Thesis Submitted for the Degree of Master of Philosophy by Research in the School of Biological and Environmental Sciences

Studies on the Activity and Composition of Soil Microbial Communities in Resource Limited Soils

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Statement of Originality

I declare that this dissertation represents my own work and that where the work of others has been used, it has been duly accredited.

Signed

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30th November 2006

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Abstract

Soils from two resource limited sites were sampled and various chemical and microbial parameters measured. The sites chosen were Signy Island in the maritime Antarctic and the southern slopes of Mt Etna in Sicily. The soil from the Antarctic was resource limited due to the severe nature of its habitat, very cold and windy. There were several soils analysed from Mt Etna from young developing soils to older more established soils.

The soils from the Antarctic had low concentrations of carbon and nitrogen and with a carbon to nitrogen ratio of 10. Initial analyses showed that they had a low microbial diversity but after the addition of plant residues the diversity increased with time.

The young soils from Mount Etna behaved in a similar way to the soils from Antarctica. They had low carbon and nitrogen levels and a low microbial biomass. When plant material was added to the soil they were able to decompose it readily. By contrast the older volcanic soils had higher levels of carbon and nitrogen and a more diverse microbial community.

Chapter 1

Introduction

1.1 Soils and the Carbon Cycle

Decomposition, defined as the "process of separating materials into their constituent parts" (Paul & Clark 1989), is an important mechanism in maintaining soil fertility i.e. the ability of a soil to provide nutrients for plant growth and soil quality, the capability of a soil to maintain key ecological functions (Joergensen & Castillo 2001), as it provides essential nutrients in usable forms for sustaining plant systems (Paul & Clark 1989). It is the breakdown of organic carbon substrates in the soil. Carbon is central to many biogeochemical reactions and is regarded as a key factor controlling microbial growth in soils (Wardle 1992, Paul & Clark 1989). The largest fraction of carbon to enter the soil is derived from plant litter, with a smaller amount coming from animals (White 1997). Should the decomposition process fail, organic matter would accumulate, plants would be unable to obtain the necessary nutrients for growth and there would be a reduction in CO_2 production (White 1997).

There are many factors that affect the decomposition process which include temperature, soil moisture, nutrient availability and soil texture (Paul & Clark 1989). In temperate climates where warm summers are followed by cold winters, the organic matter accumulates on the soil surface during the warm weather but decomposition slows down during the cold winter.

Therefore, there is high organic matter accumulation in the soil. If the temperature is cold all the year round, then less plant biomass is available, but the decomposition rate is correspondingly slow. Both plant growth and organic matter decomposition require moisture. In most cases if there is too little or too much moisture then these processes decelerate. Nutrients must also be made available for decomposition to take place. Nitrogen is especially important; if it is reduced then plant growth is also reduced, as the soil microorganisms will utilise the nutrients in dead organic matter before the roots can (Miller & Donahue 1990).

The movement of carbon in its many forms through the land, oceans and atmosphere is called the Carbon Cycle. Plants take in CO_2 from the atmosphere during a process called photosynthesis, then release it back into the atmosphere during respiration. When the plants die and start to decay some of this carbon enters into the soil (Paul & Clark 1989). Once there, carbon is utilised by a variety of soil organisms especially soil microorganisms. During decomposition, essential elements undergo a process called mineralisation, which involves a conversion from complex organic compounds to simpler inorganic forms readily available for utilisation by plants. Mineralisation, especially the release of CO_2 is essential for new plant growth. The remainder of the carbon that is used by the microorganisms is incorporated into the microbial biomass where it is immobilised and therefore rendered unavailable for plant growth. Once the organisms die, however, the carbon becomes available for mineralization, and so the process starts over again. The carbon to nitrogen ratio influences the way this system operates. A carbon to nitrogen ratio of < 25 would yield a system where net mineralization is likely and > 25 generally would be indicative of a system where net immobilisation is expected (Paul & Clark 1989). Bacterial C:N ratios are typically in the range of 3-5, and if they are respiring around 60% of their carbon then the optimal C:N ratio

would be about 10:1. Not all of the carbon in the plant material is labile, so this value is higher, probably close to the aforementioned 25:1 (White 1997; Paul & Clark 1989).

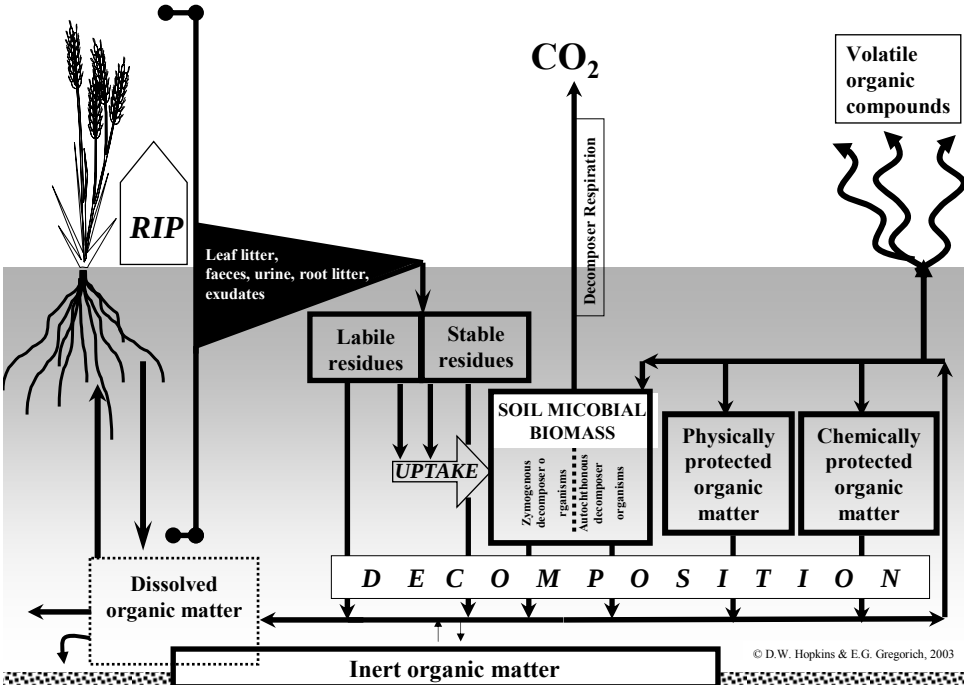


Figure 1.1 A schematic diagram of the carbon cycling through soil and the role of the soil microbial biomass. (Hopkins & Gregorich 2005)

1.1.1 Soil Microbial Community

Soil biomass is a collective name for all plant and animal material living in or on the soil surface. There are many creatures that make their home in the soil from large burrowing animals such as moles (megafauna), to smaller creatures such as worms, insect and beetle larvae and molluscs (macrofauna). There is also an extensive soil microbial community, including bacteria and fungi (microflora) (White 1997; Miller & Donahue 1990). The mixture of organic compounds that is derived from the dead and decaying remains of these plants animals and micro organisms is known as the soil organic matter (Hopkins, 2007 – in press).

The soil microbial community can be sub-divided into bacteria and fungi, each of which have different roles in decomposition. Nearly all organic matter produced passes through the microbial community during decomposition and therefore has a crucial role in global carbon cycling.

Fungi - When the plant material reaches the organic rich soil surface, saprophytic fungi, which are abundant in the litter layer, colonize the new resource first. There are fewer fungal species than bacterial and the fungal population is less variable (White 1997). Fungi grow better in lower pH environments, especially less than pH 5.5, and are more tolerant of variations in soil moisture conditions.

Bacteria - These are the smallest and physiologically the most diverse of all soil organisms. (Singer & Munnis 1996). These organisms can be as small as 1 μm in length and 0.2 μm in breadth and can be rod shaped (*Bacillus*) or spherical (*coccus*). The most common microbial species in the soil are unicellular prokaryotes; these cells lack a nuclear membrane and complex organelle structure and can live in most of the small pores in the soil (Killham 1994). Under favourable conditions, bacteria proliferate rapidly and many species can double their number in a matter of hours. Soil bacteria multiply slowly, but this can be increased by the warming or wetting of the soil, or by the addition of a suitable organic substrate i.e. glucose. These microbes respond quickly to change by multiplying and changing the species working to adapt to new conditions. Once a substrate has been utilised by one species of bacteria this species may become dormant for an indefinite period. This is when the cell ceases to be active, and remains in a state of rest until it is required for action again (White 1997).

Soils also contain a variety of small animals (less than 100 μm in dimension) called protozoa. There are three classes of protozoa in soils, these being the flagellates e.g. *Euglena,* the ciliates e.g. *Vorticella,* and amoebae e.g. *Naegleria*. Their size is limited by the pore size in the soil and they are generally restricted to the top 15-20 cm of the soil profile where their microbial food supply resides (White 1997; Lavalle & Spain 2001; Paul & Clark 1989).

1.1.2 Plant Residues

The plant residues entering the soil are the primary source of soil organic matter and come from litter and root material. As soon as they enter, there is an immediate flush of decomposition, when the more readily broken down components are utilised by the microbial community. These include mono- and polysaccharides and proteins (Killham 1994 & White 1997). This is followed by a slower, steady breakdown of the more stable constituents of the plant material, such as fats, waxes, lignin and phenolic compounds (Brady 1984). Nitrogen, phosphorus and sulphur are converted to NH_4^+, $H_2PO_4^-$ and SO_4^{2-} respectively, and almost half of the carbon is released as CO_2. Therefore mineralization is essential for the production of future plant successions (White 1997). On the assumption that the soil is reasonably well-aerated, the end product of the plant material breakdown (or soil organic matter breakdown) is carbon dioxide.

Microbial communities within soils are usually extremely diverse, with the microorganisms present playing a huge role in the quality of the soil (Schinner *et al.* 1995; Vestal & White 1989). In this thesis, soils from two environments considered to be resource poor are investigated. Those environments are Signy Island in the maritime Antarctic, and the south eastern slopes of Mount Etna on the island of Sicily. In these resource limited soils,

microbial communities are probably not so well developed and diversity may be more limited (Barrett *et al.* 2006).

Odum described a need for simple systems to study community development. He said that "besides being difficult to delineate, natural ecosystems are often large and unwieldly" (Odum 1969). These resource poor soils may provide the simple ecosystem sought after to study community development. The trends expected in the community structure were that in young developing soils the total organic matter would be small, species diversity low, biochemical diversity low and the spacial heterogeneity poorly organised. In contrast, the more mature soils would have large levels of total organic matter, higher species and biochemical diversity and a well organised spacial heterogeneity.

In this piece of work we will look at what effect non sterile plant additions to soils have on decomposition and what limitations are evident from the two resource limited field sites chosen.

1.2 Antarctica

Antarctica, the fifth largest continent, covers about one tenth of the Earth's land surface, around 13.6 million square kilometres, of which 98% of this is a permanent icecap. This icecap constitutes 70% of the world's fresh water and 90% of its ice. If this ice cap were to melt completely it is estimated that the sea level would rise by about 55 m globally. The average temperature at the polar plateau is around -50 °C, and in the costal areas this temperature is -15 °C. Precipitation in the form of snowfall is equivalent to < 22.5 mm water at the pole and 450 mm at the coast (http://www.antarctica.ac.uk/).

As a result of this cold, windy and hostile environment, species diversity is low (Gregorich *et al.* 2006) therefore making it an ideal environment for studying function and diversity relationships in soils (Malosso *et al.* 2004). Because the climate is so harsh and therefore unsuitable for habitation, it has been spared the commercialism and industrialisation found in most regions on earth, making it "the last unspoilt wilderness" (http://asoc.org/). Most of the continent is south of the Antarctic circle and here the summers very rarely get warm enough to melt what little snow falls and freezes each year. Therefore, over hundreds of thousands of years, snow has accumulated and been compressed under its own weight to form two massive ice sheets. The continental margins, however, are very different. The summers are warmer and wetter and there are many areas that are free from ice and snow (Bravo *et al.* 2001). The mean annual temperature along the west coast of the Antarctic Peninsula has risen by 2.6 °C and the mean summer air temperature by 1.5 °C over the past 40 years (Alberdi *et al.* 2002; Day *et al.* 1999). This rate has increased since 1980 making it a suitable place to study global warming (Lewis Smith 1984; Xiong *et al.* 1999; Fowbert & Lewis Smith 1994).

1.2.1 Maritime Antarctic plants

Most of the vegetation is found in the Maritime Antarctic, along the peninsula and the surrounding islands, including the South Shetland, South Sandwich and South Orkney Islands. This is where the ice and snow free areas are, and the mean air temperature remains above 0°C (Komárková *et al.* 1985). There are many cryptogams such as mosses, liverworts and lichens as well as the only two flowering plants that have colonised this cold environment (Lewis Smith 1984; Kennedy 1995; Monteil *et al.* 1999).

1.1.2 Soils from the Maritime Antarctic

The soil used for this project came from Signy Island, one of the South Orkney Islands. The soils here are similar to those found on the mainland (Lewis Smith 1984). There are very small patches of humic soil occurring beneath the limited moss and grass stands. The South Orkney Islands and the South Shetland Islands contain examples of both humic and ahumic soils. The presence of sea birds and mammals also account for high levels of chemical nutrients, making ornithogenic soils common too (Walton 1984; Lewis Smith 1984).

1.3 Mount Etna

Volcanic soils are unique natural resources, and are often very fertile but are subjected to disturbance by the way of eruptive activity (Arnalds & Stahr 2004). Mt Etna (currently 3,340 m), Europe's largest active volcano, is situated 20 km north of Catania on the island of Sicily off the southwest coast of Italy. Etna is a young but highly complex volcano with most of its activity taking place in the last 250 000 years. Some have described Etna as a strato volcano but below about 2900 m Mt Etna is a shield of layers of balsaltic magma, lava flows and tephra. The 400 m to the top is a composite volcano comprising coalesced vents and 4 main active craters (Chester & Duncan 1986). The largest recorded eruption was in 1669, when a lava flow devastated the town of Nicolosi and formed the Siamese cinder cones known as Monti Rossi (Red Mountains) 15 km from Etna's summit.

