Soil microbial stability and function: the role of diversity, composition and soil resources

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Abstract

Soil microbial function and stability may affect many other ecosystem functions, including soil carbon storage, nutrient cycling and plant productivity. However, the drivers of soil microbial stability itself are little understood. This thesis therefore aimed to develop a method and indices capable of quantifying soil microbial stability in terms of the resistance (amount of change caused by a disturbance), and resilience (rate of recovery) of the soil microbial community to a model disturbance, and to determine the role of three potential drivers of soil microbial function and stability: diversity, composition and soil resources. Initially, soil microbial stability and soil resources were measured during three chronosequences to assess whether stability changes in a natural environment and whether resources are an important driver of these changes. Although soil resources were frequently related to resistance and resilience, the direction and strength of correlations depended on the response variable and chronosequence considered. This suggested a factor related to soil resources, which varied across chronosequences, was a stronger driver of soil microbial stability than resources themselves. Two potential factors were plant species composition and diversity. A glasshouse experiment that tested these factors was harvested at 4 times throughout a 16-month period. Plant species composition, but not diversity, proved to be a strong driver of soil microbial function and stability. As different plant species may alter soil microbial function and stability by depositing different carbon substrates, a further experiment manipulated the composition and diversity of carbon substrates added to a base soil. The composition, and sometimes the diversity, of added substrates affected the soil microbial community, its function and stability. Diversity effects saturated at low levels and depended on which substrates were added. The overall conclusion from this set of experiments was that the strongest drivers of soil microbial function and stability seemed to be the composition of plant and soil microbial communities as well as soil resources.

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Chapter 1: Drivers of ecosystem function and stability

The organisms within the earth's ecosystems are responsible for maintaining our planet's atmosphere, water quality, and soil fertility, providing us with the essentials of human life: drinking water, food, and shelter. Preservation of these vital ecosystem functions or services requires an understanding of their drivers and mechanisms. Of these, the drivers and mechanisms of biogeochemical cycling are some of the most important, involving the recycling of elements essential to life (e.g. carbon (C), nitrogen (N), oxygen). Plants and soil microbes form the base of the biological component of this recycling.

The combined function of plants and soil microbes is essential for the maintenance of aboveground and belowground food webs, atmospheric quality and soil fertility. The end result of these combined functions can be measured by ecosystem properties such as plant biomass and productivity, nutrient retention, and decomposition of organic matter. Plant and soil microbial function are inextricably linked (Naeem et al. 2000, Wardle 2002). Plants contribute organic matter to the soil via litter and root exudates. This organic matter is decomposed by soil microbes, providing them with C and energy for maintenance and biomass production (Swift et al. 1979, Bremer and van Kessel 1990, Waldrop et al. 2000). Soil microbes convert the nutrients bound up in organic matter into inorganic forms, which can then be taken up by plants and used in photosynthesis and plant biomass production (Grayston et al. 1996) . The rate of decomposition, and therefore the rate of nutrient release and plant growth, is determined in part by the quality and amount of resources returned to soil by plants (Swift et al. 1979). The rate of decomposition has implications for soil C storage and therefore the global C budget – the faster and more complete the decomposition, the less C will be stored in soil (Catovsky et al. 2002). The ways in which plants and microbes interact, and the drivers behind those interactions, therefore have major implications for ecosystem services.

The delivery of ecosystem services can be disrupted by disturbances. A disturbance can be defined as any discrete event that causes a change in a response variable. Traditionally, disturbance has been measured as a reduction in biomass, such as may occur during drought, windstorms or earthquakes (Grime 1979). However, the definition of a disturbance can be expanded to include an increase in a response variable (White and Pickett 1985), such as may occur when nutrients are added to an ecosystem. The types, magnitudes and frequency of disturbances experienced by many ecosystems have been significantly altered by human activity. The response of ecosystems to disturbance has therefore become a major focus of ecological and conservation research. These ecosystem responses can be considered as indicative of ecosystem stability. Ecological stability can be divided into two parts: resistance (the amount of change caused by a disturbance) and resilience (the rate of recovery from a disturbance) (Pimm 1984). An ecosystem can be resistant but not resilient and vice versa (e.g. Herbert et al. 1999), both resistant and resilient (e.g. Kaufman 1982), or neither (e.g. Rejmánková et al. 1999), depending on the disturbance and response variables considered (Harrison 1979). This wide range of context-dependent responses in resistance and resilience suggests that many different factors may drive ecosystem stability. This thesis focuses on diversity, composition, and soil resources as potential drivers of ecosystem function and stability.

1.1 Diversity as a driver of ecosystem function and stability

The loss of species diversity has increased dramatically in recent years, partly due to human-induced changes in land use (Díaz and Cabido 2001). This has raised the issue of whether diversity will affect ecosystem function and stability (Sankaran and McNaughton 1999). Early modelling studies regarding diversity and ecosystem function looked primarily at stability. These studies came up with two opposing viewpoints, one that suggested that diversity should increase stability (e.g. MacArthur 1955), and one that suggested the opposite (May 1972). This dichotomy was resolved to some extent by the realisation that the two viewpoints come from different perspectives: a more diverse system may have a more stable average biomass precisely because the species abundances

within it fluctuate, as one species may compensate for the reduction in biomass of another (McNaughton 1977, Tilman 1996).

More recently, studies have focused on other measures of ecosystem function such as plant biomass production, nutrient retention and decomposition (e.g. Naeem et al. 1994, He et al. 2002, Hedlund et al. 2003). Many of the findings and issues highlighted by this literature are also of relevance to understanding stability. Several hypotheses have been proposed to describe the shape of the relationship between ecosystem function and diversity, of which the four main ones (Johnson et al. 1996) are presented here. The first of these, described above, is that diversity will increase ecosystem functioning, and stability (MacArthur 1955) in a linear manner (Johnson et al. 1996). The second is the rivet hypothesis, which was proposed by Ehlrich and Ehlrich (1981). This uses the analogy of an aeroplane held together by rivets. As rivets (i.e. species) are removed beyond a threshold number, the chance of the aeroplane (i.e. ecosystem) collapsing increases. This suggests that some species may be lost without any noticeable effect on ecosystem function, while others are critical. The redundancy hypothesis (Walker 1992) is similar to the rivet hypothesis, but focuses more on which species can or cannot be lost without any change in ecosystem function (Lawton 1994). It also incorporates the idea that species within functional groups can compensate for the loss of other species from within the same group (Walker 1992, Johnson et al. 1996). The fourth hypothesis is termed the idiosyncratic hypothesis (Lawton 1994). This proposes that the effect of species on ecosystem function will depend on which species is removed (i.e. species composition), so that there may be no predictable relationship between diversity and ecosystem function. This hypothesis and the redundancy hypothesis are similar to the keystone species concept, all of which suggest that some species have a disproportionately large effect on ecosystem functions, while others are largely redundant (Walker 1992, Lawton and Brown 1993).

