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**INTERACTIONS BETWEEN SOIL  
MICROBIAL COMMUNITIES,  
ERODIBILITY AND TILLAGE PRACTICES**

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## **Abstract**

The soil biota are a vital component of belowground systems, driving many key processes such as nutrient cycling, underwriting soil structural integrity and providing crucial ecosystem services to the wider environment. In agricultural systems, tillage practices are known to impact upon both the soil biota and surface erosion processes, but little is understood about the relationships between these three factors. This work addresses this issue within the framework of an EU Life/ Syngenta project “Soil and water protection for northern and central Europe” (SOWAP). Within this component of the SOWAP programme, the influence of different soil management practices on the size and overall composition of the soil microbial community was determined and related to the propensity for erosion, at a variety of spatial scales.

Microbial biomass and phenotypic structure, measured using phospholipid fatty acid (PLFA) analysis, were used to determine the effect tillage had on microbial communities at sites in Belgium, Hungary and the UK. The field sites were split into differing tillage practices on the same slope. Samples were taken prior to, and three years after, the adoption of inversion (conventional) and non-inversion tillage techniques. In addition, samples were taken periodically from two sites in the UK (Loddington, Leicestershire and Tivington, Somerset) to assess the temporal changes in microbial community size and structure under the tillage practices. Other soil, agronomic and ecological properties were measured at the field scale by SOWAP project partners. These field trials were supported by small plot rainfall simulations at the Loddington field site and by laboratory-based microcosm-scale studies using manipulated microbial communities and controlled rainfall, to further characterise microbial effects on soil erodibility.

The results showed that across the European sites microbial community size was reduced in conventionally tilled soils. However there was no effect of tillage type on microbial biomass at the Tivington site after three years. Microbial community structure showed significant seasonal changes greater than those relatable to tillage type. It was notable that the fungal biomarker PLFA 18:2 $\omega$ 6 decreased in conventionally tilled soils.

The small-scale experimentation using rainfall simulators and manipulated microbial communities was designed to specifically observe relationships between soil microbial communities, water movement and erodibility. These experiments showed that the presence of microbes in soils impacted upon both erosion processes and hydrological properties. There was a trend showing a decreased sediment concentration in runoff from soils containing a living microbial community. Propensity to runoff and infiltration was altered differentially as a result of microbial inocula derived from soils under different tillage practices. There was evidence that there was a specific and characteristic fraction of the microbial community susceptible to mobilisation by runoff and infiltrate waters, and hence potentially prone to relocation within the ecosystem. Linking the laboratory experiments to field rainfall simulations demonstrated the difficulty of controlling environmental variables, particularly at larger scales. Nevertheless, the same basic trends were observed at both laboratory and small plot scales.

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# *Section 1*

## **Chapter 1 Introduction and literature review**

### ***1.1 Introduction***

The impact of tillage practices and land management techniques on soil microbial communities has been much studied. However there is debate over whether these impacts are significant in the broader terms for the agroecosystem and what they mean for the productivity of the soil. The results of this study will further inform the debate by assessing the impact of tillage practices upon soil microbial community structure, particularly in relation to erosion processes. Sites in the UK and Europe have been used for demonstrating three different tillage and land management systems over the course of three years. The intention of this programme of work is to test the broad hypothesis that changes in the soil microbial population as a result of land management practices have implications for soil erodibility, productivity and ecology of field sites.

This work was carried out in conjunction with an EU Life/ Syngenta match funded project entitled “Soil and surface water protection in Northern and Central Europe (SOWAP)”.

## ***1.2 Soil as a habitat for micro-organisms***

Soil micro-organisms play a fundamental role in agroecosystems both above and below ground. They have a huge impact on the turnover rate and mineralization of organic materials which act as their substrates (Killham, 2001). The total fresh weight mass of organisms below temperate grassland can equal or exceed the above ground biomass, attaining of the order  $45 \text{ t ha}^{-1}$  (Ritz *et al.* 2004). It is estimated that about 5000 soil bacterial species have been described (Pace, 1997; Pace, 1999;) and around 1.5 million species of fungi on a global scale (Giller *et al.*, 1997; Pace, 1997; Hawksworth, 1991; Hawksworth, 2001). Wide ranging species diversity ensures that ecological functions and the productive capacity of soil are maintained (Folke *et al.* 1996). The greater the species diversity the wider the range of pathways for ecological processes and primary production, thus if a pathway is damaged or destroyed an alternative pathway is more likely to be available allowing continued ecosystem functioning. The microbial community diversity generally decreases in response to environmental stresses or disturbance which can upset the ecological equilibrium of population interactions within the community (Atlas *et al.* 1991). However, the true extent of the microbial influences on below- and above-ground ecosystems is unknown.

The soil biota is responsible for the breakdown of organic matter releasing organically held nutrients. The actions of micro-organisms convert organically bound nitrogen, sulphur and phosphorus into nitrates, sulphates and phosphate ions. Oxidation of iron and magnesium by autotrophic organisms to a higher valence state keeps the greater proportion of these elements insoluble and in a non-toxic form. This transformation is crucial

to the life of higher plants. Bacteria are responsible for the fixation of elemental nitrogen in soils, converting  $N_2$  to ammonium ( $NH_4$ ) and hence to a biologically-available form.

The chemical characteristics of the soil have, in turn, an influence over the associated microbial activity. Factors such as water and nutrient availability affect what grows and in what abundance (Madigan *et al.*, 2000). Soil pH has an impact on microbial growth (Fierer & Jackson, 2006). Carbon dioxide and salt concentrations, along with the cation exchange capacity, fluctuate and with them the soil pH. Soil microbial biomass carbon typically represents only 3-8 % of the total soil carbon but is of great importance to growing plants as a reservoir of nutrients, and as a mediator of nutrient transformations (Titi, 2003).

Soil water fluctuation has a dramatic effect on the soil microbial population. Soil water is categorised into four main types. These are: gravitational, wherein water drains out of soil playing a major role in the transport of materials; capillary, wherein soil water is held in the pore spaces; osmotic, wherein water is held around clay particles and humus - less available to microbes; and hydroscopic, where the water is strongly absorbed by particles forming a thin surrounding layer - this is the least available form. Water is an important aspect to consider as many microbial cells are killed by desiccation (Gray & Williams, 1971) or by anaerobiosis arising as a result of water-filled pores.

The temperature of soil is dependent on air temperature, soil type, colour, location and plant coverage, although there is also a connection between the moisture content of the soil and its heat absorbing capacity.

The composition of soil air differs from that in the atmosphere aboveground. It is usually higher in carbon dioxide and water vapour concentration and, because of this; there is net efflux of carbon dioxide from the soil and gaseous exchange of water vapour between the atmosphere and the soil. This exchange tends only to reduce the carbon dioxide levels in the upper regions of the soil. Therefore, the concentrations of gases vary from point to point within the soil. The soil micro-organisms have varying responses to gaseous change dependant on whether they are obligatory and facultative anaerobic or obligatory aerobic (Killham, 2001).

Soil pores form a network filled with liquids and gases, comprising the spaces between the soil particles. Depending on the soil treatment (i.e. land management) and makeup, the overall porosity varies. However, in most soils, it is estimated to make up as much as 50% of the total volume of soil. Pore sizes have been shown to have a marked effect on soil microbial growth. (Killham, 2001) Smaller diameter pores provide better protection from predators than larger pores therefore affecting the inter and intra species interactions (Juma, 1993).

### **1.2.1 Soil structure**

Soil structure can be defined as “the spatial heterogeneity of different components or properties of soil”, and can be considered at the ped, aggregate and micro-aggregate scale (Dexter, 1988). Soil aggregation and the stability of those aggregates dictate the structural integrity of a particular soil and its susceptibility to crusting and compaction. Soil crusting is caused by the vulnerable surface soil aggregates being destroyed by heavy rains and sealing-over, which can prevent water infiltration. When the soil dries a hard crust is often formed on the surface which can

inhibit crop emergence. Aggregate stability is influenced by the actions of soil biota and tillage. Micro-organisms are known to affect soil structural characteristics by the production of metabolic products binding soil particles together, and via physical enmeshment by filamentous organisms (Griffiths, 1965; Chenu, 1993; Degens, 1997; Young & Ritz 2004). Chenu, (1993), proposed that microbial production of extracellular polysaccharides may significantly change the physical properties of the immediate environment to influence microbial function and survival. The actions of fungal hyphae and roots in enmeshment of soil particles to increase the stability of macro-aggregates have been observed but the effectiveness is dependant on soil texture (Tisdall & Oades, 1979). The complexity of interrelationships in the soil matrix results in a “push-me-pull-you” effect marking out a clear hierarchical structure of cause and effect.

The production of hydrophobic exudates by fungi causing water repellence may influence preferential flow and structural stability of soils (Czarnes *et al.*, 2000), whereas the action of bacteria may break down these compounds thus decreasing repellency (Roper, 2004). Water repellency in soil aggregates caused by biological activity was reviewed by Wallis and Horne (1992). The inoculation of dune sand with fungi and bacteria has been shown by Forster (1989) to stabilise soil structure, and Edgerton *et al.* (1995) demonstrated a log-linear relationship between microbial biomass (as measured by ATP) and aggregate stability in restored opencast mine soils. Hallet and Young (1999) have shown in laboratory studies that the addition of nutrients increases biological activity, potentially causing severe water repellency of soil aggregates, whereas Feeney *et al.* (2006), showed no significant increase in repellency for an arable soil with artificially increased fungal biomass; they attribute this finding to the lack



of effectiveness of biocides used on different fungal species. Kiem and Kandeler (1997), showed that increased stability of soil aggregates as a result of microbial biomass was greatest in sandy soil with less than 15% clay content, and least impact was in clay soils (>35%). This stabilisation leading to sustained increases in porosity could be applicable at a number of scales.

The stability of soil structures has important implications for resilience of landscapes with respect to their ability to cope with externally forced change, such as wet-dry cycles and land management regimes. Cropping and tillage practices are already known to influence runoff and soil losses, and to influence the microbial community (Rasiah and Kay, 1995; Jackson *et al.*, 2003). The role of micro flora and fauna on soil structure formation, stabilisation and degradation at small aggregate scale has reviewed frequently (Oades, 1993; Six *et al.* 2004). Whilst single-aggregate scale research has been carried out into the effect of micro-organisms at species and community level on soil hydrological properties and aggregation, little research has been done at larger scales.

Identifying the microbial community components which contribute to this stability has never been completely successful, although fungi are often implicated, as discussed above. However, such factors may be related more to the overall configuration of the soil community rather than the properties of individual organisms. The r-K model, a microbial life-history strategy, divides the community into ‘opportunist’ (r) and ‘equilibrium’ (K) species, with the abbreviated terms derived from the logistic population growth model. The r-strategists are adapted to the fast production of offspring rapidly filling newly-realised niches in an ecosystem, whereas K-strategists

are adapted to a lower rate of reproduction but are able to utilise a wider range of substrates, and tend to remain active for longer than r-selected species, but at low rates of energy consumption. These K-strategists (typically filamentous organisms) are also more likely to invest resources in building resistant stabilising structures, in order that their low-level growth can persist.

Bass Becking (1934) postulated with regards to microbial ecology, that “Everything is everywhere, but, the environment selects” suggesting that in a given space and time most microbial species are latently present. Bass Becking (1934) and Beijerinck (1913) together with Sergei Winogradsky, are arguably the founders of modern microbial ecology (De Wit & Bouvier, 2006). They realised that micro-organisms modified their environment and that this could be important at a global scale, (De Wit & Bouvier, 2006). Thus, they understood the potential for earth system science and with that the concept of Gaia before it was fully postulated by Lovelock and Margulis (1974).

### ***1.3 Quantification of microbial communities***

The assessment of microbial communities provides valuable insights into the prevalence of various taxa, as well as specific metabolic genes within ecosystems, resulting in a greater understanding of the ecological processes (when mapped to function) occurring within those ecosystems. Changes in the microbial community structure, as a result of physical and chemical properties of the soil, may serve as an early warning indicator of degradation in soil properties (Schimel, 1995).

The study of soil microbial community structure can be based upon culture-dependent and culture-independent methods. Culture-dependent methods involve the cultivation of micro-organisms *in vitro* upon more or less defined media, and are ineffective for a large proportion of the soil microbiota. It has been reported that in a "typical" soil sample only 1 - 10% of the bacteria is cultivable (Johnston *et al.* 1996; Brock, 1987). Traditional cultivation techniques do not take into account bacteria that become uncultivable under environmental stress or that are obligatory symbiotic, species of low numbers in a sample being out-competed by more abundant or faster growing organisms, or the unavailability of selective media for many groups of microorganism (Roose-Amsaleg *et al.* 2001). The main culture-dependant methods are enrichment cultures and isolates, and most probable number (MPN) (Schinner *et al.*, 1996).

Due to the problems associated with culture dependent measurements, other methodologies that are cultivation-independent have been developed. Within the scope of the work contained within this dissertation it was essential that measures of the whole microbial community were made in order to elucidate the mechanisms of soil – microbial interactions.

### **1.3.1 Functional and community level assessment**

The characterisation of microbial communities can be considered in three main ways (Ritz *et al.* 2004): size (biomass), composition and activity.

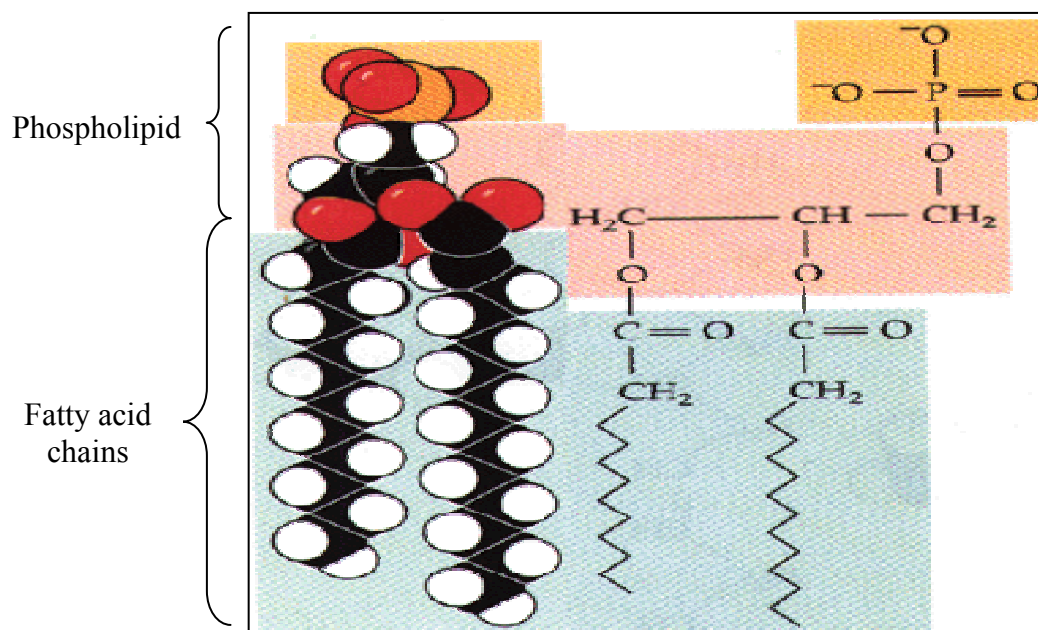
#### **1.3.1.a Microbial biomass**

Several methods for the estimation of soil microbial biomass have been developed. Jenkinson and Powlson (1976a) developed a chloroform fumigation-incubation method for the determination of microbial biomass from field soils. This method is limited to well drained soils with a pH >

4.8 that have not recently had easily decomposable material added (Martens, 1985; Beck *et al.*, 1997). Vance *et al.* (1987) developed a fumigation-extraction method which is less restricted by soil conditions. Substrate-induced respiration (SIR) is measured by monitoring the oxygen uptake or carbon dioxide production following addition of an optimal amount of glucose to the soil. SIR is a commonly used measure of microbial biomass and is less affected by soil conditions than fumigation-incubation (Anderson & Domsch, 1978). These methods provide information on the size of the microbiological population but give no indication of the species diversity or composition. There have been doubts raised about the reproducibility of microbial biomass measurements. Beck *et al.* (1997) carried out an inter-laboratory study to compare the results for biomass carbon by the above methods, they found that the 10 methods used ranked the biomass of soils in the same order, however their research was hampered by systematic error. Depending on the resolution of community analysis required the use of phospholipid fatty acid analysis or genetic techniques to assess microbial composition may be more appropriate (Zelles, 1999).

### **1.3.1.b Phospholipid fatty acid analysis**

Phospholipid fatty acids (PLFAs) are found in the membranes of all living cells. Phospholipids comprise fatty acids connected to a glycerol backbone and a polar phosphate head group (Figure 1.1).



**Figure 1.1: Phospholipid fatty acid, generic structure of a (adapted from Farabee (2000))**

The fatty acid ‘tails’ are composed of long hydrocarbon chains with carboxylic acid polar head groups. They are soluble in non-polar organic solvents such as chloroform. Fatty acids are characterised by the total number of carbon atoms, number of double bonds, the position and make up of additional functional groups and the type of isomerisation (Frostedgard *et al.* 1993). Their nomenclature is shown in Figure 1.2, where X is the length of the carbon chain, Y the number of double bonds and Z the position of the double bond in relation to either the carboxyl end ( $\Delta$ ) or the aliphatic methyl end ( $\omega$ ).



**Figure 1.2: Fatty acid nomenclature**

In this nomenclature, iso and anteiso methyl branching on the acyl chain is designated by the prefixes *i* and *ai*. Double bonds are indicated as being either *cis* (*c*) where the position of the functional groups are on the same

side, or *trans* (*t*) where the two senior groups are located on opposite sides of the double bond.

Bligh and Dyer (1959) developed a method for the rapid total extraction and purification of lipids from biological material. Variations of this method have been used to characterise a wide variety of microbial communities. The technique has been developed to provide estimates of microbial biomass, as well as of the community structure at certain group levels, e.g. it can determine both fungi and bacteria (Frostegard *et al.*, 1991; Frostegard & Baath, 1996; Schinner *et al.*, 1996). Good correlations have been shown between microbial biomass measurements and PLFA concentrations (Zelles, 1999). Zelles *et al.*, (1984), compared results from soil microbial biomass calculated on ATP content and microbial biomass calculated by CO<sub>2</sub> and found that for most soils the results were comparable.

#### **1.3.1.c Genetic assessment**

Nucleic acid-based analysis utilises nucleic acid extracted directly from soil samples (Amann *et al.* 1995). Nucleic acids are ubiquitous in cells and can act as a signature molecule for a given organism, thus providing valuable information about the species (Paul, 1996). Other methods developed employ the amplification of sub-unit rRNA genes from extracted nucleic acids. From these sequences group- and taxon- specific oligonucleotide probes can be developed making possible direct visualisation of micro-organisms in soil habitats possible (Hill *et al.*, 2000).

#### ***1.4 Limitations in studying microbial community***

The innate heterogeneity of soils and the spatial distribution of micro-organisms causes difficulty when studying microbial diversity (Trevors, 1998). Bacteria are highly aggregated in soils and their spatial distribution along with fungi is influenced by higher plants (Wall & Virginia, 1999). Inherent spatial and temporal variability in soil microorganism diversity can result in high variability between sample replicates and low power of statistical analysis (Klironomos *et al.*, 1999). Klironomos *et al.* (1999) suggest the use of geostatistical analysis to describe the spatial distribution of subsurface micro-organisms combined with power analysis to account for a greater proportion of systematic variability and produce a more representative result. Grundmann and Gourbiere (1999) suggest that when assessing the diversity of micro-organisms sampling should be carried out on a similar scale with more samples to avoid biased results and a predominant detection of dominant populations.

Franklin and Mills (2003) report that microbial communities may have several nested levels of organisation which could be dependant on different soil properties or groups of properties. Lack of taxonomic knowledge makes it difficult to study the diversity of a group of micro-organisms when it is not determined how to categorise and identify the species present (Kirk *et al.*, 2004). The study of soil microbial biomass and community is further difficult to quantify as the original quantities before any measurements are impossible to know, therefore it is difficult to determine how effective any extraction procedure is (Rondon *et al.*, 1999).

## ***1.5 Soil erosion and degradation***

The loss of soil due to erosion by either water or wind has serious implications for agricultural and non-agricultural lands. The normal erosion rate of a soil is around  $0.1 - 0.2 \text{ t ha}^{-1}\text{y}^{-1}$  (Brady, 1990), in Europe around 16% of the cultivated land is prone to erosion (Holland, 2004). When erosion rates exceed this and become unusually destructive it is termed accelerated erosion. Accelerated erosion occurs in two main stages, the detachment or loosening of material and then the transportation of material by floating, rolling, dragging and splashing (Brady, 1990). The detachment and lessening of material occurs usually through freeze-thaw cycles, flowing water and rainfall. Rainfall splash (particularly on smooth surfaces) and running water (particularly on rutted surfaces) are responsible for the carriage of loosened material (Ellison, 1947). Soil erosion and runoff can be assessed at different scales; catchment ( $>10^4 \text{ m}^2$ ), plot ( $10-10^4 \text{ m}^2$ ) or microplot ( $<10 \text{ m}^2$ ) (Barthès & Roose, 2002). The roughness of soil surface induced by tillage method affects the susceptibility of a soil to erosion and run off, on tilled soils water flow is directed along the tillage lines rather than topographic direction (Takken *et al.*, 2001). Rainfall simulation is a useful method for soil infiltration studies and the determination of hydrological properties of soil under rainfall and as well as erosion and runoff quantities (Adam *et al.*, 1957; Snelder & Bryan, 1995; Morgan *et al.* 1997; Singh *et al.*, 1999).

### **1.5.1 Rainfall**

Raindrops influence soil erosion in three ways: by detaching the soil on initial impact; by continuous beating destroying surface aggregates; and by the splash causing transportation of soil. Broken down surface aggregates can aid the formation of crusts when the soil dries which can encourage



greater runoff from subsequent precipitation. On an easily erodible soil rainfall splash can transfer as much as 255 t ha<sup>-1</sup> soil, and on a slope or in high wind splashing aids runoff translocation of soil (Brady, 1990). Three main types of water erosion are recognised:

- Sheet erosion is where soil moves uniformly from a slope, it is often accompanied by rill erosion.
- Rill erosion, where irregularly-dispersed channels (rills) form.
- Gully erosion a more dramatic form of rill erosion, here concentrated runoff causes the formation of larger channels by downward cutting.

$$A = RKLSCP \quad (1.1)$$

A=predicted soil loss  
R=climatic erosivity (rainfall & runoff)  
K=soil erodibility  
L=slope length  
S=slope gradient or steepness  
C=cover and management  
P=erosion control practice

The factors affecting accelerated erosion are expressed in the universal soil-loss equation (Equation 1.1). Snelder and Bryan (1995) suggest that the production of sediment decreases in concentration as the storm duration increases due to a decrease in splash erosion caused by ponding and exhaustion of erodible material. The inherent erodibility of a soil is indicated by the soil erodibility factor, K. The factors L and S represent the effect that topography has on erosion, for example the greater the steepness of the slope, the greater the erosion. The cover and management factor, C, shows the influence of cropping and soil management systems on erosion.

This equation does not however take into account any biological impact on the soil, merely the physical effects. However the biological component is arguably implicit in 'K'. During the course of this thesis it is intended to highlight the impact that biological systems have on soil structural integrity, a factor potentially linked with K from Equation 1.1. Morgan *et al.* (1997) found that vegetation exerted an important hydrological control by increasing the infiltration capacity of the soil which influenced the time to, and duration of, runoff and also concluded that soil loss decreased exponentially with increasing vegetation cover (Morgan *et al.* 1997). They also found that in simulated rainstorms that increasing vegetation cover affected both the total runoff and the time to run off, this can be explained by the time taken for the land to reach terminal infiltration rate (Morgan *et al.* 1997). Snelder and Bryan (1995) suggest that as vegetation cover decreases other factors such as soil biological activities and distribution of natural rainfall become important determinants of soil erosion. It is reported that increased vegetation cover reduces the amount of surface crusting, increasing the root development and therefore increasing the infiltration rate of rainfall (Snelder & Bryan, 1995; Kort *et al.*, 1998). Lastly the support practice factor, P, takes into account the benefits of strip cropping, terrace systems and contouring, plus other erosion reducing practices. Takken *et al.*. (2001) show that the inclusion of tillage induced runoff patterns when predicting soil erosion and deposition at the field scale in conjunction with detailed topographic data can greatly improve the outcome of the model. They suggest that borders between fields and linear features, such as roads, may act as water collectors or significantly change the natural flow of the water changing the normal erosion pattern of that soil (Takken *et al.*, 2001).

### 1.5.2 Aggregate stability

The mechanical strength of soil is important for ecological and agricultural stability. Soil structural stability ( $K$  in Equation 1) is a principal parameter governing soil loss (Ousser *et al.*, 1993). A widely used measure of soil structural stability is based upon the assessment of water stable aggregates (Tisdall & Oades, 1982). Increased structural stability decreases the susceptibility of the soil to erode because there is a reduction in the formation of crusts and separation of soil particles (Diaz-Zorinta *et al.*, 2002). The erosion of agriculturally cultivated soils comes from the breakdown of soil aggregates and the detachment of soil fragments by rain and wind (Le Bissonnais, 1996; Diaz-Zorinta *et al.*, 2002). Soil aggregate breakdown is caused by many factors. There is no true agreement on the best measurement of soil aggregate breakdown or on its relationship with large scale erosion (Le Bissonnais, 1996).

The measurement of aggregate stability is based on the assumption that soils possess a minimum state of aggregation under water saturated conditions (Zanini *et al.*, 1998). The wet stability of soil aggregates is important because of the effects on water entry, soil tilth and erosion (Coughlan *et al.*, 1991; Zanini *et al.*, 1998; Diaz-Zorinta *et al.*, 2002). A common measure is Yoder's (1936) 'wet sieving' where the resistance of aggregates to breakdown under mechanical abrasion in water is measured (Yoder, 1936; Zanini *et al.*, 1998). Wet sieving is dependant on the chemical composition of the water, the degree to which the aggregates are pre-moistened and the time the aggregates are agitated for (Letey, 1991). Thus there are concerns over the consistency of these measuring techniques (Coughlan *et al.*, 1991; Darbyshire *et al.* 1993; Le Bissonnais, 1996; Zanini *et al.*, 1998). When the soil aggregates are weak the wet-sieving

method discriminates poorly between soil types (Le Bissonnais, 1996). Le Bissonnais (1996) highlighted the importance of standardising initial water content of aggregates before commencing stability tests. To avoid wetting soils Darbyshire *et al.* (1993) suggest that soils could be sieved at field moisture, however finer sieves quickly become blocked causing a reduction in the size fraction able to pass through the sieve. This method would also present problems when comparing soils of different field moistures and would allow the size distribution to be created by the analyst (Darbyshire *et al.* 1993). Young and Ritz (2005) suggest that discrete soil aggregates only exist as a function of mechanical disruption of the soil and suggest that within days following disruptive events such discreteness in architecture disappears and the complex network of pores returns.

The strength of soil aggregates can also be measured by crush tests – these tests are suitable for all sizes and strengths of soil aggregates, or by shear strength, which is measured by torsional shear boxes and penetrometers (Dexter, 1988). The aggregates used in these tests should be representative of the whole soil as the behaviour of the selected aggregates may still only represent that size fraction of the soil (Le Bissonnais, 1996). Young and Ritz (2005) suggest that sieving techniques do not represent the original ped they come from as the performance of the individual aggregates does not take into account how the aggregates were originally connected. However, Barthès and Roose (2002) suggest that soil susceptibility to runoff and erosion is closely related to the stability of surface (0-10 cm) aggregates and regard it a relevant indicator of soil resistance to runoff and erosion varying from m<sup>2</sup> to ha scale.

Linn and Doran (1984) report soil structure to have a direct influence on microbial activity and community at the field scale. Lupwayi *et al.* (2001) investigated the effect of conventional and zero tillage on bacterial diversity in water-stable aggregates and found that during the cropping cycle the deterioration of soil structure is one factor that explains the adverse effect of tillage on microbial biomass and diversity as measured by catabolic potential (BIOLOG). But little information is present on the effect of biological activity on soil structural integrity.

### ***1.6 Impact of tillage on the soil microbial community***

Tillage is carried out on arable land to prepare a seed bed, incorporate plant residues and chemical amendments, for weed control and for soil and water conservation. It can be classified by the degree of soil disturbance that it causes. Primary tillage usually inverts the soil surface burying plant residues, whereas secondary tillage disturbs less soil and buries fewer residues. Newer management systems are more focused on less soil disturbance and more plant residues left on the surface of the soil to prevent loss of topsoil by erosion, and to maintain higher moisture content for the crop. Crops can be grown for several years with viable yields through the use of herbicides, genetically modified crops and implements that can plant seed with minimal soil disturbance. This change in agricultural practice is being driven by European and national legislation and guidelines on land management (Section 1.6.2).

Tillage practices involving high soil disturbance can, in the short term, provide favourable effects on soil aggregation by breaking up large clods and incorporating organic matter into the soil which in turn can stimulate the soil biota. However, tillage practices can speed up oxidation of organic

matter, break down stable aggregates and compact soils reducing the soils fertility (Brady, 1990). The scale of the disturbance to the soil compared to the scale of the microbial habitat will dictate how drastic a reaction there is, e.g. the tearing of fungal hyphae by mould-board ploughing would have dramatic effects on the fungal community whereas bacteria residing in the centre of a soil aggregate may be unaffected by the same disturbance (Young & Ritz, 2000).

Carter (1986) assessed a range of agronomic and climatic changes under a variety of different tillage systems and their effects on biomass carbon and nitrogen. The biological properties of the soil were shown to be influenced by soil mixing, incorporation of crop residues, soil moisture and root growth (Carter, 1986). Microbial biomass carbon and nitrogen were noted to react rapidly to changes in soil management and tillage, this change was observed before noticeable changes in total soil organic carbon or nitrogen (Carter, 1986). Tillage practices affect the structural architecture of soil changing the topography and distribution of pore networks and therefore the availability of substrate, water and oxygen to the microbial population (Young & Ritz, 1998).

Conventional or inversion tillage is achieved by the use of a mould board plough. Cultivation by this method results in the modification of the top 12 – 18 cm of the soil surface and typically results in a bacterially dominated soil microbial community (Titi, 2003). Conservation tillage is a broad term which encompasses a wide variety of soil management systems ranging from zero-tillage to the use of heavy discs but excludes the use of a mould board plough. It is perhaps best described as ‘non-inversion tillage’ and is practiced on some 45 million hectares worldwide (Holland, 2004).

Conservation tillage practices are primarily used as a means to protect soils from erosion and compaction, to conserve moisture and reduce production costs (Holland, 2004).

Cultivation of soil by conventional methods could stimulate mineralization of soil organic matter resulting in higher nitrate concentrations when compared to reduced tillage methods by making the carbon substrate more readily available to the soil biota. Long-term cultivation of soils tends towards higher porosity, lower bulk density and pore conductivity of the soil, therefore, the water holding capacity and saturated hydraulic conductivity decrease (Dick, 1992; Silgram & Shepherd, 1999). Longer term increases in C mineralization rate may be triggered by the effect of changes in soil physical properties on microbial populations (Jackson *et al.* 2003). Invasive land management regimes have been shown to decrease fungal activity by fragmenting hyphal networks and reducing stored soil carbon (Bailey *et al.* 2002). However, as a result of such stress conditions increased mycorrhizal sporulation has been observed (Titi, 2003).

Conservation tillage practices and the associated incorporation of crop residues have been shown to induce changes in the soil microbial biomass, particularly encouraging fungal growth and temporary immobilisation of nutrients (Drury *et al.* 1991; Pankhurst *et al.* 2002). Plant residues protect inoculated rhizobia from temperature and moisture extremes, improving microbial survival, rhizosphere colonization and increased nodulation (Titi, 2003). Conservation tillage practices can decrease soil temperature by up to 10°C, improve water availability by aggregate stability and increased number of macropores thus producing favourable conditions for symbioses (Titi, 2003).

Lupwayi *et al.* (2001) investigated the bacterial diversity in water-stable aggregates of conventional and zero-tillage loam and silt loam soils in British Columbia grown to barley. They found that whilst there were no significant effects of tillage on bacterial diversity in the whole soil, at barley heading time functional diversity (catabolic potential, BIOLOG) by Shannon's index, substrate richness and substrate evenness were significantly higher under zero tillage than under conventional tillage (Lupwayi *et al.* 2001). From the results of this experimentation Lupwayi *et al.* (2001) conclude that during a whole cropping cycle the adverse effect of tillage on microbial biomass and diversity is due to deterioration in soil structure. In another study Lupwayi *et al.* (1998) used substrate utilization by bacteria to characterise the effect of tillage and wheat crop rotation on diversity and community structure in a Gray Luvisol in Alberta.

Jackson *et al.* (2003) investigated the effect of a simulated tillage event on intensively managed vegetable crop soil and a grassland soil in California. They found that after simulated tillage the ratio of PLFA markers 19:0cyc to 18:1 $\omega$ 7 increased in both soils indicating stressful conditions for bacteria, this increase was more pronounced in the grassland soil (Jackson *et al.* 2003). They found that the PLFA composition of the soils was significantly related to the time after sieving, the grassland soil developed a different microbial community structure within one day but that there was little change in total microbial biomass. The higher microbial activity and respiration rate of tilled soils and the higher cumulative CO<sub>2</sub> flux in this experiment may be explained in part by the higher soil temperature associated with tilled soil as a result of energy input, but ultimately some compromise has to be made between the benefit of tillage for health and productivity of some crops and the decrease in soil quality resulting in



increased greenhouse gas emission and nitrate leaching potential (Jackson *et al.* 2003).

Wright *et al.* (2005) investigated the impact a 20 year cropping sequence of corn and cotton in Texas, under different tillage regimes, had on soil C and N sequestration and on microbial C and N dynamics. They found that the total microbial biomass C and N were impacted little by tillage when observed at 2.5 – 20 cm depth and that microbial biomass N was more affected by tillage under a cotton crop rather than a corn crop. The microbial biomass C and N decreased with depth of soil and was influenced by long term tillage possibly influencing the potential nutrient supply to crops. Tillage regimes maintaining crop residue at the soil surface had beneficial impacts on the supply of mineralizable nutrients and enhancement of the microbial biomass (Wright *et al.* 2005).

Steenwerth *et al.* (2002) compared soil microbial communities existing under different land use histories in both cultivated and grassland ecosystems in coastal California. They found that PLFA profiles, soil characteristics and site and management factors showed distinct groupings for land use types, and suggest that labile soil organic matter affects microbial composition. Nsabimana *et al.* (2004) also suggest a broad relationship between size, activity and diversity of the soil microbial community and soil organic matter content. They show that size, activity and diversity of soil microbes is substantially affected by land use (Nsabimana *et al.*, 2004). They also suggest that the reduced inputs of above and below ground plant litter may well have reduced the fungal/bacterial ratio favouring a greater metabolic rate (Nsabimana *et al.*, 2004). Alvarez *et al.* (1998) investigated the associations between organic matter

and soil microbial biomass in conventional, no tillage and pasture land management regimes. They found that the availability of C in the light fraction per unit of active soil microbial biomass and the respiration unit of active biomass were strongly, positively associated. They also showed that after a four year period of no-tillage the light soil fraction had accumulated carbon suggesting no-tillage as a potential treatment to improve soil fertility (Alvarez *et al.* 1998). Bending *et al.* (2004) found that biochemical and biological parameters of soil can provide contrasting indications of soil quality and suggest that microbial analyses is an effective measure of land management induced changes to soil quality. Schloter *et al.* (2003) demonstrated that the use of precision farming compared to conventional agricultural management did not influence the microbial biomass and community structure in southern Germany. However, they did show that there was a strong seasonal influence and that enzymatic activities altered with land use practice.

Alvear *et al.* (2005) investigated the effect of no tillage (with stubble burning), no tillage (without stubble burning) and conventional tillage (with stubble burning), on microbial biomass in an Ultisol from southern Chile in the third year of a wheat-lupin-wheat crop sequence. They found that the microbial biomass C and N generally increased in the no tillage systems when compared to conventional tillage, this more markedly in the winter season and the upper most soil layer (0 – 50 mm).

Feng *et al.* (2003) researched the effect of conventional and no tillage practices on microbial communities in Decatur silt loam soil under long-term continuous cotton systems. Changes in the microbial community (by PLFA) shifted over time and by depth of sample. During the growing

season the changes were attributed more to changing soil conditions (e.g. moisture and temperature), whereas prior to cotton establishment community changes associated with tillage were more pronounced. The impact of reduced, zero and conventional (with and without residue) tillage practices on microbial dynamics was studied by Spedding *et al.* (2004) on a sandy loam to loamy sand soil in Quebec under maize monoculture. The microbial biomass C was found to change very little temporally, whereas the microbial biomass N was responsive to mineral nitrogen fertilisation post crop emergence and increased total PLFA and fungal component were shown by PLFA profiles.

Changes in PLFA profiles of microbial communities under zero and conventional tillage over 25 years of wheat-fallow management in Nebraska was studied by Drijber *et al.* (2000). They suggest that there is a relationship between tillage management and long-term resilience of the microbial community as FAME profiles from the fallow plough were the most dissimilar from the cropped soils (Drijber *et al.* 2000).

Stenberg *et al.* (2000) looked at the effect of reduced tillage with and without liming compared to mouldboard ploughing with and without liming in a silty clay loam soil under a 4 year crop rotation. Under these conditions reduced tillage improved the aggregate stability, increased the organic matter and the activity of the soil micro-organisms. Liming was shown to increase the microbial activity but not impact the soil structure significantly (Stenberg *et al.* 2000). The study of long-term no tillage and conventional tillage on microbial biomass C and N in a Brazilian Oxisol, showed that whilst increases in microbial biomass, C and N mineralization were observed under no tillage systems, the microbial pool under

conventional tillage was shown to be more metabolically active (Zhang *et al.*, 2007).

Tillage simulated by sieving a sandy loam soil, from under a grassland and vegetable production, has been shown to produce rapid changes in the microbial community structure (PLFA). Respiration was shown to decrease immediately after sieving and decline over a 14 day measuring period along with the continuous accumulation of nitrogen. The PLFA profiles from soils obtained under vegetable production showed slower and more gradual changes indicating that short term responses of the microbial community to tillage may be less pronounced in soils with a history of long-term cultivation possibly due to a more resistant community (Calderon *et al.* 2000).

Frey *et al.* (1999) showed that in the top 0-5 cm of surface soil from sites under tillage treatments between 11 and 26 years, the fungal population was significantly higher under conservation tillage than in conventional tillage. In soils under conservational tillage, when compared to conventional tillage systems, nitrogen fixing bacteria (*Azospirillum* spp.), ammonifiers and micro-organisms that solubilize phosphate, showed increased numbers. It was also observed that soils under conventional tillage showed a greater population variation, density and an increase in microbial activity after harvest (Titi, 2003).

Microbial community size, structure and activity have been shown to be affected by land management as a result of perturbation and substrate distribution. Conservation tillage practices have been shown to have an

increased biomass, in particular fungal population, when compared to conventional tillage systems and there is evidence of seasonal variation in microbial populations under a variety of land management systems. Effects of tillage on microbial community size tend to occur after a period of years and there is also evidence to support community resilience to perturbation over a long history of tillage. The soil architectural environment alters significantly after tillage causing huge alterations to the habitat of soil biota, such changes in community structure may have functional consequences for soil structural properties, and hence erosion, and nutrient cycling phenomena.

#### **1.6.1 Effect of cropping on microbial community structure**

Plants are primary producers of organic matter. Their roots modify the soil they inhabit by widening pores and cracks and creating pores when they decompose. They implement stress conditions in the soil by removing water and stimulating soil aggregation. They support micro-organisms with their exudates (Killham, 2001).

Cropping influences the microbial population by the release of different organic exudates which may further stabilize soil aggregates (Brady, 1990; Titi, 2003). Legume growing adds nitrogen to the soil through  $N_2$  fixation, therefore, introduction of a legume cover crop could ensure a nitrogen reserve in the soil, however most of the legume N is harvested as grain and therefore the use of legume residues does not necessarily increase soil nitrogen levels.

In a growing global community (currently around 5.8 billion growing by 90 million people a year (Population Action International, 2005), agriculture

will continue to be urgently required to produce food, therefore understanding how this affects the landscape and determining sustainable agricultural practices will be key in the maintenance of soil resources.

### **1.6.2 Legislative drivers**

In recent years the political priorities for environmental protection have started to become more attuned to the need for a coherent approach to soil protection. In Europe the soil protection until recently has been covered under different policies such as the EC Nitrates Directive (91/676/EEC), Sewage Sludge in Agriculture Directive (86/278/EEC) and Habitats and Species Directive (92/43/EEC). In September 2006 the European Commission adopted the thematic strategy for soil protection proposing a framework directive and an impact assessment of soils in the EU (COM (2006) 231, 232). It is recognised that the long-term protection of soils in Europe requires the development of more complete soils information, monitoring and indicators (Bullock & Montanarella, 2005).

The Common Agricultural Policy (CAP) in Europe began by subsidising production of basic foodstuffs in order to ensure adequate supply. However, since its inception some 50 years ago the focus of CAP has changed to the preservation and management of our natural resources. It ensures financial security for farmers who are hit by natural disasters or animal disease, whilst ensuring compliance with set standards, e.g. rural landscape preservation, biodiversity and plant and animal health.

These changes in legislative focus mirror the changing public awareness and concern over our natural environment. The growing global population requires adequate food production so the development of sustainable

agricultural practices is paramount to the survival of our species in the long term. It is critical that scientific observation continues to provide the evidence and data to inform legislative change both at regional, national and European scales. It is already apparent that certain aspects of the environment should be considered at larger scales, for example the emission of green house gasses is not only a European concern but a global one.

Increasing amounts of green house gases such as carbon dioxide are linked to human-induced global warming (by fossil fuel burning, industrialisation and agriculture), a concept first speculated by the Swedish chemist Svante Arrhenius in 1897. Under the United Nations Framework Convention on Climate Change (UNFCCC) the Kyoto protocol is an agreement in which ratifying countries (around 160 countries) commit to reducing their emissions of carbon dioxide. However this attempt at global enforcement of environmental protection is opposed still, notably by the USA and Australia. Sceptics of global warming see the Kyoto protocol as an attempt to slow the growth of industrial democracies or to transfer wealth to developing countries in an act of so called 'global socialism'.

### ***1.7 Soil and Surface Water Protection using Conservation Tillage in Northern and Central Europe (SOWAP) Project***

The SOWAP project aims to demonstrate, at a representative and reproducible scale, an innovative solution which will protect soil resources, reduce pollution of surface water by sediment and sediment-bound contaminants, and promote sustainable land-use practices. There are two project sites located the UK, one in Hungary, and one in Belgium. The UK sites (Somerset and Leicestershire) were used to gain higher resolution data

on soil erosion losses, runoff volumes, surface water quality, and physical, chemical and biological indicators of environmental quality.

SOWAP project objectives were to demonstrate:

- The viability and effectiveness of “conservation oriented” arable land management systems in protecting soil resources, improving catchment water quality and promoting biodiversity.
- The environmental, ecological, economic and social benefits of conservation oriented land use practices.
- The environmental impacts associated with conventional arable land use practices, where intensive soil management can lead to degradation of soil resources, water pollution, reduced biodiversity and less carbon sequestration.
- How an environmentally sound land use policy can be implemented, as recommended by the EU 6<sup>th</sup> Environment Action Programme and the EC Communication on Soil Protection.
- How a database can be disseminated successfully at the local, regional, national and EU level via workshops, multimedia, field visits, publications and the Internet.

### **Aims and Objectives**

This multiple-scale study aimed to improve the mechanistic understanding of the effect of soil tillage practices on soil microbial community structure and to determine links between soil microbial communities and the propensity of soils to erode.



### 1.7.1 Hypotheses

- Different tillage practices will result in a variation in microbial community structure. It is hypothesised that conventional tillage will have a specific effect on microbial C dynamics relative to conservation tillage via the following mechanisms:
  - *Reduction in soil organic C and associated microbial-based adhesion of soil particles*
- The rate of rainfall-induced erosion at the microcosm scale will be inversely proportional to the total biomass.
  - *Increased soil aggregate stability by the presence of fungal hyphal enmeshment and the cell exudates will reduce surface aggregate breakdown and particle detachment.*
- Variation in the microbial community structure, in particular the increased ratio of fungi to bacteria, will have an impact on the propensity of soils to erode.
  - *Increased abundance of filamentous species will enmesh soil structure reducing particle detachment.*
- The presence of a microbial community will impact on water movement through soil in relation to infiltration and runoff.
  - *Increased soil aggregation and pore connectivity as a result of microbial mediation will improve soil drainage, decreasing surface flow.*

- Tillage impact on microbial community size and structure at a field scale will lead to differing erosion event outcomes across tillage treatment types.
  - *Different microbial community composition and size, as a result of tillage practice, will impact on particle detachment.*

### **1.7.2 Approaches:**

The effects of the tillage practices upon the soil microbial community size and structure was investigated at the community-level, specifically using phospholipid fatty acid analysis (PLFA) and microbial biomass carbon.

The impact of soil microbial communities *per se* on the erodibility of soils was assessed by the use of sterile and non-sterile microcosm systems.

## Chapter 2 – General methodologies

### 2.1 Introduction

Soil microbial community size and structure were the main focus of this study as indicated by biomass carbon and phospholipid fatty acids (PLFAs). In addition to the microbial measurements, it was necessary to gain some idea of the characteristics of the soil matrix itself and so other physicochemical measurements were conducted by NRM Laboratories Ltd (Bracknell, Berkshire) within the framework of the SOWAP project; the methods for this dataset are included in the appendices (Appendix I).

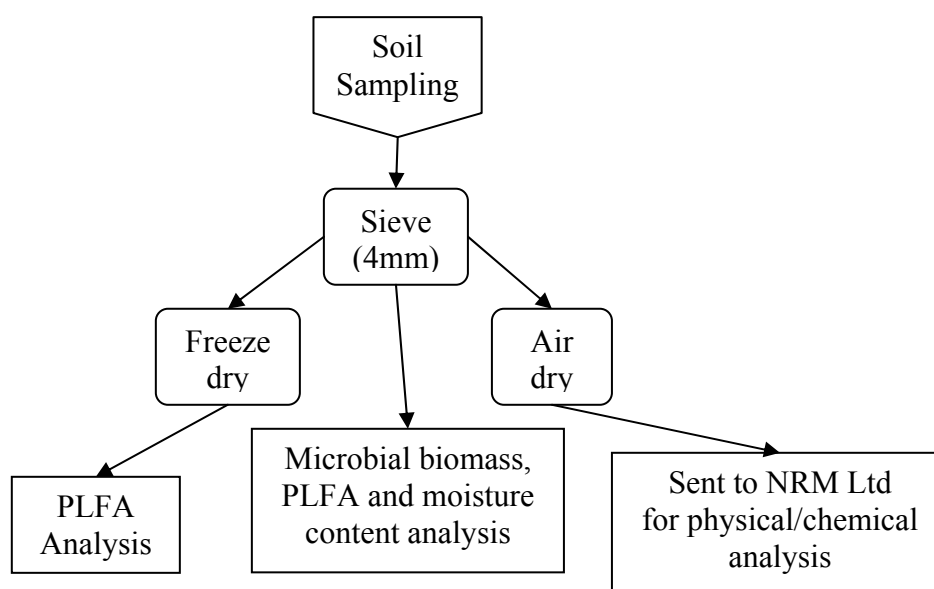


Figure 2.1: Flow chart of sample processing

### 2.2 Sample preparation and storage.

Field samples were taken in accordance with British Standard 7755-2.6:1994 (ISO 10381-6:1993). This defines general sampling methods such as sample marking, transportation and storage conditions. Soil was sampled (unless otherwise stated in the relevant chapter) using a gouge

auger to a depth of 150 mm relative to the surface following removal of superficial residues. All soil samples were homogenised by passing through a 4 mm sieve, and all obvious plant root material, stones and animals removed. Samples were then stored at 4°C and processed within two weeks of sampling.

