soil surface phenotypes. The inocula which drove the formation of such soil-surface community phenotypes, especially the photoautotrophic components, was demonstrated to derive predominantly from aerial sources.

Functionally the nature of the soil surface community was found to affect run-off generation and shear strength at the surface. There was no significant impact of the soil surface microbiota on erodibility or water infiltration rates, although whilst distinct surface phenotypes had developed in this experimental circumstance, these were relatively deficient in photoautotrophs compared to other microcosm experiments and field circumstances, and hence extrapolation of this conclusion is not sound.

This project has demonstrated that a soil surface ecological niche may exist in other unexplored soil surfaces and highlights the needs to explore this possibility and to examine any associated functional consequence should such niches be found to exist.

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Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction and Review of the Literature

1.1. Introduction

The nature of the first few millimetres of a soil horizon strongly affects water infiltration rates, generation of run-off, and soil detachment (Auzet *et al.* 2004). Whilst much is known about the physics and erosion of soil surfaces on a millimetre scale, little is known about the microbiology, particularly in temperate arable systems. Increased understanding of the microbiology of the soil surface may lead to an increased understanding of the processes behind soil erosion and those affecting water infiltration and run-off generation, which in turn may lead to improvements in making agricultural systems more sustainable.

The vast majority of research regarding microbial interactions at soil surfaces has concerned microbiotic crusts (also known as cryptogamic crusts) (Eldridge and Greene 1994; Leys and Eldridge 1998; St. Clair and Johansen 1993). However, such surface crusts take many years to form and then only on relatively undisturbed soil systems.

Arable soil surfaces are subject to relatively extreme environmental conditions, and are capable of undergoing rapid changes with regards to temperature, water status and solar radiation when compared to deeper soil zones (Hillel 1982; Robinson 1966; Strangeways 2003). These extreme environmental parameters are likely to have a large impact on the biota found at the arable soil surface when compared to that which occurs in deeper soil zones. Research into the effects of such factors on the soil biota is an area of soil microbiology which will help increase our understanding of those process that are driven by, or affected by, the microbial communities present, such as nutrient cycling (Lund 1967; Madigan *et al.* 2000; Johnson *et al.*2007) and the structural properties of the soil (Ritz and Young 2004).

1.2. The Soil Environment

The solid phase of soil is a mixture of minerals consisting of differing mixtures of clay, silt and sand, along with varying amounts and types of organic matter and Much of its formation and properties are produced and driven by biological processes. The soil environment is a dark, semi-aquatic, dynamic system of ever-changing pore networks (Lavelle and Spain 2001).

Soil texture is dependent on the proportions of clays, silts and sands, which are categorised according to particle size, which has a direct influence on their chemical and physical properties. Clays have a particle size of < 2 µm, silts have particles sizes of 2 – 20 µm, and sands have particle sizes of 20 µm to 2 mm (Brady 1990). The texture, along with the organic matter within the soil, determines many of the physical properties of the soil, including cation exchange capacity (Brady 1990), water infiltration rates (Auzet *et al.* 2004), and propensity to from crusts or seals, as well as a soil's inherent erodibility (Le Bissonais *et al.* 1995).

1.3. Overview of Soil Biology

A very wide range of organisms inhabit most soils, including all major forms of microbe and most invertebrate groups.

1.3.1. Bacteria

Bacteria are found everywhere, including the soil environment, where very large numbers are typically resident. Bacterial diversity in soil is exceptionally high compared to other ecosystems and it has been estimated that 99.9% of all soil microorganisms in typical agricultural soil are currently unculturable under laboratory conditions (Torsvik *et al.* 1990; Ritz 2007).

Garcia-Pichel *et al.* (2003) found that the top centimetre of certain soils are covered by a microbiotic crust, usually in arid desert ecosystems, and contain bacterial populations which can be ten-fold larger than in uncrusted or deeper soils. They found that the depth at which bacterial populations peaked within microbiotic crusts occurred at different depths, on a millimetre scale, depending on the maturity of the microbiotic crust. Bacterial populations, as measured by microscopic counts, extractable DNA and plate counts of viable copiotrophs, peaked at the immediate sub-surface, at a depth of 1 – 2 mm in relatively immature, light coloured crusts, and at the surface, 0 – 1 mm in mature, well developed, dark coloured crusts. Copiotrophs would be expected to be found in greater abundance near the soil surface, as this is a relatively rich carbon environment due to input of organic matter as a result of death and decay of organisms and plant matter, as well as input of carbon into the soil surface by photoautotrophs, being those organisms which are capable of synthesizing their own food from inorganic substances using light as an energy source.. Bacterial

abundance was shown to decrease with depth below these crust covered horizons (Garcia-Pichel *et al.* 2003).

Bacteria perform many important functions within the soil system including nutrient cycling. As well as breaking down organic matter and contributing to the carbon cycle (Janzen 2004), some species of bacteria also play important roles throughout the nitrogen cycle. This includes nitrogen fixation, where gaseous N_2 is fixed into nitrogen-containing organic substances by either free living bacteria such as *Azotobacter* or bacteria which function as part of a symbiotic relationship such as *Rhizobia* (Madigan *et al.* 2000). Nitrification is the process in which nitrogen, in the form of ammonium is converted firstly to nitrite, by bacteria such as *Nitrosomonas* and then to nitrate by bacteria such as *Nitrobacter*. Denitrification is performed by several species of bacteria, usually anaerobes or facultative anaerobes.It is the process whereby nitrate and nitrite are reduced back to gaseous forms of nitrogen principally N_2O and N_2 (Madigan *et al.* 2000).

1.3.2. Cyanobacteria

Cyanobacteria (previously also known as blue-green algae) are photoautotrophic bacteria. They perform a role in the carbon cycle as they fix atmospheric CO_2 through photosynthesis. Cyanobacteria have been shown to be capable of photosynthetic growth in extremely arid conditions, down to a dry limit of less than 5 mm precipitation $year^{-1}$, and to be able to resist decadal periods of no rainfall (Warren Rhodes *et al.* 2006). This clearly exceeds the sort of desiccation that would be expected to be experienced in temperate arable systems and suggests that cyanobacteria should easily cope with the levels of desiccation expected in temperate arable soil surfaces.

Similar results to those described above regarding bacteria in microbiotic crusts have been found with regard to the location of cyanobacterial populations, although Pringault and Garcia-Pichel (2004) demonstrated that the cyanobacterial populations of microbiotic crusts can migrate via hydrotaxis on a millimetre scale in response to changing moisture levels.

Montechiaro and Giordano (2006) demonstrated that when the cyanobacteria *Phormidium autumnale* were subjected to periods of darkness photosynthesis stopped

as would be expected. However, it resumed promptly upon re-exposure to light. Furthermore, they could continue to photosynthesise in conditions of very high irradiance, implying that their photo-protective pigments were either still capable of functioning after exposure to dark conditions, or were very quickly produced. This adaptation is particularly pertinent to the soil surface environment, which is highly dynamic due to the effects of wind, rain and mechanical disturbance. This means they may rapidly move from a light to a dark environment and back again.

Cyanobacteria have also been shown to play an important role in the nitrogen cycle as non-symbiotic nitrogen fixers. In some soils of England and Wales, Lockyer and Cowling (1977) found that up to 6 – 10 g of nitrogen per hour was fixed in soils that had a well developed surface film of cyanobacteria, although generally rates were found to be much lower than this. Pankratova (2006) reported that the contribution of cyanobacteria to the soil nitrogen pool could reach 30 kg ha^{-1} of nitrogen fixed, although the time-frame of this contribution was not reported. This demonstrates that in some situations, where cyanobacteria are abundant, their contribution to the input of nitrogen into the soil system and hence their influence on soil fertility can be highly significant. Further evidence of the effects of cyanobacteria on soil fertility comes from Nisha *et al.* (2006) who demonstrated that as well as increasing soil fertility in those soils where extra cyanobacteria were inoculated compared to those where they were not, as shown by an increase in crop productivity, cyanobacteria also improved the structure of the soil, significantly reducing bulk density and increasing both the water holding capacity and hydraulic conductivity of the soil. However, as the cyanobacteria were not inoculated on to the soil in a pure form but rather as a form of "biofertilizer" mixed with other compounds, as to whether these effects are directly attributable to cyanobacteria remains uncertain.

1.3.3. Algae

Recent work on algae in the soil environment is lacking, with the majority of literature on soil algae being from the middle part of the 20th century. Algae are found in soils everywhere and are generally most abundant at or near the soil surface, although they are also found in lower soil horizons (Lund 1967). Algae are photoautotrophs and as such rely on light to allow them to fix CO_2 through the process of photosynthesis. For

this reason, it would seem logical to conclude that the vast majority of algae would be found at the soil surface, where light is abundant as is the case for cyanobacteria. However, Feher (1948) found that there are nearly 700 species or algae at 15 to 20 cm depth below the surface in many parts of the world. It is believed that earthworms and rain are the main cause of vertical movement of algae through the soil (Tchan and Whitehouse 1953). Many soil algae including diatoms and Cyanophyta are motile and so are often able to return to the surface if they are not buried too deeply (Lund 1967)

Algae are an important part of the soil microflora as they act as a reserve for plant nutrients, incorporate organic carbon and nitrogen into the soil system through photosynthesis and nitrogen fixation, and influence soil structure and the activity of other edaphic organisms (Metting 1981). Algae fix nitrogen within the soil surface in a light-dependent process. Lund (1967) reported that when moist sand, containing no organic matter or other minerals apart from algae, was exposed to light there was evidence of nitrogen fixation, whereas with no light exposure there was no evident increase in the amount of fixed nitrogen. The light exposure also caused a visible greening of the surface. This provides strong evidence that algal nitrogen fixation is light dependent. Farming practices such as turnover of the soil via ploughing or drilling, an inherent part of farming practice, therefore will have an impact on algal nitrogen fixation.

Algae have been shown to withstand desiccation (Bristol Roach 1919; Petersen 1935) and this is a useful adaptation for organisms inhabiting the soil surface where desiccation is common and more severe compared to deeper soil layers. However, Petersen (1935) demonstrated that very slow drying of the soil, over a period of about a month, killed a large number of vegetative algal cells. In comparison Bristol Roach (1919) showed that algae were able to survive “intensive” desiccation. This was confirmed by Tchan and Whitehouse (1953) who found that when desiccation occurred over a period of hours the number of algae only decreased to about 65% of the pre-drying population and remained at a constant level once the soil had become air-dry. This implies that algae are more resistant to fast desiccation when compared to desiccation which takes place over a longer time frame. This resistance would be expected for organisms found at the soil surface where desiccation will normally be a relatively quick process due to evaporation, insolation and drainage.

1.3.4. Fungi

Fungi exist in two forms, holocarpic, being single-celled forms such as yeasts, and eucarpic, which are filamentous. Filamentous fungi are particularly well adapted to the soil environment because of their ability to both grow across surfaces and to bridge soil pores whilst exploring for substrates (Ritz and Young 2004).

Fungi and soil structure have a strong interdependent relationship. The growth of fungi within the soil is modulated by the pores through which it grows. The growth of filamentous fungi is affected by the spatial distribution of substrate within the soil. When substrate is sparsely distributed, fungi can change their foraging strategy to explorative growth, whereby they grow sparsely in order to explore as large an area as possible to increase the likelihood of locating suitable substrate. Upon contact with a suitable substrate fungi can change their growth form, becoming much denser when suitable substrate is available to provide nutrients (Ritz and Young 2004). Fungi also affect the structure of soil. Fungal hyphae can physically bind soil aggregates together. Medows *et al.* (1994) showed that *Penecillium chrysogenum* significantly increased the stability of sand when inclined on beds. They also reported that when the sand slopes failed, hyphal trails were visible enmeshing the sand. This suggests that the fungal hyphae were physically binding the sand together.

Otten and Gilligan (1998) reported that the fungus *Rhizoctonia solani* spread further and faster over surfaces when compared to growing through the soil pore network. This has implications for the microbiology of the soil surface since if fungi preferentially grow over surfaces, it seems likely that fungi will be found in relatively large numbers per gram of surface soil when compared to a gram of soil taken from deeper levels of the soil. Further investigation within this project will confirm whether this is the case.

Fungi, as well as many prokaryotes, excrete a wide range of compounds into their immediate environment including polysaccharides and glycoprotein mucilages. These compounds often have adhesive properties and so can provide a primary mechanism of soil aggregation (Ritz and Young 2004). Arbuscular mycorrhizal fungi, for example, excrete glomalin, which is an insoluble glycoprotein, along with other extracellular exudates such as polysaccharides and a variety of enzymes. Wright et al

(1999) showed that glomalin can greatly increase aggregate stability and thereby potentially lower rates of erosion.

1.3.5. Protozoa

Protozoa often feed on bacteria and are a vital part of the soil micro food web and they, in turn, are predated by other soil-inhabiting species. Protozoa make up a much smaller proportion of the overall microbial biomass, comprising as little as 0.07 g dw soil m^{-3} in some soils (Coleman *et al.* 2004). Protozoa have been found at depths of more than 200 m in groundwater environments although generally they are found within the upper few centimetres of soil.

The activity of protozoa has been shown to stimulate the uptake of nitrogen in the rhizosphere of living plants (Ekelund and Ronn 1994). However, protozoa are only active in relatively moist conditions so it seems likely that their activity at the surface may be restricted due to the relatively long and intense periods of desiccation experienced at the soil surface compared to deeper soil layers.

1.3.6. The mesofauna and macrofauna

Many other multi-cellular organisms such as nematodes, rotifera and earthworms also live within the soil environment, and drive many important ecological processes (Coleman *et al.* 2004). However, this project will not be investigating the mesofauna or macrofauna in depth and instead will be concentrating on the microbiota and as such, a review of the extensive literature on these subjects is not included here.

1.4. Characterising the Soil Surface

1.4.1. Definition of the soil surface

Defining the soil surface is somewhat arbitrary, meaning different things to different people working at different scales. The soil surface is often defined as being the top 10, 15 or 20 cm of soil (Calderon *et al.* 2001; Fierer *et al.* 2003; Ovreas and Torsvik 1998). However, due to the buffering effects of soil with regards to temperature, wetting and drying cycles, and the influence of light on soil, it seems likely that the greatest influences and fluctuations will be found in the top zones of the soil, with the influence of buffering effects being inversely proportional to depth.

One further problem with defining the soil surface is that due to tillage and soil erosion an arable soil surface is not a permanent surface. Morgan (1995) reported that typically UK cultivated soil erosion rates are between 0.1 and 20 t ha^{-1} y^{-1}. Whilst this soil will not be eroded evenly over the entire hectare, but will rather occur in 'erosion hot spots', it does demonstrate that a soil surface cannot be a soil surface forever and leads to the question of ‘how long is a surface a surface’.

1.4.2. Solar radiation

Solar radiation is an environmental parameter which will affect only the extreme surface of the soil system. Solar radiation can function as either a stress on biological systems, via energy from the ultra violet (UV) part of the spectrum or, an energy source in the form of photosynthetically active radiation (PAR), being those parts of the spectrum that photoautotrophs can utilise for photosynthesis.

Of the total radiation emitted by the sun, approximately 9% falls in the UV spectrum (Jong 1973) with wavelengths in the range of 0.2 - 0.4 µm (Figure 1.1). However, due to very strong absorption by oxygen and nitrogen (in both molecular and atomic form) e.g. ozone in the stratosphere, solar radiation with a wavelength of approximately 0.3 µm and below generally does not reach the Earth’s surface at all (Moeller *et al.* 2005; Robinson 1966). This means that the majority of UV reaching the earth’s surface is UV-A, which is the least energetic form being of wavelengths 400 – 320 nm, and therefore the least damaging with regards to biological systems, with only a small amount of UV-B, being of wavelengths 320 – 280 nm, and virtually no UV-C, being of wavelengths below 280 nm, reaching the Earth’s surface.

UV is believed to play an important role in the induction of mutations and damage to DNA. It also produces reactive oxygen species within cells which may also degrade other cellular components leading to lipid peroxidation or protein inactivation (Moeller *et al.* 2005). Duguay and Klironomos (2000) found that UV affected fungal respiration, reducing respiration when compared to fungi that were not exposed to UV. Endogenous pigments such as carotenoids and melanins may provide a selective advantage to microbes containing these pigments that are exposed to UV by providing shielding from environmental radiation. Viable fungal and bacterial spores collected at high altitudes of up to 77 km, where intensities of UV are much higher due to

decreased atmospheric shielding, were found to be dominated by pigmented forms (Moeller *et al.* 2005).

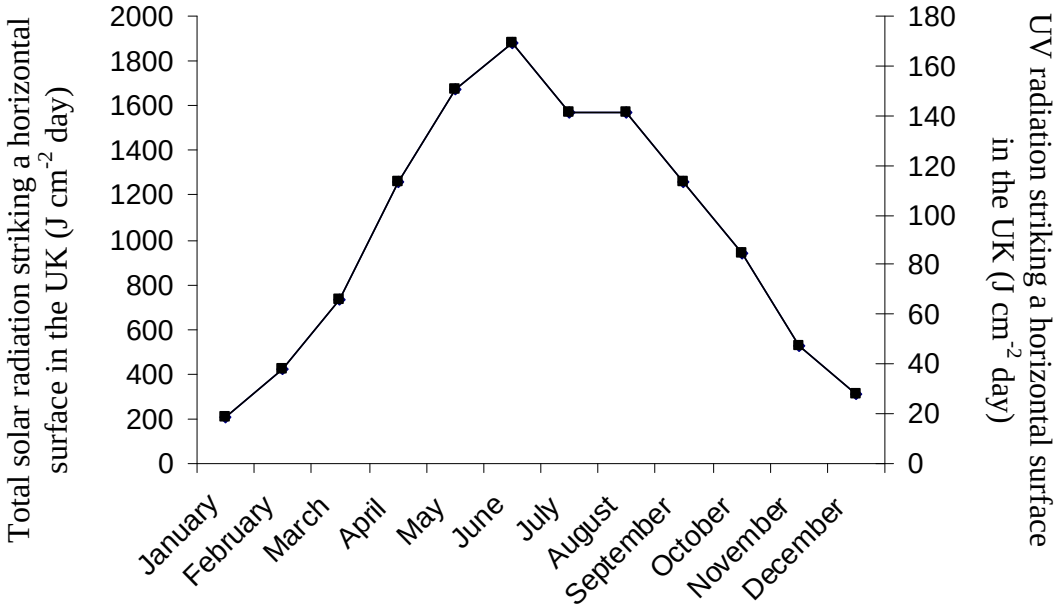


Figure 1.1. Monthly means showing the energy input from both total solar radiation (diffuse and direct) and UV incident on a horizontal surface in the UK (adapted from Jong 1973).

Light, including UV, penetrates very poorly into soil. Benvenuti (1995) found that less than 0.2% of light was transmitted through 1 mm of a silty clay soil, although he found that the amount of light transmitted varied with soil type. The soil with the highest transmittance only allowed 0.3% of light to be transmitted through to 2 mm depth. This means that even in soil with the highest transmittance, light, as a stressor in the very short UV wavelengths, and also as an energy source as PAR, is only present at the extreme soil surface.

All wavelengths of light were found to be reduced as it passed through the soil, with the shorter wavelengths of blue light, including UV being reduced to the greatest extent. This reduction in the quantity of light will have implications for any photosynthetic organisms that lie just below the soil surface as the amount of PAR will be greatly reduced, even at less than 2 mm below the surface. The significance of this is that any photosynthetic activity by soil photoautotrophs can be expected to be

great on the soil surface where there is direct exposure to sunlight, and to decline rapidly within the top 1 or 2 mm of soil as the amount of PAR rapidly decreases.

The differing effect on the wavelengths of light also meant an overall reduction in the red/far red ratio down through the soil, being the ratio of light of wavelengths 600 – 700 nm to those of wavelength 700 – 800 nm. Changes in the red/far red ratio of light have been shown to be detected by higher plants, triggering various responses including the tillering response (Casal *et al.* 1987) and morphological responses (Methy and Roy 1993). It seems possible therefore that photoautotrophic organisms could respond to changes in the red/far red ratio that occur when buried a short distance from the surface. This shift in the ratio may trigger a phototaxic response leading to movement of the buried photoautotrophic organisms back towards the surface.

Lund (1967) found that due to their reliance on light for photosynthesis, algae and other photoautotrophs are usually most abundant on the soil surface, although they may also be found in the deeper soil horizons generally due to the actions of rain and earthworms. Many soil algae are able to return to the surface if they are not buried too deeply as many species are motile possibly as a result of the phototaxic mechanism hypothesised above.

1.4.3. Temperature

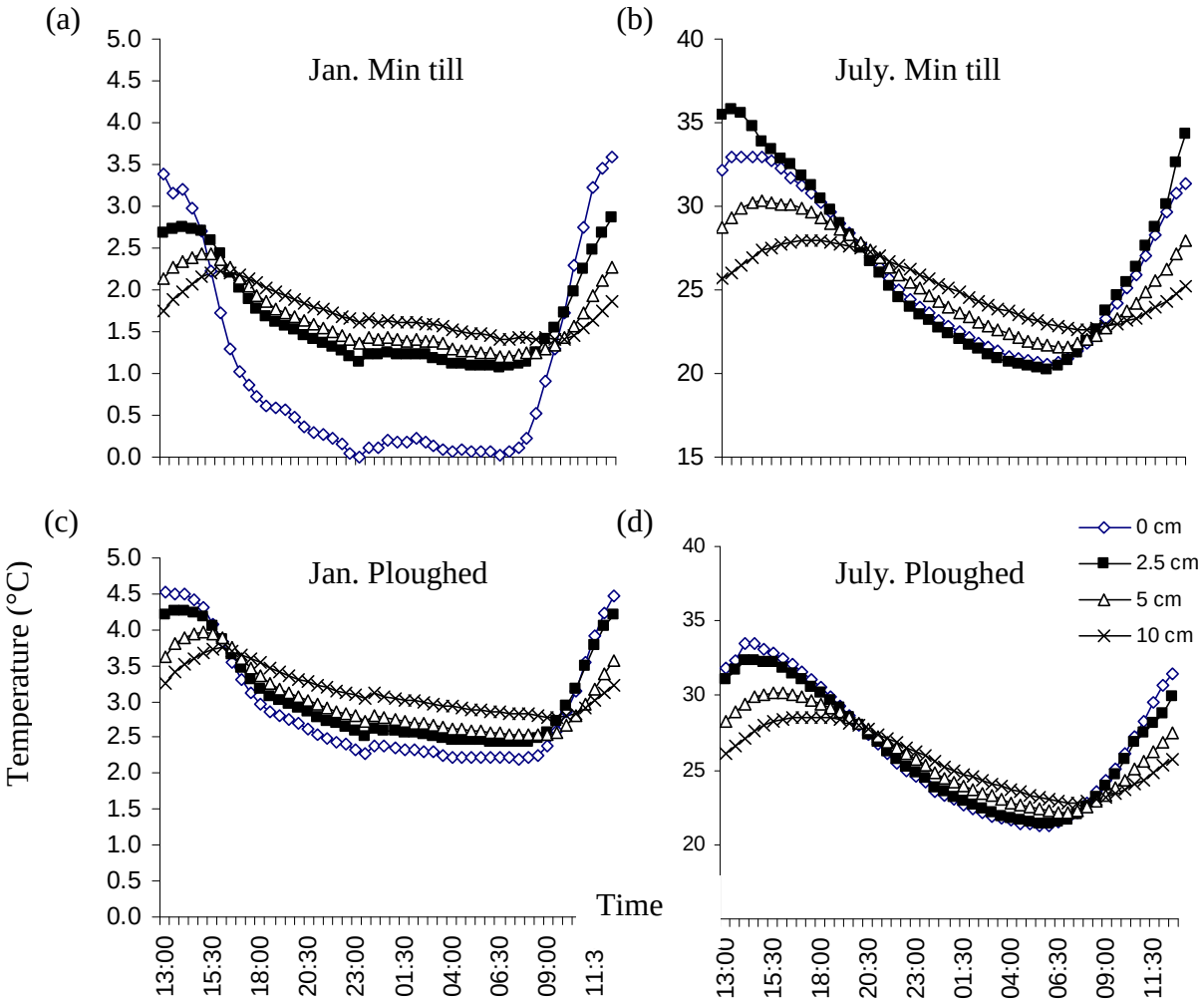


Figure 1.2. Average diurnal temperatures at 4 depths in fields under: Minimum till, (a) January 2003, (b) July 2003; and Ploughed (c) January 2003, (d) July 2003. From data provided by Scott (2005; personal communication)

Data relating to temperature fluctuations with depth in soils generally covers a range of depths to 15 cm (Warrick 2002). Surface data on a smaller scale is uncommon. Scott (2005; Personal communication) measured temperature at 4 depths moving down from the soil surface to 10 cm (Figure 1.2). Figure 1.2 (a) shows that throughout January, on average, the surface drops to freezing over night, whereas at 2.5 cm below the surface the temperature remains on average over 1°C warmer. This is due to the insulating effects of the top 2.5 cm of soil. Added to this, when a frost is formedit is likely that air flow through the pores of the surface soil will become restricted,

preventing the freezing air from entering the deeper pores. This would increase the insulating effect of the top layer of soil.

In contrast, Figure 1.2 (c) shows that the ploughed field on average did not freeze in January 2003. Both fields were very close to each other on the farm where the temperature measurements were taken, but it is still not possible to rule out topographical effects causing the disparity between results. For example, the minimum tilled field could have been lower than the ploughed field, meaning cold air would pool in the minimum till field, possibly reducing the surface temperature when compared to the ploughed field. Figure 1.2 (b) and d show relatively similar patterns in average diurnal temperature fluctuations for July 2003.

This demonstrates that with regards to temperature fluctuations, the soil surface experiences changes in temperature of up to 3.5 orders of magnitude higher than temperature fluctuations at 10 cm depth

1.4.4. Wetting and drying cycles

Drying and rewetting provides a stress for microorganisms and may induce lysis in much of the soil microbial biomass (Fierer *et al.* 2002). It has been estimated that 30-60% of the microbial biomass C that is found in soil is released during an individual rewetting event (Fierer *et al.* 2002). There are two main mechanisms which cause mineralisation of organic matter and nutrients after air drying – rewetting cycles. First, there are physical processes such as the disruption of soil aggregates and exposure of fresh surfaces to microbial attack. Second, there is a killing off of a proportion of the microbial population, which is then mineralised (Wu and Brookes 2004).

West *et al.* (1992) showed that respiration and activity declined linearly as the volumetric water content decreased. Microbial biomass C also declined but generally only after a considerable initial period of drying thereby demonstrating the stressful impact of drying on the soil microbial biomass.

However, Sparling *et al.* (1989) demonstrated that whilst drying of soils reduces respiration and hence microbial activity by 3 – 60%, a proportion of the population can generally tolerate the dry conditions. Due to the fact that the surface environment

normally experiences drier conditions than deeper soil it seems likely that the microorganisms which are more tolerant of these dryer conditions will be selected for near the soil surface. Therefore, it can be expected that the soil surface microbial community will generally be dominated by desiccation resistant microorganisms.

1.5. Why Study the Surface?

1.5.1. Soil surface physical properties

Water infiltration rates, runoff generation and soil detachment and erosion are all strongly affected by the nature of the first few millimetres of topsoil (Auzet *et al.* 2004). Soil erosion greatly damages agricultural sustainability as loss of soil leads to a loss of productivity, as well as leading to off-site effects such as increased water turbidity and eutrophication due to contaminants such as fertilizers attatched to the eroded sediments entering the waterways.

Soil crusts and seals can form at the surface and affect its physical properties. These effects include increased bulk density as well as a reduction in water infiltration rates and hydraulic conductivity which in turn leads to an increased susceptibility to surface run off generation (Cattle *et al.* 2004)

Singer and Shainberg (2004) investigated the effects of physical soil surface crusts on the impacts of wind and rain erosion, and found that the kinetic energy of raindrops was the most important factor regarding the detachment of particles leading to erosion. They also found that the presence of seals or crusts did affect the erodibility of the soils, as well as other factors such as surface roughness and time to ponding.

The amount of erosion is dependent on many factors including soil type, prevailing weather conditions and slope as well as the biology of the soil Cultivating soils increases the amount of erosion at the soil surface, and leaving bare soil exposed increases it further still. (Table 1.1).

Table 1.1 Average annual soil erosion rates under different land covers for several different countries.

Country	**Natural**	**Cultivated**	**Bare Soil**
China	0.1 - 2	150 – 200	280 – 360
USA	0.03 – 3	5 - 170	4 – 9
Australia	0.0 – 64	0.1 – 150	44 – 87
Ivory Coast	0.03 – 0.2	0.1 – 90	10 – 750
Nigeria	0.5 – 1	0.1 – 35	3 – 150
India	0.5 – 5	0.3 – 40	10 – 185
Ethiopia	1 – 5	8 – 42	5 – 70
Belgium	0.1 – 0.5	3 – 30	7 – 82
UK	0.1 – 0.5	0.1 – 20	10 – 200

All units = t ha^{-1} (from Morgan 1995)

Clearly, anthropogenic influences, whether through cultivation or exposure of soil via forest clearing, can have a very large impact on the amount of erosion from a given soil (Table 1.1). This further demonstrates the need to increase our understanding of all of the processes which occur at the soil surface so we can best limit our negative impacts on the soil. One further question is also raised by the fact that erosion is not distributed evenly over the soil surface - Why does erosion occur in some places and not others?

Leys and Eldridge (1998) found that biological crusts can dramatically reduce erosion in arid environments, meaning that areas not protected by crusts for whatever reason will erode further and faster than those protected by these crusts. These biological crusts have been studied in detail, especially in arid environments. The terminology used for these crusts is under some dispute. Kaltenecker and Wicklow-Howard (1994) reviewed much of the terminology under discussion and noted that various terms have been used to name these biological soil crusts, including microfloral (Loope & Gifford 1972), cryptobiotic (Belnap 1993), cryptogamic (Kleiner, & Harper 1972), and microphytic (West 1990; Williams 1993).

All of these terms are reasonably good descriptions of these biological soil crusts, but St. Clair and Johansen (1993), Eldridge and Greene (1994) and Kaltenecker and Wicklow-Howard (1994) all determined that microbiotic crust was the most accurate term and recommended its use. This is because cryptogamic refers to plants that do not produce seeds, including ferns which do not form a part of these crusts. The term also excludes cyanobacteria, bacteria, and fungi which are core constituents of these biological crusts. Also, not all components of the crusts are floral in nature. For example, fungi are an important constituent of these crusts and the suffixes 'phyte' and 'floral' tend to exclude fungi and the other non-floral constituents. Microbiotic refers both to the microscopic nature of the majority of the constituents of these crusts, as well as their biological nature, and for these reasons throughout this project the term microbiotic will be used to describe these crusts. This allows differentiation between these biologically based crusts and other crusts which are physically-formed from the soil itself.

As discussed previously, soil microorganisms are known to exist at the soil surface, even if their communities do not become extensive enough to be classed as microbiotic crusts as discussed above (Lund 1967, Duguay and Kironomos 2000). A high proportion of these microorganisms will produce extracellular exudates, the direct influence of which on soil erosion is poorly understood. Research into this area may lead to new insights into the biological influence on a soil surface's propensity to erode. Fertilisers and pesticides also first come into contact with vegetation cover, if a crop is present, followed by the soil surface. How these interact with the soil surface will affect their infiltration into the soil and their probability of being washed away and so will influence their overall effectiveness (Smith and Jackson 2001). Gillespie *et al.* (1995) found that the majority of organisms inoculated into soil remain on or near the surface. Combined with the possibility of using genetically modified organisms in worldwide agriculture, this highlights the importance of understanding the possible interactions of these fertilizers, pesticides and allochthonous microorganisms with the autochthonous soil surface population.

1.6. Soil Physics and Microbial Interactions

The impacts of aggregate stability, as mentioned before, are particularly important at the soil surface. Soil seals and crusts occur as a result of the breakdown of aggregates due to processes such as slaking. These can lead to reduction in water infiltration rates, and can increase run off, thereby inducing erosion (Le Bissonnais *et al.* 1995).

Singer and Shainberg (2004) stated that the tendency of a soil to form a seal or crust depends largely on its structural stability which in turn depends largely on its clay contents. They found that soils with between 20% and 30% clay content were the most susceptible to forming seals and thus had the lowest infiltration rate. They also found that when the clay content of a soil increased above 40%, structural stability was found to increase and seal formation to decrease. However, there is as yet nothing in the literature regarding the influence of the soil surface microbial community on seal formation and infiltration rates.

Soil structure inherently affects the soil biota and the soil biota itself affects the soil structure in a very closely bound relationship (Coleman et al 2004; Young and Ritz 2005). It follows logically, therefore, that whilst seal formation will affect the soil microbial community by restricting gas and water inflow into the soil via blocking pores, the soil microbial community may well affect the soils propensity to form seals. This could be due to the production of extra-cellular exudates. Many of these exudates are hydrophobic and so will have an impact on the flow and infiltration of water. Other exudates, such as glomalin, have also been shown to bind soil together to form aggregates (Wright *et al.* 1999).

Wright and Anderson (2000) demonstrated that glomalin content and aggregate stability are linearly correlated. They also showed that whilst this linear correlation exists regardless of crop, crop rotations and tillage management, the amount of glomalin, and therefore aggregate stability, varies between crops and tillage practices, with the amount of glomalin generally decreasing with increasing disturbance of the soil. This would be expected as glomalin is excreted by arbuscular mycorrhizal fungi and these are prone to breaking and other forms of damage when soil is disturbed.

1.7. Hypotheses

Five hypotheses are proposed in this thesis, as follows:

A. The biomass, community structure and physiological properties of organisms in the topmost layers of arable soil differ from those in deeper layers.

B. The microbial community will change over time as a new soil surface is uncovered after a disturbance event.

C. Light is an important factor in driving the succession of distinct microbial soil surface communities with changes in the wavelength having differing effects on the soil surface microbial community. Light will either function as an energy source (PAR) or a stressor (UV)

D. There are specific relationships between microbial communities and soil structural and hydrological properties at the soil surface, particularly in relation to infiltration, run-off generation, , crust formation, shear strength and erodibility (susceptibility to erosion).

E. The distinct microbial soil surface community originates exclusively from an aerial source.

Chapter 2: General Methodologies

This chapter describes details of methods that have been used repeatedly throughout the different stages of this project. Specific methods are detailed within each chapter where they were used.

2.1. Phospholipid fatty acid profiling

Phospholipid fatty acids (PLFAs) are a class of lipids that, along with glycolipids and cholesterols make up the majority of the constituents of biological membranes. Phospholipids are bipolar in nature, consisting of a polar (hydrophilic) phosphate "head" and a non-polar (hydrophobic) glycerol "tail", which is the "fatty acid". Many different types of phospholipids exist, each containing different numbers of carbon atoms in their tails and / or different numbers of double bonds between these carbon atoms. The nomenclature of phospholipids is derived from the molecular configuration of the tails.

Different organisms typically contain different proportions of various PLFAs and, as such, their analysis has been shown to be able to provide a 'fingerprint' of the microbial community (Griffiths *et al.* 1999). PLFA extraction and analysis will therefore provide the standard method of community level microbiological analysis throughout this project. PLFA profiling allows the microbial community phenotype to be quantified. The phenotype is the result of interactions between the microbial communities genotype, that is which genes are present in the organisms, and the environment. This means that any differences seen between communities as a result of PLFA profiling may not be due to differences in which organisms are present and in what proportions, but rather may be due to differences in the environmental conditions to which those organisms have been exposed. For example the ratio of *trans* to *cis* 16:1ω7 has been shown to be an indicator of environmental stress on the microbial community phenotype (Hedrick *et al.* 1991).

PLFA extraction from fish liver was originally described by Bligh and Dyer (1959) and later developed by others including Zelles (1997) who demonstrated an extended extraction procedure for the extraction and analysis of phospholipids extracted from soils. Zelles (1999b), in a review of PLFA analysis, concluded that the analysis of PLFAs as a means of looking at the entire microbial community of a given soil is

sensitive to changes in a wide range of soils, but also noted that the method does not, and cannot, give any indication of changes in the populations of individual species.

PLFA data generally cannot be used to classify microorganisms to a species level, as molecular methods such as nucleic acid sequencing or terminal-restriction fragment length polymorphism analysis (T-RFLP) often can. Not only is there a distinct lack of information regarding the qualitative and quantitative distribution of fatty acids but also PLFA patterns of individual species often overlap and the proportions and types of PLFAs within a species can change as a result of that organism's interactions with its environment. This means that a PLFA analysis gives rise to a different type of classification in microbial ecology where differences in the phenotype of the microbial community are quantified and less attention is generally given to individual species.

PLFA analysis can be used to overcome the difficulties posed by the unculturability of the vast majority of soil microorganisms. However, the extraction techniques do not necessarily extract all of the fatty acids present in the soil biota and so PLFA analysis cannot be said to be a complete representation of all the soil microorganisms in a given soil sample (Zelles, 1999b). That said, many studies have shown PLFA extraction and analysis to be sufficiently sensitive to detect changes in microbial populations due to different treatments such as different soil management practices (Calderon *et al.* 2001), different soil preparation techniques (Peterson and Klug 1994) and changes in the ratios of bacterial to fungal PLFA biomarkers (Frostgård and Bååth 1996).

Phospholipid fatty acid (PLFA) extraction and analysis undertaken in this project was carried out as outlined by Bligh and Dyer (1959) and extended by Zelles (1999b). All analyses were undertaken on fresh soil (i.e. not air dried).

2.2. Phospholipid fatty acid extraction and analysis protocol.

All solvents used were HiPerSolv grade unless otherwise stated. All glassware was cleaned in 10% Decon® 90 and rinsed in deionised water.

2.2.1. Soil preparation

Soil samples were stored in a refrigerator at 4°C after collection to await preparation. It has been demonstrated that different quantities of soil and different soil preparation, i.e. grinding the soil or sieving, can change the extraction efficiency of PLFAs (Peterson and Klug 1994; Allison and Miller 2005). Therefore, in all cases the soil used was sieved to 2 mm, any visible root and plant matter was removed and 10 g aliquots of soil were used from each sample for analysis.

Soil moisture contents were measured by oven drying each soil sample at 105°C for at least 12 hours and calculating the mass of water per gram of soil. All samples were placed in Bligh Dyer solution (described below) and sonicated at most 24 hours after sample collection.

2.2.2. Extraction procedure

Soil aliquots of 10.0 g were weighed into glass vials. Citrate buffer, consisting of 0.15M citric acid dehydrate (31.5 g l^{-1}) and 0.15 M trisodium citrate (44.1 g l^{-1}) in deionised water, adjusted to pH 4 using dilute acetic acid, was used to adjust the moisture content of each sample to 3 ml per 10g of soil.

To each sample, 11.25 ml of a 1:2 (v/v) mix of chloroform (HPLC grade):methanol (HPLC grade) with 0.005% w/v (50 mg l^{-1}) 2,6,di-tert butyl-4-methylphenol was added to make a 0.8:1:2 ratio of citrate buffer: chloroform: methanol (v:v:v), known as the Bligh and Dyer solvent ratio (Bligh and Dyer 1959). A layer of PTFE tape was placed over the top of each vial under the plastic lid to prevent plasticides from contaminating the lid.

Samples were sonicated for 30 minutes and then shaken on an oscillating shaker for 30 minutes followed by centrifugation at 2000 rpm for 10 minutes. The supernatant was then decanted into a clean glass vial. The supernatant was separated into two phases through the addition of 4 ml of chloroform and 4 ml of citrate buffer, then it was centrifuged at 2000 rpm for 10 minutes. This caused a clear interface to form between the two phases. The upper layer was removed and discarded leaving the organic layer behind containing all of the soil lipids. This was then dried on a sample condenser set at approximately 30°C, under a stream of nitrogen gas. The extract was then stored under nitrogen in a freezer before undertaking fractionation.

2.2.3. Fractionation procedure

Solid phase extraction (SPE) was undertaken using 3ml/ 500 mg silica Sep-pak Vac™ columns (Waters Chromatography; Elstree, Hertfordshire). Approximately 0.5 g of sodium sulphate was added to the top of each SPE column and the columns were placed in the top of an SPE manifold.

The columns, including the sodium sulphate, were washed with 2 ml of chloroform; residual solvent was removed by leaving them on the manifold for a few minutes under a vacuum. They were then conditioned by the addition of 2 ml of chloroform which was allowed to percolate through the columns. From this stage onwards the columns were not allowed to dry out. The lipid extracts were defrosted at room temperature and two aliquots of chloroform, each of 200 µl, were washed down the inside of each vial containing the lipid extracts to re-suspend the lipids. The re-suspended lipid extracts were loaded onto the top of each SPE cartridge using a glass Pasteur pipette. The columns were then washed with 5 ml of chloroform, followed by 12 ml of acetone to elute the neutral lipids (sterols) and glycol lipids respectively. The solvents containing these lipids were then discarded and clean glass vials were then placed in the SPE manifold.

The columns were then washed with 8 ml of methanol to elute the polar lipids, including the phospholipids. The methanol was then evaporated off to dryness under a stream of nitrogen on a sample condenser set at 35°C. Samples were then stored in a freezer at <-20°C.

2.2.4. Mild alkaline methanolysis

This stage was undertaken to cleave the fatty acids from the phospholipid glycerol backbone. In this process the glycerol bonds are replaced with a methyl group creating fatty acid methyl esters (FAMEs) which can then be analysed by gas chromatography. Throughout the derivation of the fatty acids it is important that no water is present as this will compete for the methanol and prevent methyl esters from forming, resulting in free fatty acids. All solvents were therefore dried over anhydrous sodium sulphate.

Samples were defrosted at room temperature and reconstituted using 1 ml of 1:1 toluene (Analar):methanol. To hydrolyse the lipids, 1 ml of 0.2M methanolic

potassium hydroxide (potassium hydroxide dissolved in methanol) was added. Samples were mixed by swirling and incubated at 37°C for 30 minutes.

The pH of the solution was raised by adding 0.25 ml of 1 M Acetic acid, to pH 6 – 7 to stop the reaction. Five ml of 4:1 v/v hexane: chloroform and 3 ml of deionised water were added to the samples. If an emulsion formed this was broken up through the addition of 100-200 mg of sodium chloride. Samples were sonicated for 30 minutes and then stored in a refrigerator overnight to allow separation into two phases.

2.2.5. Clean-up procedure

The lower layer of each sample was removed and discarded by glass Pasteur pipette and 3 ml of 0.3 M sodium hydroxide was added to each sample to increase the pH and thereby render any un-derivativised free fatty acids more polar and hence less soluble in the organic phase. Using a glass Pasteur pipette, the top layer was filtered through anhydrous sodium sulphate using Whatman no. 4 filter papers and collected in clean glassware. Samples were evaporated to dryness under a steam of nitrogen on a sample condenser set to 25°C. The dried FAMEs were stored in a freezer at <-20°C.

2.2.6. Sample preparation

The dried samples were defrosted at room temperature and reconstituted by washing 2 x 100 µl of hexane down the sides of the glass vials. Using a Pasteur pipette the reconstituted samples were transferred into gas chromatography (GC) vials (QMX Laboratories, Dunmow, UK) each containing 100 µl glass LVI/Spring inserts (QMX Laboratories, Dunmow, UK).

2.2.7. GC methodology

PLFAs in the reconstituted samples were separated using an Aligent Technologies 6890N GC (Aligent Technologies, California, USA). A splitless injector was installed to an HP5 Aligent Technologies capillary column (30 m length, 0.32 mm ID, 0.25 µm film), passing helium at a rate of 1 ml min^{-1}. Samples were injected using an auto sampler with an injector temperature of 310°C.

