Structural diversity and decomposition functions of volcanic soils at different stages of development

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Table of Acronyms

MRP	**Monti Rossi *Pinus***
MRG	**Monti Rossi *Genista***
MRc	**Monti Rossi control**
MRd	**Monti Rossi disturbed**
SDCA	**Salto del Cane A**
SDCB	**Salto del Cane B**
SDc	**Salto del Cane control**
SDd	**Salto del Cane disturbed**
PLFA	**Phospholipid Fatty Acid**
SATFAs	**Saturated Fatty Acids**
MUFAs	**Monounsaturated Fatty Acids**
PUFAs	**Polyunsaturated Fatty Acids**
OHFAs	**Hydroxy Fatty Acids**
NMR	**Nuclear Magnetic Resonance**
CRP	**Catabolic Response Profile**
SIR	**Substrate Induced Response**
PCA	**Principal Components Analysis**

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This thesis contains no other material that has been previously submitted for the award of any other degree or diploma. It contains no material previously published or written by another person except where due reference has been made in the text.

Laura- Lee Shillam

Chapter 1 Introduction

The reservoir of organic C contained in the earth's soils is larger than the atmospheric and biological compartments combined (Schimel, 1995). Globally, estimates of the total amount of organic C in soils is between 1500 and 2000 Pg (Hopkins and Gregorich, 2003). Soil microorganisms are the major direct contributors to C flux from soils to the atmosphere. Global temperatures are envisaged to rise between 1.5oC and 4.5oC by 2050 (Grogan and Jonasson, 2005). Within a limited range, a rise in temperature increases the rate of microbial activity including enzyme synthesis and activity. This may lead to an increase in the release of C from soil as CO_2 as a result of increased microbial activity. Given the huge reservoir of organic C in soils and the key role of micro-organisms within soils, it is apparent that the nature and function of the microbial community may play a part in the release of C from soil reserves. In this respect, there is a need to understand the role of microbial community diversity in soil ecosystem functioning.

Agenda 21, a document from the United Nations Conference on Environment and Development, prepared in Rio de Janeiro in 1992 called for a greater understanding of interactions between diversity and ecosystem functioning. This is due to the realisation that diversity may have an important role in maintaining functioning during times of disturbance. Further experimental work is required in order to assess whether community diversity and functioning are related in soil systems.

Soils formed on volcanic deposits present an opportunity to investigate microbial primary successional processes in an environment where soil formation and

development occurs under relatively similar climatic conditions. Upon the extrusion of lava, land is made sterile and a virgin surface is created. Repeated eruptions create areas at different stages of development which in many volcanic regions have been reliably mapped for centuries. Investigation of soil formed on these volcanic chronosequences can provide information about the nature, diversity and resource efficiency of the soil microbial community through time.

1.1 Volcanic Soils

Volcanic ash soils or Andisols (Soil Taxonomy; Andosols, FAO- WRB) are principally derived from volcanic ash or ejecta which is distinct from other types of magmatic rocks due to its rapid rate of weathering (Arnalds, 2004). Although volcanic soils are distributed throughout the world, most scientific investigations into the nature and properties of volcanic soils have been carried out in Japan, the USA and New Zealand (Bartoli et al., 2003). Although this information is useful, caution must be taken when extrapolating the results to other volcanic regions of the world. Topographic and climatic differences as well as variation in type of volcanic parent material will all have an effect on soil forming characteristics.

1.1.1 *Soil Formation on volcanic materials*

Soil formation is influenced by five key factors: climate, biological activity, parent material, topography and time. The obvious difference between volcanic soils and other soils is the nature of the parent material. Volcanic ash or tephra is often unconsolidated and different components weather at different rates. The weathering process is influenced by climatic factors which mean that different areas globally promote the genesis of different classes of volcanic soils with different chemical, physical and biological properties as discussed below.

1.1.2 ***Classification of volcanic soils***

In order to be classified as an andisol, soils must fulfil certain criteria. Soil Taxonomy is the most widely used system for the international classification of volcanic ash soils (Takahashi and Shoji, 2002). Two sets of criterion must be met in order to be classified as an Andisol although the central concept if volcanic ash derived soil is that of a soil developing on volcanic ash or ejecta. Criterion 1 requires the soil to have an *andic* horizon whereby a high degree of weathering is exhibited as determined by ammonium oxalate extractable Al plus Fe (Al+ 1/2 Fe^{o}). Typically these soils have a low bulk density and a high degree of phosphate retention. Developing volcanic soils tend to lack this *andic* horizon as weathering is not pronounced enough to create one. Criterion 2 is used to classify these less weathered volcanic materials which have a *vitric* horizon as opposed to an *andic* horizon. The bulk densities of these soils with *vitric* horizons are higher than those of *andic* horizons and the phosphate retention capacity is generally less but still greater than 25%. Criterion 2 also requires that soils contain a high concentration of volcanic glass. Andisols are further divided into seven suborders according to their physical and chemical variances: Aquands, Cryands, Torrands, Xerands, Vitrands, Ustands and Udands (Soil Survey Staff, 1999) (Table 1-1).

Table 1-1 Suborders of Andisols and their characteristics (Soil Survery Staff, 1999)

Suborder	Characteristics
Aquands	Aquic moisture regime due to restricted drainage. Found globally under a range of climatic conditions.
Cryands	Volcanic soils of cold regions. Often formed under coniferous vegetation.
Torrands	Formed in arid areas of the world. Chemical weathering is restricted under the dry conditions.
Xerands	Xeric moisture regime. Humification of organic matter is deemed to be most favourable under xeric conditions.
Vitrands	Commonly derived from pumice, cinder or scoria and is weakly weathered.
Ustands	Ustic moisture regime. Occur mainly in Mexico, western parts of the USA, the Pacific Islands and parts of Eastern Africa.
Udands	Udic moisture regime and are the most widely used for agriculture.Occur in humid temperatures so weathering rates are fast.

Emphasis will be placed on the Xerand and Vitrand suborders as the soils of the study sites are classified as such.

1.1.3 ***Chemical and physical properties of Andisols***

The very nature of volcanic ejecta i.e. the small particle size, the high porosity and permeability of tephra, enhance chemical weathering (Ugolini and Dahlgren, 2002). This rapid weathering often produces amorphous or poorly crystalline silicate materials such as allophane and imogolite (Brady and Weil, 2002). Ferrihydrite and Al- and Fe- oxides are also often distinctive chemical components of Andisols which results in the surfaces of soil colloids being positively charged and as a consequence strong covalent bonding of soil organic matter often occurs (Joergensen and Castillo, 2001). The nature of these distinctive materials give

Andisols unique properties. These include a low bulk density, high water retention and phosphate retention (Arnalds, 2004).

1.1.3.1 pH

According to Arnalds (2004), vitrands commonly have a near neutral pH. As such, it can be inferred that they will have a relatively low organic C content in comparison to soils with an andic horizon as there is a distinct trend towards lower pH with increasing organic C content (Arnalds, 2004). In an active volcanic environment, the little organic matter present in vitrisols may be further diluted by the input of volcanic ash and ejecta. Soils with a pH of less than 4.9 have been observed to inhibit allophane formation (Shoji and Fujiwara, 1984).

1.1.3.2 Crystalline and non crystalline materials

As previously noted, one of the distinctive characteristics of volcanic soils is the presence of large quantities of non crystalline materials such as allophane and imoglite. This allophane formation is inhibited not only by high pH values but by large quantities of organic matter which forms complexes with Al (Dahlgren et al., 1993).

These non crystalline materials have high surface areas, an amorphous structure and variable charge (Powers and Schlesinger, 2002). These characteristics are responsible for stabilising soil organic matter by increasing the residence time of C in soil and decreasing its availability to microorganisms by physical or chemical protection (Powers and Schlesinger, 2002).

Through time and in warmer, dryer climates; crystalline minerals form at the expense of non crystalline materials indicating a reduction in allophane with soil age (Ungoli and Dahlgen, 2002).

1.1.4 ***Biological properties of Andisols***

Few studies have been conducted regarding the soil microbial community and their activity in volcanic ash soils. As such there is relatively little information from which to extrapolate the behaviour of the Etna soil community. Much of the biological information regarding volcanic ash soils concerns topics such as: soil fertility and soil quality (Castillo and Joergensen, 2001), N cycling (Rhoades and Coleman, 1999), soil C distribution (Powers and Schlesinger, 2002) etc. As yet, no one has investigated the diversity of developing volcanic soils such as those found on Mount Etna.

1.1.4.1 Carbon contents of volcanic soils

The weathering environment to which volcanic soils are exposed, is of extreme importance as it influences the release of minerals and governs which materials are formed. As discussed, the formation of crystalline and non crystalline complexes within volcanic soil affects the entire soil environment and thus nutrient cycling and inevitably the soil community. Comparison of the soil biological properties of global volcanic soils may appear to be slightly fruitless in this respect as many biological factors are extremely variable. As well as climate being responsible for this variability, time also has a marked effect on the biological characteristics of volcanic soils (Joergensen and Castillo, 2002). In developing vitric soils created in warm, wet environments such as those of Nicaragua, soil C levels can be fairly low

(between 9 and 26.1 mg g^{-1}) (Joergensen and Castillo, 2002). This is vast in comparison with some Icelandic vitrisols which often have soil C content of less than 1% (Arnalds, 2004). Japanese volcanic soils are again in contrast with organic C contents as high as 154 mg g^{-1} in a study by Nishiyama et al (2001). Studies of similar areas on Mount Etna have shown marked contrasts in certain soil properties. Two studies conducted at sites on Monti Rossi gave markedly different results of total C and N values. Hopkins et al (2007) found total C values to range between 15.2 and 31.1 mg C g^{-1} soil where as Sanjurjo et al (2003) found organic C values to be around 69.4 mg g^{-1}. This may be due to the techniques employed to measure soil C, microscale differences in C or may be due to a dilution effect caused by the influx of fine ash and tephra from the volcano. Dilution of organic matter by recently ejected tephra particles has been reported in fields in Nicaragua (Velasquez- Pereira, 1996).

1.1.4.2 Microbial biomass in volcanic soils

Very little information is available regarding microbial biomass in developing volcanic ash soils. In a study of volcanic soils in Japan, Nishiyama et al (2001) found biomass C in volcanic soils ranging from 54.9- 917 mg kg^{-1} soil. Conversely, the young vitric soils of Nicaragua were found to contain microbial biomass C of between 44 and 233 mg kg^{-1} (Joergensen and Castillo, 2002). Hopkins et al (2007) recorded biomass C values of between 260 and 710 mg kg^{-1} at sites of varying age on Mount Etna. The variability of these results suggest that soils found in extremely dynamic environments such as those located within the regular path of volcanic ejecta are very rarely stable for long periods of time and are subject to regular disturbance.

1.2 Decomposition

During the process of decomposition, the chemical and physical states of a substrate are changed as organic matter is broken down into inorganic constituents. Decomposition in soils is mediated by the activities of microbial communities which are regulated by the physico- chemical environment and substrate quality (Heal et al., 1997). The process of decomposition is as fundamental to long term ecosystem functioning as primary production (Zak et al., 1994, Øvreås, 2000).

Three distinct but concurrent processes contribute to organic matter breakdown. These processes are leaching, comminution and catabolism (Swift et al., 1979).

1.2.1 *Decomposition processes*

1.2.1.1 Leaching

Leaching is the removal and transport of soluble materials from a substrate by the action of water. This abiotic process causes weight loss and change in chemical composition (Swift et al., 1979). The leaching action of water removes labile components from the substrate but discrete particles are often washed further down the soil profile where by they can be further degraded by the activity of microorganisms. Wachendorf et al (1997) found that leaching contributed to an 18% C loss from litter in an area characterised as a dry site while 30% C loss occurred from similar litters at a wet site. This indicates that leaching is an important factor in removing nutrients from litter and soil.

1.2.1.2 Comminution

The reduction in size of organic materials as a result of the feeding activity of microorganisms is known as comminution (Heal et al., 1997). Wetting and drying cycles, freezing and thawing are amongst some of the abiotic factors which can also be responsible for communition (Swift et al., 1979). Comminution differs from catabolism as it is a largely physical rather than a chemical process (Swift et al., 1979). As most organic matter is comminuted through ingestion and digestion of soil animals, it is almost always accompanied by catabolic reactions resulting in a product which is both physically and chemically altered.

1.2.1.3 Catabolism

Carbohydrates, proteins and lipids provide an energy rich source of nutrition for soil organisms. The biodegradation of these substances and other organic compounds is known as catabolism (Fuhrmann, 2005). In biochemical terms, catabolism describes energy yielding enzymatic reactions involving the transformation of complex organic compounds to smaller simpler molecules such as the enzyme mediated degradation of cellulose to glucose (Swift et al., 1979).

1.2.2 ***Factors controlling decomposition***

1.2.2.1 The Physico- chemical Environment

There are many factors influencing the rate of plant litter decomposition that are included within the title physico- chemical environment. At the broad scale climatic factors such as rainfall and temperature clearly have an effect on the soil community responsible for decomposition. The type of soil and associated properties such as pH, aeration and soil texture also have an effect on the decomposer community.

Temperature is one of the most important environmental factors influencing the rate of decomposition. The effect of this variable quite obviously fluctuates markedly over the surface of the Earth, and both seasonally and diurnally. Rates of biological, chemical and physical processes within the soil are greatly influenced by soil temperature. Within a limited range, chemical reactions and biological processes double for every 10°C increase in temperature (temperature coefficient Q_{10}), (Hartel , 2005; Swift et al., 1979). Microorganisms have an optimum growth temperature at which they exhibit their maximum rates of growth and reproduction. Enzymatic activity can cease for many mesophiles at extremes of temperature due to the denaturing of enzymes at high temperatures and metabolic inactivity at low temperatures (Atlas and Bartha, 1987). Below around 5°C, a standard temperature commonly referred to as biological zero, most microbial activity and decomposition ceases (Brady and Weil, 2002). Various soil microorganisms do not conform to this generality such as snow mould fungi (Killham, 1994). This particular fungus is capable of decomposing leaves buried under snow. Temperature also has many indirect effects on the soil environment and soil biological activity. As well as influencing rates of weathering and redox potentials etc, temperature also has a marked effect on the behaviour of plants which in turn affect the soil biological environment (Killham, 1994). Small changes in temperature can influence the growth of plant roots and shoots thus altering water and nutrient uptake etc.

Moisture content within soils is a particularly important physico- chemical factor due to its direct effect on the soil microbes and also its indirect effect on them through other environmental factors (pH, aeration etc). Bacteria tend to be confined to water filled pore spaces but fungal hyphae have the ability to grow across air

filled pore spaces (Killham, 1994), therefore, the presence and location of soil water creates niches within the soil habitat in which different organisms can exist.

1.2.2.2 Resource Quality

A wide range of materials are used as substrates for decomposition and according to Hopkins and Gregorich (2005), substrates form a "*continuum from recently added plant litter and carbon transferred into the soil as root exudates and via mychorrhizas, to very stable, highly altered organic matter.*" Plant litter is the primary substrate for heterotrophic decomposition in soil ecosystems. These plant litter residues i.e. leaf tissue, roots, reproductive structures, do not decompose at the same rate or as whole units. A typical dry composition of green plant material is: cellulose 45%, lignin 20%, hemicellulose 18%, protein 8%, sugars and starches 5%, polyphenols 2%, fats and waxes 2% (Brady and Weil, 2002). Composition is an important factor in determining the decomposition rate of a substrate as certain litter components are more biodegradable than others (Wolf and Wagner, 2005). This is due to the selective nature of soil microorganisms as the soil community produces many different degradative enzymes. In general, the more recalcitrant a material is, the lower the frequency of its degradative enzyme in the microbial population (Wolf and Wagner, 2005). Broder and Wagner (1988) investigated the decomposition of wheat straw and its major constituents (cellulose 50%, hemicellulose 25% and lignin 20%). Following analysis after 300 days *in situ,* it was found that certain constituents were very easily degraded by microorganisms but others persisted in the soil. Cellulose, the most abundant polysaccharide was shown to have decomposed more rapidly than the residue as a whole with only 20% remaining at the end of the investigation. Hemicellulose, another polysaccharide was completely

exhausted in the soil system after only 160 days indicating that it is a readily available resource for the microbial biomass. Lignin however decomposed at a much slower rate due to the stability of its chemical structure.

Cellulose, the most common structural polysaccharide in plants is made up of a linear chain of glucose units joined by β 1-4 linkages (Figure 1-1).

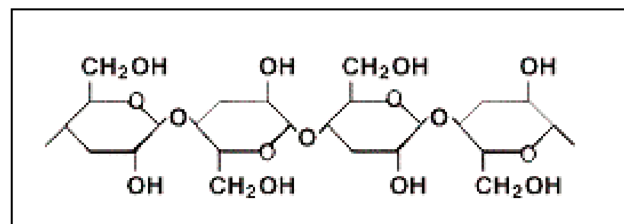


Figure 1-1 Structure of cellulose

Due to the large size of the insoluble cellulose polymer, it cannot directly enter the microbial cell. The cleavage of cellulose is achieved by three main enzymes collectively known as the cellulases (Wolf and Wagner, 2005) (Figure 1-2). The enzymatic degradation of cellulose is initiated by endo-β-1,4- glucanase (EC 3.1.2.4) which causes the loss of the crystalline- like structure followed by depolymerisation (Turner et al., 2002; Wolf and Wagner, 2005). The small chains of glucose are further degraded by the action of cellobiohydrolase also known as β-1,4- exoglucanase (EC 3.1.2.91) which results in linear chains of glucose units, normally two or three long which are called cellobiose and cellotriose respectively (Turner et al., 2002; Wolf and Wagner, 2005).

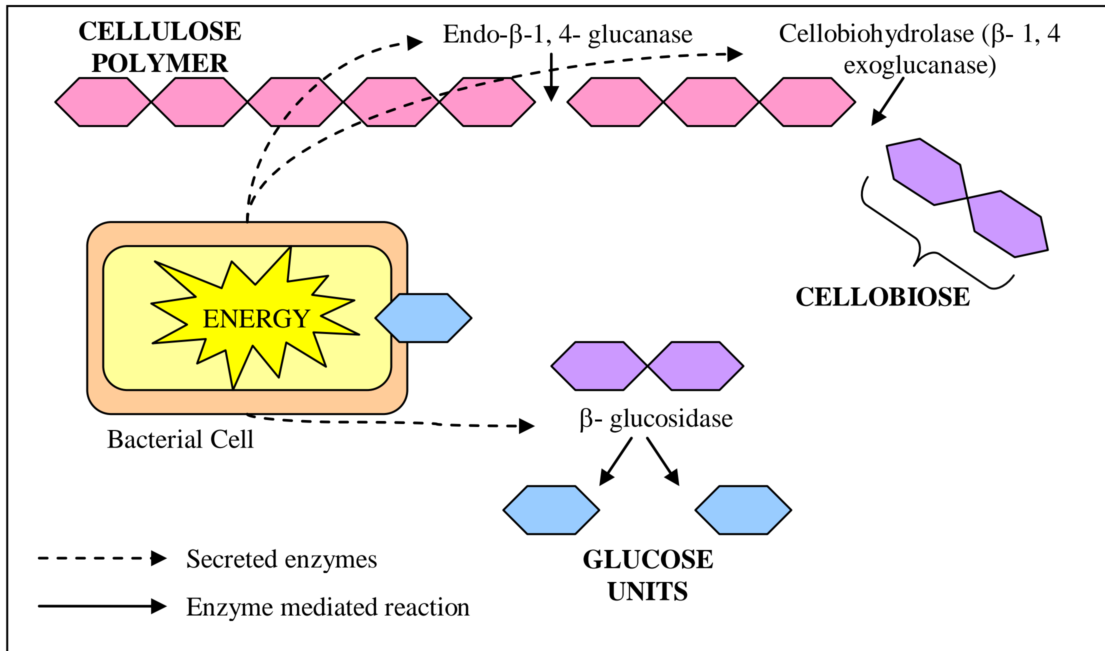


Figure 1-2 Extracellular enzyme assisted degradation of cellulose to glucose.

The reaction is completed by β-glucosidase which catalyses the breakdown of cellobiose to single glucose units. The action of β-glucosidase in hydrolysing the final step of the reaction is extremely important, allowing microorganisms which may be unable to uptake the larger cellobiose molecule to obtain energy from glucose. The synthesis of the enzyme by microorganisms is thought to be induced by the products of cellulose degradation (cellobiose) and is the most easily detectable of the three cellulase enzymes. Due to its central role in organic matter cycling, β-glucosidase is thought to be a useful enzyme in assessing soil quality (Turner et al., 2002).

The third most common component of plant reside, lignin, is the most recalcitrant (Hammel, 1997). It is an essential constituent of plant cell walls, allowing plants to stand upright and protecting their structural polysaccharides from attack (Hammel, 1997) but no specific structure is known as it varies among plant species (Wolf and

Wagner, 2005). The basic unit of lignin is known to be phenylpropene which consists of a hydroxylated 6-C aromatic benzene ring and a 3-C linear side chain. 500 to 600 of these phenylpropene units link to construct lignin (Wolf and Wagner, 2005). It is this complex aromatic structure and its relative size that makes lignin difficult for microorganisms to decompose (Figure 1-3).

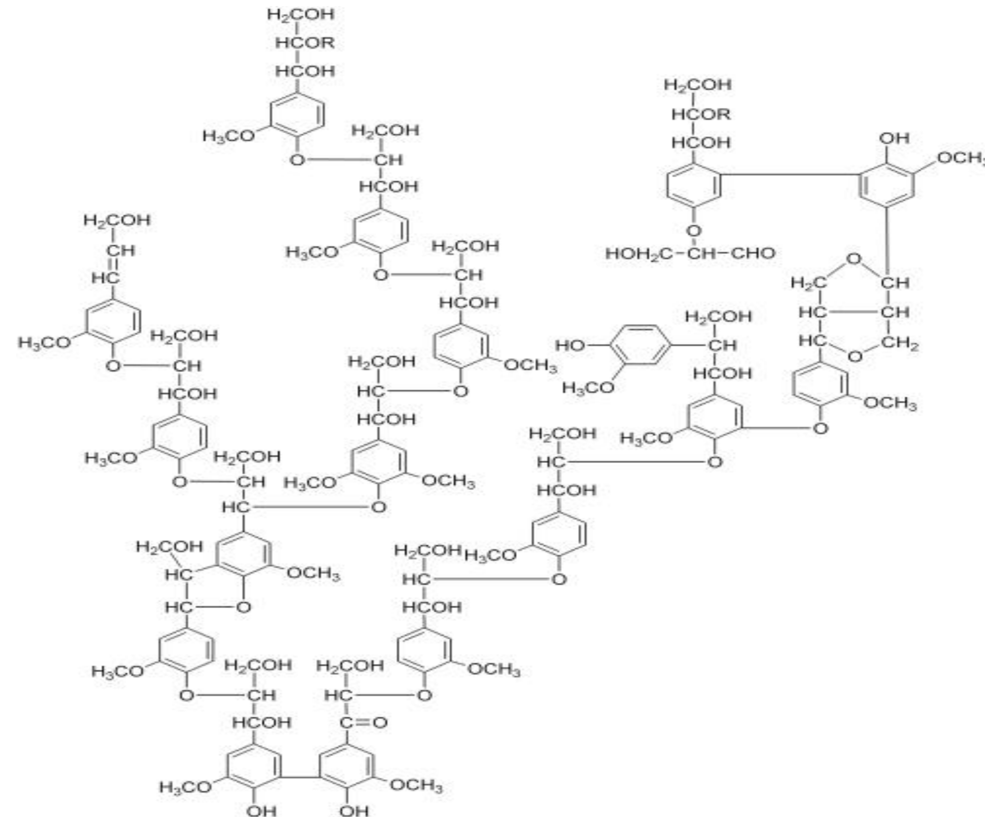


Figure 1-3 Possible structure of lignin

It is fundamental that lignin is broken down so as to maintain the global C cycle; otherwise C would eventually become locked as ligno-cellulose (Hammel, 1997). Certain organisms are adept at lignin degradation, aerobic filamentous fungi in particular basidiomycetes are the fastest lignin degraders (Kirk and Farrel, 1987). Hammel (1997) hypothesised that fungi may be more equiped at lignin degradation due to their vast network of filamentous hyphae which may be able to import scarce nutrients such as Fe and N across a distance to the nutrient poor site of lignin decomposition.

The C to N ratio (C:N) is one of the most significant ratios in determining litter quality. It is a well established ratio with reports of investigations as far back as 1916 (Jensen, 1916; cited in Heal et al., 1997). N determines the growth and turnover of the microbial community and in and therefore is noted as being one of the most common factors in limiting plant litter decomposition (Heal et al., 1997). Dead plant materials can have C:N ratios ranging from around 20 up to 500 but theoretically the optimum ratio for microbial growth is around 25 (Heal et al., 1997). If N levels are low i.e. the C:N ratio is high, then C is lost due to respiration and N is mineralised and converted into microbial protein (Swift et al., 1979). In contrast, when C:N ratios are low and N is not a limiting factor to microbial activity, other factors become more important and N becomes more available to plants (Swift et al., 1979).

1.2.2.3 The Decomposer Community

"*Soil microbial inhabitants are able to conduct almost all known metabolic reactions*" (Kunc, 1994)

The decomposer community are perhaps the most important of the three interlinking factors influencing decomposition. The importance of soil and the processes which are mediated by soil organisms should not be undervalued considering that 80-90 % of soil processes are microbe mediated reactions (Coleman and Crossley, 1996; Nannipieri and Badalucco, 2003, cited in Nannipieri et al., 2003). Killham (1994), described the soil microbial biomass as the "*driving force of most terrestrial ecosystems*". Swift et al (1979) highlighted the issues surrounding the investigation of decomposer community structure and function in that they are extremely complex. The sheer magnitude of species in one gram of soil makes it almost

impossible to highlight the individual contributions of species of microorganisms so it is often easier to quantify them into broad functional groups.

Bacteria are the most morphologically and metabolically diverse group of soil organisms given that some have the ability to switch between metabolic states as prevailing environmental conditions alter (Alexander, 2005). Bacteria are unable to engulf large particles of food and must therefore absorb dissolved nutrients from the soil solution through the cytoplasmic membrane (Alexander, 2005).

The majority of soil fungi are have a mycelial morphology (Killham, 1994) comprising of a filamentous network of hyphae (Morton, 2005). These hyphae are able to branch out in all directions, penetrating soil pores in search of food. Enzymes are produced external to the mycelium to catalyse the breakdown of large food molecules into particles that are small enough to be absorbed into the fungal cell (Raven et al*.,* 1999). As fungal enzymes are released into the environment in excess quantities, nutrients can be made available to other soil organisms (Harley, 1971). Together with bacteria, saprophytic fungi are the responsible for the decomposition of organic substances such as cellulose, hemicellulose, and pectin in plant cell walls (Morton, 2005). As well as their role in decomposition, fungi also have important functions in nitrification, denitrification and various other chemical reactions within the soil (Morton, 2005).

1.3 Ecosystem Development

"*Just as the relative importance of species varies in space, so their patterns of abundance may change with time*" (Begon et al., 1996)

Succession is defined as "*the non-seasonal, directional and continuous pattern of colonisation and extinction on a site by species and populations*" (Begon et al., 1996). Succession in a location which lacks biological residues or legacies is known as primary succession (Fagan et al., 2004) and is the starting point for ecosystem development. Clements (1907) regarded ecosystem development to be an "*ordered, directional and deterministic process*" in which a community progresses through a series of stages towards a climax community. This theory was rebuked by Gleason (1926) who argued that succession was not deterministic and community patterns occurred by chance as a product of the prevailing environmental conditions. In 1969, Odum provided a theoretical framework for the growth and development of ecosystems entitled "*The Strategy of Ecosystem Development*" (Table 1-2). This work provided the foundations for many subsequent investigations of ecosystem development on plant communities and has also been applied to soil microbial succession (Anderson and Domsch, 1985; Insam and Haselwandter, 1989, Ohtonen et al., 1999). Organisms characteristic to initial stages of development are envisaged to have very different growth strategies and metabolic characteristics than those present at later stages of development. It is thought that initial communities facilitate the colonisation of subsequent communities by altering the physical and chemical environment (Conell and Slayter, 1977). The validity of Odum's theories have received much speculation however the framework provides a basis for experimental studies as the process of ecosystem development has many complex interactions which are still poorly understood (Fagan et al., 2004).

Table 1-2 Tabular model of ecological succession: trends to be expected in the development of ecosystems adapted from Odum (1969)

Ecosystem attributes	Developing stages	Developed stages
	Community energetics	
1. Gross production/community respiration (P/R ratio)	Greater or less than 1	Approaches 1
2. Gross production/standing crop biomass (P/B ratio)	High	Low
3. Biomass supported/unit energy flow (B/E ratio)	Low	High
4. Net community production (yield)	High	Low
5. Food chains	Linear, predominantly grazing	Web like, predominantly detritus
	Community structure	
6. Total organic matter	Small	Large
7. Inorganic nutrients	Extrabiotic	Intrabiotic
8. Species diversity: variety component	Low	High
9. Species diversity: equitability component	Low	High
10. Biochemical diversity	Low	High
11. Stratification and spatial heterogeneity (pattern diversity)	Poorly organized	Well-organized
	Life history	
12. Niche specialization	Broad	Narrow
13. Size of organism	Small	Large
14. Life cycles	Short, simple	Long, complex
	Nutrient cycling	
15. Mineral cycles	Open	Closed
16. Nutrient exchange rate, between organisms and environment	Rapid	Slow
17. Role of detritus in nutrient regeneration	Unimportant	Important
	Selection pressure	
18. Growth form	For rapid growth (*r-selection*)	For feedback control (*K-selection*)
19. Production	Quantity	Quality
	Overall homeostasis	
20. Internal symbiosis	Undeveloped	Developed
21. Nutrient conservation	Poor	Good
22. Stability (resistance to external perturbations)	Poor	Good
23. Entropy	High	Low
24. Information	Low	High

1.3.1 ***Growth Strategies***

Organisms can be classified on the basis of their growth strategies. Simply, the terms *r* selection (zymogenous organisms) and *K* selection (autochthonous organisms), are used to define growth strategies of certain organisms. It is thought that *r* selection should predominate in early succession (colonisation) as *r* strategy organisms focus on high reproductive rates in low density populations so as to occupy a niche space as quickly as possible (Odum, 1969). Conversely, *K* strategists are said to predominate in environments where the population density is close to its carrying capacity. Intrinsic growth and reproduction rates of *K* strategists are finely tuned to reflect current resource limitation so are more adept at dealing with environmental stresses and nutrient limitation than r strategists (Bottomley, 2005). This concept of *r* and *K* strategists has increasingly been used as part of soil microbial research (Sigler et al., 2002) and may be applied to characterise early (*r* strategists) and mature successional stages (*K* strategists) (Odum, 1969).

1.3.2 ***Investigating Patterns of Ecosystem Development***

Ecosystem development can be investigated by observing changes through time on permanent plots of land (del Moral and Wood, 1993, cited in Kanehiro et al., 1995). Otherwise it is possible to infer changes through a sequence of closely related sites which differ only in age or time after disturbance such as glacier fore lands (Chapin et al., 1994; Ohtonen et al., 1999; Tscherko et al., 2003), volcanic chronosequences (Vitousek et al., 1993; Kanehiro et al., 1995; Schipper et al., 2001; King, 2003; Hopkins et al., 2007) or areas in which repeated fires have occurred (Haslam et al., 1998; Wardle et al., 2003).

Although primary succession is confined to a very limited number of environments i.e. receding glaciers, sand dunes and volcanic deposits; investigation of the colonisation and growth of communities on new soil sources provides valuable insight into the development and functioning of ecosystems which could not be obtained from more stable areas (Aplet et al., 1998).

1.3.3 ***Microbial Succession***

Despite the importance of the soil microbial environment to ecosystem functioning, the below ground environment has been largely neglected in the development of ecosystem development theory (Wardle and Giller, 1996). Many of the attributes outlined by Odum (1969) to describe ecosystem development can be extrapolated to include microbial communities. Nutrient availability (e.g. C, N, P) should increase with soil development. This is supported by investigations of plant successions on volcanic soils and on glacier forelands (Vitousek et al., 1993; Chapin et al., 1994). Microbial biomass and PLFA diversity have been found to increase with development (Ohtonen et al., 1999) and other microbial parameters such as the metabolic quotient (qCO^2) and biomass C to total C ratio should change accordingly. It is thought that initial pioneer (*r* strategy) microbial communities could facilitate the colonisation of subsequent (*K* strategy) microbial communities by altering the physical and chemical environment of the soil (Insam and Haselwandter, 1989; Sigler et al., 2002). With this alteration life strategy, it has also been hypothesised that the microbial community will become more efficient in their use of energy (Anderson and Domsch, 1985; Insam and Haselwandter, 1989; Ohtonen et al., 1999; Merila et al., 2002) as the amount of maintenance energy required by species declines.

1.3.3.1 Biomass and Soil Nutrient Contents

It has been predicted that nutrient availability in early stage development soils will be low (Odum, 1969). This is supported by investigations of plant successions (Vitousek et al., 1993; Chapin et al., 1994). With reduced nutrient availability, it can be proposed that community biomass will also be lower at early stages of primary succession (Odum, 1969). Several studies have assessed the trends in soil C dynamics and microbial biomass during primary succession. The majority of cases have concentrated on glacier forefield succession. Ohtonen et al (1999) found an increase in microbial biomass over a successional sequence on the forefield of the Lyman glacier in Washington, United States using three different techniques. They also found a corresponding increase in soil organic matter content. These trends of increasing microbial biomass and nutrient content with soil development have also been noted on glacier forelands by Insam and Haselwandter, (1989); Sigler et al (2002); Tscherko et al (2003) and on volcanic chronosequences by Schipper et al (2001), Hopkins et al (2007) supporting the theories of Odum (1969). Soil C (Anderson and Domsch, 1985; Insam and Haselwandter, 1989) and N (Chapin et al., 1994) limitation have been proposed as major limiting factors to the growth of the microbial community in early stages of development.

1.3.3.2 The Metabolic Quotient (qCO_2)

The flow of energy through an ecosystem allows the maintenance of its structure. The metabolic quotient (qCO_2) relates to the amount of respiration produced per unit of microbial biomass and is thought to respond promptly to disturbance and can indicate a decline in the quality of soil (Wardle and Ghani, 1995). Adapted from Odums' (1969) paper by Anderson and Domsch (1985), qCO_2 has been used as a

measure of how efficiently a microbial community is performing (Wardle and Ghani, 1995). At early stages of development, C loss due to respiration is significant but this loss is envisaged to decline over time (Ohtonen et al., 1999). Insam and Haselwandter (1989) tested the hypothesis that ecosystem succession is accompanied by a decrease in metabolic quotient of the soil microflora, proposing that microbial communities "*evolve towards less energy wasting stages*" and require less energy to maintain themselves. Their results concurred with the hypothesis that metabolic quotient would decrease with successional age and the cycling of nutrients becomes less "leaky". The concept of *r* and *K* growth strategies was employed to explain the characteristic of the data. A decrease in metabolic quotient throughout succession may be attributed to the change from initial simple substrate decomposer relationships dominated by *r* strategists, to progressively more complex food webs leading to the dominance of slower growing *K* specialists which occupy a greater number of narrow niches (Insam and Haselwandter, 1989). Decreases in qCO_2 with development have also been reported by Wardle and Ghani (1995), Ohtonen et al (1999) and Hopkins et al (2007), suggesting that communities may increase their C use efficiency with development.

1.4 Diversity

It has been hypothesised that community diversity and community equitability (evenness) should increase with ecosystem development (Odum, 1969). Few studies have investigated how the soil microbial community may change with ecosystem development however, and no one as yet has investigated microbial diversity on volcanic soils at different developmental stages.

Total biodiversity can be represented by three interrelating elements: genetic diversity, taxonomic diversity and functional diversity (Zak et al., 1994). Biodiversity is said to have a genetic foundation as community diversity arises due to evolutionary differentiation (Zak et al., 1994). This genetic diversity is then translated into taxonomic diversity although researchers are unsure how genetic diversity and taxonomic diversity are ultimately related. Even more enigmatic is the degree to which genetic and taxonomic diversity relate to functional diversity and how this affects ecosystem properties. Functional diversity describes the range of functions that can be performed within the community by the organisms which inhabit it and may be more important in terms of ecosystem functioning than taxonomic diversity.

1.4.1 *Diversity in relation to ecosystem function*

Despite the current concern over human impacts on biological diversity, the intrinsic value of biodiversity has been appreciated for centuries.

"*It is better to have a multiplicity of species than a multiplicity of one individual species*" St Thomas Aquinas (1225AD- 1274AD) (cited in McIntosh, 1985).

In 1859 Charles Darwin wrote:

"It has been experimentally proved, that if a plot of ground be sown with one species of grass, and a similar plot be sown with several distinct genera of grasses, a greater number of plants and a greater weight of dry herbage can be raised in the latter than in the former case".

Communities with increased linkages (higher taxonomic diversity) are hypothesised to contain a higher number of functions and occupy a greater number of niches therefore increasing ecosystem productivity. Many of the investigations into possible relationships between biodiversity and ecosystem function have been conducted on plant communities (Naeem et al., 1994; Tilman, 1996; Naeem and Li, 1997, Hector et al., 1999). For plant communities, it has been proposed that greater plant diversity would lead to greater access to available resources which would allow plants to increase their resource uptake and lower nutrient loses from the ecosystem thus increasing net productivity (Hooper and Vitousek, 1997).

Several athors have provided evidence to support the diversity- ecosystem function relationship. Naeem et al (1994) measured: community respiration, decomposition, nutrient retention, plant productivity and water retention in high and low diversty ecosystems. Higher diversity communities fixed more C and had more foliage cover than lower diversity communities thus utilising more CO_2 and intercepting more light than their lower diversity counterparts. Naeem et al (1994) also remarked in light of their results that a loss in biodiversity could also lead to "*a reduction in the ability of ecosystems to remove anthropogenic CO_2 from the atmosphere*". This indicates a reduction in community function with loss of diversity.

In a similar experiment Naeem and Li (1997) hypothesised that a greater species abundance should enhance "ecosystem reliability" or the "*probability that a system will provide a consistent level of performance over a given unit of time*". It was found that as the number of species increased, communities were more "*consistent in biomass and density measures*."

Naeem et al (1994) showed that higher diversity communities are more productive than lower diversity communities. This concept of productiveness is also a measurement of community function. Tilman (1996) reported on a 13 year study of plant species, abundances, diversity and production for plots of Minnesota grassland. These plots experienced great climatic variability during the study including two years of drought. Tilman (1996) described the experiment as testing the diversity-ecosystem function hypothesis by "*exploring the dependence of community productivity and plant species abundances on plant species richness*". Tilman's long term study demonstrated that "*biodiversity stabilises community and ecosystem processes, but not population processes*." He found that variability in above ground biomass was significantly lower in plots with greater complexity for both the 11 year period and the 9 drought free years. Species rich plots returned to their pre-drought biomass more quickly than species poor plots (Tilman, 1996). Tilman (1996), suggested the "*greater stability of community biomass associated with higher diversity would seem to require (1) that species differ in their susceptibility to disturbance and (2) that species compete and thus compensate via competitive release for decreased abundances of disturbance- susceptible species*." This evidence suggests that after disturbance events, higher diversity communities

may be perturbed to a lesser degree than lower diveristy communities and will in turn recover at a faster rate.

The experiments conducted by Naeem et al (1994), Naeem and Li (1997) and Tilman (1996), provide some empirical evidence to support the diversity- ecosystem function hypothesis. These experiments do not however, wholly confirm the hypothesis. Critics argue that these experiments are not accurate in their use of ecosystem productivity as a measure of function and the rise in productivity associated with diversity is a statistical artefact known as "the sampling effect" (Kaiser, 2000). "*As more species are randomly added to a plot, the odds rise that one of those species will be productive*" (Kaiser, 2000). Tilman however argues that the sampling affect is a legitimate rationale for why a complex ecosystem is more resilient or productive (Kaiser, 2000). Despite the criticisms of plot studies such as Tilman (1996), some researchers have come to the general consensus that under constant conditions at least some minimum number of species is required for ecosystem functioning while a larger number of species is probably required for ecosystem maintinence in changing environments (Loreau et al., 2001). Resilience defines a community's capacity to return to its equilibrium state after perturbation while resistance defines a community's ability to resist changes due to perturbation (Begon et al., 2000) (Figure 1-4). It has been hypothesised that lower diversity communities may be less resistant and resilient to an environmental disturbance than higher diversity communities (Pimm, 1984; Tilman and Downing, 1994) however it is not known whether these theories apply to the soil microbial community.

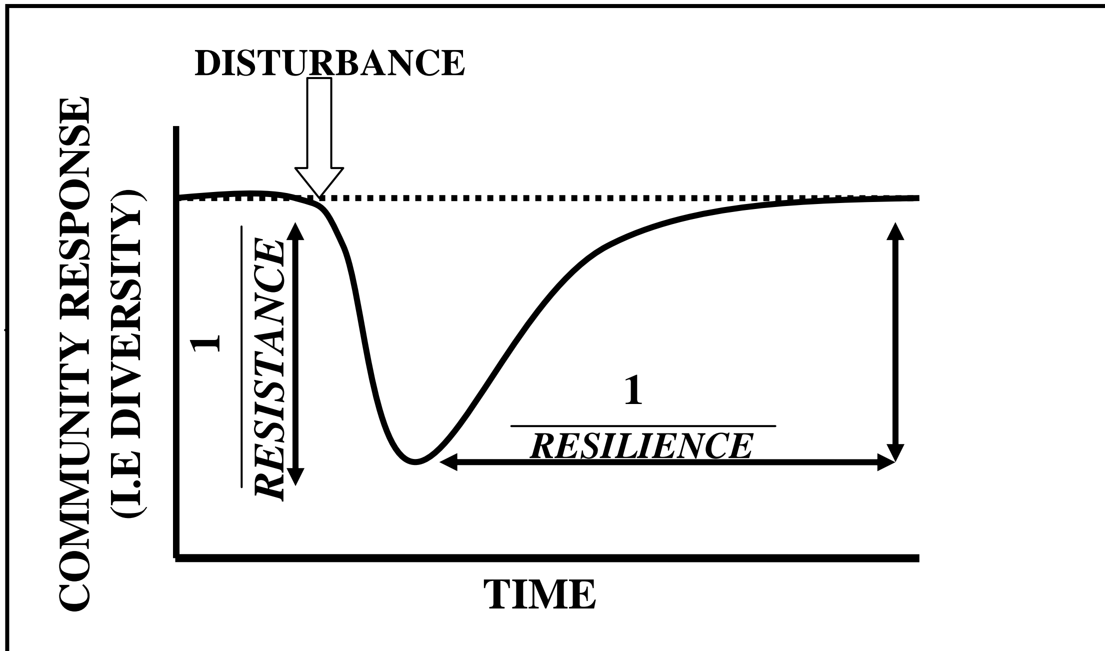


Figure 1-4 Graphical representation of community resistance and resilience after an environmental disturbance. The broken line shows the response in an control soil while the unbroken line shows community response following disturbance (Adapted from Ritz et al., 2003).

1.4.1 *Methods of Assessing Soil Microbial Species Diversity*

It has been estimated that 80- 99% of species of microorganisms have not yet been cultured (Amann et al., 1995) and indeed many may be uncultivable. Tunlid and White (1992) commented that numerous studies involving "classical" methods i.e. isolation and culture of microorganisms provide limited soil microbial diversity and function information.

Soil microbial community DNA extraction methods have paved the way for direct, non culture depenent studies of microbial diversity in soils. DNA extraction methods can be useful for a range of puposes including, providing insight into the prevelance and or activity of specific genes in soil microbial communities resulting in a better understanding of the functioning and selection of such genes under specific soil conditions (van Elsas et al., 2006). They are also useful as "signature

genes" can be used as biomarkers and overall community DNA analysis can be the basis for a description of microbial community structure. Polymerase Chain Reaction (PCR) based fingerprinting techniques give a high resolution and provide information on the whole community structure (Torsvik and Øvreas, 2002). Amplification of PCR is often followed by Denaturing Gradient Gel Eletrophoresis (DGGE). There are however some baises involved in the amplification of PCR which limits their use in the assessment of species richness (Torsvik and Øvreas, 2002).

One of the few methods currently capable of providing an unbiased proxy for soil microbial community structure is phospholipid fatty acid (PLFA) analysis which does not rely on the selective cultivation of microorganisms (Zelles, 1999). PLFAs are not found in dead cells or storage products and have a relatively rapid turnover rate in soil which means they make up a relatively constant proportion of the microbial biomass. PLFA analysis for measuring community diversity is further discussed in Section 4.1.1.

1.4.1.1 Microbial Diversity and Ecosystem Function

Investigations into biodiversity and its relation to ecosystem function have largely neglected the role of microorganisms in ecosystem processes (Wardle and Giller, 1996; Ohtonen et al., 1997; Øvreås, 2000; Wall et al., 2005). The range of biota within the soil is widely regarded as more extensive than that of any other group of organisms on the planet (O'Donnell et al., 2005). In one gram of soil several thousand bacterial species may be found (Torsvik et al., 1990) which renders the task of assessing soil microbial community diversity and functional composition

extremely difficult. Functional traits are those which influence ecosystem properties or the responses of species to environmental conditions (Hooper et al., 2005).

There is a growing interest in the role of biodiversity in the functioning of soils (Wall et al., 2005). This is mainly because the effects of environmental disturbances or perturbations on soil functioning are poorly understood and changes in the composition of biological communities may have important or long term affects (Øvreås, 2000). Microbial diversity has become an important issue due to the importance of the microbial community in organic matter transformations and nutrient cycling and knowledge about microbial community structure is vital in understanding the relationships between environmental factors and ecosystem functions (Torsvik et al., 1990). In essence, it is not microbial taxonomic diversity that is ultimately important but the consequent functional diversity that may exist within a community. With increased environmental degradation, it is important that effects on taxonomic and functional diversity are assessed to establish any links with ecosystem processes, community resilience and sustainability (Prosser et al., 2002). However there are critics of the applicability of the diversity- ecosystem function hypothesis to microbial communities (Findlay et al., 1997; Findlay, 2002). It has been suggested that as micro-organisms can potentially occur anywhere, their richness has the potential to be very high and thus microbial systems will have an extremely high rate of functional redundancy. Findlay (2002) in a study of the global dispersal of free living eukaryote species, found a large fraction of species were similar in small intensively studied plots and also found similar sets of species in widely separated locations. This concept of functional redundancy means that there is a high potential within the diverse microbial community to have many

species which perform the same functional role. This may mean that a reduction in species diversity after an environmental disturbance may not affect ecosystem functioning. There is still however some controversy over whether functional redundancy actually exists (Loreau, 2004).

1.4.2 ***Methods of Assessing Soil Functional Diversity***

1.4.2.1 BIOLOG

Several techniques have been introduced to assess decomposition functions of soil communities by investigating patterns of substrate utilisation (Garland and Mills, 1991; Degens and Harris, 1997). The BIOLOG method employed by Garland and Mills (1991) utilises microplates which were developed to test the responses of bacterial isolates to C sources in order to identify different strains (Garland and Mills, 1991). Soil suspensions are incubated in BIOLOG microtitre plates which comprise of 95 wells each containing a sole C source and a redox indicator dye (most often tetrazolium violet which is reduced to insoluble formazan) (Garland and Mills, 1991; Lehman et al., 1995; Garland, 1996). The technique potentially creates large quantities of data in a relatively small time period as many samples can be processed in a short space of time and has been used to assess microbial functional diversity in rhizosphere microbial communities (Ellis et al., 1995), soils under sugarcane plantations (Graham and Haynes, 2005), soils amended with simple C substrates and crop residues (Schutter and Dick, 2001; Bending et al., 2002), soils under different management practices (Butcher and Lanyon, 2005) as well as many other studies (Mayr et al., 1999; Collins and Cavigelli, 2003; Goberna et al., 2005).

Despite its relative popularity as a rapid measurement of microbial community functional diversity, the BIOLOG sole C source utilization method has been criticised as less than 1% of soil microorganisms can be cultured on agar plates and wells are near neutral pH which restrict the growth of fungi (Gray, 1990 cited in Degens and Harris, 1997; Campbell et al., 2003). This means that the BIOLOG method is flawed in that the results are biased towards bacteria that are readily extractable and fast growing. Data is also confounded by predation and competition effects within the plate wells. (Degens and Harris, 1997; Campbell et al., 2003). It has been noted that patterns of substrate utilisation were often more reliant on the competitive ability of organisms rather than the actual proportions of each in the mixture (Degens and Harris, 1997). Colour development in the wells is cell density dependant however, in order to obtain the rapid responses reported by the manufacturer, a large cell density is required which is often difficult to achieve in diluted samples thus applications often take between 72 and 168 hours to incubate before an adequate colour is developed (Campbell et al, 2003). The method has also been criticised for the complicated way in which data are interpreted and analysed (Howard, 1997; Insam and Hitzl, 1999; Campbell et al., 2003). Although the BIOLOG method is marketed on its quick and simple approach to assessing soil functional diversity, it appears that it is unsuitable due to the selective nature of the preparation of the plates and the long incubation times required. In developing soils these complications may be amplified and create grossly inaccurate results reinforcing the need for a whole soil approach to assessing soil microbial functional diversity.