Most studies that have manipulated species or functional group diversity have used plant species. Several recent studies have claimed that plant biomass, productivity and/or nutrient retention increases with plant species diversity (Tilman et al. 1997a, Hooper and Vitousek 1998, Symstad et al. 1998), although there are exceptions (Hooper and Vitousek 1997, Wardle et al. 2003). Some studies have also found positive effects of diversity on some aspects of the stability of plant (Frank and McNaughton 1991, Tilman and Downing

1994, Tilman 1996), aquatic (McGrady-Steed et al. 1997), and microbial (Naeem and Li 1997) communities. There are very few studies that have directly manipulated soil microbial diversity. This is partly because the soil system is extremely diverse, with several thousand species of bacteria estimated to be in 1 g of soil (Torsvik et al. 1994). It has also been estimated that only 1% of species are culturable (Degens and Harris 1997). It is therefore difficult both to grow representative species to add to the soil, and to produce a diversity gradient that is grounded in ecological reality. Studies that have attempted to do so have found that soil microbial functions such as decomposition, nitrification and stability show the full range of responses - positive, negative and neutral - to changes in diversity (Griffiths et al. 2000, Degens et al. 2001, Griffiths et al. 2001b).

Because of the difficulty of manipulating soil microbial diversity directly, most studies have investigated how the diversity of other organisms or factors, which may indirectly alter soil microbial diversity, affect soil microbes and their function. The most common of these factors have been plant and litter diversity, although the number of studies manipulating soil faunal diversity are increasing. Increases in substrate diversity such as occurs when plant species and litter are mixed have been predicted to increase the diversity of the microbial community by creating more niches (Grayston et al. 1998, Ettema and Wardle 2002), and to reduce nutrient recycling by increasing the chance of containing a recalcitrant substrate (Loreau 2001). However, studies that have varied plant and litter diversity have again reported the full range of positive, negative and neutral effects on decomposition (Briones and Ineson 1996, Nilsson et al. 1999, Spehn et al. 2000a), soil microbial activity and biomass (Wardle et al. 1997a, Spehn et al. 2000a, Gastine et al. 2003), and soil microbial community structure and diversity (Stephan et al. 2000, Wardle et al. 2003). Studies manipulating soil faunal diversity have also found variable results, with some studies showing no effect of diversity on soil microbial biomass (Laakso and Setälä 1999, Liiri et al. 2002), while others show inconsistent effects (Mikola and Setälä 1998c). In terms of ecosystem processes, one study found no effect of faunal diversity on nutrient mineralisation (Laakso and Setälä 1999), while others have found a positive effect on C mineralisation (Mikola and Setälä 1998b), and plant N uptake (Liiri et al. 2002). In combination these plant and microbial diversity studies support the diversity-function, redundant and idiosyncratic hypotheses, depending on the type of system and the response variable measured.

1.1.1 Facilitation, complementarity and the sampling effect

Where significant effects of diversity on ecosystem function and stability have been found, three main mechanisms have been used to explain the results: facilitation, complementarity, and the sampling effect. Facilitation takes place when one species enhances the contribution made to an ecosystem function by another species (Connell and Slatyer 1977, Chapin et al. 1994), such as the fertiliser effect that legumes can have on other species' biomass (e.g. Symstad et al. 1998, Hector et al. 1999). Facilitation could also occur if one species reduces the effect of a disturbance on another species, thereby increasing the stability of aggregate community properties (e.g. Mulder et al. 2001). Complementarity occurs when two or more species vary in their resource requirements, spatially, temporally, or simply by using different forms of a resource (Tilman et al. 1997b, Tilman et al. 2001). For example, when two or more plant species differ in the type of N they use, growing them together should result in a higher use of resources and therefore higher plant community productivity (Hooper and Vitousek 1997, Hooper 1998). Complementarity can also increase stability; when different species grow optimally under different conditions, one species can compensate for reductions in the growth of another species if the conditions change (McNaughton 1977, Tilman 1996). This mechanism is one of the components of the insurance hypothesis (Yachi and Loreau 1999), and suggests that although species may be redundant in the current environment, they may not be so when that environment changes.

The final mechanism, called the sampling effect, originated as a criticism of experimental design. The sampling effect can be defined as the greater probability that more diverse mixtures will include a species that has a disproportionally high contribution to the ecosystem function measured (Aarssen 1997, Huston 1997, Tilman et al. 1997b). Where this species is able to dominate, this will result in a higher value for the ecosystem function measured than the mixtures that do not contain this species (Loreau and Hector 2001, Tilman et al. 2001). This change in ecosystem function is through an effect of species composition rather than the result of increases in the number of species; the biomass of a productive species in monoculture could be just as high as the biomass of a more diverse

community containing it. This logic has also been applied to the increased probability that more diverse mixtures will contain small groups of species that are facilitative or complementary (Huston and McBride 2002); here both diversity and composition play a role in increasing ecosystem function.

For the sampling effect to operate in nature, community assembly and species loss must be random. There is some argument over whether communities are assembled at random, and therefore whether the sampling effect is a true mechanism behind diversity effects or an artefact of experimental design (Wardle 1999, Loreau et al. 2001). This disagreement has led to a need to be able to distinguish the sampling effect from facilitation and complementarity effects. This requires knowledge of how each species that occurs in a mixture behaves in monoculture, and in mixtures of lower diversity (Garnier et al. 1997b, Loreau 1998). Many studies that have claimed to provide evidence of diversity affecting function have failed to do this adequately, and re-analysis of their data has suggested that the sampling effect, rather than facilitation or complementarity, is the primary mechanism behind the reported diversity effects (e.g. van der Heijden et al. 1998 vs Wardle 1999; Hector et al. 1999 vs Huston et al. 2000; Pfisterer and Schmid 2002 vs Wardle and Grime 2003). Diversity studies that control explicitly for sampling effects (i.e. composition) tend to find that facilitation and complementarity are only occasionally important (e.g. Hooper and Vitousek 1997, Nilsson et al. 1999, Wardle et al. 2000). There is therefore still a significant debate regarding the importance of diversity in ecosystem function and stability.

Most research on diversity has focused on aboveground systems and aquatic systems. The number of studies on the effect of aboveground diversity on belowground biomass, activity and some functions (e.g. decomposition) is increasing (e.g. Hector et al. 2000, Gastine et al. 2003, Hedlund et al. 2003), but there is still very little information on how above or belowground diversity affects belowground stability (Griffiths et al. 2000, Wardle et al. 2000, Griffiths et al. 2001a). Studies that examine this question in the opposite direction, i.e. how does soil microbial diversity affect aboveground function, are also rare.

1.2 Species composition as a driver of ecosystem function and stability

Inherent in the conceptual basis of the sampling effect is the idea that species composition can be an important driver of ecosystem function and stability. In his discussion of diversity effects, Grime (1998) advanced the idea that the traits of the dominant autotrophic species will determine many ecosystem functions at any point in time. The dominant species contributes the most to biomass, and therefore its traits will have the greatest impact on the ecosystem function measured (Grime 1998). Species traits have been suggested as more important than diversity as a driver of resistance and resilience in aquatic (Sousa 1980), and plant systems (Lepš et al. 1982, MacGillivray et al. 1995). Soil community composition may also result in differences in stability. For example, fungi and bacteria vary in their ability to cope with different disturbances (Orchard et al. 1992, Allen et al. 1999, Griffiths et al. 1999). Studies that look at the interaction between plants and soil microbes have also found strong effects of the dominant plant species on soil microbial communities (Vinton and Burke 1995, Bardgett et al. 1999b), their function (Berendse 1993, Wardle et al. 1998), and stability (Wardle et al. 2000).