### **2.2.1 Air drying**

Prepared samples were laid out in a thin layer in metal trays and left exposed for a minimum of 48 h at room temperature (typically 25°C) until dry. The samples were then milled to 2 mm using a hammer mill (Glen Creston, Twickenham, UK). These samples were then sent to NRM laboratories for analytical testing.

### **2.2.2 Freeze drying**

For microcosm and field rainfall simulation experiments the samples were freeze-dried for preservation. After basic sample preparation (Section 2.2), samples were frozen at -80°C for 24 h then freeze-dried using an Alpha 1-2 LD (Christ Freeze Driers, Osterode am Harz, Germany).

## ***2.3 Soil physical parameters***

### **2.3.1 Determination of gravimetric moisture content**

The gravimetric moisture content of soils was determined by weighing prepared soils (~10 g, accurately weighed) into dried, pre-weighed and numbered drying tins. The tins were then placed in a forced circulation oven and dried at 105°C for 48 h. The tins were then placed in a desiccator to cool and weighed to four decimal places. The moisture content was then determined as follows:

$$MC = \frac{(FW - DW)}{DW} \times 100 \quad (2.1)$$

Where:

MC = moisture content (%)

FW = mass of fresh (moist) soil (g)

DW = mass of dry soil (g)

Once the percentage moisture content was calculated on fresh weight of soil it was possible to calculate the dry weight of the soil by subtracting the moisture content from the fresh weight of the original sample.

### **2.3.2 Determination of shear strength by Torvane**

Shear stress measurements were taken from the surface of soil using a Torvane or Pocket Vane Tester (Eijkelkamp Agrisearch Equipment, Town, Country), fitted with a CL 100 vane (Figure 2.1). The Torvane dial was aligned to zero using the index mark on the knob. The Torvane was pressed into the soil surface to the depth of the blades whilst maintaining a constant vertical pressure and turning the knob such that the rate of rotation was sufficient to allow failure to develop in 5 to 10 seconds. After the failure developed the remaining spring was released slowly and the value indicated by the index mark recorded. The vane was then cleaned using demineralised water and dried.

Once recorded, the readings were converted to  $\text{kg cm}^{-2}$  according to:

$$S = 0.10936xR \quad (2.2)$$

Where:

S = shear strength

R = recorded reading on Torvane dial

The constant equals 1/10 of the value of a complete revolution (1.0936  $\text{kg cm}^{-2}$ ).

## ***2.4 Chemical analyses***

Chemical analysis of all soil samples were conducted by NRM Laboratories in accordance with their standard methods (Table 2.1 & Appendix I).

**Table 2-1: Soil parameters measured by NRM laboratories Ltd.**

Determinand	Method
Particle size distribution	Pipette
Organic matter content	Wet oxidation (Walkley Black)
Cation exchange capacity	Sodium saturation
pH	In water and in calcium chloride
Total nitrogen	Dumas
Total carbon	Dumas
Available phosphorus	'Olsen's' sodium bicarbonate extractable
Available potassium	Ammonium nitrate extractable
Available magnesium	Ammonium nitrate extractable

## ***2.5 Microbiological analyses***

### **2.5.1 Determination of microbial biomass carbon by chloroform fumigation-extraction**

Preformed in accordance with British Standard 7755-4.4.2:1997 (ISO 14240-2:1997), based on the method described by Vance *et al.* (1987).

#### **2.5.1.a Methodology**

Two 10 g sub-samples of each soil were weighed into glass jars. One was labelled control (C) and the other was labelled fumigated (F). A desiccator was lined with moist filter papers and the samples for fumigation placed within. A beaker of ethanol-free chloroform (~50 ml) with anti bumping granules was placed inside the desiccator which was then sealed using

silicon grease. The desiccator was then evacuated using a pump until the chloroform had boiled for 2 min, after which the desiccator vacuum tap was closed and the desiccator left at room temperature for 24 h. After fumigation the chloroform and filter papers were removed and the chloroform vapours removed by repeated evacuation (6 times 2 min each).

Control and fumigated samples were extracted using 40 ml of 0.5 M potassium sulphate for 45 min on an end-over-end shaker at 60 revolutions  $\text{min}^{-1}$ . The samples were then filtered through Whatman No. 42 filter papers. In addition samples of the potassium sulphate were filtered for use as blanks. The extracts were then stored at  $-16^{\circ}\text{C}$  until determination.

Determination of carbon in the extracts was performed using a SFA-2000 segmented flow analyser (Burkard Scientific, Uxbridge, UK). Frozen samples were thawed at room temperature and diluted 2-fold with sodium polyphosphate (50 g in 900 ml of distilled water, adjusted to pH 2 using orthophosphoric acid made up to 1 litre with distilled water). Potassium hydrogen phthalate standards of 10, 20, 30, 40 and 50  $\text{mg C l}^{-1}$  were prepared from a stock standard solution. In this method, soil organic carbon is oxidised by the presence of acidified potassium persulphate (pH 2) and irradiated with UV light to convert any organic carbon to carbon dioxide. The carbon dioxide permeates a gas diffusion membrane into a buffered phenolphalein solution causing a change in colour measured at 550 nm.

DOC concentration in extracts and biomass C were calculated according to Equation 2.3 and 2.4.

$$C(\mu g g^{-1}) = (SC - BC) \times \frac{Ev}{Ws} \quad (2.3)$$

C = carbon

SC = value of carbon measured in the sample

BC = value of carbon measured in the blank

Ev = Extraction volume

Ws = dry weight of soil mass

$$MBC = \frac{(FOC - UOC)}{0.45} \quad (2.4)$$

MBC = microbial biomass carbon

FOC = fumigated organic carbon

UOC = unfumigated organic carbon

## **2.5.2 Determination of microbial community structure by phospholipid fatty acid analysis**

### **2.5.2.a Overview**

The method used for determination was based on the method described by Frostegard *et al* (1991), as based on the methods described by Bligh and Dyer (1959) and White *et al* (1979).

All glassware was prepared by rinsing with hexane and drying before use, and care was taken to avoid exposure of samples to light for longer than necessary.

### **2.5.2.b Methodology**

Aliquots of prepared soil (10 g) were weighed into sterile glass media bottles and a ratio of 0.8:1:2 of citrate buffer: chloroform: methanol added, with the volume of citrate buffer added altered to take into account the moisture content of the soil. All solvents used were HPLC grade throughout. Citrate buffer consists of 0.15 M citric acid dehydrate and 0.15 M trisodium citrate in deionised water and adjusted to pH 4 using dilute acetic acid. For storage purposes the 1:2 (v/v) of chloroform: methanol



was stored using 0.005% w/v butylated hydroxyl toluene as an anti-oxidant. PTFE tape was used as a barrier between the lid and contents of the media bottle in order to prevent plasticide contamination. The samples were then sonicated for 30 min and shaken on a horizontal shaker at 200 revolutions  $\text{min}^{-1}$  for 30 min. The samples were then centrifuged at 700 x g for 10 min to ensure a clean interface between the phases. The organic phase was then removed to a clean sterile media bottle, discarding the remaining soil and media bottle. A further phase separation was made by the addition of 4 ml chloroform and 4 ml citrate buffer then leaving the samples overnight, refrigerated at 4°C. The aqueous phase was then removed and discarded and the organic phase dried under nitrogen at 37°C to prevent the breakdown of unsaturated fatty acids by oxidation of double bonds. The samples were then frozen at -18°C.

Fractionation was achieved by solid phase extraction (SPE) resulting in the lipid extract being separated into neutral lipids, glycol-lipids and polar lipids. Commercially prepared SPE columns (3 ml/ 500 mg silica Sep-pak Vac™, Waters Chromatography, Milford MA, USA) were used; they have an optimal rate of elution of 2 ml  $\text{min}^{-1}$ . Active sites (silanols) on the silic acid (slightly acidic precipitated silica) contain hydroxyl groups which interact with the polar groups of the lipid classes. As the solvent polarity increases the lipid classes are selectively eluted from the solid phase.

Sodium sulphate (~0.5 g) was added to the top of the SPE cartridge to absorb any moisture left within the sample. The columns were washed with 2 ml methanol, followed by acetone then chloroform. The columns were then dried on a SPE manifold for 2 min and then conditioned by

seeping 2 ml chloroform through. From this stage onward the sorbent was not allowed to dry out.

The frozen extracts were defrosted at room temperature and 1 ml of chloroform added (washed down the sides of the bottle). The resuspended lipid extracts were then loaded into the conditioned SPE cartridges. The neutral (sterols) and glycol lipids were eluted using 5 ml chloroform and 12 ml acetone. Clean sterile media bottles were then placed under the manifold while the polar lipids containing phospholipids were eluted using 8 ml of methanol. These were then evaporated to dryness under nitrogen at 37°C and frozen and stored at -18°C.

Mild alkaline methanololysis was carried out based on the procedure of Dowling *et al.* (1986). All solvents used were dried over anhydrous sodium sulphate and all glassware oven dried for an extra 30 min beforehand. The frozen polar lipid extracts were defrosted at room temperature and reconstituted with 1 ml of 1:1 toluene: methanol. One ml of 0.2 M methanolic potassium hydroxide was added at 37°C and swirled to mix for 30 min to hydrolyse the lipids. This reaction was halted by the addition of 0.25 ml of 1 M acetic acid to neutralise the pH of the sample. Hexane: chloroform 4:1 (v/v) 5 ml was added along with 3 ml of deionised water and then the samples were sonicated for 30 min. They were then left overnight at 4°C in the dark to create clearly separated phases. The aqueous phase was then removed and discarded using a Pasteur pipette, and 3 ml of 0.3 M sodium hydroxide added, causing a further phase separation. The top phase was then filtered through sodium sulphate into a clean sterile media bottle and dried under nitrogen at room temperature (~25°C). The

fatty acid methyl esters (FAMES) were then stored at -18°C under nitrogen until determination by gas chromatography.

The dried, frozen FAMES were resuspended in 200 µl of hexane and transferred to an amber gas chromatography (GC) vial. Samples were injected into a GC (6890N, Agilent Technologies, Santa Clara CA, USA) using an auto sampler with an injection temperature of 310°C. The FAMES were separated using a temperature programme starting at 50°C for 1 min splitless hold time, increasing at 25°C per min to 160°C, then increasing at 2°C per min to 240°C and then at 25°C per min until reaching 310°C. An 6890N (Agilent Technologies, Stockport, UK) GC was used in conjunction with Agilent G2070 ChemStation for GC systems software. The carrier gas used was helium and the detection was by a flame ionisation detector operating at 320°C.

The separated FAMES were identified by comparison of GC retention times to a standard qualitative bacterial acid methyl ester mix (Supelco 26 standard) supported where necessary by gas chromatography mass spectrometry. The PLFA molecules derived from fatty acid methyl esters are shown in (Table 2.2). The results were expressed as a percentage of the total area of the identified peaks on the chromatogram (mol %). Using %mol data it is possible to calculate a fungal: bacterial ratio using the %mol 18:2 $\omega$ 6 (fungal biomarker) divided by the summed %mol of biomarkers i15:0, ai15:0, 15:0, i16:0, 16:1 $\omega$ 7t, i17:0, ai17:0, 17:0, 18:1 $\omega$ 7 and 19:0c as an expression of total bacterial abundance (Frostegard & Baath, 1996).

## ***2.6 Statistical Analyses***

Statistical analysis of all data was achieved using statistical software, Statistica<sup>®</sup> version 7.0 (StatSoft Inc, Bedford, UK) and Genstat<sup>®</sup> 9<sup>th</sup> edition (VSN International Ltd, Hemel Hempstead, Hertfordshire UK). The experimental design of each experiment and the specific analysis used is detailed in the relevant chapter.

**Table 2.2: PLFA identification by GC retention time.**

<b>PLFA</b>	<b>Peak retention time (min)</b>	<b>Putative microbial identification group</b>	<b>References</b>
14:0	18.71		
14:1 isomer a	19.27		
14:1 isomer b	20.19		
i15:0	20.86	Bacterial	(Bardgett & McAlister, 1999)
ai15:0	21.16	Bacterial	(Bardgett & McAlister, 1999)
15:1	21.92		
15:0	22.22	Bacterial	(Bardgett & McAlister, 1999)
16:1 isomer	23.85		
i16:0	24.61	Bacterial	(Bardgett & McAlister, 1999)
ai16:0	25.13	Bacterial	
16:1w7 c	25.28	Gram negative bacteria	(Wilkinson, 1988)
16:1w7 t	25.41		
16:1w5	25.65	Gram negative bacteria	(Wilkinson, 1988)
16:0	26.07	Bacteria	(Bardgett & McAlister, 1999)
cyc i17:0	28.43	Bacteria	(Bardgett & McAlister, 1999)
i17:0	28.65	Bacteria	(Bardgett & McAlister, 1999)
ai17:0	29.02		
i17:1	29.95		
17:0	30.18	Bacteria	(Bardgett & McAlister, 1999)
i17:0	30.33	Bacteria	(Bardgett & McAlister, 1999)
i18:0	31.91		
18:2w6 c	33.09	Eucaryotes, particularly fungi	(Federle, 1986)
18:1w9 c	33.33	Gram negative bacteria / eukaryotic	(Zelles <i>et al.</i> 1992; Bardgett & McAlister, 1999; Lindahl <i>et al.</i> 1997; Frostegard & Baath, 1996; Myers <i>et al.</i> 2001)
18:1w9 t	33.57	Gram negative bacteria/ eukaryotic	(Wilkinson, 1988; Frostegard & Baath, 1996)
18:1w7t	33.81	Gram negative bacteria	(Wilkinson, 1988)
i18:1	33.99		
18:0	34.41		
19:2	36.09		
cyc 19:0	38.15	Anaerobic eubacteria	(Jackson <i>et al.</i> 2003)
19:0	38.51		
20:0	42.90	Nematode	(Chen <i>et al.</i> 2001)

# *Section 2*

## **Section 2: National and trans-national field trials**

### ***Introduction***

Soil is a complex matrix governed by both positive and negative feedback systems. Processes occurring in soil affect systems beyond it, and vice versa. For example soil type, structure, pH and nutrient availability affect plant growth, which in turn affects the soil physical and chemical structure and composition, for example by root growth, exudate production, and litter decomposition.

Soils differ at both spatial and temporal scales and the factors affecting soil formation and ecosystem processes change with them depending on chemical and physical characteristics. These processes act at varying scales but in terms of agricultural legislation, anthropogenic scales are imposed in terms of regions, countries and continents. Therefore, it is important to be able to upscale insights derived from process studies to regional, national and global scales in order to better inform policy.

Human actions are now seen as a central issue in global climate change and our actions upon the earth, such as via land management, are being increasingly scrutinised. In order to fully understand “Earth systems” it is necessary to look at effects not just in isolation but at different scales to fully understand the spatial and temporal variation, for example, determining the importance between the genoform and phenoform of soil in tilled landscapes (Droogers & Bouma, 1997; Pennock & Veldkamp, 2006).

The following chapters detail field trials designed to observe trans-national and temporal effects of tillage practice on microbial community size and structure. They were designed to test the following hypotheses:

- Conventional tillage will reduce the quantity of microbial biomass.
  - *Mouldboard ploughing will reduce organic matter and break-up fungal hyphae.*
- Microbial community structure will be altered by tillage practice.
  - *Mouldboard ploughing with minimal residue incorporation results in a reduction of soil organic matter and greater system perturbation, potentially reducing the ecosystem goods and services provided by specific trophic groups.*



## Chapter 3 Experimental site setup

### 3.1 Introduction

The overall field site selection and treatment layout was determined by the SOWAP project (Chapter 1.7). The project was a pan-European demonstration project with sites located across Europe; in the UK, Hungary and Belgium (Figure 3.1). At each site there were a number of ‘Farmer’s fields’, each of which was bisected, one half conventionally tilled and the other half conservation tilled (Chapter 1.6). There was also a ‘Demonstration field’ at each site; this field was split into three plots which received conventional and two different kinds of conservation tillage, denoted SOWAP best practice and Farmer’s Choice treatments (Table 3.1). At each site on the demonstration field a weather station was installed (CWi Technical Ltd, Spalding, UK), recording wind speed, wind direction, soil temperature, air temperature, relative humidity, solar energy and rainfall.



**Figure 3.1: Location of SOWAP project sites within Europe**

**Table -3.1: Description of tillage practices applied at field sites.**

Tillage treatment	Definition
Conventional tillage	Inversion tillage using a mould board plough
SOWAP Best Practice	Tillage practice specifically intended to reduce soil disturbance during seedbed preparation, the minimum amount of tillage required to generate a viable crop.
Farmer's Choice	The site farmers own choice of conservation tillage, different at each site

### ***3.2 Loddington, Leicestershire***

The farm site at Loddington (N 052°36'53" W 00°50'31") was hosted by the Allerton Project under the direction of Dr Alastair Leake (Allerton Project Manager). The Allerton Research and Educational Trust was formed in 1992, with 136 ha for research and projects to demonstrate wildlife management alongside commercial farming. The farm has approximately 250 ha of arable cropping with main crops of winter wheat, winter oats, beans and oilseed rape.

The demonstration field at Loddington is called Upper Ponds South field, and the Farmer's fields are Stonepits, Barrow Hill and Churchills (Figure 3.2). Three fields (Stone pits, Churchills and Barrow hill) have been split into conventional and conservation tillage plots. The fields were selected because the two halves of each field drain separately, making them suitable for water quality monitoring within the SOWAP project.



**Figure 3.2: Aerial photograph of Loddington farm site with field names.**

### **3.2.1 Soil**

The basic soil characteristics for the site are detailed in Table 3.2. A soil profile from Upper Ponds South is shown in Figure 3.3. Cropping and tillage practice for the demonstration field are given in Table 3.3.

#### **3.2.1.a Soil series information**

Hanslope – Clayey to the surface and have slowly permeable subsurface horizons which are seldom seriously waterlogged. Calcareous, chalky subsurface horizon which is normally brown but can be mottled, beneath which is a dense mottled substrate containing chalk stones. Found in

moderate to strongly sloping valley sides or convex upper slopes (Hodgson, 1997).

Denchworth – Stoneless, strongly mottled and waterlogged for long periods in the winter months. Dark greyish brown soils beneath which is a grey stoneless clay (Hodgson, 1997).

Irondown - Dark yellowish brown slightly stony clay loam, beneath which is slightly stony clay loam with yellowish brown mottles and olive brown stoneless clay (Hodgson, 1997).

Bambury - Dark brown slightly stony clay loam, beneath which is dark yellowish brown very stony sandy clay loam (Hodgson, 1997).

**Table 3.2: Basic soil information for Loddington site.**

Field ID	Soil series	Textural classification	(sand/silt/clay) %w/w	pH
Stone Pits	Denchworth and Irondown	Clay	Not known	Not known
Church Hills	Denchworth and Irondown with a small amount of Bambury	Clay	30/30/40	6.1
Barrow Hill	Denchworth	Clay	14/27/59	6.8
Upper Ponds (Demonstration field)	Hanslope and Denchworth	Clay	30/23/47	7.0



Ap Horizon    Medium brown loamy soil

0-25cm

B Horizon    Light brown clay loam, slightly gleyed

25-54cm

C Horizon    Clay loam, heavily gleyed

54-120 cm

D Horizon    Gley mottled

120-173 cm

E Horizon    Sandstone - orange

173-205 cm

**Figure 3.3: Soil profile from Upper Ponds South field, Loddington.**

**Table 3.3: Land management and cropping practises for Loddington demonstration field (Upper Ponds South).**

Year	Crop	Land management
1998	Linseed	Conventional tillage
1999	Winter wheat	Conservation tillage with sub-soiling
2000	Winter barley	Conventional tillage
2001	Winter wheat	Conservation tillage
2002	Winter oil seed rape	Conservation tillage
2003	Mustard	SOWAP plot only as cover crop
2003	Winter wheat	By split plot conventional/ conservation
2004	Mustard-rye	SOWAP plot only as cover crop
2005	Spring beans	By split plot conventional/ conservation
2005	Winter wheat	By split plot conventional/ conservation

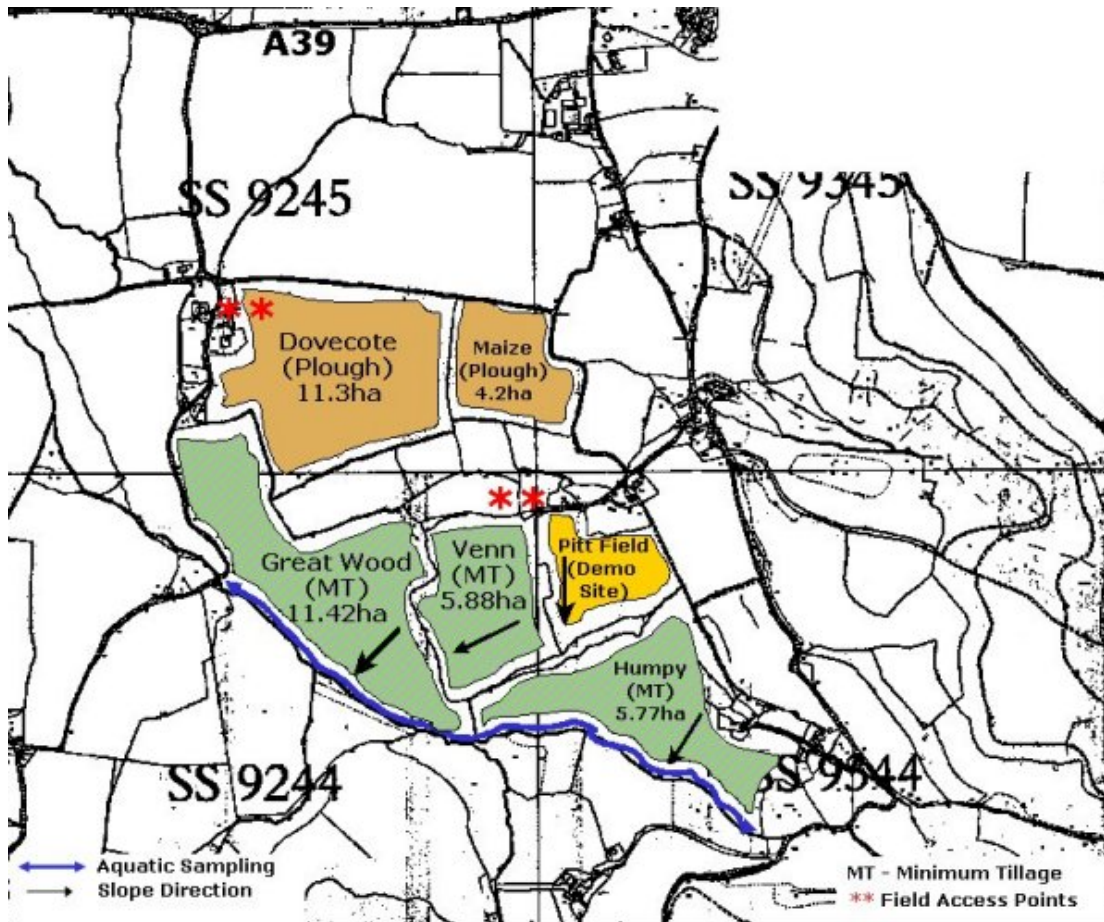
### ***3.3 Tivington, Somerset***

Tivington farm is part of the Holnicote Estate which covers 5,042 ha of Exmoor national park (N 051°11'56" W 003°31'32"). It is situated between Porlock and Minehead in Somerset. Whilst in terms of the SOWAP project there are Farmer field sites at this farm they were not used as part of this study, only the demonstration field (Pitt Field) was used (Figure 3.4). The main crops at this site were wheat and oilseed rape.

#### **3.3.1 Soil**

The soil association of the Tivington demonstration field is Worcester which is a slowly permeable non-calcareous and calcareous reddish clayey soil developed in Pero-Trassic mudstone and clay shale (Table 3.4). It is classed as having a slight risk of water erosion (Hodgson, 1997). Land management and cropping history of the demonstration field is known from 1998 (Table 3.5), during this time there has been no cover crop used on this field.





**Figure 3.4: Tivington site field setup.**

**Table 3.4: Basic soil information for Tivington demonstration field.**

Determinand	Result
Textural classification	Sandy clay loam
(Sand/silt/clay) % w/w	51/27/22 %
pH	7.0

**Table 3.5: Land management and cropping for Tivington demonstration field.**

Year	crop	soil management
1998	Not known	Conventional tillage
1999	Winter Oats	Conservation tillage
2000	Winter wheat	Conservation tillage
2001	Winter oil seed rape	Conservation tillage
2002	Winter wheat	Conservation tillage
2003	Winter oil seed rape	By split plot conventional/conservation
2004	Winter wheat	By split plot conventional/conservation
2005	Spring beans	By split plot conventional/conservation
2006	Spring beans	By split plot conventional/conservation

### ***3.4 Hungarian Site***

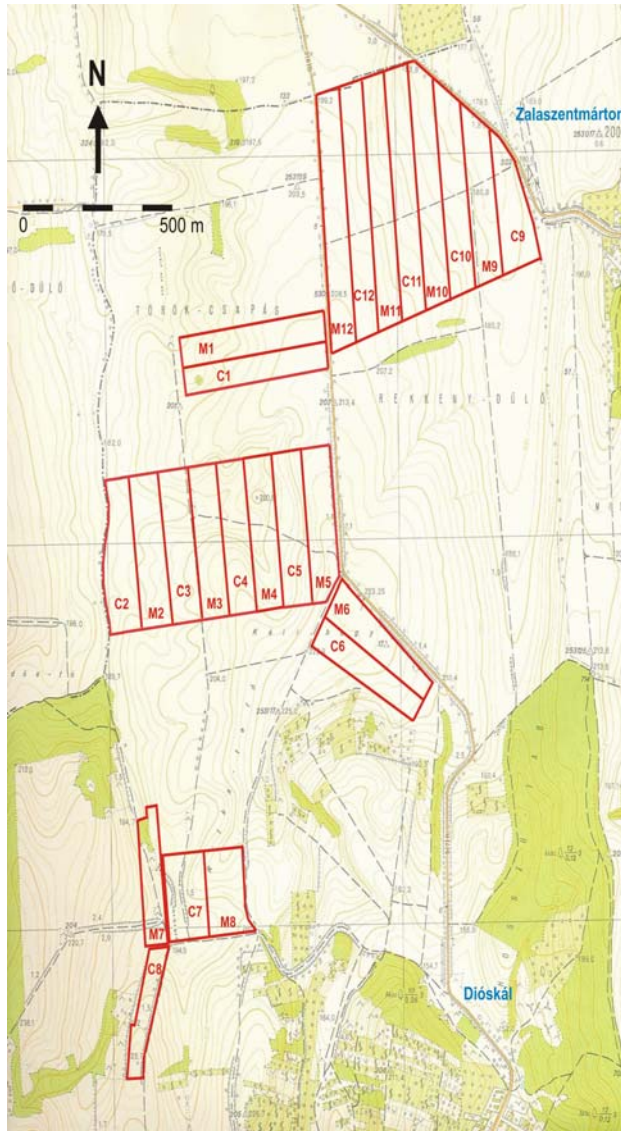
The two main sites were located near lake Balaton, near Keszthely. Szentgyörgyvár (Saint George N 46°44'90", E 17°08'81") run by the Enterprise of János Horváth, a small 2 ha farm was chosen as the site for the demonstration field and Dióskál (N 46°42'04", E 17°02'37" - N 46°41'09", E 17°02'07" and N 46°42'22", E 17°02'35" - N 46°42'07", E 17°03'11") a 107 ha farm owned by the Plótár family was chosen for the Farmer Fields. The demonstration site has 4 plots: 2 conventionally tilled and 2 minimum tilled, each plot is 24 x 50 m in size (Figure 3.5). The main crops in these areas were winter wheat, maize and potatoes.

#### **3.4.1 Soil**

Szentgyörgyvár farm site is located on rolling sandy loess–fine sand plain, at the boundary of the humid and dry continental climate types; average annual precipitation is 700 mm. The soil type is Luvisol.



The Dióskál farm site is situated in a hilly, sandy loess–fine sand area. The climate is temperate cool and humid with average annual precipitation of 700-750 mm. The soil type is luvisol, at some points strongly eroded Luvisol and Cambisol.

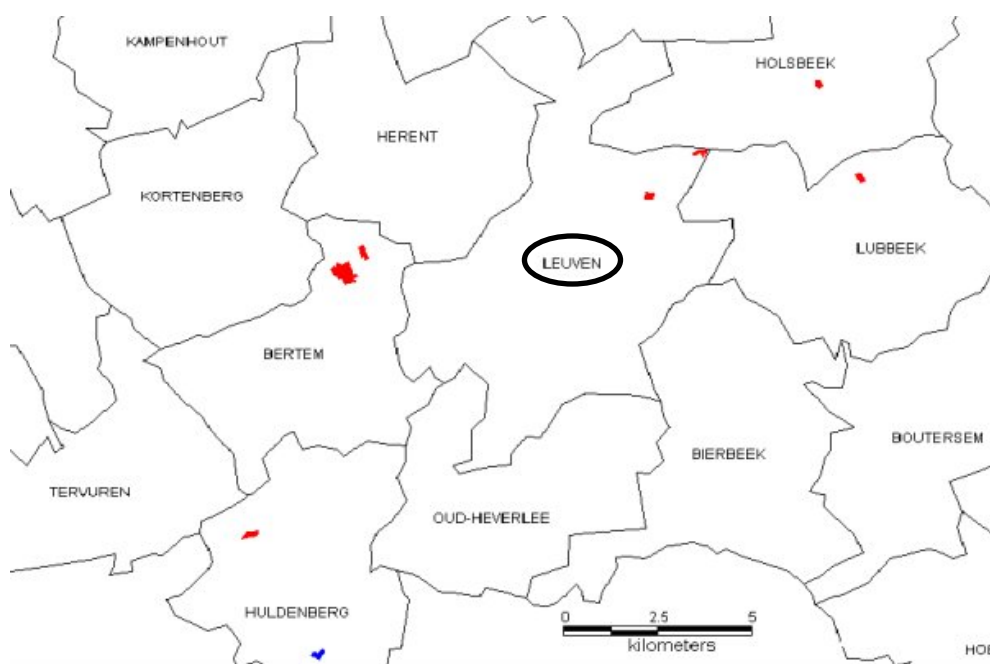


**Figure 3.5: Field sites at Dióskál, Conservation tilled (M) and Conventional tilled (C).**

### ***3.5 Belgian site***

The demonstration field site belongs to the ‘Hof ter Vaeren’ family farm, a 85 ha (55 ha cropland and 30 ha pasture) mixed farm. The demonstration

field is divided into three treatments (conventional tillage, Farmer's Choice (reduced tillage) and SOWAP best practice tillage), each with two replicate erosion plots. There were 15 designated Farmer's fields each split into conventional and conservation oriented soil management (Figure 3.6). The Farmer's fields were located in three areas; southwest of Leuven; northwest of Leuven; and Sint-Triden. There were also two fields with plots receiving no tillage.



**Figure 3.6: Location of Belgian field sites, farmer fields marked in red, demonstration field marked in blue.**

### 3.5.1 Soil

The fields used in this study are situated in the Belgian loess belt, Haplic Luvisols and some Haplic Albeluvisols. These soils have very high silt contents (70-80%), moderate clay contents (10 – 20%) and are very fertile. The main crops of this area are maize, sugar beet, potatoes and wheat.

## **Chapter 4 : Pan European assessment of microbial community change under differing tillage practices**

### ***4.1 Introduction***

Agriculture is an important industry in the European Union, shaping the landscape and supporting rural communities. It is essential to support the growing global population therefore around half the land within the European Union is farmed. This has the potential to adversely affect the landscape in terms of habitat fragmentation, reduced biodiversity, pollution and soil erosion by inappropriate land management practices. But what are inappropriate practices?

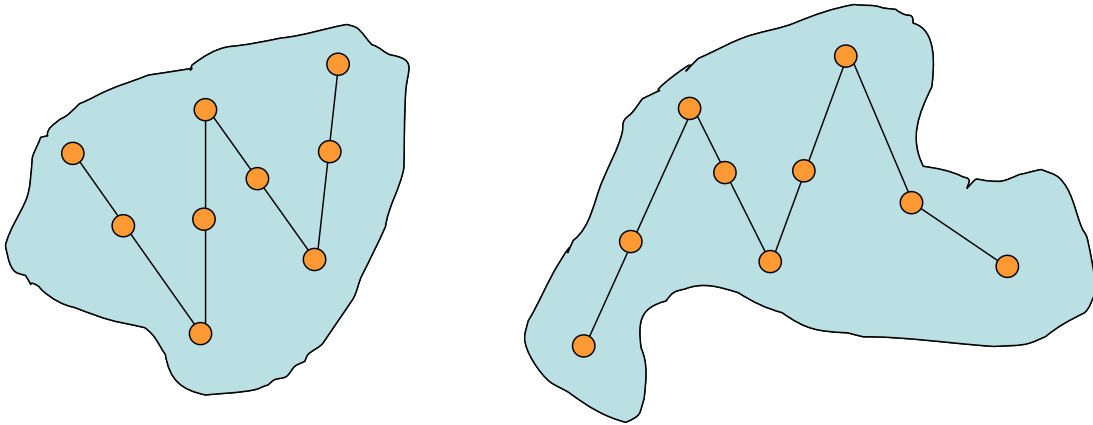
European legislation such as the Water Framework Directive, Habitats Directive and Thematic Strategy for Soils are aimed at reducing environmental degradation and yet maintaining commercially sustainable agriculture (Chapter 1.6.2). The maintenance of biodiversity and rural socio-economic stability are key concerns. However, the impacts of land management on soils and biodiversity differ from country to country, region to region, and soil type to soil type. It is therefore essential that studies and observations of these factors are made at the appropriate level to inform agricultural policy both at the European, country and regional scales.

This study was aimed at determining the effect of tillage practice on the microbial community, specifically testing the hypotheses that:

1. Microbial biomass will be reduced by conventional tillage practices at each site.
  - *Perturbation caused by ploughing will reduce soil organic matter.*
2. Microbial community structure will be significantly different between tillage practices at each site.
  - *The effect of tillage practice on soil physical parameters will alter soil habitat and therefore influence microbial community composition.*
3. Inter-site comparison will show similar changes in microbial community structure and size as a result of tillage practice.
  - *The effect of tillage treatment on the microbial community will outweigh the inherent effects of soil type and climate.*

#### **4.2 Field sampling**

Each field involved in the pan European study was sampled twice during the course of the three year SOWAP project. The first sampling occurred in the first year of the project (late 2003/ early 2004), dependant on site. The sites were finally sampled in spring 2006. Each tillage treatment plot was sampled along 'W of best fit' transects taking 9 samples in total per treatment (Figure 4.1). To reduce the sample number, five of the samples were chosen at random and bulked together, creating a total of five samples per treatment plot. Soil samples were taken to a depth of 15 cm and then shipped in cooled containers from the field sites to Cranfield University for sieving and analysis (Chapter 2.2). Specific site details can be found in Chapter 3.



**Figure 4.1: Examples of how 'W of best fit' transects are fitted to irregular field boundaries.**

### ***4.3 Measurements***

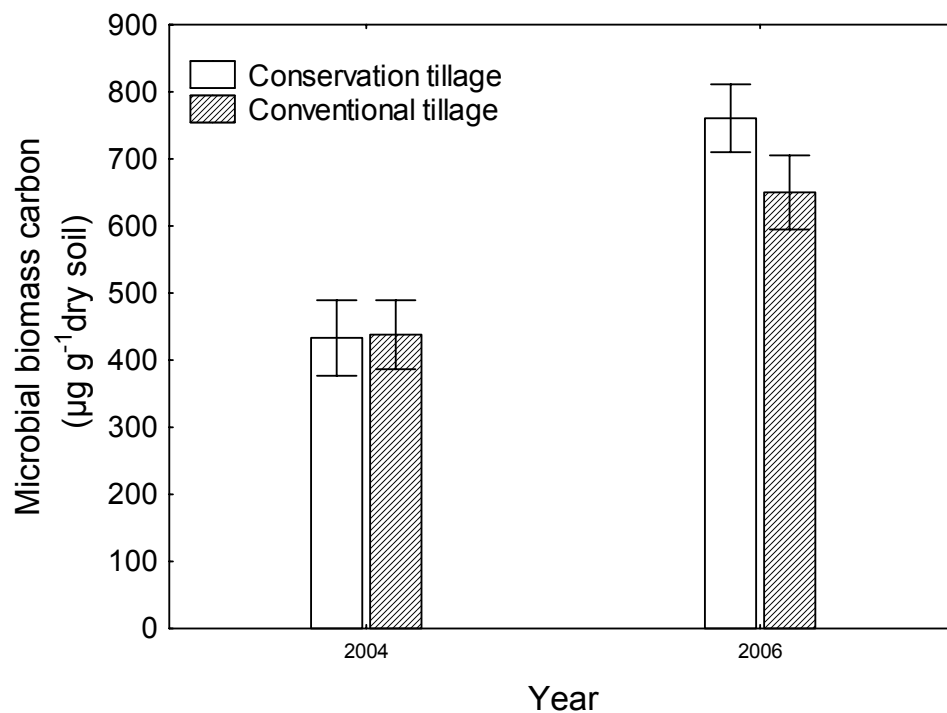
Collected samples were analysed for moisture content (Chapter 2.3.1), microbial biomass carbon (Chapter 2.5.1) and microbial community structure (PLFA) (Chapter 2.5.2). Data was analyzed by analysis of variance and principal component analysis.

### ***4.4 Results and discussion***

#### **4.4.1 Loddington, Leicestershire UK**

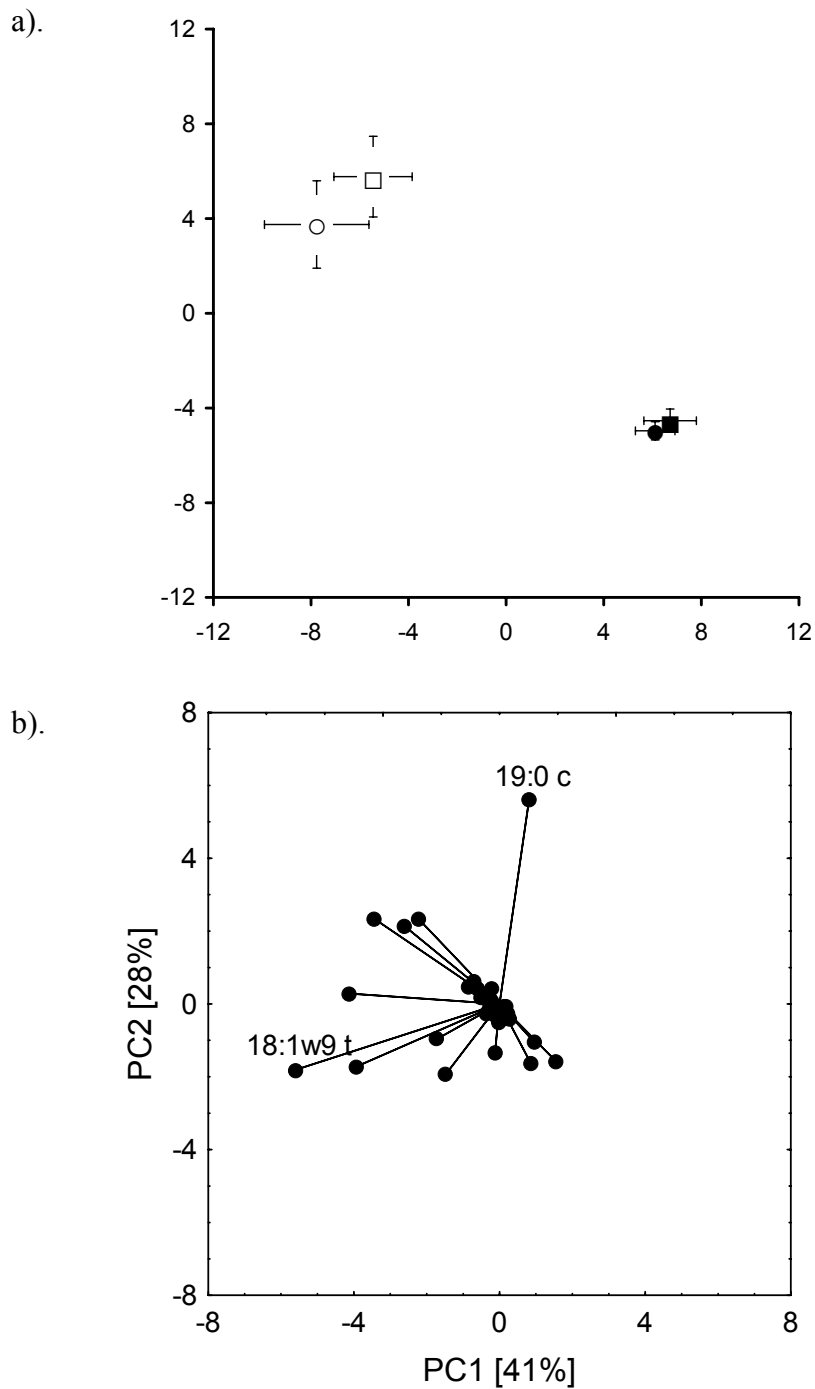
The moisture content for the Loddington field site was significantly different by year at each sampling point, samples from 2006 were significantly wetter ( $p > 0.01$ ; means of  $0.21 \text{ g g}^{-1}$  [2004] and  $0.36 \text{ g g}^{-1}$  [2006] L.S.D. 2.35 d.f. 108).

Microbial biomass carbon was not significantly different between fields before treatments were applied, but were significantly different between 2004 and 2006 (Figure 4.2). In 2006 soils under conservation tillage had a significantly higher biomass than those under conventional tillage after three years of treatment.



**Figure 4.2: Microbial biomass carbon measurements by year and tillage treatment for Loddington field sites. Bars show means, whiskers show 95% confidence interval.**

PCA of PLFA profiles shows a significant difference in microbial community structure between 2004 and 2006, however, it does not show any significant grouping by tillage treatment (Figure 4.3). In 2006 there was a significantly smaller proportion of 18:1 $\omega$ 9t and 19:0 c biomarkers than there was in 2004 (means 8.1% [2006] and 12.8% [2004] for 18:1 $\omega$ 9t; and 1.7% [2006] and 6.4% [2004] for 19:0 c). These biomarkers are all indicative of bacteria (Jackson *et al.* 2003; Frostegard & Baath, 1996; Wilkinson, 1988).



**Figure 4.3: PCA of PLFA from Loddington field sites for 2004 and 2006 a). First and second principal components, Open symbols show samples from 2004, closed symbols samples from 2006; Conventional (■) (2004 n=30, 2006 n=26) and Conservation (●) (2004 n=25, 2006 n=30) tilled plots; points show means, whiskers denote s.e. b). Loadings associated with PCs.**

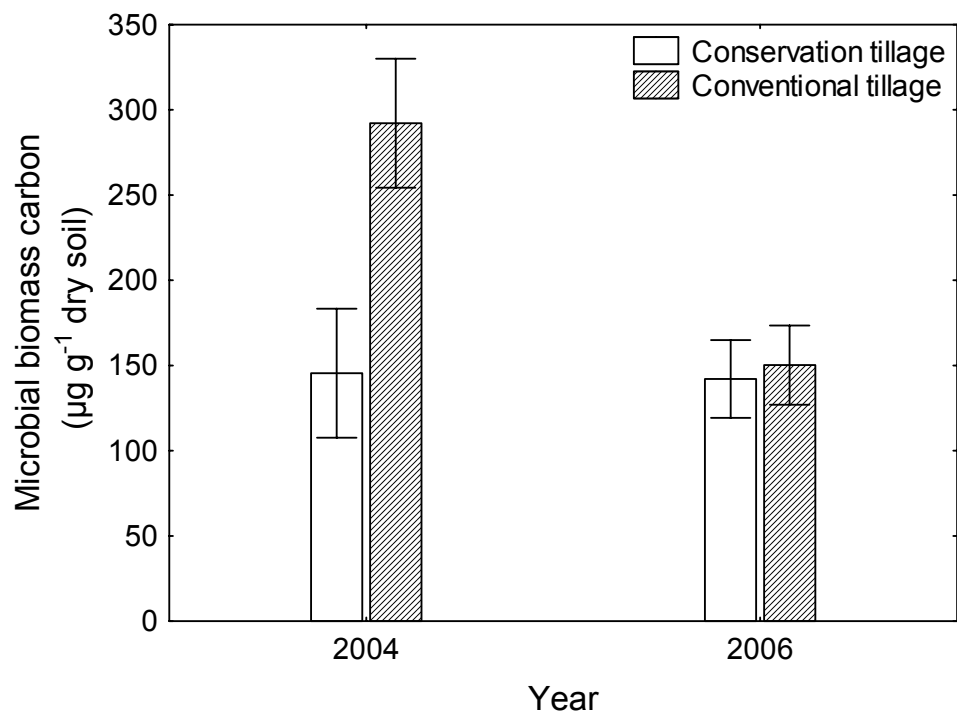
Further analysis of this data by year shows that in 2004 there was no significant effect of tillage, which is understandable as the samples were taken before the tillage treatments were applied. However, there was also no significant difference between different tilled plots after three years of application. This suggests that the community structure was more affected by other environmental parameters than it was by tillage after a 3 year period at the Loddington site.

#### **4.4.2 Hungary**

The moisture content at the Hungarian site was not significantly different by year or tillage treatment, however there was a date by tillage interaction ( $p > 0.01$ ; means of  $0.16 \text{ g g}^{-1}$  [2004] and  $0.17 \text{ g g}^{-1}$  [2006] for conventional tillage; means of  $0.17 \text{ g g}^{-1}$  [2004] and  $0.19 \text{ g g}^{-1}$  [2006] for conservation tilled L.S.D. 3.87 d.f. 144). The moisture content of the Conventional tilled plots was lower in 2004 than the Conservation plots however, in 2006 there was no significant difference between the tillage treatments. The Conventional tillage plot's moisture content had increased after the three years whilst the Conservation tilled plot had decreased.