FAMEs were separated using a temperature programme with a starting splitless hold time temperature of 50°C for 1 min. The temperature was programmed to then

increase at 25°C min^{-1} to 160°C followed by 2°C min^{-1} to 240°C and 25°C min^{-1} to a final temperature of 310°C. FAMEs were detected using a flame ionising detector (FID) operating at 320°C.

2.2.8. Peak identification and calculation

The software used for all analyses was Aligent G2070 Chemstation for GC systems. Separated FAMEs were then compared to known retention times to identify individual PLFAs based upon a Supelco 26 peak standard (Sigma-Aldrich company Ltd., Dorset U.K.). Results were normalised by expressing each peak area as a percentage of the sum of all peak areas. This gave the results on a percent mol (% mol) basis.

2.3. Determination of Soil Microbial Biomass Carbon by Fumigation Extraction.

Chloroform fumigation extraction allows the quantification of microbial biomass carbon. It functions through the release of carbon immobilised in microbial cells which are killed and lysed using chloroform fumes. The carbon released from cells can then be extracted and quantified and by comparing the carbon flush between fumigated and non fumigated samples. Fumigation extraction has advantages over other methods as its results are independent of the physiological state of the microbial community, unlike substrate induced respiration, it is rapid and is also applicable to soils with a low pH or waterlogged soils (Hofman *et al.* 2000).

Whilst extracted PLFAs have been shown to be well correlated with microbial biomass carbon (Zelles 1999a), chloroform fumigation extraction was used to quantify microbial biomass carbon throughout this project. This was done due to the fact that quantifying microbial biomass carbon through use of PLFAs requires the use of a standard, normally the PLFA 19:0. With this method pre-extracted samples are spiked with the standard to allow the extraction efficiency of the PLFAs to be calculated. However, due to the fact that the standard used is not in membrane bound form the extraction efficiency of the standard is not necessarily the same as the extraction efficiency of the other PLFAs analysed due to the fact that these are all in membrane bound form whose release and extraction relies on lysing of the cellular membranes during the extraction procedure. Also, PLFAs may have different extraction efficiencies from different soils due to the soil's properties such as

structure. A standard added to the soil at any given stage of the PLFA extraction process may not be subject to these varying extraction efficiencies as the standard is unlikely to enter micropores and other areas from which it may be difficult to extract.

One further problem is that to calculate microbial biomass via data collected through PLFA extraction and analysis, the analysis must not be selective and must cover the lipid profile of all organisms present (Zelles 1999a). This is an assumption that cannot be known with confidence. Furthermore, the fatty acids used to determine biomass vary from those which determine community structure (Zelles 1999a) and hence PLFA is not an ideal method for quantifying microbial biomass. Due to these problems, quantification of microbial biomass carbon was undertaken independently of PLFA extraction and was determined through use of chloroform fumigation extraction as described by Vance *et al.* (1987), adopted as ISO method 14240-2:1997.

2.3.1. Fumigation

All soil to be analysed was first homogenised via sieving through a 2 mm sieve. Two aliquots of each sample were (10.00g) weighed into glass jars, one as the control and the other for fumigation.

Fumigation was undertaken using implosion resistant desiccators. The desiccators were prepared by lining the base with damp tissue paper. A small beaker containing soda lime to absorb CO_2 was placed in the bottom of each desiccator. A glass beaker containing chloroform with a small quantity of anti-bumping granules was placed in the desiccators along with the soils to be fumigated in open jars. The desiccators were then sealed using silica gel, and evacuated using a vacuum pump until the chloroform had boiled for 2 minutes. The desiccator valve was then shut and the vacuum pump turned off. Desiccators were stored in a fume hood for 24 hours. The chloroform and anti-bumping granules were then removed and the desiccators vented six times, each time for a period of 2 minutes, using a vacuum pump.

2.3.2. Extraction

Each soil sample was extracted using an extraction ratio of 4:1 (v/w) of 0.5 M K_2SO_4. Samples were then placed on an overhead shaker for 45 minutes and rotated at 60 revolutions per minute. Each sample was then filtered through Whatman no. 42 filter

paper and the filtrate collected. These were then stored in a freezer at <-20°C prior to analysis.

2.3.3. Determination of carbon in extracts

A Burkard Scientific SFA-2000 Segmented Flow Analyser (Rickmansworth, Hertfordshire) was used to analyse all samples. This works by oxidising soil organic carbon to carbon dioxide in the presence of potassium persulphate and determining gas concentration by infra-red spectrometry.

Segmented flow analysis was carried out as per the standard operating procedure for the instrument. If the carbon concentration reached higher than that of the concentration curve, then the samples were further diluted with 0.5 M K_2SO_4

2.3.4. Calculation

$$\text{C}\,(\mu\text{g/g}) = (\text{sample} - \text{blank})\ \text{x}\left(\frac{\text{extraction volume}}{\text{dry weight soil mass}}\right)$$

Microbial C (μg/g) = fumigated organic C – unfumigated organic C

Microbial C was converted to biomass using the conversion factor for microbial C of 0.45 (Wu *et al.* 1990).

2.4. Chlorophyll *a* Extraction

Chlorophyll *a* extraction and quantification was used throughout this project as a measure of the abundance of photoautotrophs. Chlorophyll *a* is the most widespread of all of the chlorophylls, being ubiquitous to all plants, algae and cyanobacteria (Goodwin and Mercer 1972). The hot ethanol extraction method for quantification of chlorophyll *a* in soil was used as described by Metting (1994). A method exists where chlorophyll is extracted in acetone. However, the acetone extraction has been shown to underestimate the amount of chlorophyll present (Tada *et al.* 2004) and in a direct comparison between the hot ethanol extraction and acetone extraction, the extraction efficiency was shown to be much greater in ethanol than in acetone (Wasmund *et al.* 2006). For this reason the ethanol extraction was used throughout this project.

2.4.1. Extraction

From each homogenised soil sample, aliquots were weighed out into conical flasks (10.00g). Cold, 95% ethanol (5 ml), was added to a sample and the mouth of the flask was covered with aluminium foil. The foil was perforated with 3 small holes using a dissecting needle. The flask was placed over a steam bath and swirled occasionally until the ethanol boiled. The contents of the flask were then filtered through Whatman no. 1 filter paper using a vacuum applied to a 250 ml side arm flask. The conical flask was rinsed with an additional 5 ml of hot ethanol, which was then added to the filter. The amount of leachate was measured using a 25 ml measuring cylinder and then transferred into a 250 ml separating funnel with the stopcock closed.

An aliquot of petroleum ether equal to twice the amount of leachate was added to the separating funnel and the contents swirled for *c.* 3 minutes with the funnel held at approximately a 45° angle. A volume of deionised water equal to the total volume of leachate plus petroleum ether was added and the contents swirled for a further *c.* 3 minutes.

The lower, ethanol/water mixture and a very small part of the upper petroleum ether layer was drained and discarded. The remaining petroleum ether and dissolved chlorophyll was drained into 50 ml conical flasks containing approximately 1 g of Na_2SO_4. The contents were swirled and a fraction transferred to a UV grade disposable cuvette using a pipette.

2.4.2. Analysis and calculation

Absorbance was measured at 666 and 730 nm using a Nicolet Evolution 100 spectrophotometer (Thermo Electron Corporation, Massachusetts, USA.) unless otherwise stated, and chlorophyll *a* concentration calculated using the following equations:

$$[\text{Chlorophyll } a]\,\text{mg ml}^{-1} = \frac{(\text{OD at } 666\,\text{nm} - \text{OD at } 730)\,\text{x ml of sample x } 10}{890}$$

$$[\text{Chlorophyll } a]\,\text{mg g}^{-1}\,\text{soil} = \frac{[\text{Chlorophyll } a]\,\text{mg ml}^{-1}\,\text{x amount of petrol ume ther (ml)}}{\text{amount of soil (10g)}}$$

OD = optical density

Chapter 3: The Environmental Physics of the Arable Soil Surface

3.1. Introduction

This chapter aims to charecterise the environmental physics associated with the soil surface at a fine scale. This characterisation was undertaken in the location where the majority of the arable soil surfaces were collected from. This was done in order that the magnitude and frequency of fluctuations with regards to temperature, solar radiation and wetting and drying cycles were known and so each of their possible influences on the soil surface microbial community could be quantified.

3.1.1. Temperature

Collecting environmental physics data at a millimetre scale, which is necessary given the tenets of this study, is not a trivial task. Very little data is available regarding changes in temperature at such scales moving down through the soil profile. High-resolution data based upon 2.5 cm increments shows that the greatest fluctuations and largest range of temperatures occur at the soil surface and that the magnitude of the fluctuations and the range of temperatures decrease with depth (Scott; Personal Communication: Figure 1.2).

Thermocouples are a convenient way of collecting soil temperature data as they can be linked to a data logger which allows data to be collected with a high time resolution over extended periods of time which would not be practical using manual methods such as thermometers. Thermocouples work due to the thermoelectric effect whereby any conductor, such as a metal, will generate a voltage when subjected to a thermal gradient, as discovered by Thomas Johann Seebeck in 1821. By combining two dissimilar metals a small difference in voltage between them can be measured, which increases with temperature. For the modern range of available metal combinations this difference is typically 1-70 mV per degree Celsius.

Thermocouples are generally used in conjunction with data loggers which perform the polynomial interpolation internally and output the data as degrees Celsius.

The placement of thermocouples into the soil system necessitates some disturbance to the soil. Whilst care can be taken to keep this disturbance to a minimum, the effects of

the disturbance will increase with decreasing scale. Also, thermocouples are generally only accurate to within 1°C. For these reasons measurements of temperature differences at a very small scale in the soil profile i.e. a few millimetres, is not possible with a high level of accuracy.

3.1.2. Solar radiation

Data is available regarding the amount of solar radiation striking a horizontal surface within the UK (Jong 1973). This, combined with work done by Benvenuti (1995) allows the approximate amount of solar radiation reaching deeper soil layers to be calculated. Beyond 3 mm into the soil system, the amount of light penetration reaches 0%, with only a very small proportion, < 0.5% of light penetrating down to 2 mm, even in soils with the highest transmittance.

Much work has been undertaken in attempts to produce models of light interception by crop canopies (Anderson, 1966; Norman and Jarvis 1974; Norman 1980) but this work has been done to investigate light penetration onto leaves lower in the crop canopy as opposed to onto the soil surface itself. For this reason two pyranometers (thermal solarimeters) were used to measure the sum of the direct and diffuse solar radiation reaching the crop canopy as well as the soil surface under the canopy. These function by measuring the temperature difference between a grid of adjacent black and white surfaces within a glass sensor case, which is transparent to all wavelengths except far-UV and is relatively opaque to any terrestrial infra-red radiation, which is infra red radiation emitted from the atmosphere or other terrestrial sources (Strangeways 2003). Therefore, they allow just the solar spectral band to reach the sensor. The black elements are normally made from a lacquer containing carbon as this produces a matt finish and has a very wide spectral absorption. Magnesium oxide is often used for the white surfaces due to the fact that it very effectively reflects the solar spectrum and also absorbs wavelengths greater than 3 μm. This is important as it means that long wave infra-red radiation which originates within the glass tube is absorbed by both the black and white surfaces and so is largely cancelled out. The difference in temperature between the black and white elements within the sensor therefore is proportional to the amount of solar radiation (Strangeways 2003).

Pyranometers allow quantification of the total amount of solar energy received by the soil surface microbial community in situations where bare soil is exposed to direct

sunlight (such as when fields are fallow) and when placed under a crop canopy the amount of solar energy received by the soil surface microbial community when a crop is growing. Pyranometers detect only the total amount of solar energy and so no conclusions can be drawn regarding the quality of light, i.e. which wavelengths reach the soil surface in exposed soil or when under a crop canopy.

3.1.3. Soil moisture

Capacitance probes and neutron probes are the two common methodsnon-destructive methods for measuring soil moisture content. A capacitance probe usually has 4 pins, one central pin surrounded by 3 outer pins in a triangular formation. Theta probes work by sending a radio signal at a single frequency down the centre pin. Some of this radio signal is transmitted and some of it is reflected back. The difference in transmitted and reflected energy can be measured accurately. The soils dielectric constant is directly proportional to its moisture content and as the dielectric constant changes the ratio between the amount of signal transmitted into the soil and the amount of signal reflected back changes. The theta probe measures this change and converts it mathematically using a defined relationship to give a measure of volumetric soil moisture content. Capacitance probes rely on the use of a pre-defined calibration curve. This has implications for their accuracy as whilst the soils dielectric constant is proportional to its moisture content it can also be affected by other factors such as soil texture, pH and salinity.

Neutron probes work on the basis that when neutrons collide with hydrogen they lose energy and change from being “fast” neutrons (those with > 1eV) to “slow” neutrons (those with < 1 eV). A neutron probe is able to detect the change in ratio between fast neutrons and slow neutrons. Due to the fact that water contains two atoms of hydrogen per molecule, neutron probes can be used to measure soil moisture content.

One problem inherent in both of these instruments is the fact that they work by measuring soil moisture content in a sphere of influence around the probes. This limits the minimum scale at which they can be accurately used. As this project is looking at the surface on a millimetre scale it is pertinent to try and get a measure of changes in wetting and drying cycling on a millimetre scale, or as close to this scale as possible.

Tensiometers, which consist of a glass or plastic tube with a porous ceramic cup filled with water buried in soil, are a further method for measuring soil moisture content. However, as these require burying in the soil they are a destructive method. A partial vacuum is set up inside the tensiometer. As the soil dries the pressure inside the tube decreases with decreasing matrix potential. Therefore, by measuring the pressure within the tensiometer the soil water matric potential can be quantified.

Vetterlein *et al.* (1993) showed that a micro-tensiometer, a scaled-down version of a tensiometer, could be used to measure soil moisture content on a millimetre scale and to a soil matric potential of up to -80 kPa. Tensiometers must not be allowed to dry out completely as this releases the vacuum inside the tube, and as such they are not able to measure soil moisture content at the soil surface for extended periods of time where high levels of desiccation can occur.

Soil moisture content can also be calculated through measuring the difference in mass between soil before and after oven-drying, typically for 24 h at 105°C (Black 1965). This gives a very accurate, direct measure of the soil gravimetric moisture content, but is destructive, meaning the same volume of soil cannot be assayed twice. Also, due to being relatively labour-intensive along with the impossibilities of using an automated data logger with this technique, it is impractical to obtain high resolution data over an extended period of time using this technique.

Osborne and Jin (2004) designed a sensor for detecting soil water content at the soil-air interface. The sensor worked by measuring the resistance between two sensing elements which are placed so that they lay on the soil surface. As Soborune and Jin (2004) reported success with regards to measuring soil moisture content at the air : soil interface this sensor may be suitable to measure soil moisture content at the soil surface on a millimetre scale, and as such was investigated further. Used in conjunction with a theta probe, it may allow measurement of the frequency and intensity of wetting and drying cycles at the soil surface versus deeper soil zones.

3.2. Materials and Methods

The data was collected from Showground Field, on the Cranfield University research farm at Silsoe, Bedfordshire (National Grid reference TL075355). The soil was a sandy loam which had been fallow for at least the previous 6 months. As no herbicide had been used, some plant cover was present in the field which was cleared from the area from which environmental parameter data was to be collected. The soil had a dry bulk density of 1.48g cm^3, a pH of 6.1, %C of 1%, and %N of 0.1%.

Measurements were taken over a period of 9 months ranging from August 2005 to April 2006.

3.2.1. Temperature

Type T thermocouples which are made from copper and constantan (constantan being an alloy consisting of 60% Copper and 40% Nickel) were placed within the soil in Showground Field (Cranfield University, Silsoe; UK National Grid reference TL 075 355) at depths of 0, 2.5 and 5 cm. To do this, a hole was dug approximately 30 cm deep. Care was taken to make one side of the hole as vertical as possible. The thermocouples were then inserted horizontally into the soil profile at depths of 2.5 cm and 5 cm with each thermocouple positioned diagonally to each other at approximately a 45° angle. This was done so that any disturbance to the soil through inserting the thermocouple would affect only that thermocouple and not the others (Figure 3.1). The hole was left unfilled, as it contained the data logger. However, the thermo couples were inserted into the soil in such a way that they were much further away from the edge of the hole than they were in a direct vertical line to the surface. This was done so that the effects of any lateral thermal gradients on the thermo couples were minimised.

A Delta T DL2e data logger (Cambridge, UK) was used and was set to log data every 30 minutes and report the mean of 4 data points meaning data was recorded every 2 hours. This was done to minimise the impact of interference arising from the slight fluctuations in electrical current naturally occurring between the two metals in the thermocouples, but still maintain an adequate diurnal resolution to allow quantification of temperature fluctuations at the soil surface compared to deeper soil levels. A higher resolution was not used due to the limits of the data logger storage space.

Figure 3.1 Placement of thermocouples moving down through soil profile.

3.2.2. Solar radiation

Two pyranometers were set up in Showground Field (Cranfield University, Silsoe; UK national grid reference TL 075 355), attached to the same Delta T DL2e data logger as the three thermocouples. One pyranometer was raised horizontally above the soil by about 30 cm on wooden canes which were pushed into the soil. Any plants which grew near to this pyranometer were cut back to ensure that there was no restriction on the amount of solar radiation reaching it. The other pyranometer was placed horizontally on the soil surface and any plant growth was allowed to naturally cover this second pyranometer. The plants growing were a mixture of weeds as the field had been left fallow but not treated with herbicide. Pyranometer data was logged every 30 minutes and the mean reported every 4 data points (i.e. every 2 hours). This was done to minimise the impact of noise, as discussed previously regarding the thermocouples, from the pyranometers, but still detect fluctuations in solar radiation due to changes in cloud cover at this frequency.

3.2.3. Soil Moisture

A sensor for detecting soil water content at the soil-air interface was developed following the method described by Osborne and Jin (2004). The sensor was attached to a Campbell 21X data logger (Campbell Scientific Inc., Loughborough, U.K.) which was used to apply current of 2.5 volts alternating current (AC).

The sensor works by measuring the resistance between the sensing elements. The circuit is open, and resistance (R_s) is infinite when there is no conductance between the sensing elements. This occurs when the soil contains no water. When water is present in the soil, this completes the circuit, and resistance across the sensing elements is reduced and can be determined. By measuring the change in resistance which is higher when moisture levels are low and approaches a minimum when "free water" is present, soil moisture content can be calculated.

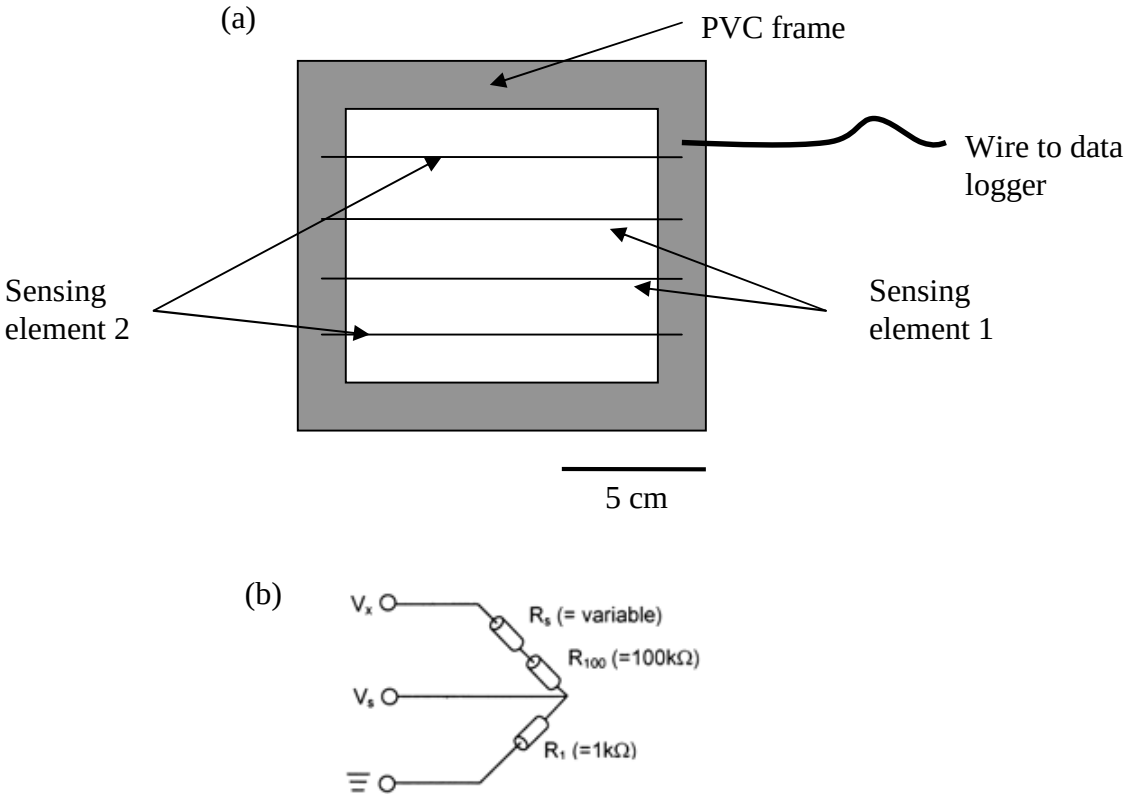


Figure 3.2 Schematic showing: (a), the main components of the soil surface wetness sensor including two stainless steel wire sensing elements, the PVC frame, and the data logger wire for excitation and voltage measurement; and (b), half-bridge circuit diagram where V_x = excitation voltage, V_s = measured voltage, and R_s = variable resistance across the plane of sensing elements (i.e., sensor resistance). R_1 and R_{100} represent integrated fixed resistors (Adapted from Osborne and Jin 2004).

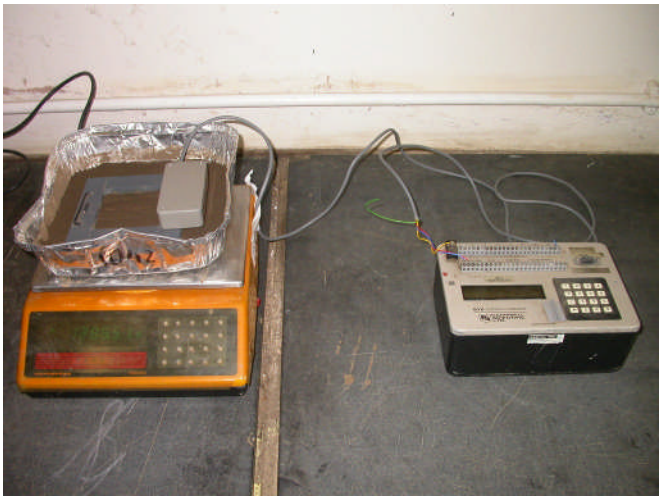


Figure 3.3 Calibration of soil moisture sensor using a Campbell data logger and balance.

The sensor was calibrated and its sensitivity tested through by placing the sensor on soil with a known moisture content on a balance. The sensor then measured change in the resistance of the soil as it dried out, whilst using the change in mass of the soil and sensor to allow calculation of the soil's moisture content.

Whilst the sensor may provide a means of measuring soil water content at the soil surface on a millimetre scale by laying on the soil surface and has the advantage that it could be left *in situ*, it should be noted that there are some disadvantages. For example, by leaving the sensor *in situ* it may restrict the free movement of water vapour or precipitation and so may not give a true reading of what the soil moisture levels would be if the sensor were not in place.

3.3. Results

Means were calculated using the diurnal data from each month. Standard error was calculated as a measure of the variation and plotted on each monthly data graph to give an indication of the variation within the diurnal data. Coefficient of variation was used to compare the variation from different months due to the differences in the scales of temperature and solar energy from different months.

3.3.1 Temperature

The data obtained from the thermocouples showed that the soil surface was exposed to a greater diurnal fluctuation in temperature than the two deeper soil depths investigated (Figure 3.4). The soil surface temperature dropped to a cooler temperature overnight and rose to a higher temperature during the daytime than that experienced at the two deeper soil depths. There was no significant difference in temperature regime between the two deeper soil layers. The temperature difference fluctuated more greatly, rising both higher and falling lower at the surface than at 2.5 cm down through the soil profile. This temperature difference was as much as approximately 8°C higher at the surface and down to as much as approximately 7°C lower, as seen in August 2005 (Figure 3.4).

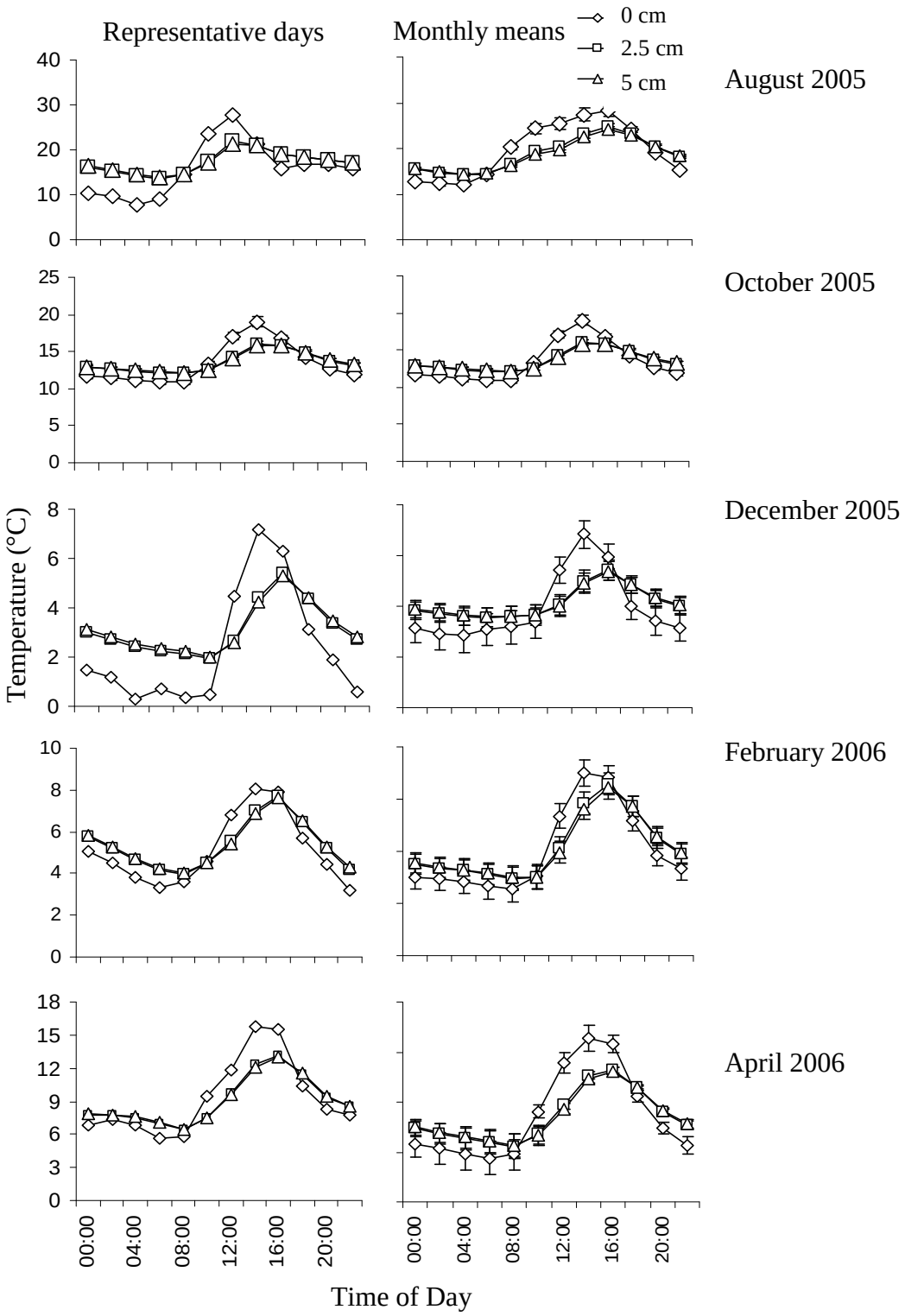


Figure 3.4 Diurnal temperature fluctuations in soil at three depths: 0 cm 2.5 and 5 cm in an arable field at Cranfield University Research Farm, Silsoe. Showing data collected on (a) representative days and (b) monthly means, bars show standard errors. Note differences in magnitude of scales on y-axes.

The coefficient of variation for the temperature at the soil surface layer in August varied between *circa* 10% and 25% whereas the range of coefficient of variation in the 5 cm layer was lower, varying from *c.* 7% up to *c.* 18%. In December, the coefficient of variation with regards to temperature at the soil surface varied from *c.* 40% at 14:00 hrs up to a maximum of *c.* 120% at 04:00 hrs. At a depth of 5 cm, the coefficient of variation ranged from *c.* 35% to 55%, again demonstrating the reduction in fluctuations and temperature ranges at this depth due to the buffering effects of the soil.

3.3.2. Solar radiation

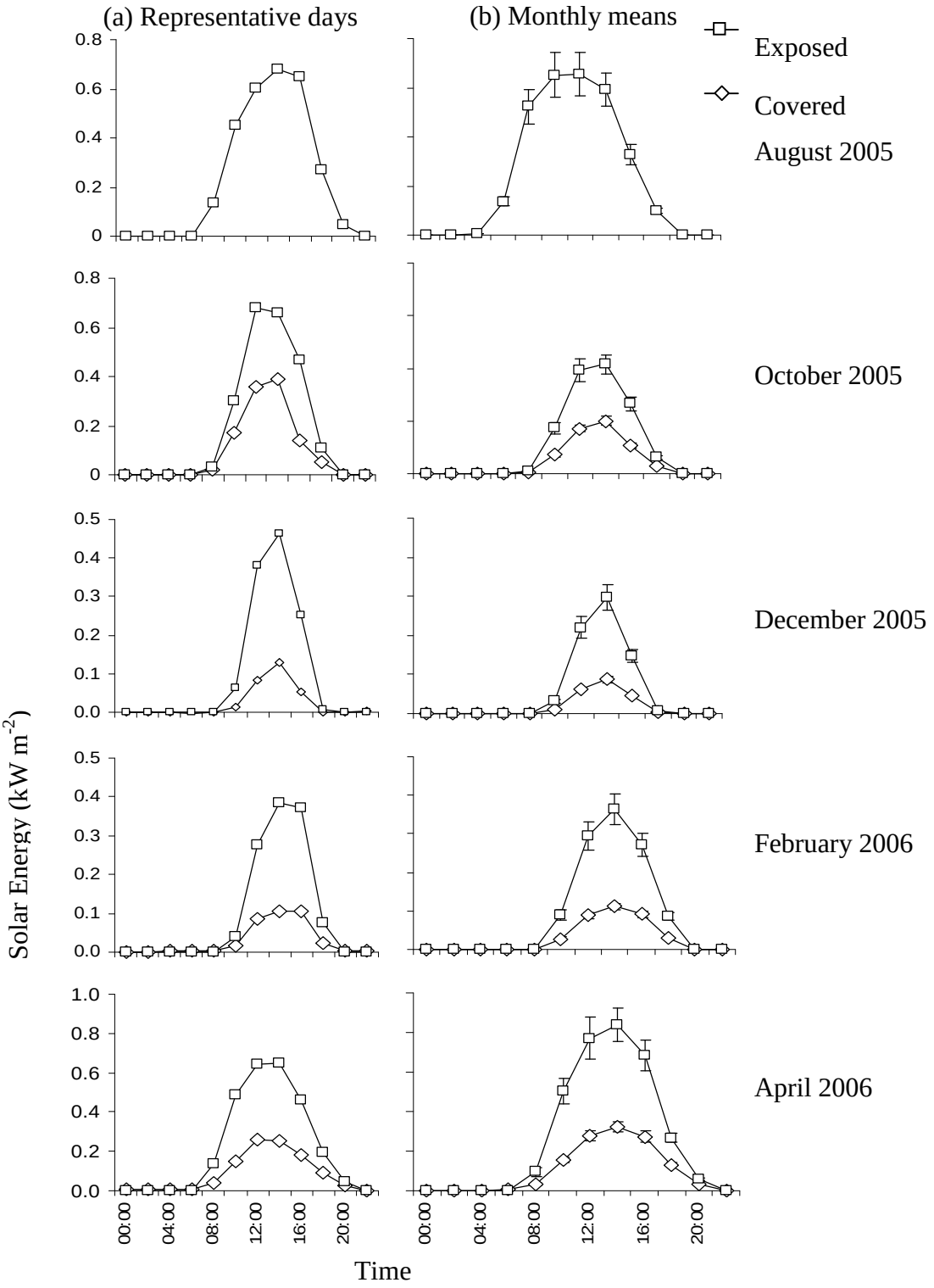


Figure 3.5 Diurnal solar energy flux density reaching a horizontal surface in an arable field at Cranfield University research Farm, Silsoe: showing data collected on (a) representative days and (b) monthly means; bars show standard errors. Note differences in scales on y-axes.

The mean input of solar energy onto a horizontal exposed soil surface varied from a maximum of approximately 0.7 kWm^{-2} in the summer down to a minimum of approximately 0.3 kW m^{-2} in the winter (Figure 3.5).

With regards to solar radiation, the coefficient of variation (CV) for August varied between approximately 56% and 70% whereas in December, the data obtained from the exposed pyranometer varied between approximately 41% and 69%. This shows that the variation in the amount of sunlight reaching the Earth's surface remained relatively similar, even though the energy from the sunlight reaching the Earth's surface dropped by more than half between August and December.

During August there was no crop which is why there are no reported values for the "Covered" treatment for this month. The establishment of a crop reduced the mean input of solar energy onto the horizontal soil surface by between three- and four-fold. The amount of light reaching the exposed soil surface was much more variable as shown by the error bars in Figure 3.5. The CV for the data obtained for the covered pyranometer varied between approximately 58% and 88% higher than that seen in the data collected from the exposed pyranometers.

3.3.3. Soil moisture

The soil moisture probe was found to have virtually no sensitivity with regards to distinguishing between gravimetric moisture contents greater than approximately 6% (Figure 3.6). This would be a problem when applying the sensor at the soil surface as the soil moisture content can be expected to greatly exceed 6%, especially after rainfall events where the soil surface may become saturated.

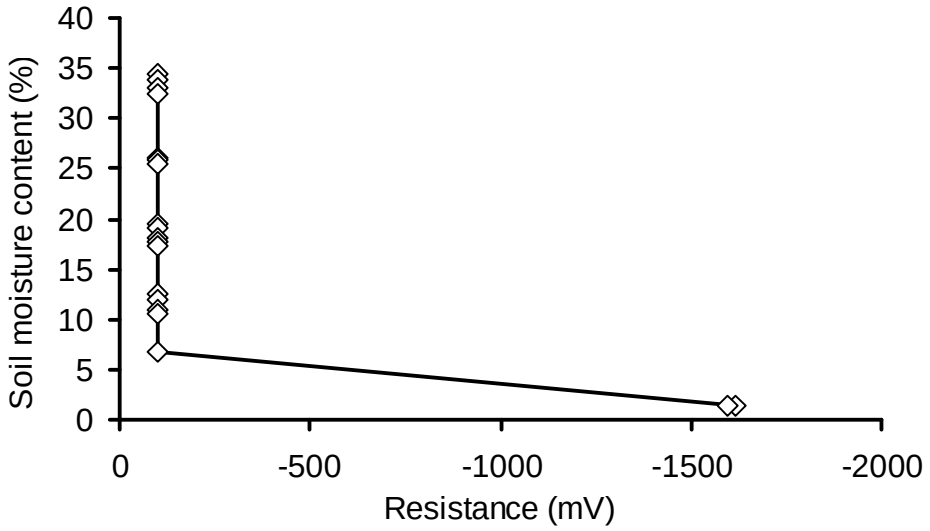


Figure 3.6 Calibration graph of the soil moisture sensor showing the resistance (mV) for soil at different moisture contents.

Due to the insensitivity of the sensor to soil moisture contents above 6% this sensor was deemed inappropriate for application in the field for surface-based studies. Due to these technical difficulties, measurements of soil moisture fluctuations were not made in this project. Further work is needed to devise a means of measuring soil moisture content at these millimetre- and surface-scales.

3.4. Discussion

There was a lag seen in the time between the maximum temperature reached at the soil surface and the maximum temperature reached in the subsequent soil layer at 2.5 cm depth. This was probably due to the fact that when the sun had passed the point where it was putting maximum energy into the surface, as shown by the declining afternoon temperature, energy was still conducting down through the soil, both through the mineral and organic parts as well as any water that was present in the soil and so the deeper layers were slower to respond to the decrease in energy.

The variation in temperature, as shown by the standard error bars on the monthly mean graphs is much greater at the surface than at deeper levels. This was due to the fact that the soil at depths responded much slower to changes in daily temperatures which arise through changing weather patterns. Changes in temperature at the soil

surface occurred as a response to radiation flux density which would have been influenced by changing weather patterns. It should be noted that much of the differences seen in the size of the error bars during different months can be attributed to changes in scale on the y-axis and are not necessarily representative of differences in variation. The range of the coefficient of variation for the soil surface layer in August was greater than at 5 cm depth. This shows that the buffering effect of the soil reduced the range of fluctuations in temperature when compared to the soil surface.

The large increase in the coefficient of variation seen in December was due to the relatively large size of the fluctuations when compared to the size of the means as the mean temperature was almost five times lower in December than in August. The fact that the coefficient of variation was found to be so large at 04:00 hrs was because this was when the greatest variation in temperatures at the soil surface occurred. This is due to the fact that the presence or absence of cloud cover has a very large effect on nocturnal temperatures, especially during the winter, as clouds “trap” warm air under them. The time before dawn is where nocturnal temperatures are the lowest on clear nights and so are affected most by remaining relatively high if there has been cloud cover all night. The coefficient of variation was lower at a depth of 5 cm, again demonstrating the reduction in fluctuations and temperature ranges at this depth due to the buffering effects of the soil. This data demonstrates that the soil had a large buffering effect with regards to temperature. This means that the arable soil surface is subject to an increased amplitude and greater and more rapid fluctuations of temperatures when compared to subsequent soil layers, even on a millimetre scale. This has possible implications for the soil surface microbiology as this data shows that surface microorganisms must be able to resist these greater and more rapid fluctuations in temperature.

With regards to solar radiation, the increase in variation with regards to the amount of energy reaching the covered pyranometer compared to the exposed pyranometer, as shown by the coefficients of variation, was attributable to the crop canopy. This is because whilst cloud cover would cause variation in the amount of solar energy reaching the earth’s surface, this would affect both pyranometers equally. The crop canopy, however, would be moving due to air currents and so would also affect the

amount of energy reaching the covered pyranometer thereby increasing the variation in the reported data.

With regard to soil moisture as well as the manufactured sensor not having the required sensitivity, an alternative approach based upon soil sampling and oven-drying to determine moisture content was also deemed impractical, due to the extremely limited resolution with regards to time and also due to the fact that when sampling in the rain, inaccurate results would be obtained from the deeper soil layers as they would be influenced by the rain as soon as the upper layers were removed.

With regards to environmental factors such as temperature and solar radiation it is clear that the surface experiences much greater extremes and faster fluctuations than deeper soil layers, even on a millimetre scale. Moving down through the soil profile, the soil matrix exerts a buffering effect with regards to temperature meaning that the soil surface temperature can be more then 5°C higher or lower at the surface when compared to 2.5 cm below the soils surface.

Solar radiation provides energy to the soil surface and is confined to the top couple of millimetres of the soil surface, as was demonstrated by Benvenuti (1995). This energy input will also function to heat the soil, but as has been shown and discussed previously, soil functions as a buffer with regards to temperature and so this temperature increase decreases with depth, even on a relatively small scale. The input of energy from solar radiation is highly variable due to changes in cloud cover. However, the biggest factor affecting the amount of solar radiation reaching the soil surface was cover by vegetation. This was found to reduce the amount of energy by up to a factor of 5.

Although no conclusive data is available nor has been collected through this project concerning wetting and drying cycling at the soil surface on a millimetre scale, some inferences can still be drawn.

During rainfall events, exposed soil surfaces will be exposed to the full intensity of the rainfall event. This may lead to the soil surface becoming saturated before the water has a chance to infiltrate down beyond the surface layer. When the rain falls onto the soil at a rate greater then the water can infiltrate the water will pond or run off depending on the slope gradient. This can lead to potential environmental

degradation at the soil surface due to erosion of soil particles if the water runs off (Morgan 1995).

Plant cover has been shown to reduce the impact energy of raindrops on the soil surface (Romkens *et al.* 1990). However, whilst the impact energy may be reduced, most of the rainfall will still reach the soil surface after running off of the plant covers. Whilst the water from the rainfall event will still reach the surface, the speed of the initial wetting at the soil surface is reduced. This means that there is a higher probability that the water will have time to infiltrate before building up to the level where it ponds or runs off. Surface roughness also has a large influence on run off generation and water infiltration rates. This is because rougher surface have more areas for water to pool, allowing more time for it to infiltrate into the pore network. The pooling of water due to increased surface roughness also means that the rate of any overland flow from run off is reduced due to increased pooling. This means that the risk of soil erosion is reduced as there is less energy in the surface water which may lead to particle detachment.. Plant roots also function to bind soil particles together and so reduce the risk of soil erosion (Morgan 1995). Through these mechanism cover crops can help to prevent the environmental degradation associated with soil erosion

Due to the influence of the pore network on the inflow of water into the soil, combined with any biological effects which can be highly heterogeneous there are normally areas of preferential flow for water moving into the soil profile. This difference in water infiltration on the horizontal plane can lead to differences in wetting and drying rates in both the x – y plane and the z plane, even on a millimetre scale.

With regards to drying, evaporation is dependent on several controlling factors. These include vapour pressure gradients where by water evaporates more slowly into air which is more humid when compared to less humid. Turbulence or wind speed at the soil surface also has a strong influence on the evaporation rate of water from soil. This is because it influences the vapor pressure gradient by transporting humid air away from the evaporating surface and replacing it with drier air. The number of photons arriving at the water surface (Roerick and Farquar 2002). The soil surface experiences greater temperatures on a millimetre scale, as well as a greater influx of photons,

therefore it can be expected that evaporation will be greatest at the soil surface, and to decline with depth.

As the soil surface dries the pressure on the remaining water within the pore spaces is reduced. This leads to a matric potential which causes water to move from areas of lower matric potential (wetter areas) to areas of higher matric potential (dry areas) (Brutsaert 2005).

Chapter 4: Investigation of the Microbiology of the Soil Surface of an Arable Field

4.1. Introduction

The soil system is home to an immense number of highly diverse microorganisms. These organisms are exposed to different stresses depending on where they exist within the soil profile. Environmental stresses as shown in Chapter 3 have the largest and most rapid fluctuations and greatest extremes at the surface, which reduce in frequency, amplitude and rate of fluctuation with depth, due to the physical buffering effects of the soil.

Microorganisms have different degrees of resistance to different environmental stresses. Some are highly resistant to radiation such as *Deinococcus radiodurans* (Mattimore and Battista 1996), whilst others are highly resistant to desiccation such as many species from the *Nostoc* genus of cyanobacteria (Scherer *et al.* 2004); *D. radiodurans* is also particularly desiccation resistant (Mattimore and Battista 1996). It seems likely that the soil surface environment will favour organisms that are capable of resisting desiccation, solar radiation and temperature fluctuations. If these organisms can resist these environmental stresses and also utilise photosynthetically active radiation (PAR) then they will have a further competitive advantage. It therefore seems probable that the arable soil surface will be inhabited by desiccation- and temperature-resistant organisms and possibly photoautotrophs. These factors, combined with the disturbances inherent in arable systems such as erosion and tillage, are likely to influence the composition of the microbial communities.