Lepš et al. (1982) and MacGillivray et al. (1995) extended theory on species composition effects on stability to incorporate specific traits based on the life history strategies described by Grime (1979, 2001). They suggested that plants adapted to nutrient stress (i.e. stress tolerators) should have characteristics that also infer a high degree of resistance (i.e. a high level of defence), but a low level of resilience (slow growth rate). In contrast, ruderal species have a fast growth rate, and therefore should recover quickly, but have a low level of defence and therefore low resistance (MacGillivray et al. 1995). MacGillivray et al. tested their hypothesis by quantifying the stress tolerance of a range of plant species, and subjecting them to fire, drought and freezing disturbances. The response of the biomass of the plants to the disturbances supported their hypothesis, with stress tolerant species showing a high resistance but low resilience. Plant life history strategies may also have implications for soil microbial function and stability. For example, plants that grow in low nutrient areas (and therefore are presumably stress tolerant) often produce low quality

litter that is difficult to decompose (Gartner and Cardon 2004), which can result in a resource-poor environment for soil microbes (Berendse 1993, Wardle et al. 1997b, Berendse 1998).

Soil microbes themselves have been divided into two groups that differ in their growth rate: r-selected or zymogenous microbial species, which have similar characteristics to ruderal species, and K-selected or autochthonous species, which are similar to stress-tolerators (Grime 1979, Gerson and Chet 1981). Therefore, the type of relationship between stress tolerance and stability that has been proposed for plants may also apply to soil microbes. There is some experimental evidence for this. It is thought that during a wetting-drying event, the r-selected, active organisms are killed by the disturbance (i.e. have low resistance), and the dormant or slower-growing, K-selected organisms survive (i.e. have high resistance) (Bottner 1985, Cortez 1989). The relationship between stress tolerance and stability is also partially supported by another study, which found that *Pseudomonas putida* coped better with osmotic and oxidative stress if it had been growing in C-starved conditions, but found no difference in its resistance or resilience to heat (Gu and Mazzola 2001).

Many studies have examined how plant or litter composition affects soil microbial function (e.g. Bardgett and Shine 1999, Nilson et al. 1999, Porazinska et al. 2003) but few studies have looked at how these factors affect soil microbial stability. It is also unclear what role soil microbial composition has in determining soil microbial stability, and whether r- and K-selection or nutrient stress tolerance are important drivers behind composition effects. Studies of how soil microbial community composition affects plant function are also rare.

1.3 Soil resources as a driver of ecosystem function and stability

Another factor suggested as a driver of ecosystem function and stability is resources. It is well known that plant and microbial function are strongly tied to soil resources. For example, the addition of N to soil usually results in higher plant growth (Tilman 1987,

Jonasson et al. 1999). The same can be applied to soil microbes; C and N addition can increase or decrease soil microbial activity and biomass (Wardle 1992, Jonasson et al. 1996a) and the decomposition of organic matter (Fog 1988, Dalenberg and Jager 1989, Wu et al. 1993, Hobbie and Vitousek 2000). Plant and soil microbial responses to changes in resource availability can also interact. For example, the addition of C to soil can enhance microbial demand for nutrients, reducing nutrient availability to plants and therefore reducing their growth (Rutherford and Juma 1992, Jonasson et al. 1996b). Alternatively, increases in nutrient availability to plants can result in increased biomass and increased litter production (Tilman 1987). This may result in increases in standing litter and immobilisation of nutrients in that litter, eventually reducing productivity (Knops et al. 2001).

It was evident from the study of MacGillivray et al. (1995) that there may be a link between nutrient availability and the resistance and resilience of plant biomass. Several other models and theorists have suggested that ecosystem stability may depend on nutrient and resource availability. DeAngelis (1980, 1989, 1992) presented models of ecosystems that included plants and soil with or without herbivores and carnivores. His results suggested that resilience should increase as the supply rate of limiting resources increases (DeAngelis 1980, DeAngelis et al. 1989, De Angelis 1992). If a system is limited by a particular resource, its supply will also limit the rate at which that system can rebuild biomass after a disturbance. Experimental tests of this are few and only involve plant and aquatic communities, but are generally consistent with this model (Steinman et al. 1991, Biggs et al. 1999, Herbert et al. 1999).

Other evidence from a variety of sources support the hypothesis that resources may be an important determinant of soil microbial stability. Higher productivity has been predicted to increase resilience (Moore et al. 1993), and found to decrease the resistance of species composition (Jenkins et al. 1992). Wardle (1998) conducted a meta-analysis to explore the relationship between temporal variability of the soil microbial biomass and various indicators of disturbance, land use, and soil resources. The variables that explained the most variation were pH, total C and total N, all of which had a negative effect on temporal variability. As a more resistant system will be less temporally variable, this suggests that higher soil resources may also increase resistance (Wardle 1998). This conclusion is

supported by several other studies that suggest that organic C can act as a reservoir of nutrients in disturbed systems (O'Neill 1976, De Angelis 1992). Another study predicted that N and C mineralisation of a C-limited system should be stable when either C or N is added (Bosatta and Berendse 1984). However, if the system is initially N limited, C and N mineralisation will only be stable under certain circumstances, and may oscillate during the system's recovery (Bosatta and Berendse 1984). Despite the implications of these theoretical studies, there have been very few empirical studies that examine how resources alter stability, and none of these include soil microbial stability.

1.4 Succession: links with potential driving factors

The above discussion revolves around three potential drivers of ecosystem function and stability: species diversity, species composition, and resource availability. These drivers all interact and may have a role in primary plant succession. Primary plant succession refers to the development of a plant community on a previously uncolonised surface (Walker and del Moral 2003), such as occurs after deglaciation or a volcanic eruption. It usually involves a build-up of organic matter (eg: Insam and Haselwandter 1989, de Kovel et al. 2000) and plant biomass towards a maximal, climax point (Odum 1969). In some successions, resources begin to decline after the climax phase, resulting in a decrease in organic matter (Crews et al. 1995) and potentially plant biomass. It has also been suggested that resistance should increase with time (Odum 1969), while resilience should decrease (Grime 1979).

1.4.1 Succession and species composition and diversity

By definition, the composition and diversity of plant species change during succession. These changes can be described in terms of r- and K-selection, and in terms of Grime's life history strategies (Grime 1979, Huston and Smith 1987). The factors that influence succession tend to change with time. Initially, the primary driver is disturbance, as an open

environment is less buffered against changes in temperature, moisture and wind than a vegetated one (Bazzaz 1979). Therefore, early colonisers tend to have the traits of r-selected, or ruderal, species (Huston and Smith 1987), with a high reproductive and growth rate, and high quality litter (Grime 1979). As succession continues, competition and then stress become more important as shading increases and nutrients are lost from the soil (Grime 1979). Later species therefore tend to be competitors, followed by species that have the traits of K-selected, or stress-tolerant, species, and have slower growth rates and lower quality litter (Odum 1969, Grime 1979, Huston and Smith 1987). This change in species composition as succession proceeds may result in differences in stability, with ruderals showing high resilience and stress tolerators showing high resistance (Lepš et al. 1982, MacGillivray et al. 1995). This change may also affect soil microbial community composition and function, by altering the amount and quality of organic matter returned to the soil. There have been few detailed studies on how soil microbial composition changes during plant succession. However, it appears that soil microbial activity and biomass increase, at least during early succession (Insam and Haselwandter 1989, Wardle and Ghani 1995, Bardgett et al. 1999a). The system also tends to shift from a bacterial-dominated system to a fungal-dominated one (Allen et al. 1999, Ohtonen et al. 1999), possibly reflecting a reduction in nutrient availability (Bardgett et al. 1999a).