Mt Etna measures 47 km from north to south and 38 km from east to west as delineated by the extent of Parco dell'Etna. The four large summit craters, these are the Voragine and Bocca Nuova in the central crater, and the North East and South East Craters. There are 200-250 surrounding cinder cones with new structures being added in nearly every flank eruption. Most of the activity manifests from the four main craters (Fernandez Sanjurjo *et al.* 2003). Mount Etna can be divided into three main parts as follows:

1. The Piedmont (cultivated region) – This is the lowest part, where on the broad slopes are many villages. Vegetation is plentiful here due to the older fertile lava soils, home to orange and lemon groves, vineyards and cereal plots.

2. Wooded Region (1000 – 2000 m a.s.l.) – This area is more or less covered in pine forests (*Pinus nigra*) and further up, Etnean broom (*Genista aetnensis*). Most of the cinder cones that have erupted are located here, indicating that some of them have well established wooded areas and others are bare.

3. Above 2000 m – This is the barren region leading steeply up to an in-filled caldera at the summit. Activity here is ongoing and constantly changing the face of the volcano. Smoke can be seen billowing out of the main crater most of the time, indicative of the continuing activity (Scarth & Tanguy 2001).

Figure 1.2 Mount Etna taken from Monti Rossi (May 2001)

1.3.1 Vegetation

Various species of oak, pine, beech, birch, chestnut and broom cover much of Etna's slopes. (Chester & Duncan 1986). *Genista aetnensis* (Etnean broom) is endemic to Mt Etna and its

vibrant yellow flowers can be seen over much of the lower slopes of the volcano. This broom was once used by locals to produce charcoal. This has ceased now and Corsican Pine (*Pinus nigra*) forests are being planted after eruptions (Certini *et al.* 2001). At higher altitudes there is a mix of evergreen and deciduous woodland before giving way to the summit where vegetation is limited and predominantly bare.

1.3.2 Soils

The soils from Mount Etna vary greatly depending on where they were collected, from very young soils from newly erupted cinder cones, to older more stable soils that have not experienced any volcanic activity for thousands of years. Seven out of the eight soils studied here are andisols. Volcanic ejecta are the parent materials for the formation of andisols (Kimble *et al.* 2000). It was only in 1960 that volcanic ash soils were identified for the first time in an international system of soil classification (Takahashi & Shoji 2002). Andisols cover about 1.2 million km^2 throughout the earth and form at all temperatures and on different landscapes thus the potential for vegetation to colonise these soils is large. The soils tend to have a low pH thereby encouraging the growth of acidophilic vegetation (Kimble *et al.* 2000).

1.4 Objectives

The objectives of this work were:

1. To characterise, in terms of biomass, community composition and activity, the properties of the soil microbial communities of soils from the Maritime Antarctic and developing volcanic soils.

2. Where possible, to compare properties of these soils both within and between the two sets of sites.

Chapter 2

Microbial Characteristics of Soils from the Maritime Antarctic

2.1 Introduction

The Antarctic is one of the most severe natural habitats in the world. Environmental conditions have restricted vegetation to lichen, mosses and two angiosperm species (Gielwanowska *et al.* 2005).

Most of the vegetation in Antarctica is found in the Maritime Antarctic, the Peninsula and surrounding islands (Alberdi *et al.* 2002). Within Antarctica, the Peninsula is a special case because it is one of the few places on the continent where there are vascular plants (Day *et al.* 1999). Here vascular plants are at the limits of their range, and this may affect the presence, diversity and activity of soil organisms. However, the factors that affect the distribution of soil organisms are not necessarily the same as those affecting aboveground organisms. For example, belowground wind speed and direction and the extremes of temperature are less likely to affect the distribution of organisms. The South Orkney Islands are subjected to a harsher climate with less sunshine and more precipitation than some sites that are further south (Convey 1996). In 1987, Campbell and Claridge noted that the main reason for restricted development of vegetation in the Antarctic was due to low temperatures and precipitation i.e. desert-like constraints. The microbial biomass is the main constituent of the living organic matter in Antarctic soils (Bolter 1995). This is mainly due to the lack of

mammalian, vertebrate and invertebrate groups with the result that the primary production is not consumed but dies and passes directly into the decomposition cycle (Heal & French 1974; Smith 2003).

2.1.1 Vegetation

Deschampsia antarctica, the Antarctic hair grass, is one of only two angiosperms (flowering plants) native to Antarctica, the other one being the Antarctic pearlwort, *Colobanthus quitensis* (Ruhland & Day 2001; Bravo et al 2001; Fowbert & Lewis Smith 1994). Both species occur sporadically in exposed areas of the maritime Antarctic, especially those with high levels of organic matter. *Deschampsia antarctica* is very similar to *Deschampsia flexuosa*, which is found in more temperate climates. It is a perennial grass and can grow from 20–100 cm high. *Deschampsia Antarctica* can grow at higher altitudes that *Colobanthus quitensis*, and with its ability to tolerate wetter soils and those that have received nutrient inputs from seabird guano, it has a wider range of habitats (Lewis Smith 1984). For many years the population of these plant species remained low: Brown, who first noted this in 1906, said:

"It is doubtful if a flowering plant could obtain the requisite amount of heat needed for its various life functions even to reach the flowering stage, while maturation of fruit would be next to impossible".

However, in recent years, the number of these plants has increased, making it a sensitive indicator of climate change (Ruhland & Day 2001; Lewis Smith 1994).

2.2 Experimental Design and Methods

2.2.1 Site and collection of soil

The soil was collected from Jane Col on Signy Island, a small island (6 km x 5 km), part of the South Orkney Islands (60° 43'S, 45° 36'W), (Fig. 2.1). The island is located in the north province towards the northern limit of the winter pack ice. The terrain is rugged and has permanent ice cover over the majority of its area, the small part that is free of ice and snow, is covered with unstable rock debris. This is formed by glacial action and frost shattering. Recent deglaciation in this area has increased terrestrial habitats by exposing areas of new soil. The Jane Col site lies 150 m above sea level. Because of poor drainage the soils are nutrient poor and remain saturated for much of the year (Weinstein *et al.* 1997). Signy Island is unique in that it has both continental and maritime environments which play host to a wide variety of vegetation (http://www.antarctica.ac.uk/). The climate in the maritime Antarctic is cold and moist and on average remains above 0°C for the summer months (December–March), fluctuating typically between -5°C to about 20°C, with much temperatures occurring in the geothermal-heated areas (Lewis Smith, 1984; Hopkins, pers comm).

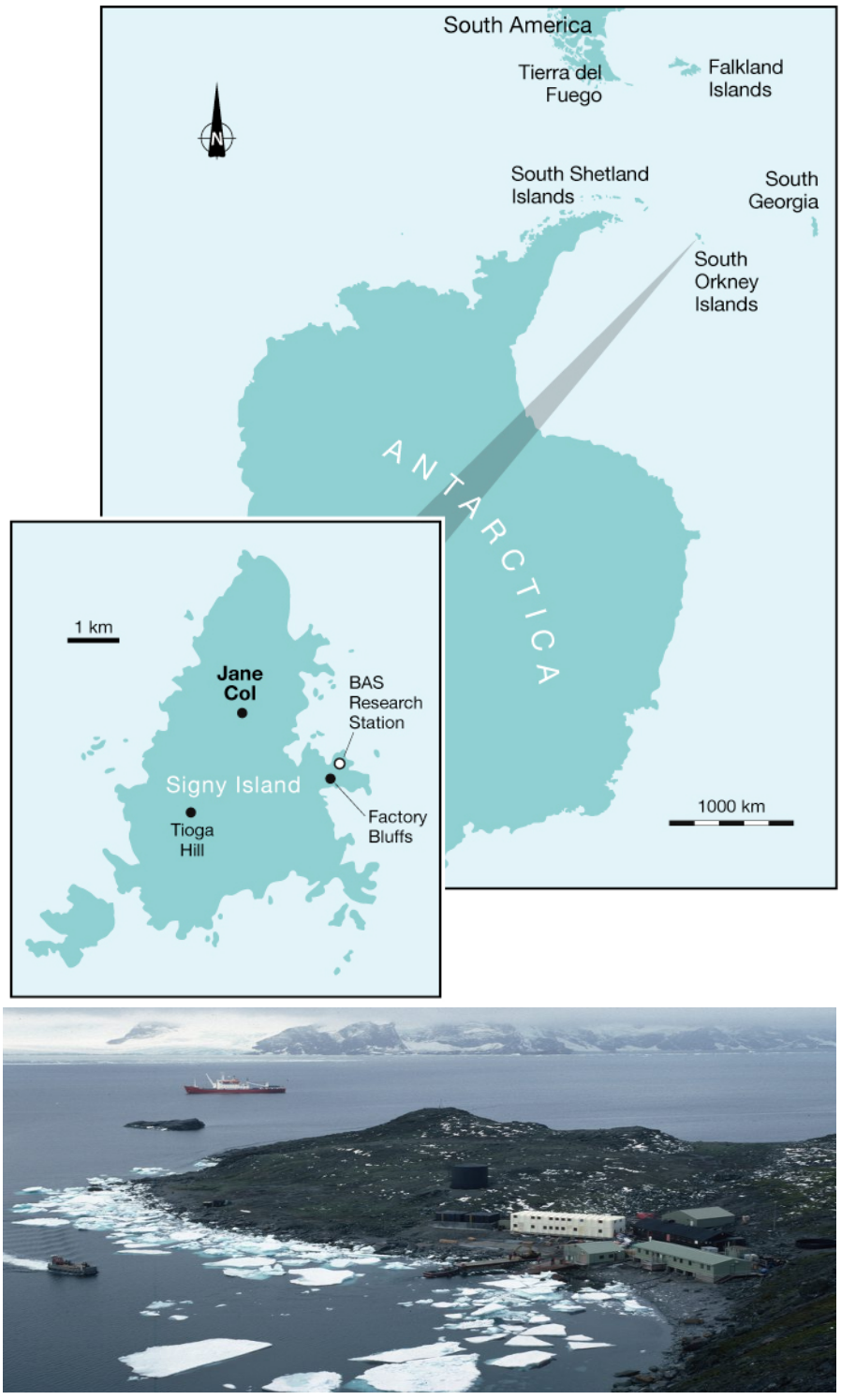


Figure 2.1 Map of Antarctic Peninsula showing Jane Col on Signy Island where the soil and plant material was collected from. (Marshall 1997 redrawn by Bill Jamieson 2006)

Soil samples were collected by Professor J I Sprent of the University of Dundee who visited the site in December 1997 as part of a tour of British Antarctic Survey bases in her capacity as chairperson of the Natural Environment Research Council's Polar Science Committee. The soil samples obtained were the result of combining three subsamples collected at Jane Col on Signy Island. The only vascular plant at the site was *Deschampsia antarctica*, which is only one of two vascular plants found in Antarctica (Lewis Smith 1994). The soil samples were sealed in polythene bags and transported back to the UK in refrigerated storage until being frozen at -20°C until required for analysis. Preliminary analysis of the soil showed that it was composed mainly of silt sized particles containing 0.915% total carbon and 0.088% total nitrogen, with a pH of 5.6.

2.2.2 Vegetation

Small *Deschampsia antarctica* plants were collected at the same time as the soil and brought back to the UK, where they were grown in 0.125 m^3 glass chambers. The plants were grown at 10°C (representative of the summer temperature in Antarctica), with 12 hours light and 12 hours dark. The airtight chambers had rubber septa on the lid, which were injected weekly with 0.25 cm^3 of labelled CO_2 ($^{13}CO_2$, 99 atom %). The atmosphere was changed every week for c. 5 weeks i.e. typical length of the Antarctic summer. Once established, the plant material was harvested, dried at 60°C and stored refrigerated until required. The plant material was chopped to approximately 1 mm lengths prior to being added to the soil.

Figure 2.2 *Deschampsia antarctica* (Antarctic hair grass)

2.2.3 Experimental Design

In the laboratory the soil was thawed and sieved through a 4 mm sieve to remove any large stony particles and the remaining soil was then weighed into 30 cm^3 glass vials (4.5 g in each vial). Each sampling time had 3 control samples and 3 samples that contained 50 mg of the chopped *D. antarctica*. The plant material was mixed thoroughly throughout the soil sample, with a glass rod. There were 9 sampling times yielding a total of 54 vials. Sterile water was added to the vials at this time to bring the water content up to 50% water-holding capacity. (The water holding capacity was pre-determined by placing 5 g fresh weight soil in a glass funnel with a sintered base, and sitting it in a beaker of water. This was left until the soil had taken up as much water as it could hold, until the soil glistened). The soil was then dried overnight in an oven at 105°C, cooled and re-weighed. This difference is then halved to give 50% water-holding capacity.

One set of samples (those to be destroyed on the final sampling occasion) was set up for CO_2 analysis in a microcosm experiment. Each of the six vials was placed in 60 cm^3 syringes (the rubber part of the plunger had been previously coated in araldite^{TM} to ensure that the accumulated gas did not leak). A three-way luer lock tap (Vygon) was attached to the syringe

to allow gas to be removed from the sample and analysed on the gas chromatograph (Hopkins & Ferguson 1994) (fig 2). The six syringes were then put in an incubator previously set to 10°C. The rest of the vials were placed in sealed boxes and put into the same incubator. To prevent the samples drying out, an absorbent sheet was placed in the bottom of the box and wetted slightly. The moisture content was checked and adjusted throughout the experiment.