1.4.2.2 Catabolic Response Profiles

Degens and Harris (1997) developed an alternative approach to assessing microbial functional diversity by modifying the substrate induced respiration (SIR) technique. A similar method had been used previously by Hopkins and Ferguson (1994) and Hopkins et al (1994) to assess SIR responses of soil microorganisms to D- and L-isomers of amino acids. Briefly, the substrate induced respiration response of microbial communities to a variety of substrates varying in complexity is used as a method of characterising functional diversity. Degens and Harris (1997) tested the SIR responses of 83 different substrates encompassing a range of substrate guilds; amino acids, organic acids, carbohydrates, alcohols, amines and amides. From this assortment, the 36 most responsive were selected for further analyses and were used as a basis for the catabolic response profiling (CRP's) of microbial communities. Unlike BIOLOG, the substrates are added directly to the soil and the response is recorded after a 6 hour incubation period. This short incubation time means that the resultant C mineralisation is characteristic of the active microbial community as opposed to a growth response (Degens and Harris, 1997). The measurement of "*in situ* catabolic potential" (ISCP) is thought to provide a proxy assessment of microbial decomposition function diversity that does not rely on the selective culturing of microorganisms (Degens and Harris, 1997; Degens, 1998a). The method has been used to assess variation in microbial communities after the addition of simple organic substrates to soil (Degens, 1998a), to investigate decomposition function under different moisture conditions (Degens, 1998b), to assess the effect of land use on functional diversity (Degens and Vojovodic-Vokovic, 1999) and to evaluate the effect of decreases in organic C resources on functional diversity (Degens et al., 2000).

Graham and Haynes (2005) compared the BIOLOG and ISCP techniques when assessing the impact of sugarcane production on catabolic diversity. It was concluded that the ISCP technique was "*more successful at separating treatments with broadly similar land uses*". This suggests that the ISCP technique of Degens and Harris (1997, 1998a) is a more sensitive measurement of microbial community decomposition function than the BIOLOG technique. Despite the relative simplicity and accuracy of the SIR technique it is still deemed to be "*laborious*" and like BIOLOG, requires high substrate concentrations and large amounts of soil to obtain a complete profile (Campbell et al., 2003).

1.4.2.3 MicroResp

In 2003, Campbell et al developed a novel microrespirometry system (MicroResp, MaCaulay Enterprises Limited) which aimed to overcome the drawbacks of BIOLOG and the SIR method. The MicroResp is a miniature respiration device consisting of two microtitre plates (one deep welled to contain soil, sediment or water samples) sealed together with a silicone gasket containing holes to allow the exchange of gas between the two microtitre plates. The plates allow 96 samples to be analysed simultaneously over a short period of time 4-6 hours thus generating large amounts of data in a relatively short space of time. Substrates are added to the deep wells before the addition of soil and the resultant CO_2 evolved from the soil is detected colorimetrically in a microtitre plate reader at 590nm (Campbell et al., 2003). As well as the advantages of generating a great deal of data in a short space of time, the MicroResp technique uses whole soil samples, responses produced are immediate responses rather than growth responses and the C source concentration is

correlated to the individual sample. Although the MicroResp is a relatively new innovation, the technique holds much promise and as well as community physiological profiling, the list of potential applications includes toxicity testing and bioremediation, soil health and quality testing and pollution induced community tolerance (Kaufmann et al., 2006).

1.5 Research Objectives

The relationship between soil microbial community diversity and functioning os poorly understood and as yet has not been assessed fully using the range of techniques in this thesis or in volcanic soils.

The main aim of this study is to investigate the structural diversity and decomposition functions of volcanic soils at different developmental stages. As outlined in the introduction section, debate still exists over the role of diversity on ecosystem functioning and whether the same principles can be applied to the soil microbial community.

The principal research objectives of this study are:

1. To investigate the long term *in situ* catabolic abilities of microbial communities in volcanic soils at different stages of development in the decomposition of litters of differing qualities and to investigate the effects of litter mixing on the rate of decomposition.

2. To investigate the PLFA diversity, community composition and community function (assessed by soil enzyme activity) of volcanic soil communities of different developmental stages and to assess the effect of an environmental disturbance on these key soil community features.

3. To investigate the functional diversity of volcanic soil microorganisms from different stages of development using a range of simple and complex

substrates and assess whether function is a product of taxonomic diversity or reflects the characteristics of the predominant vegetation type input into the soil.

Chapter 2 Site Location and Description

2.1 Study Area- Mount Etna (Sicily)

2.1.1 *Etna's location and morphology*

Located on the north eastern coast of Sicily, southern Italy (15° 0' E, 37° 43.8 N) (Figure 2-1), Etna is Europe's most active volcano (Hopkins et al., 2007). From north to south, Etna spans 47 km and east to west 38 km covering an area of approximately 1200 km^2 (Scarth and Tanguy, 2001). The volcano rises approximately 3330 m above sea level to the summit of one of its four main craters and its surface is covered with up to 200 cinder cones produced during its long and active history (Scarth and Tanguy, 2001).

Figure 2-1 Map showing the location of Sicily and Mount Etna

Locally the volcano is divided into three regions. The piedmont, or cultivated region, is the lowest part of the volcano. The slopes are broad and gentle and the weathered lavas provide excellent crop growing mediums. Between 1000 m and 2000 m lies the second wooded region. This region is characterised by its expanses of pine forests and Etnean broom. Areas of the volcano at altitudes higher than 2000

m frequently receive large amounts of pyroclastic ejecta. The constant supply of this material along with the relatively low temperatures, create poor conditions for plant growth and pedogenisis (Fernandez- Sanjurjo et al., 2003). The upper cone of Etna beginning at about 2900 m, known as Mongibello, is built upon previously active summits including Trifoglietto, Vavalaci and Ellittico.

2.1.2 ***Rock chemistry and petrography***

Historically the composition of Etna's lavas has been surprisingly consistent. The lavas have tended to be alkali basalts and trachybasalts (hawaiite and mugearite) and have tended to be porphyritic with 20-25 % plagioclase feldspar, 10% clinopyroxene and 3 % olivine as major phenocrysts (large crystals within porphyritic rocks) (Kilburn and McGuire, 2001). Typically variation in petrographic composition of Etna's lavas is concentrated on variation in size of the representative plagioclase phenocrysts. Larger sized phenocrysts indicate longer storage periods before extrusion. It has been noted by Kilburn and McGuire (2001) that the size of plagioclase phenocrysts has decreased over the past few centuries with eruptions during the late sixteenth into the seventeenth century being characterised by crystals with a length of approximately 5 mm. In recent times, ejected phenocrysts have been in the range of 2- 3 mm long.

2.1.3 ***Common Flora***

Etnean Broom [*Genista aetnensis* (Biv.) DC.] and Corsican Pine (*Pinus nigra* Arn.ssp, *laricio* Maire) are two of the most common plant species encountered on the uncultivated slopes of Mount Etna (Certini et al., 2001). They prevail until around 1900 m above sea level where the land becomes barren and uninhabitable.

Endemic to Mount Etna, *Genista aetnensis* (*Genista*) is indicative of relatively new and unstable soils where it forms N-fixing symbioses (Certini et al., 2001; Fernandez-Sanjurjo et al., 2003). Mature *Genista* trees have an average height of around 2.5 m and an average base diameter of around 10cm (Certini et al., 2001). This vascular plant is considered to be a pioneer species due to its ability to colonise both pyroclastic deposits and lava flows.

In contrast to the endemic *Genista*, the presence of *Pinus nigra* (*Pinus*) to the area is not a natural occurrence. The *Pinus* trees grow to an average height of around 8m with a base diameter of around 28 cm (Certini et al., 2001). Large areas of the landscape have been afforested with fast growing *Pinus* in order to stabilise volcanic soils. According to Certini et al (2001), further afforestation with *Pinus* has not been encouraged as its already widespread coverage has led to "*a monotonous coloured and textures landscape*" and a high fire risk.

Despite the aesthetic implications of promoting the growth of *Pinus* over *Genista* on newly formed soils, the ecological and environmental implications are relatively unknown. Certini et al (2001) and Fernandez-Sanjurjo et al (2003) were the first to investigate the effect these species have on pedogenesis. Hopkins et al (2007) investigated the decomposition of these two species in volcanic soils of different ages. As yet no other direct comparisons have been made of these two species.

2.1.4 ***Site location and details***

Two main study areas on Etna have been chosen for this investigation in localities of contrasting soil development and predominant vegetation cover (Figure 2-2). The particular field characteristics of these sites will be outlined (Table 2-1).

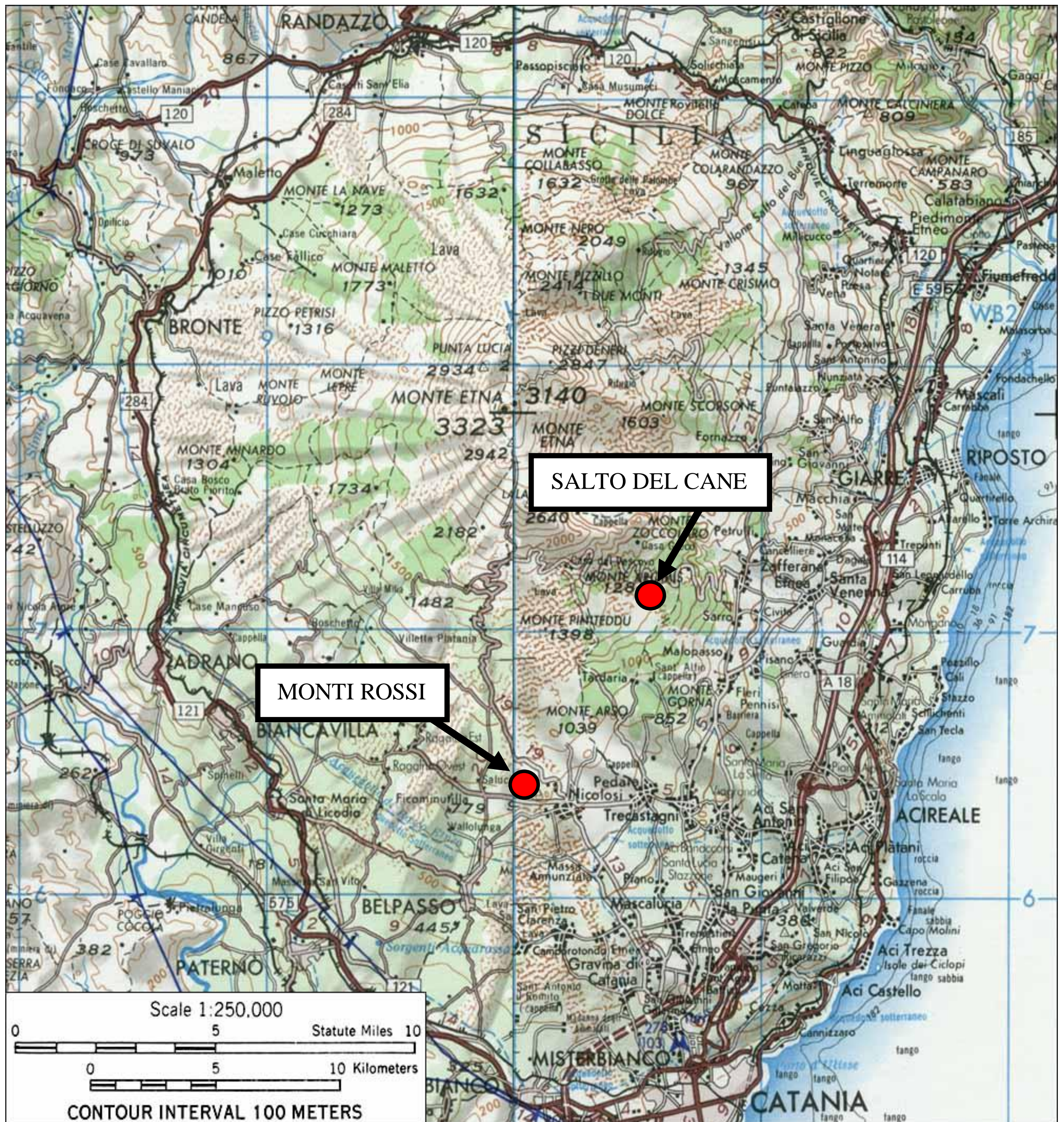


Figure 2-2 Map showing the location of study areas on Mount Etna, Sicily

Table 2-1 Details of study sites on Mount Etna, Sicily (Adapted from Hopkins et al., 2007)

Site	Acronym	Location	Altitude	Aspect	Soil Type	Dominant Vegetation	Soil forming materials and other information
Monti Rossi *Pinus*	MRP	NW of Nicolosi	850	S	Typic Vitrixerand	*Pinus nigra* L. Planted in the 1960's	Tephra from 1669 eruption (composition like group of alkalic basalts, hawaiites, phonolitic tephrites and basic mugerites)
Monti Rossi *Genista*	MRG	NW of Nicolosi	850	S	Entisol (Probably Vitrandic Xerorthent)	*Genista aetnensis* planted in the 1960's	Tephra from 1669 eruption (composition like group of alkalic basalts, hawaiites, phonolitic tephrites and basic mugerites)
Salto del Cane A	SDCA	NNW of Pedara	1330	SSE	Typic Udivitrand	Castanea sativa	Deposited 7,500 year BP, with 1634- 38 dated lava flow to the west, north and east
Salto del Cane B	SDCB	NNW of Pedara	1330	SSE	Typic Udivitrand	Castanea sativa	Deposited 7,500 year BP, with 1634- 38 dated lava flow to the west, north and east

2.1.4.1 Monti Rossi (developing sites)

2.1.4.1.1 Site description

Formed in the eruption of 1669, Monti Rossi is a large cinder cone located on Etna's southern flank near the town of Nicolosi, approximately 15 km from the summit craters. The structure itself consists of two Siamese cinder cones sitting at 949m and 875m above sea level (Ferandez- Sanjurjo et al., 2003). The area remained semi barren until the 1940s when both cones were afforested due to the erosion risk. Between 1947 and 1948 the area was afforested with plantations of *Pinus nigra* (Corsican Pine) and the endemic *Genista aetnensis* (Etnean Broom) (Ferandez- Sanjurjo et al., 2003). The mean annual precipitation at Monti Rossi is approximately 1150mm with a mean annual air temperature of 14.5^{o}C and mean annual soil temperature of 15.5^{o}C (Fernandez- Sanjurjo et al., 2003). Sites were selected under each vegetation type for analysis. The sites are referred to as Monti Rossi *Pinus* (MRP) (Figure 2-3) and Monti Rossi *Genista* (MRG) (Figure 2-4).

Figure 2-3 Monti Rossi (*Pinus*) site

Figure 2-4 Monti Rossi (*Genista*) site

2.1.4.1.2 Soil profile description

Figures 2-5 and 2-6 show the soil profiles for MRP and MRG respectively and brief descriptions of these profiles are outlined in tables 2-2 and 2-3. A more detailed soil profile for Monti Rossi has been constructed by Soil Survey Staff (1999) and can be found in Fernandez- Sanjurjo et al (2003).

Figure 2-5 Soil Profile of Monti Rossi (*Pinus*) site

Table 2-2 Soil Profile Description for Monti Rossi *Pinus* (MRP) Mount Etna, Sicily

Horizon	Depth (cm)	Colour	Texture	Roots*	Stoniness	Other observations
O	2-0	-	-	-	-	Decomposing leaf litter amalgamating with an ashy surface layer.
A	0-30	10YR 4/2	Gravely, coarse	A	Very	An abundance of volcanic tephra
B	30-50+	2.5YR 4/1	Gravely	E	Quite	Frequent large roots, smaller stones than previous horizons.

*Roots: 0- not present, V- very few, F- few, P- plentiful, A- abundant, E- extremely abundant.

Figure 2-6 Soil profile of Monti Rossi (Genista) site

Table 2-3 Soil Profile Description for Monti Rossi *Genista* (MRG), Mount Etna, Sicily

Horizon	Depth (cm)	Colour	Texture	Roots*	Stoniness	Other observations
LH	0-5	-	Very fine	-	-	Top 2cm mainly decomposing litter.
A	5-20	2.5YR 2.5/1	Gravely, coarse	P	Very	An abundance of large volcanic pumice > 1cm.
B1	20-45	2.5YR 3/1	Gravely	F	Quite	Smaller stones than above layer.
B2	45+	7.5YR 2.5/1	Gravely, course	V	Very	Larger stones than above layer

- Roots: 0- not present, V- very few, F- few, P- plentiful, A- abundant, E- extremely abundant.

The main soil forming material at both of these sites is tephra from the 1669 eruption. This tephra is composed of alkalitic basalts, Hawiites, Phonolitic tephrites

and Basic mugearites and results in soil taxonomy groups of Vitrandic Xerorthent (MRP) and Typic Vitrixerand (MRG) (Hopkins et al., 2007).

2.1.4.2 Salto del Cane (developed sites)

2.1.4.2.1 Site description

The second studied area lies approximately 0.85km south of the summit cone near the town of Pedara (Hopkins et al., 2007). Salto del Cane lies at an altitude of approximately 1330 m above sea level and was formed during an eruption of the volcano approximately 7500 years ago (Hopkins et al., 2007). Two sites were selected in this area: one site within a dense European Chestnut (*Castanea sativa*) woodland (SDCA) (Figure 2-7) and the other in an area sparsely populated with European chestnut stands (SDCB) (Figure 2-8). The mean annual air temperature of this area is around 14°C but can rise to 35°C in the peak of summer and fall to 0°C in winter (Hopkins et al., 2007). The annual precipitation at this area is between 1100 and 1300mm year^{-1} (Hopkins et al., 2007).

Figure 2-7 Salto del Cane (A) site

Figure 2-8 Salto del Cane (B) site

2.1.4.2.2 Soil profile description

The presence of European chestnut indicates a well developed soil. The first 10 cm of these two soils was dominated by a layer of fine black ash. There was a well defined boundary between the top layer of ash and the rich organic soil below. Soil profiles for SDCA and SDCB are shown in figures 2-9 and 2-10 respectively and their corresponding soil profile descriptions are outlined in tables 2-4 and 2-5:

Figure 2-9 Soil profile for Salto del Cane (A)

Table 2-4 Soil Profile Description for Salto del 1 (SDCA), Mount Etna, Sicily

Horizon	Depth (cm)	Colour	Texture	Roots*	Stoniness	Other observations
LH	0-2	-	-	-	-	-
A	2-10	7.5YR 2.5/1	Weak, Fine	A	Few	A thick black ash layer.
B	10-35+	2.5YR 3/2	Tightly bound	E	Few	A rich organic soil.

- Roots: 0- not present, V- very few, F- few, P- plentiful, A- abundant, E- extremely abundant

Figure 2-10 Soil profile for Salto del Cane (B)

Table 2-5 Soil Profile Description for Salto del Cane 2 (SDCB) , Mount Etna, Sicily

Horizon	Depth (cm)	Colour	Texture	Roots*	Stoniness	Other observations
LH	0-2	-	-	-	-	-
A	2-10	7.5YR 2.5/1	Weak, Fine	A	Few	A thick black ash layer.
B	10-50+	2.5YR 3/3	Tightly bound	A	Few	Ash incorporated through moist organic soil layer.

- Roots: 0- not present, V- very few, F- few, P- plentiful, A- abundant, E- extremely abundant.

The main soil forming materials in this area are lava from a dated flow (1634-1638) and tephra from an eruption deposited around 7500 BP (Hopkins et al., 2007). The dating of this site is primarily from field observations (Hopkins et al., 2007). These lavas and tephras are surprisingly similar in mineral content to the tephral deposits

on Monti Rossi consisting of alkalitic basalts, Hawiites, Phonolitic tephrites and Basic mugearites (Hopkins et al., 2007). The main soil group for both of these sites is Typic Udivitrand. Busa et al (1998) investigated the petrological and textual characteristics of soils formed on Salto del Cane. They found that the area had been subjected to a number of explosive events throughout its evolution. It was also found that primary chemistry appeared to be less changed in the coarse fraction (>2 mm) than in the finer-grained fraction, where elements like Si, Mg, Ca, and alkalis are preferentially extracted by weathering and hydration processes, leading to an enrichment of Al, Fe, and Ti.

These four sites represent volcanic soils at different stages of development.

Chapter 3 In situ decomposition of pure and mixed leaf litter in volcanic soils at different stages of development

3.1 Introduction

Litterfall from vegetation is subject to decomposition by a vast array of primary decomposer species whose activities and growth rates are determined by the physical and chemical characteristics of the soil microenvironment. The chemical composition of a residue influences the rate of biological degradation and during the decomposition process, composition continues to be altered resulting in differential decay rates (Coûteaux et al., 1998). The initial phase of decomposition is characterised by rapid degradation of soluble compounds and non lignified carbohydrates and is controlled by the concentrations of limiting nutrients such as N, which are required by the decomposer community (Berg, 2000). Lignin and associated constituents influence decomposition rates at later stages of decay and N concentration has been hypothesised to negatively interact with lignin during these phases, reducing the rate of decomposition (Berg, 1986). Changes in the chemical nature of residues during decomposition are accompanied by changes in the structure and activity of the decomposer community (Wardle, 1993).

It has been proposed that as soil microbial communities develop over time, C use efficiency increases (Ohtonen et al., 1999). This suggests that more developed soil communities should decompose organic residues faster than developing soil communities and incorporate a greater concentration of C into biomass.

3.1.1 ***Litter Mixtures***

Decomposition research has largely focussed on single species litters (Salamanca et al., 1998), however, litter species rarely occur alone in nature and it has been proposed that litter mixtures may interact affecting their rate of decomposition relative to single species litters (Thomas, 1968; Seastedt, 1984). When litters of several species are mixed, decomposition may be very complex. Resultant observed mass loss from mixed leaf litter decay is therefore compared to a predicted result obtained from the decomposition of single species. “Additive” effects describe those in which mass loss of mixtures can be predicted from single species litter decomposition. When observed mass loss from litter mixtures differs from predicted values and is significantly enhanced or retarded the effect is termed as “non additive”. The same nomenclature applies to all other measurements i.e. biomass and respiration.

Comprehensive reviews of litter mixing effects suggest that in most studies interactions do occur and that these interactions are often synergistic resulting in enhanced decomposition of one or more litter species (Gartner and Cardon, 2004). The mechanisms for litter mixing effects are still poorly understood but increased resource heterogeneity (Blair et al., 1990) and nutrient translocations (Seastedt, 1984) are possible explanations. The effects of litter mixing in developing volcanic soils are unknown and communities at different developmental stages may decompose mixtures at different rates.

3.1.2 ***NMR With Particular Reference to Solid State C^{13} NMR***

Nuclear magnetic resonance spectroscopy (NMR) is based upon the measurement of absorption of electromagnetic radiation in the radio frequency region of 4 to 600

MHz. Nuclei spin occurs when an odd number of protons or neutrons are present within the nucleus. This spin gives rise to a magnetic field as all nuclei possess a charge. Nuclei which spin have an angular momentum of *p*. The maximum number of spin components or values for *p* for a particular nucleus is dependant on its spin quantum number (*I*). The spin quantum number for the ^{13}C nucleus is ½ meaning two spin states exist. Nuclei with a spin quantum number of ½ become orientated in one of two directions when subjected to an external magnetic field (Bo). Nuclei precess (move in a circular path) around the magnetic field at the Larmor frequency. Nuclei in samples are irradiated with intense radio frequency radiation which is produced by a radio frequency oscillator coil. Samples are irradiated at an angle of 90 degrees from the fixed external magnetic field so as to introduce circularly polarised radiation in the proper plane for absorption thus obtaining a maximum signal for the detector. The nuclei are then relaxed by exposing them to another magnetic field at the same frequency after which they give out a discrete signal. Relaxation occurs quickly in liquids but is relatively slow for solid particles. NMR on solid specimens was long thought to be impossible due to the effects of line broadening which reduces to resolution of individual peaks on spectra. Line broadening can be caused by static dipolar interactions between ^{13}C and ^{1}H or by chemical shift anisotropy. Several thousand scans are required to construct a good quality spectrum.

Solid state ^{13}C NMR spectroscopy overcomes the problems associated with the analysis of solid specimens. Samples are spun at an angle of 54.7 degrees to the external magnetic field (Bo) to eliminate the effects of chemical shift anisotropy. This angle is known as the "magic" angle giving rise to the name magic angle spinning or MAS. The technique of cross polarisation (CP) is also used in order to reduce the time

needed for excited nuclei to return to equilibrium. ^{13}C is of low isotopic abundance in nature (1.1%) but because of its low magnetic moment, it can be useful in determining the amount of and distribution of C atoms in a sample. Although around 6000 times less sensitive than proton NMR, ^{13}C NMR provides information about the backbone of molecules rather than the periphery.

CPMAS ^{13}C NMR has the ability to obtain structural information on soil organic matter in bulk soil. The technique gives a unique insight into the biochemical make up of a specimen which is particularly useful in decomposition experiments. Spectra provide detailed information about the distribution of ^{13}C in a range of functional groups. Shift range is between 0 and 220 ppm and is generally divided into 6 regions although further differentiation of regions provides more detailed information on functional groups (Figure 3-1, Table 3-1). As noted by Hopkins et al. (2000), these functional groups are *"indicative of the main classes of biochemicals involved in the biogeochemical transformations of carbon in soil*."

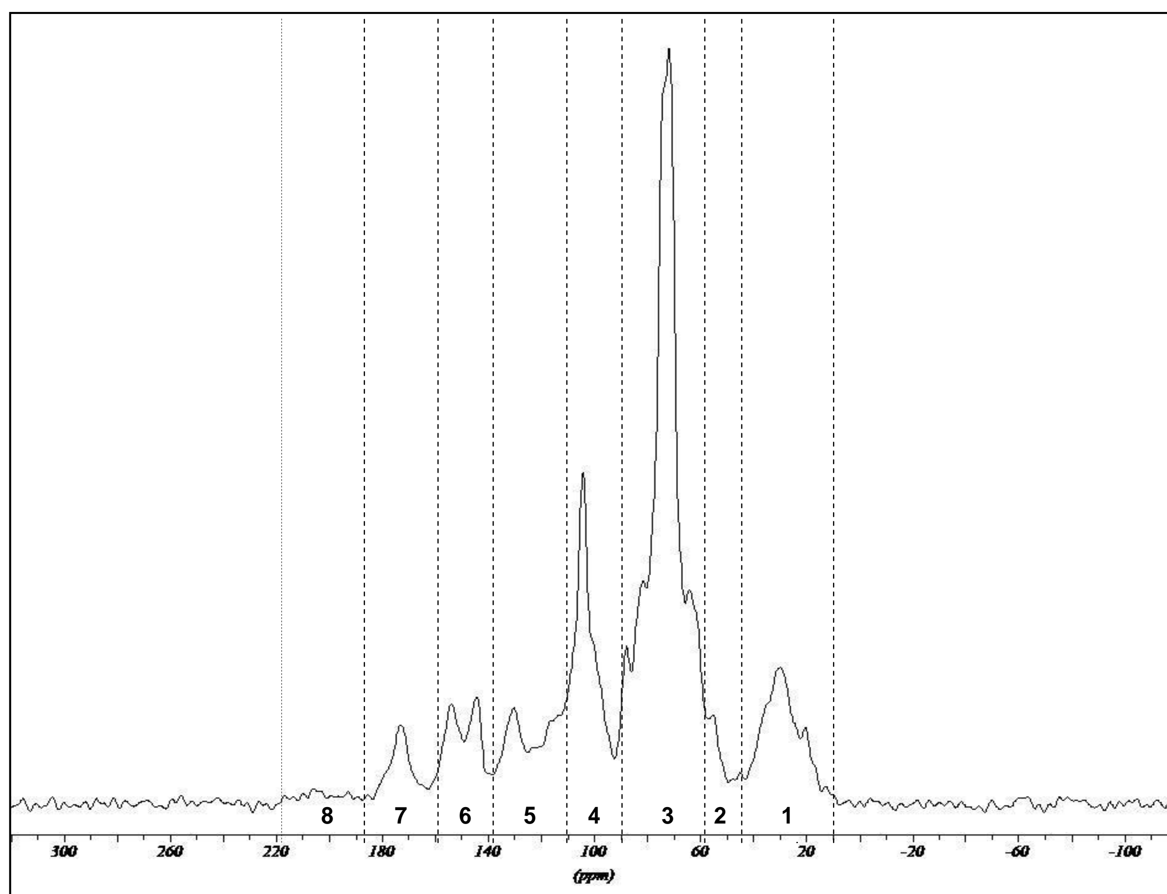


Figure 3-1 An example of a ^{13}C NMR spectrum. Eight regions are identified and the typical functional groups of these regions are outlined in Table 3-1

Table 3-1 Chemical shift ranges, functional group assignments and typical classes of biochemicals represented in ^{13}C NMR spectra.

Region	Shift Range (ppm)	Functional Groups	Typical Classes of Biochemical Represented
1	10-45	Methyl and alkyl C	Lipids, waxes and aliphatic hydrocarbons
2	45-60	O Methyl (methoxyl) and N alkyl C	Lignin substituents and amino acids
3	60-90	O alkyl C	Polysaccharides
4	90-110	Acetal and ketal C (di O alkyl C)	Polysaccharides
5	110-140	C substituted aromatic C	Lignin
6	140-160	O or N linked aromatic C	Tannins
7	160-185	Carboxyl C	Amides
8	185-220	Carbonyl C	Organic acids and peptides.

3.2 Specific Objectives and Hypotheses

The main objectives of this chapter were to compare the characteristics and activities of volcanic soils at different stages of development in the decomposition of a range of litters common to the area and to assess the effect of litter mixing on the rate of leaf litter decomposition using a range of parameters (mass loss, microbial biomass, microbial respiration, C and N contents of litters, NMR spectra of litters).

3.2.1 *Specific Objectives*

- ❖ Investigate chemical and biological characteristics of volcanic soils at different developmental stages.
- ❖ Compare the rates of decomposition of litters varying in chemical composition in soils at different developmental stages.
- ❖ Compare the size and activity of the microbial biomass found on litters buried in soils from different developmental stages.
- ❖ Investigate the effects of mixing litters of different plant species on the overall rate of decomposition compared with single species litter decomposition.
- ❖ Assess the C and N contents and NMR spectra of pure litters and mixtures after decomposition in soils of different developmental stages.

3.2.2 *Hypotheses*

- C and N content, microbial biomass and respiration will increase with soil development while the metabolic quotient ($q\mathrm{CO}^2$) should decrease.
- Mass loss from litter bags will be greatest in more developed soils.
- The microbial community in soils from Monti Rossi (*Genista*) will be able to decompose litter to a greater degree than the microbial community in the Monti Rossi (*Pinus*) soil due to the increased availability of N.

- Respiration from microorganisms present on litters buried at more developed sites will be higher due to the increased rate of decomposition.
- Observed mass loss will be greater in mixed litter bags than values predicted from single species litter due to translocation of nutrients.
- Litters decomposed in a mixture should have more similar chemical characteristics (i.e. C and N and NMR spectra) than those decomposed alone.

3.3 Materials and Methods

3.3.1 *Soil Chemical Properties*

Soils were sampled in triplicate from each site and were sealed in polythene bags and stored at 4°C. Moist soils were sieved to less than 2 mm before analysis whilst triplicate sub-samples were used to determine soil dry weights by heating to 105°C for 24 hours. Soil sub samples were also dried and ground to a fine powder in agate chambers using a Retsch MM200 ball mill before determination of C and N contents using a Carlo- Erbo CHN analyser. Soil particle size distribution for each site was determined on a 100 g sample of soil which was sieved through a nest of sieves varying from an 8 mm mesh size to a 1 mm mesh size after which each of the fractions were weighed. Soil pH was determined on a 1:2.5 (soil to water) suspension.

3.3.2 *Soil Biological Properties*

Soil was transferred into miniature incubation chambers as described by Heilmann and Beese (1992) and modified by Hopkins and Shiel (1996) (Figure 3-2).

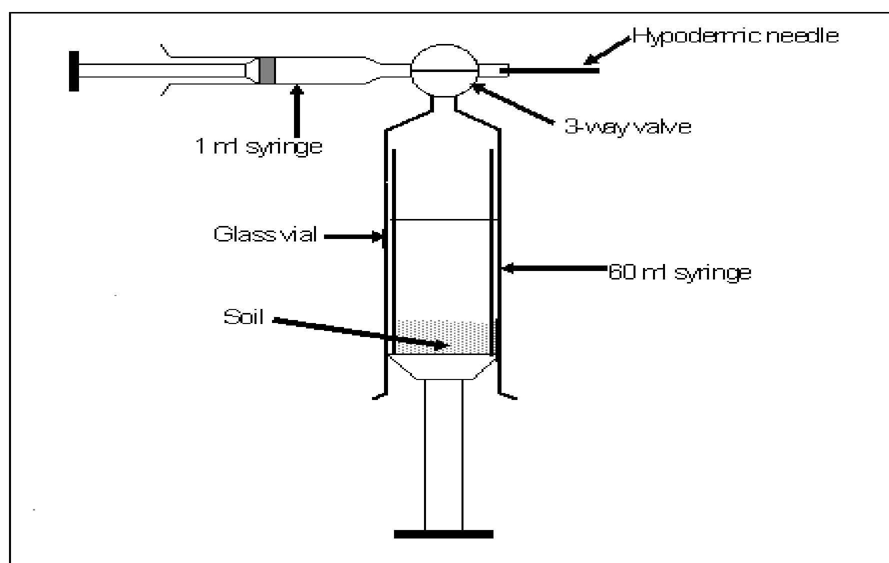


Figure 3-2 Design of miniature incubation chambers, diagram adapted from Hopkins and Shillam, (2005)

The soils were incubated in darkness at 20°C for 48 hours after which evolved CO_2 was analysed using a Varian gas chromatograph (GC). The amount of CO_2 evolved was plotted on a chart recorder and respiration calculated as (μg CO_2-C g^{-1} soil hr^{-1}). Soil microbial biomass was obtained by following the substrate induced respiration method of Anderson and Domsch (1978). The vials were left to incubate for a further six hours until the maximal period of microbial respiration was reached and the resultant CO_2 evolution analysed by GC. Substrate induced respiration was calculated by subtracting basal respiration from the maximal respiration and biomass calculated as described by Hopkins and Shiel (1996) then converted to mg biomass g^{-1} C soil.

3.3.3 ***Leaf Litter Decomposition***

Decomposition of leaf litter was calculated by mass loss from litter bags.

3.3.3.1 Litter Bag Construction and Contents

Litter bags were constructed from nylon netting, and were approximately 3 cm^2 with a mesh size of approximately 0.25 mm^2. Each bag contained 3 g of litter which had previously had all debris removed and was heated at 60°C to sterilise and then cut into strands of approximately 2 mm. Two different litter types common to the Mount Etna area were used in the experiment; Corsican Pine (*Pinus nigra*) and Etnean Broom (*Genista aetnensis*). The chemical characteristics of these two plant species indicate that *Pinus nigra* is a lower quality substrate for soil microorganisms than *Genista aetnensis* (Hopkins et al., 2007). In order to establish the effect of litter mixing on the rate of decomposition in this environment, a 1:1 mixture of the two studied species was added to litter bags and mixed thoroughly. Glass wool was used as a control specimen due to its inert properties. All litterbags were produced in triplicate.

3.3.3.2 Burial and Collection of Litter Bags

Within each of the chosen areas i.e. Monti Rossi (*Pinus*) (MRP), Monti Rossi (*Genista*) (MRG), Salto del Cane (*Castanea* woodland) (SDCA) and Salto del Cane (mixed woodland) (SDCB), a suitable site was chosen for litter bag distribution. In each case, a flat piece of land was found, the leaf litter and topsoil removed to a depth of around 5 cm and the litter bags laid out with their positions noted. The bags were then re-covered with the soil and leaf litter which had been removed. Litter bags were deposited on the 23rd October 2004 and were collected at various intervals there after. Once removed, excess soil was brushed away and the bags stored in sealed plastic bags until analysis. Litter bags were removed on three occasions for mass loss calculations and litter respiration measurements and on the first and last sampling occasions two lots of litter bags were removed in order to carry out microbial biomass analyses. C and N concentrations and NMR were carried out on litter removed on the first sampling occasion in July 2005.

3.3.3.3 Calculation of Mass Loss of Litter

Litter bags were heated to 60°C for 48 hours to remove any moisture. The bags were then re- weighed and mass loss calculated as reduction in mass from original mass. Mixture samples were treated exactly like single species litterbags and were compared with mass loss of component species. Values were adjusted to include any weight change in control samples.

3.3.4 ***Respiration of Microorganisms Associated with Litter***

Litter bags were transferred into incubation devices such as that of Heilmann and Beese (1992). The miniature devices were incubated in darkness at 20°C for 48 hours

after which evolved carbon dioxide was analysed using a Varian gas chromatograph (GC). The amount of carbon dioxide evolved was plotted on a chart recorder and respiration calculated as both (μg CO_2-C g^{-1} litter hr^{-1}) and (μg CO_2-C g^{-1} litter C hr^{-1}).

3.3.5 ***Biomass of Microorganisms Associated with Litter***

Litter microbial biomass was obtained by following the substrate induced respiration method of Anderson and Domsch (1978). Small incisions were made in the bags in order for glucose addition (40 mg g^{-1} DW litter) direct to the moist litter. Prior to addition of the glucose substrate, test measurements were made to assess the optimum rate of glucose addition for each litter type. Glucose powder was added at a rate of 10 mg g^{-1} DW litter, 40 mg g^{-1} DW litter, and 70 mg g^{-1} DW litter. The vials were left to incubate for a further six hours until the maximal period of microbial respiration was reached and the resultant CO_2 evolution analysed on a GC. Substrate induced respiration was calculated by subtracting basal respiration from the maximal respiration and biomass as described by Hopkins and Shiel (1996) , then converted to mg biomass g^{-1} C litter.

3.3.6 ***Determination of Litter Carbon and Nitrogen contents***

After calculation of mass loss, mixed leaf litter samples were separated into their component species and all litter samples were ground to a fine powder in agate chambers using a Retsch MM200 ball mill. The total C and N contents of the litters were determined using a Carlo- Erba CHN analyser and compared to single species litter samples and control samples (dried leaf litter which had been stored in a sterile environment) which had been prepared in the same manner.

3.3.7 ***NMR***

All CP MAS ^{13}C NMR spectra were obtained using a Bruker DSX 200 NMR spectrometer, Bruker, Karlsruhe, Germany operating at a frequency of 200.13 MHz for ^{1}H and 50.32 MHz for ^{13}C. Samples were spun at the magic angle in 7 mm (o.d.) zirconia rotors at 6.8 kHz. Cross polarization (CP) experiments were performed using a 7μs 90° pulse, a 1 ms contact time and a 2 s relaxation delay. Between 700 and 7000 acquisitions were collected. The ^{13}C spectra were referenced to an external tetramethylsilane standard (0 ppm). The free induction decays were transformed using a 100 Hz line broadening.

After calculation of mass loss, mixed leaf litter samples were separated into their component species after dyring and removal of soil. All litter samples were ground to a fine powder in non- metallic agate chambers using a Retsch MM200 ball mill and compared to single species litter samples and control samples (dried leaf litter which had been stored in a sterile environment) which had been prepared in the same manner.

3.3.8 ***Statistical Analyses***

The decomposition of leaf litter *in situ* was estimated using first order decay function kinetics ($y=ae^{-kt}$) by using the curve fitting function of the Sigmaplot package. This package was also used to estimate the decomposition rate constant (k) and the fraction readily mineralised material (a).

Using the statistical computer package Minitab, a series of analysis of variance (ANOVA) tests were conducted for mass loss, respiration, biomass and carbon and

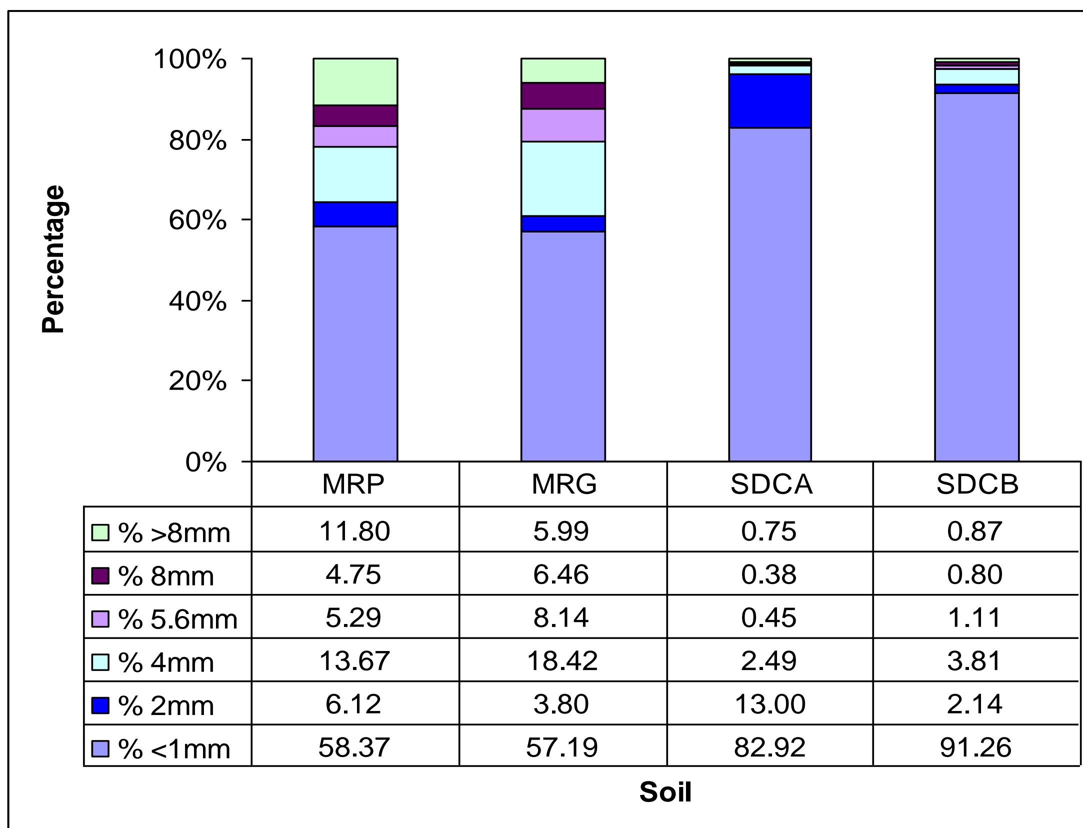
nitrogen data. ANOVA is the standard parametric test of difference in three or more samples (Ebdon, 2001). Observed and predicted values from the decomposition of litter mixtures were compared using a 2 sample t-test.

3.4 Results

3.4.1 *Soil Chemical and Physical Properties*

On the first sampling occasion, the total C and N contents of MRP, SDCA and SDCB were not significantly different but MRG had substantially greater concentrations of C and N than all other soils (Table 3-2) which may reflect the characteristics of the predominant source of litter input to this soil; N rich *Genista aetnensis*. This was also true for soils collected in May 06. However, although total C contents of MRP, SDCA and SDCB were not significantly different, total N was greater in the developed soils. Total C and N contents of MRP, SDCA and SDCB did not vary dramatically over the experimental period but MRG showed a significant reduction in both elements on the second sampling occasion although the C:N ratio was similar to other sampling dates.

Analysis of soil particle size distribution of Etna soils ranging from <1 mm to >8 mm showed some distinct differences in the morphology of the developing and developed soils (Figure 3-3). Soil particles included both inorganic material (in this case ash and tephra) and larger organic particles. Over 80 % of the soil collected from the developed sites was less than 1 mm in size while this fraction in the developing soils accounted for less than 60 % of the overall soil composition. In total, material smaller than 4 mm in size made up more than 97 % of the soil particles in the developed soils. The developing soils however contained a greater proportion of coarser grained materials with over 35 % of the total soil material made up of particles greater than 4 mm in size. Soil pH which was recorded only on the first and last sampling dates, was similar at each site and was near neutrality.



	MRP	MRG	SDCA	SDCB
% >8mm	11.80	5.99	0.75	0.87
% 8mm	4.75	6.46	0.38	0.80
% 5.6mm	5.29	8.14	0.45	1.11
% 4mm	13.67	18.42	2.49	3.81
% 2mm	6.12	3.80	13.00	2.14
% <1mm	58.37	57.19	82.92	91.26

Figure 3-3 Particle size distribution by percentage in Monti Rossi (*Pinus*) MRP, Monti Rossi (*Genista*), Salto del Cane A (SDCA) and Salto del Cane (B)

3.4.2 ***Soil Biological Properties***

Basal respiration and microbial biomass for the soils collected on the first sampling occasion showed a similar trend to total C and N values as basal respiration and biomass were significantly greater in MRG than all other soils (Table 3-2). No clear patterns in microbial biomass or basal respiration were noted on the other sampling occasions. Respiratory quotient (qCO_2) and biomass C to total C were the only biological parameters which appeared to show a trend with developmental stage. qCO_2 declined with greater soil development suggesting the developed soil community may be utilising C resources more efficiently. This parameter also appeared to decline throughout the experimental period which may indicate a recovery from a previous stress or disturbance. The ratio of biomass C to total C appeared to be greater in the more developed soil however this was not significant.

Table 3-2 Chemical and Biological Properties of Mount Etna Soils .Values are means (± SD). Abbreviations: MRP (Monti Rossi (*Pinus*)), MRG (Monti Rossi (*Genista*)), SDCA (Salto del Cane A) , SDCB (Salto del Cane B)

Sampling time	Site	Total C (mg C g^{-1} soil)	Total N (mg N g^{-1} soil)	C:N	pH	Basal Respiration (µg CO_2-C g^{-1} soil hr^{-1})	Microbial Biomass C (mg biomass C g^{-1} soil)	Respiratory Quotient (qCO_2) (mg CO_2-C g^{-1} biomass C hr^{-1})	Biomass C to total C ratio	SIR (µg CO_2-Cg^{-1} soil hr^{-1})
Oct 04	MRP	15.11 (3.74)	0.54 (0.13)	27.98	6.5	0.418 (0.046)	0.385 (0.019)	1.167	0.024	5.815 (0.214)
	MRG	32.51(1.41)	2.28 (0.44)	14.26	6.7	0.874 (0.026)	0.553 (0.015)	1.579	0.019	9.221 (0.818)
	SDCA	14.79 (1.79)	0.68 (0.15)	21.75	6.4	0.290 (0.106)	0.341 (0.029)	0.850	0.026	5.437 (0.280)
	SDCB	12.95 (1.27)	0.75 (0.09)	17.27	6.4	0.415 (0.046)	0.499 (0.030)	0.833	0.042	7.940 (0.001)
July 05	MRP	19.09 (1.31)	0.85 (0.10)	22.46	-	0.462 (0.041)	0.472 (0.053)	0.979	0.025	6.812 (0.691)
	MRG	15.37 (0.30)	1.07 (0.17)	14.36	-	0.323 (0.045)	0.419 (0.031)	0.771	0.027	5.819 (0.356)
	SDCA	14.10 (1.41)	1.06 (0.20)	13.30	-	0.327 (0.023)	0.583(0.135)	0.561	0.041	8.090 (1.373)
	SDCB	10.52 (1.50)	1.06 (0.12)	9.92	-	0.199 (0.044)	0.504 (0.060)	0.395	0.048	6.977 (0.711)
May 06	MRP	15.33 (1.22)	0.74 (0.14)	20.81	-	0.319 (0.070)	0.441 (0.053)	0.723	0.029	6.275 (0.659)
	MRG	25.45 (1.39)	1.51 (0.13)	16.85	-	0.443(0.062)	0.424 (0.038)	0.573	0.017	5.974 (0.478)
	SDCA	15.98 (0.98)	1.12 (0.11)	14.31	-	0.257 (0.046)	0.398 (0.029)	0.646	0.016	5.622 (0.352)
	SDCB	13.54 (1.43)	1.09 (0.12)	12.74	-	0.228 (0.037)	0.476 (0.042)	0.479	0.035	6.640 (0.546)
March 07	MRP	16.12 (1.09)	0.63 (0.15)	25.59	6.5	0.236 (0.032)	0.580 (0.092)	0.406	0.036	8.071 (1.214)
	MRG	23.76 (1.56)	1.31 (0.12)	18.14	6.6	0.224 (0.031)	0.500 (0.099)	0.447	0.021	6.977 (1.305)
	SDCA	16.91 (0.97)	1.20 (0.13)	14.09	6.4	0.119 (0.008)	0.351 (0.017)	0.339	0.021	4.864 (0.228)
	SDCB	12.98 (0.87)	1.14 (0.20)	11.36	6.4	0.157 (0.022)	0.435 (0.057)	0.361	0.034	6.031 (0.754)

3.4.3 ***Decomposition of Leaf Litter* in situ**

Decomposition of the different species of leaf litter in each soil was described by first order decay kinetic functions (Table 3-3).

Table 3-3 First order decomposition parameters (*k* decomposition rate constant, and *a* fraction readily mineralised material) for decomposition of leaf litters from *Pinus nigra*, *Genista aetnensis* and an equal mixture of the two species (both observed and predicted) in soils from Mount Etna

Site	Litter	*k* (day^{-1})	SD	*a* (%)	SD	R^2
MRP	*Pinus nigra*	0.0011	0.0002	96.52	6.21	0.955
	Genista aetnensis	0.0017	0.0002	99.31	5.81	0.977
	Mixture (observed)	0.0017	0.0003	96.186	8.59	0.944
	Mixture (predicted)	0.0014	0.0003	96.16	7.54	0.947
MRG	*Pinus nigra*	0.0012	0.0002	96.96	5.57	0.968
	Genista aetnensis	0.0016	0.0003	96.45	7.31	0.957
	Mixture (observed)	0.0012	0.0003	96.02	8.76	0.920
	Mixture (predicted)	0.0014	0.0002	96.16	5.82	0.969
SDCA	*Pinus nigra*	0.0013	0.0002	97.28	5.28	0.974
	Genista aetnensis	0.0019	0.0004	98.32	8.66	0.953
	Mixture (observed)	0.0017	0.0001	98.64	3.92	0.989
	Mixture (predicted)	0.0016	0.0003	97.65	6.89	0.964
SDCB	*Pinus nigra*	0.0011	0.0002	97.35	8.01	0.928
	Genista aetnensis	0.0017	0.0001	99.99	3.64	0.992
	Mixture (observed)	0.0013	0.0002	99.45	7.24	0.951
	Mixture (predicted)	0.0014	0.0002	99.08	5.61	0.974

First order decay kinetic functions describe the rate at which a substrate decayed as proportional to the substrate concentration at any given time and this rate changes as the substrate concentration changes. R^2 values for the regression of the mass loss data ranged between 0.920 and 0.992 indicating that the first order decay kinetics

described litter decay in each soil to a high degree (Table 3-3). The decomposition rate constant (*k*) indicated that the overall decomposition rate of *Pinus* in all soils was slower than the rate that *Genista* decomposed which may reflect the resource qualities of these litter species (Table 3-4).