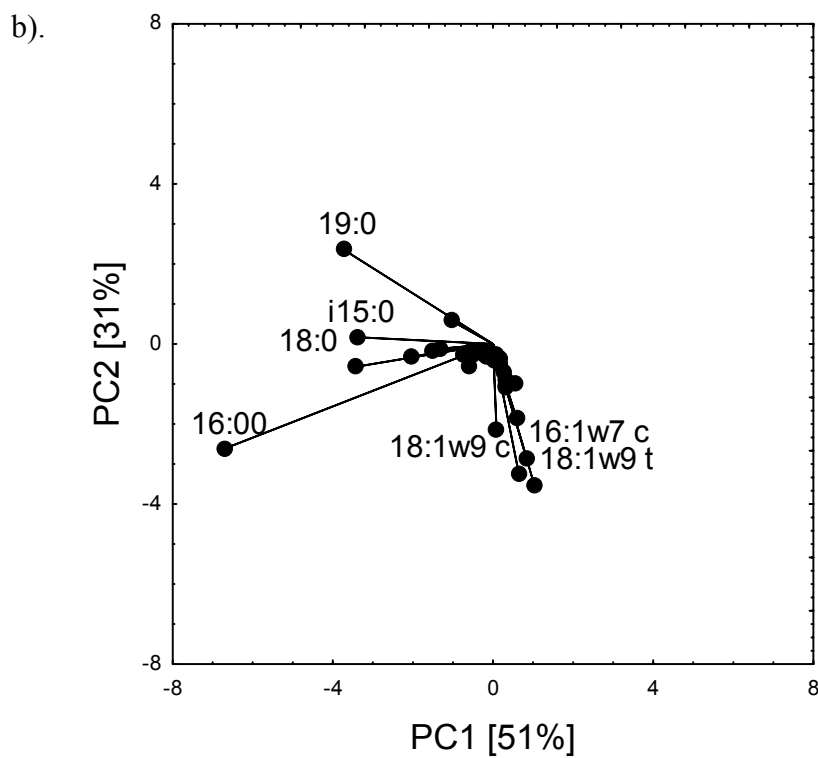
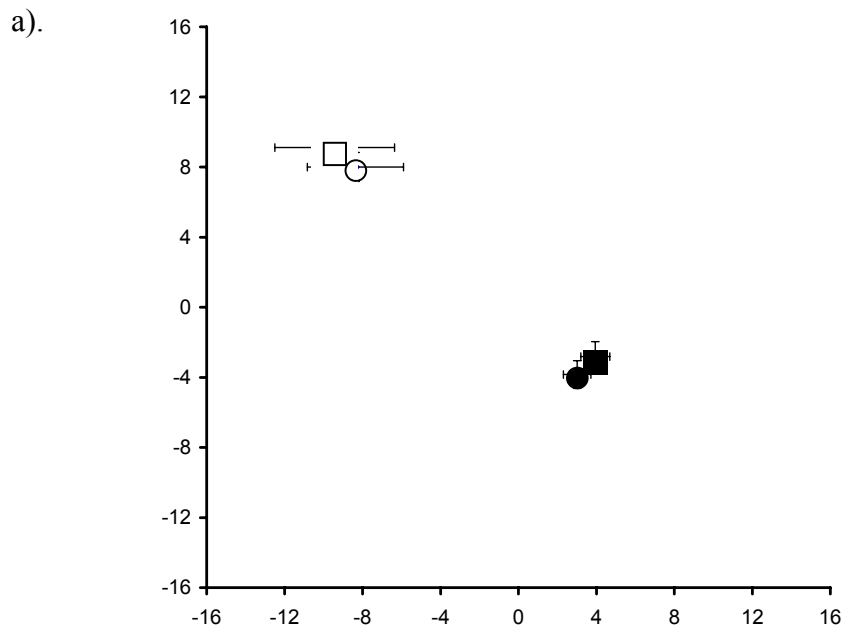
Microbial biomass carbon was significantly reduced in the Conventional plot after three years of tillage application ( $p > 0.01$ ; Figure 4.4). Samples taken in 2006 showed no significant difference in microbial biomass between treatments; however, the concentration of microbial biomass was significantly higher in the conventional plots in 2004 and has therefore declined under the Conventional tillage treatment.





**Figure 4.4: Microbial biomass carbon measurements by year and tillage treatment for the Hungarian field sites. Bars show means (2004 n=20, 2006 n=55), whiskers show 95% confidence intervals.**

PCA of PLFA profiles shows a significant difference in microbial community structure between 2004 and 2006 ( $p > 0.01$ ) in both PC 1 and 2 (Figure 4.5a). There was no effect of tillage. The loadings associated with the PCA of PLFA profiles showed biomarkers i15:0, 16:0, 19:0, 18:0, 18:1 $\omega$ 9c, 18:1 $\omega$ 9t and 16:1 $\omega$ 7c to be significantly influencing (Figure 4.5b). In 2006 there were significantly more 18:1 $\omega$ 9t, 18:1 $\omega$ 9c and 16:1 $\omega$ 7c than recorded in 2004. The biomarker 18:1 $\omega$ 9t was significantly lower in the conventional tilled soils. These biomarkers are all indicative of bacteria (Wilkinson, 1988; Bardgett & McAlister, 1999; Frostegard & Baath, 1996).

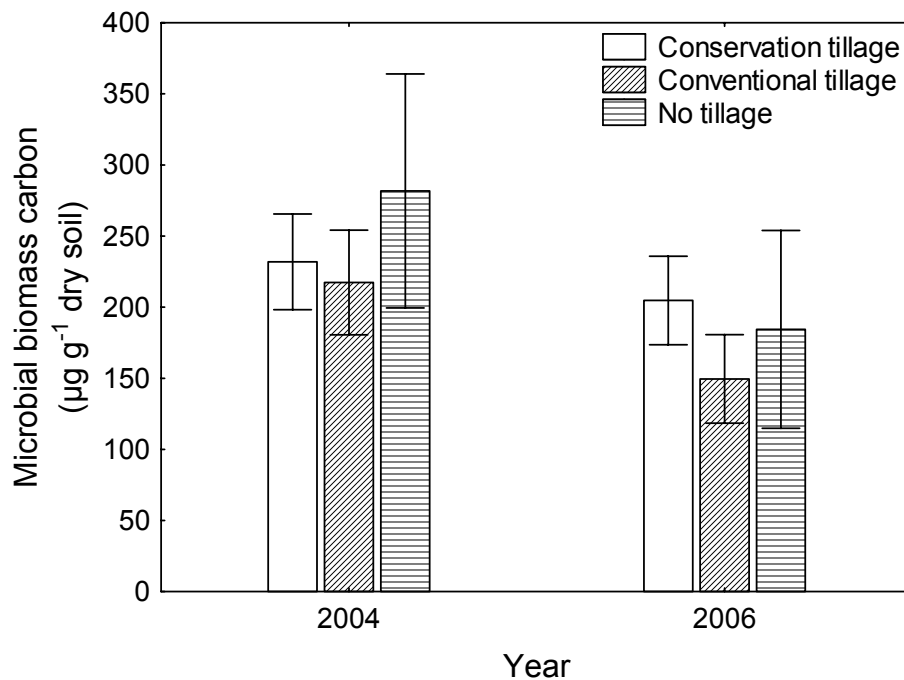


**Figure 4.5: PCA of PLFA profiles derived from Hungarian field sites for 2004 and 2006 a). 1<sup>st</sup> and 2<sup>nd</sup> principal components; Open symbols denote samples from 2004, closed symbols denote samples taken in 2006; Conventional (■) and Conservation (●) tilled plots; points show means (2004 n=20, 2006 n=55), whiskers denote s.e. b). Loadings associated with PCs.**

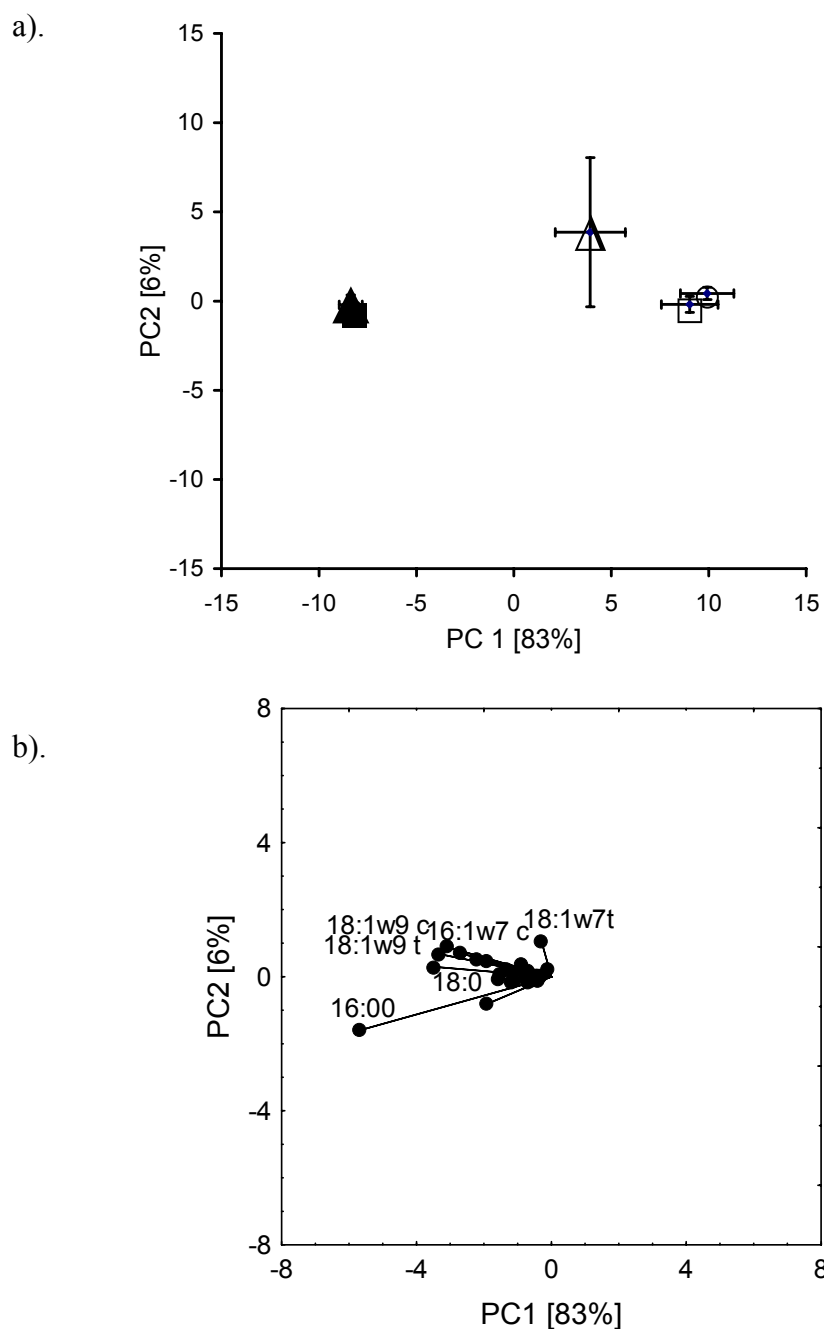
Further analysis of this data by year shows that in 2004 there was no significant effect of tillage. There was a significant difference in PC3 [variance 7%] as a result of tillage type in 2006 but this difference is masked by the variance associated with the year sampled. This suggests that the community structure was more affected by other environmental parameters (in PC1 and 2) than by tillage after a 3 year period at the Hungarian site.

#### 4.4.3 Belgium

The moisture content of the Belgian soil samples was not significantly different by time or tillage treatment. The microbial biomass was significantly different between year of sampling ( $p > 0.01$ ), samples taken in 2004 had a significantly higher biomass carbon concentration (overall means 2004  $230 \mu\text{g g}^{-1}$ ; 2006  $178 \mu\text{g g}^{-1}$  s.e. 16.0) (Figure 4.6).



**Figure 4.6: Microbial biomass carbon measurements by year and tillage treatment for the Belgian field sites. Bars show means (2004 n=30, 25, 5; 2006 n=35, 35, 7 [conservation, conventional and no tillage]), whiskers show 95% confidence intervals.**

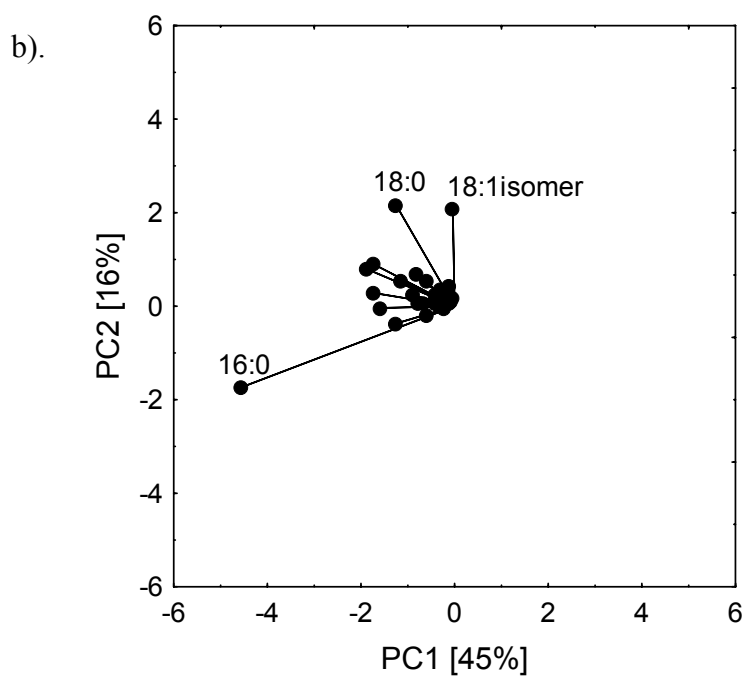
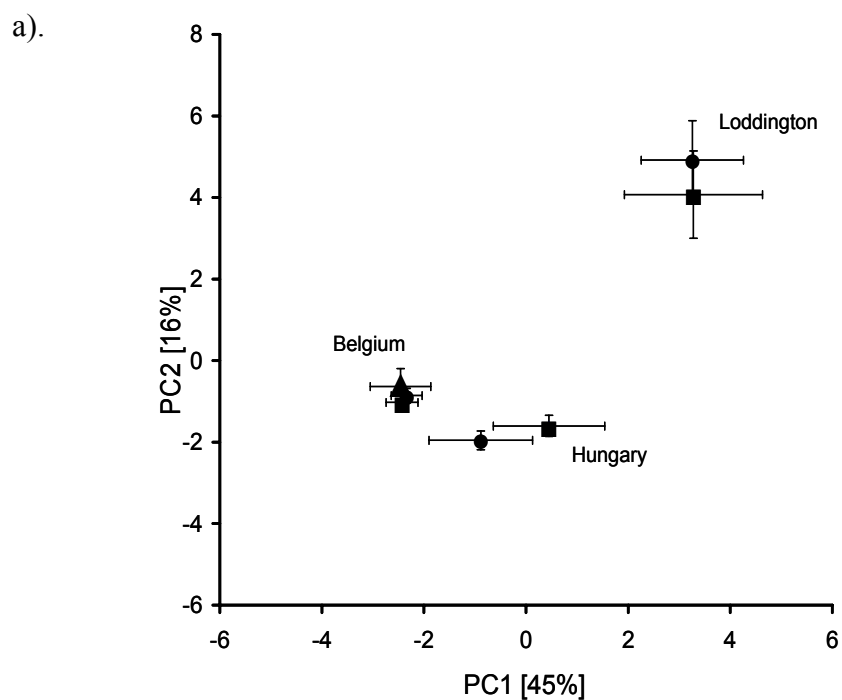


**Figure 4.7: PCA of PLFA profiles derived from Belgian field sites for 2004 and 2006 a). 1<sup>st</sup> and 2<sup>nd</sup> principal components; open symbols denote samples taken in 2004, closed symbols samples taken in 2006, Conventional (■) (2004 n=25 2006 n=35), Conservation (●)(2004 n=30, 2006 n=35) and No tilled (▲) (2004 n=5, 2006 n=7) plots; points show means, whiskers denote s.e. b). Loadings associated with PCs.**

PCA of PLFA profiles shows a significant difference in microbial community structure between 2004 and 2006 ( $p > 0.01$ ) in PC1 (Figure 4.7a). There was no significant difference between the tillage treatments at either site, note that the ANOVA performed takes into account the difference in observation number between the No till treatment and the Conventional/Conservation treatments. Biomarkers 16:0, 18:0; 18:1 $\omega$ 9c, 18:1 $\omega$ 9t and 16:1 $\omega$ 7c were significantly greater in percentage in 2006 (Figure 4.7b). The biomarkers 18:1 $\omega$ 7t and 18:1 $\omega$ 9c showed significant reduction in No-tilled plots after 3 years. These biomarkers are indicative of bacteria (Frostegard & Baath, 1996; Bardgett & McAlister, 1999).

#### **4.4.4 Pan European analysis**

ANOVA of all site data together showed that the differences in location of site are dominating over any effect of tillage on microbial biomass. Each site has a significantly different microbial biomass. However, Conservation tillage across all sites carried a higher microbial biomass when compared to Conventional tillage (overall mean Conventional 264.0  $\mu\text{g g}^{-1}$  dry soil and Conservation 318.7  $\mu\text{g g}^{-1}$  dry wt soil), and therefore the original experimental hypothesis that microbial biomass will be reduced under conventional tillage can be accepted.



**Figure 4.8: PCA of all site PLFA for 2006; a). 1<sup>st</sup> and 2<sup>nd</sup> principal components; Conventional (■) (Loddington n=26, Belgium n=35, Hungary n=53), Conservation (●) (Loddington n=30, Belgium n=35, Hungary n=55) and (▲) No tilled (Belgium n=7) plots; points show means, whiskers denote s.e. b). Loadings associated with PCs.**

PCA of all site PLFA profiles at the end of the three year period shows that microbial communities are distinct by geographic location; PC1 separates all three sites whereas PC2 separates Loddington from the other two sites (Figure 4.8a).

The biomarker 16:0 had a higher %mol in samples from the Hungarian site. The Belgian site had the lowest %mol of 18:0 biomarker and the Hungarian site the highest. The Loddington site had significantly higher %mol of 18:1 isomer biomarker than the other two sites. These biomarkers are all bacterial indicators (Frostegard & Baath, 1996; Bardgett & McAlister, 1999).

#### ***4.5 Conclusions***

In all cases conventional inversion tillage resulted in a reduction in microbial biomass carbon. It is impossible to say by community size alone whether or not the microbial community functioning in terms of ecosystem services was likely to be affected but it is conceivable that the channels that these processes occur through have been altered. The microbial community structure of each of the sites has altered in time significantly with the greatest amount of variation observed in 2004. These time differences are not significant when all the sites are grouped together in the analysis. Environmental effects such as climate and soil type seem to be separating the sites with Loddington (heavy clay soil from a maritime climate) distinct from the Hungarian and Belgian sites which both are loess soils under a continental climate. It is therefore apparent that the geographical location, climatic and environmental influences at these sites changes the microbial community phenotypic structure more predominantly than the land management practice. Therefore, the

reduction of microbial biomass as a result of tillage apparently has no effect on the phenotypic profile of the community at this scale. It is also apparent that the phenotypic community changes are strongly associated to bacterial markers in terms of site and tillage differences.



## Chapter 5 - UK temporal study

### 5.1 Introduction

Microbial communities are constantly changing and adapting to suit the complex soil environment in which they reside. The temporal change of these communities is dependant on many factors such as disturbance, vegetation, season and climate. Microbial biomass changes can be attributed to extreme events such as flash flooding rather than temporal variation of soil water and nutrient concentrations (Hamel *et al.* 2006). Microbial communities are resistant to environment changes and adapt to a wide range of soil conditions, therefore changes in community phenotypic structure may be more dramatic in the short term than changes in biomass size (Chapter 1).

The following chapter aims to assess the microbial community size and structure change over a three year period under different tillage systems, specifically testing the hypotheses that:

1. Microbial biomass will be reduced on Conventional tilled plots after a three year period compared to Conservation tilled plots
  - *Mouldboard ploughing will reduce soil organic matter and fungal biomass by breaking up mycelium*
2. Microbial biomass will be higher in spring than in autumn of each year.
  - *Higher temperatures and lower soil moisture contents associated with summer months will result in reduced microbial activity in the autumn*
3. Each site will support characteristic and distinct microbial biomass and community structures.
  - *As a result of different soil types and climate at each site*

## ***5.2 Experimental design***

Samples were taken from the demonstration fields (avoiding the erosion plots) at Loddington and Tivington, UK (Chapter 3), using a stratified randomised block design of five blocks (shown in green in Figures 5.1 & 5.2). From each block five randomly-located (using a random number generator) cores were taken, and bulked together to form a single sample (i.e. 5 samples per tillage treatment per time per site). Each treatment had a boundary of at least 2 m around it to allow for any edge effects; and tramlines were avoided using an exclusion zone of at least 2 m where possible. The stratified blocks were divided into five regions with each randomly allocated to one of the sampling occasions, circumventing problems associated with repeated-measure sampling. The precise location of the sample areas was recorded using GPS (Trimble Pathfinder GPS Pro XRS) (Figures 5.1 & 5.2).

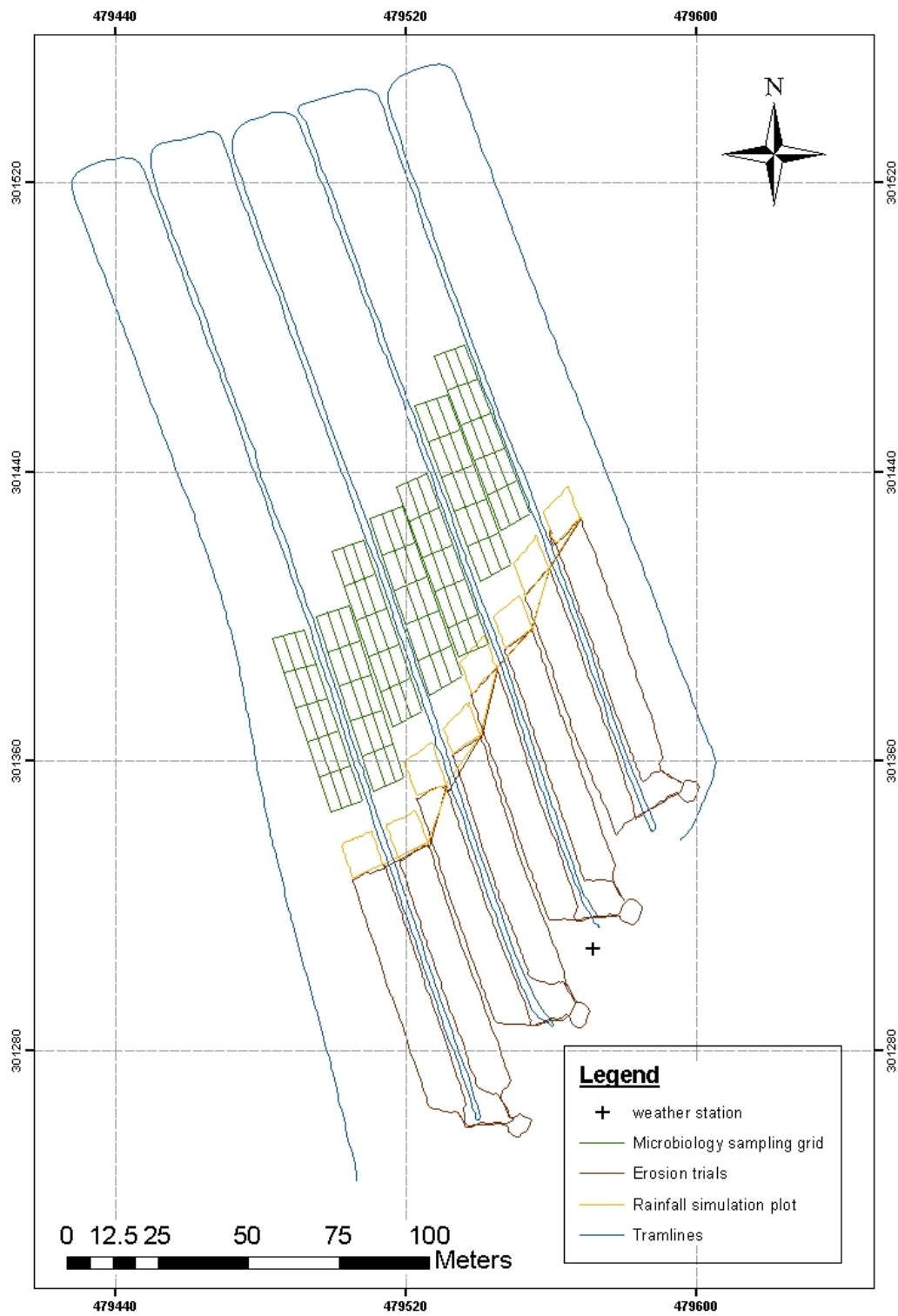
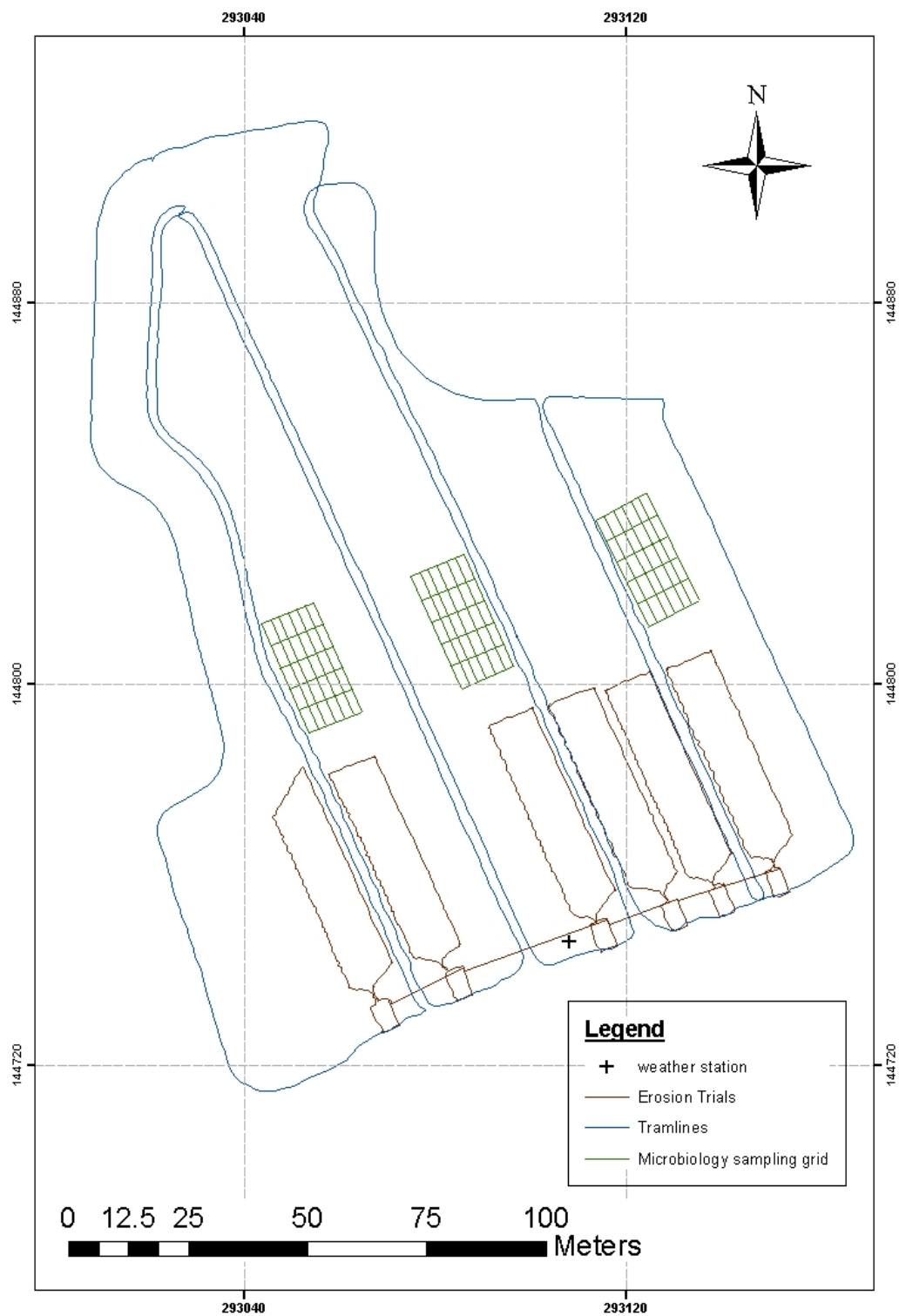


Figure 5.1: British National Grid projection of Loddington demonstration field.



**Figure 5.2: British National Grid projection of Tivington demonstration field.**

Locations within the sampling grids were randomly allocated to sampling occasions, taking into account the potential effect of the hill slope (Tables 5.1 & 5.2). Soils were extracted using a gouge auger (width 4 cm) to a depth of 15 cm. Samples were then refrigerated at 4°C until analysis (Chapter 2). Collected samples were analysed for moisture content (Chapter 2.3.1), microbial biomass carbon (Chapter 2.5.2) and microbial community structure (PLFA, Chapter 2.5.2). Data was analyzed using analysis of variance, multiple forward stepwise linear regression and principal components analysis.

**Table 5.1: Sampling strategy for Loddington demonstration field.**

Conventional Tillage						
	<b>Block 1</b>	<b>Block 2</b>	<b>Block 3</b>	<b>Block 4</b>	<b>Block 5</b>	<b>Block 6</b>
<b>Stratum<sup>1</sup> 1</b>	Spring 2006	Autumn 2006	Spring 2004	Autumn 2004	Autumn 2005	Spring 2005
<b>Stratum 2</b>	Spring 2005	Autumn 2005	Autumn 2006	Spring 2006	Autumn 2004	Spring 2004
<b>Stratum 3</b>	Spring 2004	Autumn 2004	Spring 2006	Autumn 2004	Autumn 2006	Spring 2005
<b>Stratum 4</b>	Autumn 2004	Autumn 2006	Spring 2006	Spring 2004	Spring 2005	Autumn 2005
<b>Stratum 5</b>	Autumn 2005	Autumn 2006	Spring 2006	Spring 2004	Autumn 2004	Spring 2005
SOWAP Best Practice						
<b>Stratum 1</b>	Spring 2005	Autumn 2005	Spring 2006	Autumn 2006	Autumn 2004	Spring 2004
<b>Stratum 2</b>	Spring 2004	Autumn 2005	Spring 2005	Spring 2006	Autumn 2006	Autumn 2004
<b>Stratum 3</b>	Spring 2006	Spring 2005	Autumn 2006	Spring 2004	Autumn 2004	Autumn 2005
<b>Stratum 4</b>	Autumn 2004	Spring 2005	Spring 2004	Autumn 2005	Spring 2006	Autumn 2006
<b>Stratum 5</b>	Spring 2004	Spring 2005	Autumn 2006	Spring 2006	Autumn 2005	Autumn 2004
Farmer Choice						
<b>Stratum 1</b>	Autumn 2006	Autumn 2005	Spring 2005	Spring 2006	Spring 2004	Autumn 2004
<b>Stratum 2</b>	Autumn 2006	Spring 2006	Spring 2004	Autumn 2004	Spring 2005	Autumn 2005
<b>Stratum 3</b>	Autumn 2004	Spring 2006	Autumn 2005	Spring 2004	Spring 2005	Autumn 2006
<b>Stratum 4</b>	Autumn 2005	Autumn 2006	Spring 2005	Spring 2004	Autumn 2004	Spring 2006
<b>Stratum 5</b>	Spring 2005	Autumn 2006	Autumn 2005	Spring 2006	Autumn 2004	Spring 2004

<sup>1</sup> Stratum 1 – 5 ran from bottom of slope (1) to top of slope (5).

**Table 5.2: Sampling strategy for Tivington Demonstration field.**

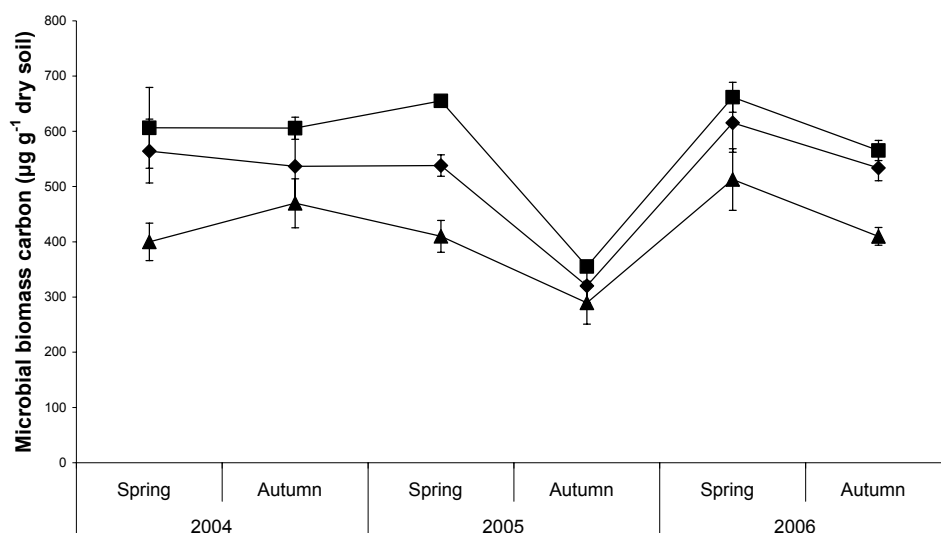
Conventional Tillage						
	<b>Block 1</b>	<b>Block 2</b>	<b>Block 3</b>	<b>Block 4</b>	<b>Block 5</b>	<b>Block 6</b>
<b>Stratum<sup>2</sup> 1</b>	Spring 2005	Spring 2006	Autumn 2004	Autumn 2006	Autumn 2006	Spring 2004
<b>Stratum 2</b>	Spring 2006	Autumn 2006	Autumn 2004	Spring 2004	Autumn 2005	Spring 2006
<b>Stratum 3</b>	Autumn 2006	Autumn 2004	Autumn 2006	Spring 2004	Spring 2006	Spring 2005
<b>Stratum 4</b>	Autumn 2004	Spring 2004	Autumn 2006	Spring 2005	Autumn 2005	Spring 2006
<b>Stratum 5</b>	Spring 2006	Autumn 2005	Autumn 2004	Autumn 2006	Spring 2004	Spring 2005
SOWAP Best Practice						
<b>Stratum 1</b>	Spring 2005	Autumn 2005	Autumn 2004	Autumn 2004	Spring 2006	Autumn 2006
<b>Stratum 2</b>	Spring 2005	Autumn 2005	Autumn 2006	Autumn 2004	Spring 2004	Spring 2006
<b>Stratum 3</b>	Spring 2006	Spring 2005	Autumn 2006	Autumn 2004	Spring 2004	Autumn 2005
<b>Stratum 4</b>	Spring 2005	Autumn 2006	Spring 2004	Spring 2006	Autumn 2005	Autumn 2004
<b>Stratum 5</b>	Autumn 2006	Spring 2005	Spring 2006	Autumn 2004	Spring 2004	Autumn 2005
Farmer Choice						
<b>Stratum 1</b>	Autumn 2005	Spring 2005	Autumn 2006	Spring 2004	Spring 2006	Autumn 2004
<b>Stratum 2</b>	Spring 2004	Autumn 2004	Autumn 2006	Spring 2006	Autumn 2005	Spring 2005
<b>Stratum 3</b>	Autumn 2004	Spring 2006	Autumn 2006	Autumn 2005	Spring 2004	Spring 2005
<b>Stratum 4</b>	Spring 2005	Spring 2006	Autumn 2004	Spring 2004	Autumn 2006	Autumn 2005
<b>Stratum 5</b>	Autumn 2004	Spring 2006	Spring 2005	Autumn 2006	Spring 2004	Autumn 2005

<sup>2</sup> Stratum 1 – 5 ran from bottom of slope (1) to top of slope (5).

## 5.3 Results and discussion

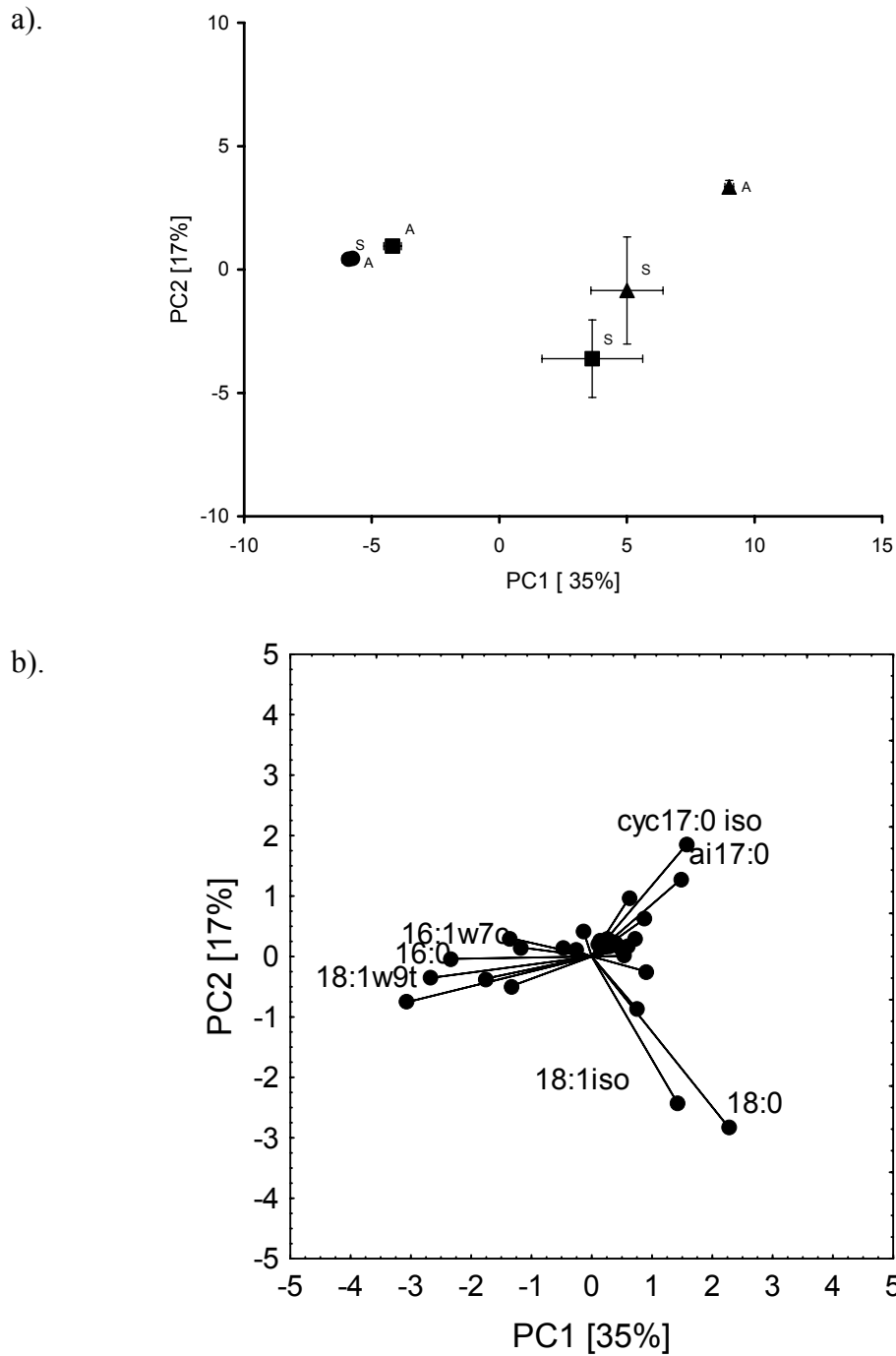
### 5.3.1 Loddington

Microbial biomass carbon was significantly higher in the SOWAP plot than in the Conventional tilled plot across all measurements (Figure 5.3). In order to ascertain the climatic impact on microbial biomass multiple forward stepwise linear regression analysis was used. Weather variables (from the on-site weather stations), soil temperature; air temperature; relative humidity; solar energy; and rainfall volume were taken for 30 days up to and including the day of sampling. Air temperature and soil temperature were positively correlated ( $r^2=0.98$ ) therefore air temperature was excluded from the analysis. The analysis of the remaining variables showed that average microbial biomass at the Loddington site was not dependant on these local climatic variables.



**Figure 5.3: Microbial biomass carbon from Loddington demonstration field; conventional (▲), SOWAP (■) and Farmer's Choice (◆) tillage treatments, points show means (n=5), whiskers show standard error.**



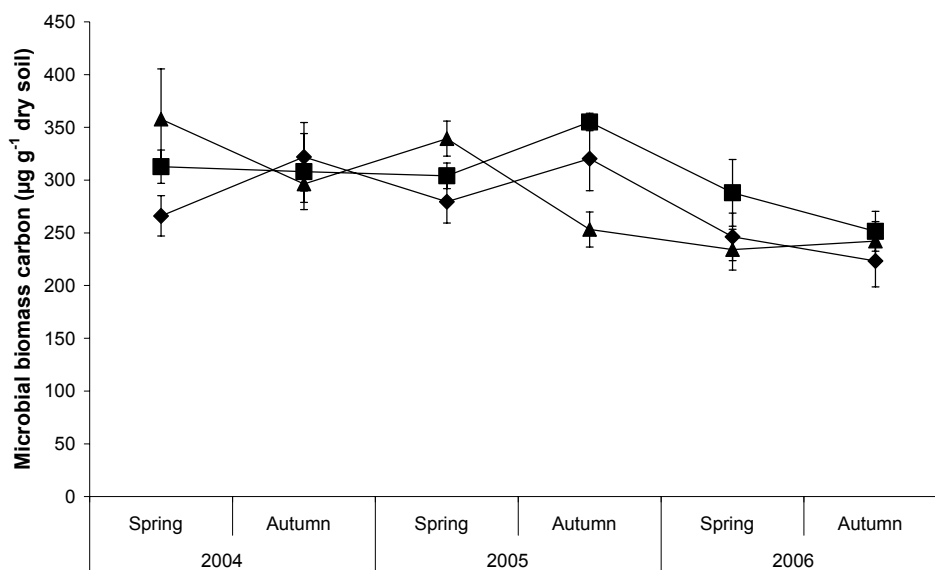


**Figure 5.4: PCA of PLFA from Loddington demonstration field a). 1<sup>st</sup> and 2<sup>nd</sup> principal components (PC); 2004 (●), 2005 (■) and 2006 (▲), spring (s) and autumn (A) samples; points show means (n= 15), whiskers show s.e. b). Loadings associated with PCs.**

Microbial community structure was significantly different between samples taken in spring and samples taken in the autumn of 2005 and 2006 (Figure 5.4). Samples taken in spring and autumn 2004 were not significantly different from each other (Figure 5.4). The biomarker 18:2 $\omega$ 6 (indicative of fungi) had greater proportions from samples taken in autumn than those from spring. Qualitative analysis of these results compared to the cropping cycle showed no obvious correlations.

### 5.3.2 Tivington

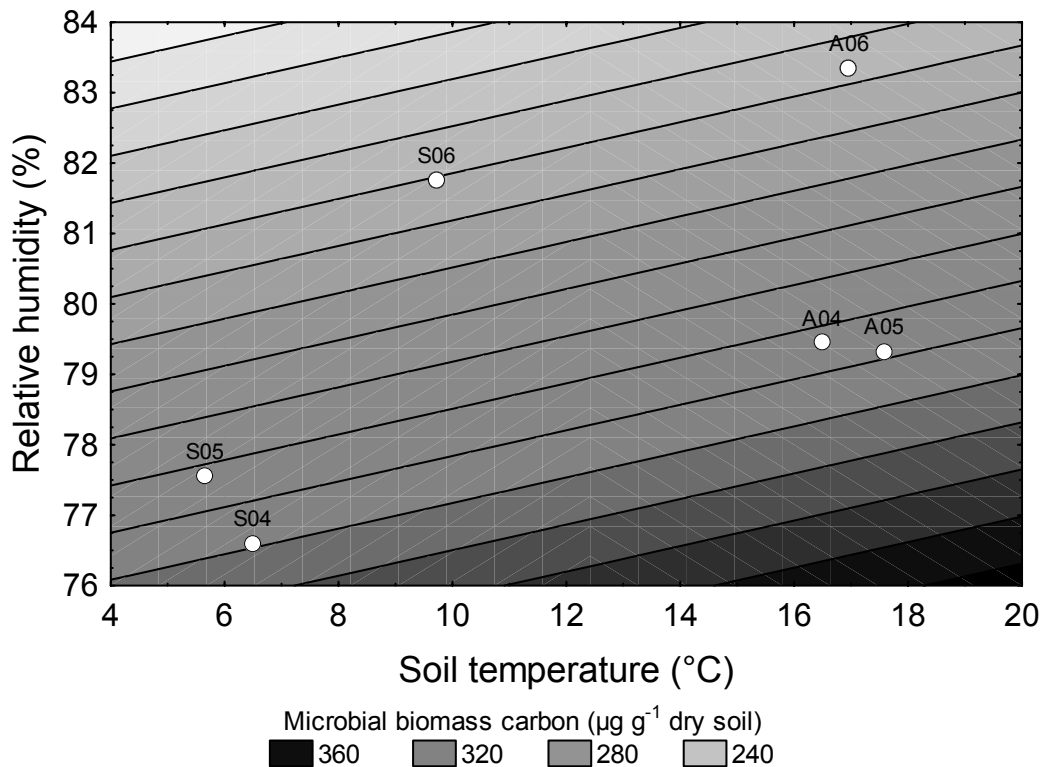
There was no significant effect of treatment at the Tivington site (Figure 5.5). ANOVA of microbial biomass showed that there was significantly smaller concentration of biomass in 2006 than the previous years.



**Figure 5.5: Microbial biomass carbon from Tivington demonstration field; conventional (▲) SOWAP (■) and Farmer's Choice (◆) tillage treatments, points show means (n=5), whiskers show standard error.**

In order to ascertain the climatic impact on microbial biomass multiple forward stepwise linear regression analysis was used. Weather variables, soil temperature, air temperature, relative humidity, solar energy, and rainfall volume were taken for 30 days up to and including the day of

sampling. Air temperature and soil temperature were positively correlated ( $r^2=0.98$ ) therefore air temperature was excluded from the analysis. Multiple forward stepwise linear regression of the remaining variables showed that soil temperature; relative air humidity and rainfall had an effect on the concentrations of microbial biomass carbon in the soils (Figure 5.6).

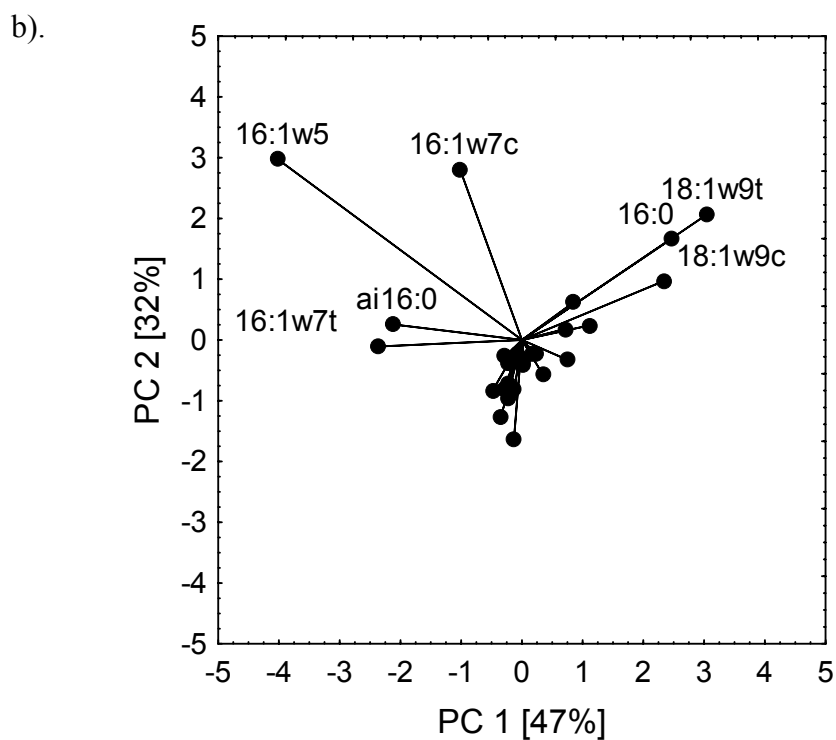
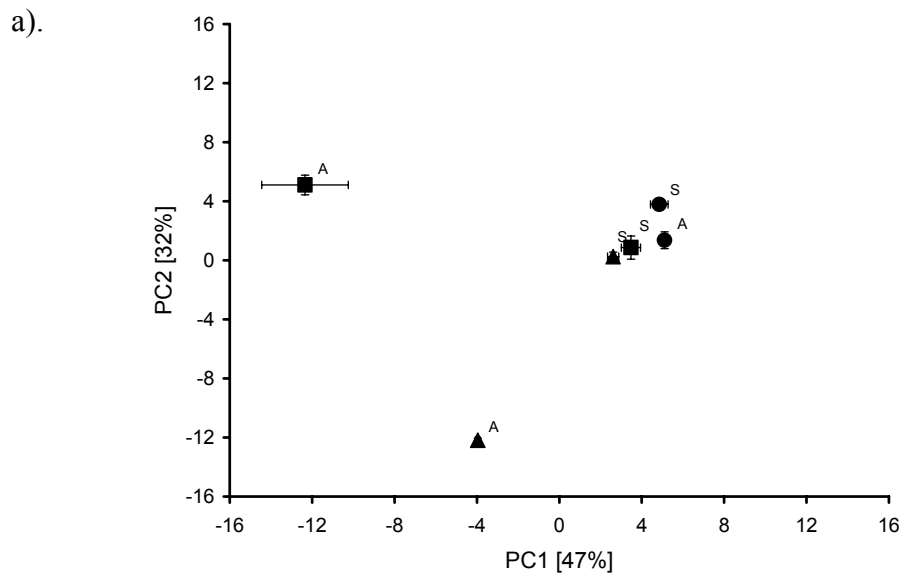


**Figure 5.6: Contour plot showing the effect of relative humidity and soil temperature on Tivington demonstration field microbial biomass samples (spring (S) and autumn (A), 2004 (04), 2005 (05) and 2006 (06) samples; points show means (n=15).**

Statistical analysis of this data using soil temperature; rainfall and relative air humidity as continuous predictors in a general linear model showed no significant effect of tillage treatment on microbial biomass carbon. Therefore local climatic changes at Tivington had a greater impact on the microbial community size than the tillage treatments imposed. Increased relative humidity during sampling times in 2006 is correlated with lower

microbial biomass carbon concentrations in samples from both spring and autumn (Figure 5.6). There was also a significant difference in soil temperature between samples taken in spring and those taken in autumn (Figure 5.6).