This chapter aims to determine whether differences exist regarding the microbiology in the uppermost layers of arable soils on a millimetre scale, and the nature of depth gradients if differences exist, by testing the following hypothesis:

Hypothesis B. *The community structure and physiological properties of organisms in the topmost layers of arable soil differ from those in deeper layers*

4.2. Materials and methods

4.2.1. Experimental design

Samples were collected from two fields from the Cranfield University, Silsoe campus on-site farm. The two fields used were Avenue Field (UK grid reference TL075357) and Ivy Ground (UK grid reference TL077352). These fields were selected because at the time of this work they were at different stages of the arable agricultural cycle. Avenue Field was fallow and the surface had not been disturbed mechanically or chemically for approximately 6 months prior to sampling This means that there was a selection of various "weeds" growing over the surface. Ivy Ground was drilled 4 months previously and planted with winter beans. These fields were chosen as this would allow some approximation of how long after a disturbance event a distinct soil surface community developed.

Areas to be sampled within each field were randomly selected via the throwing of a palette knife backwards over the shoulder and sampling the area where it fell. Three soil layers were sampled, through scraping with a palette knife; the surface layer to a depth of *circa* 1 mm, followed by two subsequent and sequential depths of *circa* 5 mm each.Any plant matter or stones that crossed through two or more soil layers were removed. Three replicate areas from each field were sampled using this technique. Due to the microtopography of the soil surface, some deviation within the depths of each sample layer may have occurred but all possible care was taken to keep this to a minimum. All samples were stored in plastic bags overnight in a refrigerator at 4°C.

Table 4.1. Chemical and physical properties of soils sampled.

Location	Field	Texture	Dry bulk density	pH	Total N %	C %	Available N %	C:N
Silsoe, Bedfordshire	Avenue Field	Sandy loam	1.48 g cm^{-3}	6.07	0.125	1.0	0.1	10.6
Silsoe, Bedfordshire	Ivy Ground	Clay loam	1.07 g cm^{-3}	6.64	0.258	2.9	0.2	13.9

Table 4.1 shows data obtained from analysis of homogenised soil collected from the top approximate 10 cm of each field.

Once it was confirmed that a distinct soil surface phenotype did exist, work was undertaken at an increased resolution to test whether other distinct microbial community phenotypes existed at a finer resolution within the two sampled deeper soil layers. This was done as it was possible that differences in community level phenotypes may not have been detected at the previous resolution where the two deeper layers were each sampled to sequential depths of 5 mm. This means that it is possible that distinct microbial community phenotypes that may have existed within these layers were diluted out and so not detected. For this reason the experiment was repeated as described above, with the top 10 mm of the soil surface sampled in five layers each to sequential depths of 2 mm. This increased resolution was used to detect whether any other distinct microbial community phenotypes exist.

4.2.2. Laboratory analyses

To investigate community level phenotypes, phospholipid fatty acid (PLFA) extraction and analysis as outlined by Bligh and Dyer (1959) and extended upon by Zelles (1999) were undertaken as described in Chapter 2. Chlorophyll *a* extraction and analysis was performed using the ethanol extraction method for quantification of chlorophyll *a* in soil (Metting 1994) as described in Chapter 2.

The Fungal: bacterial ratio was calculated using 18:2ω6 (fungal biomarker) divided by the summed %mol of biomarkers i15:0, ai15:0, 15:0, i16:0, 16:1ω7t, i17:0, ai17:0, 17:0, 18:1ω7 and 19:0c as an expression of total bacterial abundance (Frostegård & Bååth, 1996).

4.2.3. Data analysis

Principal component analysis (PCA) using covariance and *post-hoc* analysis of variance (ANOVA) was undertaken using %mol data for PLFA profiles *Post-hoc*. The ANOVA was performed on the PC coordinates to check for significance. ANOVA, using Fisher's Least Significant Difference (LSD) was used to test differences in chlorophyll *a* and microbial biomass C between samples from different soil layers. All analyses were performed using Statistica 7.1 (Statsoft Inc, 2005).

4.3. Results

When an arable soil surface was not disturbed through either mechanical or chemical means for a period of up to 6 months, a statistically significant distinct community phenotype apparently developed within the top *circa* 1 mm of the soil surface ($P < 0.001$) (Figure 4.1). Differences between microbial community phenotypes become less pronounced with depth (Figure 4.1a). Disturbance, such as through drilling, was apparently enough to destroy any distinct soil surface phenotype that may have developed so that it was not significantly different from deeper community phenotypes ($P>0.05$) and four months was not sufficient time for a distinct soil surface phenotype to become established (Figure 4.1b).

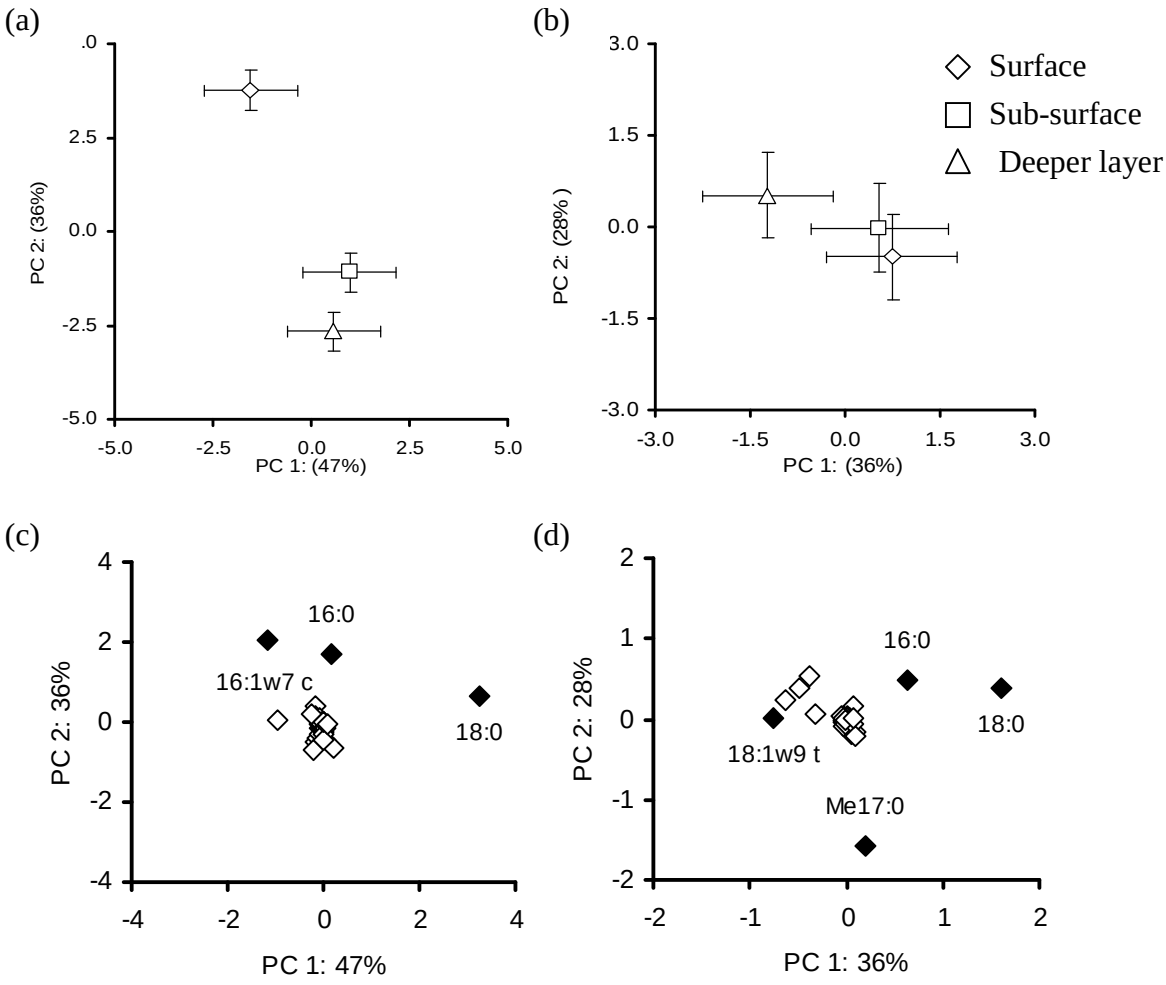


Figure 4.1. First and second principal components (PCs) derived from phospholipid fatty-acid profiles originating from surface layers of arable soils at two sites at different stages in the cropping cycle. (a) Silsoe, Avenue Field: fallow; surface not disturbed mechanically or chemically for approximately 6 months prior to sampling (b) Silsoe, Ivy Ground: drilled; mechanically disturbed 4 months previously. Points show means (n = 3), bars show standard errors. (c) and (d) show loading plots for Avenue Field and Ivy Ground respectively. Those PLFAs contributing most to the

variance seen in the PC plots are highlighted in black and named. Percentage variation accounted for by PC shown in parenthesis on each axis.

The loadings associated with the principal components which contributed most to the discrimination seen between depths in Avenue field were 16:1ω7c and 16:0. There were consistently higher proportions of 16:1ω7c and 16:0 at the soil surface which decreased with depth at the scale investigated. The PLFA 16:1ω7c is a biomarker for Gram negative bacteria and the ratio of the proportion of 16:1ω7t to 16:1ω7c is often used as an indicator of stress on the microbial community (Zelles 1999).

There is much disagreement in the literature as to the biomarker status of 16:0. Pelz *et al.* (2001) use 16:0 as a general bacteria marker. Keinanen *et al.* (2003) use 16:0 as a biomarker for microbial biomass, Jones *et al.* (2003) state that it is ubiquitous and Knief *et al.* (2006) use it as a biomarker for Type II methanotrophs.

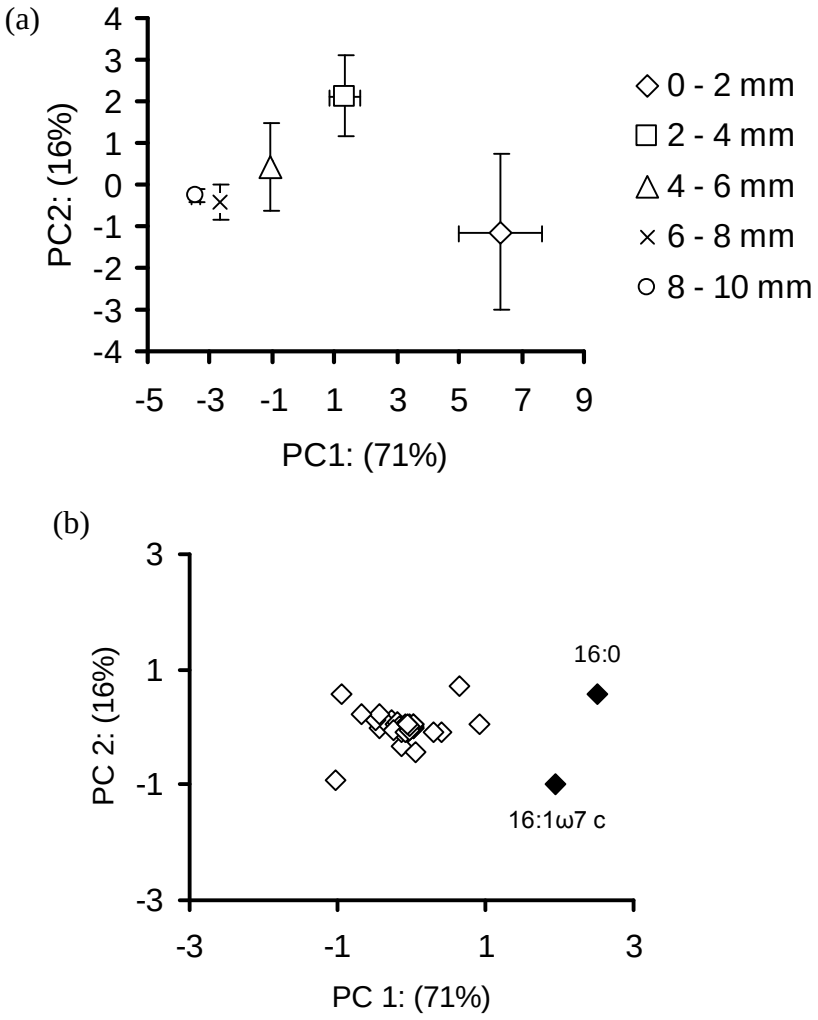


Figure 4.2. First and second principal components (PCs) derived from phospholipid fatty-acid profiles originating from surface layers of an arable field (Showground Field, Silsoe). Points show means (n = 3) ± S.E. Percentage variation accounted for by PC shown in parenthesis on each axis.

The greatest amount of discrimination with regards to microbial community phenotype between soil layers occurred between the soil surface layer and the subsequent soil layer (Figure 4.2 a). The amount of discrimination between phenotypes declined with depth, with discrimination between soil layers occurring almost exclusively in PC1.

Variation in microbial community phenotype within each soil layer occurred in both PC1 and PC2. The variance was greatest in both PC1 and PC2 in the surface layer, and this variation declines with depth. The decline in variation is probably attributable to the buffering effects of the soil meaning that environmental effects become relatively homogenous when compared to layers closer to the surface.

The PLFAs contributing most to the discrimination seen between depths are 16:0 and 16:1ω7c. These are the same PLFAs that contributed most to the discrimination seen in Figure 4.2 and this adds further evidence to the fact that these PLFAs are associated with the soil surface microbial community phenotype.

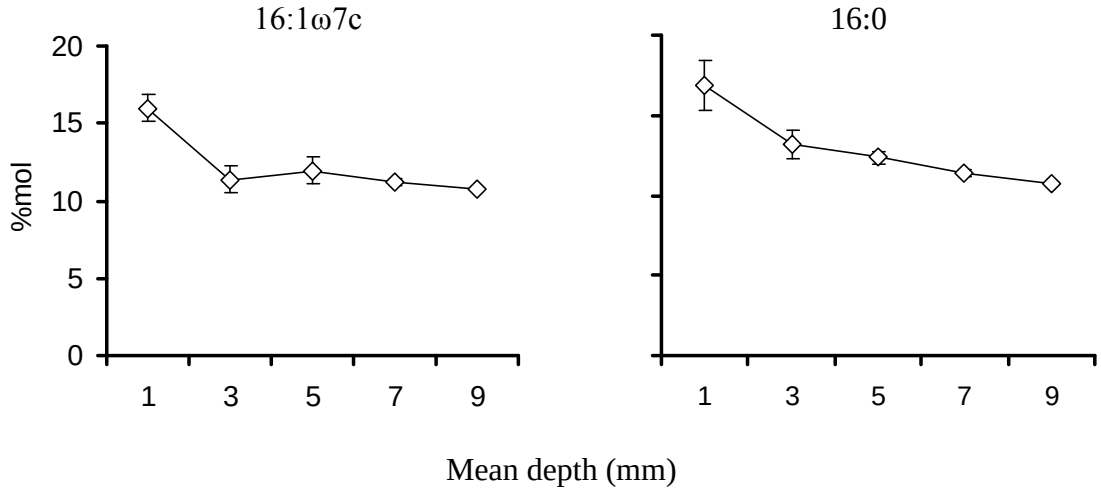


Figure 4.3. Proportion of PLFAs, 16:1ω7c and 16:0, which are most responsible for the discrimination seen between soil layers in Figures 4.1 and 4.2 in relation to depth from soil surface.

Both of the PLFAs which were most responsible for the discrimination seen between soil surface layers decreased with depth (Figure 4.3). The PLFA 16:1ω7c decreased significantly (ANOVA $P < 0.01$) between the soil surface layer (mean depth 1 mm) and the subsequent layer (mean depth 3 mm). There was no significant change in the %mol concentration of the PLFA 16:1ω7c at subsequent depths.

The PLFA 16:0 also decreased significantly (ANOVA $P < 0.01$) between the soil surface layer (mean depth 1 mm) and the subsequent layer (mean depth 3 mm). Again there was no significant change in the % mol concentration of the PLFA 16:0 at subsequent depths.

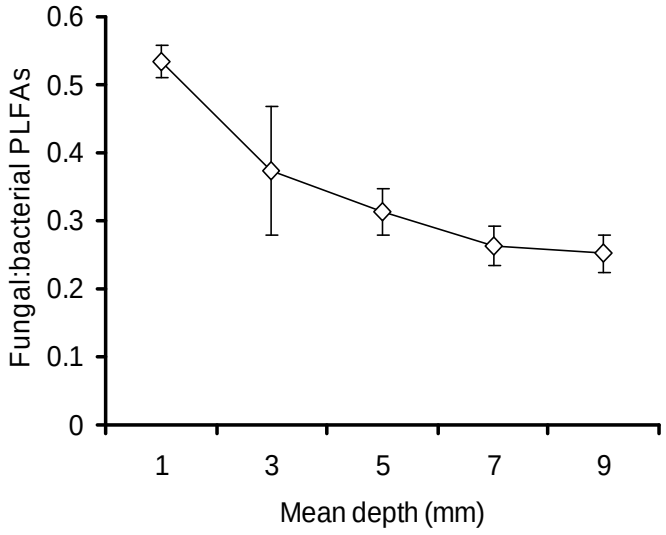


Figure 4.4 Ratio of PLFA fungal biomarker to PLFA bacterial markers from 5 depths originating from surface layers of an arable field (Showground Field, Silsoe). Points show means (n = 3) ± S.E

The mean ratio of fungal to bacterial biomass decreased significantly from the surface layer down to a mean depth of ($P = 0.01$) with a suggestion of stabilisation occurring at a ratio of approximately 25% (Figure 4.4). In the subsequent layers, differences in the fungal to bacterial biomarker ratios declined and were not shown to be significantly different. The variance, as shown by the standard error bars, was greatest at a mean depth of approximately 3 mm.

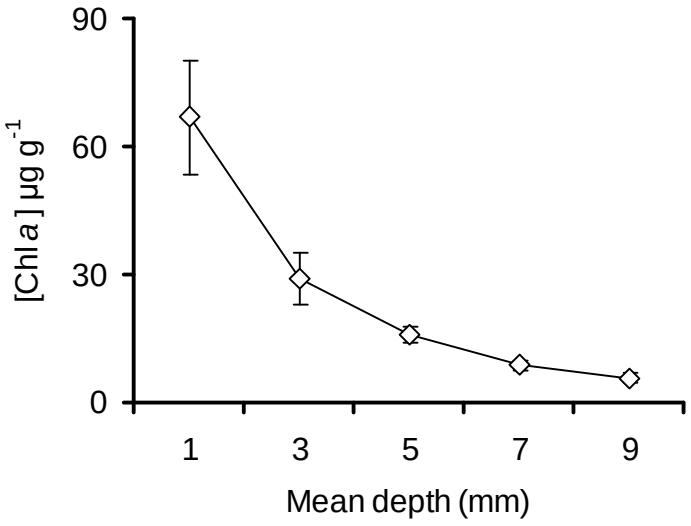


Figure 4.5 Chlorophyll *a* concentrations at different soil depths relative to the surface. Points show means (n = 3), bars show standard errors.

The concentration of chlorophyll *a* in the surface layer was significantly higher than in subsequent layers ($P < 0.01$) (Figure 4.5). The chlorophyll *a* concentration in the second layer, at a mean depth of 3 mm, was also significantly higher than in the two deepest layers investigated at mean depths of 7 and 9 mm ($P = 0.02$ and $P = 0.01$ respectively). Overall there was an approximate 10-fold decrease in chlorophyll *a* concentrations between the surface layer and the deepest layer investigated. This could equate to a 10-fold decrease in the abundance of photoautotrophs, or a decrease in the amount of chlorophyll *a* produced by photoautotrophs present in the deeper soil layer.

4.4. Discussion

PLFA analysis is an inherently destructive process. The soil must be removed and processed before the analysis can be undertaken. This raises the possibility that the preparation procedures and the analysis itself and may change the PLFA fingerprint of the soil. Obviously, if these effects were large and were not more or less consistent between samples then this could call into doubt the validity of PLFA analysis. However, Peterson and Klug (1994) found no significant changes in the concentrations of individual fatty acids over a 7-week period when the soils were stored at 4.5°C. This implies that delays between collecting samples from the field

and undertaking the analysis should not have a negative impact if the soils are refrigerated. They did however find that sieving reduced the signature fatty acid of fungal hyphae (18:2ω6), probably due to damage caused to the hyphae by the physically disruptive act of sieving. Due to the fact that sieving of soil is an inherent part of the procedure it seems likely that the reductions in the fatty acid would probably be equal between samples and as the statistical analysis compares differences between samples the effects of the reduction of this PLFA are likely to be negligible. The main analysis where a reduction in the PLFA might cause an effect is with the fungal to bacterial ratios. This is because the summed bacterial PLFA biomarkers are divided by the fungal biomarker 18:2ω6 and so a reduction of this PLFA may lead to an under reporting of the fungal to bacteria ratio. However, as this is likely to remain consistent between samples the effects regarding the statistical analysis of the data are likely to be minimal.

Both of the PLFAs 16:0 and 16:1ω7c, which were most responsible for the discrimination between soil surface layers, decreased with depth between the surface layer and subsequent layer, and no further significant reduction occurred in both soils where a distinct soil surface phenotype existed (Figure 4.1a and 4.2). This provides evidence that increases in the PLFAs 16:1ω7c and 16:0 are strongly associated with the soil surface microbial phenotype.

Chlorophyll *a* also declined most dramatically between the first two soil depths sampled. This was expected due to the fact that virtually no light, including UV and PAR penetrates down to a depth of 3 mm in any soil type (Benvenuti 1995). This implies that much of the difference seen between microbial community phenotypes within the soil surface layers is likely to be due to photoautotrophs.

It is possible that photoautotrophs are present in the same numbers in deeper soil layers but are in a dormant form and so do not contain equivalent concentrations of chlorophyll. Further work, possibly involving molecular techniques would be needed to confirm or refute this hypothesis, but such work did not come within the scope of this project.

The fact that any chlorophyll *a* is found in the deeper soil layers is probably the result of vertical transport of photoautotrophs. This could occur in the form of rainfall

events washing photoautotrophs down through the soil profile or due to co-transportation of chlorophyll-containing photoautotrophs in the guts of larger organisms such as earthworms and nematodes. It may also be the result of various forms of microbial taxis such as hydrotaxis where microorganisms such as cyanobacteria have been shown to migrate due to changes in moisture contents although this was found to occur on a smaller scale than that investigated in this chapter (Pringault and Garcia-Pichel 2003).

The data presented in this chapter supports the two hypotheses stated at the beginning of this chapter. The community structure and physiological properties of organisms in the topmost layers of the arable soils studied here differed from those in deeper layers, and there was evidence that light is an important factor in driving the succession of distinct microbial soil surface communities. Furthermore, fungi were shown to exist as a greater proportion of the microbial community at the soil surface when compared to deeper soil layers at the scale investigated here. This was expected following on from the work reported by Otten and Gilligan (1999) where the fungus *Rhizoctonia solani* spread further and faster over surfaces when compared to growing through the soil pore network. Although it is unknown which species of fungi were present at the soil surfaces investigated here, it seems unlikely that it was *R. solani*. It therefore seems probable that the preferential growth over surfaces discovered in *R. solani* also applies to other species of fungi. This is likely because when growing in their filamentous form, it is easier for fungi to grow over surfaces where they are generally supported by the soil surface beneath them but are not restricted by having to move through pores, spending energy navigating tortuous routes which may not lead to utilisable substrates. It is also possible that fungi have an increased tolerance to dessication due to their ability of transferring water along hyphae and so may be found as an increased proportion of the microbial community due to a reduction in competition from other, less desiccation-resistant organisms. Further work would need to be undertaken to confirm this hypothesis.

Chapter 5: Temporal Succession at the Arable Soil Surface

5.1. Introduction

It was reported in Chapter 4 that, given a sufficient period of time, a distinct microbial community will develop within the top *circa* 1 mm of the soil in arable systems. The process by which the microbial community develops will arguably follow the concept of an ecological succession which is characteristically observed in macroscopic ecosystems. This chapter investigates this process with regards to succession within the microbial community of the arable soil surface.

Ecological succession is a fundamental concept in ecology. It is the process whereby changes in the composition or structure of an ecological community occurs in a more or less consistent way (Cowels 1911). Succession may occur after the formation of a new, and hence unoccupied habitat, such as on new islands that have risen out of the sea following geological activity including volcanoes, termed primary succession, or after some form of disturbance such as after fire in a forest, termed secondary succession (Begon et al. 1996).

In arable systems, disturbance generally falls into three categories:

1. Mechanical disturbance such as that caused by soil management practices for example tillage and drilling;

2. Physical disturbance such as that caused by wind or rain erosion, but also by deposition of sediments eroded by wind or water;

3. Chemical disturbance, through the application of fertilisers, pesticides and herbicides;

This chapter investigates secondary succession within the arable soil surface microbial community. Chapter 3 showed that environmental parameters, specifically temperature and solar radiation, were greatest with regards to range and rate of fluctuations, at the soil surface on a millimetre scale showing that the soil surface was likely to be an ecologically distinct zone. Chapter 4 demonstrated that the arable soil surface, given a sufficient period of time, will develop into a biotically distinct zone. However, the time resolution in the field level investigations undertaken in Chapter 4

was relatively small. This means that the actual timeframe required for the formation of a distinct soil surface community determined here was only an approximation.

A microcosm experiment was designed to look at succession after mechanical disturbance. The flatness of the soil surface layer within these microcosms was artificially controlled, reducing the influence of the microtopography of the soil as a variable. This allowed greater confidence with sampling to specified depths on a millimetre scale.

This experiment was designed to test Hypothesis D: *The microbial community will change over time, as a new surface is uncovered after a disturbance event.*

5.2. Materials and methods

5.2.1. Materials

Microcosms were constructed from unplasticised polyvinyl chloride (uPVC) drainpipe (150 mm diameter), cut into 100 mm lengths. One end of each microcosm was covered with Loktek™ LS greenhouse shading (73% density with a mean mesh size 2 mm^2; Tildenet, Bristol, UK), which was attached using silicone sealant and nylon cable ties to function as the base. This was done to allow free passage of water and the microbioita but to still keep the soil in place.

A sandy loam soil as characterised in Chapter 4 was collected from Showground Field, down to a maximum depth of approximately 10 cm and sieved, fresh, to pass 2 mm and thoroughly mixed to ensure homogenisation. It was then packed into each microcosm to a dry bulk density of 1.3 g cm^{-3} so that the microcosm was full. The surface of the soil in the microcosms was flattened by gently pushing down with a cylindrical high density foam bung 145 mm diameter.

5.2.2. Experimental design

Thirty microcosms were placed in a randomised block design, allowing for 5 replicates to be sampled at each sample time, resting on grass in an open area at Cranfield University Silsoe campus. Each microcosm was ramdomly assigned to one of the 6 blocks and each block was aligned north to south to mitigate for possible effects of shadows at sun-rise and sun-set. All microcosms were uncovered at the surface and so were exposed to the prevailing elements.

Samples were collected at 1, 2, 4, 8, 16 and 32 weeks after establishment. To function as “Control” samples, 5 samples were taken from the homogenised soil before it was packed into the microcosms, and analysed. At each subsequent sample time 5 replicates were taken. The remaining samples were replaced in a randomised block design, still with microcosms aligned north to south.

Soil surface layers collected using the same sampling strategy as outline in Chapter 4, with a surface depth of 1 mm being removed named Surface, followed by two subsequent depths of 5 mm each named Sub-surface and Deeper layer respectively. This was achieved by removing the cover from the bottom of each microcosm. The microcosms were then placed on a cylindrical high density foam bung (140 mm diameter). By pushing the microcosms down on the plastic surrounding each microcosm, soil was made to extrude from the top of the plastic pipe of each microcosm. The extruding soil was then removed by scraping a large blade over the top of the microcosm and collecting the soil on a plastic tray which was placed under the microcosms. Each sample layer was stored in a fridge at 4°C prior to analysis. All samples were homogenised, divided into three aliquots, and analysed for chlorophyll *a* concentration, phenotypic community profile via PLFA analysis and microbial biomass C using the methods as described in Chapter 2.

5.2.3. Laboratory analyses

Chlorophyll *a* analyses were all performed within 6 h of sample collection. All samples for PLFA analysis were placed in Bligh Dyer solution made up to the Bligh Dyer ratio with citrate buffer, allowing for the initial soil moisture content as discussed in Chapter 2, sonicated and shaken within 18 h. All samples for microbial biomass C analyses were fumigated with chloroform within 24 h.

This sequence of analysis was adopted as chlorophyll *a* breaks down significantly after 24 h (Wasmun *et al.* 2006). Microbial communities’ phenotypic profiles are also capable of relatively rapid change (Petersen and Klug 1994). Placing the soil in Bligh Dyer solution, and sonication kills the microbial cells and effectively preserves the phenotypic profile at the time of sampling. Of the three analyses, microbial biomass carbon is likely to have the slowest rate of change. However, all chloroform fumigations were still carried out within 24 hours of sample collection.

5.2.4. Data analysis

Time 0 was excluded with regards to chlorophyll *a* concentration due to experimental error as used cuvettes had been placed back into the box with unused cuvettes. When these were unknowingly used they caused an over reporting of the concentration of chlorophyll within the extracts. Data analysis was undertaken using Statistica 7.1 (Statsoft Inc, 2005) for chlorophyll *a* microbial biomass C and PLFA data as described previously.

5.3. Results

5.3.1. Chlorophyll *a*

Chlorophyll *a* concentration within the surface community did not differ from the two deeper soil layer communities for the first 8 weeks after the disturbance event, in this case the homogenisation of the soil by sieving. After 8 weeks chlorophyll *a* production increased, becoming significantly greater than the concentration found within the two deeper soil layers ($P = 0.039$). However, the chlorophyll *a* concentrations in the sub surface and deeper layers did not become significantly different until Week 32 ($P < 0.01$).

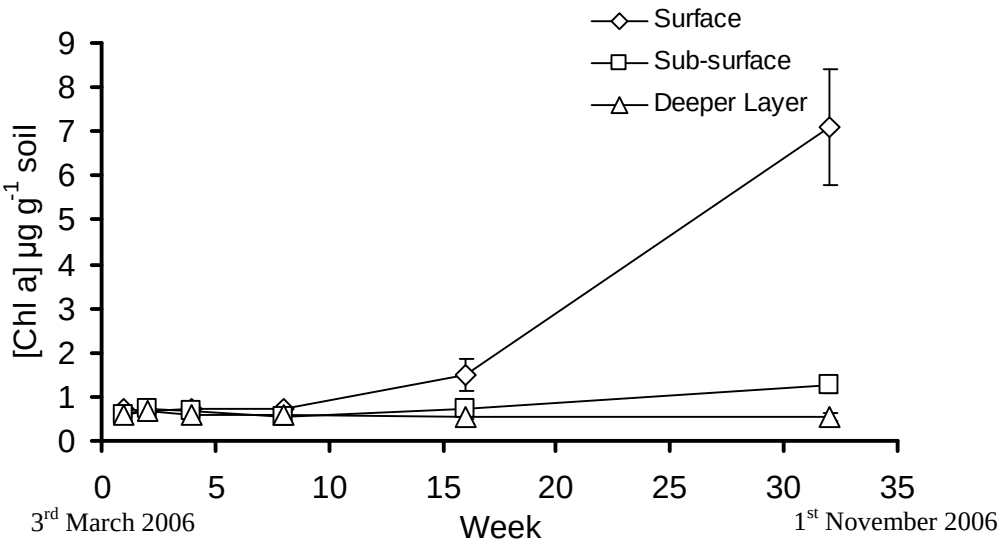


Figure 5.1 Concentration of chlorophyll *a* extracted from soil at three depths, over a time sequence including 1, 2, 4, 8, 16 and 32 weeks, from 6/3/2006 to 16/10/2006. Points show means (n = 5) Bars show standard error.

5.3.2. Microbial biomass C

Microbial biomass carbon increased during the first week at all three depths (Figure 5.2). A significant reduction in the soil surface biomass ($P < 0.01$) occurred in Week 2. By Week 4, the soil surface microbial biomass had increased so that it was not significantly different from the sub-surface or deeper layer. Microbial biomass then increased significantly by Week 8 in all three soil layers, ($P < 0.01$). At this stage there was no significant difference in microbial biomass C in all three soil layers (Figure 5.2).

From Week 16, the soil surface layers of the microcosms had reached an equilibrium, with the microbial biomass C being significantly higher in the surface layer when compared to both the Sub-surface layer ($P = 0.02$) and the deeper layer ($P = 0.01$). No significant differences developed between the sub-surface and deeper layer with respect to microbial biomass C ($P = 0.85$). This equilibrium was maintained until the final sample time of the experiment at Week 32 (Figure 5.2).

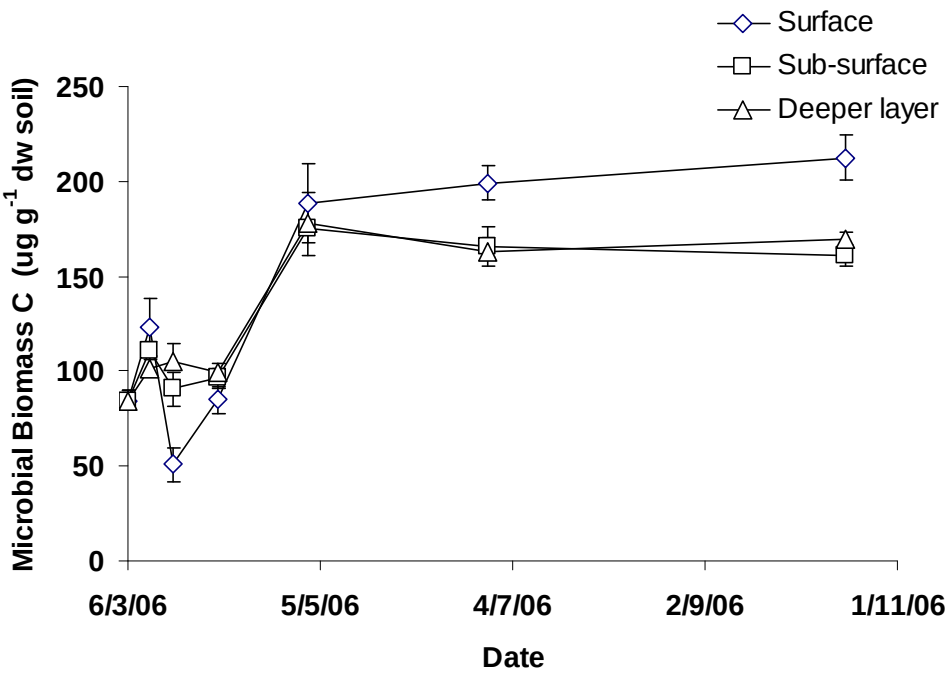


Figure 5.2 Mean concentration of microbial biomass C extracted using chloroform fumigation extraction, adjusted with a kEC value of 0.45, from soil at three depths, over a time sequence including 0, 1, 2, 4, 8, 16 and 32 weeks, from 6/3/2006 to 16/10/2006. Points show means (n = 5), bars show standard error.

5.3.3. PLFA

Figure 5.3 shows that a distinct soil surface phenotype developed by *c.* 8 weeks ($P < 0.01$). The magnitude of the difference in phenotypes between the soil surface layer and the two subsequent soil layers then increased with time until 32 weeks ($P < 0.01$) as is apparent by the increase in the distance between the surface and sub surface layers with regard to PC 1.

The PLFA 16:0 is the PLFA which contributed most to the discrimination between the depths (Figure 5.4). Discrimination between the soil surface communities at the three depths investigated occurred in PC1. The PLFA 16:1ω7 was also responsible for discrimination between microbial communities at the three depths investigated, but did not contribute to this discrimination as strongly as the PLFA 16:0. The PLFA 16:1ω5 was responsible for the majority of the discrimination seen in PC2. Separation in PC2 appeared to occur between all three depths by Week 32. The fact that there was no difference with depth in PC2 by Week 32 implied that the differences arising may be due to drift (i.e. the result of genetic drift interacting with the environment) in the phenotypic composition of the microbial populations.

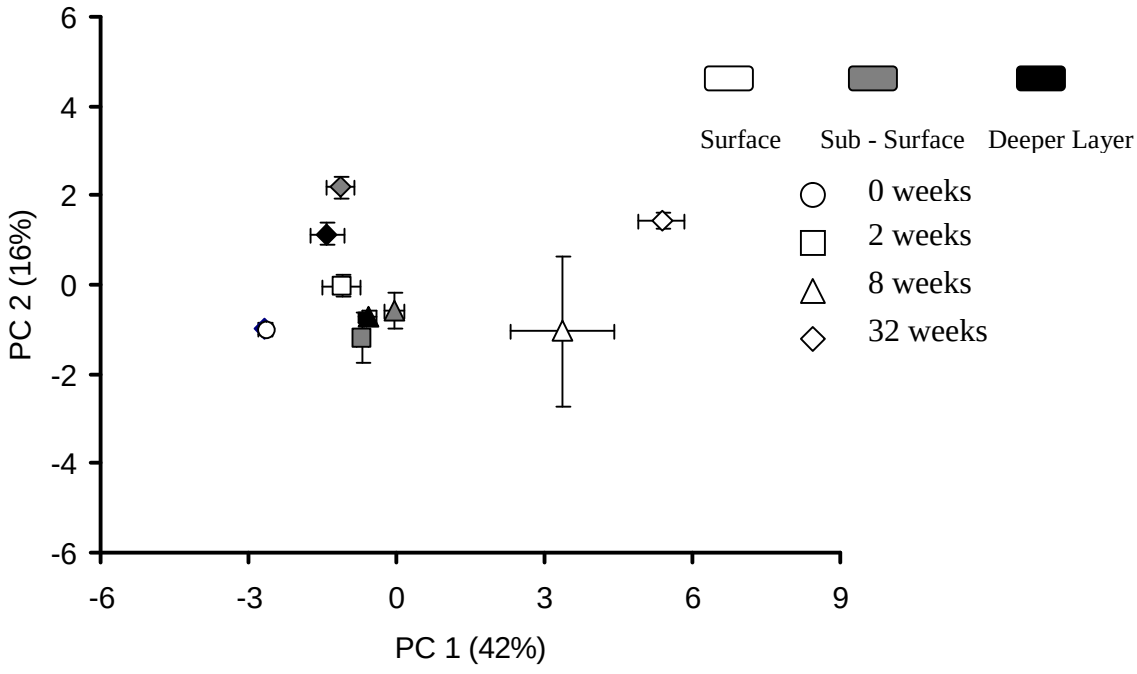


Figure 5.3 First and second principal components (PC) plots derived from phospholipid fatty-acid profiles originating from surface layers of microcosms

sampled 0, 1, 2, 4, 8, and 32 weeks after establishment. Points show means (n = 5), bars show standard errors. Percentage variation accounted for by PC shown in parenthesis on each axis. Week 16 was excluded due to clearly erroneous chromatography.

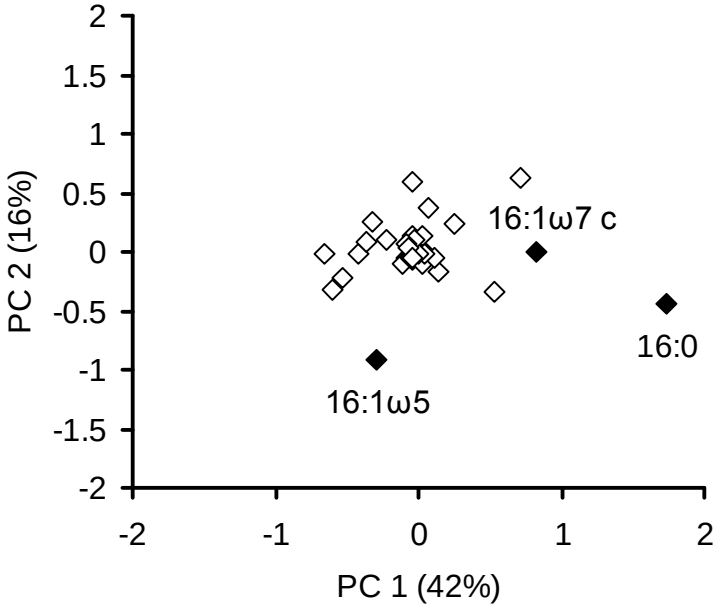


Figure 5.4 Loadings plot showing those PLFAs associated with the principal components which contributed most to the discrimination between the depths in Figure 5.3 highlighted in black and named. Percentage variation accounted for by PC shown in parenthesis on each axis.

There was a greater increase in the proportion of the fungal PLFA biomarker 18:2ω6 during the first four weeks after the microcosms were set up compared to the sum of the bacterial markers (Figure 5.5). This means that the composition of the community changed during the first four weeks with the proportion of fungi within the community increasing at all three depths investigated. The greatest increase was found to occur in the Deeper layer during that four week period where the fungal to bacterial ratio increased significantly ($P = 0.03$). The surface layer also showed a significant increase in the fungal to bacterial ratio ($P = 0.04$). The Subsurface layer did not change significantly over the initial four week period ($P = 0.21$). There was then no significant change in the fungal to bacterial ratio in either the Sub-surface or Deeper layer between Week 4 and Week 8 ($P = 0.6$ & $P = 0.1$ respectively). From Week 8 onwards the proportion of fungi when compared to the proportion of bacteria within the microbial community was found to decrease in both the Sub-surface and

Deeper layers, with the fungal to bacterial ratio falling significantly to Week 32 ($P < 0.01$). The proportion of fungal to bacterial PLFA biomarkers was found to continue to increase in the Surface layer from Week 4 until Week 8 although this increase was not statistically significant ($P = 0.13$). The fungal to bacterial ratio then reached a plateau in the Surface layer, remaining at a ratio of fungi to bacterial biomarkers of approximately 0.2.

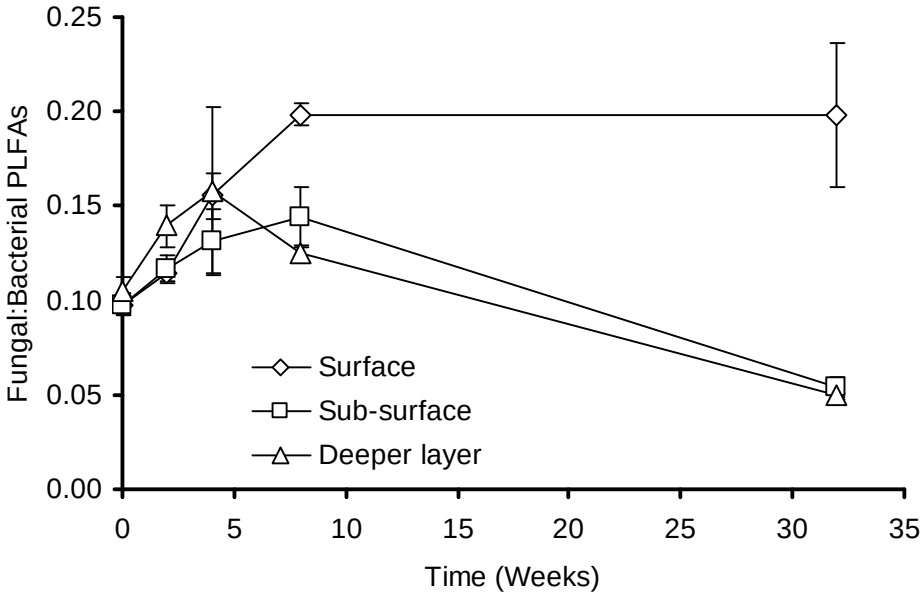


Figure 5.5 Ratio of fungal to bacterial PLFA biomarkers from soil surface layers of microcosms sampled over a 32 week period. Points show means (n = 5), bars show standard errors.

