The Role of the Soil Microbial Community in Decomposition in a Raised Mire System

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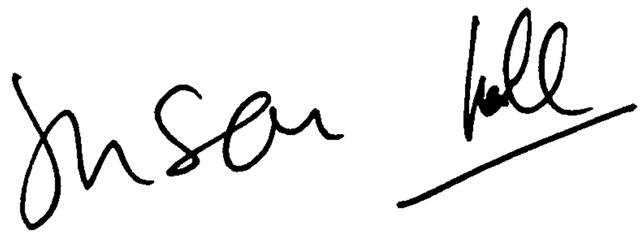
Candidates Declaration

I hereby declare that the following thesis is my own composition. This work has not been previously been accepted for a higher degree.



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Abstract

Peatlands make up 3% of the earth's land surface and contain about one third of the C contained in soils globally. The role of peatlands in the C cycle is as a net sink. Organic matter accumulates in these areas because the rate of net primary productivity (NPP) exceeds the rate of decay. Peatlands are often harsh environments, characterized by cold, wet and anoxic conditions, therefore it is not accelerated NPP which exerts the main control over the accumulation of peat, but the slow rate of decomposition. During the decomposition process nearly all organic matter passes through the soil microbial pool, and so the soil microbial community is an important factor in the decomposition process. Despite the obvious importance of the soil microbial community in decomposition in peatlands, our knowledge of their role in peatland C cycling is still largely limited. This thesis addresses some aspects of the soil microbial community and investigates their role in decomposition in a raised mire. The soils in a raised mire system may be categorized according to their nutrient input into nutrient rich, mineral soils and soils of the lagg fen, and nutrient poor, soils of the mire expanse. The soil microbial community in the three soils was characterized in terms of size, activity and composition. The size of the soil microbial community in the soils of the mire expanse was small in comparison with that of the mineral soils and soils of the lagg fen, however it was very active. The hypothesis that nutrients restrict the size of the soil microbial community in the soils of the mire expanse was tested. The data showed that nutrients did not significantly effect the size of the soil microbial community. Litterbags were used to investigate the decomposition of a range of plant species found on the different soils and mass loss and CO_2 production were used as indicators of decomposition. CO_2 production was a more sensitive and reliable measure of decomposition than mass loss. The size of the soil microbial community was an important factor in decomposition rate. Litter quality of the above ground biomass was not related to decomposition rate. The relationship between the size of the microbial community in contact with decaying plant material and decomposition was investigated. In this study, microbial colonization of decaying litter was not correlated with the measure of litter quality used. This work has provided baseline information the environmental factors that influence decomposition and future work should focus on investigating the changes in the soil microbial community during the decomposition process.

Chapter 1 Introduction

1.1 Global Carbon cycling

Carbon (C) is the major element in living organisms, making up approximately 50% of organic matter. The C cycle is closely linked to energy flow and many energy stores are in the form of reduced carbon. Indeed the majority of C is not in circulation but in sediment storage. One of the most useful ways to consider the carbon cycle is to examine the C budget and size of the pools involved (fig. 1.1).

In the global C cycle, there are three major components: atmospheric, terrestrial and oceanic. Within these individual systems there is a lot of C locked up e.g. deep sea sediments and fossil fuels, however, it is the transfer of C between these components and the consequent exchange of energy that fuels biological systems.

In 1995 there was an estimated 765 Pg C in the atmospheric C pool and this was undergoing an estimated net annual increase of 3.4 Pg yr^{-1} (Paul and Clark, 1996). The mechanisms involved in this increase are the result of a complex balance of the different components of the global C cycle. The oceanic component of the C cycle has a vast buffering capacity, hence implications for the atmospheric C component. The amount of dissolved C is an important mediator of C exchange. The turnover rate and storage of C in the oceans is greater than in the terrestrial component. The four major exchanges that occur within the terrestrial system are decomposition, respiration, and use/agriculture, which lead to a net movement of C to the atmosphere as CO_2 and, gross primary production, which transfers C from the atmospheric pool to the terrestrial system. The mean turnover rate for plant material using these figures is about 10 years. This value is much greater than in oceanic systems where the organisms account for 3-5 Pg but photosynthesis and decay are similar to the terrestrial environment. Where primary production exceeded decay on a geological time scale then fossil fuels have formed. However, through anthropogenic use there has been a transfer of these stores to the atmospheric pool. In this scenario, the oceans are important as a buffer, although some terrestrial ecosystems may be important for reclaiming lost C.

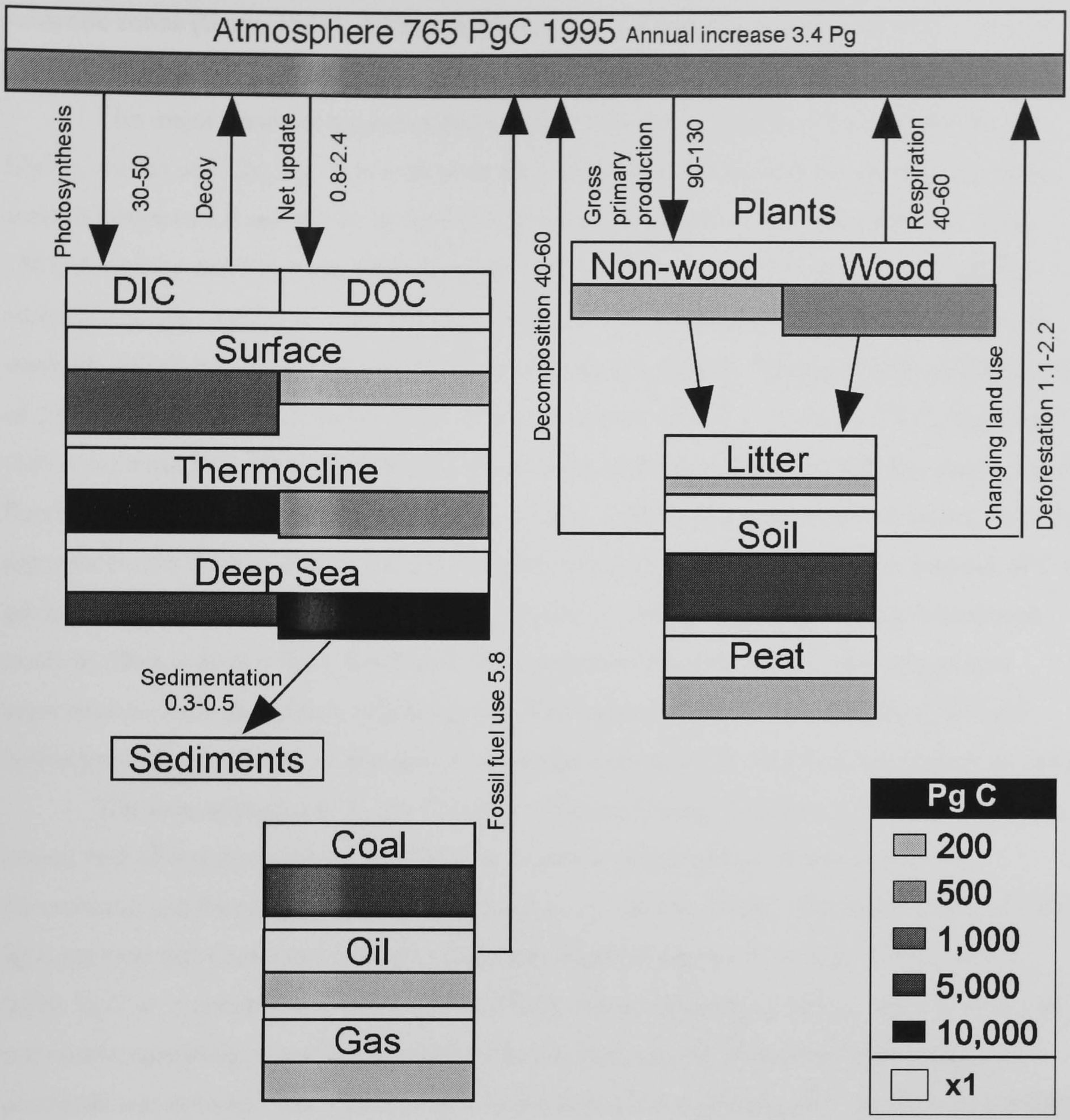


Figure 1.1: The carbon cycle budget. The key relates to the amount of carbon in each component of the C cycle, where the area of the x1 box represents the scale. DIC and DOC are dissolved inorganic carbon and dissolved organic carbon respectively. Adapted from Paul and Clark (1996).

1.1.2 The role of peatlands in the global C cycle

In most ecosystems productivity and decomposition are approximately in balance (Gorham, 1991). However, where the rate of net primary productivity (NPP) exceeds the rate of decay, organic matter accumulates as peat (Clymo, 1965; Malmer 1986; Farrish and Grigal, 1988; Vitt, 1990). Peatlands are characteristically waterlogged environments where cool and anoxic conditions contribute to an accumulation of organic matter from 30-40 cm depth to several metres. These conditions lead to extensive coverages of land surfaces especially in boreal and

subarctic zones (Gore, 1983). Such ecosystems act as a sink for C; sequestering C from the atmospheric pool (Billings *et al*, 1987; Sundh *et al*, 1997).

The major peatland expanses are found in Scandinavia, Eastern Europe, Western Siberia, Alaska and Canada. It is estimated that the total area covered by peatlands in these areas is between 2.4 and 4.1 X 10^6 km^2 (Kivinen and Parkarinen, 1981; Mathews and Fung, 1987; Aselmann and Crutzen, 1989; Gorham, 1991). The major peatlands in the Southern Hemisphere are small by comparison, for example New Zealand has a total of 3113 km^2 of wetlands, which is mainly peatland (Mitsch and Gosselink, 2000). Using an estimated total area of 3.46 X 10^6 km^2, a mean depth of 2.3 m, a bulk density of 112 g l^{-1} and 51.7 % C, Gorham (1991) estimated the total amount of C in peatlands globally to be about 455 Pg. Assuming that Post's (1982) estimation for the total soil C pool at 1395 Pg is a good approximation, then this represents one third of the total C pool in soils. Another way to consider the amount of C in peats is to compare the C in peats with that in the overlying vegetation. Using calculations made by Ohlson *et al* (1983), Gorham (1991) estimated the mean C content of peatland vegetation to be in the region of 2000 g m^{-2}. This value compares with 133000 g m^{-2} held below ground. Therefore, of the total C in peatland ecosystems 98.5 % is held below ground.

The role of peatlands in the C cycle is as a net C sink. Gorham (1991) estimated the annual rate of sequestration to be 0.096 Pg, however this could be as low as 0.05 Pg (Armentano and Menges, 1986) or as high as 0.11 Pg (Silvola, 1986). The lower value of 0.05 Pg is perhaps too conservative due to the mean depth of peatlands used (Gorham, 1991). 0.096 Pg C yr^{-1} equates to a mean depth of 0.53 mm yr^{-1} (Gorham, 1991). Sjors (1982) ruled out recent spreading of peatlands, stating that the main period of horizontal expansion of peatlands was between 5000-2000 yr BP. Considering the quantities of C involved in peatland ecosystems it is fundamentally important that the overall balance between NPP and decomposition is not altered. Indeed, it is the slow rate of decomposition that maintains the status of peatland ecosystems as a C sink (Heal *et al*., 1978; Dickinson, 1983; Farrish and Grigal, 1988; Moore, 1989). Several factors may upset this balance (Waksman and Stevens, 1929; Clymo, 1965; Coulson and Butterfield; 1978;) for example, drainage and combustion, however, because decomposition is very slow in these environments it is difficult to measure the impact that changes have on the decomposition process. Our understanding of the microbial decomposition process and the factors controlling it is fundamental to our management of peatland ecosystems and hence to the global carbon cycle.

1.2 Peatland development

There are two factors which determine the development of peatlands, a positive water balance and greater NPP than decomposition (Mitsch and Gosselink, 2000). The peatland system may develop in one of two ways when these factors are present. Terrestrialization is the name gives to the infilling of shallow basins by vegetation, and paludification is used to describe the blanketing of terrestrial ecosystems by the overgrowth of peatland vegetation. Paludification generally requires the presence of gently sloping landscapes. Three major bog forming processes are commonly seen: quaking peatland succession, paludification and flowthrough succession. Quaking peatland succession and flowthrough succession both refer to the infilling of basins; in the former organic matter builds up on the surface of a lake, and in the latter, material settles on the bottom of the basin and gradually builds up.

1.2.1 Landscape development

Developmental processes are responsible for the production of landscape features. These features may take four main forms (Mitsch and Gosselink, 2000): raised mire (peat deposits that fill entire basins and are raised above groundwater levels), aapa peatlands (long narrow strings of hummocks perpendicular to the slope), paalsa peatlands (also referred to as patterned fens are large plateaus of peat underlain by frozen peat) and blanket bogs (large expanses of peat that are much greater in area than the original coverage). Raised mires get their name from their characteristic domed shape, at the margin of which there is a rand (fig. 1.2), and a lagg fen. As a consequence of the raised nature of these mires, the raised area (mire expanse) is not connected to the groundwater supply, and so it only receives water input from precipitation i.e. it is ombrogenous. The term ombrogenous and oligotrophic are often used interchangeably because the concentration of nutrients in rainwater is low, however ombrogenic refers to the origin of the water input and oligotrophic refers to the lack of available nutrients. The lagg fen of raised bogs receives water flowing through the surrounding mineral soils, which is sometimes called telluric water. Water accumulates in this area, and in the winter months, or when rainfall is high, excess water is transported away in a temporary lagg stream.

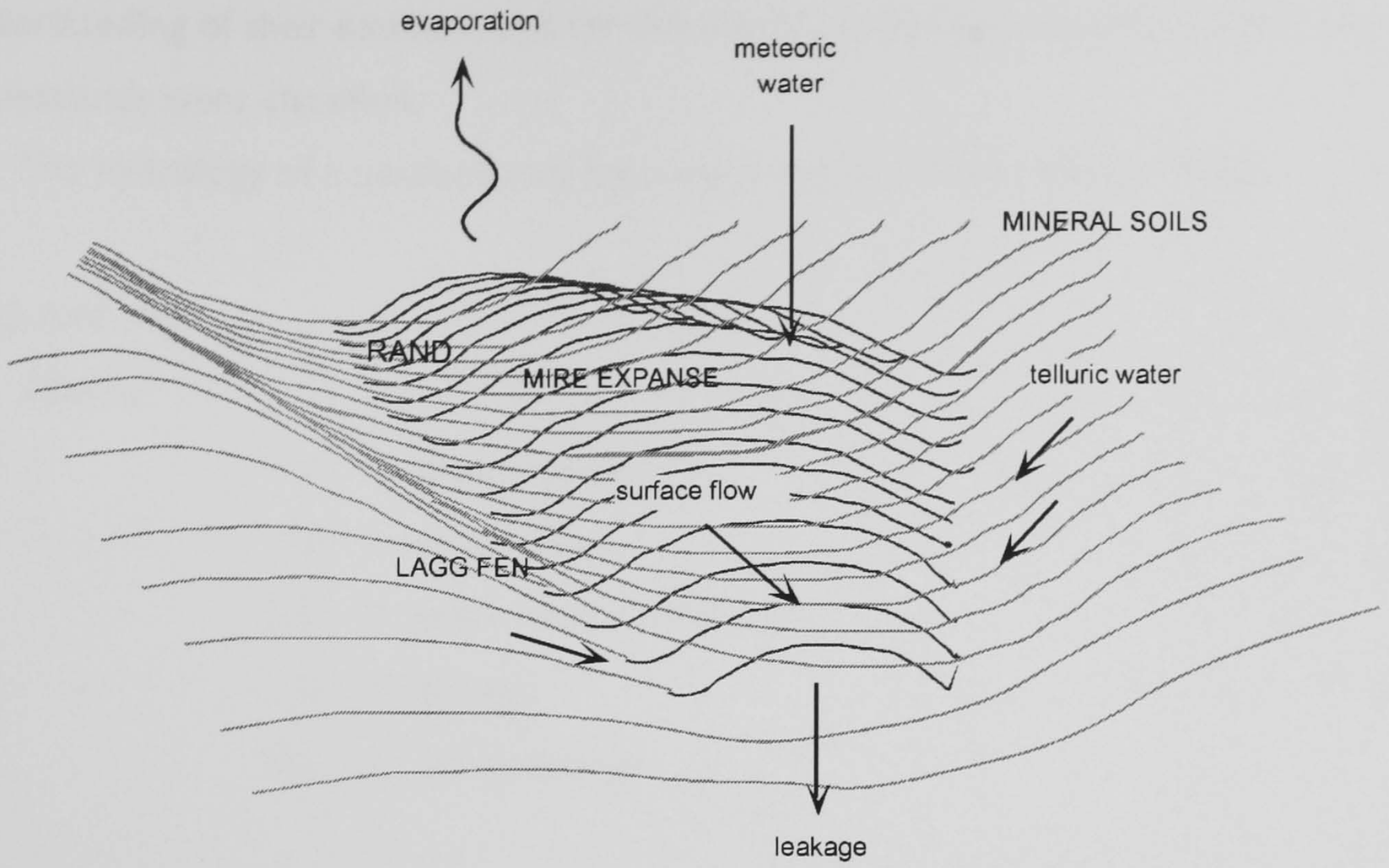


Figure 1.2: Cross section view of a raised mire showing the water flow in the system. The mire expanse is raised above the groundwater supply and joins the lagg fen at a sloping margin (rand). The mire expanse does not receive water from the surrounding soils.

1.2.2 Classification of peatlands

Peatlands have been classified according to a range of features, depending on the interests of the researcher. Moore (1984) described seven features on which classification schemes have been based; floristics, vegetation structure, geomorphology, hydrology, chemistry, stratigraphy and peat characteristics. Chemical and hydrological classification systems have been the most popular classification schemes, however the complexity of the ecosystems involved has lent itself to confusing nomenclature. Indeed the issue of classification is still topical in peatland discussion groups. In order to avoid the use of confusing terms this thesis uses the outlined below (Mitsch and Gosselink, 2000):

- *Bog*: peatland receiving water exclusively from precipitation and not influenced by groundwater; Sphagnum dominated peatlands.
- *Fen*: peatland receiving water rich in dissolved minerals; vegetation cover composed dominantly of gramnoid species and brown mosses.

1.2.3 Hydrology

Peat may contain as many solids as milk (Smit, 1996) therefore, water is an important component of peatland ecosystems. The origin of water in peatland ecosystems is crucial to

the understanding of their function, and for this reason, hydrology was one of the first ways in which peatlands were classified.

The hydrology of a peatland may be summarized as follows (Gore, 1983):

$$P-E-U-G-\Delta W = 0$$

Where:

P = precipitation
E = evaporation
U = groundwater discharge
G = leakage
ΔW = water storage

1.2.3.1 Water input

There are two possible origins of water in peatlands: water from precipitation and water that has travelled through surrounding soils. Bogs only receive water from precipitation because they are raised and are not connected to the groundwater supply (Gore, 1983). Fens on the other hand, receive water both from precipitation and from surrounding soils. Although the water input to bogs and fens is distinctly different in natural systems, the division is less clear and many gradients are involved. More importantly, it is very difficult to quantify the relative influence of the two sources accurately in the field. This thesis will use the terms meteoric and telluric to describe water from precipitation and water input from mineral soils respectively.

1.2.4 Stratigraphy

Peats differ from mineral soils in their development; mineral soils develop from the bottom of the soil profile, whereas the new material in a peat profile is added at the top. Therefore, it is not possible to describe peat soil profiles using the same schema used for mineral soils. Ingram (1978) referred to 'horizons' in the peat profile as two main layers: the acrotelm and catotelm. The basis for this classification system is the 'activity' within the layers. Activity in this sense is used to describe water movement, with the boundary of the two layers being the water table (fig 1.3). With this in mind, the depth of the layers depends on the height of the watertable, hence it is not static. The uppermost layer in the peat profile is referred to as the acrotelm, and this extends between a few centimetres and half a metre deep in the peat. In this region

there is a free movement of water in the peat horizontally and vertically. Clymo (1992) further split this into two sub-layers: a euphotic layer and a dead layer. Essentially, the euphotic layer is where photosynthesis is actively occurring and temperature fluctuations are large. There is a fairly porous structure to the peat in this region which is composed 10% water and 9% gas. Immediately below this sub-layer (still in the acrotelm) lies a darker layer, which light is not able to penetrate. The structure is still very porous, allowing water movement and gas exchange for aerobic decay, however unlike in the euphotic layer, most of the material here is dead.

The layer below the acrotelm is permanently waterlogged and allows little water movement. The catotelm is subdivided into the layer of collapse and the lower peat. The layer of collapse has a much more compact structure than the overlaying acrotelm due to the progressive decay of plant litter by microorganisms. Therefore the bulk density here is typically 5 times greater than in the acrotelm (Clymo, 1984a). This layer may range from a 2-15 cm in depth. The region below the layer of collapse is by contrast 10-1000 cm deep. In this layer increasing reducing conditions occur, rendering the environment less hospitable for biological habitation. Anaerobic processes predominate in this region and the resultant products may also accumulate here. In this region temperature fluctuations are usually small and the decay rate is much less than in the acrotelm.

Carbon balance and hence the rate of accumulation are strongly influenced by the acrotelm in peatlands (Clymo, 1983). The principal decomposers in acidic environments are fungi, and these are aerobic. Therefore, it is in the acrotelm where O_2 is available that decomposition is important in peats. Dickinson (in Gore, 1993) points out that 'the failure of microorganisms to complete decomposition of tissues which have become incorporated into the mire surface, or to destroy materials formed *in situ* within the mire surface is the main cause of peat formation'.

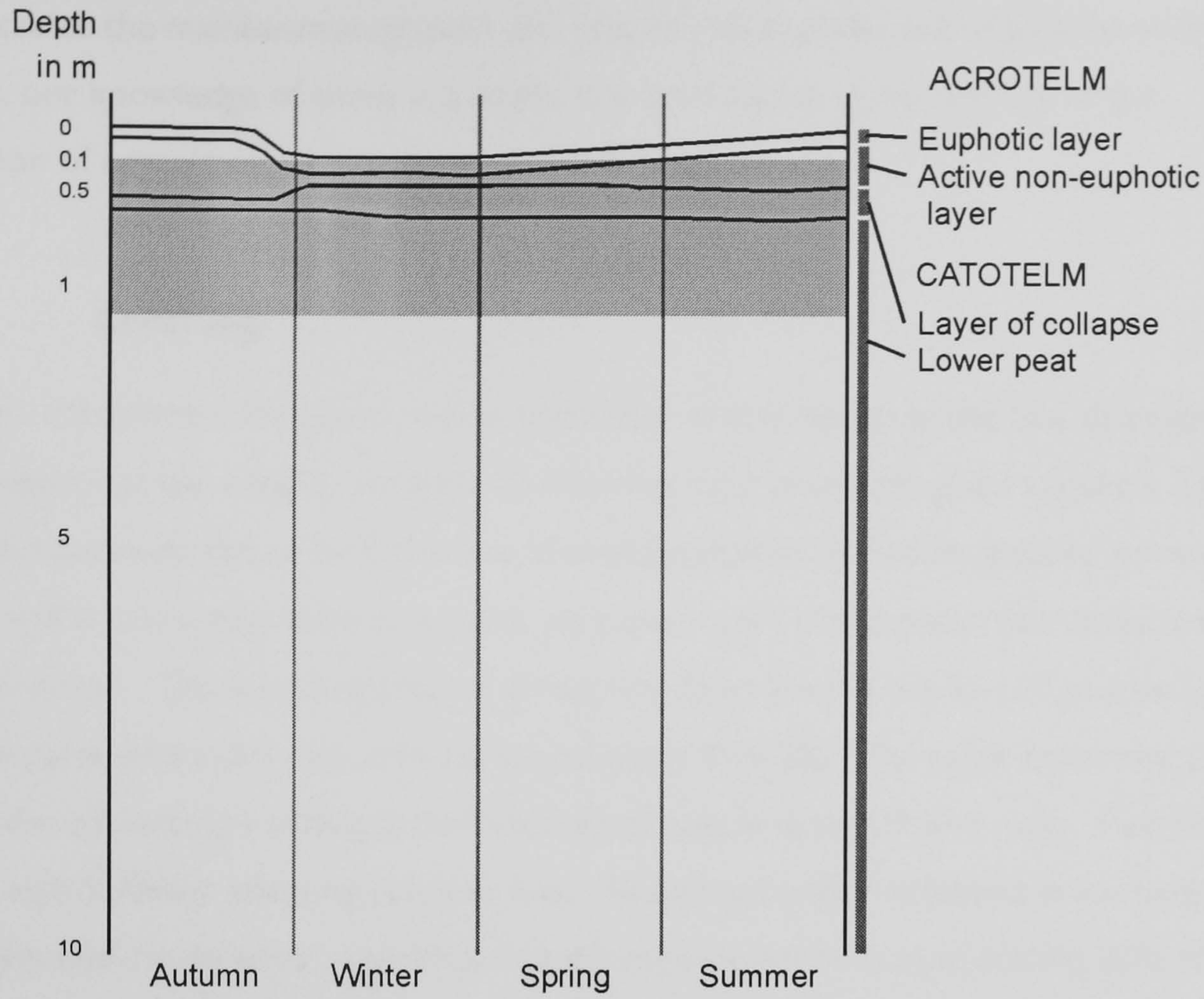


Figure 1.3: Diagramatic representaion of the peat profile showing the change in the structural zones with season. The grey region represents the limits of the watertable, which is highest in Winter. (Adapted from Clymo, 1992)

1.3 Decomposition

Decomposition is the process by which resources required for plant growth are recycled in the environment. During the decomposition process, detritus is broken down by physical and chemical means to release nutrients and other inorganic elements. Should the decomposition process fail, then organic matter would accumulate, plants would be unable to obtain nutrients for growth and there would be a depletion of CO_2. The ultimate consequence of a relapse in decomposition would be devastating: all biological processes would stop.

There are three main sub-processes involved in decomposition; leaching, fragmentation and biochemical alteration. Leaching is the initial process to affect decaying material and this involves the transportation of water-soluble compounds away from the decomposing organic matter. Next, soil animals are responsible for breaking up larger pieces of organic matter into smaller fragments that have a greater surface area, fragmentation. Then there is biochemical alteration of these small fragments, which occurs mainly due to bacteria and fungi. During these complex interrelated processes some carbon is released as CO_2 and nutrients are recycled to plants and animals. The evolutionary forces that shape decomposition are those

which maximize the maintenance, growth and reproduction of the soil organisms involved Therefore, our knowledge of these organisms is crucial to the understanding of the accumulation of organic matter in peatland ecosystems.

1.3.1 Leaching

Leaching is a rate-determining step only in fresh litter and it results in the loss of mineral ions and small water soluble organic compounds from recently senescent plant material. During senescence plants attempt to limit the loss of useful nutrients by breaking down compounds in the leaves and transporting them to a more permanent part of the plant; this process is aptly named resorption. The main importance of leaching from litter is the loss of nutrients, however in mass terms this may account for no more than 5%. The most important plant related factor influencing leaching is the ratio of cell contents to cell wall ratio. Rainfall is an important abiotic factor effecting nutrient loss. The compounds implicated in leaching are mainly sugars and amino acids. Leaching, and the consequent release of readily utilizable nutrients support a pulse of microbial activity in the autumn.

1.3.2 Fragmentation

The main consequence of fragmentation is to increase the surface area available for microbial attack. Plants produce protective layers to prevent against unwanted attack from pathogens. However, these barriers hinder the decomposition process. In leaf material this layer is called a cuticle and it is resistant to microbial attack because of lignin impregnated in the cell walls. Fragmentation serves to pierce these protective sheaths, and by increasing the surface area to volume ratio, it enhances decomposition. There are several ways in which fragmentation occurs and these can be categorized into abiotic and biotic factors (Paul and Clark, 1996). The climate can effect fragmentation by initiating freeze-thaw and wetting-drying cycles. Mammals tear apart wood and mix the soil as they search for food at the soil surface. Earthworms are a major contributor to fragmentation in grassland ecosystems. As 'ecosystem engineers' earthworms ingest and move soil, reduce bulk density and break up soil aggregates. The soil microbial community also contributes to fragmentation as a loss of physical strength may occur due to microbial attack.

1.3.3 Microbial transformations

The soil microbial community can be sub-divided into bacteria and fungi, and these have different roles in decomposition attributable to the features of the individual organisms involved and their ecology. It is important to note that nearly all organic matter passes through the soil microbial community during decomposition, and therefore the community has a crucial role in C cycling and ecosystem function. Figure 1.4 shows the main routes of C cycling. Readily decomposible C and moderately decomposible C may be assimilated directly into the microbial biomass. Resistant C, on the other hand, may become part of the plant and microbial byproducts and/or humus before it is transferred through the microbial biomass.

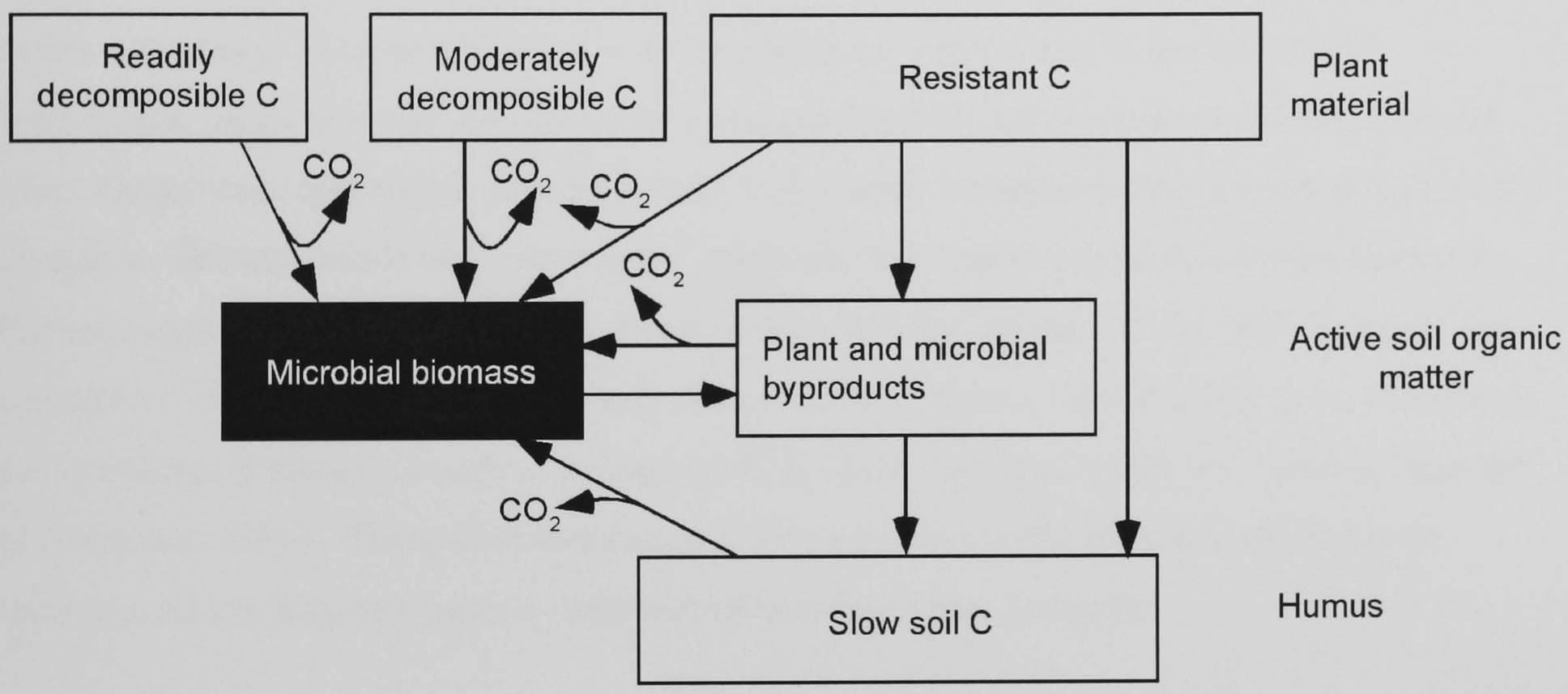


Figure 1.4: The role of living organisms in the production of soil organic matter. (Adapted from Paul and Clark, 1996)

1.3.3.1 Fungi

When plant litter falls to the soil surface, fungi are the first to colonize the new resource. Fungi have an advantage over bacteria for this job because they are able to import essential nutrients from other places in the soil. This is a significant advantage to the pioneers of fresh plant litter because not all of the nutrients needed may be available. In addition to their hyphal transport network, fungi have the ability to penetrate the cuticle of dead leaves or the suberized exterior of roots to gain access to the inside of the dead plant organ (Paul and Clark, 1996). Fungi breakdown the resistant lignin component in the cell walls in order to reach more labile compounds. In doing so, fungi make way for other organisms, in particular bacteria. It is because fungi can break down almost all classes of plant compounds that they have a

competitive edge over bacteria in the initial stages of decomposition. However, when more N becomes available, fungi are outcompeted by bacteria. Conditions become unfavourable for fungi when the pH increases and when O_2 becomes scarce.

1.3.3.2 Bacteria

The small size and large surface area to volume ratio of bacteria enable them to take full advantage of soluble substrates when they are readily available. Bacteria are particularly important in lysing and breaking down live and dead bacterial and fungal cells. However, a main limitation of bacterial cells is that they are dependent on substrates that diffuse. The bacterium governs this availability of substrates to some extent by producing exoenzymes that produce soluble substrates. However bacteria are susceptible to water flows in the soil, plant transpiration, evaporation at the soil surface and gravitational water movement after a rainfall event. Larger macropores are prone to more rapid water movement than are densely packed aggregates. Bacteria work as a 'community', each member being responsible for producing a different range of enzymes required to break down complex tissues. In the main, bacteria are non-motile. Therefore, a bacterial colony will eventually exhaust the substrate in its immediate environment and become inactive. Indeed, 50-80% of the bacteria in soils are inactive (Norton and Firestone, 1991). These dormant bacteria will reactivate in the presence of new labile substrates; hence they represent a reservoir of decomposition potential.

1.3.3.3 Soil animals

Bacteria and fungi are grazed by small soil animals. These predators are composed of the soil microfauna: protozoa, ciliates and amoeba, and mesofauna: of which the nematodes are the most abundant. When these soil animals die, they act as a fresh substrate supply to the soil microbial community.

1.3.4 Temporal pattern of decomposition

The rate of decomposition changes with time and this is best understood by outlining three important phases in the process. As soon as senescent litter hits the soil surface, amino acids, sugars and other soluble compounds are lost because of leaching. This phase is relatively rapid, and may account for up to 5% of mass loss from litter in a relatively short period after litter-fall. The second phase involves all three decomposition processes; leaching, fragmentation by

soil animals and chemical alteration by microbes. Chemical alteration and leaching dominate the third phase.

1.3.5 Spatial pattern of decomposition

Most decomposition occurs near the surface of soils, where litter inputs are concentrated. It is here where most of the initial N and P is lost. Roots also grow in the upper horizons of the soil profile, and so root litter is produced here. The rate of decomposition is greatest at the surface because of these factors and the high activity of soil animals in the surface region.

1.3.6 Factors controlling decomposition

The factors controlling decomposition can be categorized as follows: types of decomposer organisms, the quantity and quality of substrates, and the physical environment (Swift *et al.*, 1979b). Each of these factors will be discussed in turn.

1.3.6.1 Decomposer community

The decomposer community is characterized by being genetically and functionally more diverse than any other trophic group. The food webs of the soil decomposer community are more important and more complex that those of plants and are wide ranging: bacteria, fungi, Archaebacteria (obligate anaerobes), protozoa and many phyla of multicellular animals. There are three aspects of the soil microbial community to consider when determining its role in decomposition. First, information is needed about the size of the community. Second, an assessment of the activity of the soil microbial community is required. Without this information, it is impossible to determine the ecological significance of the microbial biomass on the C cycle. Third, there is a wide range of plant materials involved therefore it is useful to know which organisms are present and what their metabolic capabilities are.

It has long been known that peat soils are not sterile environments (Waksman and Stevens, 1929; Waksman and Purvis, 1932). Many researchers have attempted to quantify the microbial biomass in peat soils (Williams and Sparling, 1984; Sparling and Williams, 1986; Hart *et al.*, 1986; Groffman *et al.*, 1996; Schipper *et al.*, 1998; Brake *et al.*, 1999). Culture techniques fail to identify the whole soil microbial community, and microscopic techniques are time consuming and require very skilled work. As a consequence of these limitations, and perhaps the lack of research interest in the soil microbial communities of peatlands, data on the

taxonomical distribution of different microbial groups of wetlands are very scarce (Úlehlová, 1999).

Activity of the decomposer community in wetlands has been measured using a number of approaches. One such method is the use of litterbags to determine the decomposition of standard substrates (often cellulose) or natural plant material in the peat soils. From this experimental approach it is possible to calculate a decay constant for a given soil and plant species. Úlehlová (1999) summarizes the information that has been reported for wetlands. Despite the fact that the litterbag technique is a useful tool to determine actual decomposition rates in the field (Belyea, 1996), there is a great deal of variation in the plant species used and mesh size of the litterbags, and so comparisons are somewhat limited. Measurements of the rate of evolution of CO_2 from the soil surface provides an integral and holistic approach to determining the activity of soil microorganisms in peatlands (Úlehlová, 1999). These methods have been used successfully *in situ* and in the laboratory to estimate decomposition rates in peat soils. However in general most of the methods used for determination of microbial biomass and activity were originally developed for use with mineral soils and some limitations have been reported with the use of these methods for determining microbial biomass and activity in peat soils (Brake *et al.*, 1999).

In terms of the occurrence and distribution of functional and taxonomically groups of microorganisms, peatlands have been investigated much less thoroughly than aerated terrestrial soils (Sundh *et al.*, 1997). Many species have been identified from peats (Baker, 1970; Martin *et al.*, 1982; Williams and Crawford, 1983; Nilsson *et al.*, 1992). However, there is limited information about the quantitative contribution of the organisms involved (Sundh *et al.*, 1997). Even fewer studies have been carried out on the metabolic diversity of the soil microbial community in peat soils. This exemplifies the need for studies combining the quantitative aspects of the soil microbial community (i.e. biomass size and activity) with qualitative aspects.

The metabolic quotient of a microorganism is a measure of how active it is per unit of biomass. There are many interpretations of the metabolic quotient, one of the more common ones being CO_2 respiration per unit of biomass. When activity is presented as respiration per amount of microbial biomass it is possible to estimate the efficiency of the organisms involved. At greater metabolic quotients more C is used and released as CO_2 for the maintenance of a smaller and less efficient microbial biomass.

1.3.6.2 Substrate quality

Where moisture and temperature are favourable, substrate quality is the most important control on decomposition (Couteaux *et al.*, 1995).

The ratio of carbon to nitrogen may be used to determine the extent to which N may be limiting in soils. Typically, bacterial C:N ratio is in the region of 3-5. Assuming that they respire 60% of C then, optimal C:N conditions of bacteria would be 10:1. However, not all C in litter is labile, so this value is in fact much higher, probably somewhere around 25:1. At greater C:N ratios microbes import N to meet their needs. Most plant litter has C:N ratio that is greater than optimal for microbes, so the lower the C:N the greater the decomposition rate (Melillo *et al.*, 1989). In many studies the concentration of N in the litter was strongly correlated with litter decay rates (Coulson and Butterfield, 1978; Berg and Staaf, 1987; Taylor *et al.*, 1989). P has also been correlated with decay rates in a number of studies (Staaf and Berg, 1982; Berg *et al.*, 1987; Vitousek *et al.*, 1994) and indeed this may be a more useful indicator in low P environments.

The carbon quality of litter becomes increasingly important after the labile compounds have been broken down. It is at this stage that the more recalcitrant components, mainly lignin, exercise more control over the decomposition rate than C:N ratios (Taylor *et al.*, 1989). Indeed, nitrogen addition may inhibit decomposition at this stage by causing a surge in the bacterial community, which does not possess lignin degrading enzymes. Berg and Staaf (1989) point out that even in fresh litter which has a high lignin content, lignin has a greater influence on decomposition than C:N. There are three characteristics of compound that are associated with recalcitrance: large molecular weight, irregularity and complexity of structure and abundance of aromatic rings. Compounds with a large molecular weight, and which are also irregular in nature do not fit conveniently into the active site of an enzyme. Therefore, multiple enzymes may be required to convert these products into soluble products that can be used by microbes. Aromatic rings react with enzymes and render the active site inactive.

Lignin is recalcitrant and therefore influences the decomposition rate in soils. It forms a barrier between the soil microbial community and the more labile compounds within the plant litter. Lignin is normally analysed by using chemical extraction procedures. However these methods are non-specific and may take chitin from cell walls and other aromatic compounds into account as well. Measurements of lignin have been combined with N contents to produce a lignin:N ratio, which may be a good indicator of decomposition (Melillo *et al.*, 1989). Root exudates are at the opposite end of the scale to lignin. They represent a supply of

abundant labile substrate to the soil microbial community. Sugars, organic acids and amino acids are concentrated in root exudates, and may be absorbed by microbes directly.

Plants growing on higher quality soils generally have a higher quality litter than those growing on low-fertile soils. Fertile soils are associated with plants with high N and P concentrations, low lignin content and low level of toxic secondary metabolites.

1.3.6.3 Toxicity

Some plant litter may be toxic to the soil decomposer community. Inhibiting substances may be formed during the decay of Sphagnoid mosses (Dykyjova and Úlehlová, 1999) Preservation of bog bodies was correlated with the occurrence of alpha-keto-carboxylate groups in a glycuronoglycan '**sphagnan**' that comprises similar to 60% of the holocellulose in the hyaline cell walls of the mosses (Painter, 1991).

1.3.6.4 Vegetation (gradients/structure)

Sphagnum species are the characteristic peat-forming plants on ombrotrophic bogs, and sedges are common in fens. In the transition from the mire expanse of an ombrotrophic bog to the lagg fen region there is a chemical gradient and a consequent change in the plant structure of the above ground flora. Comprehensive studies of the relationship between above ground vegetation and the soil water chemistry have been carried out on a range of peatland types (Nicholson *et al.*, 1996). The need for these studies is justified by the extreme nature of the environment in which the plants live and the consequent adaptations of individual plant and ecosystem structure to cope with the inherent physical and chemical conditions. Bog plants are adapted to cope with waterlogging, acidic conditions and nutrient deficiency.

The complex ecosystem structure of bog flora has important implications for the soil microbial community and the decomposition process. Some researchers have noted the high resistance of mosses (Bartsch and Moore, 1985). Litter with a high initial C:N ratio may be decomposed more slowly (Taylor *et al.*, 1991; Verhoeven and Arts, 1992). The low nutrient status of ombrotrophic bogs results in a high C:N ratio of the vegetation growing on the soils there.

1.3.6.5 Soil enzymes

The activity of soil enzymes depends on the activity of the soil microbial community and the soil matrix. The microbial activity determines the rate of enzymes secretion and the soil matrix affects turnover rates of the enzymes. Depending on the type of enzyme, the residence time in soil may vary. Proteases, for example, are degraded rapidly. Some other enzymes may have a longer residence time than the parent organisms, which is accomplished through binding with soil particles. Phosphatase enzymes are particularly long lived in soils and their activity is related to environmental factors such as pH rather than to microbial activity. The group of enzymes responsible for cellulose breakdown is especially important in soil because cellulose is the most abundant component of plant litter (Paul and Clark, 1996). Three types of enzymes are employed in the breakdown of this compound. Endocellulases are responsible for breaking internal bonds to disturb the crystalline structure. The disaccharide units are cleaved from the ends of the chains by exocellulases to produce cellobiose. Finally, cellobiose is converted to glucose by cellobiase. Although bacteria posses some cellulase enzymes, fungi have the most extensive complex of these enzymes.

Lignin is the most difficult compound to break down in plant litter. The recalcitrance of this compound is due to the way in which it forms i. e. a condensation reaction with phenols and free radicals that is not completely enzyme mediated. Therefore, fungi must invest a large amount of energy into producing complex enzymes systems for the breakdown of lignin. The fungus actually expends more energy than it gains from the breakdown of lignin. The main purpose of lignin breakdown is not to gain energy from the process itself, but to reveal more labile compounds protected by the lignin.

Proteins and nucleic acids are nitrogen containing compounds in plant litter. Both bacteria and fungi are capable of degrading these compounds. During the breakdown of soil humus, proteases and peptidases are important in degradation.

1.3.6.6 The environment

Temperature affects decomposition directly by altering microbial respiration. However, soil moisture and the quality of organic matter also influences the decomposition process. Generally, an increase in temperature speeds up the mineralization of organic C to CO_2. However, whereas at moderate temperatures, the consequent energy is used for growth, at more extreme temperatures the energy is required for the general maintenance of the cell. This means that an extreme temperature rise will result in less efficient microbial metabolism.

At the opposite end of the scale, freeze thaw events kills microbes and release large amounts of labile compounds into the environment. This corresponds to a burst in activity in the Spring after winter freezing (Hobbie and Chapin, 1996). Freeze thawing also exposes surfaces for microbial colonization.

Indirect effects of temperature can increase or decrease decomposition depending on the climate. In wet climates, for example, decomposition increases as temperature increases because the soil moisture decreases, and gas exchange increased. However, in dry environments decomposition would decrease because soils would become too dry and cause moisture stress for the organisms living there.

The *moisture content* of soils is closely related to O_2 availability or redox status. Generally, the lower the redox potential of soils the less aerated the soil. The decomposition process is less susceptible to low soil moisture conditions than net primary production (NPP). However, under high soil moisture conditions decomposition is reduced, resulting in C accumulation in wet soils. If soil moisture falls below 30-50% of dry weight or exceeds 100-150% dry weight then decomposition is inhibited. In wet soils, decomposition is restricted by oxygen availability. Other factors can also effect oxygen exchange: high clay content, presence of permafrost, irrigation and rainfall, compaction by animals and agricultural equipment. The thickness of the oxidised surface depends on the supply of O_2 at the surface and the rate of O_2 consumption of the substrate. Wetlands are associated with waterlogging and low redox potentials, however, microbial processes taking place in the oxidised zone of waterlogged soils are similar to, or identical with those in moist but well aerated soils.

In oxygen limited situations the soil microbial community may turn to alternative electron acceptors for respiration. In the absence of oxygen, anaerobic metabolism proceeds with the use of sulphate, nitrate, iron and manganese as electron acceptors. However, this metabolism is not favoured because it is much less efficient than aerobic metabolism. Anaerobic metabolism produces more intermediate products and less C as CO_2 and microbial biomass (Sircar *et al.*, 1940).

Growing plants produce a whole range of labile compounds that they exude from their roots (Paul and Clark, 1996). Carbohydrates, amino acids, small molecular weight proteins, enzymes, organic acids, phytohormones and other compounds that attract, simulate or inhibit the soil microbial community are produced. By producing these compounds, the plant is able to mediate food webs and processes in its environment (Mitsch and Gosselink, 2000). In producing labile substrates the plant stimulates microbial growth and proliferation of the soil microbial community. Predators are attracted at areas of high microbial numbers, then they

lyse and consume cells. During this process, nutrients are produced and are taken up by plant roots.

Soil properties influence processes occurring in soils. In neutral soils decomposition occurs at a faster rate than in acidic soils. This corresponds to a shift in the microbial community from fungi to bacteria with a decrease in acidity. It has been suggested that decomposition is faster under bacterial domination because these organisms are less efficient than fungi, and therefore require more substrate. However, pH also effects nutrient availability. The limiting effect of the low phosphorus (P) supply in rainwater is enhanced by acidic environments, such as bogs. In acidic bogs where there is also a lot of organic matter, P forms complexes with organic compounds, Fe and Al (Patrick and Mikkelsen, 1971). Clymo (1965) reported the occurrence of toxic concentrations of Al in bogs as a result of the high acidity. This may also be the case with other substances. Plant species composition may also be affected by pH therefore, it is difficult to separate the effects of pH from other environmental factors. Indeed, it may be difficult to separate pH from the effects of plants. Sphagnum mosses may also be able to acidify surrounding water as a mechanism for gaining cations from dilute supplies in the soil water. This adaptation of Sphagnum makes it an efficient competitor in nutrient limited environments.

Clay minerals may serve to stimulate decomposition by enhancing the growth and activity of microorganisms. However, the net effect of a high clay content is to inhibit decomposition. Clay minerals can negatively effect decomposition in several ways. Clay particles increase water retention in soils which is often associated with oxygen limitation. The negative charges associated with clay particles are available as binding sites for positively charged particles. Therefore, enzymes and soluble products of exoenzyme activity may be inactivated or reduced by clay particles. The net effect of clays therefore is protection of the soil organic matter from breakdown.

Mineral nutrients are found in low concentrations in ombrotrophic mires (Pollet, 1972) which are separated from the groundwater supply and do not receive water from surrounding soils. The concentrations of nitrogen and phosphorus are small in rainwater (Gorham, 1955) resulting in a small input of inorganic nutrients to the soil. Peat soils are typically acidic; contributing to the shortage of mineral nutrients for biological processes. For example, P availability is reduced in bogs because complexes are formed with organic compounds, aluminium and iron (Patrick and Mikklesen, 1971). On the other hand, the acidity of these soils facilitates other nutrients e.g. Al (Clymo, 1965), Mn, Fe, and Ni (Dykyjov and Ulehlová, 1998) to reach toxic concentrations in peat.

Inorganic nutrients have a limiting effect in bogs (Dykyjov and Ulehlová, 1999) which has implications for everything living in them from plants and small animals to microorganisms. Peat is commonly deficient in plant available nutrients (Brady, 1990), which restricts plant growth (Verhoeven, 1996). Plants have adapted to the low nutrient status of peat bogs in a number of ways, for example the form of the plant may vary according to nutrient status of the soil. In general, the low nutrient availability in bogs is reflected by the abundance of evergreen plants in this environment (Aerts, 1995). Evergreens are better competitors in a nutrient poor environment because they are able to retain some nutrients from year to year. Some deciduous plants, for example *Eriophorum* sp., manage to retain nutrients as long as possible by translocating nutrients from dying parts of the plant to surviving parts (Goodman and Perkins, 1959). Nitrogen use efficiency (NUE), has been determined as the ratio between above ground biomass production and nutrient loss in litter fall (Vitousek, 1982). This takes into account tissue life spans, tissue chemistry and nutrient resorption by senescing tissues (Aerts and Decaluwe, 1994; Aerts, 1995). NUE for bogs is greater than for other soils (Aerts, 1997), illustrating that plants indigenous to bog ecosystems have adaptations to grow under low N conditions. Sphagnum species act as cation exchange materials. Soil water containing dilute cations and organic acid ions are retained in complex structural components in the plants (Dykyjov and Ulehlová, 1998). The enables *Sphagnum* and higher plants to make the best use of low nutrient conditions.

Decomposition is slow in peats in part due to the relative shortages of nitrogen and phosphorus for microbial growth (Coulson and Butterfield, 1978; Clymo, 1983; Brock and Bregman, 1989; Aerts *et al.*, 1995). It has been proposed that increased N and P availability would lead to increased organic matter decay and increased CO_2 production (Aerts and Toet, 1997). Indeed, the application of mineral salts has been shown to increase the rate of decomposition in bogs (Germanova, 1978).

Soil disturbance may be natural (e.g. earthworm activity) or anthropogenic (e.g. ploughing). In general the effect is to promote microbial activity by exposing new surfaces and aerating the soil. As a consequence of disturbance decomposition increases.

Peatlands are often disturbed by the means of drainage ditches and mining. Drainage of peatlands is often carried out to ameliorate the conditions for plant growth, for the management of agricultural based systems. The watertable height decreases as a result of drainage, consequently decomposition increases. These practices may present problems for peatland ecosystems as net sinks of C. Gorham (1991), in his classical review on the subject, estimated the net effect of drainage and combustion, arising from mining of peat, to be 0.0085

Pg Yr $^{-1}$ and 0.026 Pg yr $^{-1}$ respectively. Clearly, this is an important issue and steps must be taken to control the impact of management systems on peatlands and indeed their role in the C cycle.

1.4 Aims of study

Within the general remit of microbial C cycling in peatland ecosystems the specific aims of this study are outlined below.

- To characterize the soil microbial community in the aerobic surface region in soils from an ombrotrophic mire and put these into an ecological context by comparing with mineral soils
- To test the hypothesis that nutrients limit the size of the aerobic soil microbial biomass and hence decomposition, in soils from an ombrotrophic mire
- To investigate microbial decomposition in these soils with a view to explaining the relationship between the size of the soil microbial biomass and decomposition
- To test the role of plant nutrient status and litter quality on microbial decomposition and specifically on the association of the soil decomposer community with litter

1.5 Site description

Dun Moss lies in the Southern Grampian foothills about 40 kilometers north of Dundee, Scotland (3°21' W 56°41' N NO 167 558). The catchment of Dun Moss is a designated site of special scientific interest (SSSI), therefore remains relatively intact. It is for this reason that Dun Moss has been the site of many scientific studies (e.g. Ingram, 1982) and is known internationally. Many peatlands gain popularity from their location or the extent of their coverage, however Dun Moss, although an excellent text book example of a raised bog, has an area of less than 300 square meters. The mire expanse is an asymmetric dome, and is higher at the west. The surface of the mire is a mosaic of hummocks and hollows, however there are no permanent pools. The peat substrate remaining in the lowermost layers was formed from vegetation similar to that found at Dun Moss today; *Sphagnum*, *Calluna vulgaris* and *Eriophorum*. Human disturbance to the mire is not an obvious feature of the mire, however there is some evidence of peat cutting in the south west region. A 1.2 m drainage ditch divided the mire into two halves, east and west, however this is now filled with moss. Sheep graze the surrounding soils, however they tend not to graze the mire expanse itself.

Figure 1.5 shows the location of the mire (1.5a) and the small scale topography (1.5b). The objective of using transects was to incorporate all three of the soil types, mineral soils and the soils of the lagg fen and mire expanse, into the study with some consideration of replication. In choosing the location of the transects several criteria were used, these are outlined below.