The microbial community phenotypic structure showed no significant change as a result of tillage treatment (Figure 5.7a). There was a significant difference between samples taken in each year, autumn 2005 and 2006 were significantly different to all other samples (Figure 5.7). The second principal component separates samples taken in 2006 from samples taken in the previous two years this could be related to the lower biomass recorded in 2006 (Figure 5.7). Change in community structure in autumn 2005 and 2006 could have been as a result of the crop since plants are known to invoke different microbial community properties (Chapter 1.6.1). In this circumstance, the crop that had just been harvested was a legume, field beans (cultivars Wizard in 2005 and Fuego in 2006). Legume crops have specific symbiotic relationships with micro-organisms, such as rhizobia, and therefore may have influence the microbial community phenotypic structure detected.

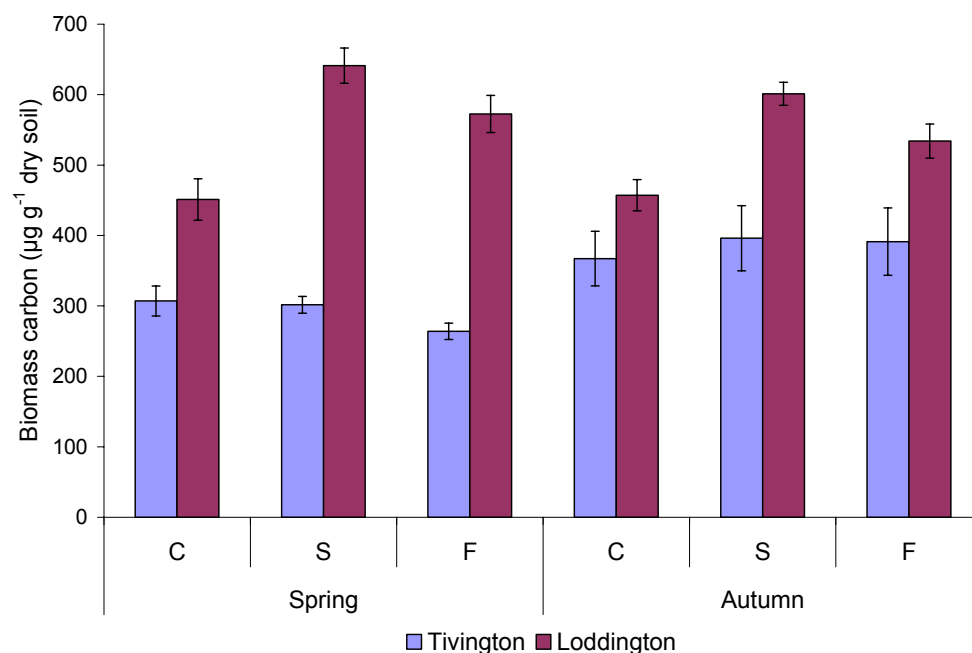


**Figure 5.7: PCA of PLFA from Tivington demonstration field; a). 1st and 2nd principal components (PC); 2004 (●), 2005 (■) and 2006 (▲), spring (S) and autumn (A) samples; points show means (n=15), whiskers show s.e.; b). Loadings associated with PCs.**

The fungal biomarker 18:2 $\omega$ 6 had significantly higher proportions in samples taken in the spring of each year; it was also significantly lower in proportion in the conventional tilled plot. The biomarkers 16:0, 18:1 $\omega$ 9t and 18:1 $\omega$ 9c (indicative of methanotroph and gram negative bacteria, Chapter 1) were significantly higher in proportion in the spring and found in greater proportion in 2004 (Figure 5.7b). The biomarkers 16:1 $\omega$ 5, ai 16:0, 16:1 $\omega$ 7c and 16:1 $\omega$ 7t (indicative of bacteria, Chapter 2.5.2) were significantly higher in proportion in autumn 2005 than at any other time.

### 5.3.3 Joint site analysis

The Loddington demonstration field supported a significantly higher biomass than Tivington under all tillage practices (Figure 5.8).

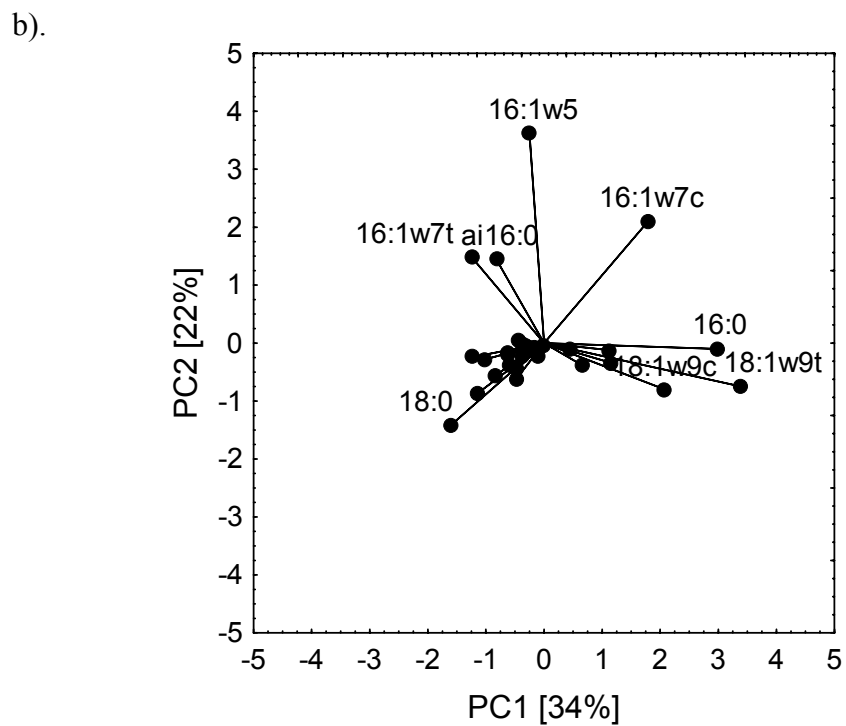
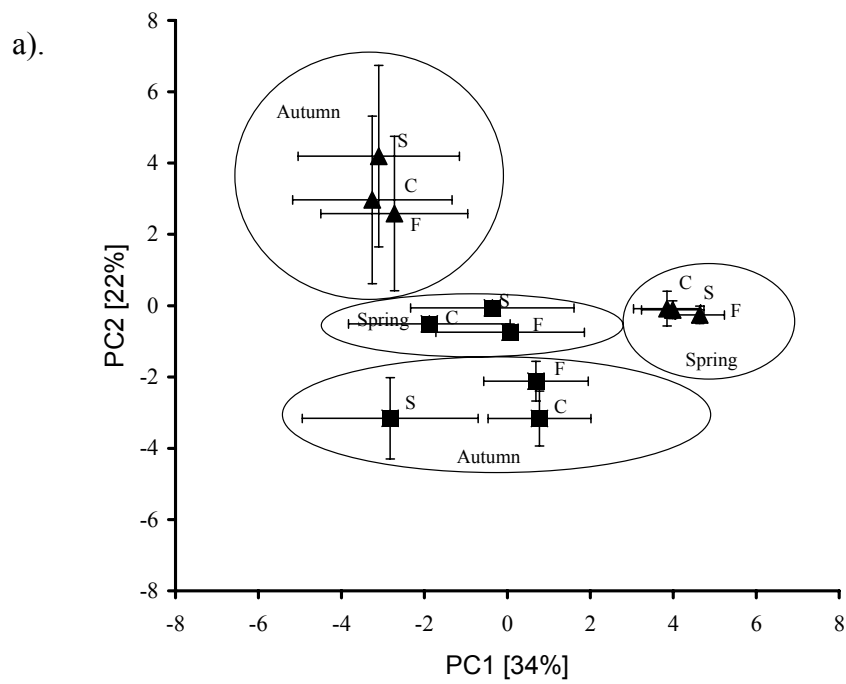


**Figure 5.8: Microbial biomass carbon under different tillage systems on the UK demonstration fields; Conventional (C), SOWAP (S) and Farmer’s Choice tillage treatments, bars show means (n=45); whiskers show s.e.**

The phenotypic microbial community was significantly different at each site (Figure 5.9). There was also a significant difference between spring and autumn samples at each site. At the Loddington site there was a

significantly different community under SOWAP tillage in the autumn, this was the only treatment difference observed.

The biomarkers 16:1 $\omega$ 5; 16:1 $\omega$ 7t; ai16:0; 16:1 $\omega$ 7c; 16:0 and 18:2 $\omega$ 6 were found in higher proportion at the Tivington site than at the Loddington site (Figure 5.9b). The markers 16:1 $\omega$ 5 and 18:2 $\omega$ 6 have been identified as markers for saprophytic fungal biomass and arbuscular fungal biomass (Olsson *et al.* 1995; Frostegard & Baath, 1996), both were found in higher proportion at the Tivington site. The biomarker 18:2 $\omega$ 6 was significantly lower in proportion in the conventional tilled plots and in samples taken in 2005, but significantly higher in spring samples. Whereas, the marker 16:1 $\omega$ 5 was higher in autumn samples and highest in samples taken in 2005. Conventional tilled plots do not provide a favourable environment for fungal species, indicated by the reduction of the biomarker 18:2 $\omega$ 6, a fungal indicator (Federle, 1986; Zelles *et al.* 1992). The potential increase of saprotrophic fungal biomass in the autumn could be as a result of residue incorporation after harvest (Frostegard & Baath, 1996).



**Figure 5.9: PCA of PLFA profiles from Tivington (▲) and Loddington (■) demonstration fields; a). 1st and 2nd principal components; points show means, whiskers show s.e.; b). Loadings associated with PCs.**



#### ***5.4 Conclusion***

Microbial biomass was reduced after a three year period only at the Loddington site and therefore the first hypothesis must be rejected. However, there is evidence for the fungal biomass being adversely affected by conventional tillage by the reduction in proportion of the PLFA biomarker 18:2 $\omega$ 6. The second hypothesis that microbial biomass will be higher in samples taken in the spring of each year must also be rejected as it was unsupported at both sites. The microbial biomass supported at the Loddington site is significantly greater than at the Tivington site therefore the third hypothesis can be accepted. Each of the sites supports different phenotypic communities which differ in spring and autumn of each year.

The effect of cropping is indeterminable from the data collected because of the rotation of crops over the three year period, therefore it is unknown to what influence the regimes had on the results obtained. Qualitative analysis suggests that the use of legumes in the Tivington rotation for two years could have masked any changes in biomass resultant from tillage practice. There was a greater proportion of fungal marker 18:2 $\omega$ 6 found at the Tivington site which was significantly reduced in the conventional tilled soils.

## **Section 2 Conclusion**

Conventional tillage resulted in a notable reduction of microbial biomass in all but the Tivington demonstration site and also there was evidence to suggest a reduction of fungal biomass. Although the actual microbial biomass size alters from site to site dependant on environmental factors such as soil type and climate. Environmental effects have been shown to also influence the phenotypic structure of microbial communities, in particular bacterial communities potentially linking in to r-K selection theory (Chapter 1) where fungal biomass would be environmentally adapted and therefore prevail.

Microbial biomass did not significantly alter over the time period observed. However, the phenotypic structure of the microbial community was significantly different between samples taken in spring compared to those taken in autumn. There is some evidence to suggest an increase in saprotrophic organisms in the autumn months which may drive these phenotypic community shifts. The experiments contained in this section were not specifically designed to investigate the effect of crop on the microbial community so much of the variation in results obtained could be contributed to the effect of different plant species interactions and the different kinds of residues produced by these plants. Legume crops have specific symbiotic relationships with soil microbes which are not present in cereal crops, therefore assessment of changes in phenotypic structure must take into account interactions with higher plants in terms of exudate production and symbioses.

# *Section 3*

### **Section 3– Small scale experimentation**

#### *Section overview*

A series of microcosm and small plot-scale experiments were designed to gain a mechanistic understanding of interactions between microbial communities, the soil matrix, and water and soil loss by water erosion. Laboratory experiments provided an opportunity for greater replication and control of variables caused by natural phenomena such as seasons, soil moisture, nutrient and pH fluctuation. It was hypothesised that the presence and composition of a living microbial community may influence loss from, and water movement through, soils.

The broad hypotheses tested were:

1. The presence and composition of a microbial community will influence the hydrology of soil.
2. The rate of rainfall and runoff erosion will be inversely proportional to the presence of a microbial community when compared to sterile controls.
3. Raindrop impact and overland flow will change the size and structure of surface soil microbial communities.
4. Variation in the microbial community structure will have an impact on the propensity of soils to erode.
5. Altering microbial communities by differing tillage practices will result in changes of response to rainfall events, due to the interaction of specific components of the microbial community that are specifically selected by each tillage practice

In order to test these hypotheses non-sterile, sterile and sterile re-inoculated systems were used to compare the effects of microbial communities.

### ***The use of rainfall simulation in erosion studies***

Rainfall simulations are frequently used in soil erosion studies to avoid having to rely on natural rainfall events, which are virtually impossible to predict and impossible to control. Natural rainfall, at a given time, may not produce the intensity and duration of rainfall sufficient to effect soil erosion at a measurable scale. Natural rainfall varies in intensity, drop size distribution and kinetic energy – no two storms are alike. This makes the study of erosion rates and processes under different treatments across different landscapes problematic and possibly misleading. Hence, the utility of simulated rainfall which is controllable in space and time, and reproducible. The use of laboratory-based simulated rainfall allows the reduction of environmental variability, such as wind speed and direction, temperature and humidity. Field-based experiments are expensive and labour intensive to set up and monitor in comparison to laboratory studies which greatly improve the speed at which research can be conducted in this topic (Bowyer-Bower & Burt, 1989; Rickson, 2006).

Simulated rainfall has been used to determine runoff and erosion rates across the globe in many studies; Morgan *et al.* (1997) explored erosion rates of rangeland in Swaziland (Morgan *et al.* 1997), whilst the runoff and erosion from paved forest roads in northern Spain was studied by Arnaez *et al.* (2004) (Chapter 1). Rainfall simulation has also been used to study factors other than particle erosion such as; the heavy metal and suspended solid movement in urban storm waters in Australia (Herngren *et al.* 2005), the effect of termites on infiltration through crusted soils in West Africa

(Mando *et al.* 1996), and the effect of cattle manure in relation to water pollution by faecal coliforms (Ramos *et al.* 2006). In the following study rainfall simulators were used to help determine the effect soil micro-organisms have on soil erodibility using sterile and non-sterile soil systems.

### ***Sterilisation of soil for use in ecological studies***

Sterilised soil is widely used in laboratory based experiments to eliminate or reduce the biological activity. The ideal sterilisation method will eliminate soil organisms and resistant spores whilst not affecting other soil properties. There are many different sterilisation methods. Generally, the smaller the volume of soil required the easier it is to achieve complete sterilisation and therefore the quantity of sterile soil required can greatly influence the choice of method.

Sterilisation by moist heat (autoclaving) is widely used in research as the equipment is readily available in most laboratories. Soils are either air-dried or have adjusted moisture content less than 60%. Soil is laid out in a thin layer to allow maximum steam penetration during the autoclave run. Shaw *et al.* (1999) found that autoclaving produced a significant increase in the concentration of water soluble organic carbon. Significant decreases in pH in clay soil have also been reported (Salonius *et al.*, 1967). In contrast, Egli *et al.* (2006) found that pH increased in soils with increasing carbonate contents and that there was a partial decrease in organic matter. It is suggested that the magnitude of pH decrease as a result of autoclaving will depend upon the acidic buffering capacity of the soil used (Shaw *et al.*, 1999). Dry heat sterilisation is achieved by laying soil in thin layers and heating it to 200°C for a minimum period of 24 hours. Trevors (1996)

suggests that wetting and incubating the soil for a few days, allowing heat resistant bacterial spores to germinate prior to dry heat sterilisation will result in more effective sterilisation. These methods destroy soil structural properties, change soil chemistry and are problematic to aseptically pack into proposed soil erosion study trays. They were therefore deemed unsuitable for the requirements of the following experiments.

Chemical sterilisation can make use of many different substances. Amongst the most common are chloroform and ethylene oxide. These are generally employed either as fumigants if volatile (chloroform, ethylene oxide) or as chemical additives (mercuric chloride, sodium azide) (Wolf *et al.* 1989). The latter of these methods poses significant risks to the environment in terms of soil disposal post experimentation and also a serious hazard to human health. Chemical additives are also impossible to completely remove from the soil which makes them inappropriate and impractical to use in an ecological study. Chloroform is used in the determination of microbial biomass carbon to lyse cell membranes releasing cell carbon. This is a simple and inexpensive sterilisation method. Fumigation by chloroform results in an immediate increase in ammonium and organic carbon (Jenkinson & Powlson, 1976b). Ethylene oxide fumigation alkylates the functional groups of proteins (Trevors, 1996). This sterilisation is generally carried out using commercially available sterilisation units to reduce user exposure. Soil is incubated before sterilisation to permit the germination of bacterial spores. Ethylene oxide boils at 11°C so must be kept cold prior to addition to fumigation vessel. It has been shown to increase soil pH due to esterification of carboxyl groups in the soil organic matter (Trevors, 1996; Kirk *et al.*, 2004). Fumigation methods have little impact on soil structural properties

but there is a risk of residual fumigant in the soil, a disadvantage where re-inoculation is required. Klose *et al.* (2006) experimented on the effect of fumigation by various biocides on microbial phenotypic profile in a sandy loam soil. They concluded that actinomycetes and Gram-positive bacteria may preferentially recover after fumigation affecting key reactions in nutrient transformations.

Sterilisation by irradiation can be achieved in a number of ways and the fact that experimental units can be assembled prior to the sterilisation process makes it a particularly useful tool to investigate structural stability and soil physical degradation processes. Microwaves are non-ionising radiation, which produce hyperthermic conditions affecting water molecules and interfering with cell membranes. Therefore, the soil must have a high moisture content to provide the most favourable conditions for cell death. This method is unsuitable for larger volumes of soil or soils packed into metal experimental trays. High moisture content and consequent heating of the soil causes too great a chemical and physical interference for use in the following experiments.

$\gamma$ - irradiation of soil is achieved by use of a  $^{60}\text{Co}$  source and can only legally be carried out at an irradiation facility. Such irradiation results in the formation of free hydrogen and hydroxyl radicals which cleave carbon-to-carbon bonds; it also causes the depolymerisation of carbohydrates. Cell death occurs in an exponential manner. Larger cells require less ionizing radiation to kill them, therefore fungi are affected by lesser doses of irradiation than bacteria (Trevors, 1996). Fungi have been shown to be affected by irradiation doses as low as 0.01 kGy whereas most bacteria require doses between 15 – 25 kGy before death and some studies have



suggested that some bacteria can survive after doses as high as 75 kGy (McNamara *et al.*, 2003).  $\gamma$ -irradiation of soil results in an overall increase in the mineral N and a decrease in  $\text{NO}_3^-$ , which has been attributed to peroxide production (McNamara *et al.*, 2003). It appears that the greater the moisture content of the soil the greater the effects of irradiation on soil mineral N, observed to increase by up to thirty times post irradiation (Bowen & Cawse, 1962). Differing soil types greatly affect the result of  $\gamma$ -irradiation on soil N, P, Mn and S, increasing extractable N, Mn and S immediately post irradiation. There appears to be no consistent trend in changes of soil pH post irradiation; however the soil pH has been shown to vary considerably following irradiation. The higher the percentage of organic matter the greater the dose of irradiation needed to achieve complete sterilisation (McNamara *et al.*, 2003). Salenius *et al.* (1967) found that there was a slight decrease in aggregate stability after irradiation.  $\gamma$ - irradiation of soil causes minimum alteration to physical properties and leaves few residual chemicals behind, making it the most suitable method for the following experimental designs and a useful tool for re-inoculation experiments.

## Chapter 6 Field rainfall simulation experiment

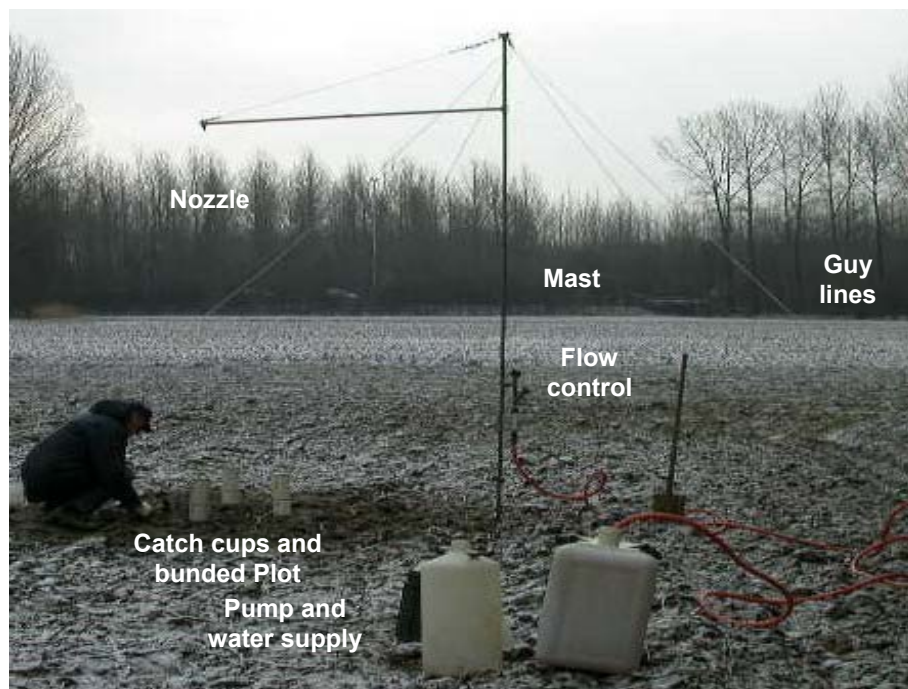
### 6.1 Introduction

Run-off occurs from soil whenever rainfall intensity exceeds the infiltration capacity and surface storage potential of the soil. The application of artificial rainfall to a defined area of study provides an experimental means to study the impact of water erosion on soils under controlled circumstances. Rainfall simulation has been used in numerous studies to assess water infiltration, run-off and erosion losses all over the world.

To supplement the field trials (Section 2), field rainfall simulations were applied in an experiment to explore the potential relationships between microbial community structure in the surface regions of soil and the propensity of soil to erode. The following specific hypotheses were tested;

1. Simulated rainfall onto soils subjected to different tillage practices will result in a variation in microbial community structure in the surface soil (top 10 mm).
  - *Raindrop impact and overland flow will physically alter soil structure and therefore soil microbial community structure.*
2. There is an association between microbial community structure and the propensity of soils to erode.
  - *Divergent microbial community configurations affect soil structural integrity by contrasting mechanisms.*
3. The microbial community structure of runoff samples will be significantly different to that of the soil before and after rainfall.
  - *Different components of the microbial community will be susceptible to detachment and carriage by overland flow.*

4. Sediment concentration will be higher in soils tilled using conventional means.
  - *Reduced surface aggregate stability and surface cover as a result of primary and secondary cultivations increases soil particle detachment.*
5. Runoff volume will be greater from conservation treated plots due to surface compaction as a result of minimal tillage practices.
  - *Minimal mechanical disturbance of soil results in greater bulk density and compaction reducing water infiltration and drainage.*



**Figure 6.1: Arrangement of rainfall simulator system in the field.**

## ***6.2 Experimental design and methods***

These experiments were conducted in conjunction with Sophie Cooper, PhD student NSRI and SOWAP project partner (Cooper, 2006). They were carried out in April 2005 at the Loddington demonstration field (detailed in

Chapter 3.2). Three replicate rainfall simulations were carried out per tillage treatment (conventional, SOWAP and Farmer's Choice conservation tillage).

### **6.2.1 Rainfall simulator**

The rainfall simulator design (Figure 5.1) was constructed by Mr J. Meersmans (K.U. Leuven, SOWAP project partner). Simulated rainfall was directed on to a 1 x 1.5 m bunded plot for 30 min. In addition to the design shown, the rainfall simulations carried out at Loddington adopted the use of a wind shield to prevent rainfall scatter away from the bunded plot due to the field's exposure. The rainfall intensity was controlled at 36 mm h<sup>-1</sup> where possible, but the final run-off values obtained were corrected for slight variation in intensities across the replicates.

### **6.2.2 Simulation installation**

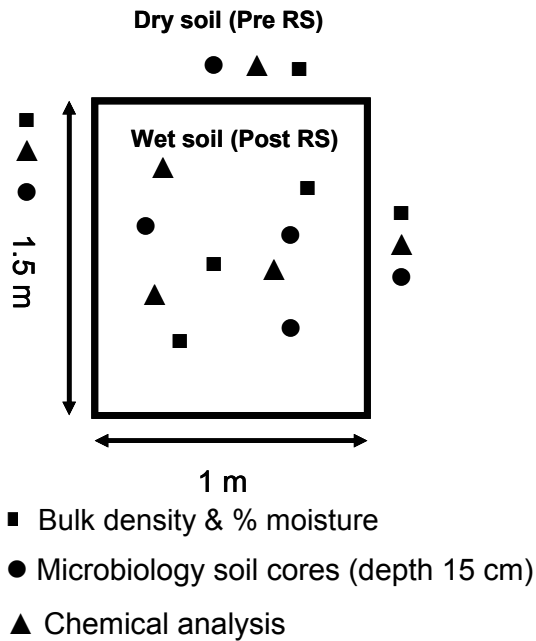
The location of each rainfall simulation was selected randomly within each tillage treatment. The bunded area was installed using sheet steel (3 mm thick, 200 mm depth) and a mallet to a depth of ~100 mm; the collection system was installed along the down-slope 1 m edge using a trowel, taking care not to disturb the surface within the 1.5 x 1 m bunded area (Figure 6.2). The vertical slope on the rainfall plot was determined using an Abney clinometer. Photographs were taken of the bunded area for analysis of percentage cover (crop, weed, residue, stone and bare soil). Rainfall gauges were installed immediately adjacent to the rainfall plot to measure the rainfall intensity (mm h<sup>-1</sup>). Copecki rings were used to sample for bulk density and soil moisture from the area immediately adjacent to the rainfall plot (Figure 6.2 & 6.3), along with soil cores at known depth for chemical analysis and undisturbed 150 mm cores for biological analysis.



**Figure 6.2: Photograph showing rainfall simulation bunded plot, rainfall catch cups and collection system.**

### **6.2.3 Rainfall simulation**

Whilst the rainfall simulation was in progress the time taken for the first run-off to appear in the collection system was noted. After this each increase of 100 ml was recorded until 1 l was collected, then every 500 ml collected run-off time was recorded. Every 5 – 10 min of the simulation running a visual assessment of the ponding (% of total surface of rainfall plot) and crusting (% of deposited sediment material across rainfall plot) were recorded. Three replicate rainfall simulations were carried out per tillage treatment (conventional, SOWAP and Farmer's Choice conservation tillage).



**Figure 6.3: Rainfall simulation (RS) plot sampling strategy showing locations of destructive sampling.**

#### **6.2.4 Post simulation**

Once the simulation was completed the collecting tube was cleaned to remove any deposited soil using collected water. The run-off water and sediment was stirred to resuspend the particulate matter and 3 sub-samples (where possible 100 ml) were taken to determine the sediment concentration, and a further sub-sample of 20 ml was also taken for PLFA analysis. Copecki rings were used to sample for bulk density and soil moisture content from the area inside the rainfall plot along with further undisturbed soil cores for chemical and biological analysis (Figure 6.3).

#### **6.2.5 Microbial sample preparation**

Run-off samples were sub-sampled by re-suspending sediment and collecting a 25 ml aliquot which was then freeze-dried (see Chapter 2.2.2). Undisturbed soil cores were sub-sampled to remove the surface 1 cm of soil, which was homogenised by chopping to remove stones, plant matter

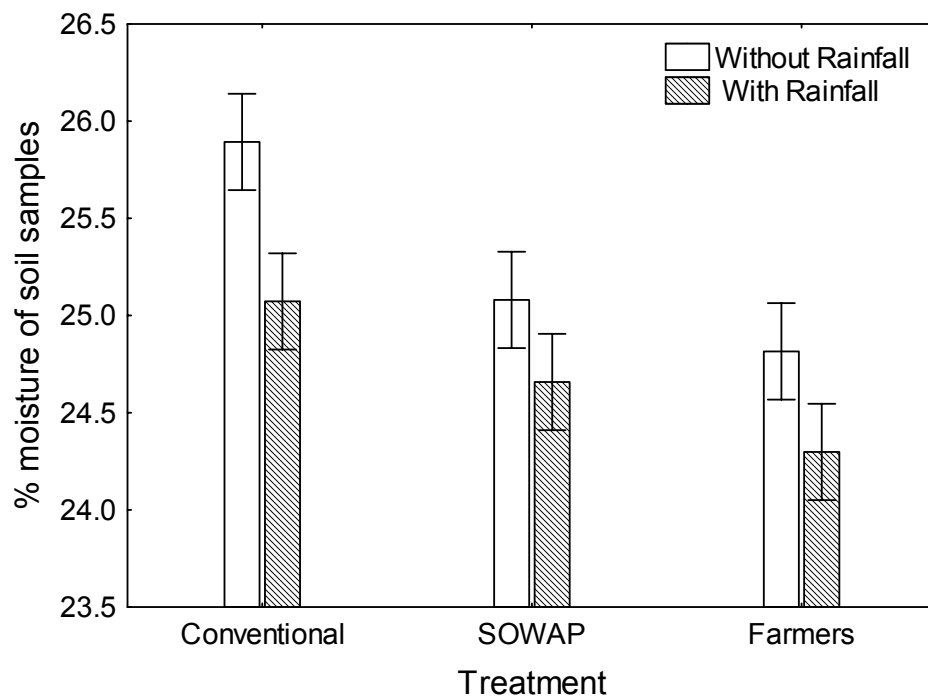
and soil animals. Homogenised samples (10 g) were freeze-dried (Chapter 2.2.2). Collected samples were analysed for moisture content (Chapter 2.3.1), microbial biomass carbon (Chapter 2.5.2) and microbial community structure (PLFA, Chapter 2.5.2).

### **6.2.6 Data analysis**

A lack of treatment replication at the demonstration field in Loddington results in this experiment adopting a nested design and statistical analysis using general linear models has taken this into account. Data analysis was achieved by principal component analysis and analysis of variance.

### ***6.3 Results and discussion***

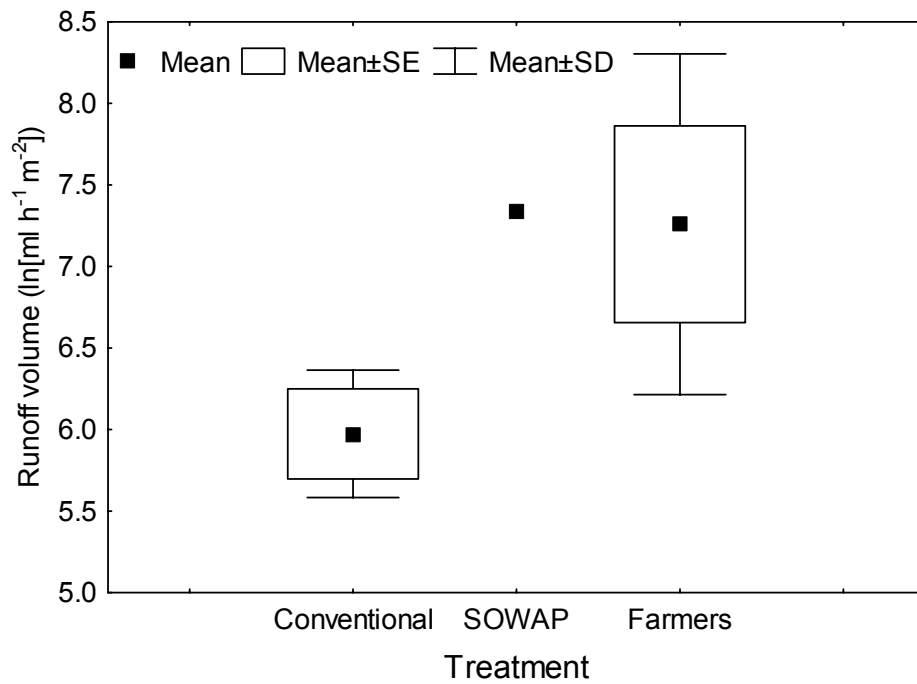
The soil moisture content was significantly greater in the conventional treatment compared to the other two treatments before simulated rainfall was applied (Figure 6.4). After simulated rainfall there was a significant overall loss in surface soil moisture, except in the SOWAP treatment which remained constant. The reduction in the moisture content of the soil surface could be as a result of infiltration to a depth below the top 1 cm or an increase in soil capping causing greater overland flow.



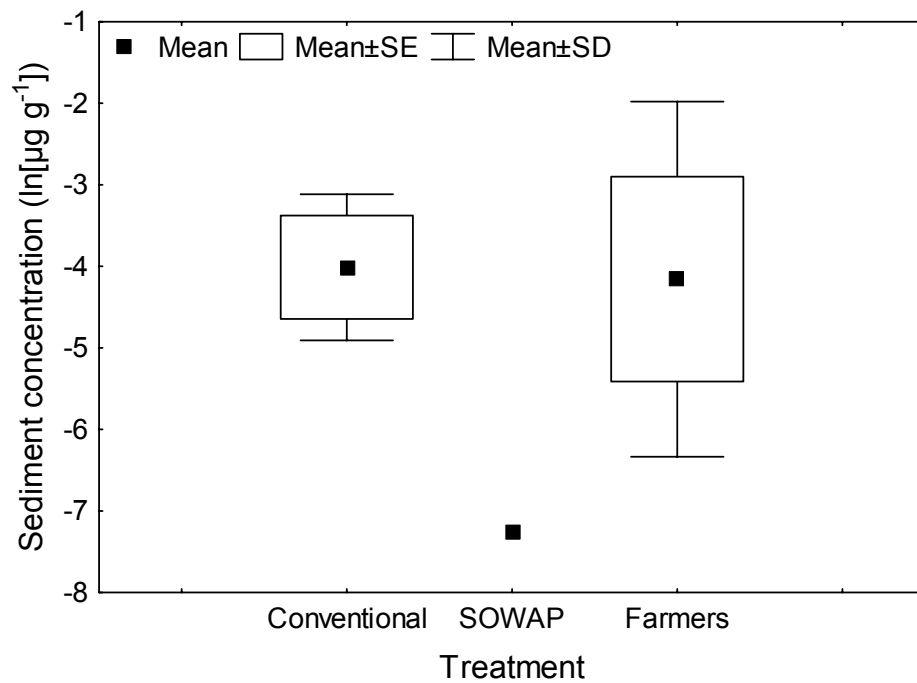
**Figure 6.4: Percentage moisture of top 1 cm of soil samples from each treatment with and without simulated rainfall: bars show means (n=3), whiskers show 95% confidence intervals.**

There was no significant difference between means of runoff and sediment concentration in relation to tillage treatment (Figures 6.5 & 6.6). However there was significantly higher variation associated with the Farmer's Choice treatment when compared to the conventional treatment using a t-test for unequal variance (Figure 6.5). There was only one runoff event from the SOWAP treatment during the three replicate rainfall simulations, therefore this was a significant finding. The SOWAP treatment in this experiment is less likely to runoff in the first place. It is not possible to include this finding in the normalised dataset due to the result being zero but the probability of runoff is definitely reduced as a result of this treatment.



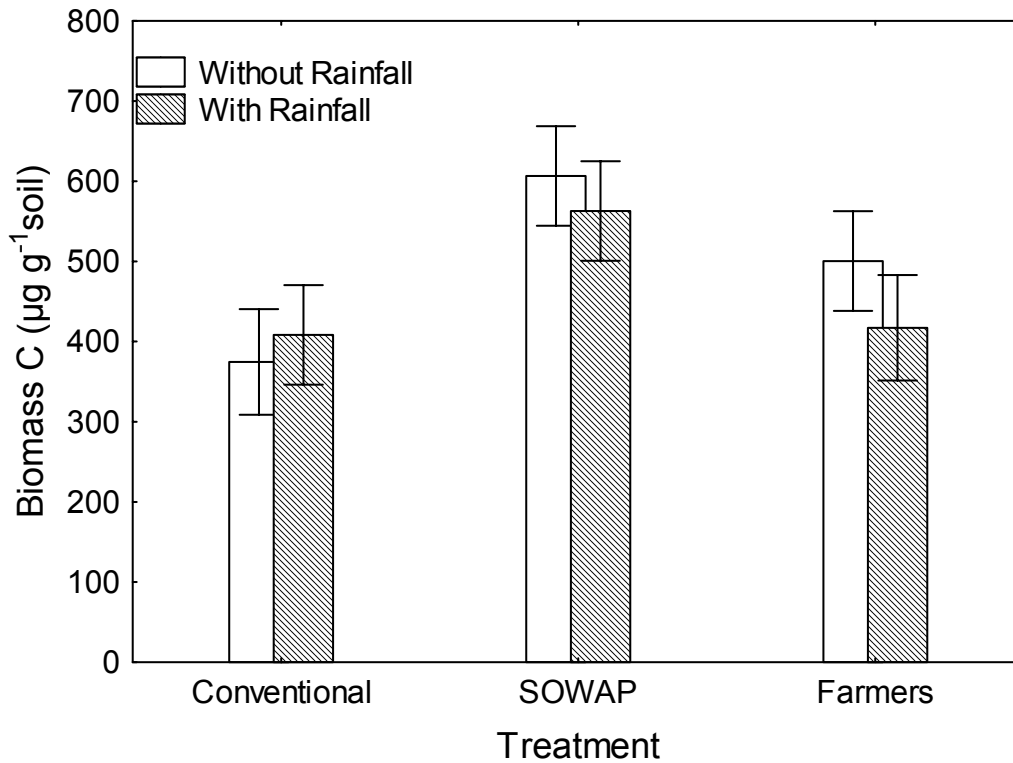


**Figure 6.5: Box and whisker plot showing runoff volume (n=3 except SOWAP where n=1).**



**Figure 6.6: Box and whisker plot showing sediment concentration in runoff (n=3 except SOWAP where n=1).**

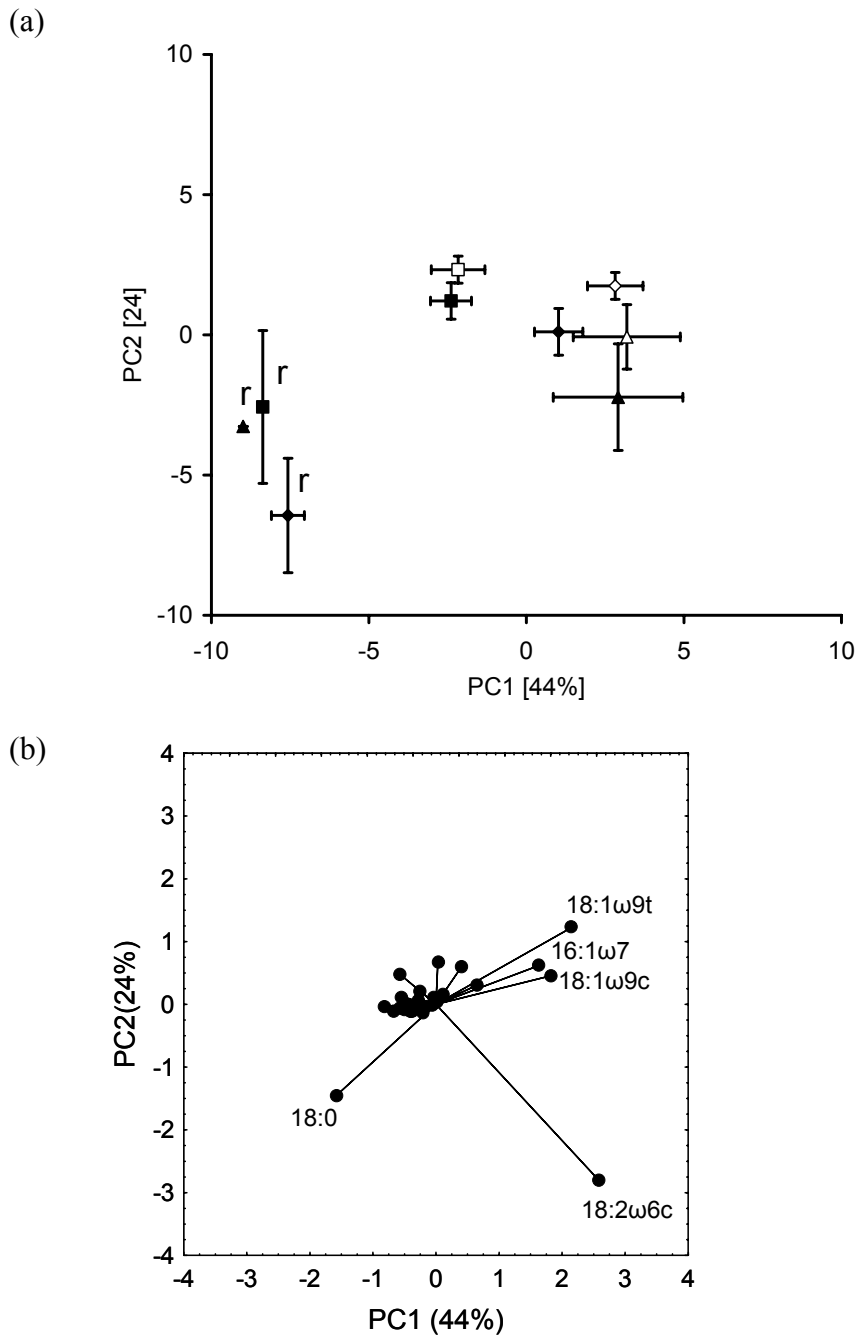
Microbial biomass C showed a significant difference between conventional and SOWAP treatments ( $p < 0.01$ ), a *post hoc* least significant difference test showed no significant difference between conventional tillage and Farmer's Choice tillage treatments. There was no significant effect of rainfall on microbial biomass C (Figure 6.7).



**Figure 6.7: Concentration of microbial biomass carbon extracted from soil in relation to the application of simulated rainfall: bars show means (n=3), whiskers show 95% confidence intervals.**

In the PCA of all PLFA profiles (Figure 6.8) the community structure of the conventional tilled plot was significantly different to the community structure of the SOWAP and Farmer's Choice tilled plots in PC1. The microbial community composition of the run-off was significantly different to that of the soil. The PCA loadings showed that the biomarkers 18:2 $\omega$ 6c; 18:1 $\omega$ 9;c 16:1 $\omega$ 7c and 18:1 $\omega$ 9t were significantly reduced in conventional samples (Figure 6.8b). The biomarker 16:1 $\omega$ 7t showed significantly lower

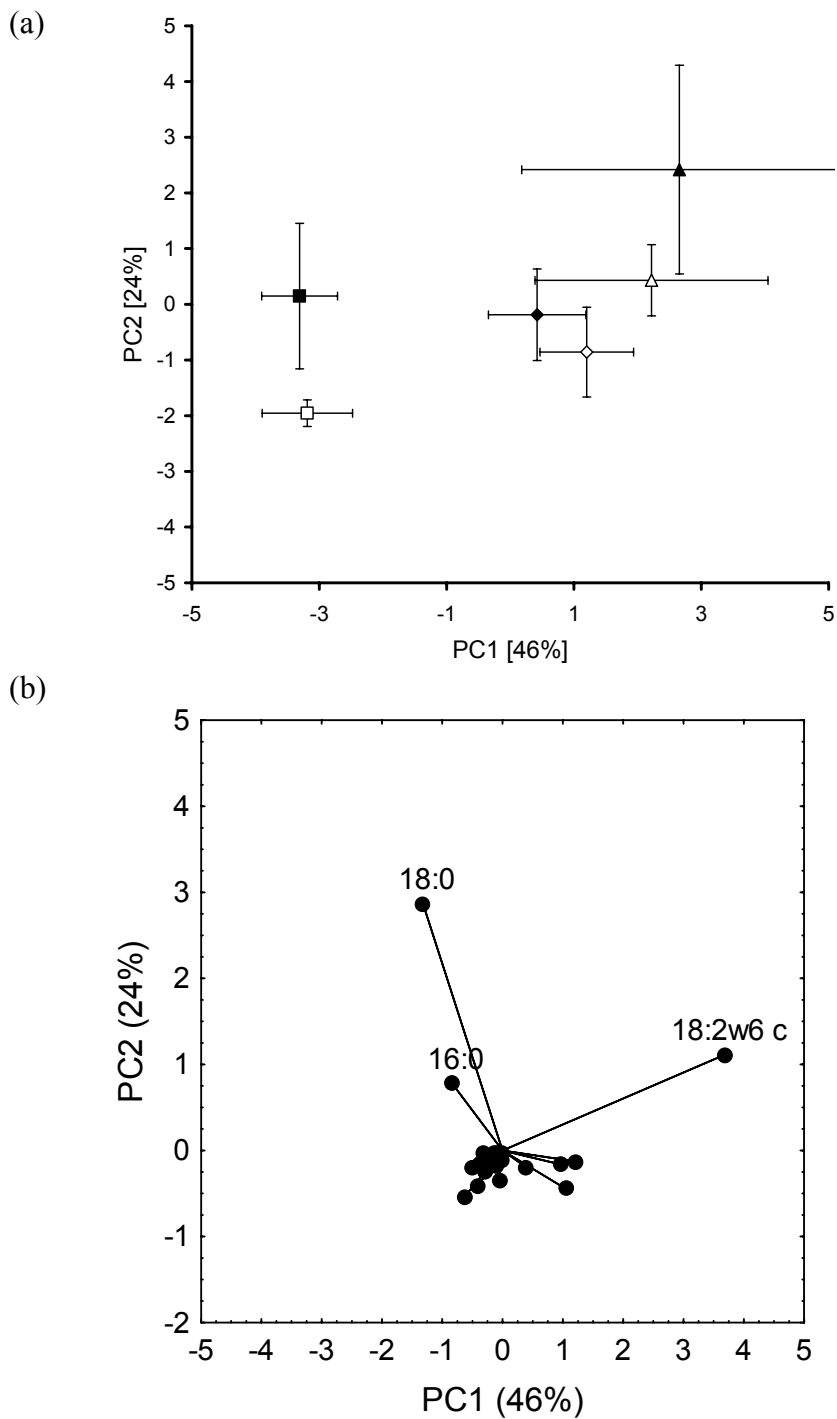
%mol in runoff samples from the conventional treatment. No significant difference in either PC1 or PC2 was observed between the PLFA profiles obtained for soil samples taken before the rainfall simulation and those taken after (data not shown).