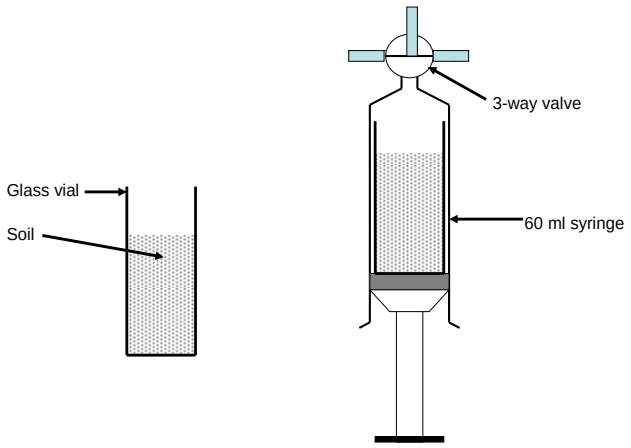


Figure 2.3 Soil respiration chambers (Hopkins 2007)

On each sampling occasion the samples were destroyed and divided as follows: 2.0 g for phospholipid fatty acid extraction (PLFA), 0.6 g for cell extraction for subsequent carbon utilisation and the remaining soil frozen.

Sampling days were assigned to days 1, 3, 7, 14, 27, 71, 105, 139 and 182. In addition to these days, CO_2 measurements were taken after days 0, 2, 4, 6, 8, 10, 13, 20, 34 and 50. Other analyses; pH, C and N analysis, total organic C and particle size distribution, were performed using the remainder of the soil.

2.2.4 Respiration Measurement

The syringes were removed from the incubator and CO_2 measurements were made using a gas chromatograph (Varian 90-P GC fitted with a 1.32 m long x 3 mm internal diameter stainless steel column packed with 80/100 mesh Porapak Q and a thermal conductivity detector). Head space gas (1 ml) was removed from the syringe, using a 1 ml syringe attached to the tap (see Fig 2.4) and injected onto the column. The chromatogram was recorded on a Servogor 102 chart recorder. The first peak on the chromatogram was nitrogen and oxygen co-eluting, followed by the carbon dioxide peak and finally the water peak. The peaks were measured in millimetres and put in a spreadsheet for further statistical analysis of variance.

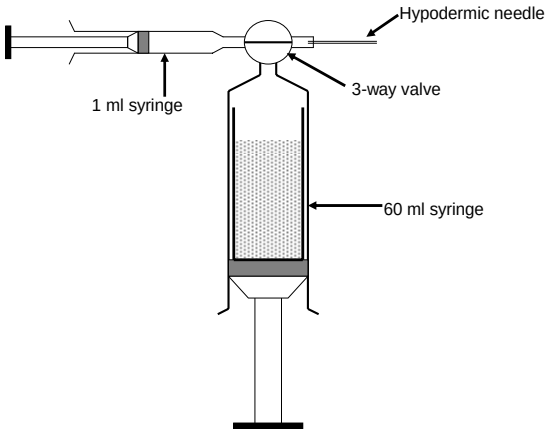


Figure 2.4 Soil respiration chambers fitted with a 1 ml syringe and needle for measuring CO_2 evolution on the gas chromatograph. (Hopkins 2007)

2.2.5 Phospholipid Fatty Acids (PLFA) Analyses

2.2.5.1 Sample Preparation

The extraction of the phospholipid fatty acids (PLFAs) were carried out using a modification of Bligh and Dyer's method (1959), which was initially designed as a single-phase technique

for extracting lipids from animal tissue. All solvents used in this procedure were HPLC grade, and chemicals used were of a suitably high grade. Citrate buffer was made up using 0.15 M trisodiumcitrate dihydrate (44.1g dm^{-3}) and 0.15 M citric acid (31.52 g dm^{-3}). To make citrate buffer with a pH of 4, 49 cm^3 citric acid were added to 41 cm^3 trisodium citrate dihydrate (This was checked with a pH meter every time a new solution was made up).

LIPID EXCTRACTION: Soil, 2 g was removed from each microcosm for PLFA extraction and analysis (3 control soil replicates and 3 amended with *Deschampsia antarctica*). The soil was placed in 30 cm^3 Pyrex tubes with Teflon™ lined caps. Added to the soil were 3.95 cm^3 chloroform and 7.80 cm^3 methanol. The residual moisture of the soil was taken into consideration by adding 0.15 M citrate buffer to the soil to re-create the same solvent proportions found in the single phase Bligh and Dyer extraction solvent (3.16 cm^3). The samples were then mixed with a vortex mixer for 15 s, sonicated in an ultra-sonic bath for 30 min to disperse the residue and redissolve the lipids and then refrigerated overnight. On day 2 the samples were centrifuged at 1200 rpm for 10 min and the supernatant decanted, using a glass Pasteur pipette into 30 cm^3 glass vials. The soil pellet was then washed with 5 cm^3 Bligh and Dyer solvent (chloroform: methanol: citrate buffer, 1: 2: 0.8, vol: vol: vol) and centrifuged at 1200 rpm for 10 min. This washing process was repeated again with one more 5 cm^3 volume of Bligh and Dyer solvent and the supernatants were combined. Chloroform and citrate buffer were added to the supernatants (4 cm^3 of each) to split the phases (organic and aqueous), and they were left in the refrigerator for up to 3 days to separate.

The upper aqueous phase was removed and discarded using a vacuum suction line fitted with a clean Pasteur pipette for every sample. The lower layer was dried under a stream of nitrogen in a water bath at 50°C and stored in the fridge.

FRACTIONATION: The method used was one that was developed in-house at the University of East London and generated reproducible results (Morris 2000). Commercially prepared silica solid phase extraction cartridges (Waters Chromatography, Silica Sep Pak Vac^{TM}) were used, these cartridges were designed to be used with a vacuum manifold. The columns were pre-washed with 2 cm^3 methanol, followed by 2 cm^3 acetone and finally 2 cm^3 chloroform (these are the three solvents used in the fractionation step). The cartridges were dried thoroughly by drawing air through them for 5 min, and then conditioned by drawing 2 cm^3 chloroform through slowly (It is important that from now on the sorbent in the cartridge does not dry out). Each sample was reconstituted in 300 µl chloroform and applied to the SPE column through a filter of anhydrous sodium sulphate supported with ashless flock, in a Pasteur pipette. This was to stop any insoluble material from blocking the top of the cartridge and prevent moisture from getting through.

The different classes of lipid were eluted from the cartridges by increasing the polarity of the solvent used. The neutral lipids were eluted with 5 cm^3 chloroform, the glycolipids were eluted with 12 cm^3 acetone and the polar lipids (including the phospholipids) were eluted with 8 cm^3 methanol.

The first two fractions were allowed to drain into the manifold trap and emptied between chloroform and acetone washes. The final methanol fraction was collected in 30 cm^3 vials placed inside the manifold. This fraction was then evaporated to dryness under a stream of nitrogen at 40°C and stored at -18°C until required for derivatisation.

DERIVATISATION (Mild Alkaline Methanolysis):

Transmethylation:- the fractionated sample was reconstituted in 1 cm^3 toluene:methanol (1:1, vol:vol, prepared with solvents dried over anhydrous sodium sulphate). Dry methanolic KOH (1 cm^3 of 0.2 M) was added; once again anhydrous sodium sulphate was used to dry the solvent. The samples were then vortexed and incubated in a water bath at 37°C for 15 min.

Extraction:- 300 µl of 1 M acetic acid was added to stop the reaction, this was followed by 5 cm^3 extraction solvent (hexane: chloroform, 4:1, vol:vol) and 3 cm^3 water. The samples were sonicated for 30 min and centrifuged at 1200 rpm for 5 min to split the phases. The lower (aqueous) phase was removed and discarded.

FINAL SAMPLE CLEAN UP: To the samples 3 cm^3 of base wash reagent (0.3 M sodium hydroxide) was added and then the samples were vortexed for 30 s (This was done in order to remove any underivatised fatty acids). The samples were then centrifuged at 1200 rpm for 15 min and the upper (organic) layer was removed to a clean vial through a glass Pasteur pipette containing anhydrous Na_2SO_4. The lower (aqueous) layer was then washed again with two successive 5 cm^3 volumes of extraction solvent. All three extractants were combined in one vial and evaporated to dryness under nitrogen at 30°C and stored at -18°C until required for GC analysis.

2.2.5.2 Gas Chromatographic analysis of PLFAs

The samples were analysed on a Pye Unicam Phillips PU4400 gas chromatograph fitted with a 30 m x 0.25 mm x 0.25 µm film (SPB^{TM}) econo cap EC5 column (Alltech) and flame ionisation detector. The detector and injector were both at 320 °C, with an initial column temperature of 60 °C and a split-less hold of 1 min. The temperature was then ramped at 25 °C/min to 145 °C, followed by another ramp at 2.5 °C/min to 250 °C and a final ramp of 10 °C/min to a maximum temperature of 310 °C where this temperature was held for 10 mins.

1.0 µl of two different standards (see fig 2.5a & b) were injected onto the column (BAME mix, Sigma and 37 FAME mix, Supelco). The standards progress through the gas chromatograph was recorded on a computer fitted with JCL600 software (Jones Chromatography). After each of the standards were run, the syringe was rinsed thoroughly with hexane ready for the unknowns to be analysed. Each of the samples were removed from the freezer one at a time and allowed to reach room temperature, they were then reconstituted in 50 µl hexane and 1 µl was injected onto the column. Each run took 63 min. The samples were run over a number of days and on each day a new set of standards were run.

2.2.5.3 Estimation of PLFA

The diversity of the PLFA data was summarised using a derivation of the Shannon Weaver index. Diversity indices are a mathematical measure of species diversity in a community. The Shannon index is normally used for species diversity estimation in which the abundance of individuals of each species in the community is considered. In the present method the concentrations of marker PLFAs were used in place of the abundances of individual species. Thus, the Shannon index of PLFA data is an indirect estimate of species diversity. As well as giving an estimation of the abundance, even-ness of the species present can also be estimated.

$$\mathbf{H = -\sum p_i \ln p_i}$$

Where p_i is the proportion of the total number of identified specimens $_i$ expressed as a proportion of the total number of species for all species in the ecosystem. The product of $p_i \ln p_i$ in the ecosystem is summed and multiplied by -1 to give **H**.

From this the species evenness index **E** can be calculated

$\mathbf{E = H/H_{max}}$ where H_{max} is the maximum possible value of H and is equivalent to ln S which is the natural log of the total number of species present.

1. Methyl undecanoate (C11:0)
2. Methyl 2- hydroxydexanoate (2-OH C10:0)
3. Methyl dodecanoate (C12:0)
4. Methyl tridecanoate (C13:0)
5. Methyl 2-hydroxydodecanoate (2-OH C12:0)
6. Methyl 3-hydroxydodecanoate (3-OH C12:0)
7. Methyl tetradecanoate (C14:0)
8. Methyl 13-methyltetrradecanoate (i-C15:0)
9. Methyl 12-methyltetradecanoate (a-C15:0)
10. Methyl pentadecanoate (C15:0)
11. Methyl 2-hydroxytetradecanoate (2-OH C14:0)
12. Methyl 3-hydroxytetradecanoate (3-OH C14:0)
13. Methyl 14-methylpentadecanoate (i-C16:0)
14. Methyl cis-9-hexadecanoate (C16:1^{9})
15. Methyl hexadexanoate (C16:0)
16. Methyl 15-methylhexadecanoate (i-C17:0)
17. Methyl cis-9,10-methylenehexadecanoate (C17:0D)
18. Methyl heptadecanoate (C17:0)
19. Methyl-2-hydroxyhexadecanoate (2-OH C16:0)
20. Methyl cis-9,12-octadienoate (C18:$2^{9,12}$)
21. Methyl cis-9-octadecanoate (C18:1^{9}cis)
22. Methyl trans-9-octadecanoate &
Methyl cis-11-octadecanoate (C18:1^{9}trans, C18:1)
23. Methyl octadecanoate (C18:0)
24. Methyl cis-9,10-methyleneoctadecanoate (C19:0D)
25. Methyl nonadecanoate (C19:0)
26. Methyl eicosanoate (C20:0)

Figure 2.5a Bacterial Acid Methyl Ester Mix (Sigma)

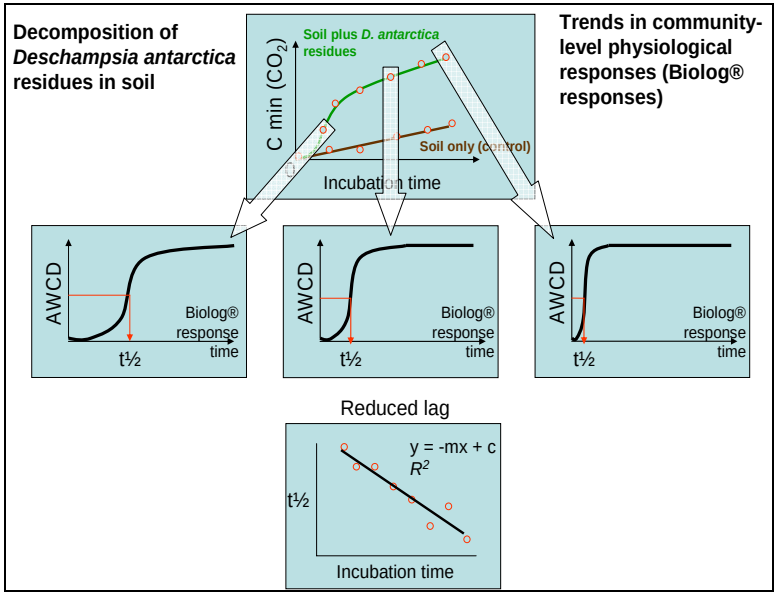
1. Methyl butyrate
2. Methyl hexanoate
3. Methyl octanoate
4. Methyl decanoate
5. Methyl undecanoate
6. Methyl dodecanoate
7. Methyl tridecanoate
8. Methyl myristate
9. Methyl myristoleate
10. Methyl pentadecanoate
11. Methyl *cis*-10-pentadecenoate
12. Methyl palmitate
13. Methyl palmitoleate
14. Methyl heptadecanoate
15. Methyl *cis*-10-heptadecenoate
16. Methyl stearate
17. Methyl oleate
18. Methyl elaidate
19. Methyl linoleate
20. Methyl linolelaidate
21. Methyl *γ*-linolenate
22. Methyl linolenate
23. Methyl arachidate
24. *cis*-11-Eicosenoic acid methyl ester
25. *cis*-11,14-Eicosadienoic acid methyl ester
26. *cis*-8,11,14-Eicosatrienoic acid methyl ester
27. *cis*-11,14,17-Eicosatrienoic acid methyl ester
28. Methyl arachidonate
29. *cis*-5,8,11,14,17-Eicosapentaenoic acid methyl ester
30. Methyl heneicosanoate
31. Methyl behenate
32. Methyl erucate
33. *cis*-13,16-Docosadienoic acid methyl ester
34. *cis*-4,7,10,13,16,19-Docosahexaenoic acid methyl ester
35. Methyl tricosanoate
36.Methyl tetracosanoate
37. Methyl *cis*-15-tetracosenoate

Figure 2.5b 37-Component FAME Mix (Supelco)

2.2.6 Cell Extraction and Carbon Utilisation Analysis

Soil, 0.6 g was removed from each microcosm to a clean pyrex 30 cm^3 tube for carbon utilisation analysis. To each sample, 6 cm^3 of sterile saline (0.85% w/w) solution was added, and then the samples were shaken on a flatbed shaker for 30 min to extract the cells. Sterile saline was used so as not to introduce any foreign microbes. They were then left to settle for up to two hours and 5 cm^3 of the supernatant was diluted 1:2 with sterile water to give a final dilution of 1:20 (Having previously established what dilution would be necessary to give a reaction within two days).