Table 3-4 Chemical Properties of plant litter common to the Mount Etna area. Means of three replicates (± SD)

	Total C (g C g-1 litter)	Total N (g C g-1 litter)	C:N
Genista aetnensis	0.490 (0.032)	0.013 (0.001)	37.4
Pinus nigra	0.465 (0.019)	0.006(0.001)	79.5

Mass loss after 254 days *in situ* showed MRG was the only soil with a higher proportion of *Pinus* litter remaining (Figure 3-4, Appendix A Table 1). At this time there were no significant differences between soils on the rate of *Pinus* decomposition (Appendix A Table 2) however *Genista* litter buried in both MRG and SDCA showed a significantly greater rate of decomposition than the same litter buried in SDCB. This suggests that between burial and 254 days *in situ*, all soil communities were able to degrade *Pinus* litter to a similar degree while the soil communities in MRG and SDCA were more able at decomposing *Genista* litter. After this initial period, no significant differences on the decomposition rate of *Pinus* or *Genista* due to soil development were noted, suggesting that either the mass loss method was too insensitive to detect slight differences in decomposition rate or that each soil community was equally capable of decomposing these two litters. After 571 days *in situ*, *Genista* litter was decomposed to a significantly greater degree than *Pinus* in soils MRG and SDCA while in MRP this was suggested but was not found to be significant. By day 878 of the experiment, SDCB was the only soil to show a

significant difference in the decomposition of *Pinus* and *Genista* litters as *Genista* was significantly more decayed.

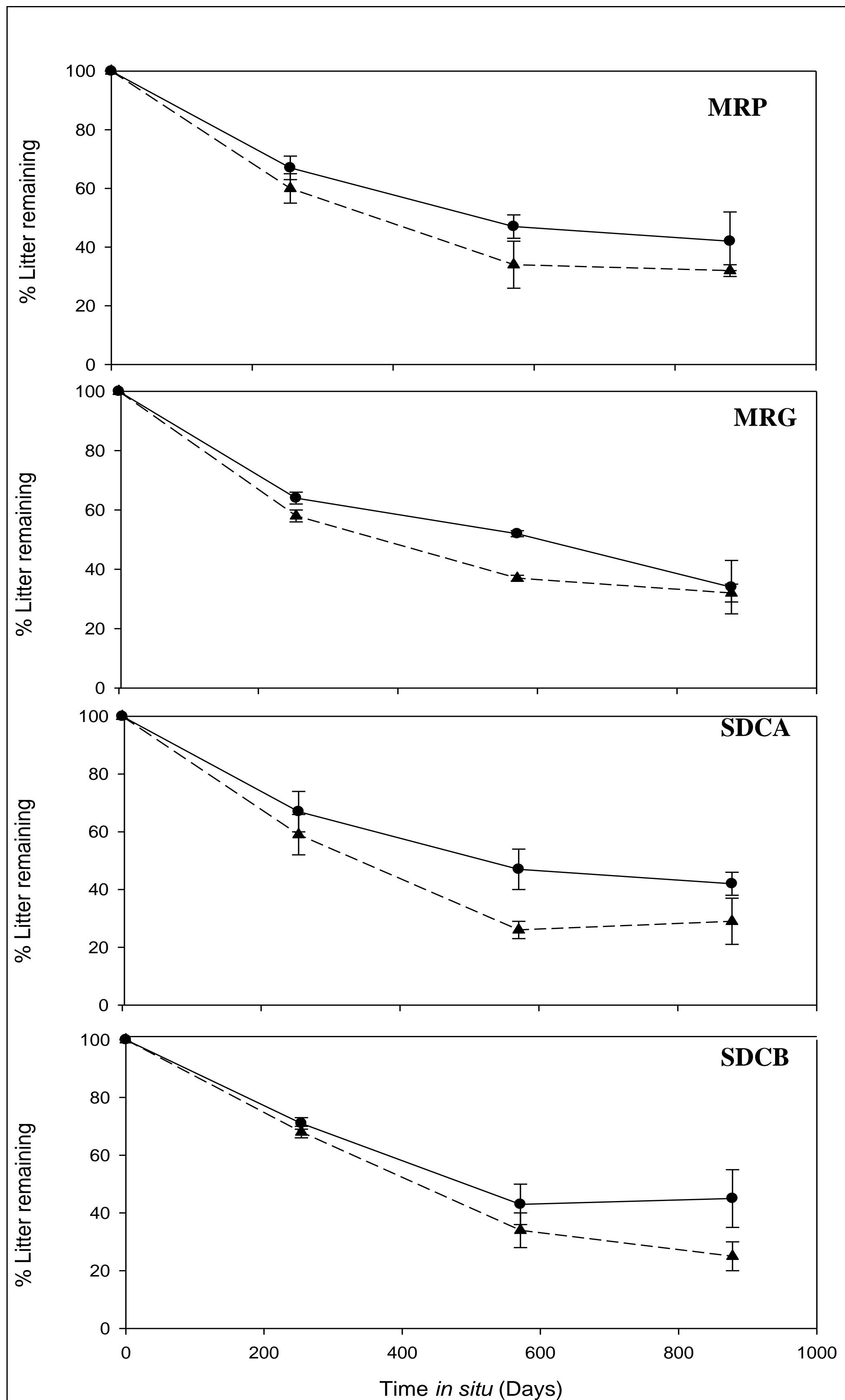


Figure 3-4 Mass loss from *Pinus nigra* (unbroken line with circle) and *Genista aetnensis* (broken line with triangle) when buried in MRP, MRG, SDCA, SDCB.

3.4.3.1 Leaf Litter Respiration

Leaf litter respiration from *Pinus* and *Genista* litter removed from each soil was recorded on three occasions (Table 3-5).

Table 3-5 Respiration from leaf litter (*Pinus nigra* and *Genista aetnensis*) buried in each soil after 254, 571 and 878 days. Values are the mean of three replicates (±SD)

Site	Litter	Respiration (μg CO_2-C g^{-1} litter hr^{-1})			Respiration (μg CO_2-C g^{-1} litter C hr^{-1})		
		Day 254	**Day 571**	**Day 878**	**Day 254**	**Day 571**	**Day 878**
MRP	*Pinus nigra*	0.38	4.43	7.27	0.18	2.02	5.07
		(0.09)	(1.78)	(4.21)	(0.04)	(0.81)	(1.91)
	Genista aetnensis	0.19	7.44	15.02	0.09	3.53	8.97
		(0.09)	(2.30)	(2.52)	(0.04)	(1.10)	(1.20)
MRG	*Pinus nigra*	0.25	6.29	8.93	0.11	3.88	4.06
		(0.06)	(1.40)	(4.46)	(0.03)	(0.64)	(2.03)
	Genista aetnensis	0.26	6.58	14.96	0.12	4.19	7.12
		(0.09)	(1.01)	(4.50)	(0.04)	(0.48)	(2.14)
SDCA	*Pinus nigra*	3.82	7.46	15.01	1.74	3.97	6.83
		(1.53)	(1.36)	(0.81)	(0.70)	(0.62)	(0.37)
	Genista aetnensis	9.78	10.68	13.78	4.65	5.67	6.54
		(1.46)	(1.48)	(5.57)	(0.70)	(0.70)	(2.65)
SDCB	*Pinus nigra*	4.43	8.26	10.03	2.02	3.76	4.56
		(2.68)	(3.55)	(5.78)	(1.22)	(1.61)	(2.63)
	Genista aetnensis	4.78	11.66	16.46	2.27	5.54	7.82
		(0.95)	(2.55)	(3.54)	(0.45)	(1.21)	(1.68)

After 254 days *in situ*, litters removed from soils MRG and SDCB were not significantly different in leaf litter respiration based on litter type (Appendix A Table 3) however microorganisms present on *Pinus* litter buried in MRP respired significantly more C than those present on *Genista* litter. When respiration was calculated per gram of litter C this was found to be insignificant (Appendix A Table 3). Microbial respiration from *Genista* litter buried in SDCA was significantly greater than from *Pinus* litter when calculated both per gram of litter and per gram of litter C (Appendix A Table 3).

One way ANOVA showed that in the initial burial period, soil development had a significant effect on microbial respiration on both *Pinus* and *Genista* litter (Appendix

A Table 4). Respiration from litter buried in the developed soils was significantly greater than respiration from litter buried in developing soils. This suggests that the microorganisms in the developed soils were decomposing each of the litter species at a faster rate than those from the developing sites. After this initial period there were no soil development effects on microbial respiration from *Pinus* litter however there was a significant development effect on respiration from *Genista* litter after 571 days *in situ* but only when calculated per gram of litter C. In this instance, microorganisms present on *Genista* litter buried in the developed soils respired significantly more C per gram of litter- C than the microorganisms present at MRG.

3.4.3.2 Leaf Litter Microbial Biomass

Microbial biomass measurements were recorded on a select number of samples to include one developing soil and one developed soil on two sampling occasions. Microbial biomass on *Pinus* litter was initially greater in litter removed from MRP (Table 3-6) although this difference was not significant. After 878 days *in situ*, microbial biomass was substantially higher on *Pinus* litter buried in SDCA (Table 3-6). This may be due to a higher proportion of microorganisms present in the developed soil which are capable of decomposing the more recalcitrant residues in *Pinus* litter leading to a higher abundance of microbial biomass.

Table 3-6 Microbial biomass on *Pinus nigra* and *Genista aetnensis* litter after 254 and 878 days *in situ* at two sites of different ages on Mount Etna. (Units are mg biomass C g^{-1} litter (±SD))

Site	Litter type	Day 254	Day878
MRP	*Pinus nigra*	10.45 (1.99)	2.03 (0.55)
	Genista aetnensis	6.35 (2.00)	6.87 (0.45)
SDCA	*Pinus nigra*	7.18 (0.87)	5.55 (1.27)
	Genista aetnensis	5.99 (0.43)	7.19 (1.21)

This is also supported by the substantial reduction in microbial biomass present on *Pinus* litter buried in the developing soil between 254 and 878 days *in situ* while microbial biomass on the same litter buried in the developed soil is not significantly different. This suggests that the microbial community present in the developing soils may have a lower abundance of organisms capable of degrading the more recalcitrant compounds which remained in the *Pinus* litter after 878 days *in situ*. Microbial biomass present on *Genista* litter did not vary significantly over time or due to soil development which suggests that both soil communities contained a group of microorganisms which were still able to sustain themselves on the degraded litter.

3.4.3.3 Leaf litter C and N

C and N analysis of *Pinus* and *Genista* leaf litter showed that litters buried in soil contained significantly more N than control samples but C content was not significantly different (Table 3-7). *Genista* litter buried in MRG, SDCA and SDCB contained significantly more C than *Pinus* litter. N concentration was consistently higher in *Genista* litter.

Table 3-7 Total carbon and nitrogen contents of *Pinus nigra* and *Genista aetnensis* litter buried alone or as a mixture after 254 days *in situ* in various soils ranging in developmental stage. Values are means (±SD)

Site	Litter type	Total Carbon (mg g^{-1} litter)	Total Nitrogen (mg g^{-1} litter)	C:N
MRP	*Pinus* alone	451.32 (7.84)	9.83 (1.38)	45.9
	Pinus mixed	444.80 (20.20)	10.81 (0.58)	41.2
	Genista alone	469.54 (32.65)	16.43 (2.16)	28.6
	Genista mixed	500.53 (28.86)	18.94 (3.06)	26.4
MRG	*Pinus* alone	434.01 (14.24)	11.02 (0.46)	39.4
	Pinus mixed	441.51 (4.39)	11.34 (1.11)	39.0
	Genista alone	458.87 (5.97)	22.13 (0.70)	20.7
	Genista mixed	475.57 (32.66)	19.44 (4.54)	24.5
SDCA	*Pinus* alone	447.77 (17.54)	9.46 (0.39)	47.4
	Pinus mixed	469.50 (7.38)	10.84 (0.93)	43.3
	Genista alone	478.92 (19.48)	19.75 (3.23)	24.2
	Genista mixed	502.24 (25.18)	18.99 (1.76)	26.4
SDCB	*Pinus* alone	430.25 (31.12)	9.91 (1.86)	43.4
	Pinus mixed	417.92 (22.39)	9.65 (0.48)	43.3
	Genista alone	493.50 (5.86)	22.55 (1.32)	21.9
	Genista mixed	507.97 (4.13)	17.75 (1.08)	28.6
Control (unburied)	*Pinus*	464.70 (19.33)	5.85(0.54)	79.5
	Genista	489.89 (32.06)	13.11 (0.82)	37.4

Genista litter buried in SDCB contained significantly more C than when the litter was buried in MRG however this was the only significant difference in C contents of litters buried in soils of different developmental stage. N content of *Pinus* litter buried in MRG was significantly greater than *Pinus* litter buried in SDCA while *Genista* litter buried at MRP contained significantly less N than when buried at both MRG and SDCB.

3.4.3.4 NMR

NMR spectra for control litters showed strong resonances in the O alkyl region (60-90 ppm) (Figure 3-5, Table, 3-8) which is indicative of high polysaccharide contents. There were distinct differences between the NMR spectra of the control *Pinus* and *Genista* litters (Figure 3-5, Table, 3-8). *Pinus* litter showed strong resonances in the aromatic regions compared to *Genista* reflecting the substrate qualities of the two litters. *Genista* litter contained stronger signals in the alkyl C region (45-10 ppm) and also the O methyl and N alkyl C region (60-45 ppm) which may reflect the higher N content in *Genista* litter. The ratio of alkyl C to O alkyl C was 0.26 in *Pinus* litter but 0.51 in *Genista* due to the increased concentrations of alkyl C in *Genista* litter.

NMR spectra of litters decomposed in soils at different stages of development were similar. After 254 days *in situ*, the concentration of O alkyl C and acetyl and ketal C decreased in both litters and to approximately the same degree for litters buried in both the developed and developing soils. Alkyl C which represents lipids and aliphatic hydrocarbons, increased in both litters with decomposition. The increases in alkyl C were similar in both litters when buried in the developing soil however when buried in the developed soil, *Genista* litter had a larger increase than *Pinus* perhaps indicating a slightly faster decomposition.

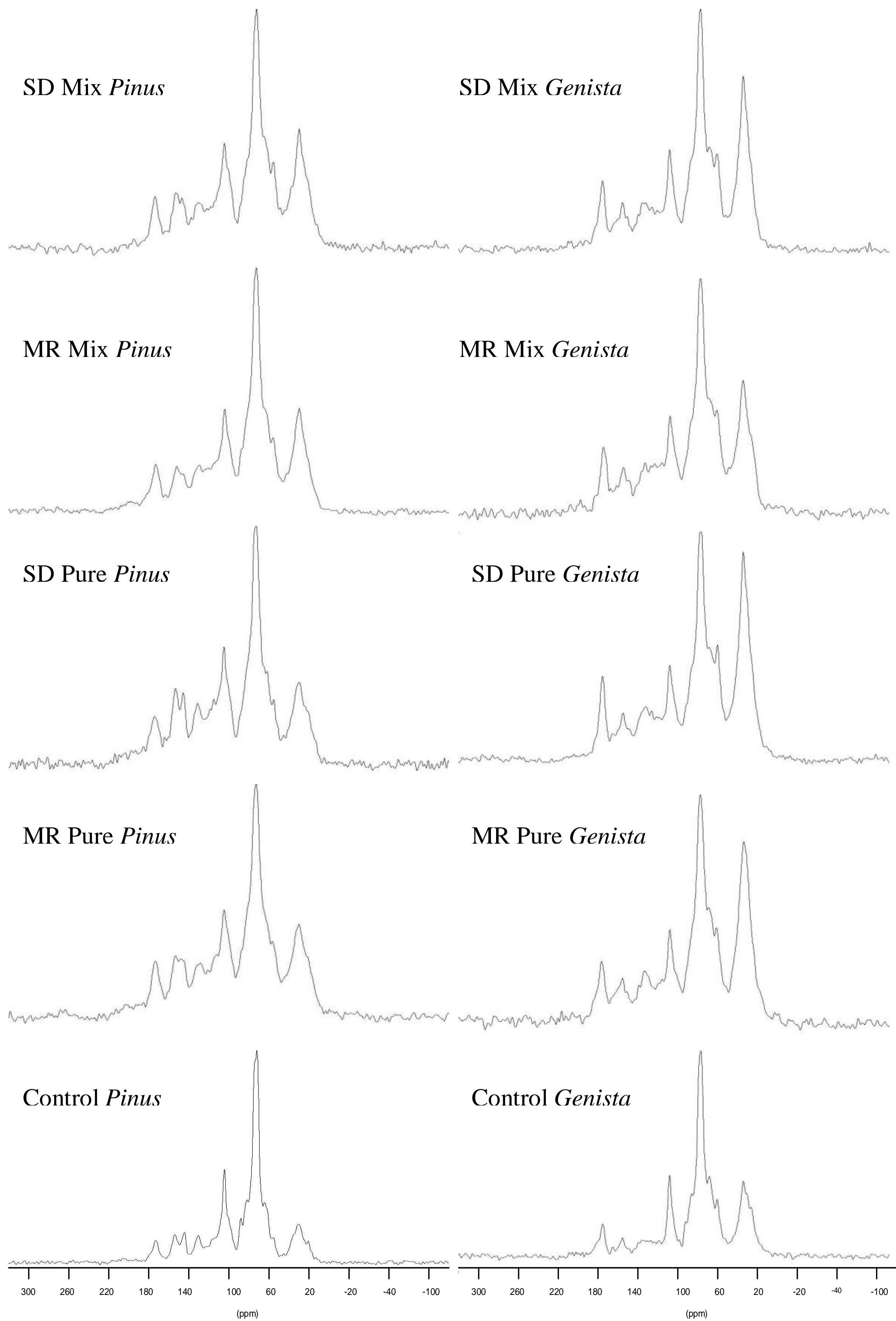


Figure 3-5 ^{13}C NMR spectra of leaf litter buried alone (Pure) or as mixtures (Mix) at Monti Rossi (MR) or Salto del Cane (SD) after 254 days *in situ.* Control samples were not buried *in situ.*

Table 3-8 Integration values and specific ratios for the major C types in the ^{13}C NMR spectra of unburied leaf litter and litter buried alone or as a mixture after 254 days *in situ*.

Sample	Carbonyl C (220-185ppm)	Carboxyl C (185-160 ppm)	O or N linked aromatic C (160-140 ppm)	C substituted aromatic C (140-110 ppm)	Acetyl and ketal C (110-90 ppm)	O alkyl C (90-60 ppm)	O Methyl and N alkyl C (60-45 ppm)	Alkyl C (45-10 ppm)	Alkyl C to O alkyl C
Control *Pinus*	1.5	4.2	7.1	10.2	14.2	45.5	5.3	11.9	0.26
MR pure *Pinus*	2.8	6.5	8.4	12.4	11.8	31.8	8.3	17.9	0.56
SD pure *Pinus*	2.9	6.7	9.8	13.7	12.0	32.0	7.3	15.7	0.49
MR mix *Pinus*	2.1	6.0	6.3	11.9	11.7	35.1	8.4	18.4	0.52
SD mix *Pinus*	0.8	5.7	7.5	11.3	11.6	32.6	9.3	21.2	0.65
Control *Genista*	1.0	5.2	3.1	7.0	10.6	42.1	9.8	21.2	0.51
MR pure *Genista*	1.8	6.9	5.3	10.1	9.0	29.9	9.2	27.7	0.93
SD pure *Genista*	1.1	7.1	5.2	10.6	8.9	28.5	10.1	28.4	1.00
MR mix *Genista*	1.6	6.7	5.6	11.5	10.2	31.9	10.5	22.0	0.69
SD mix *Genista*	1.6	7.0	5.5	10.6	9.5	31.4	9.4	25.0	0.80

Decomposition increased resonances in the aromatic regions of both litters compared with controls. For *Genista* litter this was approximately the same for both soils but *Pinus* litter showed slightly enhanced aromatic signals when buried in the developed soil compared with developing soil. The alkyl C to O- alkyl C ratio increased with decomposition as alkyl C increased and O- alkyl C decreased and this was more pronounced in *Genista* litter.

3.4.4 ***Leaf Litter Mixtures***

Predicted decay rates, respiration and microbial biomass for mixed species litters were calculated based on the values obtained from the component species and the proportion of each species present in the mixed litter bags. Observed mass loss was not significantly different from predicted mass loss on any occasion in any soil other than at MRG after 571 days *in situ* where observed mass loss was significantly lower than predicted from the component species (Table 3-9). No significant differences were noted in first order decomposition kinetics which indicates overall rates of loss (Table 3-3). Respiration or CO_2 evolution is used as a proxy for decomposer activity. It may be possible that if two or more component species are present as a mixture and produce a net positive or negative effect, this effect could be assessed by measuring respiration. Observed and predicted rates of respiration were not significantly different on any occasion in any soil which indicates that the mixed species were decomposing at the same rate as their component species. Additive effects were also found for microbial biomass measurements in both the developed and developing soils on each sampling occasion indicating that mixing of these two litter species did not elicit any positive or negative effects on the microbial biomass. No significant differences in total C or N content were found between litters decomposed alone or as

a mixture of *Pinus* and *Genista* litter in any soil (Table 3-7). NMR spectra of litters decomposed as a mixture compared with those decomposed alone showed slight reductions in the amount of aromatic C present in both soil whilst O alkyl C slightly increased. The alkyl C to O alkyl C ratio indicated a slight difference between mixtures and pure litters however it was not possible to test this statistically. *Pinus* litter decomposed in developed soils had a higher ratio compared with the pure litter but when buried in developing soils the difference between mix and pure litters was very small. *Genista* litter buried in a mixture showed reductions in the ratio compared with pure litters and the effect was slightly more pronounced for litters buried in the developed soil. The NMR spectra were not replicated however so it is not possible to test the hypothesis statistically in order to calculate significance.

Table 3-9 Comparison of observed and predicted mass loss, respiration and microbial biomass of mixed species litter based on values obtained from component species. Values are means (±SD). *p probability that observed and predicted values are not significantly different .**

Days *in situ*	Site	Mass Loss (% Litter Remaining)			Respiration (μg CO_2-C g^{-1} litter hr^{-1})			Microbial Biomass (mg C g^{-1} litter)		
		Observed	**Predicted**	***p****	**Observed**	**Predicted**	***p****	**Observed**	**Predicted**	***p****
254	MRP	65 (1)	61.5 (4.5)	NS	0.28 (0.06)	0.29 (0.09)	NS	9.54 (1.31)	8.40 (2.00)	NS
	MRG	61 (1)	61 (2)	NS	0.21 (0.00)	0.26 (0.08)	NS	-	-	-
	SDCA	62 (6)	62 (3.5)	NS	9.72 (1.34)	6.80 (1.49)	NS	5.47 (0.98)	6.59 (0.65)	NS
	SDCB	74 (4)	69.5 (2)	NS	2.91 (0.43)	4.61 (1.81)	NS	-	-	-
571	MRP	32 (1)	40.5 (6)	NS	8.06 (0.88)	5.94 (2.04)	NS	-	-	-
	MRG	57 (2)	44.5 (3)	**	6.60 (0.24)	6.43 (1.21)	NS	-	-	-
	SDCA	35 (7)	34.5 (5)	NS	9.32 (1.75)	9.07 (1.42)	NS	-	-	-
	SDCB	40 (2)	38.5 (6.5)	NS	6.36 (1.16)	9.96 (3.05)	NS	-	-	-
878	MRP	29 (8)	37 (6)	NS	8.49 (4.64)	11.14 (3.36)	NS	4.53 (0.52)	4.70 (0.50)	NS
	MRG	33 (4)	33 (6)	NS	11.32 (2.01)	11.94 (4.48)	NS	-	-	-
	SDCA	27 (8)	32.5 (6)	NS	10.11 (1.08)	14.39 (3.19)	NS	7.49 (1.14)	6.34 (1.24)	NS
	SDCB	40 (4)	35 (7.5)	NS	17.15 (3.07)	13.24 (4.66)	NS	-	-	-

NS no significant differences, * $P<0.05$, ** $P<0.01$, *** $P<0.001$

3.5 Discussion

3.5.1 *Soil Characteristics*

Total C and N contents of the soils were quite similar regardless of soil development. This is not in agreement with other studies of developmental sequences (Insam and Haselwandter, 1989; Sigler et al. 2002; Tscherko et al. 2003; Tscherko et al. 2004 and Hopkins et al. 2007) who all noted increases in C and N contents with increasing development. The main differences in soil C and N contents between the current study and Hopkins et al. (2007) who investigated the same sites 4 years previously, is increased concentrations of total N in the developing soils and declines in total C and N in the developed soils. The increase in total N concentration in the developing soils reduced the C:N ratio to similar values as the developed soils. The developed soils, despite a reduction in total C and N, have similar C:N values to Hopkins et al. (2007) indicating that this reduction was proportionally similar for both elements and could possibly be a result of nutrient dilution by fine ash and tephra deposited in the eruptions of 2001 and 2002. Similar dilution effects by volcanic ejecta have also been noted in soils in Nicaragua (Velasquez- Pereira, 1996).

Total C and N contents were lower than volcanic soils sampled in Japan (Nishiyama et al., 2001) but were similar to young volcanic ash soils of Nicaragua (Joergensen and Castillo, 2001). Microbial biomass however was higher in the Etna soils than in the young volcanic soils of Nicaragua and was in the lower range for microbial biomass C in Japanese volcanic soils. This indicates that the Etna soils are supporting a higher microbial biomass per unit of C compared with very young volcanic soils. This concurs with the theory of Anderson (1994) who suggested that the ratio of biomass C to total C should increase with soil development. Evidence for this within

the different developmental stages of Etnas' soils is lacking however. Based on Odum's (1969) theory of ecosystem development, Anderson and Domsch (1985) proposed that as soil communities develop, microbial communities become more efficient in their energy use and incorporate a higher proportion of C into biomass. The metabolic quotient (qCO_2) has been shown to decline during the first 1000 years of community succession (Insam and Haselwandter, 1989). Metabolic quotient in the Etna soils generally declined with soil development indicating a more metabolically efficient community in the developed soils. There have been several critiques of the metabolic quotient however. Wardle and Ghani (1995) criticised the effectiveness of biological ratios in determining metabolic efficiency with time as increasing stress during later succession can cause increases in metabolic quotient and Dilly and Munch (1996) suggested that the quotient was too unspecific to detect changes in ecosystem development. Care should be taken when interpreting metabolic quotient data so that the limitations are understood, however the quotient is still a very useful indication of how efficiently the microbial community is utilising C resources (Ohtonen et al., 1999).

3.5.2 ***Leaf Litter Decomposition in situ***

Ohtonen et al. (1999) concluded in a study of glacier foreland succession that C use efficiency of soil microorganisms increases with successional stage and that these changes were related to changes in microbial community structure. This may be reasonable for the short timescales over which Ohtonen et al. (1999) investigated (20-80 years since exposure) however this may not be applicable to soils which have developed over several hundred years. There were few significant differences in mass loss of leaf litter between the Etna soils at different developmental stages. This may

be because the method was not sensitive enough to detect differences in decomposition or because the microbial communities of both soils were equally able to metabolise the nutrients within both leaf litter species. Slight differences were noted in the initial phase of decomposition (254 days *in situ*) as litter bags buried at MRG and SDCA contained significantly less *Genista* litter than bags buried in SDCB soil. This suggests the microbial communities at MRG and SDCA contained a suite of microorganisms which were better adapted at decomposing *Genista* in the initial phases of degradation however after this period no significant site differences were noted.

At early stages of decomposition, water soluble substances have been found to decline quickly while concentrations of nutrients such as N, P and S start to increase (Berg, 2000). Water soluble substances may be leached from leaf litter however this is probably more of a factor in green leaf litter as opposed to the needle like *Pinus* or *Genista* litter (Berg, 1986). Increased N concentrations were noted in litter which had been *in situ* for 254 days compared with N concentrations of intact litter. At later stages of leaf litter degradation, the rate of decomposition is thought to be governed by increasing concentrations of recalcitrant materials such as lignin (Berg and Meentemeyer, 2002). NMR spectra for both litters were enhanced in the regions which represent lignin and tannin after 254 days *in situ*. As well as changes in the physical and chemical composition of leaf litter during degradation, the predominant soil community is also thought to change (Wardle, 1993) from a mainly zymogenous community to an autochthonous species dominated community (Dilly and Munch, 1996). This was inferred from an observation that during decomposition the metabolic quotient declined and it was suggested that organisms had become more efficient in

utilising C resources. In the current study, metabolic quotient of microbes on leaf litter tended to increase with the progression of decomposition and microbial communities present on *Genista* litter, regardless of soil development had lower qCO_2 values. This may indicate that the microbial communities present on the leaf litters after approximately 2 years *in situ* were stressed due to depleting C resources (Wardle, 1993) and that this stress was more apparent for *Pinus* litter. This is highlighted by the fact that microbial biomass on *Pinus* litter in both developed and developing soils declined markedly with length of litter burial however this was not apparent for N rich *Genista* litter which maintained a similar level of microbial biomass at both early and later stages of decomposition. This is not in concurrence with studies which suggest a higher initial N availability in litter retards lignin decomposition in later stages (Berg et al., 1996; Berg, 2000). It has been hypothesised that low- molecular N can react with lignin to produce more recalcitrant aromatic compounds (Berg, 2000). Sjöberg et al. (2004), however, in a study of plant litter component decomposition in relation to N content found their results contradicted the previous theories as N addition had no effect on the later stages of decay.

In general, NMR spectra of litter samples were quite similar regardless of soil development however NMR was the only method which was able to detect small differences between soils. NMR spectra showed decreases in O alkyl C and increases in the amount of alkyl C and aromatic compounds as a result of *in situ* decomposition. This is because polysaccharides such as cellulose and hemicellulose which are represented by O alkyl C are preferentially degraded during the initial stages of decomposition while plant biopolymers are selectively preserved (Haslam et al., 1998) as are recalcitrant aromatic compounds such as lignins and tannins (Quideau et

al., 2000). Increases in alkyl C were less pronounced in *Pinus* litter buried in the developed soil and this was accompanied by a slight increase in aromatic compounds. This may indicate a slightly slower rate of decomposition of *Pinus* litter in this soil which is supported by a smaller increase in the alkyl C to O alkyl C ratio. The decomposition of organic matter in soil is generally characterised by an increase in alkyl C while O alkyl C declines and it is thought that the ratio of alkyl C to O alkyl C is a good indicator of decomposition (Preston, 1996; Baldock et al., 1991).

3.5.3 ***Leaf Litter Mixtures***

It has been suggested that nutrient release from rapidly decaying litter species can facilitate the decomposition of more recalcitrant litter types (Seastedt, 1984). *Genista* litter contained significantly more N and fewer aromatic compounds than *Pinus* litter suggesting that it was a higher quality litter type (Hopkins et al., 2007). Decomposition of *Pinus* and *Genista* litter as mixtures showed additive effects for mass loss, respiration, microbial biomass and C and N contents while NMR showed slight differences but these were not statistically testable. This is not in agreement with the many reports of non additive effects of litter mixing. Gartner and Cardon (2004) in a review of the decomposition dynamics of mixed species litter reported that 67% of all mixtures tested exhibited non additive mass loss effects and most of these were synergistic. These results have been reported for mixtures of different functional groups (Wardle et al., 1997; Ganjegunte et al., 2005), when broad leaved species are mixed (Briones and Ineson, 1996) and when broad leaved species have been mixed with needle litters (Salamanca et al., 1998).

Ganjegunte et al. (2005) found that when *Pinus radiata* needles were mixed with understory species including *Cystisus scoparius* (broom), *Pinus* decomposition was retarded while broom decomposition was enhanced. These differences in decomposition rates were attributed to significant transfers of nutrients from one litter species to another. C and N contents of the mixed *Pinus* and *Genista* litters in the Etna soils were not significantly different from pure litters indicating that no translocation of major nutrients from one litter species to another had occurred. Translocation of nutrients has also been reported in several other studies such as Salamanca et al. (1998) who found that N contents of Japanese Red Pine (*Pinus densiflora)* were higher than predicted when mixed with nutrient rich Japanese Konara Oak *(Quercus serrata)* litter while the N content of *Quercus* was lower than predicted. McTiernan et al. (1997) proposed a mechanism for this translocation of nutrients as the chemical characteristics for litter mixtures were much more alike than those decomposed singly. They hypothesised that fungal hyphae could be responsible for the movement of nutrients from one litter to another as it was noted that there was a high abundance of fungal hyphal bridges between component litters of mixtures. The hyphae of fungal species can extend for long distances allowing them to retrieve nutrients from long distances and transport them to sites of decomposition. The presence of fungal PLFA biomarkers was low in the Etna soils (see section 4.4.1.5) and may indicate a possible explanation for additive effects of litter mixing. It has also been proposed that nutrient gradients could be responsible for the transport of nutrients from high to low concentrations via water films (McTiernan et al., 1997).

Recent investigations concerning the effects of mixing high nutrient status species with nutrient poor species have presented alternative results to the accepted

hypotheses. Smith and Bradford (2003) examined the effects of mixing specimens of the same species but with different nutrient concentrations to ascertain whether the nutrient status of component mixtures effects decomposition. Specimens with high N content were mixed with those with low N content. The results generated did not support the hypothesis that differences in initial N concentration between litters can cause positive, non additive effects and in fact showed the opposite effect. Hoorens et al (2003) in a similar experiment found by regression analysis that litter mixtures containing species with very different initial litter chemistry did not show any stronger interaction than those with similar initial litter chemistry. The results of this experiment support the work of Hoorens et al. (2003) and Smith and Bradford (2003) as N concentrations in *Genista* were significantly higher than those in *Pinus* but no non additive mixing effects were found. Smith and Bradford (2003) tested only one particular component of initial litter chemistry however and does not take into account differences in other chemical characteristics. Hoorens et al. (2003) suggested that their experiment "*convincingly*" proved that initial chemical composition of litters is not a useful concept to explain interactions in litter mixtures. The authors point out that some factor other than initial litter chemistry must be responsible for interactions in litter mixtures, otherwise several parameters are involved in "*such a complex manner that effects cannot be described by single litter chemistry parameters"*.

Respiration is often used as a proxy for estimating microbial activity in soils. Salamanca et al. (1998) monitored litter decomposition in a microcosm experiment and found that rates of respiration obtained from mixed litters were indicative of "*higher biological activities*" mediating the decay process. McTiernan et al. (1997) monitored respiration evolved from soils containing several pure litters and all

possible combinations of mixtures from these litters in a microcosm experiment. They found eight combinations showed a significant positive interaction for total CO_2 production whilst only one gave a significant negative interaction. It was commented that the results indicated that it was more probable for positive interactions than negative interactions to occur. Fyles and Fyles (1993) however, in an investigation of the decomposition of Douglas fir and Red Alder suggested that litters have the potential to interact in a positive or negative manner. There was no significant positive or negative effect on microbial respiration in the Etna soils when litters were decomposed as a mixture or alone which suggests mixing litters did not enhance or retard biological activities. Hansen and Coleman (1998) suggested increased structural complexity in a heterogeneous litter may have more stages of decomposition reducing competitive interactions and creating an increased number of niches possibly encouraging the growth of a more diverse soil community.

Few studies have investigated microbial biomass dynamics on leaf litter mixtures and of those studies conducted; the results have been highly variable. Bardgett and Shine (1999) examined the relationship between diversity of grass species and soil microbial biomass and found that soil microbial biomass increased with increasing grass species diversity. In contrast, Blair et al. (1990) found mixed litter generally contained less bacterial biomass and shorter fungal hyphae than single species; while McArthur et al. (1994) observed a significant reduction in bacterial diversity in some litter mixture combinations but no difference in others. The mixing of litters from different species has been hypothesised to alter not only the chemical environment in which they decompose, but also the physical environment creating an increased number of niches which allow the proliferation of a greater diversity of organisms and biomass to exist

(Hansen and Coleman, 1998). This could not be supported in the Etna soils as no effects of litter mixing were found on microbial biomass.

No direct conclusions can be drawn about the effects of litter mixing as both strong positive and negative effects have been reported over a number of parameters but any non additive effects are probably highly complex and dependant on the types and properties of litter used. Interactions between litter mixtures although common are probably not predictable (Fyles and Fyles, 1993).

3.6 Conclusions

The following conclusions have been drawn from this chapter:

- There were no correlations between soil development and microbial biomass, respiration or soil C and N content however there was a slight reduction in qCO_2 with time suggesting the developed soils may have been more metabolically efficient.
- Mass loss from litter bags was not significantly different in soils at different developmental stages which may be a result of the insensitivity of the method used or because the microbial communities were equally well adapted at decomposing both litter types.
- Respiration from leaf litter buried in soils at different stages of development was not significantly different which implies that C mineralization is equally efficient in both soils.
- Observed mass loss, respiration and microbial biomass were not significantly different from values predicted from pure litters indicating no non additive interactions between *Genista aetnensis* and *Pinus nigra*.
- The chemical components (C and N) of the litters decomposed as a mixture were not more similar than those decomposed alone suggesting there was no translocation of N from the higher quality litter *Genista aetnensis* to the lower quality litter *Pinus nigra* and this was the same for both soil developmental stages. NMR spectra showed slight but not statistically testable differences.

Chapter 4 PLFA diversity, community structure and enzyme activity in volcanic soils at different stages of development and during an environmental disturbance. [1]

4.1 Introduction

Volcanic eruptions are major natural disturbances with varied and complex consequences (Del Moral and Grishin, 1999). The extrusion of lava alters biological activity, creating virgin land surfaces which through time will be subject to soil formation and colonisation under relatively similar climatic conditions (Hopkins et al., 2007). The colonisation of volcanic substrates after an eruption have been studied with regard to plant communities (Del Moral, 1993; Vitousek et al., 1993; Kanehiro et al., 1995; Aplet et al., 1998) but less consideration has been given to the soil microbial community (Schipper et al., 2001). Microbial primary succession after a volcanic eruption however is perhaps the least well documented form of succession. Several authors have investigated microbial succession on the forelands of receding glaciers and have reported major shifts in the chemical and biological nature of soils through time including accumulation of organic matter; increases in microbial biomass, carbon metabolism efficiency, functional diversity, enzyme activities and composition shifts from bacterial to fungal dominated communities (Insam and Haselwandter, 1989; Chapin et al., 1999; Ohtonen et al., 1999; Schipper et al., 2001, Tscherko et al., 2003). Insam and Haselwandter (1989) proposed Odum's strategy of ecosystem development (Odum, 1969) to account for shifts in the efficiency of carbon utilisation by microbial communities during development, however it has become increasingly apparent that this theory may be

[1] Parts of this chapter have been accepted for publication in Soil Biology and Biochemistry, see Appendix C

incomplete (Ohtonen et al., 1999; Tscherko et al., 2003) as it does not take into account changes in microbial structure, resource utilisation or effects of competition. Waldrop et al (2000) suggested that compositional shifts in microbial communities could be linked with changes in specific enzyme activities but there are probably other important abiotic controls on enzyme activities in soils such as nutrient limitation (Allison et al., 2007). As organic matter accumulates, mineralization mediated by extracellular enzymes many of which are released by microorganisms becomes increasingly important for ecosystem functioning (Allison et al., 2007). Limitation or demand for specific nutrients is thought to lead to an increase in enzyme activities in order to mobilise resources (Allison and Vitousek, 2005). Conversely, when nutrients required for energy are readily available, corresponding hydrolysing enzymes are often suppressed (Chróst, 1991). This suggests that in soils at different developmental stages, extracellular enzymes could be useful in identifying changes in nutrient demand and soil community function.

It has long been envisaged that there is a positive relationship between species diversity and community stability (Elton, 1958) but there are several properties which define stability. Resilience defines a community's capacity to return to its equilibrium state after perturbation while resistance defines a community's ability to resist changes due to perturbation (Begon et al., 2000). It has been hypothesised that lower diversity communities may be less resistant and resilient to an environmental disturbance than higher diversity communities (Pimm, 1984; Tilman and Downing, 1994). Although some evidence for this exists for plant or animal communities (Tilman et al., 1996; Naeem and Li, 1997), it is not understood whether this may be the case for microbial communities due to the potential for microbial diversity to be

very high possibly resulting in a high occurrence of functional redundancy (Degens et al., 2001; Findlay, 2002) but maintaining community function after perturbation may be more important than maintaining taxonomic diversity. Ecosystem functioning is envisaged to require a threshold minimum number of species before ecosystem collapse although a larger number of species is probably required in order to maintain ecosystem stability in a changing environment (Loreau et al., 2001).

4.1.1 ***Phospholipid Fatty Acid Analysis (PLFA)***

Phospholipids are essential components of the membranes of all living cells and are not found in dead cells or storage products (Tunlid and White, 1992; Zelles et al., 1995; Zelles, 1999). Investigation of the decomposition of labelled phosphatidylcholine in soils showed that microbial phospholipids had a rapid turnover rate in soils (Tollefson and McKercher, 1983) suggesting they make up a relatively constant proportion of the soil microbial biomass (Lechevalier, 1989). The addition of various substrates to soils has shown that PLFA analysis can detect rapid changes in soil microbial community structure (Albers et al., 1994, cited in Zelles, 1999), providing a useful method for detecting structural changes in communities of different developmental stages.

4.1.1.1 Origins and Applications of PLFA

In 1959, Bligh and Dyer developed a method of extracting and purifying lipids of biological materials in a single operation. They hypothesised that optimum lipid extraction should result when the sample is homogenised with a mixture of chloroform and methanol. The results showed that the method was very effective to the point of almost being quantitative in separating lipids from non lipids (Bligh and

Dyer, 1959). Although Bligh and Dyer developed the method for the extraction of lipids from fish tissues, it has become useful in other areas of microbiology such as in the analysis of marine and estuarine sediments (White et al., 1979) and has more recently been applied to the study of soil microorganisms (Zelles et al., 1995; Frostegård and Bååth, 1996; Nielsen and Petersen, 2000; Steinberger et al., 1999; Bai et al., 2000; Malosso et al., 2004). The wide range of studies that have subsequently utilised PLFA profiles for the quantitative and qualitative analysis of microbial communities sanctions it as a robust method to fingerprint microbial communities (Bossio and Scow, 1998) and measure their biomass (Vestal and White, 1989).

The data obtained from PLFA analysis can be used to interpret effects at several different levels (Bossio and Scow, 1998). The whole PLFA profile can be used as a community fingerprint which allows alterations in community structure to be assessed. This can be particularly useful in assessing community change after an environmental disturbance. At a more resolute level, specific biomarkers can be used to identify specific types of microorganisms or functional groups such as Gram positive and Gram negative bacteria or fungi (Findlay and Dobbs, 1993; Zelles, 1997; Steinberger et al., 1999; Zelles, 1999). PLFAs can also be used to detect changes in the physiological state of microbial communities, either by specific biomarkers or by calculating fatty acid ratios (Guckert et al., 1985; Kieft et al., 1994; Bossio and Scow, 1998).

4.1.1.2 PLFA Biomarkers and their Interpretation

Fatty acids (FAs) of membrane phospholipids are considered to be useful biomarkers as they are often very structurally diverse and have high biological

specificity (Zelles, 1999). As well as many investigations of estuarine and marine sediments (White et al., 1979; Guckert et al., 1985; Findlay et al., 1989, Llobet-Brossa et al., 2002), PLFA profiles have been obtained from a wide range of soil environments including rice paddy soil (Bai et al., 2000), desert soils (Steinberger et al., 1999, Vishnevetsky et al., 2000), boreal forest humus (Pennanen, 2001) and peatlands (Sundh et al., 1997). Given the wide range of environmental conditions where PLFA profiles have been obtained, it is possible to characterise certain fatty acids that are present and or abundant in a particular environment into different functional groups (Table 4-1).

Table 4-1 Common phospholipid fatty acid biomarkers and their interpretations

Biomarker	Interpretation	Source	Comments
Branched chain (*i*15:0, *a*15:0, *i*16:0, *i*17:0)	Gram positive bacteria	Schinner et al (1996)	Also found in Gram negative bacteria
Polyunsaturates (18:2ω9 12, 18:2ω6c)	Fungi	Federle (1986)	Also found in plant cells
Cyclopropyl (cy17:0, cy19:0)	Stress and/ or starvation	Guckert et al (1986)	
cis/trans ratio	Stress	Guckert et al (1986)	

Certain FAs are characteristic of all organisms such as the saturated FAs 14:0 (myristic acid), 16:0 (palmitic acid) and 18:0 (stearic acid). However, branched saturates such as *i*15:0, *a*15:0, *i*16:0 and *i*17:0 are thought to be indicative of Gram positive (G+) bacteria (Schinner et al., 1996; Bossio and Scow, 1998; Tscherko et al., 2004) but are also found in Gram negative (G-) sulphate reducing bacteria (Haack et al., 1994). Cyclopropyl FAs *cy*17:0 and *cy*19:0 have been found in both aerobic (Parkes and Taylor, 1983) and anaerobic (Guckert et al., 1985) bacteria, but are also thought to be symptomatic of a physiologically stressed or starving community (Guckert et al., 1986). Identified in a number of soil fungi by Federle

(1986), polyunsaturated linoleic acid (18:2ω9,12 or 18:2ω6c) is almost exclusively considered to be an indicator of fungi (Zelles, 1999). Signature FAs or biomarkers have to be interpreted with some caution however; β-hydroxy FAs (β-OHFAs) are a major constituent of lipopolysaccharides which are one of the main components of Gram negative (G-) bacterial cell membranes. It has therefore been suggested that the presence of β-OHFAs could be a biomarker for G- bacteria (Parker et al., 1982). Zelles (1997) however found that β-OHFAs were also widely found in G+ bacteria and in plants.

4.1.2 ***Soil enzymes***

The enzymes secreted by soil microorganisms can provide a useful insight into the potential activity of the soil microbial community. By monitoring and comparing soil enzymes and their specificity, it is possible to assess some aspects of community succession, functioning and also to evaluate the effects of disturbance on ecosystem properties (Sinsabaugh et al., 1991). It has also been suggested that soil enzymes could act as "sensors" of soil degradation as they integrate information about microbial status (Sardans and Peñuelas, 2005). With the knowledge that enzymes are released in to the environment in order to degrade macromolecular and insoluble organic matter in order for cellular uptake, it may be possible to relate decomposition rates to enzyme abundance (Sinsabaugh et al., 1991). This in turn may provide information regarding the functional diversity and efficiency of the microbial community as enzyme release is species dependant and is influenced by physico- chemical factors (Fioretto et al., 2000). In this context, several types of soil enzyme are of primary interest i.e. those involved in the degradation of the main components of plant litter (cellulose, lignin, hemicellulose) as well as those

involved in the cycling of major nutrients (N, P, S) (Sinsabaugh et al., 1991; Fioretto et al., 2000). Although often studied in isolation, the enzymes involved in residue degradation probably do not operate independently despite the fact that they may originate from different organisms or species. The degradation of plant tissues to their most basic components requires the action of many different enzymes, few of which will be derived from the same organism therefore, in this respect, decomposition is a community process. Costerton et al., (1987) (cited in Sinsabaugh et al., 1991) demonstrated that the decomposition of plant fibres in rumens was maximal in the presence of the entire microflora and not when only the cellulolytic organisms were present.

A description of the role of β- glucosidase in cellulose degradation is outlined in section 1.1.2.2.

4.1.2.1 Phosphatases

Phosphatases are the group of enzymes which catalyse the hydrolysis of phosphate esters (Alef and Nannipieri, 1995) and anhydrides of phosphoric acid (Eivazi and Tabatabai, 1977). These enzymes have a broad specificity, capable of acting on a number of different structurally related substrates but at widely different rates. Their production is thought to be a response to the demand for available P. Like β-glucosidase, phosphotases are released extracellularly into the soil. The action of these enzymes causes the release of phosphate from organic phosphorus compounds to the soil solution (Mullen, 2005). Phosphomonoesterases are just one of the categories of phosphate hydrolysing enzymes. The phosphomonoesterase enzymes

hydrolyse phosphate from monoester forms of phosphorus such as phospholipids or nucleotides (Mullen, 2005). Of the two forms of phosphomonoesterase, acid phosphatase (EC 3.1.3.2) is the most widely studied and exhibits optimum activity in the pH range 4 to 6 (Eivazi and Tabatabai, 1977; Stevenson, 1986). This enzyme has been used in many studies including the investigation of effects of fire on soil properties (Boerner et al., 2005), assessment of relationships between nutrient inputs and enzyme response (Allison and Vitousek, 2005) as well as the study of enzyme response to drought in Mediterranean soils (Sardans and Peñuelas, 2005).

4.1.2.2 Sulfatases

Sulfatases catalyse the hydrolysis of organic sulphate esters and have been classified according to the type of ester in arylsulfatases, alkylsulfatases, steroid sulfatases, glucosulfatases, chondrosulfatases and myrosulfatases (Alef and Nannipieri, 1995). Arylsulfatase (EC 3.1.6.1) plays an important role in S cycling and is one of the extracellular enzymes that catalyses the mineralization of sulfur (Li and Sarah, 2003a). Arylsulfatases catalyse the hydrolysis of aromatic sulphate esters (R-O- SO_3^-) to phenols (R-OH) and sulphate SO_4^{2-} in the reaction:

$$\mathbf{R\text{-}O\text{-}SO_3^- + H_2O \longrightarrow R\text{-}OH + (SO_4)^{2-} + H^+}$$

Due to the large proportion of sulfur being present as sulphate esters, arylsulphatases could be extremely important for the mobilisation of inorganic SO_4^{2-} for plant nutrition (Elsgaard et al., 2002; Tabatabai and Bremner, 1970).