- To avoid areas of disturbance e.g. peat cutting in the south west of the mire expanse, drainage ditch and erosion
- To consider ease of relocation by choosing easily definable bearings such as buildings
- To avoid landform features e.g. glacial erratics are particularly abundant on Drumderg and these could effect the soils in the surrounding area
- To avoid scientific equipment – Dun Moss has been the site of a number of hydrological studies and many of the rain gauges and dip wells are still in place

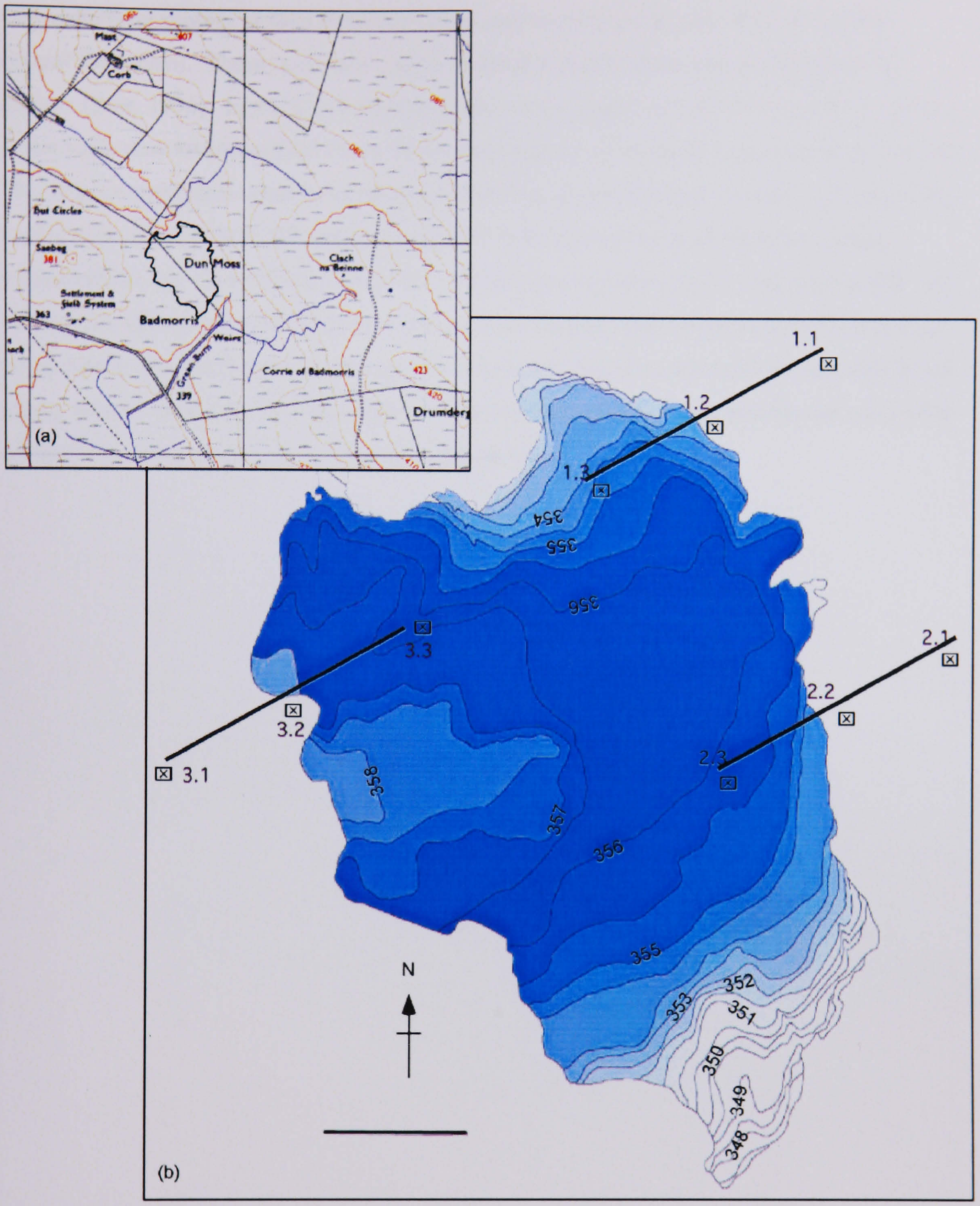
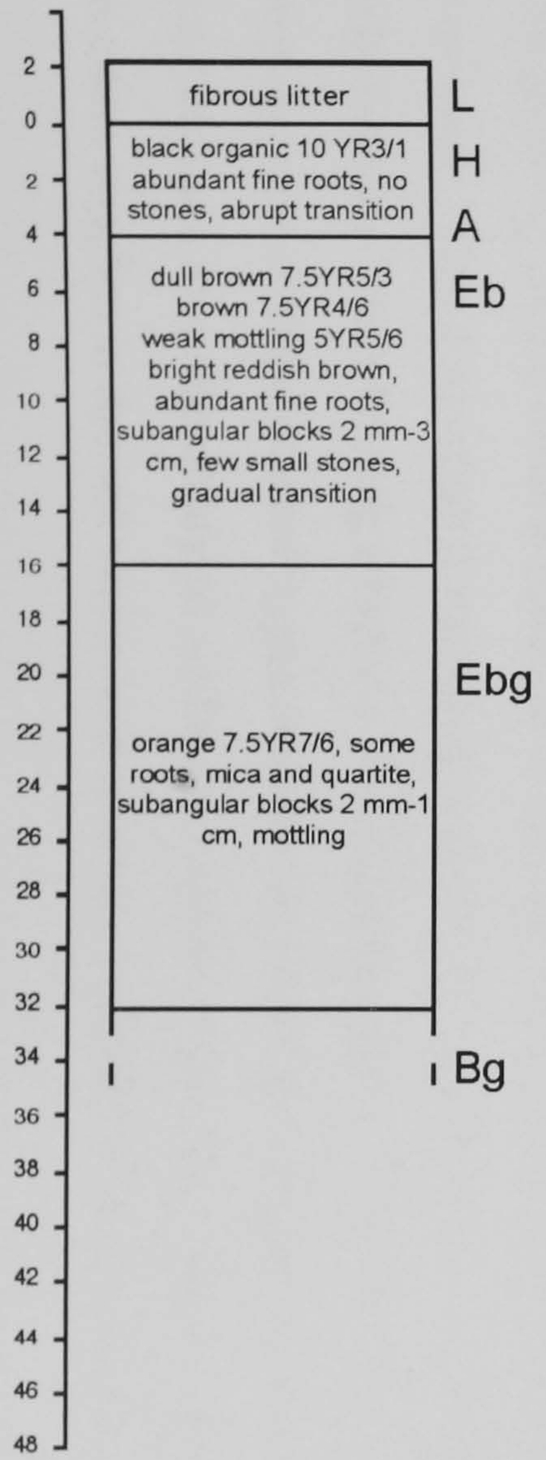


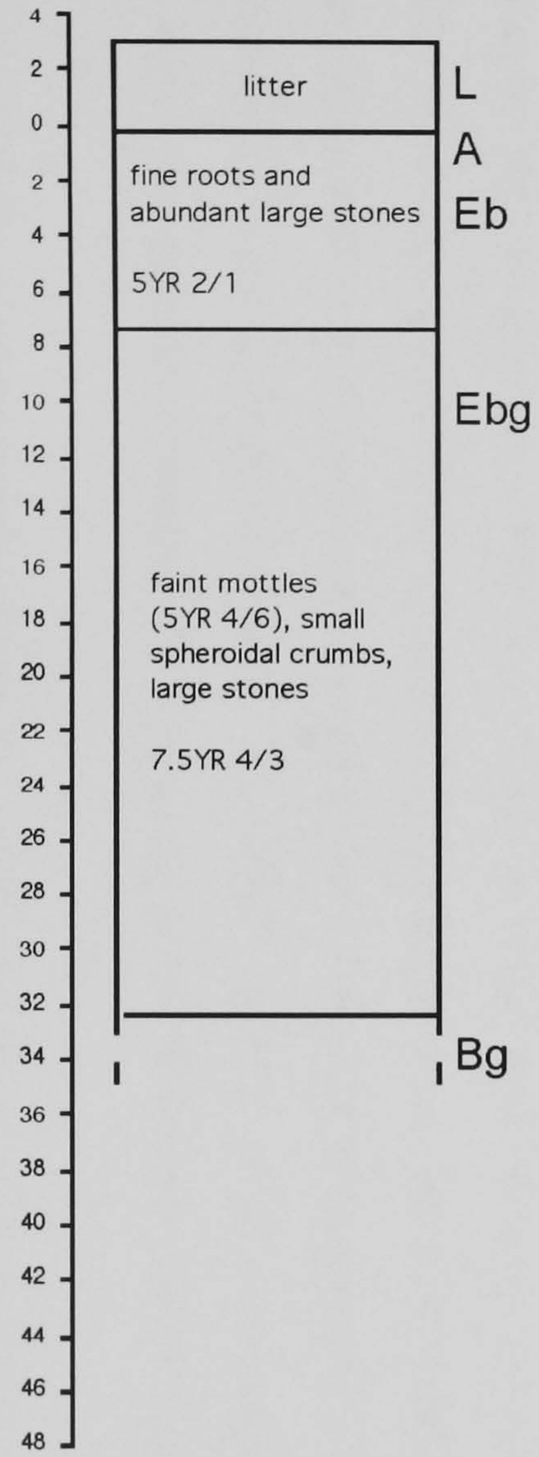
Figure 1.5: (a) Location of Dun Moss. Ordnance survey Pathfinder 310 Bridge of Cally (NO 05/15). (b) Diagramatic representation of Dun Moss showing contours in 0.5 m. The scale bar is 100 m. Numbered bars show the transects chosen for sampling, and the ☒ marker indicates individual sampling points. Notation: x.y where x is the transect number and y is the soil type (1 is mineral soil; 2 is lagg fen; 3 is mire expanse). (Adapted from Ingram, 1982).

The underlying geology at Dun Moss comprises quartz-mica-schist, grit, slate and phyllite (Upper Dalradian). These rocks are mostly covered with moranic material deposited by glaciers (Smit, 1996). In the 5 to 6 thousand years since organic matter first started to collect in the basin now known as Dun Moss, 10 m of peat have accumulated in the centre of the mire. This organic material or peat is sometimes referred to as soil, however it does not look like or function like mineral soils. Whereas mineral soils have distinct horizons which are visibly distinguishable and have different functions, the horizons in peat soils are not easily visible. For this reason, the criteria which are normally used to describe mineral soil profiles do not apply to peat soils, and the distinction between the two layers, the acrotelm and the catotelm, is not clearly visible. However, for the purpose of the soil descriptions I will describe the soil profile of both the mineral soils and the peat soils (figure 1.6).

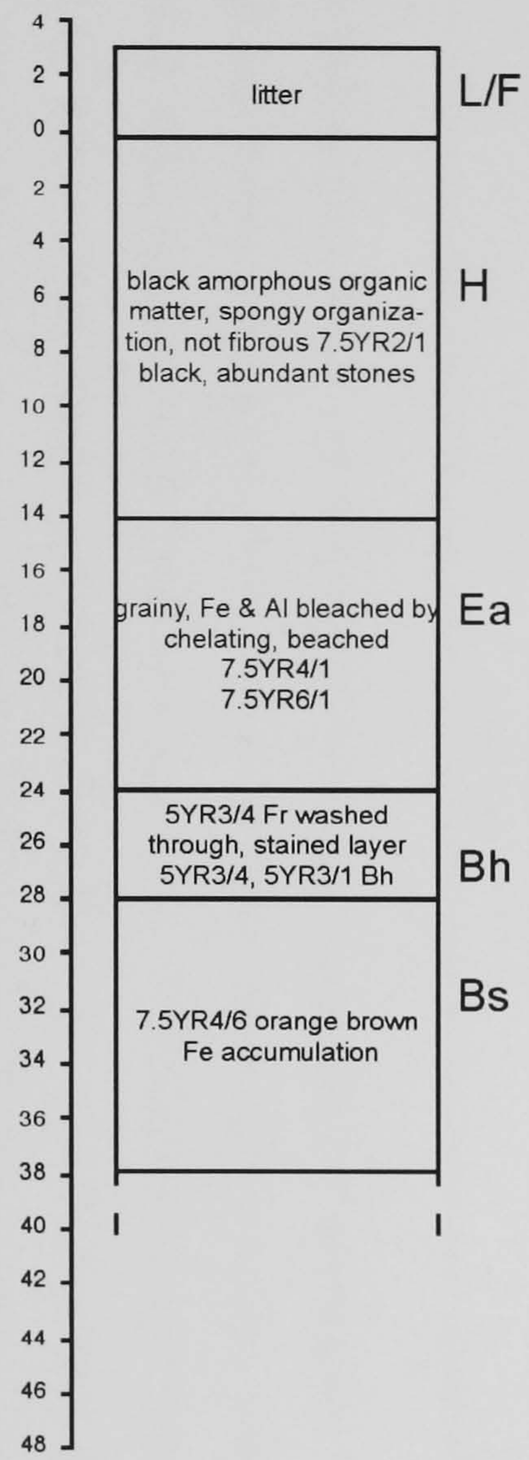
Soil: 1.1
Slope: 4°
Veg: *Calluna vulgaris, Festuca ovina*



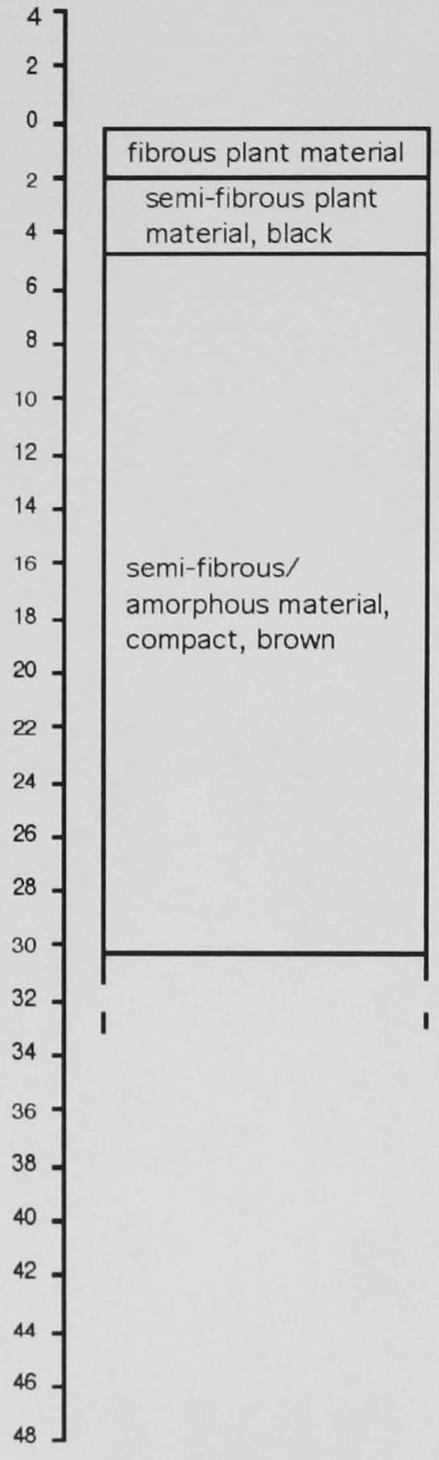
Soil: 2.1
Slope: 11°
Veg: *Calluna vulgaris, Festuca ovina*



Soil: 3.1
Slope: 15°
Veg: *Calluna vulgaris, Hypnum jutlandicum*



Soil: x.2
Slope: 0°
Veg: *Juncus effusus, Sphagnum*



Soil: x.3
Slope: 0°
Veg: *Calluna vulgaris, Sphagnum*

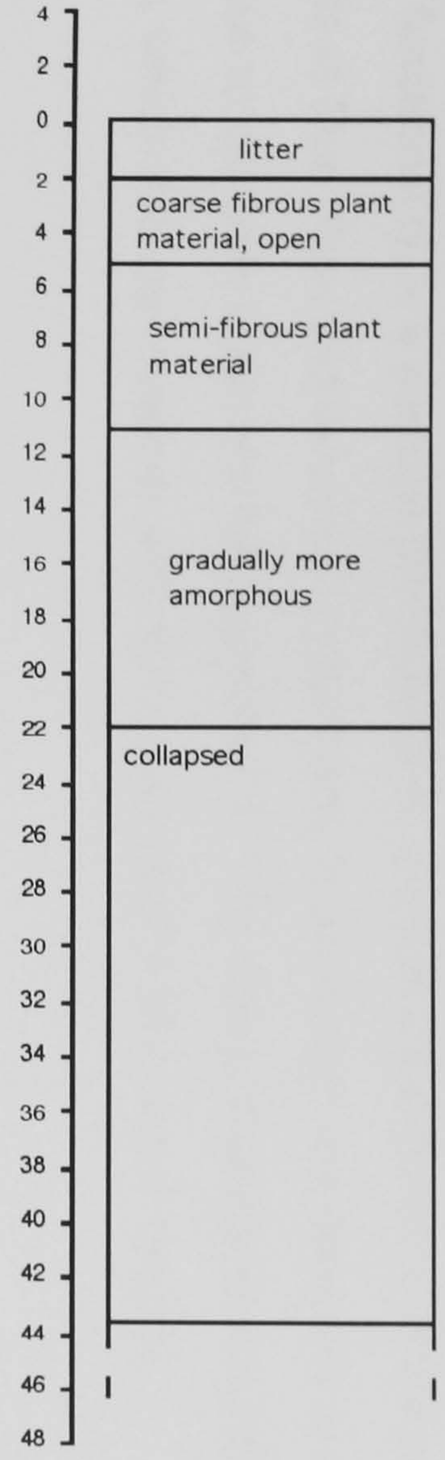


Figure 1.6: Profiles for the soils at Dun Moss.

In terms of functionality, it is perhaps more useful to present the difference in the water inputs into the soils at Dun Moss and other soil properties. In particular, the presence of oxygen and nutrient input to the soils is summarized in table 1.1. The soils are grouped according to their water input, which also corresponds with the above ground biomass at the site.

Table 1.1: Properties of the soil types at Dun Moss.

Soil	Origin of water input	Nutrient input	Vegetation	OM	pH	O_2 status
Mineral	Telluric and meteoric	++	Grasses and sedges, *Calluna vulgaris*	+	+++	Seasonally waterlogged, with aerobic pockets
Lagg fen	Telluric and meteoric	++	Sphagnum mosses, Juncus spp.	++	++	Waterlogged
Mire expanse	Meteoric	+	Sphagnum mosses, *Calluna vulgaris*	++	+	Waterlogged

The mineral soils at Dun Moss can be categorized as peaty podzols and peaty gleys. Although these upland soils have a large proportion of organic matter in comparison with arable soils, they have much more mineral matter than true peat soils. The mineral content has important implications for the nutrient content of the soils; more dissolved mineral nutrients (N, P, K, Ca, Mg, Fe etc.) are present in these soils. The soils of the lagg fen receive water that has flowed through these soils, therefore they receive some of the mineral nutrients from the mineral soils (telluric water). The mire expanse does not receive this nutrient rich water because it is raised, it is not connected to the groundwater supply and it does not have vascular plant with a root system capable of tapping the groundwater. Therefore the soils of the mire expanse only receive water from precipitation, consequently the supply of mineral nutrients to these soils is nutrient poor.

The nature of the nutrient supply effects the vegetation cover at the sites, therefore the vegetation growling on the mineral soils and in the lagg fen is much richer than that growing on the mire expanse. This distinction is especially apparent in the summer months when climatic conditions have a less limiting effect on the vegetation growth than in the winter. The plants growing on each of the three soil types and the nutrient input influences the pH of the soils. Sphagnum mosses have the ability to acidify surrounding water as a mechanism for gaining cations from dilute supplies in the soil water, and this may contribute to the acidity of the soils

of the mire expanse. The presence of mineral cations in the lagg fen reduces the acidity of the soils, therefore these soils are a less acidic environment than the soils of the mire expanse.

Perhaps the most obvious difference between the soils at Dun Moss is the moisture content. The mineral soils have stones, roots and larger soil organisms (e.g. earthworms) and are relatively well drained because they are on a slope. The peat soils, on the other hand, have negligible amounts of stones and mineral matter therefore the pore size is much smaller; this helps to retain water, these soils are also at the bottom of the slope so water accumulates here. The consequence of these factors is the peat soils have a moisture content greater than 90%, and the mineral soils have an average moisture content of <50%. Therefore the peat soils are waterlogged and oxygen availability in these soils is smaller.

The hydrology and soils of Dun Moss make it a suitable site to carry out the work outlined in section 1.7 and test the hypotheses. Additionally, the data already documented for the site provided a good background to the ecosystem ecology found there.

Chapter 2

Characterization of the soil microbial biomass

2.1 Introduction

2.1.1 Soil microbial ecology

In studies of soil microbial ecology, three important aspects must be addressed. First, the size of the soil microbial community must be determined. Second, information about the activity of this community is sought. Third, the composition of the soil microbial community is investigated. There are several methods available to estimate these three aspects of the soil microbial community; these are outlined in figure 2.1.

This chapter deals with the ecological aspects of the soil microbial community. Size and activity will be discussed together first, followed by the composition of the microbial community in the soils at Dun Moss. The overall aim of the chapter was to characterize the soil microbial community in the peat and mineral soils at Dun Moss using a range of methods.

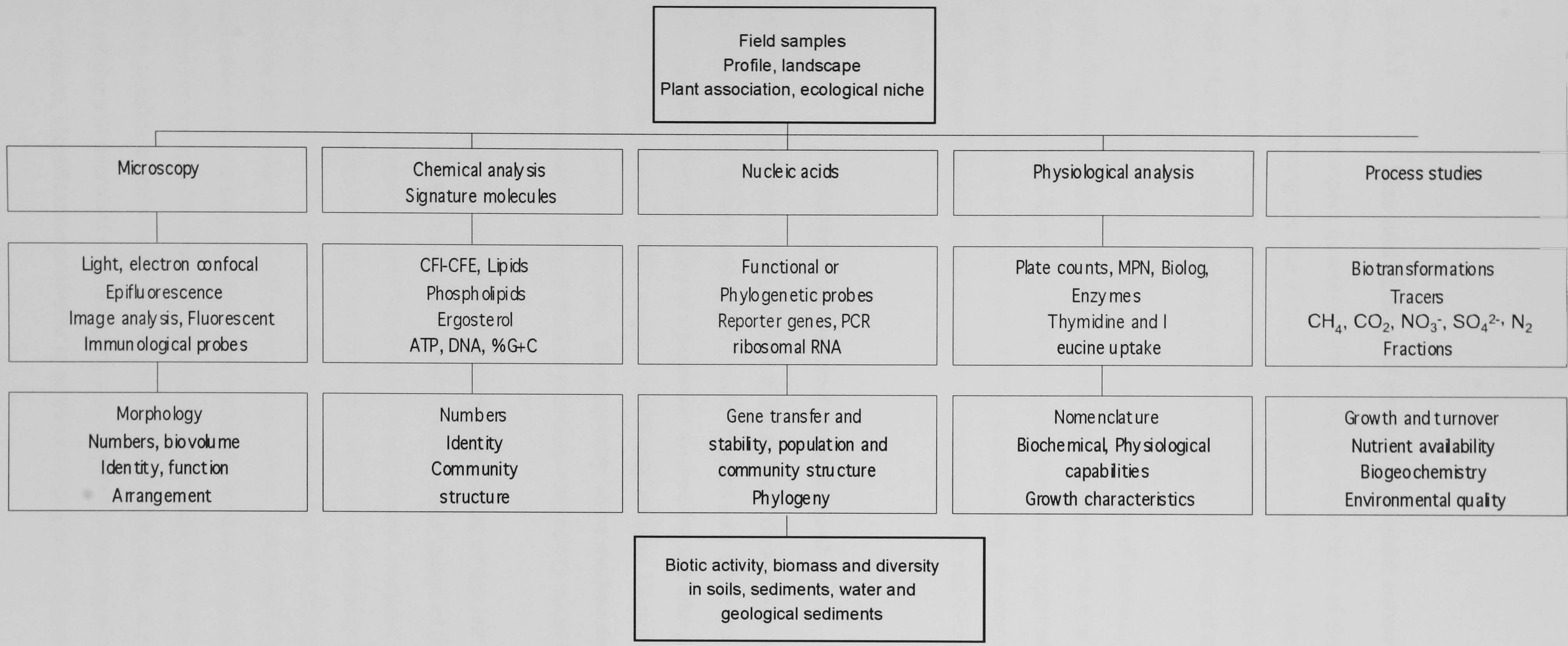


Figure 2. 1: Current methods used in the study of microbial ecology in soils.

2.1.1.1 Size and activity of the soil microbial community

One important aspect quantifying the ecological importance of the soil microbial community in soils is determining the size of the soil microbial community. There are only a few data available on the size and activity of soil microbial communities in peat soils (e.g. Williams and Sparling, 1984; Hart *et al.*, 1986; Sparling and Williams, 1986; Groffman *et al.*, 1996; Schipper *et al.*, 1998; Brake *et al.*, 1999.)

The aim of this chapter is to address the issue of biomass C size and activity in peat soils. Several methods have been devised for determining the size of the soil microbial community in soils, however, some limitations have been reported with the use of these methods in peat soils (Brake *et al.*, 1999). Consequently, development and testing of methods was required before the determination of the size of the soil microbial community could be carried.

2.1.1.2 Method selection and development

Most methods for estimating the size of the soil microbial community and activity in soils have been developed for use with mineral soils. Mineral soils differ from peats in a number of ways that may affect the suitability of a particular method for use with peat soils. In contrast to mineral soils, peats are highly organic, containing less than 1% mineral material with predominantly water filled pores. Consequently, some method development was required before the size and activity of the soil microbial community could be determined for the raised mire soils.

The methods available for determining the size of the soil microbial biomass are wide ranging. Classical studies concentrated on direct observation of the soil using microscopy. This methods requires skilled work and it is very labour intensive, however, it remains a standard for determining the size of the soil microbial community in soils. One limitation of this approach is the failure to be able to distinguish between the active proportion and the inactive proportion of the soil microbial community. Additionally, soil particles and dead organisms are not easy to detect. Culture methods have the advantage of being able to isolate and enrich for specific groups of bacteria within the soil. However, even with complex media it is not possible to culture the entire soil microbial community. It has been estimated that only 1% of the soil microbial community may be cultured in the laboratory (Paul and Clark, 1996). In addition, identification problems arise due to changes in the morphology and in the

metabolic capabilities of microorganisms. The alternatives to these methods have centred on the measurement of metabolic capabilities and cell components in which the soil is treated as a whole system.

The measurement of adenosine triphosphate in cells has been used as an indicator of both biomass size and activity in soils. ATP is present in actively metabolising cells and is quickly degraded when released into the environment, therefore it is a good indicator of the active soil microbial community (Jenkinson and Oades, 1979). The extraction efficiency depends on the exctractant used and the soil under analysis. Additionally, some substrates can inhibit the action of luciferase, the key enzyme involved in the extraction procedure. Substrates known to cause an inhibitory effect are NO^{3-}, Mg^{2+}, Ca^{2+}, Cl^{-}, Br^{-} and humic substances. There is a high concentration of humic substances in peat (Clymo, 1992) and Mg^{2+} and Ca^{2+} in fen peat therefore it was decided to avoid the use of this method.

In the same way, fumigation extraction measures cell components (Jenkinson and Powlson, 1976). In this method, chloroform is used to kill and lyse microbial cells, then the cytoplasm is released into the environment and the cell material can be extracted. Several authors have reported problems with using this method with acidic soils (Jenkinson and Oades, 1979) because acidity may limit the rate of decomposition of killed microbial cells in highly acid soils. It is also well documented that fumigation extraction does not target the soil microbial community but the chloroform extraction releases material from larger organisms and plant roots in the soil (Williams and Sparling, 1984). In the surface area of peat soils where larger organisms, for example algae and protozoa, may be abundant, therefore the fumigation extraction method would over-estimate the size of the microbial community. Indeed, Williams and Silcock (1997) reported an increased size of the soil microbial community on biomass measured using the substrate induced respiration method.

Other methods measure metabolic processes, for example respiration. Respiration may be measured by oxygen consumption, CO_2 production or using a redox reaction (dehydrogenase). The dehydrogenase method uses the electron transport system involved in respiration to measure the activity of the soil microbial community. A marker electron acceptor (tetrazolium salt TTC or INF) is used to estimate activity, which is represented by a colour change. The reaction is pH dependent and some chemical reduction of the INF could occur in acid soils (Sparling in Alef and Nannipieri, 1995). Additionally, the high levels of humic substances in peats and the consequent colour of the soil solution may cause problems reading relatively small activities. It has been also suggested that soil water content and temperature influence the dehydrogenase activity indirectly by affecting the soil oxidation-reduction status

(Brzezinska *et al.*, 1998). These factors may explain the lack of correlation between previously reported measurements of microbial community in peat soils (Gammelgaard *et al.*, 1992).

Respiration measurements using CO_2 as an indicator of respiration are widely used method for determining microbial activity and size of the aerobic soil microbial community. The first extensive work using respiration as a measure of the size of the soil microbial community was done by Anderson and Domsch in 1978. They proposed a method by which the size of the soil microbial biomass could be estimated by adding an available substrate to soil samples, then measuring the CO_2 produced. This method has been widely used to estimate levels of microbial biomass in mineral and organic soils (Ross *et al.*, 1984; Sparling and Williams, 1986; Martens, 1995; Alef *et al.*, 1988). The theory behind this substrate induced respiration (SIR) approach, is that when provided with an available substrate in excess, the soil microbial community will respire maximally. The procedure assumes that the entire soil microbial community has a uniform maximum rate of respiration. D-glucose is widely accepted as being the most available substrate to microorganisms. When this substrate is added to soil at saturating concentrations for the soil microbial community maximal respiration is achieved. The method, conducted at 22°C, was calibrated for biomass C in soils by Anderson and Domsch (1978).

Substrate induced respiration requires few pieces of specialised equipment; gas chromatograph and closed vessels for incubating soil samples. It is simple and rapid to perform (Ritz and Wheatley, 1989) and many samples may be tested at once. Furthermore, SIR measures the amount of soil biomass C in soil, which is especially relevant to decomposition studies.

The substrate induced respiration method was originally developed for use with mineral soils. The method relies on uniform mixing of the substrate, which could be difficult to achieve in a peaty soil. West and Sparling (1986) used the SIR method to examine soils of varying water contents. They proposed the addition of D- glucose as a solution and following static incubation, vigorous mixing of samples prior to sampling the headspace gas. Heilmann and Beese (1992) proposed a scaled down version of this experiment using airtight syringes and about 10 g soil. West and Sparling (1986) suggested that this slurry method could be used to estimate the size of the soil microbial community in soils of pH <6.5. Soil more alkaline than pH 6.5 may be problematic due to the retention of CO_2 in soil solution as bicarbonate at higher pH values (Martens, 1995). To overcome this problem Ritz and Wheatley (1989) suggested acidification of the soil solution before measuring CO_2.

The activity of the soil microbial community may also be measured by monitoring respiration. Respiration measurements have been used extensively with mineral soils and peat soils as indicators of decomposition. A range of techniques has been employed to measure respiration. Field measurements have been used to determine the CO_2 evolution from a given surface area using chambers (e.g. Silvola *et al.*, 1996b; Rayment and Jarvis, 2000), and from different depths in the peat using membrane inlet mass spectrometry (e.g Lloyd *et al.*, 1998; Clymo and Pearce, 1995). However, studies on peat decomposition made in the field are affected by root respiration (Chapman and Thurlow, 1998). Laboratory measurements have the potential to target the soil microbial community in a more specific way. CO_2 production during laboratory incubations has been measured using an array of techniques. Chapman and Thurlow (1998) used an automated respirometer to measure CO_2 production from samples. In other studies an infra-red mass spectrometer has been used to quantify CO_2 evolution from soil samples (e.g. Bridgham and Richardson, 1992 and Anderson and Domsch, 1993). Zak *et al.*, 1994, Freeman *et al.*, 1996; Moore and Dalva, 1997 used gas chromatography to measure CO_2 from soil samples incubated in the laboratory.

Some authors measured respiration in conjunction with the SIR method for determining soil microbial biomass (Ritz and Wheatley, 1989; Hopkins and Shiel, 1996). This approach was selected to estimate the activity of the soil microbial community in this project for the following reasons. Firstly, it could be carried out at the same time as biomass C determinations, thereby increasing the efficiency of the procedure and enabling many samples to be measured. Secondly, respiration measurements select for the aerobic proportion of the soil microbial community. Lastly, the method could be customized for use with small sample sizes. Calibration of the slurry method was required for activity measurements.

2.1.1.3 Microbial diversity

A measure of the microbial diversity is required in order to determine what the active microbial biomass in soils is and what processes it is involved in. Two distinct approaches have emerged to evaluate diversity in microbial ecology (Garland and Mills, 1991). Some researchers have concentrated on identifying organisms in soils and producing lists of species. The other extreme has been to use a functional approach whereby microbial processes are studied. Successful work that combines the two has not been reported (Garland and Mills, 1991).

2.1.1.4 Methods available for investigating the diversity of the soil microbial community

Most studies that have been carried out on the microbial diversity of peat soils have utilized direct counts or plate counts (Parkinson and Coleman, 1991). Borga *et al.*, (1994) point out that the direct count approach fails to provide information on the physiological differences within a microbial community. Plate counts can be misleading due to the use of selective media and the formation of colonies from aggregates of single cells. This approach is time consuming and requires skilled work, additionally microbial mutations may present themselves as changes in the physiological properties, structural properties and other traits which are used as unique identifiers of a particular species or group.

Other approaches to determine microbial diversity and function in soils using metabolic capabilities, microbial biomarkers and nucleic acid based studies have become popular. One method used to determine the metabolic diversity of the soil microbial community is the Biolog™ system. This system employs a redox reaction to measure the metabolic activity of a microbial inoculum in response to a range of carbon substrates. Reproducible profiles of microbial communities are produced, providing a 'fingerprint' of the substrate utilization of the soil microbial community. However, because this technique was originally developed for the identification of medically important isolates there are limitations with this technique for use with environmental samples. As is generally the case with culture based methods, the Biolog™ technique relies on the utilization of substrates at high concentrations. Slower growing microbes may not be able to utilize substrates at concentrations much greater than those found in the environment. Biolog™ assumes a transparent and C free medium for the sample. However, this is difficult to achieve in peat soils. The choice of substrates included in the pre-prepared Biolog™ plate may also be inappropriate. Although some of the substrates are found in the environment (e.g. N-acetyl glucosamine that exists in fungal cell walls) the ecological significance of the range of substrates used in the Biolog™ technique has yet to be established. Furthermore, Biolog™ was developed for use with bacterial isolates all at the same stage in the growth cycle. Microorganisms in the environment exist in different states, ranging from dormant to fully active. Therefore, the fixed incubation period used with isolates is not suitable for environmental samples. This has lead to much debate in the literature, and universal agreement remains to be arrived at. It must be noted that there is little documentation of the use of Biolog™ with peat soils (Timonen *et al.*, 1998). However, as discussed earlier in this chapter, there are problems associated with using redox reactions with acidic soils.

Phospholipid fatty acid (PLFA) analysis has been used to determine groups of organisms present in soils. PLFAs occur in all microorganisms. PLFAs vary qualitatively and quantitatively between groups of organisms (Lechevalier and Lechevalier, 1989), therefore it is possible to use a PLFA profile or marker to identify a group of organisms. PLFA analysis has been used to characterize isolates grown in the laboratory (Germida *et al.*, 1998), however the technique can also be used to assess the microbial diversity of soils (Tunlid *et al.*, 1989; Frostegård *et al.*, 1993). Overlapping PLFA patterns of individual species mean that only broad groups of microorganisms can be classified (Zelles, 1999; Steer and Harris, 2000). Ester linked fatty acids (EL-PLFA) in soils are regarded as the most useful signature in soils (Guckert *et al.*, 1986; Tunlid and White, 1990). However, these PLFAs do not include fatty acids indicative of methanogenic bacteria (Sundh *et al.*, 1997). Therefore, it is suggested that the extended method in which non-ester linked PLFAs (NEL-PLFAs) are extracted is used for studies of deep peat.

Nucleic acid based studies have also been used to assess the diversity of the soil microbial community. Using species specific markers it is possible to characterize the DNA of the soil biota. However, molecular methods to determine the contribution to ecosystem function are still in their infancy and current techniques may fail to recognise ecologically important organisms (McDonald *et al.*, 1999). Furthermore, the method requires direct extraction from the soil. This can be complicated by humic substances contained in the extracts, therefore this may pose problems in peat soils.

Most bacterial or fungal cells contain fatty acids between 10 and 20 carbon atoms in length. Fatty acid nomenclature is based upon the number of carbon atoms in the acyl chain, the degree of unsaturation, the type of isomerization and the position and type of additional functional groups. The shorthand notation: Cx:yωz will be used throughout this work: x is the total number of carbon atoms, y is the number of double bonds and z indicates the position of the first double bond from the methyl end of the molecule. Prefixes iso- and anteiso- indicate a methyl branch on the acyl chain at either the penultimate or third carbon. Branching occurring elsewhere on the chain is indicated by quoting the relevant carbon (from the carboxylic end of the molecule). The prefix cyc- refers to a 3-carbon ring on the acyl chain. α- and β- refer to an additional alcohol group on the acyl chain (bonded to the second or third carbon respectively). Cis- and trans- prefixes indicate the arrangement of the functional groups attached to double bonds.

There is no single technique that provides the definitive description of the diversity of the soil microbial community because each technique described is a measure of a different aspect of the microbial community. The information provided by each technique almost

certainly varies independently. Therefore, it is beneficial to use more than one of the methods when attempting the characterization of the soil microbial community. One drawback with all of the methods described is the failure to detect which organisms are active at the time of sampling. More fundamental problems remain; the meaning of species diversity in soils has not yet been properly defined. It has been suggested that the microbial community is a sensitive ecological marker of stress situations in soils caused by pollution, agricultural management practices and recovery of disturbed areas (Kunc, 1994). However, the impact of 'natural' environmental stress on the species diversity of the soil microbial community is not known.

2.1.3 Aims and hypotheses

The aim of this chapter was to describe the microbial ecology of the soils at Dun Moss with a view to quantifying and qualifying the difference between the soil microbial community of peat and mineral soils.

The following hypotheses were tested:

2.1.3.1 Size and activity

- The size of the soil microbial community in the mire soils is smaller than in the soils of the lagg fen and the mineral soils, which have a greater nutrient input.
- Waterlogging imposes a stress on the soil microbial community of the mire and lagg fen soils, therefore the metabolic quotient is greater in these soils.
- There is a relationship between the ratio of C:N, as a measure of litter quality, and the size of the soil microbial biomass.

2.1.3.2 Diversity of the soil microbial community

2.1.3.2.1 Metabolic diversity

- There is a significant difference between the metabolic diversity of the soil microbial community of the different soils. The microbial communities in the lagg fen and mineral soils are more diverse metabolically than those in the mire expanse because botanical diversity is greater on these soils

2.1.3.2.2 *Functional diversity*

- There is a significant difference between the soils in terms of functional diversity. The soils of the surrounding slopes and lagg fen have a greater microbial diversity than the soils of the mire expanse.
- There is a high population of fungi (indicated by C18:2ω6) in all of the soils due to the acidity.
- Metabolic and functional diversity indicators of the soil microbial communities of Dun Moss are similar.

2.2 Materials and methods

2.2.1 *Biomass size calibration experiment*

2.2.1.1 Sampling

A version of the original method Heilmann and Beese (1992) and a modified method with the incubation of samples as a slurry were used to measure the size of the soil microbial community in 7 soils. A summary of the soils used for this experiment is made in table 2.2. The soils were selected because they had a range of physical and chemical properties.

pH was determined in water using 4 g moist soil and 8 ml distilled water. Moist soil was mixed with distilled water and then was shaken for 30 minutes. After shaking, the samples were allowed to settle for 30 minutes before the pH was read. Moisture content of the peat soils was determined after oven drying the soils at 70° C for 3 days. Similarly, the mineral soils samples were dried at 105° C for 24 h. Bulk density was measured by collecting soil samples using a cylinder of known volume. The samples were weighed at field moisture and after drying. C and N analysis was carried out on the soil samples after oven drying. This was done using an elemental analyser (Appendix A).

Table 2.1: Soils used for the comparison of methods for the determination of size and activity of the soil microbial community in soils.

Soil	Details
Aldbar	0-15cm Collected Jan 1998, stored for 3 weeks
Vinny	Stored at 4° C for 18 months
Gley DM1.1	Dun Moss soil (transect 1), top 0-15 cm, Feb 1998
Podzol DM3.1	Dun Moss soil from mire expanse (Appendix B)
Redesdale Wilcocks, 2.1	Lime treated upland soil. 6 t ha^{-1} 0-4 cm
Redesdale Wilcocks, 2.3	Lime treated upland soil. 6 t ha^{-1} 8-12 cm
Dun Moss Peat	Peat, 0-10 cm

2.2.1.2 Original method (Heilmann and Beese, 1992)

Soil was sieved at field moisture content and stored at 4°C for less than one week before analysis. 5 g fresh weight soil was mixed with D-glucose and 0.5 g talcum powder. The talcum powder served as an inert carrier for the glucose. This was necessary because only small

amounts of D-glucose were required. D-glucose and talcum powder was added (0, 0.5, 1, 2, 4 mg g^{-1} soil) to triplicate samples of soil in glass vials. The vials were placed in 60 ml syringes and the carbon dioxide in the headspace gas was measured prior to incubation. The samples were incubated at 22°C for 6 hours. CO_2 in the headspace gas was measured using a gas chromatograph (Appendix A).

2.2.1.3 Slurry method

Modifications to the original method were made for use with highly organic and waterlogged peat soils. The modifications were

- adding the substrate as a solution, to ensure adequate mixing
- incubating the soil as a slurry
- continuous shaking of the samples (350 rpm on a flatbed shaker)

These are explained in detail below.

The substrate (D-glucose) was added to 1 g soil samples as a solution (0, 20, 40, 80, 160 mg l^{-1}). Distilled water was added to saturate the soils in quantities of 2 ml per 1 g fresh weight soil. In this way, it was unnecessary to correct for the moisture content of the soils, because the soils were saturated with solution. A vortex mixer was used to mix the samples for approximately 5 seconds. Samples were then incubated for 4 hours. Carbon dioxide evolving from soil respiration was trapped in the syringes and measured by gas chromatography.

The relationship between maximum initial respiration and microbial biomass carbon was calculated according to Hopkins and Shiel (1996) which is based on Anderson and Domsch (1978). The relationship between substrate concentration and respiration observes Michaelis-Menten kinetics. Hopkins and Shiel (1996) used a Hanes-Woolf[1] plot it to describe the rate of increase, which they calibrated for biomass C size empirically using a range of different soils. The relationship between V_{max} ([S] / V_o) and biomass C was: biomass C (mg C_{mic} g^{-1} soil) = 0.9021 Vmax – 0.0031. It was possible to use this relationship to estimate the size of the soil microbial community in the soils from Dun Moss because saturation levels were achieved for

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[1] If [S]/Vo is plotted against [S] a straight line is obtained where the slope is equal to 1/Vmax and the y-intercept is equal to Km/Vmax (Han and Levenspiel, 1988).

every determination. Figure 2.1 is a typical saturation curve. By using five glucose concentrations it was possible to ensure that saturation point had been reached.

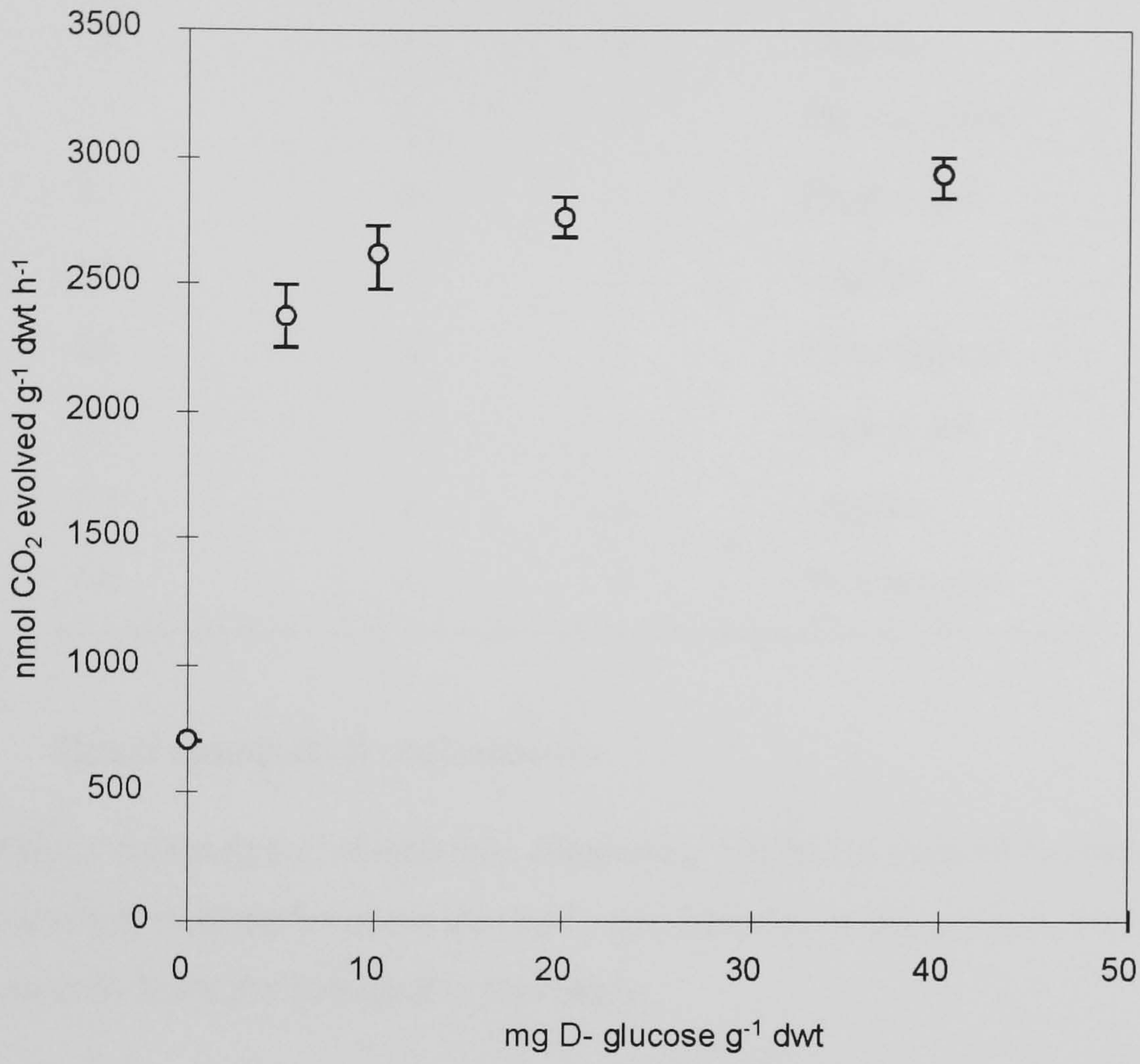

Figure 2.2: Response of the soil microbial community to glucose additions.

2.2.2 *Size of soil microbial community*

2.2.2.1 Sampling

Soil sampling took place on 16 July 1999. The samples were sieved (10mm) in the field moist state and stored in polythene bags 1-3 weeks prior to analysis. Six transects were sampled therefore 54 biomass determinations were done in total (6 transects x 3 soils x 3 replicates). Properties for each of these soils are listed in Appendix B2.

2.2.2.2 Method

Soil microbial biomass C of the Dun Moss soils was estimated using the modified method.

Table 2.2: Numbering system used for soils from Dun Moss.

Sampling I.D. of Dun Moss (DM) soils	Transect	Soil type	Origin
1.1	1	1	Mineral soil
1.2	1	2	Lagg fen
1.3	1	3	Mire expanse
2.1	2	1	Mineral soil
2.2	2	2	Lagg fen
2.3	2	3	Mire expanse
3.1	3	1	Mineral soil
3.2	3	2	Lagg fen
3.3	3	3	Mire expanse

2.2.3 Basal respiration calibration

In order to make a comparison between the laboratory method proposed by Heilmann and Beese (1992) and the modified method that had been adopted in this project, both methods were tested on soils from the site used in this study.

2.2.3.1 Sampling

The calibration of the modified basal respiration technique was carried out on the samples collected on 16 July 1999.

2.2.3.2 Original method (Heilmann and Beese, 1992)

Soil was sieved using a 10 cm sieve to mix the soil. The samples were stored for up to two weeks at 4° C. 10 g samples of moist soil were placed into glass vials, which were then placed into 60 ml syringes. The syringes were stored at 14° C and CO_2 was measured and syringes were flushed at 24 h intervals. CO_2 production was recorded until a steady rate of CO_2 evolution was reached. 14° C was used because this represents the annual average temperature for upland soils in Scotland. Stabilisation of CO_2 evolution occurred after 31 hours. Mean CO_2 production rates were then calculated for each soil using 6 subsequent time

points. These data were used for comparison with the values obtained from the modified method.

2.2.3.3 Slurry method

Samples of moist soil (1 g) were weighed into culture tubes (15 ml). 2 ml distilled water was added to each tube and the tubes were sealed so that they were airtight. The tubes were placed on a flatbed shaker for 4 h at 350 rpm. The tubes were kept at room temperature (22°C). This method could also be carried out in conjunction with the substrate induced respiration method for the determination of biomass carbon (Ritz and Wheatley, 1989). CO_2 accumulation in the headspace of the tubes was measured using a gas chromatograph. The data were expressed as CO_2 g^{-1} soil h^{-1}.

2.2.4 *Basal respiration of soils*

2.2.4.1 Sampling

Soil sampling took place on 16 July 1999. The samples were sieved (10mm) in the field moist state and stored in polythene bags 1-3 weeks prior to analysis.

2.2.4.2 Method

The basal respiration of the Dun Moss was determined using the original method.

2.2.5 *Metabolic diversity*

2.2.5.1 Procedure

Inoculation A range of approaches has been used in the inoculation of Biolog™ microtitre plates with environmental samples. Some researchers have used colony forming units as a way of standardizing the inoculum size (Zak et al., 1994) however, this is reliant on the culturing the soil microbial community. Average well colour development (AWCD) was suggested by Garland and Mills (1991). In this procedure, the average well colour development is subtracted from each well separately before analysis. However, it is probably more relevant to this study to standardize for respiration of the soil microbial community as this takes into account both

the size and the active component of the soil microbial community in an ecological context. Respiration is appropriate because Biolog™ also measures respiration as a redox reaction.

Approximately 5 g moist soil was added to 10 ml dH_2O (adjusted for moisture content). The amount of sample varied according to the basal respiration of the soils (see table 2.3).

Table 2.3: Sample size and water amendment for Biolog™ assay inoculation.

Soil	Basal respiration (μ mol CO_2 g soil h^{-1})	Distilled water cm^{-3}	Sample in g fresh wt
1.1	90.02	11.56	5.00
2.1	124.23	12.80	3.37
3.1	102.45	12.80	4.16
1.2	980.86	13.14	1.96
2.2	384.14	13.25	2.12
3.2	499.44	13.33	2.12
1.3	402.31	13.31	1.90
2.3	274.11	10.50	5.01
3.3	400.36	10.44	4.81

Samples were sonicated for 30 minutes (Appendix A). Ten fold serial dilutions were made aseptically using distilled water. 150 µl of 10^{-3} dilution was used to inoculate the wells of the microtitre plate. The plates were incubated in the dark at 22° C for 10 days. The colour change at 560 nm in the wells was recorded using a plate reader (Appendix A). Absorbance was measured twice daily for the first 72 hours and every 24 hours thereafter. Table 2.4 shows the substrates used in the Ecoplate assay.

2.2.5.2 Data analysis

Two overall measurements were obtained per sample for each substrate. Maximum substrate utilization was calculated using the maximum absorbance recorded. An indicator of the rate of substrate utilization was arrived at using the time taken for the soil microbial community in the wells to produce maximum colour change for the individual wells.

The data were reduced using principal components analysis (PCA) in Minitab™ version 12. PCA is a multivariate statistical method used to reduce the dimensionality of data sets containing many interrelated variables while retaining as much as possible of the systematic

variation in the original data set (Joliffe, 1986). PCA was used to analyse the similarity between soil samples (observations) and substrates (variables). Factor analysis was also carried out on the data. The data are presented for each plot within a site because of the variability within the soils.

Table 2.4: Substrates used in the Biolog™ Ecoplates.

Amino acids (AA)	Carbohydrates (C)
L-arginine	α-keto butyric acid
L-asparagine	D-malic acid
L-phenylalanine	D-celloblose
L-serine	α-D-lactose
L-threonine	β-methyl-D-glucoside
glycyl-L-glutamic acid	D-xylose
phenylethyl-amine	I-erythritol
	glucose-1-Phosphate
Amines (Am)	D,L-a-glycerol phosphate
N-acetyl-D-glucosamine	
putrescine	Polymers (P)
	Tween 40
Alcohol (A)	Tween 80
D-Mannitol	α-cyclodextrin
	glycogen
Carboxylic acids (CA)	
pyruvic acid	
D-glucosaminic acid	
D-galactonic acid	
D-galacturonic acid	
2-hydroxy benzoic	
4-hydroxy benzoic acid	
γ-hydroxybutyric acid	
itaconic acid	

2.2.6 *Functional diversity*

2.2.6.1 Procedure

Extraction of lipids from soil samples

3 g moist soil was added to Bligh and Dyer solvent (Bligh and Dyer, 1959) comprising chloroform:methanol:citrate buffer 1:2:0.8 (v/v/v) + 0.005% butylated hydroxy toluene. The citrate buffer volume was adjusted for the moisture content of the soil. Samples were sonicated for 30 minutes prior to overnight refrigeration at 4°C.

Samples were centrifuged at 1200 rpm for 10 minutes, after which the supernatant was collected. 5 ml Bligh and Dyer solvent was added to the sample then the samples were spun at 1200 rpm for 10 minutes. Again, the supernatant was removed and combined with the previous supernatant. Phases were split using 8 ml chloroform:citrate buffer 1:1 (v/v). Samples were incubated 24 hours at 4°C.

Lower phase was collected and dried under liquid N_2 at 50°C and stored at 4°C.

Fractionation of lipids

Pre-wash

Sicilic acid columns (Appendix A) were washed using 2 ml of each of the extraction solvents (methanol, acetone, chloroform).

Conditioning and fractionation

2 ml chloroform was allowed to travel through the column before fractionation. Neutral lipids, glycolipids and polar lipids were eluted from the column using 5 ml chloroform, 12 ml acetone and 8 ml methanol respectively.

The final, methanol fraction containing the phospholipids was collected and dried under N_2 at 40°C. The samples were stored at –18°C prior to derivitization.

Derivitization

The fractionated sample was dissolved in 1 ml toluene:methanol (1:1). 1 ml methanoic KOH was added and the sample was incubated at 37°C for 30 min. Acetic acid (0.3 ml) was added to stop the reaction. 5 ml hexane:chloroform (4:1 v/v) and 3 ml distilled water were then added. The samples were sonicated for 30 min, and spun at 1200rpm for 10 min. The aqueous layer was discarded.

Final sample clean-up

3 ml NaOH (12 g l^{-1}) was added to wash the sample. The supernatant was collected in a clean vessel. This wash was repeated 3 times. The combined supernatants were dried under N_2 at 30°C. Samples were stored at –18°C until gas chromatography.

Gas chromatography was carried out using a gas chromatograph set up to measure fatty acids (Appendix A). Bacterial acid methyl ester (BAME) mixture (Sigma-Aldrich, UK) was

used as a standard for the identification of individual fatty acid methyl esters (FAMEs). Table 2.5 shows the FAMES identified in this assay.

Table 2.5: Fatty acid methyl esters (FAMEs) identified using Bacterial Acid Methyl Ester standard.

1. Me undecanoate (C11:0)
2. Me 2-hydroxydecanoate (2OH-C10:0)
3. Me dodecanoate (C12:0)
4. Me tridecanoate (C13:0)
5. Me 2-hydroxydodecanoate (2OH-C12:0)
6. Me 3-hydroxydodecanoate (3OH-C12:0)
7. Me tetradecanoate (C14:0)
8. Me 13-methyltetradecanoate (iso-C15:0)
9. Me 12-methyltetradecanoate (anteiso-C15:0)
10. Me pentadecanoate (C15:0)
11. Me 2-hydroxytetradecanoate (2OH-C14:0)
12. Me 3-hydroxytetradecanoate (3OH-C14:0)
13. Me 14-methylpentadecanoate (iso-C16:0)
14. Me cis-9-hexadecenoate (C16:1ω7*cis*)
15. Me hexadecanoate (C16:0)
16. Me 15-methylhexadecanoate (iso-C17:0)
17. Me cis-9,10-methylenehexadecanoate (cyc-C17:0)
18. Me heptadecanoate (C17:0)
19. Me 2-hydroxyhexadecanoate (2OH-C16:0)
20. Me cis-9,12-octadecadienoate (C18:2ω6,*cis*)
21. Me cis-9-octadecenoate (C18:1ω9*cis*)
22. Me trans-9-octadecenoate (C18:1ω9*trans*) and Me cis-11-octadecenoate (C18:1ω7*cis*)
23. Me octadecenoate (C18:0)
24. Me cis-9,10-methyleneoctadecanoate (cyc-C19:0)
25. Me nonadecanoate (C19:0)
26. Me eicosanoate (C20:0)

2.2.6.2 Data analysis

The data were presented as percentage of total FAMEs identified by gas chromatography. An internal standard was not used therefore, the data were not quantitative.