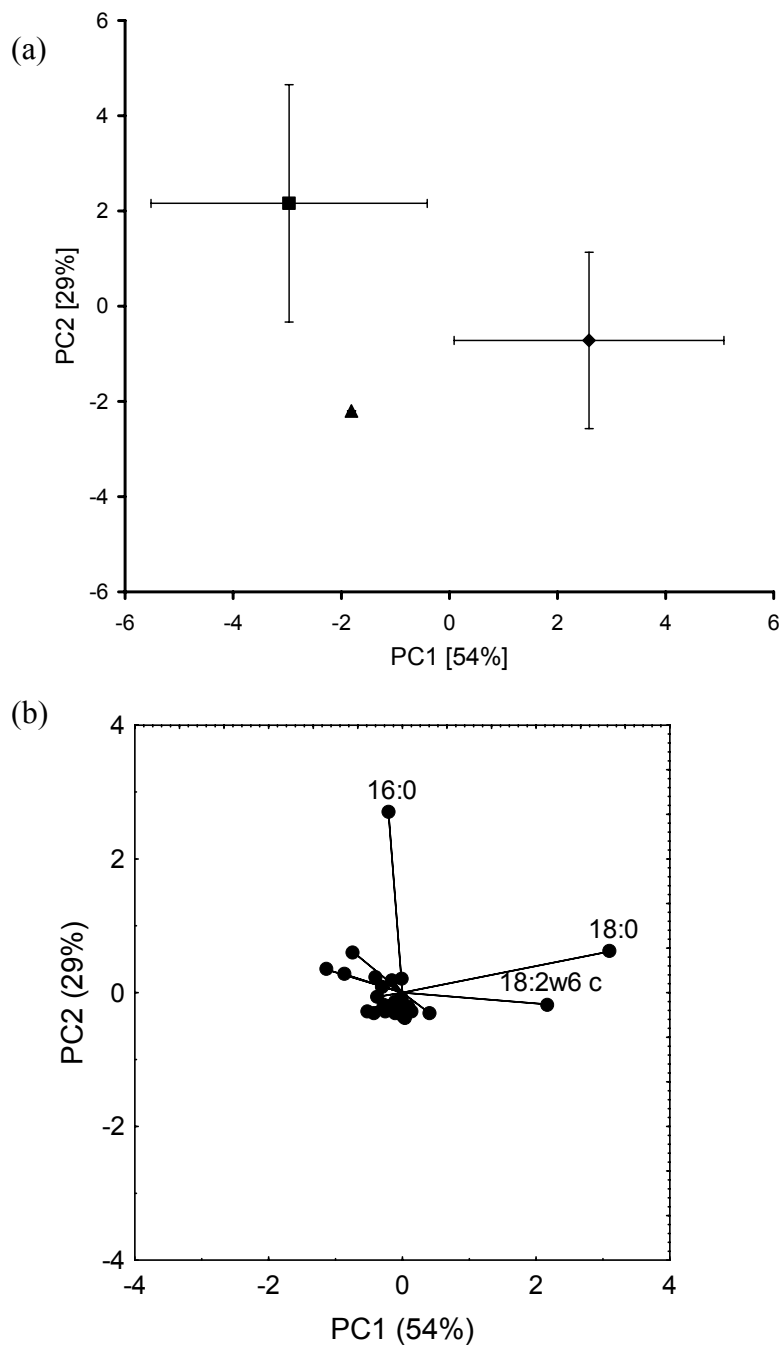
**Figure 6.8: Phenotypic structure of samples as described by principal component analysis (PCA) of PLFA profiles; (a) first and second principal components (PC); Conventional (■); Farmer's Choice (◆); and SOWAP (▲) treatments; open symbols denote samples before rainfall, closed symbols denote samples taken after simulated rainfall, runoff samples (r); points show means (sediment n=3 (n=1 for SOWAP), soil n=6), whiskers show s.e. Percent variation accounted for by PCs shown in square parentheses. (b) Loadings associated with PCs.**

PLFA profiles derived from conventional tilled soil samples were significantly discriminated by PC1 to soils under the Farmer's Choice and SOWAP tillage treatments (Figure 6.9). The loadings associated with the PCA of PLFA profiles showed biomarkers 18:2 $\omega$ 6c, 18:0 and 16:0 to be significantly influencing (Figure 6.9b). 18:2 $\omega$ 6c (fungal biomarker (Federle, 1986)) was significantly lower in conventional treated soil (2.2% Conventional; 4.3% Farmer's Choice, 6.5% SOWAP, s.e. 0.1). The biomarker 16:0 had greater %mol in conventional tilled soils compared to the other treatments (11.71% Conventional; 10.08% Farmer's Choice, 11.15% SOWAP, s.e.0.03).

The PLFA profiles of the conventional soil runoff were significantly different to that of the Farmer's Choice tillage soil runoff (Figure 6.9). The loadings associated with the PCA of PLFA profiles indicated that the biomarkers 18:2 $\omega$ 6c, 18:0 and 16:0 accounted for the majority of variance. ANOVA of these indicated biomarkers showed no significant treatment difference (Figure 6.10b).



**Figure 6.9: Phenotypic structure of samples as described by principal component analysis (PCA) of PLFA profiles; (a) first and second principal components (PC); Conventional (■); Farmer (◆); and SOWAP (▲) treatments; open symbols denote samples before rainfall, closed symbols denote samples taken after simulated rainfall; points show means (n=3), whiskers show s.e. Percent variation accounted for by PCs shown in square parentheses. (b) Loadings associated with PCs.**



**Figure 6.10: Phenotypic structure of runoff samples as described by principal component analysis (PCA) of PLFA profiles; (a) first and second principal components (PC); Conventional (■); Farmer (◆); and SOWAP (▲) treatments; points show means (n=3 except SOWAP where n=1), whiskers show s.e. Percent variation accounted for by PCs shown in parentheses. (b) Loadings associated with PCs.**

#### **6.4 Conclusions**

Simulated rainfall onto the different tillage practices did not result in a significant change in the microbial community structure of the soil after rainfall, as determined by PLFA, however there was a significant difference between the soil PLFA profiles associated with conservation tillage practices when compared to conventional. Thus the first hypothesis, that rainfall onto soils subjected to differing tillage practices would result in a change in microbial community structure, is accepted.

There was no significant difference in erosion between tillage treatments; however there was a significantly higher variation in runoff volume and sediment concentration from the Farmer's Choice tilled plot. The microbial community structure of the conventional tilled plot was significantly different to the Farmer's Choice tillage treatment therefore the difference in variation could be attributed to a different microbial phenotypic structure.

The microbial community structure of the runoff was significantly different to that of the soil samples. This would indicate that a specific fraction of the microbial community was either associated with eroded fine particulate matter or was less tightly bound to the soil matrix (Väisänen *et al.*, 2005). If the PLFA profiles associated with the runoff were derived from minute quantities of fine-particle sediments this could result in a massive disparity between initial sample sizes of soils and runoff. The hypotheses that the microbial community structure will differ between run-off and soil samples can be accepted; however, further experimentation is required to understand the causal mechanisms behind such observations. There was no



significant effect of run-off or sediment concentration in relation to tillage treatment. Therefore, it is impossible to connect these community profiles with soil erodibility and as such the initial hypotheses are rejected. One explanation for the lack of treatment significance could be the variation in field conditions, i.e. soil moisture across the different treatments. The soil texture at the Loddington demonstration site is clay which is not susceptible to large-scale erosion events: however, it has been associated with large run-off events possibly due to surface sealing. Greater replication and the use of additional fields would provide a stronger experimental design to test these hypotheses. These field conditions cannot be easily manipulated within the constraints of the SOWAP project, therefore further justifying the need for laboratory based microcosm scale experimentation where variation is minimised and potentially contributory factors more precisely controlled.

The more extensive field rainfall simulations carried out by Cooper (2006) showed that the percentage of crop and other cover in the plot is important in the development of surface seals and ponds. In the majority of rainfall simulations carried out on the Loddington site; the runoff and soil losses were greatest from the conventional plot. Soil seal formation positively correlated with the organic matter content of the soil. Seals form as a result of a breakdown and reforming of aggregates however, higher organic matter is usually associated with an increased aggregate stability (Chenu *et al.* 2000), and so this was an unexpected finding. It is possible that this result could be due to a decrease in fungal biomass which has been shown to increase aggregate stability (Cosentino *et al.* 2006). Soil moisture modulated by precipitation and temperature regimes and tillage treatment have both been implicated as altering fungal biomass (Frey *et al.*, 1999),

however no direct measure of fungal biomass was made during this experiment. The biomarker 18:2 $\omega$ 6 has been implicated as a fungal PLFA marker (Federle, 1986); in this study the %mol was significantly less in conventional tilled soil than in the SOWAP tilled soil.

This investigation indicated that microbial community size and structure has implications for runoff and erodibility at the field scale but in this case the inherent variation in natural systems concealed the dynamics of these interactions. In order to further explore the mechanisms underlying microbial community and soil water movement, laboratory experiments are required. This would allow greater control over rainfall intensity, duration and drop size using a gravity fed rainfall simulator. A laboratory based experiment at small scale would allow for quantitative control treatments in order to more completely assess system variability.

## **Chapter 7 The impact of microbial communities on soil hydrology**

### ***7.1 Introduction***

The role of micro flora and fauna on soil structure formation, stabilisation and degradation at small aggregate scale has been reviewed frequently (Oades, 1993; Six *et al.* 2004; Chapter 1). Research on the scale of single aggregates has been carried out into the effect of micro-organisms at species- and community- levels on soil hydrological properties and aggregation, little research has been done at larger scales, such as the microcosm scale involving many soil aggregates.

Identifying the microbial community components which contribute to this structural stability has never been completely successful, although fungi are often implicated. However, such factors may be related more to the overall configuration of the soil community rather than the properties of individual organisms. There may also be a relationship to microbial life-history strategies e.g. the r-K model (Chapter 1.2.1). A clear understanding of the processes governing a microbiologically active soil and the relationships between the soil biota, soil structure, hydrology and erodibility has implications for soil and water management, and the dispersal of micro-organisms at the field scale. The aim of this experiment was to ascertain the effect of the soil microbial community on hydrological processes in relation to water holding capacity, run-off and infiltration both immediately post irradiation and two weeks later with the inclusion of a system re-inoculated with microbes.

Specific hypotheses were:

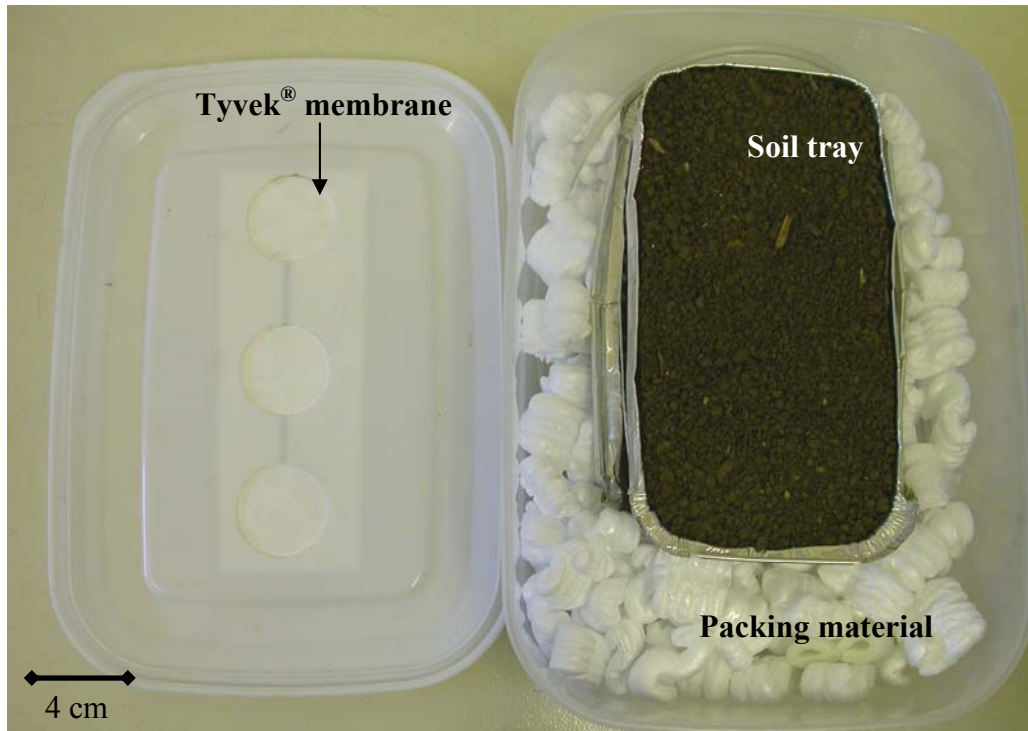
1. The presence of a living microbial community will increase the soil water holding capacity, in comparison to sterilised controls.
  - *Microbial community action on soil structural formation and pore connectivity will alter the movement of moisture through soil.*
2. The presence of a living microbial community will decrease the propensity of a soil to generate run-off and increase infiltration of water through the soil profile.
  - *Increased soil aggregation and pore connectivity as a result of microbial mediation will improve soil drainage, decreasing surface flow.*
3. Variation in the microbial community structure, in particular the ratio of bacteria to fungi, will have an impact on water movement through the soil.
  - *Modulation of soil structure by microbial communities affects soil hydrological properties.*
4. Raindrop impact and overland flow will change the size and structure of surface soil microbial communities.
  - *Different components of the microbial community will be susceptible to detachment and carriage by overland flow.*

## ***7.2 Experimental Design***

In order to test the experimental hypotheses,  $\gamma$ -irradiated sterile systems were used requiring the construction of sample storage boxes capable of keeping microbes alive but also keeping sterilized soil sterile. It also required a post irradiation and post incubation experiment in order to ensure that any differences observed were not as a result of the sterilisation process.

### **7.2.1 Microcosm design and preparation**

Surface soil (0 – 150 mm) was collected from Upper Ponds Field, on the Allerton Estate in Loddington, Leicestershire, U.K. (National Grid reference SK479301). The soil is from the Hanslope and Denchworth series (5.2% organic matter, 34% sand, 24% silt, 42% clay, pH 6.7. Soil was homogenised by sieving to 4 mm and packed into 60 x 110 x 200 mm (d, w, l) foil trays with an internal volume of 707 cm<sup>3</sup> to a bulk density of 1.2 g cm<sup>-3</sup>. The foil trays had a nylon mesh at the bottom to allow infiltrate water through. The packed trays were placed into specially designed containers, comprising of a 4 litre plastic storage container with three 40 mm diameter holes bored into the lid (Figure 7.1).



**Figure 7.1: Microcosm storage container.**

The holes were covered with a Tyvek® medical grade membrane secured using silicone sealant in order to allow gaseous exchange. The packed soil trays were placed into another foil tray with 250 g of 1 cm<sup>-3</sup> gravel in the bottom and a tube outlet for passage of infiltrate water. These trays were then placed into the storage box and packed around the sides with polystyrene packing material. The lids were sealed using Parafilm® laboratory film and refrigerated at 4°C until the treatments were applied.

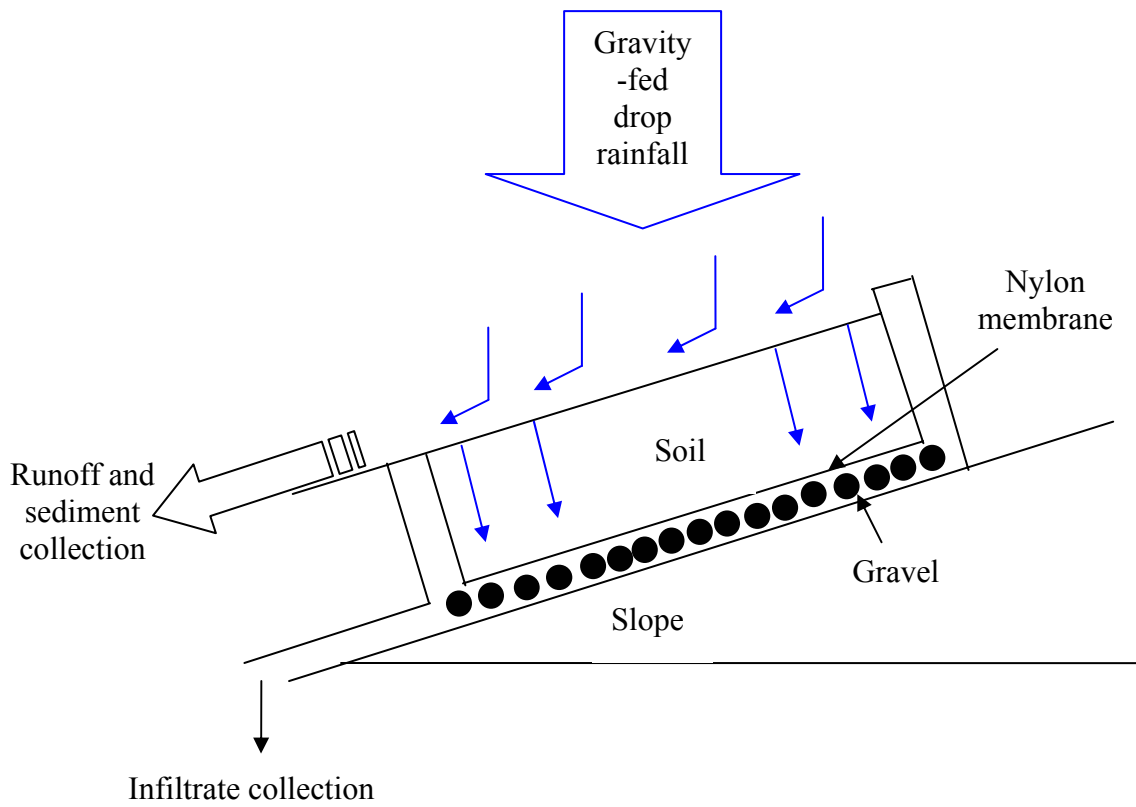
### **7.2.2 Microcosm treatments**

Two treatments were applied to the microcosms creating three different experimental set ups; a non-sterile field condition soil, a sterilised soil and a sterilised soil re-inoculated with a field soil slurry. Each treatment was replicated for each experiment five times both with and without rainfall addition.

Soil trays were sterilised in their storage boxes by  $\gamma$ - irradiation at a minimum of 25 k Gy at Isotron plc (Swindon). Replicate trays of field soil and sterilised soil immediately post sterilisation were used in the initial experiment to study any immediate effect of sterilisation. Sterilised soil was re-inoculated by mixing 100 g of sieved field soil in 200 ml of de-ionized water to create a slurry; 10 ml of the slurry was then applied to the soil tray surface using a four-channel multi pipette in 200  $\mu$ l aliquots. To maintain the homogeneity of soil moisture content between treatments, 10 ml of sterile water was added to the field and sterile soils. Once the treatments were implemented replicate trays of each treatment were incubated for two weeks at room temperature (approx.  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) in low light conditions on the bench to allow the establishment of microbial communities in the re-inoculated trays and to sustain those in the field soil.

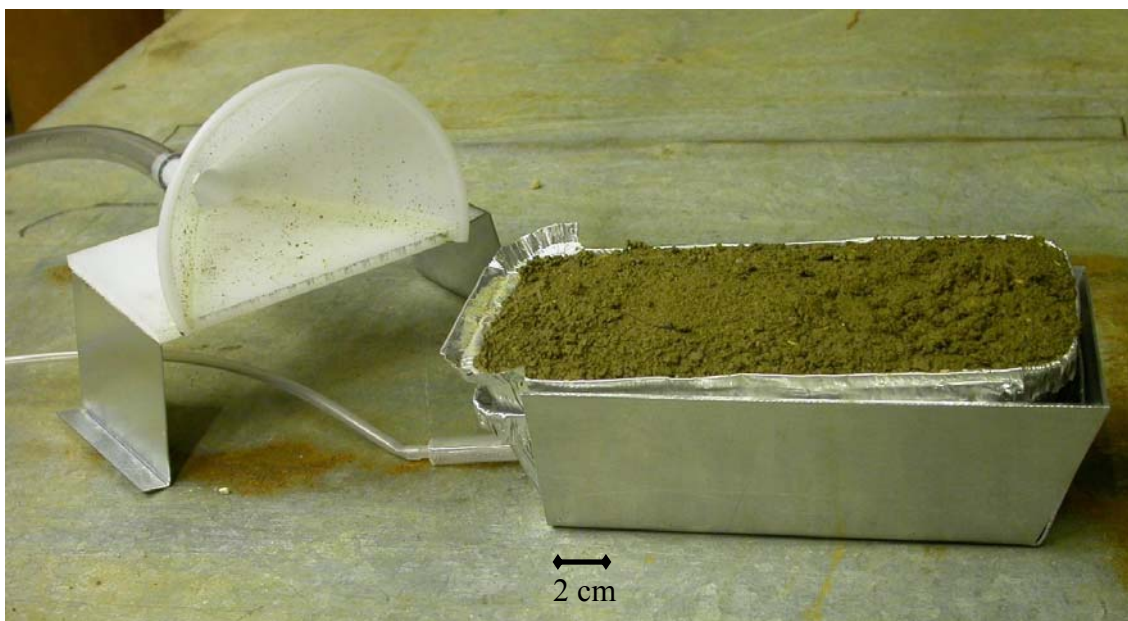
### **7.2.3 Rainfall simulation**

Five randomly-selected replicates of each treatment were subjected to simulated rainfall generated by a gravity fed, hypodermic needle rainfall simulator. Trays were inclined at  $12^{\circ}$ , and a rate of  $60 \text{ mm h}^{-1}$  for 30 minutes was applied, which represents a 1 in 70 year storm (NERC, 1975). Throughout the rainfall simulation, run-off and infiltrate were collected using a sterile funnel collection system (Figures 7.2 & 7.3). The total volumes of run-off and infiltrate were recorded once rainfall ceased, and 25 ml aliquots were removed for phospholipid fatty acid (PLFA) analysis (Chapter 2.5.2). Five replicates of each treatment were not subjected to simulated rainfall, in order to provide a control. These soil trays were sampled in the same manner as the soil trays receiving rainfall with the exception of run-off and infiltrate samples.



**Figure 7.2: Schematic diagram of microcosm experimental design. Not drawn to scale.**





**Figure 7.3: Microcosm tray and collection system.**

#### **7.2.4 Sampling**

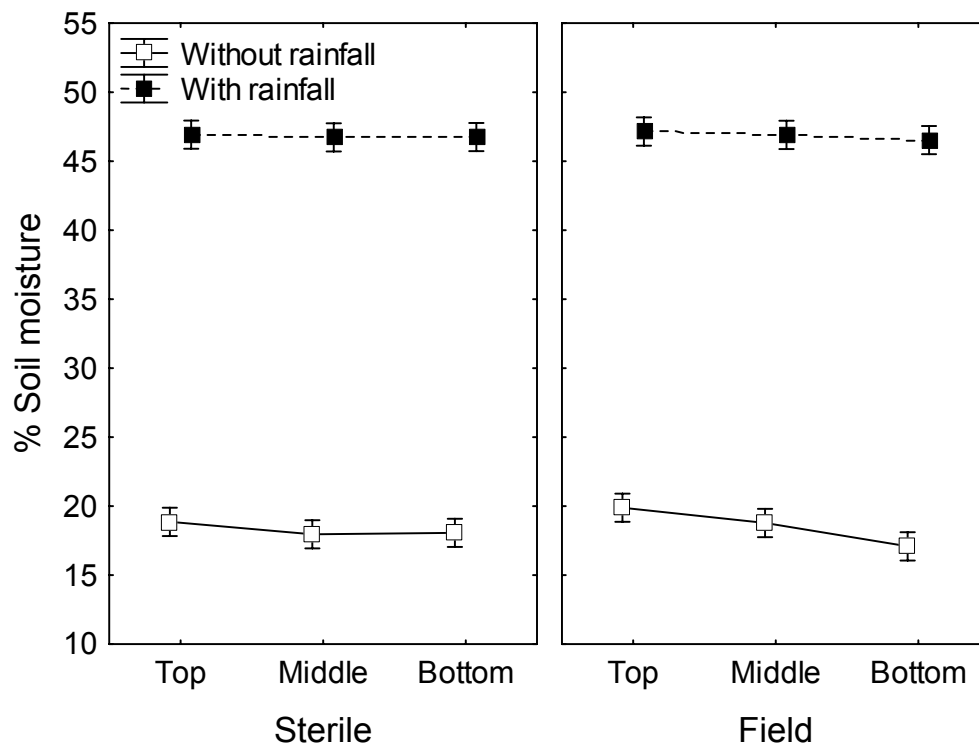
Soil from each tray was sampled at the top, middle and bottom regions of the slope, relative to the incline. Before destructive sampling occurred Torvane measurements were taken at each region of the slope (Chapter 2.3.2). The surface 10 mm of each sample zone (66 x 110 mm, l, d) was removed using a palette knife and homogenised by chopping, then sub-samples weighed for determination of moisture content, microbial biomass and PLFA analysis (Chapter 2). Data was analyzed using analysis of variance and principal components analysis.

### ***7.3 Results and discussion***

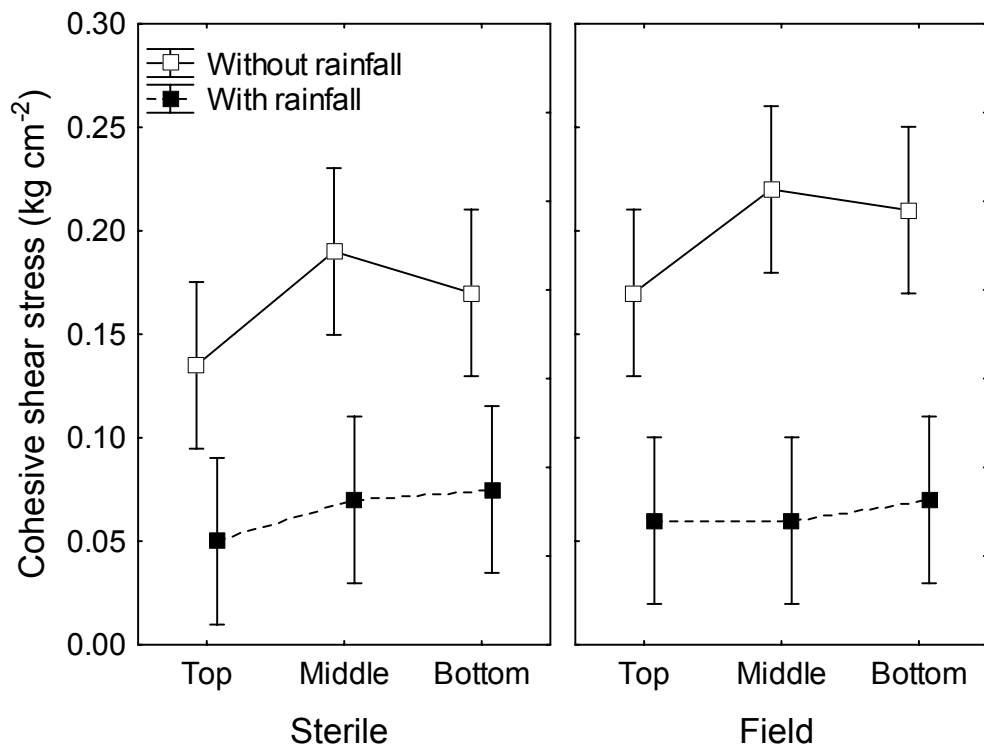
#### **7.3.1 Immediately post irradiation sampling**

The mean gravimetric moisture content was not significantly different between the sterile and field soil treatments prior to the application of simulated rainfall (Figure 7.4). After the simulated rainfall was applied the moisture content of the soil increased by around 10% across the treatments

and there were no significant differences (Figure 7.4). This finding does not support the hypothesis that the presence of an living microbial population will increase the water holding capacity of the soil. Measurements of cohesive shear stress also showed no significant treatment effect before or after simulated rainfall, but were significantly lowered with an increase in moisture content (Figure 7.5).

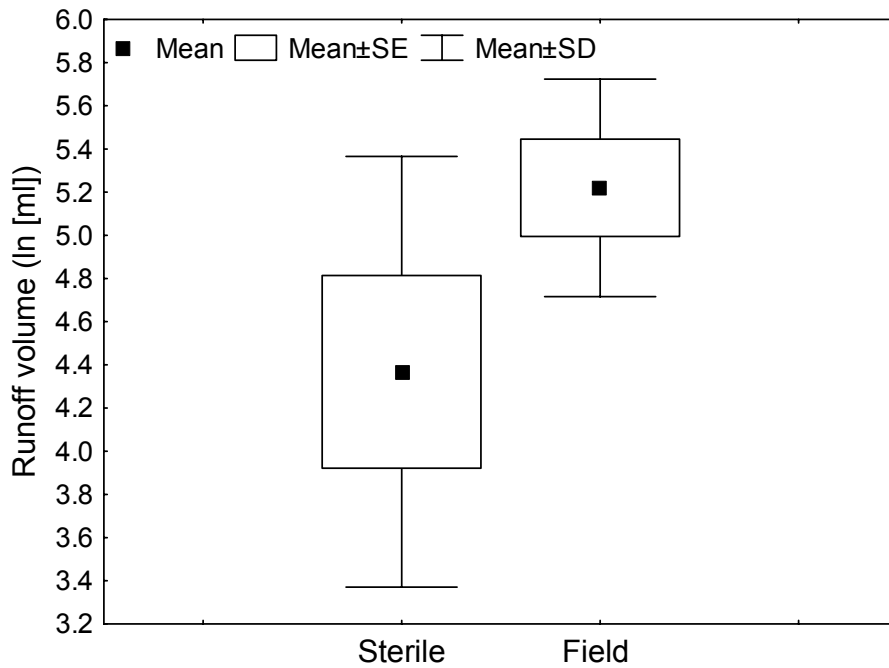


**Figure 7.4: Soil moisture content before and after simulated rainfall application in relation to sampling position; top; middle; or bottom of slope. Points show means (n=5); whiskers show 95% confidence intervals.**

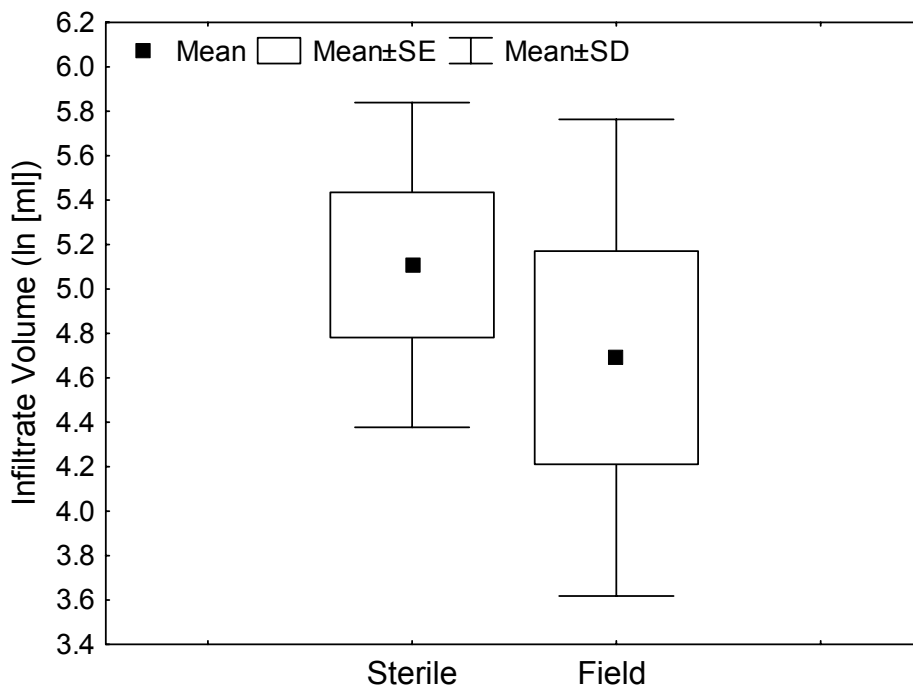


**Figure 7.5: Cohesive shear strength measured by Torvane before and after simulated rainfall application in relation to sampling position; top; middle; or bottom of slope. Points show means (n=5); whiskers show 95% confidence intervals.**

The runoff and infiltration volumes collected were not a normally distributed dataset and were therefore transformed by natural log to reduce the skewness and kurtosis. There was no significant difference between the treatments with respect to either runoff or infiltrate volume (Figures 7.6 & 7.7). This does not support the hypothesis that a living microbial community in soil will influence runoff and infiltration volumes. However, variances were unequal; variation between replicates in the field soil were significantly less than the sterile soil ( $p=0.05$ ). This suggests that the microbial community within the field soil treatment may be influencing the potential surface flow from the soil.

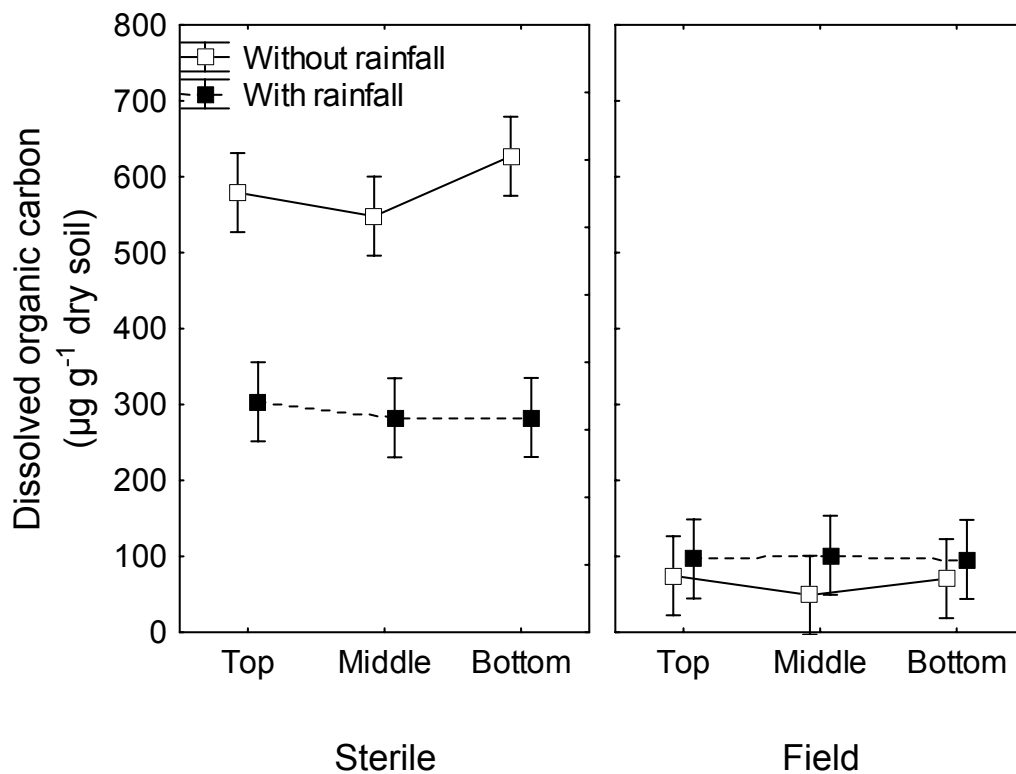


**Figure 7.6: Box and whisker plot showing the log volume of runoff of simulated rainfall collected for soil treatments. Points show means (n=5); whiskers show 95% confidence intervals.**



**Figure 7.7: Box and whisker plot showing the log volume of infiltrate of simulated rainfall collected for soil treatments. Points show means (n=5); whiskers show 95% confidence intervals.**

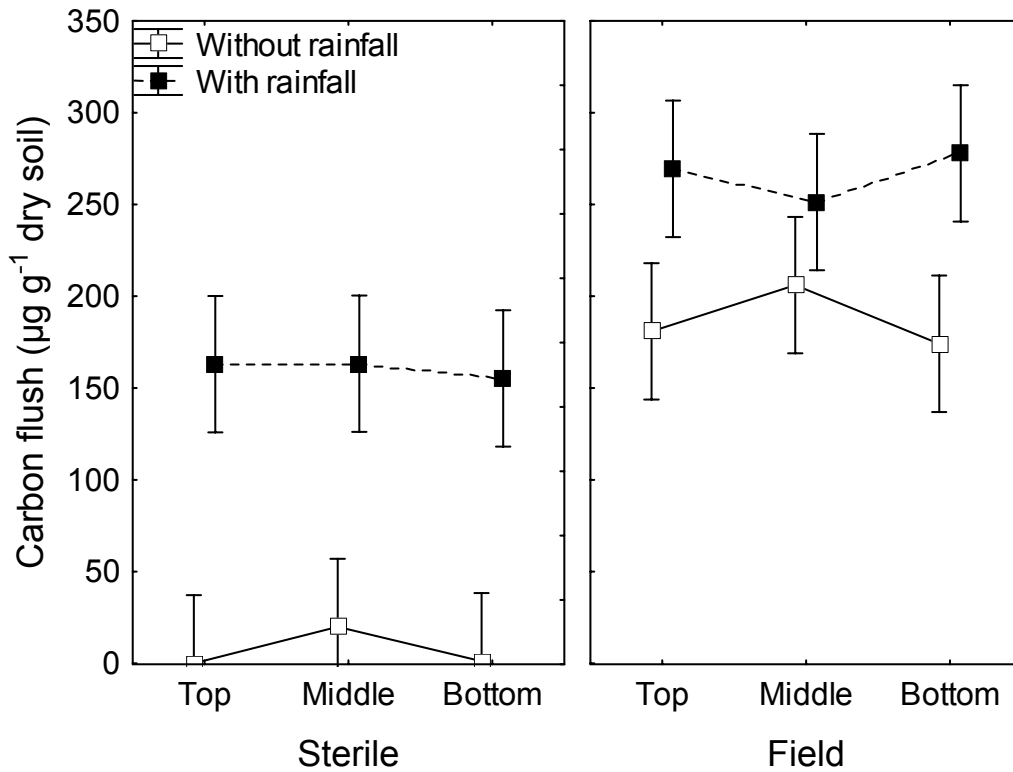
The concentration of dissolved organic carbon (DOC) was significantly different between the treatments both before and after simulated rainfall was applied. The concentration of DOC in the field soil treatment was not significantly different after simulated rainfall, whereas, the sterile treatment showed a significant decrease (Figure 7.8). In the sterile treatment, before simulated rainfall was applied, there was a 5-fold increase in the DOC extracted when compared to the field soil. Gamma irradiation is well known to increase the concentration of DOC in soils arising from the cleaving of carbon-to-carbon bonds (Trevors, 1996). After simulated rainfall the concentration of DOC in the sterile treatment dropped by greater than 3-fold but was still significantly higher than that extracted from the field soil (Figure 7.8). This is evidence that the simulated rainfall addition acted as a “pre-extraction” procedure for DOC in the sterile treated soil. However it would not have affected the biomass determination by fumigation-extraction because DOC was extracted from associated control soil samples.



**Figure 7.8: Concentrations of dissolved organic carbon (DOC), in relation to their position on the slope of the microcosms; top, middle and bottom of slope. Points show means (n=5), whiskers show 95% confidence intervals.**

Carbon flushes, and hence estimated microbial biomass C concentrations were significantly different between treatments both before and after simulated rainfall (Figure 7.9). The carbon flush of the field soil treatment was significantly different after simulated rainfall addition in the top and bottom sampled areas of the slope. However, the sterile treatment showed an increase in microbial biomass carbon from zero to around  $150 \mu\text{g g}^{-1}$  after simulated rainfall addition. This implies that there was some microbial biomass contained within the rainfall applied, and this biomass was retained within the upper layer of the microcosms. The possible addition of biomass by the simulated rainfall does not affect the field soil treatment across all sample areas; this could be due to the samples only being taken from the surface 10 mm of soil. The field soil treatment also

had a trend towards higher runoff losses; therefore, biomass contained in the simulated rainfall may have just run off with surface waters. There was no effect of sample position on the slope with respect to either treatment, therefore not supporting the hypothesis that rainfall and surface flow may modify surface soil microbial community size.

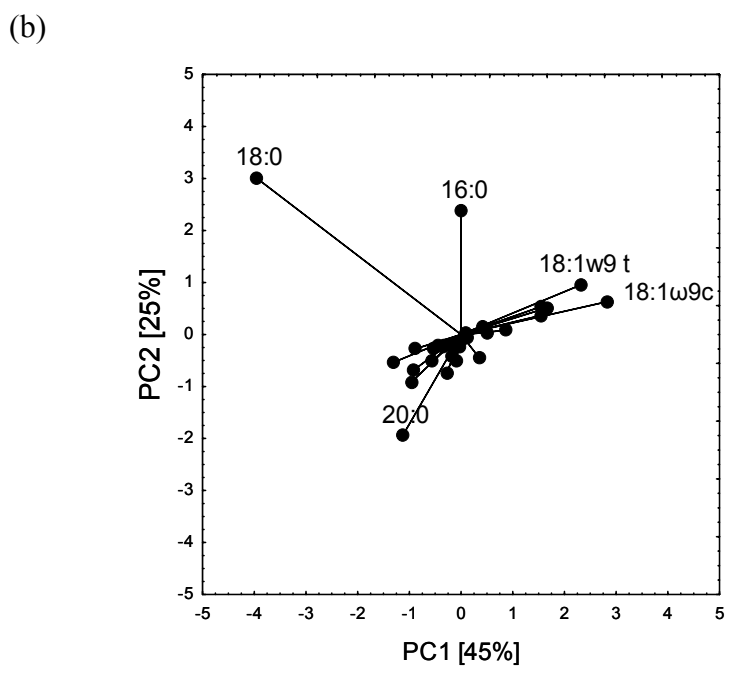
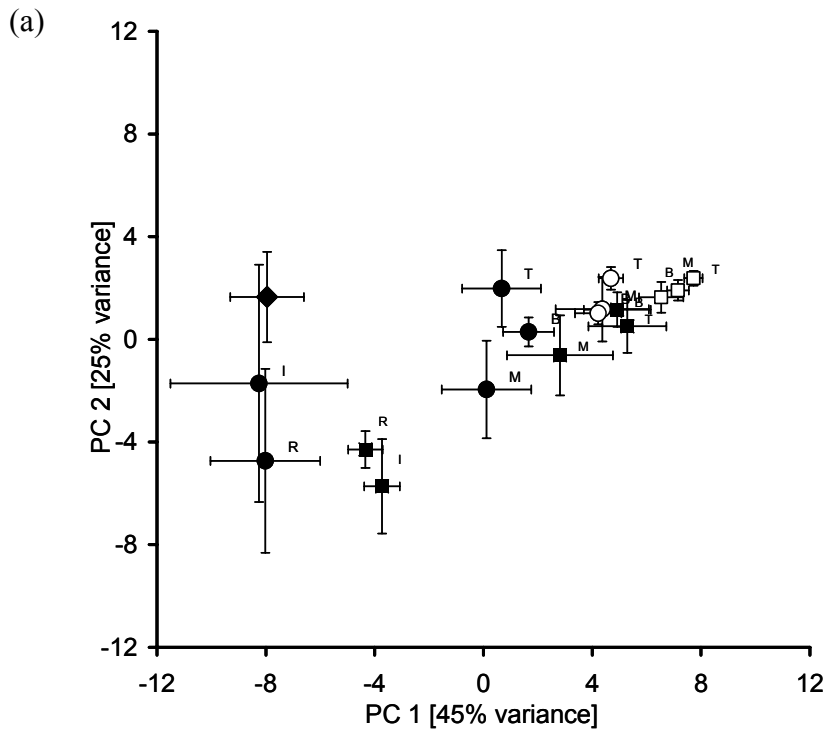


**Figure 7.9: Concentrations of microbial biomass carbon extracted from soil in relation to their position on the slope; top, middle or bottom of slope. Points show means (n=5), whiskers show 95% confidence intervals.**

In the PCA of PLFA profiles, PC1 significantly discriminated both soil treatments and the simulated rainfall profiles whereas PC2 significantly discriminated soil samples taken before and after rainfall with each treatment ( $p < 0.01$ , Figure 7.10). PC1 discriminated soil samples from runoff and infiltrate samples. The runoff and infiltrate samples collected from the sterile treated soil were not significantly different to the profile of the simulated rainfall applied. The loadings attributed to the PCA analysis

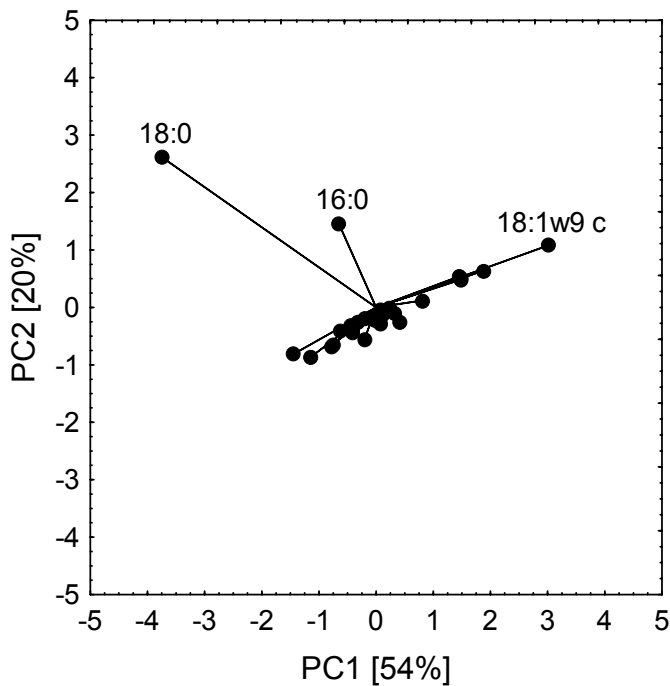
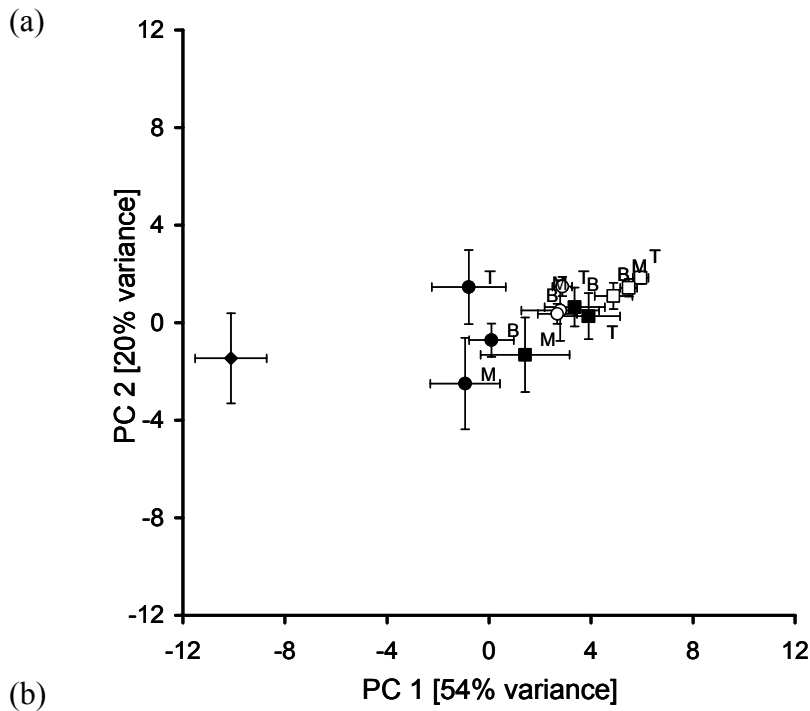
(Figure 7.10), showed the biomarkers 18:0, 16:0, 18:1 $\omega$ 9c, 18:1 $\omega$ 9t and 20:0 to be significantly dominant in the PCA analysis. Simulated rain water was significantly different from the soil both before and after rainfall, the biomarkers 18:0 and 16:0 increased in %mol and the biomarkers 20:0 and 18:1 $\omega$ 9t decreased after rainfall. The biomarker 18:1 $\omega$ 9c was not found in the simulated rainfall and was significantly more abundant in %mol in field soil compared to sterile soil. The runoff and infiltrate samples contained significantly less 16:0, 18:1 $\omega$ 9t and 18:1 $\omega$ 9c biomarkers but had a higher %mol of 20:0 biomarker compared to soil samples (Table 7.1). There was no significant difference in fungal: bacterial ratio between treatments, sample location or sample type (data not shown).