Early theories suggested that plant diversity should increase with time (Odum 1969). However, if other theories are combined a more complex story appears. During succession, the system shifts from disturbance to competition to stress controlled. After a disturbance, diversity increases as plants begin to colonise. However, if there are no further disturbances, competitive exclusion may reduce diversity (Connell 1978, Huston and Smith 1987). Increases in stress towards the end of succession may increase diversity again, as competitive interactions, and therefore competitive exclusion, become less important (Grime 2001). This range of scenarios may explain why changes in plant species richness over time show variable patterns across studies (e.g. Crews et al. 1995, Berendse et al. 1998, Bellingham et al. 2001). Changes in plant species diversity will also affect the diversity of substrates within soil, with potential flow-on effects to soil microbial diversity (Ettema and Wardle 2002). There have been no direct studies on how substrate diversity affects soil microbial diversity during succession, but some studies have found that soil

microbial diversity shows variable trends with time (Bardgett et al. 1999a, Schipper et al. 2001).

1.4.2 Succession and soil resources

Soil resources change significantly during primary plant succession. Soil C and N increases as plants colonise new surfaces and grow (Chapin et al. 1994, De Kovel et al. 2000, Schipper et al. 2001). Both of these resources are of biological origin (McLaren and Cameron 1990, Catovsky et al. 2002), therefore their availability and rate of increase in soil during succession will be determined by the types of plants that colonise, and the rate of nutrient cycling (Berendse 1990, Mao et al. 1992, Cote et al. 2000). For example, plants that produce poor quality litter will result in a faster build up of soil C than plants that produce high quality litter, as poor quality litter takes longer to decompose (Swift et al. 1979). Concurrent with this increase in C and N is a decrease in pH (e.g. Chapin et al. 1994, De Kovel et al. 2000), as acids within organic matter are one of the main sources of H^+ ions (McLaren and Cameron 1990).

The primary source of phosphorus (P) is from the rocks, or parent material, that the system develops on (Walker and Syers 1976). Over time, the total amount of P and especially its availability declines. This is due to two main processes, leaching and occlusion (Walker and Syers 1976). Occlusion refers to the binding of P ions to clays in such a way that they cannot be released again (McLaren and Cameron 1990). As P is lost from the parent material or is bound in unavailable inorganic forms, its availability becomes more reliant on recycling through organic matter.

The different processes involved in regulating C, N and P availability across successional gradients has lead to the suggestion that early succession is primarily N and C limited, and late succession is primarily P limited (Scheu 1990, Crews et al. 1995). These changes in resources have been suggested as important drivers of plant succession (Tilman 1987, De Kovel et al. 2000). Overall, primary plant succession incorporates changes in plant and soil microbial composition and diversity, and soil resources. Successional gradients therefore

provide ideal systems to test how these factors influence ecosystem function and stability. However, few studies have extended these ideas to soil microbial function or stability.

1.5 Thesis aims and objectives

The above discussion identifies a large gap in our current knowledge as to what factors control soil microbial function and stability, and how soil microbial function and stability interact with plant composition, diversity and function. My research aims to contribute to filling this gap. The primary goals of my research were to determine which factors drive soil microbial resistance and resilience, and to explore the link between plant and soil microbial function. This research is presented in four chapters, each written as a journal article. Each chapter containing experimental work is introduced with an abstract and a flow diagram describing the links between the concepts examined in that chapter.

My first objective was to develop a technique that was capable of distinguishing the resistance and resilience of soil microbial activity, biomass and mineral N contents. I chose to use a wetting-drying event for this, as it involves both a negative (drying) and a positive disturbance (the release of nutrients when dry soil is rewet) (Birch 1958, Birch 1959, Turner and Haygarth 2001), and is an important driver of soil microbial turnover, and therefore the release of nutrients for plant uptake (Fierer and Schimel 2002). The method was tested with three different soils. Part of this data is presented in Chapter 2. To compare different soils statistically it is necessary to summarise the response of the soil community to a disturbance into a single number. I therefore developed two indices that quantify resistance and resilience respectively; this is presented in Chapter 2. This chapter has been published in *Soil Biology and Biochemistry*.

My second objective was to test my method further by determining whether soil microbial resistance and resilience change in a natural environment: during primary plant succession. Several potential factors that may control soil microbial stability undergo change during succession, including plant and microbial diversity and composition, and soil resources. I

focused on changes in soil resources as these may be important drivers of changes in plant species composition and diversity during succession, and because they reflect changes in plant litter quality and quantity. I measured soil microbial resistance and resilience to a wetting-drying event during three primary plant successions, and related this to soil resources and time within each succession. These data and results are presented in Chapter 3. This manuscript has been submitted for publication in *Oikos.*

The results from this experiment depended on which succession was measured. This suggested that some factor that differed between each succession was a stronger driver of soil microbial resistance and resilience than soil resources. Given the strong link between plant and soil microbial function it seems likely that plant community properties may be this driver. I used a glasshouse experiment in which the composition and diversity of plant species were manipulated to determine the effect of plant communities on soil microbial stability and function (decomposition) in different seasons and at different stages of plant community development. Plants may affect soil microbial function and stability by altering either soil resources or the soil microbial community; therefore I measured several variables that represented changes in these factors. The experiment focuses on the effect of plants on soil microbes, and is presented in Chapter 4. This manuscript has been submitted for publication in *Plant and Soil.*

The results from this experiment also indicated context-dependent effects of plant species on soil microbial function and stability, but showed that the composition of the plants had a much greater role in determining soil microbial properties and function than plant diversity. One potential reason for this may be that plants vary considerably in the types of substrates they return to the soil, in their amount, quality and diversity (Vinton and Burke 1995, Gastine et al. 2003). A large amount of research has been conducted on C:N ratios and the effect of different amounts of substrates, but very few studies have looked at the effects of the quality of C substrates, and none to date have explicitly varied substrate diversity. My fourth and final experiment therefore examined how C substrates and their diversity affected soil microbial stability and ecosystem function. I also measured the effect of the C substrates on soil microbial community structure and related this to measurements of ecosystem function and stability. Substrates can also alter soil resources, which may have flow-on effects to soil microbial function. I therefore measured some

aspects of soil chemistry. To examine the effect of soil microbes on plants, I looked at how soil communities that had adapted to the C substrate treatments affected plant growth. This research therefore completes the link between plant and soil microbial function. It is presented in Chapter 5. This manuscript will be submitted for publication to *Oikos*.

The final chapter of this thesis discusses the likely importance of diversity, composition and soil resources in driving soil microbial function and stability, and the relationships between plant and soil microbial function. It will also examine how successful my approach was in assessing these interactions.

Chapter 2: New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances

2.1 Abstract

The stability (resistance and resilience to disturbance) of a soil system is a key factor influencing ecosystem properties and processes. To compare the stability of different systems, it is necessary to have indices that provide a relative quantitative measure of both the resistance and resilience of a response variable in all possible scenarios. However, the indices currently in use are frequently unable to do this, or are difficult to interpret. Here we present new indices that avoid these problems. We compare our indices with previously published indices of stability, and test their performance by using a real data set. We show that our indices accurately represent the response of soil properties (e.g. soil microbial biomass) to a disturbance, and that they are capable of determining differences in stability between contrasting soils.