PCA (Minitab™ ver 12) was used to analyse the similarity between soils (observations) and FAMEs (variables).

Fames have been used as indicators of groups within bacteria and fungi. For example the detection of iso-C15:0 in FAME sample suggests the presence of Gram positive bacteria (Sundh *et al.*, 1997). The FAMEs identified by the BAME™ (Sigma Aldrich) standard which have been connected with particular groups of organisms are outlined in table 2.6.

Table 2.6: FAMEs as indicators of soil microorganisms.

FAME	Organisms	Reference
Iso-C15:0, anteiso-C15:0, C15:0, C16:0, C16:1w9, iso-C16:0, cyc-C17:0, C17:0, cyc-C19:0	Bacteria	Frostegård *et al.*, 1996
Iso-C15:0, anteiso-C15:0, iso-C17:0, iso-C16:0	Gram positive bacteria	Sundh, *et al.*, 1997 Zelles, 1997
cyc-C17:0, cyc-C19:0	Gram negative bacteria	Zelles, 1997
C18:2ω6	Fungi	Lechevalier and Lechevalier, 1988 Frostegård and Bååth, 1996
C20:0	Plant fatty acids, mosses and eukaryotes	Zelles, 1999

2.3 Results

2.3.1 *Soil properties*

Some basic properties of the mineral soils and soils of the lagg fen and mire expanse are shown in table 2.7. The pH ranged from 3.32 to 4.75, the soils of the mire expanse were more acidic than the those of the lagg fen and the mineral soils. The amount of carbon in the soils differed significantly between soil types, ranging from 4.4 to 51 %. Similarly, nitrogen contents varied considerably (0.04 to 1.5%). The bulk density was greatest in the mineral soils and did not exceed 0.1 g cm^{-3} in the peat soils.

Table 2.7: Summary of soil properties. Numbers in brackets are standard deviation of 3 replicates. ANOVA was used to determine *P*. Similar letters correspond to similar soils.

	%N	%C	C:N	C_{mic}:C_{org}	pH	Bulk density (dwt cm^{-3})
P	0	0	0.001	0	0	0
Mineral soil	1.17a	25.44a	21.22a	0.006a	3.53a	0.26a
SD	(0.46)	(13.33)	(7.48)	(0.003)	(0.18)	(0.07)
Lagg fen	1.85b	43.47b	25.44a	0.001b	3.45a	0.098b
SD	(0.48)	(6.67)	(9.35)	(0.007)	(0.17)	(0.06)
Mire expanse	1.51a	46.33b	31.6b	0.003b	3.31b	0.06b
SD	(0.29)	(1.39)	(6.01)	(0.01)	(0.09)	(0.016)

2.3.2 *Calibration of the modified biomass C method*

The comparison between the original substrate induced respiration method, proposed by Anderson and Domsch (1978) and the modified method was made using a range of soils. The relationship between the two methods was analysed using least squares regression statistics.

There was a significant difference between the soils in terms of biomass C ($p<0.001$). Table 2.8 shows the smallest biomass C was found in the Redesdale 2.3 (0.09 mg g^{-1} soil) soil using both methods and the greatest biomass C was found in the gley mineral soil (1.36 mg g^{-1} soil) using the modified method. There was a positive linear relationship (original = 0.9743modified + 0.0745) between the two methods and this was significant ($r^2 = 0.89$).

Table 2.8: Biomass C sizes determined by the original and modified method.

Soil	Biomass C Original SIR method (mg C_{mic} g^{-1}) $\overline{x}$	Biomass C Modified SIR method (mg C_{mic} g^{-1}) $\overline{x}$
Aldbar	0.45	0.28
Vinny	0.10	0.09
Gley	1.34	1.36
Podzol	1.00	0.67
Redesdale Wilcocks 2.1	0.49	0.37
Redesdale Wilcocks 2.3	0.05	0.10
Peat DM 3.2	0.60	0.72

2.3.3 Size of soil microbial community

In total 54 different analyses were carried out for this section, and the results may be found in appendix B2. The biomass C of the soil microbial community was greatest in the mineral soils. This was significantly different from the biomass size in the peat soils, which were similar. The size of the soil microbial community in the mineral soils was approximately 3-4 times greater than in the peat soils (table 2.9).

Table 2.9: Biomass C sizes for Dun Moss soils. Numbers in brackets indicate standard deviation of 18 field and 4 analytical replicates. Statistical analysis using ANOVA was used to determine p. Similar letters correspond to similar populations.

	P	Mineral soil	SD	Lagg fen soil	SD	Mire expanse peat soil	SD
Biomass C (mg C_{mic} cm^{-3} soil)	<0.001	0.33a	(0.17)	0.08b	(0.03)	0.09b	(0.08)

Best-subsets regression analysis revealed that soil properties pH, %N, %C and C:N did not correlate to microbial properties, basal respiration and soil microbial biomass size. However, soil properties were correlated to microbial properties for individual soils. In the mineral soils a correlation was seen between basal respiration and the size of the soil microbial biomass (r^2 = 0.68). Nitrogen was also correlated with biomass size in these soils (r^2 = 0.51). However in the peats none of the environmental variables tested showed a good correlation with the size of the soil microbial community.

2.3.4 *Calibration of modified basal respiration method*

CO_2 production in the original method stabilized after 31 hours. Therefore, a mean of the 6 time points after 31 hours was taken as the rate of basal respiration of the soils. These data are compared with the slurry method for determining basal respiration in figures 2.4 to 2.7. In the slurry method basal respiration was calculated from the CO_2 production during 4 hours incubation in slurry form.

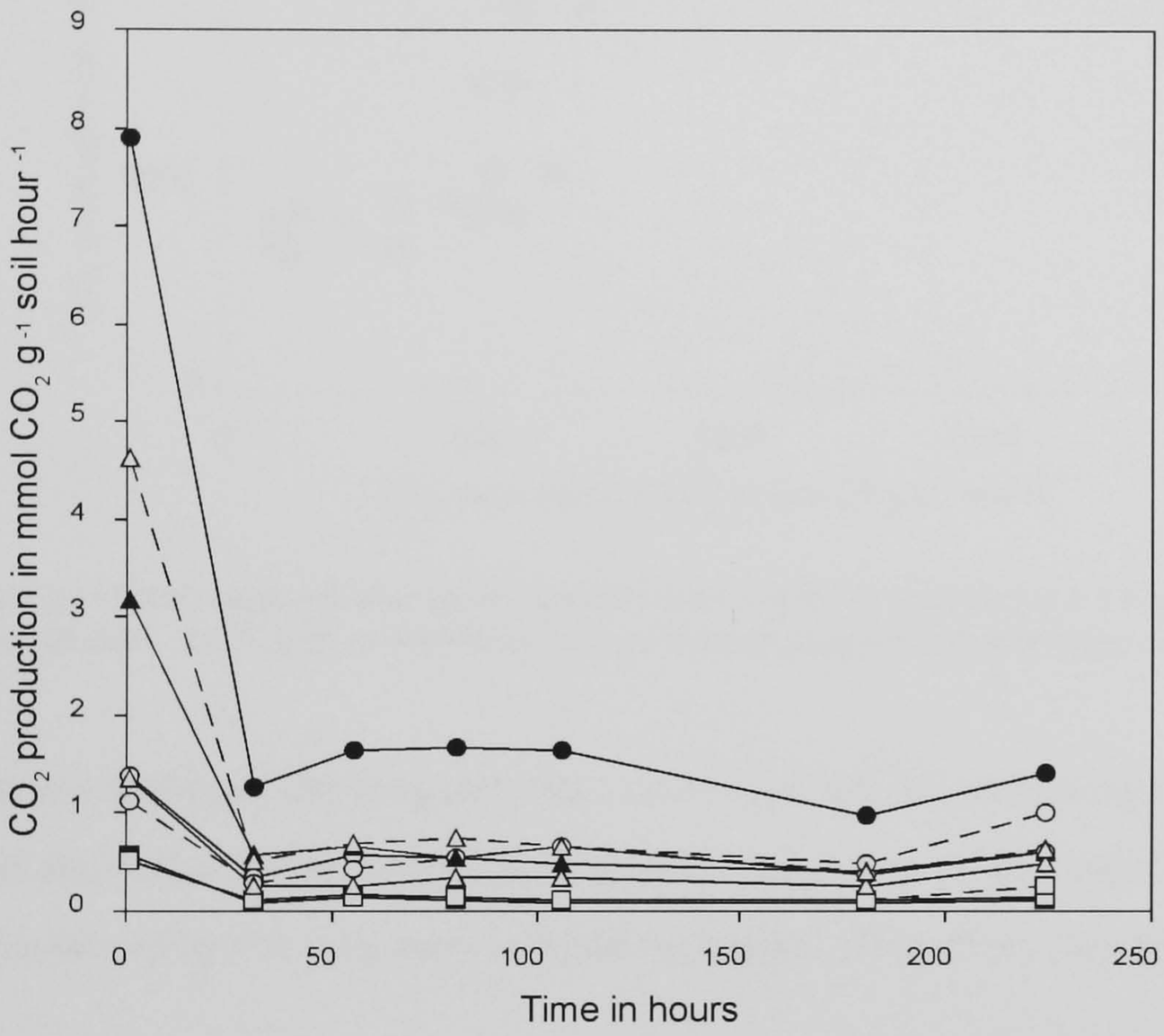

Figure 2.3: CO_2 production from soils incubated at 14°C. The square, circular and triangular markers represent the mineral soils, the soils of the lagg fen and the soils of the mire expanse respectively. Closed markers are transect 1, open markers are transect 2 and open markers with a dashed line are transect 3.

Figure 2.4 shows the relationship between the CO_2 production rates measured using syringes and soils in slurry. The CO_2 production rates from the short term slurry experiment were much greater than from the syringe experiment and there was no correlation between the two methods. The data were not normally distributed (Anderson-Darling test for normailty; P = 0.0358). Soil 1.2 from the lagg fen had a greater respiration than the other soils. The data are also presented by soil type in figures 2.5 to 2.7.

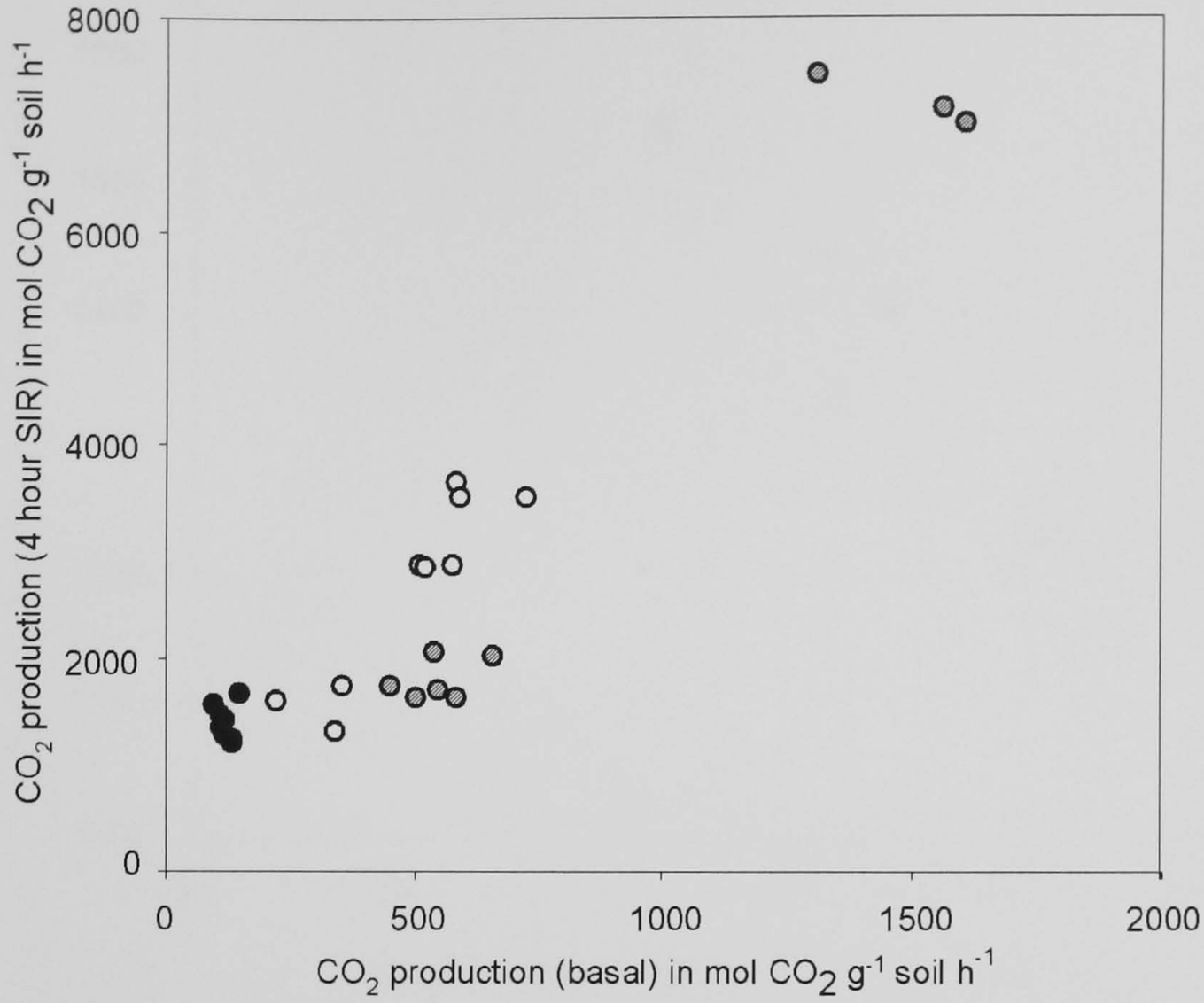

Figure 2.4: Basal respiration measured after an initial stabilization period (x axis) and in a 4 hour incubation in a slurry (y axis): all soils. Black, grey and white circles are mineral, lagg fen and mire expanse respectively.

Comparison between the long and short term methods for estimating respiration in the mineral soils showed a decrease in CO_2 production measured by the short term method, as respiration measured by the long term method increased. This effect may have been a result of clustering.

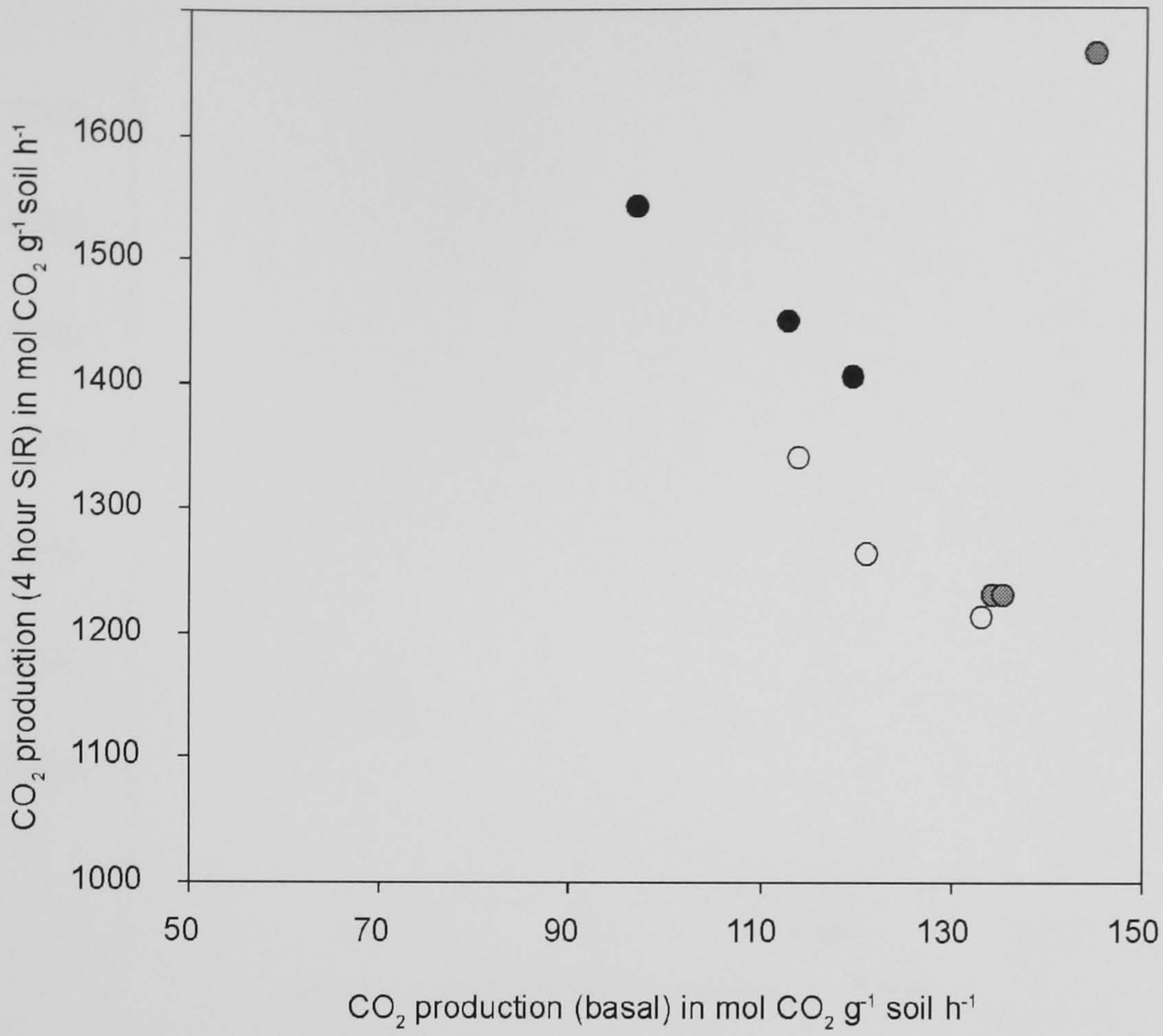

Figure 2.5: Basal respiration measured after an initial stabilization period (x axis) and in a 4 hour incubation in a slurry (y axis): mineral soils. Black, grey and white circles are from transects 1, 2 and 3 respectively.

The CO_2 rates for the soils of the lagg fen were much greater than those of the mineral soils. The data were not normally distributed; there were two distinct groups, one showing unusually high rates of respiration. The group of samples with high respiration rates all originated from soil 1.2, therefore this set may be an outlier.

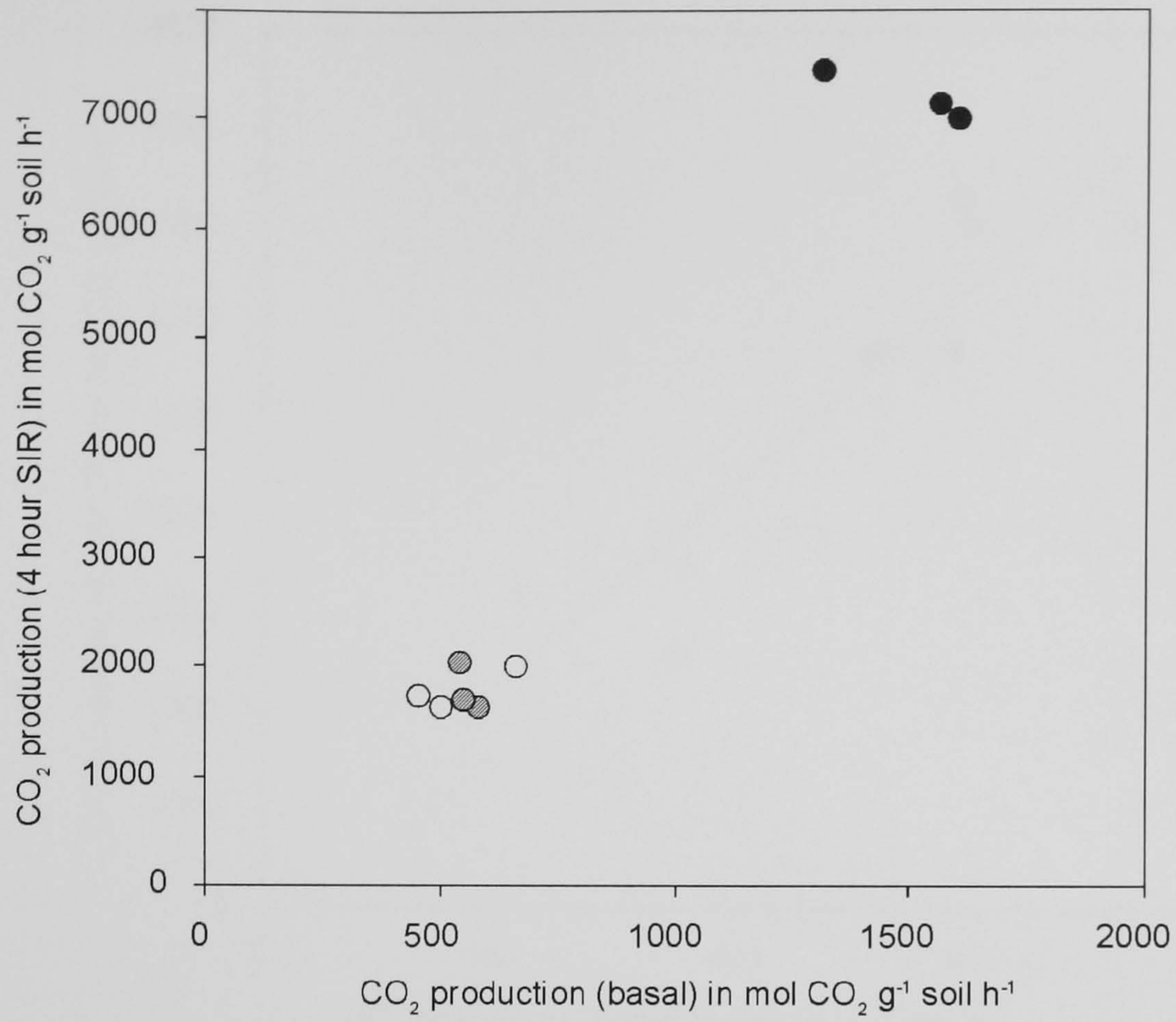

Figure 2.6: Basal respiration measured after an initial stabilization period (x axis) and in a 4 hour incubation in a slurry (y axis): soils of the lagg fen. Black, grey and white circles are from transects 1, 2 and 3 respectively.

Respiration from the soils of the mire expanse fell between the levels measured for the mineral soils and the soils of the lagg fen. The plot of CO_2 production rates for comparison between the two methods showed a normal distribution and 82.75 % of the data were described by a linear equation ($y = 0.1598x + 66.127$).

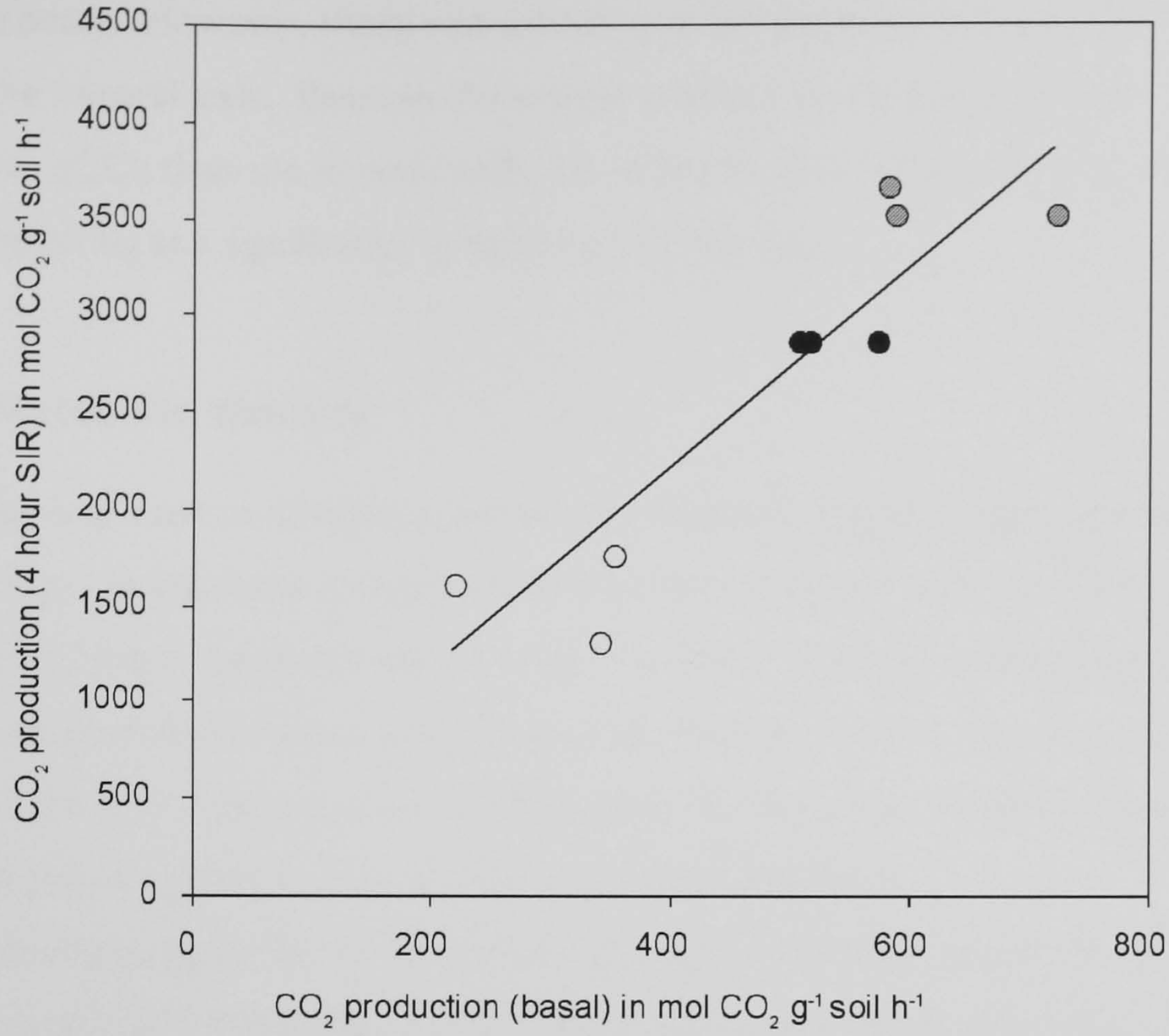

Figure 2.7: Basal respiration measured after an initial stabilization period (x axis) and in a 4 hour incubation in a slurry (y axis): mire expanse soils. Black, grey and white circles are from transects 1, 2 and 3 respectively.

2.3.5 Basal respiration soils

There was a significant difference in respiration between the mineral soils and the mire expanse peat (table 2.10). Indeed the mean soil respiration for the mineral soils was more than twice that of the peat soils from the mire expanse. The respiration of the lagg fen soils was between that of the mire expanse soils and of the mineral soils, and not significantly different from either.

Table 2.10: Basal respiration and metabolic quotients for soils at Dun Moss.

	P	Mineral soil	SD	Lagg fen soil	SD	Mire expanse peat soil	SD
Basal cm^3 (n mol CO_2 cm^{-3} h^{-1})	0.005	109.66	(53.60)	76.81	(48.83)	58.44	(33.91)
qCO_2 (mol CO_2 mg^{-1} C_{mic} h^{-1})	0.005	0.38×10^{-6}a	(0.15×10^{-6})	1.12×10^{-6}b	(0.82×10^{-6})	1.10×10^{-6}b	(1.14×10^{-6})

Soil respiration was also expressed per unit of microbial biomass C as determined by SIR. This provides a measure of specific metabolic activity or qCO_2. The qCO_2 differed significantly between the mineral soils and the peats, with little difference between the lagg fen peats and

the mire expanse peats. However, there was a much greater variation in the qCO_2 of the peats soils than the mineral soils. Because the smaller biomass in the peat soils has a significantly greater qCO_2 than the mineral soils, this indicates that the smaller microbial community was respiring at a significantly greater rate in the peats.

2.3.6 *Metabolic diversity*

The Biolog™ assay was used to describe some of the metabolic characteristics of the soil microbial community. In this assay a single carbon source is provided and the microbial respiration related to this is measured using a redox reaction. This is possible using a tetrazolium dye, which changes colour producing a result which may be measured optically. The respiration of the microbial inoculum is proportional to the colour intensity produced. Absorbance at 550 nm was used as a measure of substrate utilization.

There was a large variability within soils for maximum absorbance and time elapsed to maximum absorbance in all of the soils. For this reason, the plots within soil types are presented separately in tables 2.12 and 2.13.

Table 2.11 contains the *P* values for analysis of variance tests on the maximum absorbance values and the time taken for maximum absorbance to be reached in the soils. The analysis revealed that the Biolog™ system was able to differentiate between soils (Tukey's pairwise comparison) using 10 different substrates. Significant differences were found between the soils in the utilization of 7 substrates. The utilization of glcyl-L-glutamic acid and γ-hydroxybutyric acid was significantly different between the soils of the mire expanse and the other two soils; utilization was much less in the soils of the mire expanse. The soil microbial community of the mire expanse and lagg fen soils were significantly different in their utilization of D-mannitol, 2-hydroxy benzoic acid, α-keto butyric acid and β-methyl-D-glucoside; the utilization was also smallest in the soils of the mire expanse. The utilization of β-methyl-D-glucoside and D,L-α-glycerol phosphate was significantly different between the mineral soils and the soils of the lagg fen; the utilization was smallest in the mineral soils and soils of the lagg fen respectively.

Only one substrate differentiated soils in terms of colour development and time taken before maximum absorbance was reached therefore the time taken for maximum utilization to be reached may be less useful than the actual value of maximum utilization for distinguishing between soil types. The utilization of D-mannitol was significantly different in the soils of the mire expanse, which took the longest time to attain greatest substrate utilization (333 h). This

was also true for D-malic acid, for which the mean substrate utilization was 285 h for the bog soil. Utilization of D-galactonic acid was significantly different between the lagg fen and mire expanse, 78 and 279 hrespectively. D-xylose was able to distinguish between the mineral soils and the soils of the mire expanse. The soil microbial community of the mire expanse took longer to attain maximum colour development of this substrate.

The maximum utilzation of 7 substrates (out of the 31 tested) was different between two or more soils. The time taken to reach maximum absorbance was less successful at distinguishing between soils, only 4 differences were seen. These results suggest that the Biolog ™ assay is not suitable for distinguishing between the soils tested, and the possible reasons for this will be outlined in the discussion section of this chapter.

Table 2.11: *P* values relating to significant differences between soils according to maximum absorbance and time elapsed before maximum absorbance was reached in wells.

Well number		Abs *P* value	Tukey's pairwise comparison (0.05)	Time of max. absorbance *P* value	Tukey's pairwise comparison (0.05)
12	L-arginine	0.845		0.178	
29	L-asparagine	0.83		0.502	
30	L-phenylalanine	0.143		0.303	
31	L-serine	0.3		0.444	
32	L-threonine	0.156		0.067	
22	glycyl-L-glutamic acid	0.013	1+3, 2+3	0.095	
23	phenylethyl-amine	0.74		0.216	
24	N-acetyl-D-glucosamine	0.067		0.264	
25	putrescine	0.119		0.076	
26	D-mannitol	0.04	2+3	0.007	1+3, 2+3
11	pyruvic acid	0.055		0.154	
9	D-glucosaminic acid	0.134		0.616	
13	D-galactonic acid	0.089		0.02	2+3
27	D-galacturonic acid	0.232		0.854	
28	2-hydroxy benzoic	0.032	2+3	0.258	
4	4-hydroxy benzoic acid	0.031		0.072	
15	γ-hydroxybutyric acid	0.004	1+3, 2+3	0.541	
16	itaconic acid	0.415		0.452	
17	α-keto butyric acid	0.019	2+3	0.115	
18	D-malic acid	0.192		0.024	1+3, 2+3
19	D-celloblose	0.512		0.483	
20	α-D-lactose	0.061		0.758	
21	β-methyl-D-glucoside	0.025	1+2, 2+3	0.579	
2	D-Xylose	0.744		0.044	1+3
3	l-erythritol	0.266		0.625	
4	glucose-1-phosphate	0.174		0.079	
5	D,L-α-glycerol phosphate	0.017	1+2, 1+3	0.58	
6	Tween 40	0.514		0.622	
7	Tween 80	0.773		0.221	
8	α-cyclodextrin	0.179		0.596	
10	glycogen	0.256		0.503	

Table 2.12: The maximum absorbance at 550nm of Biolog™ substrates

Substrate	L-arginine	L-asparagine	L-phenylalanine	L-serine	L-threonine	Glcyl-l-glutamic acid	Phenylethyl-amine	N-acetyl-D-glucosamine	putrescine	D-mannitol	pyruvic acid	D-glucosaminic Acid	D-galactonic acid	D-galacturonic Acid	2-hydroxy benzoic acid	4-hydroxy benzoic acid	γ-hydroxybutyric acid	itaconic acid	α-keto Butyric acid	D-malic acid	D-celloblose	α-D-lactose	β-methyl-D-glucoside	D-xylose	L-erythritol	Glucose-1-phosphate	D,L-α-glycerol phosphate	Tween 40	Tween 80	α-cyclodextrin	glycogen
Soil	Absorbance at 550 nm																														
1.1	1.57	1.53	1.34	1.31	1.67	0.87	0.29	1.38	1.00	1.88	0.81	0.93	1.11	1.18	0.46	1.07	0.74	1.09	0.53	0.69	1.84	1.47	1.11	1.99	1.57	0.76	0.49	1.52	1.53	0.89	1.71
2.1	0.59	1.22	0.40	1.24	0.59	0.50	0.64	0.91	0.80	1.79	1.00	0.80	1.12	1.30	0.43	0.75	0.45	1.18	0.39	0.72	0.99	0.98	0.14	0.91	0.72	0.51	0.40	1.06	1.36	0.81	1.00
3.1	0.76	0.82	0.77	0.73	0.99	0.77	0.56	0.81	0.82	1.70	0.78	1.17	0.59	0.99	0.24	0.39	0.11	0.67	0.31	0.40	1.31	0.53	0.87	1.18	1.01	0.44	0.41	1.40	1.25	0.35	1.50
1.2	1.78	1.70	0.88	1.50	0.67	1.16	1.14	1.58	1.29	1.86	1.05	1.43	1.33	1.48	0.27	1.56	0.91	1.29	0.12	0.39	1.59	1.72	1.16	1.84	1.56	0.79	0.56	1.68	1.49	0.29	0.22
2.2	1.58	1.44	1.17	1.38	1.60	1.17	1.59	1.42	0.98	1.89	0.74	1.12	1.10	0.92	0.05	1.23	1.56	1.26	0.62	0.65	1.39	1.88	1.11	1.28	2.13	0.66	0.47	1.76	1.55	0.21	1.96
3.2	1.23	1.45	0.99	1.36	1.37	0.89	0.07	1.45	0.56	1.96	0.77	0.94	1.10	1.17	0.42	0.94	0.86	0.85	0.04	0.27	1.61	0.62	1.23	2.05	1.42	0.67	0.26	1.50	1.73	0.07	0.14
1.3	0.35	0.64	0.68	0.77	0.44	0.85	0.30	1.43	0.57	1.91	0.77	1.02	0.61	1.07	0.25	0.21	0.16	0.11	0.27	0.06	1.74	0.16	1.55	0.93	1.05	0.12	0.49	1.68	1.43	0.13	0.38
2.3	1.18	1.08	0.56	0.91	1.05	0.82	0.31	1.20	0.64	2.20	1.09	0.92	0.52	0.50	0.35	0.52	0.20	0.55	0.17	0.08	1.37	0.68	1.16	1.25	0.80	0.27	0.16	1.21	1.53	0.12	0.82
3.3	0.46	0.68	0.42	0.87	1.38	0.26	0.28	1.15	0.41	1.25	0.98	0.72	0.47	0.84	0.54	0.76	0.36	0.06	0.79	0.37	1.43	0.67	1.19	1.15	0.68	0.02	0.24	1.55	1.47	0.06	1.37

Table 2.13: Time taken for maximum absorbance (table 2.11) to be reached in Biolog ™ plates.

Substrate		L-arginine	L-asparagine	L-phenylalanine	L-serine	L-threonine	glcyl-l-glutamic acid	phenylethyl-amine	N-acetyl-D-glucosamine	putrescine	D-mannitol	pyruvic acid	D-glucosaminic Acid	D-galactonic acid	D-galacturonic Acid	2-hydroxy benzoic acid	4-hydroxy benzoic acid	γ-hydroxybutyric acid	itaconic acid	α-keto Butyric acid	D-malic acid	D-celloblose	α-D-lactose	β-methyl-D-glucoside	D-xylose	L-erythritol	Glucose-1-phosphate	D,L-α-glycerol phosphate	Tween 40	Tween 80	α-cyclodextrin	glycogen
Soil	Time in hours																															
1.1		155	169	352	133	352	169	352	126	101	296	169	146	123	36	352	60	352	165	352	280	148	352	76	126	241	250	194	352	352	296	352
2.1		140	23	184	23	187	225	131	211	58	178	23	134	23	23	267	115	185	192	241	167	241	241	163	216	241	296	130	187	296	352	185
3.1		154	154	352	296	296	296	211	241	200	352	136	219	219	194	296	352	242	241	240	296	185	352	185	296	352	242	240	296	296	117	280
1.2		63	194	296	47	352	154	186	101	47	200	275	93	352	55	352	68	296	248	352	352	352	352	216	160	352	281	194	296	352	272	352
2.2		148	90	272	85	296	352	140	94	148	169	133	146	219	138	281	148	90	143	352	296	352	110	28	275	352	242	250	281	352	352	352
3.2		197	139	352	272	352	241	275	41	272	187	173	248	176	44	352	248	241	248	352	296	63	352	143	97	352	93	250	352	352	352	296
1.3		184	296	235	235	352	352	281	191	296	209	352	216	235	209	235	154	267	235	235	235	352	352	74	134	296	179	156	352	352	169	194
2.3		272	352	352	352	352	275	240	296	296	352	241	352	296	352	235	235	235	352	235	235	352	352	250	352	352	296	240	275	352	235	352
3.3		267	352	235	275	272	352	352	352	296	352	296	352	296	296	352	275	352	352	352	296	352	352	275	352	352	352	173	352	352	352	352

2.3.6.1 Principal components analysis (PCA)

For comparison within the PCA plots the distance between samples of soils from the same area was used as an indicator of variability. Using the maximum absorbance data produced by the Biolog ™ assay presented in figure 2.8 as an example, the distance ratio between the three soil types is 14:8:7. This figure was arrived at by measuring the total distance between 1.*, 2.* and 3.* on the PCA plot. This was done for all three soil types, which produced a ratio.

Maximum absorbance was not able to differentiate between the three soil types (figure 2.8). The greatest distance within soils was observed within the mineral soils 1.1, 2.1, 3.1. There was no overlap between soils. The mire expanse peats (1.3, 2.3, 3.3) formed the closest grouping. The ratio of distances between soils was 14:8:7 for the mineral soils, soils of the lagg fen and mire expanse respectively.

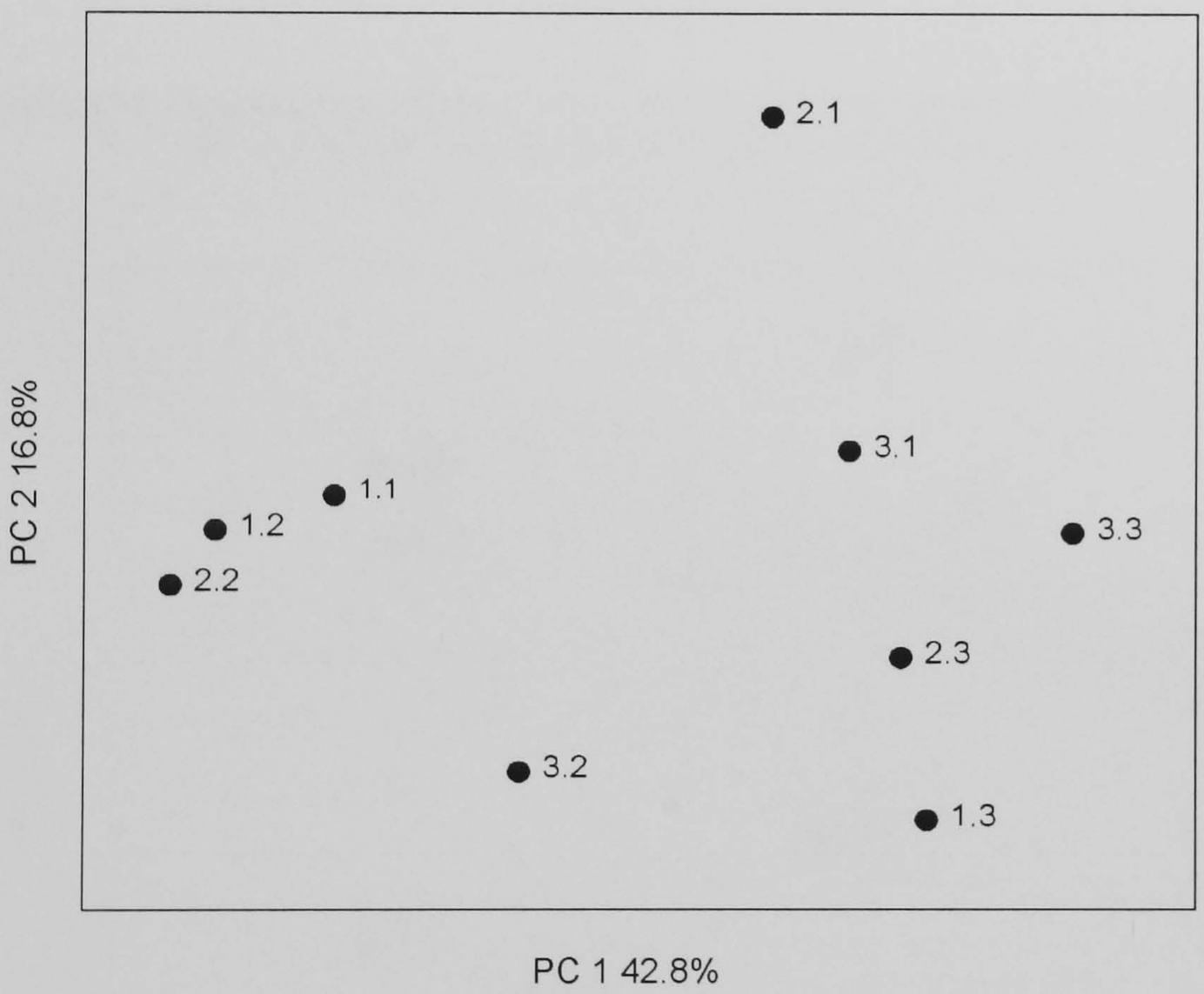

Figure 2.8: PCA analysis of soils using maximum absorbance values of Biolog™. PC 1 and 2 account for 42.8 % and 16.8 % of the variability respectively.

PCA using time taken for maximum substrate utilization was not able to differentiate between the three soils (figure 2.8). There was no overlap between the peats of the mire expanse and the soils of the lagg fen and surrounding slopes. The ratio of distances between soils was 16.5:4:7 for the mineral soils, soils of the lagg fen and mire expanse respectively.

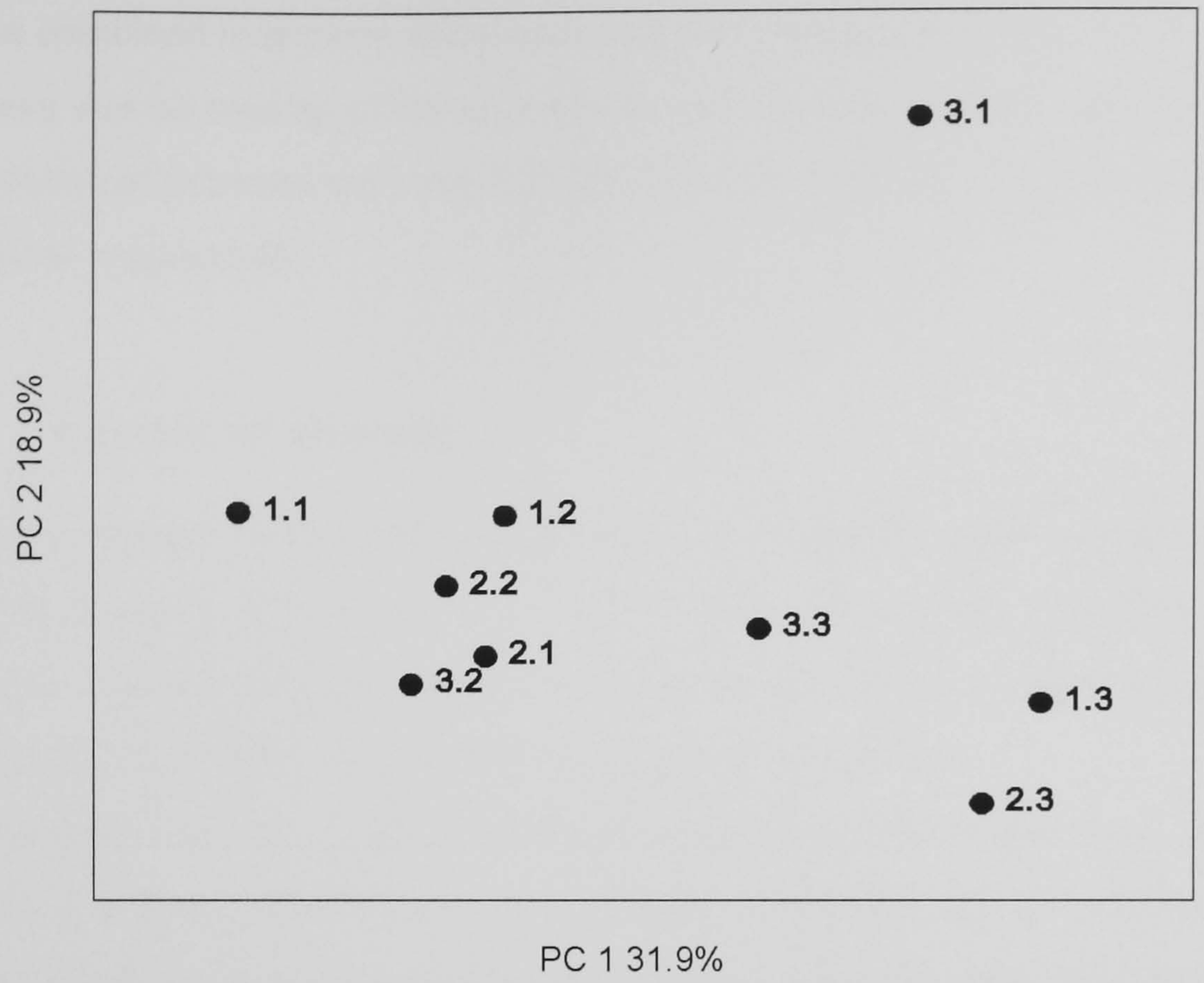

Figure 2.9: PCA analysis of soils using time elapsed before maximum substrate utilization occurred in Biolog™ plates. PC 1 and 2 account for 31.9 % and 18.9 % of the variability respectively.

Figure 2.10: PCA analysis of soils using maximum absorbance and time elapsed before maximum substrate utilization occurred in Biolog™ plates. PC 1 and 2 account for 35.0 % and 13.9 % of the variability respectively.

PCA using the combined maximum absorbance and time factors did not reveal any clustering patterns. There was no overlap of the soils of the mire expanse with the other two soil types. The ratio of distances between soils was 20:10.5:11 for the mineral soils, soils of the lagg fen and mire expanse respectively.

2.3.7 *Functional diversity*

It was possible to identify 16 of the fatty acid methyl ester (FAME) peaks found in the soils using the BAME standard. There were a number of other peaks produced by gas chromatography, however it was not possible to identify these peaks using the BAME standard. The distribution of identifiable peaks will be discussed in this section.

The presence of eicosanoate in samples is commonly interpreted as an indicator of plant fatty acids, and this was by far the most abundant FAME in all the soils (table 2.14). Plant fatty acids typically accounted for between two and eight times the next most abundant fatty acid in all soils. Pentadecanoate and 15-methylhexadecanoate, which are both indicators of bacterial fatty acids were the next most abundant fatty acids identified. Although both these fatty acids were present in the mineral soils, 15-methylhexadecanoate was always present in greater quantities. In contrast, pentadecanoate was the predominant of the two fatty acids in the soils of the lagg fen. The distribution of these two fatty acids in the soils of the mire expanse was mixed.

The remaining fatty acids, 12-methyltetradecanoate, 14-methylpentadecanoate, cis-9-hexadecenoate, hexadecanoate, cis-9,10- methylenehexadecanoate, heptadecanoate and nonadecanoate were detected in small amounts, and generally indicate bacterial presence in the samples. On the other hand, fungal distribution was not ubiquitous; cis-9,12-octadecadienoate, a commonly used marker for fungi, was found in the mineral soils and the soils of the lagg fen, however FAME analysis did not identify this group in the peats from the mire expanse. The fungal marker made up only a small proportion of the fatty acids identified. Soil 1.1 had significantly greater amounts of identifiable FAMEs than the other soils. There were five FAMEs that were common to all soils: 3-hydroxydodecanoate, pentadecanoate, cis-9-hexadecenoate, hexadecanoate and 15-methylhexadecanoate. There were five FAMEs which were peculiar to the mineral soils: 12-methyltetradecanoate, 14-methylpentadecanoate, cis-9,10-methylenehexadecanoate, heptadecanoate, trans-9-octadecenoate, octadecanoate and nonadecanoate. Tridecanoate was only found in one soil from the lagg fen region. Generally,

fatty acids that were only present in one soil types were not found in all the samples tested from a particular site, as such these results may not be statistically significant.

Table 2.14: Summary of FAMEs identified from soil samples. Values presented as percentage of total FAMEs identified using BAME standard.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Sample number	11:0 undecanoate	2-OH 10:0 2-hydroxydecanoate	12:0 dodecanoate	13:0 Tridecanoate	2-OH 12:0 2-hydroxydodecanoate	3-OH 12:0 3-hydroxydodecanoate	14:0 Tetradecanoate	i-15:0 13-methylpentadecanoate	a-15:0 12-methyltetradecanoate	15:0 Pentadecanoate	2-OH 14:0 2-hydroxytetradecanoate	3-OH 14:0 3-hydroxytetradecanoate	i-16:0 14-methylpentadecanoate	16:1w9 cis-9-hexadecenoate	16:0 Hexadecanoate	i-17:0 15-methylhexadecanoate	17:0 delta cis-9,10-methylenehexadecanoate	17:0 heptadecanoate	2-OH 16:0 2-hydroxyhexadecanoate	18:29'12 cis-9,12-octadecadienoate	18:1w9' cis-9-octadecenoate	18:1w9 trans-9-octadecenoate	18:0 octadecanoate	19:0 delta cis-9,10-methyleneoctadecanoate	19:0 nonadecanoate	20:0 eicosanoate
1.1						0.20			0.03	0.69			0.01	0.46	0.68	1.52	0.03			0.16	0.03	0.15	0.04			2.17
SD						0.06			0.03	0.49			0.01	0.23	0.46	0.98	0.05			0.08	0.06	0.16	0.06			0.85
2.1						0.03				0.09			0.04	0.10	0.13	0.15		0.01		0.00		0.00				0.09
SD						0.05				0.16			0.08	0.09	0.12	0.26		0.01		0.01		0.00				0.16
3.1						0.02				0.19				0.16	0.32	0.63				0.02		0.11			0.02	0.91
SD						0.03				0.16				0.12	0.07	0.05				0.04		0.02			0.03	0.83
1.2			0.02			0.02				0.23				0.04	0.50	0.07					0.04					1.67
SD			0.03			0.03				0.22				0.05	0.21	0.12					0.06					0.47
2.2										0.16				0.03	0.00	0.04										0.57
SD										0.19				0.03	0.01	0.08										0.50
3.2						0.01				0.44				0.16	0.12	0.18				0.04	0.05					1.01
SD						0.01				0.43				0.15	0.11	0.32				0.06	0.08					0.60
1.3										0.06				0.16	0.06	0.35										0.61
SD										0.10				0.12	0.10	0.10										0.59
2.3						0.01				0.04				0.01	0.07	0.05										0.87
SD						0.01				0.07				0.02	0.01	0.09										0.08
3.3						0.04				0.07																1.86
SD						0.06				0.11																0.16

2.3.7.1 Principal components analysis

Soil 1.1 was significantly different from the other soils, therefore it was excluded from the PCA plot as an outlier (figure 2.11). No clustering was evident from the PCA plot.

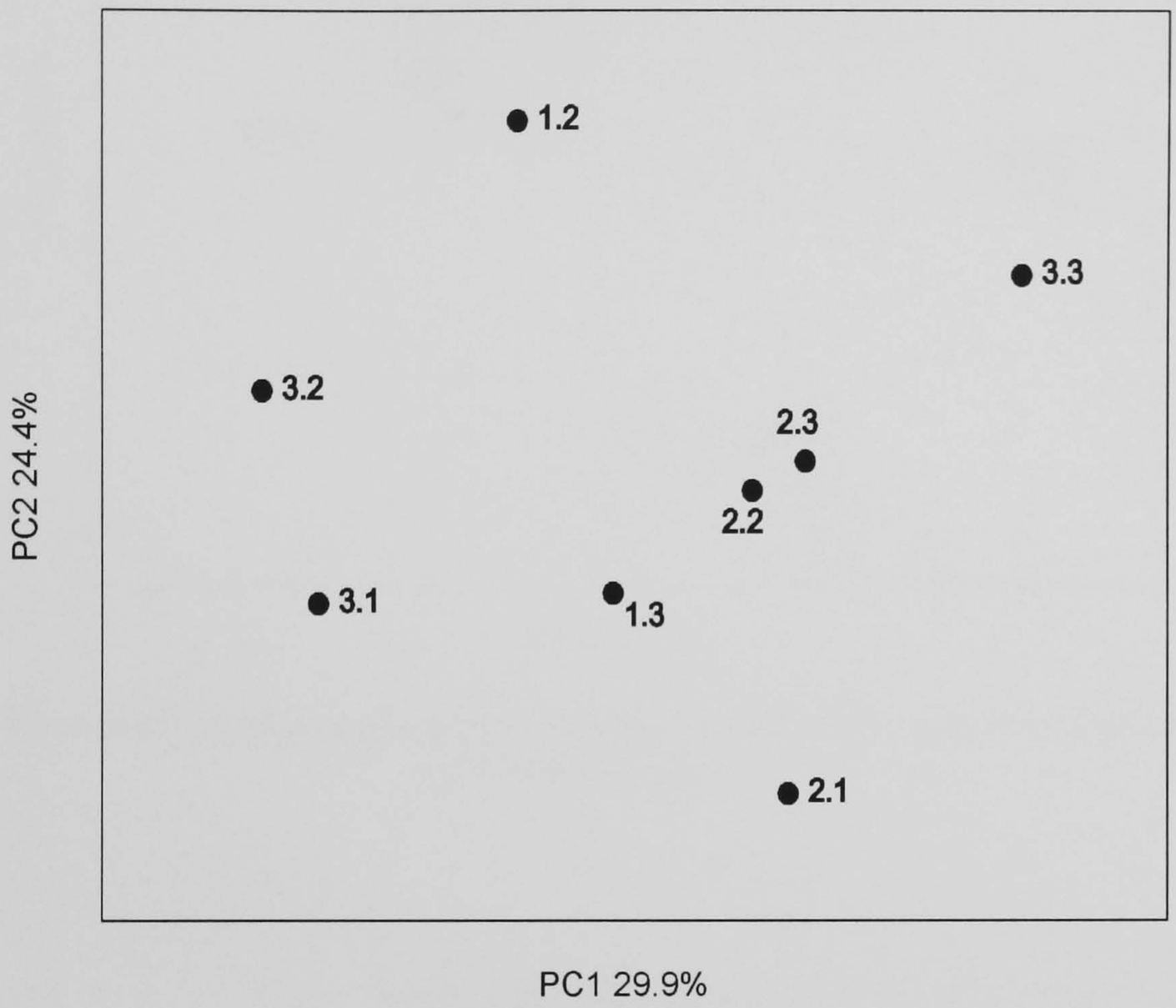

Figure 2.11: PCA analysis of soils using proportion of identified FAMEs. Soil 1.1 was excluded from the analysis.