**Figure 7.10: Phenotypic structure of samples as described by principal component analysis (PCA) of PLFA profiles; (a) first and second principal components (PC); open symbols denote samples before rainfall, closed symbols denote samples taken after rainfall; simulated rainfall (♦), sterile (●) and field soil (■) treatments, sample positions indicated, top of slope (T), middle of slope (M), bottom of slope (B), runoff (R) and infiltrate (I); points show means (n=5), whiskers show s.e. Percent variation accounted for by PCs shown in square parentheses. (b) Loadings associated with PCs.**





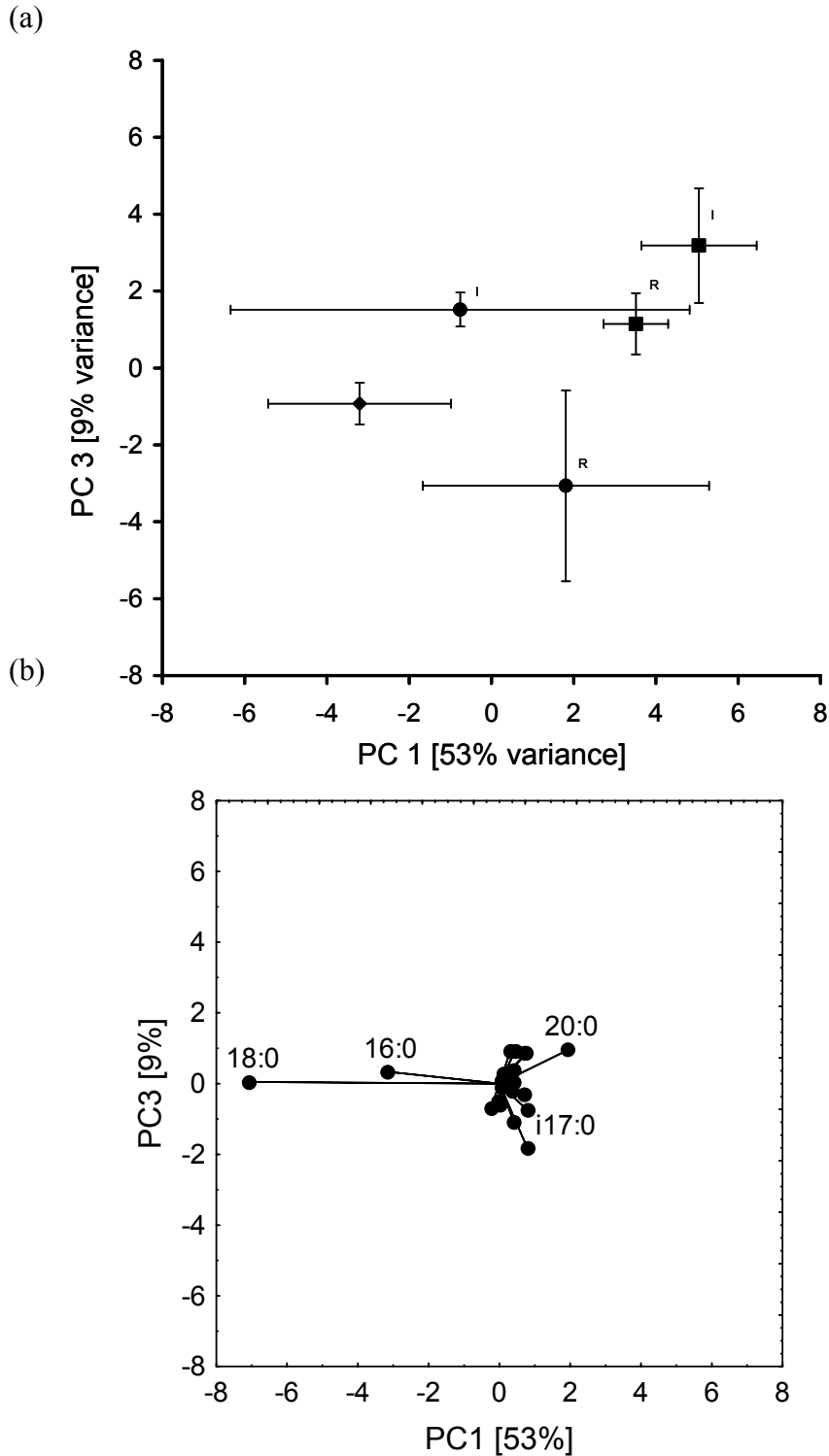
**Figure 7.11: Phenotypic structure of soil samples as described by principal component analysis (PCA) of PLFA profiles; (a) first and second principal components (PC); open symbols denote samples before rainfall, closed symbols denote samples taken after rainfall; Simulated rainfall (♦), sterile (●) and field soil (■) treatments, sample positions indicated, top of slope (T), middle of slope (M) and bottom of slope (B). Points show means (n=5), whiskers show s.e. Percent variation accounted for by PCs shown in square parentheses. (b) Loadings associated with PCs.**

**Table 7.1: Mean biomarker values, %mol, (n=5) and individual standard error with respect to treatment.**

Treatment	Rainfall	Sample position	16:0	17:0	18:2ω6c	18:1ω9c	18:1ω9t	18:0	20:0
Sterile	No	Top	11.1±	2.3±	2.2±	7.4±	10.1±	4.1±	2.5±
			0.4	0.1	0.3	0.2	0.7	0.1	0.2
Sterile	No	Middle	9.6±	2.7±	2.4±	7.8±	9.9±	4.0±	2.5±
			1.0	0.4	0.2	0.6	0.7	0.4	0.5
Sterile	No	Bottom	9.1±	2.7±	2.3±	7.5±	9.9±	4.1±	2.7±
			0.5	0.1	0.2	0.3	0.4	0.4	0.2
Sterile	Yes	Top	10.5±	2.8±	2.3±	6.9±	11.6±	7.0±	4.1±
			0.6	0.2	0.1	0.4	1.4	1.8	0.6
Sterile	Yes	Middle	8.3±	3.2±	2.7±	6.7±	9.5±	4.7±	5.2±
			1.5	0.5	0.2	0.9	1.7	0.4	1.0
Sterile	Yes	Bottom	10.8±	3.1±	2.8±	6.2±	10.7±	4.3±	3.7±
			0.5	0.2	0.5	0.7	1.1	0.2	0.4
Sterile	Yes	Runoff	5.9±	7.1±	4.4±	2.4±	4.3±	8.5±	6.7±
			1.7	2.6	1.5	0.7	0.5	2.9	2.0
Sterile	Yes	Infiltrate	8.3±	2.6±	2.6±	2.9±	5.3±	10.3±	7.6±
			2.2	0.5	0.2	0.6	0.4	5.1	1.4
Field	No	Top	10.0±	2.3±	2.4±	9.0±	10.9±	2.9±	1.4±
			0.2	0.0	0.1	0.2	0.3	0.1	0.1
Field	No	Middle	9.7±	2.3±	2.8±	8.9±	10.5±	3.0±	1.5±
			0.4	0.0	0.1	0.1	0.4	0.1	0.1
Field	No	Bottom	9.7±	2.3±	2.7±	8.4±	9.7±	3.1±	1.7±
			0.6	0.1	0.1	0.3	0.5	0.2	0.2
Field	Yes	Top	9.1±	2.5±	3.2±	8.3±	10.7±	3.4±	3.0±
			0.8	0.2	0.3	0.4	0.7	0.4	0.5
Field	Yes	Middle	9.4±	2.4±	2.3±	6.1±	8.8±	3.5±	3.6±
			1.2	0.2	0.2	0.8	1.2	0.3	0.7
Field	Yes	Bottom	10.0±	2.4±	3.0±	7.6±	10.3±	3.6±	3.0±
			0.4	0.2	0.2	0.9	0.7	0.2	0.5
Field	Yes	Runoff	6.7±	3.0±	2.9±	3.5±	5.3±	6.0±	6.1±
			0.7	0.4	0.1	0.3	0.4	0.7	0.7
Field	Yes	Infiltrate	6.8±	2.5±	2.3±	3.9±	5.6±	5.1±	9.4±
			1.3	0.4	0.4	0.4	0.6	0.7	2.9
None	Rainwater		11.2±	1.9±	2.9±	0.0±	6.2±	9.8±	2.2±
			0.8	0.1	0.2	0.0	0.4	2.2	0.1

PLFA profiles derived only from soil samples (Figure 7.11), showed a significant difference between the soil treatments and the simulated rainfall in PC1. There was also a significant difference between soil samples taken prior to and after simulated rainfall. There was no significant effect of sample location, therefore not supporting the hypothesis that runoff and surface flow will affect microbial community structure as it flows down slope. The biomarker 18:1 $\omega$ 9c had a higher %mol in field soil compared to sterile soil and decreased in both soils after simulated rainfall. The biomarker 16:0 which appears significant in the loadings plot had no significant effect with respect to the parameters tested. The biomarker 18:0 increased in %mol after rainfall and was higher in sterile soil than in field soil (Table 7.1).

The PCA of PLFA profiles contained within the runoff and infiltrate samples showed no significant effect of treatment in PC1 but a significant difference between the simulated rainfall and both soil treatments in PC2 (19% variance, data not shown). PC3 significantly discriminated the simulated rainfall from the field soil treatment (Figure 7.12). Simulated rainfall and runoff samples were not significantly different in PC3, implying minimal microbial interaction between the soil and simulated rainfall. The PCA loadings indicated that biomarkers 18:0; 16:0; i17:0 and 20:0 are were responsible for such discrimination, however, statistical analysis of %mol data did not prove significant for the experimental parameters (Table 7.1).

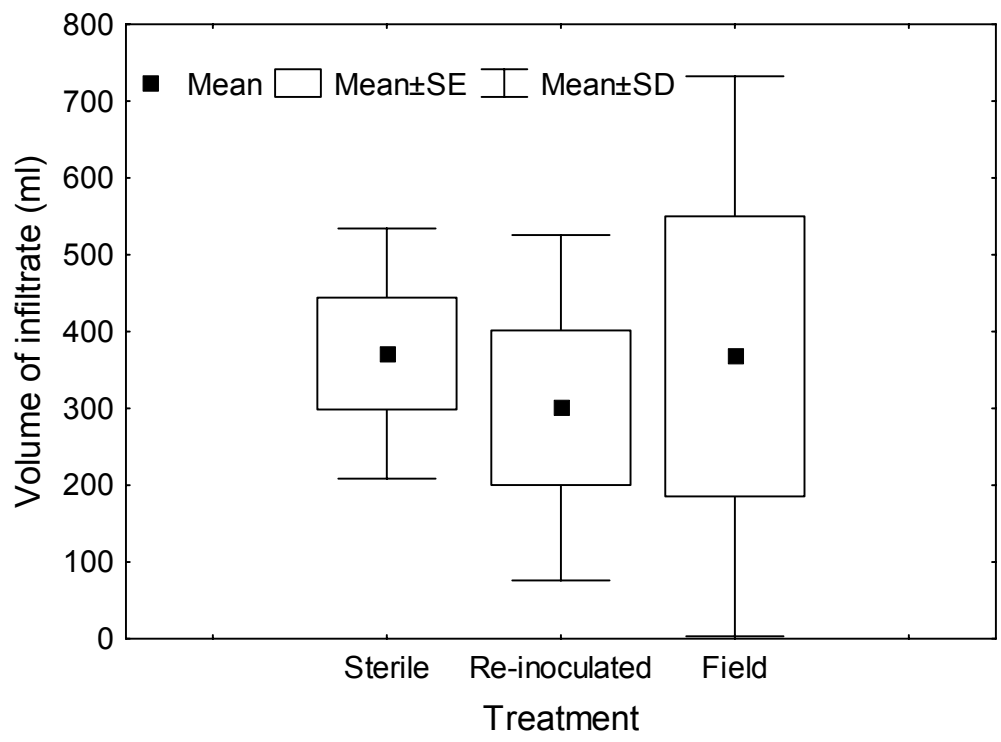


**Figure 7.12: : Phenotypic structure of runoff and infiltrate samples as described by principal component analysis (PCA) of PLFA profiles; (a) first and second principal components (PC); open symbols denote samples before rainfall, closed symbols denote samples taken after rainfall; simulated rainfall (◆), sterile (●) and field soil (■) runoff samples (R) and infiltrate samples (I). Points show means (n=5), whiskers show s.e. Percent variation accounted for by PCs shown in square parentheses. (b) Loadings associated with PCs.**

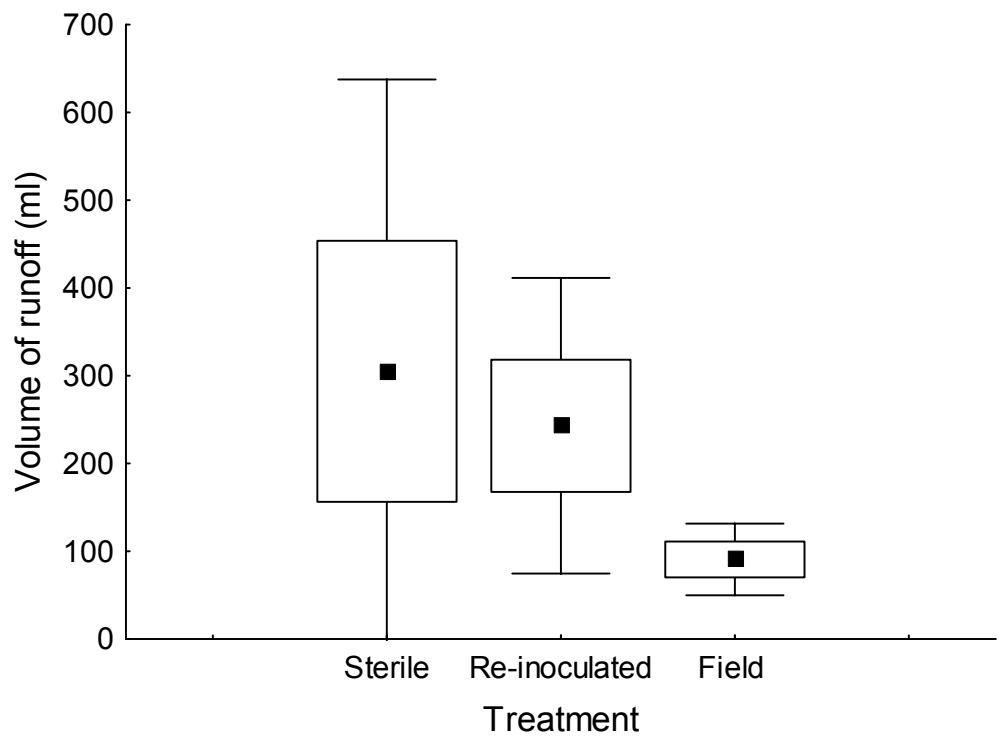
### 7.3.2 Post incubation sampling

The mean gravimetric soil moisture content was not significantly different between the three treatments prior to application of the simulated rainfall (mean  $0.19 \text{ g g}^{-1}$  dry wt; pooled s.e.  $0.02$ ,  $p > 0.05$ ). After rainfall, this increased to  $0.35$ ,  $0.29$  and  $0.31 \text{ g g}^{-1}$  dry wt for field, re-inoculated and sterile soil respectively, with field soil holding significantly more water than the other two treatments (pooled s.e. =  $0.03 \text{ g g}^{-1}$ ;  $p < 0.05$ ). This supports the hypothesis that the presence of an established, living, microbial community increases the soil water holding capacity.

There was no significant difference between any of the treatments with respect to either volume of infiltrate or runoff (Figures 7.13 & 7.14), which does not support the hypothesis that the presence of a microbial community will influence the propensity of a soil to generate run-off and the volume of water infiltrating the soil at this level of replication. However, variances were unequal, being markedly different between the treatments. The variation in volume of infiltrate was similar in sterile and re-inoculated treatments, but greater in field soil (Figure 7.14). This trend was reversed with respect to volume of run-off, where the variation within sterile soils was some two orders-of-magnitude greater than within field soils, with re-inoculated samples ranking in the middle (Figure 7.15). These results, whilst not conclusive, suggest that the presence and size of the microbial community may influence the potential run-off or surface flow from a soil.



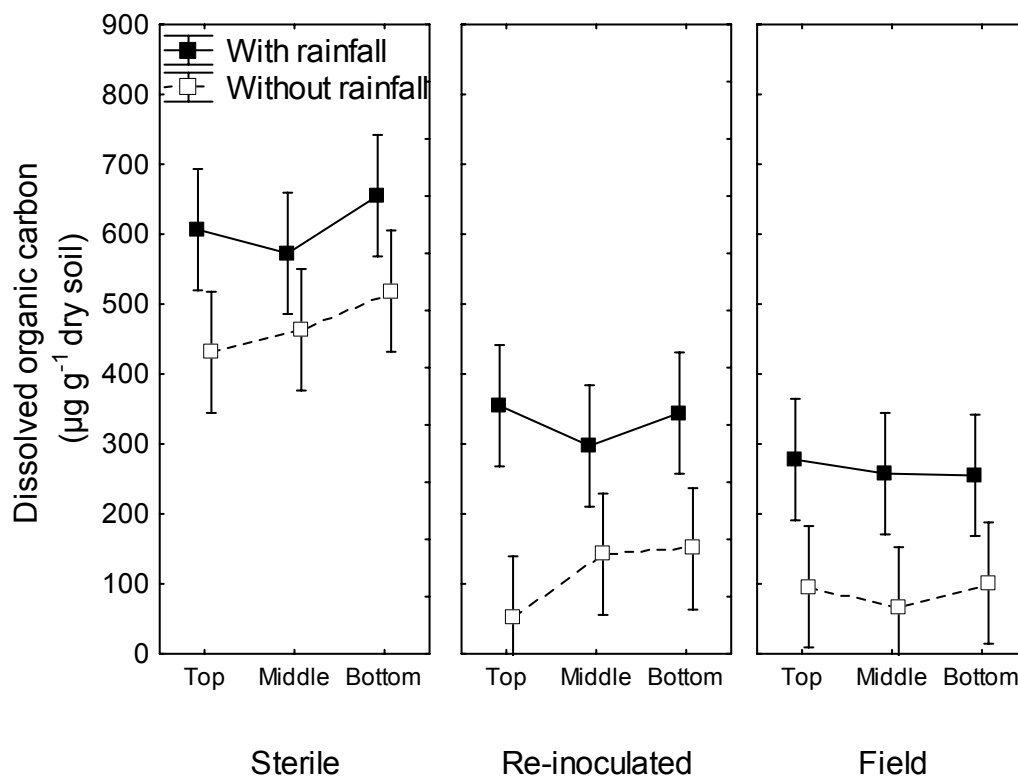
**Figure 7.13: Box and whisker plot (n=5) showing volume of infiltrate collected for soil treatments.**



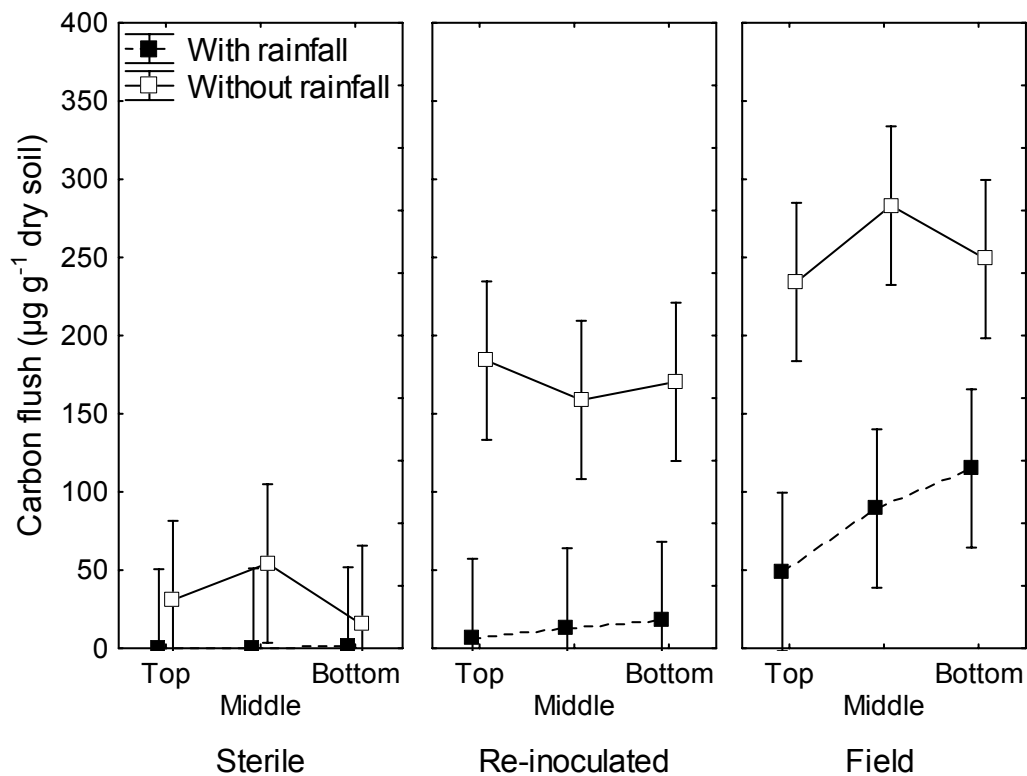
**Figure 7.14: Box and whisker plot (n=5) showing volume of runoff collected for soil treatments.**



Concentrations of DOC in sterile soils were significantly higher than those in the re-inoculated field soils both prior to and after simulated rainfall (Figure 7.15). There was a significant increase in DOC concentration in all soils after simulated rainfall was applied. Thus the rainfall was supplying DOC to the surface horizon of the microcosms. Carbon flush was significantly affected by rainfall application in the re-inoculated and field soils (Figure 7.16). Re-inoculated soils after rainfall were not significantly different from the sterile soil therefore the biomass within these soils must be mobile and was washed out of the surface layer of the soil. The field soil had the highest C-flush both before and after rainfall of all soil treatments (Figure 7.16).



**Figure 7.15: Concentration of dissolved organic carbon (DOC) extracted from soil treatments in relation to their position on the slope; top, middle and bottom; before and after rainfall. Points show means (n=5), whiskers show 95% confidence intervals.**



**Figure 7.16: Carbon flush extracted from soil treatments in relation to their position on the slope before and after rainfall. Points show means (n=5), whiskers show 95% confidence intervals.**

The basic relationships between the microbial community structures in simulated rain water, run-off and infiltrate, and soils were similar for all three treatments (Figures 7.17; 7.18 & 7.19). Both PC1 and PC2 significantly discriminated the soils (pre- and post- rainfall) from both the run-off and infiltrate samples and from the simulated rainfall. There was no significant discrimination between the run-off and infiltrate samples in any treatment. This supports the hypothesis that the microbial community profiles of run-off and infiltrate will be significantly different to both pre- and post-rainfall soil profiles. Therefore, there appears to have been an inherently mobile phase of the microbial community which had a different structure than that of the sessile phase of the community. Prior to rainfall, the PLFA profiles of the soil were not significantly different ( $p>0.05$ ).

Soil derived from the middle regions of the slopes within the re-inoculated treatment carry the greatest variation, whilst those from the top of the slopes differ from those derived from the bottom after rainfall has been applied (Figure 7.17). The loadings associated to the PCs showed that the biomarkers 16:0 and 18:1 $\omega$ 9c were strongly influencing experimental variation. The biomarkers 16:0 and 18:1 $\omega$ 9c decreased in %mol in soil samples after simulated rainfall addition. The biomarker 18:1 $\omega$ 9c was not found in the simulated rainfall therefore, it is significant that it is later found in runoff and infiltrate samples as it demonstrates the mobile phase of the soil microbial community. Both these markers were found in higher %mol in soil samples than in the runoff or infiltrate samples. The biomarker 18:0 increased in soils sampled after simulated rainfall was applied (Table 7.2).

The PLFA profile associated with the sterile treatment was not significantly different in relation to position on the slope pre- and post- rainfall (Figure 7.18a). This implies that the sampling position of the field soil was not significantly different pre-rainfall, but there was a significant difference in PC1 between the community structure in soils derived from the top and bottom of the slopes (Figure 7.18). This trend was also seen in the re-inoculated treatment (Figure 7.17). This indicates that the microbial community structure was influenced by rain flowing down the slope. The loading values associated with PCs indicated significant biomarkers. ANOVA of these biomarkers showed that 16:0; 18:1 $\omega$ 9t and 18:1 $\omega$ 9c all decrease in %mol in soil after the addition of simulated rainfall (Figure 7.18b). Runoff and infiltrate samples contained significantly less %mol of

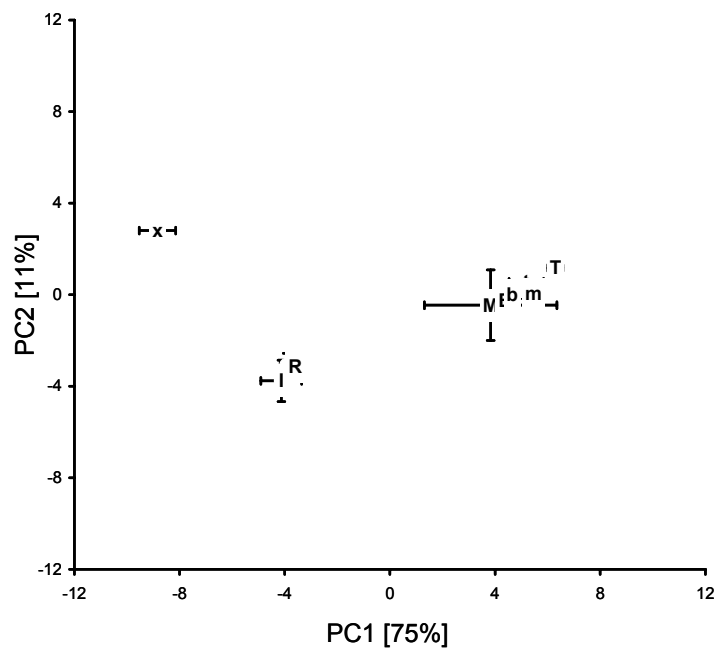
these markers than the soil samples. The proportion of phospholipids ai16:0 and 18:0 increased in soils after simulated rainfall application and were found in greater proportion in the runoff and infiltrate samples compared to the soil samples (Table 7.2).

The PLFA profile associated with the field soil treatment showed both PC1 and PC2 significantly discriminated the soils (pre- and post- rainfall) from both the run-off and infiltrate samples, and from the simulated rainfall (Figure 7.19). The loadings associated with the PCA of PLFA profiles indicated the biomarkers 18:1 $\omega$ 9c; 18:1 $\omega$ 9c; 18:0 and 20:0 to be making a significant contribution (Figure 7.19b). These biomarker proportions showed that markers 18:1 $\omega$ 9c and 18:1 $\omega$ 9t decreased in soils subjected to simulated rainfall, and runoff and infiltrate samples contained significantly less %mol of these biomarkers. The biomarkers 18:0 and 20:0 increased in soils subjected to simulated rainfall and the %mol of these biomarkers were significantly higher in runoff and infiltrate samples when compared to soil samples (Table 7.2).

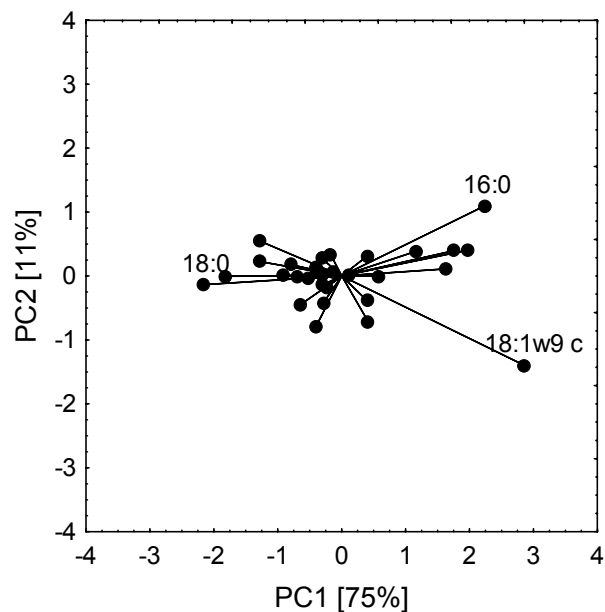
In all cases the biomarker 18:1 $\omega$ 9c was found to be absent from the simulated rainfall. This is a particularly significant observation as it demonstrated the presence of a water mobile fraction of the microbial community, which was not present in the rainfall but was present in both runoff and infiltrate samples. The relative proportions of biomarkers 18:1 $\omega$ 9c; 18:1 $\omega$ 9t and 16:0 in all treatments decreased in soils subjected to simulated rainfall and were found in a significantly greater proportion in soil samples compared to runoff and infiltrate samples. These biomarkers are implicated as bacterial biomarkers specifically gram negative and eukaryotic biomarkers (Wilkinson, 1988; Zelles *et al.* 1992; Lindahl *et al.*

1997). In all treatments the relative proportion of biomarker 18:0 increased in soils subjected to simulated rainfall and was significantly greater in runoff and infiltrate samples when compared to soil samples (Table 7.2).

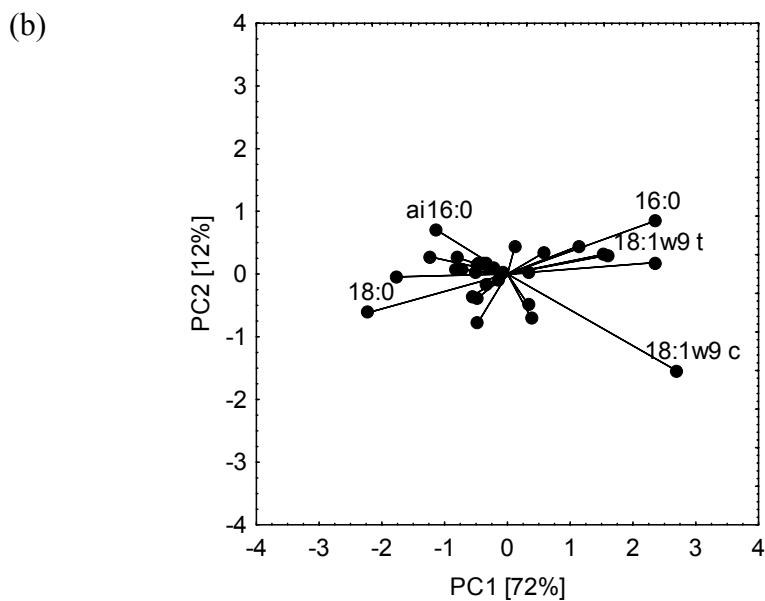
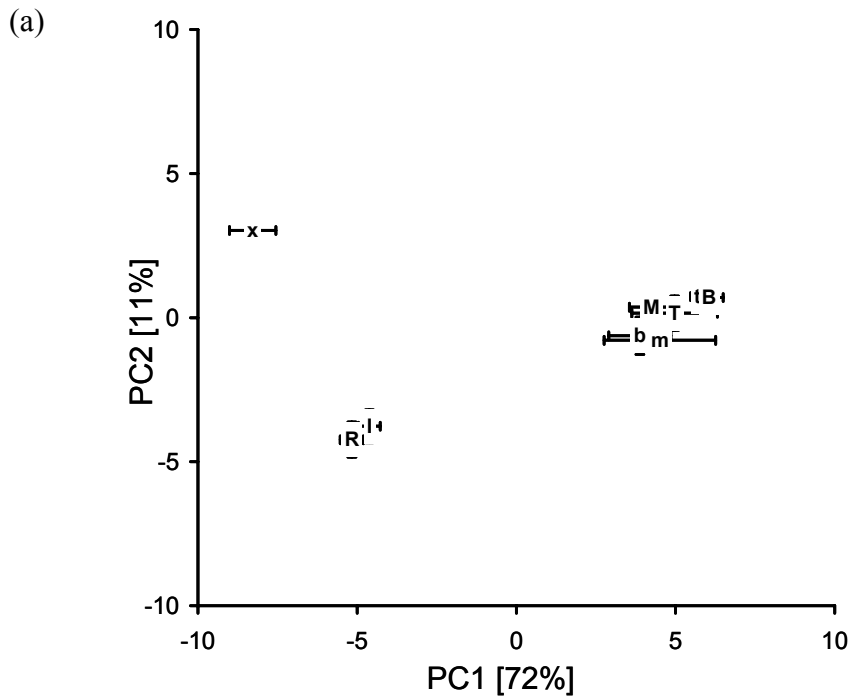
(a)



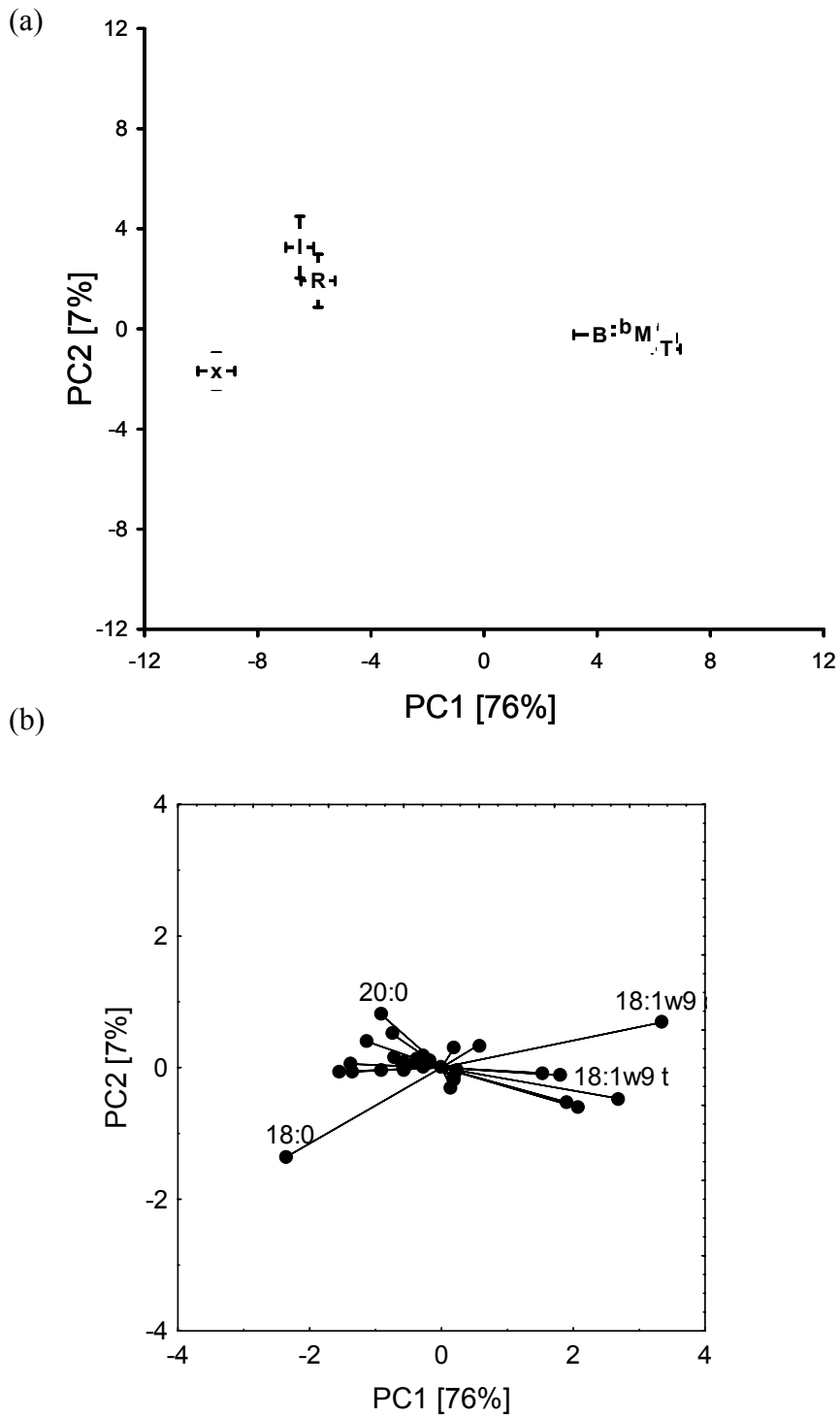
(b)



**Figure 7.17: Phenotypic structure of re-inoculated samples as described by principal component analysis (PCA) of PLFA profiles; (a) first and second principal components (PC); (Upper case) with rainfall; (Lower case) without rainfall; (x) Rainfall; (R) run-off; (I) infiltrate; (T) top of slope; (M) middle of slope; (B) bottom of slope. Points show means (n=5), whiskers show s.e. Percent variation accounted for by PCs shown in square parentheses. (b) Loadings associated with PCs.**



**Figure 7.18: Phenotypic structure of sterile samples as described by principal component analysis (PCA) of PLFA profiles; (a) first and second principal components (PC); (Upper case) with rainfall; (Lower case) without rainfall; (x) rainfall; (R) run-off; (I) infiltrate; (T) top of slope; (M) middle of slope; (B) bottom of slope. Points show means (n=5), whiskers show s.e. Percent variation accounted for by PCs shown in square parentheses. (b) Loadings associated with PCs.**



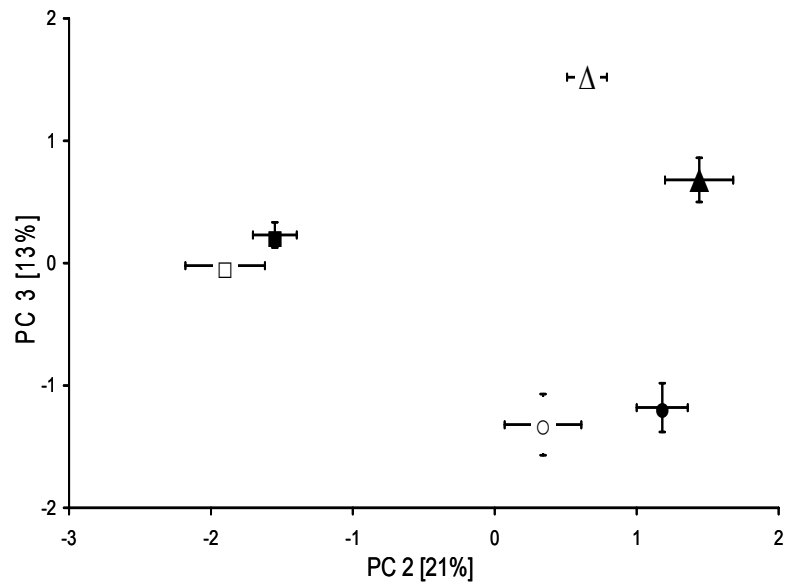
**Figure 7.19: Phenotypic structure of field soil samples as described by principal component analysis (PCA) of PLFA profiles; (a) first and second principal components (PC); (Upper case) with rainfall; (Lower case) without rainfall; (x) rainfall; (R) run-off; (I) infiltrate; (T) top of slope; (M) middle of slope; (B) bottom of slope. Points show means (n=5), whiskers show s.e. Percent variation accounted for by PCs shown in square parentheses. (b) Loadings associated with PCs.**



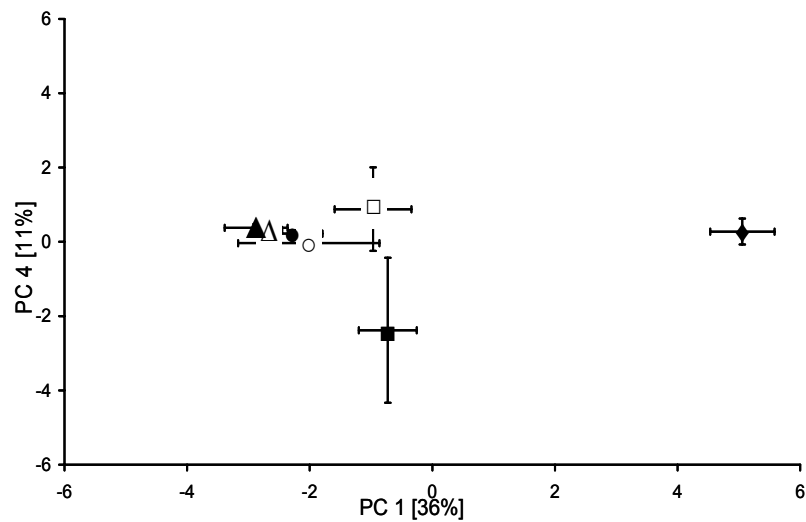
**Table 7.2: Mean biomarker values, %mol, and associated individual standard error.**

Treatment	Rainfall	Sample position	ai16:0	16:0	18:2 $\omega$ 6c	18:1 $\omega$ 9c	18:1 $\omega$ 9t	18:0	20:0
Sterile	Yes	Top	2.7 $\pm$ 0.1	10.8 $\pm$ 0.9	2.3 $\pm$ 0.3	7.3 $\pm$ 0.3	10.1 $\pm$ 0.6	4.0 $\pm$ 0.3	2.1 $\pm$ 0.3
			2.7 $\pm$ 0.2	15.3 $\pm$ 4.0	2.2 $\pm$ 0.3	6.7 $\pm$ 0.6	9.2 $\pm$ 0.8	3.8 $\pm$ 0.4	1.8 $\pm$ 0.2
Sterile	Yes	Middle	2.6 $\pm$ 0.1	11.3 $\pm$ 0.5	2.0 $\pm$ 0.2	7.4 $\pm$ 0.2	10.5 $\pm$ 0.3	3.9 $\pm$ 0.1	1.7 $\pm$ 0.1
			2.6 $\pm$ 0.1	6.1 $\pm$ 1.6	4.0 $\pm$ 0.4	5.5 $\pm$ 0.4	5.9 $\pm$ 0.3	8.7 $\pm$ 1.8	3.8 $\pm$ 0.2
Sterile	Yes	Bottom	3.5 $\pm$ 0.1	5.9 $\pm$ 0.8	4.0 $\pm$ 0.2	5.6 $\pm$ 0.3	6.2 $\pm$ 0.1	7.6 $\pm$ 0.4	3.6 $\pm$ 0.1
			3.1 $\pm$ 0.1	6.1 $\pm$ 1.6	4.0 $\pm$ 0.4	5.5 $\pm$ 0.4	5.9 $\pm$ 0.3	8.7 $\pm$ 1.8	3.8 $\pm$ 0.2
Sterile	Yes	Runoff	2.6 $\pm$ 0.1	11.3 $\pm$ 0.5	2.0 $\pm$ 0.2	7.5 $\pm$ 0.2	10.5 $\pm$ 0.7	4.1 $\pm$ 0.1	2.4 $\pm$ 0.2
			2.6 $\pm$ 0.2	9.6 $\pm$ 1.0	2.4 $\pm$ 0.2	7.8 $\pm$ 0.6	9.9 $\pm$ 0.7	4.0 $\pm$ 0.4	2.5 $\pm$ 0.5
Sterile	No	Middle	2.5 $\pm$ 0.1	9.1 $\pm$ 0.5	2.3 $\pm$ 0.2	7.5 $\pm$ 0.3	9.9 $\pm$ 0.4	4.1 $\pm$ 0.4	2.7 $\pm$ 0.2
			2.5 $\pm$ 0.1	9.1 $\pm$ 0.5	2.3 $\pm$ 0.2	7.5 $\pm$ 0.3	9.9 $\pm$ 0.4	4.1 $\pm$ 0.4	2.7 $\pm$ 0.2
Field	Yes	Top	2.3 $\pm$ 0.1	10.5 $\pm$ 0.3	2.6 $\pm$ 0.2	8.5 $\pm$ 0.2	10.5 $\pm$ 0.2	3.0 $\pm$ 0.2	1.3 $\pm$ 0.1
			2.3 $\pm$ 0.1	10.5 $\pm$ 0.3	2.6 $\pm$ 0.2	8.5 $\pm$ 0.2	10.5 $\pm$ 0.2	3.0 $\pm$ 0.2	1.3 $\pm$ 0.1
Field	Yes	Middle	2.5 $\pm$ 0.1	9.9 $\pm$ 0.3	2.5 $\pm$ 0.1	8.5 $\pm$ 0.1	10.2 $\pm$ 0.2	3.0 $\pm$ 0.1	1.6 $\pm$ 0.1
			2.5 $\pm$ 0.1	9.9 $\pm$ 0.3	2.5 $\pm$ 0.1	8.5 $\pm$ 0.1	10.2 $\pm$ 0.2	3.0 $\pm$ 0.1	1.6 $\pm$ 0.1
Field	Yes	Bottom	2.6 $\pm$ 0.2	9.9 $\pm$ 0.6	2.7 $\pm$ 0.1	8.2 $\pm$ 0.2	9.9 $\pm$ 0.3	3.1 $\pm$ 0.1	1.6 $\pm$ 0.3
			2.6 $\pm$ 0.2	9.9 $\pm$ 0.6	2.7 $\pm$ 0.1	8.2 $\pm$ 0.2	9.9 $\pm$ 0.3	3.1 $\pm$ 0.1	1.6 $\pm$ 0.3
Field	Yes	Runoff	4.4 $\pm$ 0.2	6.2 $\pm$ 0.3	3.1 $\pm$ 0.3	4.4 $\pm$ 0.6	4.6 $\pm$ 0.3	6.5 $\pm$ 1.3	3.6 $\pm$ 0.2
			4.4 $\pm$ 0.2	6.2 $\pm$ 0.3	3.1 $\pm$ 0.3	4.4 $\pm$ 0.6	4.6 $\pm$ 0.3	6.5 $\pm$ 1.3	3.6 $\pm$ 0.2
Field	Yes	Infiltrate	4.2 $\pm$ 0.2	7.0 $\pm$ 0.9	2.6 $\pm$ 0.5	4.8 $\pm$ 0.5	4.3 $\pm$ 0.1	6.4 $\pm$ 1.1	5.0 $\pm$ 1.0
			4.2 $\pm$ 0.2	7.0 $\pm$ 0.9	2.6 $\pm$ 0.5	4.8 $\pm$ 0.5	4.3 $\pm$ 0.1	6.4 $\pm$ 1.1	5.0 $\pm$ 1.0
Field	No	Top	2.4 $\pm$ 0.1	10.0 $\pm$ 0.2	2.4 $\pm$ 0.1	9.0 $\pm$ 0.2	10.9 $\pm$ 0.3	2.9 $\pm$ 0.1	1.4 $\pm$ 0.1
			2.4 $\pm$ 0.1	10.0 $\pm$ 0.2	2.4 $\pm$ 0.1	9.0 $\pm$ 0.2	10.9 $\pm$ 0.3	2.9 $\pm$ 0.1	1.4 $\pm$ 0.1
Field	No	Middle	2.5 $\pm$ 0.1	9.7 $\pm$ 0.4	2.8 $\pm$ 0.1	8.9 $\pm$ 0.1	10.5 $\pm$ 0.4	3.0 $\pm$ 0.1	1.5 $\pm$ 0.1
			2.5 $\pm$ 0.1	9.7 $\pm$ 0.4	2.8 $\pm$ 0.1	8.9 $\pm$ 0.1	10.5 $\pm$ 0.4	3.0 $\pm$ 0.1	1.5 $\pm$ 0.1
Field	No	Bottom	2.6 $\pm$ 0.2	9.7 $\pm$ 0.6	2.7 $\pm$ 0.1	8.4 $\pm$ 0.3	9.7 $\pm$ 0.5	3.1 $\pm$ 0.2	1.7 $\pm$ 0.2
			2.6 $\pm$ 0.2	9.7 $\pm$ 0.6	2.7 $\pm$ 0.1	8.4 $\pm$ 0.3	9.7 $\pm$ 0.5	3.1 $\pm$ 0.2	1.7 $\pm$ 0.2
Re-inoculated	Yes	Top	2.3 $\pm$ 0.0	11.8 $\pm$ 0.1	2.7 $\pm$ 0.4	7.8 $\pm$ 0.0	9.0 $\pm$ 0.4	3.5 $\pm$ 0.1	1.7 $\pm$ 0.1
Re-inoculated	Yes	Middle	2.5 $\pm$ 0.2	10.2 $\pm$ 1.9	2.8 $\pm$ 0.5	7.6 $\pm$ 0.4	8.4 $\pm$ 0.7	4.0 $\pm$ 0.7	2.2 $\pm$ 0.5
Re-inoculated	Yes	Bottom	2.4 $\pm$ 0.1	10.0 $\pm$ 0.2	2.7 $\pm$ 0.2	7.7 $\pm$ 0.1	8.7 $\pm$ 0.2	3.9 $\pm$ 0.2	2.1 $\pm$ 0.1
Re-inoculated	Yes	Runoff	3.6 $\pm$ 0.2	7.0 $\pm$ 0.7	3.9 $\pm$ 0.1	5.5 $\pm$ 0.2	6.5 $\pm$ 0.2	6.3 $\pm$ 0.2	3.7 $\pm$ 0.0
Re-inoculated	Yes	Infiltrate	3.5 $\pm$ 0.2	5.1 $\pm$ 0.8	3.9 $\pm$ 0.3	5.9 $\pm$ 0.4	5.9 $\pm$ 0.5	6.2 $\pm$ 0.4	3.5 $\pm$ 0.2
Re-inoculated	No	Top	2.3 $\pm$ 0.1	10.5 $\pm$ 0.4	3.0 $\pm$ 0.1	7.6 $\pm$ 0.3	8.7 $\pm$ 0.2	3.2 $\pm$ 0.0	1.6 $\pm$ 0.1
Re-inoculated	No	Middle	2.4 $\pm$ 0.1	9.4 $\pm$ 0.3	2.7 $\pm$ 0.2	8.1 $\pm$ 0.2	9.0 $\pm$ 0.3	3.1 $\pm$ 0.1	1.5 $\pm$ 0.1
Re-inoculated	No	Bottom	2.5 $\pm$ 0.1	9.6 $\pm$ 0.3	3.1 $\pm$ 0.1	7.9 $\pm$ 0.2	8.6 $\pm$ 0.2	3.4 $\pm$ 0.1	1.6 $\pm$ 0.0
None	Rainwater		3.9 $\pm$ 0.4	9.3 $\pm$ 0.7	2.9 $\pm$ 0.1	0.0 $\pm$ 0.0	5.9 $\pm$ 0.3	9.1 $\pm$ 1.4	2.4 $\pm$ 0.1

In the PCA of the soil-derived PLFA profiles from all treatments, PC1 accounted for 42% of the variation, and there was no significant difference in this component attributable to any of the soil or rainfall treatments (data not shown). PC2 significantly discriminated field from sterile and re-inoculated soils, and pre- and post-rainfall soils for both the latter soil treatments (Figure 7.20). PC3 further separated pre- and post-rainfall samples for sterile soils. Soils after rainfall contained increased proportions of 16:0 and ai16:0; these biomarkers are mostly associated with bacterial populations and decreasing amounts of cyc 19:0 (an anaerobic eubacterial marker (Jackson *et al.* 2003)) and 18:1 $\omega$ 9c which been implicated as a eukaryotic biomarker (Bardgett & McAlister, 1999; Lindahl *et al.* 1997). Field soil had significantly higher %mol of 16:1 $\omega$ 7c, cyc 19:0 and 18:1 $\omega$ 9t whereas i16:0 was significantly lower in field soil compared to sterile, and highest in re-inoculated soil. This showed that the application of simulated rainfall changed the microbial community profile in all treatments. In the case of the hydrological samples, PCA revealed a highly distinct PLFA profile for the rain water via PC1, and phenotypic community profiles in waters derived from the field samples were distinct from sterile and re-inoculated soils (Figure 7.21). PC2 and PC3 (22 and 13% of variance respectively) showed no significant difference attributable to origin of the water samples. However, PC4 further discriminated runoff from infiltrate for the field soil samples only (Figure 7.21). The biomarker 18:1 $\omega$ 9c (bacterial biomarker (Zelles *et al.* 1992)) significantly discriminated sterile and re-inoculated soil from field soil which contained less. This is perhaps a more dominant biomarker in r selected communities (Chapter 1) than in more stable communities.