Using a multistepper pipette, 100 µl of the solution was added to EcoPlates™ (Biolog, USA) in triplicate (3 control and 3 samples amended with *Deschampsia antarctica*, plates per sample). Within each plate the 31 carbon substrates were also replicated 3 times (Table 2.1). The plates were then incubated at 22 °C, and absorbance readings were taken every day at 550 nm for 7 days on a plate-reader (MRXII Dynex Technologies). The formation of a purple colour occurs when the organisms present in the soil suspension utilise the carbon source and start to respire. The respiration causes the reduction of a tetrazolium dye that is included with the carbon source, causing a colour change (Biolog USA). The data were removed regularly from the disk, as only 100 readings could be stored at a time, and then downloaded onto a computer and converted to Excel files (Microsoft™ USA) where the data could be manipulated and interpreted. The estimated time for half maximum amount of Average Well Colour Development (AWCD) to have occurred was calculated (**t½**). This was done by plotting the Absorbance at 550 nm against time and then working out how long it would take for half the AWCD to occur. This was repeated for each sampling time i.e days 1, 3, 7, 14, 27, 71, 105, 139 and 182 days.)



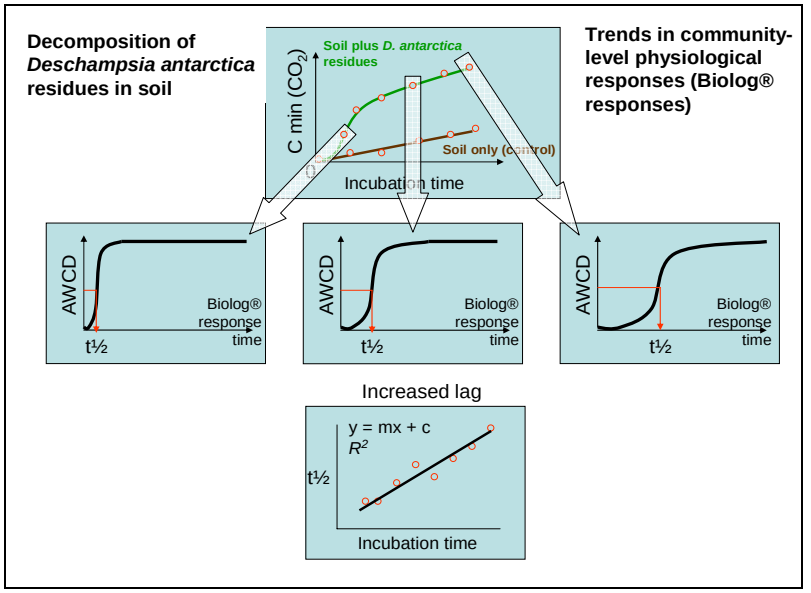


Figure 2.6 Figure showing the possible responses from the Biolog EcoPlates™ . 1st diagram shows a negative response for t½ vs incubation time. 2nd diagram shows a positive t½ vs incubation time relationship.

Table 2.1 The 31 Substrates represented in the Biolog EcoPlate™ (Biolog USA)

POLYMERS	CARBOHYDRATES	ALCOHOLS	AMINES	CARBOXYLLIC ACIDS	AMINO ACIDS
Pyruvic acid methyl ester	D-cellobiose	i-erythritol	n-acetyl-D-glucosamine	D-glucosaminic acid	L-arginine
Tween 40	α-D-Lactose	D-mannitol	Putrescine	D-galactonic acid g-lactone	L-asparagine
Tween 80	β-methyl-D-glucoside		Phenylethyl amine	D-galacturonic acid	L-phenylalanine
α-cyclodextrin	D-xylose			2 - hydroxy benzoic acid	L-serine
Glycogen	D-L-α-glycerol phosphate			4 - hydroxyl benzoic acid	L-threonine
	glucose-1-phosphate			γ-hydroxy butyric acid	glycyl – L-glutamic acid
				α-keto butyric acid	
				D-malic acid	

2.3 Results

2.3.1 Respiration Measurement

The analysis showed that there was a more marked increase in respiration in the soil amended with *Deschampsia antarctica* than in the control (Fig 2.7). This could indicate a "priming" effect, whereby the amount of CO_2 produced exceeds the amount of carbon added in the plant material. In the soil amended with plant material there is a rapid period of CO_2 production until day 27 and then the rate slows and starts to plateau. There is very little activity by the way of CO_2 production in the control soil.

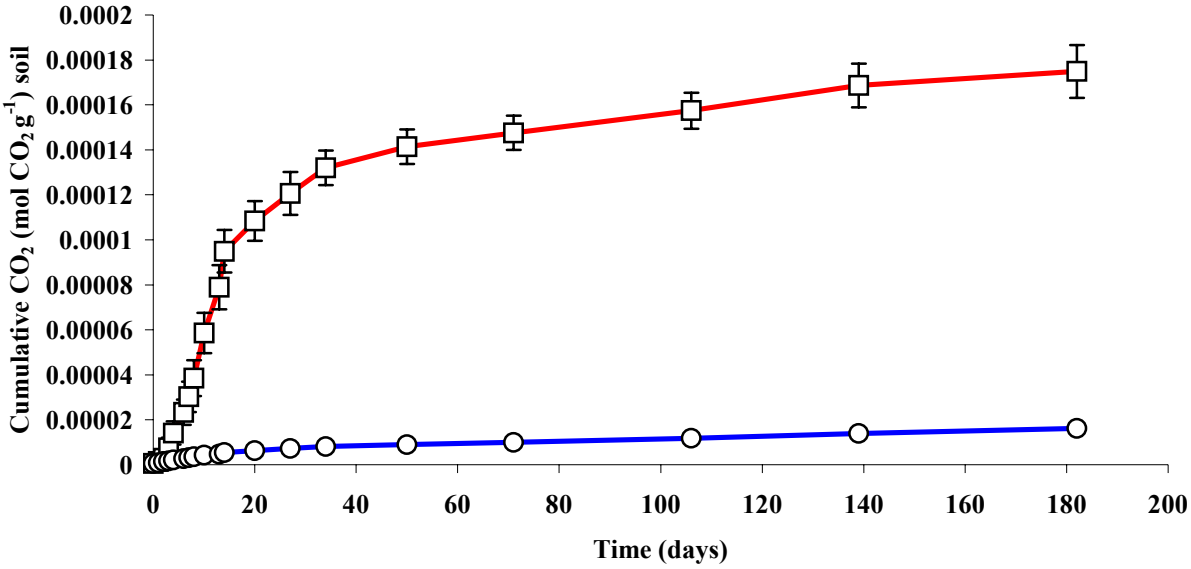


Figure 2.7 Respiration /residue decomposition of Jane Col soil with and without the addition of *Deschampsia antarctica* residues. n=3. Open circle with blue trendline represents the control and the open square with the red trendline being the Jane Col soil amended with *Deschampsia antarctica.*

2.3.2 Phospholipid Fatty Acid Analysis

There are many ways that PLFA data can be interpreted. One way is to look at the diversity. For this the Shannon Weaver Index was used. It can be seen from the graph in Fig. 2.8 that the control samples showed no trends.

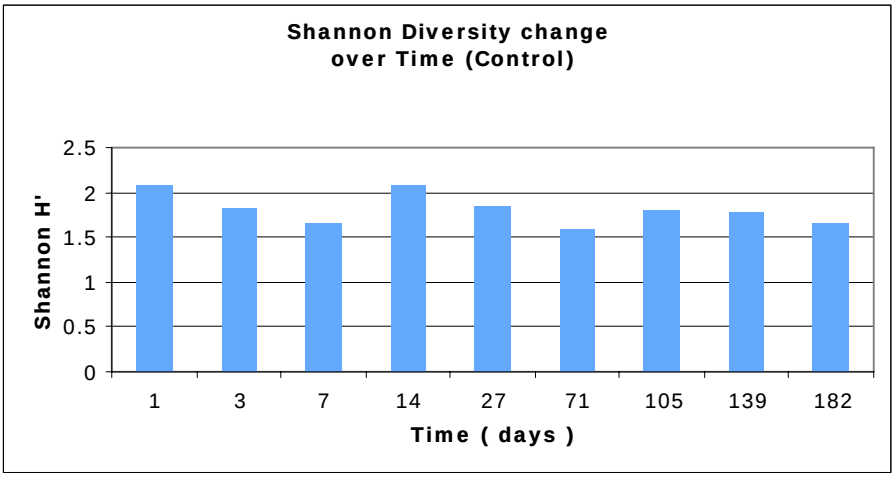


Figure 2.8 Graph showing Shannon diversity change with time in the control soil. n=1. These are observational data rather than statistical data

The amended samples started off low and showed a steady increase until day 27 where they plateaued, and started to decline.

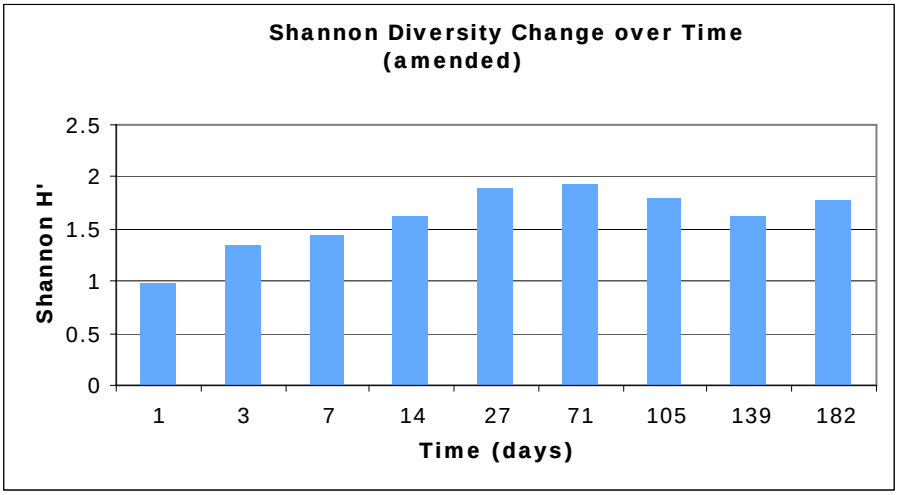


Figure 2.9 Graph showing Shannon diversity change with time in the soil that had been amended with plant material. n=1. These are observational data rather than statistical data

Even-ness

The even-ness data from the control soil once again showed no significant trends.

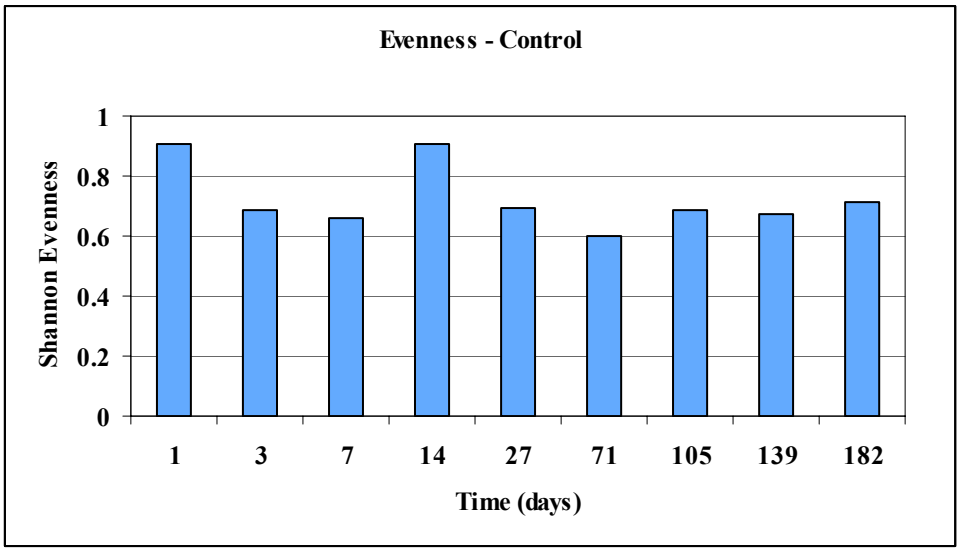


Figure 2.10 Graph showing Shannon Even-ness change with time in the control soil. n=1 (These are observational data)

The soil that had been amended with the plant material showed an increase in even-ness until day 27 then it maintains a more steady value.