Excluding plant FAMEs and soil 1.1 from the PCA analysis did not reveal any clustering patterns (figure 2.12). However, the distance between the soils of the mire expanse was lass than that for the mineral soils and the soils of the lagg fen. The ratio of distances between soils plotted on the PCA output was 20:12:8 for the mineral soils, soils of the lagg fen and mire expanse respectively.

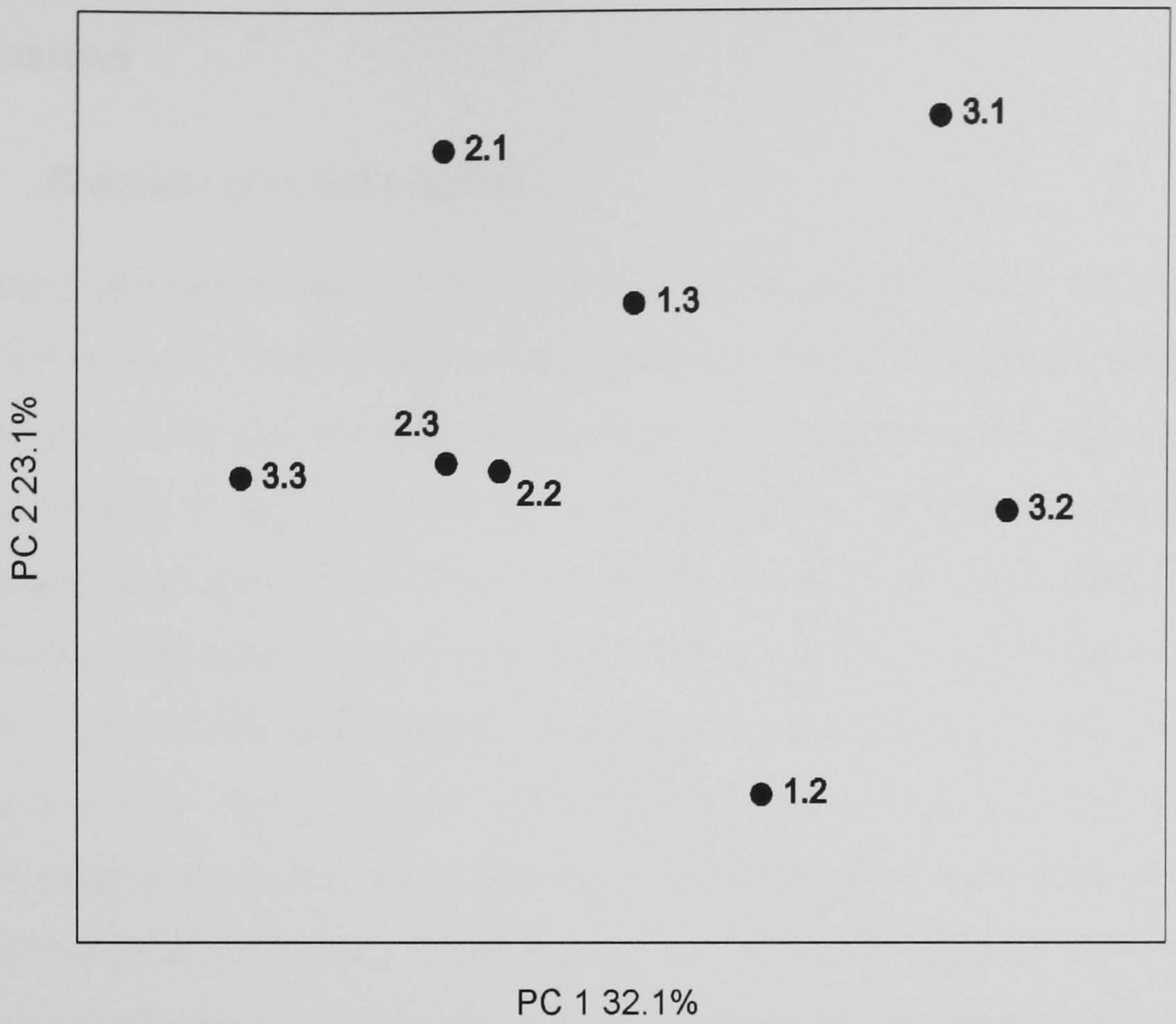

Figure 2.12: PCA analysis of soils using proportion of identified FAMEs. Soil 1.1 and plant FAMEs were excluded from the analysis.

2.4 Discussion

2.4.1 Biomass size calibration

There was a good correlation between the biomass size data produced by the original method and the modified method. Therefore, it was acceptable to use the modified method as an indicator of the size of the soil microbial community. High concentrations of glucose in water may impose an osmotic stress on the soil microbial community. Kroeckel and Stolp (1986) observed inhibited respiration above 60 mg l^{-1} probably due to rises in soil water osmotic potential. However, this effect was not seen in the SIR determinations carried out on the soils from Dun Moss. Lin and Brookes (1999b) observed that shaking removed the inhibition of oxygen limitation in soils with high water contents (1.2 water holding capacity). West and Sparling (1986) suggested that the slurry method that they devised was suitable for estimating the size of soil microbial community. Furthermore, soil microbial biomass determinations on heath soil using microscopy and slurry SIR showed good agreement (Sparling and Williams, 1986).

2.4.2 Biomass size

The size of the soil microbial community in the Dun Moss soils was greater than the mean value of 42 g C m^{-2} observed by Williams and Silcock (1997) when measuring microbial biomass in the surface region of a raised bog. However, the values for the Dun Moss soils were within the range measured by Williams and Silcock (1997), 20 g C_{mic} m^{-3} to 250 g C_{mic} m^{-3}. A comparison was made with the fumigation extraction method and this was found to overestimate the size of the soil microbial biomass in the peat soils tested. The size of the microbial biomass in the surface layer of a blanket bog measured by Collins *et al.* (1978) was much smaller (30 g C m^{-3}). This was measured by direct observation, and no comparison was made with other methods. Brake *et al.*, (1999) reported biomass C sizes for the soil microbial community of raised bog soils which were in the range of 2.36 to 4.60 mg C_{mic} g^{-1}.

To enable comparison between peat and mineral soils it was necessary to measure the bulk density of the soils (Schipper *et al.*, 1998). Peat soil in the acrotelm is typically 0.02 to 0.04 g cm^{-3} and 0.12 to 0.15 cm^{-3} in the deeper catotelm (Clymo, 1992) however mineral soils range from 0.9 to 1.3 g cm^{-3} (Paul and Clark, 1996). Therefore, bulk density units should be quoted in order to place biomass C estimates into ecological context, and to make comparisons between

mineral and peat soils. This prerequisite is not satisfied in the all of the data previously published and so it is not possible to make comprehensive comparisons between data.

The size of the soil microbial community in the mineral soils at Dun Moss was greater than that of the peat soils. Brake *et al.*, (1999) reported that the C_{mic} values measured in peat soils tested were high in comparison with grassland soils, but lower than the range known from arable and grassland soils. The peat soils were similar in terms of the size of the soil microbial community. Peat soils are predominantly waterlogged. Oxygen is around 300 times less soluble and 10,000 times less diffusible (Monteith, 1973) in water than in air. Therefore, oxygen may be limiting as an electron acceptor for respiration in these soils. Thus, this may pose a restriction on the size of the soil microbial community in the peats soils. Wheatley *et al.*, (1976) described a negative correlation between moisture content and bacterial numbers in the peat soils.

Additionally, the pH of the bog soils was significantly lower than in the lagg fen and the mineral soils. Sphagnum mosses are responsible for acidifying peat waters (Clymo, 1983). These plants were found on the lagg fen and the mire expanse. All biological processes are pH dependent; bacteria have about 1000 enzymes which are pH dependent (Paul and Clark, 1996). Therefore increased acidity may be responsible for restricting the size of the microbial biomass in the soils of the mire expanse. This may also influence the diversity of the soil microbial community in these soils.

The ratio of C:N in soils has been used as an indicator of the capacity of an ecosystem to support nutrient cycling and biodegradation functions. N content of litter has been implicated as an important factor influencing decomposition rates in peat soils (Coulson and Butterfield, 1978) and therefore availability to the soil microbial community. The C:N ratio significantly different between the soils ($p < 0.001$) and greatest in the mire soils. This is consistent with the nutrient input to the raised mire system. Therefore, N may have been limiting biomass size in the soils of the mire expanse. Litter quality may also be quantified as proportions of soluble and insoluble C components. Low molecular weight compounds such as amino acids and peptides are rapidly recycled in the soil. However, the breakdown of recalcitrant compounds such as lignin takes longer and is less energy efficient for the soil microbial community (Paul and Clark, 1996). Lignin is found in the woody component of *C. vulgaris*. *C. vulgaris* is one of the dominant plant species on the mire expanse and makes a large contribution to the soil organic matter. Therefore, the breakdown of this, less available substrate, may impose an energetic constraint on the size of the soil microbial biomass.

It has been noted that the breakdown of some plant materials may cause a negative effect on the soil microbial community (Brake *et al.*, 1999). During decomposition, toxic or antimicrobial compounds may be released, therefore limiting microbial processes. Sphagnum decay has been associated with this phenomenon. A compound called Sphagnan may be produced during the breakdown of Sphagnum in the environment (Painter, 1991). This is a complex, pectin like material which is covalently linked to cellulosic and amyloid-like chains in living Sphagnum moss. Sphagnan can also supress microbial activity by reacting with exo-enzymes and sequestering essential, multivalent cations (Painter, 1991). Sphagnum is dominant in soils of the lagg fen and the mire expanse. Therefore, Sphagnum breakdown may restrict the size of the soil microbial community in the peat soils.

In peat soils the activity of small animals is low. Soil animals aerate the soil by creating channels that air passes through. Additionally, it has been shown that direct contact between soil organic matter and soil animals increases decomposition (Coulson and Butterfield, 1978), therefore, the release of nutrients to the soil microbial community. The mineral soils at Dun Moss would have more soil invertebrates which aerate the soil, cycle nutrients and cause physical breakdown of organic matter.

2.4.3 Basal respiration calibration

After the initial disturbance to the soil, caused during the preparation of the experiment the basal respiration of the soils settled (after 31 hours) and the soil microbial community achieved basal respiration. The CO_2 evolution rates from the slurry experiment were greater than from the syringe experiment.

After disturbance to soil, there is a sharp increase in microbial activity. This was observed in the original method 0-31 hours. Similarly, constant agitation in the slurry experiment would have increased the CO_2 evolution above the basal rate. Therefore the true basal rate would not have been measured by the slurry experiment. Ritz and Wheatley (1989) observed high variability in estimates of basal respiration using the slurry experiment. They attributed this to the length of the experiment and found increased accuracy by increasing the duration of the experiment from 6 hours to 24 hours.

The slurry experiment was conducted at room temperature (22 °C) whereas the syringe experiment was incubated at 14 °C. This difference in temperature would have had a positive affect on the physiological reaction rates of microbial processes, hence the activity of the soil microbial biomass. Additionally, the slurry experiment involved constant agitation.

This shaking would have served to increase the contact between the soil microbial community and resources in the soils, therefore, the number of unavailable microsites for microbial activity would have been reduced.

Comparing the data separately for each soil type, different responses were found for the peat soils and the mineral soils. In the mineral soils, there was a decrease in the size of the soil microbial biomass as measured by the original method when CO_2 evolution measured by the slurry experiment increased. This suggests that the slurry method overestimates the size of the soil microbial community in the mineral soils. There are two possible reasons for this; that the overall activity of the soil microbial community increases or, that dormant cells are become active in the slurry experiment. In the peat soils the slurry method overestimated the activity of the soil microbial community and the correlation between the two methods was positive. The soils of the lagg fen had a wide range of basal respiration rates. These data may not be characteristic of the soils at Dun Moss. The basal respiration rates for the soils of the mire expanse were normally distributed and there was a good correlation between the two methods, thus suggesting that the slurry method is a good indicator of basal respiration in these soils. Much more testing of the slurry method would be required for accurate comparisons between soils. The slurry method overestimated the basal respiration and there was a lot of variability between the soils.

2.4.4 Basal respiration

The basal respiration for the three soils was significantly different when presented volumetrically. The mire expanse soils were significantly less than the mineral soils.

The soil respiration per unit of microbial biomass (metabolic quotient) was greater in the peats than in the mineral soils. This may be due to stress imposed on the soil microbial community in the peat soils. Under stressed environmental conditions a microorganism may have to carry out additional functions to cope with the stress, for example pumping toxic metal ions out of the cell against a concentration gradient. As a consequence, a greater maintenance energy is required by the organism. In the soils at Dun Moss, substrate quality may impose a stress on the soil microbial community. For breakdown of recalcitrant compounds (e.g lignin) more energy is required to release C from litter resulting in less energetically efficient metabolism (Paul and Clark, 1996). This could explain the greater metabolic quotient of the soil microbial community in the peat soils. Other stresses for the soil microbial community may be toxicity of Sphagnum during breakdown, acidity and waterlogging, as discussed earlier in this

chapter. There was a greater variation in metabolic quotients in the peat soils than in the mineral soils, suggesting that the peats are more heterogeneous than the mineral soils. Brinson (1993) stated that variations in microbial properties may very considerably within a given wetland type.

2.4.5 Metabolic diversity

Overall, where there was difference in substrate utilization between soils the soil microbial community from the soils of the lagg fen had the greatest maximum absorbance and that of the soils of the mire expanse had the smallest maximum absorbance. This suggests that although the microorganisms in the soil of the mire expanse were able to utilize the substrates present in the Biolog™ plate, they were not as proficient as those were from the soils of the lagg fen. The soil microbial community of the mineral soils was able to utilize substrates more efficiently than that of the mire, but less than that of the lagg fen soils. One possible explanation for this is the botanical diversity of the soils of the lagg fen. Microbial species diversity has been tied with botanical diversity (Borga *et al.*, 1994), therefore botanical diversity may also effect metabolic diversity of the soil microbial community. The nutrient input to the lagg fen is also greater than in the mire expanse, hence providing mineral nutrients for the proliferation of a more metabolically diverse microbial community. In both of these factors, the mineral soils lie between the lagg fen and mire expanse. They have a more diverse plant cover than the mire but not as diverse as the lagg fen. Furthermore, whilst they receive mineral nutrients from water travelling through mineral soils, this nutrient rich water does not accumulate in this region, as it does in the lagg fen.

2.4.5.1 PCA

The principal components analysis of the maximum absorbance data did not show distinct clustering patterns (figure 2.8). However, the difference between the soils of the mire expanse was less than that within the lagg fen soils and the mineral soils. The mineral soils had the most diverse in substrate utilization pattern. There was no crossover between the soil types. This suggests that there is more variation in the soil microbial community within the mineral soils than the peat soils.

Analysis of the time elapsed before maximum substrate utilization occurred showed less distinct grouping of the soil microbial community within the mineral soils and the soils of

the lagg fen. However there was no overlap with these soils and the soils of the mire expanse. This suggests that the soil microbial community of the mire expanse is distinct from those of the lagg fen and mineral soils.

Combining the two sets of data (time elapsed before maximum substrate utilization and maximum substrate utilization) increased the distance between the soils of the mire expanse. However, there was no overlap between this group and the other two soil types. An overlap was seen between the mineral soils and the soils of the lagg fen. Clustering of the soils of the mire expanse soils is consistent with the nutrient input to the soils at Dun Moss. The lagg fen and mineral soils receive a similar nutrient input, which is different from that of the mire expanse.

2.4.5.2 Limitations of Biolog™

Several authors have noted that there are limitations with using the Biolog™ substrate utilization technique and substrate utilization patterns must be interpreted with caution (Degens, 1997). Differences in the 'fingerprint' obtained from Biolog™ substrate utilization patterns are probably not solely due to differences in the types of organic C available, but also to the physical and chemical properties of the soil. Degens (1997) points out that comparison between substrate utilization patterns of the soil microbial community of the same soil over time may be more robust. In addition, Campbell *et al.*, (1997) stated that the substrates included in the Biolog™ GN plates were not ecologically relevant. He proposed the use of microtitre plates with buffer and tetrazolium dye to which substrates could be added. In this way, more ecologically relevant substrates about which more is known could be used.

Negative values of substrate utilization are often obtained. It is common practice to ignore this result and substitute negative values with zero (Kunc, 1994). Howard (1999) stated that it is not acceptable to do this without understanding why the negative values occurred. He proposes two possible explanations for a negative absorbance value: i) the control value is erroneous; or ii) that some of the C-sources have an inhibiting effect on some organisms. Indeed, Benefield *et al.* (1977), and Cook and Garland (1997) discussed the toxicity of tetrazolium salts to microorganisms. Furthermore, the inclusion of particulate matter in the wells of the microtitre plate may cause further complications. This is especially important during the early stages of substrate utilization when the colour change is small. Additionally some soils may be more problematic than others depending on the range of absorbance values of the soil particles and size of the soil particles. It has also been noted that not all organisms

are able to use tetrazolium salts (Winding, 1997). In contrast, free soil enzymes included in the soil extract may reduce tetrazolium violet. Benefield *et al.*, (1977) stated that there is no simple relationship between soil microbial activity measured by dehydrogenase activity. Reduction of tetrazolium salts is a redox reaction, which requires a buffered environment. This buffered environment may present complications when working with acidic soils. Typical incubations last for 7-10 days before the ultimate OD is reached. This buffering will undoubtedly have an effect on the soil microbial community.

2.4.6 Functional diversity

Soil 1 had significantly more identifiable FAMEs than the other soils. This soil had a greater number of FAMEs and greater concentrations. This suggests that the soil microbial community in this mineral soil had a more diverse structure and that the size of the soil microbial biomass with the representative FAMEs was greater in this soil. This soil is aerobic and has a good supply of mineral nutrients from the water travelling through surrounding soils. Therefore, it is possible that this soil had a more diverse and bigger biomass than in the peat soils. The same pattern was not observed in the other mineral soils (soils 2.1 and 3.1). One possible explanation for this is the heterogeneity of the soil system. Often in soils microsites may be found where the conditions are quite different from the surrounding area. It appears that this soil is an outlier, therefore it has been excluded from the analysis.

Table 2.13 contains data of the 6 FAMEs identified in almost all of the soils. Pentadecanoate, 14-methylpentadecanoate, cis-9-hexadecenoate and hexadecanoate are indicative of the presence of bacteria in the soils (Frostegård, 1996). Pentadecanoate has been found in marine sediments and is indicative of anaerobic eubacteria (Rajendran *et al.*, 1994). However, others have noted that pentadecanoate has also been found in mineral soils where anaerobes are unlikely to occur (Zelles *et al.*, 1995). Indeed it is interesting that this fatty acid was found in greater amounts in the mineral soils than in the soils of the lagg fen. Cis-9-hexadecenoate has been attributed to bacteria. It was found in all of the soils, with the exception of soil 3.3 from the mire expanse. 15-methylhexadecanoate is indicative of Gram positive bacteria (Zelles, 1997). This FAME was detected in all of the soil samples with the exception of soil 3.3 from the mire expanse.

An indicator of fungal biomass (cis-9,12-octadecadienoate) was detected in soils from the lagg fen and the mineral soils, however not in the soils from the mire expanse. This FAME

has been suggested as a biomarker for fungi by a number of authors (e.g. Lechevalier and Lechevalier, 1988; Frostegård and Bååth, 1996). However C18:2ω6 may not be suitable as a biomarker in all soils. Dembitsky and Renzanka (1995) noted the occurrence of this FAME in the top shoots of Sphagnum while Lechevalier and Lechevalier (1988) and Palojarvi *et al.* (1997) identified it in cyanobacteria and straw respectively. Caution must therefore be taken when interpreting PLFA profiles from peat soils where contamination from Sphagnum mosses is inevitable. However, with this in mind it is interesting to note the absence of this FAME in the soils of the mire expanse where Sphagnum mosses are dominant, and this may indicate further problems with the use of C18:2ω6 as a standard for fungi .

Long saturated, straight chain fatty acids (e.g. eicosanoate) are commonly found in higher plants, mosses and eukaryotes (in small amounts) Sundh *et al.* (1997). Eicosanoate was detected in all of the soils examined. This FAME accounted for the greatest proportion of FAMEs in the soils. This suggests that a large proportion of the FAMEs identified are not of microbial origin, but of plant origin.

2.4.6.1 PCA

Principal components analysis identified an outlier, soil 1.1. This was excluded as discussed earlier. In this way, it was possible to determine the differences between the other soils.

Figure 2.12 illustrates the differences between the soils as determined by the FAME profile. In order to analyse the data representative of the microbial population it was necessary to exclude FAMEs unique to plants (eicosanoate). Clustering is not obvious in these data, however the mean distance between the soils of the mire expanse is smaller than that between the soils of the lagg fen and surrounding slope. This suggests that there is less variation in the soil microbial community of the mire expanse than the soils of the lagg fen and the surrounding slopes. Borga *et al.* (1994) compared PLFA profiles with botanical composition in Carex and Sphagnum dominated peatlands. They found that as the botanical diversity increases (Sphagnum peats in their study area) there was a larger variation in the PLFA composition. This is consistent with the findings at Dun Moss, where the botanical composition is greater in the lagg fen and mineral soils than on the mire expanse. Therefore, this suggests that the botanical composition plays a key role in determining the structure of the soil microbial community in these soils.

2.5 Conclusions

The original hypotheses were:

2.5.1 Size and activity

- The size of the soil microbial community in the mire soils is smaller that of the soils of the lagg fen and the mineral soils.

Using the substrate induced respiration method the size of the soil microbial community in the mire soils was smaller than in the mineral soils. However, there was not a significant difference in the size of the soil microbial community between the soils of the lagg fen and the mire expanse. From this result, it cannot be concluded that nutrient supply has a significant beneficial effect on the soil microbial community of the soils of the lagg fen over those of the mire expanse. However, without testing the effect of adding mineral nutrients to the soils of the mire expanse it is not possible to rule out the significance of nutrients to the microbial ecology in these soils.

- Waterlogging and other environmental factors imposed a stress on the soil microbial community of the mire and lagg fen soils, therefore the specific metabolic quotient in greater in these soils.

The qCO_2 was much greater in the soils of the lagg fen and the mire expanse than the mineral soils. Therefore, the energy required to maintain a unit of biomass was greater in the peats. This suggests that soil conditions were imposing a stress on the soil microbial community.

- There is a relationship between the ratio of C:N, as a measure of litter quality, and the size of the soil microbial biomass.

There was not a significant relationship between the size and activity of the soil microbial community and litter quality in the peats of the lagg fen and the mire expanse. This suggests that some other factor was limiting the size and activity of the soil microbial community in these soils. However, there was a correlation between the N content of litter and the size of the soil microbial community in the mineral soils. To a lesser extent, there was an effect of N

on basal respiration. Therefore, in the mineral soils nitrogen may have been limiting to the soil microbial community.

2.5.2 *Diversity*

2.5.2.1 Metabolic diversity

- There is a significant difference between the metabolic diversity of the soil microbial community of the different soils. The microbial communities in the lagg fen and mineral soils are more diverse metabolically than those in the mire expanse because botanical diversity is greater on these soils

Principal components analysis did not show distinctive clustering of the soil types. Although PCA may not be directly used to measure metabolic diversity it can be used to show how similar a set of samples may be. It is not possible to use the FAME data from this work as indicators of microbial diversity because so few fatty acids were identified. Increasing the number of standards used would help to identify a greater range of fatty acids.

There was not a significant difference between the number of substrates utilized by the soil microbial community of the different soil types. Biolog™ was not able to detect metabolic differences between the soils. Although there are differences in the biochemical make-up of different plant species or of the same plant species growing under varying conditions the general array of biochemicals encountered by soil microorganisms decomposing them is not greatly different from ecosystem to ecosystem. However, the metabolic profile should be different for soils with contrasting physical and chemical properties. It is likely that the aforementioned limitations of the assay and its unsuitability for use with acidic soils, as well as the heterogeneity of the soil ecosystem affected the accuracy of the substrate utilization patterns.

2.5.2.2 Functional diversity

- There is a significant difference between the soils in terms of functional diversity. The soils of the surrounding slopes and lagg fen have a greater microbial diversity than the soils of the mire expanse.

It was not possible to identify individual groups of organisms from the PLFA analysis carried out. Further work would need to be carried out on the samples to reveal differences in the fatty acid profiles. Suggested improvements are i) using a greater range of standards to identify fatty acids and ii) using gas chromatography-mass spectrometry (GC-MS) to identify and confirm fatty acid profiles.

- There is a high population of fungi (indicated by C18:2ω6) in all of the soils due to the acidity.

The fungal marker, C18:2ω6, was found in the mineral soils and in the lagg fen, but not in the soils of the mire expanse. Although, C18:2ω6 is often used as a fungal marker because it is common in many species of fungi (Lechevalier and Lechevalier, 1988), the absence of this fatty acid can not be interpreted as the complete lack of fungi in the mire expanse region of Dun Moss The presence of fungi in the aerobic region of the bog soils is highly likely as fungi have been found in abundance in raised bogs and blanket bogs. Considering the scope for misinterpretation of fatty acid markers, it would be necessary to carry out other tests for fungal markers, for example ergosterol, before it is possible to rule out the presence of fungi in the soils of the mire expanse.

- Metabolic and functional diversity indicators of the soil microbial communities of Dun Moss are similar.

Palojarvi *et al.*, (1997) found a good correlation between PLFA and Biolog™; PLFA profiles produced clearer groupings. The data from PLFA and Biolog™ analyses were similar in that they did not produce clustering. However, in both analyses the distance between the mineral soils and the lagg fen soils was greater than that between the mire expanse soils. This suggests that the soil microbial community of the soils of the mire expanse is less diverse than the soils of the lagg fen and the surrounding slopes.

This chapter discussed the microbial ecology of peat and mineral soils from a raised mire. By determining the size, activity and composition of the microbial communities in peat and mineral soils from the same area, it was possible to put the soil microbial community of the peat soils into an ecological context with mineral soils. These comparisons are important when studying

the role of the soil microorganisms in C cycling in peat soils because it provides an idea of the relative importance of environmental characteristics and litter quality. The initial hypothesis proposed that mineral nutrients were an important limiting factor to the decomposer community in peat soils, and that the soil microbial community of the mire soils would be less than of the lagg fen and the mineral soils. However, it appears that the soils of the lagg fen and the mire expanse have very similar biomass sizes. Even after this unexpected result we can not rule out the importance of mineral nutrients as mediators of the size of the soil microbial community in these soils, and testing the effect of the addition of mineral nutrients on the microbial community would provide additional evidence to backup the results found in this chapter.

Chapter 3

Effect of mineral nutrient addition on the soil microbial community

3.1 Introduction

Mineral nutrients are essential to microbial growth and maintenance. Nitrogen for example, is required for the synthesis of proteins, cell wall components and nucleic acids (Paul and Clark, 1996). Although, N is a major component of plants, and therefore is available in organic forms, it is far more available to the soil microbial community in inorganic forms. Consequently, the inorganic N input to a system has implications for the processes carried out by the soil microbial community during growth and maintenance. In a similar way, inputs of phosphorus and other nutrients in mineral forms may be important limiting factors to microbial processes such as decomposition, which is a key process in the nutrient cycles of terrestrial ecosystems (Vitousek *et al.*, 1994; Aerts and De Caluwe, 1997). Many of the investigations into the effects of nutrients on decomposition in bogs has focused on the uptake of nutrients by plants, and the subsequent effect on litter quality (e.g. Couslon and Butterfield, 1978; Berg and Staff, 1980; Staff and Berg, 1987; Vitousek *et al.*, 1994). Less attention has been paid to the direct effect of mineral nutrients on the soil microbial community, and microbial decomposition in these environments.

The low ash content of organic soils means that mineral nutrients are in limited supply. Additionally, ombrotrophic bogs are not connected to the groundwater supply and do not receive water from surrounding soils. The combined result of these factors is that mineral nutrients are found in small concentrations in raised mires (Pollet, 1972). P and N concentrations in precipitation are small (Gorham, 1955), and so these inputs do not amount to a significant increase in the levels of nutrients in raised mires. Wetland biogeochemistry also has an important role in the supply of mineral nutrients to the biological component of the system. This topic is a complex and intricate one, which is beyond the scope of this chapter. However, it is sufficient to note that the high water and organic content of peat soils may limit the supply of nutrient to plants and microorganisms, for example, P availability may be reduced in bogs due to the complexes formed with organic matter.

Despite the obvious implications of added mineral nutrients to microbes outlined in the introduction to this thesis, the picture is still not clear. Many studies have shown that inorganic nutrients have little effect on microbe numbers and microbial processes occurring in peat soils (Waksman and Stevens, 1929; Waksman and Purvis, 1932; Knowles, 1957; Clymo, 1965). Although there is a large pool of organic matter available in peats, this is less available to the soil microbial community (Jörgensen and Richter, 1992; Bridgham and Richardson, 1992). This has been illustrated by experiments which have involved the addition of an available C and N source to peats, resulting in increased microbial activity (e.g. Holding, 1965). Therefore, it may be argued that not only are inorganic nutrients unavailable to the soil microbial community, but also that organic nutrients may be locked up in recalcitrant organic matter in the soil.

In the literature, there is more information available about plant-related processes than those associated with microbe-related processes occurring in mire ecosystems. The majority of investigations have focused on the change in botanical abundance and diversity in mire ecosystems (e.g. Hayati and Proctor, 1991; Kirkham *et al.*, 1996) while other studies have concentrated on specific physiological functions of the plants involved e.g. nitrogen use efficiency (Aerts *et al.*, 1999). In microbial studies the activity of microorganisms in response to nutrient addition has been investigated as well as specific microbial processes e.g. nitrogen mineralization (Williams and Wheatley, 1989; Williams, 1992). However, for the most part our knowledge of the relationship between microbial processes and mineral nutrients in bogs is patchy, and it is far outweighed by the botanical informational that is available. One reason for this imbalance is a logistical one, as it is much easier to measure the changes in plant growth at an individual and community level because these changes are often obvious to the naked eye. Furthermore, botanical characteristics are well documented and there is a set of common standards for measuring plant growth. On the other hand, microbial studies require much more sophisticated methods that often have logistical difficulties associated with them, partly because they have been developed and tested for use with mineral soils.

Despite the inherent difficulties associated with microbiological methods, the requirement for further knowledge into the microbiology of peatland soils, and the effect of mineral nutrients on the soil microbial commmunity, is greater than ever. There is a very well developed knowledge base on the nutrient effects on the above ground aspect of raised mires, yet very little is known about the microbiological component. Therefore, it is important that baseline information is sought from a range of systems in order to provide the foundations for future work. In this chapter an attempt is made to make some of these fundamental determinations of the effect of mineral nutrients on the soil microbial community of three

different soil types. The importance of comparative studies incorporating mineral soils from the same study area and at the same time cannot be overestimated and this information is useful to our understanding of how increased mineral nutrients effect microbial processes in bog ecosystems.

3.1.1 Aim and hypothesis

The aim of this chapter is to determine the effect of mineral nutrients on the size and activity of the soil microbial biomass. Specifically, the role of mineral nutrients on the ombrotrophic soils will be tested with a view to testing the limiting effect of these nutrients on the soil microbial community in peat soils from ombrotrophic sites.

- The size of the soil microbial community in the mire expanse is limited by mineral nutrient supply. Increasing the supply of mineral nutrients to the soil of the mire expanse will increase the size of the soil microbial community in these soils.

3.2 Materials and Methods

3.2.1 Sampling

Soils were collected from 9 points on the three transects illustrated in figure 1.4 on 12th February, 1999. The samples were collected from the upper 10 cm^{-3} of the peat soils and upper 15 cm^{-3} of the mineral soils. The soils were sieved using a 10 mm sieve and stored in polythene bags at 4° C for 15 days prior to the setup of the experiment.

The soils were dried at 70° C and sub-samples were ground using a pestle and mortar for carbon and nitrogen analysis (Appendix A). The pH of the soils was determined using a ratio of 4:1 distilled water to moist soil (pH meter). The biomass carbon size of the soil microbial community was determined using slurry SIR as described in chapter 2. These data are presented in table 3.1.

Table 3.1: Soil properties of soils used in the nutrient amendment experiment. Standard deviations are of 3 replicates.

	Mean biomass C in mg C_{mic} cm^{-3} soil	SD	Mean C %	SD	Mean N %	SD
Mineral soils	0.76	(0.34)	29.3	(17.4)	1.0	(0.5)
Lagg fen	0.14	(0.03)	46.0	(6.8)	1.1	(1.1)
Mire expanse	0.20	(0.09)	45.8	(7.2)	1.1	(0.2)

3.2.2 Stock nutrient solution

A stock nutrient solution was made up in accordance with the determination of tracer concentrations after rainfall events. This information was collected from Dun Moss in 1995. The data (Smit, 1996) were recorded for; the 'mixed streams', which comprise water from both the mire and surrounding soils; the mire; and the 'environs', water that had travelled through the surrounding mineral soils. The elements tested for are listed in table 3.2 with corresponding, mean concentrations for 8 rainfall events.

A mineral nutrient stock solution was made up to equal the recorded concentrations in the streams from the mineral soils as much as possible. NaCl, $CaSO_4.2H_2O$, $MgCl_2$ and KNO_3 were used at 200, 100, 100 and 100 µM dm^{-3} respectively. The amount of stock to be added to each soil was calculated according to the rate of drying that occurred during the experiment. This was determined by incubating the soils over a one week period and recording the amount

of drying occurring in each soil. The aim was to maintain field water capacity throughout the experiment. Table 3.3 lists the amount of water loss observed over one week.

Table 3.2: Concentrations of minerals after 8 rainfall events at dun Moss between 1994-1996 (Smit, 1996). The concentrations are for the streams containing water from the surrounding mineral soils.

Mineral	Concentration in µmol L^{-1}
Calcium	106.2
Magnesium	100.3
Sodium	197.3
Potassium	70.6
Chloride	174.2
Sulphate	66.4
Nitrate	9.0

The nutrient addition was also standardized for carbon content (table 3.1). The carbon content in the mineral soils was taken as the standard concentration of carbon. The mineral soils were used as a standard because mineral nutrients were added at the concentration found in these soils. The mean carbon content for the mineral soils was 29%, therefore the nutrients for a soil with an organic carbon content of 42% would be 1.45 times greater than in that in the mineral soil. In effect, the mineral nutrients added to the soils of the mire expanse and the lagg fen were greater than the concentrations found in the mineral soils because of the greater carbon content.

Table 3.3: Water loss from samples during incubation in vials, enclosed in syringes, at 14°C for 1 week.

Soil	Mean water loss (g^{-1} per g dwt^{-1} soil) SD of 6 replicates in brackets
Mineral soil	0.06 (9.31×10^{-10})
Lagg fen	0.063 (0.015)
Mire expanse	0.053 (0.006)

3.2.3 Nutrient addition

A factorial (3 x 2 x 3) design was used. Three replicates of the three soils (detailed in appendix B3) were set up to test the effect of three time points and two nutrient treatments on the size and activity of the soil microbial community. 10 g moist soil was weighed into 10 ml vials. The soils were allowed to acclimatize at 14° C for 24 hours before the first treatments were added and the first measurements were made.

Nutrients were pipetted on to the soils and mixed in the amounts previously described. The vials were then enclosed in gas-tight syringes (60 ml). CO_2 production was determined daily for the first week by injecting 1 ml samples into a gas chromatograph set-up to measure CO_2 (appendix A) The syringes were flushed every day.

The experiment was set up in a staggered fashion to enable biomass measurements of the soils to be made at weekly intervals. The size of the soil microbial community was measured using the modified SIR technique outlined in chapter 2. On day 1 of the experiment, soils 1.1 and 2.1 received the necessary nutrient addition and 3 replicates with and without nutrients (6 vials) were enclosed in syringes. The remaining vials were incubated at the same temperature. On day 2, the CO_2 was recorded from soils 1.1 and 1.2 and soils 3.1 and 1.2 received nutrients and so on. By day 5 all of the soils had received the required treatments. On the seventh day of the experiment the first measurements of the size of the soil microbial community were made, and these were carried out on soils 1.1 and 2.1. From this point on the frequency of the CO_2 measurements decreased to every 48 hours and the size of the soil microbial biomass for soils incubated for 7 days was determined every day. This staggered design avoided the need for storage of the soils after incubation before the size of the soil microbial community was measured.

3.3 Results

3.3.1 CO_2 production

The mean values for respiration of the mineral soils ranged from 34-44 mol CO_2 cm^{-3} $hour^{-1}$ and 29-44 mol CO_2 cm^{-3} $hour^{-1}$ in the control soil and amended soil respectively (fig. 3.1). There was no significant change in CO_2 production within the first 144 hours of the incubation period. Nutrient treatment did not have a significant effect on the rate of the CO_2 production.

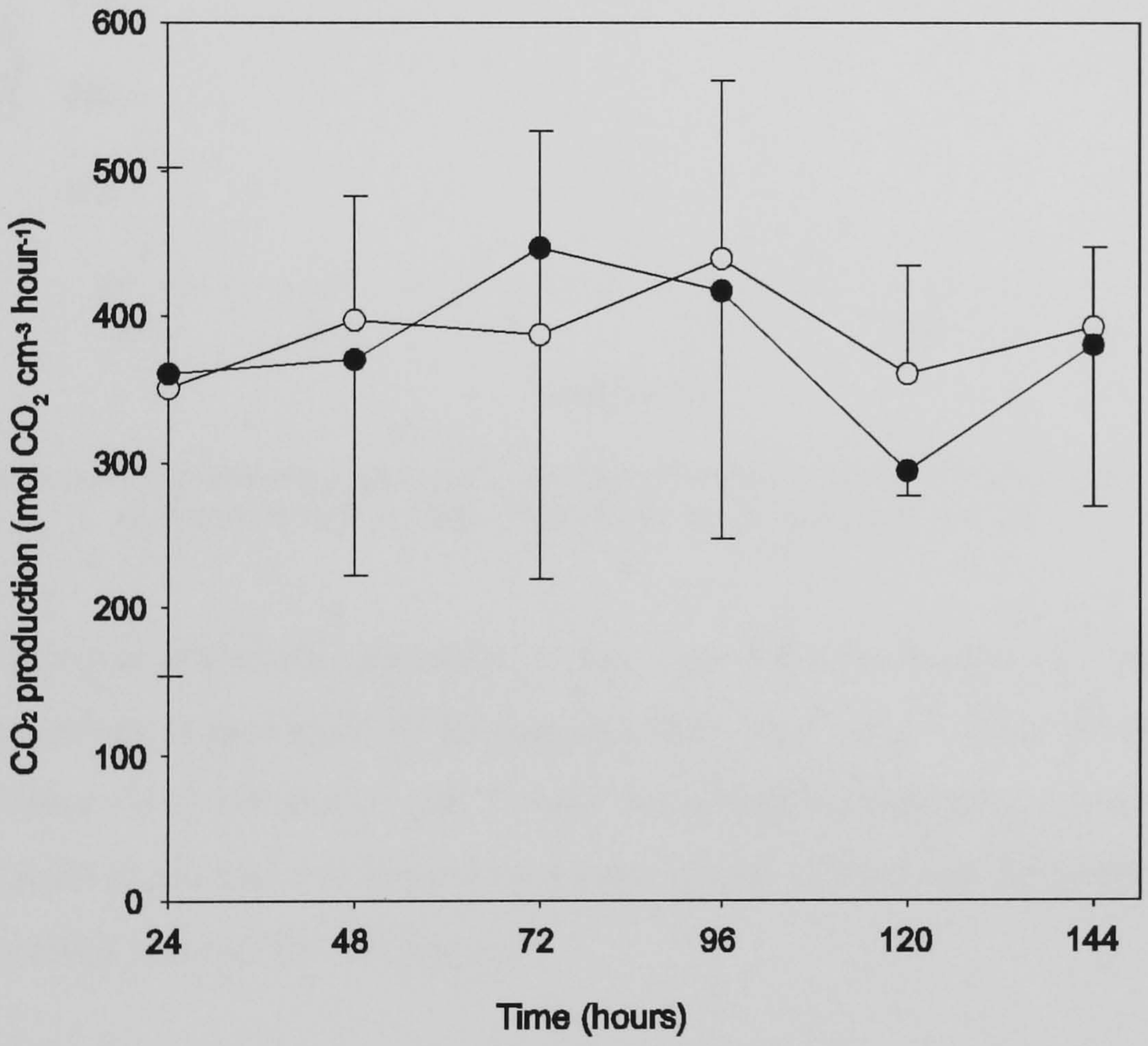

Figure 3.1: Mean rate of respiration in the mineral soils between 0 and 144 h incubation. Open circles represent control soil and closed circles are nutrient amended soil.

The range of mean values for the respiration of the lagg fen soils was 49-65 mol CO_2 cm^{-3} $hour^{-1}$ for the control and 39-51 mol CO_2 cm^{-3} $hour^{-1}$ for the treated soil (fig.3.2). There was no significant effect of time on the CO_2 production in the soils. Nutrient amendment did not have a significant effect on soil respiration. However, CO_2 production from the control soil was greater than that from the amended soil.

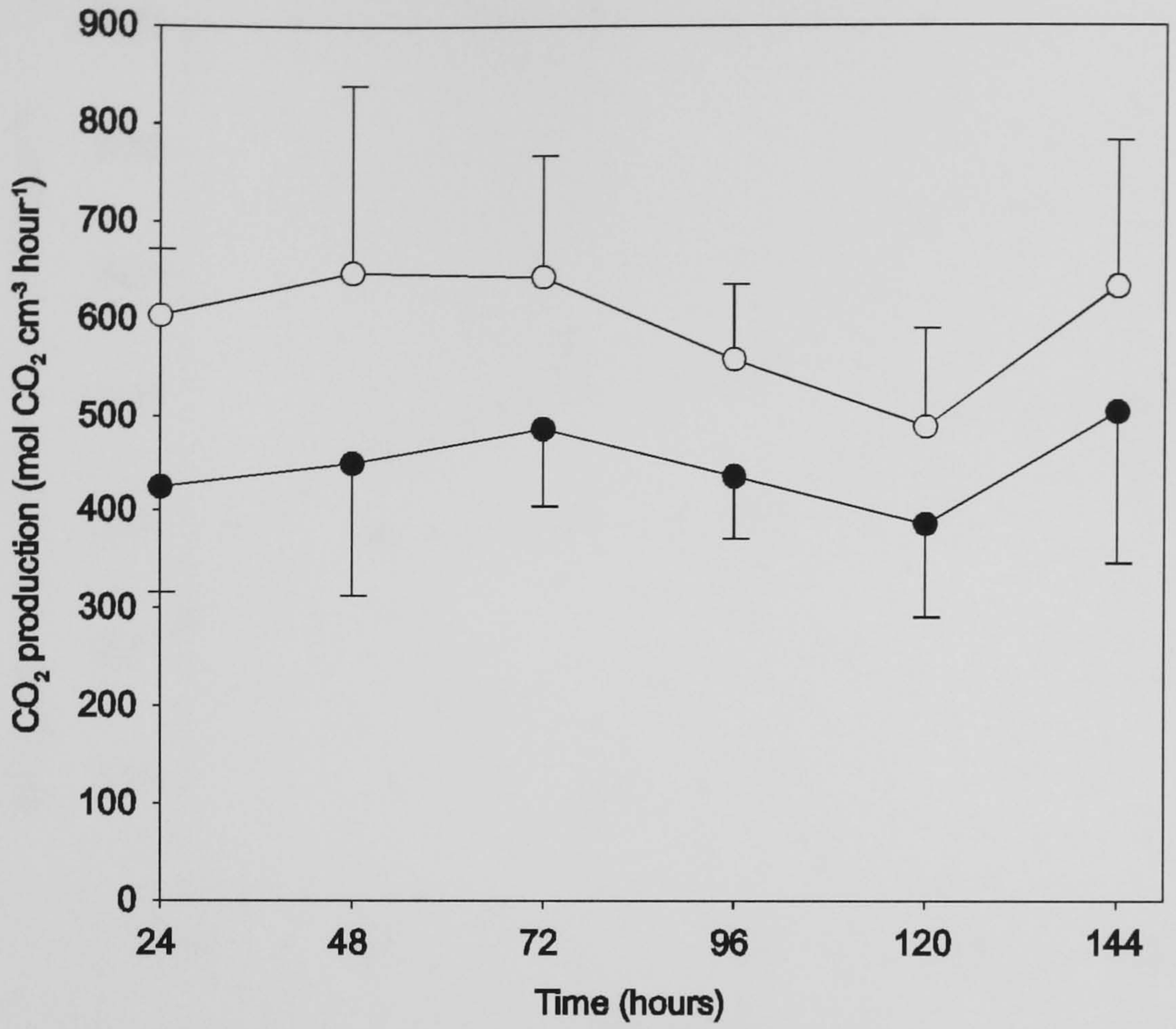

Figure 3.2: Mean rate of respiration in the soils of the lagg fen between 0 and 144 h incubation. Open circles represent control soil and closed circles are nutrient amended soil.

There was an increase in the soil respiration of the soils of the mire expanse with time (fig. 3.3). The mean values ranged from 27-55 mol CO_2 cm^{-3} $hour^{-1}$ in the control soil, and 22-44 mol CO_2 cm^{-3} $hour^{-1}$ in the amended soil. There was a significant difference between the rate of respiration at 24 hours and 144 hours in the control soil. There was no significant difference between the treated soil and the control soil.

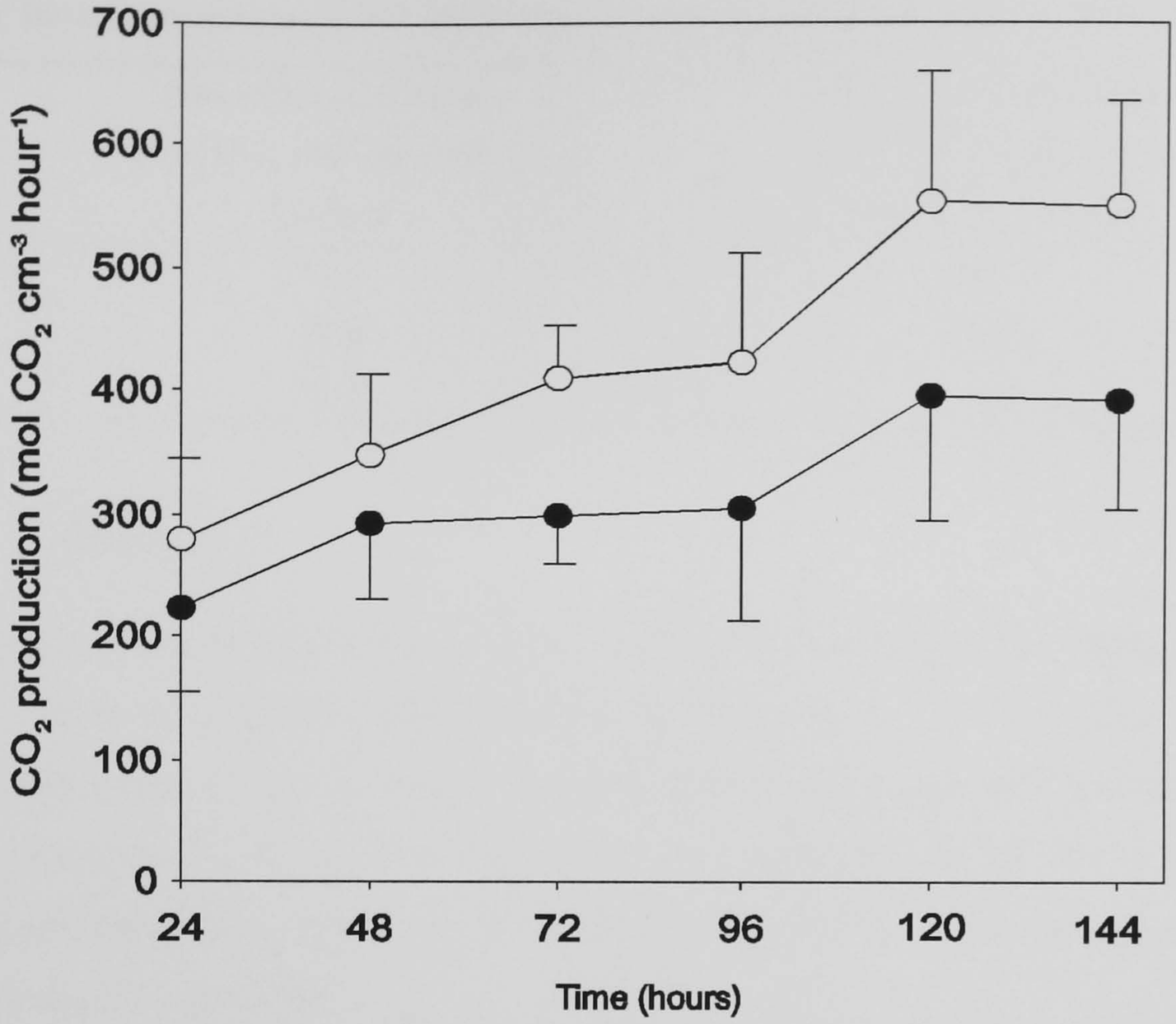

Figure 3.3: Mean rate of respiration in the soils of the mire expanse between 0 and 144 h incubation. Open circles represent control soil and closed circles are nutrient amended soil.

Table 3.4 lists the mean rate of respiration for the first 144 hours of the nutrient addition experiment. After 6 days incubation there was a significant difference in the CO_2 production between soil types in the control soil ($p < 0.005$). The soil from the lagg fen had a significantly greater CO_2 production rate than the soils of the mire expanse and the mineral soils. However, there was no significant difference between the treated soils.

Table 3.4: Soil respiration in the first 144 h of the experiment. Standard deviations are of 3 replicates.

Soil type	Mean CO_2 production (mol CO_2 cm^{-3} soil $hour^{-1}$) Control	SD	Mean CO_2 production (mol CO_2 cm^{-3} soil h^{-1}) Nutrient amended	SD
Mineral soil	38.8	(8.6)	35.9	(17.2)
Lagg fen	59.9	(10.3)	45.6	(9.7)
Mire expanse	43.6	(13.3)	32.3	(7.4)

In the control soils, there was a significant difference between the mean soil respiration between the soils between 144-288 hours (table 3.5). The respiration was greatest in the soils of the lagg fen. However, there was no significant difference in the respiration between the treated soils.

Table 3.5: Soil respiration between 144-288 h of the experiment. Standard deviations are of 3 replicates.

Soil type	Mean CO_2 production (mol CO_2 cm^{-3} soil $hour^{-1}$) Control	SD	Mean CO_2 production (mol CO_2 cm^{-3} soil $hour^{-1}$) Nutrient amended	SD
Mineral soil	41.2	(6.89)	39.2	(11.2)
Lagg fen	55.4	(24.6)	46.0	(9.9)
Mire expanse	35.3	(10.1)	34.8	(12.3)

3.3.2 Biomass C

Figure 3.4 illustrates the change in the size of the soil microbial community during the experiment. There was a significant difference in the size of the soil microbial community between soil types after 6 days incubation ($p=0.01$). The mire expanse soils had the greatest biomass size at this time; 0.49 mg C cm^{-3} soil in the mire compared with 0.29 and 0.21 mg C cm^{-3} soil in the mineral soil and the soils of the lagg fen respectively. This was not true for the nutrient amended soils ($p=0.084$).

At 12 and 18 days there was no significant difference in the size of the soil microbial community between soil types in the control soils or soils with nutrient amendment.

Nutrient amendment and time had no significant effect of the size of the soil microbial community.

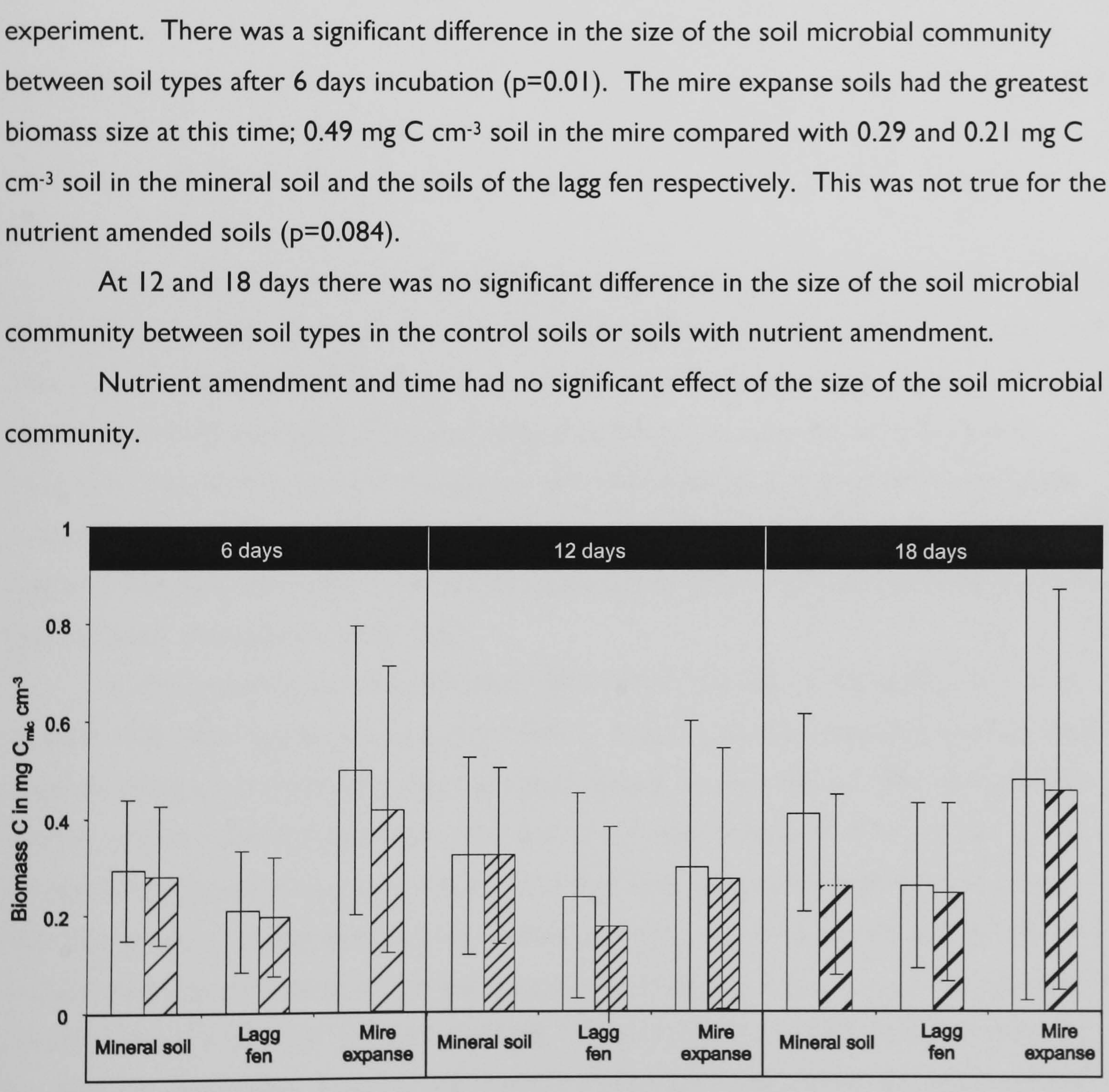

Figure 3.4: Size of the soil microbial community in the soils after nutrient addition. Control soils are represented by blank columns and amended soils are illustrated but shaded columns.

3.4 Discussion

Mineral nutrient addition did not affect the respiration of the soil microbial community of any of the soils. The nutrient concentration in the mineral soil was the same as the nutrient concentration added to the soils, therefore a change in the respiration of the soil microbial community would not be expected in these soils. However, in the soils of the mire expanse the concentration of nutrients was smaller than in the mineral soils, so nutrient addition should have alleviated nutrient limitation in these soils.