The enzyme group has been detected in microorganisms, plants and animals. Of the sulfatases, arylsulfatase is the most studied with respect to its role in soil sulfur cycling (Germida, 2005).

4.2 Specific Objectives and Hypotheses

The main aims of this study were to investigate PLFA diversity, community composition and enzyme activity in volcanic soils at different stages of development and to evaluate the effects of an environmental disturbance on these characteristics over a six month period.

4.2.1 *Specific Objectives*

- ❖ Compare community structure, PLFA diversity, PLFA evenness and total PLFA content in volcanic soils at different developmental stages.
- ❖ Compare the total and specific activities of three enzymes (β-glucosidase, acid phosphatase and arylsulfatase) in volcanic soils at different stages of development.
- ❖ Identify changes in community composition, PLFA diversity, PLFA evenness, total PLFA and enzyme activity after an environmental disturbance and monitor enzyme recovery over a period of 6 months in volcanic soils of different developmental stages.
- ❖ Establish whether enzyme activity and recovery are linked to soil community composition, biomass (as assessed by total PLFA content) and soil C content.

4.2.2 *Hypotheses*

- The developing soil community will have a lower total PLFA content and a lower PLFA diversity compared with the developed soil community.
- The developed soil will contain a higher abundance of fungal biomarkers than the developing soil and the bacterial to fungal PLFA will decline with site age.
- Enzyme activities will be lower in the developing soil due to its relative age.

- Total PLFA, PLFA diversity and enzyme activity will be more affected in the developing soil due to a reduced number of functional linkages within the soil.
- Enzyme activities will be more related to total PLFA than community composition or soil C content.

4.3 Materials and Methods

4.3.1 *Sample Preparation and Experimental Design*

One developing soil, Monti Rossi *Genista* (MRG) and one mature soil, Salto del Cane A (SDCA) were chosen at random to undergo investigation. Samples were weighed in to small glass vials on a 15 g dry weight equivalent basis and incubated at 20°C for 10 days prior to analysis to allow the microbial biomass to equilibrate. The samples were then divided into four groups:

- Developing soil (control)
- Developing soil (disturbed)
- Mature soil (control)
- Mature soil (disturbed)

The disturbance treatment involved the soils being heated in an oven at 60°C for 48 hours while the control samples were kept incubating. Once removed from the oven, the dried samples were allowed to cool and were re-adjusted to their initial moisture content and then all samples were adjuster to 50% water holding capacity. The vials were then stoppered and samples stored in the dark at a constant temperature of 20°C. On days 2, 16, 33, 54, 81, 115 and 148 selected vials were sampled destructively to monitor the activity of β-glucosidase, arylsulphatase and acid phosphatase using one gram of soil for each assay. On days 2, 33, 81 and 148, 1 gram of soil was also used to monitor changes in soil microbial community diversity by PLFA analysis.

4.3.2 ***Phospholipid Fatty Acid Analysis***

4.3.2.1 Lipid Extraction

Solutions and solvents were prepared using HPLC grade solvents and high quality chemicals. A 1:2 ratio of chloroform and methanol was prepared with 0.005% butylated hydroxyl toluene (Cresol). Citrate buffer solution (100ml pH 4) was made up using 59 ml citric acid solution (0.15 M) and 41 ml tri sodium citrate solution (0.15 M). Bligh and Dyer solvent was prepared as chloroform: Methanol: Citrate buffer in a (1:2:0.8) ratio and contained 0.005% butylated hydroxyl toluene.

Microbial community composition within soil samples was estimated qualitatively with phospholipid fatty acid analysis (PLFA) according to an established laboratory protocol adapted from that of Bligh and Dyer (1959). On days 2, 30, 80 and 150, soil samples were destructively sampled and one gram of fresh weight soil was removed and transferred into pyrex tubes with Teflon lined screw caps. 11.9 ml of Chloroform: Methanol in a 1 to 2 ratio was added to the soil. Citrate buffer (pH 4) was then added in order to bring the soil samples up to a moisture content of 3.16 ml. The samples were then sonicated for 30 minutes and left to refrigerate overnight for extraction to take place. Each sample once removed from refrigeration was centrifuged for 10 minutes at 1200 rpm. The supernatant was decanted from the vials using a glass Pasteur pipette and dispensed into a clean, labelled vial. 5ml of Bligh and Dyer solvent was added to the remaining soil which was vortexed for 30 seconds and once again centrifuged for 10 minutes at 1200 rpm. The resultant supernatant was decanted and added to the supernatant previously removed. To the combined supernatants, 4 ml of chloroform and 4 ml of citrate buffer was added to split the phases and the vials were left to refrigerate overnight. Once the phases had

separated, the upper layer was removed and discarded using a vacuum suction line fitted with a clean glass Pasteur pipette. The remaining phase was dried down under N_2 at 50°C and then refrigerated.

4.3.2.2 Separation of Phospholipids

0.5 g of silicic acid which had been activated for 1 hour at 100°C was mixed with 3 ml of chloroform in a small drum vial until it became slurry. The silicic acid-chloroform slurry was then added to a commercially prepared column and the excess chloroform let to drain. This was repeated for the desired number of columns. The columns were then pre- washed with 2 ml methanol, 2 ml acetone and 2 ml chloroform and allowed to dry out. After pre washing, each column was conditioned with 2 ml chloroform after the addition of which, the column was not allowed to dry out. The lipid extracts which had been dried down under N_2 the previous day were reconstituted in 300 µl chloroform and vortexed for 30 seconds. The reconstituted sample was applied to the column and filtered through a depth of approximately 2.5 cm of sodium sulphate anhydrous (Na_2SO_4) which was contained within a Pasteur pipette plugged with ashless floc. 5 ml of chloroform was then added also through the Pasteur which removed the neutral lipids. The glycolipids eluted with the addition of 12 ml of acetone and finally, the polar lipids (including the phospholipids) were eluted by addition of 8 ml of methanol. The methanol containing the phospholipids was collected in a clean vial and dried down at 40°C under N_2. The samples were then capped and frozen until required for derivatisation.

4.3.2.3 Derivitisation (Mild Alkaline Methanolysis), Acidic Hydrolysis and Final Sample Clean Up

The fractioned sample was dissolved in a 1 ml Toluene and Methanol mixture (1:1) (dry solvents) and then 1 ml of 0.2 M methanolic potassium hydroxide added. The samples were vortexed after the addition of each 1 ml. The samples were then incubated at 37°C for 30 minutes. The reaction was stopped with the addition of 0.3 ml 1M acetic acid. This was followed by the addition of 5 ml hexane: chloroform (4:1) (extraction solvent) and 3 ml of water. The samples were then sonicated for 30 minutes and then centrifuged for 5 minutes at 1200 rpm to split the phases. The bottom layer was removed by pipette and discarded. 3 ml of sodium hydroxide solution (12g/L) was added and vortexed for 30 seconds. The samples were then centrifuged for 15 minutes at 1200 rpm and the top layer of each decanted to clean vials through a Pasteur pipette with anhydrous sodium sulphate, plugged with ashless floc. The aqueous layer was washed with 3 ml hexane: chloroform (4:1) and centrifuged again for 15 minutes at 1200 rpm and the top layer collected. This was repeated and all three extractants combined in one vial. The extractants were then dried down under nitrogen at 40°C and stored at -18°C until required for gas chromatography. All measurements were carried out in triplicate.

4.3.2.4 Identification of PLFAs

Gas chromatography was used to separate the PLFAs in each sample. Prior to injection of the purified extract, an internal standard was added which would allow quantification of molar PLFA values. Nonadecanoic acid methyl ester (19:0) (Supelco) 0.01 g was dissolved in 1 ml of hexane which was diluted to give a 1:100 concentration of the original stock. 50 µl of the 1:100 nonadecanoic acid solution

was added to reconstitute the sample with a final sample volume of 1 μl being added to the gas chromatograph (GC). Preliminary analyses showed that (19:0) was not present naturally in the PLFA extracts. Analyses were conducted on a Pye Unicam Philipps PU4400 GC with a 30 m x 0.25 m x 0.25 μm column and a flame ionisation detector. Prior to analysis, the GC was prepared according to a pre-established program for PLFA separation. The detector and injector parts of the GC were set to an initial temperature of 320°C whilst the column was programmed to heat the column in three stages from 60°C to 310°C. Firstly the column was set to reach 145°C at a rate of 25°C per minute; the next phase required the column to rise to 250°C at a rate of 2.5^{o}C per minute and finally a temperature of 310°C at 10°C per minute with a hold time of ten minutes at this temperature. The obtained peaks were recorded on Clarity software (Prague, 2003) and identified in reference to a qualitative standard of bacterial acid methyl esters (BAME, Supleco).

4.3.2.5 PLFA nomenclature

When naming a fatty acid, the first number is designated by the total number of carbon atoms. The degree of unsaturation is indicated by a number separated from the chain length number by a colon. The degree of unsaturation is followed by Δ*x*, where *x* indicates the position of the double bond nearest to the carboxyl end,Δ , or in a few cases nearest to the aliphatic end, ω. The prefixes *a*, *i*, cy and d refer to *anteiso*, *iso*, cyclopropyl branching and dicarboxylic fatty acids respectively and the suffixes *c* and *t* indicate *cis* and *trans* forms respectively (Zelles, 1999). For example, the mono unsaturated oleic acid is denoted by the symbol 18:1ω9c which means there are 18 carbon atoms, the degree of unsaturation is one, the double bond

is at the 9th carbon atom nearest to the aliphatic end of the chain and it is a *cis* isomer.

4.3.3 ***Soil Enzyme Activities***

All soil enzyme activities were based upon the colorimetric determination of *p*-nitrophenol which is yellow in alkaline solutions but is clear in acid solutions or when present as a form of *p*-nitrophenyl in acid or alkaline solutions (Tabatabai and Bremner, 1970). $CaCl_2$ was required to prevent dispersion of clays and extraction of organic matter during treatment with NaOH whilst NaOH was required to extract the colour due to *p*-nitrophenol (Tabatabai and Bremner, 1970).

4.3.3.1 β-glucosidase activity

The β-glucosidase assay was performed according to the protocol set out in Alef and Nannipieri (1995). The method was based on that first performed by Tabatabai (1982) and modified by Eivazi and Tabatabai (1988). The method is based on the determination of released *p*-nitrophenol after the incubation of soil with *p*-nitrophenyl β-D-glucopyranoside solution for 1 hour at 37°C (Alef and Nannipieri 1995). 1 g of moist soil was placed inside a 30 ml glass vial and to this 4 ml Modified Universal Buffer (MUB) (pH 6) was added. MUB was compiled as follows: MUB stock solution- dissolve 12.1 g of Tris (hydroxyl methyl) amino methane, 11.6 g of maleic acid, 14 g of citric acid and 6.3 g of boric acid in about 500 ml of NaOH (1 M) and dilute the solution to 1000 ml with distilled water. Store at 4°C. MUB pH 6.0- Titrate 200 ml MUB stock solution to pH 6.0 under continuous stirring with HCl (0.1 M) and dilute to 1000 ml with distilled.

After the addition of MUB, 1 ml of *p*-nitrophenyl-β-D-glucopyranoside solution (PNG) was added and the vials capped, mixed thoroughly and incubated for 1 hour at 37°C. 25 mM PNG solution was made by dissolving 0.377 g of PNG in 40 ml of MUB pH 6.0 and diluted to 50 ml with the same buffer. The solution was stored at 4°C.

Once incubated, 1 ml of 0.5 M $CaCl_2$ solution was added along with 4 ml of Tris buffer, pH 12. The flasks were then swirled to mix and then filtered immediately through Whatman 2v filter paper. 0.1 M, pH 12 Tris buffer was produced as follows: dissolve 12.2 g of Tris (hydroxyl methyl amino methane) in 800 ml of distilled water, adjust to pH 12 with NaOH (0.5 M) and bring to 1000 ml with distilled water.

The colour intensity of the filtered material was then measured on a spectrophotometer at 400 nm. Control samples were prepared by adding the substrate PNG immediately after the incubation before adding $CaCl_2$ and Tris buffer. Samples were produced in triplicate.

4.3.3.2 Acid Phosphatase activity

Acid phosphatase assays were performed according to Alef and Nannipieri (1995), bases on the methods of Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977). 1 g of moist soil was placed inside a 30 ml glass vial and 4 ml Modified Universal Buffer (MUB) (pH 6.5) was added (see β-glucosidase for MUB production). 1 ml of *p*-nitrophenyl phosphate solution was added and the vials

capped and contents mixed and incubated at 37°C for 1 hour. *p*-nitrophenyl phosphate solution (PNP, 0.115 M) was made by dissolving 1.927 g of disodium *p*-nitrophenyl phosphate tetrahydrate in 40 ml MUB (pH 6.5) and diluting to 50 ml with the same buffer (Tabatabai and Bremner, 1969).

Once incubated, 1 ml of $CaCl_2$ (0.5 M) and 4 ml of NaOH (0.5 M) were added and mixed to stop the reaction. The flasks were then swirled to mix and then filtered immediately through Whatman 2v filter paper and the colour intensity of the filtered material was then measured on a spectrophotometer at 400 nm. As a control, 1 ml of PNP solution was added after the addition of $CaCl_2$ and NaOH and immediately before filtration. Samples were produced in triplicate.

4.3.3.3 Arylsulphatase activity

The arylsulphatase assays were performed according to the protocol of Alef and Nannipieri (1995), which had been based on that of Tabatabai and Bremner (1970). One gram of soil was placed in a 30 ml glass vials to which 4 ml of 0.5 M, pH 5.8 acetate buffer and 1 ml of 25mM *p*-nitrophenyl sulphate solution was added. The contents were then mixed and the vials capped and incubated at 37°C for 1 hour. The acetate buffer was made by dissolving 68 g of sodium acetate trihydrate in 700 ml of distilled water with the pH adjusted to 5.8 with concentrated acetic acid and brought up to 1000 ml with distilled water. The *p*-nitrophenyl sulphate solution was produced by dissolving 0.312 g of potassium *p*-nitrophenyl sulphate in 40 ml acetate buffer and diluted to 50 ml with the same buffer. The solution was stored at 4°C.

Once incubated, 1 ml of $CaCl_2$ (0.5 M) and 4 ml of NaOH (0.5 M) were added and mixed to stop the reaction. The contents were then filtered through Whatman 2v filter paper and the optical density measurements conducted at 400 nm on a spectrophotometer. Control samples were treated the same as other samples except *p*-nitrophenyl sulphate was added immediately before the filtration of the soil. All assays were carried out in triplicate.

4.3.4 ***Data handling and Statistical Analyses***

All statistical tests were carried out using Minitab version 14. A Kolmogorow-Smirnoff goodness of fit test was carried out to test the normality of the data whilst Levene's test was used to test the homogeneity of the variances (Tscherko et al., 2004).

Principal components analysis (PCA) was used to summarise PLFA profiles. PLFA diversity was used as a proxy for species diversity and was calculated using Shannon's diversity index:

$$\boldsymbol{H} = -\sum_{i=1}^{n} pi \ln pi$$

where n represents the number of species (in this case fatty acids) and pi is the abundance of the each fatty acid in the total sum (ln= log $base_n$). The evenness of the fatty acids was calculated according to Shannon's evenness:

$$\boldsymbol{E} = H/\ln(s)$$

where s represents the total number of fatty acids tested in the community. One way ANOVA was used to assess the effects of soil type and disturbance through time on PLFA diversity, evenness, total PLFA and enzyme activities. The effects of soil

type, disturbance and time on PLFA diversity, evenness, total PLFA and enzyme activities was assessed using a general linear model or 3 way ANOVA. Specific enzyme activities were calculated as total enzyme activities normalised to a per nmol PLFA basis. All calculations were carried out on an oven dry weight basis.

Monti Rossi control soils and disturbed soils are referred to as MRc and MRd respectively and the same terminology applies to Salto del Cane control (SDc) and disturbed soils (SDd).

4.4 Results

4.4.1 *Phospholipid Fatty Acids*

4.4.1.1 Entire Community Profiles

PCA of PLFAs revealed clear spatial and temporal differences in community PLFA composition (Figure 4-1). Component weights were plotted for the first two variables in the PCA. The first variable accounted for 29% of the variation while the second variable accounted for 17%. PCA 1 may have been dominated by PLFA composition while total PLFA concentration may have dominated PCA 2.

The control soils MRc and SDc were significantly different in their PLFA composition (Table 4-2, Figure 4-1) throughout the experiment indicating that community structure was different. However in some instances such as on day two, 6 of the 7 PLFAs found in MRc were also found in SDc with no significant differences in abundance. Therefore the divergence of these two soils at this time is a product of the eight additional PLFAs found in SDc which were not present in MRc. MRc and SDc showed similar broad patterns of change in community structure over time.

Disturbance affected the PLFA contents of both soils although SD soils were affected to a greater extent (Table 4-2). Figure 4-1 shows that two days after disturbance, PLFA composition and total were fairly similar in both disturbed soils (MRd and SDd). By 81 days after disturbance, MRc and MRd were indistinguishable from one another in both composition and total PLFA contents indicating that any effect of disturbance had been recovered from. SDc and SDd did not show any similarities in PLFA composition or total until the end of the

experiment where they were similar, however, by this point MRc and MRd showed differences in their PLFA content perhaps revealing a residual effect of the disturbance treatment.

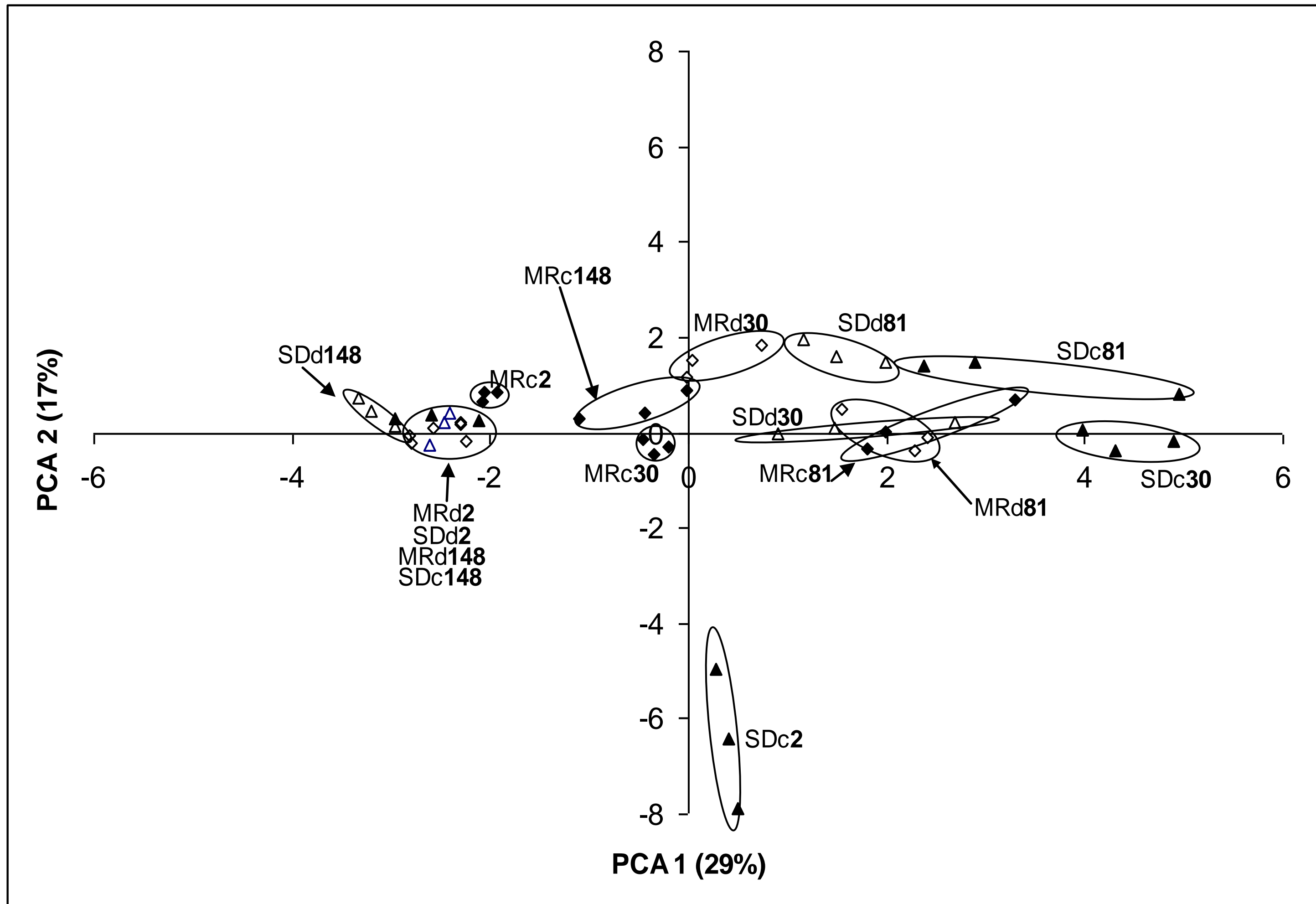


Figure 4-1 Score plot from principal component analysis of PLFA profiles according to soil development and disturbance treatment on sampling days 2, 33, 81 and 148 after disturbance.

4.4.1.2 Major Groups of Fatty Acids

Certain FA's can be grouped together to assess community structural changes through time and to identify differences between soil microbial communities (Zelles, 1999). Figure 4-2 shows the differences in the subfractions of total PLFA content for each soil and treatment. Saturated fatty acids (SATFA) were most abundant in all soils followed by monounsaturated fatty acids (MUFA) while polyunsaturated fatty acids (PUFA) and hydroxy fatty acids (OHFA) made the smallest contribution to total PLFA content in these volcanic soils.

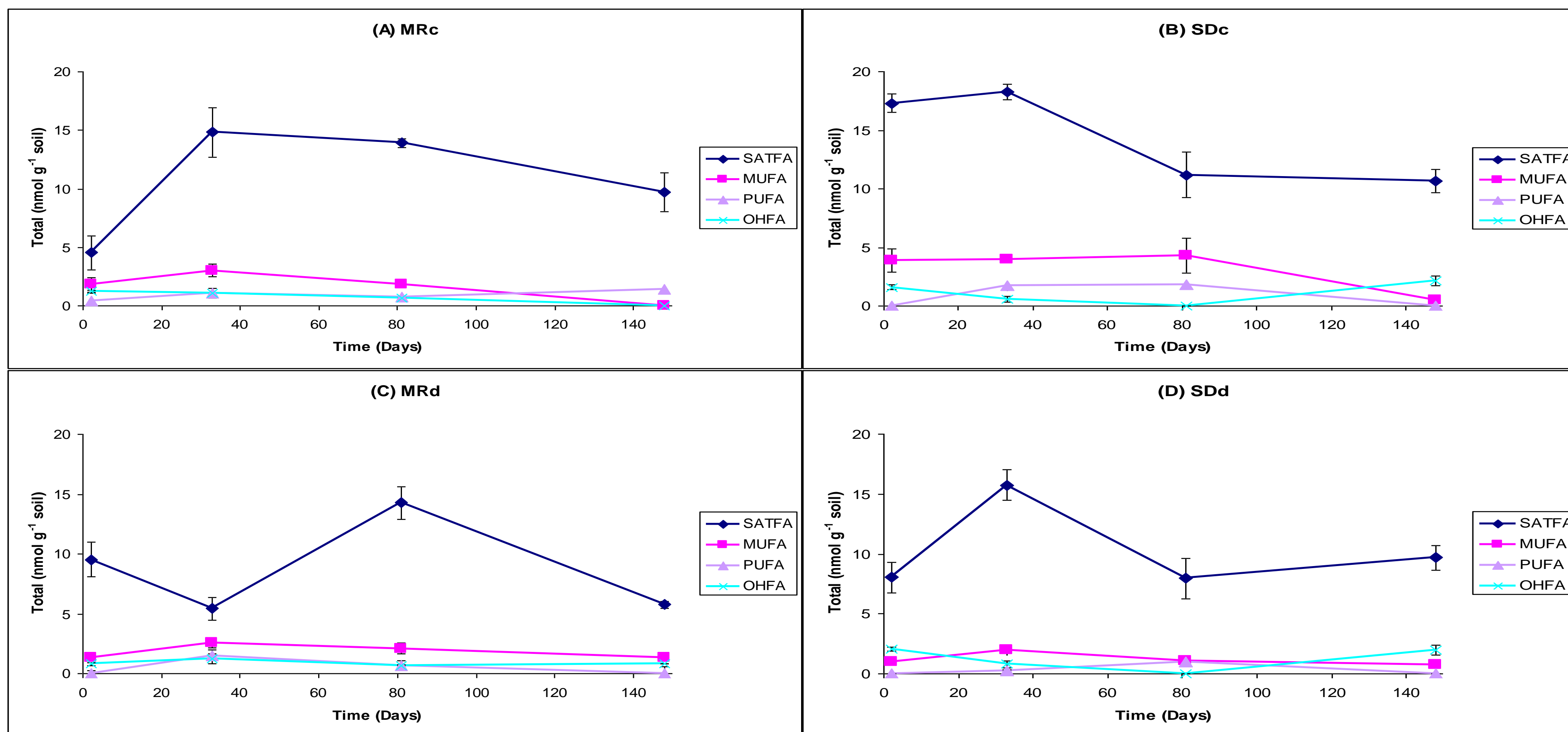


Figure 4-2 Differences in the subfractions of total PLFA content in (A) MRc, (B) SDc, (C) MRd and (D) SDd.
SATFA denotes Saturated Fatty Acids, MUFA denotes Monounsaturated Fatty Acids, PUFA denotes Polyunsaturated Fatty Acids and OHFA denotes Hydroxy fatty acids

MRc initially contained a very low concentration of SATFAs (approximately 4.8 nmol g^{-1} soil) in comparison with SDc (17.3 nmol g^{-1} soil). Both soils had 4 straight chained SATFAs in common with no significant differences in concentration (Table 3-2). These were lauric acid (12:0), palmitic acid (16:0), stearic acid (18:0) and arachidic acid (20:0). SDc contained 5 other SATFAs not found in MRc which were straight chained margaric acid (17:0), branched chained SATFAs (*i*15:0, *a*15:0, *i*17:0) and cyclopropyl fatty acid (cy19:0).

The SATFA content of MRc increased to approximately 12.4 nmol g^{-1} soil on day 33 with the appearance of branched chain SATFA *i*15:0, cyclopropyl fatty acid cy19:0 and straight chained SATFA 17:0 which along with increases in 16:0 and 18:0 increased the degree of saturation within the community profile. The concentration of shorter chained SATFAs (14:0 and 15:0) in SDc increased on day 33 but the concentration of longer chained SATFAs (18:0 and 20:0) had significantly ($p<0.05$) declined.

MRc showed few compositional differences between days 33 and 81 (Figure 4-2 and Table 4-2). The concentration of short chained SATFA 12:0 was significantly reduced while 14:0, 15:0 and branched chain SATFA *i*15:0 significantly increased. After day 81 a steady decline to 10 nmol SATFA g^{-1} soil was experienced in MRc. SATFA levels in SDc declined after day 33 and were not significantly different from those of MRc by the end of the experiment. Several PLFA's were lost from the SDc community profile after day 33. These included straight chained SATFA 12:0, 14:0 and 15:0 as well as branched chain SATFAs *a*15:0, *i*16:0 and *i*17:0 indicting a

shift in community composition away from a largely G+ community. This is supported by an increase in concentration of cyclopropyl fatty acid cy17:0 and the high abundance of cy19:0 which are thought to be produced in times of stress.

On the first three sampling occasions SDc contained significantly ($p<0.05$) greater MUFA levels than MRd with approximately 4 nmol g^{-1} soil and 2 nmol g^{-1} respectively. The main contributors to MUFA content in SDc and MRc were oleic acid (18:1ω9c) and palmitoleic acid (16:1ω9c) although SDc also contained a small amount of the *trans* isomer elaidic acid (18:1ω9t) which was never found in MRc.

Levels of OHFAs fluctuated throughout the experiment in both soils but were mainly attributable to the presence of 3 OH 14:0. PUFAs were only present in small amounts on days 3 and 81 in SDc but were found on each sampling occasion in MRc. The PUFA content of the soils is solely attributable to linoleic acid (18:2 9 12) which is thought to be indicative of the presence of fungi. This suggests that MRc provides a better habitat for fungal existence.

Table 4-2 Abundance of fatty acids through time according to soil development and disturbance treatment. Values are means (± SD) and are given in (nmol g^{-1}). The effect of soil development on PLFA abundance on each separate day is denoted by the suffix a : while the effect of disturbance treatment is denoted by the suffix b. Significance levels: NS no significant differences, * P<0.05, ** P<0.01, *** P<0.001. Continued on next page.

	Monti Rossi (control)				Salto del Cane (control)			
Day	**2**	**33**	**81**	**148**	**2**	**33**	**81**	**148**
12:0	1.25(0.69)aNS, bNS	2.25(2.04)aNS, bNS	0.00(0.00)a*,b*	2.20(1.39)aNS, bNS	1.32(0.36)aNS,bNS	0.86(0.26)aNS, bNS	0.00(0.00)	1.87(0.17)aNS, bNS
3-OH 12:0	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
14:0	0.00(0.00)	0.00(0.00)	0.91(0.29)a**,bNS	0.00(0.00)	0.00(0.00)	1.03(0.49)a*, bNS	0.00(0.00)	0.88(0.12)a***,b***
*i*15:0	0.00(0.00)	0.00(0.00)	1.16(0.27)aNS, bNS	2.01(0.13)a***,b***	2.71(0.70)a**,b**	1.81(0.09)a***,b*	2.46(1.16)aNS, bNS	0.00(0.00)
*a*15:0	0.00(0.00)	0.00(0.00) bNS	1.66(0.39)a**, bNS	1.78(0.17)a***,b***	1.66(0.52)a**, bNS	1.56(0.61)a*, bNS	0.00(0.00)	0.00(0.00)
15:0	0.00(0.00)	0.00(0.00)	0.92(0.14)a***,b*	0.00(0.00)	0.00(0.00)	0.93(0.22) a**, bNS	0.00(0.00)	0.00(0.00)
3-OH 14:0	1.22(0.12)aNS,b**	1.09(0.39) aNS, bNS	0.64(0.05)a***, bNS	0.00(0.00)	0.97(0.24)aNS, bNS	0.56(0.38) aNS, bNS	0.00(0.00)	2.15(0.49)a**, bNS
*i*16:0	0.00(0.00)	0.00(0.00)	0.60(0.15)a**, bNS	0.00(0.00)	0.00(0.00)	0.80(0.16) a***,b*	0.00(0.00)	0.00(0.00)
16:1 ^{9}c	0.00(0.00)	1.12(0.09)aNS,b*	0.58(0.20) aNS, bNS	0.00(0.00)	1.03(0.27)a**,b**	1.23(0.19) aNS,b**	1.47(0.63)aNS, bNS	0.00(0.00)
16:0	2.31(0.78)	5.00(1.12) aNS,b***	2.86(0.37) bNS	3.72(0.50)aNS, bNS	4.06(0.80)a*, bNS	5.25(1.6) aNS, bNS	3.89(0.39)a*,b***	5.12(1.10)aNS, bNS
*i*17:0	0.00(0.00)	0.76(0.21)b**	1.04(0.03)a***, bNS	0.00(0.00)	3.10(0.44)a***,b***	1.50(0.29)a*,b*	0.00(0.00)	1.90(0.57)a**,b*
*cy*17:0	0.00(0.00)	0.00(0.00)	0.83(0.74) aNS, bNS	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.83(0.05) aNS	0.00(0.00)
17:0	0.00(0.00)	1.12(0.14)aNS,b***	0.87(0.08) aNS, bNS	0.00(0.00)	1.66(0.47)a**, bNS	1.71(0.58) aNS,b**	0.94(0.29)aNS, bNS	0.00(0.00)
2-OH16:0	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.62(0.19)a**,b**	0.00(0.00)	0.00(0.00)	0.00(0.00)
18:2 $^{9\ 12}$	0.43(0.26)a*,b*	1.11(0.43)aNS, bNS	0.75(0.35) bNS	1.44(0.59)a*,b*	0.00(0.00)	1.70(0.42) aNS,b**	1.79(0.38)a*,b*	0.00(0.00)
18:1ω^{9}c	1.79(0.66)aNS, bNS	1.87(0.47) bNS	1.24(0.03) bNS	0.00(0.00)	0.99(0.50)aNS, bNS	2.75(0.19)a* ,b***	2.82(0.84)a*,b**	0.47(0.08)a**
18:1ω^{9}t	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	1.87(0.35)a**,b**	0.00(0.00)	0.00(0.00)	0.00(0.00)
18:0	0.44(0.09)aNS,bNS	1.01(0.47)a*,b*	0.63(0.07)a***, bNS	0.00(0.00)	0.81(0.39)aNS, bNS	0.00(0.00)	0.00(0.00)	0.92(0.45)a*, bNS
*cy*19:0	0.00(0.00)	1.78(0.55) aNS, bNS	2.08(0.85) aNS, bNS	0.00(0.00)	1.32(0.74)a*,b*	2.83(0.82) aNS, bNS	3.07(0.51) aNS	0.00(0.00)
20:0	0.55(0.08) aNS, bNS	0.44(0.11) a*,b**	0.35(0.09) a**, bNS	0.00(0.00)	0.66(0.10) aNS,b*	0.00(0.00)	0.00(0.00) bNS	0.00(0.00)

	Monti Rossi (disturbed)				Salto del Cane (disturbed)			
Day	**2**	**33**	**81**	**148**	**2**	**33**	**81**	**148**
12:0	2.32(0.70)bNS	1.24(0.81) bNS	0.39(0.21)	0.89(0.32) bNS	2.37(0.30)bNS	0.74(0.29) bNS	0.00(0.00)	3.02(1.01) bNS
3-OH 12:0	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	1.25(0.34)b**	0.00(0.00)	0.00(0.00)	0.00(0.00)
14:0	0.00(0.00)	0.00(0.00)	1.12(0.10) bNS	0.00(0.00)	0.00(0.00)	0.79(0.24) bNS	0.00(0.00)	0.00(0.00)
*i*15:0	1.77(0.69) b*	1.45(0.15)b***	1.32(0.21) bNS	0.00(0.00)	1.07(0.76)	1.04(0.42)	1.34(0.24) bNS	0.00(0.00)
*a*15:0	0.00(0.00)	1.11(1.25) bNS	1.22(0.17) bNS	0.00(0.00)	0.00(0.00) bNS	1.07(0.23) bNS	0.00(0.00)	1.62(0.95)b*
15:0	0.00(0.00)	0.00(0.00)	0.66(0.07)	0.00(0.00)	0.00(0.00)	1.18(0.43) bNS	0.00(0.00)	0.00(0.00)
3-OH 14:0	0.80(0.10)	1.23(0.16)	0.68(0.08) bNS	0.81(0.15)b***	0.81(0.27) bNS	0.70(0.15) bNS	0.00(0.00)	1.99(0.39) bNS
*i*16:0	0.00(0.00)	0.00(0.00)	0.51(0.16) bNS	0.00(0.00)	0.00(0.00)	0.36(0.13)	0.00(0.00)	0.00(0.00)
16:1 ^{9}c	0.00(0.00)	0.81(0.11)	0.65(0.19) bNS	0.00(0.00)	0.00(0.00)	0.62(0.10)	0.51(0.15) bNS	0.00(0.00)
16:0	4.17(0.55)b*	0.00(0.00)	2.82(0.50) bNS	3.17(0.29) bNS	2.88(1.25) bNS	4.99(1.55) bNS	0.77(0.12)	4.08(1.08) bNS
*i*17:0	0.00(0.00)	0.00(0.00)	1.25(0.18) bNS	0.00(0.00)	0.00(0.00)	0.79(0.24)	1.22(0.60)b*	0.00(0.00)
*cy*17:0	0.00(0.00)	0.00(0.00)	0.54(0.08) bNS	0.00(0.00)	0.00(0.00)	0.75(0.26)b**	1.11(0.15)b*	0.00(0.00)
17:0	0.00(0.00)	0.00(0.00)	0.68(0.24) bNS	0.00(0.00)	0.88(0.16) bNS	0.62(0.14)	0.51(0.07) bNS	0.00(0.00)
2-OH16:0	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
18:2 $^{9\ 12}$	0.00(0.00)	1.53(0.26) bNS	0.70(0.12) bNS	0.00(0.00)	0.00(0.00)	0.24(0.06)	0.98(0.17)	0.00(0.00)
18:1$\omega^{9}c$	1.36(0.26) bNS	1.75(0.26) bNS	1.45(0.30) bNS	0.78(0.13)b***	1.02(0.11) bNS	0.93(0.24)	0.57(0.15)	0.77(0.06)b**
18:1$\omega^{9}t$	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.51(0.03)b***	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
18:0	0.67(0.36) bNS	0.00(0.00)	0.70(0.23) bNS	1.47(0.67)b*	0.45(0.13) bNS	0.55(0.13)b**	0.00(0.00)	1.00(0.23) bNS
*cy*19:0	0.00(0.00)	1.63(0.43) bNS	2.65(0.51) bNS	0.00(0.00)	0.00(0.00)	2.54(0.28) bNS	3.00(0.57) bNS	0.00(0.00)
20:0	0.55(0.08) bNS	0.00(0.00)	0.41(0.11)	0.23(0.06)b**	0.41(0.12)	0.33(0.01)b***	0.00(0.00)	0.00(0.00)

The levels of SATFAs in MRd fluctuated throughout the experiment and did not show any consistent disturbance effects. Immediately after the disturbance event, the majority of PLFA's were unaffected however there were significant increases in the *iso* branched chain SATFA *i*15:0 and the straight chained SATFA 16:0 in the disturbed soil (Table 4-2). By day 33 the two MR communities showed differences in their composition. MRd contained a significantly higher concentration of the branched chain fatty acids *i*15:0 and *a*15:0 but significantly lower concentrations of 16:0, *i*17:0, 17:0 and 18:0 than MRc. There was no significant difference in SATFA contents of MRc and MRd on day 81 but MRd contained significantly lower levels of SATFA on day 148.

MUFAs were unaffected by the disturbance treatment however by the end of the experiment MRd still contained a small concentration of MUFAs while MRc contained none.

Disturbance had a marked effect on the levels of PUFAs in MR soils. Fungal biomarker linoleic acid (18:2 9 12) was completely lost from the MRd PLFA profile two days after disturbance indicating that the fungal biomass had not survived. Levels of PUFAs in MRd recovered quickly however and were not significantly different from the levels in MRc after 33 and 81 days of incubation.

OHFAs were also subject to a very small but significant reduction two days after disturbance but were not significantly different from MRc on days 33 and 81.

Figure 4-1 shows that SDc and SDd were initially very different in their community composition indicating the disturbance seriously altered the PLFA composition of the SD soil. SATFAs, MUFAs and PUFAs were all significantly affected by the disturbance treatment. The SATFA content was significantly reduced after the disturbance until day 81 where no significant difference was noted between SDc and SDd. MUFA and PUFA contents were significantly lower than in the control until day 148. OHFAs were not significantly different in the disturbed soil compared with the control through-out the experiment.

Four PLFA's were initially lost from the Salto del Cane soil (Table 4-2). These were SATFAs *i*17:0 and cy19:0 and MUFAs 16:1ω9c and 18:1ω9t. Two PLFAs increased in abundance as a result of the disturbance (12:0 and 3-OH 12:0). After 33 days of incubation the soils had diverged further with significant differences in a number of PLFAs. In particular, there was a marked reduction in the branched chain PLFAs (*i*15:0, *i*16:0 and *i*17:0) as well as reductions in straight chained acid 17:0, MUFAs 16:1ω9c and 18:1ω9t and fungal indicator 18:2 9 12. The only significant increase in the disturbed soil was the cyclopropyl fatty acid cy17:0. Similar PLFA profiles were identified on day 81 of the incubation where SDc and SDd had 8 PLFAs in common although SDc contained significantly higher amounts of 4 of these PLFAs indicating that although the community was similar, SDd still contained a significantly lower microbial biomass.

4.4.1.3 Biomarkers

The fatty acids *i*15:0, *a*15:0, 15:0, *i*16:0, 17:0, *i*17:0, cy17:0, 18:1ω9^{c} and cy19:0 were chosen to represent total bacterial PLFAs (Tscherko et al., 2004) and 18:2$^{9\ 12}$ to represent fungal PLFA (Frostegård and Bååth, 1996). G+ specific fatty acids *i*15:0, *a*15:0, *i*16:0 and *i*17:0 and G- specific fatty acids cy17:0, 18:1ω9^{c} and cy19:0 were chosen as a measure of the ratio between G+ and G- bacterial PLFAs (Tscherko et al., 2004).

4.4.1.3.1 <u>Bacterial: Fungal PLFA ratio</u>

Figure 4-4 shows the ratio of bacterial to fungal PLFAs. MRc was the only soil to contain fungal biomarkers on day 2 and also contained the lowest total bacterial PLFA (approximately 1.79 nmol g-1 soil) content. MRd and SDd contained similar types of bacterial PLFAs but SDc contained significantly higher ($p<0.001$) concentrations of these (approximately 11.4 nmol g-1 soil) indicating that the disturbance had caused substantial bacterial cell death in the SD soil. There was no significant difference in bacterial to fungal PLFA ratios between MRc and SDc on day 33, however, there was a marked increase in the ratio in SDd. This resulted from the extremely small fungal PLFA presence in SDd. SDc contained significantly more of both bacterial ($p<0.05$) and fungal PLFAs ($p<0.01$) on day 33 compared with SDd which suggests the bacterial species had still not recovered from the disturbance while the soil environment had been altered in such a way to restrict fungal colonisation. SDc had the smallest ratio on day 81 due to a reduction in bacterial biomass but an unchanged fungal biomass. MRc showed no significant difference in total bacterial PLFA but a significantly smaller quantity of fungal PLFA than SDc which lead to MRc having a significantly greater ratio value.

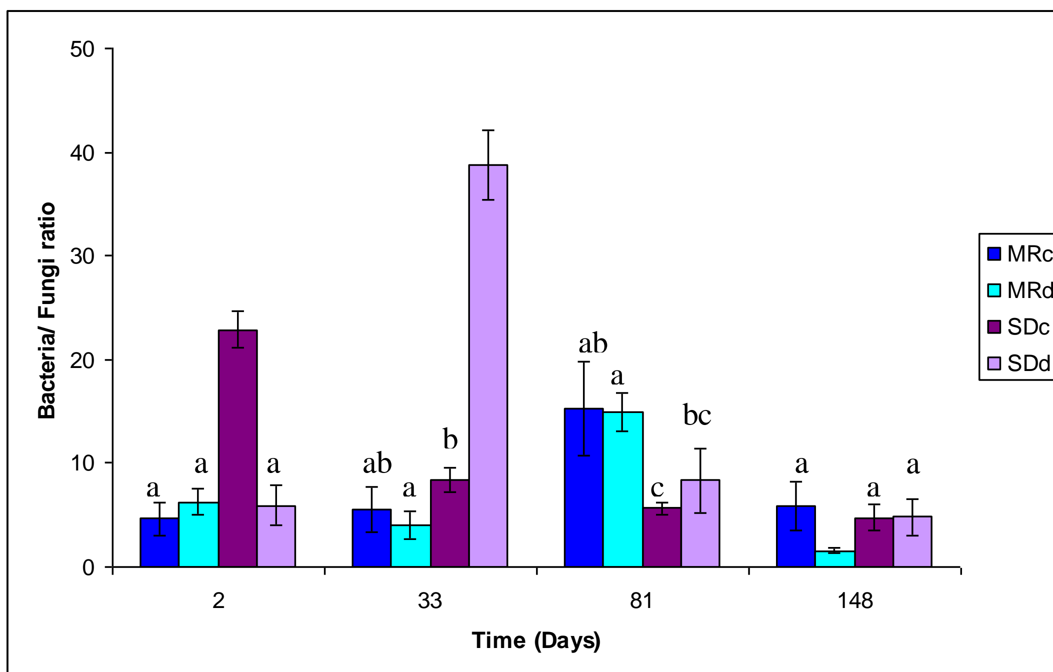


Figure 4-3 Ratio of bacterial to fungal PLFA for Monti Rossi and Salto del Cane soils both unaltered (MRc and SDc) and disturbed (MRd and SDd) over 148 days. Bars with the same letter at each time period are not significantly different

By day 148, fungal biomarkers had been lost from each soil and treatment other than MRc. MRd had a significantly smaller ratio than MRc due to a substantial reduction in bacterial PLFAs in MRd. SDc and SDd showed no significant differences.

4.4.1.3.2 Gram positive to Gram negative bacteria ratio

Each soil initially had similar G+/G- ratios although the ratio in MRc was significantly smaller than the other soils due to the lack of G+ biomarkers and only one G- biomarker was present in a small quantity (Figure 4-5). The ratio in MRc increased over time until day 148 where the community became G+ dominated however this could not be shown in the figure due to the absence of G- biomarkers. SDc initially showed the opposite pattern to MRc although on day 148 the soil community was also G+ dominated. No significant differences were found between disturbance treatment and control in either soil on days 33 and 81 although MRc and MRd showed differences in their primary bacterial types on day 2 and day 148.

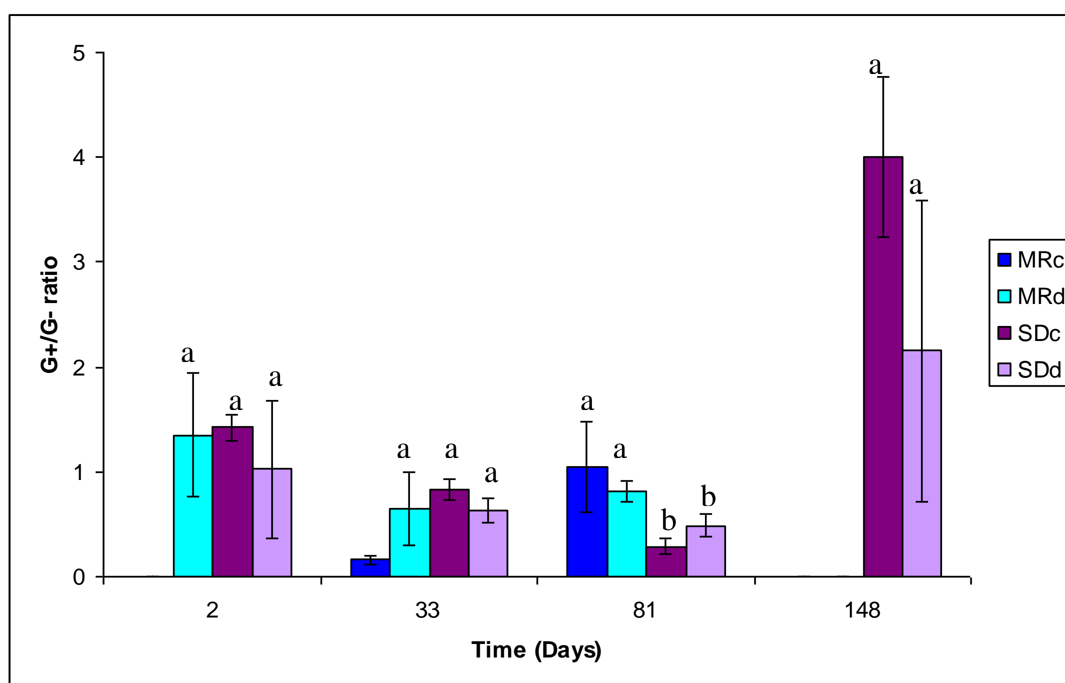


Figure 4-4 Gram positive to gram negative bacteria ratio for Monti Rossi and Salto del Cane soils both unaltered (MRc and SDc) and disturbed (MRd and SDd) over 148 days. Bars with the same letter at each time period are not significantly different

4.4.1.3.3 Cis/Trans ratio of Monounsaturates

The *cis/trans* ratio of SDc was initially significantly lower than any other soil/ treatment combination due to the presence of the *trans* fatty acid, elaidic acid (18:1ω9^t) which did not appear in any other soil. However on days 33 and 81, SDc had a significantly higher *cis/trans* ratio than each of the other soils as the number of *cis* isomers had increased whilst there was a reduction in the *trans* isomer (Figure 4-6). MRc and MRd did not show any significant differences in their cis/trans ratio's until day 148 where MRd contained both *cis* and *trans* isomers but MRc contained neither.