2.2 Introduction

Ecological stability consists of two components: resistance (the amount of change caused by a disturbance, and resilience (the speed with which a system returns to its pre-disturbance level following a disturbance) (Pimm 1984). A system's stability determines its ability to continue functioning under changing conditions, as might occur through either natural processes or human-driven disturbances. The stability of the soil community in particular affects its turnover rate and hence the ecosystem processes driven by the soil biota (Wardle and Parkinson 1990). There are therefore many situations where it is highly desirable to be able to quantify stability. Although several indices of both resistance and resilience have been used in the literature, most of these have problems that make interpretation difficult, or they cannot be used in some situations. The purpose of this work is to present indices that we believe have advantages over previous indices when belowground systems are considered, and to assess their performance in comparison with other indices.

The resistance and resilience of organisms or processes is best quantified by comparing their performance in the disturbed soil against that in an undisturbed soil (which can be thought of as a control) (Fig. 1). For an index of resistance or resilience to work properly, several criteria should be met. Firstly, the index should increase monotonically as resistance or resilience increases, so that it is easy to interpret. Secondly, the index should give an identical value when the disturbed soil is the same relative distance away from the control, regardless of direction. This allows the resistance of soils to be compared without subjectively judging whether a soil that responds positively to a disturbance is more resistant than one that responds negatively. Because the definition of resilience does not include direction, neither should the index of resilience. Thirdly, the index should be bounded for both positive and negative values and not tend to infinity, and it should be constructed in such a way that it is not possible for zero to appear in the denominator. This means that any response to the disturbance can be quantified by the indices, and that index values can be easily compared statistically. Finally, resistance should be standardised by an undisturbed control soil and resilience by the amount of change caused by the disturbance initially.

2.3 Resistance index

The index that we propose for resistance (RS) is:

$$\mathrm{RS}\ (t_0) = 1 - \frac{2 \times |D_0|}{C_0 + |D_0|}$$

where D_0 is the difference between the control (C_0) and the disturbed soil (P_0) at the end of the disturbance (t_0) (see Fig. 1). This index is standardised by the control soil, as this takes into account differences in the amount of change that a disturbance could cause. For example a soil variable that starts at 10 units can potentially decrease by 10 units, compared with a soil that starts at 5 units, which can only decrease by 5 units. A reduction of 5 units in soil A only results in a 50% reduction in the response variable, whereas a reduction of 5 units in soil B results in a 100% reduction in the response variable, which is a more severe response. Our index accommodates such differences.

This index of resistance is bounded by -1 and +1, with a value of +1 showing that the disturbance had no effect (maximal resistance), and lower values showing stronger effects (less resistance) (Fig. 2a). If the value of the disturbed soil (P_0) is between 0 and 2 x C_0 (i.e. $|D_0| \leq C_0$), the index will give values between 0 and 1. An index value of 0 indicates either a 100% reduction or increase in the value of the disturbed soil. If, however, the value of P_0 is higher than 2 x C_0 (i.e. where $|D_0| > C_0$), the index will give a negative value (Fig. 2a). This may occur, for example, when a pulse of glucose is added to soil.

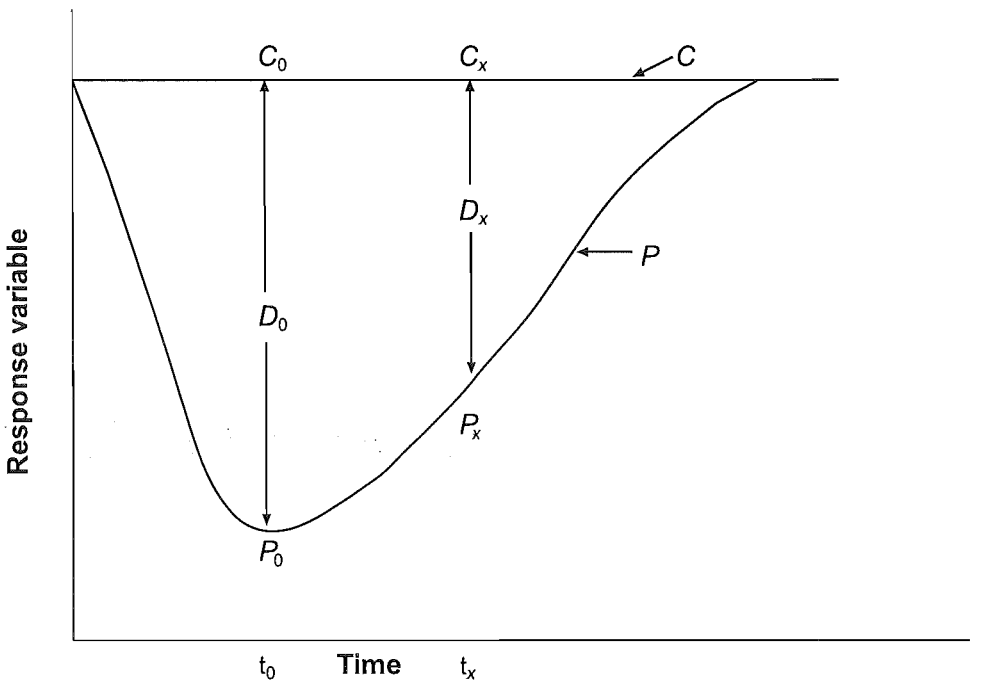
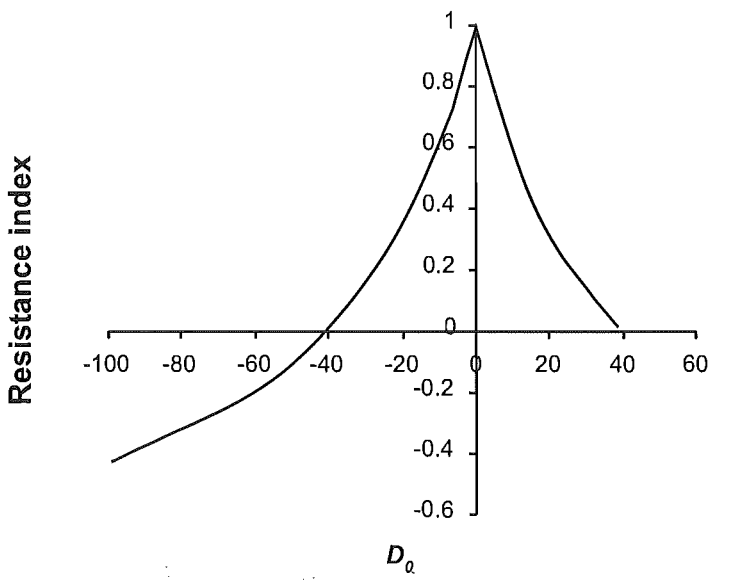


Fig. 1: An example of the resistance and resilience of a response variable to a disturbance. Here, a response variable can be any biotic or abiotic soil variable that responds to a disturbance. The upper line represents the undisturbed control soil (C) and the lower line represents the disturbed soil (P). For resistance (i.e. time 0 or t_0), the value for the control soil is C_0; the value for the disturbed soil is P_0; and $C_0 - P_0 = D_0$. An example of the data used to show resilience is given at t_x, with the value for the control soil as C_x, the value for the disturbed soil as P_x and the difference between the two as D_x. Time x can be any time point beyond t_0.