5.4. Discussion

The above results show that Hypothesis C as stated above should be accepted. The microbial community does change over time as a new surface is uncovered after a disturbance event leading to the formation of a distinct soil surface phenotype within the top *circa* 1 mm of the soil surface following a disturbance event.

As previously stated, Benvenuti (1995) demonstrated that less than 0.3% of light is transmitted through to 2 mm depth, even in soils with the highest transmittance of light. This lack of light is almost certainly responsible for the differences observed in

chlorophyll *a* concentration in this data. The fact that a significant difference between the two deeper layers analysed over the 32-week time period studied developed in the chlorophyll data implies that some vertical transport of photoautotrophs from the surface layer down through the soil occurred, on a millimetre scale at least. Tchan and Whitehouse (1953) stated that rainfall and earthworms were the most significant factors in vertical transport of photoautotrophs, but it seems likely that vertical transport in the guts of nematodes and other grazing organisms may also have an effect. As stated in Chapter 1, it is also possible that some mechanisms of phototaxis cause photoautotrophs to move back towards the surface. Due to the fact that some light, albeit a very small amount, penetrates through the surface layer and into the first millimetre of the second layer it seems possible that a trigger for phototaxis would reach the second layer in the microcosms. Lund (1967) stated that algae are capable of moving back to the surface if "they are not buried too deeply", although he did not hypothesise about a possible mechanism for this movement. This movement may contribute towards the difference in photoautotroph concentration between the two deeper layers. Further work is needed to test whether phototaxis occurs and whether it plays a significant part in movement of photoautotrophs within the soil surface layers.

Roveria and Greacen (1957) reported that microbial biomass can increase after disruption due to utilisation of organic carbon which was previously trapped and has been released. Therefore the initial increase in microbial biomass seen in Figure 5.2 was probably a response to the released organic carbon due to the breakdown of aggregates in the sieving process. However, the microbial biomass then experienced a significant reduction, with the reduction being greatest at the soil surface. The reason for this is unclear. Frost events did occur between the measurements taken at Weeks 1 and 2. However, there were also frost events between Weeks 0 and 1, and also 2 and 4 but these did not produce the same reduction in soil surface biomass. Data which was collected showed that the soil surface temperature on average in Week 2 was 1.9°C, compared to 3.0°C in Week 1 and 6.3°C in Weeks 3 and 4. It is therefore possible that the mean nocturnal temperature dropped below an as yet unknown critical threshold which caused a proportion of the soil surface microbial biomass to die. However, this is conjecture at the moment and further work is needed to test this hypothesis.

Another possible reason for the reduction in biomass at the surface is that it takes time for the soil surface community to adapt to the soil surface environmental conditions. The influence of UV at the soil surface may lead to a proportion of the soil surface community which are not UV-tolerant dying. Repeating the experiment and investigating whether this event occurs again would help to test this hypothesis. This could be done by filtering out UV from the light reaching the soil surface and comparing the results from this treatment with a control.

The increase in chlorophyll *a* concentration within the top *circa* 1 mm implies that photoautotrophs were present in higher abundance at the soil surface compared to the two deeper soil layers analysed. It is, therefore, probable that the difference in proportion of photoautotrophs within each soil layer was responsible for the difference seen in PLFA profiles between soil layers. However, there are as yet no PLFAs which have been identified as distinct markers of photoautotrophs, and as such it is not possible to test this hypothesis using the data presented in this chapter. The PLFA 16:0 was responsible for most of the discrimination seen between layers. As discussed previously there is much disagreement in the literature as to whether the PLFA 16:0 functions as either a distinct or a general biomarker, and this is true of several other PLFAs.

The available data suggests that photoautotrophs were responsible for the discrimination seen in PLFA profiles between soil layers. This was demonstrated by the relatively high levels of chlorophyll found in the soil surface layers (Figure 5.1). Whilst the lack of photoautotrophic PLFA biomarkers means that it is not possible to calculate the ratio of photoautotrophs to bacteria as was done with the fungal to bacteria ratio, molecular techniques such as transcription restricted fragment length polymorphism (T- RFLP), when undertaken with primers associated with photoautotrophs, could be performed in the future to further confirm this conclusion.

Due to the fact that the soil surfaces within the microcosms were exposed to wind and rain, physical disturbance may have continued throughout the time that the microcosms were in the field. However, any physical disturbance which may have occurred was not sufficient to interrupt the formation of a distinct microbial phenotype within the top *circa* 1 mm of the soil surface.

The fact that the ratios of the proportions of fungal to bacterial biomarkers increased during the first 4 weeks implies that the fungi within the microbial communities were better able to utilise the substrate freed during the homogenisation of the soil by sieving, when compared to the bacterial fraction of the microbial community. After the initial flush of substrate was utilised by week 4, the bacterial proportion of the microbial community became able to compete better with the fungal proportion within the two sub-surface soil layers. However, at the soil surface, the fungal proportion of the microbial community was better able to compete, establishing itself as a greater proportion of the microbial community in the soil surface layer when compared to the subsequent soil layers investigated. This agrees with work published by Otten and Gilligan (1998) who found that fungi preferentially grew over soil surfaces than within the pore matrix. Further work could be undertaken to confirm that this increase was a result of the fungal biomass increasing at the soil surface, as opposed to resulting from a relative decrease in the bacterial biomass. This could be done by analysing the concentrations of an additional fungal biomarker such as ergosterol as a measure of the fungal biomass (Mille-Lindblom et al 2004).

Chapter 6: The Effects of Light Wavelengths on the Development of Soil Surface Microbial Communities

6.1. Introduction

All previous work undertaken in this investigation has demonstrated that distinct microbial communities develop at different soil depths at the millimetre scale. The notion of a biologically distinct 'surface phase' in arable soils appears confined to an order of 1 mm depth.

As has been discussed previously, soil acts as a buffer with respect to environmental factors. Therefore, environmental factors such as temperature, wetting and drying cycles and light will both fluctuate more frequently and be relatively more extreme at the soil surface than at deeper soil levels. However, Benvenuti (1995) found that less than 0.2% of light was transmitted through 1 mm of a silty clay soil, although he found that the amount of light transmitted varied with soil type. The soil with the highest transmittance studied allowed 0.3% of light to be transmitted through to 2 mm depth. Therefore, it seems probable that the factor that will show the most extreme differences between the surface and deeper soil level at the millimetre scale is light.

Light can have several different effects on organisms. It can function either as an energy source, in the form of photosyntetically active radiation (PAR), or as a stress, in the form of UV.

Photoautotrophs are organisms that use energy from sunlight along with carbon dioxide and water to synthesise organic materials which are then used in various cellular functions. Microbial photoautotrophs are unicellular and can be either prokaryotic, such as cyanobacteria, or eukaryotic such as algae. All photoautotrophs use pigments such as chlorophylls to capture photons emitted by the sun and convert them into useable energy. Chlorophyll exists in several forms, viz. *a, b, c1, c2* and *d* (Figure 6.1).

(a)

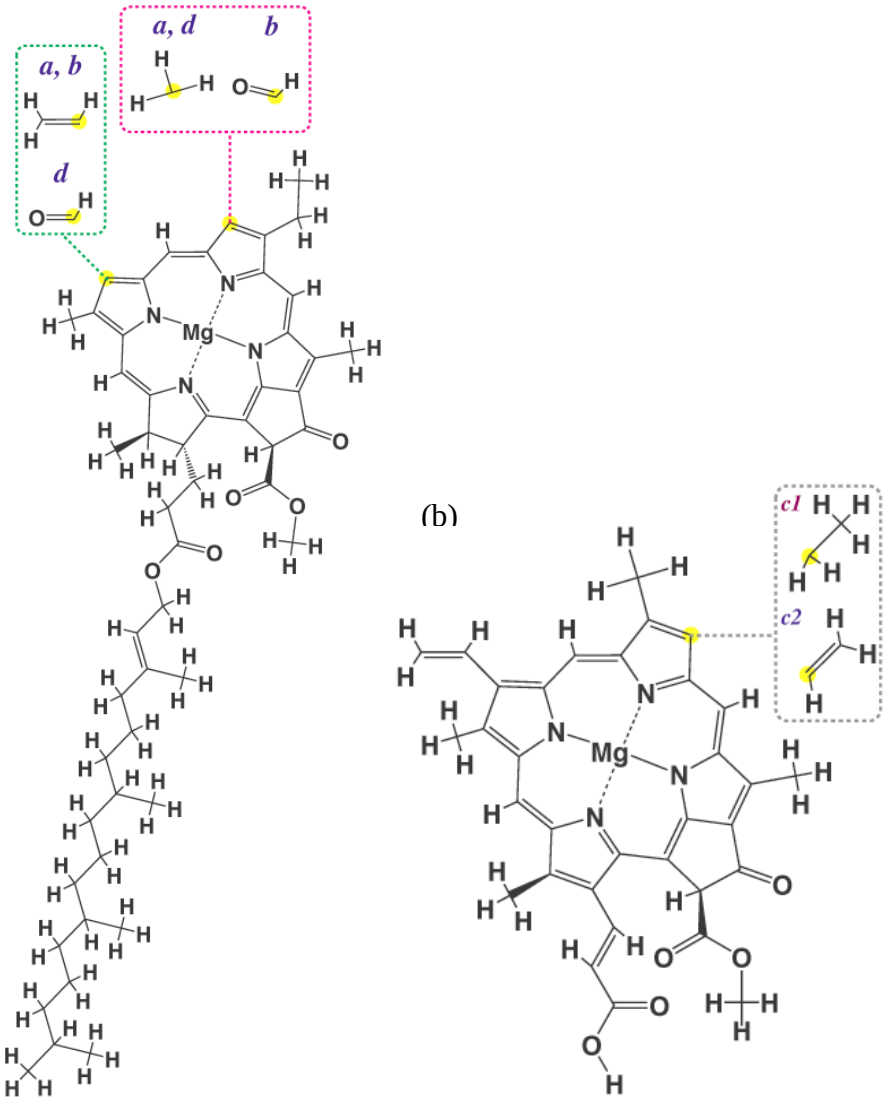


Figure 6.1 The molecular structure of (a): chlorophylls *a*, *b* and *d*; and (b), chlorophylls *c1* and *c2* (Richfield 2005).

Photoautotrophs also contain other pigments as well as chlorophylls such as carotenoids and phycobiliproteins. Some of these are also capable of absorbing light energy and transferring it to photosystems where it is converted into energy (Campbell et al.1999). Carotenoids, as well as some other accessory pigments also function to absorb and dissipate excess light energy thereby helping to prevent damage from over exposure to solar radiation. They can also function as antioxidants, helping to protect organisms from the damaging effects of free radicals (Campbell et al 1999).

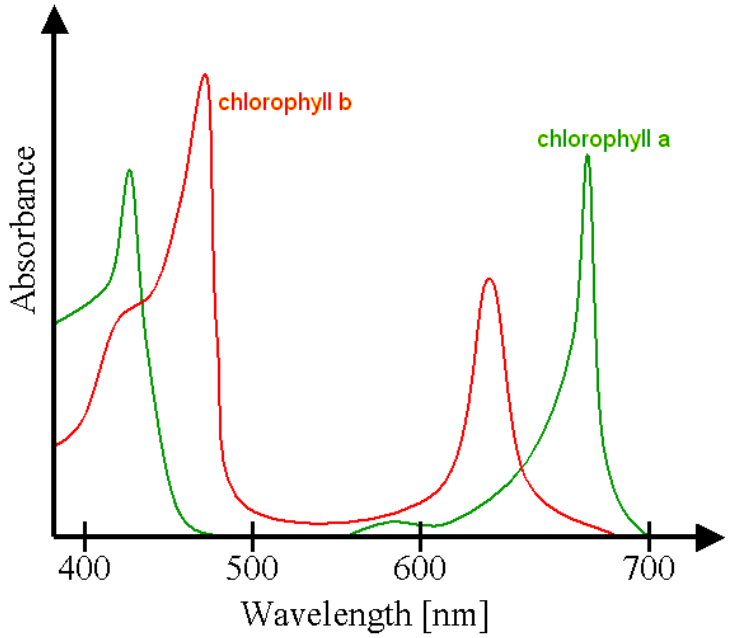


Figure 6.2. Absorption spectra of free chlorophyll *a* (green) and *b* (red) in solvent (García 2005).

Chlorophylls *a* and *b* each have a slightly different absorption spectrum as shown in the schemtic Figure 6.2. These absorption spectra may be slightly different *in vivo* due to interactions with cellular protein complexes. However, chlorophyll *a* and *b* still have sufficiently different absorption spectra *in vivo* that organisms which contain both are able to utilise a wider range of solar energy as photosynthetically active radiation (PAR) (Campbell et al., 1999).

Chlorophyll *a* is ubiquitous to all plants, eukaryotic algae and cyanobacteria, meaning it is the most widespread of all of the chlorophylls. Chlorophyll *b* is absent from all algae except the Chlorophyceae (green algae) and Euglenophyceae, but is found in all higher plants. Chlorophyll *c* is found only in the photosynthetic members of the chromista and dinoflagellates (Goodwin and Mercer 1972). Chlorophyll *d* has been found in red algae and has been shown to be the primary light-harvesting molecule in the cyanobacterium *Acaryochloris marina*. Both red algae and *Acaryochloris marina* are normally found in the relatively light-restricted environment a few meters below the sea surface (Larkham and Kühl 2005).

Cyanobacteria used to be referred to as blue-green algae. However, they are not algae and nor are they necessarily blue-green in colour. Cyanobacteria contain an accessory pigment called phycocyanin which has a blue colour which when combined with the green of the chlorophyll can make them look blue-green. However, many cyanobacteria have a different accessory pigment called phycoerythrin which is red. When this red colour combines with the green of the chlorophyll it gives these cyanobacteria a brown colour (Madigan et al. 2000).

6.1.1. The influence of light on soil surface communities

Only relatively limited work has been done looking that the effects of light on soil microorganisms. Arrage *et al.* (1993) found that, contrary to what may be expected, many of the subsurface microorganisms they studied exhibited UV resistance to the same level as surface microorganisms. However, their study was based on microorganisms in pure culture. Whilst their analysis was carried out on microorganimsms in the stationary phase, care must still be taken when extrapolating pure culture data as microorganisms may behave very differently when part of microbial communities.

Hughes *et al.* (2003) found that solar radiation with a wavelength of >400 nm had no effect on hyphal growth on fungi in the Antarctic. However, they found that lower wavelength radiation inhibited hyphal growth demonstrating that UV functions as an environmental stress to fungi that are exposed to it. They also demonstrated that this inhibition of growth increased with decreasing wavelength. This is as would be expected, as the increasing energy of lower wavelengths of solar radiation means increased potential of harmful effects to fungal cells.

Downing and Selkirk (1993) found that light levels played a significant role in the distribution of bryophytes in microbiotic crusts in Australia where they showed that high light levels increased the abundance of bryophytes and low light levels caused a decrease in bryophyte abundance. This follows what would be expected as light is needed for the bryophytes to photosynthesise. In this example, the beneficial effects of being exposed to PAR outweigh the negative effects of being exposed to UV. This will generally be the case for photoautotrophs as they gain benefits from solar radiation that in most cases can be assumed to outweigh the negative effects of UV.

However, non-photosynthetic organisms such as fungi cannot utilise solar radiation as an energy source and so for these organisms it functions as a stress.

Research into the effects of different wavelengths of solar radiation on soil surface microbial populations is a relatively depauperate area. Work done here will help to increase the understanding of the roles of different wavelengths of light (as well as its presence or absence) on arable soil surface microbial communities.

This chapter has the aim of determining whether light plays a part in driving the formation of a distinct soil surface phenotype by testing hypothesis E:

> *Light is an important factor in driving the succession of distinct microbial soil surface communities with changes in the wavelength having differing effects on the soil surface microbial community either functioning as an energy source (PAR or a stress (UV)*

6.2. Materials and Methods

6.2.1. Materials

Microcosms were constructed from unplasticised polyvinyl chloride (uPVC) drainpipe (150 mm diameter) cut into 100 mm lengths. One end of each microcosm was covered with Loktek™ LS greenhouse shading (73% density with a mean pore size 2 mm^2; Bristol, U.K.), which was attached using silicone sealant and cable ties as discussed previously in Chapter 5.

Light filters attached to baffles were made by gluing Lee lighting filters (Lee Filters, Andover, U. K.) to the 100 mm sections of uPVC drainpipe (200 mm diameter). Small pieces of garden cane were attached to the bottom of each baffle so that they stood approximately 50 mm off the ground, to allow circulation of air.

Three different types of filters, along with opaque PVC which was used to make a no light control treatment, were used to establish 4 treatments (Table 6.1).

Table 6.1. Properties of filters used to control quality of light reaching soil surfaces in the microcosms.

Treatment	Filter	Filter properties
Restricted photosyntetically active radiation (PAR)	DS 124 dark green	<5% transmission of wavelengths up to 460 nm and <5% transmission of wavelengths between 610 and 700 nm, therefore <5% transmission of PAR.
Restricted UV	DS 226 ultra violet	0% transmission of wavelengths below 380 nm, >85% transmission of all wavelengths above 420 nm.
With UV A	DS 130 clear	> 70% transmission of all wavelengths above 320 nm
No light	White opaque PVC	No wavelengths transmitted

A sandy loam soil characterised in Chapter 5 was collected from Showground Field, again from the top approximate 10 cm, sieved fresh to pass through a 2 mm sieve and thoroughly mixed to ensure homogenisation, and packed into each microcosm to a dry bulk density of 1.3 g cm^{-3} a typical bulk density for an arable field.

6.2.2. Experimental design

Microcosms were packed and randomly allocated to a treatment. Microcosms with their respective filters were then placed in a randomised block design, using 5 replicates, on grassin an open area at Cranfield University Silsoe campus (OS grid reference: TL 082 352, Silsoe, U.K.), with each block aligned north to south to mitigate for possible effects of shadows at sun-rise and sun-set. They were weighed periodically (a maximum of every 2 weeks) to check moisture content and tap water was added through use of a watering can and a fine rose to maintain them at ≈ 12% (± 2%) moisture content.

Figure 6.3. Microcosms and light filters in a randomised block design.

Microcosms were left in the field for 6 months between November 2005 and May 2006. This has been shown to be sufficient time for distinct microbial communities to develop within the soil surface (Jeffery *et al.* 2007).

Temperatures at the soil surface in each microcosm were recorded at hourly intervals using iButton® data logging thermocouples (DS1921G - Thermochron® iButton - 40°C to 85°C: Maxim Integrated Products Inc., Sunnyvale, CA, U.S.A.) which were wrapped in polythene and located at the edge of each microcosm.

After 6 months, the greenhouse shading was removed from the bottom of the microcosms and a high density foam bung, 140 mm in diameter, was used to push the soil up from the bottom of the microcosms. This allowed soil to be extruded from the top of the microcosms. The first layer, approximately 1 mm in depth was extruded and removed with a knife. Two subsequent layers, each of approximately 5 mm in depth, were then extruded and further removed. All samples were placed in sealed plastic bags and stored in a refrigerator at 4°C prior to analysis.

6.2.3. Laboratory analyses

Analyses were performed in the sequence: The first stage of PLFA extraction into Bligh Dyer solution and sonication; chlorophyll *a* analysis; microbial biomass C analysis; followed by the remainder of the procedure for PLFA analysis for reasons

discussed in Chapter 5. The methods were all undertaken as described in Chapter 2. Data analysis was performed using the same statistical analyses and software as described in Chapter 4

6.3 Results

Differences did exist between the temperature regimes experienced by the soil surface microbial communities under different light treatments (Figures 6.3a and b). The greatest difference in temperature regime experienced by microbial communities in the soil surface layers existed between the no light treatment, and all other treatments. If this temperature difference was responsible for driving the formation of distinct microbial communities it would be hypothesised that the results from the no light, and with UV A treatments would be different from each other whereas the restricted PAR and restricted UV treatments would exhibit very similar results.

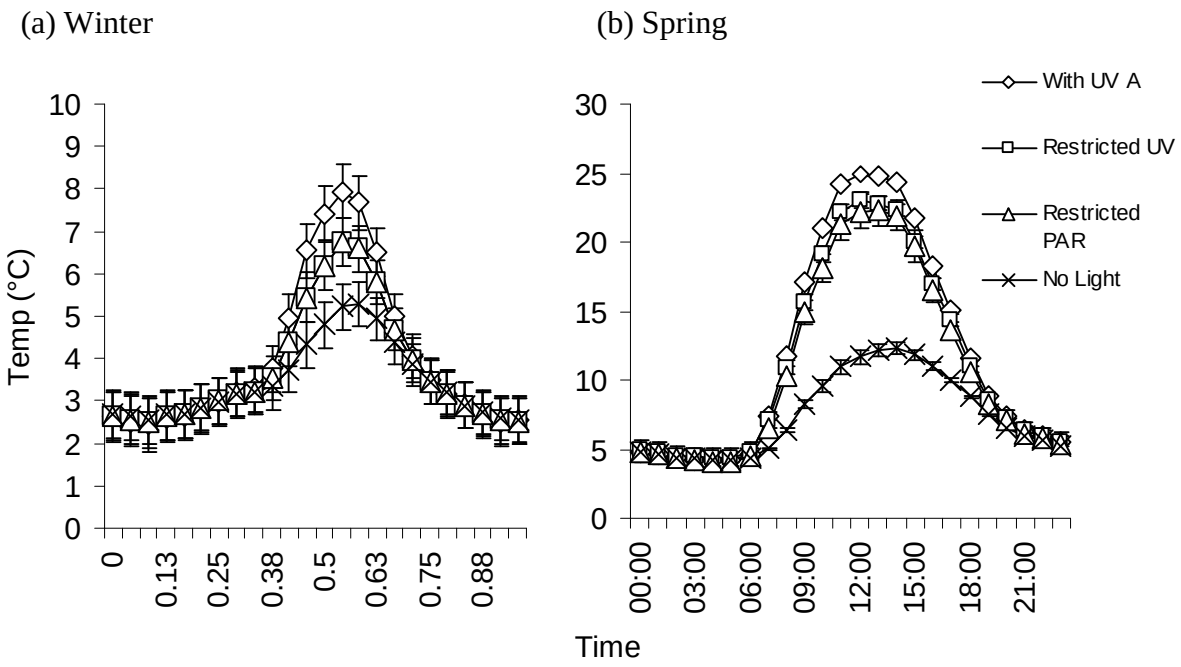


Figure 6.4. Mean diurnal temperature fluctuations under a range of light treatments for the two different seasons: (a) Winter (combined data from 21/12/05 to 20/01/06); (b) Spring (combined data from 21/03/06 to 20/04/06). Points show means (n = 30), bars show standard errors. Note difference in scales on Y axis.

With regards to the surface layer, to a mean depth of approximately 1 mm, the restricted UV treatment showed a significantly higher concentration of chlorophyll *a* compared to the with UV A treatment (P = 0.018). The with UV A treatment showed

a significantly higher concentration of chlorophyll *a* concentration than both the restricted PAR and no light treatments ($P < 0.01$). However, the restricted PAR treatment was not significantly different to the no light treatment with regards to chlorophyll *a* concentration (Figure 6.5).

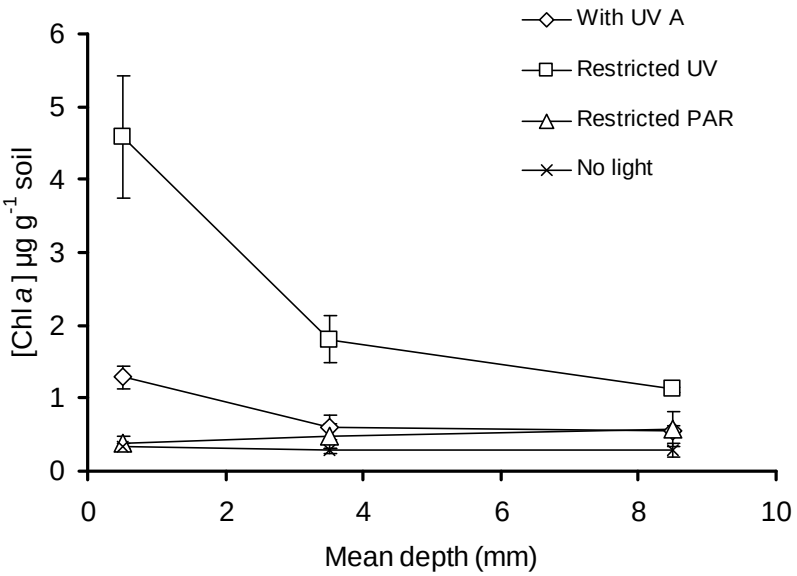


Figure 6.5 Mean concentration of chlorophyll *a* extracted from soil at three depths, from four different light treatments. (n = 3; bars show standard error).

With regards to microbial biomass carbon the only treatment which showed any significant difference from the other treatments was the restricted UV treatment. The microbial biomass C in the surface of the restricted UV treatment, to a mean depth of approximately 1 mm, was significantly different to that found in the surface layers in the restricted PAR treatment ($P < 0.01$) and the with UV A treatment ($P < 0.01$). However, it was not found to be significantly different to the no light treatment ($P = 0.28$). The restricted UV treatment showed a significant decline with depth from the surface layer to a depth of approximately 9 mm ($P < 0.01$). No other treatment showed any statistically significant change with depth in microbial biomass C at the scale investigated (Figure 6.6).

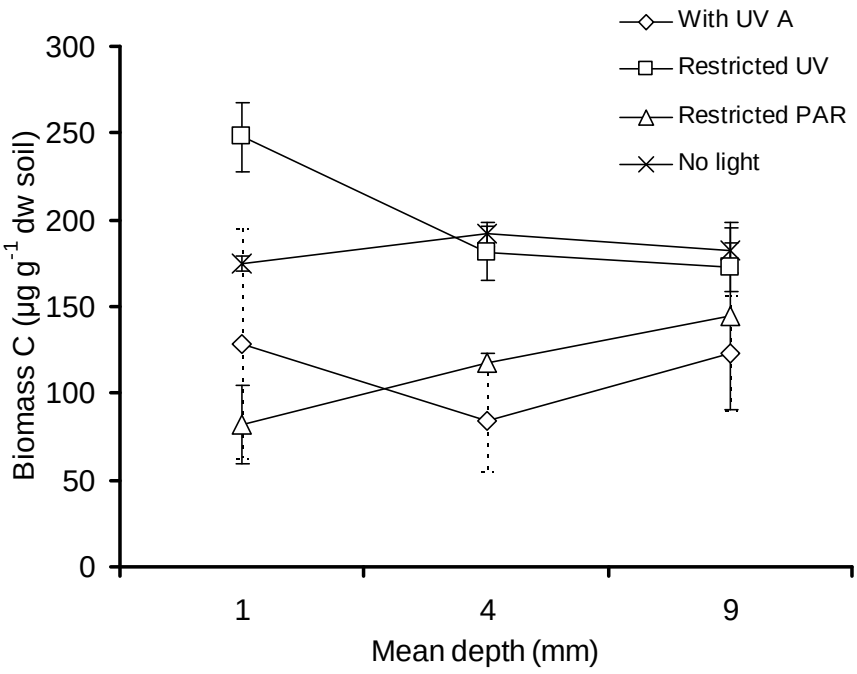


Figure 6.6. Microbial biomass C extracted from soil at 3 different depths from 4 light treatments. Points show means (n = 3), bars show standard error.

Analysis of all all of the PLFA data collated into one principal component analysis showed that in the no light treatment, all three depths group together. In the restricted PAR treatment some increase in microbial biomass moving from the surface layer to the two deeper layers could be seen.However, this increase was not found to be statistically significant (Linear regression: $R^2 = 0.1$; $P = 0.2$). In the restricted UV treatment the microbial biomass C decreased significantly between the Surface and the Deeper layer (Linear regression: $R^2 = 0.48$; $P < 0.01$). There was no significant effect with regards to depth in either the "with UV A" treatment or the "no light" treatment. This demonstrates that light is the key factor in the development of a distinct microbial community within the soil surface, and that PAR and UV are the most important wavelengths governing this process (Figure 6.5).

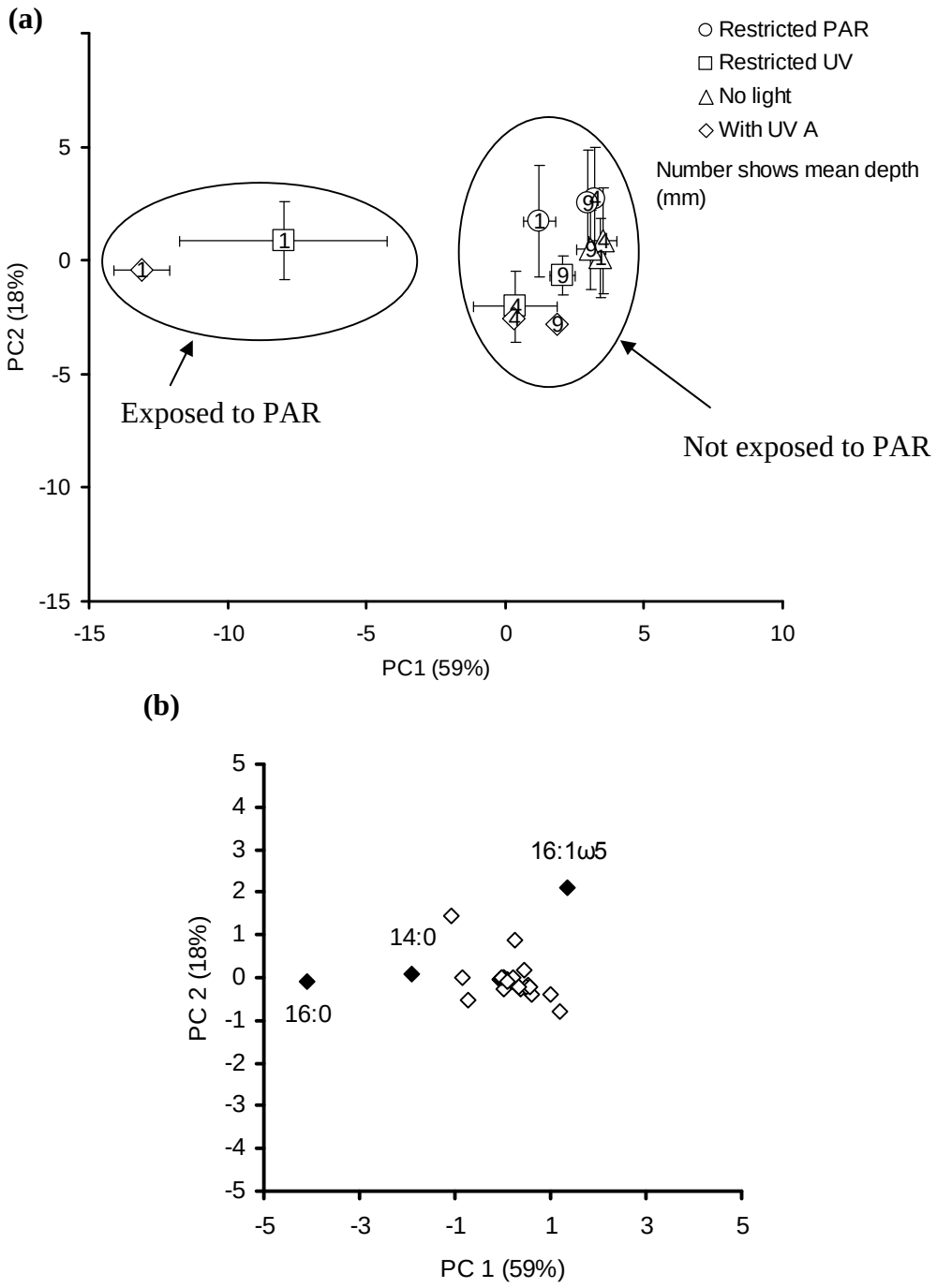


Figure 6.7 (a), First and second principal components (PC) plots derived from phospholipid fatty-acid profiles originating from surface layers of microcosms from all treatments Points show means (n = 3), bars show standard error. Percentage variation accounted for by PC shown in parenthesis on each axis. Mean depth of each sample mean in millimetres given by number within point: (b), loading plots with those PLFAs contributing most to the variance seen in the PC plots highlighted in black and named.

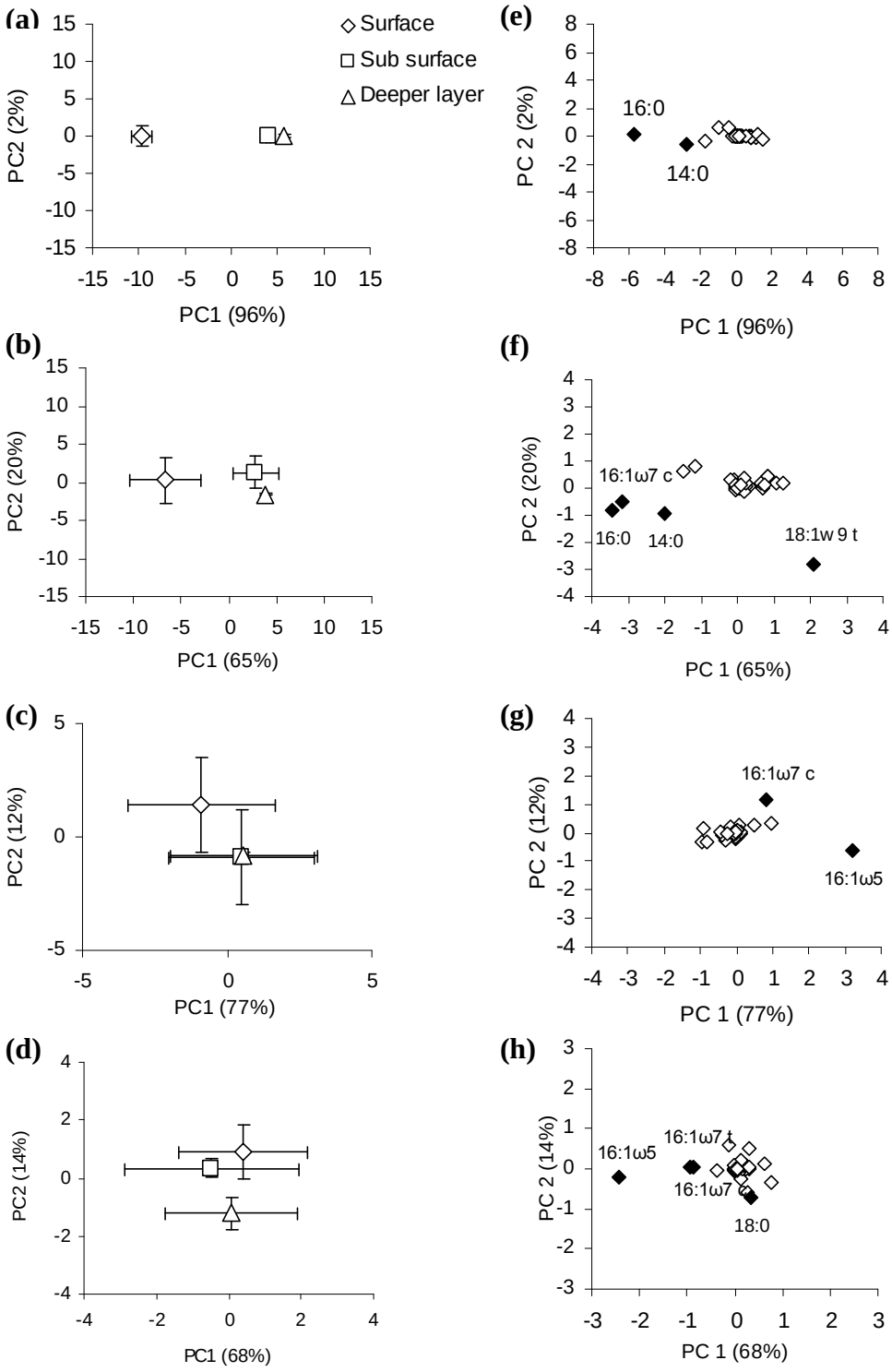


Figure 6.8. (a – d) First and second PC plots derived from phospholipid fatty-acid profiles originating from surface layers of microcosms showing the mean plots of data derived from three depths (*circa* 0-1 mm, 1-6 mm and 6 – 11mm) from 4 treatments: (a) = with UV A; (b) = restricted UV; (c) = restricted PAR; (d) = no light. Points show means (n – 3), bars show standard errors. (e – h) shows the respective loading plots with those PLFAs contributing most to the discrimination seen in the PC plots highlighted in black and named. Percentage variation accounted for by PC shown in parenthesis on each axis.

When principal component analysis was applied to each treatment, the surface layer in the with UV A treatment was found to be significantly different to the two deeper soil layers in the With UV A treatment, ($P < 0.001$), which were not significantly different to each other in PC1. No significant differences existed in PC2 ($P = 0.14$) (Figure 6.7a). The PLFAs 16:0 and 14:0 contributed most to the discrimination seen in PC1 (Figure 6.7b). The surface layer in the restricted UV treatment was significantly different to the Sub-surface ($P = 0.039$) and the Deeper layer ($P = 0.026$) (Figure 6.6b) in PC1. However, the Sub-surface layer was not found to be significantly different to the Deeper layer ($P = 0.14$) in PC1. The PLFAs 16:0 and 16:1ω7c were responsible for the majority of the discrimination seen in PC1. There were no significant differences with regards to PC2 between any of the depths investigated. The PLFA 18:1ω9t was responsible for the majority of the variation seen between replicates in PC2 but did not contribute to any discrimination between the soil depths investigated. No Significant differences were found to exist between depths in either the restricted PAR or the no light treatments (PC2; $P = 0.14$ Figure 6.6c) and (PC2; $P = 0.11$ Figure 6.6d). The PLFAs highlighted in Figure 6.6 g & h were responsible for variation seen between replicates at each depth sampled but did not cause any discrimination between soil depths in either treatment.

6.4. Discussion

This experiment was designed to minimise the effects of all variables apart from light. However, with the experimental design used it was not possible to control the temperature at the microcosm soil surface. Therefore, this data was collected so that if differing temperature regimes between treatments existed they would be known and could be accounted for in the interpretation of the results. Temperature differences did occur at the soil surface between microcosms within different treatments (Figure 6.1 a & b). As stated previously the greatest difference in temperature regime experienced by microbial communities in the soil surface layers existed between the no light treatment, and all other treatments. If this temperature difference was responsible for driving the formation of distinct microbial communities we would expect the results from the treatments no light, and with UV A to be different from each other whereas restricted PAR and restricted UV should exhibit very similar results. However, this did not occur, and so although it is possible that differing temperature regimes did have some effect on the soil surface microbial communities, this effect was negligible

when compared to the effect of differing wavelengths of light on the soil surface microbial communities.

Chlorophyll *a* concentrations increased at the soil surface in the with UV A treatment when compared to the deeper soil levels within this treatment, although less chlorophyll *a* was produced in the with UV A treatment when compared to the restricted UV treatment (Figure 6.5). This confirms that photoautotrophic microorganisms are able to produce chlorophyll *a* while exposed to UV A, as they do within the natural environment. However, due to the harmful effects of UV A some energy must be invested in the production of protective pigments such as carotenoids (Krinsky 1989). Further investigation into the amounts of carotenoids produced within each treatment would test the hypothesis that this energy investment in protective pigments means there is less energy available to make chlorophyll *a*. There was no statistical difference between the restricted PAR treatment and the no light treatment in relation to chlorophyll *a* production. This shows that it is PAR that is the main driving factor and photoautotrophs are responsible for the majority of the differences found within the microbial communities at the soil surface with regards to photoautotrophs, as would be expected.

The fact that chlorophyll *a* concentration was highest in the restricted UV treatment (Figure 6.5) implies that the soil surface microorganisms within this treatment were able to save energy by not having to produce as many protective pigments as microorganisms exposed to higher levels of UV. This means that these organisms could invest more energy into chlorophyll *a* production, thereby ensuring maximum utilisation of available PAR. This would allow them to fix carbon at an extended rate. In natural environments, the ability to change the ratio of photosynthetic to protective pigments would be highly pertinent as this would allow those photoautotrophs capable of changing this ratio in response to environmental stimuli to maximise their photosynthetic rate whilst minimising damage from UV. This may allow them to gain a competitive advantage over other photoautotrophs that are not capable of changing their pigment ratio.

Alternatively, it is possible that the increase in chlorophyll *a* concentrations in the restricted UV treatment arose due to an increase in the number of photoautotrophs present at the surface. This may have occurred due to the lack of UV meaning that any

photoautotrophs at the soil surface could grow more readily. Further work would need to be undertaken to investigate which of these two hypothesis, or whether a mixture of the two occurred.

The effects of different light treatments on microbial biomass can be seen to diminish with depth by the fact that the lines from all treatments are starting to converge with depth (Figure 6.6). Jordan and Beare (1991) and Vangestel *et al.* (1992) demonstrated that microbial biomass generally declines with depth. However, on the millimetre scale as studied in this experiment, this decline in microbial biomass was only apparent in the restricted UV treatment which decreased significantly at the scale investigated. Whilst the restricted PAR treatment showed a general trend to increase in microbial biomass C with depth, this increase was not found to be statistically significant. Although the apparent increase in microbial biomass C with depth was not significant it seems logical that microbial biomass would be lowest at the surface in this treatment and to increase with depth. This is because in this treatment, the beneficial effect of PAR is removed but the environmental stress of UV A still remains.

Mean microbial biomass carbon concentrations from each treatment can be seen to be converging with depth, as shown by the differences between microbial biomass C decreasing with depth, at the scale investigated. This is likely to be because the "buffering effect" of soil increases with depth, diminishing any differences in treatment effects. This disagrees with results obtained by Rinnan *et al* (2005) who demonstrated that differing levels of UV in arctic systems had no significant effect on microbial biomass carbon. It is possible that this disagreement has arisen because the microbial communities found in the arctic are more resistant to UV, but further work would need to be undertaken to confirm this hypothesis. Alternatively, it is possible that this disagreement occurred because of differing levels of UV found at the arctic when compared to the latitude (52°N) where this project was carried out. For example, as sunlight must travel through more atmosphere before reaching the earth's surface at the poles when compared to the equator due to the angle at which the light enters the atmosphere (ignoring the effects of any holes in the ozone layer), less UV reaches the surface at the higher latitudes than it does at lower latitudes (United States Environment Protection Agency 1999). Increasing the levels of UV in a repeat of the

arctic experiment may mean that significant effects of UV with regards to microbial biomass are found.

Where statistically significant differences existed between microbial community phenotypes expressed in the soil surface when compared to deeper soil layers, the PLFA 16:0 was consistently associated with the discrimination seen between soil depths (Figures 2 & 3). This confirms the work reported in Chapters 4 and 5 and adds further evidence to the hypothesis that the PLFA 16:0 is associated with soil surface microbial community phenotypes. Although the apparent reduction in proportion of 16:0 with depth has been shown not to be significantly different at the scales investigated, it is possible that this is due to the relatively high variation in proportion of 16:0 at the soil surface, combined with the small number of replicates. Further work needs to be undertaken with an increased number of replicates to confirm the hypothesis that the PLFA 16:0 functions as a biomarker of the microbial community phenotype expressed within soil surface microbial communities.