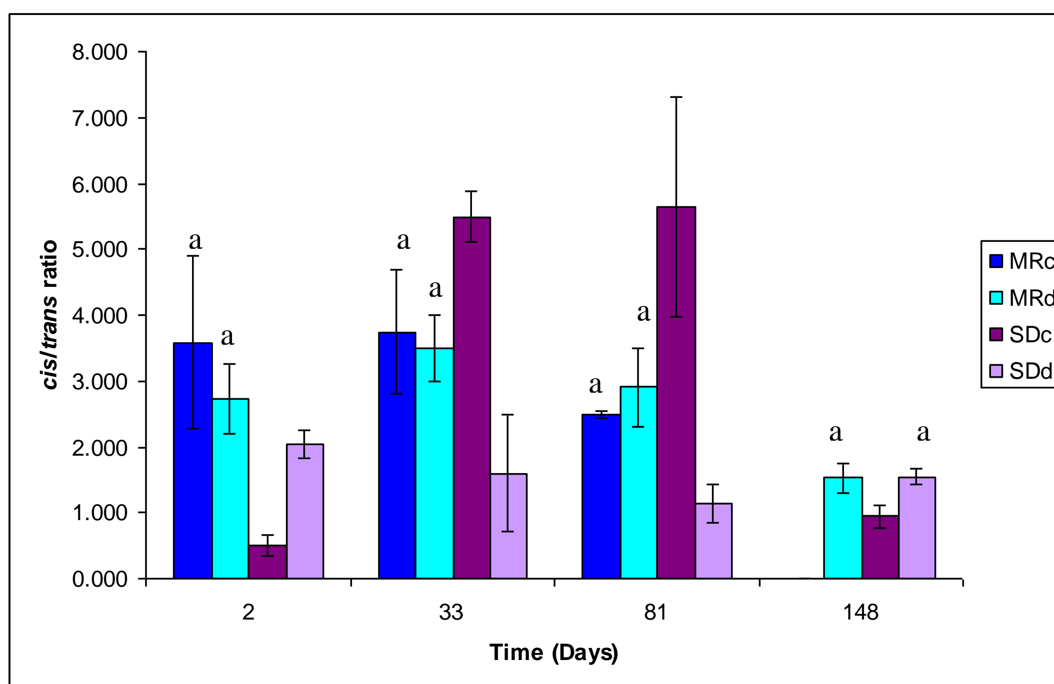


Figure 4-5 *Cis/ trans* ratio for Monti Rossi and Salto del Cane soils both unaltered (MRc and SDc) and disturbed (MRd and SDd) over 148 days. Bars with the same letter at each time period are not significantly different

4.4.1.3.4 Cyclopropyl Fatty Acids

The concentration of cyclopropyl fatty acids in each soil was highly variable (Figure 4-7). SDc was the only soil to contain cyclopropyl fatty acids initially although every soil/ treatment combination contained them on days 33 and 81. On these two occasions, no significant differences were noted between the two control soils or either control/ treatment combination. No traces of cyclopropyl fatty acids were found in any soil on day 148.

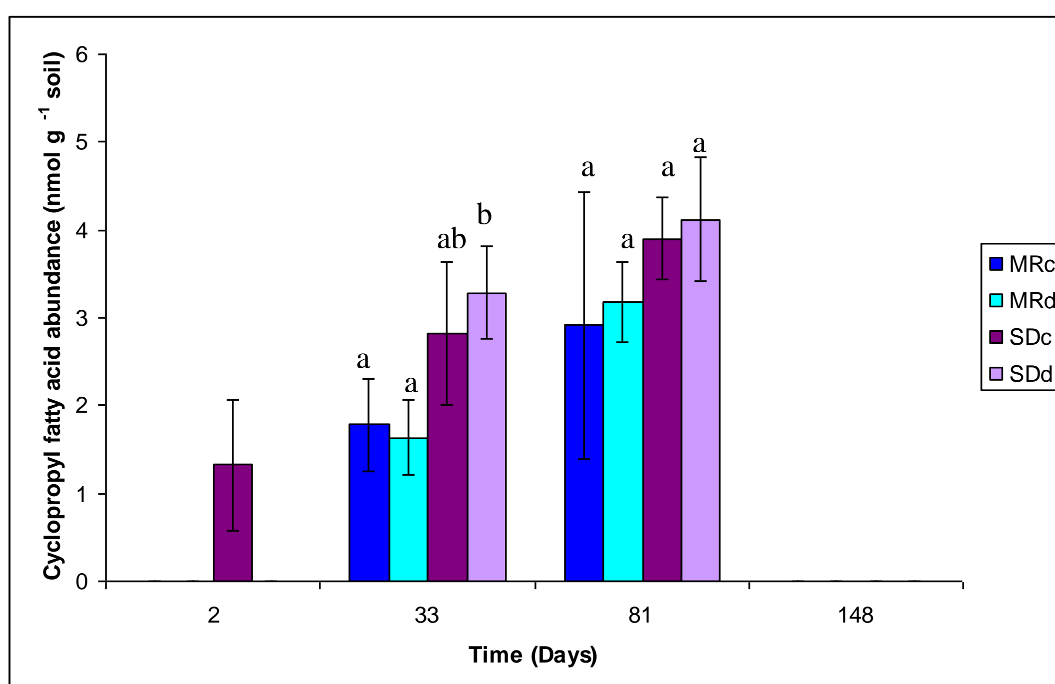


Figure 4-6 Cyclopropyl fatty acid content for Monti Rossi and Salto del Cane soils both unaltered (MRc and SDc) and disturbed (MRd and SDd) over 148 days. Bars with the same letter at each time period are not significantly different

4.4.1.4 PLFA Diversity and Evenness

Soil development was a significant factor in determining microbial PLFA diversity (Shannon's Diversity (*H*)) and evenness ($p<0.05$) (Appendix B Table 1). SDc initially contained a greater PLFA diversity and evenness was greater than MRc (Figure 4-8). However, as the incubation progressed, PLFA diversity in SDc declined. PLFA diversity was maximal (approximately 2.5) in SDc on days 2 and 33 of the incubation after which it steadily declined to approximately 1.6 on day 148 (Figure 4-8). Diversity in MRc throughout the incubation was variable and began low at approximately 1.6 on day 2, rose to a maximum of 2.6 on day 81 before declining to its lowest level, 1.5 on day 148.

The effect of disturbance on diversity was different for each soil. There was initially a significant reduction in the PLFA diversity of SDd but this returned to control levels by the second sampling occasion while MRd showed no significant effects from disturbance on PLFA diversity (Figure 4-8). Three factorial ANOVA encompassing the variables of soil development, disturbance and time, showed that PLFA evenness was not significantly affected by disturbance alone but was significantly affected by soil type and time in conjunction with disturbance (Appendix B Table 1).

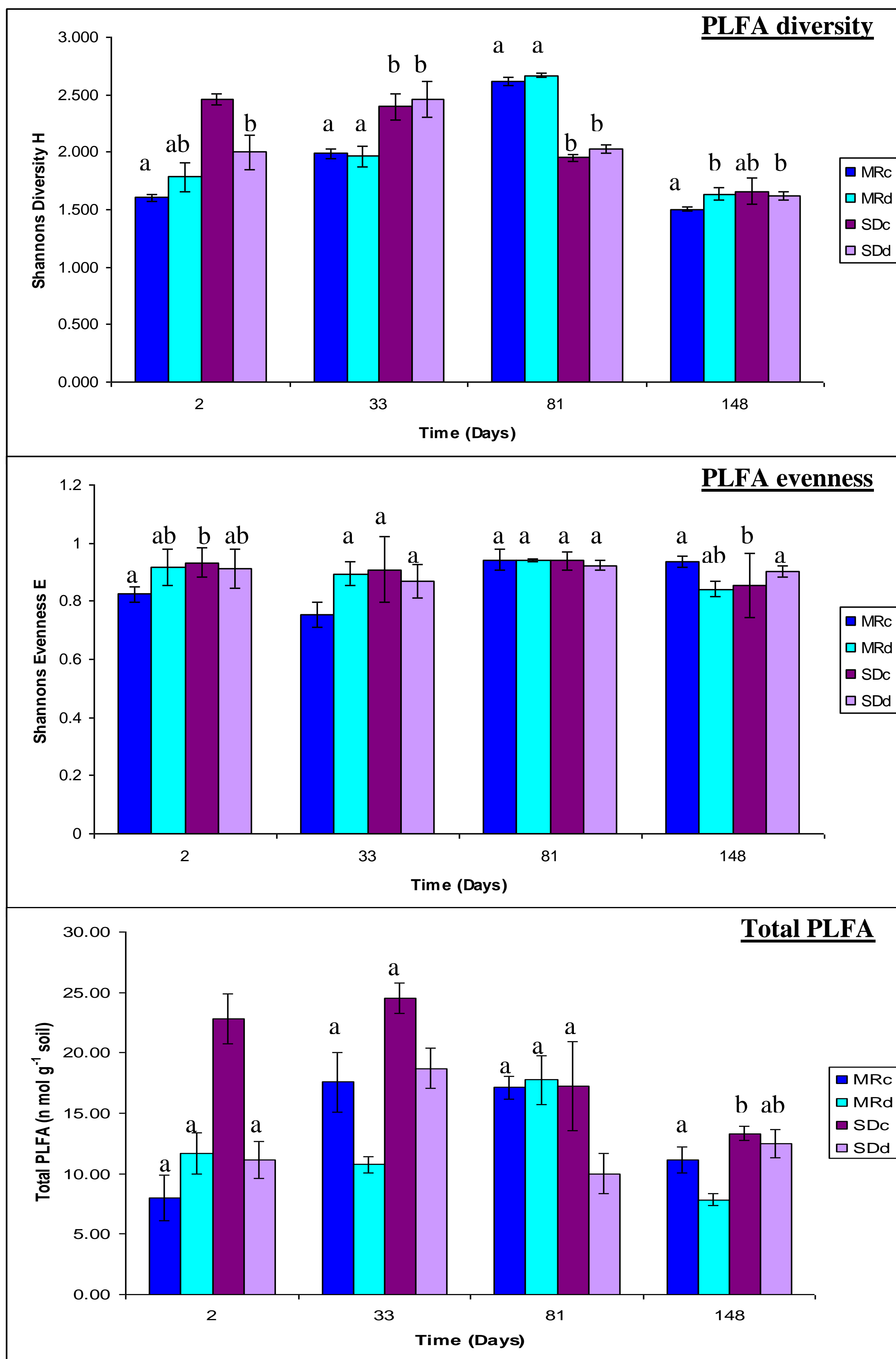


Figure 4-7 PLFA diversity, PLFA Evenness, and Total PLFA, of Monti Rossi and Salto del Cane soils both unaltered (MRc and SDc) and disturbed (MRd and SDd) over 148 days. Values are means (±SD). Bars with the same letter at each time period are not significantly different

4.4.1.5 Total PLFA content

The total PLFA content of a given quantity of soil has been used as a proxy for microbial biomass (Bååth et al., 1992; Steinberger et al., 1999; Zelles et al., 1995; Zelles, 1999). Zelles et al (1992, 1994 and 1995) found significant correlations between total PLFA content and microbial biomass measurements derived from the substrate induced respiration technique.

Soil, disturbance and time were all significant factors in determining total PLFA content (Appendix B table 1). SDc initially contained the greatest PLFA content indicating a larger microbial biomass than the MRc soil (Figure 4-8). There was a gradual decline in PLFA content in both soils toward the end of the experiment indicating a gradual reduction in microbial biomass.

Disturbance had a marked effect on the mature soil as SDd was initially reduced by almost 50% and remained significantly lower until day 148 when it was not significantly different from SDc. Disturbance had a less well defined affect on MR soils as MRd was not initially significantly different from MRc but was significantly lower than the control on days 33 and 148.

4.4.2 ***<u>Enzyme Activity and Recovery</u>***

4.4.2.1 β-glucosidase

Soil development significantly affected total and specific β- glucosidase activity (Appendix B Table 2, Appendix B Table 3) as MRc had both significantly higher total and specific ($p<0.05$) β-glucosidase activities than SDc at all time periods (Appendix B Table 3, Appendix B Table 4). Total β-glucosidase activity in MRc in most instances (i.e. days 2, 16, 33, 115 and 148) was at least double that of SDc while specific activity was consistently greater in MRc indicating the Monti Rossi soil community perhaps had a higher carbon demand than the Salto del Cane soil community or that the soil contained a high residual level of the β-glucosidase enzyme (Figure 4-9).

The effect of disturbance was highly dependent on soil development and community size as total β-glucosidase activity in MRd was not significantly affected by disturbance (Figure 4-9) but specific activities showed no clear trend (Appendix B Table 7). In contrast, the total β-glucosidase activity in SDd declined by approximately 50% after disturbance and remained lower than SDc until day 115 when values were similar. Specific activity in SDd however was almost identical to SDc indicating that any reduction in β-glucosidase activity was linked to a reduction in the size of the biomass (Figure 4-9).

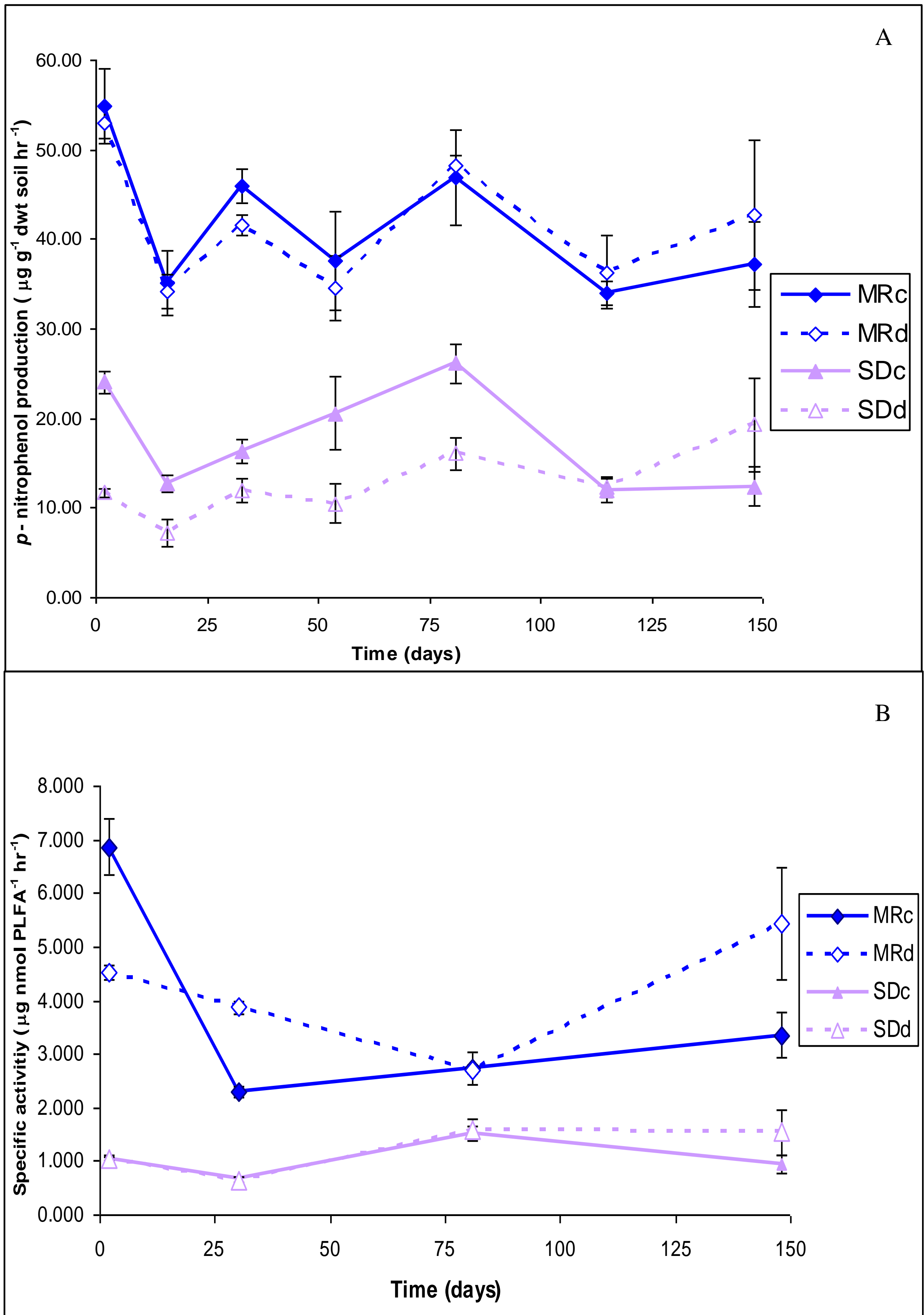


Figure 4-8 Total (A) and Specific (B) β- glucosidase activity in Monti Rossi and Salto del Cane soils both unaltered (MRc and SDc) and disturbed (MRd and SDd) over 148 days.

4.4.2.2 Acid Phosphatase

Total and specific acid phosphatase activity were significantly affected by soil development ($p \leq 0.001$), disturbance ($p \leq 0.005$) and time ($p \leq 0.001$) however in combination the effects were found to be insignificant on total acid phosphatase activity (p=0. 560). MRc and SDc were found not to be significantly different at any time period (Figure 4-10, Appendix B Table 2, Appendix B Table 3). MRc however had a higher specific activity (Appendix B Table 5) indicating that this soil had greater residual acid phosphatase content.

The effects of disturbance on acid phosphatase activity were less well defined than with β- glucosidase. MRc and MRd were not significantly different 2 days after disturbance but thereafter until 115 days of incubation, the altered soil had a significantly lower total acid phosphatase activity than the control (Appendix B Table 6) while specific activities showed no clear trend (Appendix B Table 7). Total acid phosphatase activity in SDd was also significantly lower than the control soil throughout the experiment but to a greater extent than the MR soil while specific activity was slightly higher in SDd (Figure 4-10).

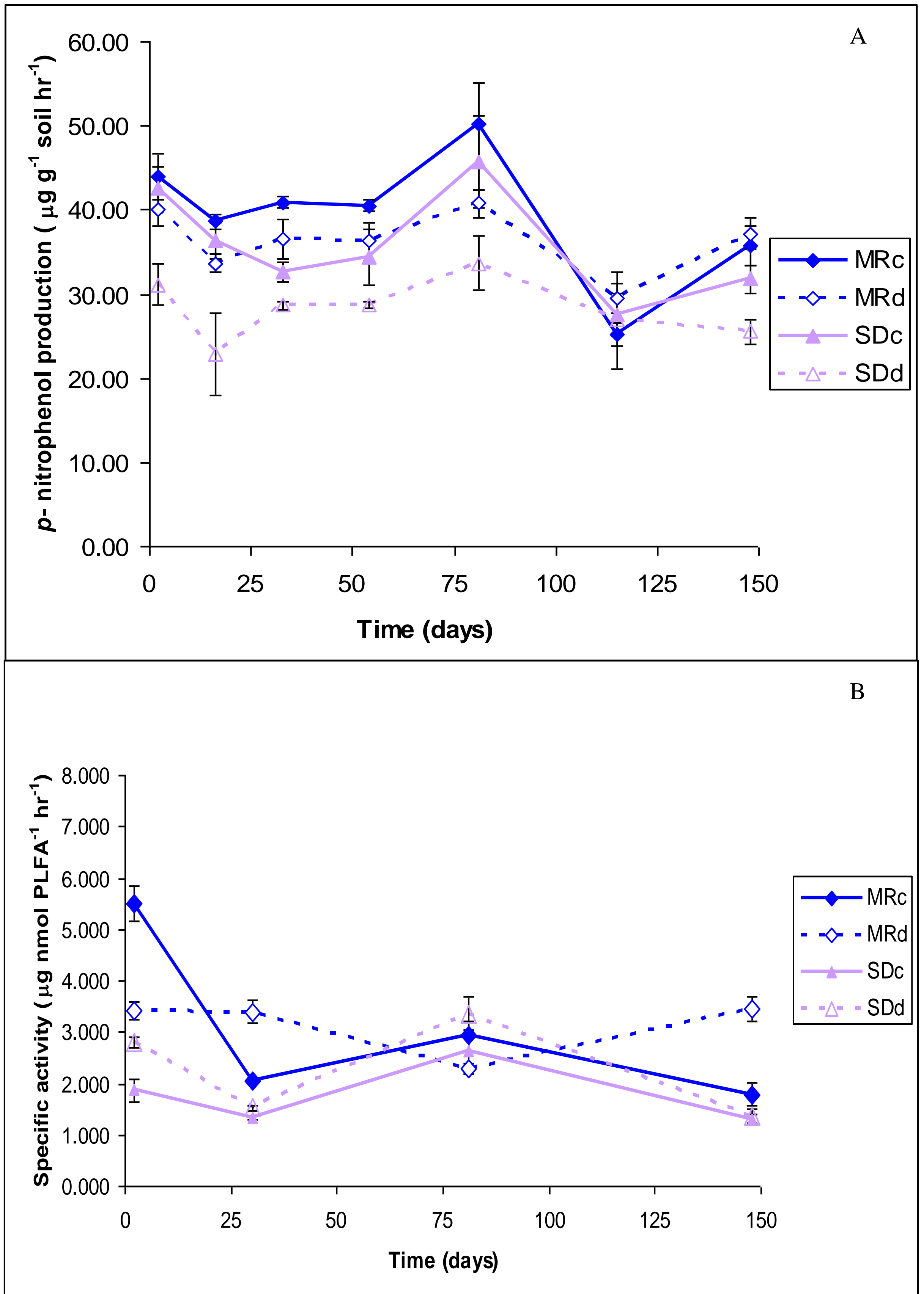


Figure 4-9 Total (A) and Specific (B)Acid phosphatase activity in Monti Rossi and Salto del Cane soils both unaltered (MRc and SDc) and disturbed (MRd and SDd) over 148 days

4.4.2.3 Arylsulfatase

Of the three enzymes assayed, arylsulfatase consistently showed the lowest total activity in both the developing (MR) and developed (SD) soils. The period of incubation significantly affected total and specific arylsulfatase activity ($p \leq 0.001$) as did disturbance treatment ($p \leq 0.001$) and soil development ($p \leq 0.001$) (Appendix B Table 2, Appendix B Table 3). Total and specific arylsulfatase activity was consistently higher in SDc than MRc throughout the experimental period (Figure 3.10) perhaps indicating a higher demand for sulphur in the more mature soil (Appendix B Table 4, Appendix B Table 5).

Two days after disturbance total arylsulfatase activity in MRd and SDd declined by approximately 30% and 35% respectively (Figure 4-11), Appendix B Table 6). Total arylsulfatase activity in MRd was not significantly different from control levels by day 115 while specific activities showed no clear trend. In the SDd soil however, total arylsulfatase activity was still significantly lower than the control 148 days after disturbance (Appendix B Table 6, Appendix B Table 7) while specific activity was similar to the control soil.

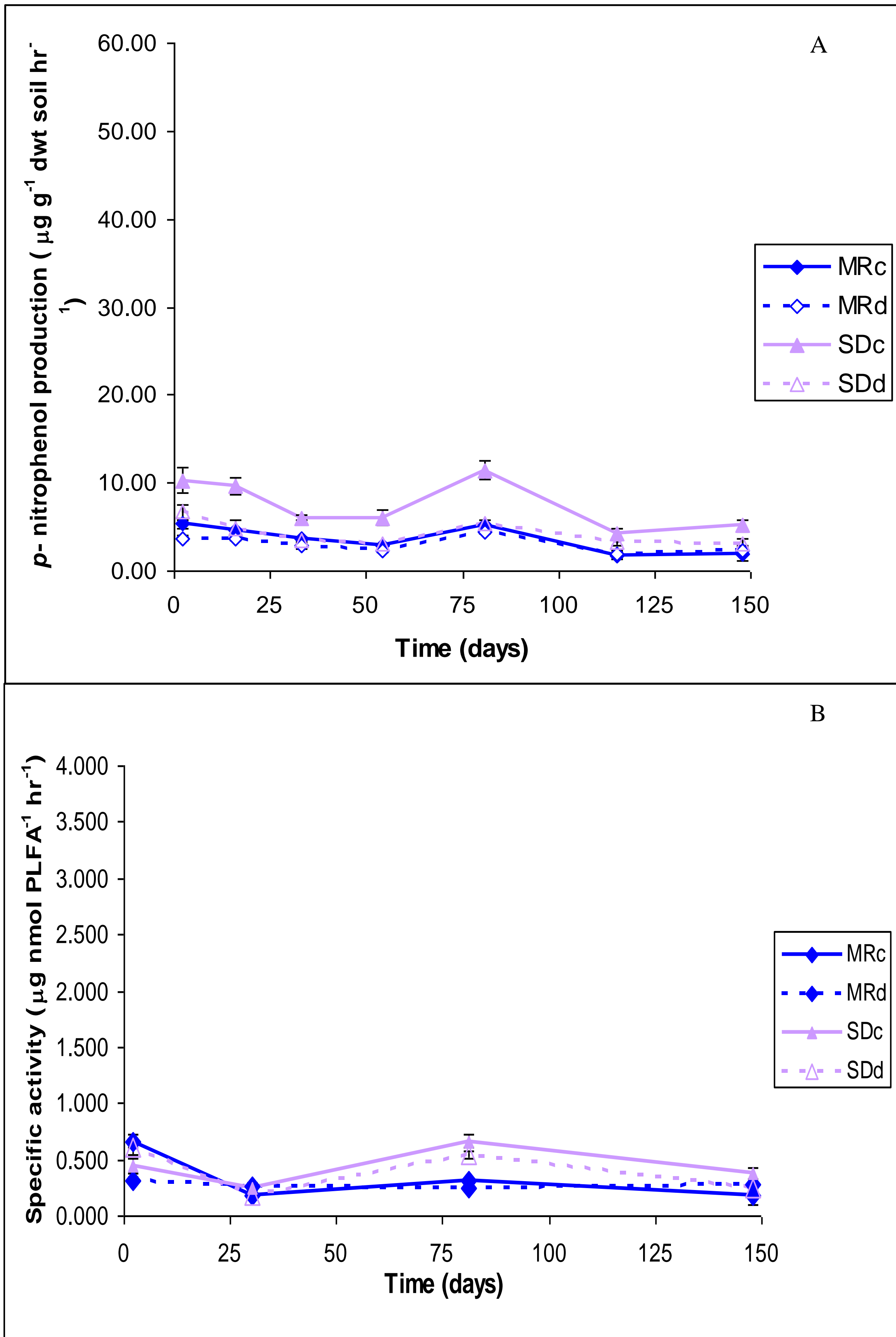


Figure 4-10 Total (A) and Specific (B) Arylsulfatase activity in Monti Rossi and Salto del Cane soils both unaltered (MRc and SDc) and disturbed (MRd and SDd) over 148 days

4.4.2.4 Relating composition to function

It has been suggested that soil organisms may produce extracellular enzymes when resources are scarce (Koch et al., 1985 cited in Allison and Vitousek, 2005). Shifts in microbial community composition have also been used to explain influences in enzyme production (Waldrop et al., 2000). Total carbon content did not correlate with any enzymes total or specific activities. No correlations existed between total β-glucosidase or total acid phosphatase activity and total PLFA, total bacterial PLFA, total fungal PLFA, SATFA content, MUFA content, OHFA content, G+ bacterial content, G- bacterial content, *cis* fatty acids, *trans* fatty acids or any of the individual PLFAs. Total arylsulfatase correlated with several PLFA groups; total bacterial PLFA content ($p<0.05$, $R^2=32.6$), G- bacterial content ($p<0.005$, $R^2= 47.7$) and most significantly MUFA content ($p<0.001$, $R^2=54.7$). This enzyme also correlated with individual fatty acids *cy*19:0 ($p<0.05$, $R^2=26.8$), 18:1ω9^c ($p<0.05$, $R^2=32$), *i*15:0 ($p< 0.05$, $R^2=34.8$), 16:1ω9^c ($p<0.01$, $R^2=42.8$) and 17:0 ($p<0.005$, $R^2=34.8$). Specific enzyme activities (enzyme activity per unit total PLFA) however provided different results to total enzyme activities. Specific arylsulfatase activity did not correlate with any individual PLFAs or PLFA groups. Total PLFA correlated with specific β-glucosidase ($p<0.001$, $R^2= 34.09$) and acid phosphatase activity ($p<0.001$, $R^2= 66.7$). Saturated fatty acid content and total bacterial content also correlated with specific β- glucosidase activity ($p<0.005$, $R^2=43.5$; $p<0.05$, $R^2=26.4$ respectively) and specific acid phosphatase activity ($p<0.001$, $R^2=70.9$; $p<0.05$, $R^2= 36.3$ respectively). This indicates that while β-glucosidase and acid phosphatase are produced by a wide range of soil microbial species, in this volcanic environment, arylsulfatase is produced mainly by MUFA containing species. SDc contained significantly higher amounts of MUFAs than MRc and also SDc soil also

contained a significantly higher concentration of the arylsulfatase enzyme. There was also a significant reduction in MUFA content of SD after the disturbance treatment which was accompanied by a reduction in arylsulfatase activity. As previously noted, although MUFA's are found in both G+ and G- bacteria, their low contribution to total PLFA content in G+ means they can be roughly used as G- bacteria biomarkers.

4.5 Discussion

4.5.1 *Soil Development Effect*

4.5.1.1 Community Structure, Major Groups of Fatty Acids and Biomarkers

Individual PLFAs are found in a wide variety of organisms however the abundance of certain PLFAs can provide insight into the microbial community as their occurrence varies among different groups of microorganisms (Steinberger et al., 1999). Only a few studies of microbial community development have utilised PLFA analysis to track changes in microbial community structure through time (Ohtonen et al., 1999; Tscherko et al., 2004). Several studies have employed the technique to assess the effects of drastic anthropogenic disturbance on microbial communities and to assess their recovery (Mummey et al., 2002a; Mummey et al., 2002b; DeGrood et al., 2005). Soils at different developmental stages generally have different community structures as identified through PLFA analysis (Ohtonen et al., 1999; Tscherko et al., 2004). This is also true of Etna's volcanic soils. PCA analysis showed clear differences in PLFA composition and therefore community structure between developed and developing soils.

SATFA content was initially the main difference between the two Etna soils as the developing soil contained a very small SATFA content. SATFAs can be divided into straight chained, branched and cyclopropyl fatty acids. Although straight chained fatty acids possess a low biomarker capacity and provide only superficial information about community structural change, they are useful indicators of biomass (Zelles et al., 1992; Bai et al., 2000). Most of the extra SATFA content found in the developed soil resulted from the presence of branched chained fatty

acids *i*15:0, *a*15:0 and *i*17:0 which are characteristic of G+ bacteria (Zelles and Bai, 1994). The developing soil also contained significantly higher amounts of MUFAs which are thought to be characteristic of the anaerobic desaturase pathway of G- heterotrophic bacteria (Steinberger et al., 1999). According to Zelles et al (1992) however, MUFA oleic acid ($18:1\omega9^{c}$) can be produced anaerobically but can also be produced by direct oxygen- dependant desaturation of 18:0. Although MUFAs can occur in both G+ and G- bacteria, their relative contribution to total PLFA content in G+ bacteria is less than 20% so they can be roughly used as biomarkers for G- bacteria (Bai et al., 2000).

There were also significant differences in PUFA content between the developed and developing soils. PUFAs generally indicate the presence of eukaryotic organisms in soil (Zelles and Bai, 1994; Zelles et al., 1995) and the PUFA linoleic acid ($18:1^{9\ 12}$) has been found to be abundant in a wide variety of fungal groups (Federle, 1986). In an investigation of cultivated and forest soils, Frostegård and Bååth (1996) found the concentration of linoleic acid correlated with the concentration of the fungal biomarker ergosterol suggesting that linoleic acid may be used as a general indicator for fungi. However, Sundh et al (1997) in an investigation of boreal peatlands suggested that excesses of linoleic acid may have originated from *Sphagnum* mosses or cyanobacteria rather than from fungi indicating that this biomarker may not be suitable for all environments. This information suggests that linoleic acid can be a reliable fungal biomarker in the absence of plant cells. The developing soil was the only soil to consistently show low traces of linoleic acid but the overall concentration in each soil was fairly low indicating that fungi are not particularly active in these volcanic soils.

The ratio of bacterial to fungal biomass is commonly used as a measure of the relative abundance of fungi and bacteria in the microbial community (Bardgett and Walker, 2004) and has been used to measure shifts in community structure in a tallgrass prairie chronosequence (Allison et al., 2005), under different management regimes (Frey et al., 1999) and in soils following deglaciation (Bardgett and Walker, 2004). It has been hypothesised that after a disturbance, the bacterial to fungal ratio should decline through time as fungi become more prevalent in undisturbed soils (Allison et al., 2005). Initial bacterial to fungal ratios in the control developed soil showed the opposite trend as no fungal biomarker was present. However throughout the experimental incubation, the developed soil showed a decline in bacterial to fungal ratio as fungi became more dominant in the community. The developing soil in contrast showed a slight increase in bacterial to fungal ratio before a decline although this was due to a very low bacterial biomass initially which increased through time concurrently with a slight reduction in fungal biomass. The lack of fungal activity in the developed soil does not concur with other studies of bacterial and fungal abundance during ecosystem development. Pennanen (2001) found an increase in fungal abundance with successional stage and suggested that fungal abundance was linked to organic matter accumulation and increasing C/N ratios of plant material. Ohtonen et al (1999) also found an increase in the prevalence of fungi through time on a glacial chronosequence and suggested that C use was more efficient in fungal dominated communities. Conversely, Klein et al (1995) and Allison et al (2005) found the opposite trend. Allison et al (2005) suggested that in addition to cessation of disturbance, changes in vegetation characteristics and nutrient concentrations are also important in determining the principal members of the soil community. These studies indicate that site

development probably does not act alone in influencing soil microbial community structure and other physical and chemical factors also have significant effects. The volcanic soils of Mt Etna in Sicily are often subject to periods of disturbance which deposit volcanic ejecta on the soil surface. Ejected material can dilute organic matter and has been shown to do so in volcanic soils in Nicaragua (Velasquez-Pereira, 1996). This dilution effect probably has a different consequence for fungal activity than bacterial activity due to the differences in morphology and feeding strategies and may be responsible for the reduced abundance of fungal species.

4.5.1.2 PLFA Diversity

Little or no information exists regarding microbial species diversity in developingvolcanic soils or how soil microbial diversity changes with soil development. Theories suggest species number and equitability should increase through the early stages of community development due to the increasing availability of nutrients and niche specificity (Odum, 1969). There has been some evidence to suggest this relationship in plant communities (Aplet et al., 1998) but little verification for soil microbial communities.

The results of this study show that the developed soil community had greater PLFA diversity and was more even than the developing soil community. These results concur with those of Tscherko et al (2004) who found similar diversity values to this study from PLFA analysis of rhizosphere and bulk soils across an Alpine chronosequence. Rhizosphere soils showed a trend towards increasing diversity through successional stage but there was no such pattern with the bulk soil. Schipper et al (2001) also noted an increase in heterotrophic evenness along various

successional gradients although no differences in heterotrophic richness were observed. This indicates that once newly formed surfaces such as volcanic lavas and tephras are exposed, they are rapidly colonised by an extensive range of heterotrophic microorganisms which are supported on relatively little organic matter (Schipper et al., 2001).

Through time both volcanic soil communities in the present study experienced a distinct reduction in diversity. This is probably due to the decreasing availability of resources in the soil. The developing soil behaved in a more idiosyncratic manner as diversity was initially low and then increased before an eventual decline. This increase in diversity may reflect a rapid change in community composition in the developing soil and is supported by the increased degree of saturation on day 33. SATFAs cannot be formed by *in situ* modification of existing fatty acids and must be a consequence of biomass turnover (Petersen and Klug, 1994). These results suggest although there were significant differences in PLFA diversity with site development, species diversity in soil communities is extremely complex and is probably highly variable through time. It is also unlikely that microbial diversity behaves in the same manner during soil development in every ecosystem.

4.5.1.3 Total PLFA

Soil microbial biomass plays a crucial role in ecosystem nutrient cycling due to the release of readily available soil nutrients (Roy and Singh, 2003). Several authors have predicted that biomass will be low in the initial stages of ecosystem development (Clements, 1916; Odum, 1969) due to low nutrient availability. Total PLFA, a proxy for microbial biomass was initially substantially greater in the mature soil compared to the developing soil which concurs with Odum’s theory of

ecosystem development and other studies of successional sequences (Insam and Haselwandter, 1989; Schipper et al., 2001; Sigler et al., 2002; Tscherko et al., 2003). However, by 81 days of incubation total PLFA in the developing soil was comparable to total PLFA in the developed soil. This was attributable mainly to a decrease in total PLFA content of the developed soil. Increasing microbial biomass with soil development has been noted in studies of glacier forelands (Insam and Haselwandter ,1989; Ohtonen et al., 1999; Sigler et al., 2002; Tscherko et al., 2003), volcanic chronosequences (Hopkins et al., 2007) and blast furnace wastes (Roy and Singh, 2003). No correlation between microbial biomass and stage of development has also been reported (Tscherko et al., 2003) indicating that soil microbial biomass is an indicator sensitive to perturbation by other biotic and abiotic factors. PLFA analysis may be more reliable than other methods of estimating the concentration of viable biomass as PLFAs are not found in dead cells or storage products (Vestal and White, 1989).

Total PLFA values in soils have a large range. Forest soils have been shown to have larger total PLFA values than arable soils of a similar region with approximately 1350 nmol PLFA g^{-1} soil and 65 nmol g^{-1} soil respectively (Frostegård et al., 1993). The soils in this experiment are in the lower range of these values but are similar to those found in soils disturbed by burning and flooding (Bossio and Scow, 1998), arable soils in Sweden (Frostegård et al., 1993) and in harsh Antarctic soils (Malosso et al., 2004). This suggests that despite the timescale over which these volcanic soils have existed, the soil microbial biomass has been subjected to numerous disturbance events which have stunted any pattern of succession.

4.5.1.4 Enzyme Activity

Perhaps the most fundamental function of soils is the capacity to mineralize soil organic matter and degrade plant material (Trasar-Cepeda et al., 2000). The microbial degradation of plant litter is mediated by extracellular enzymes which change nutrients into a form where they can be assimilated into the microbial biomass (Sinsabaugh et al., 1991). Enzymes are key in regulating the flow of energy through an ecosystem and soil microbial communities are envisaged to become more efficient in their energy use though time (Odum, 1969). Soil nutrient dynamics are also envisaged to change through time and C and N have been found to accumulate as soils develop (Ohtonen et al., 1999; Schipper et al., 2001; Tscherko et al., 2003; Hopkins et al., 2007). Conversely, P availability declines (Crews et al., 1995) possibly according to the model of Walker and Syers (1976) where P is envisaged to become occluded by Fe and Al hydrous oxides which renders it largely unavailable to plants and soil microorganisms (Bardgett, 2005) or by leaching from the soil.

Extracellular enzymes require a substantial energy and N investment by soil microorganisms which means large quantities of enzymes should only be produced at the expense of growth and metabolism when available nutrients are limited (Fontaine et al., 2003; Allison and Vitousek, 2005). β-glucosidase is thought to be the rate limiting enzyme in the degradation of cellulose and its synthesis by microorganisms is thought to be induced by the products of cellulose breakdown (Turner et al., 2002). β-glucosidase activities in the Etna soils were similar to those found in agricultural soils under different cropping systems (Bandick and Dick,1999); in those of a tall grass prairie soil (Ajwa et al., 1999) and in semi arid

agricultural soils (Acosta- Martínez et al., 2003). β-glucosidase activity was consistently higher in the developing soil than in the developed soil which concurs with the findings of Allison et al (2007) and Tscherko et al (2004) on other successional sequences. Waldrop et al (2000) found that β-glucosidase activity correlated with monosaturated fatty acids typical of G- bacteria which are known to compete for simple substrates and have high growth and reproduction rates. In the present study, no correlation existed between any biological parameters and total β-glucosidase activity however total PLFA content, total bacterial content and saturated fatty acid content correlated with specific β- glucosidase activity. This suggests that in the soils of Mount Etna, β-glucosidase is produced by a wide range of microorganisms. The quantity and quality of litter inputs are important in regulating the microbial biomass and inducing enzyme synthesis (Swift et al., 1979). Different plant species have been shown to elicit different effects on enzyme production in soil (Rutigliano et al., 2004) both due to the quality and quantity of litter inputs to the soil. The developing soil receives a greater litter input of *Genista aetnensis* than the developed soil which receives predominantly *Castanea sativa*. *Genista aetnensis* is also thought to be a higher quality litter due to its lower C to N ratio and lower predominance of aromatic compounds (Hopkins et al., 2007). This would suggest that the developing soil receives a greater quantity of easily degradable material. Fontaine et al (2003) suggest that zymogenous species are adapted to grow and reproduce rapidly when easily degradable substrates are present. This makes it difficult for slower growing autochthonous species to compete over easily assimilable residues. It may be possible that higher quality litter inputs from *Genista aetnensis* at Monti Rossi encourage the growth of a higher proportion of simple substrate utilising zymogenous species which require the

cleavage of cellobiose to glucose units in order to satisfy carbon demand. The organisms in the developed soil may have a similar potential for the synthesis of this particular simple enzyme but due to the developed nature of the soil and higher PLFA diversity, there may be less competition over carbon resources reducing the need to produce large quantities of simple sugar releasing enzymes. In effect, the microbial community of the developed soil could be more niche specific and therefore degrade a greater range of substrates including more recalcitrant compounds for energy.

Acid phosphatase activity in the Etna soils was fairly high in comparison with other studies in similar Mediterranean environments (Krämer and Green, 2000; Sardans and Peñuelas, 2005) although both Etna sites had similar total acid phosphatase activities. Phosphatases are known to be inducible and are produced predominantly under conditions of low P availability (McGill and Cole, 1981; Schinner et al., 1996). Allison et al (2007) found the activity of P hydrolysing enzymes increased with soil development while soil P concentration declined. Increasing P limitation with site age has also been demonstrated to occur with plant communities (Crews et al., 1995; Wardle et al., 2004). P contents were not investigated in the present study, but Fernandez- Sanjurjo et al. (2003) found that P availability at Monti Rossi was scarce. This implies that P may also be limiting at Salto del Cane and that both communities may have a similar P demand and are therefore investing similar amounts of energy and resources into producing P hydrolysing enzymes to gain a sufficient P supply. This may be due to the inherent nature of volcanic soils which have high phosphate retention capabilities even in early stage soils (>25%) (Arnalds and Kimble, 2001; Takahashi and Shoji, 2002).

In both soils arylsulfatase was found to have the lowest activity of each of the three enzymes studied and the developed soil consistently contained significantly greater quantities than the developing soil. This concurs with other studies which have shown arylsulfatase to have lower activities than acid phosphatase and β-glucosidase in other environments (Bandick and Dick., 1999; Acosta-Martinez and Tabatabai, 2000; Acosta- Martínez et al., 2003). Acosta- Martínez et al (2003) suggested that the lower predominance of arylsulfatase in a range of environments indicated that despite soil property effects on enzyme activities, the predominance and ecological role among enzymes do not change in different soils and vegetation. Arylsulfatase is known to be controlled by demand for S (Dilly and Nannipieri, 2001) and it is possible that in these volcanic soils the weathering of volcanic minerals may provide a source of inorganic S reducing the need for the hydrolysis of SO_4^{2-} esters. Baligar et al (2005) found that increasing levels of soil applied inorganic S reduced arylsulfatase activity while Trasar- Cepeda et al (2000) suggested that a low arylsulfatase activity in Galician soils was a result of the S requirement of microorganisms being fulfilled. Sulfatase activity has previously been found to correlate well with G- bacterial abundance (Waldrop et al., 2000) which concurs with the findings of the current study.

4.5.2 ***Disturbance Effects***

4.5.2.1 Community Structure, Major Groups of Fatty Acids and Biomarkers

The microbial communities of Etnas volcanic soils responded differently to disturbance and recovered at different rates. The developed soil showed less resistance to disturbance initially although both soils showed the greatest degree of

perturbation 33 days after the disturbance. Temperature is thought to be the environmental factor that most affects PLFA composition due to its affect on cell membrane function. Increase temperature increases the fluidity of the lipid bilayer and may affect membrane permeability by formation of non-bilayer phases (Russell and Fukunaga, 1990). Increased temperature caused an immediate reduction in the amount of *anteiso* branched chain fatty acid *a*15:0 in the developed soil and increased the amount of *iso* branched fatty acid *i*15:0 in the developing soil. Pure culture tests have shown a relative increase of *iso*- over *anteiso* fatty acids with an increase in temperature due to the lower melting point of the *anteiso* type (Kaneda, 1991). Another response to increasing temperature is to reduce the degree of unsaturation as *cis*- unsaturated fatty acids have a low transition temperature and counteract membrane crystallisation at lower temperatures (Petersen and Klug, 1994). The developed soil showed a significant reduction in the *cis* MUFA 16:1ω9^{c} which persisted until the end of the experiment and the developing soil appeared to show a reduction in 18:1ω9^{c} but due to high variability this was not significant. This reduction in MUFAs which are indicative of G- bacteria could also be attributed to the relative thinness of G- cell walls which are thought to be vulnerable to desiccation (Van Gestel et al., 1993). Both communities initially showed an increase in straight chained fatty acid 12:0 although this was not significant in the developing soil however a significant increase in straight chained SATFA 16:0 after disturbance was also noted. Straight chained SATFAs have a higher phase transition temperature than *cis* unsaturated fatty acids and reducing the degree of saturation at higher temperatures may act to prevent excess leakage (Petersen and Klug, 1994). The mechanisms involved in creating new SATFAs are very complicated however and few organisms have the ability to remove a double bond by hydrogenation of

intact membrane lipids (Gurr and Harwood, 1990). Biosynthesis of new SATFAs generally results from growth or turnover of existing membranes (Petersen and Klug, 1994).

Cyclopropyl fatty acids have been shown to increase in concentration under stressful conditions such as decreasing pH, oxygen depletion, nutrient deprivation (Guckert et al. 1986) and also higher temperatures (Petersen and Klug, 1994; Zogg et al., 1997). The total concentration of cyclopropyl fatty acids in the Etna soils was not significantly different between soils or between controls and disturbance treatments. However individual cyclopropyl fatty acids varied with disturbance. The developing control was shown to contain cy19:0 on day two but this was not found in the corresponding disturbed soil. This appears to contradict other studies which suggest microorganisms create cyclopropyl fatty acids as they enter a stationary phase in response to stressful conditions. However it may be that the microorganisms in the developed control soil are growing in nutrient limited conditions due to the dilution of organic matter by volcanic ash. Cyclopropyl fatty acids are also thought to be indicative of starvation (Guckert et al, 1986) and it is probable that the organisms containing cy19:0 were in a weakened state and died as a result of the disturbance leading to the absence of this particular fatty acid in the developing disturbed soil. At longer periods after the disturbance, the quantity of cy17:0 increased in the developing disturbed soil relative to the control indicating that the surviving organisms were under stress and/or starvation either as a result of the disturbance or the disturbance amplified previously limited conditions. No significant differences were noted in the cyclopropyl fatty acid content of the developing control and disturbed soil.

Immediately after the disturbance, the fungal biomarker linoleic acid significantly declined in the developing soil but recovered quickly and no significant differences were noted after 33 days of incubation. Conversely fungal biomass in the developed soil was significantly reduced and did not recover by the end of the experiment. The reduction in fungal biomass may be due to the fact that active fungi are less resistant to heating and moisture loss than bacteria (Dunn et al., 1985; D'Ascoli et al., 2005; Jiménez Esquilín et al., 2007) which may have more tolerant propagules.

4.5.2.2 PLFA Diversity

A system's stability determines its ability to continue functioning under changing conditions (Orwin and Wardle, 2004). Disturbance causes dramatic changes in soil properties (DeGrood et al., 2005) and diversities of microbial communities generally decrease in response to environmental stress or disturbance (Odum, 1985; Dunn et al., 1985; Atlas et al., 1991). The effects of disturbance can be long lasting (Mummey et al., 2002a) but developed communities have been hypothesised to be more stable than developing communities (Odum, 1969) and may be more resilient to environmental perturbation. The results of this investigation show that the diversity of the developed soil was initially reduced to a greater degree than the diversity of the developing soil which was seemingly unaffected. Despite this initial reduction, the soil community recovered quickly and diversity was not significantly different throughout the rest of the experiment. Atlas et al (1991) found that diversity was seriously reduced when soils were exposed to various chemical pollutants. Although no measures of recovery were made, it was noted that disturbed soils tended to have increased physical tolerances and nutritional versatility. Diversity was not as significantly affected by disturbance in this

experiment which may be a reflection of the type of disturbance applied to the soil system. Van Gestel et al (1993) suggested that soils that have a climatic history of intermittent drying may contain microorganisms that have adapted to desiccation and become drought tolerant. Given that these soils may have at some point been subjected to a similar type of disturbance naturally, it may be possible that they have increased physical tolerances.

4.5.2.3 Total PLFA

There is increasing evidence that changes in soil microbial biomass could act as indicators for soil degradation or improvement due to their sensitivity to environmental change (Gelsomino et al., 2006). Significant degradation of the microbial community can occur following a disturbance both to the size and composition of the microbial community (Insam and Domsch, 1988; Harris et al., 1993) but responses to disturbance largely depend upon the mechanism of disturbance. Mummey et al (2002a) found that microbial biomass was markedly reduced in a reclaimed mine site compared to an undisturbed soil while total PLFA was found to be unaffected in a study of the effects of alkaline dust pollution (Bååth et al., 1992) and in soils that had been exposed to heavy metals (Frostegård et al., 1993).

In this investigation, total PLFA in the more developed soil was initially reduced by approximately 50% and did not recover to control levels until the end of the experiment. The developing soil was initially unaffected by the disturbance treatment although this appeared to be related to the low biomass in the control soil as after 33 days of incubation, the disturbed soil had a significant reduction in

biomass compared to the control. This reduction in biomass was probably a result of metabolic stress at higher temperatures. A similar disturbance treatment on a Devonshire semi- improved grassland showed a reduction in microbial biomass of around 40% with heating to 60°C for 2 hours but no recovery was witnessed in the eight week study period (Degens, 1998b). In an investigation of the effects of elevated temperature on microbial biomass, Joergensen et al (1990) found that incubation at 35°C for 10 days reduced microbial biomass to less than a third of its original content. This suggests that in fact, the volcanic soils may be more resilient to temperature and desiccation effects considering the high temperature at which the soils were incubated (60°C) and the length of disturbance (48 hours).