In the first week of incubation at 14° C the respiration of the soils remained constant. The exception to this was the control soils from the mire expanse, in which the rate of respiration increased. It has been well documented that increasing temperature increases respiration in soils Bridgham and Richardson (1992). The soils were moved from storage at 4° C to incubation at 14° C, which should have induced a more active microbial community. However, in all but the mire soils, there appeared to be no effect of a 10 °C temperature increase on the soils.

There was a difference in the mean rate of respiration of the control soils for both the first and second week. The CO_2 production in the lagg fen was greater than in the other soils. This contrasts with the characterization of the soil microbial community in chapter 2. Data already presented showed that the mineral soils of the surrounding slopes had a greater respiration rate than the soils of the lagg fen and mire expanse. It is possible the incubation conditions provided a more favourable environment for microbial respiration in the soils of the lagg fen. The soils were kept at 14 °C for a sustained period and this may have contributed to the increased respiration in some soils.

In the first week nutrients inhibited respiration in the soils of the lagg fen and mire expanse. No effect was seen on the mineral soils. Many researches have also failed to find a significant effect, or an inhibitory effect of added mineral nutrients (Fog, 1988). In my study, nutrient addition to the mineral soils was made at the same concentration as existed in the mineral soils, therefore we would not expect a significant increase in respiration of the soil microbial community. However, we would have expected an increase in the rate of respiration in the soils of the mire expanse, if these were nutrient limited. It may be that the nutrient salt solution added to the soils did not include the correct range of inorganic nutrients required, for example phosphorus, however this would not necessarily reduce the rate of respiration.

A change in community structure may also bring about changes to the microbial community that would in turn affect respiration. Increasing nutrient availability or pH may

stimulate the bacterial proportion of the microbial community as bacteria are able to outcompete fungi when there is a readily available nutrient source. Therefore, a possible avenue for further study would be to look at the community structure of the soil microbial community in response to changes in mineral nutrients.

The size of the soil microbial community was different in the three soils after 6 days. This difference was not seen in the nutrient amended soils. We would expect to see a difference in the size of the soil microbial community between the soils. However, the size of the microbial community in the soils of the mire expanse was greater than in the other soils after 6 days incubation, which suggests that incubation conditions had the effect of improving the environment for microbial growth in these soils. The effect of nutrient addition may have been masked by the heterogeneity of the soils, and consequent variability of microbial processes. However, because there was not a significant effect on the respiration rate of the soils either, this suggests that the solution of mineral nutrients added did not have a significant effect on the microbial processes measured.

The size of the soil microbial community was not different between soils after 2 weeks. Mineral nutrients did not effect the size of the community. In the first week the size of the soil microbial community in the soils of the mire expanse was greater than in the soils of the lagg fen and surrounding slopes. However, the size of the soil microbial biomass in the soils of the mire expanse may have been limited by the exhaustion of another resource in the soils, for example, carbon. It has been stated that although there is an abundance of available carbon in peats soils, not all of this is readily available for microbial attack during nutrient cycling.

The overall insignificant effect of mineral nutrients on the size and activity of the soil microbial community presented in this experiment may not be a sufficiently detailed account of the changes that occurred in the soils. Although some researchers (Williams, 1992) have documented changes in soil respiration over similar time periods, in reality the soil microbial community may react much more quickly to changes in nutrient supply. The length of the incubation time may also have contributed to the 'inhibitory effect' seen. Although the individual syringes were flushed every 48 h, over a sustained period the build up of secondary metabolites and CO_2 in the syringes may have had an adverse effect on the soil microbial community. This being the case, the sampling regime used in this experiment would not have been suitable for detecting these changes. Therefore, further work should incorporate some shorter term incubations. In addition, proposals for further work should include increasing the range of mineral nutrients added to soils. This work has provided some information about the effect of mineral nutrients similar to those found in situ, however it would be interesting to

explore the effect of other nutrients on the soils. This avenue could be explored further by including C sources in the nutrient amendment. In this particular way, the hypothesis that C is limiting in these soils could be tested.

3.5 Conclusions

The hypothesis restated:

- The size of the soil microbial community in the mire expanse is limited by mineral nutrient supply. Increasing the supply of mineral nutrients to the soil of the mire expanse will increase the size and activity of the soil microbial community in these soils.

The addition of mineral nutrients did not have a significant positive effect on the size and activity of the soil microbial community in the soils of the mire expanse. This result suggests that mineral nutrients are not limiting to the soil microbial community in these soils. The basis for further work has been set, and clearly there is scope for further investigation. It would be useful to test a wider range of nutrients, especially C sources, as they were not tested in this experiment. As well as measuring respiration and the size of the soil microbial community, some measure of the community structure at different stages during the incubation period would be useful. As a starting point, fatty acid methyl ester analysis could be used as a tool to characterize groups of microbes, with the possible application of detecting shifts in the microbial community during treatments. Further work should, however, target the early stages of treatment, as longer term incubations may not be sensitive to the changes that take place in the soil microbial community in response to nutrient addition.

This chapter provided some preliminary investigations into the effect of nutrients on the soil microbial community, and as has been outlined there are several ways in which the hypothesis could be expanded. However, the application of such laboratory studies to field situations are questionable and so subsequent experiments were transported to field conditions.

Chapter 4

Decomposition of a range of plant species

4.1 Introduction

Decomposition is the process by which dead plant and animal material is broken down to release nutrients for recycling. The importance of the soil microbial community in the decomposition process was illustrated in fig 1.4. In this chapter the microbial contribution to the decomposition process, and factors controlling it in peat and mineral soils, are investigated.

Decomposition in soils has been studied using a number of different approaches that normally target specific processes that contribute to the decomposition process. However, in terms of the organisms involved in the transformations, research to date has been less specific and the role of fractions within microbial communities has not been identified (Úlehlová, 1999). This gap in the research is indicative of the complexity of the processes involved in decomposition and the inherent difficulties in studying these processes. Of the research that has been carried out on the role of soil microorganisms in decomposition and activity in peat soils, approaches are wide ranging and involve the use of laboratory and field experiments.

4.1.1 Litterbag experiments

The most common approach to studying decomposition in peatlands, indeed in a range of soil ecosystems, has been with the use of litterbag studies. Simply, the decay of plant litter or a standard material which have been enclosed in mesh bags and buried *in situ*, is measured as ml. The method's popularity is attributed to its ability to measure the combined effects of all the decomposition processes and ease of manipulation for *in situ* studies (Belyea, 1996). By altering the size of the mesh it is possible to selectively exclude roots and larger soil organisms from the decomposition study, thereby targeting the microbial influence on decomposition. On this basis Coulson and Butterfield (1978) used the litterbag method to determine the role of the microbial population and that of larger soil organisms such as protozoa and nematodes in the decomposition in peat and mineral soils.

The litterbag method lends itself to further manipulation with the use of different substrates in the litterbags. It is possible to use a standard substrate, such as cellulose, which is a primary structural component in plants, comprising up to one half of the world net primary

production (Ljungdahl and Eriksson, 1985) to compare decomposition at different sites. Cellulose has been used extensively to compare decomposition between sites (Heal *et al.*, 1978; Fox and van Cleve, 1983; Lieffers, 1988). Usually, the loss of tensile strength from cellulose is used to measure decomposition. However, the use of cellulose as a standard perhaps over simplifies the decomposition process, and although cellulose is the main component of plant material, it is not the most important rate-determining component. It could be argued that a lignin substitute as a substrate would be a better alternative because it is the recalcitrant lignin component of plant material that resists decomposition in peatlands and contributes to the accumulation of organic matter. It remains that plant litter is a complex substrate that is the product of a range of enzyme regulated and chemical reactions which are often random, therefore it may not be possible to artificially synthesise a so-called standard substrate. The ecological significance of using a single standard substrate is yet to be proven, and is probably not a useful surrogate for plant litter in decomposition studies. For these reasons, most litterbag studies have incorporated natural substrates, often in accordance with the vegetation structure already in place at the site under study. Therefore, in peatland studies plant litter from Sphagnum mosses, Carex species and ericaceous shrubs are often used.

Litterbags are usually made up to suit the requirements of the experiment. An inert material, such as nylon, is used to make the bags, which can vary in size from a few cm square to tens of cm square. The dimensions of the litterbags depend on the amount of plant litter available and the size of the experiment. It is usual to make 3-5 replicates of each treatment and this must be taken into consideration when the size of the litterbags is decided upon. The size of the mesh diameter has greater implications for the decomposition processes occurring, and therefore this must be considered carefully at the outset of the experimental design. Bearing in mind that the mesh size effects the transport of matter in and out of the bag, a suitable mesh size must be chosen for the experiment. In microbial studies the mesh diameter needs to be big enough to allow the passage of microbes through the mesh barrier. Bacteria are typically no more than 10 μm in diameter and fungi no larger than 80 μm in diameter (Killham, 1994). Therefore, mesh sizes greater than 80 μm are suitable for decomposition studies targeting microbial processes. For examining the role of larger organisms on decomposition, for example, nematodes and small mites or plant roots, it is more difficult to select specific components of the ecosystem because a range of soil organisms may fall into the same size bracket. In addition, allochotonous material such as fibrous roots and small particles may enter the litterbags thereby causing a gain of mass into the bags. Under these circumstance extensive sorting must be carried out to separate the original plant litter from

foreign material that has been imported. There are four approaches to this problem, each having their merits and drawbacks. Most researchers have chosen to selectively pick out imported material from the litterbags to remove debris. In the latter stages of decomposition this task is very challenging and indeed may not be possible due to the visual similarity between the original plant material and any imported material. Another option has been to 'wash' the litterbags non-selectively, thereby getting rid of all the unattached material in the bags. This method does indeed get rid of foreign material that may have been imported into the bags, however some of the original material may be lost also. In these circumstances, mass loss from the bags is not an indicator of the complete breakdown and recycling of organic matter, but mainly fragmentation of litter in the early stages of decomposition. It is important to be aware of this in the interpretation of the results because mass loss in this case is not necessarily related to the accumulation of organic matter. The use of control litterbags in the experiment may be a useful indicator of how much material is imported into bags during burial. These bags are often made of the same material as the bags containing litter and can be used to estimate the amount of material that becomes attached to the litterbag itself. This approach is not without its drawbacks, and in many cases may not represent a true control. As foreign matter is transported through the litterbag its inclination to become trapped is increased by the texture of litterbag contents and occurrence of small pore spaces. Just as plant litter varies in its chemical composition, it varies greatly in its structural makeup, and so the task of designing the contents for a suitable control litterbag is not to be underestimated. By-passing the use of a simulated texture inside the bag is the usual approach, however the drawbacks of this must be considered in the interpretation of the results. Additionally, much of the material that becomes incorporated into litterbags may not be there by chance, and the presence of a newly added substrate attracts soil microorganisms, protozoa, nematodes and roots growth which all contribute to the gain of mass; the attractive forces of the substrate in the bags cannot be quantified with the use of an inert control. The other alternative is to consider the plant material in the litterbags as a resource. In this way, gain of mass into the litterbags is measured at the same time as decomposition and a net value may be used to give an indication of how attractive the decaying litter is as a resource for the soil microbial community and other soil organisms. Litterbag experiments are normally used in field decomposition studies, but some researchers have used the litterbag technique for controlled experiments in the laboratory. In the same way that the litterbag method may be manipulated in the field, it can be used for complex experiments in the laboratory with the added advantages of being able to regulate the environment and monitor changes more closely.

4.1.2 *Respiration measurements*

Organic matter is decomposed to CO_2 under aerobic conditions, and so decomposition may be measured by monitoring the rate of CO_2 evolution from soils. Problems arise when carrying out respiration measurements in the field because it is difficult to separate the respiration by larger soil organisms and roots from that of the soil microbial community. Using laboratory incubations, it is possible to overcome this problem, and therefore measure the microbial component of soil respiration (Bridgham and Richardson, 1992). The measurements of CO_2 in such experiments represent the complete decomposition of organic matter in soils and the transfer of C from the terrestrial pool to the atmospheric pool. These data are useful in studies that relate to the exchange of greenhouse gases in the environment and the status of peatlands as a C sink, consequently respiration experiments have become increasingly popular in recent years. Coupled with litterbag experiments, measurements of respiration can give more information about decomposition and the different processes involved. On the one hand mass loss measures fragmentation and loss of material by respiration, whereas respiration represents the complete breakdown of C within the system. Therefore the combination of the two techniques can provide a powerful tool in decomposition studies.

4.1.3 *Factors controlling decomposition*

4.1.3.1 Litter quality

The amount of readily available C and nutrients in litter are important controls of decomposition. The greater the amount of available C in litter the faster decomposition takes place. Similarly, litter with low C:N ratios requires less microbial energy for breakdown than does litter with higher C:N ratios and so is degraded more readily in soils. The approximate C:N ratio of bacterial cells is 10:1, and so plant litter with a ratio much higher than that will be poorer quality for the soil microbial community, hence less degradable. Information about these aspects of litter composition as it enters the decomposition cycle can provide an insight into the role of substrate quaility in controlling the decomposition process. CP MAS ^{13}C NMR spectrometry may be used to estimate the quality of litter for the soil microbial community. In much the same way that C:N ratios are used to determine the amount of nitrogen available per unit of C, the relative amount of aromatics (lignin, waxes and other recalcitrant components etc) and lignin:N can be used to determine the amount of N available to degrade

the recalcitrant component in the litter. The greater the lignin:N ratio, the greater the external N required to degrade the plant litter.

4.1.3.2 C quality using Nuclear Magnetic Resonance (NMR) spectroscopy

C in litter is distributed among a number of different structural components, and these vary in 'quality' for decomposition depending on their physical structure and associated components. The more regular and smaller the structural components the easier it is for microbes to attack and therefore the rate of degradation is faster. No single variable has consistently proven to be the best predictor of decay rates over a broad range of plant residues studied (Taylor *et al.*, 1989) therefore it is useful to measure a range of structural variables in studies of decomposition. The different structural components of plant material have traditionally been analysed using degradative techniques such as wet chemistry. Cellulose, lignin and hemicellulose contents may be determined using chemical extraction. However, this approach has limitations and may not be representative of the target components in the plant material. Lignin extractions, for example, in most chemical analyses are non-specific and generally include chitin from cell walls and many other complex aromatic compounds as well as true lignin. Additionally, during chemical extractions the analytical procedure may cause some structural transformation, thereby invalidating the results (Kinchesh *et al*, 1995b). Therefore the search for techniques to quantify the individual components in plant litter has not yet been exhausted.

Using NMR it is possible to separate the structural components of C within plant material and soils. There has been a range of applications to-date, often based on topical areas of research. Since the early eighties (Wilson, 1984) management practices have been monitored using NMR, and with the growing interest in the technique it became useful for identifying different components of organic matter. The range of usage increased further when research diversified into investigating decomposition processes in soils.

4.1.3.2.1 NMR as a tool for investigating decomposition

NMR can be summarized as the interaction between matter and electromagnetic radiation such that energy is absorbed or emitted according to the Bohr frequency condition (Harris, 1983). NMR is concerned with the magnetic energy of nuclei when they are placed in a magnetic field, and this is calculated according to the equation below.

$$\nabla E = h\nu$$

Where ∇E = the energy difference between the initial and final states of matter

h = Planck's constant

ν = frequency of the electromagnetic radiation

4.1.3.2.2 Brief background to NMR

In this section a brief explanation of the theory behind NMR will be given in order to show how different C components may be identified from NMR studies.

Nuclei spin due to an odd number of protons or neutrons in the nuclei, and as a consequence all nuclei are charged and will spin when placed in a magnetic field. The nucleus takes up orientations according to the direction of the field, which in turn produces a signal. A receiver, the NMR spectrometer detects this signal. The signal can be maximised by subjecting the charged particle to a 90° pulse. It is the speed at which the nuclei become relaxed that enables the spectrometer to determine the relevant properties of the nuclei, and this is called the free induction decay (FID). Many FIDs are collected and a mean value is taken, which is transformed into the more familiar NMR spectra using a fourier transform. Depending on where the functional group is in an organic molecule then the protection from electron varies. The greater the protection from electrons, the longer it takes for a nuclei to become relaxed. C atoms are well protected in the molecule, and therefore take a long time to relax. However, the time taken for relaxation depends on the relative protection within the molecule.

4.1.3.2.3 Interpretation of NMR spectra

Transformed spectra for CP MAS ^{13}C NMR spectrometry fall in the range 0-200 ppm (figure 4.1). It is possible to categorize these signals into 6 broad regions according to functional groups (see table 4.1). To the right of the NMR spectra (nearer the 0 end) the shielding of the nuclei is less and so the relaxation time is shorter. This end of the spectra represents the more 'exposed' C. It is important to note that different soils and indeed different organic matter have different 'background' NMR signals it is not possible to compare spectra from different soils or substrates (Baldock *et al.*, 1991).

Table 4.1: Shift ranges used to determine the C groups in plant material from NMR scans.

Shift range (ppm)	Type of C	Main class of compounds included
0-45	Alkyl- and methyl- C	Lipids, waxes and aliphatic hydrocarbons
45-60	Methoxyl-C	Lignin substituents, amino acids and amino sugars
60-90	O-alkyl-C	Carbohydrates
90-110	Acetal- and ketal-C	Carbohydrates
110-160	Aromatic-C	Phenyl-propylene sub-units of lignin
160-200	Carbonyl-C	Organic acids including amino acids and peptides

An example of a transformed ^{13}C NMR spectra is shown in figure 4.1. The regions of interest are split into bands according to table 4.1. In figure 4.1 there is a relatively large peak in the 90-60 ppm region, which represents carbohydrates. The height of this peak may be taken as an estimate of the proportion of a particular type of carbon present. More accurately, the area under the peak may be calculated to show the distribution of different types of carbon within a single sample, and these measurements are often quoted. Although theoretically there is a scale on the y axis of the spectra this may not be used to compare samples of different origin because the background signal for each sample is different. As a consequence of this C13 NMR may not be used as a quantitative tool to compare different samples, but it may be used to examine the ratio of different types of C components in samples.

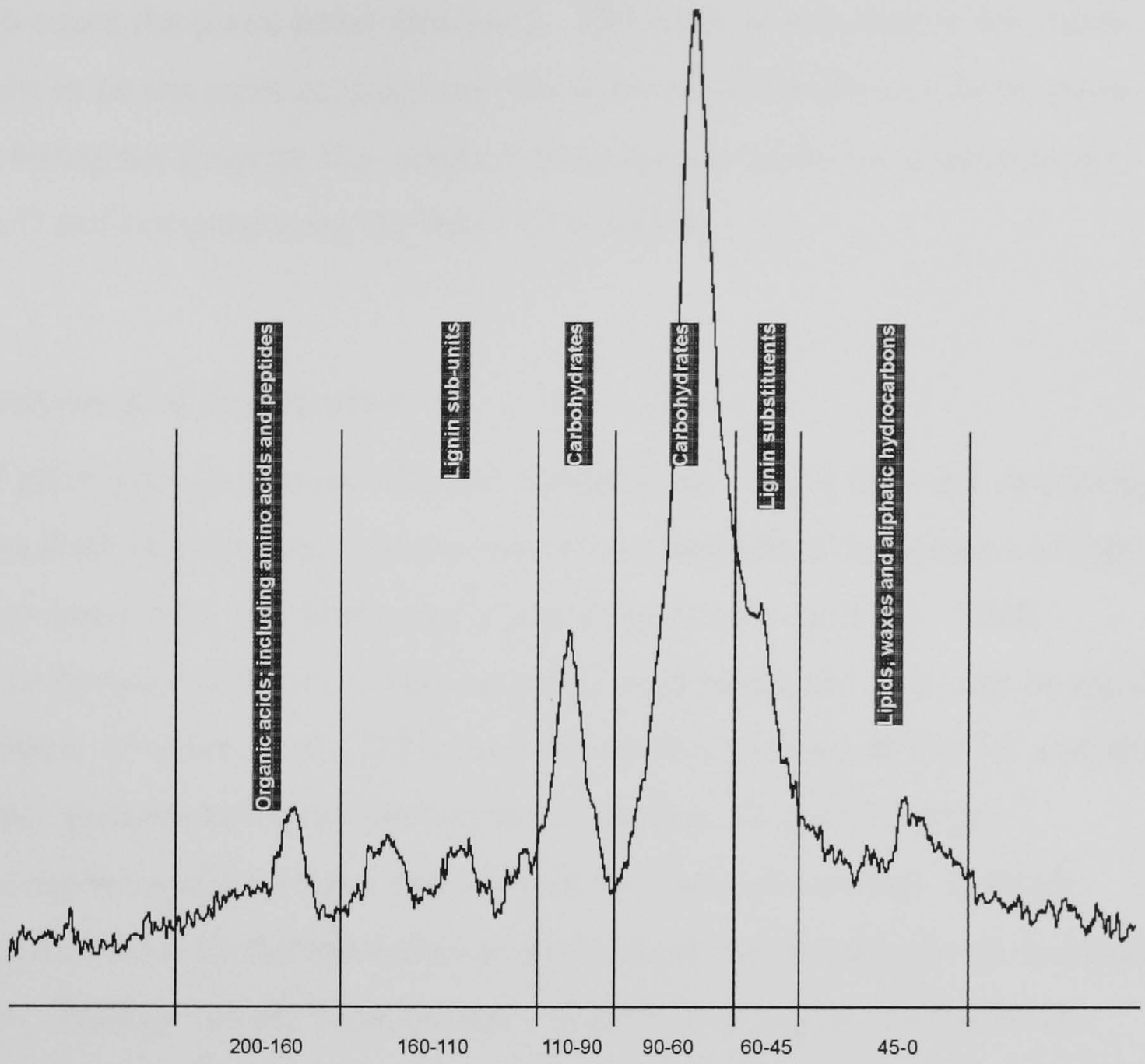

Figure 4.1: Typical ^{13}C NMR spectra showing the 6 different shift ranges that represent different C groups

In the literature several methods have been used to measure the areas underneath the curves assigned by the shift ranges in table 4.1. The whole process of determining peak areas is problematic because there are contasting views on how sidebands should be accounted for in ^{13}C NMR spectra of soils. For the purposes of this thesis sidebands will be ignored, and measure only the area specified within the ranges stated in table 4.1. Measuring the area under the peak is not always straight forward and often depends on the software used to control the NMR spectrometer. By manually calculating the area under the peaks of test spectra and comparing with calculations made by the software (Spinsight for UNIX) the applicability of the software was tested. This test revealed that the software was not suitable for determining the areas under the peaks using the interpretation adopted in this thesis. Alternative techniques to software integration are often adopted, and the most common and simple of these is the weighing technique. This method involves cutting out the area under the peak and weighing it, and using the density of the paper the area is calculated. More sophisticated approaches have been to digitize the output from the software using either a digitizing tablet or scanner. After

the data is in a digital format it is possible to apply greyscales to the shift bands and use a histogram function to count the pixels under the peaks. This method was used in this study because it was thought to be the most accurate and therefore reproducible way to measure the peak areas. The histogram program that counted the grayscale pixels from the scanned image was written in C and compiled using Borland's C++ Builder.

4.1.3.2.4 Measurements of decomposition

As decomposition of plant litter progresses different components of plant litter are selectively degraded according to their litter quality. For example, the carbohydrate component of litter decomposes at a much faster rate than lipids, waxes and aliphatic hydrocarbons. NMR spectroscopy is able to measure different components of C in samples, and so it may be applied to decomposition studies. Webster *et al.* (2001) used the relative amounts of O-alkyl and, alkyl and methyl C to reflect decomposition of different plant materials. O-alkyl C (largely carbohydrates) is lost more rapidly during decomposition than alkyl and methyl- C (lipids, waxes and aliphatic hydrocarbons), therefore the relative ratio of these components decreases during decomposition. Thus by making measurements of these two regions on the NMR spectra and presenting as a ratio it may be possible to obtain some information about decomposition. Indeed, in the first instance these figures could provide baseline information about the quality of the C for microbial use.

In addition to between O-alkyl:alkyl and methyl- C ratios, it may also be possible to compare the amount of lignin components with more degradable carbohydrate components in plants. There is potential for these measurements to be used both in the context of resource quality and as an indication of decomposition. It is not possible to quantify all lignin components in a given sample using the NMR technique, therefore 'lignin' must be interpreted with caution. The guaiacyl lignin peak is often used as a representative of lignin in samples and that falls in the 154 ppm region of a typical spectra.

4.1.4 Aim and Hypotheses

The overall aim of this chapter is to investigate the decomposition process in soils from a raised mire system in order to put decomposition in peat soils into context. Special attention will be paid to litter quality as a control of decomposition in order to measure its importance as a control in the three different soil types of a raised mire system.

Litter quality

- The soil microbial community should decompose litter with higher quality faster than it would decompose lower quality material. C is not limiting in peatland ecosystems, therefore the ratio of C:N will be used is an indicator of litter quality in these soils.

- Sphagnum litter should decompose more slowly than other litters.

Soil type

- The indigenous soil microbial community of a particular soil type is able to carry out faster decomposition on litter types with which they have previously been associated. For example, the soil microbial community of the mire soil should be able to decompose plant material that had grown on the mire faster than the soil microbial community of the mineral soils would be able to do so.

- Decomposition in the mineral soils should be fastest because the size of the soil microbial

Time

- Mass loss is fastest in the initial stages of the decomposition process because leaching makes large amounts of nutrients available to the soil microbial community which is greatest in these soils.

4.2 Materials and methods

4.2.1 Experimental design

The dominant plant species on the soils from each transect (table 4.2) were different, therefore, the three transects will be analysed individually in this chapter. The two most dominant plant species on each soil were used: two species from the mineral soils and two species from the soils of the lagg fen and mire expanse, six species per transect in total. Litterbags for each plant species were made up for each soil in the transect (6 plant species x 3 soils). The effect of time and season on decomposition was investigated by sampling three time points (4, 7 and 10 months). Three replicates were used for statistical purposes.

4.2.1.1 Control litterbags

Control litterbags were set-up in order to measure material moving into the litterbags. Making up bags without plant material and weighing these after the relevant incubation times comprised the controls. The material accumulating in the control bags was taken away from the experimental bags in the first stage of the calculation of mass loss from the bags.

4.2.2 Measures of decomposition

Decomposition is the general name give to a number of processes that combine to bring about the breakdown of organic material. The two main ways of measuring decomposition in soils are by examining changes in mass loss and respiration from newly added plant matter. It had often been presupposed that C mass loss from decomposing litter was entirely a loss of C to the atmosphere, and indeed good correlations between mass loss and CO_2 evolution have been observed (e. g. Updegraff *et al*, 1995). However, Domisch *et al.* (1998) demonstrated that a significant amount of C originating from newly added plant matter may be incorporated into the peat substrate, and not released directly to the atmosphere. It was for this reason that both methods were used in this study.

4.2.3 Plant species

The two most dominant plant species (% area covered) on each of the three soils were collected from the field on (17th May 1999). The dominant species were obvious at each site.

Samples were sorted by hand, separated and laid out in aluminium trays to allow them to dry at room temperature (22°C) for three weeks. Air-dried plant material was manually cut into lengths of 1-2 cm using scissors and mixed manually. Samples were kept separate at all times. The samples were returned to the trays and covered with aluminium foil for storage before the litterbags were made.

The moisture content of the air-dried plant material was measured by drying sub-samples at 70°C for 24 hours. Drying at 70°C facilitated the grinding procedure which was carried out in preparation for the C and N analysis. Sub-samples of dried material were ground using a pestle and mortar and the total C and N content was done using an elemental analyser (Carlo-Erbo analyser). The difference in moisture content between the air-dried samples and the 70°C dried samples was used in mass loss determinations. This was required because decomposed plant material from the litterbags was dried at 70°C for 24 hours prior to weighing to standardize the procedure.

4.2.4 Litterbags

Litterbags were made of nylon mesh (BDH) with a hole diameter of 100µm (lab experiments) and 200µm (field experiments). Mesh size was measured using a calibrated microscope (Olympus BX, UplanFl optics; Olympus Optical Co. Japan) and AnalySIS image processing software (Soft-Imaging System; Muenster, Germany) at a nominal 10x magnification. Mean values of horizontal and vertical mesh size were calculated from 10 measurements. The bags were made by cutting sections from a large sheet of mesh, folding and sealing two sides to leave one side open, so that plant material could be inserted at a later date. The mesh was sealed using a Bunsen burner and two pieces of metal plate adapted for sealing the bags. The litterbags in the laboratory experiment were 10 mm by 30 mm and for the field experiment dimensions of 50 mm by 50 mm were used.

Plant material was added to the bags to achieve a standard volume of material in the litterbags. Therefore the total dry weight of plant material in the bags varied between bags and species.

4.2.5 Relocating and marking the samples

Often in litterbag experiments samples are lost or misidentified. Several steps were taken to avoid this in the experimental procedure described here. In the laboratory experiment

precautions were taken to label vials and syringes correctly, thereby ensuring that sampled were not mixed up. However in the field experiment, there was a greater risk of mixing up samples because they were placed directly into the soil, and not in individual containers. Also, labelled had to be able to withstand soil physical and chemical processes for up to 10 months. Therefore a plastic marker was put into each bag before sealing by heat. The marker did not effect the decomposition process and would remain intact in the soil for a number of years.

The top 10 cm of peat soils normally falls within the acrotelm. The acrotelm is so named because this is the area of the soil profile in which activity is occurring. Water and other physical processes contribute to movement of material within in this layer. Therefore, it was important to fix the bags in position so that they would remain at the correct place in the profile i.e. that they would neither migrate to the anaerobic layer below or migrate horizontally. The litterbags were placed in a larger bag (150 mm x 300 mm) made of plastic coated wire and this was fixed to a wooden stake (450 mm in length) in the mineral soils or hollow plastic pole (1 m) in the peat soils. The poles were used to anchor the larger bags in position, and also to aid relocation of the sites.

4.2.6 Summary

In total 567 litterbags were made up for each part of the decomposition experiment and this is summarized below.

Plant species	6 + 1 (control)
Soils	3
Sampling occasions	3
Replicates	3
Total	189 litterbags per transect

The litterbags were filled with plant material according to the dominant aboveground biomass *in situ*. The dominant species found on each of the three transects is shown in table 4.2, and these were used to fill the litterbags to similar volumes.

Table 4.2: The dominant plant species on each soil type at Dun Moss.

	Litter no.	Transect 1	Transect 2	Transect 3
Mineral soil	1	*C. vulgaris*	*S. recurvum*	*C. vulgaris*
	2	*F. ovina*	*F. ovina*	*H. jutlandicum*
Lagg fen	3	*S. palustre*	*J. effusus*	*J. effusus*
	4	*J. effusus*	*C. vulgaris*	*F. ovina*
Mire expanse	5	*C. vulgaris*	*C. vulgaris*	*C. vulgaris*
	6	*S. capilifolium*	*S. papillosum*	*S. capilifolium*

4.2.7 Soil sampling

The laboratory experiment comprised microcosms of soil taken from the study site. Soil was sampled from the aerobic horizon of the mire expanse soil (0-10cm) the lagg fen soil (0-10cm) and the mineral soils (0-15cm) of Dun Moss on 17th July 1999. The soil was sieved and sorted then stored in polythene bags at 4°C for 2 weeks before the laboratory incubation was set up. Details of these soils are listed in appendix B4.

4.2.8 Field experiment

Litterbags were buried *in situ* at the surface (0-10cm) in the aerobic region in each of the soils. One set of litterbags was collected after 4, 7, and 10 months following burial. After extraction from *in situ* conditions, the bags were stored at 4°C for not more than 2 days. The bags were weighed at field moisture content because the material in the bags was needed as a sample for the measurement of the soil microbial community associated with the decaying litter. The surface of the bags was cleaned with tissue prior to weighing and then a sub-sample of material was dried at 70°C overnight to determine the moisture content. The water held within the mesh of the bags was taken into account using the control bags and this value was different depending on the soil. The mass loss of plant material due to decomposition was calculated. The litterbags were then stored at 4°C for not more than 3 weeks until soil microbial biomass analysis.

4.2.9 Laboratory experiment

The second part of the litter decomposition experiment was set up 1 month after the field experiment. This experiment took place entirely in the lab where a constant temperature of 14°C was maintained using an incubator (Appendix A).

Nylon mesh bags (1 X 2cm) were made up as above. Mesh diameter was 100µm. The bags were weighed prior to insertion of air dried plant material, reweighed, then sealed. A coded plastic marker was not used in the laboratory experiment, instead each bag was given a number using a permanent marker. Moist soil (6g) was placed into glass vials . Litterbags were then placed in the vials containing soil so that they were covered with soil. The design of the experiment was set up in parallel with the field experiment, (3 sampling occasions x 3 transects x 3 soils x 7 vegetation types (6 + control) x 3 replicates. Vials were contained in 60 mass loss syringes to measure CO_2 evolution from the samples. The headspace gas in the syringes was analysed continually for the first month of the experiment using a gas chromatograph (Appendix A). The syringes were flushed after each measurement was taken.

At any one time there were 189 samples incubated in syringes (all combinations for 1 sampling time). The remaining samples were also incubated at 14°C, however these were contained in plastic tubs with lose fitting lids. These tubs were selected because they simulated the same headspace as the syringes when calculated per sample vial. To prevent drying out of the samples and reduce the maintenance time required for these samples, 3 vials of distilled water were placed with 12 sample vials in a tub. However, from time to time some samples were subject to drying so the moisture status was monitored regularly. This procedure was crude and did not involve any measurements of the moisture content. Drying was estimated by visually comparing the sample with field moist soil of the same type. Distilled water was added where there was evidence of drying.

4.2.9.1 Mass loss

The mass loss of material from the litterbags was measured after 4, 7 and 10 months. The surface of the bags was cleaned prior to drying (at 70°C). The bags were then weighed and the mass loss calculated.

4.2.10 C quality - Solid state ^{13}C-NMR

300 MHz ^{13}C-NMR spectra of samples with crosspolarization and magic-angle spinning (CPMAS NMR) were obtained using a Chemagnetics CMX LITE Spectrometer operating at 300 Hz. The samples were spun at 4 kHz. A contact time of 2 ms was used for cross polarization of 3000 scans. This was sufficient to achieve a good signal-to-noise ratio for the samples. Chemical shifts are reported relative to tetramethylsilane (TMS) at 0 ppm.

4.3 Results

4.3.1 Mass loss

The mean data from the mass loss experiments are illustrated in appendix C1-C6. A negative mass loss value represents a gain of mass in the litterbags. The results are grouped in transects because the litter used for each transect varied in species and origin. For the purposes of interpretation, one-way ANOVA tests were used to examine the effect of soil type, vegetation type and time on mass loss from the litterbags. The results of these analyses are presented in tables 4.3-4.6. Tukey's family error rate (MINITAB ver 12) was used to test the difference between significant ($p < 0.05$) responses. An example of these statistical tests is given in the appendix (Appendix C). The effect of each of these factors is discussed in sections 4.3.1.1 to 4.3.1.3.

4.3.1.1 Effect of plant type on mass loss

4.3.1.1.1 Transect 1

In the field experiment there was a net gain of material in the *C. vulgaris* (ME) litterbags (table 4.3), in all of the soils. The net gain was greater in the lagg fen (352%) than in the mire expanse soils (261%). The mass loss from the other litter types did not differ significantly. In the lab experiment (table 4.4), there was a significant difference in the mass loss of material from the litterbags in the soils of the lagg fen and mire expanse after 7 months. The mass loss from the *C. vulgaris* bags was much smaller than from the other litterbags. This was also true for the 10 months that litterbags were buried in the peat soils.

4.3.1.1.2 Transect 2

The *in situ* experiment showed that the mass loss from the litterbags buried for 4 months did not differ between vegetation types. After 7 months, there was a significant gain of mass in the *C. vulgaris* (SS) (97%) and *C. vulgaris* (ME) (127%) bags buried in the mire expanse. After 10 months of burial in the lagg fen, there was a significant mass gain in the *F. ovina* (SS) and *C. vulgaris* (ME) bags. *C. vulgaris* (SS) and *C. vulgaris* (ME) litter buried in the soil of the mire expanse had a mass gain, suggesting that material had moved into the bags. In the microcosm experiment the mass loss of *S. papillosum* (ME) litter after 4 months was much smaller than of the other vegetation types in the peat soils of transect 2. After 7 months in the mineral soils,

the greatest mass loss was from *F. ovina* (SS) (75%). There was no difference between mass loss in the soils of the lag fen. In the soils of the mire expanse there was a mass gain in the *C. vulgaris* bags, which was significantly different from the other vegetation types.

4.3.1.1.3 Transect 3

In the field experiment there was no significant difference in the mass loss of the litter between the vegetation types in any soil type after 4 months. After 7 months in the mineral soil, however, *C. vulgaris* (ME) gained an average mass of 59 % while *H. jutlandicum* (SS) and *J. effusus* (LF) lost 64% and 45% respectively. Mass gain of material into the litterbags buried in the lagg fen was seen in the *C. vulgaris* (SS) (91% gain) and *C. vulgaris* (ME) (91% gain). After 10 months, there was no significant difference between the vegetation buried in the mineral soils and the soils of the lagg fen. However, there was a significant gain (150%) of material into the *C. vulgaris* (ME) litterbags buried in the mire expanse.

4.3.1.1.4 Summary

- there was a significant mass gain of material into the litter bags containing *C. vulgaris* originating from the mire expanse (*in situ*). Although this result was ubiquitous in transect one, there were also examples in transects two and three.
- significant mass gain was observed in *C. vulgaris* (SS), however, this was to a lesser extent (*in situ*)
- in transect 1 the mass loss was least from the litterbags containing *C. vulgaris* (ME), and these values were negaitve i.e. there was a mass gain of material (lab)
- there were significant differences between the mass loss of some treatments in transects two and three, however no obvious patterns emerged (lab)

Table 4.3: Summary of the effect of vegetation type on mass loss from litterbags (*in situ*), analysed using one way ANOVA. *P*= 0.05 Similar letters indicate similar populations where a is greater than b and c, and b is greater than c.

Transect 1	Time (month	*C. vulgaris* (SS)	*F. ovina* (SS)	*S. palustre* (LF)	*J. effusus* (LF)	*C. vulgaris* (ME)	*S. capilifolium* (ME)
Mineral soil	4	a	a	a	a	b	a
	7	a	a	a	a	b	a
	10	a	a	a	a	b	a
Lagg fen	4	a	a	a	a	b	a
	7	a	a	a	a	b	a
	10	a	a	a	a	b	a
Mire	4	a	a	a	a	b	a
	7	a	a	a	a	b	a
	10	a	a	a	a	b	a

Transect 2		*C. vulgaris* (SS)	*F. ovina* (SS)	*J. effusus* (LF)	*S. recurvum* (LF)	*C. vulgaris* (ME)	*S. papillosum* (ME)
Mineral soil	4						
	7						
	10						
Lagg fen	4						
	7						
	10						
Mire	4						
	7	b	a	a	a	b	a
	10	b	b	a	a	b	a

Transect 3		*C. vulgaris* (SS)	*H. jutlandicum* (SS)	*J. effusus* (LF)	*F. ovina* (LF)	*C. vulgaris* (ME)	*S. capilifolium* (ME)
Mineral soil	4						
	7	a	a	a	a	b	a
	10						
Lagg fen	4						
	7	b	a	a	b	a	a
	10						
Mire	4						
	7	b	a	a	a	b	a
	10	a	a	a	a	b	a

Table 4.4: Summary of the effect of vegetation type on mass loss from litterbags (lab incubation), analysed using one way ANOVA. *P*= 0.05 Similar letters indicate similar populations where a is greater than b and c, and b is greater than c.

Transect 1	Time (months)	*C. vulgaris* (SS)	*F. ovina* (SS)	*S. palustre* (LF)	*J. effusus* (LF)	*C. vulgaris* (ME)	*S. capilifolium* (ME)
Mineral soil	4						
	7						
	10						
Lagg fen	4						
	7	a	a	a	a	b	a
	10	a	a	a	a	b	a
Mire	4						
	7	a	a	a	a	b	a
	10	a	a	a	a	b	a
Transect 2		***C. vulgaris***	***F. ovina***	***J. effusus***	***S. recurvum***	***C. vulgaris***	***S. papillosum***
		(SS)	(SS)	(LF)	(LF)	(ME)	(ME)
Mineral soil	4						
	7	b	a	b	b	b	b
	10						
Lagg fen	4						
	7						
	10						
Mire	4	a	a	a	a	a	b
	7	a	a	a	a	b	a
	10						
Transect 3		***C. vulgaris***	***H. jutlandicum***	***J. effusus***	***F. ovina***	***C. vulgaris***	***S. capilifolium***
		(SS)	(SS)	(LF)	(LF)	(ME)	(ME)
Mineral soil	4	a	a	b	a	a	a
	7						
	10						
Lagg fen	4	a	a	a	b	a	a
	7						
	10						
Mire	4	a		a	a	b	
	7						
	10						

4.3.1.2 Effect of time on mass loss

4.3.1.2.1 Transect 1

In transect 1 (*in situ*) mass loss of *F. ovina* buried in the mire expanse was greater after 10 months (82%) than after 7 months (39%) (table 4.5). However, the mass loss of *S. palustre(LF)* was greater after 4 months (50%) than after 10 months (-19%). In the microcosm experiment, mass loss from the *S. palustre* (LF) litterbags was greater after 7 months (38%) and 10 months (54%) than after 4 months (–22%) burial in the mineral soils ($p<0.05$). There was no significant difference in the mass loss of the other vegetation types buried in the mineral soils ($p>0.05$). There was a greater mass loss from litterbags containing *S. palustre* (LF) in the lagg fen after 10 months (30%) than after 4 months (-101%). However, there was a mass gain of material into the *C. vulgaris* bags, and this was greatest after 10 months burial in the lagg fen when 171% of the initial material remained in the bags after incubation. In the soils of the mire expanse there was no significant difference in the mass loss of material from the litterbags.

4.3.1.2.2 Transect 2

The mass loss of *S. papillosum* buried in the mineral soil *in situ* was greater after 4 months (62%) decay than after 7 months decay (30%). The microcosm experiment showed that in the mineral soils, there was a smaller mass loss from the *F. ovina* (SS) litterbags after 10 months (75%) than after 7 months (39%). However, the mass loss of *S. recurvum* (LF) was greater after 10 months (41%) than after 4 months (18%). *S. papillosum* (ME) litterbags had a progressively greater mass loss with time weighing 12%, 44% and 55% of the initial mass, after 4, 7 and 10 months respectively. In the mire expanse, *S. recurvum* (LF) mass loss was greater after 10 months than 4 months. This was also true for *S. papillosum* litterbags, which had a greater mass loss with time. However, in the *C. vulgaris* litterbags buried in the mire expanse there was a decrease in mass loss with time

4.3.1.2.3 Transect 3

In transect 3, more mass loss of *F. ovina* buried in the lagg fen occurred after 10 months (13%) than after 7 months (-12%). *S. capifollium,* on the other hand lost more mass after 4 months (21%) than after 10 months (4%). In the laboratory experiment, there was an increase of mass loss with time of *J. effusus* (LF) buried in the mineral soils. This was also seen in the lagg fen

site, but not in the mire expanse. Mass loss of *F. ovina* (LF) and *C. vulgaris* (ME) increased with time in this soil. In the mire expanse the mass loss of *C. vulgaris* (ME) was smaller after 10 months (83%) than after 4 months (68%).

4.3.1.2.4 Summary

- there were no examples of crossover between the field and lab experiments
- time only effected very few treatments in the field experiment
- in the lab experiment there were more significant differences than in the field experiment, and in the main these resulted in greater mass loss with time

Table 4.5: Summary of the effect of incubation time on mass loss from litterbags analysed using one way ANOVA. $P = 0.05$
Similar letters indicate similar populations where a is greater than b and c, and b is greater than c.

			In situ			Lab		
			4 months	7 months	10 months	4 months	7 months	10 months
Transect 1								
Mineral soil	*C. vulgaris*	(SS)						
	F. ovina	(SS)						
	S. palustre	(LF)					a	b
	J. effusus	(LF)						
	C. vulgaris	(ME)						
	S. capilifolium	(ME)						
Lagg fen	*C. vulgaris*	(SS)						
	F. ovina	(SS)						
	S. palustre	(LF)				b		a
	J. effusus	(LF)						
	C. vulgaris	(ME)						
	S. capilifolium	(ME)						
Mire expanse	*C. vulgaris*	(SS)						
	F. ovina	(SS)		b	a			
	S. palustre	(LF)	a		b			
	J. effusus	(LF)						
	C. vulgaris	(ME)						
	S. capilifolium	(ME)						
Transect 2								
Mineral soil	*C. vulgaris*	(SS)						
	F. ovina	(SS)					a	b
	J. effusus	(LF)						
	S. recurvum	(LF)				b		a
	C. vulgaris	(ME)						
	S. papillosum	(ME)	a		b			
Lagg fen	*C. vulgaris*	(SS)				b	b	a
	F. ovina	(SS)						
	J. effusus	(LF)						
	S. recurvum	(LF)						
	C. vulgaris	(ME)						
	S. papillosum	(ME)				c	b	a
Mire expanse	*C. vulgaris*	(SS)						
	F. ovina	(SS)						
	J. effusus	(LF)						
	S. recurvum	(LF)				c	b	a
	C. vulgaris	(ME)						
	S. papillosum	(ME)				c	b	a
Transect 3								
Mineral soil	*C. vulgaris*	(SS)						
	H. jutlandicum	(SS)						
	J. effusus	(LF)				c	b	a
	F. ovina	(LF)						
	C. vulgaris	(ME)						
	S. capilifolium	(ME)						
Lagg fen	*C. vulgaris*	(SS)						
	H. jutlandicum	(SS)						
	J. effusus	(LF)				c	b	a
	F. ovina	(LF)		b	a	c	b	a
	C. vulgaris	(ME)				c	b	a
	S. capilifolium	(ME)						
Mire expanse	*C. vulgaris*	(SS)						
	H. jutlandicum	(SS)						
	J. effusus	(LF)						
	F. ovina	(LF)						
	C. vulgaris	(ME)						
	S. capilifolium	(ME)	a		b	b		a

4.3.1.3 Effect of soil type on mass loss

4.3.1.3.1 Transect 1

In the soils of transect 1 after 4 months burial *in situ*, there was a significant difference in the mass loss of three vegetation types (table 4.6). Three species lost more mass in the mineral soils than in the lagg fen soils: *C. vulgaris* (SS) lost 16% mass in the mineral soils compared with 105% gain in the mire expanse, *F. ovina* (SS) 63% loss in mineral and only 21% in the mire expanse, and *C. vulgaris* (ME) had 8% gain in the mineral soils in contrast to 352% gain in the mire expanse.

After 7 months *in situ* more mass loss was observed in the mineral soils than in the lagg fen for some species (*C. vulgaris* (SS), *F. ovina* (LF), *S. palustre* (LF) and *S. capilifolium* (ME). *F. ovina* (LF) and *S. palustre* (LF) lost more mass in the soils of the mire expanse than in those soils of the lagg fen. Also in this transect, there was 16% *S. capilifolium* (ME) mass lost from the litterbags incubated in the mineral soils compared with 1% in the soils of the mire expanse. There was no significant effect of soil type on the mass loss from litterbags after 7 months incubation in the laboratory.

After 10 months burial *in situ* 82% mass was lost from the *F. ovina* (LF) in the soils of the mire expanse in contrast to 1% in the soils of the lagg fen. *S. palustre* (LF) lost more mass in the mineral soils (61%) than in the soils of the mire expanse after 10 months (-19%). *C. vulgaris* (ME) incubated in the laboratory lost more mass in the mineral soils (5%) than in the soils of the lagg fen (-170%) and *S. capilifolium* (ME) lost more mass in the mineral soils (40%) than in the soils of the mire expanse (2%).

4.3.1.3.2 Transect 2

Only one significant effect on mass loss was observed in transect 2 after 4 months burial in the field. *S. papillosum* (ME) broke down slowest in the soils of the mire expanse in which there was a net agin of 10%. 57% mass was lost form the *C. vulgaris* (SS) litterbags incubated in microcosms containing soils of the lagg fen, while 69% was lost from the species in the mire expanse, however, there was only 15% mass lost from the mineral soils.

After 7 months burial in the field the mass loss of the *S. recurvum* (SS) was slowest in the mineral soils at 4%. *C. vulgaris* (ME) mass loss was greater in the soils of the lagg fen (57%) than in the soils of the mire expanse (-5%). The mass loss of *S. papillosum* (ME) was smallest in the soils of the mire expanse at –4%, similar to the result after 4 months. In the lab

experiment *F. ovina* (LF) lost 21% mass in the soils of the lagg fen, which was greater than in the other two soils. *C. vulgaris* (ME) on the other hand, had the smallest mass loss from the soils of the mire expanse at –129%.

After 10 months burial *in situ* there was a significant effect on all species except *S. recurvum* (SS). In the litterbags containing *C. vulgaris* (SS), *S. recurvum* (ME) and *S. papillosum* (ME) mass loss was smallest when incubated in the soils of the mire expanse. *F. ovina* (SS) mass loss was smaller in the soils of the lagg fen (-43%) than in the soils of the mire expanse or the mineral soils. *J. effusus* (LF) lost 34% mass when buried in the mire expanse compared with 1% in the soils of the lagg fen. In the microcosm experiment *F. ovina* (SS) and *C. vulgaris* (ME) lost more mass when incubated in the soils of the mire expanse (54% and 5% respectively) than in the mineral soil 61% and 35% respectively).

4.3.1.3.3 Transect 3

H. jutlandicum (SS) lost 14% mass in the soils of the lagg fen in contrast to 49% in the soils of the mire expanse and 50% in the mineral soils after 4 months in the field experiment. *J. effusus* (LF) lost more mass in the mineral soils (44%) than in the soils of the lagg fen (10%). In the laboratory experiment *C. vulgaris* (SS) and *C. vulgaris* (ME) lost more mass from incubation in the soils of the lagg and mire expanse than in the mineral soils. *J. effusus* (LF) and *F. ovina* (LF) had a greater mass loss in the soils of the mire expanse than in the mineral soils and soils of the lagg fen respectively.

After 7 months burial in the field there was only one significant effect of soil type on mass loss, this was from the *H. jutlandicum* (ME) which lost more material when buried on the mineral soils (65%) than in the soils of the lagg fen (19%). In the microcosm experiment, the mass loss of *C. vulgaris* (SS) was 24% in the mineral soils compared with 25% mass gain in the soils of the lagg fen.

In the field experiment, after 10 months in transect 3 the mineral soils increased mass loss of three species. *C. vulgaris* (SS) lost more mass in the mineral soils (37%) than in the soils of the lagg fen (-42%), and *C. vulgaris* (ME) and *S. capillifolium* (ME) lost more mass in the mineral soils (-10% and 8% respectively) than in the soils of the mire expanse (-30% and –28% respectively). In the microcosm experiment *J. effusus* (LF) lost 79% mass from the mineral soils in contrast to 49% in the soils of the lagg fen.

4.3.1.3.4 Summary

- soil type had a greater effect on the in situ experiment than the lab experiment.
- In general, there was more mass loss from the mineral soils than the soils of the lagg fen and mire expanse
- Taking into account all the significant differences between mass loss from litterbags buried in the soils of the lagg fen and mire expanse, there was no overall effect of soil type

Table 4.6: Summary of the effect of soil type on mass loss from litterbags analysed using one way ANOVA. $P = 0.05$ Similar letters indicate similar populations where a is greater than b and c, and b is greater than c.

			In situ			Lab		
			Mineral soil	Lagg fen	Mire expanse	Mineral soil	Lagg fen	Mire expanse
Transect 1								
4 months	*C. vulgaris*	(SS)	a	b				
	F. ovina	(SS)	a	b		a	b	
	S. palustre	(LF)						
	J. effusus	(LF)						
	C. vulgaris	(ME)	a	b	b			
	S. capilifolium	(ME)						
7 months	*C. vulgaris*	(SS)	a	b				
	F. ovina	(SS)	a	b	a			
	S. palustre	(LF)	a	b	a			
	J. effusus	(LF)						
	C. vulgaris	(ME)						
	S. capilifolium	(ME)	a	b	b			
10 months	*C. vulgaris*	(SS)						
	F. ovina	(SS)		b	a			
	S. palustre	(LF)	a		b			
	J. effusus	(LF)						
	C. vulgaris	(ME)				a	b	
	S. capilifolium	(ME)				a		b
Transect 2								
4 months	*C. vulgaris*	(SS)				a	b	b
	F. ovina	(SS)						
	J. effusus	(LF)						
	S. recurvum	(LF)						
	C. vulgaris	(ME)						
	S. papillosum	(ME)	a	a	b			
7 months	*C. vulgaris*	(SS)						
	F. ovina	(SS)				a	b	a
	J. effusus	(LF)						
	S. recurvum	(LF)	b	a	a			
	C. vulgaris	(ME)		a	b	a	a	b
	S. papillosum	(ME)		a	b			
10 months	*C. vulgaris*	(SS)	a	b	b		b	a
	F. ovina	(SS)	a	b	b	b		a
	J. effusus	(LF)		b	a			
	S. recurvum	(LF)						
	C. vulgaris	(ME)	a	b	b	a		b
	S. papillosum	(ME)	a	b	b			
Transect 3								
4 months	*C. vulgaris*	(SS)				b	a	a
	H. jutlandicum	(SS)	a	b	a			
	J. effusus	(LF)	a	b		b		a
	F. ovina	(LF)					b	a
	C. vulgaris	(ME)				b	a	a
	S. capilifolium	(ME)						
7 months	*C. vulgaris*	(SS)						
	H. jutlandicum	(SS)		a	b		a	b
	J. effusus	(LF)						
	F. ovina	(LF)						
	C. vulgaris	(ME)						
	S. capilifolium	(ME)						
10 months	*C. vulgaris*	(SS)						
	H. jutlandicum	(SS)		a	b			
	J. effusus	(LF)					a	b
	F. ovina	(LF)						
	C. vulgaris	(ME)		a	b			
	S. capilifolium	(ME)		a	b			

4.3.2 Respiration

Figures 4.2 to 4.10 show the respiration rate in the first 4 months from the microcosms containing soil and litterbags. The rate of respiration was calculated according to the amount of litter substrate that was used as treatment. The amount of litter added varied, therefore the recorded values were adjusted for the equivalent of 1g litter. The control values were not adjusted, therefore, can not be used as a direct comparison but to give an indication of fluctuation in the basal CO_2 from the soils over the incubation period.

4.3.2.1 Change in respiration rate 0-4 months

4.3.2.1.1 Transect 1

In all of the treatments there was an initial surge in activity (0-10 days) proceeded by a gradual decrease to a more stable respiration level. All vegetation treatments had a significant positive effect on the respiration of the soil microbial community. In the mineral soil there was a peak of respiration in some treatments between 1010-1517 hours.

Mineral soil *C. vulgaris* (SS) had a significantly greater respiration rate than *F. ovina* (SS), *J. effusus* (LF), *S. papillosum* (LF), and *S. capilifolium* (ME) after 48 hours (fig. 4.1). *C. vulgaris* (ME) was also greater than *S. capillifolium* (ME) after 48 hours. Between 48 and 1010 hours there was not a significant difference between CO_2 evolution rate. However, after that time there was an increase in the respiration rate of treatments *C. vulgaris* (SS), *S. papillosum* (LF) and *F. ovina* (SS). *C. vulgaris* (SS) maintained a significantly greater respiration than from *C. vulgaris* (ME) and *S. capilifolium* (ME) treatments up to 3 months.