There is currently much disagreement in the literature regarding as to which taxonomic groups the PLFA 16:0 functions as a biomarker (Pelz *et al.* 2001; Jones *et al.* 2003; Keinanen *et al.* 2003; Knief *et al.* 2006). The PLFA 14:0 also discriminated between the treatments with UV A and restricted UV. The PLFA 14:0 is not often cited in the literature as a biomarker of any taxonomic group although it has previously been associated with the pathogenic oomycete *Aphanomyces euteiches* (Larsen and Bodker 2001). The PLFA 16:1ω7c, was responsible for much of the discrimination seen between soil depths in the Restricted UV treatment. 16:1ω7c is a biomarker for Gram negative bacteria (Zelles 1999). The PLFA 18:1ω9t which was responsible for some of the discrimination seen in PC1 in the restricted UV treatment and the majority of the variation seen in PC2 is a general a prokaryotic biomarker (Wardle *et al.* 2003). There is currently no general photoautotroph marker in the primary literature. The PLFAs 14:0, 16:0 18:0 and 18:1ω9c have all been reported as being found in plants, green algae cyanobacteria and brown algae, but these are also sometimes found in fungi and some bacteria (Harwood and Russell 1984). It may be that either no general biomarker exists, or because it or they have not yet been identified. Further work would need to be done to investigate if a photoautotrophic PLFA biomarker exists, but this work did not fall under the scope of this project.

What this chapter highlights is the importance of light, and specifically PAR in the development of distinct communities within the soil surface. The logical conclusion of this is that photoautotrophs play the primary role in the discrimination of the soil surface microbial community from those found in the deeper soil zones.

The functional consequences of these results may include effects on soil surface stability and water infiltration. It is generally accepted that the soil microbiota has an impact on soil structural integrity (Wright *et al.* 1999; Ritz and Young 2004). Effects upon structural integrity at the soil surface are likely to have an impact on the soil's tendency to erode and the implications of this warrant further study. Due to the fact that microorganisms, especially algae, excrete extracellular compounds such as polysaccharides, many of which can be hydrophobic, it also seems likely that the soil surface microbial community may have a direct impact on water infiltration rates. The effects of this also warrant further investigation as manipulation of environmental factors such as light at the soil surface may allow some control over the composition of the microbial communities at the soil surface, and hence the soil's infiltration rates and propensity to erode.

Chapter 7: The Effects of the Soil Surface Microbial Community on Soil Surface Physical and Hydrological Properties

7.1. Introduction

Much is understood about the effects of soil texture on the soil surface's physical properties (such as propensity to form crusts, generate run-off and erode: Le Bissonnais 1995; Cattle *et al.* 2004). However, beyond the work on microbiotic crusts, very little is known about the effects of surface-dwelling soil biota on these soil physical properties.

It is widely accepted that microbiotic crusts can affect the soil surface's physical properties. One of the most important roles of microbiotic crusts is the stabilisation of the soil surface, so reducing the soil's tendency to erode (St Clair and Johansen 1993). It has been shown that soil biota can also affect soil physical properties, even when microbiotic crusts are not present (Edgerton *et al.* 1995, Ritz and Young 2004). Fungi have been shown to be particularly important in affecting a soil's physical properties with regards to erosion (Medows *et al.* 1994). As such, quantification of fungal biomass within the soil may give an indication of soil erodibility (namely susceptibility to erosion processes). Nisha *et al.* (2007) demonstrated that cyanobacterial populations within the soil can affect the soil with regards to bulk density, water holding capacity and hydraulic conductivity, and Falchini *et al.* (1996) found that cyanobacteria from the genus *Nostoc* also affected soil structure, in a manner which maintained porosity and protected the soil structure from the damaging effects of water addition when inoculated onto the surface.

It was shown in Chapter 6 that by manipulating the wavelengths of light which reach the soil surface can change the microbial community phenotype which develops there. Through changing the microbial community phenotype again in this way (producing different soil surface microbial communities) this chapter investigates the effect of different soil surface microbial communities on the soil surface's physical and hydrological properties, by testing Hypothesis E:

There are specific relationships between microbial communities and soil structural properties at the soil surface, particularly in relation to crust formation, shear strength and erodibility (susceptibility to erosion).

The soil microbiota have been shown to affect soil physical properties (Young and Ritz 2005), as well as hydrological properties (Nisha *et al.* 2007). Crust formation can occur by two mechanisms. Either microbiotic crusts can form, as discussed previously, or physical crusts can form in which raindrop impacts can detach soil particles which are then carried in splash trajectories and re-deposited on the soil surface, sometimes forming a surface seal (Farres 1978; Le Bissonnais 1990). When these seals dry they form crusts which can affect soil structural and hydrological properties such as porosity, infiltration rates, generation of surface run-off and soil shear strength at the soil surface. Through use of a Torvanean approximate measure of crust formation can be obtained, due to the concurrent increase in dry bulk density which occurs when a crust forms (Bresson *et al.* 2004). Also, shear strength is likely to be correlated with a soil detachment rate and therefore erodibility as it is a measure of the energy required to shear soil particles from each other (Rauws and Govers 1998).

Whilst the impacts of the soil surface microbiota have been studied from an erosion perspective with regards to microbiotic crusts, this work has not been undertaken in arable systems. Also, due to the mechanisms by which the soil surface microbiota can affect erodibility, some selectivity may occur in the size of the particles which are detached and eroded. For example, due to the physical binding of soil particles by fungal hyphae, small particles may be more susceptible to erosion as they may pass through the "mesh" of fungal hyphae at the soil surface. Particle size analysis of eroded sediment would allow this hypothesis to be tested.

Porosity at the soil surface is reduced when physical crusts are formed (Le Bissonnais 1990). Using X-ray computer tomography, soil can be scanned to produce a series of 2-dimensional slices which can then be reconstructed to produce 3-dimensional images by a computer (Nunan *et al.* 2006; Rogasik *et al.* 1999). Analysis of the pore spaces can then be undertaken using software to gain information regarding porosity within the soil matrix. Using this method, the porosity at the soil surface can be measured to help determine whether any changes in crusts formed at the soil surface

are physical or biological in nature. This is because the shear strength of the surface may be increased under a biological crust, but the porosity is unlikely to be thus affected.

7.1.1. Ergosterol

PLFA is a method for determining differences in community level microbial phenotypes, and so was used in this chapter to quantify differences in community level phenotypes between light treatments, as discussed in Chapters 2 and 6. However, PLFA analysis is not an ideal method for determining total microbial biomass. Because PLFAs are expressed on a percent mol basis, they are not ideal for determining the biomass of specific parts of the microbial community such as fungi. As fungal biomarkers exist in the form of 18:3ω3 (Ebersberger *et al.* 2004) and 18:2ω6 (Zelles 1999a), it is possible to quantify changes in *relative* abundance of these biomarkers, but it is not possible to quantify *absolute* fungal biomass in terms of gram of fungi per gram of soil.

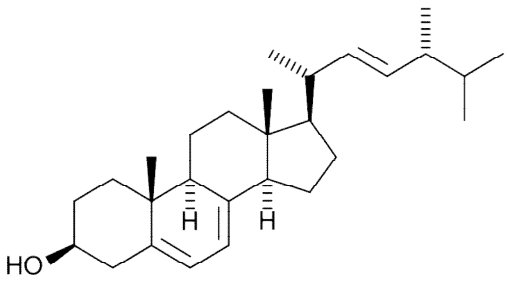


Figure 7.1 Molecular structure of ergosterol

Ergosterol is a fungal-specific sterol (Figure 1). It is found in the majority of fungi, with the exception of some aquatic types, and in very low concentrations in a few algae and protozoa (Mille-Lindbolm, *et al.* 2004). Ergosterol is frequently used as an indicator of living fungal biomass, based on the assumption that it is labile and so is degraded rapidly following the death of fungal hyphae, although a recent study has suggested that ergosterol may be more recalcitrant than had been initially assumed (Mille-Lindbolm, *et al.* 2004). Nonetheless, for most practical purposes, ergosterol quantification is still able to provide a relatively accurate measure of fungal biomass (Ruzicka *et al.* 1995) and so was used to quantify fungal biomass in this chapter.

7.2. Materials and Methods

7.2.1. Materials: erosion trays

Rectangular erosion trays (dimensions 60 x 110 x 200 mm - d, w, l) were constructed from steel and painted with Hammerite™ metal paint. Approximately 5 mm above the base of each erosion tray a steel mesh, covered with a nylon mesh (gauge = 1mm) was placed, which allowed the infiltration of water, but not the passage of soil aggregates greater than 1 mm in size.

Figure 7.2 Erosion tray packed with soil as described below.

A sandy loam soil was collected from Showground Field, Silsoe Experimental Farm as described in Chapter 4, again with soil being collected from the top approximately 10 cm. The area from which the soil was collected had been under set aside from at least the previous 6 months. The soil was sieved to pass through a 2 mm sieve and thoroughly mixed to ensure homogenisation. It was then packed to fill each erosion tray at a dry bulk density of 1.3 g cm^{-3}, deemed typical for an arable soil.

In order to generate different microbial communities, the same strategy as employed in Chapter 6 was used. This included three different types of filters plus a no light control treatment, to make 4 treatments as described in Table 6.1. The light filters were attached to pinewood frames (with dimensions of 80 x 150 x 300 mm, so that they could be placed over each field microcosm). The frames were varnished to protect them from decomposition. The frames were made to stand 80 mm high, although on two opposite sides the wood was 20 mm shorter, creating a gap between

the ground and the frame, providing a baffle which allowed the circulation of air, but ensured that no light reached the soil surface without passing through the light filter first.

7.2.2. Materials: microcosms for X-ray computed tomography scanning

The homogenised soil was used to pack the erosion trays as described above, as well as 12 plastic cylinders (internal diameter 40 mm, length 100 mm) which were to be used for the X-ray computed tomography (CT) scanning. One end of each cylinder was covered with Loktek™ LS greenhouse shading (73% density with a mean pore size 2 mm^2), which was attached using silicon sealant and nylon cable ties. This allowed the passage of water and the free movement of the microbiota, but kept the soil in place. Soil was again packed into the cylinders to a dry bulk density of 1.3 g cm^{-3} to produce 12 replicate microcosms.

7.2.3. Experimental design

Each erosion tray was randomly allocated to a filter through use of a random number generator. Each erosion tray and filter where then randomly placed in a block, with one replicate of each treatment to a block. They were then placed in the field in a randomised block design to produce 8 replicates of each treatment. Blocks were aligned to run north to south to minimise any effects arising from shadows at sunrise and sunset (Figure 7.3).

Figure 7.3 Erosion trays covered by light filters in randomised block design.

All erosion trays and their corresponding light filters were left in the field for a period of approximately 6 months from November 2006 until May 2007. The erosion trays were irrigated regularly (a maximum of every 2 weeks) through the use of a watering can and a fine rose to maintain a constant moisture content of approximately 12% as in Chapter 6. However, due to an unusually hot and sunny April 2007, and logistical difficulties in getting into the field, the moisture content in the soil surface layer in the field microcosms sometimes dropped to below 8%, as was the case at time of harvesting.

The microcosms for the X-Ray CT scanning were also placed in another randomised block design beside erosion trays and filters described above, with 3 microcosms placed under each light filter namely: with UV A, restricted UV, restricted PAR and no light as described in Table 6.1.

7.2.4. Laboratory analyses

As the soil in each erosion tray was homogenised and packed to the same bulk density, with moisture content kept approximately constant between treatments, it is logical to conclude that any differences observed in soil surface physical and hydrological properties such as shear strength, run-off , infiltration and soil loss must be the result of differences in soil surface biological community which develop when exposed to the effects of different treatments (e.g. wavelengths of light, as shown in Chapter 6). To confirm that different soil surface microbial communities had again developed under the different light treatments, and to allow interpretations of the causal relationships between different microbiological communities and soil surface physical properties, three randomly chosen replicates from each light treatment were used for the microbiological assays.

All laboratory analyses were performed on the soil surface layer of the microcosms, which was removed down to a depth of *c.* 1 mm by scraping with a palette knife. The subsurface layer was then removed using the same technique down to a further depth of *c.* 5 mm. Only two depths, the surface and the sub-surface, were used as all previous experiments had shown that light treatment effects diminished with depth,

becoming not statistically significant by the deeper layer beyond approximately 6 mm deep.

7.2.4.1. Chlorophyll *a* analysis

Analyses were again performed in the order as described in Chapter 5. Chlorophyll *a* extraction and analysis was used to quantify photoautotrophs in the different treatments and was again undertaken using the ethanol extraction method for quantification of chlorophyll *a* in soil (Metting 1994) as described in Chapter 2. Analysis of extracted chlorophyll *a* was undertaken using a SPECTRAmax 386 spectrophotometer (Molecular Devices Corporation, Sunnyvale California, USA) and chlorophyll *a* concentration calculated using the equations described in Chapter 2:

7.2.4.2. PLFA analysis

PLFA extraction and analysis was undertaken to quantify differences in the community level phenotypes developed in the surface layers of the erosion trays under the four different light treatments. The analysis was performed as discussed in Chapter 2.

7.2.4.3. Ergosterol

Ergosterol extraction and analysis was undertaken to quantify the absolute fungal biomass in the soil surface layers of the erosion trays from each light treatment. Ergosterol was extracted via the rapid ultrasonication method described by Ruzicka *et al.* (1995). Homogenised soil, collected from each layer of the soil erosion trays was divided into 3 laboratory replicates, each of 5.00 g, and placed in 50 ml Falcon™ tubes and 10 ml methanol : ethanol (4:1 v/v) was added. To find the extraction efficiency of ergosterol from the soil, 5 further replicates of the same soil used to pack the erosion trays were spiked with 1 ml of ergosterol standard made to a ratio of 10 mg ergosterol in hexane : propan-2-ol (98:2 v/v). All samples were incubated in a fridge for 2 hours. 20 ml of hexane : propan-2-ol (98:2 v/v) was then added to each of the microcosm samples and 19 ml of hexane : propan-2-ol (98:2 v/v) was added to the spiked samples. All samples were then ultrasonicated at 150 W using a sonic probe (VirSonic 600, model s3000-210; VirTis, New York, U.S.A.) for 200 seconds while

kept on ice. The samples were then allowed to settle for 30 seconds and approximately 2 ml of the top layer (hexane : propan-2-ol) was transferred into a microfuge tube and centrifuged at 10,000 rpm for 10 minutes.

Analysis was performed using a Waters HPLC system (Massachusetts, U.S.A.). Each sample (20 µl) was injected into 150 mm (4.6 mm i.d.) Phenomenex (California U.S.A.) Luna 5 µm silica column preceded by a Phenomenex security guard column and eluted with n-hexane-propan-2-ol (98:2 v/v) at 1.5 ml min^{-1}. A UV absorbance detector was used to measure the absorbance at 282 nm.

7.2.5. Rainfall simulation

In order to observe the effect of different soil biota on soil physical and hydrological properties (namely crust formation, shear strength and soil erodibility), five randomly selected replicate erosion trays from each light treatment were selected. These erosion trays were then subjected to simulated rainfall, generated by a gravity fed, hypodermic needle rainfall simulator at a rate of 60 mm h^{-1} for 20 minutes. This storm intensity and duration were chosen to be representative of a typically erosive rainfall event in the UK, with a return period of approximately 1 in 15 years. The rainfall intensity and texture of the soil used were also chosen to encourage the formation of a surface seal, whereby raindrop impact would first detach soil particles from the soil surface. These particles would then be carried in splash trajectories and re-deposited on the soil surface, so forming a surface seal (Farres, 1978; Le Bissonnais, 1990).

The microcosms were placed on a table approximately 8m below the drop formers of the rainfall simulator to ensure the raindrops reached their terminal velocity (Rickson, R.J. personal communication). The microcosms were inclined at 9° (representing a relatively steep arable field; Figure 7.4). This ensured surface run-off was generated. Infiltration was also measured after collecting the amount of water infiltrating through the erosion trays in plastic bottles.

Figure 7.4 Microcosms under rainfall simulator. Run-off was collected by a funnel at the down-slope edge of the microcosm, and then directed through the larger gauge plastic pipes attached to the surface of the microcosms. Infiltration was collected from the thinner gauge plastic pipes at the base of each microcosm.

The runoff generated during the storm was collected in 1 l plastic bottles, and then filtered through Whatman No. 1 filter papers which had been pre-dried over night in an oven set to 55°C and weighed. After the runoff had been filtered, the filter papers were again dried overnight (at 55°C) and weighed. The difference in filter paper weight was taken to be the sediment which had been eroded by the runoff from the soil surface of the microcosms.

7.2.6. Run-off and infiltrate

Run-off and infiltrate were both collected from the erosion trays via plastic tubing which carried the water to collection bottles (Figure 7.4). Run-off was then measured by measuring cylinder. The soil erosion trays were found to contain different initial moisture contents. However, statistical analysis of the starting moisture contents suggested that the variation was not due to treatment effects ($P = 0.18$) and as such these differences would not introduce any bias into the final analysis of the amount of infiltrate collected from each light treatment.

7.2.7. Soil shear strength

Shear strength (kg cm^{-2}) at the soil surface was measured using a Torvane (Soiltest, ELE International, Colorado, U.S.A.). After exposure to simulated rainfall, the microcosms were dried overnight in an oven at 105°C to dry any seals which may

have developed at the soil surface, so creating a crust. Also, this ensured that all replicates were at the same moisture content prior to the measurement of shear strength. After leaving the microcosms to cool for several hours the Torvane (diameter 25 mm) was used to measure the soil shear strength at 3 randomly chosen, discrete locations on the soil surface of each microcosm, giving 15 replicates of shear strength for each treatment. The Torvane was gently pushed into the soil surface to a depth of 5 mm, and rotated at a slow and steady rate until the soil failed in shear. At that point, a reading of the Torvane was taken.

7.2.8 X-ray CT scanning

During the 6 months that the microcosms for CT scanning were in the field, the restricted PAR filter was damaged by an unknown source and removed from the pinewood frame approximately 1 month into the experiment. In order that some data was still retrieved from this experiment these microcosms were left in the field and the experiment plan changed to make this a new “Open” treatment. These microcosms were exposed to natural rainfall events as well as wind and natural sunlight. The effects of these conditions on the microbial communities and soil properties with regards to porosity, could then be compared to those microcosms which were not exposed to rainfall or wind as they has remained under filters. Therefore, this modified experimental design allowed the quantification of the effects of different microbial communities which developed under different environmental conditions with regards to the presence or absence of UV A by comparing the two treatments covered by the different filters. Added to this, by comparing the open microcosms to the covered microcosms, when grouped together to make one treatment, the effects of being covered versus being exposed to wind and rain could also be quantified.

In April 2007, with approximately 2 weeks left before the microcosms were due to be scanned, ants built a nest in the no light treatment (Figure 7.5), rendering these microcosms useless for their intended purpose.

Figure 7.5 Impact of ants on microcosms under the no light treatment.

To investigate the structural effects of the soil surface microbiota specifically with regards to porosity, X-ray CT scanning was undertaken at Abertay University, Dundee, UK. The scanner used was a X-Tek Real-time X-ray benchtop CT scanner (Model type BT160UF), using the software CT Pro, Version 1.0.2582.18543 (X-Tek Systems Ltd. Tring, Herts, U.K.). Image analysis was undertaken using VG Studio Max 1.2.1 Volume Graphics GmbH (Heidelberg, Germany).

Analyses of scans were performed at a resolution of 73 μm. Each layer was an amalgamation of 10 slices (each layer was 0.73 mm deep). The soil surface layer was taken as close to the surface as permitted by the soil surface microtopography, this being the first layer at which a 300 x 300 pixel square could be drawn on the scans with no false pores due to the effects of the soil surface microtopography. Subsequent layers were analysed 7.3 mm (100 slices) from the bottom slice of the previous layer. VG Studio Max 1.2.1 Volume Graphics GmbH calculated porosities on greyscale images using a shading threshold of 35.

7.2.9. Data analysis

Data analysis was undertaken using Statistica 7.1 (Statsoft Inc, 2005) as described previously. *Post-hoc* ANOVA, using Fisher's LSD, was used to test differences in ergosterol concentrations and fungal to bacterial PLFA biomarker ratios, run-off, infiltrate, soil loss, and soil shear strength, as well as on data obtained from the CT scans.

7.3. Results

7.3.1. Microbial analyses

PC plots are shown in Figure 7.6. The soil surface layers for both the with UV A and restricted UV treatments were differentiated from other soil depths in PC1 (Figure 7.6a) Again, the PLFA 16:0 was responsible for much of the discrimination seen in PC1 between the 2 soil depths. The PLFA 18:1ω9c was also responsible for much of the discrimination seen between depths in PC1 as well as discriminating in PC2. The subsurface layer of the with UV A treatment was significantly different to all other treatments and depths with the PLFA 18:2ω6 being responsible for much of the discrimination seen in the With UV A subsurface layer (Figure 7.6b).

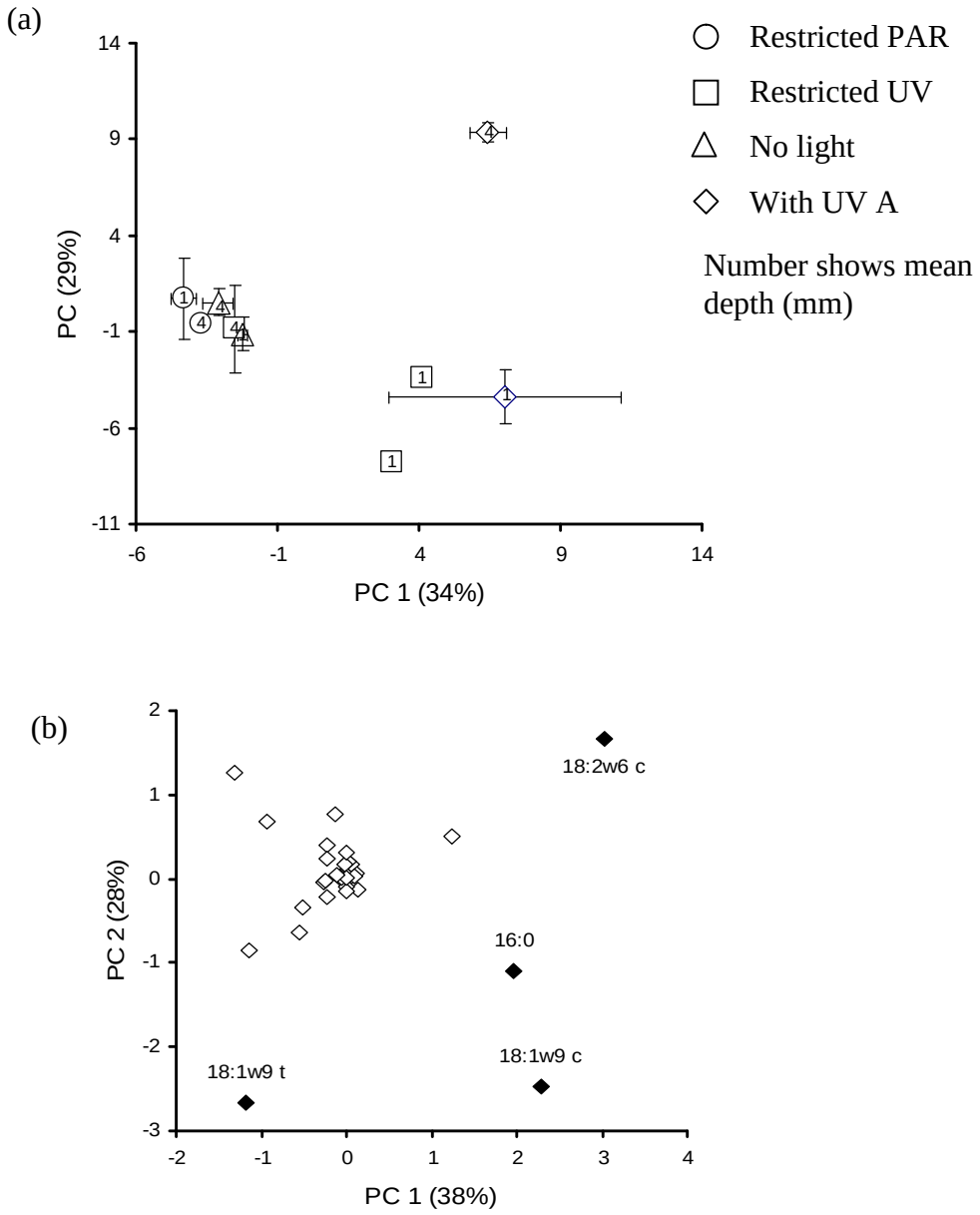


Figure 7.6 (a) First and second principal component (PC) plots derived from PLFA profiles originating from surface layers of erosion trays from all treatments. Points show means (n = 3), bars show standard error. Percentage variation accounted for by PC shown in parenthesis on each axis. Mean depth of each sample given in millimetres within point: One of the values for the surface layer of the Restricted UV treatment was excluded from the principal component analysis as an outlier. For this reason the two remaining values have been plotted individually instead of as a mean; (b) loading plots with those PLFAs (highlighted in black and named) contributing most to the variance seen in the PC plots.

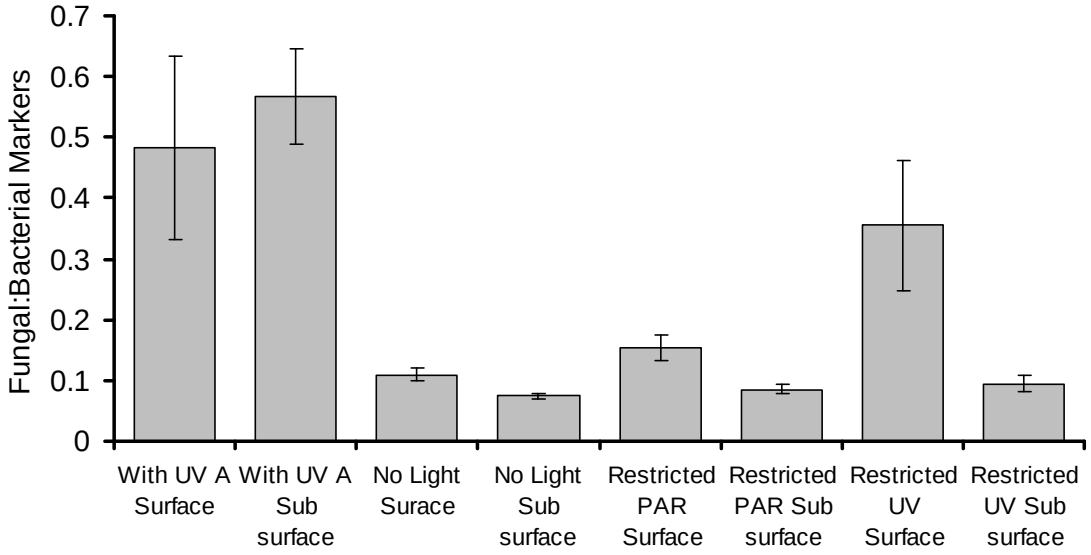


Figure 7.7 Ratio of fungal to bacterial PLFA biomarkers, from two different depths, under the four different treatments, with UV A, no light, restricted PAR and restricted UV. Columns show means (n = 3), bars show standard error

Significant differences were found to exist between light treatments with respect to fungal to bacterial PLFA biomarker ratios ($P < 0.01$) (figure 7.7). The only treatment which showed significant differences between depths was the restricted UV treatment, which was found to have a significantly higher ratio of fungal to bacterial PLFA biomarkers in the surface layer compared to the sub-surface layer ($P = 0.02$). No significant difference was found in relation to fungal-to-bacterial biomarker ratios with regards to the interaction between treatment and depth ($P = 0.15$). Whilst there was no significant difference between depths in the with UV A treatment, both depths in this treatment were found to have a significantly higher ratio of fungal-to-bacterial PLFA biomarkers compared to all treatments, with the exception of the restricted UV surface treatment ($P < 0.01$). For the restricted PAR and no light treatment, no significant differences were found in the fungal to bacterial PLFA biomarker ratio at either depth between ($P = 0.4$).

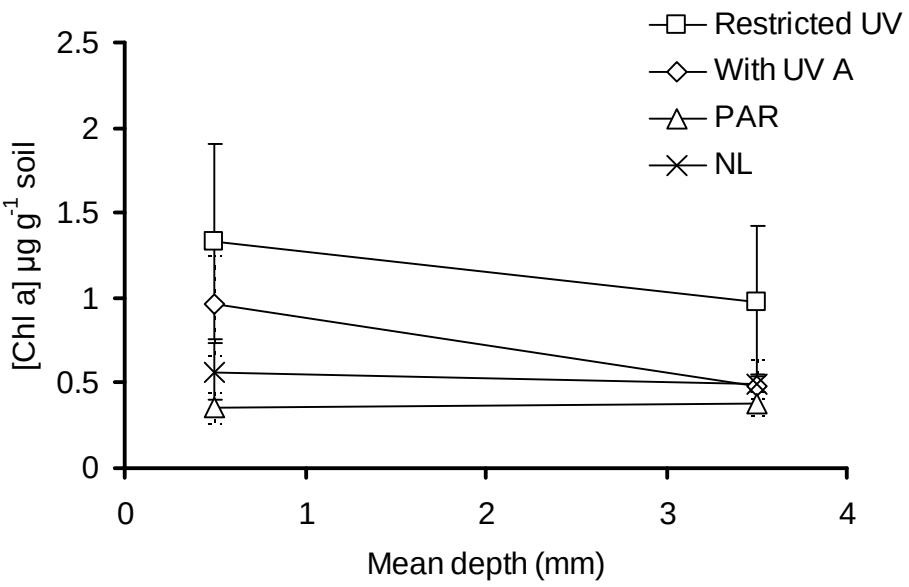


Figure 7.8 Mean concentration of chlorophyll *a* extracted from soil at two depths, from four different light treatments. Points show means (n = 3), bars show standard error).

Analysis of chlorophyll *a* concentrations extracted from the two depths under the four different light treatments showed that there was no significant difference between treatments (P = 0.08) (Figure 7.8). There was also no significant difference between depths (P = 0.3) or with regards to the interactions between depth and treatment (P = 0.8). Chlorophyll *a* concentrations in this experiment were considerably lower than those found in the previous experiment described in Chapter 6 (Figure 6.2). The reason for this disparity in results warrants further investigation.

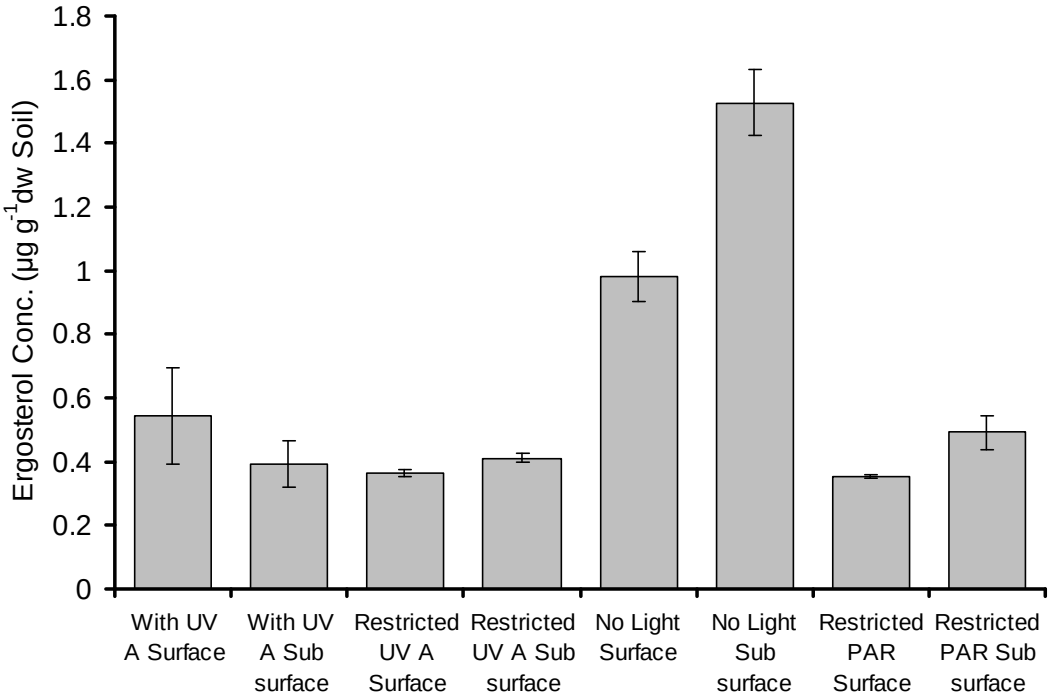


Figure 7.9 Mean ergosterol concentrations obtained from erosion trays under four different treatments, with UV A, no light, restricted PAR and restricted UV. Columns show means (n = 3), bars show standard error.

With regards to ergosterol there were significant differences in fungal biomass between treatments ($P < 0.01$), between depths ($P = 0.02$) and in the interactions between treatments and depths ($P < 0.01$). The ergosterol concentrations in the no light treatment were significantly higher than those of all the other treatments at both depths investigated ($P < 0.01$). The mean ergosterol concentration in the no light, sub-surface samples was significantly higher than that found in the no light, surface ($P < 0.01$) samples. No other significant differences were found between light treatments at the depths investigated. However, the fungal to bacterial PLFA biomarker ratio was lowest in the no light treatment (Figure 7.7).

This data shows that the different light filter treatments did again drive the formation of distinct soil surface microbial phenotypes and as such any significant differences seen between light treatments with regards to soil surface physical and hydrological properties must be attributable to the differences in microbial communities in the surface layers of the erosion trays.

7.3.2. Simulated rainfall experiment

Significantly more run-off was generated from the erosion trays under the no light treatment (P = 0.01) (Figure 7.10). No significant differences were found in the amount of run-off generated by the other three light treatments (P > 0.05).

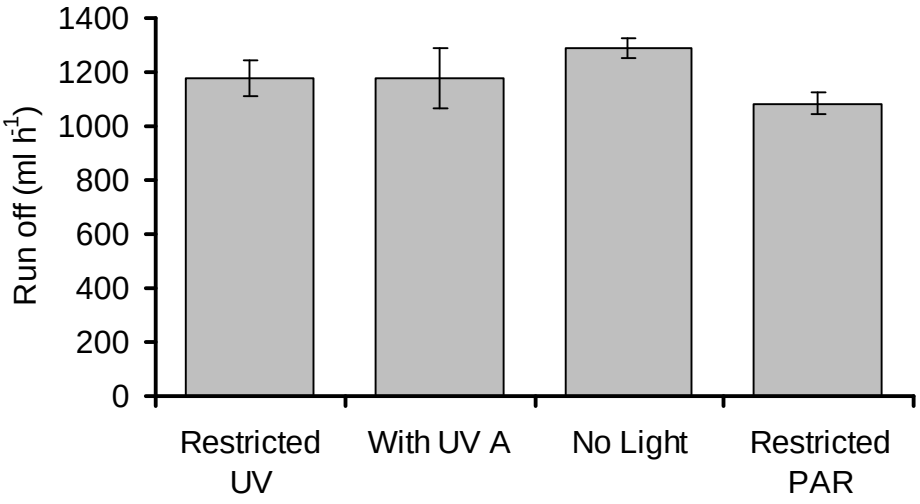


Figure 7.10 Amount of run-off generated from erosion trays under four different treatments, with UV A, no light, restricted PAR and restricted UV. Simulated rainfall was applied for 20 min at a rate of 60 mm h^{-1}. The slope gradient was 9°. (n = 5; bars show standard error).

There were no significant differences in infiltrate from the erosion trays under the different light treatments (P = 0.48) (Figure 7.11). It is notable that the variation between replicates, as shown by the standard error bars for each treatment was considerably higher for infiltration when compared to the variation between run-off generation replicates. It was this relatively high variation which affected the results of the statistical analysis of the amount of rainfall infiltrating into each erosion tray, meaning that whilst the mean amount of rainfall infiltrating in the no light treatment was less than half of the amount of infiltrate in the restricted UV and restricted PAR treatments there was found to be no statistically significant difference.

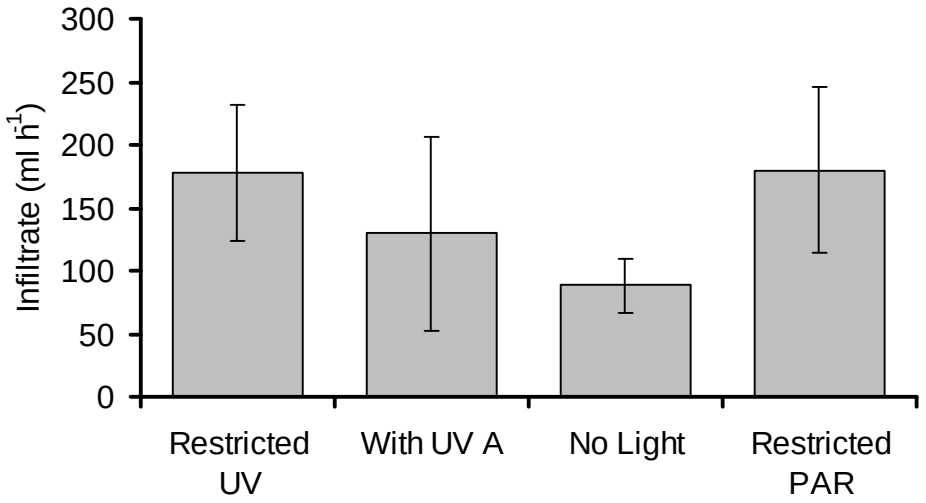


Figure 7.11 Amount of infiltrate from erosion trays under four different treatments, with UV A, no light, restricted PAR and restricted UV. Simulated rainfall was applied for 20 min at a rate of 60 mm h^{-1}. The slope gradient was 9°. Columns show means (n = 5); bars show standard error.

There was no significant difference in either soil loss from the erosion trays under the four different light treatments (P = 0.86; Figure 7.12) or the sediment concentration in the run-off generated under each light treatment (P = 0.53; Figure 7.13).

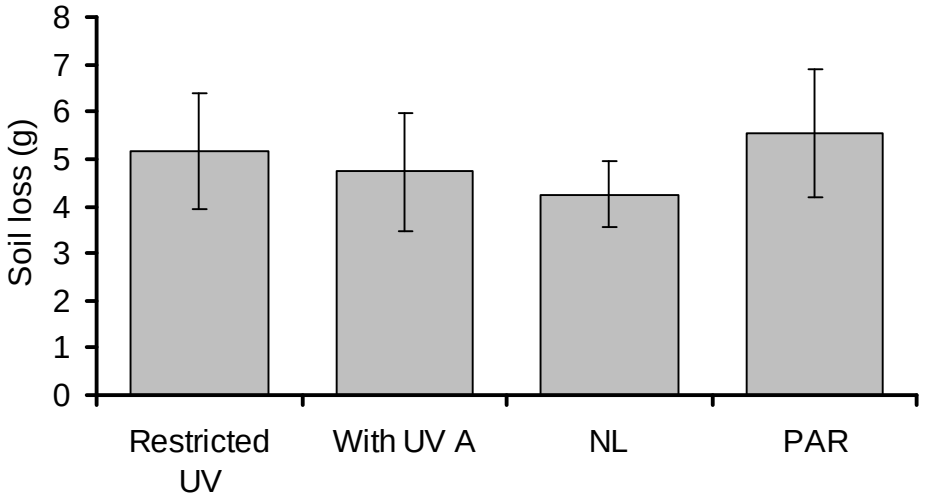


Figure 7.12 Soil losses from erosion trays under four different treatments, with UV A, no light, restricted PAR and restricted UV. Simulated rainfall was applied for 20 min at a rate of 60 mm h^{-1}. The slope gradient was 9°. Columns show means (n = 5); bars show standard error.

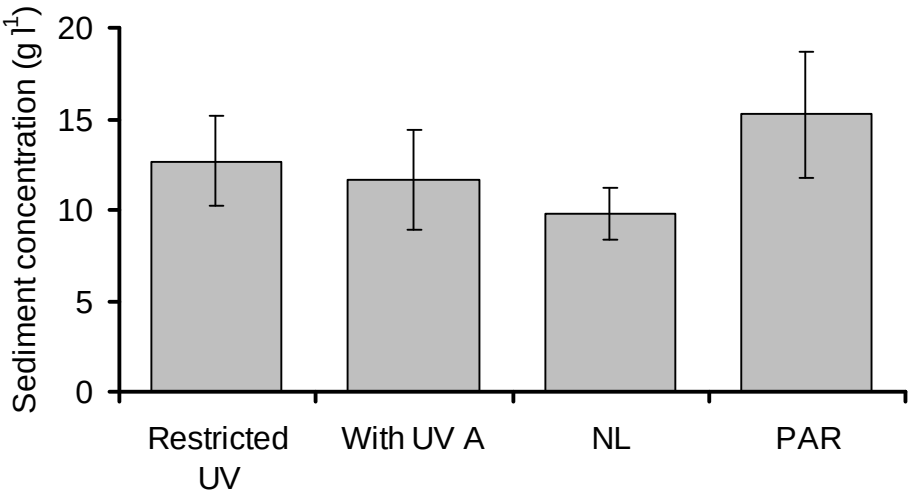


Figure 7.13 Sediment concentrations in run-off generated from erosion trays under four different treatments, with UV A, no light, restricted PAR and restricted UV. Simulated rainfall was applied for 20 min at a rate of 60 mm h^{-1}. The slope gradient was 9°. Columns show means (n = 5); bars show standard error.

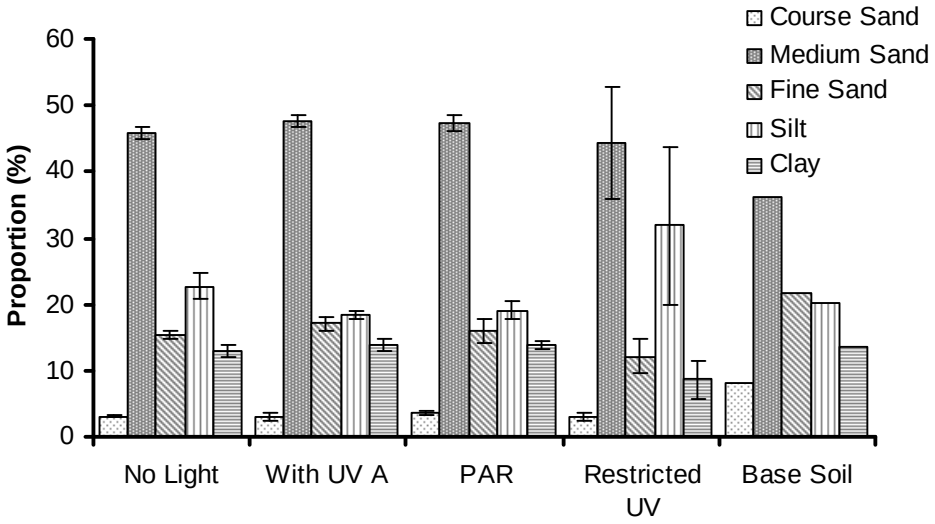


Figure 7.14 Particle size distributions (determined by pipette method) of eroded soil from erosion trays under four different treatments, with UV A, no light, restricted PAR and restricted UV. Simulated rainfall was applied for 20 min at a rate of 60 mm h^{-1}. The slope gradient was 9°. Columns show means (n = 5); bars show standard error.

A factorial ANOVA showed that there was no significant interaction between light treatment and the particle size distribution of the sediment from the run-off (P = 0.4) (Figure 7.14). This means that despite the different light treatments giving different

microbial communities, this had no significant effect on the selectivity of the particle size eroded.