(a)



(b)

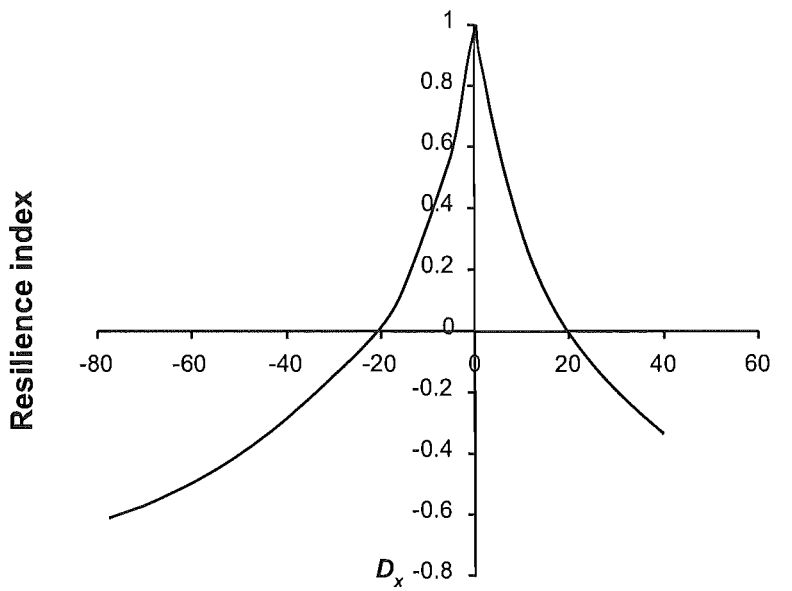


Fig. 2: The distribution of values obtained from the indices under different scenarios. (a) Changes in the resistance index with changes in D_0 (i.e. $C_0 - P_0$), when C_0 is fixed at 40. (b) Changes in the resilience index with changes in D_x (i.e. C_x - P_x), when C_x is fixed at 40 and D_0 at 20. See Fig. 1 for notation

2.4 Resilience index

The index that we propose for resilience (RL) at time x is:

$$\text{RL at } t_x = \frac{2\times|D_0|}{|D_0|+|D_x|} - 1$$

where D_0 is as above and D_x is the difference between the undisturbed control (C_x) and the disturbed soil (P_x) at the time point (t_x) chosen to measure resilience (see Fig. 1). This index is standardised by the amount of change initially caused by the disturbance (D_0), as this determines the state from which it has to recover. For example, consider two soils that have been disturbed; the value of the response variable of one soil has been reduced by 8 units, while the other has been reduced by 2 units. Both soils show a 70% recovery in the response variable 3 days after the end of the disturbance. This means that their relative rate of recovery is the same, and the time required to reach full recovery if they continue at this rate will also be the same. Therefore, the index assigns identical values of resilience to them.

This index of resilience is also bounded by -1 and +1 (Fig. 2b). A value of 1 at the time of measurement indicates full recovery (maximal resilience), and lower values indicate a slower rate of recovery. If the absolute value of D_x is between 0 and the absolute value of D_0, the index will give values between 0 and 1. An index value of 0 indicates that the disturbed soil has either not recovered at all since the disturbance ended (i.e. $D_0 = D_x$), or that at t_x it is the same distance away from the control as it was when the disturbance ended at t_0, but in the opposite direction (i.e. if $D_0 = 20$, and $D_x = -20$, or vice versa, the index will give a value of 0). If the absolute value of D_x is higher than the absolute value of D_0, the index will give a negative value (Fig. 2b). This might occur when the disturbance initially reduces the response variable being measured, but also increases substrate availability which subsequently causes a large increase in the response variable (e.g. Birch 1959, Bottner 1985).

2.5 Comparison with other indices

Several indices of resistance and resilience have been suggested. The performance of our proposed indices and that of previous indices is shown in Table 1 and Table 2. Our index of resistance satisfies the five criteria described above. It is the only index that increases monotonically as resistance increases, and gives identical values for positive and negative effects that are of the same magnitude (Table 1). Although the reciprocal of the absolute of one of the other indices (i.e., that used by Sousa, 1980, Biggs et al. 1999, Herbert et al. 1999) would also result in this pattern, 100% resistance would become indefinable, as 0 would appear in the denominator. Our index of resistance also has an advantage over most of the other indices in that it remains bounded even when extreme values are encountered (e.g. when P_0 is vastly in excess of C_0, as might occur when a pulse of glucose is added to soil).

Our index of resilience also satisfies the above criteria. In comparison with the other indices (Table 2), our index is the only one that shows a monotonic increase with an increase in resilience. While an index of resilience that does increase with increasing resilience could be derived from taking the reciprocal of one of the other indices (O'Neill 1976), this would again result in problems of dividing by 0 when the values of C_x and P_x are the same. Our index avoids such problems by being standardised in such a way as to ensure that 0 values cannot appear in the denominator. It also gives identical values for positive and negative effects that are of the same magnitude, giving it an advantage over most of the other indices (Steinman et al. 1991, Griffiths et al. 2001a). Finally, our index of resilience is the only one that is bounded even when extreme situations are considered, for example, when the effects of the disturbance continue to cause changes in the response variable even after the disturbance has ended (i.e. when $|C_x - P_x| > |C_0 - P_0|$).

Table 1: Comparison of the performance of different indices of resistance

		Values of parameters					
		P_0:	200	80	60	40	20	0
Source	Formula	C_0-P_0:	-160	-40	-20	0	20	40
Griffiths et al. 2000; 2001	$\left(C_0 - \frac{P_0}{C_0}\right) \times 100$		3500	3800	3850	3900	3950	4000
Kaufman 1982, MacGillivray et al. 1995	$\frac{P_0}{C_0}$		5	2	1.5	1	0.5	0
Sousa 1980, Biggs et al. 1999, Herbert et al. 1999	$\frac{D_0}{C_0} \times 100$		-400	-100	-50	0	50	100
Wardle et al. 2000	$\frac{D_0}{P_0}$		-0.8	-0.5	-0.33	0	1	∞
Our index	$1 - \frac{2 \times \lvert D_0 \rvert}{C_0 + \lvert D_0 \rvert}$		-0.6	0	0.33	1	0.33	0

Indices calculated assuming that C_0 (the value of the response variable for the undisturbed control soil at the end of the disturbance) = 40. P_0 = the value of the response variable for the disturbed soil at the end of the disturbance. $D_0 = C_0 - P_0$. See Fig. 1 for details.

Table 2: Comparison of the performance of different indices of resilience

Source	Formula	Values of parameters					
		P_x:	200	80	60	40	20	0
		C_x - P_x:	-160	-40	-20	0	20	40
O'Neill 1976[a]	$\sqrt{\sum_{t=i} \frac{D_x^2}{C_x}}$		25.3	6.32	3.16	0	3.16	6.32
Kaufman 1982	$\frac{P_x}{C_0}$		5	2	1.5	1	0.5	0
Griffiths et al. 2000; 2001	$\left(C_x - \frac{P_x}{C_x}\right) \times 100$		3500	3800	3850	3900	3950	4000
Sousa 1980, Tilman 1996[b], Herbert et al. 1999	$\frac{D_x}{D_0}$		-8	-2	-1	0	1	2
Our index	$\frac{2 \times \lvert D_0 \rvert}{\lvert D_0 \rvert + \lvert D_x \rvert} - 1$		-0.78	-0.33	0	1	0	-0.33

Indices calculated assuming that $C_0 - P_0 = 20$ and that the undisturbed, control soil $C_x = 40$. P_x = value for the response variable in the disturbed soil at time x after the end of the disturbance. $D_x = C_x - P_x$. Other symbols are the same as in Table 1. See Fig. 1 for details.