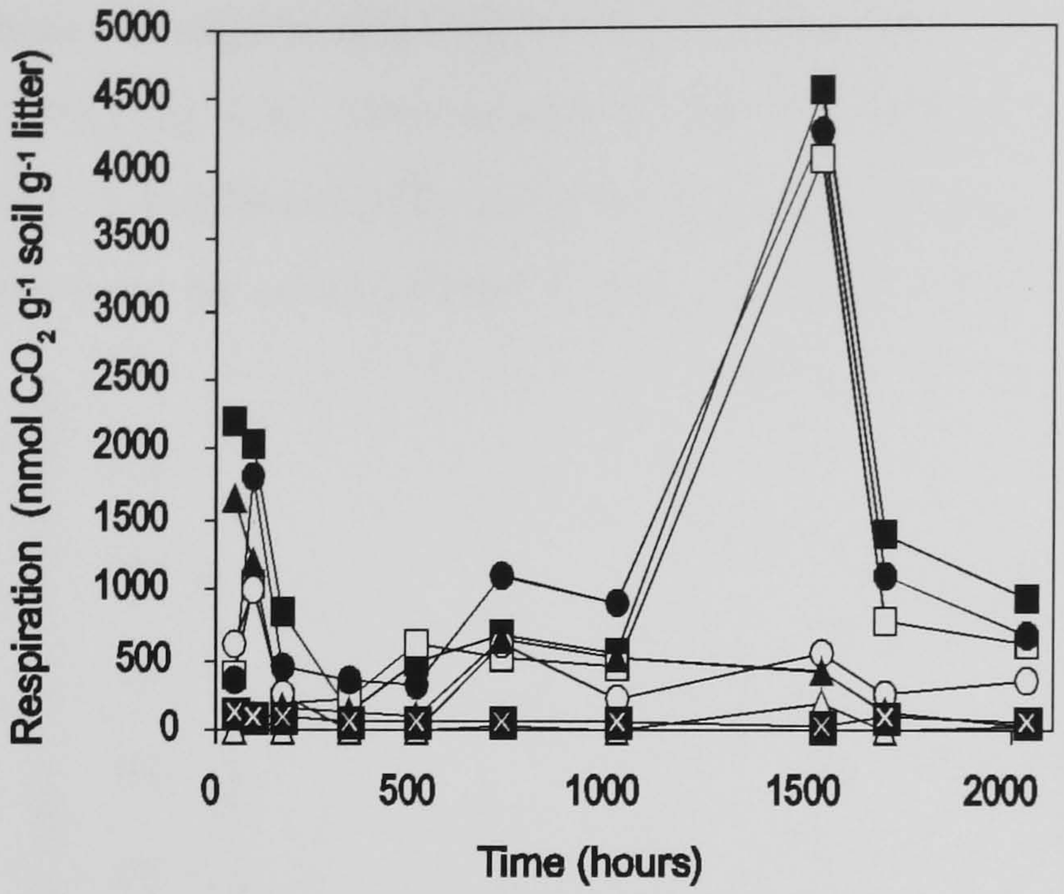

Figure 4.2: Respiration from the laboratory microcosm experiment in the mineral soils, transect 1. Closed square, open square, closed circle, open circle, closed triangle and open triangle are plant species 1 to 6 where 1-6 are *C. vulgaris* (SS), *F. ovina* (SS), *S. palustre* (LF), *J. effusus* (LF), *C. vulgaris* (ME) and *S. capilifolium* (ME). The closed square with a cross represents the control litterbag (without plant material).

Lagg fen Vegetation treatments had a significant effect on the respiration rate of the soils (fig. 4.2). The maximum value was from the *J. effusus* treated sample at 905 mol CO_2 g^{-1} soil g^{-1} litter at 48 hours. After 107 hours there was a levelling of respiration rates and there was no significant difference between the treatments.

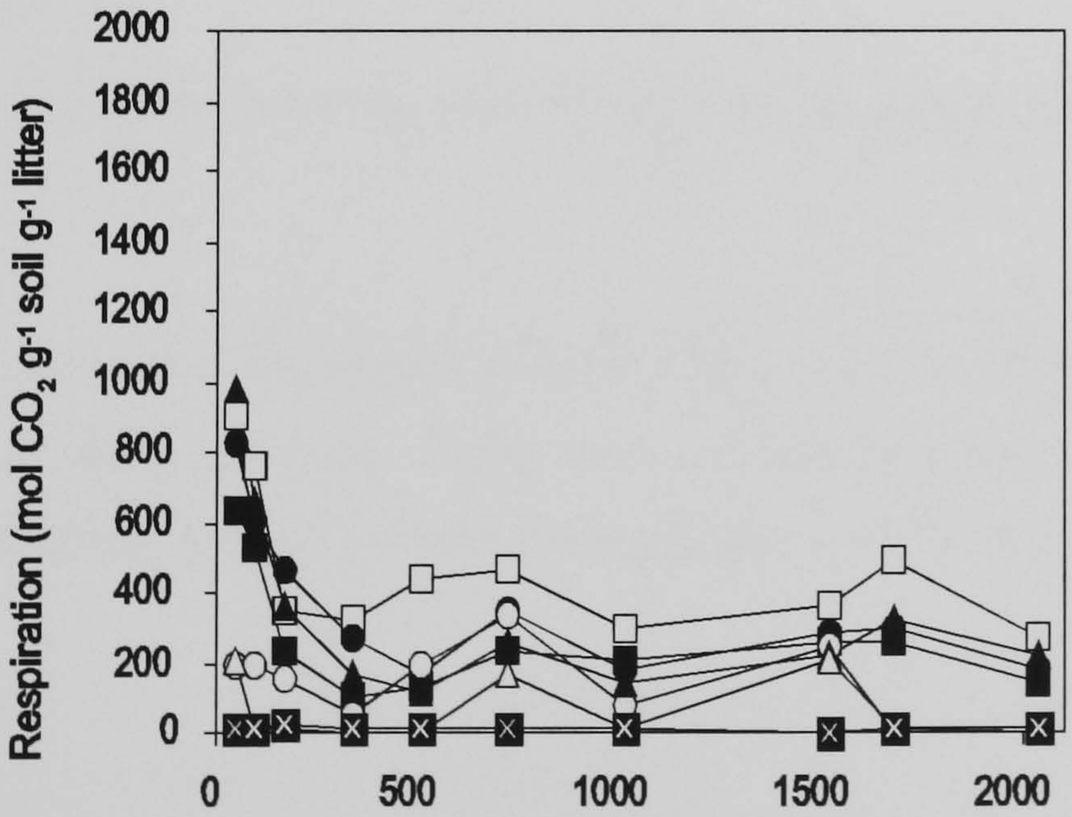

Figure 4.3: Respiration from the laboratory microcosm experiment in the soils of the lagg fen, transect 1. Closed square, open square, closed circle, open circle, closed triangle and open triangle are plant species 1 to where 1-6 are *C. vulgaris* (SS), *F. ovina* (SS), *S. palustre* (LF), *J. effusus* (LF), *C. vulgaris* (ME) and *S. capilifolium* (ME). The closed square with a cross represents the control litterbag (without plant material).

Mire expanse At 48 hours *C. vulgaris* (SS) induced significantly greater respiration than *F. ovina* (SS) and *S. capilifolium* (ME) (fig. 4.3). The respiration from *S. papillosum* (ME) was greater than from *S. capilifolium* (ME). *S. papillosum* (ME) had greater respiration than *J. effusus* (LF) at 719 and 1517 hours. There were no other differences between respiration rates of the treatments.

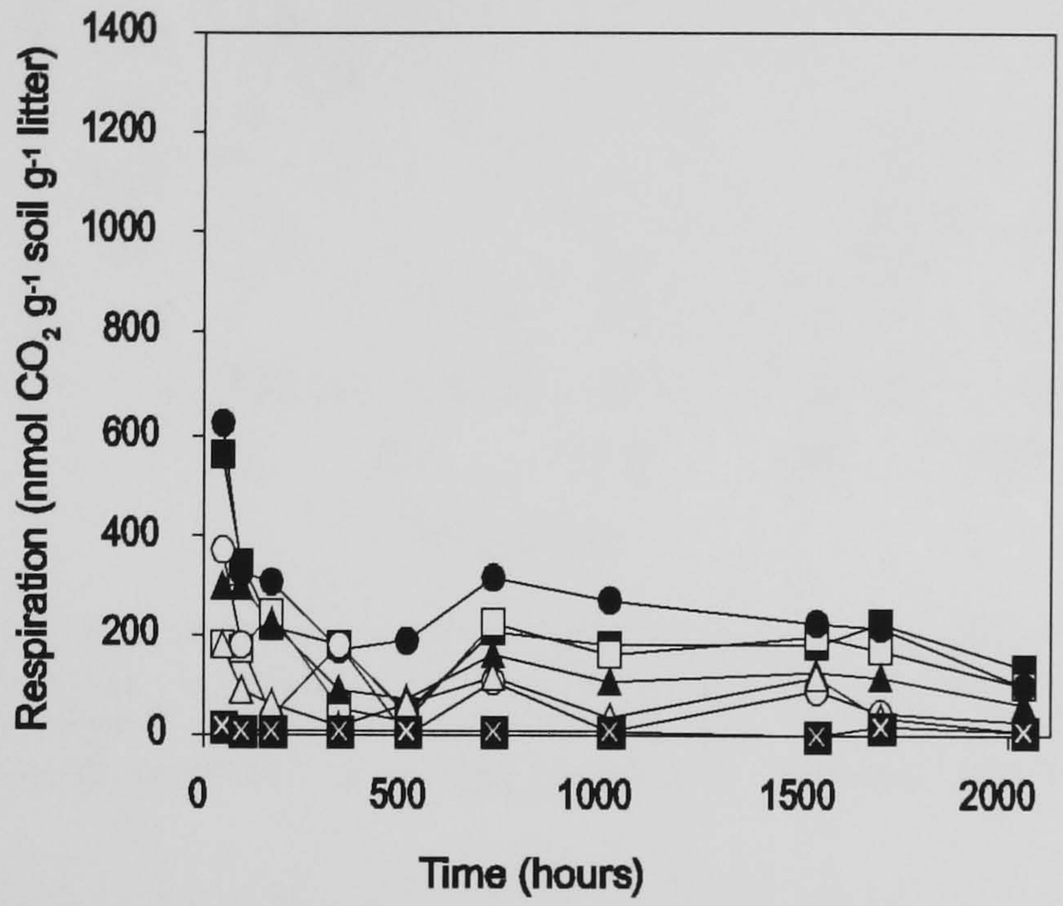

Figure 4.4: Respiration from the laboratory microcosm experiment in the soils of the mire expanse, transect 1. . Closed square, open square, closed circle, open circle, closed triangle and open triangle are plant species 1 to 6 where 1-6 are *C. vulgaris* (SS), *F. ovina* (SS), *S. palustre* (LF), *J. effusus* (LF), *C. vulgaris* (ME) and *S. capilifolium* (ME). The closed square with a cross represents the control litterbag (without plant material).

4.3.2.1.2 Transect 2

The data from the microcosms containing plant material and soil from transect 2 are presented in figures 4.5 to 4.6.

Mineral soil Figure 4.5 shows that the maximum initial respiration was 4932 mol CO_2 g^{-1} soil g^{-1} from the *H. jutlandicum* (SS) treated soil. There was a considerable variation in the respiration rates, however *H. jutlandicum* treated samples were greatest at six different sampling points.

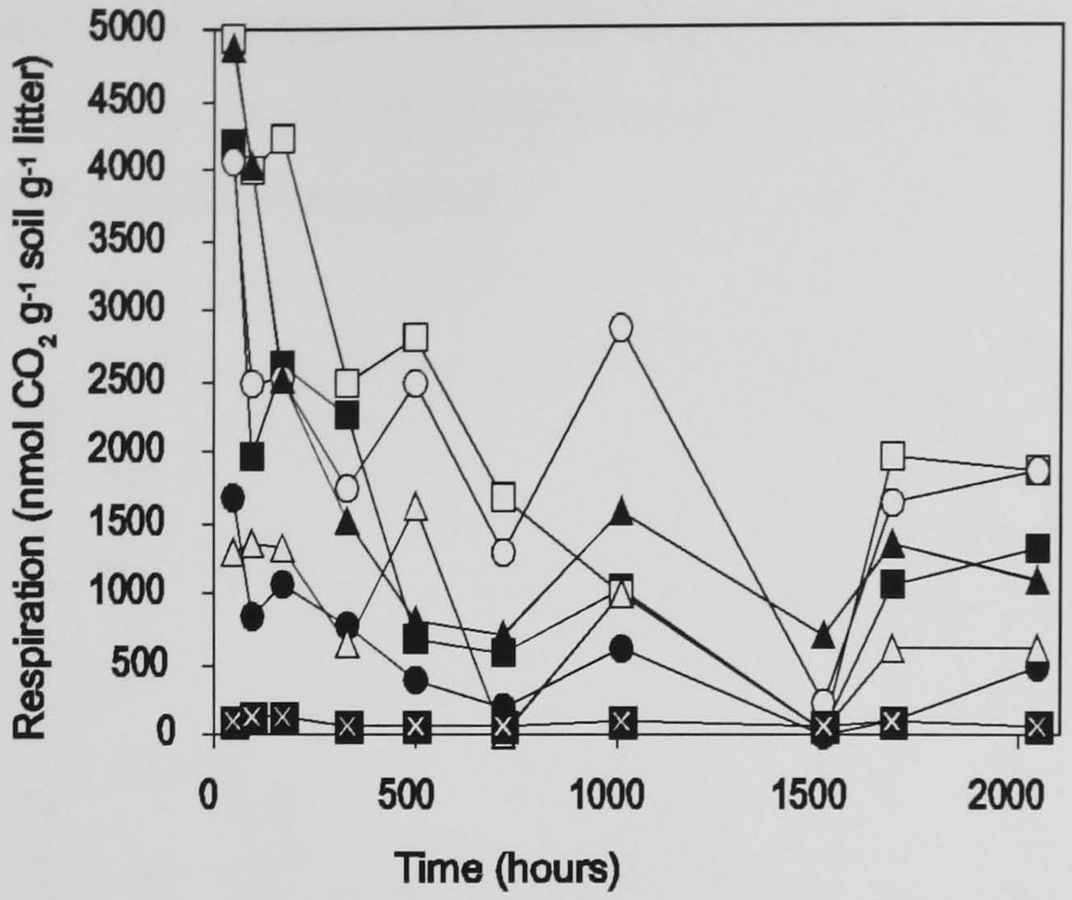

Figure 4.5: Respiration from the laboratory microcosm experiment in the mineral soils, transect 2. . Closed square, open square, closed circle, open circle, closed triangle and open triangle are plant species 1 to 6. Where 1-6 are *S. recurvum* (LF), *F. ovina* (SS), *J. effusus* (LF), *C. vulgaris* (SS), *C. vulgaris* (ME), *S. papillosum* (ME). The closed square with a cross represents the control litterbag (without plant material).

Lagg fen After the initial disturbance of the soil and consequent fluctuation in respiration rates, the rate of respiration levelled out (fig 4.5). *H. jutlandicum* (SS) treated soil had the greatest respiration rate consistently.

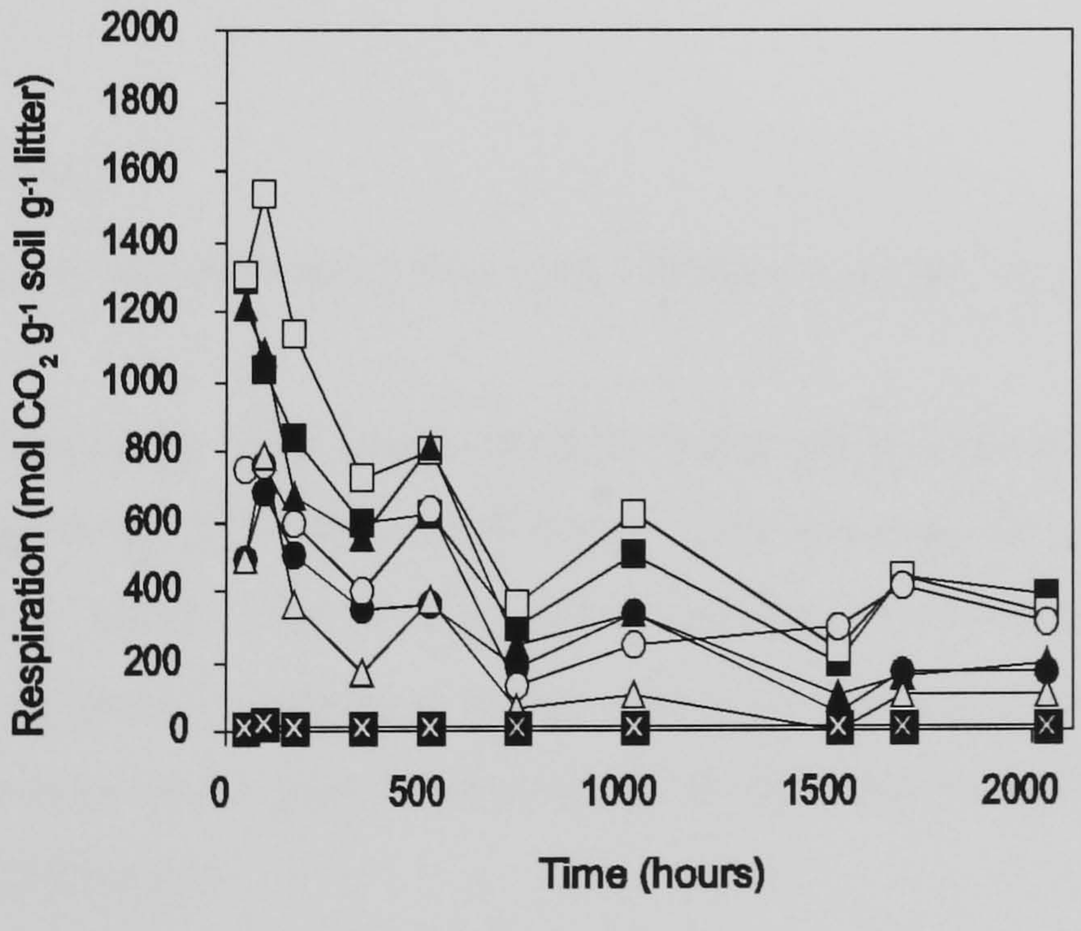

Figure 4.6: Respiration from the laboratory microcosm experiment in the lagg fen, transect 2. . Closed square, open square, closed circle, open circle, closed triangle and open triangle are plant species 1 to 6. Where 1-6 are *S. recurvum* (LF), *F. ovina* (SS), *J. effusus* (LF), *C. vulgaris* (SS), *C. vulgaris* (ME), *S. papillosum* (ME). The closed square with a cross represents the control litterbag (without plant material).

Mire expanse There was a large variation in the vegetation treatments and over time, within treatments (fig. 4.6). However, *H. jutlandicum* (SS) treated samples had significantly greater respiration rates than all the other treatments.

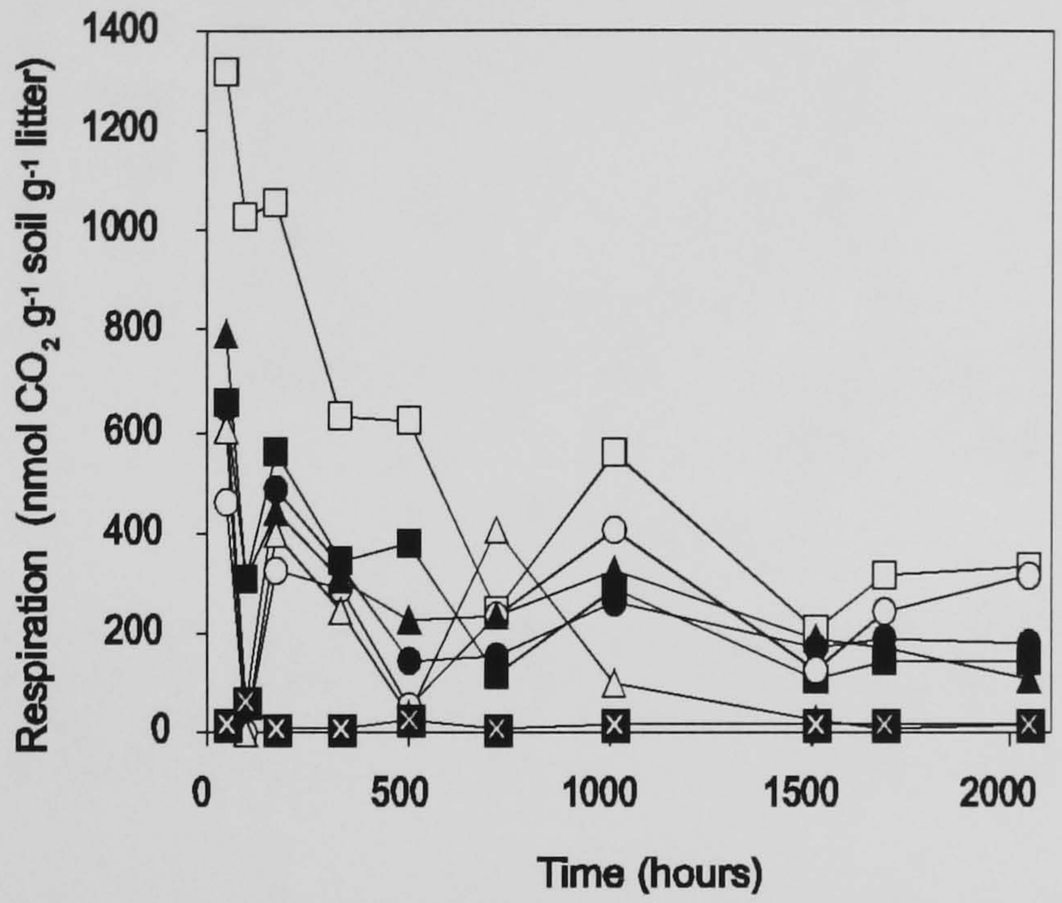

Figure 4.7: Respiration from the laboratory microcosm experiment in the mire expanse, transect 2. . Closed square, open square, closed circle, open circle, closed triangle and open triangle are plant species 1 to 6. Where 1-6 are *S. recurvum* (LF), *F. ovina* (SS), *J. effusus* (LF), *C. vulgaris* (SS), *C. vulgaris* (ME), *S. papillosum* (ME).. The closed square with a cross represents the control litterbag (without plant material).

4.3.2.1.3 Transect 3

Figures 4.8 to 4.10 show the respiration from soils and plant species from transect 3.

Mineral soil At the outset of the experiment there were high respiration rates, followed by a decrease and levelling off (fig. 4.7). After 1680 hours there was a significant increase (between 1000-2000 nmol CO_2 g^{-1} soil g^{-1} litter) in the respiration rate, corresponding to that observed in the mineral soil of transect 1. However, in this case all of the soils showed an increase in respiration rate. The respiration rates decreased rapidly after 1680 hours to values similar to those measured at 1010 hours.

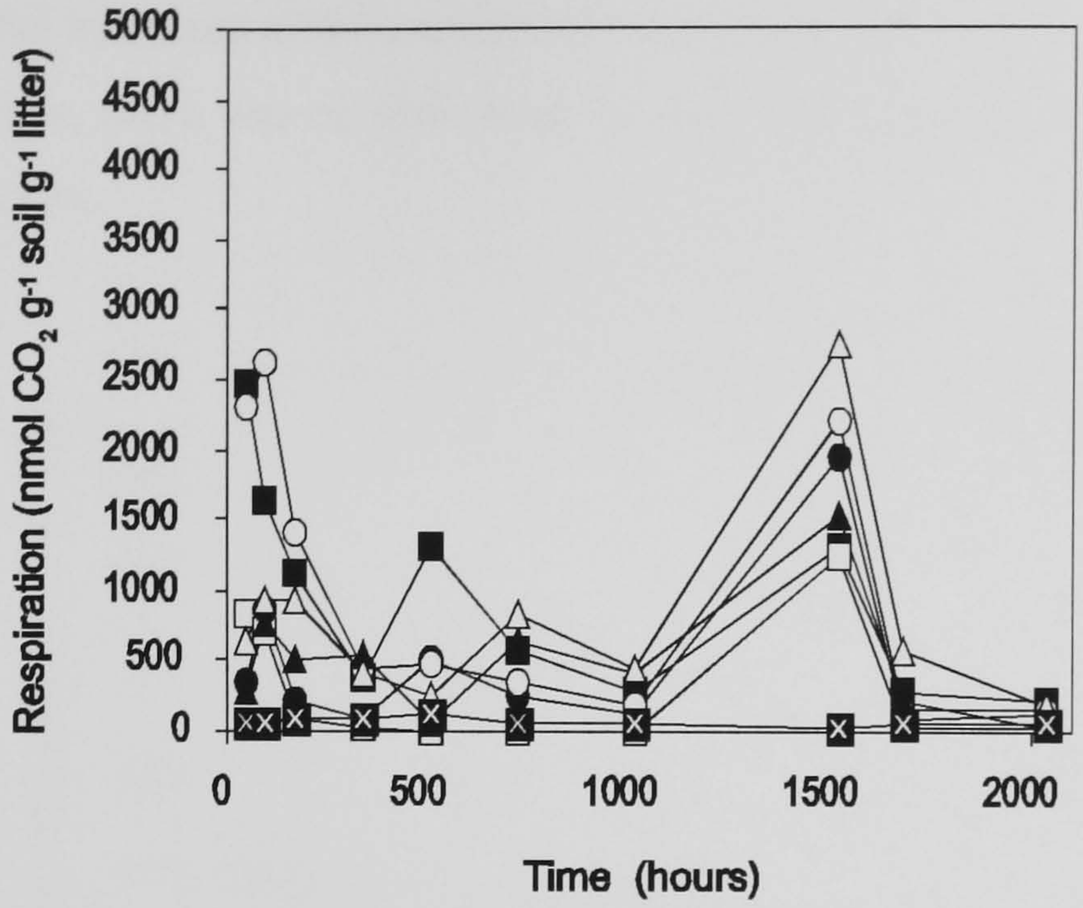

Figure 4.8: Respiration from the laboratory microcosm experiment in the mineral soils, transect 3. . Closed square, open square, closed circle, open circle, closed triangle and open triangle are plant species 1 to 6. Where 1-6 are *C. vulgaris* (SS), *H. jutlandicum* (SS), *J. effusus* (LF) *F. ovina* (LF), *C. vulgaris* (ME) and *S. capilifolium.* (ME). The closed square with a cross represents the control litterbag (without plant material).

Lagg fen Figure 4.9 shows that the greatest respiration rate was in the *F. ovina* (LF) treated soil, at 1649 nmol CO_2 g^{-1} soil g^{-1} litter hour $^{-1}$. This treatment induced the greatest respiratory response from the soil microbial community throughout the experiment. *C. vulgaris* (SS) treated soil was greater than *H. jutlandicum* (SS) and *J. effusus* (LF) in the initial 167 hours, after which there was not difference between the treatments.

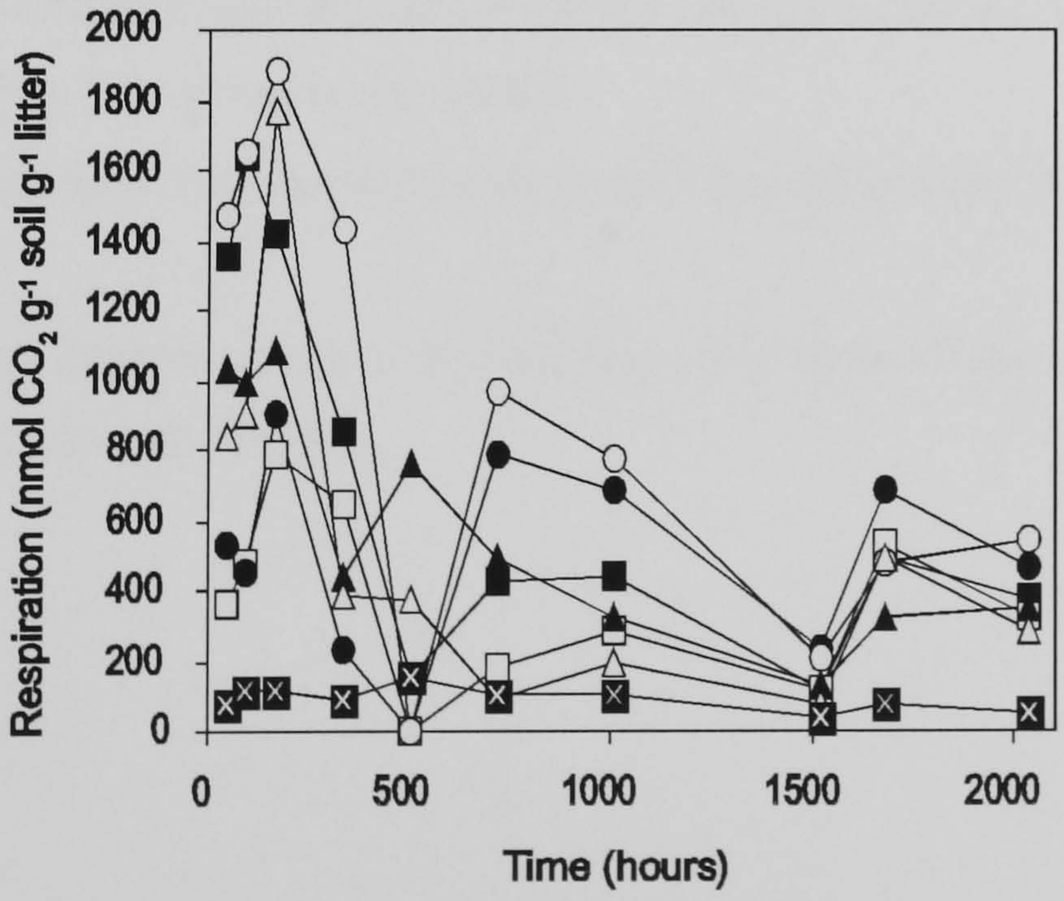

Figure 4.9: Respiration from the laboratory microcosm experiment in the lagg fen, transect 3. . Closed square, open square, closed circle, open circle, closed triangle and open triangle are plant species 1 to 6. Where 1-6 are *C. vulgaris* (SS), *H. jutlandicum* (SS), *J. effusus* (LF) *F. ovina* (LF), *C. vulgaris* (ME) and *S. capilifolium.* (ME). The closed square with a cross represents the control litterbag (without plant material).

Mire expanse Two initial increases in the respiration rate were observed in all of the treated soils (fig. 4.9). However, there was no difference between the treatments. Levelling off occurred after 719 hours.

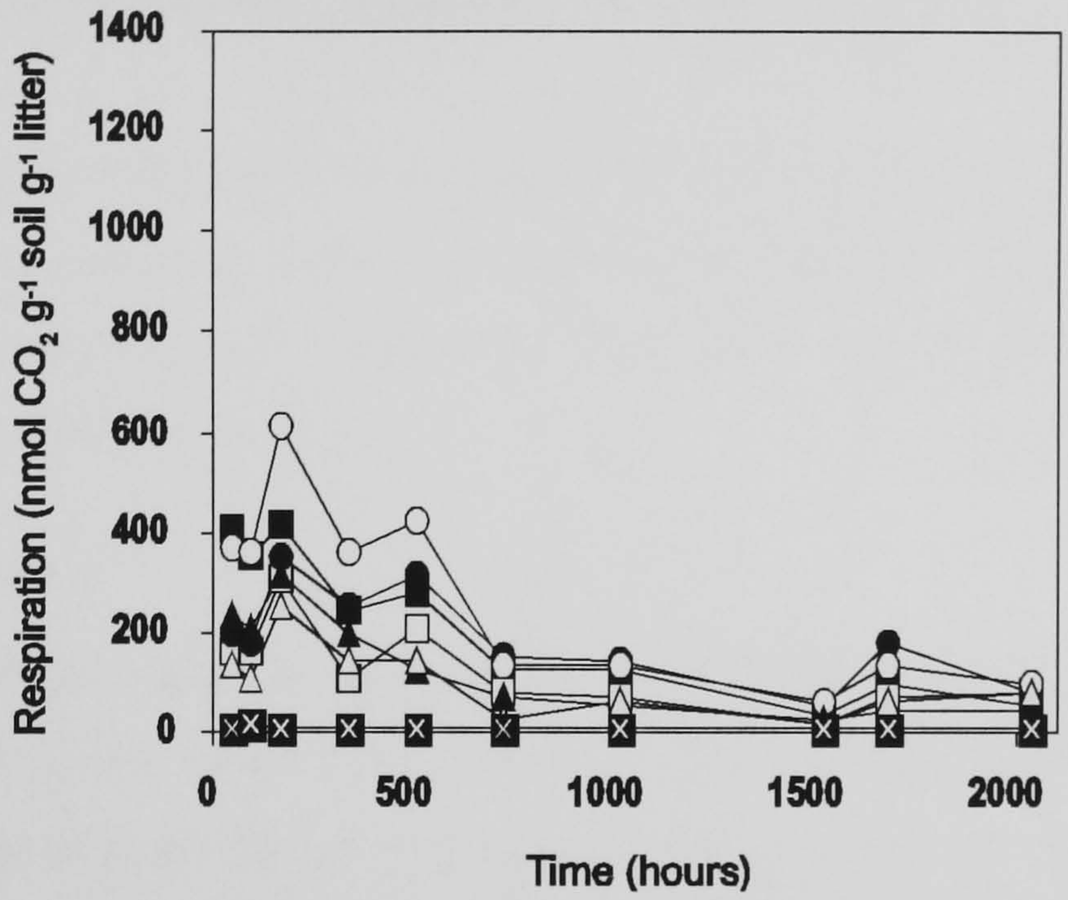

Figure 4.10: Respiration from the laboratory microcosm experiment in the mire expanse, transect 3. Closed square, open square, closed circle, open circle, closed triangle and open triangle are plant species 1 to 6. Where 1-6 are *C. vulgaris* (SS), *H. jutlandicum* (SS), *J. effusus* (LF) *F. ovina* (LF), *C. vulgaris* (ME) and *S. capilifolium*. (ME). The closed square with a cross represents the control litterbag (without plant material).

4.3.2.1.4 Summary

- a similar pattern was observed for each treatment and soil type; there was a sharp increase in respiration when the resource was added
- in most cases, this spike decayed to less than half the original CO_2 rate within the incubation time
- unusual peaks and troughs occurred in the CO_2 rate in some of the soils, these were recorded at approximately 1500 h

4.3.2.2 Cumulative respiration, 0-4 months

The respiration data from the initial 4 months of the laboratory experiment are presented as a mean rate for this period so that they can be compared with the results from the first set of data from the mass loss experiment. These data are presented in table 4.7.

In transects 1 and 2 the mineral soils were significantly more active than the soils of the lagg fen and the mire expanse. This is consistent with the characterization of the size and activity of the soil microbial community reported in chapter 2. There was a significant positive effect of plant material on the respiration of the soils.

4.3.2.2.1 Transect 1

The greatest rate of CO_2 in the mineral soil (1703 μmol CO_2 g^{-1} litter h^{-1}), was induced by the *C. vulgaris* (SS) plant material. The *F. ovina* (LF) and *J. effusus* (LF) treated samples also had high respiration rates accounting for 29 % and 24 % of the total. In the soils of the lagg fen the greatest rate of respiration was from the *J. effusus* (SS) treated sample. However this was much smaller than the equivalent in the mineral soils (373 compared to 1406 μmol CO_2 g^{-1} litter h^{-1}). In the soils of the mire expanse *F. ovina (LF)* induced the maximum rate, which was smaller than in the soils of the lagg fen and mineral soils.

In the mineral soil the ratio of *C. vulgaris* (SS):*C. vulgaris* (ME) is 5:1, however in the soils of the mire expanse and the lagg fen, this value is closer to 1:1.

4.3.2.2.2 Transect 2

F. ovina (SS) had the greatest effect on the respiration of all the soils, and *S. papillosum* (ME) had the smallest effect. The respiration of the slope soils was much greater than for the soils of the mire expanse and the lagg fen. The ratio between *C. vulgaris* from the mire and from the mineral soils was close to 1:1 for each soil type.

4.3.2.2.3 Transect 3

In the lagg fen *F. ovina* (LF) had the greatest respiration, closely followed by *J. effusus* (LF). These two species were also the most productive in the soils of the mire expanse, however the order was reversed. *S. capilifolium* (ME) had the greatest respiration rate in the mineral soils (1011 μmol CO_2 g^{-1} litter h^{-1}) which was unusually large compared to the same

species in the soils of the lagg fen (249 μmol CO_2 g^{-1} litter h^{-1}) and mire expanse (65 μmol CO_2 g^{-1} litter h^{-1}). The ratios of *C. vulgaris* (SS) to *C. vulgaris* (ME) were 8:7 in the mineral soils, 17:15 in the soils of the lagg fen, and 9:5.5 in the soils of the mire expanse.

4.3.2.2.4 Summary

- in all three transects *F. ovina* (LF), *J. effusus* (SS) and *C. vulgaris* (SS) treatments produced greater CO_2 production than the other vegetation types, and this pattern was irrespective of soil type
- the respiration in the mineral soils was greater than that of the soils of the lagg fen and mire expanse

Table 4.7: Mean rate of CO_2 production for laboratory microcosm experiment. Numbers in bold are the percentage of the total CO_2 evolution for all the plant species for a particular soil type in a transect.

	Transect 1					
μmol CO_2 g^{-1} litter h^{-1}	*C. vulgaris* (SS)	*J. effusus* (SS)	*F. ovina* (LF)	*S. palustre* (LF)	*C. vulgaris* (ME)	*S. capilifolium* (ME)
Mineral soil	1703	1406	1624	426	348	101
SD	1098	665	4195	1091	831	92
Lagg fen	**209**	**373**	**259**	**183**	**227**	**86**
SD	21	202	323	157	60	14
Mire expanse	**173**	**142**	**215**	**78**	**111**	**64**
SD	27	62	69	21	19	14
	Transect 2					
μmol CO_2 g^{-1}	*C. vulgaris*	*F. ovina*	*S. recurvum*	*J. effusus*	*C. vulgaris*	*S. papillosum*
Mineral soil	**919**	**1468**	**476**	**1564**	**1175**	**554**
SD	0	5083	8392	2905	2125	1772
Lagg fen	**409**	**470**	**209**	**330**	**312**	**123**
SD	689	220	55	52	81	74
Mire expanse	**200**	**399**	**199**	**234**	**221**	**108**
SD	55	74	42	42	43	43
	Transect 3					
μmol CO_2 g^{-1}	*C. vulgaris*	*H. jutlandicum*	*J. effusus*	*F. ovina*	*C. vulgaris*	*S. capilifolium*
Mineral soil	**731**	**535**	**639**	**846**	**607**	**1011**
SD	0	829	60	664	1332	251
Lagg fen	**428**	**287**	**455**	**589**	**362**	**249**
SD	266	164	191	152	126	245
Mire expanse	**117**	**78**	**137**	**160**	**69**	**65**
SD	93	13	39	45	14	7

4.3.3 *Litter quality*

4.3.3.1 C:N

The C:N values for the vegetation types used in the litterbag experiment are shown in table 4.8. Using C:N as an indicator of litter quality, *S. papillosum* (LF) and *J. effusus* (LF) have the most favorable litter quality from transect 1 at 32.9 and 32.7 respectively. The litters originating from the mire expanse have the poorest litter quality. In transect 2 the litters from the mire expanse also have the highest C:N values, making them the poorest in quality. *S. recurvum*, from the lagg fen has the smallest C:N and so is the best litter in this transect. In transect 3 the difference between the C:N values of the different species is not as great as in the other two transects. The smallest C:N, therefore best litter quality, was found from *J. effusus* (LF), and the greatest was found from *S. capilifolium (*ME). There was some variation between similar litter types growing on different soils and transects, however in general *C. vulgaris* had a high C:N ratio and *J. effusus* had a comparatively low C:N ratio out of the litter types tested.

Table 4.8: Quality of the vegetation used in the litter experiment, expressed as C:N ratio.

Vegetation type	C%	N%	C:N
Transect 1			
C. vulgaris (SS)	51.02	1.04	49.05
F. ovina (SS)	42.69	1.03	41.44
J. effusus (LF)	46.77	1.43	32.7
C. vulgaris (ME)	52.25	0.98	53.32
S. capilifolium (ME)	45.52	0.82	55.51
Transect 2			
C. vulgaris (SS)	49.65	1.05	47.29
F. ovina (SS)	43.12	1.2	35.93
S. recurvum (LF)	45.12	1.61	28.02
J. effusus (LF)	47.07	1.27	37.06
C. vulgaris (ME)	51.24	0.97	52.82
S. papillosum (ME)	45.80	0.91	50.33
Transect 3			
C. vulgaris (SS)	51.05	1.08	47.27
H. jutlandicum (SS)	44.95	1.14	39.43
J. effusus (LF)	47.50	1.38	34.42
F. ovina (LF)	44.73	0.98	45.64
C. vulgaris (ME)	51.63	1.10	46.94
S. capilifolium (ME)	45.42	0.91	49.91

4.3.3.2 NMR spectra

The data presented in this section are a summary of the NMR spectroscopy analyses carried out on control samples (undecomposed) and material extracted from that remaining in the litterbags after 10 burial in situ. The number of analyses carried out was restricted by cost, and also by the amount of material available in the litterbags. For these reasons the analyses were only carried out on the samples from transect 2, and the individual replicates were bulked. The description of the NMR spectra will first address some general shift ranges, then specific properties will be examined.

Figure 4.11 shows the NMR spectra of the six plant litters (*C. vulgaris* (SS), *F. ovina* (SS), *S. recurvum* (LF), *J. effusus* (LF), *C. vulgaris* (ME) and *S. papillosum* (ME)) at the outset of the experiment. This figure therefore presents a measure of resource quality. However, it must be noted that it is not possible to compare between samples, only within samples i.e. it is not viable to compare the proportions of similar C components between samples, yet spectra may be used to compare the relative proportions of different C components within one sample. Working from 0 ppm to 200 ppm, the first C component is alkyl- and methyl- C, which is composed mainly of lipids, waxes and aliphatic hydrocarbons. This region represents a relatively recalcitrant component of the plant. The methoxyl C (40-60 ppm shift range) is not pronounced in any of the samples, on the other hand the O- alkyl C (60-90 ppm) is a major component. This peak may be combined with the 90-100 ppm shift range under the general heading of carbohydrates, and these represent a more available C pool than the 0-45 ppm region (alkyl- and methyl- C). In the *F. ovina* (SS), *S. recurvum* (LF), *J. effusus* (LF) and *S. papillosum* (ME) spectra the relationship between these two regions is similar; the carbohydrate region is approximately 3 times greater than the alkyl- and methyl- C. However in the *C. vulgaris* samples this value is significatly smaller. The 110-160 ppm shift range is aromatic C, the phenyl-propylene sub-units of lignin, therefore this is recalcitrant C. Again, this region is predominant in the *C. vulgaris* samples. It is worth note at this point that although there appears to be a lot of aromatic C in *F. ovina* (SS) and *S. papillosum* (ME) this is probably due to noise, and this will be discussed later in this section.

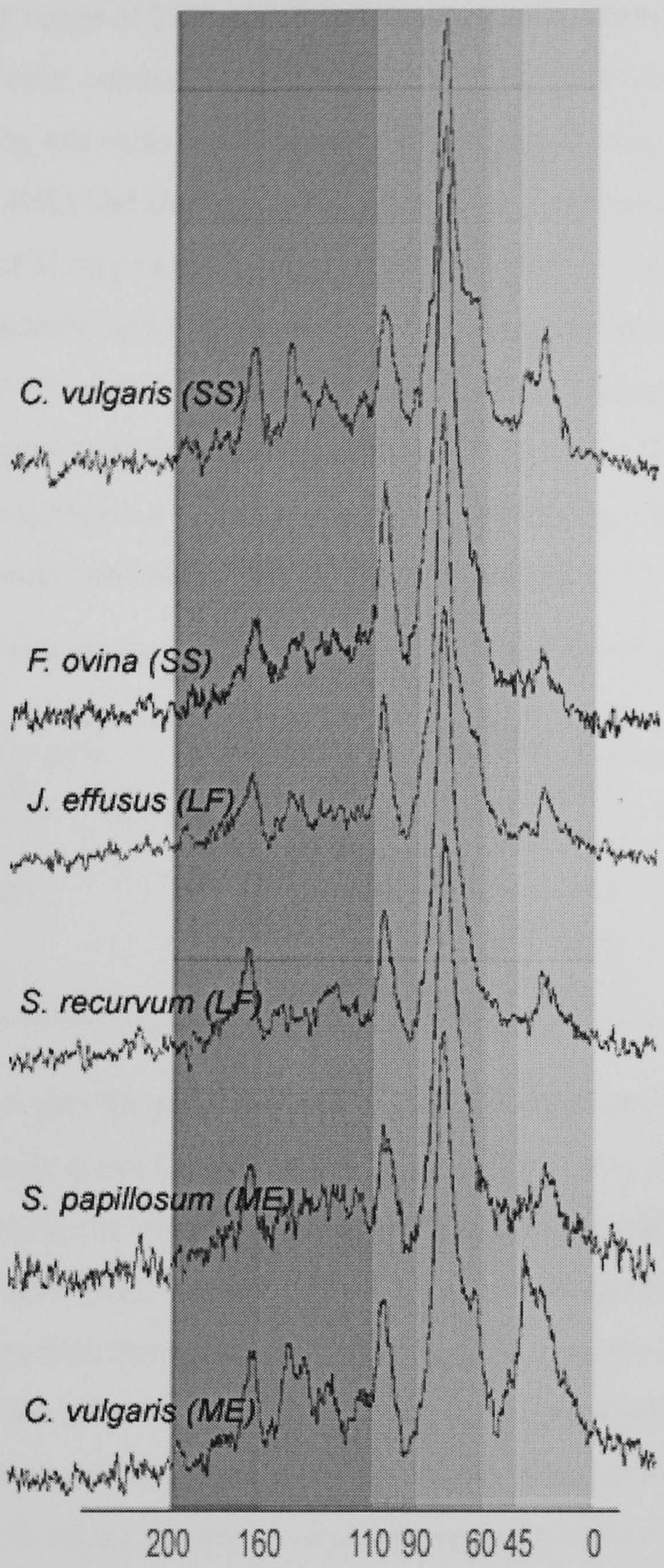

Figure 4.11: A comparison of ^{13}C NMR spectra of *C. vulgaris* (SS), *F. ovina* (SS), *S. recurvum* (LF), *J. effusus* (LF), *C. vulgaris* (ME) and *S. papillosum* (ME) plant material at the outset of the decomposition experiment.

In the introduction to this chapter the application of O- alkyl:alkyl- C ratios to decomposition studies was outlined. These ratios give indications of resource quality and degree of decomposition. The data in table 4.9 are a summary of these ratios for the plant material used in the litterbag experiment. The O- alkyl:alkyl- C of the plant litter used in the

experiment was in the range of 2.88 to 3.7; the larger number should represent a better quality litter for the soil microbial community because there is a greater carbohydrate component to lignin and waxes. Using this ratio as an example of C quality *S. recurvum* (LF) had the best litter quality, and *C. vulgaris* (ME) had the poorest litter quality. Differences in the O- alkyl:alkyl C ratios of litter after burial may relate to decomposition. In this case, a decrease in the ratio would signify decomposition and the greater the difference between the starting and 'decomposed' material would indicate more decomposition. However, after 10 months burial *in situ* there were no significant differences between O- alkyl:alkyl C ratios of the decomposed material and the starting material in any of the plant litters tested. Indeed, the O- alkyl:alkyl C ratio of *J. effusus* (LF) increased after burial in the mire expanse.

Table 4.9: O- alkyl:alkyl- C ratio of plant litter before burial (control) and after 10 months in the mineral soil, lagg fen and mire expanse.

Litter	*C. vulgaris* (SS)	*F. ovina* (SS)	*S. recurvum* (LF)	*J. effusus* (LF)	*C. vulgaris* (ME)	*S. papillosum* (ME)
Control	3	3.38	3.7	3.45	2.88	3.3
Mineral soil	3.25	3.4	3.18	3.6	2.27	2.5
Lagg fen	3	3.18	3.25	2.58	3.22	3.16
Mire expanse	3	3.54	2.53	3.7	2.90	3.27

NMR spectroscopy can also be used to measure lignin in plant samples. Again, this measure is not quantitative, however it can be used to compare between like samples, and in this example it may be used to examine the resource quality of the plant material just as the more general C:N ratio is applied. Table 4.10 shows that the *C. vulgaris* from both sites had the greatest ratio of lignin to N, indicating that these species had the least N available for breaking down lignin, and consequently had the poorest resource quality. *S. papillosum* (ME) was approximately half of *C. vulgaris* at 19.6, demonstrating a much better quality in Lignin:N terms. Lastly, the two sphagnum mosses and *F. ovina* had similar values for lignin:N, which made these species the best resources for microbes.

NMR spectra are not quantitative, and therefore it may not be accurate to take the signal at 154 as a measure of lignin. For this reason, the ratio of 'aromatics' to N were calculated to complement the lignin:N data. The 'aromatics' region refers to a general region of C components rather than the more specific 154 ppm guaiacyl lignin peak. The term aromatics is used here to describe a broad range of recalcitrant aromatic compounds, and may be a better measure of the recalcitrant fraction of plant material. The aromatics:N ratio shows that *C. vulgaris* had the poorest litter quality. *S. papillosum* (ME) was also high in aromatics:N. *S.*

recurvum (LF) has the lowest aromatics: N ratio, and so using this indicator, this species had the best quality litter for decomposer microorganisms.

Table 4.10: The ratio of lignin to N in the initial plant material. The lignin was calculated by measuring the size of the guaiacyl lignin peak (154 ppm) in relation to the largest peak from ^{13}C NMR.

	C. vulgaris (SS)	*F. ovina* (SS)	*S. recurvum* (LF)	*J. effusus* (LF)	*C. vulgaris* (ME)	*S. papillosum* (ME)
Lignin:N	40.8	12.2	9.8	8.0	36.7	19.6
Aromatics :N	18.1	15.0	9.9	12.6	19.6	22.0

Detailed analysis and close observation of NMR spectra can often reveal information about samples, which would not be easily obtained using other analytical methods. Within the six main shift ranges outlined earlier there are a number of other ways to make inferences from NMR spectra. These are comprehensively documented in Hopkins *et al.* (2000). The description of NMR spectra is not within the scope of this chapter, instead the issues relevant to decomposition of Dun Moss samples will be discussed.

Background noise is considered problematic in NMR spectra because it can mask effects that would be otherwise revealed by the analysis. This phenomenon was an issue in the analyses carried out for this chapter, but it was not ubiquitous. Interestingly, the occurrence of background noise could neither be linked to soil type, or to vegetation type, with examples exhibited by *J. effusus* (LF) in the lagg fen (fig. 4.12), *S. recurvum* (LF) in the lagg fen (fig. 4.13) and *C. vulgaris* (ME) in the mineral soil (fig. 4.14).

Microbial activity, specifically that of anaerobes, sometimes presents itself in NMR spectra by a swap in the CH_2 and CH_3 peaks, at 27 ppm and 18 ppm respectively. In the methyl and alkyl C region (0-45) of the *C. vulgaris* (ME) spectra a swapping of the CH_2 and CH_3 peaks occurred (fig. 4.14). The material buried in the lagg fen showed a greater proportion of CH_3 groups to CH_2 groups, this is the opposite to the orientation of these groups for the same plant material at the outset and buried in the mineral soils and the mire expanse. There were no other significant effects of decomposition on plant material after 10 months burial in soils from Dun Moss.

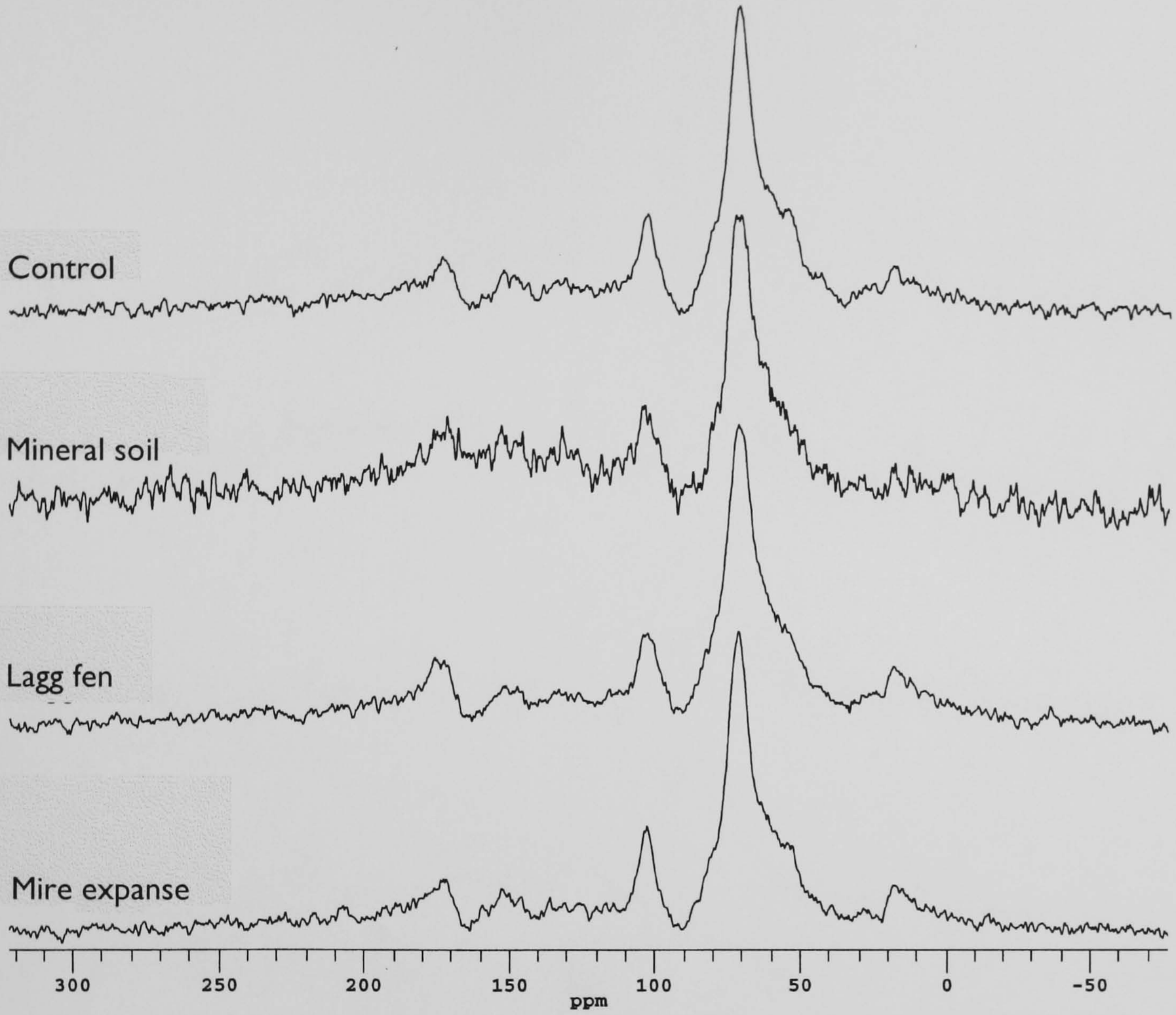

Figure 4.12: NMR spectra of *J. effusus (LF)* plant litter before burial (Control) and after 10 months in the mineral soils, lagg fen and mire expanse.