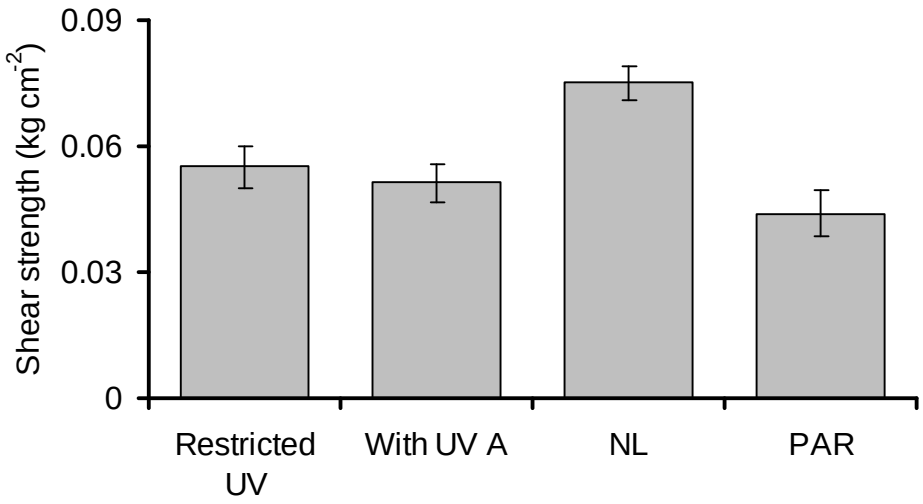


Figure 7.15 Mean shear strength of oven dried soil surfaces under four different treatments, with UV A, no light, restricted PAR and restricted UV. Simulated rainfall was applied for 20 min at a rate of 60 mm h^{-1}. The slope gradient was 9°. Columns show means (n = 5); bars show standard error.

A nested ANOVA with a *post hoc* LSD test showed that the shear strength at the soil surface in the no light treatment was significantly higher when compared to the other light treatments ($P < 0.01$). There was no statistically significant difference between the other three light treatments.

7.3.3. X-ray CT scanning experiment

Data analysis obtained through X-ray scanning CT allowed porosities at different depths moving down from the soil surface to be quantified. The three depths used, as discussed previously were amalgamations of 10 layers, each 73 μm in depth, so that the layers analysed for porosity totalled 0.73 mm in depth. The three layers analysed were as close to the surface as possible (Surface layer), followed by two further layers each 73 mm down from the previous layer analysed (Deeper and Deepest layers).

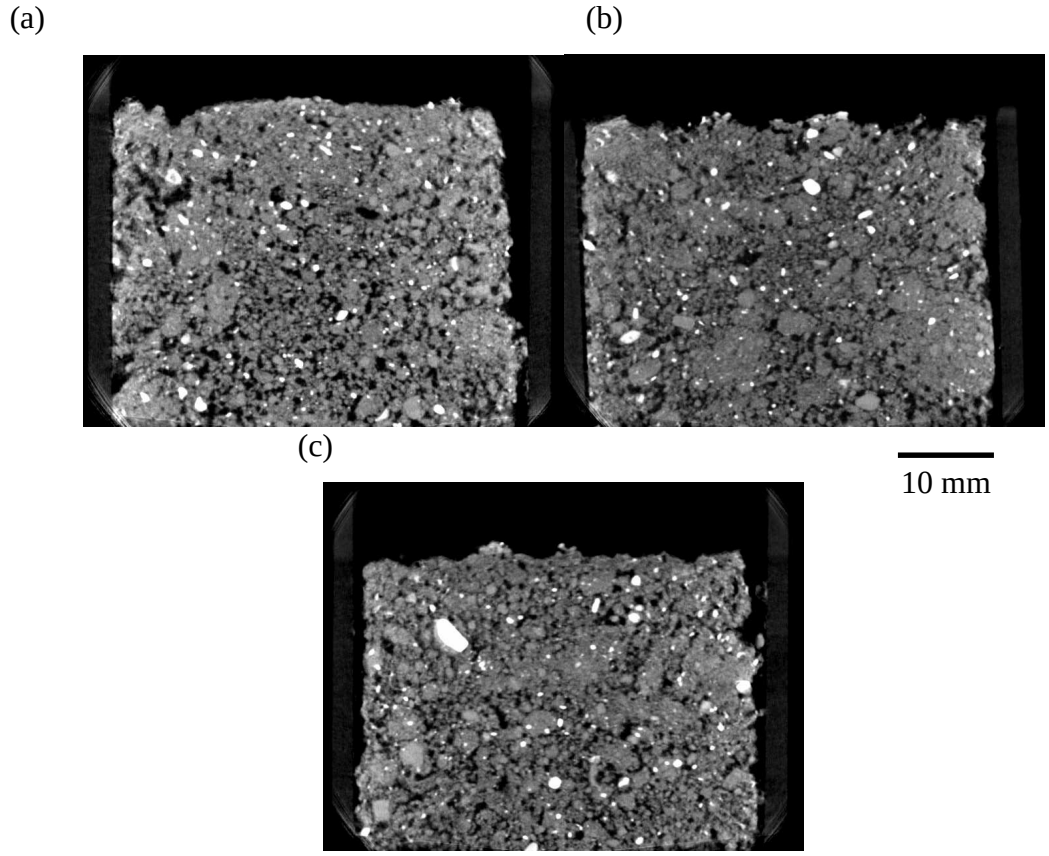


Figure 7.16. Vertical plane cross sections through soil core within microcosms produced by X-ray scanning showing three different treatments, (a) open, (b) restricted UV and (c) with UV A.

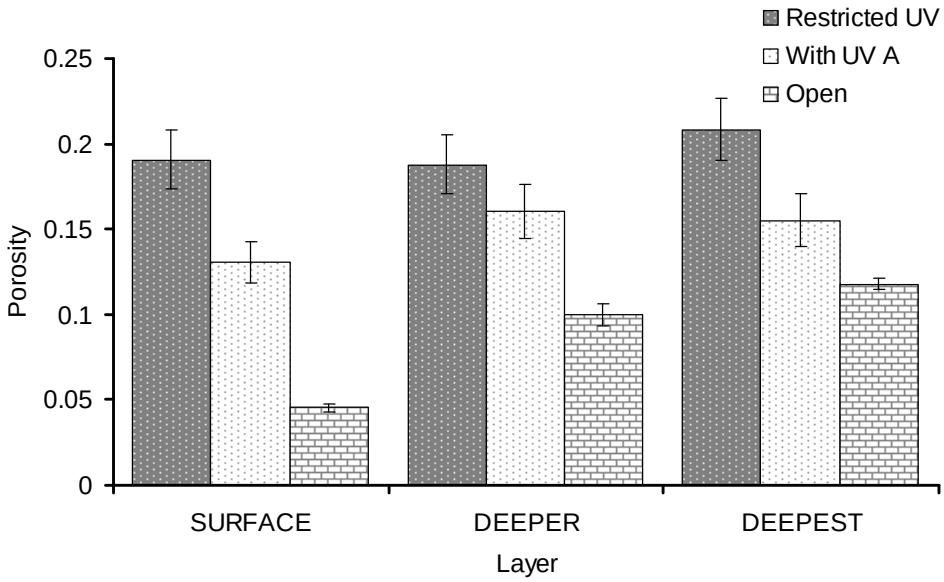


Figure 7.17 Porosity at three depths from microcosms under the three treatments restricted UV, with UV A and open, at a resolution of 73 μm. Columns show means (n = 3), bars show standard error.

There were significant differences in porosity between treatments ($P < 0.01$) and between depths ($P < 0.01$), although treatment and depth interactions were not found to be significant ($P = 0.13$). There were no depth effects with regards to porosity within the restricted UV treatment ($P > 0.05$) or With UV treatments. In contrast, porosity within the Open treatment decreased significantly in the surface layer, compared to the deeper layer ($P < 0.01$), but there was no significant change in porosity between the deeper and deepest layers (Figure 7.17).

Between all treatments, porosity in the surface layers was significantly different ($P < 0.01$), although differences between the restricted UV and with UV treatments diminished with depth and were not significantly different in either the deeper layer or deepest layer ($P > 0.05$) (Figure 7.17).

7.4 Discussion

The different "light" treatments were again sufficient to drive the formation of different microbial community phenotypes at the surface of the erosion trays (Figure 7.6). This result is very similar to that found and described in Chapter 6 and so this data verifies the data reported there. The PLFA 18:1ω9c, considered to be a biomarker for Gram positive bacteria (Frostegård *et al.* 1993; Kelly *et al.*2003) was responsible for much of the discrimination between depths. The PLFA 18:2ω6, a biomarker for fungi (Frostegård & Bååth 1996) was responsible for discriminating the sub-surface layer of the with UV A treatment from the other subsurface layers, and was due to the fact that it contained a much higher proportion of 18:2ω6 than in the subsurface layers of the other treatments. This is shown by the relatively high ratio of fungal to bacterial PLFA biomarkers in both soil layers of the UV A treatment (Figure 7.7). However, this result does not agree with the ergosterol data shown in Figure 7.9 where the total fungal biomass was found to be highest in the no light treatment. This apparent discrepancy in the results must be due to an overall reduction in microbial biomass in the with UV A treatment. This means that whilst the fungal portion of the microbial community was relatively high when compared that in the other treatments (as shown by the fungal to bacterial ratio) the total microbial biomass may have been relatively low. This is because the fungal biomass in the UV A treatment was not significantly different when compared to the restricted PAR and restricted UV treatments. This implies that whilst UV functions to reduce the microbial biomass in the soil surface

layers, fungi within the microbial community are better able to withstand the negative effects of the UV and so come to be relatively dominant within the soil surface communities.

As the same homogenised soil was used to pack the erosion trays, and all other variables such as watering regime were kept the same any differences in either soil surface structural or hydrological properties must have occurred as a result of differences in the soil surface microbial communities.

Microorganisms are known to produce a wide variety of extra cellular exudates, many of which are hydrophobic, including various polysaccharides and glycoproteins. Any difference in the composition of hydrophobic extra-cellular exudates as a result of different microbial community phenotypes within the soil surface may affect the soil surface's hydrological properties. It is also possible that changes in the physical nature of the soil surface occurred due to the influence of the microbial communities. This could include changes in porosity, either through the protection of porosity at times when the erosion trays were watered, as was reported to occur in the presence of cyanobacteria by Falchini *et al.* (1996) or through the movement of soil particles due to fungal hyphae physically binding soil particles together (Ritz and Young 2004). Either way it is possible that this will have an effect on the soil surface's hydrological properties. Unfortunately, as the microcosms relating to the no light and restricted PAR treatments which were initially set up for scanning with the X-ray CT scanner were destroyed before being analysed, it was not possible to test the direct influence on porosity of the soil surface microbial communities which developed under these light treatments during this project.

Whilst there were significant differences in run-off generated from the soil surfaces exposed to the different light treatments, there was no significant difference in the amount of infiltrate collected from each treatment. In terms of a simple water balance, this implies that more water must be stored within the soil matrix of those treatments with lower runoff volumes, i.e. their water holding capacities were increased. As less water must have entered the soil matrix of the no light treatment, demonstrated by the fact that larger amounts of run-off was generated by this treatment the water holding capacity of this treatment must have been reduced. This is because although less water entered the soil matrix, there was no significant difference with regard to infiltration.

The only way this can have occurred is for more water to have remained in the pores of the soil from the no light treatment. As stated previously, any differences in either physiological or hydrological properties arising from this experiment must have occurred as a result of differences in the microbiota of the soil. With regards to water holding capacity it is possible that the change occurred due to changes in the pore structure of the soil, with an increase occurring in the number of pores of a size that they would hold onto water when under low tension. Alternatively, differences in the make up of extra-cellular exudates, specifically the quantities of hydrophobic to hydrophilic compounds within the pore structure will affect both the hydraulic conductivity and water holding capacity of the soil. An increase in the amount of hydrophobic compounds would mean that water would generally be flushed more readily through the pore network decreasing the water holding capacity of the soil. The decrease in water holding capacity in soil under the No light treatment may have occurred for either of these reasons, or a mixture of the two. However, further work analysing either the organic compounds within the soil pore space or the porosity and pore size distribution would need to be carried out to test this hypothesis.

Another factor which may have influenced the infiltration of water through the soil in each microcosm is the presence or absence of preferential flow pathways. For example, errors in the initial packing of the microcosms may have lead to localised variability in bulk density, which will affect the flow of water. Biological activity has been shown to affect the pore structure of soils (Feeny *et al.* 2006). As light has been shown to only affect the communities within the top millimetre or so of the soil system, it is possible that different community effects, not dependent on the light treatments may have occurred in the deeper soil levels which may affect soil infiltration rate and capacity in a way not dependent on the soil surface microbial community. The relatively high variance within treatments as shown by the standard error bars in Figure 7.7 implies that variation in the soil hydrological properties between replicates within treatments may have been due to some or all of these factors.

No differences were observed in either the amount of soil eroded, the sediment concentration in the run-off from the erosion trays, or the particle size distribution of the eroded sediment. This implies that the different soil surface microbial phenotypes

resulting from the different light treatments do not affect the soil surfaces structural integrity with regards to particle detachment by rainfall.

This result disagrees with results reported concerning microbiotic crusts which have shown repeatedly that microbiotic crusts increase soil surface stability and reduce the erodibility of the soil surface (Loope and Gifford 1972; Johansen 1993). However, microbiotic crusts take many years to reach maturity, where they achieve their full effects with regards to affecting soil erodibility (St Clair and Johansen 1993) and it may be that the soil surface microbial community described in this chapter would change as it matured into a surface community which does affect the erodibility of the soil surface. Nisha *et al.* (2007) reported that cyanobacteria can have a significant impact on the soil surface with regards to improving the structural stability of the surface in organically poor soils. This may also be true of soils which are not organically poor, such as those used in this experiment, although further work would be required to test this hypothesis. However, Chlorophyll *a* levels were found to be very low compared to those found in previous chapters, particularly when compared to the field soil reported in Chapter 4. This is possibly because photoautotrophs come from an aerial source and so were unable to reach the soil surface due to the light filters and frames. Further work is required to test whether increased levels of photoautotrophs in the soil surface layers in arable systems would affect the erodibility of the soil surface.

The fact that there was a 75% reduction in chlorophyll levels compared to those reported in Chapter 6 may be due to an increased initial concentration of photoautotrophs in the homogenised soil used to pack the microcosms. An investigation in to the main source of the inoculum for the soil surface will test this hypothesis in Chapter 8.

Sediment collected from the run-off from all treatments had a tendency to contain a higher proportion of silt than was present in the *in-situ* soil (Figure 7.14). This concurs with the erosion literature which shows the silt fraction to be the most susceptible to detachment and transport, as it has low cohesion (unlike the clay fraction) and relatively low mass (unlike the sand fraction) (Hjulström, 1935; Poesen, 1985). The restricted UV treatment showed the greatest variation between replicates and appeared to be subject to greater loss of silt in the run-off. However, these data

were highly variable between replicates and as such the differences were not found to be significant.

Soil shear strength was found to be significantly higher in the no light treatment when compared to the other treatments. Although this may have been expected to have an effect on the soil loss from the no light treatment, there was no significant difference in soil loss between the treatments. The higher levels of ergosterol found in the surface layers of this treatment imply that the fungal biomass is directly correlated with shear strength. This is likely to be a result of soil fungi binding soil particles together as they have been shown to do (Medows *et al.* 1994), as well as sometimes producing extra-cellular exudates which can cause soil particles to stick together (Wright and Anderson 2000; Wright *et al.* 1999).

The microcosms were oven dried in order to encourage surface crust formation from any seals which had formed under raindrop impact. It was then hypothesised that soil surface shear strength as measured by a Torvane would then give a measure of crust formation due to the increased dry bulk density of crusts increasing shear strength (Brady 1990; Zhang et al 2001). Crust formation occurs as a result of the destruction of soil surface aggregates which are then re-dispersed over the soil surface (Brady 1990). Soil aggregate destruction often occurs as a result of slaking caused by advancing wetting fronts increasing the pressure of soil air trapped within aggregates, which is then suddenly released (Morgan 1995). It would have been expected that those soils with a relatively high fungal biomass would have an increase in aggregate stability meaning a reduction in aggregate destruction due to raindrop impact and slaking, and therefore less seal and crust formation. This is because fungi normally function to bind soil aggregates together as previously discussed. However, due to the physical effects of fungi binding soil particles together it was not possible to distinguish with this methodology whether the increased shear strength was a result of increased bulk density due to the formation of a physical crust, or was a result of a biological crust where the fungi were binding the soil particles together, both physically and chemically and hence increasing the soil shear strength. Further work would need to be done to confirm the cause of the increased shear strength. Analysing the dry bulk density of the soil surface layer would be one way to achieve this.

There was a general trend in the ergosterol data for the highest result to come from the soil surface layers, except for the no light treatment, although these differences were not statistically significant. Fungal biomass would be expected to be higher in the soil surface layers, as fungi have been shown to grow preferentially over surfaces as previously discussed in this thesis and reported by Otten and Gilligan (1998).

The fungal biomass was found to be highest in the no light treatment, and within the no light treatment, highest in the sub-surface layer. This implies that the changes in community structure due to placing the soil surface in darkness favours the growth of fungi. This may be because the development of some elements of the microbial community which would compete with fungi were restricted by the removal of light (i.e. photoautotrophs), and/or that the fungal proportion of the soil surface microbial community is better adapted to growing in the dark. However, the increase in fungal biomass in the sub-surface layer of the no light treatment is counter to work reported in the literature (Otten and Gilligan 1998) and previously in this project which has shown that fungi generally grow preferentially over surfaces. Further work would be needed to test this hypothesis and to investigate what other changes may have occurred to caused the fungi to grow preferentially in the sub-surface layer.

The data collected from the PLFA analysis agree with the data presented in Chapter 6 This adds further evidence that light, and specifically PAR, is the factor most responsible for the driving of a distinct soil surface microbial community phenotype. However, in the present experiment, the subsurface layer in the with UV A treatment was also discriminated by PC2 (Figure 7.13a). This was due to a relatively larger proportion of the fungal PLFA biomarker 18:2ω6 when compared to the other samples, as shown by the PLFA loadings plot (Figure 7.13b) and further confirmed by the increased ergosterol content shown in Figure 7.12. There was no clear mechanism as to why this occurred, and the time-constraints of this project curtailed further investigation. However, soil is a highly complex system and many factors could have been responsible for this occurrence such as some organic matter being present in that soil layer which failed to become distributed through the soil during the homogenisation process which encouraged the growth of fungi.

The reduced porosity found in the X-ray CT scans for the open treatment was almost certainly a result of crust formation at the soil surface. This demonstrates the

importance of rainfall in crust formation at the soil surface, even though the natural rainfall intensities experienced at the experimental site would be relatively low. The presence of UV A appears to have changed the soil surface microbial community and in such a way that this microbial community reduced the porosity of the soil within the microcosms of this treatment (Figure 7.17). The same homogenised soil was used for all three treatments, and the only differences in the two covered treatments were the wavelengths of light reaching the surface. Differences in wavelength of light reaching the soil surface cannot directly affect soil structure, but these differences do change the soil surface microbial community, which in turn can affect soil surface properties. However, the mechanisms by which porosity at the soil surface is reduced by the soil surface microbial community are unclear and warrant further study.

Chapter 8. Investigation Of The Source Of The Inoculum Leading To The Development Of The Distinct Soil Surface Phenotype

8.1. Introduction

There are two possible sources of inoculum that upon contact with the soil surface environment may develop and grow into a distinct soil surface phenotype. It is possible that the source of the inoculum is within the underlying soil microbial community, which adapts when exposed to the soil surface environment to develop into a distinct phenotype. Alternatively, it is possible that aerial microorganisms, the aerospora, predominantly comprise the inoculum. The hypothesis that "*Everything is everywhere but the environment selects*" was originally described by Bass Becking (1934). The idea behind the hypothesis is that essentially all microbial life is distributed worldwide, but in a given environmental setting the majority of the microbial species are only present in a latent form. The implication of this is that on a small scale, the majority of the microbial biodiversity exists below the limits of detection and so is hidden from observation. Baas Becking (1934) posited that the mechanism which would lead to homogenous distribution of the microbiota was passive transport of microorganisms by air currents.

Bacteria are known to exist in natural clouds and rainfall. Amato *et al.* (2005) found there to be in the region of 30,000 cells of both eukaryotic and prokaryotic forms per cubic metre of cloud, in clouds sampled at Puy de Dome (altitude 1465 m, Massif Central, France) and noted that less than 1% of the microbial population was culturable. This is a similar percentage to the percentage of culturable microorganisms typically found in the soil environment (Torsvik *et al.* 1990; Ritz 2007), although this is not evidence that the two populations are the same when considered at a macro scale.

This chapter aimes to test the source of inocula which could lead to the development of a distinct soil surface phenotype by testing Hypothesis F:

The distinct microbial soil surface community originates exclusively from an aerial source.

8.2. Materials and Methods

8.2.1. Materials

Opaque PVC pots (150 mm internal diameter, 500 ml volume) were apportioned into 'open' and 'closed' treatments. A hole was cut in the lids of each pot, leaving an approximately 2 mm lip around the circumference. This meant that the lids could still be screwed down firmly onto the pots. A neutral density filter (Lee filter number DS 130 Clear, as used and detailed in Chapter 6), was attached to half of the lids using epoxy resin.

A hole was drilled below each rim into the side of each pot, and a Hepa-Vent™ (Whatman®, Maidstone, Kent) glued therein. Hepa-Vents™ are bi-directional filters housed in a polypropylene casing. The filters are made of laminated, hydrophobic treated glass micro-fibre. These filters retain particles of 0.3 μm and larger and allow air to pass. This means that in the air in the closed microcosms will still be exchanged between the microcosms and the external environment, but microorganisms will be severely restricted from entering the microcosms.

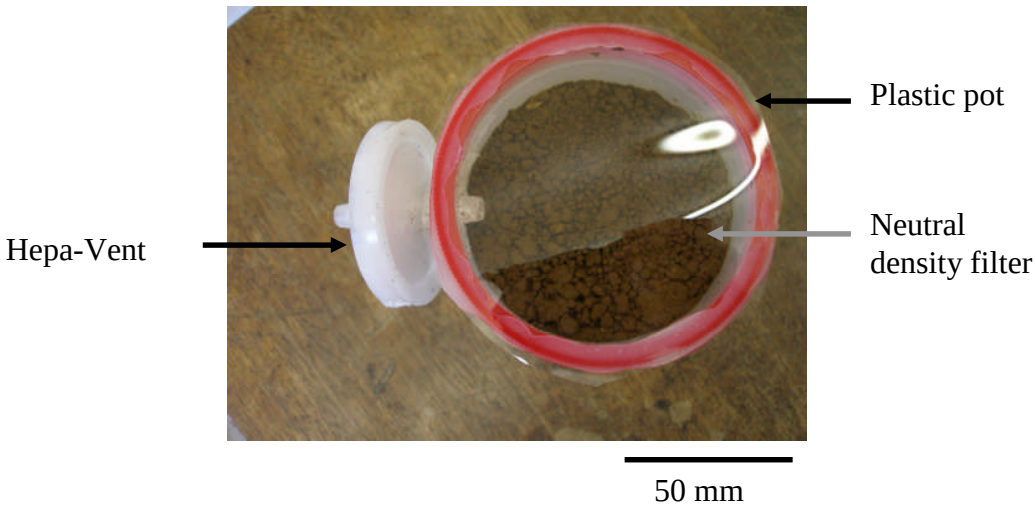


Figure 8.1. Microcosm arrangement showing the neutral density filter and Hepa-Vent™ used to allow air circulation without allowing entry of external microorganisms.

8.2.2. Methods

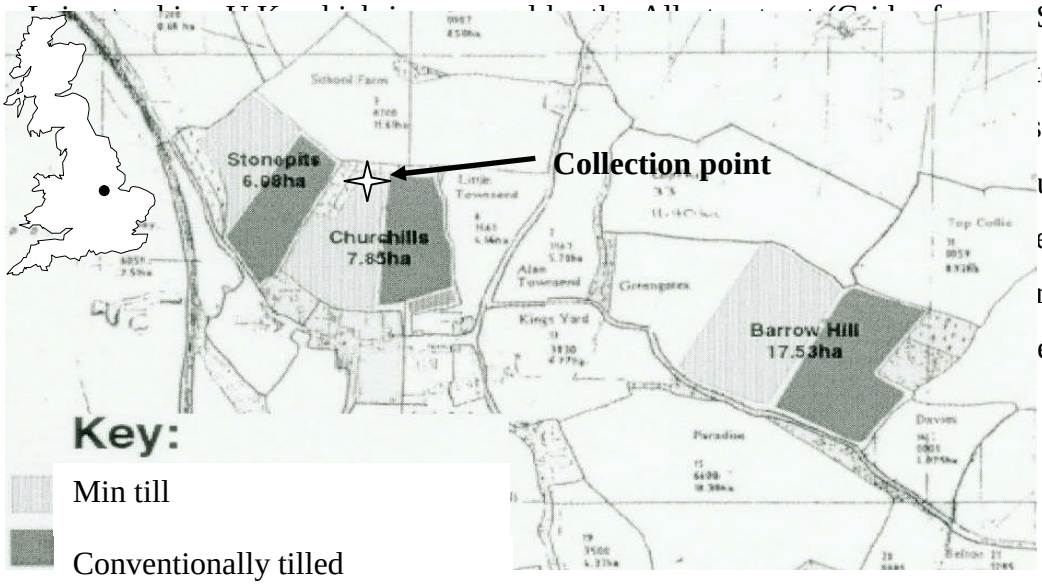
Arable soil was collected from Churchills Field on the Allerton Estate in Loddington,


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Figure 8.2 Map showing place of soil collection within Allerton Trust research farm.

Surface soil was collected to a depth of approximately 5 cm. This soil is hereafter denoted the “Surface layer”. The spade was then cleaned with 75% industrial mentholated spirits (IMS) and the soil removed to approximately 30 cm in depth and placed to one side of the hole. The spade was then cleaned again with 75% IMS. Soil was then taken from approximately 30 cm in depth down to approximately 40 cm in depth. This soil is denoted the “Deep layer”. Care was taken at all times to prevent soil from any of the top layers being mixed in with soil removed from the deeper layer.

Salt Marsh soil was collected from Abbotts Hall Farm, Essex, close to Salcott Creek (Grid reference TL 963 135). Soil here was undisturbed salt marsh and was regularly inundated with sea water. This soil was used to provide a highly contrasting system to arable systems.

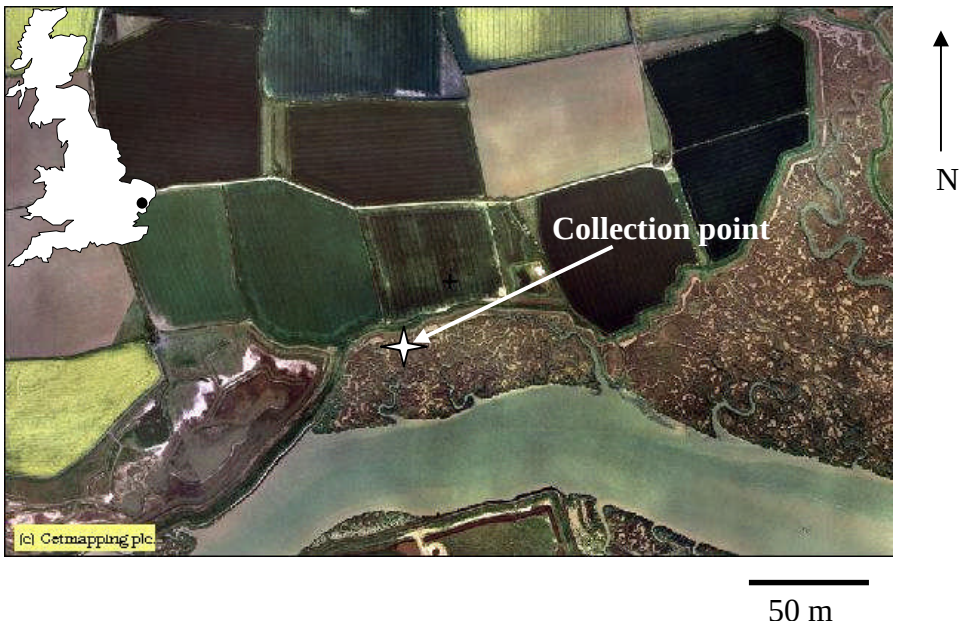

Figure 8.3 Aerial photograph showing location of soil collection from salt marsh.

The same sampling strategy as outlined about was used to collect soil from the surface, down to approximately 5 cm in depth, the "Surface layer" and again from a depth of between approximately 30 and 40 cm, the "Deep layer". As soil was taken from mature salt marsh, there is a relatively high confidence that soil taken from the Deep layer had not been exposed to the surface environment for a number of years, although the exact time frame is unknown.

8.2.3 Experimental design

The arable soil was a clay soil and was sieved to 2 mm and packed into pots at a dry bulk density of 1.4 g cm^{-3}. Approximately 1 cm was left at the top of each pot to allow air to circulate from the Hepa-Vents™ attached to the side of the pots. Due to its high moisture content and silty nature it was not possible to sieve the salt marsh soil. Instead this was mixed thoroughly using a trowel. The salt marsh soil was packed into the microcosms to a dry bulk density of 0.8 g cm^{-3}. This relatively low dry bulk

density was the maximum that could be achieved due to the high moisture content of the soil and it's inherently sticky nature.

Sterile microcosms were sterilised by exposure to gamma radiation at a minimum of 25 KGy at Isotron Ltd. (Swindon, UK). All microcosms were left outside, uncovered at Cranfield University, Silsoe, in a randomised block design, with the blocks running from north to south to mitigate the effects of any shadows at sunrise or sunset and left for a period of 18 weeks.

Pots were divided into 10 treatments each of three replicates, which factorially combined the depth and status regarding isolation from inocula, denoted as follows:

1. Arable surface layer, open microcosm;
2. Arable surface layer, closed microcosm;
3. Arable deep layer, open microcosm;
4. Arable deep layer, closed microcosm;
5. Salt marsh surface layer, open microcosm;
6. Salt marsh surface layer, closed microcosm;
7. Salt marsh deep layer, open microcosm;
8. Salt marsh deep layer, closed microcosm;
9. Sterile arable soil, open microcosm;
10. Sterile arable soil, closed microcosm.

Using this experimental design, there is a chance that some of the differences seen in community level phenotypes, between surface layers of the open and closed microcosms, if found, may be attributable to differences in environmental conditions arising due to restricted airflow in the closed microcosms. However, work presented in this thesis has shown a strong suggestion that light is the most important environmental parameter affecting the formation of a distinct soil surface community phenotype and as such was the most important parameter to prevent variation of

between treatments. Through use of neutral density filters variation in light between treatments was kept as small as possible.

The sterile microcosms were used for two reasons. The sterile soil in the closed microcosms was used to check the robustness of the microcosm design and to ensure that they remained sealed with regards to external inoculation. The sterile soil in the open microcosms was used to allow quantification of the aerospora without the influence of any underlying soil microbial effects.

Soil was then removed from the surface layers of the microcosms, to a depth of approximately 1 mm, followed by a subsequent layer, removed to a depth of a further approximately 5 mm. These sample layers will hereafter be referred to by the mean depth at which they were sampled, i.e. the 1-mm layer and the 4-mm layer respectively. This was done by placing the microcosms on their side and carefully scraping the surface with a palette knife and removing the soil onto a clean plastic trays. The soil was then removed to clean plastic bags and stored in a fridge at 4°C prior to analysis.

After 18 weeks the closed sterile microcosms were analysed for sterility via D-glucose substrate induced respiration as described by Degens and Harris (1997). This analysis works through detecting any increase in devolved CO_2 in the presence of a substrate compared to in the presence of water. Any increase in the presence of glucose is due to increased respiration as the glucose is used as a substrate by the microbiota.

Six 5.0 g wet weight aliquots of soil were taken from each of two of the three sterile closed microcosms and placed in sterile Suba-sealed glass bottles. The third sterile microcosm was not sampled as it had been clearly disturbed by some unknown mechanism, and the neutral density filter had been removed from the top of the microcosm, meaning it was no longer closed and hence sterile. Deionised water (to function as a control) and 75 mM glucose were each added to three randomly-selected aliquots from each sterile microcosm. Sufficient liquid was added in each case to just cover the soil at the bottom of each glass bottle. These were then incubated at 25°C for 4 h. The amount of CO_2 emitted from the soil was then analysed by gas

chromatography using a Csi200 series gas chromatograph with thermal conductivity detector and the software Clarity Csi 200 TCD.

8.2.4 Data analysis

Principal component analysis (PCA) using covariance and *post-hoc* analysis of variance (ANOVA) was undertaken using %mol data for PLFA profiles. *Post-hoc* ANOVA, using Fisher's least significant difference (LSD) was used to test differences in chlorophyll *a* and microbial biomass C between samples from different soil layers. All analyses were performed using Statistica 7.1 (Statsoft Inc, 2005).

8.3 Results

There was no significant difference between the amounts of CO_2 present within the Suba-sealed glass bottles upon the addition of water when compared to the amount of CO_2 present upon the addition of 75 mM D-glucose ($P = 0.62$). This means that the steile microcosms had remained sterile after the 18 weeks in the field.

8.3.1.Arable Soil

Analysis of PC1 of the principal component analysis of the PLFA data, via *post hoc* factorial ANOVA shows that there were no significant differences in phenotypic community structure in relation to the original depth from which the soil was taken in the arable field ($P = 0.2$). There were significant differences between microcosms left open or closed ($P < 0.01$), and the depth from the microcosm that the soil was taken from for analysis ($P < 0.01$). There was also a statistically significant interaction between whether the microcosm was open or closed, and the depth from the microcosms that the soil was taken for analysis ($P < 0.01$; Figure 8.5).

With regards to PC1, which accounted for a substantial proportion of the variance in the analysis, the points can be aggregated into three apparent groups. The data from the 1-mm layer inthe open microcosms group together and are significantly different to all other layers from all other treatments ($P < 0.01$), but are not significantly different to each other ($P > 0.5$). The 4-mm layers in the closed microcosms were significantly different to all other treatments, although there was no significant difference with regards to the depth from the field that the soil was originally collected. The 1-mm layers, from the closed microcosms and the 4-mm layers from

the open microcosms group together. They were significantly different to the two groups described above but not significantly different from each other in PC1. It is notable that both the 1-mm and 4-mm layers from within the sterile treatment were found to not be statistically different from the same layers of the open microcosm treatment with regard to PC1.

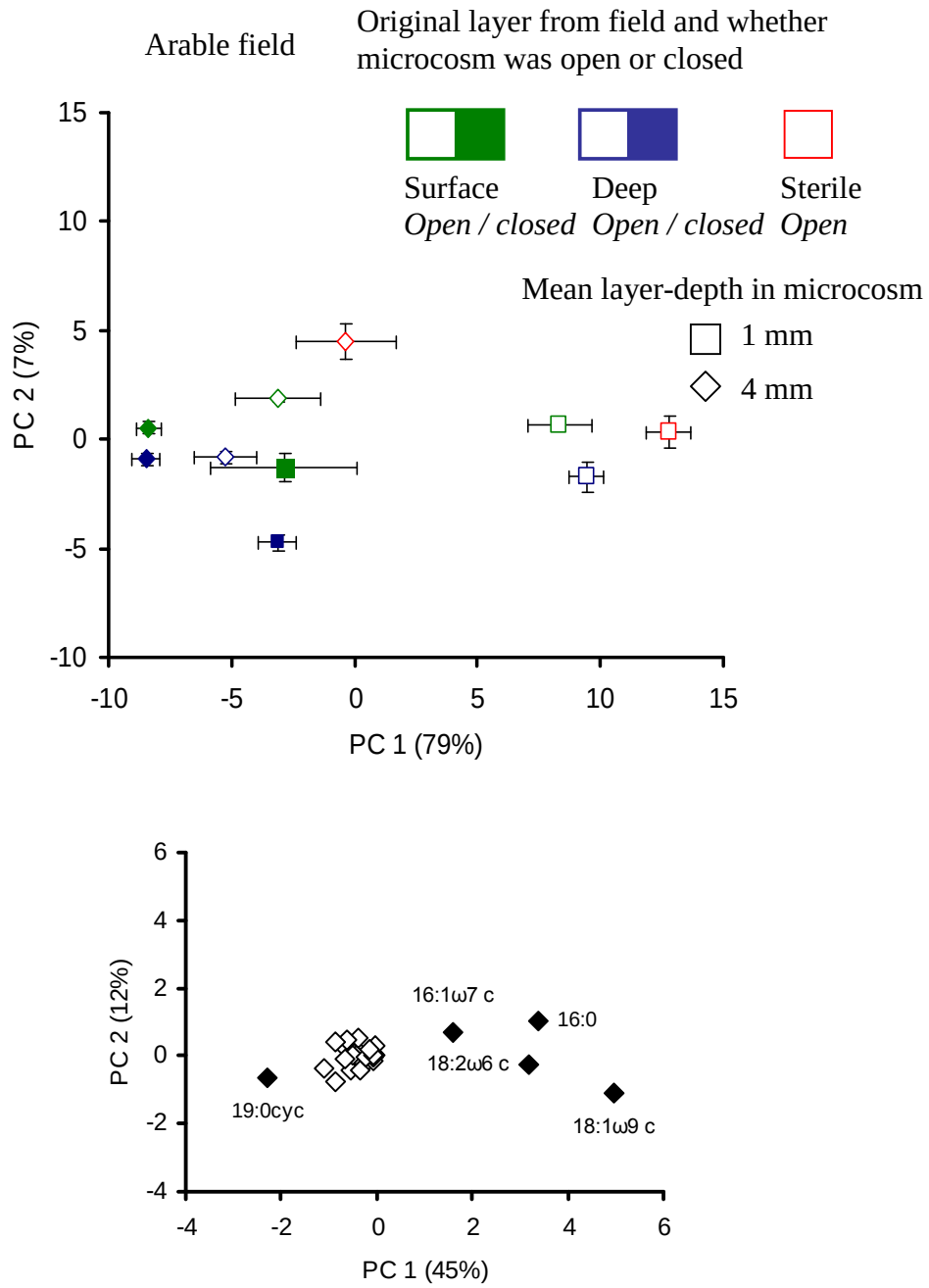

Figure 8.5 First and second principal components plots (PCs) and loading plots with those PLFAs contributing most to the discrimination seen in the PC plot highlighted in black and named. Data was derived from PLFA profiles originating from surface layers of microcosms from soil taken from two depths of an arable field which had

been under min till for at least 10 years. Microcosms were either sealed to prevent influx of external inocula, or left open to the environment. Points show means (n = 3), bars show standard errors. Percentage variation accounted for by PC shown in parenthesis on each axis.

The phenotypic community structure in the 1-mm layer from each microcosm was significantly different to the 4-mm layer with regards to PC1 ($p < 0.01$), in all microcosms with the exception of surface layer soil in the closed microcosm ($P = 0.3$) (Figure 8.5). There was tentative evidence for some discrimination between depths with regards to PC2 in the Surface layer in the closed microcosms but this was not found to be statistically significant at $P = 0.05$ (Figure 8.5; $P = 0.09$). No significant differences were found to exist between depths with regards to PC2 in any other of the microcosms (Figure 8.5)

A factorial ANOVA found that there was a significant difference in community level phenotypes between open and closed microcosms ($p < 0.01$) and the depth from each microcosm from which soil was analysed ($p < 0.01$). However, there was no significant difference as to where the original soil was taken from ($p = 0.4$). The interaction between whether the microcosm was open or closed, and the depth from which the soil was taken from each microcosm was found to be statistically significant ($P < 0.01$). No other interactions were found to be statistically significant.

8.3.2. Salt Marsh Soil

A factorial ANOVA showed there to be a significant treatment effect with regards to PC1 depending on whether the microcosms were open or closed ($p < 0.01$) (Figure 8.6). There was found to be no significant difference in community level phenotypes between the depth from each microcosm that the soil was sampled ($p = 0.68$) or the depth that the initial soil came from ($p = 0.41$). No interaction effects were statistically significant and no significant effects were found to occur with regards to PC2.

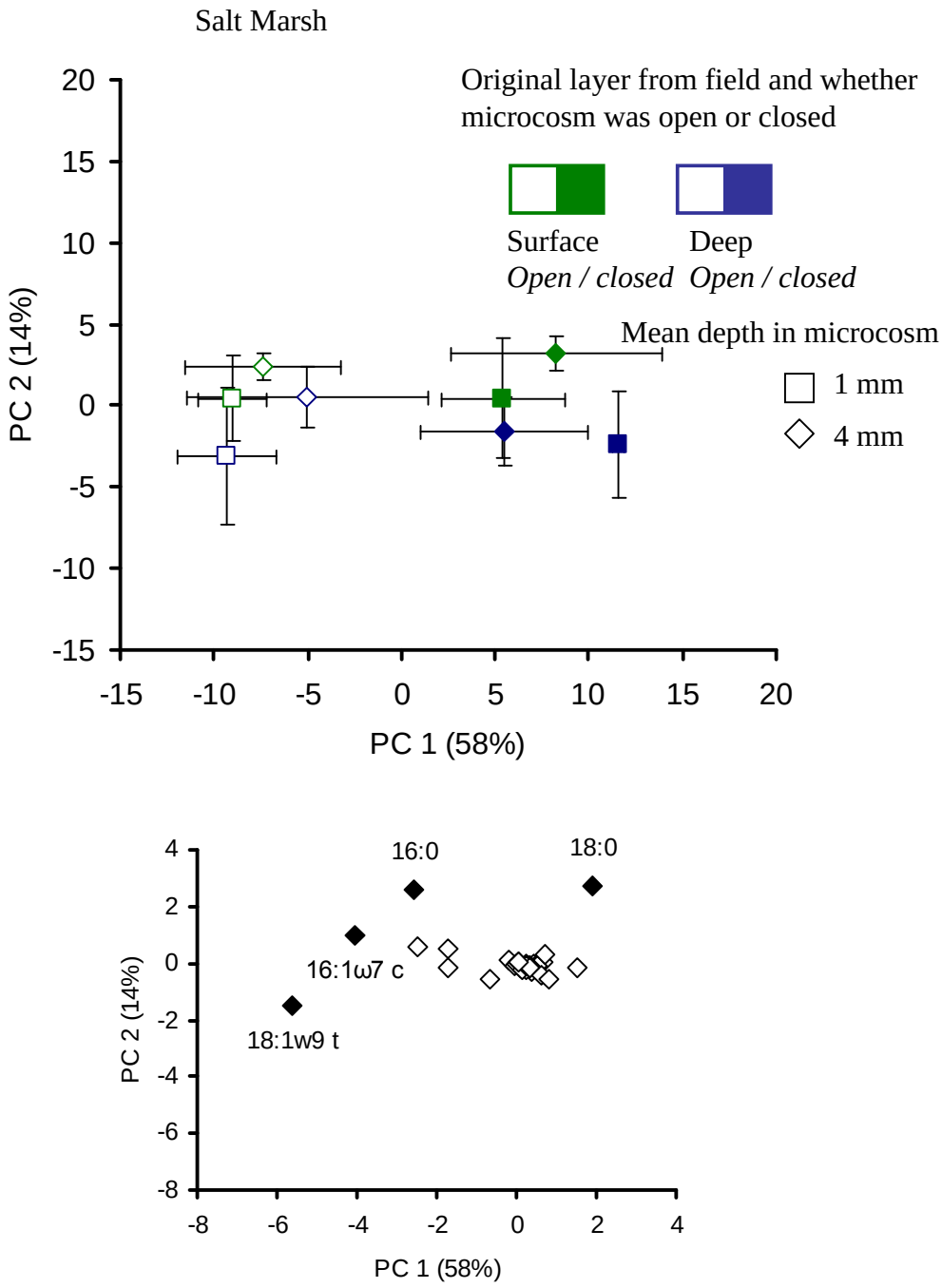

Figure 8.6 First and second principal components plots (PCs) and loading plots with those PLFAs contributing most to the discrimination seen in the PC plot highlighted in black and named. Data was derived from phospholipid fatty-acid profiles originating from surface layers of microcosms from soil taken from two depths of a salt marsh. Microcosms were either sealed to prevent influx of external inocula, or left open to the environment. Points show means (n = 3), bars show standard errors. Percentage variation accounted for by PC shown in parenthesis on each axis.