4.5.2.4 Enzyme Activity

Soil enzyme activities are thought to be very useful indicators of soil quality as they are very sensitive to disturbance (Zornoza et al., 2006). No other studies have been conducted into the effects of disturbance on enzyme activities of volcanic soils of different ages. Disturbance significantly affected the activity of each of the three enzymes in both the developing and developed soil on at least one sampling occasion. This reduction in enzyme activity concurs with other studies where soils have been subjected to either substantial heating by a forest fire (Ajwa, 1999; Boerner et al., 2000) or to drought (Li and Sarah, 2003a; Li and Sarah, 2003b; Sardans and Peñuelas, 2005). Arylsulfatase was the enzyme most affected by disturbance in both soils but while the developing soil eventually regained complete arylsulfatase activity, the altered mature soil remained significantly lower than the control by the end of the experiment. This may suggest that the arylsulfatase enzyme is less resistant to denaturing from high surface temperature or lack of moisture than the other two enzymes. Alternatively, the lack of recovery in

arylsulfatase activity may indicate that of the three nutrients studied, sulfur is the least required and while resources have been allocated to growth and repair, they have been diverted from sulfur hydrolysing enzymes. Other studies have reported differential effects of disturbance on enzyme activities. Boerner et al (2000) reported little impact of fire on β-glucosidase activity after a prescribed burn but found a significant reduction in acid phosphatase activity (arylsulfatase activity was not investigated). In contrast, Saa et al (1993) found no significant differences in acid phosphatase activity after a low intensity fire while Eivazi and Bayan (1996) found a significant reduction in β-glucosidase activity in an oak forest soil after annual or periodic burning. This indicates that frequency and intensity of disturbance affects microbe response and therefore enzyme synthesis. In the present study total β-glucosidase activity was significantly less in the developing disturbed soil than in the control only once indicating that this enzyme was affected very little in this soil however specific activity was highly variable. In the developed soil, total β-glucosidase activity significantly declined after the disturbance while acid phosphatase also declined but to a lesser degree. Specific β-glucosidase and acid phosphates activities in the more developed soil were not affected by disturbance to the same degree as total activity however and these enzymes were found to be correlated with total bacterial PLFA indicating that any reduction in activity was based on a reduction in the microbial biomass rather than reduced community function.

4.5.2.5 Effects of reduced diversity on functioning

The consequence of greater diversity in soils is not fully understood although it has been proposed that greater diversity communities may be more resistant and resilient when exposed to environmental perturbations (Giller et al., 1997). In this

respect it may be surprising that PLFA diversity, total PLFA, and total enzyme activities in the later stage soil experienced significantly greater reductions than in the earlier stage soil as a result of disturbance. However, specific enzyme activities (i.e. enzyme activity per unit PLFA) indicated that reduced total enzyme activities in the more developed soil were not the result of reduced function, but was proportionally related to the reduction in biomass. This suggests that while PLFA diversity significantly declined in the more developed soil, function was maintained. The earlier stage soil behaved in a more idiosyncratic manner but disturbance still did not result in functional loses. This concurs with the insurance hypothesis which suggests that a high species diversity acts as insurance in times of environmental disturbance ensuring functioning is maintained (Naeem and Li, 1997). Setälä and McLean (2004) reported higher diversity fungal communities had increased abilities to maintain higher respiration after a period of drought however their findings could partially be explained by a statistical artefact known as the "sampling effect" (Aarssen, 1997). This may also be true of this experiment as higher diversity soils may have a higher probability of containing organisms which can withstand rapid drying and heating. The "sampling effect" however has been proposed as a legitimate explanation for diversity- stability relationships (Tilman et al., 1999).

It is not known whether a reduction in community structural diversity or functional diversity will affect rates of decomposition. Andrén et al (1995) suggested that the rate of organic matter decomposition should increase with the number of trophic levels however the increase in the rate decomposition should be reduced with each trophic level added. In a study of leaf litter decomposition, soil microbial diversity did not affect decomposition rates of leaf litter and this was attributed to a high

degree of functional redundancy within the soil (Andrén et al., 1995). Findlay et al (1997) suggested that as micro-organisms can potentially occur anywhere, their richness has the potential to be very high and thus microbial systems will have an extremely high rate of functional redundancy. In a study of the global dispersal of free living eukaryote species, found a large fraction of species were similar in small intensively studied plots and also found similar sets of species in widely separated locations (Findlay, 2002). This concept of functional redundancy suggests there is a high potential within the diverse microbial community to have many species which perform the same functional role within an ecosystem so that an alteration in species diversity may not affect ecosystem functioning. There is still however some controversy over whether functional redundancy actually exists (Loreau, 2004). It can be assumed that the Etna soils have a high degree of functional redundancy as enzyme function was maintained after the disturbance despite loses in PLFA diversity. It is probable that once key functional groups are present, increasing diversity only has an effect on function when new functional groups are introduced (Andrén et al., 1995). The main PLFA components of the two soils were similar and these groups generally survived the disturbance although concentrations were in some cases depleted. It is possible that these few key functional groups alone were able to maintain soil functioning indicating that community diversity itself may not be important but the presence and abundance of key functional groups are.

4.6 Conclusions

The main aims of this study were to investigate PLFA diversity, community composition and enzyme activities in volcanic soils of different stages and to assess changes in these components after an environmental disturbance. The following conclusions were drawn from this chapter:

- PLFA diversity, evenness and total PLFA were greater in the developed Salto del Cane soil suggesting this soil had a higher number of functional organisms than the developing Monti Rossi soil.

- Fungal PLFA biomarkers were low in both soils indicating that these volcanic soils provide a poor habitat for fungal colonisation and development.

- The soil communities showed differences in enzyme activities with a higher proportion of simple C hydrolysing enzymes present in the earlier stage soil indicating a potentially greater demand for C resources possibly due to a more metabolically inefficient community or to high levels of community competition for simple C sources.

- Both soils showed a similar high demand for P which suggests both soils were P limited probably as a result of high P retention in these volcanic soils.

- Disturbance affected PLFA diversity, total PLFA and community structure to a greater degree in the developed soil.

- Reductions in enzyme activities after disturbance were not found to be related to soil carbon content but were closely related to changes in total biomass indicating that while PLFA diversity and total PLFA were reduced, soil function was maintained possibly due to high functional redundancy.

Chapter 5 Catabolic responses and functional diversity of volcanic soils at different developmental stages

5.1 Introduction

Little is known about the role of soil microbial diversity in mediating fundamental ecosystem processes such as decomposition (Zak et al., 1994; Garland, 1997, Giller et al., 1997). Much of the recent work investigating soil microbial diversity has concentrated on the assessment of PLFA profiles which are a useful proxy for estimating community species diversity but provide relatively little information about soil microbial community functional diversity (Degens and Harris, 1997) which may be more ecologically relevant (Zak et al., 1994). The diversity of decomposition functions performed by soil microbial communities represents only one component of their functional diversity (Degens et al., 2000) but decomposition and cycling of nutrients is arguably the soil microbial community's most important function.

Changes in land use, vegetation and management have been found to alter various soil biological properties such as genetic composition (Nusslein and Tiedje, 1999) and bacterial to fungal ratio (Bardgett and McAlister, 1999). Differences in functional diversity have also been noted to occur between soils under different land uses but the factors responsible for these differences are unknown (Degens and Harris, 1997). One possible mechanism for alterations in community function with land use change is the quantity and quality of plant material incorporated into soil organic matter (Quideau et al., 2001). Given that plant material can alter soil organic matter properties which in turn may affect the decomposition functions of the soil microbial community; it is probable that soils of different developmental

stages which receive different quantities and qualities of organic inputs will have different functional capabilities.

It has been noted that an important component of community functional diversity may be the increased resistance and resilience to stress and disturbance (Elliot and Lynch, 1994). Similarly, a reduction in functional diversity may lead to a decline in a community's ability to resist stress and disturbance although an important soil quality factor would be a soils ability to retain decomposition function after a stress or disturbance event (Giller et al., 1997; Degens et al., 2001). However, as noted by Degens et al (2001) it may be impossible to extrapolate such theories to the soil microbial community which contains such a high diversity of organisms. Stress events can be defined as an environmental condition (biological, chemical or physical) which has an adverse effect on microbial communities such as high soil pH or salinity, while disturbance may be defined as an environmental event that is constrained in time but may have lasting positive or negative effects such as freeze-thaw or rapid drying (Degens et al., 2001). Consequences of such stresses and disturbances have been found to have differing effects on microbial decomposition functions and it is important to note that declines in microbial functional diversity have not been proved to result in decreased soil functioning (Degens, 1998b). Few studies have investigated the effects of an environmental disturbance on a community decomposition functions (Degens et al., 2001) but it has been suggested that decomposition functions of soil communities at earlier stages of ecosystem development may be more vulnerable to stress or disturbance than more developed soil communities (Schipper et al., 2001). The influence of plant litter addition on the functional capacities of soils is also unknown but it may be possible that catabolic

responses of disturbed soils may reflect the chemical compositions of added material more than undisturbed soils amended with litter.

5.2 Specific Objectives and Hypotheses

This chapter consists of four main experiments:

1. *Calibration of the MicroResp rapid microtitre method.*

   Objectives: The main objective of this experiment was to provide a calibration system for the MicroResp soil respiration device and compare the precision of the MicroResp with that of another method of respiration measurement.

2. *Field testing of the MicroResp method and the effects of soil removal and storage on community respiration and simple substrate utilisation.*

   Objectives: To assess the effects of soil removal and storage on some community biological parameters by comparing field state characteristics with those of the same soils which had been transported and stored for 1 week at varying temperatures.

3. *Catabolic response profiles and functional diversity of volcanic soils at different stages of development in relation to PLFA diversity.*

   Objectives: To create catabolic response profiles in order to identify specific catabolic abilities of each soil community along with their respective community functional diversities and to compare these functions with PLFA diversity.

Hypotheses:

- Soils from different developmental stages will have different catabolic response profiles.
- Developing soils will be less catabolically diverse than developed soils.
- Catabolic diversity will increase with PLFA diversity.

4. *The effects of pre- incubation with different leaf litter types on the catabolic response profiles of volcanic soils at different stages of development both disturbed and undisturbed.*

Objectives: The purpose of this experiment was to determine the influence (if any) of pre- incubation with different leaf litter types on the catabolic response profiles of developing and developed soils and to establish whether incubation with different leaf litters promote particular decomposition functions in soils which have been subject to disturbance.

Hypotheses:

- Pre- incubation with different leaf litter species will alter the catabolic response profiles of soils.
- Soils pre- incubated with particular leaf litter will have a higher affinity to decompose the same litter when re- applied.
- The microbial community of the developed soils will be less influenced by leaf litter addition and will remain more similar to the control soil than the developing soils with litter added.

5.3 Materials and Methods

5.3.1 *Calibration of the MicroResp rapid microtitre method*

5.3.1.1 Experimental Procedure

Each of the four soils were subject to a disturbance treatment as in section 3.1.1 and along with undisturbed samples were given two C substrates, Glucose and L-Arginine as well as a control consisting of distilled H_20, to give 24 treatments (Table 5-1).

Table 5-1 Sample layout according to Soil Type, disturbance and added substrate

Soil Type	Undisturbed (U)/ Disturbed (D)	Substrate
MRP	U	Distilled H_20
MRP	U	Glucose
MRP	U	L- Arginine
MRP	D	Distilled H_20
MRP	D	Glucose
MRP	D	L- Arginine
MRG	U	Distilled H_20
MRG	U	Glucose
MRG	U	L- Arginine
MRG	D	Distilled H_20
MRG	D	Glucose
MRG	D	L- Arginine
SDCA	U	Distilled H_20
SDCA	U	Glucose
SDCA	U	L- Arginine
SDCA	D	Distilled H_20
SDCA	D	Glucose
SDCA	D	L- Arginine
SDCB	U	Distilled H_20
SDCB	U	Glucose
SDCB	U	L- Arginine
SDCB	D	Distilled H_20
SDCB	D	Glucose
SDCB	D	L- Arginine

C substrates were added at a rate of 2 mg C g^{-1} dry weight soil and resultant respiration was assessed using a multi- chamber Respirometer (Respicond IV, Nordgren Innovations, Umea, Sweden) and the MicroResp system so as to provide a calibration system for measuring community functional diversity. Prior to the investigation, approximately 35 g DW soil for Respirometer samples and a total of 50 g DW soil for MicroResp analysis, from each soil type and disturbance treatment

was moistened and incubated at 20°C for 10 days prior to the experiment in order for the biomass to re-establish and stabilise.

5.3.1.2 Preparation of MicroResp detection plates

MicroResp detection plates were prepared according to the protocol provided by MicroResp TM. 15 g of Noble Agar was dissolved in 500 ml of de- ionised H_20 then autoclaved at 121°C for 20 minutes to ensure complete dissolution. The liquid agar was then dispensed into ten 50 ml batches and stored at 4°C until required. The indicator solution was prepared by dissolving 16.77 g potassium chloride, 0.315 g sodium bicarbonate and 18.75 mg cresol red in approximately 900 ml de- ionised H_20 over a low heat. The solution was then diluted to 1000 ml in a volumetric flask and dispensed into 10 batches of 100 ml solution which were also stored at 4°C until required. Each of the 100 ml indicator and agar solutions were equivalent to 8 microplates. The detection gel was created by heating one aliquot of the indicator solution to 65°C on a hotplate under constant stirring and adding one aliquot of noble agar solution which had been completely melted in a microwave. 150 µl of detection solution was allotted into microplates using a multi- channel pipette after which plates were covered with Parafilm and stored at room temperature in a desiccator containing soda lime and a beaker of water to maintain a moist, CO_2 free environment.

5.3.1.3 Preparation of MicroResp deep- well plates

Deep well plates were prepared by adding the C substrates in solution to selected wells after which the filling device was put in place and the selected soil type added

until each of the filling cells were full and level. The plastic divider was then removed and the soil samples lightly shaken into the wells. Before the experiment, several grams of each soil were loaded into the MicroResp filling device and added to a pre- weighed deep well plate and re- weighed in order to establish the mass of soil in each well.

5.3.1.4 MicroResp assembly

After addition of substrates and soil to deep well plates, the specially adapted seal was fixed to the deep well plate and a microplate which had been pre- read at 590 nm in a Versa max tuneable spectrophotometer was fixed to the top of the gasket and the whole device clamped together. The fully assembled MicroResp device was then incubated at 20°C in darkness for 6hrs after which the microplate was re- read in the spectrophotometer.

5.3.1.5 MicroResp data handling

Files were exported from the spectrophotometer program to Microsoft Excel in order to sort the data into columns with the initial microplate readings (t0) and post-incubation readings (t6) for each well. Data were required to be normalised and the resultant CO_2 production rate (μg CO_2-C g^{-1} soil) calculated as per the MicroResp technical manual.

5.3.1.6 Respirometer set-up

The multi- chamber Respirometer allows almost continuous monitoring of CO_2 production from soil samples according to decreases in electrical conductivity as

CO_2 is absorbed in a Potassium Hydroxide (KOH) trap and carbonate ions are produced (Nordgren, 1988). 35 g DW soil was amended with 2 mg C g^{-1} DW soil of each of the two C substrates. The substrates were added in solution and control samples were amended with the equivalent amount of distilled water so as all samples (both Respirometer and MicroResp) were maintained at the same moisture content. The KOH traps contained 10 ml 0.6M KOH which was added fresh immediately after the addition of substrates to soil samples. Triplicate samples were incubated in the dark at 20°C whilst electrical conductivity, CO_2 production rate and accumulated CO_2 were monitored at one hour intervals. Accumulated CO_2 data allowed for comparison with CO_2 evolved from MicroResp samples over 6 and 24 hours.

5.3.1.7 Respirometer data handling

Accumulated CO_2 data was transferred from mg CO_2 to μgCO_2-C g^{-1} soil for comparison with MicroResp results. These data were then compared and subsequently used as calibration data for further investigations using the MicroResp device.

5.3.2 ***Field testing of the MicroResp method and the effects of soil removal and storage on community respiration and simple substrate utilisation***

5.3.2.1 Soil sampling and preparation

Soils were collected from each field site on 5th May 2006 and sieved through a 2 mm sieve to remove course tephra and large particles of organic matter. MicroResp detection plates were pre- assembled (see section 5.3.1.4) and stored in a CO_2 free environment for a maximum of 48 hours prior to use. A glucose substrate was added to the deep well plate according to the protocol outlined in the MicroResp technical manual whereby substrates are applied as 30 mg per gram of soil water in each well. Substrates were applied in solution in 25 μl aliquots before the addition of soil (as per section 5.3.1.1). Colour development after 6 hours was recorded using a digital camera.

One week after soil collection and subsequent transport to the laboratory, the experiment was repeated however detection plates were read in a tuneable microplate reader. The digital photographs obtained in the field study were analysed by assessing colour development in microplates under known CO_2 concentrations. The disturbance effect of removal from the field was assessed by change in community respiration from field state to 1 week.

5.3.3 ***Catabolic response profiles and functional diversity of volcanic soils at different stages of development in relation to PLFA diversity***

5.3.3.1 Substrate selection and preparation

26 C substrates from 6 different guild structures (chemicals characterised according to their chemical composition) were chosen in consultation with other such studies

(Degens and Harris, 1997; Degens, 1998a; Degens, 1998b; Degens et al., 2001; Campbell et al., 2003; Stevenson et al., 2004) in order to encompass a wide range of different chemical structures ranging in their decomposability. In addition to these chemical substrates, 8 substrates common to the study environment were also included so as to provide a further, more realistic insight into the decomposition functions of the soil communities studied. The 34 substrates used are outlined in Table 5-2.

Table 5-2 Substrates selected to create catabolic response profiles

Amino Acids:	***Amines:***	***Carbohydrates:***
1. L- Alanine	8. D- Glucosamine	13. D- Fructose
2. L- Asparagine*	9. L- Glutamine	14. D Galactose
3. L- Arginine	10. N acetyl D- Glucosamine	15. Glucose
4. D- Glutamic Acid*		16. D- Mannose
5. L- Glutamic Acid*	***Aromatics:***	17. Sucrose
6. Glycine	11. Inosine*	18. D- Xylose
7. L- Leucine*	12. Urocanic Acid*	19.D- Arabinose
Carboxylic Acids:	***Polymers:***	30. Pinus+ Genista
20. Fumaric Acid*	26. Tween 80	31. Pinus+ Castanea
21. Ascorbic Acid		32.Genista+ Castanea
22. D- Glucuronic Acid	***Environmental Substrates:***	33. All 3 litters
23. Maleic Acid	27. Pinus Extract	34. Ash
24. Oxalic Acid*	28. Genista Extract	
25. α- Ketoglutaric Acid	29. Castanea Extract	

Each substrate was added as 30 mg g^{-1} soil water (MicroResp technical manual) in 25 μl aliquots. Less soluble substrates (marked *) were added as 7.5 mg g^{-1} soil water (Campbell et al., 2003). Water soluble extract was used as a representation of litter samples. C content of the soluble litter extracts were calculated according to data obtained from Hopkins et al (2007) whereby litter was weighed to represent a known amount of soluble C and mixed with 20 ml distilled water for 20 minutes under constant shaking after which liquid and litter samples were passed through a cheesecloth mesh and resultant liquid filtered through a Whatman number 1 filter

paper. In order to assess the effects of any ash and tephra leachate on microbial respiration, 10 g of coarse tephra which had been heated to 60°C for 24 hrs previously, was shaken with 20 ml distilled water for 20 minutes with the resultant leachate passed through a Whatman number 1 filter paper.

5.3.3.2 MicroResp assembly

Soils from all four sites were compared in this experiment and had been incubated at 20°C for 10 days prior to the experiment. The components of the MicroResp were assembled as in section (5.3.1.4) and incubated in darkness at 25°C for 6 hours before the detection plates were read again. The MicroResp vessels were then incubated in the same conditions until 24 hours from the commencement of the experiment when the detection plates were once again read. Respiration was calculated as a mean of four replicates.

5.3.3.3 Phospholipid Fatty Acid Analysis (PLFA)

PLFA was used to determine microbial taxonomic diversity in each soil. The analysis was carried out according to the protocol outlined in section 4.2.2.

5.3.4 ***The effects of pre- incubation with different leaf litter types on the catabolic response profiles of volcanic at different stages of development both disturbed and undisturbed***

5.3.4.1 Experimental Procedure

One developing and one developed soil were chosen at random to undergo investigation. These soils were the same soils investigated in chapter 4, Monti Rossi *Genista* (MRG) and Salto del Cane A (SDCA). The soils labelled as disturbed were

heated in an oven at 60°C for 48 hours while the control samples were kept refrigerated. Once removed from the oven, the dried samples were allowed to cool and were re- adjusted to their initial moisture content. All samples were then adjusted to 50% water holding capacity.

Table 5-3 Sample layout according to Soil Type, disturbance and added litter

Soil Type	Undisturbed (U)/ Disturbed (D)	Litter
MRG	U	Pinus
MRG	U	Genista
MRG	U	Mix
MRG	D	Pinus
MRG	D	Genista
MRG	D	Mix
SDCA	U	Pinus
SDCA	U	Genista
SDCA	U	Mix
SDCA	D	Pinus
SDCA	D	Genista
SDCA	D	Mix

Small samples of *Pinus* and *Genista* litter were milled to less than 2 mm in agate chambers using a Retsch MM200 ball mill. The litters were then added to selected soil samples either individually or as a mix of both litters (Table 5-3) so that the final concentration added was approximate to 10 mg litter Cg^{-1}soil. Soils were then left to incubate in a moist environment for three weeks at 20°C.

5.3.4.2 Substrate selection and preparation

Of the 34 substrates used in section 5.3.3.1, 17 were chosen to test the functional capabilities of the soils in this experiment. The substrates were selected in order to include a full suite of environmental substrates as well as to include the aromatic substrates and a general selection of other substrates (Table 5-4). Substrates were prepared according to section 5.3.3.1.

Table 5-4 Substrates selected to test the functional capabilities of soils pre incubated with different leaf litters

Carbohydrates	Environmental Substrates	Amino Acids
1.D- Galactose	7.Pinus Extract	14.L- Arginine
2.D- Xylose	8.Genista Extract	
3.D- glucose	9.Castanea Extract	***Aromatics***
	10.Pinus+Genista	15.Inosine
Carboxylic Acids	11.Pinus+Castanea	16.Urocanic Acid
4.D- Glucuronic Acid	12.Genista+ Castanea	
5.Oxalic Acid	13.All 3	***Amines***
6.α- Ketoglucaric Acid		17.D-Glucosamine

5.3.4.3 MicroResp Assembly

Each MicroResp was loaded and assembled according to the protocol in section 5.3.1.4 and incubated in darkness at 25°C for 6 hours before the detection plates were read again. The MicroResp vessels were then incubated in the same conditions until 24 hours from the commencement of the experiment when the detection plates were once again read. Respiration was calculated as a mean of four replicates.

5.4 Results

5.4.1 *Calibration and comparison of the MicroResp rapid microtitre method*

The MicroResp rapid microtitre system requires calibration in each individual laboratory to account for differences in the types of spectrophotometers, experimental techniques and incubation conditions. Regression analysis showed that at both 6 (R^2=0.6871) and 24 hours (R^2=0.8117) of incubation, CO_2 evolution recorded from a range of samples in the MicroResp system was comparable to that of the Respirometer (Figure 5-1).

3 way ANOVA of respiration from a range of samples as recorded by the MicroResp rapid microtitre method and from the SIR method showed that after 6 hours of incubation, the SIR method had a higher resolution when distinguishing between disturbance and substrate effects (Table 5-5). Both methods detected that soil development and substrate alone were significant factors in determining respiration rate after 6 hours as well as interactions between soil development and disturbance and disturbance and substrate. While the SIR method discerned an interaction between soil development, disturbance and substrate ($p<0.05$) after 6 hours, the MicroResp system appeared to be unable to detect this ($p=0.230$) but similarities in the F values suggests that any interaction was weak. After 24 hours of incubation both methods were able to detect significant differences in respiration however the SIR method was more resolute (Table 5-5). These findings suggest that while the SIR method has a higher resolution, the MicroResp can also be used to discern differences in respiration from soils that have had different substrates added and have been exposed to a disturbance.

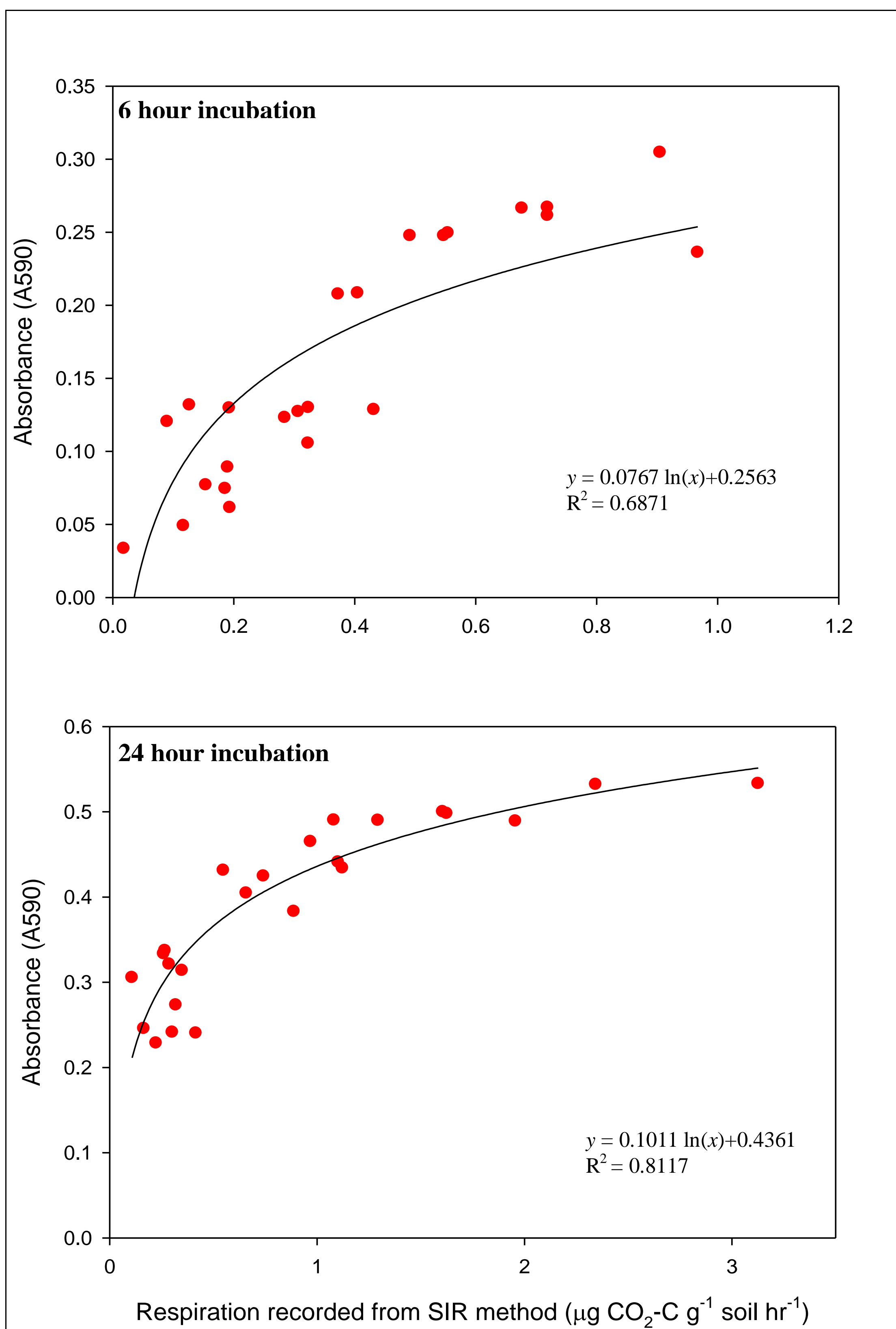


Figure 5-1 Regression analysis of respiration recorded using the SIR method and absorbance using the MicroResp rapid microtitre method after 6 hours and 24 hours of incubation

Table 5-5 Three Way Analysis of Variance to highlight sources of variance in MicroResp and SIR techniques after 6 and 24 hours incubation

Source of Variance	*MicroResp 6 hours*		*SIR 6 hours*		*MicroResp 24 hours*		*SIR 24 hours*	
	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value
Soil development	3.25	0.025	11.87	0.000	10.26	0.000	119.84	0.000
Disturbance	1.08	0.302	19.89	0.000	11.84	0.001	78.99	0.000
Substrate	16.47	0.000	61.35	0.000	125.20	0.000	602.43	0.000
Soil development *Disturbance	3.38	0.021	9.29	0.000	7.04	0.000	90.22	0.000
Soil development * Substrate	1.47	0.197	6.60	0.000	2.98	0.016	43.95	0.000
Disturbance*Substrate	5.50	0.005	4.70	0.014	7.13	0.001	24.68	0.000
Soil development *Disturbance*Substrate	1.38	0.230	3.35	0.008	3.19	0.007	20.96	0.000
R^2	46.08%		85.10%		79.20%		98.00%	

5.4.2 ***Field testing of the MicroResp method and the effects of soil removal and storage on community respiration and simple substrate utilisation***

Soil basal respiration after the six hour incubation period was greatest for those derived from MR with MRP showing the largest basal respiration (Figure 5-2). The SD soils had lower basal respiration rates (Figure 5-3) with SDCB having the lowest overall although differences in basal respiration between MRG, SDCA and SDCB were probably not statistically significant. The MR soils also showed the highest respiratory responses (although probably not statistically significant) to added glucose.

One week after the soils were removed from field conditions basal respiration was less than when the soils were initially removed from the field while glucose induced respiration was probably not significantly different (Table 5-6). Glucose addition had a significant effect on the rate of respiration while soil development had no effect (Table 5-7). There were no significant differences between soils on the rate of basal respiration ($p=0.922$ $F=0.16$) or glucose induced respiration ($p=0.205$ $F=1.71$) with all soils exhibiting similar responses.

Table 5-6 Basal and substrate (glucose) induced respiration in soils from Monti Rossi *Pinus* (MRP), Monti Rossi *Gensita* (MRG), Salto del Cane A (SDCA), Salto del Cane B (SDCB) one week after removal from field conditions

Soil	Basal Respiration (µg CO_2-C g^{-1} soil hr^{-1})	Substrate induced respiration (µg CO_2-C g^{-1} soil hr^{-1})
MRP	0.797 (0.146)	3.134 (0.594)
MRG	0.816 (0.120)	2.105 (0.386)
SDCA	0.885 (0.128)	1.597 (0.449)
SDCB	0.906 (0.117)	2.034 (0.421)

Table 5-7 Two- way ANOVA to determine the effects of substrate and soil on respiration

Source of variation	*p* value	F value
Glucose addition	0.000	81.51
Soil development	0.241	1.47
Interaction	0.175	1.76

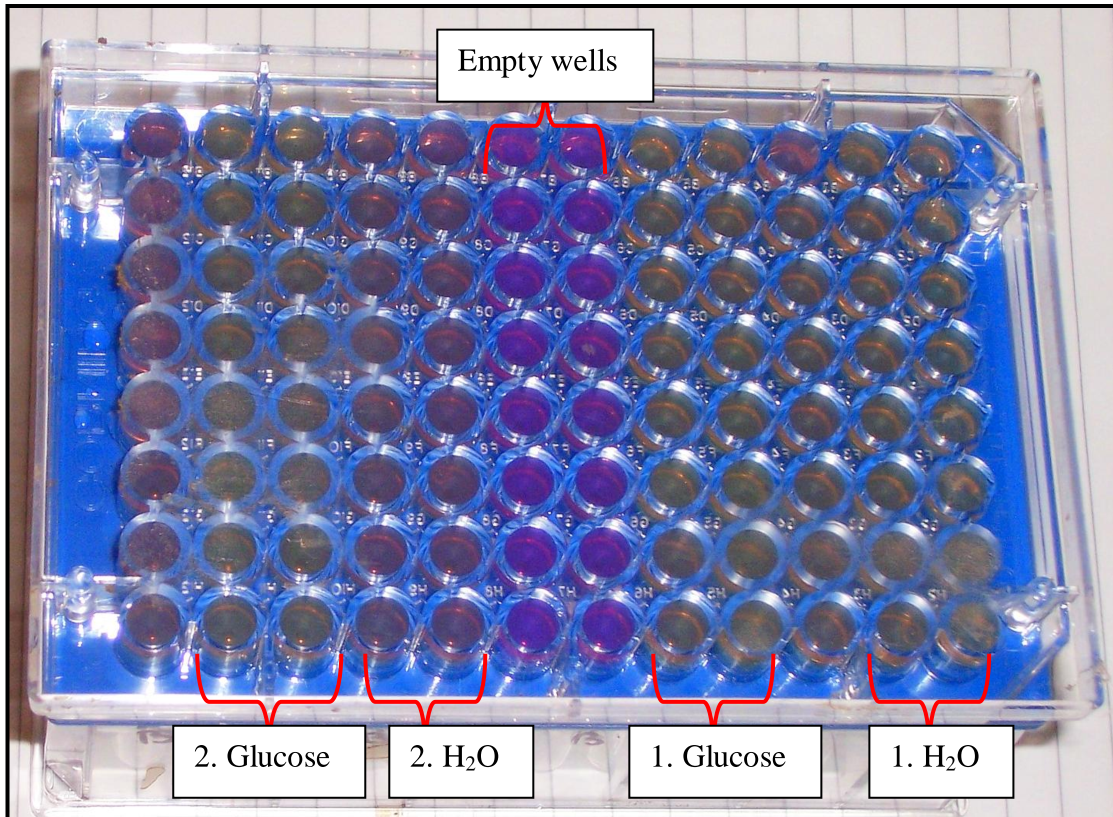


Figure 5-2 MicroResp colour development after six hours incubating soils obtained from field state at Monti Rossi 1.*Pinus* and 2. *Genista* with either water or glucose solution

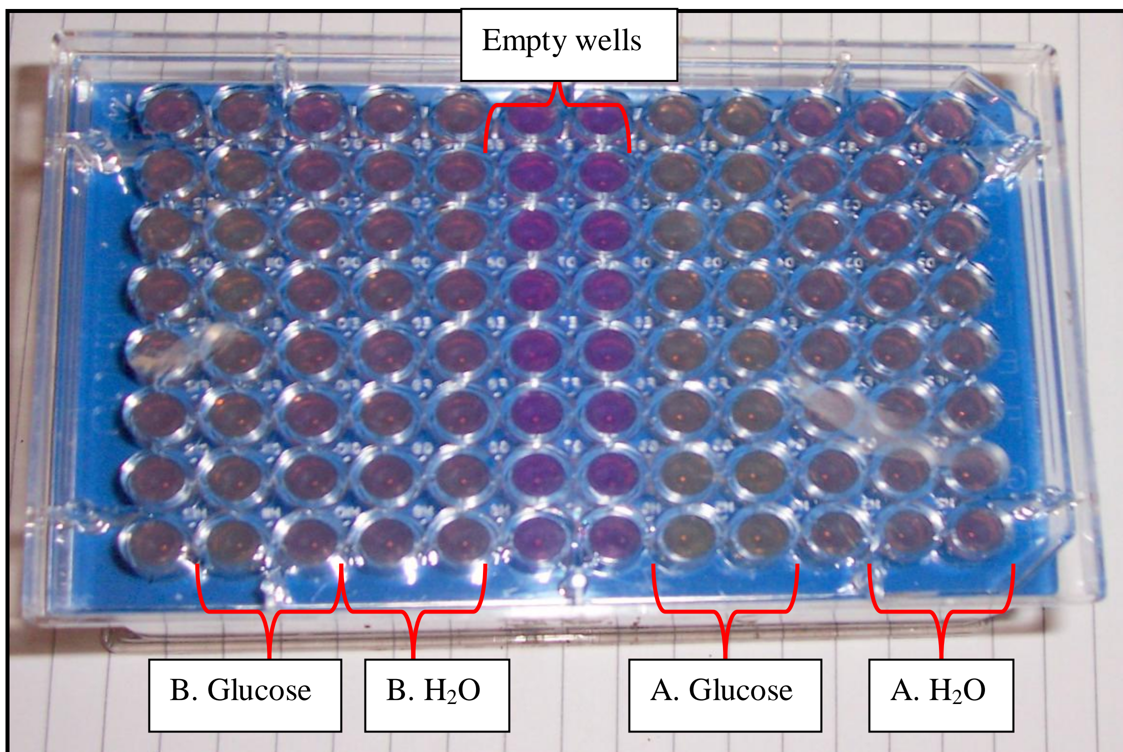


Figure 5-3 MicroResp colour development after six hours incubating soils obtained from field state at Salto del Cane A and B with either water or glucose solution

5.4.3 ***Catabolic response profiles and functional diversity of volcanic soils at different stages of development in relation to PLFA diversity***

5.4.3.1 Catabolic Responses

Of the seven substrate guilds investigated (i.e. amino acids, amines, aromatic compounds, carbohydrates, carboxylic acids, polymers and environmental substrates), no entire group was able to sufficiently distinguish between soils at different developmental stages. Catabolic response profiles after 6 hours incubation with substrates showed that of the two MR soils, MRP had greater responses to D- and L- Glutamic acids, Glycine, Urocanic acid, Inosine, D- Galactose, Sucrose, Fumaric acid, Tween 80, *Castanea* extract and *Pinus* + *Genista* extract while MRG had greater responses to D- Fructose, Glucose, D- Glucuronic acid, Maleic acid, *Pinus* extract, *Genista* + *Castanea* extract and All 3 plant extracts (Figure 5-4).

Developed soils showed much less variability in substrate utilisation after 6 hours incubation (Figure 5-4). SDCA had higher responses to L- Glutamic acid, D- Xylose and All 3 plant extracts while SDCB had higher responses to Ascorbic acid, Maleic acid, and four of the environmental substrates; *Genista* extract, *Castanea* extract, *Pinus* + *Genista* extract and *Genista* + *Castanea* extract.

Due to the high variability in the catabolic responses of the MR soils, after 6 hours incubation the only substrates which definitively differentiated between the two developmental stages were L- Leucine, D- Glucosamine and All 3 plant extracts to which the SD soils exhibited the highest respiratory responses.

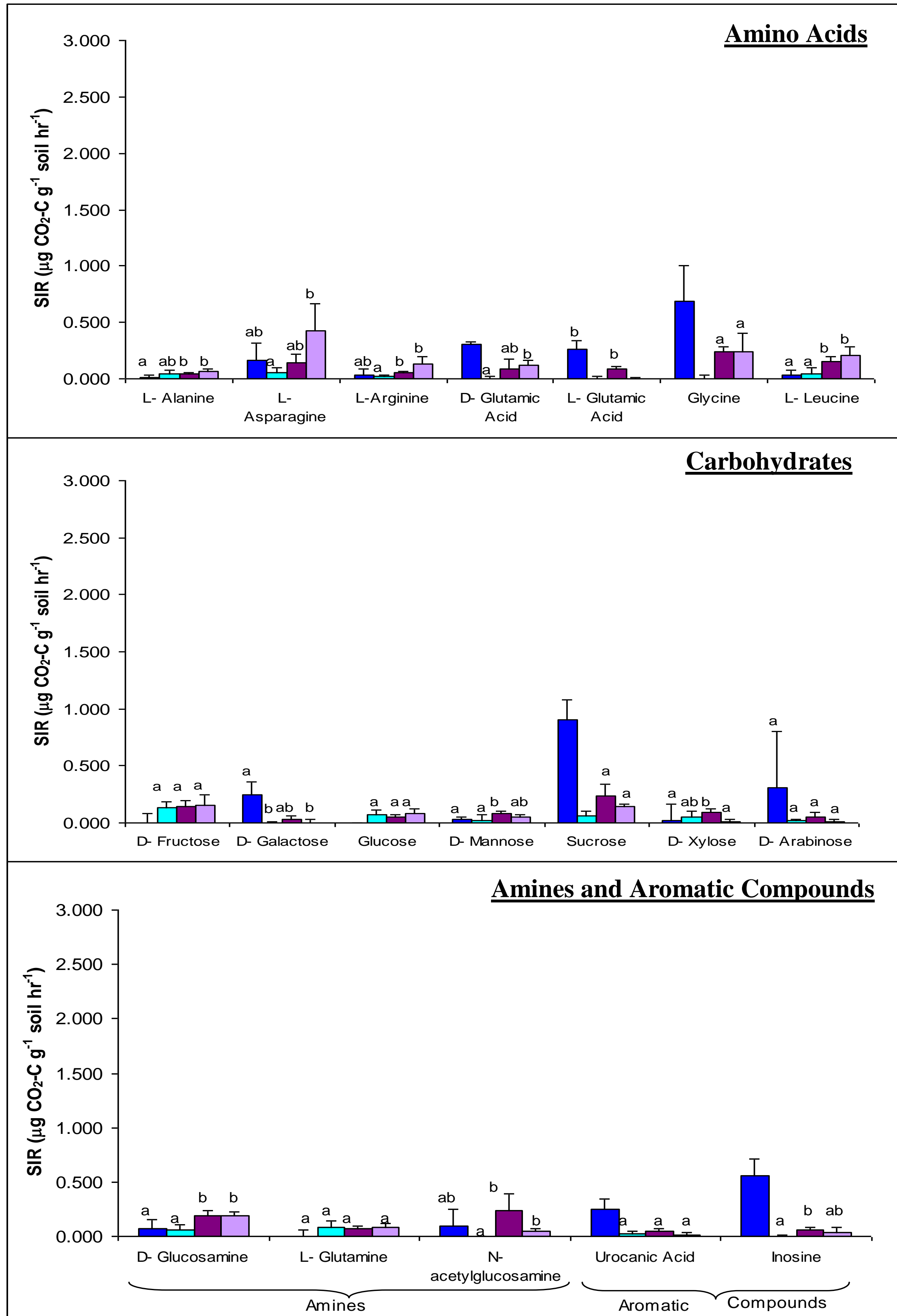


Figure 5-4 Mean catabolic responses (±SD) of MRP (dark blue), MRG (light blue), SDCA (dark purple), SDCB (lilac) to a range of substrates after 6 hours of incubation. For site effect bars with the same letter are not significantly different at the (p<0.05) level.

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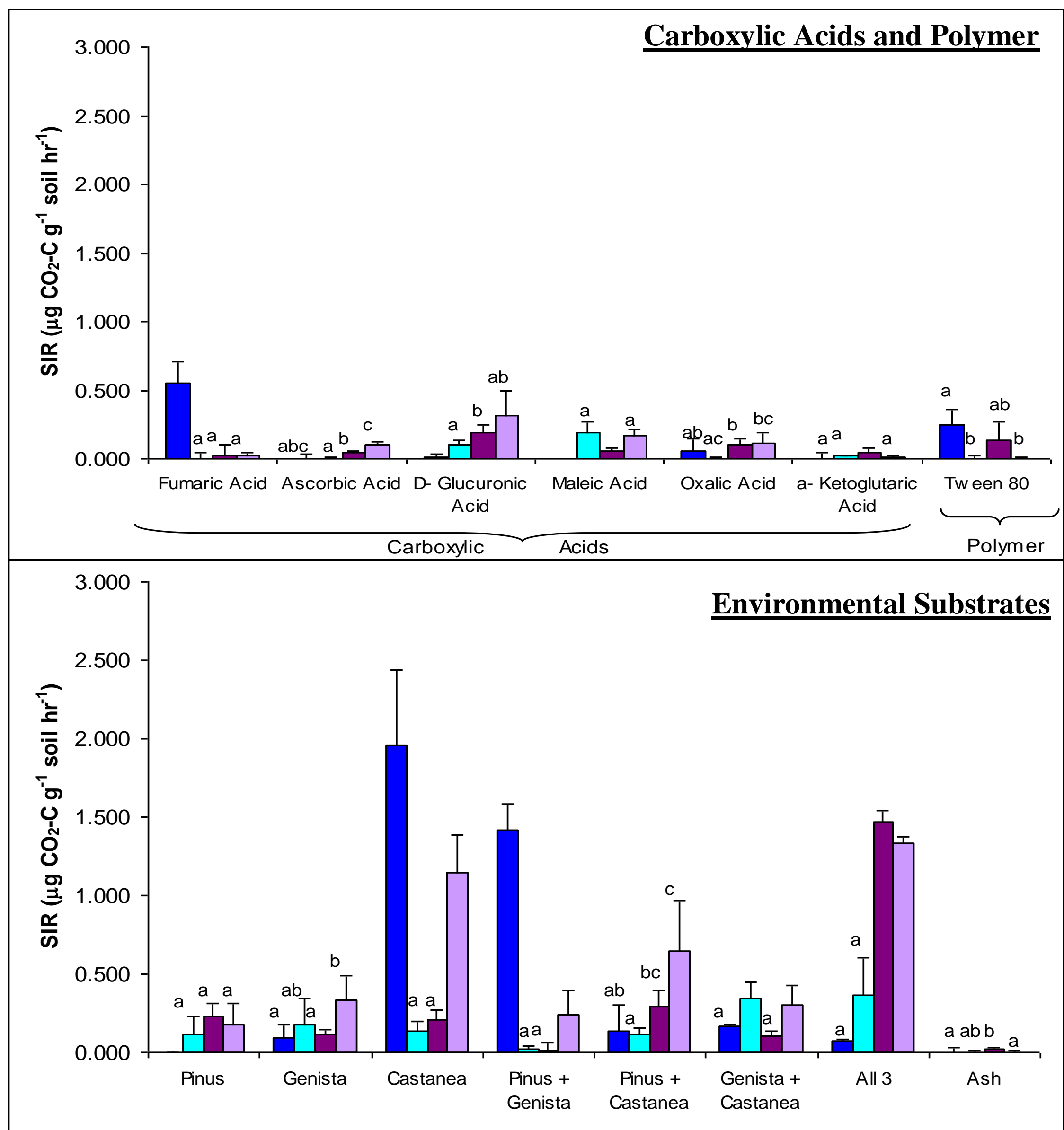

Figure 5-4 (continued from previous page) Mean catabolic responses (±SD) of MRP (dark blue), MRG (light blue), SDCA (dark purple), SDCB (lilac) to a range of substrates after 6 hours of incubation. For site effect bars with the same letter are not significantly different at the ($p<0.05$) level.

After 24 hours incubation, two of these three substrates were no longer capable of distinguishing between the two developmental stages however the SD soils had significantly greater responses to D- Glucosamine, L- Alanine, D- Glucuronic acid, Maleic acid and *Genista* + *Castanea* extract in comparison with the MR soils (Figure 5-5).

The MR soils again exhibited a high degree of variability. Responses to amino acids showed the highest variability and MRP had higher responses to 4 of the 7 amino acids tested indicating a preference for amino acids. After 24 hours MRG had greater responses to the carbohydrates D- Fructose, and Sucrose, while MRP showed a preference for D- Galactose and D- Mannose. MRP also had higher responses to Ascorbic acid, Oxalic acid, *Genista* extract, *Pinus* + *Genista* extract, *Genista* + *Castanea* extract.

SD soils were more variable after 24 hours incubation than 6 hours incubation with SDCA having significantly greater responses to L– Alanine, L– Asparagine, D-Galactose, Oxalic acid, *Pinus* extract, *Pinus* + *Genista* extract, *Genista* and *Castanea* extract and All 3 plant extracts while SDCB exhibited greater respiratory responses to D- mannose, Sucrose and *Castanea* extract.

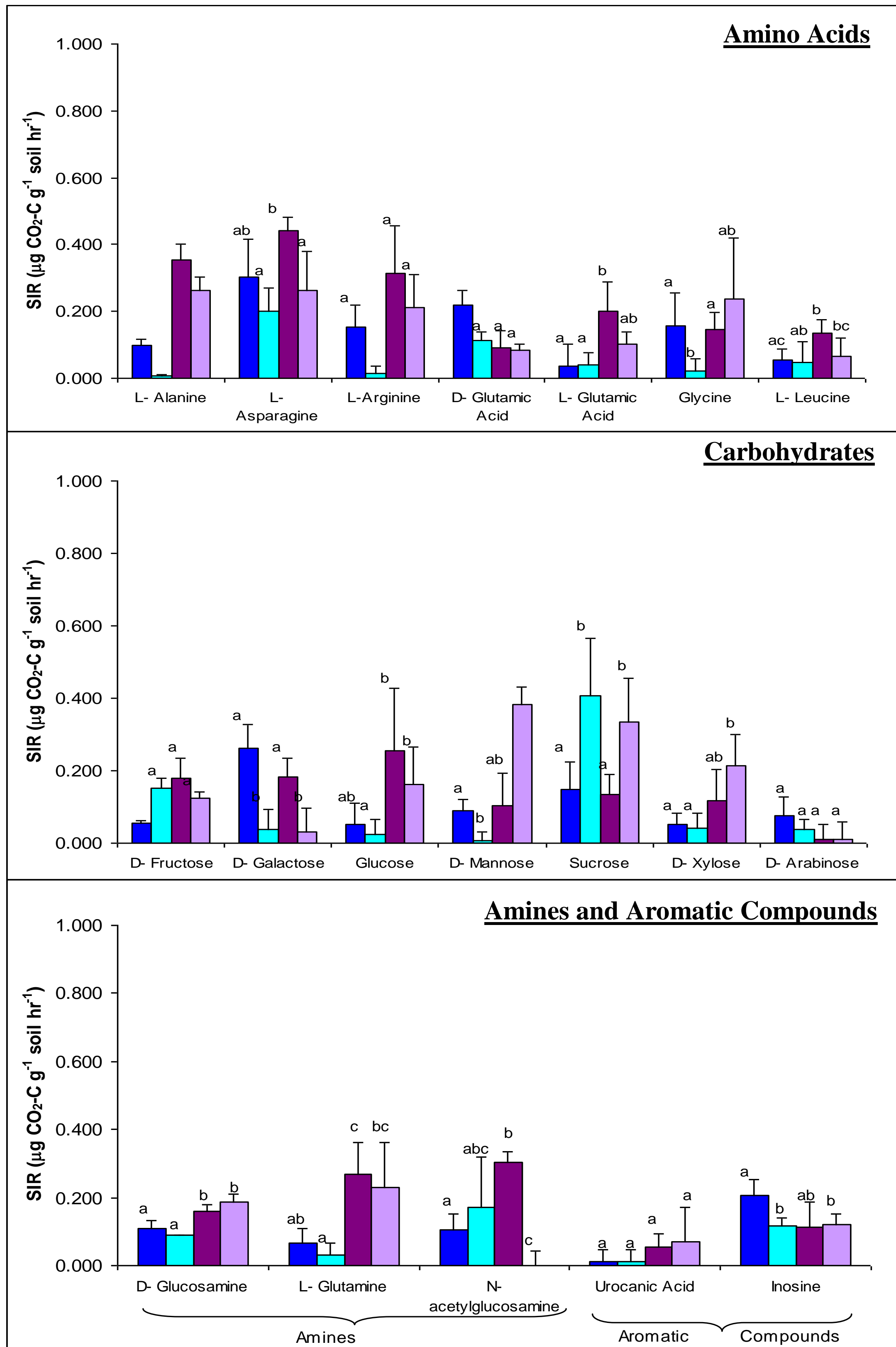

Figure 5-5 Mean catabolic responses (±SD) of MRP (dark blue), MRG (light blue), SDCA (dark purple), SDCB (lilac) to a range of substrates after 24 hours of incubation. For site effect bars with the same letter are not significantly different at the ($p<0.05$) level.