**Figure 7.20:** Phenotypic structure of soil samples as described by principal component analysis (PCA) of PLFA profiles; (□) field soil; (△) re-inoculated soil; (○) sterile soil. Open symbols denote profiles before rainfall, closed symbols after rainfall; points show means (n=5), whiskers show s.e. Percent variation accounted for by PCs shown in square parentheses.



**Figure 7.21:** Phenotypic structure of runoff and infiltrate samples as described by principal component analysis (PCA) of PLFA profiles; (□) field soil; (△) re-inoculated soil; (○) sterile soil. Open symbols denote runoff samples, closed symbols infiltrate samples; points show means (n=5), whiskers show s.e. Percent variation accounted for by PCs shown in square parentheses.

The hydrological processes that were measured were not statistically different between sterile and re-inoculated treatments, this could be attributed to the composition and associated properties of the nascent community developing in the re-inoculated treatments, although there was a tentative negative relationship between increasing biomass and decreasing run-off. There was certainly a much greater intact biomass in re-inoculated soils, and of different composition. The nascent communities that developed in the re-inoculated system would be predominantly r-strategists, and it appeared that they did not affect soil structure in such a way as to affect the hydrology of the system but they contained a particular mobile phase.

## ***7.4 Conclusions***

### **7.4.1 Post-irradiation sampling**

Sterilisation of soil had no immediate effect on water holding capacity, infiltration volume, runoff volume or cohesive shear strength. Thus the living microbiology of the soil was not influencing water movement at this stage and water movement was potentially more reliant on the physicochemical parameters. It is conceivable that the structures created by microbial cells such as exudates and mycelia remain intact without being decomposed following  $\gamma$ -irradiation, thus even when dead, are influencing soil structure and water movement. The runoff and infiltration characteristics of the soil were not significantly different however the variances within the treatment were suggesting that the presence of a living soil biota could affect the potential of surface flow by increasing the probability of infiltration and decreasing the probability of runoff.

Sterile soils contained much higher concentrations of DOC than the field soil, which can be attributed to the release of organic carbon by irradiation of microbial cells. The rainfall appears to have a strong pre-extraction effect on DOC within the sterile system and not in the field soil. The dissolved organic carbon released by irradiation therefore appeared to be water labile, perhaps in the sterile system it was free in the soil matrix and not attached to organic matter, and therefore would be more easily removed from the system. The C-flush of the sterile system significantly increased after the application of simulated rainfall suggesting the presence of biomass in the rainfall. The C-flush also increased in the top and bottom of the field soil trays after rainfall. The biomass apparently contained in the rainfall had an effect on the community size of the both soils. Components of the microbial system affecting soil structure and associated hydrological properties such as runoff and infiltration rates were influenced by irradiation. There was no effect of surface flow in either treatment on microbial community size or structure.

The microbial community structure differed in soil when compared to aqueous samples. In all cases the biomarker 18:0 appears to be associated with the simulated rainfall. Soil samples showed significantly higher %mol of 18:1 $\omega$ 9c than runoff and infiltrate samples. 18:1 $\omega$ 9c has been indicated as a fungal biomarker (Frostegard & Baath, 1996; Myers *et al.* 2001) and as a bacterial marker (Zelles *et al.* 1992). The rainwater contained no 18:1 $\omega$ 9c biomarker, but it was shown to be contained in the runoff and infiltrate samples proving that it is carried through the soil matrix by rainwater, although it was more prevalent in field soil than sterile soil. The biomarker 20:0 increased in runoff and infiltrate samples compared to rainwater and soil samples which can be interpreted as a preferential

movement of this marker out of the soil. The biomarker 20:0 is indicative of a nematode dominated community (Chen *et al.* 2001); and these organisms are characteristically free living in the aqueous phase and so would naturally tend to accumulate in runoff and infiltrate water. The suggestion that the simulated rainfall contains its own unique microbial community is entirely plausible as the system used in these experiments stores reverse osmosis water in large tanks as a head of water for the simulator. These tanks are not kept in sterile conditions and the throughput of water is dependant on the number of users of the simulator. These experiments where run over the Christmas period, therefore the water head had been stored for up to two months without use. In these conditions it is very likely that a microbial community would be abundant in the stored water and supply pipes, this makes the analysis of data from experiments utilising sterile controls complex as the biological interference cannot be quantified by the methods used here. In natural rainfall there is a high possibility of microbial biomass being present as airborne cells are carried down to earth; the simulated rainfall in this case could be more akin to natural rainfall than at first perceived, however natural rainfall would not contain the same mass or phenotype of microbial community. The water chemistry and physical attributes of simulated rainfall are frequently recorded and presented along side experimental results as these parameters are known to affect erosion rates and soil-water interactions; it is perhaps pertinent to some studies to assess the biological component as well in order to fully understand all parameters of this “closed system”.

#### **7.4.2 Post incubation experiment**

After incubation and rainfall simulation the field soil treatment had significantly higher moisture content than the other treatments indicating an

established microbial community within the soil had an impact on water holding capacity. The mean runoff and infiltration characteristics of the soil were not significantly different, however the variances within each treatment were so, suggesting that the presence of soil biota could increase the probability of infiltration and reduce the probability of runoff.

Concentrations of DOC were much higher in the sterile treatment before rainfall and after rainfall than the other two treatments. There was evidence of an increase in DOC after application of simulated rainfall in all treatments, further evidence of microbial biomass being present in the rainwater. There was no effect of overland flow on C-flush and hence movement of microbial biomass down slope. There was no significant C-flush from the sterile soil indicating that after two weeks of incubation there was no membrane-bound carbon remaining in the soil. The re-inoculated and field soil treatments showed a decrease in C-flush after the application of simulated rainfall, this indicates the movement of microbial cells through or out of the upper 10 mm horizon of the soil.

The microbial community structure differed in soil when compared to aqueous samples in all treatments. There was also evidence of community structure change down slope after rainfall in the re-inoculated and field soil treatments. There was no significant difference in microbial community structure between runoff and infiltrate samples. Biomarker proportions showed rainfall contained higher %mol of 18:0 and ai16:0 which are bacterial biomarkers (Wilkinson *et al.* 2002). The application of rainfall changes the microbial community profile in all treatments. Phenotypic community profiles in waters derived from the field samples were distinct from sterile and re-inoculated soils. The biomarker 18:1 $\omega$ 9c (bacterial

biomarker (Zelles *et al.* 1992)) was again absent from the rainfall but present in runoff and infiltrate samples indicating its movement in the aqueous phase.

### ***7.5 Overall conclusions***

Sterilisation, by  $\gamma$  – irradiation, of soil does not immediately affect its water holding capacity but it does affect the susceptibility to infiltration and surface flow. After two weeks of incubation soils which have not been irradiated can increase in water holding capacity, where as sterile soil which had been re-inoculated showed no significant effect. The results suggest that there may be a trend to a lower probability of run-off losses from a soil with higher microbial biomass and a greater potential for infiltration of water, although there was no statistically significant effect of community size or structure on run-off and infiltration volumes. The apparently “mobile” phase of the soil communities was, however, remarkably similar across all three treatments. That the run-off from the sterile soil had the same phenotypic signature as the field and re-inoculated soils, suggests that there was something inherently mobile about the constitutive organisms, since the signal was apparent even after they had been killed. The biomarker 18:1 $\omega$ 9c, which was absent from the rainfall samples in both experiments, was shown to be present in runoff and infiltration samples in all treatments indicating it as a water labile PLFA of soil origin. The PLFAs contained in the rainfall are already known to be water labile due to their origin, so their increase in proportion after contact with soils is perhaps expected, however PLFAs that are not present in the rainfall but are present in the runoff and infiltrate are indicative of soil based PLFAs which are mobile as a result of rainfall.



These experiments therefore suggest that there may be a specific water-mobile fraction of the microbial community, which is susceptible to dispersal and is prone to relocation. This has implications for the types of microbes entering the hydrological cycle, and consequent impacts on water quality. There are also wider implications for our understanding of dispersal mechanisms important for evolution. If Bass Becking's principle that "everything is everywhere, the environment selects" (Martiny *et al.* 2006) holds true, then a universal "dispersal phase" would be a potential mechanism consistent with this, with the environment simply selecting for what is manifest in the active, dominant components of soil communities. In this paradigm, the non-active component would become visible only upon dispersal, when those resting propagule signals in PLFA profiles in run-off are no longer masked by the predominant signals in the bulk soil.

## **Chapter 8 The effect of contrasting soil microbial communities on erodibility**

### ***8.1 Introduction***

Soil erosion by water principally occurs due to particle detachment and transport as a result of rainfall and runoff (Ellison, 1947). Breakdown of surface soil aggregates as a result of rainfall affects infiltration, surface sealing and soil detachment (Reichert & Norton, 1994). Many factors affect this process; in the case of arable farming systems, tillage is one of the main influences. Zhang *et al.* (2007) showed that higher soil aggregate stability and macro-porosity of the surface (1-5 cm) soil as a result of conservation tillage practices and residue management significantly reduced the runoff and soil losses when compared to conventional tillage with stubble burning. Conventional tillage practices have been shown to reduce soil organic matter and degrade soil structure accelerating the propensity of soil to erode (Tisdall & Oades, 1982; Elliott, 1986). Cropping and tillage practices are known to affect runoff and soil losses, and to also influence the microbial community (Rasiah & Kay, 1995; Jackson *et al.* 2003). Micro-organisms are known to affect soil structural characteristics by the production of metabolic products binding soil particles together, and via physical enmeshment by filamentous organisms (Griffiths, 1965; Chenu, 1993; Degens, 1997; Young & Ritz 2004). The production of hydrophobic exudates by fungi causing water repellence may influence preferential flow and structural stability of soils (Czarnes *et al.*, 2000), whereas the action of bacteria may break down these compounds thus decreasing repellency (Roper, 2004). Edgerton *et al.* (1995) demonstrated a log-linear relationship between microbial biomass (as measured by ATP) and aggregate stability in restored opencast mine soils,

and Hallet and Young (1999) have shown in laboratory studies that the addition of nutrients increases biological activity, potentially causing severe water repellency of soil aggregates. Increases in pore connectivity, aggregate stability and water repellence have implications for soil erodibility in systems where there are perturbances in micro flora structure and function.

A clear understanding of the processes governing a microbiologically active soil and the relationships between the soil biota, soil structure and erodibility has implications for soil and water management, and the dispersal of micro-organisms at the field scale. The aim of this study was to ascertain the effect of the soil microbial community on soil erodibility, by testing the following hypotheses:

1. The presence of a microbial community will decrease the sediment concentration of run off, in comparison to sterilised controls.
  - *Increased soil aggregate stability by the presence of fungal hyphal enmeshment and the cell exudates will reduce surface aggregate breakdown and particle detachment.*
2. The presence of a microbial community will increase the volume of infiltration through the soil.
  - *Increased soil aggregation and pore connectivity as a result of microbial mediation will improve soil drainage, decreasing surface flow.*
3. Sterile soils inoculated with microbial communities from conservational and conventional tilled soil will support different microbial community sizes and structures.
  - *As a result of the nature of their respective inocula.*

4. Microbial responses will influence soil erodibility and hydrology differently.
  - *Different community compositions and numbers will respond differently to rainfall events.*
5. Raindrop impact and overland flow will change the size and structure of surface soil microbial communities.
  - *Different components of the microbial community will be differentially susceptible to detachment and carriage by overland flow.*

## ***8.2 Microcosm design and preparation***

Surface soil (0 – 150 mm) was collected from conventional tilled plots and SOWAP conservation tilled plots (Chapter 3.2) from Upper Ponds Field, on the Allerton Estate in Loddington, Leicestershire, U.K. The soil is from the Hanslope and Denchworth series (5.2% organic matter, 34% sand, 24% silt, 42% clay, pH 6.7). The soils were sieved to 5 mm, and 30 kg of soil from the conservational tilled plot was combined with 30 kg collected from the conventionally tilled plot and thoroughly homogenised. The combined soil was packed into 60 x 110 x 200 mm (d, w, l) steel trays to a bulk density of  $0.8 \text{ g}^{-1} \text{ cm}^3$ . The steel trays (Figure 8.1) had a steel mesh at the bottom which was covered with a nylon mesh to allow infiltrate water through but not the passage of large soil aggregates. The packed trays were placed into storage containers as described in Chapter 7.2.1.



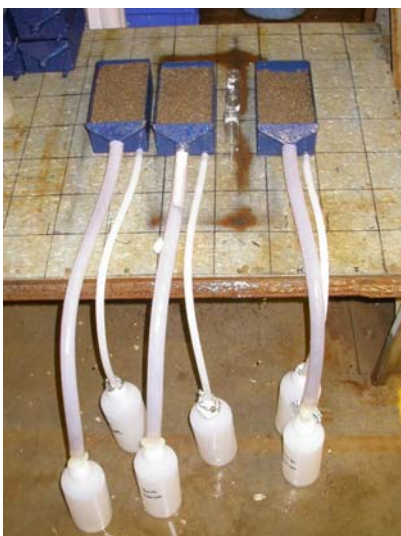
**Figure 8.1: Metal microcosm partially filled with sieved soil sample.**

### **8.2.1 Microcosm treatments**

Soils were sterilised by  $\gamma$ - irradiation at a minimum doses of 25 k Gy (Istotron plc., Swindon, UK). Sterilized soil was re-inoculated by mixing 100 g of prepared field soil (derived from conventional plot or SOWAP conservation plot) in 200 ml of de-ionized water to create a slurry; 10 ml of the slurry was then applied to the soil tray surface using an 4-channel multi pipette across five evenly-distributed places on the soil surface. These re-inoculated treatments are subsequently referred to as Conventional or Conservation respectively. The control treatments (remaining sterile) had 10 ml of sterile water added to ensure consistency of moisture content over the experimental period. Each treatment was replicated ten times in order to produce five replicates of each treatment to undergo simulated rainfall and five replicates to receive no rainfall. Trays of each treatment were subsequently incubated for two weeks at room temperature (approx. 25°C) on the bench.

### 8.2.2 Rainfall simulation

Five randomly-selected replicates of each treatment were subjected to simulated rainfall generated by a gravity fed, hypodermic needle rainfall simulator. Trays were inclined at 12°, and a rate of 60 mm h<sup>-1</sup> rainfall for 45 minutes was applied. Throughout the rainfall simulation runoff and infiltrate were collected using a sterile funnel collection system (Figure 8.2). The total volumes of run-off and infiltrate were recorded once rainfall ceased, and 25 ml aliquots were removed for phospholipid fatty acid (PLFA) analysis (Chapter 2.5.2). The remaining runoff and infiltrate samples were filtered through pre-dried and tared Whatman 542 filter papers. Once filtered the total volume of liquid was recorded and the wet filter papers dried at 55°C for 24 hours until a constant weight was reached. Sediment concentration in the originally collected volume was then calculated. Five replicates of each treatment were not subjected to simulated rainfall, in order to provide a control. These soil trays were sampled in the same manner as the soil trays receiving rainfall with exception of run-off and infiltrate samples.



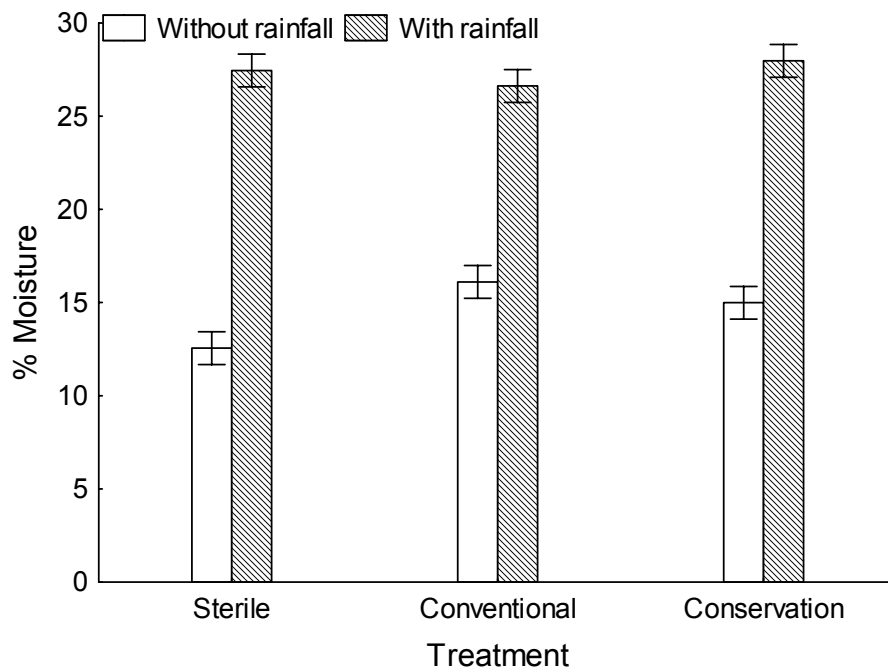
**Figure 8.2: Experimental arrangement enabling collection of runoff and infiltrate during rainfall simulation.**

### **8.2.3 Sampling**

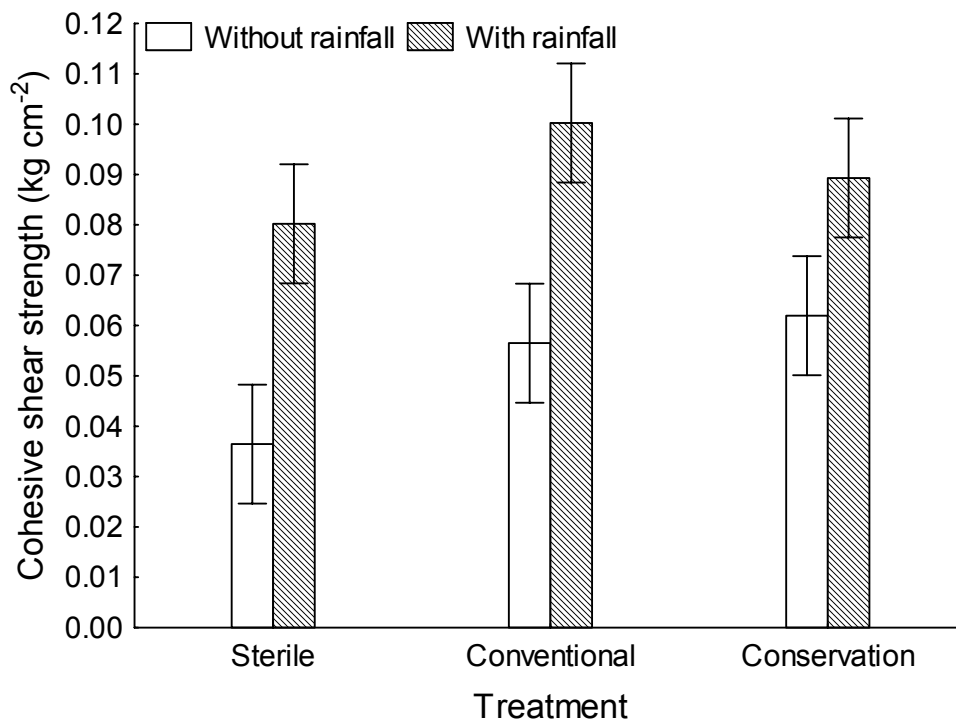
Soil from each tray was sampled at the top, middle and bottom regions of the slope, relative to the incline. Before destructive sampling occurred Torvane measurements were taken at each region of the slope Chapter 2.3.2). The surface 10 mm of each sample zone (66 x 110 mm, l, d) was removed using a palette knife and homogenised by chopping, then sub-samples weighed for determination of moisture content, microbial biomass and PLFA analysis (Chapter 2). Data was analyzed using analysis of variance and principal components analysis.

### ***8.3 Results and discussion***

The mean moisture content of the sterile soil was significantly different to that of the Conventional and Conservation re-inoculated treatments before rainfall (Figure 8.3). After simulated rainfall was applied there was no significant difference in mean moisture content between the treatments. The difference in moisture content before the simulated rainfall was applied may have impacted the results in terms of comparison of moisture balance, shear strength and erosivity. Measurements of cohesion shear strength mirror those of the moisture content in that the sterile treatment had a significantly lower shear strength before rainfall application and was not significantly different afterwards (Figure 8.4).



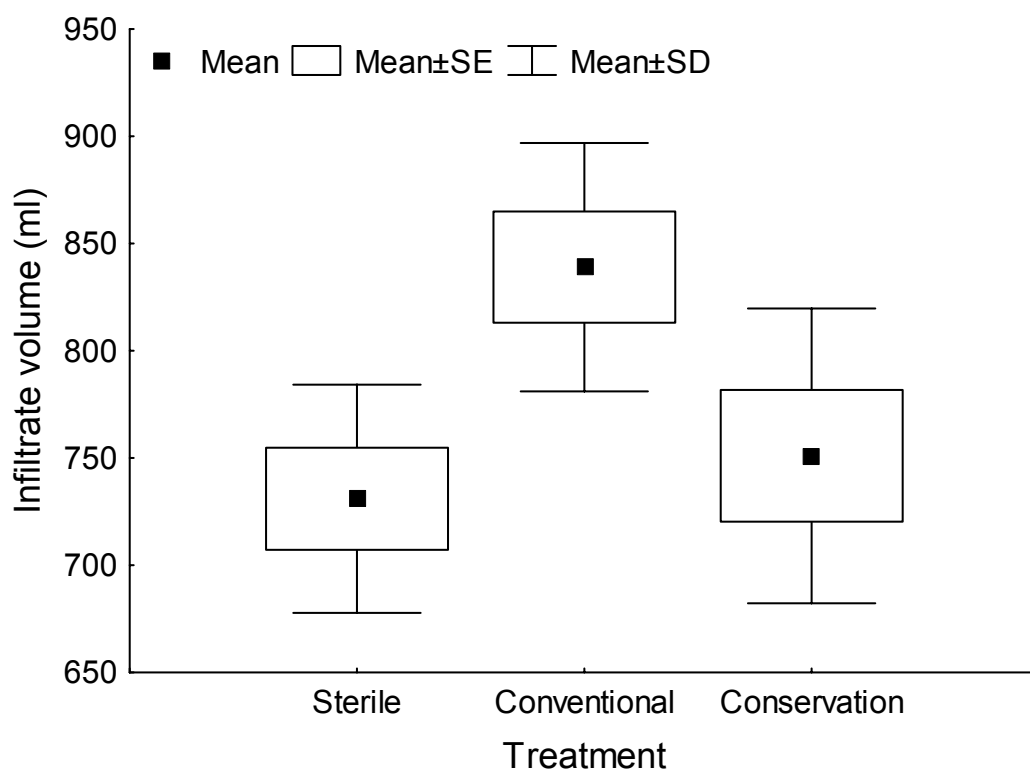
**Figure 8.3: Soil moisture content with and without application of simulated rainfall on sterile and re-inoculated soils; bars show means (n=5), whiskers show 95% confidence intervals.**



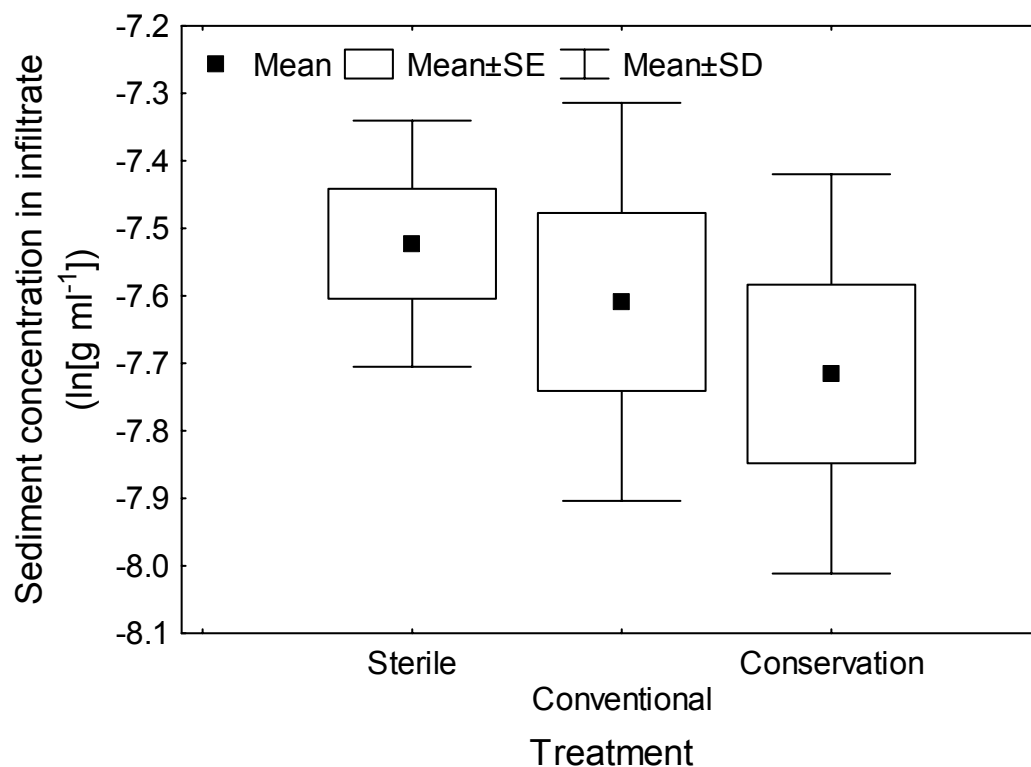
**Figure 8.4: Cohesional shear strength of sterile and re-inoculated soils measured by Torvane with and without application of simulated rainfall; bars show means (n=5), whiskers show 95% confidence intervals.**



The infiltrate volume collected was significantly greater for the Conventional treatment compared to the other treatments (Figure 8.5). The concentration of sediment contained within the infiltrate showed no significant difference in means (Figure 8.6). However, there was a significant difference in variance between the sterile and the other treatments.



**Figure 8.5: Box and whisker plot showing infiltrate volume collected for sterile and re-inoculated soil treatments (n=5).**



**Figure 8.6: Box and whisker plot showing the concentration of sediment contained in the infiltrate collected (n=5).**

There was no significant difference between treatments in relation to runoff volume (Figure 8.7). There was an indication of a trend of higher runoff volumes associated with soils containing a microbial inoculum. There were no significant differences in mean between treatments in relation to sediment concentration of runoff (Figure 8.8). However, the re-inoculated treatment from conservation tilled soil was significantly less than the other treatments. The largest variation in sediment concentration in runoff was associated with the sterile treatment. These results suggest that whilst the inoculated soils were most likely to generate runoff, the concentration of sediment contained within that runoff was likely to be lower than in a sterile system. This supports the hypothesis that an increase in microbial biomass will decrease the sediment concentration within runoff.

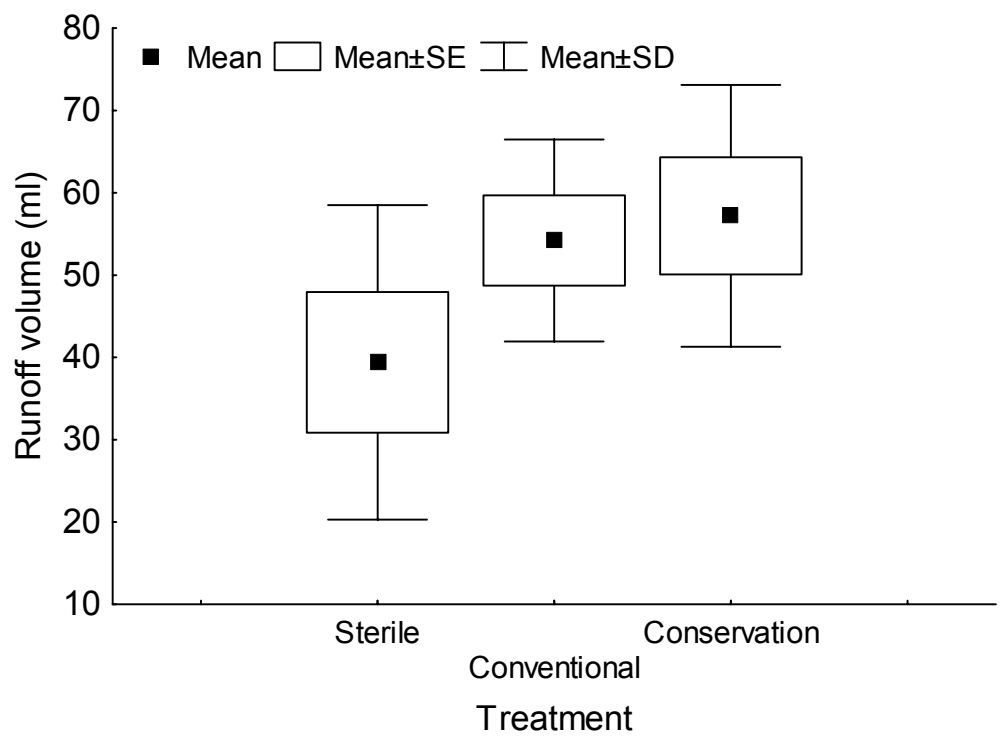


Figure 8.7: Box and whisker plot (n=5) showing the volume of runoff collected from soil treatments.

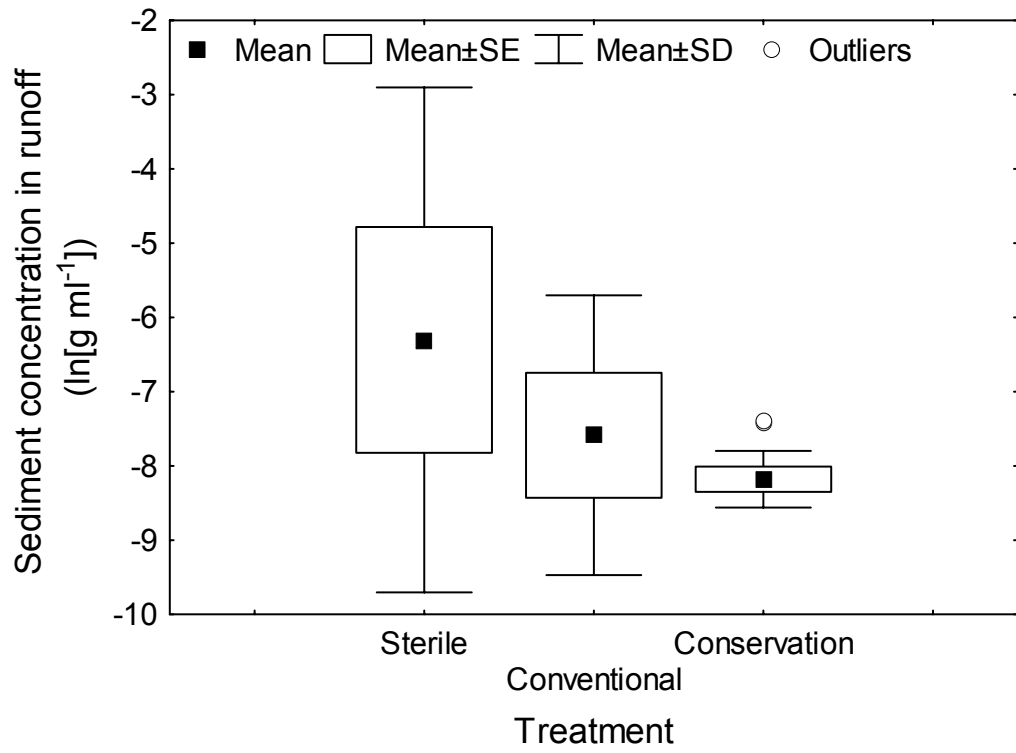
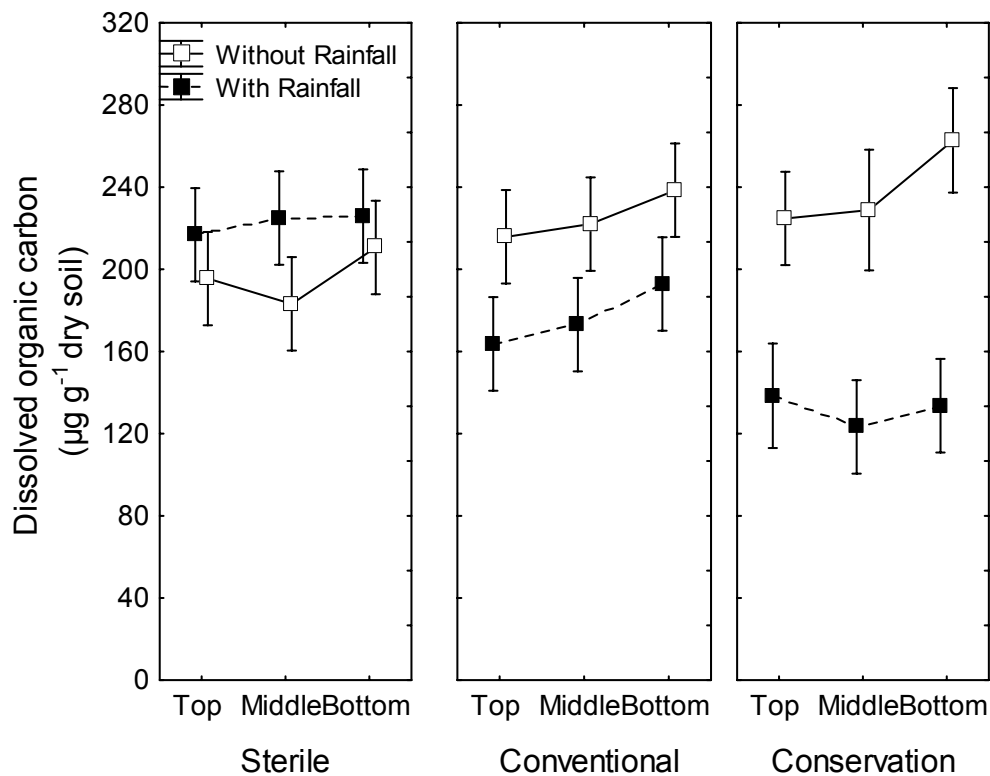


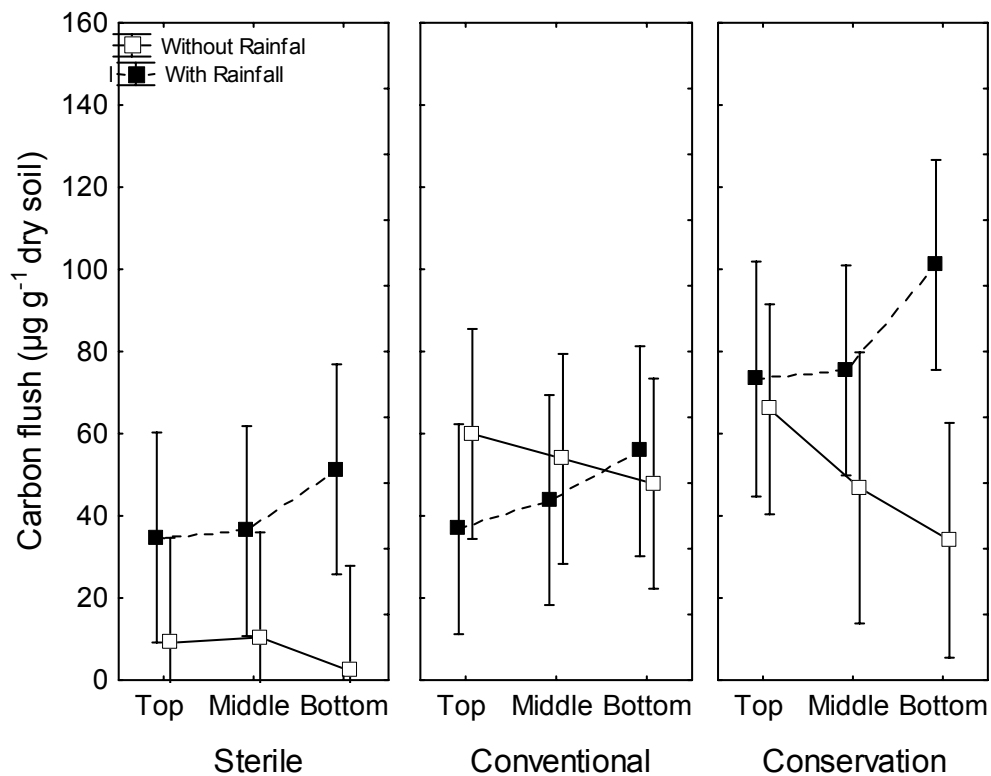
Figure 8.8: Box and whisker plot (n=5) showing the concentration of sediment contained in runoff collected from soil treatments.

The concentration of dissolved organic carbon (DOC) in the sterile treatment did not alter as a result of rainfall, however in the re-inoculated soils there was a significant decrease after simulated rainfall was applied (Figure 8.9). There was a lower concentration of DOC in the Conservation re-inoculated plot after rainfall than in the sterile treatment. This partially supports the hypothesis that increased microbial biomass will increase the volume of infiltration through a soil but it also suggests that the community composition as well as the presence of a microbial community is important. There was no effect of slope on either DOC or C-flush, thus rejecting the hypothesis that raindrop impact and overland flow will change microbial community size. After simulated rainfall there is a weak trend to suggest increasing C-flush at the bottom of the slope which implies movement of microbial biomass down slope, however, there is also a weak reverse trend in C-flush before the application of simulated rainfall. This trend of decreasing C-flush down slope cannot be explained by the experimental design.



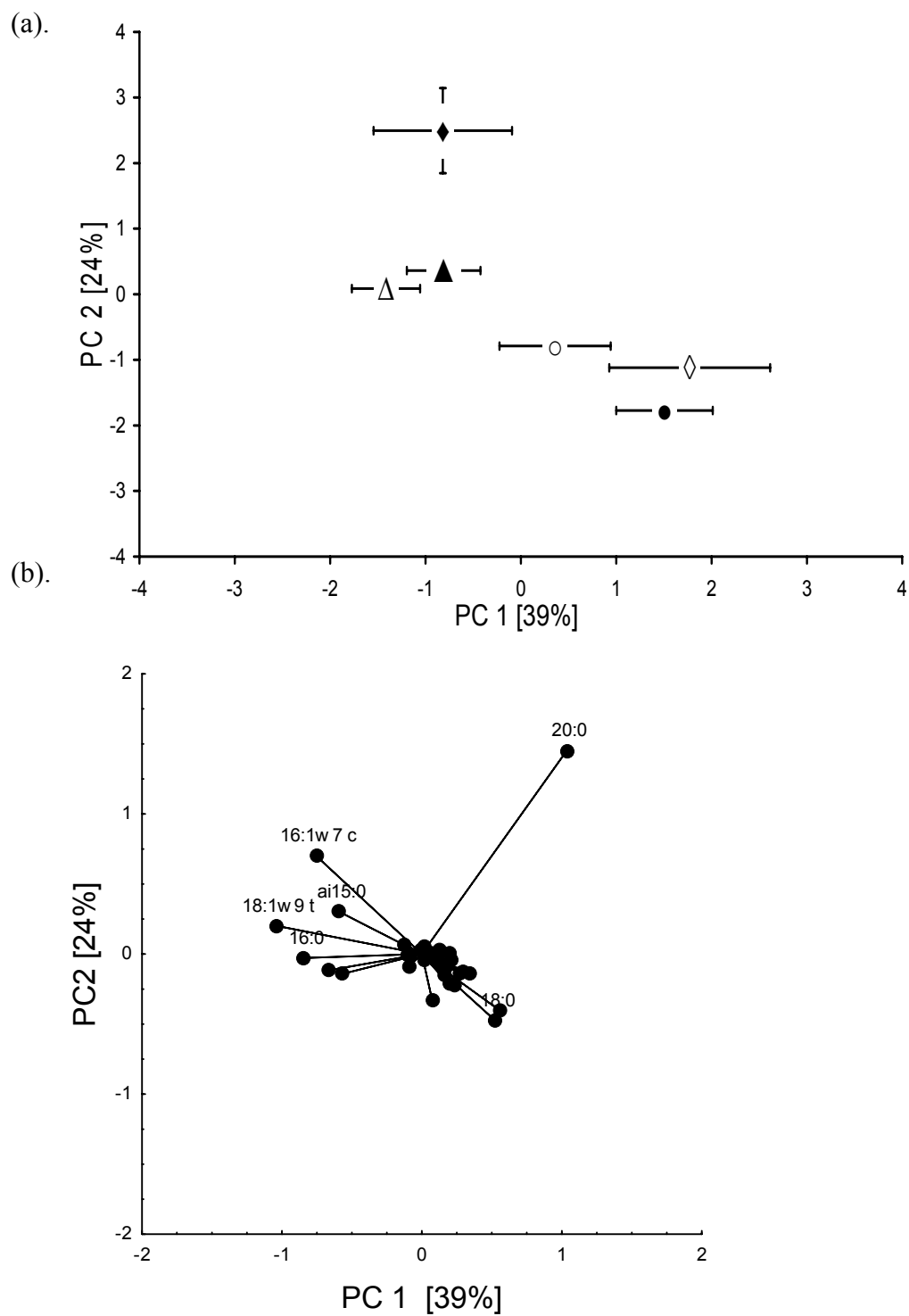
**Figure 8.9: Concentrations of dissolved organic carbon (DOC), in relation to soil treatment; points show means (n=5), whiskers show 95% confidence intervals.**

Carbon- flush, and hence microbial biomass, was significantly higher in the re-inoculated treatments than in the sterile. After the application of simulated rainfall there was an increase in the concentration of fumigated carbon extracted implying contamination of microbial cells through the rainfall (Figure 8.10). There was no change in C-flush from the conventional re-inoculated soils, where as in the conservation re-inoculated and sterile treatments there was an increase in C-flush after simulated rainfall application.



**Figure 8.10: Concentrations of microbial biomass carbon extracted from soil treatments; points show means (n=5), whiskers show 95% confidence intervals.**

The microbial community structure of soil samples was significantly different to those of the runoff, infiltrate and rainfall samples by PCA. There was no significant effect of sample location on the soil PLFA profiles hence only average data are described (Figure 8.11). The conventional re-inoculated treatment showed no change in PLFA profile after the addition of rainfall and was significantly different to the other treatments. After rainfall the conservation re-inoculated treatment has a significantly different phenotypic profile, which correlates with the increase in biomass after rainfall.



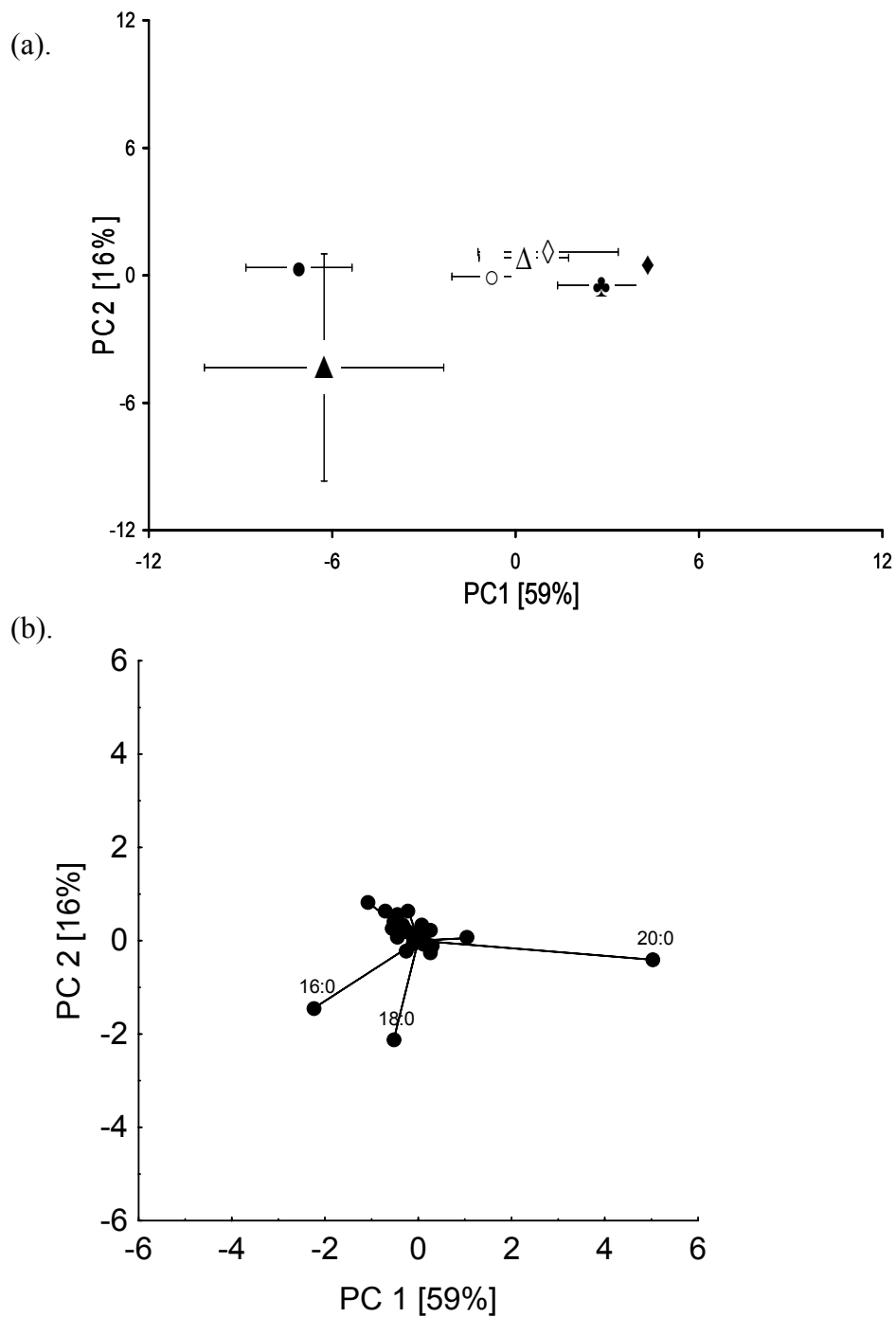
**Figure 8.11: Phenotypic structure of soil samples as described by principal component (PC) analysis of PLFA profiles. a) 1<sup>st</sup> and 2<sup>nd</sup> principal components; (C) sterile soil; (◇) minimum re-inoculated soil; (△) conventional re-inoculated soil. Open symbols denote profiles before rainfall, closed symbols after rainfall; points show means (n=5), bars show s.e. Percent variation accounted for by PCs shown in square parentheses. b) Loadings values associated with PCs.**

In the PCA of the runoff and infiltrate derived PLFA profiles, the Run off and Infiltrate samples are significantly different from each other. The infiltrate samples were not significantly different to the simulated rainfall. The phenotypic profile of the Minimum re-inoculated treatment runoff is significantly different to the runoff of the other treatments in PC1 (Figure 8.12).