In the arable soil, there was no significant difference in the proportion of fungal to bacterial PLFA biomarker ratios with regards to the depth of the soil that was used to pack the microcosms ($P = 0.4$). Significant treatment effects were found to exist both in terms of the depth at which the soil was sampled in each microcosm ($P < 0.01$) and in terms of the microcosms were open or closed ($P < 0.01$) (Figure 8.7 a). A statistically significant interaction was found to occur between the depth at which the soil was sampled from each microcosm and whether the microcosm was open or closed ($p < 0.01$). A significantly greater ratio of the proportion of fungal to bacterial PLFA biomarkers was found to exist in the surface layer to a mean depth of *c.* 1 mm in the sterile microcosm treatment when compared to a soil sampled from a mean depth of *c.* 4 mm ($P < 0.01$) (Figure 8.7 a).

The salt marsh soil showed a general tendency to have a lower proportion of fungal to bacterial PLFA biomarkers than in the arable soils (Figure 8.7 b). The depth that the salt marsh soil was collected from which was used to pack the microcosms had no significant effect on the proportion of fungal to bacterial PLFA biomarkers, although this result was close to being significant ($P = 0.06$). Whether the microcosms were open or closed did significantly affect the ratio of the proportions of fungal to bacterial PLFA biomarkers ($P = 0.04$). The interaction between the depth at which the original salt marsh soil used to pack the microcosms was collected and whether the microcosms were open or closed was found to be statistically significant ($p = 0.03$). The interaction between where the sample depth from within each microcosm and whether the microcosm was opened or closed was also found to be statistically significant ($P = 0.02$).

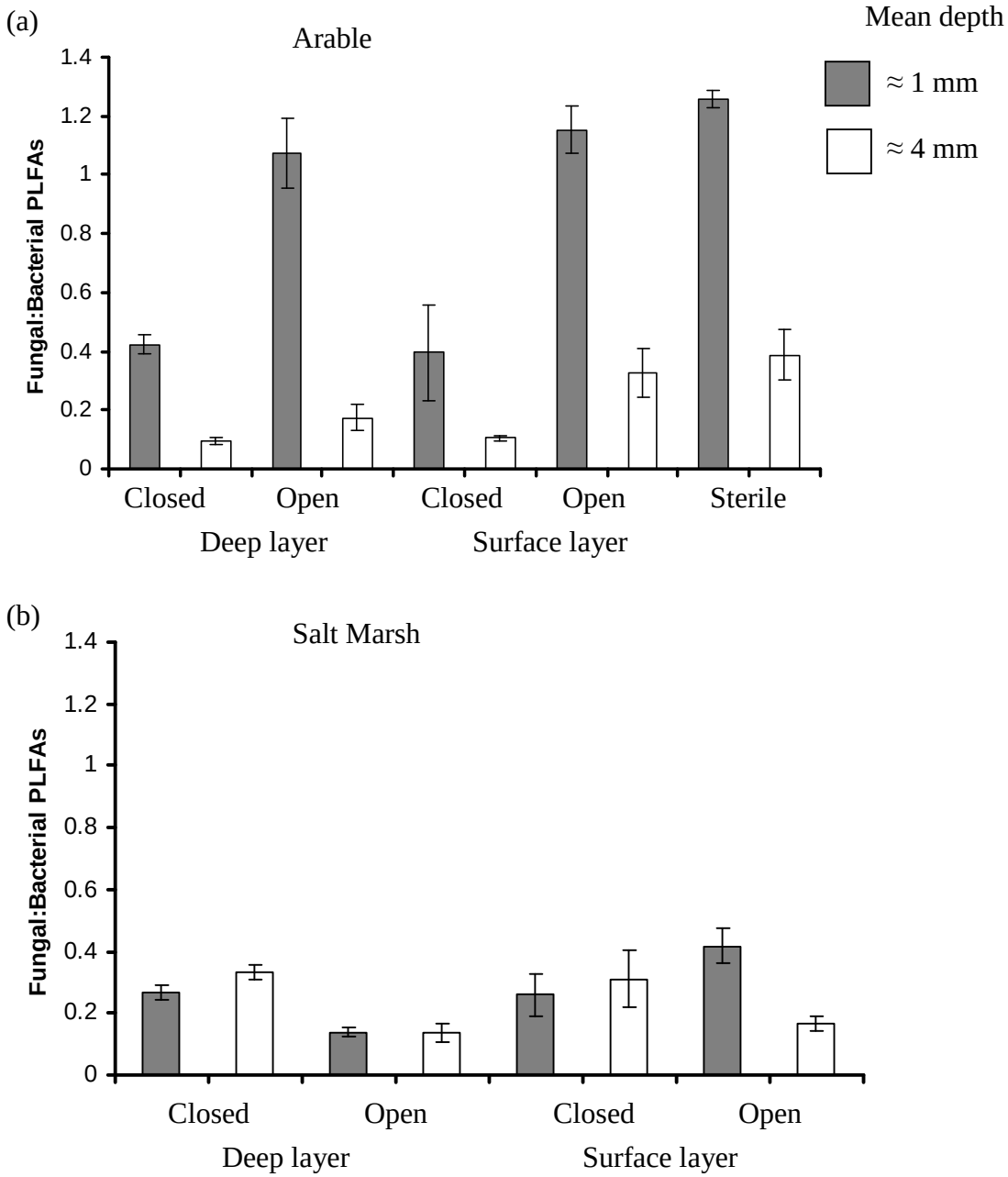

Figure 8.7 Ratio of fungal to bacterial PLFA biomarkers from soil surface layers of microcosms from soil taken from two depths of both (a) an arable soil and (b) a salt marsh. Microcosms were either sealed to prevent in flux of external microbiota, or left open to the environment. n = 3, bars show standard errors.

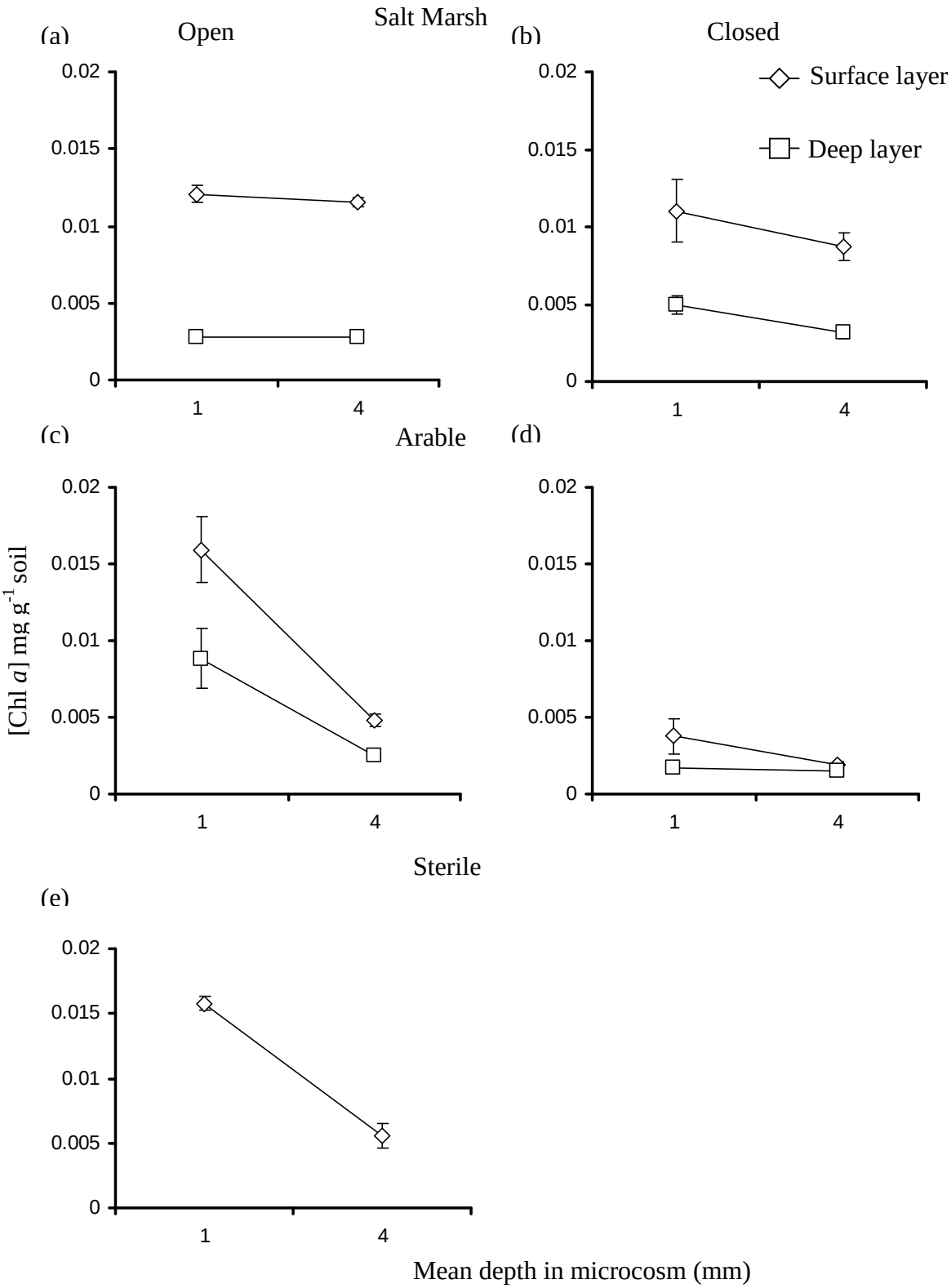

Figure 8.8 Mean chlorophyll concentrations extracted from two surface layers from microcosms packed with soil collected from a salt marsh (a & b) an arable field (c & d), and sterile arable soil (e). The microcosms were left open to the environment or were closed and were left in the field for a period of 18 weeks. n = 3, bars show standard errors (bars fall within confines of points in some instances).

Significantly higher concentrations of chlorophyll were found in the Surface layer of the salt marsh soils when compared to the Deep layer in both the open and closed treatments ($p < 0.01$) (Figure 8.8 a & b). There was no significant difference with regards to chlorophyll concentrations in either the Surface layer or the Deep layer with regards to depth of soil layer within the microcosms at the scale investigated in the salt marsh soils ($p= 0.5$) (Figure 8.8 a & b). Significantly higher concentrations of chlorophyll were found to a depth of 1 mm in the open microcosms containing arable soils in both soil from the Surface layer and the Deep layer ($p < 0.01$ and $p = 0.03$ respectively; Figure 8.8 c). There was no significant difference in chlorophyll concentrations obtained from either the Surface layer or the Deep layer from the arable soil in the closed microcosms at the scale investigated ($p = 0.18$ and $p = 0.69$ respectively) (Figure 8.8 d). The sterile soil showed a significant increase in chlorophyll concentrations in the 1-mm layer compared with the 4-mm layer ($p < 0.01$; Figure 8.8 e).

8.4. Discussion

As stated previously, the sterile arable soil in the closed microcosm was set up as a control to test that no microorganisms from the external environment could enter into the closed microcosms. By measuring respiration of these sterile microcosms at the time of analysing the other microcosms it was possible to test that no microorganisms had managed to enter the microcosms so that any changes in the closed microcosms can be assumed to have happened without the influence of the external microbial population. As there was found to be no significant difference in respiration between the addition of water and the addition of D-glucose it can be concluded that the microcosms had remained sterile for the 18 weeks that they were left in the field. This means that the robustness of the microcosm design was sufficient to ensure that no external inoculum entered the microcosms during the 18 weeks that they were in the field.

With regards to the sterile open microcosm, this treatment was designed to allow the analysis of the aerospora. The aerospora, entering the sterile soil system, developed the same community level phenotype as when the aerospora fell onto a soil surface which was already inhabited by microorganisms, i.e. the non sterile microcosms. This shows that the conditions at the soil surface are such that they drive the formation of

the same community phenotype regardless of the microorganisms already present at the soil surface. A genetic analysis comparing the aerospora with the soil surface microbial community in the 1-mm layer would provide insight into the development of the soil surface phenotype. It may be that conditions are such that relatively different genotypes produce similar phenotypes under the environmental conditions found at the soil surface, or it may be that as conditions regarding environmental factors such as solar radiation are similar either at the soil surface or some distance above it, the community level genotype of the soil surface community may be very similar to that of the aerospora. Further work isneeded to be done to test this hypothesis.

The environmental conditions at the soil surface were generally sufficient to drive the formation of a distinct microbial soil surface phenotype, as measured by PLFA analysis within all treatments, whether exposed to inoculation from external microorganisms or not, with the exception of Surface layer soil in the closed microcosms (Figure 8.4). Community structure in the 1mm layer sampled from all of the open microcosms containing arable soils was found to be statistically more significantly different than when compared to the subsequent 4-mm layer than a comparison between the two layers in the closed microcosms. This is evident from the amount of variation attributable to PC1 and the distance between points from each layer. This implies that whilst the light arriving at the soil surface in the closed microcosms was sufficient to drive changes in the soil surface microbial phenotype, this change was decreased through the admission of aerial biota onto the soil surface.

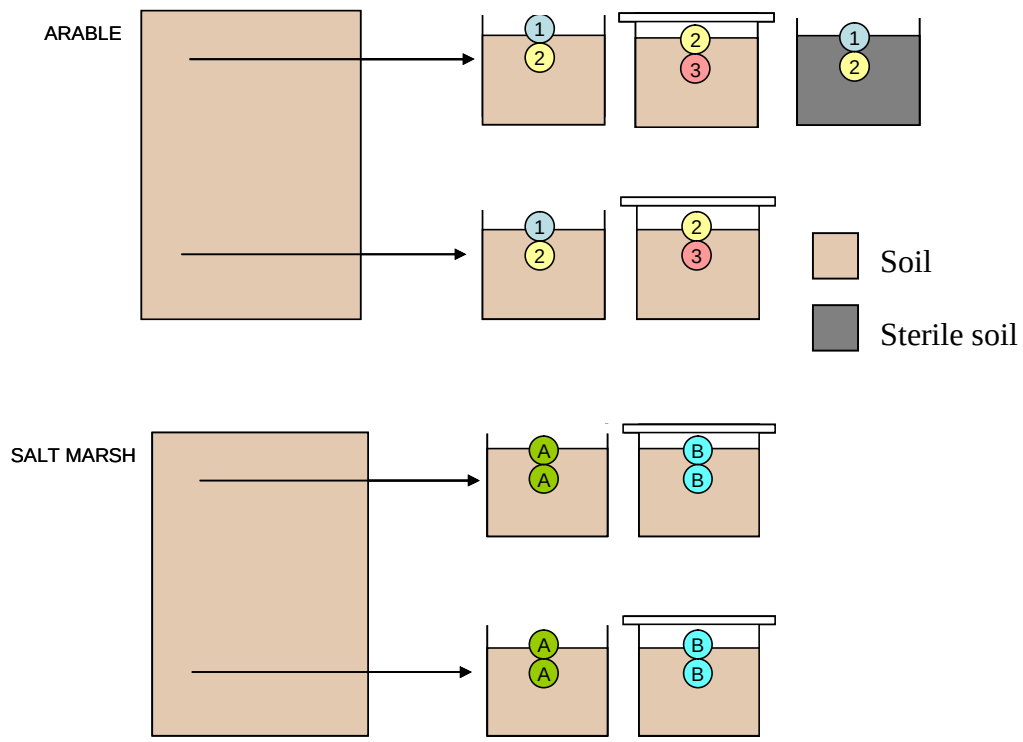

Figure 8.9. Schematic diagram of summarising relationships between phenotypic structure of soil communities shown in Figures 8.5 and 8.6 Numbers and letters represent similar community phenotypes.

These results show that Hypothesis F, *The distinct microbial soil surface community originates exclusively from an aerial source*, should be rejected (Figure 8.9). This is because a distinct soil surface phenotype developed in the 1-mm layer of the closed microcosms. Therefore, it appears that both the aerobiota and the soil biota play a part in the establishment of a distinct soil surface community level phenotype. It appears that when the influence of an aerial source is removed from an arable soil surface the environmental parameters at that soil surface are sufficient that a distinct soil surface community will still develop. However, the make up of that soil surface microbial community may well be different to what would have developed under the influence of an aerial source of microbial inoculum.

With regards to the salt marsh soil, the fact that the only significant difference occurred between open and closed microcosms implies that the aerobiota are responsible for the discrimination seen between phenotypes from each treatment. However, as no significant depth effects were found, any aerobiota falling onto the

surface must also have either migrated, or been washed down through the soil profile at the millimetre scale investigated. One alternative hypothesis is that rainfall events changed the salinity of the soil in the open microcosms which then affected the phenotype expressed by the microbial community. Further work would be needed to test this hypothesis by measuring the salinity of the soils from both the open and closed microcosms and looking for any differences which may have developed.

The PLFAs generally responsible for causing discrimination between depths within each microcosm were 18:1ω9c, 18:2ω6c and 16:0. The PLFA 16:0 has been found to be responsible for much of the discrimination found between soil layers in previous experiments and has already been extensively discussed. The PLFA 18:2ω6c is considered to be a fungal biomarker and is the biomarker used for calculating the fungal proportion of the fungal to bacterial PLFA ratios. This experiment is the first time in this project that the PLFA 18:1ω9c has been found to be responsible for much of the discrimination seen between soil layers. The PLFA 18:1ω9c has been found to exist within the cellular membranes of plants, green algae, cyanobacteria, fungi and brown algae (Harwood and Russell 1984) and as such it is not possible to say with confidence and without further work which group or groups were responsible for the discrimination seen between treatments.

With regards to chlorophyll concentrations and therefore photoautotrophs, the soil surface community appears to exist within at least the top 4 mm of the soil surface as demonstrated by the lack of change in chlorophyll concentrations moving down through the soil profile at this scale. This is possibly an adaptation on the part of the salt marsh soil surface microbial community as the salt marsh soil surface can be expected to be a more disturbed environment compared to an arable environment due to the periodic inundation with water. Buried photoautotrophs would need to retain relatively high levels of chlorophyll to be able to maximise their utilisation of light if they can make it to the surface environment at a point where PAR penetrates. Evidence exists of the vertical movement of photoautotrophs within the soil surface in inter-tidal zones (Kingston 1999) as well as other soils where there is an excess of water (Davey and Clarke 1991). The lack of difference in chlorophyll concentrations may be attributable to the migration of photoautotrophs to and from the salt marsh surface.

The lack of any significant difference inchlorophyll concentrations between the open and closed salt marsh microcosms implies that any photoautotrophs falling onto the surface of the open salt marsh soils were unable to establish themselves. This is possibly due to the relatively high salinity which the salt marsh soils can be expected to have compared to arable soils and would result if any photoautotrophs in the aerobiota were not halotolerant or halophilic. Further research would need to be done to test this hypothesis, but it seems likely that the main source of inoculum of photoautotrophs onto the salt marsh soil surface would be the sea, as this hosts a wide variety of halotolerant and halophilic photoautotrophs.

There is some evidence that Hypothesis F is correct for photoautotrophs at least. There was a significant increase in the concentration of chlorophyll *a* extracted from the surface layers of the open microcosms compared to the closed microcosms containing the arable soil (Figure 8.5 c & d). This increase must be a result of photoautotrophs falling onto the soil surface, or being washed onto it from rain. The increase in chlorophyll *a* concentration in soils collected from the Surface layer compared to soils collected from the Deep layer is likely to be a result of a greater prevalence of photoautotrophs in the soil from the Surface layer initially. This hypothesis is backed up by the data presented in Chapter 4 which demonstrated that a higher concentration of chlorophyll *a* was present in the soil surface of arable systems compared to deeper soil layers, even on a millimetre scale (Figure 4.4).

The concentrations of chlorophyll *a* extracted from the top soil layers of the sterilised microcosms are very similar to those obtained from the Surface layer soil from the open microcosms.These data suggest that photoautotrophs are derived aerially, with changes in community level phenotypes at the soil surface being supplemented by the underlying soil. This also provides evidence against the hypothesis that “everything is everywhere”, since if this were the case similar phenotypes would have formed whether the microcosms were open or closed. This did not happen, especially with regards to photoautotrophs, and can only have resulted from differences in the proportions of photoautotrophs in the aerospora when compared to the soil system.

Chapter 9: General Discussion, Experimental Considerations and Future Work

9.1. Summary

The original objective of this project was to investigate whether a distinct soil surface microbial community is generally apparent within the surface of arable soils, and if this was the case, then to investigate how and why such communities develop as well as some of the functional consequences of such phenomena.

Environmental factors such as temperature and solar radiation were shown to be greater at the soil surface with regards to both amplitude and frequency of fluctuations (Chapter 3), and as such the hypothesis that environmental factors underwent a greater range of larger and faster fluctuations was accepted. These environmental factors were shown to be sufficient to drive the formation of a distinct soil surface phenotype within the surface of arable soils on a millimetre scale if there was sufficient time between disturbance events. This led to the acceptance of the hypothesis that the environmental factors at the arable soil surface would drive the formation of a distinct soil surface phenotype (Chapter 4).

A controlled microcosm experiment then showed that the distinct surface phenotype developed after approximately 8 weeks in undisturbed systems, with the ratio of fungal to bacterial PLFA biomarkers increasing to become significantly higher in the surface layer after approximately 8 weeks, and the amount of chlorophyll *a* increasing to become significantly higher in the soil surface layer compared to subsequent layers after 16 weeks. Therefore, the hypothesis that the soil surface microbial community would change overtime after a new surface is exposed was accepted (Chapter 5). Light, specifically PAR, was then demonstrated to be the environmental factor most responsible for driving the formation of a distinct soil surface phenotype and so the hypothesis regarding light being the most important factor in driving the development of a distinct soil surface microbial community was also accepted (Chapter 6).

With regards to the functional consequences of the distinct soil surface community level phenotype, generation of rainfall run-off and shear strength at the soil surface were found to be affected, although these results did not translate into any statistically significant difference with regards to water infiltration rates or the propensity of the

surface to erode. For this reason the hypothesis regarding the effects of the soil surface microbiota on soil surface physical and hydrological properties was accepted with regards to run-off, shear strength and crust formation, but was rejected with regards to water infiltration and erodibility (Chapter 7). The effects of the presence or absence of UV A on the soil surface microbial community was found to alter that community in such a way that in turn the community affected the structure of soil surface so that porosity was reduced. However, this reduction in porosity lower than the reduction in porosity bought about by physical crust formation due to the erosion trays being exposed to rainfall events (Chapter 7).

The source of inocula for photoautotrophs in the arable soil surface layer was found to be aerial as shown by chlorophyll *a* concentrations in Chapter 8. However, environmental parameters at the soil surface were still sufficient to drive the formation of a distinct soil surface community level phenotype even when cut off from aerial inocula. Therefore the hypothesis that the distinct soil surface community originated exclusively from an aerial source was rejected (Chapter 8).

This study has demonstrated that the relatively extreme environmental conditions which exist at the soil surface layer when compared to subsequent soil layers on a millimetre scale are sufficient to drive the formation of a distinct soil surface microbial community phenotype which appears confined to an order of 1 mm depth. This community level phenotype takes approximately 8 weeks to become established in undisturbed systems, and PAR is the main factor in driving its development. Much of the inoculum with regard to photoautotrophs comes from an aerial source, but removing the aerial source of inoculation is not sufficient to prevent a distinct soil surface phenotype from forming, albeit it with a significant reduction in the proportion of photoautotrophs.

9.2. Functional Consequences of the Soil Surface Phenotype

The functional consequences of the soil surface community level phenotype included effects on the hydrological properties of the surface as well as the surface's propensity to form crusts, although this study did not find any significant differences in soil erodibility due to different soil surface microbial communities. That said, work undertaken in Chapter 8 demonstrated that by isolating the soil surface from aerial

inocula, the soil surface community phenotype which developed was different from that which would develop upon exposure to aerial inoculum with regards to the proportion of photoautotrophs present. Therefore, extrapolation of the functional consequences of the soil surface phenotype from Chapter 7 is restricted. Maxwell and Neuman (1994) found that photoautotrophic microorganisms were important to particle aggregation with regards to surface stabilisation against wind erosion. It is likely that the reported surface stabilisation may also have an effect, reducing erosion from raindrop impact and overland flow due to the reported increased surface stabilisation although this was not investigated in the study. In the experiment described in Chapter 7, the lack of statistically significant differences found in erosion rates under the same conditions between treatments may have been partially due to the lack of significant differences found in photoautotrophic communities as demonstrated by similar chlorophyll concentrations under the four different treatments.

The fact that significant differences were found with regards to photoautotrophic communities under the different light treatments in Chapter 6, where microcosms were also cut off from aerial inocula, may have been a result of a higher inherent photoautotrophic population in the homogenised soil which was used to pack the microcosms. Evidence for this hypothesis comes from the fact that whilst the chlorophyll concentrations reported in Chapter 6 were three times greater than those reported in Chapter 7, they were still less than 10% of those found at the arable soil surface analysed in Chapter 4. This implies that a greater concentration of chlorophyll a would have been found in the surface layers of the microcosms analysed in Chapters 6 and 7 if those microcosms had been exposed to aerial inocula of photoautotrophs. The soil surface layers of microcosms analysed in Chapter 5, which were uncovered and so exposed to photoautotrophic aerial inocula, had chlorophyll concentrations almost twice those reported in Chapter 6, but were still found to have approximately seven times lesschlorophyll concentrations compared to the field soil analysed in Chapter 4. The disparity in chlorophyll concentrations reported between those from the field soil in Chapter 4 and from the final sampling occasion in Chapter 5 may be a result of environmental conditions at the time of sampling, or the fact that the soil surface used for the chlorophyll analysis used in Chapter 4 had been undisturbed for longer than the surfaces used in Chapter 5. This would mean that they had been

exposed to photoautotrophic inocula from an aerial source for a longer period of time and so would be expected to have a higher concentration of photoautotrophs in the soil surface layer.

Further work is required to discern the true effects on the soil surface's erodibility of a soil surface community phenotype which has a proportion of photoautotrophs similar to that which may be found in arable systems. This is because cyanobacteria have been shown to increase aggregate stability in soils (Falchini *et al.* 1996; Issa *et al.* 2007; Nisha *et al.* 2007). Barclay *et al.* (1985) also reported that algae can increase soil surface aggregate stability in agricultural soils through the production of extracellular polysaccharides. They also reported that this polysaccharide production was confined to approximately the top 1 mm of the soil surface, where 99% of the algal cells were found in their study, further confirming work undertaken in this study that the notion of a biologically distinct surface phase, particularly with regard to photoautotrophs appears confined to the top millimetre or so of the arable soil surface. As aggregate stability is known to be correlated with a soil's erodibility (Farres 1987) due to reducing detachment by rainfall impact and slaking, it seems likely that increases in the photoautotrophic biomass in those erosion trays which were exposed to PAR may have lead to significant effects with regards to erodibility.

9.3. Why study the phenotype?

The phenotype was used for community level analysis throughout this project as it integrates the genotype with the environment. This is a rational choice since this project was focused on both the interactions of the environment with the microbial communities found at different depth and how this interaction drives differences in the community level phenotype. Regarding the effects of the soil surface microbiota on soil surface physical properties and hydrology, again it is the phenotype that is most pertinent. PLFA analysis allows an assessment of community level phenotypes. Through statistical analysis, for quantification of differences between community level phenotypes to be made.

Phospholipid fatty acids do not yield taxonomic information with regard to community composition, for example to genus or species. However, whether taxonomic information regarding community composition at the soil surface is

pertinent is contingent upon the issues being considered. The aim of this project was to investigate whether a distinct soil surface microbial community could develop in arable systems, and if it did develop, then what were the main environmental factors which drove its formation and what were its functional consequences?

However, now that it has been demonstrated that a relatively consistent phenotype exists at the soil surface which appears confined a depth of approximately 1 mm further work could be undertaken to investigate whether the community level genotype, or metagenome, at the soil surface is also consistent.

PLFA analysis showed both replicably within experiments and repeatedly between them, throughout this project, that given sufficient time between disturbance events, a distinct and characteristic soil surface microbial community phenotype developed. Therefore, it was worthwhile investigating the functional consequences of that distinct surface phenotype even without ascertaining firm information with regard to the genetic make up of that surface community. Indeed, although no information with regard to genus or species can be ascertained through PLFA analysis, information regarding the presence, absence and proportions of various groups such as bacteria or fungi can be discerned. This information was sufficient to drive the formation of further hypotheses as the functional consequences of various groups of microorganisms have been well documented.

Molecular genetic studies would allow a greater understanding of the groups of microorganisms that exist at the arable soil surface to be identified to a higher taxonomic resolution. This information could be pertinent for studies which target more specific issues such as the behaviour of specific organisms and their movements within the soil system, or when examining the role of symbiotic relationships within soil systems. Information regarding the presence or absence and tracing of species would also be applicable when investigating biotechnological processes which are performed by very specific microorganisms, such as in bioremediation studies of recalcitrant compounds, where only very few microorganisms are capable of breaking the compounds down. Molecular genetic techniques would also be pertinent for tracking functional genes or gene groups within the soil microbiota to uncover information such as whether the majority of nitrogen fixation takes place at the soil surface or in deeper soil layers by looking for the greatest concentrations of *Nif* genes,

those genes which encode enzymes involved in nitrogen fixation such as the nirogenase complex.

Ecological theory states that in general, communities in early succession will be dominated by fast-growing, well-dispersed species (r strategists) and as succession proceeds, these species will tend to be replaced by slower growing species (k strategists) that are more able to utilise complex carbon compounds as an energy source. Due to the relatively high amount of disturbance at the arable soil surface when compared with deeper soil layers, for example because of erosion events caused by wind and rain it could be hypothesised that r strategists may be expected to dominate at the surface. Whilst it is conceivable that distinct phenotypes may be found between communities dominated by either r or k strategists it seems likely that the multitude of other variables that may affectthe phenotypes, such as different environmental conditions, different substrate availability and different taxonomic composition, it is unlikely that PLFA would be able to distinguish these ecological phenotypes. However, this information could be acquired through the use of MicroResp™ (Campbell *et al.* 2003) or Biolog™ (Garland and Mills 1991) experiments which focus on the functional properties of microbial communities with regard to substrate utilisation. Using these techniques, soil sampled from random areas of the surface could be tested for their respiration responses to different substrates. If the above hypothesis is correct then the soil surface microbial community would be expected to respond most to glucose soon after a disturbance event when r strategists dominate the microbial population, and for the response to change over time to show utilisation of more complex carbon compounds as k strategists come to dominate the microbial population.

9.4. Nutrient cycling.

9.4.1. Nitrogen

Some species of cyanobacteria and algae fix N_2 as well as CO_2. The fixing of N_2 by these organisms is a light-dependent process (Lund 1967) and so will only be performed at the soil surface.

Herbicides, including gyphosphate, have been shown kill cyanobacterial populations in the aquatic environment (Lopez –Rodas *et al.* 2007). It, therefore, follows that

cyanobacteria could be susceptible to herbicides in arable systems. By reducing the abundance of cyanobacteria at the soil surface the amount of carbon and nitrogen being fixed at the soil surface will also be reduced. Cullimore and McCann (1977) showed that herbicide application affected the make up of algal communities within the top centimetre of the soil surface. They found that whilst susceptible species were killed, this removal of competition meant that more tolerant species came to dominate and no overall reduction in cell numbers was reported. However, they also reported that there was an apparent reduction in the number of potential nitrogen fixers, which could have implications regarding soil fertility.

Understanding the functional consequences of the arable soil surface phenotypes with regards to nutrient cycling will increase our overall understanding of arable ecosystems. Witty et al (1979) reported that during years of average rainfall on field plots which received 48 kg ha^{-1} of nitrogen, algae were estimated to fix between 25 and 28 kg ha^{-1}. In plots which received no fertiliser, algae were estimated to fix between 13 and 19 kg ha^{-1} demonstrating that algae make a substantial contribution to soil fertility in unfertilised plots. Witty et al (1979) also reported that relatively high applications of N fertiliser of 196 kg ha^{-1} (compared to the UK average of 150 kg ha^{-1}) inhibited the fixation of nitrogen by soil algae. This demonstrates the importance of understanding soil microbial process as fully as possible so that the efficiency and sustainability of agriculture can be maximised.

This project has demonstrated that the soil surface community contains a high proportion of photoautotrophic organisms, including cyanobacteria and algae. Many of these organisms are known to fix N_2. This leads to the hypothesis that the photoautotrophic community is responsible for significant inputs of nitrogen into the soil system. This could possibly be tested using SIP with ^{15}N as described above and could allow for the investigation of gross scale measures of nitrogen fixation by soil surface microorganisms.

Alternatively, by having two controlled field areas this could be tested by having two soil surfaces that have been left undisturbed for sufficient amounts of time for a soil surface phenotype to develop i.e. at least 8 weeks. One could be covered (so that air could still circulate but no light could reach the soil surface) and the other left uncovered. Quantification of ammonium, nitrite and/or nitrate within the soils of the

two different treatments will allow for any treatment effects to be determined. Any differences occurring would be due to the fact that N fixation by algae is a light dependent process (Lund 1967). This hypothesis could also be tested by spraying herbicides onto a soil surface to prevent the growth of nitrogen fixing cyanobacteria and algae and examining the difference between this treatment and a control, thereby allowing the inputs of fixed nitrogen into the soil system by cyanobacteria and algae to be determined.

9.4.2. Carbon

This project has demonstrated that photoautotrophs exist in relatively high numbers at the soil surface in arable systems which have been undisturbed for sufficient time for a distinct surface microbial community to have formed compared to deeper soil layers on a millimetre scale. This has possible implications for carbon cycling. However, as the photosynthetic potential has been shown to be confined to an order of depth of approximately one millimetre, it seems unlikely that much carbon fixation potential exists compared to that provided by vascular plants. That said, only by investigating and understanding a variety of feedback mechanisms can the accuracy of models which aim to predict the effects of increased CO_2 be improved, and if the results are extrapolated to all soil surfaces, whilst still likely to be very small, the amount of CO_2 fixed by microorganisms in the soil surface layer may have some effect on the concentration of atmospheric CO_2.

It is now widely accepted that the concentration of CO_2 in the atmosphere is increasing due to anthropogenic influences (Forster *et al.* 2007). The soil surface is the main interface between the atmosphere and the lithosphere and as such it will play an important part in the exchange of gases between these two highly contrasting environments. It can be hypothesised that increased levels of CO_2 will lead to increased photosynthesis by photoautotrophs within the soil surface zone and hence lead to higher input of carbon into the soil system. Further work needs to be undertaken to test this hypothesis. As discussed previously, testing this hypothesis would provide pertinent information with regard to the functioning of the soil surface layer on a millimetre scale as a feedback mechanism. The work could be further scaled up to take into account other soil surfaces, for example in grassland or forest ecosystems which would provide more information for models which attempt to

predict the impacts of increased levels of CO_2 as well as increasing our understanding of the effects of interactions at the soil: air interface has on both atmospheric and soil CO_2 concentrations. This would be relatively simple to test experimentally through the use of small sealed, greenhouse type covering made from neutral density filters on soils on which a soil surface microbial phenotype has developed. The CO_2 levels within these coverings could then have their atmosphere controlled with artificially higher CO_2 levels, designed to coincide with the predictions of CO_2 increase over the next century or so. Any effects of the increased levels of CO_2 could then be measured (e.g.via increased biomass at the soil surface or an increase in production of chlorophyll by photoautotrophs to maximise their utilisation of CO_2 for photosynthesis).

Through use of stable isotope probing (SIP), as described by Boschker and Middelburg (2002), using CO_2 labelled with ^{13}C it would be possible to trace the movement of CO_2 into the soil surface and trace the percentage of CO_2 that moves into the soil system This would allow investigation of the movement of CO_2 once in the soil system to find what proportion is retained in the long term, and what proportion is fixed into sugars and then used and respired back into the atmosphere. This information may provide evidence as to whether increased levels of CO_2 will

9.5. Other soil surfaces

This project has focused on arable systems. However, environmental parameters are likely to drive the formation of a distinct soil surface phenotype in other ecosystems such as forests and grasslands. The phenotype and associated functional capabilities of the microbial communities at the soil surface will be dependent on the environmental factors driving its formation as well as being dependent on the complexity of the available substrates. The soil surface in forest and grassland ecosystems will receive much less PAR than arable systems due to the extensive canopy or grass cover. This hypothesis could be tested through repeated use of many of the techniques that have been used throughout this project.

Although the grassland soil surface will have a lower input of PAR, areas exist where grass is not present for various reasons, for example in areas previously affected by poaching by cattle. In these areas the surface is likely to be relatively undisturbed

compared to arable systems which are often ploughed seasonally and, as such, it is probable that there will be sufficient time for soil surface communities to become well established. One factor which will affect the soil surface in many grassland systems which is not present in arable systems, is manure and urine from sheep and cattle. These will create patches of high nutrient content and may contribute to increased microbial heterogeneity at the soil surface.

Due to the change in complexity and availability of substrate at the forest soil surface compared to arable systems due to events such as leaf fall it is probable that the soil surface microbial community in forest ecosystems will be more fungal dominated compared to deeper soil layers (Young and Ritz 2005). Higher levels of faunal activity occur in forest ecosystems meaning that there may be a greater turnover at the soil surface, and this combined with burying during leaf fall events leads to the question "how long is a forest soil surface a surface?" Work would need to be done to answer this question and to investigate whether the soil surface in forest systems is the surface for sufficient time for a distinct soil surface phenotype to develop

This thesis highlights that, at the previously unreported millimetre-scale, distinct soil surface microbial communities develop within the arable soil surface. Furthermore, the phenotypic composition of such communities appears to be between soil types. Such communities were shown to have functional consequences with regard to the soil surface physical and hydrological properties, and are likely to do so in other soil systems. The soil surface in any given terrestrial environment is the main interface between the atmosphere and soil, affecting hydrology as well as other factors such as gas exchange. Only by increasing our knowledge of this relatively poorly investigated zone can our understanding and our ability to predict the possible effects of various anthropogenic activities as well as the predicted increased severe weather events associated with climate change be enhanced.

Reference List

Allison, V.J. and Miller, R.M. (2005) Soil grinding increases the relative abundance of eukaryotic phospholipid fatty acids. *Soil Science Society of America Journal* **69**, 423-426.

Amato, P., Menager, M., Sancelme, M., Laj, P., Mailhot, G. and Delort, A.M. (2005) Microbial population in cloud water at the puy de dome: implications for the chemistry for the chemistry of clouds. *Atmospheric Environment* **39**, 4143-4153.

Anderson, M.C. (1966) Stand structure and light penetration: ii. A theoretical analysis. *Journal of Applied Ecology* **3**, 41-54.

Arrage, A.A., Phelps, T.J., Benoit, R.E. and White, D.C. (1993) Survival of subsurface microorganisms exposed to UV radiation and hydrogen peroxide. *Applied and Environmental Microbiology* **59**, 3545-3550.

Auzet, A.V., Poesen, J. and Valentin, C. (2004) Editorial: soil surface characteristics: dynamics and impacts on soil erosion. *Earth Surface Processes and Landforms* **29** , 1063-1064.

Barclay, W.R. and Lewin, R.A. (1985) Microalgal polysaccharide production for the conditioning of agricultural soils. *Plant and Soil* **88**, 159-169.

Bardgett, R.D., Bowman, W.D., Kaufmann, R. and Schmidt, S.K. (2005) A temporal approach to linking aboveground and belowground ecology. *Trends in Ecology and Evolution* **20**, 634-641.

Béard, A., Rimet, F., Capowiezand, C. and Leboulanger, C. (2004) Procedures for determining the pesticide sensitivity of indigenous soil algae: a possible bioindicator of soil contamination? *Archives of Environmental Contamination and Toxicology* **46**, 24-31.

Begon, M., Harpur, J, and Townsend C. R. (1996) Ecology. Individuals, Populations and Communities. 3rd edition. Blackwell Science ltd.

Belnap, J. (1993) Recovery rates of cryptobiotic crusts: inoculant use and assessment methods. *Great Basin Naturalist* **53**, 89-95.

Belnap, J. and Gardner, J.S. (1993) Soil microstructure in soils of the colorado plateau: the role of the cyanobacterium *Microcoleus vaginatus*. *Great Basin Naturalist* **53**, 40-47.

Belnap, J.H.K.T. (1995) Influence of cryptobiotic crusts on elemental content of tissue in two desert seed plants. *Arid Soil Research and Rehabilitation* **9**, 107-115.

Benvenuti, S. (1995) Soil light penetration and dormancy of jimsonweed. *Weed Science* **43**, 389-393.

Black, C. A. (1965) *Methods of Soil Analysis: Part I Physical and mineralogical properties.* American Society of Agronomy, Madison, Wisconsin, USA.

Bligh, E.G. and Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **37**, 911-917.

Bochner, B.R. (1989) Sleuthing out microbial identities. *Nature* **339**, 157-158.

Boschker, H.T.S., Kromkamp, J.C. and Middelburg, J.J. (2005) Biomarker and carbon isotopic constraints on bacterial and algal community structure and functioning in a turbid, tidal estuary. *Limnology and Oceanography* **50**, 70-80.

Boschker, H.T.S. and Middelburg, J.J. (2002) Stable isotopes and biomarkers in microbial ecology. *FEMS Microbial Ecology* **1334** , 1-15.

Bottomley, P. (1994) Light microscopic methods for studying soil microorganisms. In: Weaver, R.W., Angle, J.S. and Bottomley, P.S., (Eds.) *SSSA Book Series 5: Methods of Soil Analysis: Part 2; Microbiological and Biochemical Properties*, Wisconsin, USA: Soil Society of America Inc.

Brady, N.C. (1990) *The Nature and Properties of Soils*, 10th edn. London: Macmillan Publishers.

Bresson, L.M., Moran, C.J. and Assouline, S. (2004) Use of bulk density profiles from x-radiography to examine structural crust models. *Soil Society of America Journal* **68**, 1169-1176.

Bristol Roach, B.M. (1919) *New Phytologist* **18**, 92

Brutsaert, W. (2005) *Hydrology: An Introduction*, Cambridge, UK: Cambridge University Press.

Burgess, A. and Raw, F. (1967) *Soil Biology*, London: Academic Press.

Calderon, F., Jackson, L., Scow, K. and Rolston, D. (2000) Microbial responses to simulated tillage in cultivated and uncultivated soils. *Soil Biology and Biochemistry* **32** , 1547-1559.

Calderon, F., Jackson, L., Scow, K. and Rolston, D. (2001) Short-term dynamics of nitrogen, microbial activity, and phospholipid fatty acids and tillage. *Soil Science Society of America Journal* **65**, 118-126.

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S. and Potts, J.M. (2003) A rapid microtiter plate method to measure carbon dioxide evolved from carbon amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. *Applied and Environmental Microbiology* **69**, 3593-3599.

Campbell, N. A., Reece, J. B. and Mitchell, L. G. (1999) Biology. 5th Edition. Addison Wesley, Harlow, U. K.

Casal, J.J., Sanchez, R.A. and Deregibus, V.A. (1987) Tillering responses of *Lolium*

multiflorum plants to changes of red/far-red ratio typical of sparse canopies. *Journal of Experimental Botany* **38**, 1432-1439.

Chambers, B.J., Garwood, T.W.D. and Unwin, R. (2000) Controlling soil and water erosion and phosphorous losses from arable land in England and Wales. *Journal of Environmental Quality* **29**, 145-150.

Coleman, D., Crossley Jr, D. and Hendrix, P. (2004) *Fundamentals of Soil Ecology*, Second edn. USA : Elsevier Academic Press.