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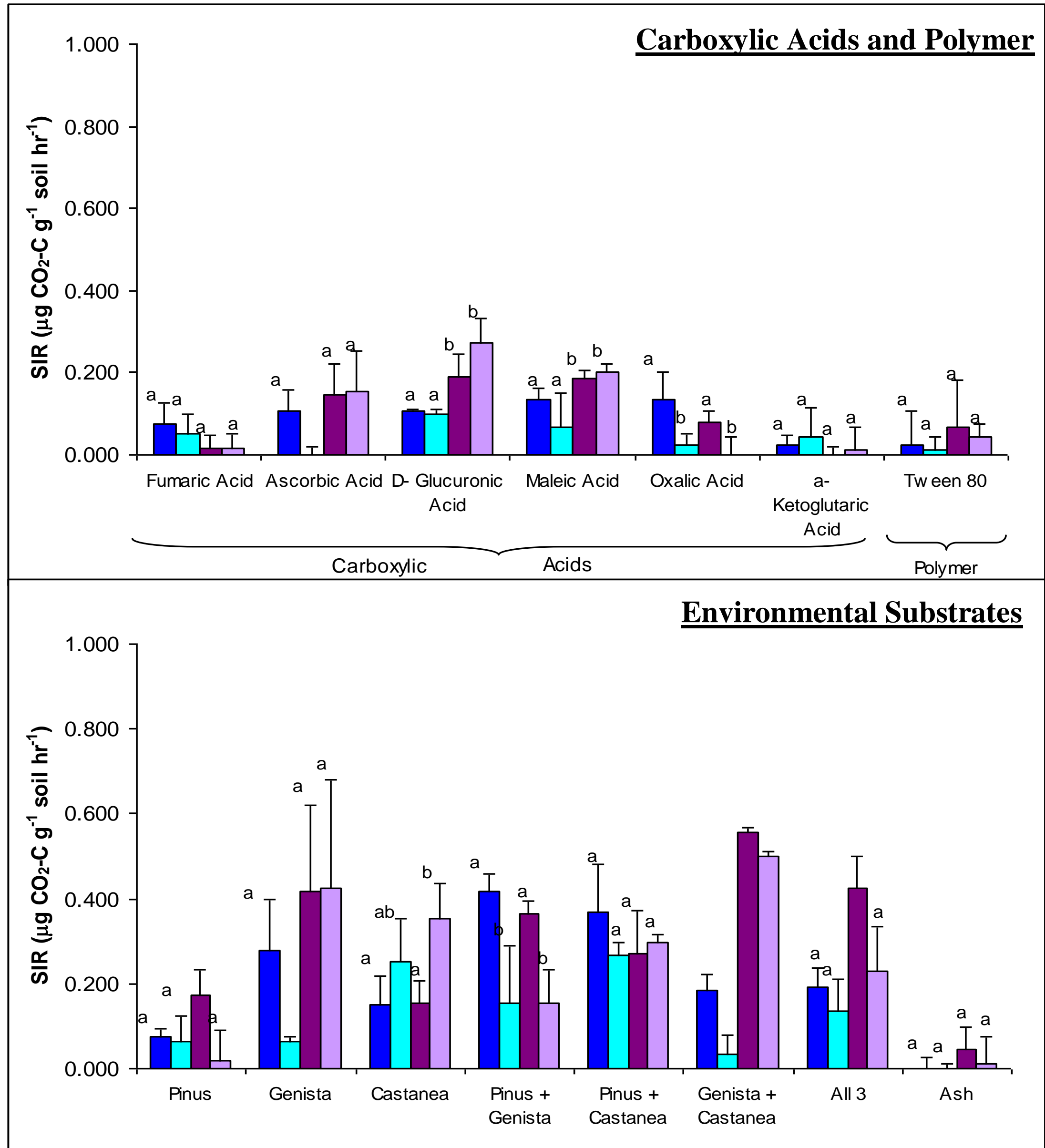


Figure 5-5 (continued from previous page) Mean catabolic responses (±SD) of MRP (dark blue), MRG (light blue), SDCA (dark purple), SDCB (lilac) to a range of substrates after 6 hours of incubation. For site effect bars with the same letter are not significantly different at the ($p<0.05$) level.

The first two components of PCA for catabolic responses over the 6 hour incubation accounted for 38% and 16% of the total variance respectively (Figure 5-6). The PCA was not able to discriminate between different developmental stages as MRG and SDCB were very similar. This was confirmed by canonical variate analysis (Table 5-8). MRP was significantly different from all other soils on the first component ($p<0.001$, $F=162.68$) while SDCA was separated from all other soils along the second component ($p<0.01$, $F=8.43$). Component 1 was dominated by Fumaric acid, D- Galactose, D- Arabinose and L- Alanine (Figure 5-7). *Genista* + *Castanea* extract, *Genista* extract and L- Asparagine dominated component 2.

The PCA for catabolic responses over the 24 hour incubation successfully differentiated between the two developmental stages separating the groups along axis 1 ($p<0.001$, $F=50.62$). Component 1 accounted for 33% of the total variance and was dominated by L- Alanine, L- Arginine, *Genista* + *Castanea* extract and Fumaric acid while component 2 accounted for 21% and was dominated by oxalic acid, *N* acetylglucosamine, D- Galactose and D- Xylose (Figure 5-8, Figure 5-9). After 24 hours incubation, canonical variate analysis confirmed that the distance between the two MR soils was minimal as was the distance between the two SD soils however the two developmental stages were very different (Table 5-8).

Table 5-8 Canonical variate analysis: squared distance between groups of catabolic responses of Etna soils after 6 and 24 hours incubation

Incubation period (hours)	Squared distance between groups	MRP	MRG	SDCA	SDCB
6	**MRP**	0.000	244.364	187.446	261.168
	MRG	244.364	0.000	11.698	0.603
	SDCA	187.446	11.698	0.000	17.608
	SDCB	261.168	0.603	17.608	0.000
24	**MRP**	0.000	3.980	174.001	165.577
	MRG	3.980	0.000	228.293	215.634
	SDCA	174.001	228.293	0.000	4.375
	SDCB	165.577	215.634	4.375	0.000

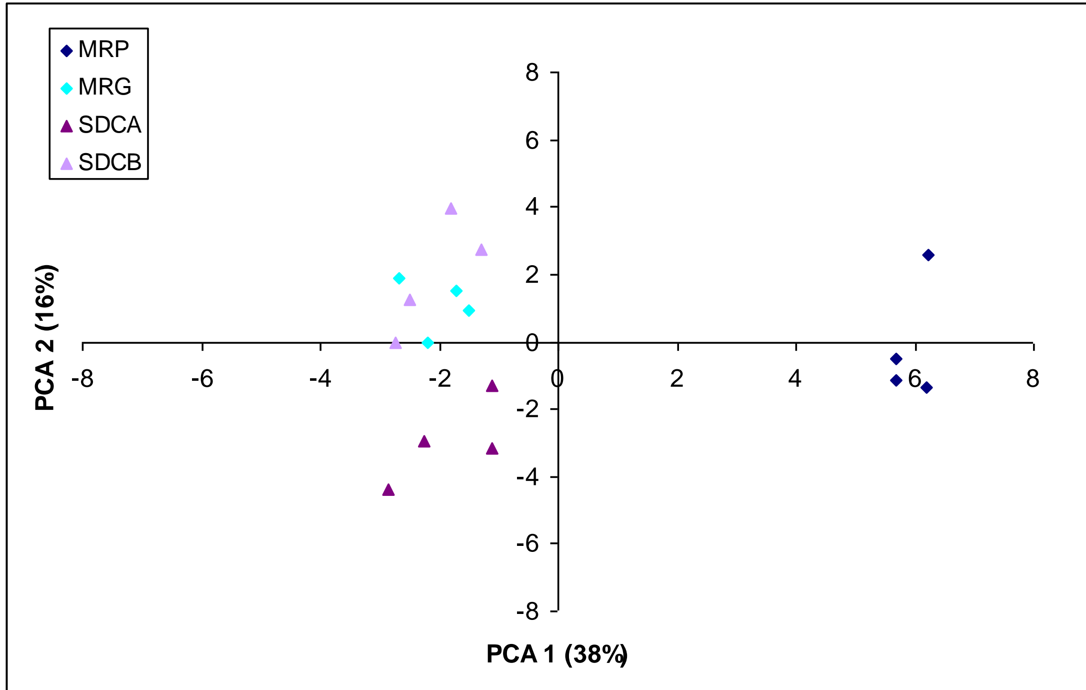


Figure 5-6 Component scores for principal components analysis of catabolic response profiles using all 34 substrates after 6 hours of incubation for Monti Rossi *Pinus* (MRP), Monti Rossi *Genista* (MRG), Salto del Cane A (SDCA) and Salto del Cane B (SDCB)

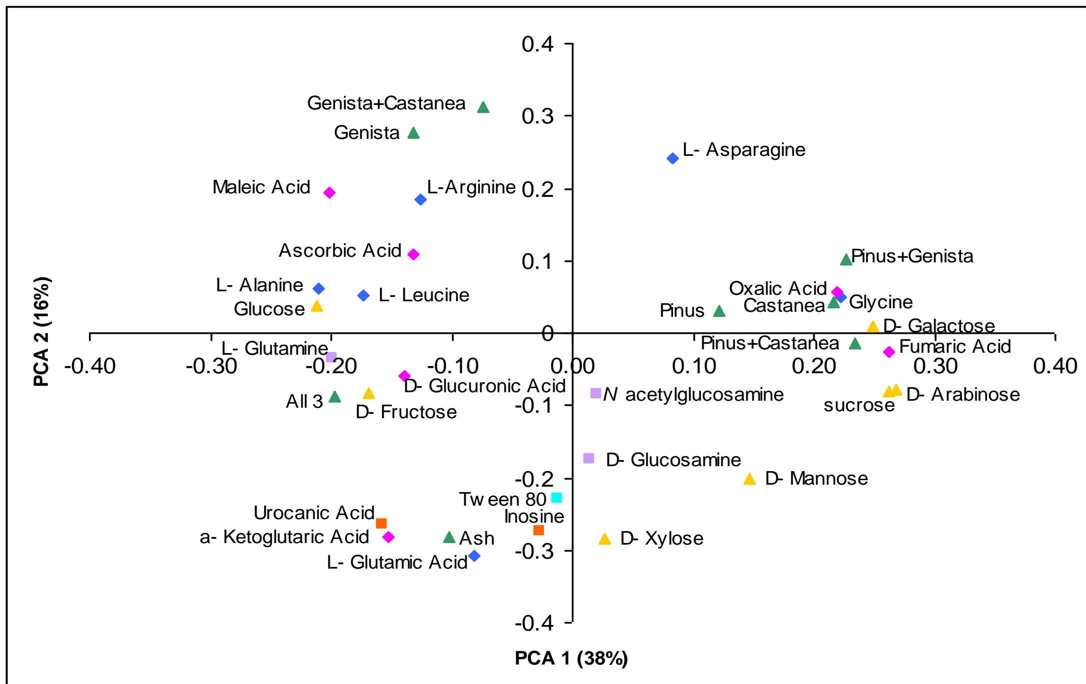


Figure 5-7 Loading plot from principal components analysis of catabolic response profiles including all 34 substrates after 6 hours of incubation. Blue diamonds denote amino acids, gold triangles denote carbohydrates, Purple squares denote amines, red squares denote aromatic compounds, pink diamonds denote carboxylic acids, blue squares denote polymers, green triangles denote environmental substrates

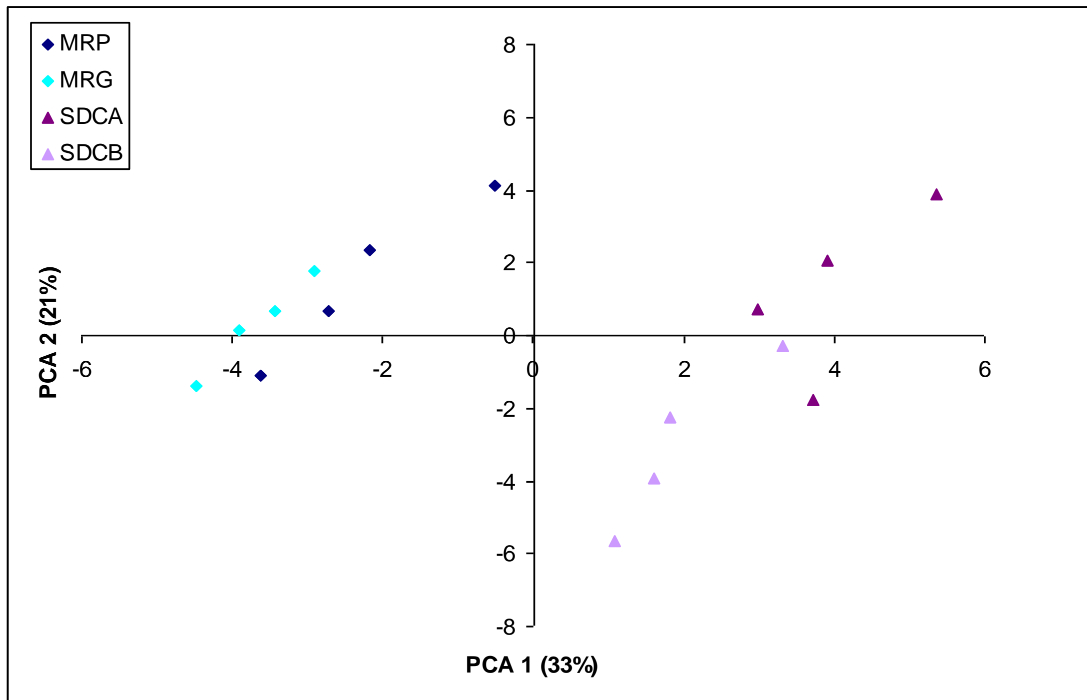


Figure 5-8 Component scores for principle components analysis of catabolic response profiles using all 34 substrates after 24 hours of incubation for Monti Rossi *Pinus* (MRP), Monti Rossi *Genista* (MRG), Salto del Cane A (SDCA) and Salto del Cane B (SDCB)

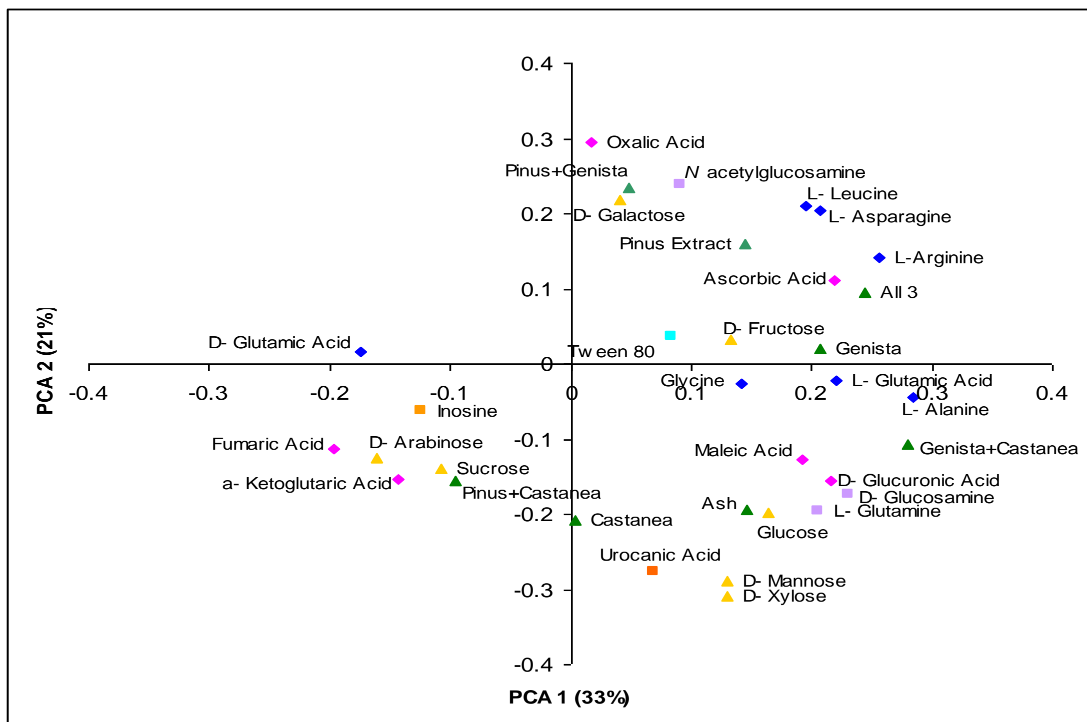


Figure 5-9 Loading plot from principle components analysis of catabolic response profiles including all 34 substrates after 6 hours of incubation. Blue diamonds denote amino acids, gold triangles denote carbohydrates, Purple squares denote amines, red squares denote aromatic compounds, pink diamonds denote carboxylic acids, blue squares denote polymers, green triangles denote environmental substrates

5.4.3.2 Catabolic Diversity

After 6 hours, combined catabolic diversity was significantly greater in the SD soils ($p<0.001$, $F=43.38$). MRP was the least diverse followed by MRG, SDCB and SDCA respectively. SDCA had the greatest catabolic evenness after 6 hours but was only significantly greater than the MRP soil (Table 5-9).

Catabolic diversity indices were less well defined after 24 hours of incubation. MRP and SDCA were similarly diverse but MRG and SDCB were significantly less diverse. Catabolic evenness after 24 hours followed the same pattern of catabolic diversity with MRP and SDCA having the greatest catabolic evenness values (Table 5-9).

Table 5-9 Catabolic diversity (H) and evenness (E) of Monti Rossi *Pinus* (MRP), Monti Rossi *Genista* (MRG), Salto del Cane A (SDCA) and Salto del Cane B (SDCB) after 6 and 24 hours of incubation. Values are means (±SD) n=4

Time (hours)	Site	Catabolic Diversity (H)	Catabolic Evenness (E)
6	MRP	2.425 (0.077)	0.773 (0.024)
	MRG	2.566 (0.058)	0.807 (0.018)
	SDCA	2.882 (0.049)	0.817 (0.014)
	SDCB	2.730 (0.034)	0.803 (0.010)
24	MRP	3.166 (0.072)	0.908 (0.021)
	MRG	2.796 (0.150)	0.807 (0.043
	SDCA	3.167 (0.050)	0.908 (0.014)
	SDCB	2.915 (0.050)	0.814 (0.015)

5.4.3.3 PLFA Profiles and Diversity

PCA of PLFA profiles showed that the first two components accounted for 73% of total variation. All soils other than MRG and SDCB were successfully separated along the first component which accounted for 53% (Figure 5-10). The separation of MRP and SDCA along component one indicated that they were different in their PLFA composition. The loading plot of PLFAs indicated that MRP was associated with increased concentrations of PLFAs 18:1 9 12 and 16:1ω9c while SDCA was associated with increased concentrations of *a*15:0, *cy*17:0, *i*17:0 and 17:0 (Figure 5-11). Component two successfully separated all soils other than MRP and SDCA which were not significantly different. The plot of loading scores showed that component two was dominated by *cy*17:0, *i*17:0, 14:0, 18:0 and 3:OH14:0. PLFA diversity (H) of the four soils ranged from 2.706 in SDCA to 1.618 in MRG (Table 5-10). SDCA was significantly more diverse than the developing MR soils however SDCB was only significantly greater than MRG. PLFA evenness was not significantly affected by soil development (P>0.05 F=4.07). The soil with the greatest PLFA diversity (SDCA) also had the greatest catabolic diversity after both 6 and 24 hours. A positive correlation was found between catabolic diversity and PLFA diversity for both 6 (R^2= 0.528) and 24 (R^2= 0.4006) hours. Removal of MRP from the data set increased the correlation substantially for both time series with R^2 values changing to 0.7982 and 0.9039 for 6 hours and 24 hours incubation respectively.

Table 5-10 Phospholipid fatty acid (PLFA) diversity (H) and evenness (E) of Monti Rossi *Pinus* (MRP), Monti Rossi *Genista* (MRG), Salto del Cane A (SDCA) and Salto del Cane B (SDCB). Values are means (±SD) n=3

Site	PLFA Diversity (H)	PLFA evenness (E)
MRP	1.925 (0.072)	0.886 (0.079)
MRG	1.618 (0.080)	1.000 (0.049)
SDCA	2.706 (0.276)	1.000 (0.115)
SDCB	2.173 (0.185)	0.989 (0.083)

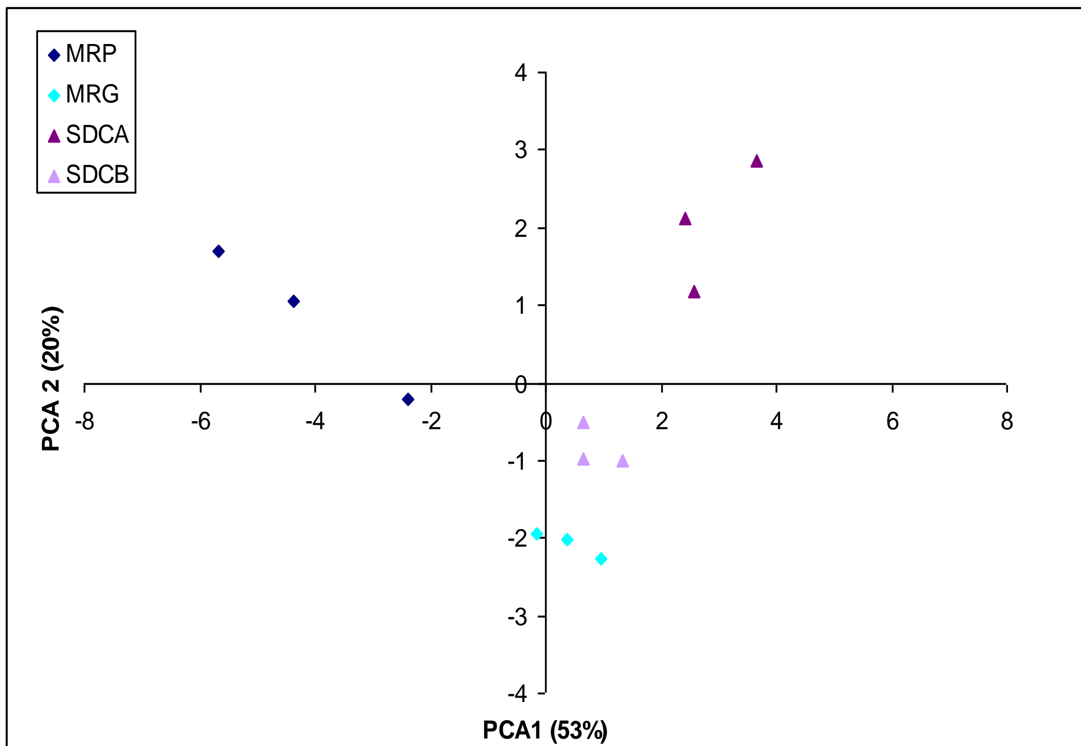


Figure 5-10 Score plot of phospholipid fatty acid profiles for Monti Rossi *Pinus* (MRP), Monti Rossi *Genista* (MRG), Salto del Cane A (SDCA) and Salto del Cane B (SDCB)

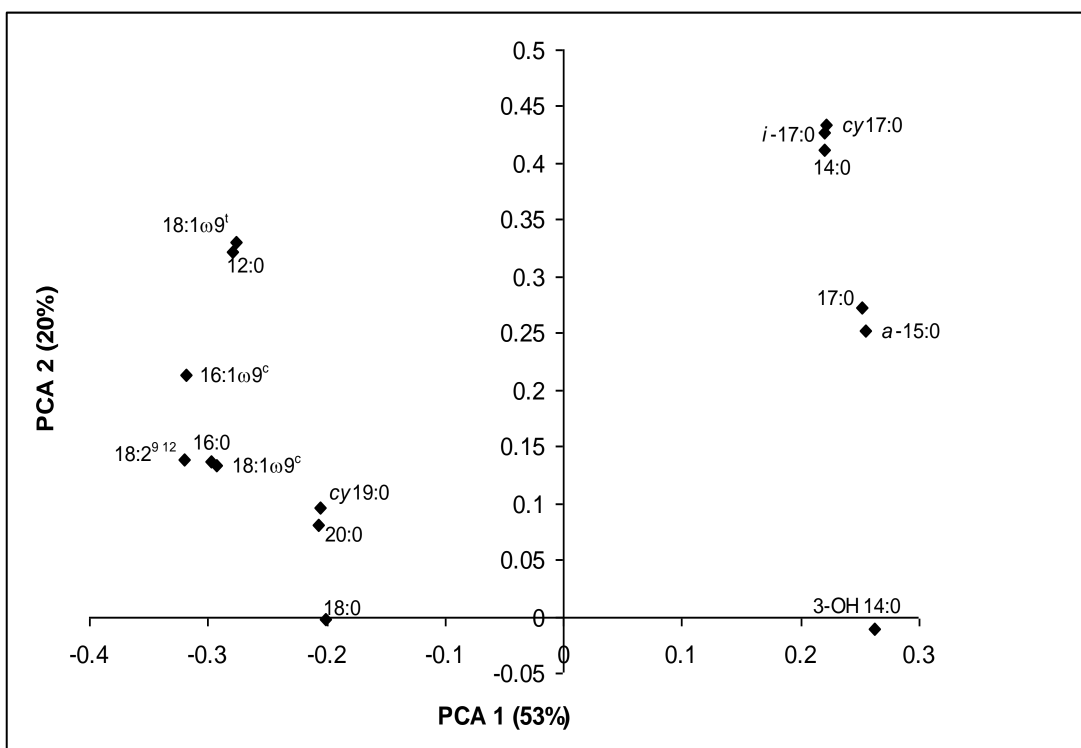
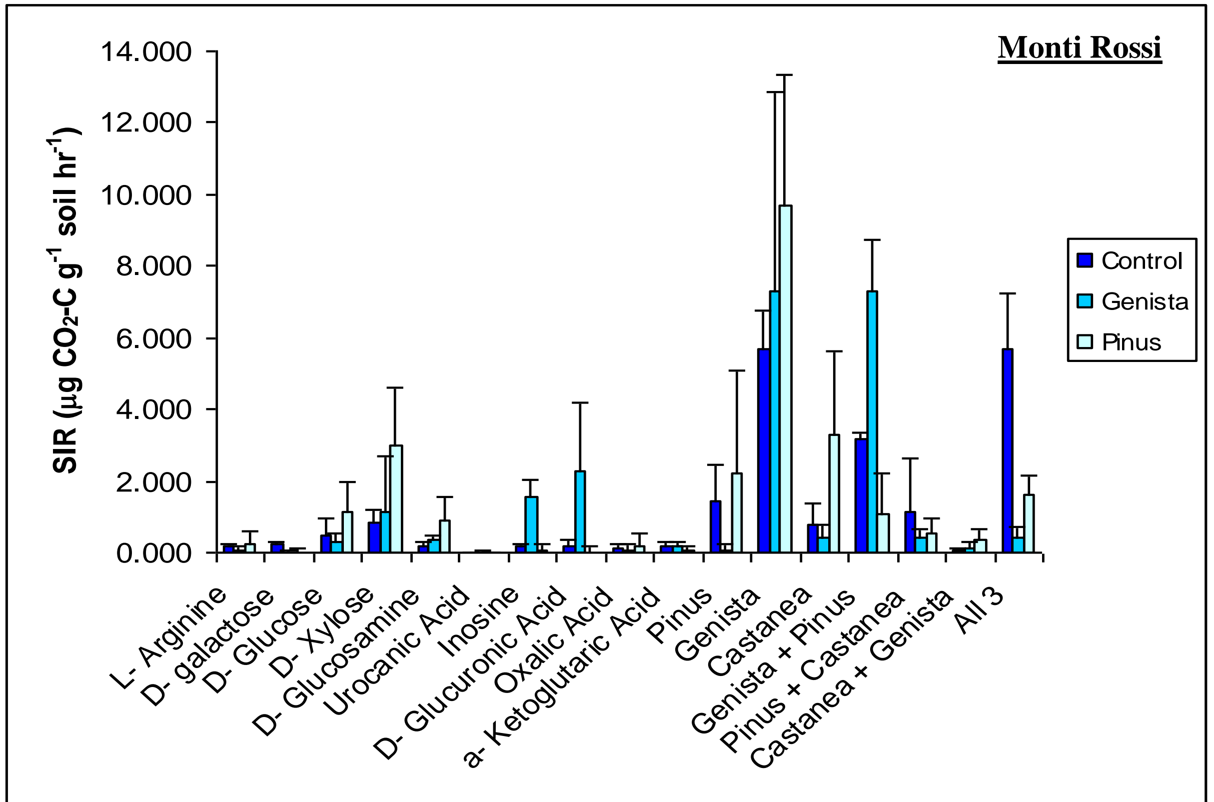


Figure 5-11 Loading plot of phospholipid fatty acids on the first two factors

5.4.4 ***The effects of pre- incubation with different leaf litter types on the catabolic response profiles of volcanic soils from different developmental stages both disturbed and undisturbed***

5.4.4.1 Effect of pre- incubation with different leaf litters on catabolic response

Effects of pre- incubation were litter species dependant and effects on individual catabolic responses to substrates varied with time and soil development. After 6 hours incubation with the 17 substrates, the MR soils had significantly greater responses to almost all substrate additions in comparison with the SD soils, however over 24 hours incubation, the soils responded to a similar level (Figures 5-12 and 5-13). After 6 hours the MR soil amended with *Genista aetnensis* litter showed eight significant differences to the control soil although only three of these were positive differences (Figure 5-12, Table 5-11). After 24 hours incubation, the number of responses significantly different from the control soil was reduced to six but of these; five were positive responses (Figure 5-13, Table 5-12). When amended with *Pinus nigra* litter, the MR soil showed a weak positive response to only one substrate (D- Xylose) while stronger negative responses were shown for 4 other substrates after 6 hours incubation (Figure 5-12, Table 5-11). Negative responses with *Pinus* litter amendment were more pronounced in the MR soil after 24 hours incubation with six substrates showing significant negative responses and only one substrate showing a weak positive relationship (D- Glucuronic Acid) (Figure 5-13, Table 5-12). The *Pinus* amended SD soil showed very similar responses to those found in the *Pinus* amended MR soil after six hours incubation (Table 5-11). Despite similarities to MR *Pinus* after 6 hours, the *Pinus* amended SD soil showed significant positive responses to 9 of the 17 substrates and only 2 significant negative responses after 24 hours incubation (Figure 5-13, Table 5-12).


Monti Rossi
SIR (µg CO2-C g-1 soil hr-1)
14.000
12.000
10.000
8.000
6.000
4.000
2.000
0.000
Control
Genista
Pinus
L- Arginine
D- galactose
D- Glucose
D- Xylose
D- Glucosamine
Urocanic Acid
Inosine
D- Glucuronic Acid
Oxalic Acid
a- Ketoglutaric Acid
Pinus
Genista
Castanea
Genista + Pinus
Pinus + Castanea
Castanea + Genista
All 3

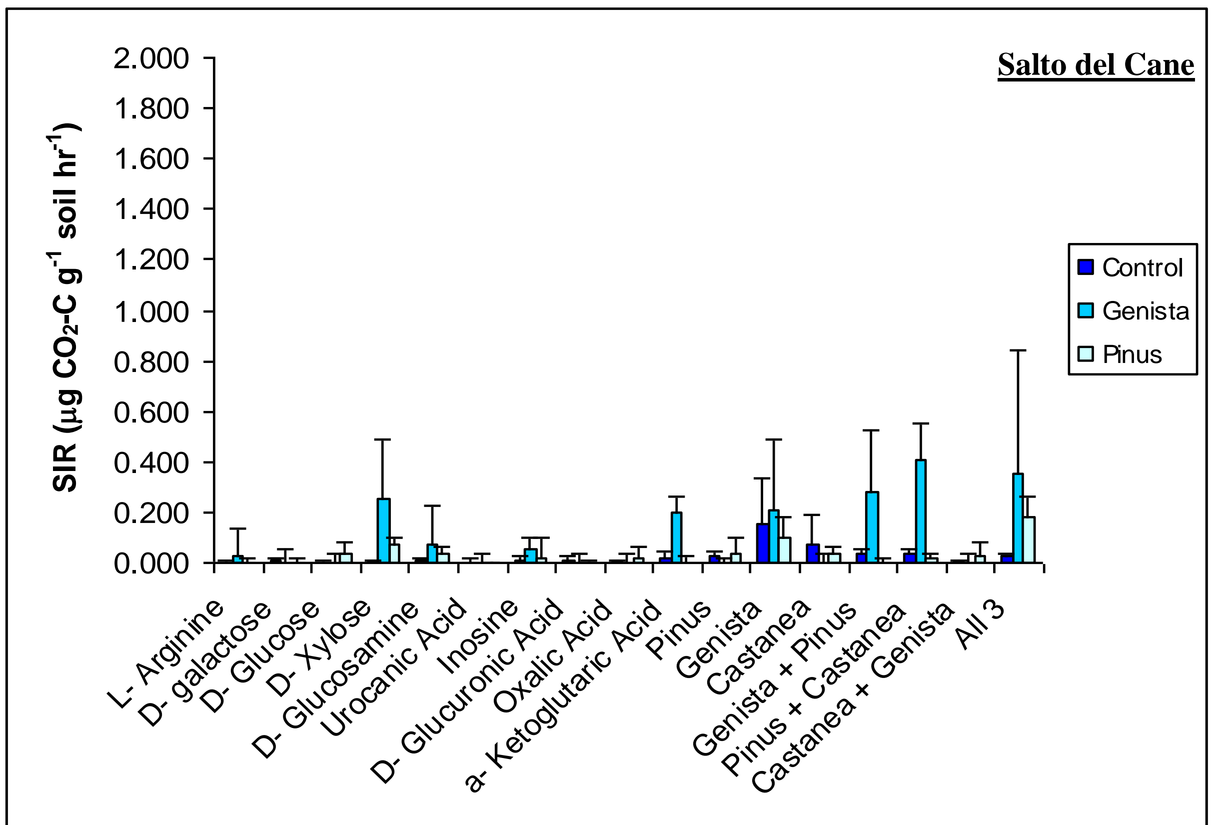
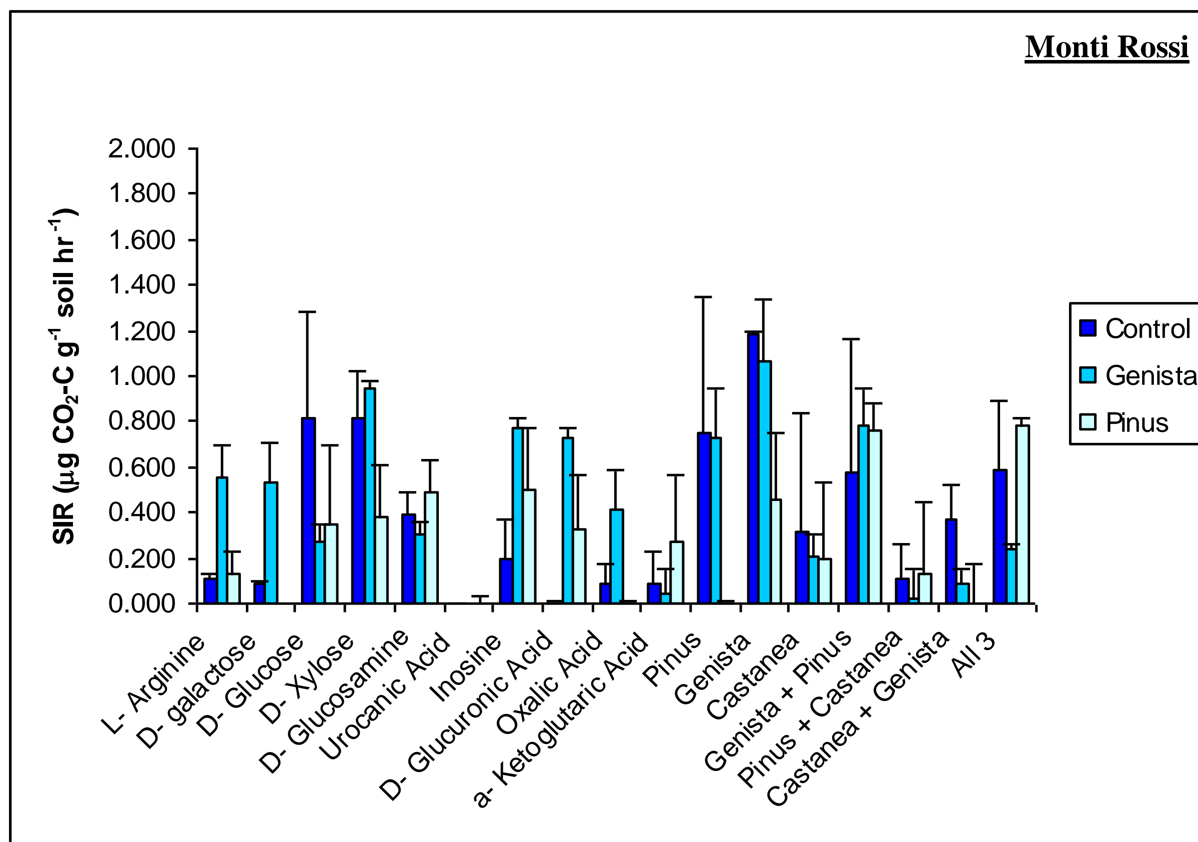

Salto del Cane
SIR (µg CO2-C g-1 soil hr-1)
2.000
1.800
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Control
Genista
Pinus
L- Arginine
D- galactose
D- Glucose
D- Xylose
D- Glucosamine
Urocanic Acid
Inosine
D- Glucuronic Acid
Oxalic Acid
a- Ketoglutaric Acid
Pinus
Genista
Castanea
Genista + Pinus
Pinus + Castanea
Castanea + Genista
All 3

Figure 5-12 Catabolic responses of Monti Rossi soils and Salto del Cane soils to a range of substrates after 6 hours incubation. Soils were either unamended, pre- incubated with *Genista aetnensis* or pre- incubated with *Pinus nigra*). Values are means of 4 replicates (±SD)



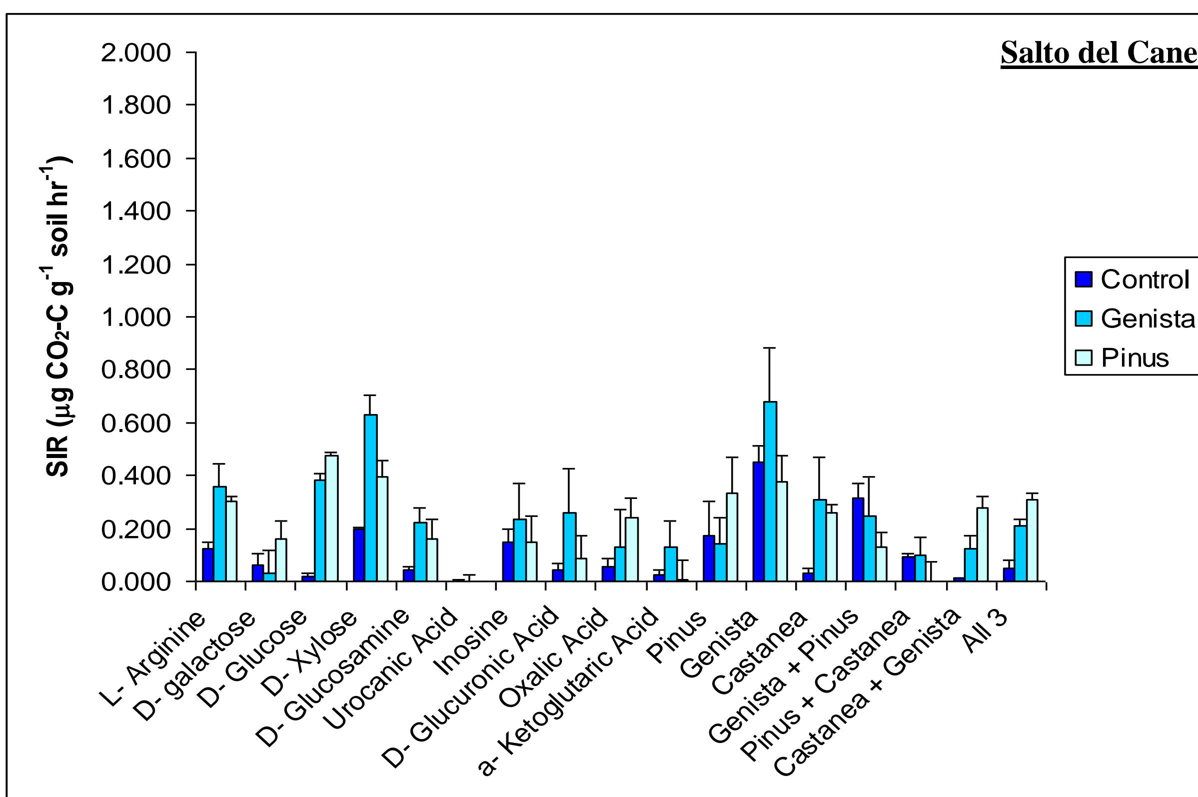


Figure 5-13 Catabolic responses of (A) Monti Rossi soils and (B) Salto del Cane soils to a range of substrates after 24 hours incubation. Soils were either unamended, pre- incubated with *Genista aetnensis* or pre- incubated with *Pinus nigra.* Values are means of 4 replicates (±SD)

Table 5-11 Significance of litter amendment effects and the effect of disturbance on control samples in analysis of variance of individual catabolic responses resulting from addition of different substrates to soils after 6 hours incubation

Substrates	Effect of Litter Amendment Only				Effect of Disturbance Only	
	Genista aetnensis		*Pinus nigra*		Control	
	Monti Rossi	Salto del Cane	Monti Rossi	Salto del Cane	Monti Rossi	Salto del Cane
L- Arginine	* (−)	NS	NS	NS	*** (−)	*** (+)
D- Galactose	*** (−)	* (−)	** (−)	** (−)	*** (−)	* (+)
D- Glucose	NS	* (−)	NS	NS	NS	NS
D- Xylose	NS	NS	* (+)	** (+)	* (−)	NS
D- Glucosamine	* (+)	NS	NS	NS	NS	** (−)
Urocanic Acid	* (−)	NS	*** (−)	*** (−)	NS	NS
Inosine	* (+)	NS	NS	NS	* (−)	NS
D- Glucuronic Acid	NS	NS	NS	** (+)	NS	* (+)
Oxalic Acid	NS	** (−)	NS	NS	NS	NS
α- Ketoglutaric Acid	NS	** (+)	NS	NS	NS	NS
Pinus	* (−)	** (−)	NS	NS	* (−)	NS
Genista	NS	NS	NS	NS	*** (−)	NS
Castanea	NS	NS	NS	NS	NS	NS
Pinus + Genista	** (+)	NS	** (−)	* (−)	*** (−)	NS
Pinus + Castanea	NS	** (+)	NS	NS	NS	NS
Genista + Castanea	NS	** (−)	NS	NS	* (−)	* (+)
All 3	*** (−)	NS	** (−)	** (+)	*** (−)	NS

***: Significant at P<0.05**
****: Significant at P<0.01**
*****: Significant at P<0.001**
NS: Not significant at P<0.05
(−) Negative effect
(+) Positive effect

Table 5-12 Significance of litter amendment effects and the effect of disturbance on control samples in analysis of variance of individual catabolic responses resulting from addition of different substrates to soils after 24 hours incubation

Substrates	Effect of Litter Amendment Only				Effect of Disturbance Only	
	Genista aetnensis		*Pinus nigra*		Control	
	Monti Rossi	Salto del Cane	Monti Rossi	Salto del Cane	Monti Rossi	Salto del Cane
L- Arginine	*** (+)	** (+)	NS	*** (+)	NS	** (+)
D- Galactose	** (+)	NS	*** (−)	* (+)	*** (+)	* (+)
D- Glucose	NS	*** (+)	NS	*** (+)	* (−)	** (+)
D- Xylose	NS	*** (+)	* (−)	*** (+)	** (−)	*** (+)
D- Glucosamine	NS	*** (+)	NS	* (+)	*** (−)	*** (+)
Urocanic Acid	NS	NS	NS	NS	NS	NS
Inosine	*** (+)	NS	NS	NS	NS	NS
D- Glucuronic Acid	*** (+)	* (+)	* (+)	NS	* (+)	NS
Oxalic Acid	* (+)	NS	* (−)	** (+)	NS	NS
α- Ketoglutaric Acid	NS	NS	NS	NS	NS	NS
Pinus	NS	NS	* (−)	NS	NS	*** (+)
Genista	NS	NS	** (−)	NS	*** (−)	NS
Castanea	NS	* (+)	NS	*** (+)	NS	* (+)
Pinus + Genista	NS	NS	NS	** (−)	NS	NS
Pinus + Castanea	NS	NS	NS	* (−)	NS	NS
Genista + Castanea	* (−)	** (+)	* (−)	*** (+)	** (−)	** (+)
All 3	NS	*** (+)	NS	*** (+)	* (−)	* (+)

***: Significant at P<0.05**
****: Significant at P<0.01**
*****: Significant at P<0.001**
NS: Not significant at P<0.05
(−) Negative effect
(+) Positive effect

The SD soil amended with *Genista* litter initially responded positively to only two substrates after 6 hours and negatively to 5 (Figure 5-12, Table 5-11), however after 24 hours incubation, all of the eight responses significantly different from the control sample were positive responses (Figure 5-13, Table 5-12).

Factor analysis of catabolic responses after 6 hours incubation showed that the MR soil amended with *Genista* litter responded quite differently to the control soil (Figure 5-14). The same was also true of the *Pinus* amended MR soil but to a lesser degree. Canonical variate analysis showed that after 6 hours incubation, the responses of the MR control soil were most similar to the SD control closely followed by the SD soil amended with *Pinus* litter and then SD amended with *Genista* litter as opposed to any of the MR soils amended with litter (Table 5-13). This suggests that pre- incubation with litters had a more pronounced effect on the short term metabolic preferences or decomposition abilities of the MR soil.

Table 5-13 Canonical variate analysis: squared distance between groups of catabolic responses of Monti Rossi soils (MR) and Salto del Cane soils (SDC) pre- incubated with *Genista aetnensis* and *Pinus nigra* litter after 6 and 24 hours incubation with substrates

Incubation period (hours)	Squared distance between groups	MR control	MR Genista	MR Pinus	SDC control	SDC Genista	SDC Pinus
6	**MR control**	0.00	72187.9	43161.0	6409.7	7841.2	6837.1
	MR Genista	72187.9	0.00	10669.4	54470.8	46767.9	51649.3
	MR Pinus	43161.0	10669.4	0.00	28560.8	21295.9	25586.4
	SD control	6409.7	54470.8	28560.8	0.00	1507.5	142.6
	SD Genista	7541.2	46767.9	21295.9	1507.5	0.00	889.3
	SD Pinus	6837.1	51649.3	25586.4	142.6	889.3	0.00
24	**MR control**	0.00	12635.7	13125.7	1554.4	3146.4	2246.3
	MR Genista	12635.7	0.0	5929.5	13970.1	3770.6	6875.0
	MR Pinus	13125.7	5929.5	0.0	19545.5	7661.9	8969.9
	SD control	1554.4	13970.1	19545.5	0.0	3730.4	2871.6
	SD Genista	3146.4	3770.6	7661.9	3730.4	0.0	660.2
	SD Pinus	2246.3	6875.0	8969.9	2871.6	660.2	0.0

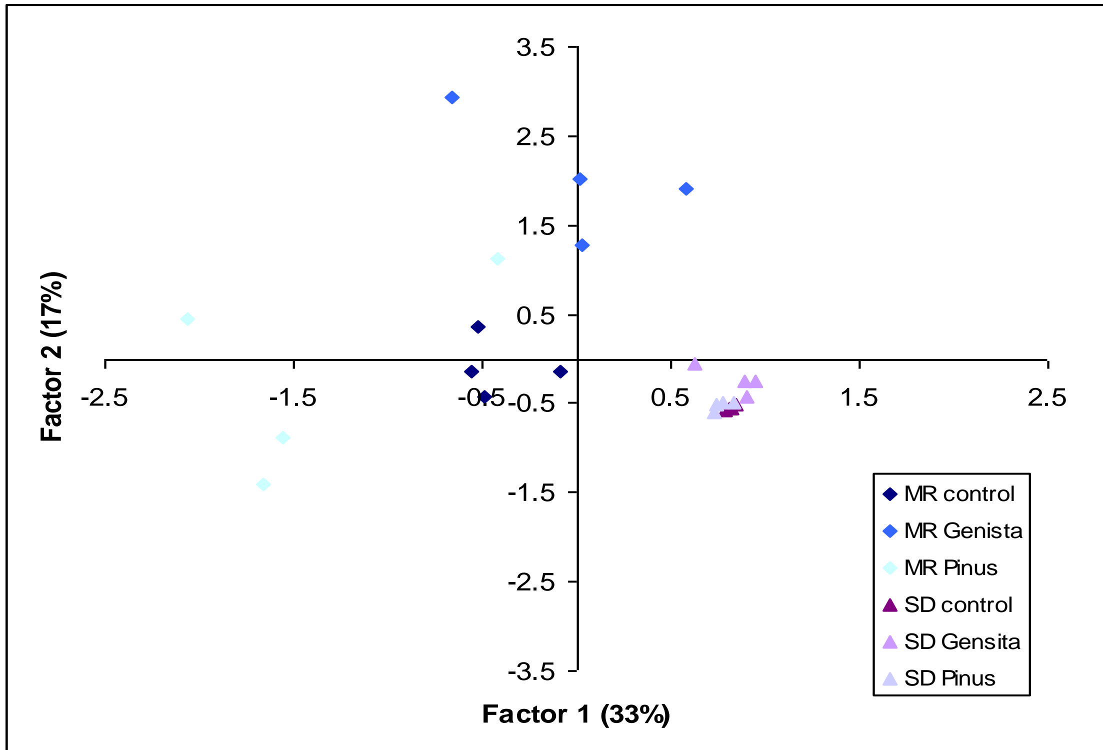


Figure 5-14 Factor scores for catabolic responses of Monti Rossi soils (MR) and Salto del Cane soils (SD) amended with *Genista aetnensis* and *Pinus nigra* litters to 17 different substrates after 6 hours incubation.