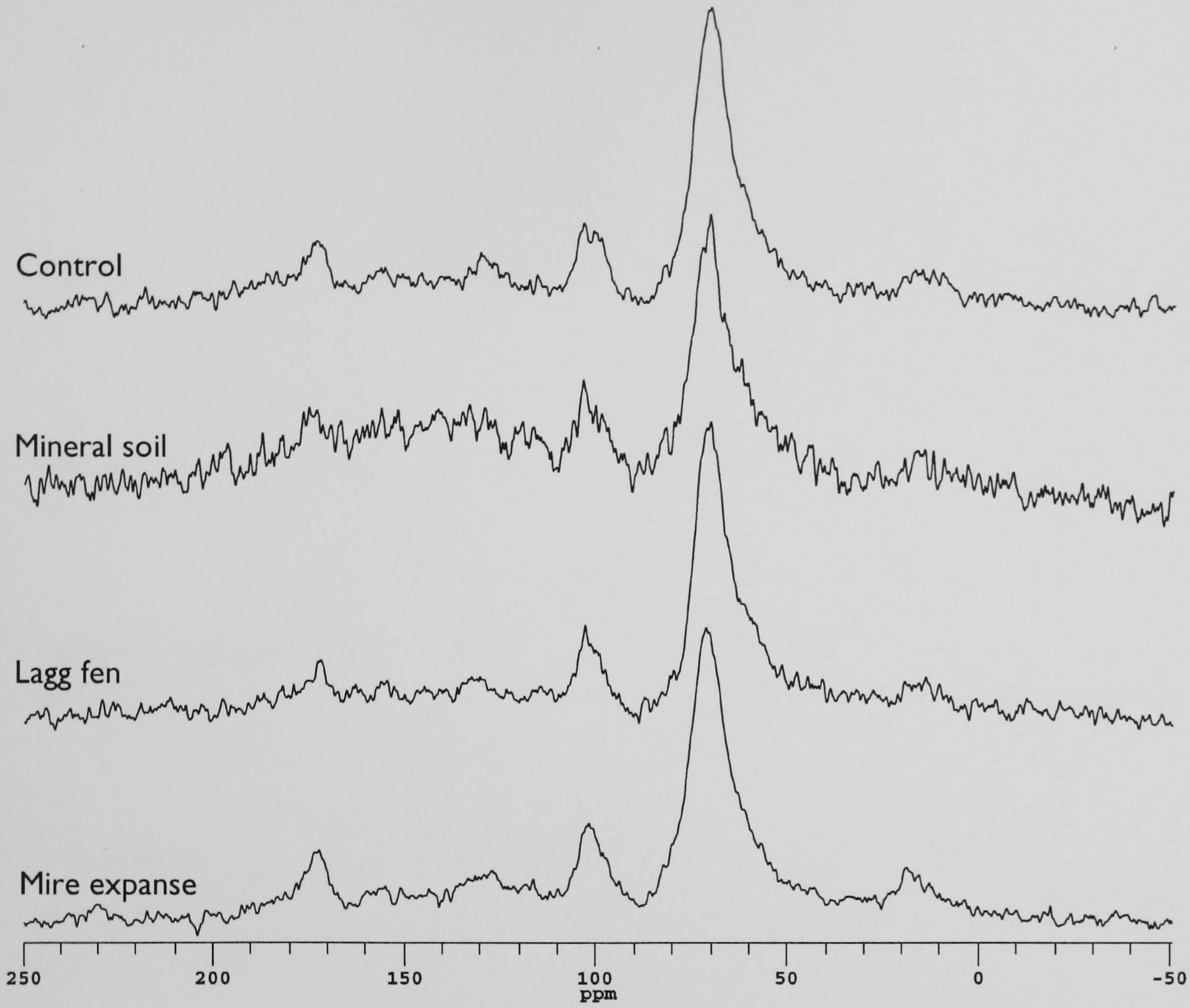

Figure 4.13: NMR spectra of *S. recurvum (LF)* plant litter before burial (Control) and after 10 months in the mineral soils, lagg fen and mire expanse.

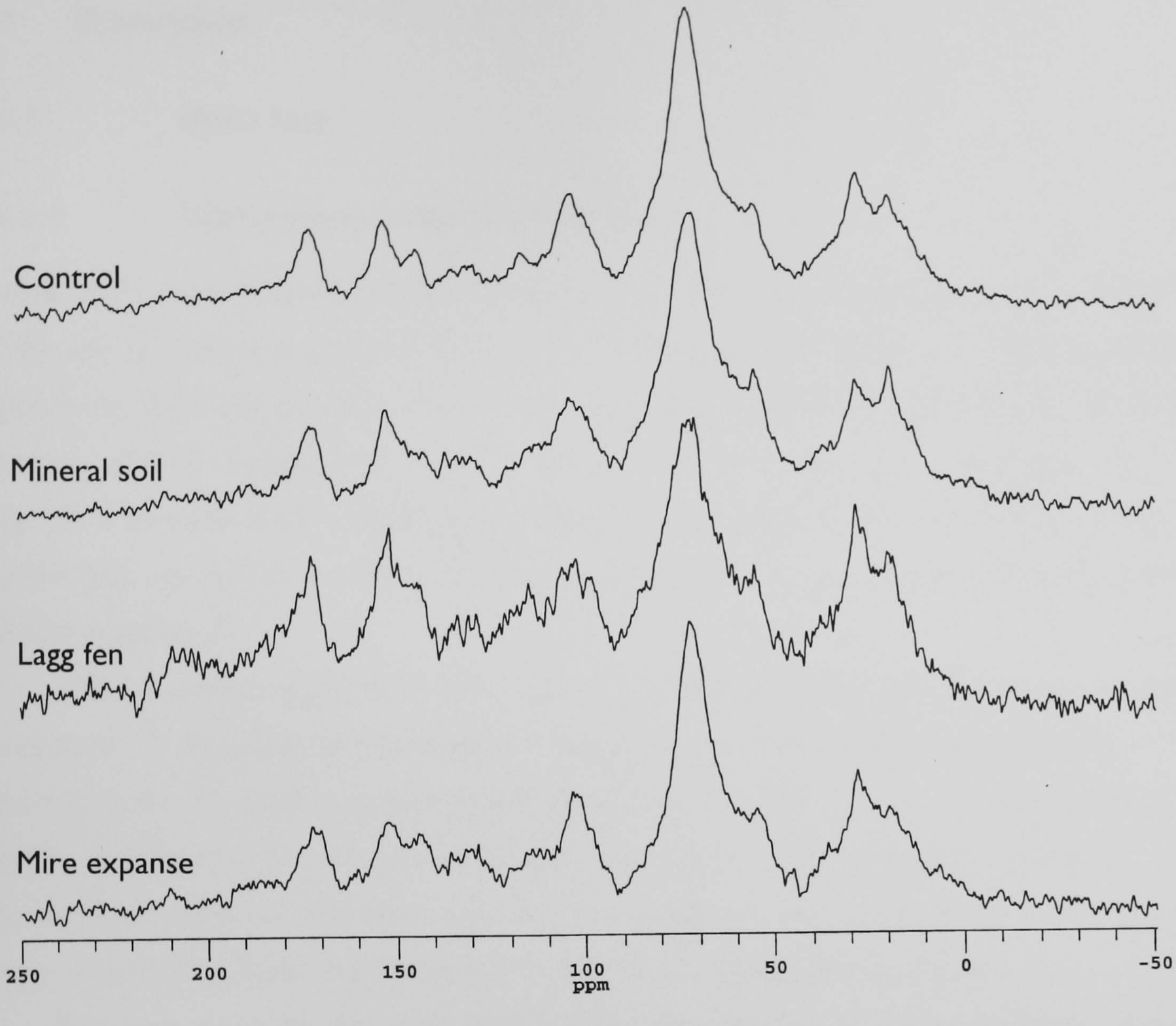

Figure 4.14: NMR spectra of *C. vulgaris (ME)* plant litter before burial (Control) and after 10 months in the mineral soils, lagg fen and mire expanse.

4.4 Discussion

4.4.1 Mass loss

4.4.1.1 Comparison of lab and field data

In total there were 28 significant differences ($p < 0.05$) between the vegetation types in both the lab and field experiment. Of these results, 12 were similar between the lab and the field experiment. All these similarities were associated with a significant net gain of material into the litterbags of the *C. vulgaris* (ME), mainly in the soils of the mire expanse. There were no instances where there was a direct conflict between the results of the laboratory experiment and the field experiment i. e. where a species was smaller in the lab experiment, but greater in the field experiment.

These results suggest that there was a good correlation between the lab and the field experiment. In particular, in relation to the net gain of material into the *C. vulgaris* (ME) litterbags there was good consistency between the experiments. There was also a greater number of differences between the litterbags buried *in situ* compared to those buried in laboratory microcosms. The following reasons may account for the difference in mass loss of the plant species between the microcosm experiment and the field experiment.

The mesh size of the litterbags buried in the microcosms was smaller than those placed in the field. The experiment was designed so that a range of microbes would have access to the material held within the bags and bacteria, actinomycetes, fungi and protozoa would have been able to gain entry to both the 100 µm and 200 µm bags. Most microbes have a diameter smaller than 10 µm and protozoa are generally between 10-80 µm. However, the 100 µm difference in the mesh diameter between the laboratory and field experiment would have made a difference in the amount of other matter that entered the bags. It is unlikely that soil animals would have been able to enter the field bags. However, fine roots and root hairs would have been able to get into the field bags more readily than into the microcosm bags. Also, these roots would have featured more *in situ* because the experiment was not cut off from external factors, as was the laboratory experiment.

The movement of non-living material into the bags would have been more important under *in situ* conditions because there is an active exchange of matter going on in the acrotelm continuously (Clymo, 1965). Therefore, soil particles and mineral matter would have been interacting with litterbags during these processes. This situation would not arise in the microcosm experiment set up in the laboratory because each vial of soil was isolated. The

most likely outcome of this would be a net transport of material into the litterbags buried in the field. Indeed, this has been commonly referred to in the literature and is recognised as a drawback of the technique. Often allocthonous debris that has accumulated in the litterbags is removed by a manual sorting procedure (Coulson and Butterfield, 1978; Belyea, 1996; Anderson and Hetherington, 1999). Even after intricate sorting procedures some foreign material may still remain in the bags, however it is generally assumed that all litterbags are equally affected thus minimizing the overall influx of material into the bags. Anderson and Hetherington (1999) noted that this influx increased with incubation time.

The temperature of the lab experiment was regulated at 14 °C. *In situ* conditions would have fluctuated greatly during the 10 month period and this would have affected biological and physical processes occurring in the soils. Biological processes operate more slowly at lower temperatures, therefore it is possible that the lower field temperatures slowed the decomposition processes attributable to biological action, and so decomposition under field conditions would be slower than under regulated laboratory conditions. In contrast, Hobbie (1996) recorded greater mass loss rates in winter in Alaskan Tundra, which she attributed to the physical process of freeze-thaw on the vegetation. During the winter months, freezing is common at Dun Moss and so freeze-thaw may have positively effected the decomposition rates of the litter. The action of freeze-thaw would have been greater on the wetter soils, namely the peats.

The mineral nutrient source for the active microbial community would have been greater in the field experiment. Once nutrients were depleted from the laboratory soils, then the only new source of nutrients was the decaying plant material itself. However, in the field there was a constant supply of mineral nutrients in the form of precipitation and in the soils of the lagg fen and mineral soils; groundwater and water which had travelled through the mineral soils. So, in the soils of the mire expanse where the external supply of mineral nutrients is limited in the natural environment, the laboratory conditions would have been a good reproduction of *in situ* conditions. However, in the mineral soils and the soils of the lagg fen there would have undoubtedly been a significantly greater supply of mineral nutrients to the soils.

4.4.1.2 Effect of plant species

In the litterbag, experiment the mass loss data must be interpreted with caution because in many samples there was a net mass gain. One possible reason for this accumulation of material

is the cleaning procedure adopted. In many other accounts of the litterbag technique, attempts have been made to remove all of the foreign litter that accumulates in the litterbags during the incubation period (Coulson and Butterfield, 1978; Beylea, 1996; Anderson and Hetherington, 1999). There is one major problem with this approach; it is not possible to identify unequivocally the foreign material that has entered the bags without using complex labelling experiments. Additionally, there is some merit in determining the amount of the mass gain in the litterbags because this can indicate the interactions between the decaying vegetation and external components in the decay process.

To analyse the affect that litter type has on the mass loss in the soils the data were analysed using one-way analysis of variance to test for significant differences between mass loss from the different litter types.

There was only one example of the mass loss of a particular test where the mass loss was significantly greater. The mass loss from *F. ovina* (SS) in transect 2 in a mineral soil microcosm which had been incubated for 7 months was greater than the other litter types buried under the same conditions. Although this vegetation has one of the smallest C:N ratios (36%) for the transect this result was unusual in the experiment because it was not repeated.

Net mass gain, on the other hand is common, especially of *C. vulgaris* (ME). In total, between the field and laboratory experiment, there were 28 tests in which a significant net mass gain occurred, and out of these, 21 were associated with *C. vulgaris* (ME) and 3 were associated with *C. vulgaris* (SS). It appears, therefore that there was a significant difference between the *C. vulgaris* from the mire region and *C. vulgaris* from the soils of the surrounding slopes. *C. vulgaris* is spiky in its nature, and therefore may be more efficient at trapping foreign material as it passes through the bags. However, this does not explain the significantly greater net mass gain of the *C. vulgaris* from the mire expanse. The microbial community (responsible for decomposition, hence mass loss) may be attracted by litter quality (low C:N). At the same time, root colonization of could be induced by a release of nutrients from the decaying litter, thereby causing mass gain. It is the interaction of the complex suite of variables which complicates the interpretation of mass loss data from litterbags, and this is demonstrated by the difference between *C. vulgaris* (ss) and *C. vulgaris* (ME). One possible solution to this problem is isotopic labelling, and this has been successfully used in decomposition studies to trace the route of C in the system (e.g. Domisch *et al.*, 1998; Jones and Darrah, 1994).

4.4.1.3 Effect of time

There were 8 occasions on which the mass loss significantly decreased with time i. e. net increase of mass with time. This clearly indicates movement of material into the litterbags, and is therefore supportive of earlier comments on net mass gain. Anderson and Hetherington (1999) observed an increase in variability of mass loss data from litterbags buried in peats soils, which they attributed to movement of foreign material into the litterbags. There were only 13 occasions when there was a significant increase in the mass loss with time. There are two possible reasons for this: i) the rate of mass loss was slow and the experiment was not sensitive enough to detect changes in mass loss, ii) other processes were counteracting the mass loss. In the initial stages of decomposition there is a rapid loss of material where water and alcohol soluble compounds are lost, and this has been shown using CO_2 flux experiments (Berg, 1984b; Domisch *et al.*, 1998). Therefore we would have expected to see a significant mass loss in all the bags initially however, this stage may have been masked by a flux of material into the litterbags.

4.4.1.4 Effect of soil type

Between the lab and field experiments, there were 6 instances when the results from the two experiments matched each other and there were 4 cases where the field experiment contradicted the laboratory experiment. This is a poor match between the experiments, and the aforementioned comments of the differences between the experiments apply. In the field experiment there were more significant differences between the soil types than in the lab experiment, however the same trends emerged.

The mineral soils were associated with the greatest mass loss. Coulson and Butterfield (1978) observed similar rates of mass loss for a range of peatland species buried in mineral soils and soils of blanket bog. In view of the size of the microbial biomass at each of their sites, which was six times greater in the mineral soils (Latter *et al.*, 1967), this result was entirely unexpected. However, the ecological significance of these biomass measurements is not know because they were not accompanied by measurements of activity. Nonetheless, these mass loss results contradict the findings from this study.

Farrish and Grigal (1998) and Verhoeven *et al.* (1990) both found that mass loss of a common substrate (cellulose) to be greater in fen soils than in bog soils. Other researchers have investigated the decomposition of a common substrate in nutrient rich fen and nutrient poor fen soils. Verhoeven and Arts (1992) found cellulose decomposition to be greater in the

rich fen, however Ohlson (1987) reported less mass loss of Carex species in a rich fen. This conflict of results may be due to differences in the substrates used (Szumigalski and Bayley, 1996). However, it is clear that nutrients in soils effect decay (Martin and Holding, 1978; Williams and Crawford, 1983; French, 1988; Melillo *et al.* 1989).

Other environmental factors that have been implicated as regulators of the decomposition process in peat soils are water table level, pH and soil temperature. Szumigalski and Bayley (1996) found a good correlation between weight loss of *Carex lasiocarpa* litter and the height of the water table; drier sites tended to have the greatest weight losses. Lieffers (1988) found similar results. Reducing conditions (Brinson *et al.* 1981; Bridgham and Richardson, 1992) and low soil temperatures (Clymo, 1965; Brinson *et al.*, 1981; Farrish and Grigal, 1985; Lieffers, 1988; Bridgham and Richardson, 1992; Hogg, *et al.*, 1992; Santelmann, 1992) associated with higher water tables are the likely inhibitors of decay. Acidic environments are also unfavourable for decomposition (Farrish and Grigal, 1988; Tóth and Zlinszky, 1989; Verhoeven *et al.*, 1990), although this may be restricted to anaerobic decay (Farrish and Grigal, 1988; Bridgham and Richardson, 1992).

The mineral soils were drier, and consequently would have a higher soil temperature. The nutrient status of the mineral soils was also greater than the bog soils. Therefore, greater rates of decay would be expected in the mineral soils. It must be noted that, our understanding of contrast between the processes occurring on peat and mineral soils is hindered by the lack of investigations comparing decomposition on both soil types.

4.4.1.5 Mass loss as an indicator of decomposition

Mass loss has been used extensively as an indicator of decomposition in peat soils (Clymo, 1965; Coulson and Butterfield, 1978; Beylea, 1996). However, two criteria must be satisfied before the litterbag technique may be used as an indicator of decomposition. First, the litterbag technique must produce accurate measurements of mass loss and, second, mass loss must be a reliable measure of decomposition. These criteria will be discussed in this section.

In both the field and the laboratory experiment, there were anomalies in the mass loss from the litterbags. The results of litterbag experiments may be affected by processes other than decomposition: erosion or influx of particles finer than the mesh, colonization of experimental material by roots bacteria and fungi (Beylea, 1996). There were several examples of increased mass gain with time. Indeed, mass gain has been well documented by other

researchers (Clymo, 1965; Rochefort *et al.*, 1990; Johnson and Damman, 1991; Anderson and Hetherington, 1999).

Whether mass loss is representative of decomposition is also questionable. What is perhaps more important is how the data are interpreted. 'Earlier decomposition studies presuppose that the loss from the litterbags is also entirely a loss of C to the atmosphere' (Domisch *et al.*, 1998) however they proved this inaccurate using ^{14}C labelling experiments. Beylea (1996) rightly points out, however, that the litterbag method is the only one by which mass losses due to all decomposition processes of comminution, leaching and respiration can be measured in manipulative field experiments. Further complications arise in comparisons made between litterbags studies. Decomposition is controlled by a complex suite of variables (Bridgham *et al.*, 1991a) and slight differences in experimental design may have a major impact on any one of these. In particular, degree of senescence and prior leaching (Ohlson, 1987), drying temperature used (Clymo, 1965), depth of placement (Farrish and Grigal, 1988), differences in regional climate (Santelmann, 1992), parts of plants included (Clymo, 1965) and differences in mesh sizes of the litterbags (Brinson *et al.*, 1981) may complicate the interpretation of the results.

Decaying plant material is an invaluable resource for the soil biological community, and has implications for the rhizosphere and soil microbial community. The importance of decomposing litter as a resource has not been explored to any great extent in peatland studies. The litterbag technique lends itself to extensive manipulation and may be used for investigations of plant litter as a resource both *in situ* and in the laboratory.

4.4.2 CO_2 production

4.4.2.1 Change in respiration rate 0-4 months

In the initial stages of decomposition water-soluble compounds and compounds of low molecular weight are released. These compounds are released rapidly and correspond to an initial respiration peak (Berg, 1984a; Domisch *et al*, 1998). The temporal rate at which fresh litter decomposes probably reflects a heterogenous chemical composition: a labile fraction decays rapidly, while a more recalcitrant fraction decays at a much slower rate (Clymo, 1965; Johnson and Damman, 1991; Upedegraff *et al*, 1995; Beylea, 1996). Depending on the percentage of rapidly degraded compounds in the plant material there may be a lesser or greater initial CO_2 response (Domisch *et al*, 1998).

In transect 1, *C. vulgaris* (SS) had significantly greater CO_2 production at the start of the experiment than the other plant types, in the mineral soils and the soils of the mire expanse. *H. jutlandicum* (SS) had the greatest CO_2 in transect 2, and *F. ovina* (LF) was greatest in the lagg fen of transect 3. It is possible that these plant type have a higher proportion of readily decomposable compounds than do the other plant types.

In the mineral soils of transects 1 and 3 there was a large increase in the rate of respiration after 1517 hours. One possible explanation for this is an increase in the availability of readily utilizable substrates for the soil microbial community. Distilled water was added to the microcosms systematically to maintain field moisture conditions throughout the experiment. This addition may have induced the higher rates of respiration which were recorded after 1517 hours.

4.4.2.2 Cumulative respiration 0-4 months

For analysis, the CO_2 data were summed to give an average rate for the first 4 months of the experiment. This was done so that it would be possible to compare the CO_2 data with that of the mass loss experiment as comparative measures of decomposition.

The same plant materials decayed fastest irrespective of soil type; thus suggesting that litter properties are important in determining the rate of decomposition. There was no link between soil type and origin of the vegetation, which may mean that the soil microbial community in the three soils was not significantly different in its metabolic diversity.

In all three transects *F. ovina* (LF), *F. ovina* (SS), *J. effusus* (SS) and *C. vulgaris* (SS) produced the greatest increase in respiration. These plant species originated from nutrient rich soils and therefore may be richer in mineral nutrients. Internal nutrient concentrations are important in determining relative decay rates of different plant litters (Martin and Holding, 1978; Melillo *et al.*, 1989). The plants growing on mineral rich soil would have a greater nutrient content than those growing on mineral poor soils. It is therefore likely that these materials decomposed faster than plant species from the mire expanse. *J. effusus* (SS) in the soils of the lagg fen from transect 1 had the greatest respiration rate and smallest C:N ratio, however C:N ratio does not appear to mediate in the other soils. Therefore, other nutrients e.g. phosphorus, may be important factors in regulating the respiratory activity of the soils.

The alternative scenario is that the decomposition of transects *F. ovina* (LF), *F. ovina* (SS), *J. effusus* (SS) and *C. vulgaris* (SS) is greater because there were inhibitory effects on the other vegetation types. In particular, Sphagnum spp. are known to produce a compound called

sphagnan, which has been associated with nutrient limitations (Bergman *et al*, 1999). Sphagnan may induce nutrient limitation of decomposers by chelating essential metal ions or by binding and rendering insoluble ammonium, amino acids and proteins. Additionally, sphagnan may induce C limitation by tanning (binding C) extracellular enzymes produced by decomposers (Painter, 1991). Indeed, several researchers have found that mosses are more resistant to decomposition in peatlands (Reader and Stewart, 1972).

Soil type was important in the release of carbon from the litter. The slope soils had significantly greater respiration rates under litter amendments than the other two soils. Of the peats, the soils of the lagg fen had a greater respiration than the soils of the mire expanse when plant material was added. These findings are consistent with the data reported in chapter 2; the soil microbial community in the slope soils has a greater respiration than in the peat soils. The less favourable conditions in the peats e.g. water level and consequent reducing conditions (Brinson *et al.*, 1981; Bridgham and Richardson, 1992; Szumigalski and Bayley, 1996), lower soil temperatures (Clymo, 1965; Brinson *et al.*, 1981; Bridgham and Richardson, 1992; Hogg *et al.*, 1992 and Santelmann, 1992) at wetter sites (Farrish and Grigal, 1985) undoubtedly play a role in these results. The soils of the lagg fen also had a greater respiration when new plant material was added than did the soils of the mire expanse. The main difference between the two peat soils is the supply of nutrients to the soils, therefore this result suggests that the nutrient supply may govern the breakdown of plant material in these soils.

4.4.3 NMR spectra

4.4.3.1 Litter quality measured by NMR

Samples from transect 2 were also used for NMR analysis to determine the quality of the C in the litter. Lignin: N and aromatics:N ratios may be used as indicators of litter quailty because lignin and other aromatic compounds are difficult for microbes to breakdown. Not only does lignin breakdown require specialized sets of enzymes, only found in the fungi, but it is not energetically efficient for microorganisms, and so more nutrients are required. Therefore the ratio of lignin:N is an important indicator of litter quality.

The ratio of O- alkyl:alkyl- C may be used as a measure of litter quality because this represents the amount of readily available carbohydrates to more recalcitrant waxes and lignin components (Webster *et al.* 2001). The litter used in this experiment did not very greatly, and therefore it may not be possible to use this relationship as an indicator of litter quality.

However, of all the plant types *J. effusus* (LF) had the greatest O- alkyl:alkyl- C, and so this had the best litter quality. The *C. vulgaris* samples had the smallest ratios, therefore these plant materials were poorest in terms of resource quality. Lignin:N may also be used as a measure of litter quality, as this indicates the amount of N available to help the soil microbial community breakdown the relatively recalcitrant lignin fraction of the plant litter. Therefore, the smaller this value, then the better the quality of the litter for decomposition. *C. vulgaris* was significantly greater than all the other plant litters, with *F. ovina (SS), S. recurvum (LF), J. effusus (LF)* having a much smaller ratio. These data suggest that *F. ovina (SS), S. recurvum (LF), J. effusus (LF)* contain much more N to help the soil microbial community breakdown recalcitrant lignin compounds in the later stages of the decomposition process.

The cumulative CO_2 data showed that the *F. ovina* (SS) had the greatest decomposition rate when buried in all soils. The lignin:N ratio indicated this plant material to be favorable for decomposition by the soil microbial community. However, all three methods suggested that *S. recurvum* (LF) would have the best litter quality for microbes, yet this litter type had the second smallest loss of CO_2 in all of the soils. This result suggests that another factor or factors is important in influencing the decomposition rate of *S. recurvum* (LF) in these soils.

4.4.3.2 Evaluation of the use of ^{13}C NMR for decomposition studies in peat soils

The usefulness of ^{13}C NMR as a technique for assessing the litter quality and changes in plant C components during composition was limited in this study. Although C was abundant in the samples, which is a desirable property for NMR spectroscopy, some of the samples produced noisy spectra. This attribute is normally caused by a lack of carbon, a difference in the number of scans taken by the spectrometer or the presence of higher concentrations of paramagnetic species (Hopkins *et al.* 2000) (Fe, Mn, Zn etc.) in the samples. The quantity of sample did not vary and the same number of scans was taken for each sample, therefore it is possible that the influence of paramagnetic species caused some spectra to have more noise. However, a test for paramagnetic species was not carried out. It is possible to test for these species and in some cases to remove them, however in this work there was only a very small amount of sample available, and so it was not possible to carry out the analyses required. However, there is little evidence to suggest that extraction procedures are successful when carried out, furthermore they involve the use of chemicals which may modify the C in the samples. The other problem associated with the use of ^{13}C MAS NMR for these samples to monitor

decomposition in the soils over the period under study is the sensitivity of the technique. The rate of decomposition in upland soils is slow, and perhaps too slow for the ^{13}C MAS NMR to detect changes in the plant material over short time periods. However the technique may be useful for looking at longer term decomposition in these soils.

4.5 Conclusions

4.5.1 Litter quality

- The soil microbial community should decompose faster litter with higher quality than it would decompose lower quality material. C is not limiting in peatland ecosystems, therefore the ratio of C:N will be used is an indicator of litter quality in these soils.

When the C:N content was measured *C. vulgaris* had the highest C:N values, indicating that there was less N supplied by the plant for breaking down the C present. The decomposition as measured by mass loss was difficult to interpret because of the net mass gain in many samples, however the CO_2 data provided useful information about the decomposition of the plant species in the laboratory. These data contradicted the hypothesis with respect to *C. vulgaris* because they showed that decomposition of this plant species was relatively fast. On the other hand, the species with the smallest C:N ratios i.e. *J. effusus* also decomposed quickly. These contradictory results suggest that C:N is not a reliable measure of litter quality on its own, or that litter quality is not the main limiting controlling factor of decomposition in these soils. Indeed, it may not be possible to simplify the C to N content by measuring the total amounts in plant material. The NMR data showed that C varies in composition considerably, and this may have exerted a greater influence on decomposition than total C:N ratios.

- Sphagnum litter should decompose more slowly than other litters.

With one exception, the sphagnum species decomposed the most slowly in all of the laboratory experiments using CO_2 as a measure of decomposition. This occurred irrespective of the soil type, suggesting that it is particular qualities of the litter that restrict its decomposition and not the soil conditions in which it grows or the decomposer communities of the soils.

4.5.2 Soil type

- The indigenous soil microbial community of a particular soil type is able to carry out faster decomposition on litter types with which they have previously been associated. For example, the soil microbial community of the mire soil should be able to decompose plant

material that had grown on the mire faster than the soil microbial community of the mineral soils would be able to do so.

This hypothesis suggested that a memory effect exists between the soil microbial community and plant litter with which it has already been associated. However, the test carried out here is not a true test of this hypothesis because soil conditions may effect the results. Irrespective of this there was no relationship between the soil type and the decomposition of litter from that origin. The most likely explanation for this is the adaptation of the soil microbial community, which can occur over relatively short time periods. However, a link between soil conditions with respect to decomposition and plants growing on the soils can be ruled out as a result. This observation is an interesting one, in that it is in the interests of the plant to promote decomposition, hence nutrient cycling. Therefore it is suprising that plants adapted for growth on a particular soil type do not also appear to be adapted for decomposition on a particular soil type. This observation could provide the basis for hypotheses in future work.

- Decomposition in the mineral soils should be fastest because the size of the soil microbial community is greater in these soils.

Decomposition was greatest in the mineral soils without exception, and in most cases decomposition was greater in the soils of the lagg fen than in those of the mire expanse. This is entirely consistent with the original hypothesis, and is probably due to the size of the soil microbial community in these soils.

4.5.3 Time

- Mass loss is fastest in the initial stages of the decomposition process because leaching makes large amounts of nutrients available to the soil microbial.

The mass loss data was not a good indicator of decomposition in this experiment. There were 8 examples where the mass of material in the litterbags increased with time, 33 examples of no change and only 8 instances of mass loss with time. These data highlight the unsuitability of mass loss experiments with litterbags for use in decomposition studies. Respiration data would provide a better indicator of decomposition in these soils.

4.5.4 *General conclusions*

In carrying out both lab and field experiments it was possible to test the viability of laboratory experiments, however no new information on decomposition was yielded from this experiment. Field experiments have greater ecological significance than laboratory experiments, however laboratory experiments are clearly amenable to much greater manipulation. Further research in this area should concentrate on the strong points of both these approaches. The laboratory experiment produced the most sensitive and applicable measurement of decomposition; CO_2 evolution rates. On the other hand, *in situ* studies should never be replaced by laboratory studies because they take other environmental variables into account. In consideration of these factors, a compromise between the two approaches would be ideal, and this would involve scaling up the laboratory experiment by using greater quantities of soil. By adapting the incubation conditions better simulation of in situ conditions could be achieved, for example, using open containers to allow free exchange of gases, and only sealing when measurements are needed. The water content of the samples could also be regulated to mimic environmental conditions by creating an artificial water table by using semi-permeable containers. Using laboratory-based experiments, it would also be possible to combine the future work proposed in chapter three with that of this chapter. Testing a range of different nutrients on decomposition processes could expand our understanding of the role of nutrients in the decomposition process in these soils. These examples are just some of the ways that the incubations in this chapter could be taken further, and there is much greater scope for further research, clearly adaptations should be based on recreating *in situ* conditions in the laboratory, where it is possible to make more frequent and more detailed measurements of changes occurring.

The suitability of using litterbags and mass loss experiments in decomposition studies must be questioned. In the work described in this chapter there were fundamental problems with mass loss measurements, i.e. movement of allochotounous material into the bags, and it is remarkable that the technique accounts for so much of the literature published on decomposition in peatlands. Notwithstanding this limitation, the interpretation of 'mass loss' in decomposition is often simplified too much, when in fact mass loss from decaying litter does not equal complete decomposition of that component but fractionation. Without being able to trace the route of the mass loss component it is inaccurate to use the term decomposition. However consideration of these issues does not necessarily render the litterbag redundant,

indeed litterbags are amenable to manipulation and may be used in conjunction with defined substrates and isotopic labelling studies. The use of defined substrates, e.g. cellulose was discussed in the introduction to this chapter. The technique was rejected because it involves the use of a specific plant component and was considered too simple, however used in conjunction with chemical analyses, it may be more useful as a baseline experiment than the mass loss experiment used in this work. Isotopic analysis will only be mentioned briefly here, but will be discussed in more detail in chapter 6. This technique uses isotopic labelling to trace the route of specific components in process studies. In such a way it would be possible to account for 'mass loss' and be more specific about the decomposition processes occurring. The possibilities for this approach will also be discussed in the concluding chapter.

The use of solid state ^{13}C NMR is becoming increasingly familiar to soil scientists, however the use of the technique with peat soils is still largely unexplored. As such almost any work done in this area is new, and therefore adds to the knowledge base. In this work the lack of significant changes in NMR spectra signifies slow decomposition. There are two factors which undoubtedly made a contribution to this result: plant species employed and length of incubation. The plant litters used were all characteristic of resistant plant materials, and it may have been useful to investigate the changes in more degradable litters and plant components. For example, cellulose could be used as a standard substrate. Also, there are numerous possibilities for the enrichment of substrates. Such modifications should be targeted in future research. Without doubt, time is also a crucial factor, and 10 months may have been too short to detect some of the changes in plant components using NMR. Increasing the duration of the experiment would be advantageous in further studies, and this will be discussed in the concluding chapter.

Chapter 5

Microbial community associated with decomposing plant material

5.1 Introduction

The soil microbial community is important in litter transformation and mineralization, however little is known about changes in microbial biomass during the decomposition process (Dilly and Munch, 1996). A better understanding of the changes in both microbial biomass and community structure is required in order to put the importance of litter quality into context. "Although relationships between resource quality and decomposition are well known, few studies include the dynamics of primary decomposers, especially with respect to the physiologically active biomass" (Neely *et al.*, 1991). There are many references in the literature to the microbial component of peat soils, indeed there is a great deal of information available on the factors that effect decomposition. However, the role of the microbial community has not been explored. Certainly, there have been no studies reported that have focused on the changes in the soil microbial community during decomposition in peatlands. Consequently, the work discussed in this chapter provides new information on the colonization of plant litter by the soil microbial community in peatland environments.

5.1.1.1 Temporal distribution of resources

Before plant material becomes dissociated from the plant it is already supporting a microbial community; a relationship which takes place in the phyllosphere (Paul and Clark, 1996). Plant related and climatic factors mediate the concentration of microorganisms on the leaf surface, on stems and bark. During senescence leaves fall and eventually become incorporated into the aboveground litter. It is here that the phyllosphere organisms become the primary decomposer organisms of the plant litter. In the initial stages of decomposition fungi take the lead in colonising newly available plant material because they are able to translocate external nutrients through their hyphal networks (Ingold and Hudson, 1993). In this particular way, fungi are able to attack substrates that may not contain all of the nutrients they require, and in doing so they make other nutrients available to other components of the soil microbial community (Campbell, 1977). These changes occur throughout the decomposition process,

and as leaching, fragmentation and microbial attack cause the release of different substances, the temporal distribution of available nutrients changes (Paul and Clark, 1996). The size, activity and structure of the soil microbial community associated with decaying plant litter shifts as a consequence of these changes.

5.1.1.2 Spatial distribution of resources

In the initial stages of decomposition, most of the plant nutrients are still locked up in the plant material until these are released by leaching and eventually, fragmentation and microbial attack (Paul and Clark, 1996). Therefore, in this early stage the value of this resource to the soil microbial community may be estimated by characteristics of the soil microbial community in the vicinity of the decomposing litter (Neely *et al.* 1991). The size of the active proportion of the soil microbial community throughout decomposition may therefore be able to give an indication of the importance of the decaying plant in the whole process. Eventually, as the plant material becomes incorporated into the surface litter and the soil beneath, at this point the newly added plant becomes a resource to the soil microbial community. The dynamics of the microbial communities associated with decomposing plant material may supply important information about the temporal release of resources from plant material in the initial stages of decay (Beare *et al.* 1990). By extending analyses to the soil surrounding the decaying plant material it may be possible to detect the release of nutrients from decaying litter and their availability in the external environment. In the first instance this release of nutrient will mainly be a result of leaching (Berg, 1984b).

5.1.1.3 Active soil microbial community

In the soil up to 90% of the total microbial community may be inactive at any one time. When conditions for microbial life improve to a satisfactory level, e.g. acidity is reduced or essential nutrients become available, the inactive or dormant microbes become active. Microbes can survive in this dormant state for long periods at a time, during which they do not contribute to processes occurring in the soil. Therefore, the active proportion of the soil microbial community is important to decomposition. Some measure of the size of the soil microbial community take the inactive part of the soil biomass into account as well as the active fraction. Substrate induced respiration (SIR) has been employed as a method for examining the active

soil microbial community because it is simple and rapid (Beare *et al.*, 1990). The technique has been successfully used to obtain biomass sizes on plant material e.g. Beare *et al.*, 1990 and Neely *et al.*, 1991. Some researchers have incorporated the use of antibiotics with SIR determinations (e.g. Lin and Brookes, 1999a) in an attempt to estimate the relative proportions of the bacterial and fungal components of the soil microbial community. There are several drawbacks with the methods involved (Parkinson, 1971) and the results obtained are probably not representative of the respective microbial communities.

The SIR approach was chosen for this study because it targeted the active fraction of the soil microbial community. The size of the active microbial community associated with decomposing litter as measured by SIR will be referred to as C_{mic}litter.

5.1.2 Aim and Hypotheses

The aim of this chapter is to investigate the changes in the size of the microbial community associated with plant litter during the decomposition process. The effect of leaching on the microbial community in the soil surrounding the decaying litter in the early stages of decomposition is also addressed.

- Higher quality litter supports a greater microbial community.

- The size of C_{mic}litter (size of the soil microbial community in contact with litter) changes with time. The greatest community is found at the onset of decomposition when leaching of readily available compounds occurs. .

- The soil microbial community in the immediate vicinity of the decaying vegetation will be affected according to the quality of the vegetation.

5.2 Materials and methods

5.2.1 Size of the soil microbial community in the microcosms

The size of the microbial community of the soil in the microcosms was measured after 4 months incubation. The modified substrate induced respiration experiment (as described in chapter 2) was used. Glucose was added in solution, to soil samples (1g moist weight). The samples were incubated at 22°C for 4 hours, then the headspace CO_2 was measured using a gas chromatograph (Appendix A). However, 4 different glucose concentrations (0, 40, 80 and 160 mg g^{-1} dwt) were used and these were replicated twice; there was only a small amount of material (<6g moist weight) available for analysis. 4 different glucose concentrations were used to ensure saturation of the microbial biomass. The size of the soil microbial biomass was calculated using the relationship between the maximum substrate induced respiration described by Anderson and Domsch (1978).

5.2.2 Size of the soil microbial community associated with decomposing plant litter

The microbial biomass C associated with the decomposing plant material from the litterbags incubated *in situ* was measured. After the litterbags were retrieved from the study site they were stored in a cool environment (4 °C) for at least 48 hours. They were cleaned by wiping the surface with tissue, and weighed. A sub-sample was taken and dried for 24 hours at 70°C to determine the moisture content. Control bags were used to determine the amount of material trapped in the mesh of the bags. These bags were also dried at 70°C and a correction factor was taken away from the sample bags before the total mass was calculated. The litter remaining in the sample bags was used to determine the size of the soil microbial community associated with the decomposing litter. The size of the microbial community associated with the decaying litter (C_{mic}litter) was determined using modifications of the SIR technique described in chapter 2. There were two main modifications of this technique; a smaller amount of sample was used and the concentration of substrate was increased.

0.2g of field moist plant material was used instead of 1g moist soil. For analysis, this was corrected per gram dry weight of material to standardize the measurements. Due to the limited material available in the litterbags, analytical replicates were not made, however because the C_{mic}litter of three field replicates was measured it was possible to carry out statistical

analysis on the data. The concentrations of glucose used for estimating the C_{mic}litter were greater than those used for the substrate induced respiration method to determine the size of the soil microbial community. The amount of freshly available resource in plant litter is far greater than in soil, therefore the size of the soil microbial community associated with litter is much greater (Beare *et al*, 1990). The concentrations of glucose used were 0, 40, 80, 160 mg g^{-1} dry residue. This range covered the concentration of glucose used by Beare *et al.*, (1990): 80 mg g^{-1} dry residue, measuring the bacterial and fungal biomass on plant residues using the SIR method. Four concentrations of glucose were used to ensure saturation of the soil microbial community associated with the plant material. Microbial respiration increased with increasing glucose concentration, and in all cases the community was saturated within the range of glucose concentrations used: demonstrated by a sigmoid curve.

5.3 Results

5.3.1 Size of the soil microbial community in the microcosms

In chapter 2 it was reported that there were distinct differences between the soils in terms of the size of the soil microbial community. However, after 3 months incubation at 14°C in the laboratory there was no significant difference between the size of the soil microbial community in the soils ($p > 0.05$ in transects 1, 2 and 3) (table 5.1).

Vegetation treatments appeared to have an effect on the size of the soil microbial community. In transect 1 the soil of the mire expanse amended with *C. vulgaris* (ME) had a greater soil microbial community than did the mineral soil and the soil of the lagg fen. The lagg fen had the greatest soil microbial community when treated with *S. palustre* (LF) and *J. effusus* (LF). In nearly all cases the size of the soil microbial community increased when a newly available resource was introduced.

In transect 2 the effect of vegetation was not always positive. In all but the *S. capilifolium* (ME) treated mineral soils there was a decrease in the soil microbial community. *J. effusus* (LF) significantly increased the size of the soil microbial community in the mire soil in comparison with the mineral soil and the soils of the lagg fen. *S. capilifolium* (ME) had a significant positive effect on the size of the soil microbial community in the soil of the lagg fen. *H. jutlandicum* (SS), *J. effusus* (LF), *C. vulgaris* and *S. capilifolium* (ME) negatively effected the size of the soil microbial community. The soil microbial community of the lagg fen was increased in comparison with that of the mineral soil under *J. effusus* (LF) and *C. vulgaris* (ME) treatments.

Table 5.1: Size of the microbial biomass in the soil microcosms containing decomposing litter. Numbers in brackets represent the standard deviation of 3 replicates. *** indicate missing data.

Transect 1	*C. vulgaris*	*F. ovina*	*S. palustre*	*J. effusus*	*C. vulgaris*	*S. capilifolium*	Control
Soil type	mg C_{mic} g^{-1} dwt litter						
Mineral soil	1.51	0.74	1.66	2.54	2.33	1.19	0.79
SD	(0.7)	(0.2)	(0.6)	(0.4)	(1.0)	(0.5)	(0.2)
Lagg fen	6.29	5.64	6.17	6.59	3.29	3.61	1.91
SD	(2.2)	(1.9)	(2.1)	(0.3)	(2.7)	(4.0)	(1.2)
Mire expanse	11.48	20.92	2.88	2.91	4.96	3.91	0.83
SD	(4.3)	(15.6)	(1.5)	(1.4)	(2.5)	(0.7)	(0.1)

Transect 2	*S. recurvum*	*F. ovina*	*J. effusus*	*C. vulgaris*	*C. vulgaris*	*S. papillosum*	Control
Mineral soil	1.57	1.52	0.81	2.62	2.23	6.22	5.02
SD	(1.7)	(1.1)	(0.3)	(0.3)	(0.3)	(6.7)	(3.6)
Lagg fen	2.66	7.99	1.93	9.37	14.38	13.41	1.56
SD	(0.6)	(4.7)	(1.2)	(9.4)	(1.8)	(4.3)	(0.3)
Mire expanse	8.76	7.33	12.52	5.62	4.87	2.17	1.46
SD	(9.4)	(7.0)	(6.6)	(2.7)	(2.5)	(0.9)	(0.5)

Transect 3	*C. vulgaris*	*H. jutlandicum*	*J. effusus*	*F. ovina*	*C. vulgaris*	*S. capilifolium*	Control
Mineral soil	6.47	2.71	1.68	5.39	1.73	3.66	4.06
SD	(4.8)	(2.0)	(0.5)	(4.7)	(0.6)	(3.1)	(4.0)
Lagg fen	20.04	10.42	8.62	6.64	22.26	4.13	3.86
SD	(15.7)	(11.3)	(2.4)	(3.5)	(9.3)	(2.3)	(0.9)
Mire expanse	2.72	5.40	4.71	4.12	8.96	3.38	***
SD	(1.0)	(2.1)	(2.5)	(2.5)	(8.2)	(1.4)	***

5.3.2 *Size of the soil microbial community associated with decomposing plant litter*

The data in apendix C7-C9 show the mean values for the size of the microbial community associated with decomposing litter from the field experiment. These data were analysed using one-way ANOVAs at the 0.05 level of significance to examine the effect of time, soil type and vegetation type on the size of the microbial community in contact with decaying litter. The results from these analyses are described in sections 5.3.2.1 to 5.3.2.3.

5.3.2.1 Effect of vegetation

5.3.2.1.1 Transect 1

In the first sampling period (4 months) there was no significant difference in the size of the microbial community associated with decaying vegetation (table 5.2). After 7 months, however, *S. palustre* (LF) had a greater C_{mic} associated with it than *J. effusus* (LF), *C. vulgaris* (ME) and *S. capilifolium* (ME) in the lagg fen. After 10 months *S. palustre* (LF) had a greater C_{mic} than *J. effusus* (LF), *C. vulgaris* (ME) and *S. capilifolium* (ME) in the mineral soil and *J. effusus* and *S. capilifolium* (ME) in the mire expanse.

5.3.2.1.2 Transect 2

There was no significant difference in the size of the soil microbial community associated with the vegetation after 4 months. However after 7 months *F. ovina* (LF) was greater than *S. recurvum* (SS), and *J. effusus* (LF) was greater than *S. recurvum* (SS) in the mineral soil. There was no difference between the vegetation Cmic after 10 months.

5.3.2.1.3 Transect 3

In transect 3 there was no significant difference between the size of the soil microbial community associated with any of the decaying litters. This was true of all soils and all sampling points.

5.3.2.1.4 Summary

- in transect 1 the soil microbial community associated with *S. palustre* (LF) was greater than that associated with most other plant species
- there were relatively few significant effects

Table 5.2: Summary of the effect of vegetation type on the size of the soil microbial community associated with decaying litter from litterbags (*in situ*), analysed using one way ANOVA. *P*= 0.05 Similar letters indicate similar populations where a is greater than b and c, and b is greater than c.

Transect 1	Time (month	*C. vulgaris* (SS)	*F. ovina* (SS)	*S. palustre* (LF)	*J. effusus* (LF)	*C. vulgaris* (ME)	*S. capilifolium* (ME)
Mineral soil	4						
	7						
	10			a	b	b	b
Lagg fen	4						
	7			a	b	b	b
	10						
Mire	4						
	7						
	10			a	b		b

Transect 2		*C. vulgaris* (SS)	*F. ovina* (SS)	*J. effusus* (LF)	*S. recurvum* (LF)	*C. vulgaris* (ME)	*S. papillosum* (ME)
Mineral soil	4						
	7		a	a	b		
	10						
Lagg fen	4						
	7						
	10						
Mire	4						
	7						
	10						

Transect 3		*C. vulgaris* (SS)	*H. jutlandicum* (SS)	*J. effusus* (LF)	*F. ovina* (LF)	*C. vulgaris* (ME)	*S. capilifolium* (ME)
Mineral soil	4						
	7						
	10						
Lagg fen	4						
	7						
	10						
Mire	4						
	7						
	10						

5.3.2.2 Effect of time

5.3.2.2.1 Transect 1

The size of the soil microbial community associated with the decaying litter was not significantly different in the mineral soil of transect 1, in any of the decomposing vegetation types (table 5.3). In the lagg fen there was a significant difference between the size of the microbial community associated with *C. vulgaris* (SS) between 4 and 7 months, where the microbial community was smaller at 4 months. *S. capilifolium* (ME) buried in the mire expanse also had a greater microbial community after 7 months than 4 months.

5.3.2.2.2 Transect 2

Time did not significantly effect vegetation C_{mic} in any vegetation buried in the mineral soil. In the lagg fen, *S. recurvum* (LF) had a greater microbial community after 10 months than after 7 months. In the mire expanse, the size of the microbial community associated with *C. vulgaris* (ME) decreased between 4 and 7 months.

5.3.2.2.3 Transect 3

Overall in transect 3, time significantly effected the size of the microbial community associated with decaying plant material. In most cases the C_{mic} of decaying vegetation was greatest after 10 months. However, there was one exception; *H. jutlandicum* (SS) buried in the mineral soil had a greater microbial community associated with it after 7 months than after 4 and 10 months. Time did not significantly effect the size of the microbial community of *C. vulgaris* (ME), *J. effusus* (LF), *C. vulgaris* (ME) and *S. capilifolium* (ME) burred in the mineral soil; *J. effusus* (LF) buried in the lagg fen; or, *C. vulgaris* (SS) and *S. capilifolium* (ME) buried in the mire expanse.

5.3.2.2.4 Summary

- in general the size of the soil microbial associated with decaying litter increased with time

Table 5.3: Summary of the effect of incubation time on the size of the soil microbial community associated with decaying litter from litterbags analysed using one way ANOVA. $P = 0.05$ Similar letters indicate similar populations where a is greater than b and c, and b is greater than c.

			In situ		
			4 months	7 months	10 months
Transect 1					
Mineral soil	*C. vulgaris*	(SS)			
	F. ovina	(SS)			
	S. palustre	(LF)			
	J. effusus	(LF)			
	C. vulgaris	(ME)			
	S. capilifolium	(ME)			
Lagg fen	*C. vulgaris*	(SS)	b	a	
	F. ovina	(SS)			
	S. palustre	(LF)			
	J. effusus	(LF)			
	C. vulgaris	(ME)			
	S. capilifolium	(ME)			
Mire expanse	*C. vulgaris*	(SS)			
	F. ovina	(SS)			
	S. palustre	(LF)			
	J. effusus	(LF)			
	C. vulgaris	(ME)			
	S. capilifolium	(ME)	b	a	
Transect 2					
Mineral soil	*C. vulgaris*	(SS)			
	F. ovina	(SS)			
	J. effusus	(LF)			
	S. recurvum	(LF)			
	C. vulgaris	(ME)			
	S. papillosum	(ME)			
Lagg fen	*C. vulgaris*	(SS)			
	F. ovina	(SS)			
	J. effusus	(LF)			
	S. recurvum	(LF)		b	a
	C. vulgaris	(ME)			
	S. papillosum	(ME)			
Mire expanse	*C. vulgaris*	(SS)			
	F. ovina	(SS)			
	J. effusus	(LF)			
	S. recurvum	(LF)			
	C. vulgaris	(ME)	a	b	
	S. papillosum	(ME)			
Transect 3					
Mineral soil	*C. vulgaris*	(SS)			
	H. jutlandicum	(SS)	b	a	b
	J. effusus	(LF)			
	F. ovina	(LF)	b	b	a
	C. vulgaris	(ME)			
	S. capilifolium	(ME)			
Lagg fen	*C. vulgaris*	(SS)	c	b	a
	H. jutlandicum	(SS)	c	b	a
	J. effusus	(LF)			
	F. ovina	(LF)	c	b	a
	C. vulgaris	(ME)	c	b	a
	S. capilifolium	(ME)	c	b	a
Mire expanse	*C. vulgaris*	(SS)			
	H. jutlandicum	(SS)	c	b	a
	J. effusus	(LF)	c	b	a
	F. ovina	(LF)	c	b	a
	C. vulgaris	(ME)	c	b	a
	S. capilifolium	(ME)			

5.3.2.3 Effect of soil type

5.3.2.3.1 Transect 1

In transect 1 after 4 months there was a significantly greater microbial community associated with *C. vulgaris* (SS) and *C. vulgaris* (ME) vegetation buried in the mineral soil relative to the other two soil types (table 5.4). However soil type did not significantly effect the size of the vegetation microbial community after 7 months. There were several differences in the microbial community after 10 months burial. *C. vulgaris* (ME) had a greater microbial community in the soil of the mire expanse than in the mineral soil. However the reverse was the case for *F. ovina* (SS). *S. palustre* (LF) had the greatest size in the mineral soil, and *J. effusus* (LF) was greater in the mineral soil than in the soil of the mire expanse. The microbial community associated with *S. capilifolium* (ME) was smallest in the mire soil.

5.3.2.3.2 Transect 2

There was no significant effect of soil type on the size of the microbial community associated with decaying vegetation after 4 and 10 months. However, after 7 months *F. ovina* (SS), *C. vulgaris* (ME) and *S. papillosum* (ME) had a significantly greater microbial community in the mineral soils than in the other two soil types.

5.3.2.3.3 Transect 3

After 4 months there was no significant effect of soil type on the vegetation microbial community. After 7 months, however, soil type effected all but one of the vegetation types. *C. vulgaris* (SS), *H. jutlandicum* (SS) and *F. ovina* (LF) had a greater microbial community in the mineral soil than in the mire soil and the lagg fen soil. *C. vulgaris* (ME) had a greater microbial biomass in the mineral soil than in the lagg fen soil. The vegetation microbial community associated with *S. capilifolium* (ME) was greater in the mineral soil than in the mire soil, and greater in the lagg fen than in the mire. There was no significant difference between the lagg fen and mineral soil. After 10 months, *F. ovina* (LF) had a greater microbial community in the soil of the mire expanse than in the mineral soil.

5.3.2.3.4 Summary

- the soil microbial community associated with decaying litter was greater in the mineral soils than in the soils of the lagg fen and mire expanse

Table 5.4: Summary of the effect of soil type on the size of the soil microbial community associated with decaying litter from litterbags analysed using one way ANOVA. $P = 0.05$ Similar letters indicate similar populations where a is greater than b.

			In situ		
			Mineral soil	Lagg fen	Mire expanse
Transect 1					
4 months	*C. vulgaris*	(SS)	a	b	b
	F. ovina	(SS)			
	S. palustre	(LF)			
	J. effusus	(LF)			
	C. vulgaris	(ME)	a	b	b
	S. capilifolium	(ME)			
7 months	*C. vulgaris*	(SS)			
	F. ovina	(SS)			
	S. palustre	(LF)			
	J. effusus	(LF)			
	C. vulgaris	(ME)			
	S. capilifolium	(ME)			
10 months	*C. vulgaris*	(SS)			
	F. ovina	(SS)	a	b	b
	S. palustre	(LF)	a	b	b
	J. effusus	(LF)	a	b	b
	C. vulgaris	(ME)	b		a
	S. capilifolium	(ME)	a	a	b
Transect 2					
4 months	*C. vulgaris*	(SS)			
	F. ovina	(SS)			
	J. effusus	(LF)			
	S. recurvum	(LF)			
	C. vulgaris	(ME)			
	S. papillosum	(ME)			
7 months	*C. vulgaris*	(SS)			
	F. ovina	(SS)	a	b	b
	J. effusus	(LF)			
	S. recurvum	(LF)	a	b	b
	C. vulgaris	(ME)	a	b	b
	S. papillosum	(ME)	a	b	b
10 months	*C. vulgaris*	(SS)			
	F. ovina	(SS)			
	J. effusus	(LF)			
	S. recurvum	(LF)			
	C. vulgaris	(ME)			
	S. papillosum	(ME)			
Transect 3					
4 months	*C. vulgaris*	(SS)			
	H. jutlandicum	(SS)			
	J. effusus	(LF)			
	F. ovina	(LF)			
	C. vulgaris	(ME)			
	S. capilifolium	(ME)			
7 months	*C. vulgaris*	(SS)	a	b	b
	H. jutlandicum	(SS)	a	b	b
	J. effusus	(LF)			
	F. ovina	(LF)	a	b	b
	C. vulgaris	(ME)	a	b	
	S. capilifolium	(ME)	a	a	b
10 months	*C. vulgaris*	(SS)			
	H. jutlandicum	(SS)			
	J. effusus	(LF)			
	F. ovina	(LF)	b		a
	C. vulgaris	(ME)			
	S. capilifolium	(ME)			

5.4 Discussion

5.4.1 *Size of the soil microbial community in the microcosms*

The size of the soil microbial community in the soils surrounding the decaying plant litter was measured from the soils in the laboratory experiment. In all transects *J. effusus* (LF) had a significant positive affect on the size of the soil microbial community from peat soils. However, *J. effusus* (LF) had a negative effect on the mineral soils in transect 3. *C. vulgaris* (ME) also had a positive effect on the soils of the mire expanse and lagg fen in transects 1 and 3 respectively. These results suggest that in the first 4 months of incorporation into the soil *J. effusus* (LF) and *C. vulgaris* (ME) were a good source of nutrients for the soil microbial community and as a consequence the soil microbial community increased.