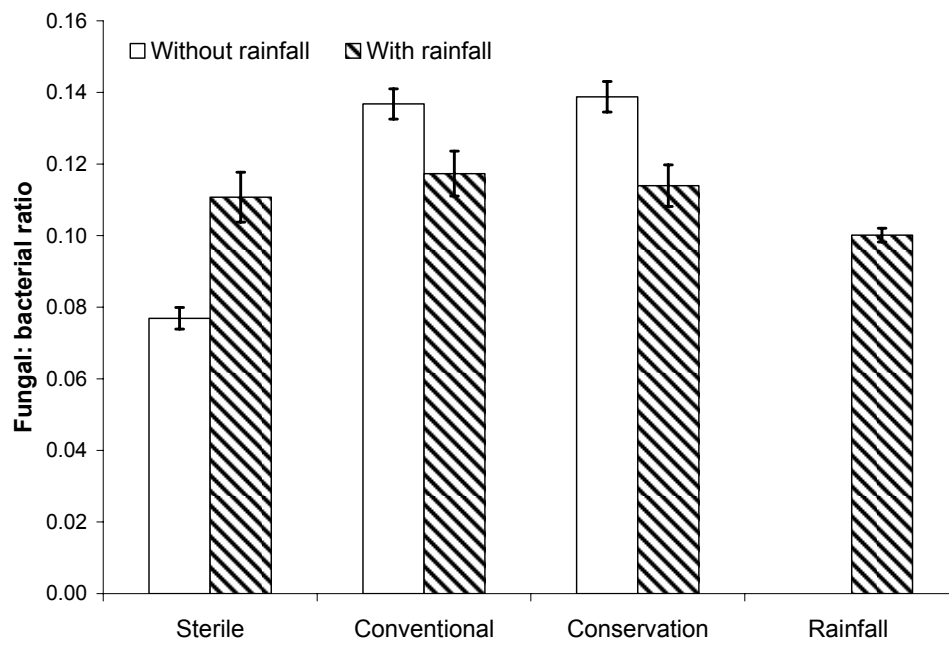
The biomarkers 20:0 and 18:0 were found in higher proportions in the water derived samples than the soil samples, with the highest proportion in the rainwater. The biomarkers 18:ω9t, 16:0 and 18:1ω9c significantly discriminated the runoff samples from the rainfall and infiltrate samples which contained lower proportions of these markers (Table 8.1).

The sterile treatment contained a lower fungal: bacterial ratio (Chapter 2.5.2.b) than the other treatments before the application of simulated rainfall (Figure 8.13). After the application of simulated rainfall there was no significant difference in the fungal: bacterial ratios of any of the soil treatments. After the application of simulated rainfall the %mol of 18:2ω6 increased in the sterile treatment and decreased in the re-inoculated treatments. These results tentatively support the hypotheses that the ratio of fungal: bacterial biomass will alter the propensity of a soil to erode.





**Figure 8.12: Phenotypic structure of run-off and infiltrate samples as described by principal component (PC) analysis of PLFA profiles. a) first and second principal components (PC); (○) sterile soil; (◇) minimum re-inoculated soil; (Δ) conventionally re-inoculated soil; (♣) simulated rainfall. Open symbols denote run-off profiles, closed symbols infiltrate; points show means (n=5), bars show s.e. Percent variation accounted for by PCs shown in square parentheses. b) Loadings values associated with PCs.**



**Figure 8.13: Fungal: bacterial ratio based on %mol in PLFA profiles of soil treatments with and without the application of simulated rainfall.**

**Table 8.1: Mean biomarker values , %mol, and associated standard error (n=5, except rainfall where n=15).**

Treatment	Rain?	Sample position	ai15:0	16:1ω7c	16:00	18:2ω6c	18:1ω9t	18:00	20:0
Sterile	Yes	Top	4.8±	5.3±	11.2±	2.7±	9.9±	4.3±	2.8±
			0.4	0.3	0.5	0.3	0.6	0.3	0.3
Sterile	Yes	Middle	4.1±	4.9±	12.3±	3.4±	9.8±	6.9±	2.8±
			0.1	0.1	0.7	0.7	1.0	1.6	0.1
Sterile	Yes	Bottom	5.1±	5.8±	11.3±	2.4±	10.8±	3.9±	2.4±
			0.2	0.3	0.3	0.2	0.4	0.2	0.2
Sterile	Yes	Runoff	2.8±	4.1±	8.3±	3.3±	5.4±	6.4±	5.4±
			0.5	0.4	0.8	0.3	1.4	0.8	1.4
Sterile	Yes	Infiltrate	2.0±	3.4±	6.0±	3.5±	3.7±	6.0±	11.0±
			0.2	0.3	0.2	0.2	0.1	0.5	1.5
Sterile	No	Top	5.6±	5.9±	11.4±	2.3±	10.7±	4.0±	2.7±
			0.2	0.1	0.4	0.1	0.3	0.3	0.2
Sterile	No	Middle	5.3±	5.8±	11.1±	2.5±	10.9±	4.0±	3.7±
			0.4	0.3	0.6	0.1	0.6	0.2	1.4
Sterile	No	Bottom	5.8±	6.3±	10.9±	2.1±	11.3±	3.7±	2.4±
			0.2	0.2	0.3	0.2	0.3	0.2	0.2
Conventional	Yes	Top	6.6±	7.4±	11.1±	3.7±	10.7±	3.5±	3.0±
			0.0	0.2	0.5	0.6	0.6	0.2	0.5
Conventional	Yes	Middle	6.0±	7.1±	11.2±	3.8±	11.0±	3.3±	2.8±
			0.2	0.1	0.3	0.1	0.2	0.2	0.4
Conventional	Yes	Bottom	6.2±	7.0±	10.9±	3.6±	10.7±	3.4±	3.0±
			0.2	0.3	0.2	0.2	0.6	0.1	0.5
Conventional	Yes	Runoff	2.9±	2.9±	10.6±	2.7±	3.4±	9.4±	6.5±
			0.0	0.5	4.5	0.4	0.1	3.9	2.0
Conventional	Yes	Infiltrate	3.2±	3.9±	5.3±	2.6±	3.7±	5.4±	11.2±
			0.1	0.2	0.3	0.2	0.2	0.5	1.7
Conventional	No	Top	7.0±	6.6±	11.5±	4.1±	11.7±	3.0±	2.6±
			0.2	0.2	0.2	0.2	0.2	0.3	0.2
Conventional	No	Middle	6.4±	6.5±	11.2±	4.1±	10.7±	3.5±	2.8±
			0.3	0.3	0.5	0.2	0.3	0.3	0.3
Conventional	No	Bottom	6.3±	6.7±	11.4±	4.0±	11.7±	3.1±	2.9±
			0.0	0.5	0.2	0.3	0.3	0.1	0.1
Conservation	Yes	Top	6.3±	8.6±	11.5±	3.5±	11.6±	3.1±	3.6±
			0.2	0.2	0.2	0.3	0.3	0.2	0.6
Conservation	Yes	Middle	6.5±	8.1±	11.2±	3.2±	11.0±	2.9±	6.2±
			0.3	0.4	0.8	0.3	0.6	0.1	2.5
Conservation	Yes	Bottom	6.5±	7.8±	11.5±	3.1±	11.5±	2.8±	3.8±
			0.4	0.3	0.3	0.5	0.5	0.1	0.5
Conservation	Yes	Runoff	3.0±	3.5±	4.3±	2.8±	3.8±	5.0±	15.5±
			0.1	0.3	0.5	0.3	0.1	0.3	0.1
Conservation	Yes	Infiltrate	3.1±	3.8±	5.0±	2.9±	4.4±	4.7±	12.3±
			0.4	0.2	0.3	0.5	0.2	0.3	2.4
Conservation	No	Top	5.9±	5.6±	8.9±	3.8±	9.3±	3.9±	2.9±
			0.5	0.5	0.9	0.1	1.0	0.4	0.5
Conservation	No	Middle	5.9±	5.8±	9.9±	4.2±	10.4±	3.4±	2.4±
			0.2	0.3	0.2	0.2	0.3	0.1	0.1
Conservation	No	Bottom	6.0±	5.4±	9.3±	4.4±	9.2±	3.8±	2.7±
			0.5	0.6	0.9	0.3	0.6	0.4	0.4
None	Rain water		3.2±	3.5±	6.1±	2.6±	3.3±	5.5±	14.6±
			0.4	0.3	0.7	0.1	0.2	0.5	1.2

#### ***8.4 Conclusions***

These results show that whilst there was no significant difference in mean runoff sediment concentration, there was evidence to suggest that the presence of a microbial community affects the soil particle loss and the community structure in that the variance of sediment concentration was significantly reduced in soil re-inoculated with a microbial community from conservation tilled soil. The inherent properties of that community may affect the concentration of sediment lost. This effect was particularly manifest where the microbial communities, derived from the conservation-tilled soil, produced the least variation in sediment concentration when compared to the sterile control. There was also a possible trend in the runoff characteristics of the soil, with re-inoculated treatments showing a trend to a higher volume loss. There was a significantly lower fungal:bacterial ratio in the sterile treatment before the application of simulated rainfall but no significant difference between the re-inoculated treatments. It is notable that there was also a trend in that they contained the least sediment concentration. This suggests that these phenomena may be predominantly surface-based, the soils could be capped by micro-organisms creating bio-films and stronger surface aggregates, preventing the runoff loss of soil particles. These results partially support the hypothesis that the presence of a microbial community will decrease the sediment concentration of runoff.

The infiltration of water through the soils was significantly greater in the Conventional re-inoculated treatment than in the other treatments. This showed that the presence of a microbial community may increase the infiltration rate through the soil but because the minimum tilled re-inoculated soil did not differ from the sterile control, it suggests that the

community composition was also important, not just its presence, therefore supporting the initial hypothesis. Before rainfall there was no significant difference between the microbial biomass of the two re-inoculated treatments; this is evidence that the difference in infiltration may be due to a change in microbial community structure. There was also some evidence of biomass contained in the rainfall water which also had implications for the phenotypic community changes after a rainfall event.

No effect of position on slope was observed after rainfall on microbial community size, not supporting the hypothesis that surface flow and raindrop impact will alter microbial community size. There was also an increase in C-flush from the Conservation re-inoculated and sterile treatments but a lack of change in Conventional re-inoculated treatments. The lack of change in biomass of the Conventional re-inoculated treatment could be as a result of the large infiltration volume of this soil causing less interaction between the rainfall and soil matrix. Before rainfall there was no significant difference in DOC between the treatments, however after rainfall the re-inoculated treatments showed a reduction in DOC. The greatest reduction in DOC was found in the Conservation re-inoculated treatment, which ties in with the associated slower infiltration rate.

The phenotypic structure of the soil microbial community was significantly different to those in the aqueous samples. The community structure was not significantly different in relation to the position of sample on the slope. After rainfall the conventional re-inoculated soil treatment showed no change in community structure, further linking with the biomass carbon results. The sterile and Conservation re-inoculated treatment had significantly different microbial community structures after rainfall than

before. The Conservation re-inoculated treatment showed a significantly different community structure in the runoff compared to other treatments, associated to the rainfall and associated with the 20:0 PLFA biomarker. This biomarker has been linked to soil containing nematodes which would be more susceptible to filtration effects by the soil matrix (Chen *et al.* 2001). The biomarker 20:0 was found in significantly higher proportion in the rainfall and least proportion in the soil, larger organisms such as nematodes would be more prone to filtration and retention in the soil surface of the microcosms than microbes. This suggests that nematodes may be a candidate organism for rainfall ‘contamination’ in these experiments.

The presence of biomass in simulated rainfall has obvious experimental considerations particularly in the case of these experiments were the methodology used does not support quantitative detection of this biomass contaminant. Bacteria are known to exist in natural clouds and rainfall (Evans *et al.*, 2006; Amato *et al.*, 2005), the composition of these natural rainfall components is dependant on water chemistry, temperature etc. These factors all affect soil hydrological and erosion response therefore, the interactions between rainfall components could prove key in understanding the interactions between soil-water movement and microbial communities. The presence of biomass in artificial rainfall simulators may not in itself be detrimental to the overall experimental design although it is impossible to know from these experiments how different these communities are to natural rainfall biomass.

### **Section 3 - Summary**

The experiments described in this section were aimed at providing greater understanding of the mechanisms driving microbial, soil and water interactions. The results have shown that microbial communities can impact on soil hydrology and erodibility at small scales. Linking the laboratory experiments to field rainfall simulations proved the difficulty of controlling environmental variables, however the same trends were observed at both scales. Further experimentation and the use of more resolute community analysis techniques such as activity measures would help to further elucidate these mechanisms. For example gross respiration studies of these systems would provide functional information about the microbial community which cannot be determined using PLFA. Further experimentation into the fraction of microbial community leaving the soil system could provide fundamental information on the dispersal of species in terms of the hydrological cycle, potentially providing further information for water quality, pesticide breakdown and pathogen transport.

# *Section 4*



## Chapter 9 Synergy

### 9.1 Introduction

The aim of this thesis was to gain information on the effect of soil tillage practices on soil microbial communities and to formulate links between microbial communities and the propensity of soils to erode. In order to achieve this aim, experiments were carried out at different spatial scales. At the field scale experiments were designed to investigate spatial and temporal variation in microbial communities under different tillage practices. Smaller scale experiments were designed to investigate some of the mechanisms that relate soil loss and microbial communities. The original hypotheses driving this research are shown in Table 9.1, with the associated chapters which address them.

A review of the literature in Chapter 1 identified key areas of research where soil microbial communities, land management and erodibility could be linked and where research was lacking. Literature showing the effect of land management practices on soil microbial communities indicate that changes are apparent but are also dependant on climate, seasonality and soil type among other factors (Carter, 1986; Dick, 1992; Drijber *et al.*, 2000; Bending *et al.* 2004). Much work has been carried out on the effect of microbial communities on aggregate stability, particularly in relation to exudate production and hyphal enmeshment (Linn & Doran, 1984; Lupwayi *et al.*, 2001). However, research linking soil microbial communities to soil structural stability are mainly limited to aggregate scale studies and seldom involve collections of aggregates. The mechanistic processes occurring at such small scales may prove to affect processes such as water erosion and particle detachment at wider landscape

scales. Research at these different scales, whilst problematic, provides valuable insight into the causal mechanisms behind structure/ biota interactions and also the implications of these to the landscape and its associated management.

**Table 9.1: Overall hypothesis, key findings and chapters addressing them.**

<b>Relevant chapter</b>	<b>Hypotheses</b>	<b>Key findings</b>
Chapter 4 Chapter 5 Chapter 6	Different tillage practices will result in a variation in microbial community size and structure.	Conventional tillage produces a net reduction in the size of the microbial community. There is no consistent overall trend in phenotypic community change as a result of tillage practice
Chapter 8	The rate of rainfall-induced erosion at the microcosm scale will be proportional to the total biomass.	There was no significant difference in mean erosion losses. However, there was a significant difference in variation between treatments with a living microbial biomass and those without suggesting increased biomass reduces the potential of a runoff event.
Chapter 8	Variation in the microbial community structure, in particular the ratio of bacteria to fungi, will have an impact on the propensity of soils to erode.	There were significant differences in sediment concentration and runoff volumes lost from soil re-inoculated with different inocula. The fungal: bacterial ratio was not significantly different between re-inoculated treatments.
Chapter 7 Chapter 8	The presence of a microbial community will impact on water movement through soil in relation to infiltration and runoff.	Different microbial inocula onto sterile soils were shown to change the infiltration and water holding capacity of the soil. There was an inherent phenotypic microbial community associated to the runoff and infiltrate samples in all rainfall simulation studies.
Chapter 6 Chapter 9	Tillage impact on microbial community size and structure at a field scale will lead to differing erosion event outcomes across tillage treatment types.	The SOWAP project as a whole shows soil loss to be reduced under conservation tillage practices, these field plots are shown to have an increase microbial biomass, whilst phenotypic community structure shows no distinct pattern relative to tillage.

## ***9.2 The effect of tillage practices on soil microbial community size and structure***

The experiments carried out within Section 2 provide information on changes in microbial community structure and size as a result of tillage practice in a European context (Chapter 4) and in a UK temporal context (Chapter 5). In arable agricultural land even though cropping, climate and soil characteristics may change there is a common decreasing effect of conventional tillage on the size of the soil microbial community (Section 2). This is consistent with resource-ratio theory assuming that substrate contained within organic matter is the primary limiting factor with respect to the microbial biomass (Tillman, 1982). This decrease may have dramatic effects on the soil biogeochemical cycling activities of the micro-organisms. Bacteria utilise several survival strategies for dealing with low nutrient conditions either by being able to grow at low substrate levels or by becoming temporarily inactive. Thus, whilst biomass numbers may diminish as a result of land management the associated biogeochemical processes may not necessarily decrease. The high species diversity observed in soil micro-organisms is thought to ensure that the ecological functions remain active even after perturbation. The tilling of soil not only changes the soil structure but also the distribution of organic substrates and therefore impacts upon the spatial distribution of micro-organisms (Young & Ritz, 1998). The microbial community phenotypic structure did not alter annually as a result of tillage, but changed as a whole seasonally (Chapter 5) and after three years (Chapter 4). These results demonstrate the effect of climate and seasonality on the phenotypic expression of the microbial community. However, immediate changes in phenotypic structure after perturbation (not investigated in this thesis) may result in a transient changes in ecological pathways resulting, for example, in an increased

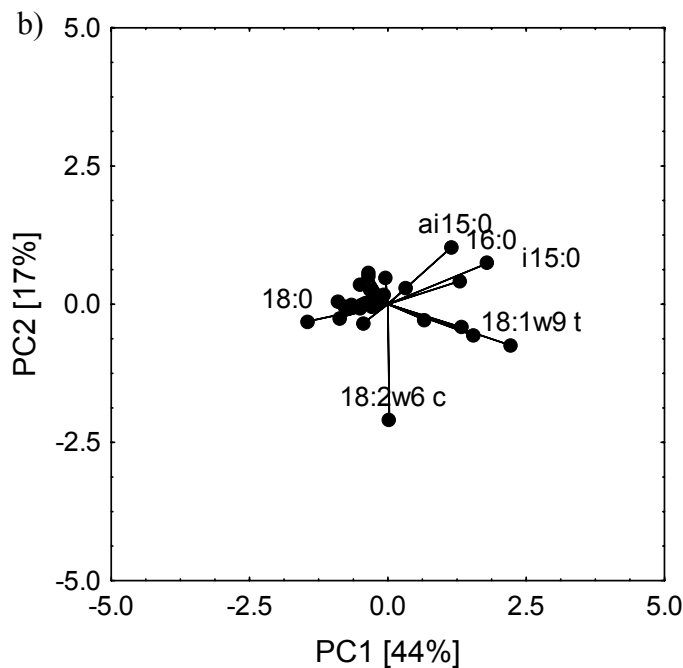
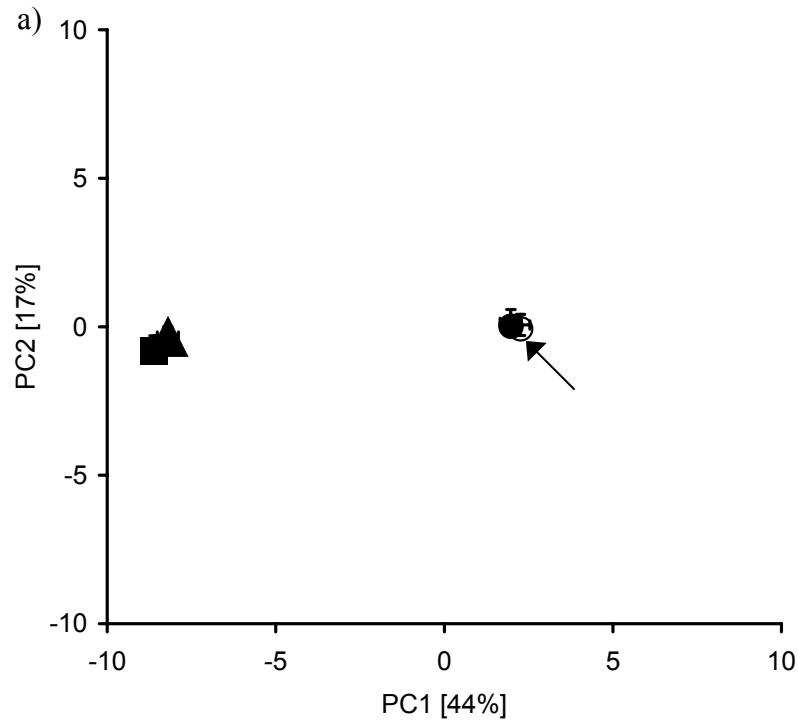
emission of greenhouse gases or reduced nutrient cycling leading to a loss of yield. Cropping practices and associated residue management also impact on the quality and quantity of substrate available and therefore again influence the soil biota (Titi, 2003). Within the field experimental design it was not possible to investigate the effect of cropping within this thesis due to the nature of the individual farm rotations (Chapters 4 & 5). As primary decomposers the quantity and composition of the microbial population will have an impact on higher trophic levels in terms of predation and wider ecosystem services.

There was evidence to suggest that conservation tillage provides a more favourable environment for earthworms than conventional tillage, increasing both numbers and biomass (personal communications; A. Rothwell, SOWAP project partner). This increase in earthworm numbers could be attributed to an increase in microbial biomass providing substrate for a larger earthworm population. An increase microbial biomass has also been shown to correlate to earthworm species diversity (Bartlett, 2006), hence this increase demonstrates the wider ecological consequences of changes in microbial populations affecting higher trophic levels.

### ***9.3 The impact of a soil microbial community on soil water movement and particle erosion.***

Small-scale experimentation has shown that microbial communities can impact on soil hydrology and erodibility at such scales (Section 3). Rainfall simulation in the field did not alter the microbial community size or structure of the surface soils and the erosion losses from these plots were not significantly different from each other (Chapter 6). However the

microbial community phenotypic structure of the runoff samples were significantly different to that of the soil before and after rainfall. This indicated that there was a specific fraction of the microbial community which was susceptible to carriage in the runoff. This result was also observed in laboratory-scale experiments (Chapters 7 & 8). In the laboratory experiments samples of the rainwater were taken as a control to ensure the microbial signals picked up were not associated with the rainwater alone. When microbial community structure (PLFA) data from the laboratory experiments and the field rainfall experiment were combined and analysed by principal component analysis, the microbial community structure contained in the runoff and infiltrate samples was significantly different to that of the soil samples in both experiments (Figure 9.1). This indicates that the microbial community leaving the soil has the same phenotypic structure whether the experiment was run in the field or in the laboratory. The loading values associated to the PCs indicate a number of biomarkers are responsible for such discrimination (Figure 9.1b). Analysis of %mol data for these biomarkers showed that the markers i15:0, ai15:0, 16:1 $\omega$ 7c, 16:0 and 18:1 $\omega$ 9t are found in higher concentrations in the soil samples. The biomarkers 18:0 and 16:1 $\omega$ 7t were found in higher concentrations in the runoff and infiltrate samples (Table 9.2).



**Figure 9.1: Phenotypic structure of samples as described by principal component analysis (PCA) of PLFA profiles; a) first and second principal components (PC); Soil profiles before (○) arrowed, and after rainfall (●), runoff (▲), and infiltrate (■). Points show means (soil n=70, runoff n=20, infiltrate n=15), whiskers show s.e. Percent variation accounted for by PCs shown in square parentheses. b) Loadings values associated with PCs.**

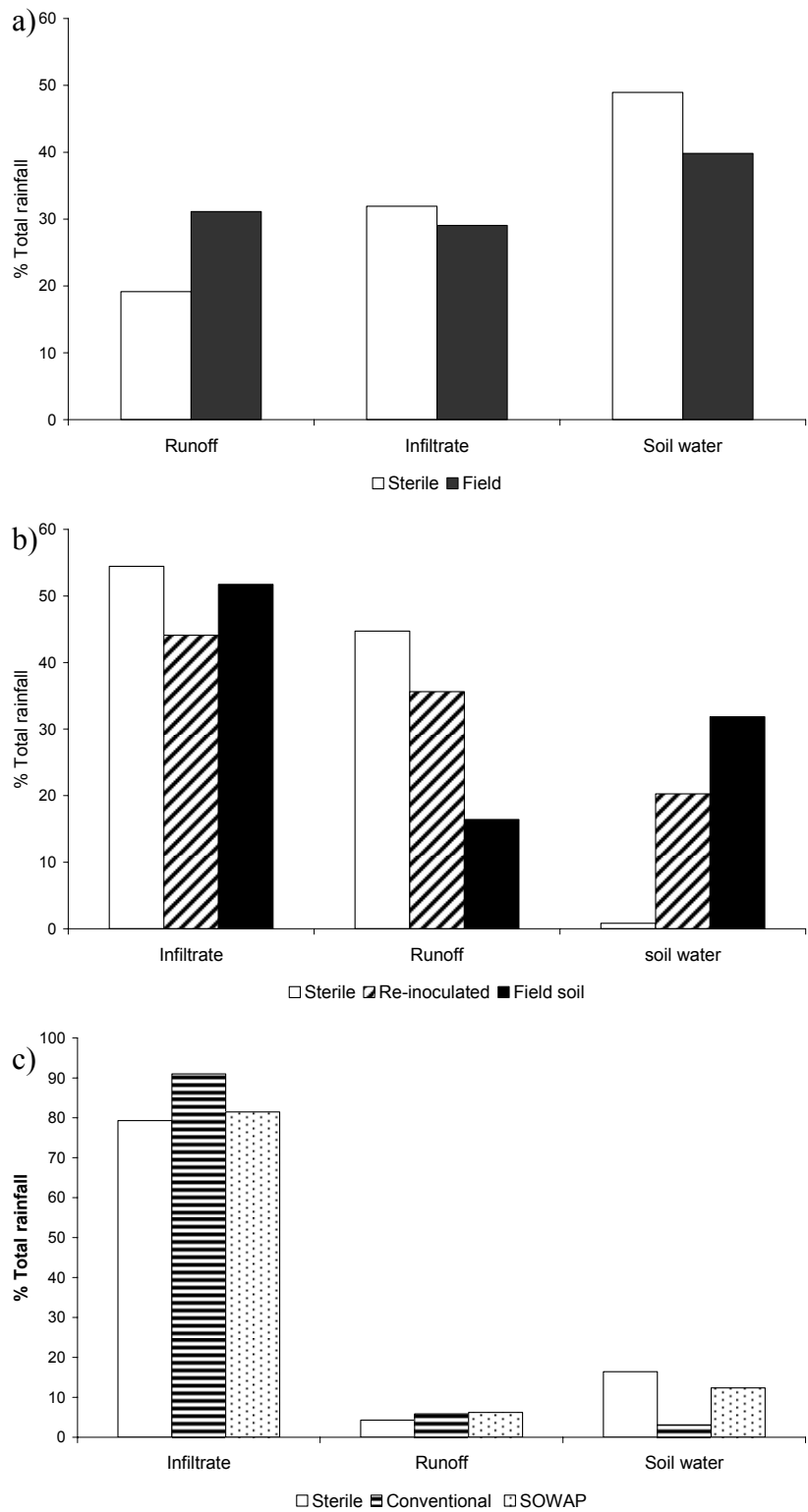
**Table 9.2: Mean biomarker values (% mol) and associated individual standard error (soil n=70, runoff n=20, infiltrate n=15)**

Sample	i15:0	ai15:0	16:1w7c	16:1w7t	16:0	18:2w6c	18:1w9c	18:1w9t	18:0
Soil samples before rainfall	5.5 ± 0.1	6.2 ± 0.2	5.9 ± 0.2	0.7 ± 0	10.2 ± 0.2	3.1 ± 0.3	8.1 ± 0.2	10.6 ± 0.2	3.6 ± 0.2
Soil samples after rainfall	5.4 ± 0.1	6.1 ± 0.2	5.7 ± 0.1	0.7 ± 0	11.0 ± 0.4	3.3 ± 0.4	7.8 ± 0.1	10.2 ± 0.2	4.3 ± 0.3
Runoff samples	2.5 ± 0.1	3.2 ± 0.1	2.4 ± 0.2	1.2 ± 0.1	7.5 ± 0.6	3.7 ± 0.3	4.7 ± 0.3	5.5 ± 0.2	7.0 ± 0.6
Infiltrate samples	2.2 ± 0.1	3.5 ± 0.3	2.9 ± 0.2	1.3 ± 0.2	6.1 ± 0.5	3.4 ± 0.3	5.4 ± 0.3	5.4 ± 0.3	6.8 ± 0.4

The experiments contained in Section 3 were designed to investigate the effects of micro-organisms on soil hydrology. Immediately prior to soil sterilisation there was no statistically significant effect of  $\gamma$ -irradiation on soil water holding capacity (Chapter 7). However, when the results are displayed as a water balance (Figure 9.2), where the data are calculated as a percentage of the total rainfall received by the experimental unit, there appears to be an increase in the moisture contained in soil and a decrease in the runoff % in the sterile soil treatment. The water balance for samples taken post incubation (Chapter 7) showed a dramatic increase in water holding capacity in the field soil but a decrease in the sterile treatment (Figure 9.2). The water balance chart for sterile soils re-inoculated with soil from different tillage practices (Chapter 8) demonstrated the impact of different microbial inocula (Figure 9.2). Soil re-inoculated from a conventional tilled soil showed a higher infiltration rate and lower water holding capacity (Figure 9.2). This data supports the hypothesis that micro-organisms will alter soil water movement; there is also evidence that the components within the microbial community will also influence water

movement (Figure 9.4), and that there are inherent properties associated with communities arising as a result of tillage that impact upon soil hydrology. These results link to the soil erodibility factor (K) within the universal soil loss equation (Chapter 1.5.1), enforcing the implicit biological component affecting soil erosion losses. The implications of microbial communities on soil hydrology also suggest that biological mediations also affect the climate erosivity factor influencing the runoff and infiltrate characteristics of the soil. The alteration of these properties by land management practice clearly influences the resultant soil loss within the confines of this equation, suggesting that this requires further consideration by erosion scientists.





**Figure 9.2: Histograms showing water balances expressed as a percentage of original rainfall received by the soil; a). Post irradiation microcosm experiment (Chapter 7); b) Post incubation microcosm experiment (Chapter 7); c) Second microcosm experiment (Chapter 8).**

This investigation indicated that microbial community size and structure has implications for runoff and erodibility at the field scale but in this case the inherent variation in natural systems concealed the dynamics of these interactions.

#### ***9.4 Experimental limitations***

The work contained within this dissertation was part of a larger EU / Syngenta project “Soil and water protection for northern and central Europe” and as such the field experimental design was prescribed and rigid. The experimental layout of the demonstration plots provided no independent replication of land management treatment and only pseudo-replication for the erosion plot study, as a result of this the demonstration field study carried out was treatment replicated in time. There was greater field replication within the farmer fields, where each field was bisected (Chapter 3). This provided a much more representative sampling for the effect of land management on microbial communities (Chapter 4), but was only able to be realised twice during the project due to the large number of samples accrued. In field trials, the ability to test hypotheses at the appropriate scale reduces the possibility of replication, so there is always a trade-off between the absolute experimental plot size and the number of replicates possible. The cost associated with this kind of field study are high, and there are inevitably compromises in terms of experimental design to balance ‘value for money’ in terms of representation of project aims. In most cases, a compromise of representative scale and replication number must be found. The use of laboratory based experiments provides greater flexibility in the number of replicates obtainable but limits the potential plot size, scale of the experiment and therefore direct applicability to the field situation. In this thesis, both field and laboratory scale experiments

were used to gain understanding of mechanistic processes occurring at small scales and how they may impact on larger scale events. The microbial phenotypic structure was shown to alter principally as a result of time and not as a result of tillage (at least over the time scale of the study), which raises the question of whether the short-term consequences of land management are of any importance at a system level. In this study there was no net change of microbial community structure in response to land management, however this study did not look at the crop interaction with the microbial community. It is conceivable that the crop effect on the microbial community masks any net effect of tillage practice. A longer time series of sampling would allow the testing of this hypothesis under the implemented crop rotations. The seasonal variation in microbial community structure may be more pertinent in terms of crop yield than the annual variation in this context however, after this 3 year study there was no apparent trend in phenotypic community structure as a result of tillage. If there is inherent resilience in the microbial community to change then the timescale of such disturbance effects may be greater than 3 years. An assessment of tillage impact on soils has been tested in the laboratory by Jackson *et al.* (2003), who used sieving as a form of simulated tillage. The sieving of soil is used in soil analytical terms to create a homogenous sample with which to analyse for a given determinand. However, sieving the soil will change the microbial phenotypic community so it is conceivable that changes as a result of sieving mask changes induced by tillage and therefore the phenotypic community expressed is that which is resilient to sieving. The use of intact soil cores for microbial analysis would also be problematic as it would require greater sample numbers in order to be representative of the original field plot and much more prone to depth effects than bulked homogenised samples. The spatial variation of

microbial communities is known to be high, strongly affected by soil texture, depth and cultivation practices (Young & Ritz, 2000). Therefore the representative sampling of such a system requires large sample sizes and numbers. Experimentation into the impact of sieving on the apparent microbial community phenotypic structure would help to understand the inherent error associated with sample preparation.

The use of more resolute community analysis techniques such as activity measures would help to further elucidate the mechanisms driving the relationships between microbial communities, soil loss and land management. For example gross respiration studies of these systems would provide functional information about the microbial community which cannot be determined using PLFA. The functionality of the microbial community could provide insight into the activity of microbial communities under different tillage systems (Lupwayi *et al.* 1998). The rate of substrate use and therefore organic matter turnover within the soil has implications for soil structural stability and productivity (Young & Ritz, 1998). Increased microbial activity as a result of residue incorporation or tillage could reduce the soils inherent structural integrity. If a microbial community under conventional tillage has an inherent resilience to perturbations then the rate of activity may be minimal in comparison to a community not used to such disturbance; this could produce a greater effect on the microbial community. This would give a clearer understanding of the impact of land management on microbial community functioning and how these changes alter the propensity of soils to erode.

The discovery that there appears to be a fraction of the microbial community that is apparently consistent and prone to mobility is a novel and potentially important finding both in fundamental ecological and environmental quality terms. It relates to the dispersal of organisms in terms of the hydrological cycle. This is pertinent in many contexts, such as, potentially providing further information for water quality, pesticide breakdown and pathogen transport. The dispersal of organisms into the hydrological cycle from terrestrial systems provides a mechanism for the colonisation of waters and of new terrestrial sites by differing microbial communities. If the community structure is altered as a result of land management or soil type then the potential community structure leached into the hydrological cycle is altered having much wider implications for our understanding of dispersal mechanisms important for evolution. If land management and soil type impact on the dispersal of microbial cells into water bodies then there is potential for further understanding pathogen transport in soils. Different community phenotypes leaving the soil system could provide different levels of ecosystem functioning in the water phase. Nutrient cycles, such as nitrogen which has stages reliant on anaerobic aqueous phases, e.g. denitrification, could be affected by the change in microbial phenotypic composition.

From the work carried out within this dissertation it is unclear whether the microbial community associated to the runoff and infiltrate are bound to soil particles or planktonic. The organisms leaving the soil may not be involved in soil structural genesis and therefore not bound to the soil matrix. However if these organisms are bound to soil particles then it could be that specific microbial communities are distinct to certain particle sizes and so

the change in microbial structure reflects the particle size distribution of erosion. The use of microbial biomass quantification techniques such as ATP determination instead of fumigation-extraction (as used in this dissertation) would provide a measure of the quantity of biomass contained in aqueous samples. Therefore, allowing the hypotheses that land management practice and history affect the quantity of biomass contained in runoff to be tested.

The mechanistic processes behind biota, soil structure and land management are complex, but by understanding these fundamental links such knowledge could be applied to all areas of land-use beyond just arable agriculture.

### ***9.5 Future research***

The research carried out within this dissertation suggests a substantive body of future research requirements at a variety of scales;

Field / plot scale:

- The impact of tillage on microbial community activity and how this impacts on the erodibility of the soil.
- The effect of residue management (as a conservation measure) on microbial communities and the impact of this on runoff and erosion of soil and microbial cells.
- Microbial community dispersal in runoff from differing soil type classifications under different land management systems.
- The impact of different microbial communities leached from soil on water quality, particularly pathogen transport, from differing land management systems, in particular the impact of livestock grazers on microbial community dynamics.
- The impact of community composition on the breakdown of agricultural amendments and their mitigation through soils in aqueous form.
- The impact of saturation, e.g. peat land soils on mobile microbial communities, how water management of these sites alters the microbial community and associated biogeochemical cycling.

Small-scale/ mixed scales:

- Assessment of mode of transport for microbial communities associated with runoff and infiltrate.
- Investigation into the effect of eroded particle size distribution and the associated microbial community size and structure.
- The effect of rainfall kinetic energy on microbial community size and structure associated with runoff.
- Stepwise isolation of soil chemical properties, such as, pH, cation exchange capacity and organic matter content to determine their effect on soil structure, erodibility and microbial community interactions.
- Time series phospholipid fatty acid  $^{13}\text{C}$ -labelling study to determine nutrient cycling properties of the microbial community associated with soil runoff and eroded soil systems.



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# **Appendix I**

## **Soil analysis methods**

**Supplied and performed by NRM Laboratories,  
Ltd (Bracknell, UK)**



### Cation exchange capacity

**Matrix:** Sample as received or air dried then sieved to pass 2mm screen

#### **9.5.1.a Principle**

Soil is saturated with Sodium Acetate (pH 7.0), and the excess acetate removed by washing with water and ethanol. The sodium ions absorbed onto the cation exchange sites of the soil are displaced with 1.0N Ammonium Acetate, and their concentration determined using a Flame Photometer (Chapman 1965).

#### **References**

- MAFF Reference Bulletin RB427, 'The Analysis of Agricultural Materials', 3<sup>rd</sup> Edition, Method 16, pp50-58.
- Chapman, H.D.,(1965). In Methods of Soil Analysis, Chapter 57, pp891-901. Editor C.A.Black, Winsconsin Am. Soc.Agronomy.

#### ***Procedural Quality Controls***

AQC - SSS series

GLP

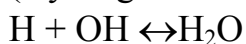
#### ***Standard Operating Procedures***

JAS-094

## Determination of the pH and Lime Requirement of Soil.

### **Introduction**

The concept of pH is based on water being made up of an acid part (Hydrogen or H ions) and an alkaline part (Hydroxyl or OH ions).



If the moisture present in the soil contains more H ions than OH ions, then the soil is described as acidic and if the OH ions are present in excess of the H ions, then the soil is alkaline. Acidity is measured on a scale ranging from 0-14 where a pH value of 7.0 represents neutrality.

The lime requirement of a soil is defined as the number of tonnes of calcium carbonate calculated to raise the pH of a hectare of soil 200mm deep (cultivated land) or 150mm deep (grassland), under field conditions, to, and maintain at, optimum pH.

Matrix: Sample as received, or air-dried at a temperature not greater than 30°C and sieved to pass a 2mm screen.

### **Principle**

The pH of soil is defined as the pH, measured potentiometrically, of the suspension obtained by stirring soil with water. The ratio of soil to water is 1:2.5. Temperature is one of the factors that affects the measurement of pH so the measurement is carried out in a controlled temperature environment.

### **References**

- The Analysis of Agricultural Materials, DEFRA Reference Book RB427
- Fertiliser Recommendations for Agricultural and Horticultural Crops, DEFRA Reference Book RB209
- ISBN 0 11 242762 6
- NRM Ltd SOP JAS-398 (Manual method) JAS-010 (Automated method)

### ***Procedural Quality Controls***

The Laboratory is fully GLP Compliant and holds UKAS Accreditation to ISO/IEC 17025 for pH on non-routine soil samples (Manual Method)

There is a minimum 5% inclusion rate of AQC.

AQC – SSA series

AQC – SSB series

AQC – SSC series

AQC – SSD series

## Determination of pH IN 0.01M calcium chloride

**Matrix:** Sample as received or air dried and sieved to pass 2mm screen

### **Principle**

The pH of soil is defined as the pH, measured potentiometrically, of the suspension obtained by stirring soil with water under controlled conditions. However, inherent and variable in every soil type is a small suspension effect, relating to soil cation exchange capacity and electrolyte concentration as well as soluble salts. This effect is generally not more than +0.3 of a pH unit. This effect is minimised by determining the pH with the soil suspended in 0.01M Calcium Chloride instead of water. The pH values determined in this manner tend to be slightly lower than, but highly correlated with, those determined in water. 0.1M and 1.0M Chloride can be used for similar reasons.

A known volume of soil is mixed with Calcium Chloride, allowed to stand for 10 minutes and then the pH measured potentiometrically using a calibrated Russell K-Series Electrode.

### **References**

- The Analysis of Agricultural Materials, MAFF Reference Book RB427 ISBN 0 11 242762 6

### ***Procedural Quality Controls***

AQC – SSA series

AQC – SSB series

AQC – SSC series

AQC – SSD series

AQC – COS series

AQC – SGS series

AQC – SMS series

GLP

### ***Standard Operating Procedures***

JAS-0135

## Determination of organic matter

**Matrix:** Sample air dried sieved to pass 0.5mm screen

### **Principle**

Soil organic matter is almost completely oxidised by with a solution of potassium dichromate, sulphuric acid and orthophosphoric acid. Excess dichromate is determined by titrating with ferrous sulphate solution.

This procedure is known as wet oxidation or Walkley Black.

### **References**

- Walkley, A, and Black, I.A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 34:29-38.
- Tinsley, J., 4<sup>th</sup> Int. Congr. Soil.Sci, 1950, 1,161.

### ***Procedural Quality Controls***

AQC – SSS series

AQC- SSA series

AQC-SSB series

AQC-SSC series

AQC-SSD series

AQC- SMS series

GLP

### ***Standard Operating Procedures***

JAS-093

## Determination of Olsen's Extractable Phosphorus in Soil

### **Introduction**

Soil Tests for Potassium, Magnesium and Phosphorus are designed to rapidly assess the available nutrient status of soils and serve as the basis for making recommendations for the addition of plant nutrients needed to achieve optimum yields.

'Available' Phosphorus is defined as the portion of the total phosphorus-containing constituent of a soil that could become nutritionally available to the plant in the soil solution.

**Matrix:** Sample as received, or air-dried at a temperature not greater than 30°C and sieved to pass a 2mm screen.

### **Principle**

A variety of chemical extractants have been developed to mimic the soil situation, thereby obtaining an assessment of the potentially plant-available phosphorus. One of the most commonly used extractants is 0.5M sodium bicarbonate known as Olsen's Reagent

The available phosphorus is extracted from the soil at 20°C by shaking with 0.5M sodium bicarbonate solution at pH 8.5. Inorganic phosphorus then reacts with acid ammonium molybdate to form the phosphomolybdate ion, which, when reduced with ascorbic acid, forms a blue coloured complex. The blue colour is measured spectrophotometrically at 880nm.

### **References**

- The Analysis of Agricultural Materials, DEFRA Reference Book RB427 ISBN 0 11 242762 6
- Olsen SR, Cole CV, Watanabe FS and Dean LA. US Dept Agric. Circ. No 939, 1954.
- NRM Ltd SOP JAS-400

### ***Procedural Quality Controls***

The Laboratory is fully GLP Compliant and holds UKAS Accreditation to ISO/IEN 17025 for Available Phosphorus on non-routine soil samples.

There is a minimum 5% inclusion rate of AQC.

AQC – SSA series

AQC – SSB series

AQC – SSC series

AQC – SSD series

## Determination of Ammonium Nitrate Extractable Magnesium in Soil

### **Introduction**

Soil Tests for Potassium, Magnesium and Phosphorus are designed to rapidly assess the available nutrient status of soils and serve as the basis for making recommendations for the addition of plant nutrients needed to achieve optimum yields.

**Matrix:** Sample as received or air-dried at a temperature not greater than 30°C and sieved to pass a 2mm screen.

### **Principle**

A variety of chemical extractants have been developed to mimic the soil situation, thereby obtaining an assessment of the potentially plant-available magnesium. One of the most commonly used extractants is Molar Ammonium Nitrate.

The available magnesium is extracted from the soil by shaking with M ammonium nitrate at 20°C for 30 minutes. After filtration, the concentration of magnesium in the extract is determined by atomic absorption spectroscopy. The addition of a releasing agent to the sample before analysis eliminates interference by phosphate.

### **References**

- The Analysis of Agricultural Materials, DEFRA Reference Book RB427 ISBN 0 11 242762 6
- NRM Ltd SOP JAS-399

### ***Procedural Quality Controls***

The laboratory is fully GLP Compliant and holds UKAS Accreditation to ISO/IEN 17025 for Available Magnesium on non-routine soil samples.

There is a minimum 5% inclusion rate of AQC

AQC – SSA series

AQC – SSB series

AQC – SSC series

AQC – SSD series

## Determination of nitrogen, carbon, hydrogen and sulphur

**Matrix:** Samples dried and ground to pass 0.5mm screen

### **Principle**

Samples are totally combusted in an oxygen enriched atmosphere in a reaction tube.

Nitrogen, carbon, hydrogen and sulphur products are carried by a constant flow of carrier gas (helium) through an oxidation catalyst, and then through reduced copper wires, where excess oxygen is removed and nitrogen oxides are reduced to elemental nitrogen.

The nitrogen, carbon, hydrogen and sulphur products are separated through a chromatographic column. As the products are eluted from this column they pass through a T.C.D detector, which generates an electrical signal proportional to the amount of nitrogen, carbon, hydrogen and sulphur present. Various products can be eliminated if required using various traps, such as a magnesium perchlorate trap to eliminate hydrogen. Peak elimination reduces the risk of overlapping peaks and shortens run times.

### **References**

- AOAC Official Methods of Analysis (1990) Method 949.12.

### ***Procedural Quality Controls***

CRM Plant Tissue

AQC – GST series

GLP

### ***Standard Operating Procedures***

JAS-373

JAS-371

JAS-370