[a] For reasons of simplicity it was assumed that $t_x = 1$ day after the end of the disturbance.

[b] Tilman 1996 uses C_0 - P_x rather than C_x - P_x (i.e. D_x) but we have combined this index with the index of Sousa 1980 and Herbert et al. 1999 as all three indices would give identical values under the conditions that we have defined.

2.6 Example using real data

To illustrate the use of our indices with real data, we used a wetting-drying event as a model disturbance. Wetting-drying events are a common occurrence in soils (Kieft et al. 1987, Fierer and Schimel 2002). They are recognised as one of the major drivers of soil microbial turnover and therefore have an impact on soil function (West et al. 1988a, Wardle and Parkinson 1990, Fierer and Schimel 2002). To illustrate the ability of the indices to distinguish between different soils, we used three soils with different characteristics. Two of the soils had low organic matter contents, and were of the same origin (pasture soil (C = 4.5%, N = 0.3%, pH = 5.2) from Lincoln, New Zealand (43° 30' S)), but had been planted with either clover (*Trifolium repens*) or plantain (*Plantago lanceolata*) for a 15-month period. The third soil was a humus soil (C = 38%, N = 1.6%, pH = 3.7) formed under podocarp forest in the Westland province of New Zealand (43° 20' S). As the pasture and humus soils have different abilities to hold water, we standardised the disturbance by air-drying each soil from 55% of its water holding capacity (WHC) to 10% of its WHC, incubating it in that state for 18 h at 25°C and then returning it to 55% WHC. One hundred percent WHC was defined as the amount of water held in each soil after it had been saturated and then allowed to drain overnight. A control soil was maintained in the incubator at 55% WHC throughout the experiment. We measured substrate-induced respiration (SIR) (0.02 g glucose/g d.w.) (Anderson and Domsch 1978) over a 3 h time period every 12 h for 6 days and then every 24 h for a further 3 days in the case of the pasture soils. For the humus soils, SIR was measured every 12 h for 7 days to allow for the extra time required to dry the soil, and then at 24 h or longer intervals over the following 9 days.

The disturbed soil subsample for both the clover and plantain soils showed an initial decrease in SIR relative to the control soil subsample (Fig. 3a, b at 24 h), but subsequently returned to, or near to, control levels in the next 4 days. The SIR of the humus soil did not on average decrease much following the disturbance when differences between the control and dried soil on day 0 are compared (Fig. 3c at 48 h), but took much longer to recover from it. We calculated resistance and resilience using our indices for each of these soils

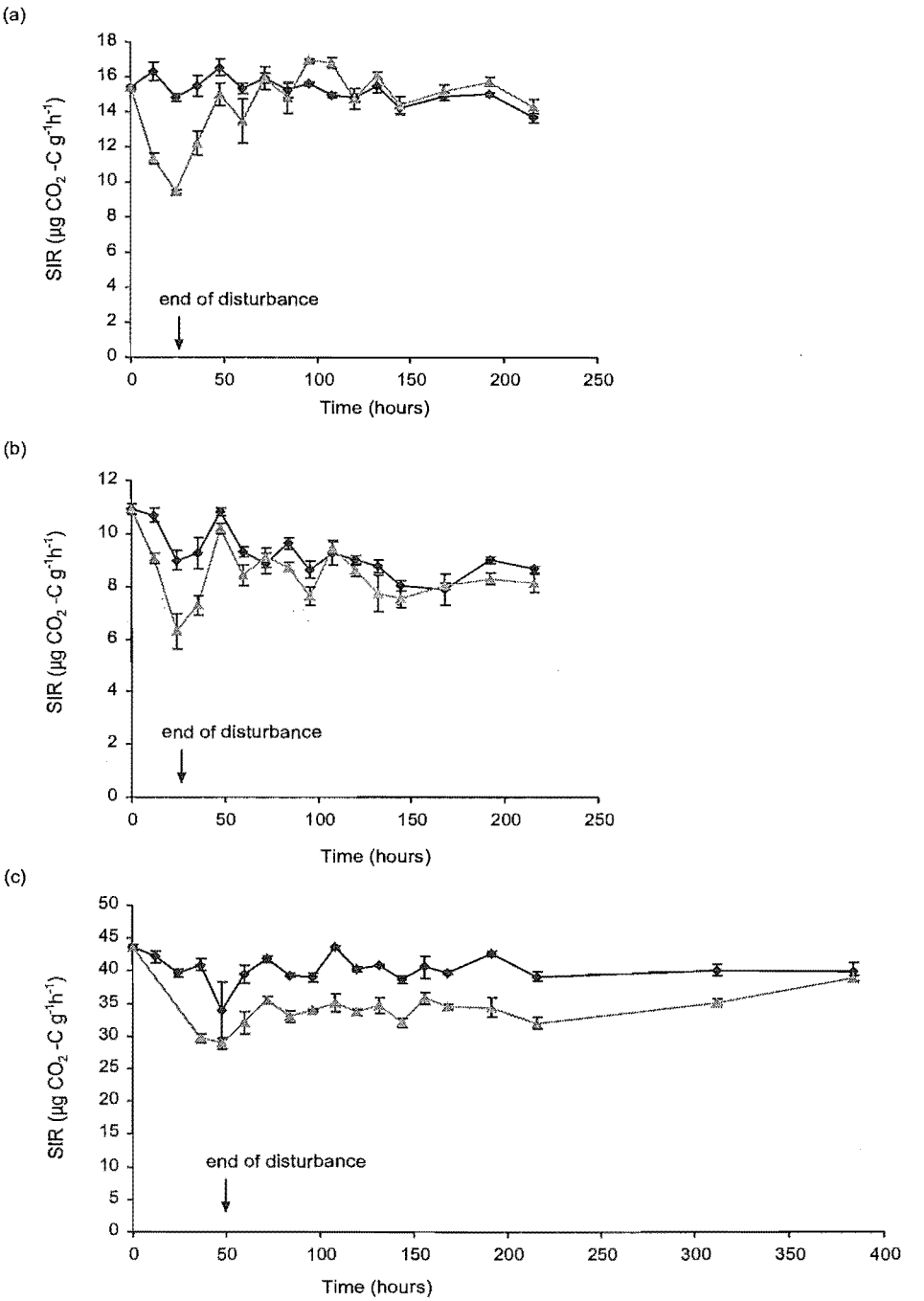


Fig. 3: Response of SIR to air-drying from 55% water holding capacity (WHC) to 10% WHC for (a) soil that had been planted with plantain, (b) soil that had been planted with clover and (c) humus soil. The soil was returned to 55% WHC at 24 h after the initiation of the disturbance for the plantain and clover soils and at 48 h for the humus soil; this represents t_0. The dark line is the control soil (*C*) and the disturbed soil is the pale line (*P*). Vertical bars represent standard errors.