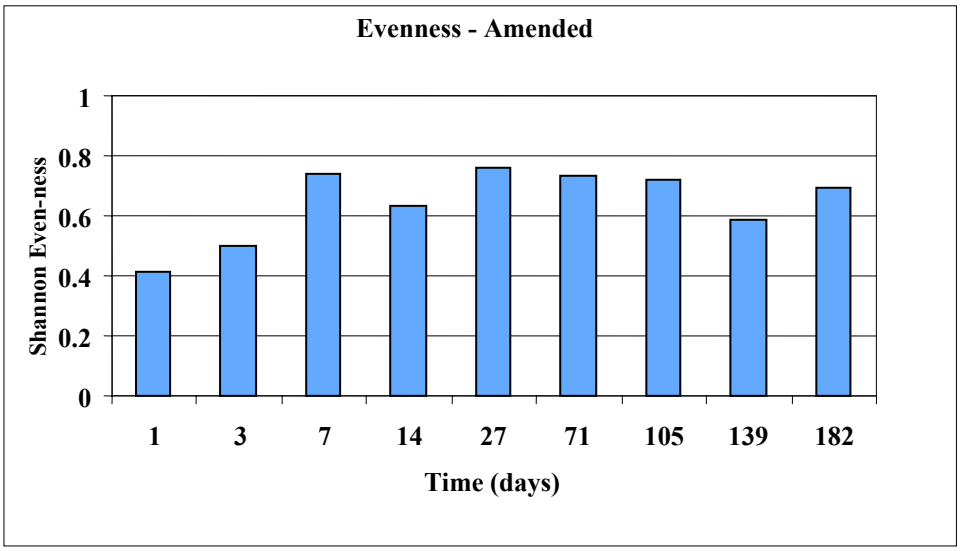


Figure 2.11 Graph showing evenness in the soil amended with *Deschampsia Antarctica* n=1 (These are observational data)

Biomarkers are commonly used to assess the types of bacteria present in a system (Zelles 1999). There were several PLFAs in the profile (data not shown) and C16:0 a general indicator of Gram positive bacteria (Findlay & Dobbs 1993) was chosen as it was present in all samples and its change in abundance could be followed throughout the time course of this study. 18:3ω6 a well documented fungal biomarker (Frostegård and Bååth 1996) was chosen to give an indication of the fungal community present. The C16:0 control samples showed no trend but the amended samples were consistent till day 27 then abundance dropped.

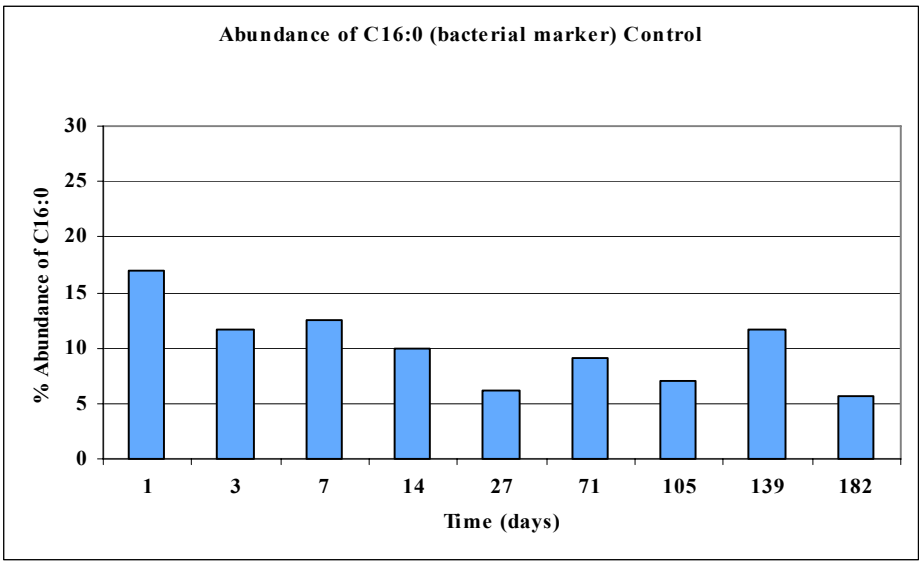


Figure 2.12 Graph showing the abundance of the C16:0 bacterial marker in the control soil

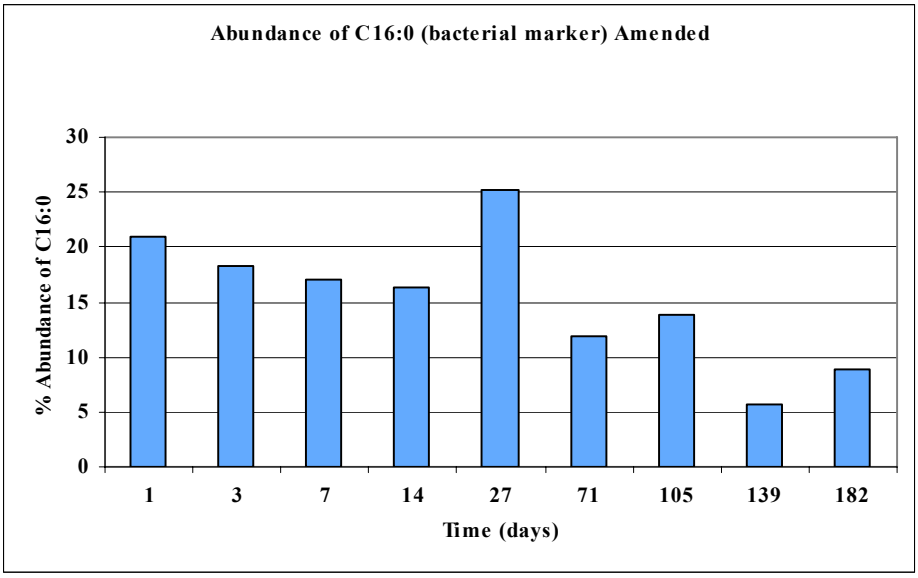


Figure 2.13 Graph showing the abundance of the C16:0 bacterial marker in the amended soil

The fungal biomarker was absent from the control samples and in the amended samples decreased steadily till day 27 and was then reduced to less that half that of the original abundance.

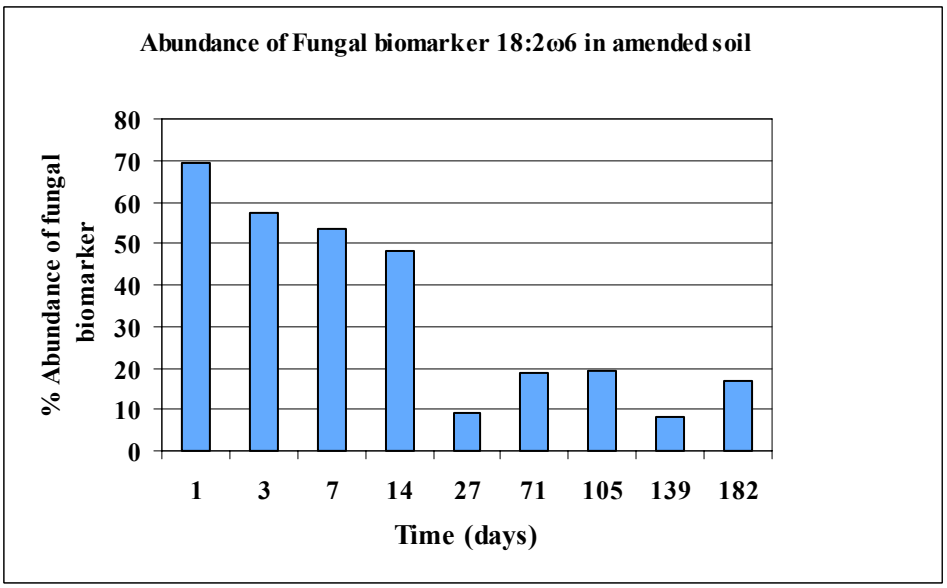


Figure 2.14 Graph showing the abundance of the fungal biomarker 18:2ω6 in amended soil

2.3.3 Cell Extraction and Carbon Utilisation

A trend in the t½ values was sought. There were 31 substrates altogether (see Table 2.1) of those 31 two did not react. There was no trend in the control soils. In the amended soils 11 gave a significant t½ vs incubation time relationship, and 18 gave no significant relationship. Of all that gave significant relationships all were positive slopes. There were no particular groups of substrates that gave a significant trend.

Table 2.2 Results of Biolog expt. t½ versus incubation time.

Grey shaded boxes indicate a significant result (P < 0.05)

	UNAMENDED				AMENDED			
Substrate	**Slope**	**S.Dev**	**R**	**P < 0.05**	**Slope**	**S.Dev**	**R**	**P < 0.05**
Pyruvic acid methyl ester	-0.0034	0.0027	-0.4351	0.24193	**0.01244**	**0.00424**	**0.74269**	**0.02189**
Tween 40	-0.0017	0.0049	-0.155	0.73955	0.00155	0.00381	0.16402	0.69793
Tween 80	0.0232	0.0204	0.42118	0.29871	0.00098	0.00343	0.11598	0.78448
α-cyclodextrin	-0.0004	0.0285	-0.0065	0.98783	-0.0008	0.00649	-0.0452	0.90812
Glycogen	0.00435	0.03825	0.05077	0.91393	**0.03415**	**0.01431**	**0.69773**	**0.04434**
D-cellobiose	-0.0021	0.00485	-0.3923	0.74335	**0.00389**	**0.00136**	**0.75935**	**0.02885**
α - D – Lactose	-	-	-	-	-0.0041	0.00722	-0.2078	0.59156
β-methyl-D-glucoside	-	-	-	-	0.0003	0.00301	0.03801	0.92266
D-xylose	-	-	-	-	**0.00451**	**0.00164**	**0.72061**	**0.02851**
l-erythritol	-	-	-	-	0.00147	0.01121	0.0587	0.90052
D-mannitol	-0.0009	0.00709	-0.0568	0.90374	**0.0034**	**0.00096**	**0.82167**	**0.01235**
N-acetyl-D-glucosamine	0.00102	0.00393	0.1156	0.80507	**0.00395**	**0.00103**	**0.84269**	**0.00862**
D-glucosaminic acid	-0.0077	0.00595	-0.5021	0.25081	**0.00375**	**0.0015**	**0.71465**	**0.04637**
Glucose-1-phosphate	-	-	-	-	**0.00364**	**0.00201**	**0.5631**	**0.011394**
D,L-a-glyercol phosphate	-0.0040	0.00284	-0.5334	0.21757	0.00015	0.0012	0.05439	0.9078
D-galactonic acid γ-lactone	-0.0025	0.00119	-0.6506	0.08063	**0.00392**	**0.00116**	**0.78656**	**0.01192**
D-galacturonic acid	0.00053	0.002	0.10795	0.79916	**0.00327**	**0.00161**	**0.60886**	**0.05183**
2-hydroxy benzoic acid	-	-	-	-	-	-	-	-
4-hydroxy benzoic acid	-0.0070	0.00804	-0.3135	0.41136	-0.0046	0.00383	-0.412	0.27054
γ-hydroxybutyric acid	-	-	-		0.00581	0.00848	0.26962	0.51843
Itaconic acid	-0.0484	0.02079	-0.8025	0.10217	-0.0106	0.00684	-0.5065	0.1641
D-malic acid	-0.0028	0.01849	-0.0683	0.88424	-0.0015	0.00473	-0.1287	0.76137
L-arginine	-0.0031	0.00284	-0.3796	0.31359	0.00198	0.00164	0.41449	0.26735
L-asparagine	-0.0002	0.004	-0.0212	0.96029	0.00058	0.00278	0.07803	0.84185
L-phenylalanine	-0.0089	-0.0095	-0.4769	0.4167	-0.0066	0.00648	-0.4145	0.35511
L-serine	-0.0009	0.00152	-0.2547	0.58143	**0.01721**	**0.00383**	**0.87789**	**0.00414**
L-threonine	-	-	-	-	-	-	-	-
glycyl-L-glutamic acid	-0.0163	0.00851	-0.5171	0.29351	0.00529	0.00577	0.35025	0.39502
Phenylethyl amine	-	-	-	-	-0.0147	0.00868	-0.5676	0.14227
Putrescine	-0.0034	0.00614	-0.2439	0.59817	-0.0010	0.0021	-0.1796	0.64385

2.4 Discussion

2.4.1 Respiration/ Residue Decomposition

Although the biological activity of these soils is low, addition of plant material resulted in CO_2 concentrations increasing from 5.95 x 10^{-7} mol CO_2 g^{-1} to 1.62 x 10^{-5} mol CO_2 g^{-1} after 182 days incubation. This increase in CO_2 evolution exceeded the amount of carbon that was added to the soil by the way of plant residue addition, demonstrating a probable significant priming effect resulting in enhanced mineralisation of the soil organic matter. Kuzyakov *et al.* (2000) said that priming effects were "strong short term changes in the turnover of soil organic matter caused by comparatively moderate treatments of the soil". It is possible that these priming effects are characteristic of soils from extreme environments where the soils are restricted by lack of nutrition or low diversity. This supplementation of fresh plant residues alleviates this and the added carbon causes a rapid increase in respiration with CO_2 efflux increasing to 17 times those of the control rates after 27 days. This tentatively suggests that the community has adapted to periodic inputs of organic carbon and is able to respond rapidly to this input that is unavailable during the long Antarctic winter. De Nobli *et al.* (2001) proposed that some microorganisms respond to sporadic substrate additions by reacting to small quantities of "trigger" molecules including glucose and some amino acids. These low carbon soils would provide and excellent laboratory for testing this hypothesis further. Unfortunately due to a lack of soil, this was not possible on this occasion.

2.4.2 Phospholipid Fatty Acid Analysis

Phospholipid fatty acids are present in all living cells as essential membrane components. They are not found in dead cells so can be used to measure the living biomass in a community (Zelles 1999). Phospholipid fatty acid analysis can be interpreted in many ways to give an

understanding of the structure and diversity of the microbial community (Cavigelli *et al.* 1995).

The control soils showed no trend with regards to change in diversity over time. Most of the values were between 1.8 and 2.0. The amended soils did show a trend of a gradual increase from 1.0 on day 1 to 2.0 after 27 days. It is worth noting that the value on day 1 for the amended soil was half that of the control soil. The microbial community seems to have been swamped by the addition of the plant material and over the first 27 days starts to utilise it steadily, until it maintains a steady H value of about 2.0. This indicates that the soil microorganisms are probably not used to receiving such a high quantity of plant material at once and they were unable to utilise it immediately, indicative of a kind of biochemical dormancy.