5.4.2 *Size of the microbial community associated with decomposing plant litter*

5.4.2.1 Effect of vegetation

In the period of 4 months, there were no significant differences in the size of the soil microbial community associated with the decaying plant material in the litterbags. This was the outset of the decomposition process and therefore it would be expected that the fastest breakdown of material would occur in this time, therefore give rise to some of the biggest differences. However there are two possible reasons why this may not be the case. First, the nutrients made available by all the newly added substrates were sufficient to remove nutrient limitation on the soil microbial community. Considering that there were differences in both the mass loss data and CO_2 data at this stage; reflecting differences in the properties of the plant material, then we would also expect to see differences in the C_{mic}litter data. Second, it is possible that the soil microbial community of the soil surrounding the decomposing litter was not sensitive to the newly available substrate after 4 months. It is likely that both of these factors played a role in the absence of vegetation effects in the first few months of the experiment.

In transect 1 *S. palustre* (LF) has a significantly greater soil microbial community associated with it than *J. effusus* (LF), *C. vulgaris* (ME) and *S. capilifolium* (ME). *S. palustre* (LF) had a small C:N ratio, suggesting that N may be limiting in the other substrates. This conflicts with suggestions that Sphagnum mosses are toxic to soil microbes. If *S. palustre* (LF) produced toxins then it is unlikely that there would not be such a significant microbial community

associated with it. However, it also seems improbable that the aforementioned sphagnan properties would be conducive to such high microbial activity, indeed a sphagnan induced nutrient limitation would limit the size and activity of the soil C_{mic}litter.

Litter quality did not seem to influence the size of the soil microbial community associated with decaying litter in transect 2 after 7 months. Therefore, there were other factors influencing the fact that there were so few differences in the C_{mic}litter suggests that there are few preferences at this stage in the decomposition process. It is also possible that the experimental design did not afford significant differences in the associations being measured, indeed the bags were all buried together in close contact and there would have been interaction between the physical and biological environment. Anderson and Hetherington (1999) refer to a 'synergistic interaction' when they combined litters in a decomposition experiment; they described enhanced decomposition under such conditions. Therefore, it is probably that there were several interactions between the microbial community and products thereof. It must be noted, however, that this situation will be closer to the natural state, indeed plants are added to the soil at similar times, and so the decomposition of one plant type will not be isolated from the decay of other plant types.

5.4.2.2 Effect of time

In transects 1 and 2 there are only four examples of significant differences in the size of C_{mic} associated with the decaying litter. This suggests that in the period under study that there was not a significant change in the decaying material. In the third transect on the other hand, there were several differences in the size of the soil microbial community associated with decaying plant material, suggesting that newly added plant litter in this transect in more susceptible to change. In each of these differences the soil microbial community associated with the litter after 10 months was greater than at 7 months, and the value for 7 months was greater than 4 months. This suggests that as the decay process progresses it is more open to microbial attack, and that this becomes increasingly microbe intensive, i.e. requiring a greater amount of microbial energy. In the initial stages of decay solutes are released from the plant litter readily. In the more advanced stages, microbial attack is the precursor to the break-up of structural components like lignin. Therefore, the increased activity of microbial organisms with time is predictable.

5.4.2.3 Effect of soil type

The differences between soils occur mainly after 7 months into the experiment. This is most likely to be a result of the slow rate of decay during the winter months. The initial release of labile compounds (within the first fourth months of this experiment) from plant material also does not require the intensive microbial activity that is required by compounds which breakdown later (e.g. cellulose and lignin).

The soil microbial community associated with plant material in the mineral soils was much greater than that of the vegetation buried in the soils of the mire expanse and the lagg fen. There was no preference for vegetation in the data. These data suggest that the soil type is important in the initial 10 months of decomposition, and that microbial attack of vegetation is greater in mineral soils than in peat soils because of better soil conditions.

The range of plant species effected by soil type suggests that litter quality (C:N) may play a role in determining the size of the soil microbial community, however this was not universal. Therefore, further study would be required to explore these possibilities.

5.5 Conclusions

- Higher quality vegetation supports a greater microbial community.

There were few significant differences between the C_{mic}litter values for different litter types. The significantly greater C_{mic}litter of *S. palustre* (LF) was unexpected and cannot be explained with the data available from this study. *S. palustre* (LF) had a slow rate of decomposition and so we would have expected it to have a small C_{mic}litter.

- The size of C_{mic}litter changes with time. The greatest community is found at the onset of decomposition when leaching of readily available compounds occurs.

In the Autumn a new resource is added to the soil when leaves fall. As decaying matter becomes more decayed the more available nutrients are depleted first, and the more difficult to degrade are left behind and may linger in the soil for some time. Therefore, the size of the C_{mic}litter would be expected to be greatest at the outset of decomposition. However, the addition of this substrate coincides with dropping atmospheric temperatures and consequently soil temperatures, so the conditions for microbial growth may be limited by temperature. In this study samples were taken out of the ground late in November, mid February and May. It is likely that the temperature had more control of microbial activity in the first two sampling points, which may explain the greater size of C_{mic}litter as the litter gets older.

- The soil microbial community in the immediate vicinity of the decaying vegetation will be affected according to the quality of the vegetation.

J. effusus (LF) had a positive effect on the soil microbial community in the soil surrounding the decaying plant material which suggests that C:N is important. However, *C. vulgaris* (ME), which has a high C:N content, also had a beneficial effect on the soil microbial community. Therefore, C:N may not be a good measure of the leaching quality of the litter.

This chapter attempted to examine the effect of soil type, time and plant species on the soil microbial community associated with decaying litter *in situ*. There was a clear effect of soil type; the mineral soils provided better conditions for the microbial community than the other

two soils, and this result backs up data from chapter 2. The effect of time and plant species may not be interpreted as easily and therefore will be discussed separately.

Time effected the litter microbial community in different ways, and there were no clear trends observed. This suggests that there are other effects that interact with this variable. Although this work concentrated on relatively long term temporal effects, the initial stage of decomposition is perhaps more interesting. At the outset of decomposition, when senescent litter is added to the soil, colonization by the soil microbial community will occur, therefore the dynamics of the microbial community will be most sensitive to resource quality. Further work should be directed to examining these relatively short term effects e.g. those occurring in the first 7 days. In addition more frequent measurements should be made in this region, which would make useful comparisons with what we already know about leaching of nutrients. At the other end of the scale, it would also be useful to have information about the dynamics of the microbial community associated with decaying plant litter from all seasons. Due to time restraints summer was excluded from the work reported in this chapter, however this would undoubtedly form a good comparison with the other data. Plant species did not seem to have a significant effect on the microbial community associated with it, and this was suprising. Clearly, there is much scope for developing this area of the research. More replicates should be used, but also a more defined substrate may be useful in obtaining baseline information. This is especially important because this work is the only work available on the size of the microbial community associated with senescent plant material in peat soils. The use of a standard substrate e.g. would lay the foundations for comparison with more complex natural substrates. To add an extra dimension to further work the composition of the plant microbial community should be considered. This is a substantial task, therefore it was not within the scope of this chapter. Used in conjunction with the size of the microbial community and measurements of the decomposition processes occurring, this information would make a significant contribution to the existing knowledge base of microbes in peatlands.

In conclusion, these data show that information about the colonization of decaying plant litter by the soil microbial community is useful in our understanding of decomposition, with respect to the microbial community. Indeed, SIR of litter provides information about the active proportion of the microbial community associated with plant material (Neely *et al.*, 1991), and is distinct from the microbial community in the surrounding soil. However, Neely *et al.* (1991) stress the need for a greater number of sample dates and a greater array of resources, which previous studies had not satisfied (Broder and Wagner, 1988; Beare *et al*, 1989; Parmelee *et al.*, 1989).

Chapter 6

General discussion

This chapter will address the objectives for the thesis, as they were outlined in the introductory chapter. Each section will briefly state the context for the specific objectives and summarize the contributions made by the data reported in the experimental chapters of the thesis. The overall results of each chapter will be summarized with recommendations for related research.

6.1 Characterization of the soil microbial community

The soil microbial community has a fundamental role in decomposition; almost all C passes through this component during the decomposition process. In peatland ecosystems much more C accumulates than does in forest and grassland ecosystems. It is therefore important that we have an understanding of the C cycling processes occurring in peats, many of which are carried out by the soil microbial community.

Some of the first research on microorganisms in peats was published more than 70 years ago (Waksman and Stevens, 1929), yet our understanding of the role of these organisms in peat soils is still somewhat limited. Research since then has targetted the size of the soil microbial community (e.g. Brake *et al.*, 1999), the organisms present (e.g Borga *et al.*, 1994) and the activity of the soil microrganisms (e.g. Wheatley, 1978). However, due to the lack of standardized techniques available and perhaps due to an overall lack of interest in peatland microbiology (Ulehlova, 1999) no single study has addressed the three aspects of microbial ecology, outlined in chapter one: size, activity and composition of the soil microbial community. The aim of chapter 2 of this study was to describe the ecology the soil microbial community of peat soils from a raised mire, and make comparisons with soils from mineral soils from the same region.

- The size of the soil microbial community in the mire soils is smaller than in the soils of the lagg fen and the mineral soils, which have a greater nutrient input.

The size of the soil microbial biomass in the mineral soils was much greater than that in the soils of the lagg fen and mire expanse. This observation is consistent with the hypothesis, and it is probably a result of a complex range of factors that effect the soil microbial community.

Without doubt, mineral nutrients effect the soil microbial community, however, the system is much more complex than it first appears. The size of the soil microbial community in the peat soil from the lagg fen and the mire expanse were not significantly different, yet the nutrient supply to these soils is different – the lagg fen receives water from mineral soils and the mire expanse does not. Mineral nutrients may have other implications for soil properties, such as pH (Clymo, 1965) or for plant litter quality (Verhoeven, 1996) and botanical diversity (Aerts, 1995). These indirect effects have implications for other components of the ecology of the ecosystem. Furthermore, size is just one attribute of the soil microbial community and understanding the effects of external factors on activity and community structure is also crucial to understanding the ecology of the microbial component of the soil ecosystem.

- There is a significant difference between the metabolic diversity of the soil microbial community of the different soils. The soil microbial community in the lagg fen and mineral has a greater metabolic diversity that the soils of the lagg fen and mire expanse because botanical diversity is greater on these soils

The respiratory activity was also greatest in the mineral soils, reflecting a greater turnover of C in these soils. However, the respiratory quotient (qCO_2) was greatest in the soils of the mire expanse. qCO_2 has been used as a measure of efficiency of microbiological components because it reflects the amount of maintenance energy required by the microbial community. In the soils of Dun Moss, the peats of the mire expanse had the greatest qCO_2 suggesting that the microbial community in this soils uses more energy for cell maintenance than growth, and that this may be a result of environmental stress. This interpretation of the data supports the suggestions by many peatland researchers that the anoxic, waterlogged and cold environment of peat soils with its nutrient poor vegetation is a harsh environment for microbial growth. However, the structure of the soil microbial community may provide clues about the effect of external factors on microbial processes in peat soils. Different components of the microbial community have different efficiencies in the natural environment; fungi have a smaller respiration rate per unit of biomass than bacteria. Therefore, it is impossible to attribute a change in qCO_2 to stress, and it is more likely to suggest a shift in the soil microbial community.

- There is a significant difference between the soils in terms of functional diversity. The soils of the surrounding slopes and lagg fen have a greater microbial diversity than the soils of the mire expanse.

The Biolog™ and PLFA analyses provided information on the metabolic and functional diversity of the soil microbial community. This data provided indices of the similarities between the soils (using principle components analyses), which is summarized in table 6.1. In the table '+' shows the closeness of soil groupings, where the more '+' represent more widely distributed groupings, therefore a more diverse population. The soils of the mire expanse have the closest groupings, suggesting that the microbial population in this soil was the least diverse. This observation is consistent with the above ground botanical diversity on these soils. However, both the methods used in the determination of the community structure had limitations. The Biolog™ method uses a select range of substrates and the incubation conditions may not be suitable for acidic soils, hence substrate utilization does not necessarily represent the actual metabolic capabilities of the soil microbial community (Degens, 1997). The PLFA method boasts the ability to identify fungi and components of the bacterial population, however some researchers have noted the misidentification of fungi from substances produced by the apical tips of some mosses (Dembitsky and Renzaka, 1995). With these limitations in mind, only a tentative interpretation of the analyses of community structure could be made. These analyses provide an outline of the limitations of these methods for use with peat soils, indeed for use with ecological studies of soil systems in general. The ecological aspect of the microbial community provides a clear opening for further research and development, and must be done before we can attempt to fully describe the role of the soil microbial community in decomposition in peatland ecosystems.

6.1.1 Summary and recommendations for future research

This chapter showed that the aerobic soil microbial community of peat soils is smaller than in mineral soils, but it is more active in peat soils. The diversity of the soil microbial community is also greater in mineral soils than in peat soils. Together, these observations provide new information to our knowledge of microbes in peats. In addition, there are several recommendations that arose from chapter 2, and these are summarized in this section. There are several methods documented for estimating the size of the soil microbial community. Culture techniques do not take into account the entire microbe population because not all organisms can be grown in the laboratory. Other techniques, such as fumigation-extraction are not specific, and so it would be difficult to target the aerobic soil microbial community. Substrate induced respiration, on the other hand, targets the aerobic soil microbial community

and may be customized depending on the soil (substrate may be added as a solution to facilitate mixing). Additionally, it is important to present these data as volumetric measurements, as this provides a basis for comparing between different soil types. Two methods were used to examine the composition of the soil microbial community: Biolog™ and fatty acid methyl ester (FAME) analysis. Of these methods FAME showed the most promise for future work. Although this method has some limitations for use with peat soils, there is a great deal of scope for development, namely the inclusion of a wider range of standards and quantification of FAMEs. By incorporating a wider range of standards it may be possible to target FAMEs in peats and to quantify them, indeed there is very little research that has been documented using these techniques with peats. However, it must be emphasized that it is not sufficient to apply a relatively new technique to peat soils without carrying out some standard analyses. It could be argued that in this case it is the requirement for standard determinations that is greater than for new techniques. Therefore future work should provide basic size and activity measurements as well as novel approaches to characterizing the soil microbial community.

- Use of standard techniques, and volumetric measurements
- Development of FAME analysis for composition of the soil microbial community
- Holistic approach

6.2 Effect of added mineral nutrients on the soil microbial community

In a raised mire system the sole water input to the mire expanse is from precipitation; there is no runoff from mineral soils and because this area is raised, there is no input from the groundwater. Inorganic nutrients such as N and P are required by microorganisms to help them release the nutrients locked up in plants, and in this way biogeochemical cycles progress. Inorganic nutrients are found in low concentrations in ombrotrophic mires (Pollet, 1972) and this has a limiting effect on everything living in them (Dykjov and Ulehlová, 1998). Research into the limiting effects of nutrients in bogs has largely been restricted to the above ground biomass i.e. plants. Microbial processes have been measured in response to mineral nutrient addition (e.g. Williams, 1992), however such studies have used an 'optimum' as the target for mineral nutrient cocktails. In this work, the aim was to test the effect of 'naturally' occurring nutrient concentrations, from the mineral soils which support a greater microbial community size.

- The size of the soil microbial community in the mire expanse is limited by mineral nutrient supply

The addition of nutrients to the soils of Dun Moss did not significantly increase the size of the soil microbial community or the CO_2 efflux from the soils, indeed in most cases a decrease was observed. The simplest interpretation of this result is that the mineral nutrients that were added did not effect the soil microbial community. If the soil microbial community in the ombrotrophic soils is nutrient limited then we would expect the addition of mineral nutrients to have a beneficial effect on the size and activity of the microbial biomass. However, if the soil microbial community is operating within its natural levels of activity, indeed for some microorganisms slow growth is optimal, then the addition of mineral nutrients would not increase the rate of respiration. The cocktail of mineral nutrients that was used to treat the soils was the equivalent of the soil solution in the mineral soils. The structure and activity of the soil microbial community in the mineral soils was different from the soils of the lagg fen and the mire expanse, and so the addition of these nutrients may not necessarily have the same effect on the soil microbial community in the mineral soils as in the peats.

The slightly inhibitory, yet statistically insignificant effect of the treatment suggested a toxic effect of mineral nutrients on the soil microbial community, and this may have been a consequence of a change of the structure of the soil microbial community. The addition of mineral nutrients may have been coupled with a change in community structure. When nutrients are available in abundance, faster growing organisms may outcompete slower growing microbes and dominate. Such a change in the community structure of the soil microbial biomass would undoubtedly have an effect on the physiological processes occurring in the soil. For example, a faster growing and more efficient population (using less C for maintenance and more for cell growth) would have a greater biomass but slower respiration comparatively. However, with the data available it is not possible to speculate on a change in structure of the soil microbial community.

6.2.1 Summary and recommendations for future research

This work showed that additional mineral nutrients do not necessarily have a beneficial effect on the soil microbial community. One explanation for this result is that soil properties limit the soil microbial community, and this overrides the effect of increased mineral nutrients. However, there are a number of possibilities for future research arising from these results in

order to further our understanding of the processes involved. As stated earlier, the aim of chapter 3 was to test the effect of nutrients naturally occurring in soils of the same region, however by breaking the nutrient cocktail down and adding specific nutrients individually and in different combinations of concentrations, it would be possible to test the effect of specific mineral nutrients. A wider range of nutrients could also be tested, and this should include some C sources because these may be limiting biogeochemical cycling in these soils. Ideally, basal respiration and measurements of the size of the soil microbial community should both be used to test the effect of nutrients. In addition, attention should be directed towards the changes in the structure the soil microbial community that take place when mineral nutrients are added. However, the microbial community may be susceptible to these changes on a shorter timescale than was used in this experiment. Indeed, microbial responses are relatively rapid, taking place over a number of hours rather than days, and so shorter timescales should be used to detect changes in the soil microbial community in future work.

- Use short experiments, maximum 1 week in duration
- Expand the range of nutrients and concentrations used, and include C sources
- Monitor changes in the structure of the soil microbial community

6.3 Decomposition of a range of plant species

The decomposition process is fundamental to biogeochemical cycling in the environment. During decomposition microbes break down organic matter, in order to obtain building blocks for their own growth and maintenance, and thereby releasing nutrients which would otherwise stay locked up in dead plant matter. There is a good knowledge base on decomposition in grassland and forest ecosystems, with respect to the role of the soil microbial community in comparison with peatland ecosystems. Although the importance of understanding the decomposition process in these ecosystems is appreciated, the emphasis for research remains on the above ground biomass, the plants. There have been several studies on the decomposition of plant material, and in general these have looked at the mass loss of plant material from litterbags buried *in situ*. Some research has attempted to describe the rates of the processes involved (e.g. Clymo, 1984; Heal *et al.*, 1997) while other research has concentrated on specific environmental paramenters such as litter quality (e.g. Aerts, 1995; Verhoeven, 1996). In most of the literature available on decomposition in peatlands the role of

the soil microbial community is mentioned, however very few researchers have included microbial studies in their analyses. Consequently, the role of the microbial component in C cycling in peat soils still remains largely unexplored, and so virtually any information that is contributed to this area is new.

Mass loss has been used extensively to measure the decomposition of plant material in peat soils, and for this reason the techniques was adapted in this chapter. However, the results produced by this analysis did not provide a useful measure of decompsoition in the soils tested. The main problem with litterbags, and the use of mass loss for determining rates of decompositon lies with the movement of allochotonous material in the litterbags, and although it is common to sort the material after the burial period (Coulson and Butterfield, 1978; Belyea, 1996; Anderson and Hetherington, 1999) it is difficult to see why this approach is accepted because it is impossible to distinguish between the original material and allochotous material after several months burial in soil. The literature on the subject can often be misleading, and mass loss experiments using litterbags should be avoided.

The CO_2 measurements reported in chapter 4 are a much more useful measure of decomposition, not only because it is possible to measure the loss of C accurately, but because C loss as CO_2 represents a complete loss of CO_2 from the system. This issue was a problem with mass loss studies because such studies so not measure the complete breakdown of C, instead they attempt to measure the relocation of C from the original plant material. In this case, relocation of C could mean fragmentation and incorporation into the passive soil organic matter, so interpreting mass loss as C breakdown would grossly overestimate the actual loss from the soil. Taking these considerations into account, the CO_2 production will be used as the principal measure of decomposition in this discussion.

- Decomposition in the mineral soils should be fastest because the size of the soil microbial community is greatest in these soils

Soil type had an important influence on decomposition in the soils at Dun Moss. The respiration was greater in the mineral soils than in the soils of the lagg fen and the mire expanse, which corresponds with the size of the soil microbial community, reported in chapter 2. This suggests that the size of the soil microbial community is related to C cycling in the soils at Dun Moss. In the laboratory decomposition experiment, CO_2 loss was a direct consequence of microbial activity, therefore it is no surprise that the size of the soil microbial community and CO_2 efflux are related. The implications of this relationship for our understanding of C

cycling in the environment are less straightforward. Other factors such as leaching and fragmentation undoubtedly make a major contribution in the initial stages of decomposition, and may exert more significant controls than those associated with the soil microbial biomass on the decomposition process. Indeed, it is the physical breakdown of litter and incorporation into the catotelm where anoxic conditions prevail that protect organic matter from decomposition and contribute to peat accumulation.

- The soil microbial community should decompose higher quality litter faster

There were three plant species which decomposed faster than the others irrespective of soil type. *F. ovina* (LF), *J. effusus* (SS) and *C. vulgaris* (SS) treatments produced the greatest CO_2 during the laboratory incubation. These plants origniated from comapratively nutrient rich soils, i.e. the mineral soils and the soils of the lagg fen, and so the resource quality was better for the soil microbial community. However, when resource quality was measured using C:N (N available for C breakdown) ratios and NMR (for C components) there was no clear relationship between these measures of resource quality and decomposition rates. There are technical limitations that may explain the lack of relationship between resource quality and decompositon: C:N does not take the 'availability' of the C into account, and NMR is not quantitative. Without doubt, resource quality presents many unknowns in peatland decomposition and further work is needed to reveal the importance of this generalization to the while decomposition process in these ecosystems.

The experiments reported in chapter 4 made some useful contributions to our understanding of the role of the soil microbial community in decompositon in peatlands. However, the experimental design may be improved by reducing the number of treatments i.e. plant species, and by increasing the number of replicates. This could have been achieved easily by choosing three transects with similar plant species at each site, thereby reducing the treatments by one third, yet increasing the number of samples. Future design strategies should also target increasing the number of sampling occasions and the inclusion of standard substrates.

6.3.1 Summary and recommendations for future research

CO_2 measurements produced some interesting data, which were sensitive and representative of the complete breakdown of C, therefore CO_2 should be used as a baseline measurement in

decomposition studies. On the other hand, measuring mass loss from litterbags was prone to inaccuracies and was not representative of the complete cycling of C. Therefore mass loss of litter is not recommended for decompositon studies. Some interesting results were yielded from measurements of resource quality, however these approaches may not be sufficient to describe the overall quality of the plant as a resource to the soil microbial community. This is clearly a problem area, which may not have one simple solution. Chemical extraction methods involve the use of harsh chemicals which may alter the state of the C under investigation (Kinchesch *et al.*, 1995a); NMR leaves the sample intact, however so far it has not been successfully interpreted in a quantitative manner and it is not possible to compare spectra from different soils or substrates (Baldock *et al.*, 1991); and, C:N ratios may be too simplistic. In this scenario, where there is no obvious solution, the best solution may be to use a combination of approaches. Another approach to this problem would be to provide a known substrate quality, or standard substrate for the soil microbial community. Cellulose is often used as a standard substrate, but equally it is possible to substitute other substrates with a predefined quality for the soil microorganisms. In using such an approach the ecological significance of the experiment may be lost somewhat, as outlined in the introduction to chapter 4, however, by incorporating a few selected substrates into the experimental design it would be possible to get some baseline data from the experiment.

The emphasis so far has been on the material remaining following microbial transformations of plant litter. Taking the opposite viewpoint, and by tracing the route of the material that undergoes transformation, it may be possible to learn more about the role of the soil microbial community in decomposition. One possible solution to this problem is isotopic labelling, and this has been used in decomposition studies to trace the route of ^{13}C in the system (e.g. Jones and Darrah, 1994; Domisch *et al.*, 1998). However, it may only be possible to achieve 20% labelling of plant material. When 'substrate' (usually at a ratio not exceeding 1:100, plant material:soil) is added to organic soils with a typical C content of 50%, the C in the soil heavily outweighs the labelled C, and it is unlikely that it would be possible to detect it. For this reason, it would not be possible to incorporate labelling with NMR studies in peat soils.

- Avoid the use of litterbags and mass loss measurements
- Use a standard substrate to provide baseline information
- CO_2 may be reliably used as a measure of decomposition
- NMR may be useful in longer-term studies to show dramatic changes in plant material

6.4 Microbial community associated with decomposing plant material

The soil microbial community is important in litter transformation and mineralization, however, little is known about changes in the microbial biomass during the decomposition process (Dilly and Munch, 1996). Moreover, few studies have included the dynamics of the physiologically active biomass in contact with litter during decomposition, yet this component may exert important controls on the whole decomposition process. It is no surprise that such studies have not been carried out on peat soils in consideration of the general lack of microbiological studies on peatland ecosystems. Therefore this work represents an opening for a whole new area of research, as such there is a requirement for general baseline information as well as specific data. This discussion will therefore address both of these considerations, however there will be a greater emphasis on the scope of this work for further development, with recommendations for future research.

The difference between the C_{mic}litter in the different soils was significant, and the mineral soil environment supported greater C_{mic}litter than the soils of the mire expanse and the lagg fen. This observation supports those made in chapters 2 and 4 with respect to the size of the indigenous microbial community in the soils and the rates of decomposition of plant litter in the three soils respectively. These results suggest that soil properties, and possibly the size of the indigenous soil microbial community, exert greater controls of the microbial community associated with decaying litter than litter resource qulaity (measured using C:N ratios).

- Higher quality litter supports a greater microbial community

Nutrient status (C:N) of the litter did not necessarily reflect its decomposability and availability to the soil microbial community. This observation is best illustrated using *C. vulgaris* as an example. *C. vulgaris*, regardless of origin induced the greatest CO_2 effluxes, suggesting that the microbial community found abundant and available resources in this litter. However, *C. vulgaris* had the greatest C:N values, which are normally associated with poor nutrient supply. Similarly the relationship between the nutrient status of litter and C_{mic}litter was not predicted. There were only very few examples where litter type was a significant factor, and in most of these *S. palustre* had a greater C_{mic}litter than other litters. Sphagnum mosses are one of the major peat

forming plants and are associated with slow decomposition, therefore this result was unexpected.

6.4.1 Summary and recommendations for future research

The data from chapter 5 indicate that the importance of the soil type (external environmental factors) far outweighs that of resource quality with respect to the soil microbial community in contact with dacaying plant litter. This result is not completely unexpected because previous chapters fail to show a strong relationship between resource quality, and the size of the soil microbial community and rates of decomposition. However, this result is an interesting one and deserves further investigation. This work represents an opening into a new area for decompositon studies in peat soils therefore there are many opportunities for development. It would be possible to combine this approach with the recommendations for furture reasearch outlined for previous sections, e.g. testing the effect of added nutrients on characteristics of the microbial community associated with decaying litter (C_{mic}litter). Additionally, it would be very useful to monitor changes in the size of the C_{mic}litter using a range of standard substrates to replace plant material. Ideally, some measure of community structure should also be used, and this would require development of the FAME analysis as suggested earlier in this chapter. The soil microbial community is very sensitive to changes in its environment, and often changes take place over much shorter timescales than would occur in other components of the ecosystem, therefore shorter timescales are recommended for future studies to extract the most meaningful information from experiments.

- More effective use of litterbags than mass loss
- Increase sampling points, and reduce timescale
- Integrate with other experiments e.g nutrient amendments
- Use a range of standard substrates
- Monitor all aspects of the soil microbial community i.e. size, activity and structure

6.5 Concluding remarks

The data presented in this thesis are summarized in table 6.1. This table provides an overview of all the analyses carried out in this thesis and it is presented here to give a comparison of all the different aspects of the work, and show how they relate to each other.

Table 6.1: Summary of data collected in experimental chapters.

Data	Mineral soil	Lagg fen	Mire expanse
Soil properties			
pH	++	++	+
C:N soils	+	++	+++
Characterization of the soil microbial community			
Size and activity			
C_{mic}	+++	+	+
Respiration	+++	++	+
qCO_2	+	++	+++
Metabolic and functional diversity			
Biolog PCA groupings (variation)	++	+++	+
PLFA PCA groupings (variation)	++	+	+
Effect of added mineral nutrients			
C_{mic}	0	0	0
Respiration	0/-	0/-	0/-
Decomposition			
Mass loss	++	+	+
CO_2 efflux	+++	++	+
Litter microbial community			
C_{mic}soil	+	+++	++
C_{mic}litter	+++	+	+

This thesis showed that the soil substrate, and the soil microbial community that it supports, is an important control of decomposition in soils of a raised mire. However, our knowledge of the soil microbial community in peatlands is limited, so further work on this component of the ecosystem is necessary to increase our understanding of C cycling in these ecosystems. Some fundamental data on the size of the soil microbial community and its activity in peats was provided, however, the characterization of the soil microbial community in terms of structure and functional diversity is a much more complex problem, and this requires the investment of more time and the development of techniques for use with peat soils. In the first instance the

emphasis should be on collecting baseline information like size, activity and structure of the soil microbial community for different peatland ecosystems. From there it would be useful to monitor the dynamics of the microbial community in response to environmental parameters at different stages in the decomposition process. Decomposition is a complex process in which a number of factors control the rates of breakdown involved. By targeting the active component of the soil microbial community (e.g. using CO_2 studies) in future studies it would be possible to learn more about the role of soil microorganisms in decomposition.

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Appendices

A Apparatus

Gas chromatography

CO_2

Varian 90-P GC fitted with a 1.32m long X 3mm internal diameter stainless steel column packed with 80/100 mesh Porapak Q and a thermal conductivity detector

Fatty acid methyl esters (FAMEs)

PU440 Gas Chromatograph (Philips, UK) fitted with a flame ionization detector. Wall coated open tubular (WCOT) column with a layer of stationary phase 30m x 250 µm diameter, SE 54, temperature range 0-325/350°C (J & W Scientific, UK)

Software: JCL 6000 for Windows version 2.0 rev 27; Jones Chromatography, UK

Sicilic acid columns: Sep-Pak, SPE Cartridges; Waters Chromatography Inc., UK

FAME standard: BAME 47080-U; Supelco, USA

Nuclear magnetic resonance

Chemagnetics CMX LITE 300 MHz Spectrometer

Software: Spinsight 4.1 for UNIX

Biolog

Biolog EcoPlates™

Dynex MRX II plate reader fitted with 550nm filter

Miscellaneous

pH meter, analogue pH meter model 81; Griffin & George UK

Carlo-Erbo CHN Analyser; Italy

Jouan Mini Arctic; Jouan, France

B Soil properties

B.1: Properties of soils for SIR calibration experiment, section 2.2.1.

Soil	Series	% C	pH
Vinny 0-15 cm	Vinny series (Laing, 1976), iron podzol from Seaton near Arbroath (NO 666416)	4.4 %	4.8
Aldbar 0-15cm	Humus podzol from the Montreathmont forest, Angus (NO 563543)	24.1 %	3.7
Redesdale 2.1 0-4 cm	Wilcocks series Lime treated upland soil. 6 t ha^{-1}	11.5 %	4.0
Redesdale 2.3 8-12 cm	Wilcocks series Lime treated upland soil. 6 t ha^{-1}	24.2 %	3.8
Dun Moss 1.1 0-10 cm	Peaty gley	33.3 %	3.7
Dun Moss 3.1 0-10 cm	Peaty pozdol	44.8 %	3.8
Dun Moss 2.3 0-10 cm	Organic peat	54.1 %	3.3

B.2 Properties for determinations of the size of soil microbial community in section 2.2.2. Soils were sampled from the mineral soils, the lagg fen and the mire on 6 different transects. The soils are labelled x.y, where x is the transect and y is the soil type. The standard deviations for %C and %N are of two replicates.

Soil	Replicate	Biomass C (mg C_{mic} cm^3)	%N	SD	%C	SD	pH
1.1	a	0.38	1.1	0.1	14.5	1.4	3.50
1.1	b	0.78	1.8	0.0	25.6	0.0	3.50
1.1	c	0.21	0.6	0.0	8.5	0.5	3.65
1.2	a	0.08	2.4	0.2	41.7	4.7	3.70
1.2	b	0.12	2.2	0.0	48.2	0.2	3.65
1.2	c	0.16	2.4	0.0	46.2	0.5	3.80
1.3	a	0.11	1.8	0.2	48.2	0.2	3.30
1.3	b	0.04	1.5	0.0	48.0	0.3	3.35
1.3	c	0.03	1.7	0.2	48.1	0.1	3.30
2.1	a	0.27	0.6	0.0	8.2	0.2	3.80
2.1	b	0.32	0.8	0.0	10.4	0.0	3.80
2.1	c	0.25	0.7	0.0	9.3	0.2	3.90
2.2	a	0.08	2.1	0.0	49.4	0.0	3.20
2.2	b	0.10	1.9	0.0	49.6	0.2	3.20
2.2	c	0.10	2.2	0.1	47.6	0.3	3.20
2.3	a	0.03	1.8	0.1	46.9	0.8	3.20
2.3	b	0.03	1.8	0.0	46.3	0.1	3.20
2.3	c	0.03	1.5	0.0	47.5	0.0	3.25
3.1	a	0.33	1.4	0.0	36.8	1.0	3.40
3.1	b	0.11	0.7	0.1	20.8	1.5	3.40
3.1	c	0.44	1.4	0.2	33.7	2.7	3.50
3.2	a	0.07	2.1	0.1	40.8	1.4	3.40
3.2	b	0.06	1.5	0.0	29.5	0.5	3.45
3.2	c	0.05	2.2	0.0	43.0	0.6	3.30
3.3	a	0.17	1.9	0.0	46.4	0.2	3.40
3.3	b	0.03	1.1	0.0	43.4	0.7	3.40
3.3	c	0.01	1.3	0.0	44.6	0.4	3.45
4.1	a	0.53	2.1	0.0	36.2	0.1	3.85
4.1	b	0.30	1.1	0.1	15.5	1.4	3.50
4.1	c	0.24	1.6	0.1	25.9	0.8	3.60
4.2	a	0.04	2.0	0.0	35.7	0.2	3.45
4.2	b	0.08	2.4	0.0	47.8	0.1	3.40
4.2	c	0.05	2.3	0.0	26.6	21.8	3.40
4.3	a	0.04	1.9	0.0	47.0	0.3	3.40
4.3	b	0.06	1.8	0.1	47.4	0.3	3.20
4.3	c	0.16	1.5	0.0	45.4	0.2	3.45
5.1	a	0.52	1.5	0.0	47.6	0.4	3.35
5.1	b	0.14	1.2	0.0	33.8	0.4	3.40
5.1	c	0.32	1.6	0.0	44.2	1.8	3.30
5.2	a	0.08	1.2	0.0	44.8	0.1	3.55
5.2	b	0.04	1.0	0.0	44.3	0.2	3.60
5.2	c	0.03	1.7	0.0	48.8	0.2	3.40
5.3	a	0.13	1.1	0.0	45.5	0.0	3.30
5.3	b	0.04	1.3	0.0	45.0	0.6	3.40
5.3	c	0.30	1.1	0.1	45.7	0.1	3.40
6.1	a	0.18	0.8	0.0	24.5	0.5	3.40
6.1	b	0.40	1.5	0.0	45.8	0.1	3.35
6.1	c	0.05	0.6	0.1	16.8	2.0	3.50
6.2	a	0.13	1.4	0.0	47.2	0.5	3.35
6.2	b	0.03	1.0	0.0	43.2	0.3	3.65
6.2	c	0.05	1.6	0.0	48.0	0.0	3.55
6.3	a	0.07	1.7	0.0	47.4	0.1	3.10
6.3	b	0.12	1.4	0.0	45.8	0.3	3.30
6.3	c	0.32	1.0	0.0	45.5	0.3	3.30

B3: Properties of the soils used in chapter 3 section 3.2.1, testing the effect of mineral nutrients on the soil microbial community. Standard deviations are of 2 replicates.

Soil	%C	SD	%N	SD	pH
1.1	33.34	0.7	1.54	0.0	3.50
1.2	50.90	12.0	2.34	0.6	3.75
1.3	37.58	11.0	1.03	0.0	3.30
2.1	10.24	0.1	0.51	0.1	3.80
2.2	41.29	0.3	0.19	0.1	3.25
2.3	51.14	7.7	1.47	0.2	3.20
3.1	44.48	0.2	1.06	0.0	3.55
3.2	41.21	0.2	0.80	0.0	3.40
3.3	48.77	0.2	0.98	0.1	3.40

B4: Properties of the soils used in laboratory incubations reported in chapters 4 and 5. Standard deviations are of 3 replicates and 2 replicates for the biomass and C/N determinations respectively.

	Biomass C (mg C_{mic} cm^3)	SD BioC	%N	SD	%C	SD	pH
1.1	0.46	0.29	1.2	0.6	16.2	8.7	3.6
1.2	0.12	0.04	2.3	0.1	45.4	3.4	3.7
1.3	0.06	0.04	1.7	0.1	48.1	0.1	3.3
2.1	0.28	0.04	0.7	0.1	9.3	1.1	3.8
2.2	0.09	0.01	2.1	0.2	48.9	1.1	3.2
2.3	0.03	0.00	1.7	0.1	46.9	0.6	3.2
3.1	0.29	0.17	1.2	0.4	30.4	8.5	3.4
3.2	0.06	0.01	1.9	0.4	37.8	7.3	3.4
3.3	0.07	0.09	1.4	0.4	44.8	1.5	3.4

C Analyses

C1: Percentage mass loss of litter from litterbags buried in transect 1 (field experiment). Numbers in brackets are standard deviations of 3 replicates.

Time	*C. vulgaris*	*F. ovina*	*S. palustre*	*J. effusus*	C.vulgaris	*S. capilifolium*
4 months						
Mineral soil	16.56	63.06	45.76	89.59	-7.85	45.58
SD	(-18.33)	(2.18)	(21.29)	(92.35)	(20.18)	(22.86)
Lagg fen	-105.44	21.54	-130.81	15.26	-352.50	6.05
SD	(43.90)	(20.59)	(213.06)	(28.08)	(144.45)	(30.01)
Mire expanse	-9.73	38.76	50.22	-10.80	-261.35	-1.19
SD	(48.74)	(7.28)	(11.93)	(7.51)	(55.68)	(30.43)
7 months						
Mineral soil	1.60	46.33	58.62	20.95	-37.37	33.50
SD	(11.63)	(13.78)	(11.43)	(19.73)	(39.59)	(19.19)
Lagg fen	-118.11	-16.22	-4.43	-15.19	-301.41	-48.89
SD	(57.93)	(15.16)	(27.54)	(30.54)	(93.40)	(22.13)
Mire expanse	-8.34	19.84	40.33	10.19	-294.93	-11.75
SD	(37.47)	(5.33)	(6.05)	(26.50)	(181.21)	(2.60)
10 months						
Mineral soil	18.00	57.52	61.05	25.37	-11.69	38.33
SD	(7.71)	(10.30)	(3.48)	(17.80)	(30.91)	(6.40)
Lagg fen	26.56	0.87	16.68	5.69	-218.40	-27.15
SD	(145.66)	(43.40)	(16.70)	(15.08)	(82.82)	(31.27)
Mire expanse	-25.58	82.38	-18.54	-119.12	-88.00	-284.88
SD	(18.69)	(31.15)	(41.10)	(142.45)	(229.42)	(407.98)

C2: Percentage mass loss of litter from litterbags buried in transect 2 (field experiment). Numbers in brackets are standard deviations of 3 replicates.

Time	*S. recurvum*	*F. ovina*	*J. effusus*	*C. vulgaris*	*C. vulgaris*	*S. papillosum*
4 months						
Mineral soil	-45.90	-0.29	-29.58	10.13	26.90	62.12
SD	(129.02)	(44.83)	(39.63)	(22.52)	(23.27)	(6.01)
Lagg fen	55.90	-8.45	27.61	-30.97	-110.46	49.41
SD	(19.13)	(26.96)	(7.15)	(30.72)	(170.07)	(11.70)
Mire expanse	69.22	9.68	5.65	5.39	-115.18	-9.68
SD	(17.97)	(23.95)	(34.85)	(123.61)	(52.74)	(13.96)
7 months						
Mineral soil	3.52	-23.75	-17.57	-5.66	-5.37	29.87
SD	(6.31)	(26.21)	(13.32)	(17.68)	(25.88)	(13.77)
Lagg fen	46.86	0.83	9.31	-51.13	56.83	42.36
SD	(5.96)	(32.73)	(27.58)	(82.85)	(90.91)	(11.08)
Mire expanse	47.44	-6.57	34.72	-97.23	-127.37	-3.95
SD	(22.07)	(11.01)	(23.03)	(15.93)	(62.04)	(11.72)
10 months						
Mineral soil	15.66	24.47	10.19	-6.09	-3.15	50.83
SD	(17.15)	(22.66)	(12.04)	(16.71)	(32.81)	(3.58)
Lagg fen	35.47	-43.41	1.20	4.36	-39.48	43.70
SD	(22.93)	(22.44)	(13.88)	(31.60)	(40.54)	(19.56)
Mire expanse	50.80	14.92	34.27	-242.92	-182.23	-19.28
SD	(7.06)	(0.86)	(8.76)	(118.39)	(44.54)	(11.84)

C3: Percentage mass loss of litter from litterbags buried in transect 3 (field experiment). Numbers in brackets are standard deviations of 3 replicates.

Time	*C. vulgaris*	*H. jutlandicum*	*J. effusus*	*F. ovina*	*C. vulgaris*	*S. capilifolium*
4 months						
Mineral soil	38.20	50.06	43.74	3.34	-3.73	69.08
SD	(55.36)	(9.71)	(12.81)	(19.12)	(64.45)	(56.12)
Lagg fen	-31.74	14.84	9.87	4.36	-66.76	20.52
SD	(74.34)	(6.07)	(5.89)	(12.37)	(45.46)	(17.37)
Mire expanse	-4.45	49.19	28.80	-29.66	-137.16	7.20
SD	(29.01)	(16.08)	(10.94)	(8.10)	(99.76)	(3.46)
7 months						
Mineral soil	8.05	64.78	45.29	-18.58	-59.20	1.95
SD	(10.18)	(11.39)	(41.52)	(40.24)	(56.28)	(17.83)
Lagg fen	-91.33	18.68	-7.64	-12.74	-91.01	-13.03
SD	(60.41)	(7.16)	(13.05)	(10.42)	(23.47)	(26.24)
Mire expanse	-53.87	39.72	26.08	-33.35	-149.76	-2.25
SD	(55.75)	(20.31)	(12.37)	(26.47)	(85.13)	(14.63)
10 months						
Mineral soil	36.78	53.24	36.32	-3.76	-10.03	7.78
SD	(20.58)	(5.59)	(10.54)	(25.56)	(41.94)	(15.04)
Lagg fen	-42.34	15.97	-263.02	13.30	-61.66	3.96
SD	(35.17)	(21.60)	(490.00)	(4.93)	(24.01)	(10.40)
Mire expanse	-20.46	32.20	-9.02	-26.25	-129.68	-28.38
SD	(10.57)	(17.54)	(29.51)	(15.30)	(19.25)	(13.53)

C4: Percentage mass loss of litter from litterbags buried in transect 1 (lab experiment). Numbers in brackets are standard deviations of 3 replicates.

Time	*C. vulgaris*	*J. effusus*	*F. ovina*	*S. palustre*	*C. vulgaris*	*S. capilifolium*
4 months						
Mineral soil	17.09	58.62	-39.74	-22.22	3.98	10.00
SD	(2.26)	(23.67)	(16.16)	(75.41)	(19.08)	(16.15)
Lagg fen	34.31	5.84	-4.99	-100.78	2.46	-225.67
SD	(27.62)	(51.92)	(16.66)	(79.43)	(49.45)	(293.46)
Mire expanse	53.93	63.87	28.64	6.43	17.25	-6.48
SD	(4.38)	(1.51)	(26.99)	(14.05)	(69.14)	(6.16)
7 months						
Mineral soil	15.04	20.52	62.83	38.11	-44.83	16.14
SD	(12.19)	(20.23)	(10.37)	(23.37)	(73.69)	(25.32)
Lagg fen	-19.74	38.02	-6.30	6.27	-142.86	17.22
SD	(9.16)	(11.23)	(70.87)	(32.45)	(47.56)	(45.79)
Mire expanse	20.43	52.11	70.37	-17.52	-168.61	0.74
SD	(40.62)	(9.01)	(19.96)	(37.08)	(137.07)	(7.32)
10 months						
Mineral soil	35.05	32.93	47.30	53.72	4.59	45.86
SD	(8.24)	(23.20)	(27.36)	(32.27)	(48.47)	(5.91)
Lagg fen	15.03	59.12	43.64	29.88	-170.73	18.82
SD	(22.77)	(9.09)	(10.85)	(15.47)	(63.36)	(23.28)
Mire expanse	39.84	52.55	60.38	33.39	-96.90	1.88
SD	(31.66)	(12.12)	(12.85)	(56.70)	(15.10)	(1.66)

C5: Percentage mass loss of litter from litterbags buried in transect 2 (lab experiment). Numbers in brackets are standard deviations of 3 replicates.

Time	*C. vulgaris*	*F. ovina*	*S. recurvum*	*J. effusus*	*C. vulgaris*	*S. papillosum*
4 months						
Mineral soil	15.36	39.23	17.79	-21.88	-54.92	-14.11
SD	(5.65)	(19.40)	(123.60)	(38.50)	(96.69)	(39.65)
Lagg fen	57.43	20.28	1.00	3.86	38.65	-170.39
SD	(15.91)	(0.25)	(20.77)	(16.36)	(4.79)	(131.59)
Mire expanse	68.98	42.09	12.66	11.74	55.34	0.90
SD	(19.66)	(8.26)	(21.99)	(29.07)	(8.84)	(4.83)
7 months						
Mineral soil	14.54	75.30	28.99	9.99	-1.43	4.89
SD	(16.08)	(9.14)	(23.31)	(13.29)	(15.23)	(37.91)
Lagg fen	19.59	-20.55	-27.29	14.35	-51.85	34.64
SD	(68.60)	(23.62)	(57.16)	(24.14)	(10.48)	(11.68)
Mire expanse	44.79	60.67	44.32	-31.47	-129.37	1.67
SD	(2.78)	(9.94)	(4.72)	(83.13)	(26.40)	(4.61)
10 months						
Mineral soil	35.87	4.82	41.08	67.06	17.25	37.34
SD	(5.65)	(14.15)	(15.17)	(17.63)	(7.46)	(34.60)
Lagg fen	5.51	21.37	31.92	39.75	5.07	39.89
SD	(34.64)	(23.88)	(25.06)	(6.31)	(27.45)	(34.78)
Mire expanse	60.53	54.72	54.90	10.20	-52.64	14.68
SD	(1.26)	(15.37)	(5.15)	(71.93)	(21.49)	(1.53)

C6: Percentage mass loss of litter from litterbags buried in transect 3 (lab experiment). Numbers in brackets are standard deviations of 3 replicates. *** indicates missing values.

Time	*C. vulgaris*	*H. jutlandicum*	*J. effusus*	*F. ovina*	*C. vulgaris*	*S. capilifolium*
4 months						
Mineral soil	22.67	43.72	-44.65	1.03	22.29	5.42
SD	(2.03)	(21.61)	(27.31)	(24.95)	(20.66)	(7.89)
Lagg fen	43.81	-12.19	-23.22	-79.20	69.39	-73.49
SD	(9.24)	(43.34)	(17.31)	(37.50)	(9.88)	(56.29)
Mire expanse	42.82	21.49	45.11	13.77	67.79	-7.89
SD	(9.02)	(25.85)	(43.41)	(34.70)	(7.15)	(11.82)
7 months						
Mineral soil	24.73	30.43	50.05	48.52	-7.74	48.39
SD	(5.18)	(43.52)	(12.38)	(28.24)	(19.07)	***
Lagg fen	-25.46	-47.38	8.80	17.08	-32.58	17.44
SD	(7.97)	(27.08)	(31.65)	(14.82)	(45.01)	***
Mire expanse	***	***	***	***	***	***
SD	***	***	***	***	***	***
10 months						
Mineral soil	46.37	28.36	78.96	50.13	-22.11	27.27
SD	(16.32)	(46.35)	(9.68)	(5.03)	(13.09)	(11.03)
Lagg fen	-13.20	27.95	48.53	44.87	-21.70	35.69
SD	(56.46)	(34.40)	(10.37)	(11.10)	(8.96)	(40.08)
Mire expanse	26.32	72.92	55.34	29.28	-83.11	20.92
SD	(20.45)	(12.95)	(11.14)	(36.40)	(54.05)	(2.05)

C7: The size of the soil microbial community associated with decaying litter from litterbags buried in transect 1 soils

Soil type	*C. vulgaris*	*F. ovina*	*S. palustre*	*J. effusus*	*C. vulgaris*	*S. capilifolium*
4 months						
Mineral soil	2.97	3.51	2.29	1.62	2.70	1.33
SD	(1.7)	(0.2)	(1.4)	(0.8)	(0.8)	(0.4)
Lagg fen	0.72	1.50	1.80	1.02	0.85	0.95
SD	(0.1)	(0.6)	(1.1)	(1.4)	(0.2)	(0.8)
Mire expanse	0.91	0.93	1.09	0.95	0.57	0.87
SD	(0.2)	(0.2)	(0.5)	(0.5)	(0.5)	(0.3)
7 months						
Mineral soil	2.49	2.15	4.53	5.53	1.37	5.05
SD	(0.8)	(1.2)	(2.5)	(3.1)	(1.2)	(3.4)
Lagg fen	1.49	1.69	3.00	0.75	0.71	0.57
SD	(0.4)	(0.2)	(1.2)	(0.7)	(0.2)	(0.3)
Mire expanse	1.62	1.66	2.62	1.37	0.71	2.20
SD	(0.5)	(0.5)	(1.6)	(0.4)	(0.4)	(0.8)
10 months						
Mineral soil	2.38	3.06	3.99	2.19	1.75	1.67
SD	(0.3)	(1.0)	(0.4)	(1.0)	(0.6)	(0.0)
Lagg fen	0.70	1.23	1.68	1.22	0.56	1.30
SD	(0.1)	(0.4)	(0.6)	(0.1)	(0.3)	(0.6)
Mire expanse	1.38	1.02	1.89	0.65	0.71	0.44
SD	(0.5)	(0.7)	(0.4)	(0.2)	(0.5)	(0.1)

C8: The size of the soil microbial community associated with decaying litter from litterbags buried in transect 2 soils

Soil type	*S. recurvum*	*F. ovina*	*J. effusus*	*C. vulgaris*	*C. vulgaris*	*S. papillosum*
4 months						
Mineral soil	2.57	2.91	3.73	2.37	1.58	2.96
SD	(1.6)	(1.7)	(0.4)	(0.9)	(0.5)	(1.0)
Lagg fen	1.07	1.73	1.15	0.91	0.80	1.24
SD	(0.3)	(0.5)	(0.5)	(0.4)	(0.4)	(0.2)
Mire expanse	4.92	1.69	2.74	1.62	1.55	1.94
SD	(5.2)	(0.7)	(2.2)	(1.0)	(0.1)	(1.1)
7 months						
Mineral soil	1.21	4.64	4.63	2.35	2.60	3.24
SD	(0.9)	(0.4)	(2.0)	(0.3)	(0.6)	(0.3)
Lagg fen	0.74	1.63	1.84	0.81	1.29	1.00
SD	(0.1)	(1.0)	(0.4)	(0.3)	(0.4)	(0.3)
Mire expanse	2.71	1.97	4.89	2.09	0.61	1.54
SD	(1.4)	(0.3)	(0.6)	(1.0)	(0.3)	(0.8)
10 months						
Mineral soil	2.01	###	2.02	2.29	1.67	1.69
SD	(0.2)	###	(0.7)	(0.7)	(0.5)	(1.3)
Lagg fen	3.06	1.43	2.64	1.21	1.30	1.10
SD	(1.4)	(0.3)	(1.0)	(0.4)	(0.4)	(0.5)
Mire expanse	1.18	1.60	1.84	1.48	1.13	1.67
SD	(0.2)	(0.4)	(0.6)	(0.5)	(0.5)	(0.1)

C9: The size of the soil microbial community associated with decaying litter from litterbags buried in transect 3 soils

Soil type	*C. vulgaris*	*H. jutlandicum*	*J. effusus*	*F. ovina*	*C. vulgaris*	*S. capilifolium*
4 months						
Mineral soil	2.85	2.10	2.53	2.33	2.72	1.65
SD	(1.8)	(0.8)	(1.8)	(1.5)	(1.7)	(0.7)
Lagg fen	0.86	1.12	1.80	1.01	0.83	0.66
SD	(0.1)	(0.6)	(0.7)	(0.1)	(0.1)	(0.6)
Mire expanse	1.08	1.11	0.98	1.05	0.38	0.84
SD	(0.1)	(0.6)	(0.4)	(0.6)	(0.0)	(0.5)
7 months						
Mineral soil	2.80	3.52	10.31	5.76	2.76	2.09
SD	(0.7)	(0.2)	(10.8)	(1.9)	(1.5)	(0.4)
Lagg fen	0.73	1.47	1.81	1.97	0.83	1.50
SD	(0.3)	(0.1)	(1.1)	(1.1)	(0.1)	(0.2)
Mire expanse	0.51	0.72	0.83	0.88	0.48	0.58
SD	(0.0)	(0.2)	(0.3)	(0.5)	(0.1)	(0.1)
10 months						
Mineral soil	1.64	1.46	1.88	1.90	1.54	1.22
SD	(0.6)	(0.1)	(0.5)	(0.7)	(0.7)	(0.3)
Lagg fen	5.91	8.63	13.91	7.83	5.37	31.85
SD	(3.4)	(4.0)	(17.9)	(2.1)	(1.7)	(20.2)
Mire expanse	11.70	24.00	19.21	19.38	9.29	16.29
SD	(14.2)	(14.9)	(2.6)	(8.6)	(5.3)	(14.7)

D Statistics

D.1: Output of One-way Analysis of Variance using Minitab. The ANOVA shows that there was not a significant difference between the soils tested ($p>0.001$). The 'Mean' and 'SD' values were presented in the results tables and figures. Where $p<0.001$ the Intervals from Tukey's pairwise comparisons were used to determine which samples were greater.

Analysis of Variance for 1

Source	DF	SS	MS	F	P
C1	2	495	248	1.40	0.317
Error	6	1062	177		
Total	8	1557			

Level	N	Mean	SD	Individual 95% CIs For Mean Based on Pooled SD
1	3	16.56	18.34	(-----------*----------)
2	3	1.60	11.63	(-----------*----------)
3	3	18.00	7.71	(-----------*----------)

Pooled SD = 13.30 -15 0 15 30

Tukey's pairwise comparisons

Family error rate = 0.0500s
Individual error rate = 0.0220

Critical value = 4.34

Intervals for (column level mean) - (row level mean)

	1	2
2	-18.37 48.30	
3	-34.77 31.90	-49.73 16.93