Cowles, H. C. (1911) The causes of vegetational cycles. *Annals of the Association of American Geographers*, **1** (1): 3-20

Cullimore, D.R. and McCann, A.E. (1977) Influence of four herbicides on the algal flora of a prairie soil. *Plant and Soil* **46**, 499-510.

Davey, M.C. and Clarke, K.J. (1991) The spatial distribution of microalgae on antarctic fell-field soils. *Antarctic Science* **3**, 257-263.

Decaens, T. (2000) Degradation dynamics of earthworm casts in grasslands of the eastern plains of Columbia. *Biology and fertility of Soils* **32**, 149-156.

Denef, K., Six, J., Bossuyt, H., Frey, S., Elliott, E., Merckx, R. and Paustian, K. (2001) Influence of dry wet cycles on the interrelationship between aggregate, particulate matter, and microbial community dynamics. *Soil Biology and Biochemistry* **33**, 1599-1611.

Drenovsky, R.E., Elliott, G.N., Graham, K.J. and Scow, K.M. (2004) Comparison of phospholipid fatty acid (PLFA) and total soil fatty acid methyl esters (tsfame) for characterising soil microbial communities. *Soil Biology and Biochemistry* **36**, 1793-1800.

Duguay, K. and Klironomos, J. (2000) Direct and indirect effects of enhanced uv-b radiation on the decomposing and competitive abilities of saprophytic fungi. *Applied Soil Ecology* **14**, 157-164.

Ebersberger, D., Wermbter, N., Niklaus, P.A. and Kandeler, E. (2004) Effects of long term co_2 enrichment on microbial community structure in calcareous grassland. *Plant and Soil* **264**, 313-323.

Edgerton, D.L., Harris, J.A., Birch, P. and Bullock, P. (1995) Linear relationship between aggregate stability and microbial biomass in three restored soils. *Soil Biology and Biochemistry* **27**, 1499-1501.

Ekelund, F. and Ronn, R. (1994) Notes on protozoa in agricultural soil with emphasis on heterotrophic flagellates and naked amoebas and their ecology. *FEMS Microbiology Reviews* **15**, 321-353.

Eldridge, D.J. and Greene, R.S.B. (1994) Microbiotic soil crusts: a review of their roles in soil and ecological processes in the rangelands of Australia. *Australian Journal of Soil Research* **32**, 389-415.

Evans, A.C. (1948) Studies on the relationship between earthworms and soil fertility. *Applied Biology* **35**, 1-13.

Falchini, L., Sparvoli, E. and Tomaselli, L. (1996) Effect of *Nostoc* (cyanobacteria) inoculation on the structure and stability of clay soils. *Biology and Fertility of Soils* **23**, 346-352.

Farres, P. (1978) The role of time and aggregate size in the crusting process. *Earth Surface Processes* **3**, 243-254.

Farres, P.J. (1987) The dynamics of rainsplash erosion and the role of aggregate stability. *Catena* **14**, 119-130.

Feeney, D., Crawford, J.W., Daniell, T.J., Hallett, P.D., Nunan, N., Ritz, K., Rivers, M. and Young, I.M. (2006) Three-dimensional micro-organisation of the soil-root-microbe system. *Microbial Ecology* **52**, 151-158.

Feng, Y., Motta, A.C., Reeves, D.W., Burmester, C.H., van Santen, E. and Osborne, J.A. (2003) Soil microbial communities under conventional-till and no-till continuous cotton systems. *Soil Biology and Biochemistry* **35**, 1693-1703.

Fierer, N., Schimel, J.P. and Holden, P.A. (2003) Influence of drying-rewetting frequency on soil bacterial community structure. *Microbial ecology* **45**, 63-71.

Forster, P.M., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R.A., Fahey, D.W., Haywood, J., Lean, J., Lowe, D.C., Myhre, G., Nganga, J., Prinn, R., Raga, G., Schulz, M. and Van Dorland, R. (2007) Chapter 2:changes in atmospheric constituents and radiative forcing. *IPCC Fourth Assessment Report: The Physical Science Basis.*

Frostegård, A. and Bååth, E. (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biological Fertility of Soils* **22**, 59-65.

Frostegård, A., Tunlid, A. and Bååth, E. (1993) Phospholipid fatty acid composition, biomass and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Applied and Environmental Microbiology* **59**, 3605-3617.

Garcia-Pichel, F., Johnson, S.L., Youngkin, D. and Belnap, J. (2003) Small-scale vertical distribution of bacterial biomass and diversity in biological soil crusts from arid lands in the colorado plateau. *Microbial Ecology* **46**, 312-321.

Garland, J. L. and Mills, A. L. (1991) Classification and characterisation of heterotrphic microbial communities on the basis of patterns of community-level sole carbon source utilisation. *Applied and Environmental Microbiology* **57,** 2351-2359

Gillespie, K.M., Angle, J.S. and Hill, R.L. (1995) Runoff losses of *Pseudomonas aureofaciens* (*lac*ZY) from Soil. *FEMS Microbiology Ecology* **17**, 239-246.

Gitay, H., Wilson, J.B. and Lee, W.G. (1996) Species redundancy: a redundant

concept? *Journal of Ecology* **84**, 121-124.

Goldberg, D.E. and Werner, P.A. (1983) Equivalence of competitors in plant communities - a null hypothesis and a field experimental approach. *American Journal of Botany* **70**, 1098-1104.

Goodwin, T.W. and Mercer, E.L. (1972) *Introduction to Plant Biochemistry*, Exeter, UK: Pergamon Press.

Griffiths, B.S., Ritz, K., Ebblewhite, N. and Dobson, G. (1999) Soil microbial community structure: effects of substrate loading rates. *Soil Biology and Biochemistry* **31**, 145-153.

Gugger, M., Lyra, C., Suominen, I., Tsitko, I., Humbert, J., Salkinoja-Salonen, M. and Sivonen (2002) Cellular fatty acids as chemotaxonomic markers of the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nostoc* and *Planktothrix* (cyanobacteria). *International Journal of Systematic and Evolutionary Microbiology* **52**, 1007-1015.

Harper, K.T. and Pendleton, R.L. (1993) Cyanobacteria and cyanolichens: can they enhance availability of essential minerals for higher plants? *Great Basin Naturalist* **53**, 59-72.

Harris, K., Crabb, D., Young, I.M., Weaver, H., Gilligan, C.A., Otten, W. and Ritz, K. (2002) *In situ* visualisation of fungi in soil thin sections: problems with crystallisation of flurochrome fb28 (calcofluor m2r) and improved staining by scri renaissance 2200. *Mycological Research* **106**, 293-297.

Harwood, J.L. and Russell, N.J. (1984) *Lipids in Plants and Microbes*, London, U.K.: George Allen & Unwin.

Hedrick, D.B., Richards, B., Jewell, W., Guckert, J.B. and White, D.C. (1991) Disturbance, starvation and overfeeding stresses detected by microbial lipid biomarkers in high-solids high-yield methanogenic reactors. *Journal of Industrial Microbiology and Technology* **8**, 91-98.

Hill, G.T., Mitkowski, N.A., Aldrich-Wolfe, L., Emele, L.R., Jurkonie, D.D., Ficke, A., Maladonado-Ramirez, S., Lynch, S.T. and Nelson, E.B. (2000) Methods for assessing the composition and diversity of soil microbial communities. *Applied Soil Ecology* **15**, 25-36.

Hillel, D. (1982) *Introduction to Soil Physics*, San Diego, CA: Academic Press.

Hjulström, F. (1935) The morphological activity of rivers as illustrated by the river Fyris. *Bulletin Geological Institute Upsala* **25**, 221-527.

Hofman, J., Dusek, L., Klanova, J., Bezchlebova, J. and Holoubek, I. (2003) Monitoring microbial biomass and respiration in different soils from the Czech Republic - a summary of results. *Environmental International* **30**, 19-30.

Hughes, K.A., Lawley, B. and Newsham, K.K. (2003) Solar UV-b radiation inhibits the growth of antarctic terrestrial fungi. *Applied and Environmental*

Microbiology **69**, 1488-1491.

Issa, O.M., Defarge, C., Le Bissonnais, Y., Marin, B., Duval, O., Bruand, A., D'Acqui, L.P., Nordenberg, S. and Annerman, M. (2007) Effects of the inoculation of cyanobacteria on the microstructure and the structural stability of a tropical soil. *Plant and Soil* **290**, 209-219.

Jackson, L.E., Calderon, F.J., Steenworth, K.L., Scow, K.M. and Rolston, D.E. (2003) Responses of soil microbial processes and community structure to tillage events and implications for soil quality. *Geoderma* **114**, 305 317

Janzen, H.H. (2004) Carbon cycling in earth systems - a soil science perspective. *Agriculture, Ecosystems and Environment* **104**, 399-417.

Johansen, J.R. (1993) Cryptogamic crusts of semiarid and arid lands of North America. *Journal of Phycology* **29**, 140-147.

Johnson, S.L., Neuer, S. and Garcia-Piche, F. (2007) Export of nitrogenous compounds due to incomplete cycling within biological soil crusts of arid lands. *Environmental Microbiology* **9**, 680-689.

Jones, W.B., Cifuentes, L.A. and Kaldy, J.E. (2003) Stable carbon isotope evidence for coupling between sedimentary bacteria and seagrasses in a sub-tropical lagoon. *Marine Ecology - Press Series* **255**, 15-25.

Jong, B. (1973) *Net Radiation Received by a Horizontal Surface at the Earth*, the Netherlands: Delft University Press.

Jordan, D. and Beare, M.H. (1991) A comparison of methods for estimating soil microbial biomass carbon. *Agriculture, Ecosystems and Environment* **34**, 35-41.

Kaltenecker, J. and Wicklow-Howard, M. (1994) Microbiotic soil crusts in sagebrush habitats of southern Idaho. Boise State University.

Kautz, T., Wirth, S. and Ellmer, F. (2004) Microbial activity in a sandy arable soil is governed by the fertilisation regime. *European Journal of Soil Biology* **40**, 87-94.

Keinanen, M.M., Korhonen, L.K., Martikainen, P.J., Vartiainen, T., Miettinen, I.T., Lehtola, M.J., Nenonen, K., Pajunen, H. and Kontro, M.H. (2003) Gas chromatographic-mass spectrometric detection of 2- and 3-hydroxy fatty acids as methyl esters from soil, sediment and biofilms. *Journal of Chromatography B* 443-451.

Keinanen, M.M., Martikainen, P.J., Korhonen, L.K. and Suutari, M.H. (2003) Microbial community structure in biofilms and water of a drinking water distribution system determined by lipid biomarkers. *Water Science and Technology* **47**, 143-147.

Kelly, J.J., Haggblom, M.M. and Tate, R.L. (2003) Effects of heavy metal contamination and remediation on soil microbial communities in the vicinity

of a zinc smelter as indicated by analysis of microbial community phospholipid fatty acid profiles. *Biology and Fertility of Soils* **38**, 65-71.

Kennedy, A.C. (1999) Bacterial diversity in agroecosystems. *Agriculture, Ecosystems and Environment* **74**, 65-76.

Killham, K. (1994) *Soil Ecology*, Cambridge, UK: Cambridge University Press.

Kingston, M.B. (1999) Effect of light on vertical migration of *Euglena proxima*. *Journal of Phycology* **35**, 245-253.

Kleiner, E.F. and Harper, K.T. (1972) Environment and community organization in grasslands on canyonlands national park. *Ecology* **53**, 299-309.

Kneif, C., Kolb, S., Bodelier, P.L.E., Lindri, A. and Dunfield, P.F. (2006) The active methanotrophic community in hydromorphic soils changes in response to changing methane concentration. *Environmental Microbiology* **8**, 321-333.

Krinsky, N.I. (1989) Antioxidant function of carotenoids. *Free Radical Biol Med.* **7**, 617-635.

García L., F. (2005) Chlorophyll ab spectra.png. http://en.wikipedia.ord/wiki/ image: chlorophyll ab spectra.png. Accessed August 2007

Larsen, J. and Bodker, L. (2001) Interactions between pea root-inhabiting fungi examined using signature fatty acids. *New Phytologist* **149**, 487-493.

Lavelle, P. and Spain, A.V. (2001) *Soil Ecology*, Dordrect, Netherlands: Kluwer Academic Publishers.

Le Bissonnais, Y. (1990) Experimental study and modelling of a soil surface crusting process. *Catena Supplement* **17**, 13-28.

Le Bissonnais, Y., Renaux, B. and Delouche, H. (1995) Interactions between soil properties and moisture content in crust formation, runoff and interrill erosion from tilled loess soils. *Catena* **25**, 33-46.

Lechevalier, H. and Lechevalier, M.P. (1988) Chemotaxonomic use of lipids - an overview 869-897. In: Ratledge C. and Wilkinson S. G. (eds.) *Microbial Lipids*. *Volume 1*. USA: Academic Press

Leys, J. and Eldridge, D. (1998) Influence of cryptogamic crust disturbance to wind erosion on sand and loam rangeland soils. *Earth Surface Processes and Landforms* **23**, 963-974.

Lockyer, D.R. and Cowling, D.W. (1977) Non-symbiotic nitrogen fixation in some soils of england and wales. *Grass and Forage Science* **32**, 7-11.

Loope, W.L. and Gifford, G.F. (1972) Influence of a soil microfloral crust on select properties of soils under pinyon-juniper in southeastern Utah. *Journal of Soil and Water Conservation* **27**, 164-167.

Lopez-Rodas, V., Flores-Moya, A., Manerio, E., Perdigones, N., Marva, F., Garcia, M.E. and Costas, E. (2007) Resistance of glyphosphate in the cyanobacterium *Microcystis aeruginosa* as a result of pre-selective mutations. *Evolutionary Ecology* **21**, 535-547.

Lund, J.W. (1967) Soil algae. In: Burgess, A. and Raw, F., (Eds.) *Soil Biology*, pp. 129-147. London: Academic Press

Lupwayi, N.Z., Rice, W.A. and Clayton, G.W. (1998) Soil structure and microbial diversity and community structure under wheat as influenced by tillage and crop rotation. *Soil Biology and Biochemistry* **30**, 1733-1741.

Macalady, J.L., McMillan, A.M.S., Dickens, A.F., Tyler, S.C. and Scow, K.M. (2002) Population dynamics of type I and type II methanotrophic bacteria in rice soils. *Environmental Microbiology* **4**, 148-157.

Madigan, M., Martinko, J.M. and Parker, J. (2000) *Brock: Biology of Microorganisms.* 9th edn, Prentice Hall, New Jersey, USA

Maron, P.A., Ranjard, L., Mougel, C. and Lemanceau, P. (2007) Metaproteomics: a new approach for studying functional microbial ecology. *Microbial Ecology* **53**, 486-493.

Mattimore, V. and Battista, J.R. (1996) Radioresistance of *deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. *Journal of Bacteriology* **178**, 633-637.

Medows, A., Medows, P.S., Wood, D.M. and Murray, J.M.H. (1994) Microbiological effects on slope stability. *Sedimentology* **41**, 423-435.

Meeting, B. (1981) The systematics and ecology of soil algae. *The Botanical Review* **47**, 195-312.

Methy, M. and Roy, J. (1993) Morphogenetic changes induced by a low red : far-red ratio and their growth consequences in water hyacinth (*Eichhornia crassipes*). *Journal of Experimental Botany* **44**, 1275-1280.

Metting, F.B. (1994) Algae and Cyanobacteria. In: Klute, A. (1994) *SSSA Book Series: 5 Methods of Soil Analysis, Part 2 - Microbiological and Biochemical Properties*, pp. 427-458. Wisconsin USA: Soil Science Society of America Inc.

Mikha, M.M., Rice, C.W. and Milliken, G.A. (2005) Carbon and nitrogen mineralisation as affected by drying and wetting cycles. *Soil Biology and Biochemistry* **37**, 339-347.

Mille-Lindblom, C., von Wachenfeldt, E. and Tranvik, L.J. (2004) Ergosterol as a measure of living fungal biomass: persistence in environmental samples after fungal death. *Journal of Microbiological Methods* **59**, 253-262.

Moeller, R., Horneck, G., Facius, R. and Stackebrandt, E. (2005) Role of pigmentation in protecting *Bacillus* Sp. endospores against environmental UV

radiation. *FEMS Microbiology Ecology* **51**, 231-236.

Montechiaro, F. and Giordano, M. (2006) Effect of prolonged dark incubation on pigments and photosynthesis of the cave-dwelling cyanobacterium *Phormidium autumnale* (oscilliatoriales, cyanobacteria). *Phycologia* **45**, 704-710.

Morgan, R. (1995) *Soil Erosion and Conservation*, second edn. Harlow, Essex: Longman Group Limited.

Nisha, R., Kaushik, A. and Kaushik, C.P. (2006) Effects of Indigenous Cyanobacterial Application on Structural Stability and Productivity of an Organically Poor Semi-Arid Soil. *Geoderma* **138**, 49-56.

Norman, J. M. (1980) Interfacing leaf and canopy light interception models. Hesketh, J. D. and Jones, J. W. (eds.) *Predicting Photosynthesis for Ecosystem Models.* 49-84. Boca Raton, FL., CRC Press.

Norman, J.M. and Jarvis, P.G. (1974) Photosynthesis in Sitka spruce (*Picea sitchensis)* iii. measurements of canopy structure and interception of radiation. *Journal of Applied Ecology* **11**, 375-398.

Nunan, N., Ritz, K., Rivers, M., Feeney, D. and Young, I.M. (2006) Investigating microbial micro-habitat structure using x-ray computed tomography. *Geoderma* **133**, 398-407.

Osborne, L. and Jin, Y. (2004) Development of a resistance-based sensor for detection of wetness at the soil-air interface . *Agronomy Journal* **96**, 845-852.

Otten, W. and Gilligan, C.A. (1998) Effect of the physical condition on the spatial and temporal dynamics of the soil bourne pathogen *Rhizoctonia solani*. *New Phytologist* **138**, 629-637.

Ovreas, L. and Torsvik, L. (1998) Microbial diversity and community structure in two different agricultural soil communities. *Microbial Ecology* **36**, 303-315.

Pankratova, E.M. (2006) Functioning of cyanobacteria in soil ecosystems. *Eurasian Soil Science* **39**, s118 - s127

Pelz, O., Chatzinotas, A., Andersen, N., Bemasconi, S.M., Hesse, C., Abraham, W.R. and Zeyer, J. (2001) Use of isotopic and molecular techniques to link toluene degradation in denitrifying aquifer microcosms to a specific microbial population. *Archives of Microbiology* **175**, 270-281.

Pendleton, R.L., Pendleton, B.K., Howard, G.L. and Warren, S.D. (2003) Growth and nutrient content of herbaceous seedlings associated with biological soil crusts. *Arid Land Research and Management* **17**, 271-281.

Petersen, J.B. (1935) Studies on the biology and taxonomy of soil algae. *Dansk Botanisk Arkiv* **8**

Petersen, S. and Klug, M. (1994) Effects of sieving, storage, and incubation

temperature on the phospholipid fatty acid profile of a soil. *Applied and Environmental Microbiology* **60**, 2421-2430.

Poesen, J. (1985) An improved splash transport model. *Zeitschrift fur Geomorphologie* **29**, 193-211.

Pringault, O. and Garcia-Pichel, F. (2004) Hydrotaxis of cyanobacteria in desert crusts. *Microbial Ecology* **47**, 366-373.

Rajendran, N., Matsude, O. and Urushigawa, Y. (1992) Distribution of polarlipid fatty- acid biomarkers for bacteria in sediments of a polluted bay. *Microbios* **72**, 143-152.

Rauws, G. and Govers, G. (1988) Hydraulic and soil mechanical aspects of rill generation on agricultural soils. *Journal of Soil Science* **39**, 111-124.

Richfield, D (2005) The structure of Chlorophyll http://en.wikivisual.com/images /6/63/Chlorophyll_structure.png. Accessed August 2007

Rinnan, R., Keinanen, M.M., Kasurinen, A., Askikainen, J., Kekki, T.K., Holopainen, T., Ro-Poulson, H.M.T.N. and Michelsen, A. (2005) Ambient ultraviolet radiation in the arctic reduces root biomass and alters microbial community composition but has no effects on microbial biomass. *Global Change Biology* **11**, 564-574.

Ritz, K. and Young, I.M. (2004) Interactions between soil structure and fungi. *Mycologist* **18**, 52-59.

Ritz, K. (2007) The plate debate: cultivable communities have no utility in contemporary environmental microbial ecology. *FEMS Microbiology Ecology* **60**, 358-362.

Robinson, N. (1966) *Solar Radiation*, Amsterdam: Elsevier Publishing Company.

Rogasik, H., Crawford, J.W., Wendroth, O., Young, I.M. and Ritz, K. (1999) Discrimination of soil phases by dual energy X-ray tomography. *Soil Science Society of America Journal* **63**, 741-751.

Roijackers, R.M.M. (1981) A comparison between two methods of extracting chlorophyll-a from different phytoplankton samples. *Aquatic Ecology* **15**, 179-183.

Romkens, M.J.M., Prasad, S.N. and Whisler, F.D. Surface sealing and infiltration. In: Anderson, M.G. and Butt, T.P., (Eds.) *Process Studies in Hill Slope Hydrology*, Chichester: John Wiley and Sons, Ltd.

Rovira, A.D. and Green, E.L. (1957) The effect of aggregate disruption on the activity of microorganisms on the soil. *Australian Journal of Agricultural research* **8**, 659-673.

Ruzicka, S., Norman, M.D.P. and Harris, J.A. (1995) Rapid ultrasonication method to determine ergosterol concentration in soil. *Soil Biology and Biochemistry* **27**,

1215-1217.

Scherer, S., Ernst, A., Chen, T. and Boger, P. (1984) Rewetting of drought resistant blue-green algae: time course of water uptake and reappearance of respiration, photosynthesis and nitrogen fixation. *Oecologia* **62**, 418-423.

Singer, M. and Shainberg, I. (2004) Mineral soil surface crusts and wind and water erosion. *Earth Surface Processes and Landforms* **29**, 1065-1075.

Smith, K. A., Jackson, D. R and Pepper, T. J. (2001) Nutrient losses by surface run-off following the application of organic manures to arable land. *Environmental Pollution* **112**, 41-51.

Sparling, G.P., West, A.W. and Reynolds, J. (1989) Influence of soil moisture regime on the respiration response of soils subjected to osmotic stress. *Australian Journal of Soil Research* **27**, 161-168.

St. Clair, L.L. and Johansen, J.R. (1993) Introduction to the symposium on soil crust communities. *Great Basin Naturalist* **53**, 1-4.

Stenberg, B. (1999) Monitoring soil quality of arable land: microbiological indicators. *Acta Agriculture Scandinavica. Section B, Soil and Plant Science* **49**, 1-24.

Stradling, D.A., Thygerson, T., Walker, J.A., Smith, B.N., Hansen, L.D., Criddle, R.S. and Pendleton, R.L. (2002) Cryptogamic crust metabolism in response to temperature, water vapor, and liquid water. *Thermochimica Acta* **394**, 219-225.

Strangeways, I. (2003) *Measuring the Natural Environment*, Second edn. UK: Cambridge University Press.

Tadal, K., Yamaguchi, H. and Montani, S. (2004) Comparison of chlorophyll a concentrations obtained with 90% acetone and n,n-dimethylformamide extraction in coastal seawater. *Journal of Oceanography* **60**, 259-261.

Tchan, Y.T. and Whitehouse, J.A. (1953) The variation of the algal population in sandy soils. *Study of Soil Algae* 160-170.

Torsvik, V., Goksoyr, J. and Daae, F. (1990) High diversity in DNA of soil bacteria. *Applied Environmental Microbiology* **56**, 782-787.

United States Environment Protection Agency (1999) Air and Radiation 6205J. www.epa.gov/sunwise. Accessed September 2007

Vance, E.D., Brookes, P.C. and Jenkinson, D.S. (1987) An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* **19**, 703-707.

Vangestel, M., Ladd, J.N. and Amato, M. (1992) Microbial biomass responses to seasonal change and imposed drying regimes at increasing depths of undisturbed topsoil profiles. *Soil Biology and Biochemistry* **24**, 103-111.

Vatterlein, D., Marschnet, H. and Horn, R. (1993) Microtensiometer technique for *in situ* measurement of soil matric potential and root water extraction from a sandy soil. *Plant and Soil* **149**, 263-273.

Wardle, D.A., Yeates, G.W., Williamson, W. and Bonner, K.I. (2003) The response of a three trophic level soil food web to the identity and diversity of plant species and functional groups. *Oikos* **102**, 45-56.

Warren-Rhodes, K.A., Rhodes, K.L., Pointing, S.B., Ewing, S.A., Lacap, D.C., Gomez-Silva, B., Amundson, R., Friedmann, E.I. and McKay, C.P. (2006) Hypolithic cyanobacteria, dry limit of photosynthesis and microbial ecology in the hyperarid Atacama Desert. *Microbial Ecology* **52**, 389-398.

Warrick A. (2002) *Soil Physics Companion*, Florida, USA: CRC Press LLC.

Wasmun, N., Topp, I. and Schores, D. (2006) Optimising the storage and extraction of chlorophyll samples. *Oceanologia* **48**, 125-144.

Wertz, S., Degrange, V., Prosser, J.I., Poly, F., Commeaux, C. and Freitag, T. (2006) Maintenance of soil functioning following erosion of microbial diversity. *Environmental Microbiology* **8**, 2162-2169.

West, A.W., Sparling, G.P., Feltham, C.W. and Reynolds, J. (1992) Microbial activity and survival in soils dried at different rates. *Australian Journal of soil Research* **30**, 209-222.

West N.E. (1990) Structure and function of microphytic soil crusts in wildland ecosystems of arid to semi-arid regions. *Advances in Ecological Research* **20**, 179-223.

West, N.E. and Skujins, J. (1977) The nitrogen cycle in North American cold-winter semidesert ecosystems. *Oecologia Plantarum* **12**, 45-53.

Wiesenberg, G.L.B., Schwark, L. and Schmidt, M.W.I. (2004) Improved automated extraction and separation procedure for soil lipid analyses. *European Journal of Soil Science* **55**, 349-356.

Williams J.D. (1993) Influence of microphytic crusts on selected soil physical and hydrologic properties in the Hartnet Draw, capitol reef national park, Utah. Utah State University.

Witty, J.F., Keay, P.J., Frogatt, P.J. and Dart, P.J. (1979) Algal nitrogen fixation on temperate arable fields - the broadbalk experiment. *Plant and Soil* **52**, 151-164.

Wright, S.F. and Anderson, R.L. (2000) Aggregate stability and glomalin in alternative crop rotations for the central great plains. *Biology of Fertile Soils* **31**, 249-253.

Wright, S.F., Starr, J.L. and Platineanu, I.C. (1999) Changes in aggregate stability and concentration of glomalin during tillage management transition. *Soil Science Society of America journal* **63**, 1825-1829.

Wu, J. and Brookes, P.C. (2004) The proportional mineralisation of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil. *Soil Biology and Biochemistry* (In Press)

Wynn-Williams, D.D. (1996) Response of pioneer soil microalgal colonists to environmental change in antarctica. *Microbial Ecology* **31**, 177-188.

Yeager, C., Kornosky, J., Housman, D., Grote, E., Belnap, J. and Kuske1, C. (2004) Diazotrophic community structure and function in two successional stages of biological soil crusts from the colorado plateau and chihuahuan desert. *Applied and Environmental Microbiology* **70**, 973-983.

Young, I. M. and Ritz, K. (2005) The habitat of soil microbes in: Bardgett, R. D. Usher M. B. and Hopkins, D. W. (eds.) *Biological Diversity and Function in Soils* pp. 31-43. Cambridge University Press, Cambridge.

Zelles, L. (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biology and Fertility of Soils* **29**, 111-129.

Zelles, L. (1999) Identification of single cultured micro-organisms based on their whole-community fatty acid profiles, using an extended extraction procedure. *Chemosphere* **39**, 668-682.

Zelles, L. (1997) Phospholipid fatty acid profiles in selected members of soil microbial communities. *Chemosphere* **35**, 275-294.

Zhang, B., Zhao, Q.G., Horn, R. and Baumgartl, T. (2001) Shear strength of surface soil as affected by soil bulk density and soil water content. *Soil and Tillage Research* **59**, 97-106.

Appendix I

Microbial community phenotypic profiles change markedly with depth within the first centimetre of the arable soil surface

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Microbial community phenotypic profiles change markedly with depth within the first centimetre of the arable soil surface

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Abstract

The nature of the first few millimetres of a soil horizon strongly affects water infiltration rates, generation of run-off, and soil detachment. Whilst much is known about the physics and erosion of soil surfaces at this scale, little is known about their microbiology, particularly in temperate arable systems. This investigation aimed to discover whether any early colonisation stages of microbiotic crusts exist within the soil surface of temperate arable systems. The phenotypic structure of the microbial community was measured by means of phospholipid fatty acid analysis (PLFA) in soils sampled from the surface of arable fields that had been either cultivated 4 weeks previously or left undisturbed for 4–6 months. Within the top *circa* 1 mm of the soil that had been undisturbed for 6 months or more, distinct microbial communities were found to be present, which were statistically significantly different from the communities found in subsequent depths to *circa* 10 mm, where differences between communities were less pronounced. The PLFA responsible for the majority of the variation seen between depths was 16:0, the proportion of which was shown to decrease with depth. This was not the case in the recently cultivated soils, where communities were more homogeneous with respect to depth.

Keywords: Depth profile; Microbial community structure; PLFA; The soil surface

Soil surface is an ambiguous concept, meaning different things to different investigators working at different scales and depths. Much of the research to date regarding the arable soil surface has used the top 10, 15 or 20 cm as a working definition (Ovreas and Torsvik, 1998; Calderon et al., 2001; Fierer et al., 2003). Environmental factors such as temperature, wetting and drying cycles, and solar radiation fluctuate more rapidly and have greater extremes at the surface compared with deeper soil layers.

Water infiltration rates, run-off generation, and soil detachment, are all strongly affected by the nature of the first few millimetres of topsoil (Auzet et al., 2004). An increased understanding of the processes that affect erodibility may lead to novel methods of controlling and reducing soil erosion and hence enhance the environmental sustainability of agriculture, for example by reducing the off-site effects of erosion such as increased water turbidity and eutrophication.

The majority of research regarding microbial interactions at soil surfaces has been concerned with microbiotic crusts (also known as cryptogamic crusts) which occur primarily in arid ecosystems (St. Clair and Johansen, 1993; Eldridge and Greene 1994; Leys and Eldridge, 1998). These microbiotic crusts take many years to form, through a series of steps of colonisation and succession, and only occur in relatively undisturbed soil systems. They perform a number of important functions which would be pertinent in arable systems, including nitrogen fixation, as well as reducing the erodibility of the soil on which they grow (Lund, 1967; Leys and Eldridge, 1998). As these crusts are very susceptible to disturbance, mature crusts will not be able to form in agricultural systems, as these tend to be disturbed via cropping cycles. Despite this, it seems likely that some of the earlier stages of microbiotic crusts will be capable of performing some of the environmental processes performed by mature crusts. It is also likely that some of these earlier stages will have time to develop within arable systems, for example, at times when the fields are left fallow. This investigation aimed to discover whether any

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such early colonisation stages of microbiotic crusts exist within the soil surface of temperate arable systems.

Soils were sampled from a number of fields at Silsoe College Farm, Bedfordshire, and Tivington, Somerset (Tables 1 and 2). Three soil layers were removed, through scraping with a palette knife; the surface layer to a depth of *circa* 1 mm, followed by two further depths of *circa* 5 mm each. Due to the microtopography of the soil surface, some deviation within the depths of each sample layer may have occurred but all possible care was taken to keep this to a minimum. All samples were stored in plastic bags overnight in a refrigerator at 4 °C.

Phospholipid fatty acid (PLFA) extraction and analysis as outlined by Bligh and Dyer (1959) and extended upon by Zelles (1999) were undertaken. Extracted PLFAs were analysed by gas chromatography (Aligent Technologies, California, USA, Model 6890N).

There was a general result that sites that were left undisturbed for a period of approximately 6 months or more developed microbial communities with distinct phenotypic structures in the uppermost 1-mm surface layer (ANOVA of principal components: $P<0.001$; Fig. 1.). However in systems that had been disturbed in the 4 months prior to sample collection there were no significant differences between the 3 depth layers of soil at these scales (ANOVA: $P>0.05$; Fig. 1). The PLFA responsible for the majority of the variation seen between depths was 16:0. There was consistently more 16:0 at the soil surface and it decreased with depth at the scale investigated, in those fields where a distinct soil surface microbial community had developed. However, there is conflicting data in the literature as to the biomarker status of 16:0. Pelz et al. (2001) attribute 16:0 as a general bacteria marker, whilst Keinanen et al. (2003) use 16:0 as a biomarker for microbial biomass, Jones et al. (2003) state that it is ubiquitous and Kneif et al. (2006) use it as a biomarker for Type II methanotrophs.

The factors that drive the formation of a distinct microbial soil surface community must affect the extreme soil surface and change rapidly with depth, even at the millimetre scale. This results in distinct community phenotypes within 6 months. Further, any physical disturbance to the soil surface, for example through cultivations is sufficient to interrupt the formation of, or destroy, a distinct soil surface microbial community. Table 1 shows that different fields were at different stages of the cropping cycle. This, combined with the information presented in Fig. 1, demonstrates that only fields that had been left undisturbed for a period of at least 6 months develop the distinct microbial community phenotype within the top 1 mm of the soil surface. Evidently such microbial community phenotypes are relatively susceptible to change when subjected to mechanical disturbance. Cropping cycles will therefore greatly influence the longevity of these microbial soil surfaces. However, the impact of environmental events such as rain storms and frost events on these microbial communities is not yet known. What is demonstrated in this study is that distinct microbial communities develop at different soil depths at this small, previously unreported, scale, and that the notion of a biologically distinct 'surface phase' in arable soils appears confined to an order of 1 mm depth. It is, however,

Table 1
Site location and sampling information of fields sampled

Location	Site	UK National Grid reference	Time since last disturbance to surface	Place in cropping cycle	No. of replicates sampled	Date of sample collection
Silsoe	Avenue field	TL075357	≈6 months	Fallow	3	March 2005
	Ivy ground	TL077352	≈4 months	Sown, winter beans	3	March 2005
	Showground field	TL075355	>6 months	Fallow	5	July 2005
Tivington	Field 1, Area 1	SS929447	≈4 weeks	Conservation tilled	3	May 2005
	Field 1, Area 2	SS929447	≈4 weeks	Conventional tilled	3	May 2005

Table 2
Chemical and physical properties of soils sampled

Location	Field	Texture	Dry bulk density ($g\,cm^{-3}$)	PH	Total N ($g\,kg^{-1}$)	C (%)	N (%)	C:N
Silsoe	Avenue field	Sandy loam	1.48	6.1	1.25	1.0	0.1	10.6
	Ivy ground	Clay loam	1.07	6.6	2.58	2.9	0.2	13.9
	Showground	Sandy loam	1.48	6.1	1.25	1.0	0.1	10.6
Tivington	Field 1, Area 1	Clay loam	1.25	7.8	n.d.	1.1	0.6	8.8
	Field 1, Area 2	Clay loam	1.25	7.8	n.d.	1.1	0.6	8.8

n.d.: not determined.

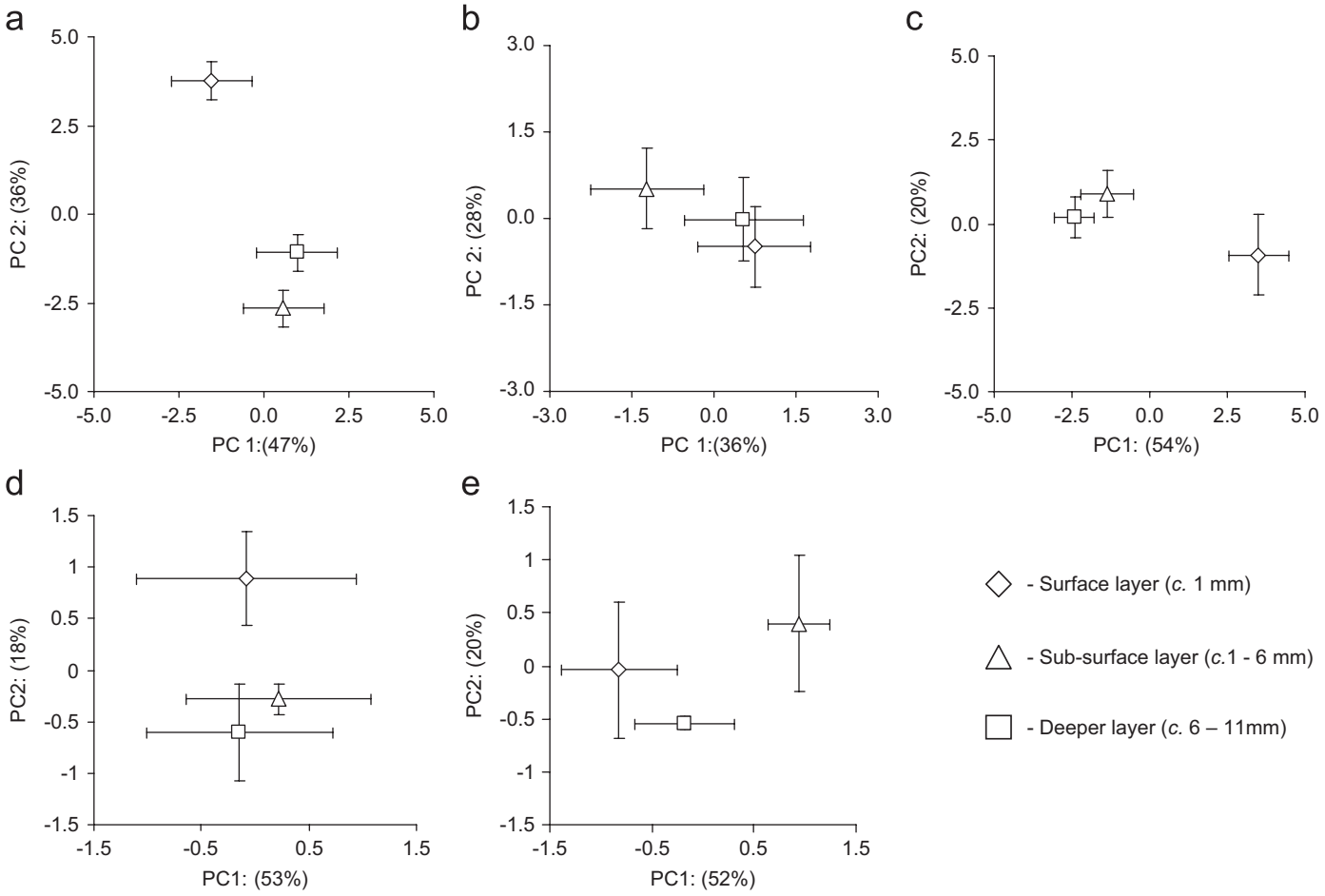

Fig. 1. First and second principal components (PCs) derived from phospholipid fatty-acid profiles originating from surface layers of a range of arable soils at two sites. (a) Silsoe, Avenue Field; (b) Silsoe, Ivy Ground; (c) Silsoe, Showground Field; (d) Tivington, conservation tilled; and (e) Tivington, conventional tilled. Points show means ± SE. Percentage variation accounted for by PC shown in parenthesis on each axis.

possible that there are other phenotypic profiles within the top 1 mm of the soil surface, probably dependent on its microtopography, or that different phenotypic profiles exist within the subsequent layers that have not been detected due to dilution effects. Further work is needed at increased resolution to determine if this is the case.

Due to the change in phenotypic profile apparently occurring so abruptly at the surface it is logical to conclude that the factors driving the formation of a distinct microbial community at the soil surface must also change abruptly at the surface. It is unlikely that different soil textures are responsible for the results as a there is no clear mechanism which would drive the formation on a sandy loam but not on a clay loam at the scale investigated, but further work is needed to confirm this hypothesis.

We hypothesise that the factor most likely to affect only the surface on such a small scale is light, as it has been demonstrated that light penetrates very poorly into soil (Benvenuti, 1995) and that photoautotrophs will therefore be contributing to much of the difference seen between the surface community and those found at deeper levels. There may also be a significant contribution from fluctuations in moisture content and temperature.

It is generally accepted that the soil microbiota will have an impact on soil structural integrity and hence the propensity for erosion (Wright et al., 1999; Ritz and Young, 2004). Through manipulation of the environmental variables that drive the formation of soil surface microbial communities, mechanisms for controlling soil erosion in arable systems, with its associated problems such as diffuse pollution and sediment losses may be found.

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References

Auzet, A.V., Poesen, J., Valentin, C., 2004. Soil surface characteristics: dynamics and impacts on soil erosion. Earth Surface Processes and Landforms 29, 1063–1064.

Benvenuti, S., 1995. Soil light penetration and dormancy of Jimsonweed. Weed Science 43, 389–393.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911–917.

Calderon, F., Jackson, L., Scow, K., Rolston, D., 2001. Short-term dynamics of nitrogen, microbial activity, and phospholipid fatty acids and tillage. Soil Science Society of America Journal 65, 118–126.

Eldridge, D.J., Greene, R.S.B., 1994. Microbiotic soil crusts: a review of their roles in soil and ecological processes in the rangelands of Australia. Australian Journal of Soil Research 32, 389–415.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Influence of drying–rewetting frequency on soil bacterial community structure. Microbial Ecology 45, 63–71.

Jones, W.B., Cifuentes, L.A., Kaldy, J.E., 2003. Stable carbon isotope evidence for coupling between sedimentary bacteria and seagrasses in a sub-tropical lagoon. Marine Ecology—Press Series 255, 15–25.

Keinanen, M.M., Martikainen, P.J., Korhonen, L.K., Suutari, M.H., 2003. Microbial community structure in biofilms and water of a drinking water distribution system determined by lipid biomarkers. Water Science and Technology 47, 143–147.

Kneif, C., Kolb, S., Bodelier, P.L.E., Lindri, A., Dunfield, P.F., 2006. The active methanotrophic community in hydromorphic soils changes in response to changing methane concentration. Environmental Microbiology 8, 321–333.

Leys, J., Eldridge, D., 1998. Influence of cryptogamic crust disturbance to wind erosion on sand and loam rangeland soils. Earth Surface Processes and Landforms 23, 963–974.

Lund, J.W., 1967. Soil Algae. In: Burgess, A., Raw, F. (Eds.), Soil Biology. Academic Press, London, pp. 129–147.

Ovreas, L., Torsvik, L., 1998. Microbial diversity and community structure in two different agricultural soil communities. Microbial Ecology 36, 303–315.

Pelz, O., Chatzinotas, A., Andersen, N., Bemasconi, S.M., Hesse, C., Abraham, W.R., Zeyer, J., 2001. Use of isotopic and molecular techniques to link toluene degradation in denitrifying aquifer microcosms to a specific microbial population. Archives of Microbiology 175, 270–281.

Ritz, K., Young, I.M., 2004. Interactions between soil structure and fungi. Mycologist 18, 52–59.

St. Clair, L.L., Johansen, J.R., 1993. Introduction to the symposium on soil crust communities. Great Basin Naturalist 53, 1–4.

Wright, S.F., Starr, J.L., Platineanu, I.C., 1999. Changes in aggregate stability and concentration of glomalin during tillage management transition. Soil Science Society of America Journal 63, 1825–1829.

Zelles, L., 1999. Identification of single cultured micro-organisms based on their whole-community fatty acid profiles, using an extended extraction procedure. Chemosphere 39, 668–682.