The evenness confirmed this observation by behaving in a similar fashion. The control soil showed no real change with time, all values staying around 0.6-0.8 with 2 exceptions. The amended soil once again started low at 0.4 and increased steadily till day 27 where it maintained a value of 0.7-0.8.

One of the other ways of looking at PLFA data is to look at specific biomarkers. C16:0 was chosen as it is a well documented general bacterial biomarker (Leckie 2005; Drissner *et al.* 2006; Zelles 1999; Bartelt-Ryser *et al.* 2005) Caution has to be exercised here when looking at the results as they are not very clear. The control soil is showing no real trend but the amended soil shows a fairly stable abundance of about 15 – 20 % then dropping off after day 27 to 10 %. The drop in abundance of this biomarker could suggest that the groups of microorganisms utilising the plant material change over the course of the experiment, or

perhaps that they use all of the material and start to die themselves. The C16:0 biomarker could also be present in the plant material (Buyer & Drinkwater 1997), and this could account for the higher levels in the amended soil samples.

The 18.2ω6 biomarker was chosen as it is a well documented fungal biomarker (Leckie 2005; Frostegård & Bååth 1996; Bartelt-Ryser *et al.* 2005). This PLFA was not present at all in the control soils, which suggests that it was introduced along with the plant material. In the amended soils the abundance is initially 70 % dropping to 10% after 27 days, where it varies between 10 and 20% till the end of the incubation. The drop in abundance after 27 days is quite dramatic suggesting that at this time the community has become dominated by a smaller diversity of organisms i.e. the initial phase of decomposition had finished and a new, relatively stable community had developed. If the fungi were indeed added along with the plant material then the fungal community declines as they deplete the organic carbon source i.e. the plant material. The decline cannot be due to depletion of oxygen inducing anoxic conditions, because the total amount of oxygen in the chambers was far in excess of that needed to oxidise completely, all the organic carbon

2.4.3 Carbon Utilisation Analyses

The EcoPlate™ was created specifically for community analysis and microbial ecological studies. It contains 31 of the most useful carbon sources for studying microbial communities. The 31 substrates include a mixture of those that are present in the GN and GP microplates (testing for Gram negative and Gram positive microorganisms) (Biolog USA).

There has been a lot of debate over the years about the usefulness of Biolog analyses (Oka *et al.* 2000). It is common practice to ignore negative values but Kunc in 1994 stated that it is unacceptable to do this without first understanding why negative results occurred. Two hypotheses were put forward, the first one being that the control value could be erroneous and secondly some of the carbon sources represented in the plates could have inhibiting effects on the organisms. Data from this type of analysis should be evaluated with care and other methods used to validate results.

All the substrates that gave significant relationships, gave positive relationships, meaning that the time taken for the half the AWCD to have occurred got longer as the experiment proceeded. This is illustrated in the second part of Fig. 2.6. This could be to do with the fact that the organisms in the soils are less able to utilise these substrates as they have consumed what they required and have perhaps moved onto some of the other more complex substrates that are not represented in the EcoPlate™. Or perhaps the organisms that have been utilising these particular substrates are starting to die off.

2.5 Conclusions

In this chapter the chemical and microbial properties of a resource-limited Antarctic soil were investigated. The soil was found to contain very low carbon and nitrogen contents, and a low microbial diversity. When plant residues were added to the soils the CO_2 production increased to more than 7 times that of the control soil after 27 days. The bacterial biomarker stayed relatively stable throughout the incubation but the fungal biomarker, which seemed to have been added with the plant material, reduced significantly with time. Microbial diversity increased too and seemed to reach its maximum and level off after 27 days. Carbon substrate

analysis showed that one third of the substrates were utilised by the microbial community but as time progressed they became slower to decompose the plant material suggesting a change in the microbial community perhaps.

Chapter 3

Mt Etna

3.1 Introduction

Volcanoes provide a useful environment for studying primary succession of plant and microbial material. Every time they erupt the lava flows and ash (tephra) deposits create a virgin soil that is ready for soil development and colonisation of vegetation and microbes.

The processes of C and N accumulation in soils, N-fixation and the dynamics of soil P, and the ways in which these environmental processes influence soil microbial community development have been widely investigated (e.g. Torn *et al.* 1997; Neff *et al.* 2000). Major shifts in the functional diversity of heterotrophic soil organisms, respiratory activity, accumulation of soil organic matter and nutrient limitation with succession (Halvorsen & Smith 1995; Vitousek 1999; Schipper *et al.* 2001; Crews *et al.* 2001; Morisada *et al.* 2002) have all been reported. In addition to soils in volcanic systems, successional processes have been investigated on chronosequences produced by repeated fires (Haslam *et al.* 1998; Wardle *et al.* 2003) and by retreating glaciers (Klingensmith *et al.* 1993; Kohls *et al.* 1994; Chapin *et al.* 1994; Ohtonen *et al.* 1999; Schipper *et al.* 2001; Hodkinson *et al.* 2003; Tscherko *et al.* 2003; Bardgett and Walker 2004). These studies have provided detail on the increase in size, activity and diversity of soil communities that largely support the classic work on primary succession (Crocker and Major 1955) and provided estimates of organic matter accumulation (Haslam *et al.* 1998; Wardle *et al.* 2003), and pointed to the importance

of allochthonous organic inputs for facilitating succession (Frenot *et al.* 1995; Haslam *et al.* 1998; Hodkinson *et al.* 2003).

Here it is hypothesised that the amount of readily mineralizable C in plant residues is greater when the residues decompose in more developed soils, compared with younger resource limited soils. This is based on the observation that the amount of C incorporated into microbial biomass from added substrate declined with community development in soils from a glacier forefield (Ohtonen *et al.* 1999). We hypothesised, further, that the microbial communities in soils at an early stage of development would be less efficient at mineralizing C from low quality (water insoluble fraction) plant residues compared with microorganisms in soils at a later stage of development. In this context, we propose that the distinction between intact and the insoluble fraction of plant residues is made on the basis of the amount of easily labile organic components, such as sugars and amino acids (Marstorp 1996a; 1996b; Webster *et al.* 2000; Webster *et al.* 2005).

Changes in the diversity of the microbial community were measured using the well documented method of phospholipid fatty acid analysis (Zelles 1999) interpreted using the Shannon Weaver index and fatty acid biomarkers.

3.2 Experimental Design and Methods

3.2.1 Site and Collection of Soil and Vegetation

The soil was collected from the southern and eastern slopes of Mount Etna in May 2001 (Fig 3.1) near the towns of Nicolosi, Sant'Alfio and Zafferana. The sites were carefully selected to include soils at different stages of development. The Monti Rossi site is a relatively young site with the cinder cones having formed after the major eruption in 1669. The predominant

vegetation here was *Genista aetnensis* (Etnean broom). It is an r-selective species, i.e. capable of rapid growth. It also has nitrogen fixing symbioses thus contributing to its fitness as a pioneer on nutrient limited soils. In the second site from Monti Rossi (soil 2) there was a pine forest (*Pinus nigra*). These pine forests are not indigenous and have been planted to try to stabilise unconsolidated soil materials (Fernandez Sanjurjo *et al.* 2003). By contrast the soils from Mt Salto Del Cane are thought to be in the region of 7500 years old. This area is covered by mature deciduous woodland cover, dominated by *Quercus cerris* (oak) and *Castanea sativa* (chestnut) suggesting a more developed and stable soil structure. *Quercus cerris* is also found on the lower slopes along with the pine and broom. As the altitude increases the plant species become deciduous indicative of a cooler more temperate climate. The upper limit of *Castanea sativa* is about 1400 m although in desirable areas it can be found at over 1700 m above sea level. Above 1700 m the vegetation is predominantly larch, beech and pine. Table 3.1 contains more information about all 8 soils collected.

A 30 cm deep spit of soil was removed from each site, the turf was removed and approximately 500g was collected from the A horizon. Two further samples were collected within 3 m of the original site. The annual precipitation (which falls as rain at the altitudes sampled) on the south and east of the volcano is between 1100 and 1300 mm y^{-1}. The warmest month is July with a daily mean temperature of 24-26°C rising to a maximum of 35°C. The coldest month is January with a mean daily temperature of 8-12°C falling to a minimum of 0°C.

Table 3.1. Details of the sites and soils from Mount Etna[a]

	Site	Age (years)	Altitude (m)	Soil type (USDA group)	Dominant vegetation	Soil forming materials and other information
1	Monti Rossi near Nicolosi	332	850	Entisol	*Ginesta aetnensis*	Tephra from 17th to 12th century eruptions – alkalic basalts, hawaiites, phonolitic tephrites and basic mugearites, with 1669 dated lava flow to the south
2	Monti Rossi near Nicolosi	332	850	Andisol	*Pinus nigra* L planted in 1960s	Tephra from 17th to 12th century eruptions – alkalic basalts, hawaiites, phonolitic tephrites and basic mugearites, with 1669 dated lava flow to the south
3	Salto del Cane c. 0.85 km south of the summit of cone	~7500	1300	Andisol	*Castanea sativa*	Lava from dated 1634-1638 flow
4	Salto del Cane c. 0.85 km south of the summit of cone	~7500	1300	Andisol	*Castanea sativa*	Upper Tuffs – yellow, fine grained tuffs showing a coarse layering (and sometimes plant remains). Deposited 7500 year BP, with the dated 1634-1638 lava flow to the west, north and east
5	Giarrita NW of Sant' Alfio east of Mt Frumento delle Concazze	5000	1450	Andisol	*Quercus cerris*	Products of the Leone eruptive centre. Sequence of mainly lavas exposed walls of Valle del Leone – hawaiites, phonlitic tephrites, mugarites and benmoreites
6	Giarrita NW of Sant' Alfio east of Mt Frumento delle Concazze	5000	1450	Andisol	*Quercus cerris*	Undated, but mainly historical tephra from 7th to 12th century BC – alkaline basalts, hawaiites, phonolitic tephrites and basic mugearites. 1865 dated lava flow to the north
7	Cassone WNW of Zafferana west of Mt Monaco	9700	1150	Andisol	*Castanea sativa*	Upper tuffs – yellow, fine-grained tuffs showing a coarse layering and sometimes including plant remains. Deposited 9700 years BP, with 1792 dated lava flow to the south
8	Cassone WNW of Zafferana west of Mt Monaco	3600	1400	Andisol	*Castanea sativa*	Lavas and associated tephra, alkalic basalts, hawaiites, phonolitic tephrites and basic mugearites. Deposited 3600 years BP.

[a]Most of the soil and vegetation data were obtained from Antonio Ioppolo (University of Catania)

3.2.2 Soil Chemical Properties

3.2.2.1 Carbon and Nitrogen Analyses

The soil that had been dried at 105°C to determine the moisture content of the soils, was ground in an MM200 ball mill (Retch) and sent to the Scottish Crop Research Institute in Invergowrie for carbon and nitrogen analysis using a Carlo Erba CHN analyser.

3.2.2.2 pH

Fresh soil, 2 g, was added to 10 cm^3 of distilled water and shaken at room temperature for 2 hr on a flat bed shaker. The soil solution was then allowed to settle for 1 hour before being measured on a pH meter with a glass electrode (Rowell 1994).

3.2.3 Soil Microbial Properties

3.2.3.1 Biomass Measurement (Substrate Induced Respiration)

Soil microbial biomass C and respiration activity (C mineralization rate) were determined using the glucose induced respiration approach of Anderson and Domsch (1978), as described by Hopkins and Shiel (1996).

3.2.3.2 Decomposition of Plant Litter

3.2.3.2.1 Preparation of the plant litter

The vegetation was collected in the Autumn of 2001 from the surface of each site. It was bagged and kept refrigerated until required for analysis. It was dried overnight in an oven set at 40°C. It was then roughly chopped with scissors before being more finely ground in a ball mill (Retch MM200), this was done to increase the surface area to enable a thorough extraction. Some (10 g) of the vegetation was "washed" to remove the water soluble fraction. This was done by placing the chopped plant material into a 500 cm^3 glass bottle to which 100 ml of distilled water was added. The bottles were agitated on a flat bed shaker for 3 h and filtered with the aid of a vacuum. The soluble fraction was collected and frozen at -18°C for

further analysis. The insoluble fraction was dried in an oven at 50°C for 48 hours. Once dry the insoluble and the intact vegetation were weighed into glass drum vials.

3.2.3.2.2 Experimental design

The soil was sieved to 2 mm. The water content was re-established as the soils may have dried out whilst in storage. Water was then added to the soil to bring the water content up to 50% water holding capacity. 20 g dry weight equivalent of soil was incubated with 0.4 g (2% by weight) of either intact or the water insoluble fraction of the vegetation. *Castanea sativa* was tested in soils 1-4, *Genista aetnensis* in soils 1 and 3 and *Pinus nigra* in soils 2 and 4. Each vial was labelled as shown by the legend in Table 3.2, and was placed in a 60 cm^3 syringe fitted with a 3-way luer lock tap (Vygon, UK). These were then incubated at 20°C in a temperature-controlled incubator. CO_2 evolution measurements were made on day 0, 1, 4, 8, 14, 28, 42, 56, 82 and 162 using a gas chromatograph (Varian 90-P GC fitted with a 1.32m long 3 mm internal diameter stainless steel column packed with 80/100 mesh Porapak Q and a thermal conductivity detector).

The decomposition of litter was estimated as the difference in cumulative CO_2 between soil amended with litter and the relevant control soil. The decomposition of soluble C, assuming no interactions with insoluble components, was estimated by the difference between the decay curves of the intact and the water insoluble fractions of the litter. First-order decay functions ($y = ae^{-kt}$) were fitted to all the curves using the curve-fitting function with the Sigmaplot package. The rate constant (k), mean residence time (MRT = $1/k$) and the readily mineralizable fraction (a) of the plant litter were all estimated.