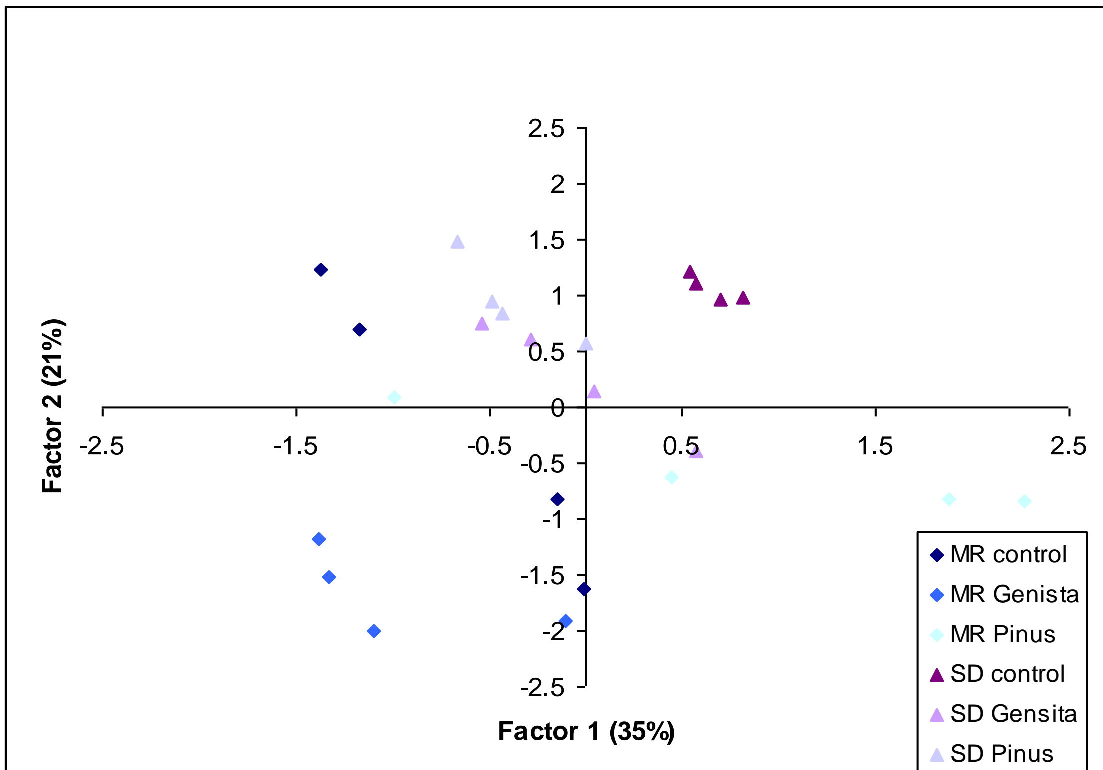


Figure 5-15 Factor scores for catabolic responses of Monti Rossi soils (MR) and Salto del Cane soils (SD) amended with *Genista aetnensis* and *Pinus nigra* litters to 17 different substrates after 24 hours incubation.

The factor scores for the SD soil both litter amended and un-amended were closely clustered together after 6 hours incubation with substrates (Figure 5-14). The SD *Pinus* amended soils had the most similar catabolic responses to the control sample and were also shown to be the most similar by canonical variate analysis (Table 5-13). For both soils, amendment with *Genista* litter caused the greatest divergence in catabolic response from the control over the shorter term incubation indicating that the addition of this litter had altered soil microbial community to a greater degree than the addition of *Pinus* litter.

After 24 hours incubation canonical variate analysis indicated that the MR control soil was once again more closely related to the SD soils (Table 5-13) although due to high variability in the MR control soil it is difficult to extrapolate this from the factor score plot (Figure 5-15). Factor analysis showed that over the 24 hour time period *Genista* litter amendment again was less similar to the control samples indicating a greater affect on the functions of the soil microbial community.

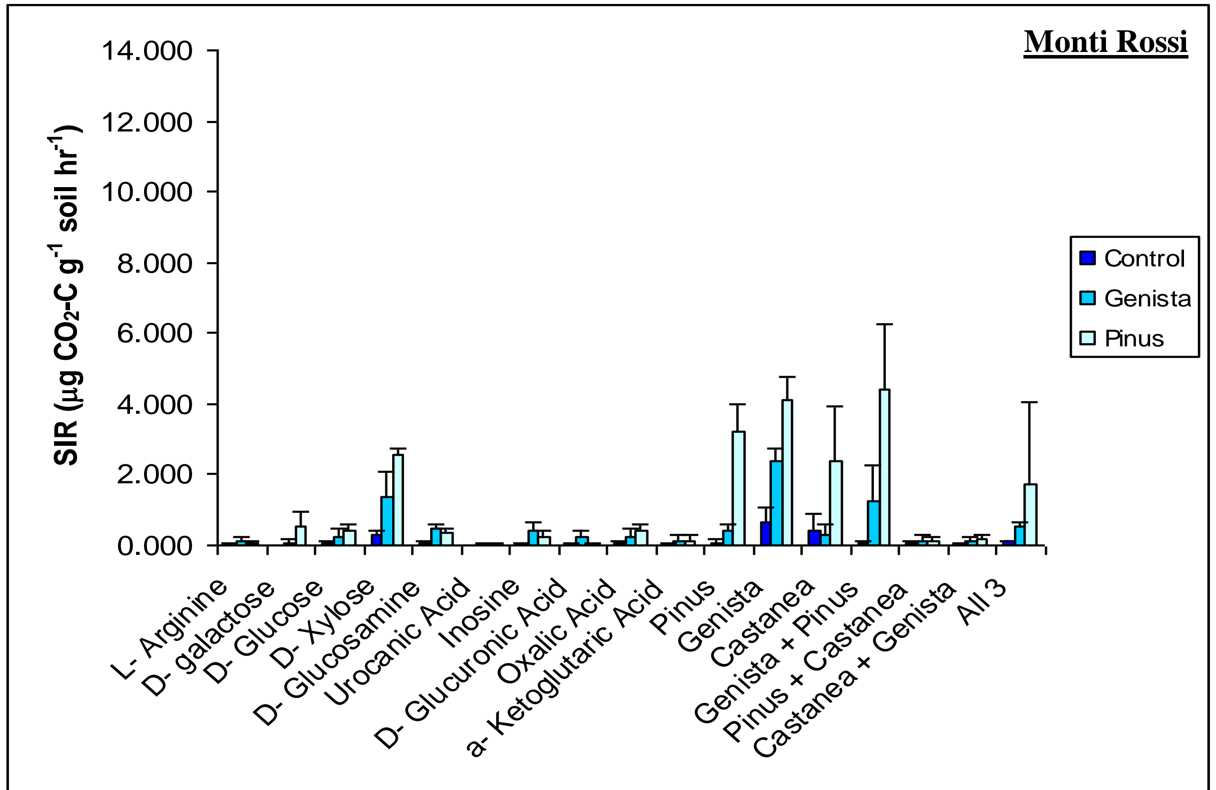
5.4.4.2 Effect of disturbance only on catabolic response profiles

After 6 hours incubation, disturbance effects were more pronounced in the MR soil than in the SD soil. 9 catabolic responses were significantly less in the MR disturbed soil than in the MR control soil indicating a significant reduction in catabolic potential with disturbance (Table 5-11). 5 significant differences in catabolic responses between the control and disturbed SD soils were noted, however, only one of these was a negative effect of disturbance (Table 5-11). This pattern was repeated for the 24 hour incubation period with the SD soil showing enhanced responses to 9 out of the 17 substrates as a result of disturbance while the

catabolic responses to 6 substrates were retarded by the disturbance in the MR soil and only 2 enhanced (Table 5-12).

5.4.4.3 The effects of leaf litter addition on the catabolic responses of volcanic soils at different stages of development after a disturbance

Litter addition to soils subject to disturbance caused variation in the catabolic responses of the Etna soils (Figure 5-16). In the MR soil, both *Genista* and *Pinus* litter addition interacted in a positive manner with disturbed soils in decomposition of an equal number of substrates. Of these substrates, three were the same for both litter additions (D- Galactose, *Pinus* and *Genista* and All 3) and no negative interactions were found. *Genista* addition to the SD soil interacted positively with disturbance in the degradation of 4 substrates over the incubation period (Oxalic acid, *Pinus, Genista* and *Castanea* extracts) indicating an enhanced ability to decompose litter extracts within this soil community. 3 negative interactions were noted however as the community appeared to have a reduced capacity for the degradation of carboxylic acids D- Glucuronic acid and α- Ketoglutaric acid as well as the aromatic compound Urocanic acid. These negative interactions were not found in the SD soil amended with *Pinus* litter after the disturbance however as *Pinus* litter interacted positively with disturbance in the degradation of 4 of the 17 substrates (D- Xylose, D- Glucosamine, *Genista* extract and *Pinus* and *Genista* extract). Interactions between disturbance and litter addition were mostly positive for both soils and both types of litter added (Table 5-14). This suggests that litter addition after the disturbance enhanced the soil communities' ability to rapidly respond to certain substrates relative to the disturbed soils without litter addition.



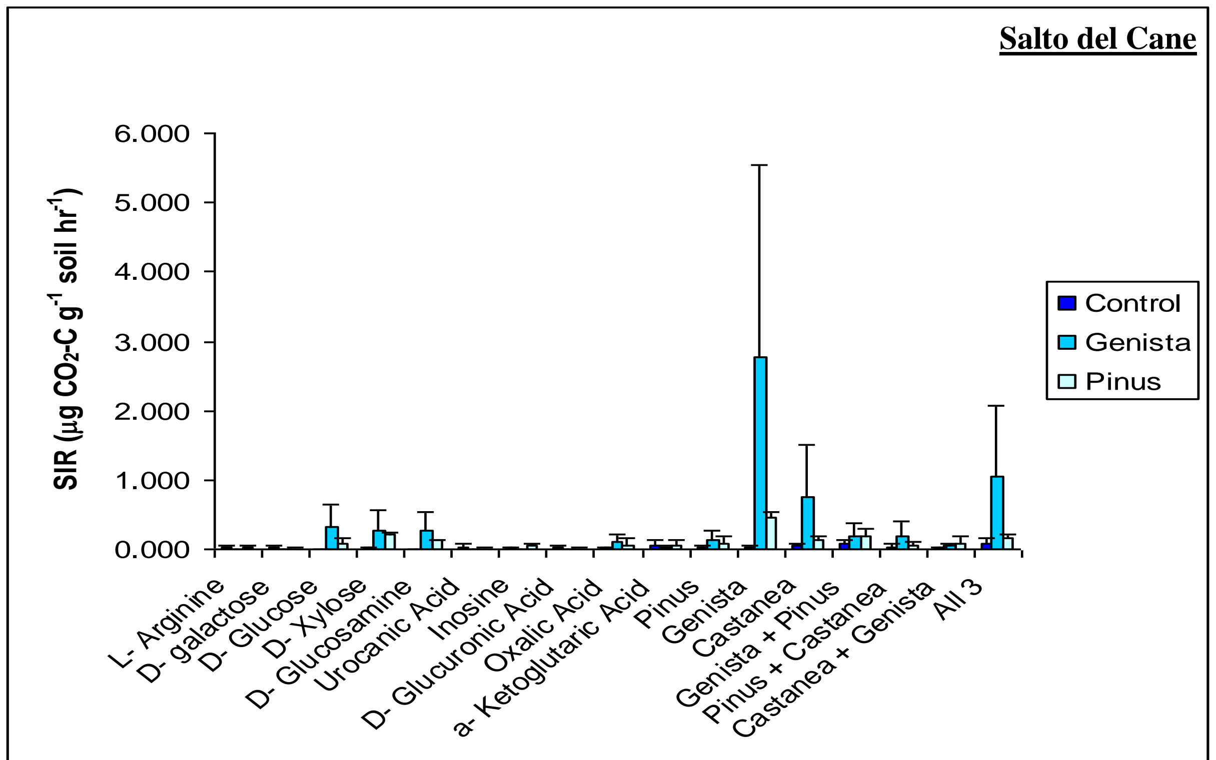


Figure 5-16 Catabolic responses of (A) disturbed Monti Rossi soils and (B) disturbed Salto del Cane soils to a range of substrates after 6 hours incubation. Soils were either unamended, pre-incubated with *Genista aetnensis* or pre- incubated with *Pinus nigra*. Values are means of 4 replicates (±SD)

Table 5-14 2 Way ANOVA for the effect of litter addition, disturbance and interactions between litter addition and disturbance on individual catabolic responses resulting from addition of different substrates to soils after 6 hours incubation for soils which had been disturbed and then amended with either *Genista aetnensis* or *Pinus nigra*

Substrates	Effect of disturbance and litter amendment											
	Genista aetnensis						*Pinus nigra*					
	Monti Rossi			Salto del Cane			Monti Rossi			Salto del Cane		
	L	D	L*D	L	D	L*D	L	D	L*D	L	D	L*D
L- Arginine	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	**	NS
D- Galactose	**	NS	***	***	*	NS	NS	NS	**	***	NS	NS
D- Glucose	NS	NS	NS	NS	NS	NS	NS	*	NS	*	NS	NS
D- Xylose	NS	NS	NS	**	NS	NS	***	NS	NS	***	***	***
D- Glucosamine	***	NS	NS	*	NS	NS	*	NS	NS	***	**	***
Urocanic Acid	**	NS	NS	***	NS	*	***	***	***	***	NS	NS
Inosine	**	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS
D- Glucuronic Acid	*	*	NS	***	NS	*	*	NS	NS	***	***	NS
Oxalic Acid	NS	NS	NS	NS	**	*	***	NS	NS	NS	NS	NS
α- Ketoglutaric Acid	NS	NS	NS	*	NS	**	NS	NS	NS	NS	NS	NS
Pinus	NS	NS	**	NS	***	**	*	NS	NS	NS	NS	NS
Genista	NS	**	NS	***	***	***	**	***	NS	**	NS	***
Castanea	NS	NS	NS	***	***	***	**	NS	NS	NS	NS	NS
Pinus + Genista	***	***	**	*	NS	NS	NS	NS	***	NS	**	*
Pinus + Castanea	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS
Genista + Castanea	NS	NS	NS	NS	*	NS	*	NS	NS	NS	NS	NS
All 3	***	***	***	**	NS	NS	NS	**	**	**	NS	NS

*: Significant at P<0.05
**: Significant at P<0.01
***: Significant at P<0.001
NS: Not significant at P<0.05
L- effect of adding litter, D- effect of disturbance, L*D- interaction

5.5 Discussion

5.5.1 ***Calibration and comparison of the MicroResp rapid microtitre method***

Campbell et al (2003) compared the MicroResp rapid microtitre system with that of the BIOLOG sole carbon source method and concluded that the use of whole soil samples such as the SIR method developed by Degens and Harris (1997) was more advantageous than soil extract in determining catabolic response profiles. For the Etna soils, the MicroResp system was comparable to the SIR method after both 6 and 24 hours incubation. Despite the increased precision of the SIR method, the MicroResp may be superior when a large number of samples are required to be processed such as when generating catabolic response profiles where treatments are applied and the catabolic responses to a large number of substrates are required. This miniaturised method is not only more cost effective but resource and time efficient.

5.5.2 ***Field testing of the MicroResp method and the effects of soil removal and storage on community respiration and simple substrate utilisation***

Goberna et al (2005) found that storage of Mediterranean forest soils affected their catabolic response profiles. This is not surprising as removal of soils from field conditions is a major disturbance in itself. The Etna soils which had been removed from field conditions and stored for one week had lower rates of basal respiration than the fresh field soils while glucose induced respiration was probably not significantly different. Removal and storage of soils undoubtedly has an effect on the soil microbial community and its activity however it is necessary in order to undertake laboratory measurements. The MicroResp method is probably useful in

assessing true and unbiased catabolic response profiles of soils under almost field conditions but further investigations would have to take place into how to transfer colorimetric data from photographs into actual data.

5.5.3 ***Catabolic response profiles and functional diversity of volcanic soils at different stages of development in relation to PLFA diversity***

Catabolic response profiles can be used to differentiate between different soil microbial communities (Campbell et al., 1997; Stevenson et al., 2004). PCA of microbial responses to the range of substrates added after 6 hours incubation was not able to adequately differentiate between the different developmental stages. SIR responses over a 4-6 hour incubation generally give an indication of the initial microbial community before organisms begin to grow on the added substrate (Degens and Harris, 1997). Variation in the substrate responses of the two developing soils over the 6 hour incubation was high, indicating that these soils have different functional capabilities. Myers et al (2001) suggested that as resource availability for microbial communities in soil is controlled by the quantity and composition of organic matter entering the soil, soil microbial communities should differ with dominant vegetation type. This may be true for the Etna soils as variation in substrate utilisation of the two later stage soils was lower than in the earlier stage soils which may be a result of more similar plant residue inputs in the more developed soils. Stevenson et al (2004) found that pasture and forest soils were easily distinguishable in their catabolic responses to 25 different substrates and noted that vegetation type was a significant factor in determining CRPs. Myers et al (2001) also noted a distinction in CRPs of different forest types while Degens and Vojvodić-Vuković (1999) noted differences between pine forests and pasture

responses as well as differential responses for different land uses. This may mean that for short term responses the quantity and composition of naturally available residues to soil is more important than temporal differences between soils which have been developing for at least several hundred years. Schipper et al (2001) investigated microbial heterotrophic diversity along five successional sequences using the method of Degens and Harris (1997) and found that in very young successional sequences (0-120 years) heterotrophic evenness increased with soil development. However, in a more developed glacial fore land sequence ranging from 40 years to 22000 years, evenness slightly declined. Tscherko et al (2003) also noted an increase in functional diversity of two glacier forelands up to the age of 50 years, however more developed soils were reported to have reached a "*temporary steady state*".

As noted by Stevenson et al (2004), very little comparative information is available for individual substrates. It has been noted however that certain substrates may be more ecologically relevant than others in discriminating between different soil communities. Campbell et al (1997) suggested that substrates naturally found in some root exudates may be more representative of substrates naturally available to soil microbes. The substrates utilised in the present experiment represent a mixture of both substrates identified from root exudates and those not known to exist in root exudates (Campbell et al., 1997). The PCA of the 6 hour incubation separated Monti Rossi (*Pinus*) from all other groups along component one which was dominated by 6 main substrates, 5 of which were know to be found in root exudates. After 24 hours there may have been some microbial growth on substrates which may have attributed to overall catabolic responses however PCA of CRPs successfully

separated the two developmental stages along component one which was dominated by 4 known root exudates and one of the environmental substrates. These results concur with the theory that known root exudates may provide a more ecologically relevant discrimination between different soil communities.

It has been suggested that when comparing soil microbial communities, greater response to a substrate may indicate that the community is more adapted to use that resource (Degens, 1998a). In this respect it would be expected that the soil communities would show enhanced responses to water soluble leaf litter extracts of litters of predominant vegetation at that site. This was not the case however as responses varied and no soil showed a preference for the soluble extract of that sites predominant vegetation type. Degens (1998a) found that after prior addition of a specific substrate to soil, responses to that substrate temporarily increased however the response was short term. This may indicate that while soil microbial communities may be influenced by predominant vegetation type in their catabolic response, increased response to the same substrate when re-applied is probably only short term.

Catabolic diversity was significantly greater in the developed soils after 6 hours incubation indicating that initial microbial communities were able to decompose a wider range of substrates. After 24 hours incubation, catabolic diversity in each soil had significantly increased after which there were no significant developmental stage differences. Schipper et al (2001) suggested that components of heterotrophic diversity were lower in developing sites but rapidly increase with organic matter inputs and eventually decline in later stage successions.

PCA of PLFA profiles did not entirely discriminate between soils at different developmental stages. Monti Rossi (*Genista*) and Salto del Cane B were most similar in their PLFA composition reflecting the results of PCA of catabolic responses after 6 hours. Monti Rossi (*Pinus*) and Salto del Cane A were fairly different in their PLFA composition indicating that different functional groups may be present in these soils. In particular, Monti Rossi (*Pinus*) was dominated by monounsaturated fatty acids 18:1ω9^c, 18:1ω9^t, 16:1ω9^c, which are indicative of Gram negative bacteria (Bai et al., 2000) while Salto del Cane A had a higher concentration of branched chain fatty acids which are thought to represent Gram positive bacteria (Schinner et al., 2006). PLFA diversities generally reflected the catabolic diversities of each of the soils although any correlation was significantly higher with the removal of Monti Rossi (*Pinus*). This shows that despite a lower PLFA diversity, the microorganisms within Monti Rossi (*Pinus*) were still able to degrade a similar range of materials to those with higher PLFA diversities indicating that there may be a high degree of functional redundancy within these soils.

5.5.4 ***The effects of pre- incubation with different leaf litter types on the catabolic response profiles of volcanic soils from at different stages of development both disturbed and undisturbed***

The catabolic responses of soils pre-incubated with different litter species showed that substrate utilisation could be influenced by litter addition and that different litter species elicited different effects on resource use in comparison with unamended samples. Both positive and negative changes in substrate use with litter

amendment were observed with more negative effects on the CRPs after 6 hours incubation. Pre- incubation with *Genista* litter altered community substrate utilisation to a greater degree than *Pinus* litter however soils amended with a specific litter did not show a higher affinity to decompose the soluble extract of that litter. This is in agreement with Degens (1998a) who noted that changes in CRPs did not consistently reflect the composition of the type of added organic material. The effect of pre- incubation with each of the litters was also different depending on soil development. Developing soils amended with *Pinus* and *Genista* litter species were less similar to the control than the amended developed soils to their corresponding control. Developing soils pre- incubated with *Pinus* litter showed a higher number of negative responses than developed soils amended with *Pinus* litter however there was no clear pattern to suggest any groups of substrates were affected. This suggests that soil developmental stage may also have had an influence on the effects of pre- incubation with different litter species on the short term decomposition abilities of the soils. Considering the length of the incubation time with the added plant material (3 weeks), effects of litter addition were still pronounced indicating the profound impact on the functional capabilities of soil microbial community. Degens (1998a) found that a single addition of a substrate caused long- term (>20 weeks) effects on the CRPs of an arable soil however it was noted that small changes in CRPs due to substrate addition do not necessarily result in detectable changes in organic matter decomposition in soils.

The effects of disturbance on CRPs were highly soil developmental stage specific. The developing soil was severely retarded in its ability to decompose a large number of substrates while the catabolic response of the developed soil was actually

enhanced. Soils which were disturbed and were then incubated with leaf litter showed enhanced catabolic responses to certain substrates relative to the disturbed soils without added litter. This indicates that litter addition had an influence on the growth and composition of the microbial community subsequent to disturbance. As before, addition of litters even after disturbance did not consistently result in the growth of a microbial community which had an increased capacity to utilise the added substrate.

5.6 Conclusions

The main conclusions of this chapter were:

1. *Calibration of the MicroResp rapid microtitre method.*

- The MicroResp rapid microtitre method although not as precise as other methods of estimating microbial respiration, is superior in its ability to conserve time, resources and money.

2. *Field testing of the MicroResp method and the effects of soil removal and storage on community respiration and simple substrate utilisation.*

- Removal and storage of soils had an effect on the activity of the soil community.
- The MicroResp device is easily transportable and can be used in field conditions to assess more true and unbiased measurements of soil microbial catabolic diversity.
- The device shows promise for field use however further work is required in order to interpret photographical reproductions of microplate colour development when a microplate reader is not available.

3. *Catabolic response profiles and functional diversity of volcanic soils at different stages of development in relation to PLFA diversity.*

- Catabolic response profiles were not able to adequately distinguish between soils at different stages of development.

- Over the shorter incubation the two developing soils had different functional capabilities probably relating to the composition of the predominant source of organic matter regularly incorporated into the soils.
- Soil communities did not show enhanced catabolic abilities for the soluble extracts of the predominant vegetation inputs to the soil.
- Root exudates were more discriminatory and may provide more ecologically relevant information.
- Catabolic diversity was significantly greater in the later developmental stage soils after 6 hours incubation but not after 24 hours incubation.
- Increased PLFA diversity generally correlated with an increase in catabolic diversity although any correlation was significantly higher with the removal of Monti Rossi (*Pinus*). This shows that despite a lower PLFA diversity, the microorganisms within Monti Rossi (*Pinus*) were still able to degrade a similar range of materials to those with higher PLFA diversities which may indicate a high degree of functional redundancy.

4. *The effects of pre- incubation with different leaf litter types on the catabolic response profiles of volcanic soils at different stages of development both disturbed and undisturbed.*

- Pre- incubation with leaf litter altered the catabolic responses of soils relative to the control soils.
- The developed soils amended with leaf litter were more similar to the corresponding control soil than the developing soils perhaps indicating a greater influence of leaf litter addition on the earlier stage soils.

- Different leaf litter species elicited different effects on the substrate utilisation patterns of the microbial communities of each soil however there was no preferential metabolism of the soluble component of the leaf litter when re- applied.
- Soils which were disturbed and then pre- incubated with leaf litters had enhanced abilities to metabolise certain substrates although there was no particular pattern to this and again there was no preference for re-application of the same leaf litter extracts.

Chapter 6 Concluding Discussion

6.1 <u>Introduction</u>

The aims of this discussion chapter are to draw together the results and conclusions obtained from the investigations outlined in chapters 3, 4 and 5 and to place them in the context of the research objectives outlined in chapter 1. A summary of the main results are outlined in table 6-1.

Table 6-1 Summary of the main attibutes of the two different developmental stages from the results outlined in chapters 3, 4 and 5. Sites are in comparison with one another and do not include comparison with other studies.

Attributes	Developmental Stage	
	Early (Monti Rossi)	***Later (Salto del Cane)***
Chapter 3		
Soil pH	Near neutral	Near neutral
Particle size	Coarse	Fine
Soil total C	No difference	
Soil total N	No difference	
Soil respiration	No difference	
Soil microbial biomass	No difference	
Metabolic quotient	Higher	Lower
Biomass C: Total C ratio	No difference	
Rate of litter decomposition	No difference	
Chapter 4		
PLFA diversity	Lower	Higher
PLFA evenness	No difference	
Total PLFA	Lower	Higher
Community structure	Simple	Complex
Fungal: bacterial PLFA ratio	Low	High
Total β- glucosidase activity	Higher	Lower
Total Acid Phosphatase activity	No difference	
Total Arylsulfatase activity	Lower	Higher
Specific β- glucosidase activity	Higher	Lower
Specific Acid Phosphatase activity	Higher	Lower
Specific Arylsulfatase activity	Lower	Higher
Effect of disturbance on PLFAs	Low	High
Effect of disturbance on total enzyme activity	Low	High
Effect of disturbance on specific enzyme activity	Low	Low
Chapter 5		
Variability of catabolic responses	High	Low
Catabolic diversity	Lower	Higher
Catabolic evenness	No difference	
Influence of leaf litter addition on microbial community metabolism	Higher	Lower
Effect of disturbance on catabolic responses	High	Low

In order to further the understanding of structural diversity and decomposition functions of volcanic soils at different stages of development, three main research objectives were outlined at the beginning of this thesis.

6.2 <u>Research Objective 1</u> *To investigate the long term* **in situ** *catabolic abilities of microbial communities in volcanic soils at different stages of development in the decomposition of litters of differing qualities and to investigate the effects of litter mixing on the rate of decomposition.*

No correlation was found between soil C and N contents, microbial, biomass or soil respiration and soil developmental stage however there was a slight reduction in metabolic quotient and particle size with development. This was not in agreement with Hopkins et al (2007) who investigated the same study sites as C and N concentrations and microbial biomass at the developed sites were significantly reduced. This highlights the difficulties of investigating soils on an active volcano as fine particulate volcanic ejecta is often released and incorporated into soil diluting soil nutrient stocks. Prevailing winds obviously do not evenly distribute ejecta uniformly over the volcanoes slopes resulting in sites which have little or no new input and other sites which receive substantial inputs. At least two major volcanic eruptions occurred between the sampling of Hopkins et al (2007) and the first sampling date of this investigation which have caused measurable soil chemical and physical changes and probably also substantial biological changes.

It was hypothesised that the developed soil community would be able to decompose plant litters *in situ* to a greater degree than developing soil communities. This hypothesis was based on the observation that C use efficiency increases with developmental stage and is related to microbial community structure (Ohtonen et al., 1999). The results of PLFA analysis in chapter 4 confirmed that the developing soil had a more diverse PLFA content (a proxy for species diversity) and a more complex community structure. Catabolic diversity was also shown to be greater in the developed soil (chapter 5). This increased structural complexity and

decomposition function did not appear to affect the decomposition of either *Genista aetnensis* litter or *Pinus nigra* litter in litter bags as mass loss, litter microbial biomass and litter respiration for litters buried in soils of different developmental stage were not significantly different. The litter bag method may have been too insensitive to detect differences in decomposition rates or it is also possible that the soil microbial communities resident in these soils were equally well equip at decomposing both of the litter species.

Soils which receive regular inputs of a particular organic residue have been envisaged to become conditioned to the decomposition of that material and may have increased metabolic preferences or enzymatic abilities to decompose it (Degens, 1998a). This would suggest that for example MRG would be more equiped at decomposing *Genista aetnensis* as it is the predominant vegetation input to that soil. This was not apparent from the results of the litter bag experiment or the catabolic responses of the soil communities outlined in chapter 5 which showed that soils communities did not have enhanced abilities to decompose soluble extracts of the predominant vegetation type.

No effects of litter mixing were noted, indicating that there were no interactions between *Genista aetnensis* and *Pinus nigra* litter during decomposition. This may in part be due to the lack of fungal activity in these soils as noted in chapter 4. Fungal activity has been proposed as a method for nutrient translocation between different litters (McTiernan et al., 1997). Although no interactions were noted between litter species in this investigation it is clear that interactions do exist however the mechanism of these interactions is still poorly understood.

6.3 **<u>Research Objective 2</u>** ***To investigate the PLFA diversity, community composition and community function (assessed by soil enzyme activity) of volcanic soil communities of different developmental stages and to assess the effect of an environmental disturbance on these key soil community features.***

As predicted, the more developed soil contained the greatest PLFA diversity, evenness and a larger quantity of PLFAs. This is in concurrence with traditional successional theory (Odum, 1969). As noted previously, this increased diversity did not appear to have any affect on the *in situ* decomposition of leaf litters common to the area (chapter 3) which indicates a degree of functional redundancy within the soil communities of the volcanic soils of Mount Etna. The results of chapter 4 also suggest a degree of functional redundancy as the catabolic response profiles of the two different soil stages were not entirely distinguishable from one another and the soil microbiota in MRP were able to decompose a similar range of substrates to the more developed soils despite being significantly less diverse in PLFA composition. Loreau (2004) suggested that functional redundancy is not compatible with stable co- existence according to the Lotka- Volterra competition model. However, it is unlikely that given the huge range of microbial species in soils (<1000 species per 1 gram of soil (Torsvik et al., 1994)) that some degree of functional redundancy does not exist. Findlay (2002) suggested that there is a high potential within the diverse microbial community to have many species which perform the same functional role within an ecosystem so that an alteration in species diversity may not affect ecosystem functioning. This suggests that in the event of an environmental disturbance, a reduction in species diversity would not severely alter the functional capacity of a community. Disturbance caused a decline in PLFA diversity of the developed soil community however specific enzyme activities were unaffected indicating that the reduction in total enzyme potential of the more developed soil

was not as a result of lower PLFA diversity but simply a result of a reduction in biomass. This shows that despite a reduction in PLFA diversity, function was maintained. This was less well defined for the developing soil which behaved in an idiosyncratic manner with regards to enzyme activity. Enzyme activities provide information about potential functional capacity; catabolic response profiles provide a more accurate indication of actual decomposition function. The results of chapter 5 concur with the conclusions of chapter 4 as after disturbance the developed soil was largely unaffected in its decomposition function.

6.4 **Research Objective 3** ***To investigate the functional diversity of volcanic soil microorganisms from different stages of development using a range of simple and complex substrates and assess whether function is a product of taxonomic diversity or reflects the characteristics of the predominant vegetation type input into the soil.***

The developed soils showed higher catabolic diversities compared with the developing soils. Catabolic diversity generally correlated well with PLFA diversity however as previously noted, the lower PLFA diversity soil MRP, was still able to degrade a similar range of substrates to the higher PLFA diversity soils. This suggests that perhaps the diversity- ecosystem function model is only applicable to very early stage soil sequences due to the high rate of functional redundancy when soils are more developed and species of soil organisms are numerous. This suggests that after an initial developmental period, the soil communities' decomposition function is governed not by developmental stage but by some other external factor such as the quality and quantity of litter inputs to the soil.

It was shown that pre- incubation with leaf litter could alter the catabolic responses of soil communities and that different species of leaf litter elicited different effects on resource utilisation. This suggests that there may be some link between the composition of the added organic material and the resultant catabolic responses. While pre- incubation with leaf litter altered catabolic responses, the organisms which proliferated within the altered soil did not appear to have a preference for the added material or have higher catabolic abilities to decompose it. This may be due to the time period over which the litters were added into the soil as almost all of the easily degradable material (similar to the water soluble extract which was re-applied) will have been metabolised in the first few days after incorporation and the microorganisms primarily responsible for its mineralization will have ceased to exist early on in the decomposition sequence. The PLFA composition of control soils was shown to change over a six month incubation period (chapter 4) and reflects changes in community structure. In soils incubated with leaf litter, structural changes may have occurred in the microbial community living on existing (background) organic matter and successional changes may also have occurred in the microbial community metabolising the incorporated leaf litter. Catabolic responses therefore probably do not reflect the chemical nature of the added residue but are influenced by it and the metabolites produced during its decomposition.

The factors affecting community function are probably very complex given the variety of microorganisms which can exist within soils. Predominant vegetation type appears to be an important factor in determining catabolic diversity however there appears to be no pattern of related substrate utilisation. PLFA diversity broadly correlates with functional diversity but functional redundancy is probably

an important ecological commodity in microbial communities. More developed soils however appeared to be less susceptible to the influence of leaf litter addition, maintaining similar catabolic responses to control soils. This indicates a more stable microbial community.

6.5 Final Remarks

The overall conclusion of this research is that the volcanic soils of Mt Etna in Sicily contain a large diversity of microorganisms, most of which are functionally redundant because:

- Despite greater PLFA diversity and functional diversity in the developed soil, residue decomposition *in situ* was unaffected.
- Reduced PLFA diversity and community complexity did not result in a reduction in soil function (specific enzyme activities).
- Soils at different developmental stages had similar catabolic responses and were able to degrade simple and more complex substrates to a similar degree.

While plant and animal communities have a limited biodiversity, the diversity of soil communities is enormous. For this reason it is unlikely that the diversity-ecosystem function model is entirely applicable to soil communities as functional redundancy probably plays a major role in their ecology. There may be some scope for the theory in very early soil successions however. Improvement in techniques used to assess the structural and functional diversity of soil microbial communities is required in order to provide better empirical evidence to test the hypothesis.

Trends of increasing biomass, diversity, nutrient accumulation and functional responses have been noted in glacier foreland sequences (Insam and Haselwandter, 1989; Ohtonen et al., 1999; Sigler et al., 2002; Tscherko et al., 2003). This was not the case with the volcanic soils of Mount Etna and may have been due to the period over which the soils were studied. The Etna soils ranged from approximately 378 years old to approximately 7500 years old while the glacier foreland successions were generally more recent. It may also have been a result of the dynamic environment on volcanic slopes where soils are often disturbed. This dynamicity is generally not experienced on glacier sequences allowing the assessment of community development to be assessed without confoundation from external factors.

PLFA profiling has been regularly used to assess microbial succession in glacier foreland sequences and soils at different stages of development have been shown to have different community profiles (Ohtonen et al., 1999; Tscherko et al., 2004). This was also true of the Etna volcanic soils. Total PLFA concentrations in the volcanic soils were similar to those of young glacier foreland soils between 0 and 100 years old. In general it would be expected that soils developing in very cold climates such as on glacier forelands would contain a lower biomass than those developing in relatively warm Mediterranean climates. Moisture was not a limiting factor in the Etna soils and as such it is probable that community development in the volcanic soils was disrupted by an external factor such as a further volcanic eruption.

Mediterranean volcanic soils have not previously been analysed using PLFA profiling. Low PLFA recovery was achieved from the Etna soil samples in this study. This may have implications for other studies of soil community composition in volcanic soils as several soil extractions may have to be combined in order to achieve a full spectrum of PLFAs. Volcanic soils are renowned for their ability to bind soil C to organic clays and colloids. It is possible that poor PLFA retention resulted from adsorption of PLFAs to organic colloids. P retention is another key property of volcanic soils which often results in a P limitation. It is possible that PLFAs had a very high turnover rate in these soils attributing to the low recovery rate in soils.

Volcanic soils are subject to frequent natural disturbances which in turn affect the resident soil microbial community. This study has shown that different soil communities respond in different manners to disturbance and that the effect of disturbance can be monitored by assessing community catabolic response profiles. It was also shown that when disturbed soils were incubated with leaf litters they had greater catabolic responses than disturbed soils with no leaf litter addition. This suggests that in order to minimise the effect of disturbance on volcanic soils, slopes should be afforested.

Volcanic soils such as those of Mount Etna require stabilisation by afforestation. This in turn promotes pedogenesis and adds nutrients to soil for further plant uptake. In light of the recent efforts to maintain global C reserves in soil, this study suggests that *Pinus nigra* is a better litter species for maintaining C reserves in the soils on the slopes of Mount Etna due to the slower release of organic C. However given the

contrasting effects of *Pinus nigra* and *Genista aetnensis* on soil pedogenesis, this is probably not a viable option in the longer term. *Genista aetnensis* promotes organic matter formation and forms N fixing symbioses with graminae, while *Pinus nigra* causes acidification via the release of oxalic acid and reduces the availability of exchangeable aluminium (Certini et al., 2001). Pre- incubation with *Genista aetnensis* also increased the catabolic responses of the soil community following a disturbance event. This suggests that for the management of similar volcanic soils, afforestation with a litter of low C:N ratio and low lignin content should be adopted in order to promote the functional diversity and resilience of the soil microbial community.

Further improvements in the design of PLFA extraction techniques are required for volcanic soils. This study would have benefitted from increased PLFA recovery and resolution which would have aided the use and interpretation of multi- variate statistical techniques.

Enzyme assays using artificial substrates were probably not appropriate for assessing soil community function as they are conducted under optimized conditions which over estimate the amount of enzyme available within the soil. In addition to this, enzymes are quantified which are not of microbial origin and others are bound to clay particles which prolongs their lifespan in soils while they are unable to perform enzymatic functions.

This study highlights the perils of conducting research on an active volcano as already dynamic soil communities are subject to transient and often unreported

volcanic influence. Soil microbial communities present in volcanic soil chronosequences provide a unique opportunity to study organisms which are frequently perturbed and their recovery mechanisms. This study would have benefitted from an increased number of research sites of different ages starting from younger volcanic substrate. The inclusion of a greater number of study sites/ data points would have allowed for a more realistic assessment of the relationship between soil development and diversity and decomposition functions. Including a set of sites affected by more recent volcanic eruptions (0- 100 years since eruption) would have allowed for an assessment of the pioneer and build- up phases of the soil community and may have provided a more accurate representation of processes and soil community characteristics during soil development. This would also have allowed for better comparison of results to other successional sequences such as glacier forelands which generally have a larger range of temporal coverage.

While it is obvious soil microbial communities will be affected to some extent by the direct influence of volcanic deposits diluting carbon etc, it is not known what effect this may have on the plant communities of volcanic soils.

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Appendix A Statistical tables from chapter 3

Table 1 One Way Analysis of Variance to assess the effect of litter type on mass loss after 254, 571 and 878 days *in situ*. NS no significant differences, * P<0.05, ** P<0.01, *** P<0.001

Site	July 05 (Day 254)		May 06 (Day 571)		March 07 (Day 878)	
	p value	*F* value	*p* value	*F* value	*p* value	*F* value
MRP	NS	3.28	NS	7.17	NS	2.84
MRG	*	15.43	***	147.03	NS	1.07
SDCA	NS	2.04	*	17.34	NS	1.83
SDCB	NS	3.86	NS	2.84	*	7.99

Table 2 One Way Analysis of Variance to assess the variance between soils on the mass loss of *Pinus nigra* and *Genista aetnensis* after 254, 571 and 878 days *in situ*.

	Pinus nigra		*Genista aetnensis*	
Days *in situ*	*p* value	*F* value	*p* value	*F* value
254	0.282	1.52	0.007	8.75
571	0.212	1.88	0.151	2.33
878	0.578	0.70	0.354	1.25

Table 3 One Way Analysis of Variance to assess the effect of litter type on respiration after 254, 571 and 878 days *in situ*.

Site	Respiration (μg CO_2-C g^{-1} litter hr^{-1})						Respiration (μg CO_2-C g^{-1} litter C hr^{-1})					
	Day 254		Day 571		Day 878		Day 254		Day 571		Day 878	
	p	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*
MRP	*	8.07	NS	3.21	NS	7.45	NS	7.14	NS	3.73	*	8.80
MRG	NS	0.04	NS	0.09	NS	2.72	NS	0.13	NS	0.45	NS	3.20
SDCA	**	23.87	*	7.73	NS	0.21	**	26.31	*	9.97	NS	0.04
SDCB	NS	0.05	NS	1.82	NS	2.70	NS	0.11	NS	2.33	NS	3.27

- *p* denotes P value, NS no significant differences, * P<0.05, ** P<0.01, *** P<0.001
- *F* denotes F value.

Table 4 One Way Analysis of Variance to assess the variance between soils on litter respiration from *Pinus nigra* and *Genista aetnensis* after 254, 571 and 878 days *in situ*.

	Respiration (μg CO_2-C g^{-1} litter hr^{-1})				Respiration (μg CO_2-C g^{-1} litter C hr^{-1})			
	Pinus nigra		*Genista aetnensis*		*Pinus nigra*		*Genista aetnensis*	
Days *in situ*	*p* value	*F* value	*P* value	*F* value	*p* value	*F* value	*p* value	*F* value
254	*	6.16	***	82.06	*	6.16	***	82.07
571	0.127	2.57	0.057	3.84	0.146	2.37	*	5.42
878	0.716	0.46	0.513	0.83	0.365	1.22	0.737	0.43

- P value, NS no significant differences, * P<0.05, ** P<0.01, *** P<0.001

Appendix B Statistical tables for chapter 4

Table 1 General linear model (3 way analysis of variance) for distinction of effects of soil development, disturbance and time on PLFA diversity (*H*), evenness (*E*) and total PLFA

	Diversity (*H*)		Evenness (*E*)		Total PLFA	
Source of Variance	*P*-value	*F*- Value	*P*-value	*F*- Value	*P*-value	*F*- Value
Soil	0.000	18.08	0.032	5.00	0.000	49.30
Disturbance	0.920	0.01	0.195	1.75	0.000	60.29
Time	0.000	173.98	0.000	10.09	0.000	32.35
Soil* Disturbance	0.001	13.69	0.056	3.92	0.000	24.07
Soil*Time	0.000	128.57	0.024	3.59	0.000	26.86
Disturbance*Time	0.019	3.83	0.066	2.64	0.039	3.13
Soil*Disturbance*Time	0.000	11.82	0.000	11.13	0.000	16.98

Table 2- General linear model (3 way analysis of variance) for distinction of effects of soil development, disturbance and time on total Arylsulfatase, Acid Phosphatase and β-glucosidase activity

	Arylsulfatase		Acid Phosphatase		β-Glucosidase	
Source of Variance	*P*-value	*F*- Value	*P*-value	*F*- Value	*P*-value	*F*- Value
Soil	0.000	437.43	0.000	80.21	0.000	988.20
Disturbance	0.000	247.08	0.000	71.78	0.004	9.13
Time	0.000	99.86	0.000	35.65	0.000	21.27
Soil* Disturbance	0.000	113.10	0.000	16.06	0.006	8.08
Soil*Time	0.000	8.04	0.039	2.40	0.000	5.38
Disturbance*Time	0.000	10.99	0.000	6.74	0.001	4.53
Soil*Disturbance*Time	0.001	4.37	0.560	0.82	0.260	1.33

Table 3 General linear model (3 way analysis of variance) for distinction of effects of soil development, disturbance and time on specific Arylsulfatase, Acid Phosphatase and β-glucosidase activity

	Arylsulfatase		Acid Phosphatase		β-Glucosidase	
Source of Variance	*P*-value	*F*- Value	*P*-value	*F*- Value	*P*-value	*F*- Value
Soil	0.000	83.62	0.000	369.41	0.000	554.13
Disturbance	0.001	14.97	0.002	10.98	0.047	4.28
Time	0.000	126.84	0.000	83.29	0.006	31.61
Soil* Disturbance	0.560	0.35	0.019	6.12	0.539	0.39
Soil*Time	0.000	36.25	0.000	81.28	0.000	35.75
Disturbance*Time	0.006	4.96	0.000	23.90	0.000	20.22
Soil*Disturbance*Time	0.001	46.45	0.000	85.31	0.000	14.21

Table 4 One way analysis of variance of soil development effects at each time period for total Arylsulfatase, Acid Phosphatase and β-Glucosidase activity

	Arylsulfatase		Acid Phosphatase		β-Glucosidase	
Day	*P*- value	*F*- value	*P*- value	*F*- value	*P*- value	*F*- value
2	0.005	30.58	0.577	0.41	0.001	99.1
16	0.001	91.14	0.162	2.93	0.001	105.73
33	0.000	188.67	0.079	5.52	0.000	375.10
54	0.003	42.65	0.072	5.89	0.012	18.80
81	0.001	101.05	0.352	1.10	0.003	39.20
115	0.003	39.7	0.21	2.22	0.000	419.58
148	0.007	25.79	0.078	5.53	0.001	68.58

Table 5 One way analysis of variance of soil development effects at each time period for specific Arylsulfatase, Acid Phosphatase and β-Glucosidase activity

	Arylsulfatase		Acid Phosphatase		β-Glucosidase	
Day	*P*- value	*F*- value	*P*- value	*F*- value	*P*- value	*F*- value
2	0.012	18.88	0.000	295.22	0.000	237.35
33	0.001	80.51	0.005	237.45	0.000	535.52
81	0.001	99.60	0.316	1.31	0.003	40.08
148	0.017	15.76	0.005	32.93	0.001	84.75

Table 6 One way analysis of variance of disturbance effects on each soil at each time period for total Arylsulfatase, Acid Phosphatase and β- Glucosidase activity

	Arylsulfatase				Acid Phosphatase				β-Glucosidase			
	Monti Rossi		Salto del Cane		Monti Rossi		Salto del Cane		Monti Rossi		Salto del Cane	
Day	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*
2	0.006	29.11	0.020	13.75	0.121	3.84	0.005	31.11	0.577	0.37	0.000	241.04
16	0.001	60.63	0.002	45.93	0.004	35.84	0.012	19.11	0.715	0.15	0.005	30.54
33	0.011	19.86	0.000	106.6	0.034	10.10	0.006	29.16	0.03	10.87	0.016	16.27
54	0.015	17.00	0.003	39.98	0.036	9.58	0.044	8.38	0.469	0.64	0.019	14.41
81	0.012	18.67	0.001	89.70	0.037	9.40	0.031	10.75	0.695	0.18	0.003	39.19
115	0.588	0.35	0.057	7.01	0.026	11.95	0.85	0.04	0.377	0.98	0.685	0.19
148	0.641	0.25	0.016	16.02	0.453	0.69	0.007	25.15	0.382	0.96	0.369	1.03

Table 7 One way analysis of variance of disturbance effects on each soil at each time period for specific Arylsulfatase, Acid Phosphatase and β-Glucosidase activity

	Arylsulfatase				Acid Phosphatase				β- Glucosidase			
	Monti Rossi		Salto del Cane		Monti Rossi		Salto del Cane		Monti Rossi		Salto del Cane	
Day	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*
2	0.001	104.66	0.044	8.49	0.001	84.97	0.003	41.06	0.004	36.82	0.948	0.00
33	0.007	25.58	0.007	25.30	0.000	109.15	0.239	1.91	0.000	339.10	0.973	0.00
81	0.006	27.56	0.039	9.19	0.024	12.61	0.052	7.51	0.907	0.02	0.519	0.50
148	0.074	5.76	0.025	12.14	0.001	66.01	0.024	12.65	0.034	10.03	0.322	1.28

Appendix C

Structural diversity and enzyme activity of volcanic soils at different stages of development and response to experimental disturbance