Table 3.2 Set up of Decomposition Experiment

	Soil	Vegetation	Pre-treatment of litter
1	Mt Rossi NW flank	None	None
1	Mt Rossi NW flank	*Castanea sativa*	Intact
1	Mt Rossi NW flank	*Castanea sativa*	Water Insoluble
1	Mt Rossi NW flank	*Genista aetnensis*	Intact
1	Mt Rossi NW flank	*Genista aetnensis*	Water Insoluble
2	Mt Rossi W Flank	None	None
2	Mt Rossi W Flank	*Castanea sativa*	Intact
2	Mt Rossi W Flank	*Castanea sativa*	Water Insoluble
2	Mt Rossi W Flank	*Pinus nigra*	Intact
2	Mt Rossi W Flank	*Pinus nigra*	Water Insoluble
3	Mt Salto del Cane	None	None
3	Mt Salto del Cane	*Castanea sativa*	Intact
3	Mt Salto del Cane	*Castanea sativa*	Water Insoluble
3	Mt Salto del Cane	*Genista aetnensis*	Intact
3	Mt Salto del Cane	*Genista aetnensis*	Water Insoluble
4	Mt Salto del Cane	None	None
4	Mt Salto del Cane	*Castanea sativa*	Intact
4	Mt Salto del Cane	*Castanea sativa*	Water Insoluble
4	Mt Salto del Cane	*Pinus nigra*	Intact
4	Mt Salto del Cane	*Pinus nigra*	Water Insoluble

3.2.3.3 PLFA Analysis

This was carried out on all twenty soils. Soil, 2 g, was extracted and analysed using the same method described in Chapter 2.

3.3 Results

3.3.1 Soil Chemical Properties

The pH values were very similar for all 8 soils. They were all near neutral ranging from 6.2 to 6.9. The amount of carbon present in the Monti Rossi and Cassone soils were lowest with the older soils from Salto del Cane giving the highest. The C:N ratios showed a marked difference too with the biggest difference between the Monti Rossi sites and the Salto del Cane sites. Soils 7 and 8 were from well developed sites but when they were sampled they had just received a substantial input of tephra from a recent eruption. This was visible in the top layer of the soil sample taken and could account for the reduced carbon and nitrogen values. Soils 3, 4, 5 and 6 all had well established woodland cover and the high levels of carbon and nitrogen reflect this (see Table 3.3).

Table 3.3 Chemical Properties of the soils (n=3, SD in brackets)

	Site	Total C (mg C g^{-1} soil)	Total N (mg N g^{-1} soil)	C:N	pH
1	**Monti Rossi**	15.2 (1.06)	0.2 (0.40)	76.0	6.9 (0.10)
2	**Monti Rossi**	31.1 (2.16)	0.4 (0.45)	77.8	6.6 (0.06)
3	**Salto del Cane**	89.7 (2.09)	5.2 (1.06)	17.3	6.4 (0.12)
4	**Salto del Cane**	40.6 (12.9)	2.7 (0.71)	15.0	6.6 (0.21)
5	**Giarrita**	34.3 (8.29)	1.4 (0.29)	24.5	6.2 (0.20)
6	**Giarrita**	37.2 (5.47)	1.5 (0.13)	24.8	6.3 (0.16)
7	**Cassone**	27.8 (8.56)	1.6 (0.50)	17.4	6.6 (0.06)
8	**Cassone**	19.4 (7.32)	1.0 (0.32)	19.4	6.5 (0.23)

3.3.2 Soil Microbial Properties

The microbial biomass C contents showed that soils 1 and 2 had the smallest biomass (as was predicted as they are young developing soils) and soils 3 and 4 had the largest microbial

biomass (these being the older more developed soils) (Table 3.4). The basal respiration and the respiratory quotient did not differ much between the soils.

Table 3.4 Microbial properties of the soils

	Site	SIR (µg CO_2-C g^{-1} soil $hour^{-1}$)	Biomass C (mg biomass C g^{-1} soil)	Biomass C: total C ratio	Basal respiration (µg CO_2-C g^{-1} soil $hour^{-1}$)	Basal respiration (µg CO_2-C g^{-1} soil C $hour^{-1}$)	Respiratory quotient (qCO_2) (mg CO_2-C g^{-1} biomass C $hour^{-1}$)
1	**Monti Rossi**	3.5 (0.83)	0.26 (0.062)	0.017	0.37 (0.29)	24.3	1.4
2	**Monti Rossi**	3.6 (0.58)	0.26 (0.043)	0.0084	0.22 (0.091)	7.1	0.84
3	**Salto del Cane**	9.5 (0.46)	0.71 (0.034)	0.0079	0.56 (0.35)	6.2	0.79
4	**Salto del Cane**	6.9 (0.28)	0.51 (0.022)	0.013	0.34 (0.19)	8.4	0.67
5	**Giarrita**	3.2 (0.64)	0.23 (0.048)	0.0067	0.19 (0.099)	5.5	0.82
6	**Giarrita**	4.1 (0.67)	0.31 (0.050)	0.0083	0.27 (0.073)	7.3	0.87
7	**Cassone**	4.3 (0.43)	0.32 (0.032)	0.012	0.29 (0.062)	10.4	0.91
8	**Cassone**	2.7 (0.83)	0.20 (0.062)	0.010	0.15 (0.065)	7.7	0.75

3.3.3 Plant litter decomposition

3.3.3.1 Plant litter characteristics

The carbon contents of the 3 types of plant litter were similar but the nitrogen contents differed greatly, resulting in C:N ratios ranging from 38 for *Ginesta aetnensis* to 145 for *Pinus nigra*. Extraction of the water soluble fraction had little effect on the C:N ratio in *Pinus nigra*. In the *Ginesta* and *Castanea* plant litter the C:N ratios both increased, from 38 to 54 and 66 to 84 respectively (Table 3.5).

Table 3.5 Characteristics of plant litter. Each value is the mean of three replicates and the standard deviations are shown in brackets

Plant species	Total C (g C g^{-1} litter)	Total N (g N g^{-1} litter)	C:N of litter	Insoluble mass[1] (g g^{-1} litter)	Insoluble C (g C g^{-1} litter)	Insoluble N (g N g^{-1} litter)	C:N of insoluble fraction	Soluble mass (g g^{-1} litter)	Soluble C (g C g^{-1} litter)	Soluble N (g N g^{-1} litter)
Genista aetnensis	0.459 (0.0094)	0.012 (0.0005)	38	0.53	0.266 (0.0154)	0.0049 (0.00032)	54	0.47 (0.0076)	0.193	0.0071
Pinus nigra	0.463 (0.012)	0.0032 (0.0003)	145	0.61	0.270 (0.0116)	0.0018 (0.00018)	148	0.39 (0.0029)	0.193	0.0014
Castanea sativa	0.438 (0.0153)	0.0066 (0.0002)	66	0.60	0.269 (0.012)	0.0032 (0.00024)	84	0.40 (0.0073)	0.169	0.0034

3.3.3.2 Plant litter decomposition

The intact litter decomposes faster than the water insoluble fraction except for 1 case but even then the insoluble fraction of the litter is not significantly faster that of the intact (Fig. 3.2). The difference in the two fractions is significant in soils with the *Pinus* and *Ginesta* addition. The removal of the water soluble fraction has virtually no effect on the decomposition of *Castanea* plant litter with both the intact and insoluble fractions giving similar values. Looking at the different ages of the soils the difference between the litter types is greater in 3 than 1.

The decomposition of the plant material in the soil all obeyed first order kinetics and the results are detailed in Table 3.6.

Table 3.6 First–order decomposition parameters (*k*, decomposition rate constant, and *a*, fraction readily mineralized) for decomposition of litter and the insoluble fraction from litter of *Castanea*, *Ginesta* and *Pinus* in soils from Mt Etna. The R^2 values are for the regression of the experimental data

	Soil	Litter	Litter treatment	k ($days^{-1}$)	sd	a (%)	sd	R^2
1	Monti Rossi	*Castanea sativa*	Intact litter	0.030	0.0029	17.0	0.62	0.989
			Insoluble fraction	0.023	0.0026	9.1	0.45	0.987
		Ginesta aetnensis	Intact litter	0.056	0.0041	19.1	0.42	0.993
			Insoluble fraction	0.027	0.0019	10.1	0.28	0.995
2	Monte Rossi	*Castanea sativa*	Intact litter	0.063	0.0091	16.8	0.70	0.971
			Insoluble fraction	0.040	0.0056	7.4	0.35	0.975
		Pinus nigra	Intact litter	0.061	0.0106	13.1	0.65	0.958
			Insoluble fraction	0.074	0.0073	5.1	0.14	0.987
3	Salto del Cane	*Castanea sativa*	Intact litter	0.088	0.0067	18.6	0.366	0.992
			Insoluble fraction	0.075	0.0084	12.0	0.360	0.984
		Ginesta aetnensis	Intact litter	0.074	0.0086	21.6	0.665	0.983
			Insoluble fraction	0.073	0.0165	6.4	0.390	0.935
4	Salto del Cane	*Castanea sativa*	Intact litter	0.073	0.0108	25.0	1.00	0.970
			Insoluble fraction	0.073	0.0098	14.1	0.51	0.976
		Pinus nigra	Intact litter	0.098	0.0144	19.1	0.71	0.968
			Insoluble fraction	0.060	0.0083	9.9	0.40	0.974

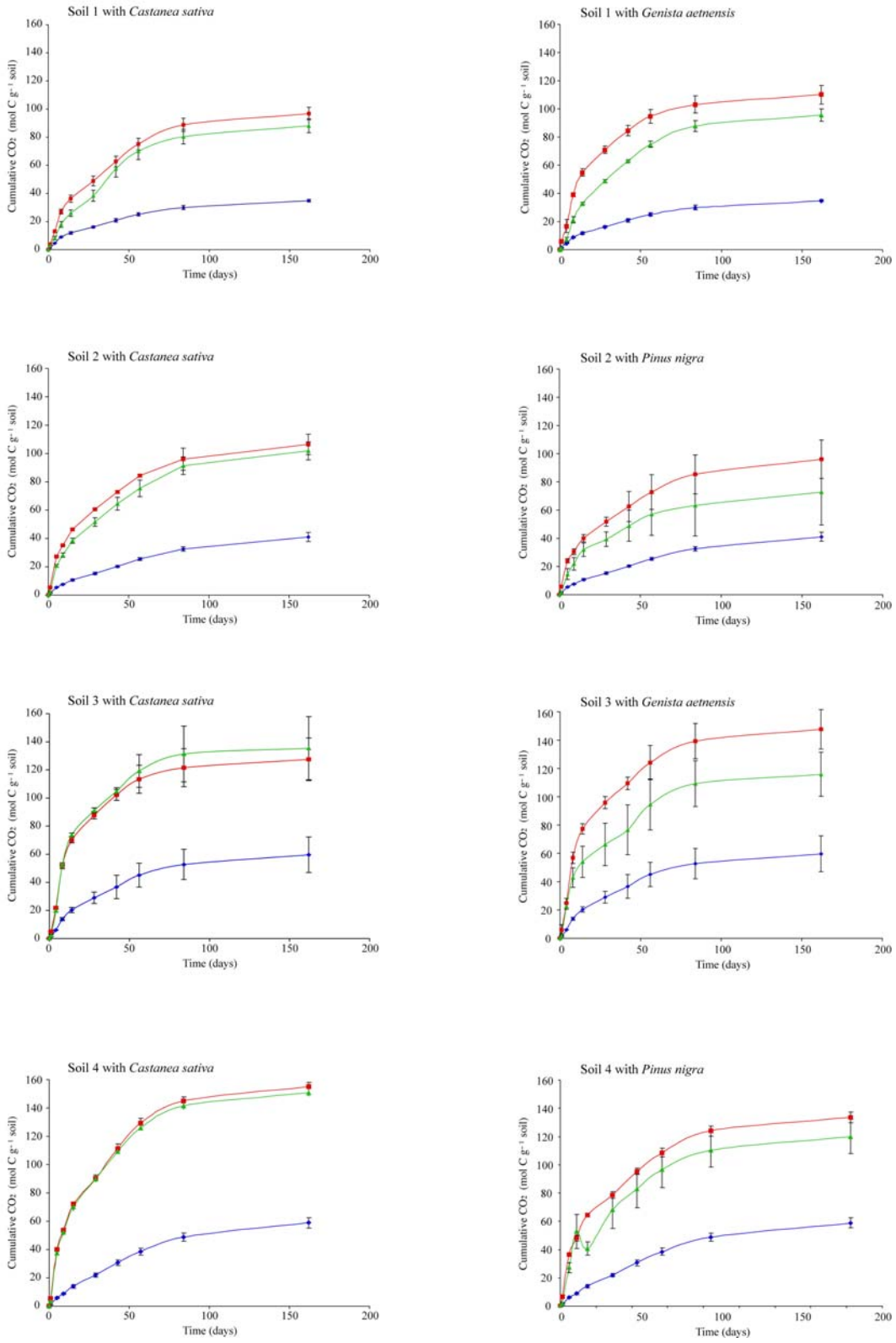


Figure 3.2 Graphs showing decomposition of intact and water insoluble plant residues in soils 1-4 The blue line is the control soil, the green line is the water insoluble fraction of the plant litter and the red line is the intact litter n=3.

3.3.4 PLFA analyses

Once again we looked at the Shannon Weaver index as an indicator of diversity and looked at individual biomarkers in the soils. Soil 1 had the lowest diversity with both soils 5 and 8 being low as well. As predicted soils 3 and 4 had the largest diversity, with 4 being slightly higher that 3.

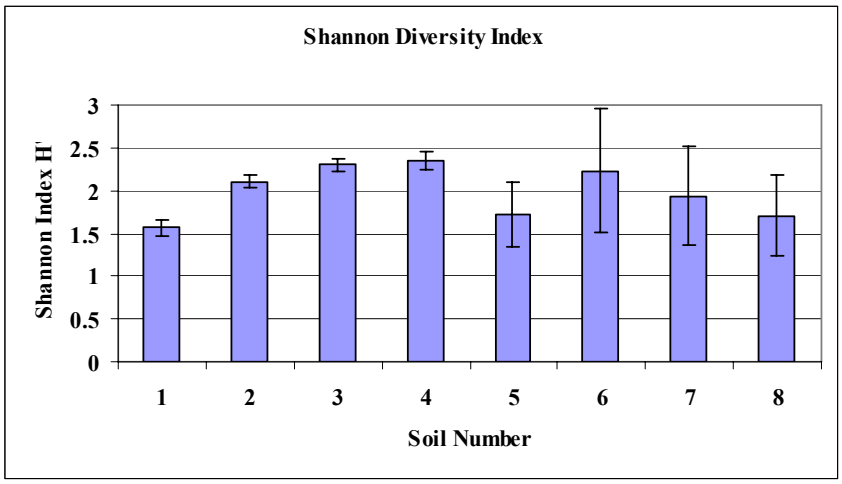


Figure 3.3 Graph showing Shannon Diversity in the eight soils from Mt Etna. n=3

We then looked at the evenness data from the soils and noticed again that soils 1, 5 and 8 had the lowest evenness scores but this time 1 was not the lowest. Soil 4 has the most even diversity with soil 2 and 3 both having a high score.

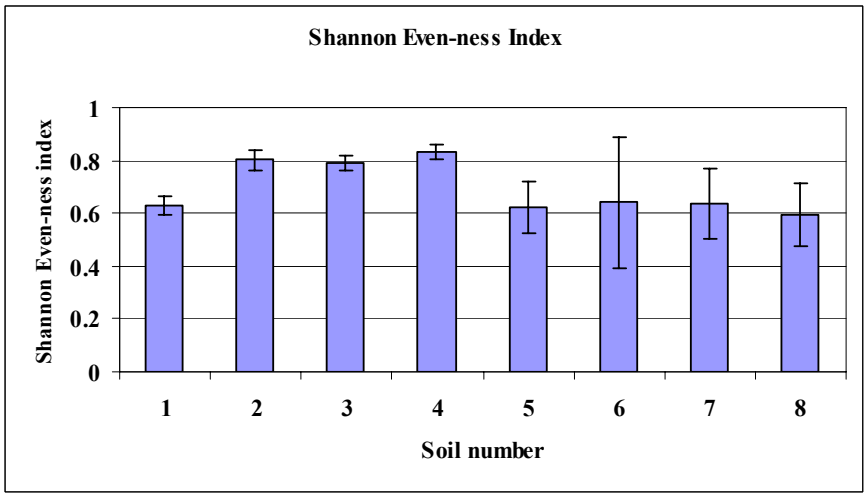


Figure 3.4 Graph showing Shannon Diversity Even-ness in the eight soils from Mt Etna. n=3

We looked at the bacterial biomarker i-C15:0 this time and found that it was lower in soils 1, 7 and 8. Soil 6 has the highest abundance of the biomarker with soils although there was only a 5 % difference between the lowest and highest values.

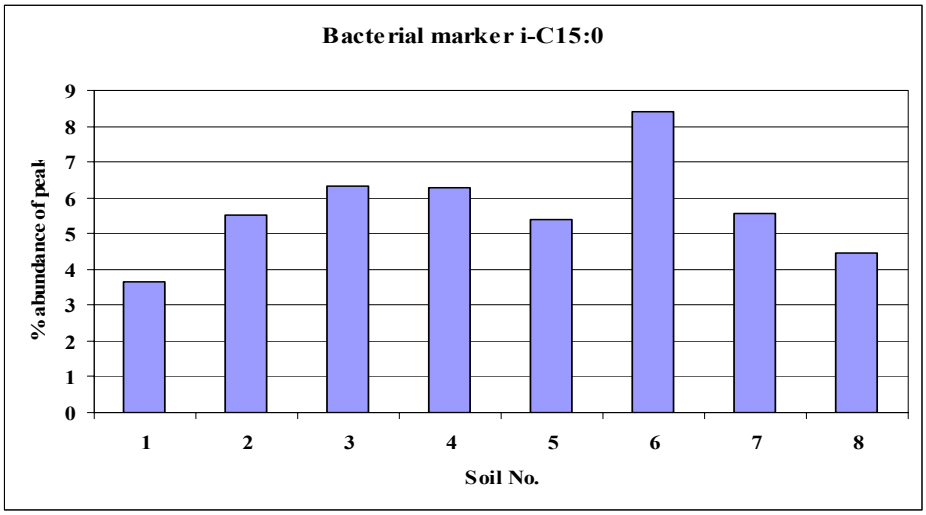


Figure 3.5 Graph showing the abundance of the bacterial biomarker i-C15:0 in the soils.

The fungal biomarker is much lower than the bacterial marker in soils 3 and 4 and much higher that the bacterial marker in soils 1 and 2. The fungal marker and the bacterial marker were similar for soils 5, 6, 7 and 8.

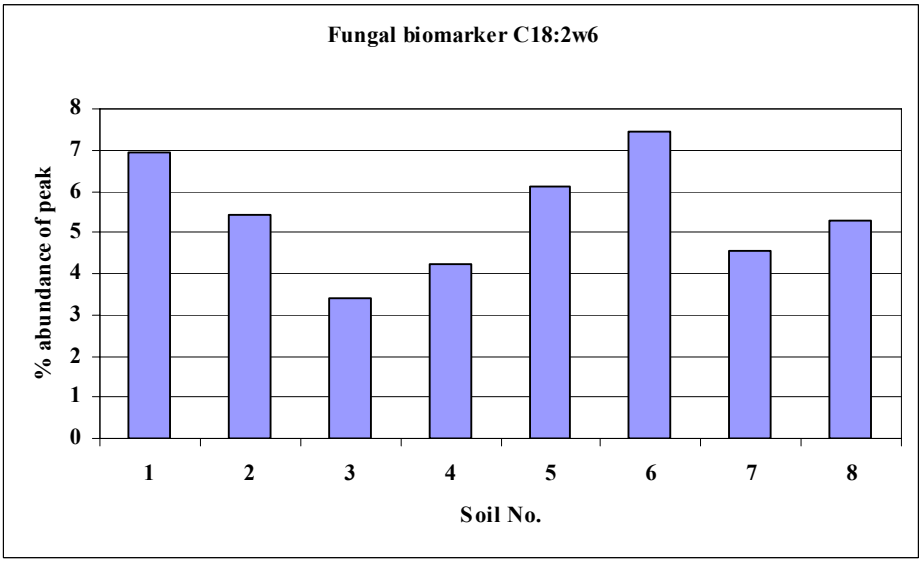


Figure 3.5 Graph showing the abundance of the fungal biomarker C18:2ω6 in the soils

3.4 Discussion

3.4.1 Soil Chemical and Microbial Properties

The soils chosen for this study encompassed a range of soils from different stages in their development, from the young soils 1 and 2 to the more developed older soils 3 and 4. There was no absolute definitive dating for these soils, these classifications are made primarily on field observations. The soils are all of a similar neutral pH. According to Certini *et al*. 2001 the soil pH under pine was slightly lower than that found under broom. Soil 1 was from beneath *Genista aetnensis* and soil 2 was from under pine. The pH of soil 1 was higher than that of soil 2, confirming what had been previously documented. The less well developed soils 1 and 2 had low carbon and nitrogen levels and but they were greater in 5, 6, 7 and 8 with the highest values for soils 3 and 4 which were the more mature, well established soils. This trend followed for the microbial properties of the soils. The microbial biomass, respiration rates and respiratory quotients of the Etna soils are similar to and comparable with other volcanic soils (Wardle & Ghani 1995; Joergensen & Castillo 2001; Nishiyama *et al.* 2001).

3.4.2 Plant litter decomposition

The intact litter always had a higher rate of decomposition than the insoluble fraction in all but one case showing that the removal of the labile water soluble component had an effect on decomposition. *Castanea sativa* is the only plant material tested in all 4 soils and showed only a small difference between the intact and insoluble fraction of the plant litter between the soils, but there was a marked difference between the decomposition in the younger soils 1 and 2 compared with the older soils 3 and 4. The decomposition rate constant (k) is 0.030 and 0.027 for soils 1 and 2, respectively, but is 0.088 and 0.073 for soils 3 and 4 (rate for

decomposition of intact litter). The total amount of CO_2 produced is also much higher in the older soils than the younger soils, suggesting that there is either a more active or larger biomass in the older soils.

The removal of the water soluble fraction from the plant litter had virtually no effect on the decomposition of *Castanea sativa* in all the soils. This suggests that either the stable fraction of the plant litter is actually quite labile or removal of the water soluble portion removes some of the stable material too. *Genista aetnensis* and *Pinus nigra* showed a more significant difference between the decomposition of the intact and insoluble fraction with the latter being lower. Perhaps the stable material was inaccessible to the soil organisms present. This does not seem to be age related as the differences between the intact and insoluble fraction of the plant litter were the same in both the old and young soils.

The Shannon Weaver index showed that soil 1 had the lowest diversity, consistent with the observational data that soil 1 is an entisol and all the others are andisols. According to Nordt *et al.* 2000, the term entisol is used to describe a "mineral soil that has the ability to support life". This is the only thing that distinguishes it from material that is not soil e.g. rock. These soils are found in areas where soil formation has been restricted which may be explained by the recent deposition of volcanic ejecta in this region. Soil 2, although the same age as soil 1 is described as an Andisol and therefore has a slightly higher diversity. Soils 3 and 4, as predicted, have the highest diversity as the microbial biomass is probably larger and perhaps more active.

3.5. Conclusion

The hypotheses tested in this chapter were to find out whether there is a difference between decomposition processes in young developing volcanic soils compared with older more established volcanic soils, and to assess what effect the removal of the water-soluble fraction of the plant litter had on the decomposition processes.

The experimental work has shown that young volcanic soils have lower levels of carbon and nitrogen as well as a smaller diversity (from the analysis of PLFAs) compared with the older soils. The microbial biomass, respiration rates and respiratory quotients of the soils increased with age with the exception of one of the soils that had recently been subjected to new ash deposits.

The decomposer communities of the younger soils find it more difficult to utilise the insoluble fraction of the plant litter compared with the older soils. This is probably because the microbial biomass is not so well equipped to deal with the larger, less labile, material than the more established biomass in the older soils which can do this more efficiently.

These data suggest that young volcanic soils, though less efficient at decomposing organic matter, nonetheless have the capacity to assimilate rapidly a more complex microbial biota and in turn increase decomposition rate as a function of time.

Chapter 4

Concluding Discussion

The objectives set out at the beginning of this thesis were to characterise, in terms of biomass, community composition and activity, the properties of the microbial communities of soils from the Maritime Antarctic and developing volcanic soils, and, where possible, to compare properties of these soils both within and between the two sets of sites. This research has covered two contrasting soils types and ecosystems, which limits the scope of the interpretation. However, in summary, this work has shown the following:

Unsurprisingly, the soil from Antarctica contained very little carbon and nitrogen, and had a low microbial diversity by comparison with most temperate soils. The principal reason for this is the extreme environmental conditions and the resource-limitation. There was, however, a large potential for microbial activity as indicated by the fact that when plant residues were added to the soils, decomposition (CO_2 production) increased by more than 7 fold over the relatively short period of 27 days. Evidence from the PLFA biomarkers indicated that the bacterial community stayed relatively stable throughout this period of decomposition, but that the fungi initially increased very rapidly and then declined as the readily mineralizable C was exploited. It seems likely that the rapid increase in fungal biomass was due, in part at least, to the introduction along with the plant residues, rather than growth of indigenous fungi. This would, however, require further study which is beyond the scope of the current project. During plant residue decomposition the diversity of the microbial community increased, as indicated by the Shannon index; microbial diversity increased to its maximum at 27 days, coincidental with the rapid phase of CO_2 production.

The carbon substrate analyses showed that the range of compounds used rapidly declined during plant residue decomposition indicating a functional shift in the microbial community. The details of this shift will need to be investigated in a subsequent study. In addition, it is worth pointing out that the alternative approach to interpreting Biolog data based on the response time to the substrate utilization, rather than the maximum rate at which the total C mineralized, is proposed. This too could be the subject of subsequent investigation.

The project on Mt Etna posed slightly different hypotheses. These were, first, to find out whether there was a difference between decomposition processes in young developing volcanic soils compared with older more established volcanic soils, and second, to assess what effect the removal of the water-soluble fraction of the plant litter had on the decomposition processes.

The experimental work presented in Chapter 3 showed that young volcanic soils contained less carbon and nitrogen compared with the older soils. Results from the PLFA analyses also indicated lower microbial diversity in the younger soils. The microbial biomass, respiration rates and respiratory quotients of the soils increased with age, with the exception of one of the soils that had recently been subjected to new ash deposits. This observation illustrated one of the recurrent difficulties with site selection and experimental work on the volcano: the fact that repeated, small volcanic inputs, which often went unreported because they were not the result of major eruptions, led to 'uncontrolled' changes in the soil. Nevertheless, it was possible that the decomposer communities in the younger soils were less able to utilise the insoluble fraction of the plant litter compared with the older soils. This is probably because the microbial community was not so well equipped to deal with the more resistant plant residues compared to the better established and larger communities in the older soils.

Although the conclusions that can be drawn from the work presented here are constrained, to some extent, by the availability of experimental materials, the novel aspect of this work is that a comparison could be made from two such diverse field sites such as Antarctica and Mt Etna. Both of these systems were resource-limited but in different ways, and both showed similar properties.

Future work on the Antarctic project could include further analysis of the *Deschampsia antarctica* plant material to see if the fungi are indeed present on the leaves. This did not present itself until the data was analysed some time after the incubation experiment. It would also be interesting to look at a change in soil temperature change in addition to measuring the change in air temperature.

A methodological development that would improve the PLFA analysis in both field sites would be to use a quantitative standard. This would give an indication of the amount of PLFAs present, and therefore offer more information about the microbial community present.

In conclusion, perhaps the soils from the Antarctic and the younger volcanic soils could be the simple ecosystem proposed by Eugene Odum in 1969 for assessing microbial community development.

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