Table 3: Effect of the three different soils on the ability of SIR to resist and recover from a drying disturbance, as assessed by analysis of variance.

	Average index value				
Recovery time (h)	Plantain	Clover	Humus	F *statistic*	P *value*
0[1]	0.47	0.56	0.65	3.27	0.0735
72	0.60a	0.37ab	-0.01b	5.22	0.0255
108[1]	0.70a	0.37ab	0.04b	5.76	0.0176
144	0.80a	0.37ab	-0.09b	7.74	0.0069

Resistance (recovery time = 0 h) was expressed as $1-\frac{2\times|D_0|}{C_0+|D_0|}$ and resilience as $\frac{2\times|D_0|}{|D_0|+|D_x|}-1$ (recovery times = 72, 108, 144 h). See Fig. 1 for symbols in equations. Plantain and Clover refers to soils that had been planted with these plants for 15 months. Within each row, means associated with the same letters are not significantly different from each other at $P = 0.05$ according to the Least Significant Difference test.

[1]Kruskal-Wallis ANOVA used for analysis as variances were not homogenous, parametric ANOVA were used for the other analyses.

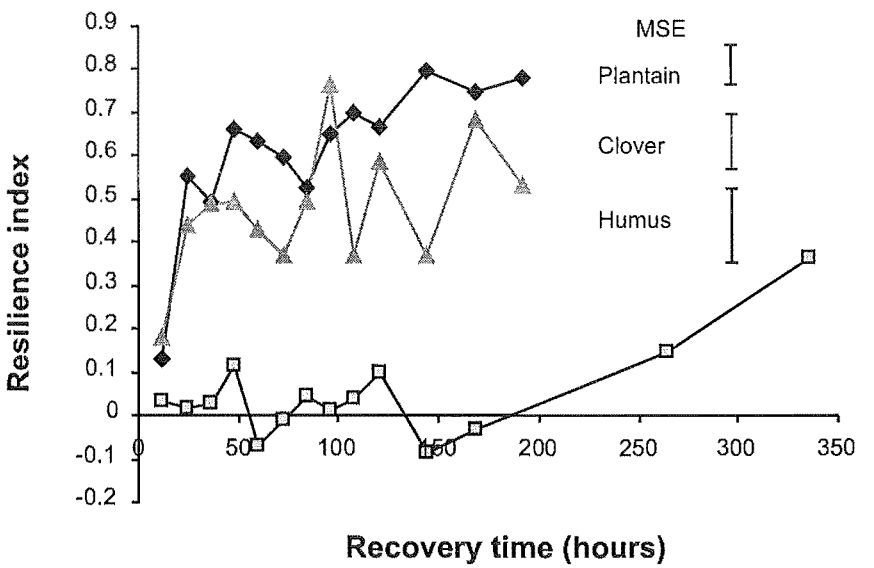


Fig. 4: Change in the resilience index for SIR of the three soils depicted in Fig. 3 over time. Black diamonds = soil that had been planted with plantain, grey triangles = soil that had been planted with clover, and dark squares = humus soil.

and compared them using ANOVA (Table 3). Resistance was not significantly different between soils at alpha = 0.05, although the general trend showed that humus > clover > plantain. It should be noted that for the humus soil, the SIR value used for the control on day 0 (48 hours) was lower than its SIR values both before and after that time point. This suggests that it may be more accurate to use an average from several time points to quantify the SIR of the control soil. The resilience index showed that the rate of recovery of the soil microbial SIR in the plantain and clover soils was initially rapid, and then decreased (Fig. 4). It suggests that the SIR of the humus soil did not begin to show clear signs of recovery until 144 h after the disturbance (Fig. 4). The resilience index never reached 1 (full recovery) in any of the soils, despite the average SIR values suggesting full recovery in many instances. This is because all five replicates never showed full recovery at any one time, resulting in an average index value of less than one at each measurement point. The resilience index was able to distinguish statistically between the resilience of the three soils at various time points. For example, at t = 72, 108 and 144 h, the resilience of the SIR of the plantain soil was different to that of the humus soil but not of the clover soil (Table 3).

2.7 Conclusion

We have shown that our indices give an accurate description of how a soil community responds to a disturbance, and that they can distinguish between different soils. We have used a disturbance involving drying soil as an example, but the indices could equally be applied to the response of soil biotic properties to other disturbances such as cultivation, pollution, pesticide addition, heating and freezing. For some of these disturbances the response of the soil community may be gradual (e.g. pesticide addition). This may make it difficult to predict the point where the difference between the control and disturbed soil will be the greatest, and therefore make it difficult to measure resistance accurately. In this situation it may be best to measure the response variable at several points during the disturbance, and apply the resistance index to the maximum recorded deviation between the control and disturbed soil. For all disturbances, it is important to precisely define the

disturbance used and the way that resistance or resilience was measured. It is also important to carefully consider the appropriateness of the response variables used. We envisage that these indices could be useful in studies that, for example, compare soils across fertility or disturbance gradients, or examine the role of changing environments and climates in ecosystem function. Response variables could include anything from diversity indices to soil respiration or soil chemical properties. The resilience index can be used to quantify resilience at either one or more specific time points after the end of the disturbance or to create a curve of relative recovery, as was presented here. The former has advantages if time is a limiting factor or for large-scale experiments, but requires some preliminary work to determine a suitable point of time for the measurement of resilience. Choosing a specific time point or points will not be meaningful when the system is likely to oscillate significantly during recovery. Neither will the two proposed indices be suitable where information is required on the direction of the community's response to the disturbance. In conclusion, we have shown that our indices have several advantages over other indices, and that they function well with a real data set. We anticipate that these indices will enable different soils and different studies to be quantitatively compared more easily than is currently possible.

Chapter 3: Context-dependent changes in the resistance and resilience of soil microbes to an experimental disturbance for three primary plant chronosequences

3.1 Abstract

The extrinsic factors that regulate soil microbial stability (resistance and resilience) are little understood, even though soil microbes are important drivers of ecosystem function and their stability is likely to affect soil carbon storage and plant nutrient availability. Soils were collected across three primary plant chronosequences (two in New Zealand and one in Hawaii) that differed in climate, parent material and time spans to test the following hypotheses: i) there is a trade-off between the resistance and resilience of key soil microbial response variables, ii) this trade-off is related to the relationship of soil microbial resistance and resilience to soil resources, iii) resources change predictably during different primary plant chronosequences, and iv) if the first three hypotheses hold and are consistent for all three chronosequences, then soil microbial resistance and resilience should change predictably during different primary plant chronosequences. Results showed that although there was a trade-off between resistance and resilience, the role of resources in determining this was unclear. Within each chronosequence, resources that were positively related to resistance were negatively related to resilience and vice versa, consistent with our second hypothesis. However, the direction and strength of correlations between stability and soil resources depended strongly on which soil microbial response variable was measured, and the chronosequence it was measured in. Total amounts of resources often showed consistent trends with ecosystem development for each chronosequence, but the way that resource quality changed varied between chronosequences. At least partly because of the variable nature of these relationships, the trajectory of resistance and resilience during ecosystem development varied considerably across chronosequences. Thus, although consistent trends were found within each chronosequence, the relationships

between the stability of different soil microbial response variables, resources and ecosystem development depended strongly on which chronosequence was considered.

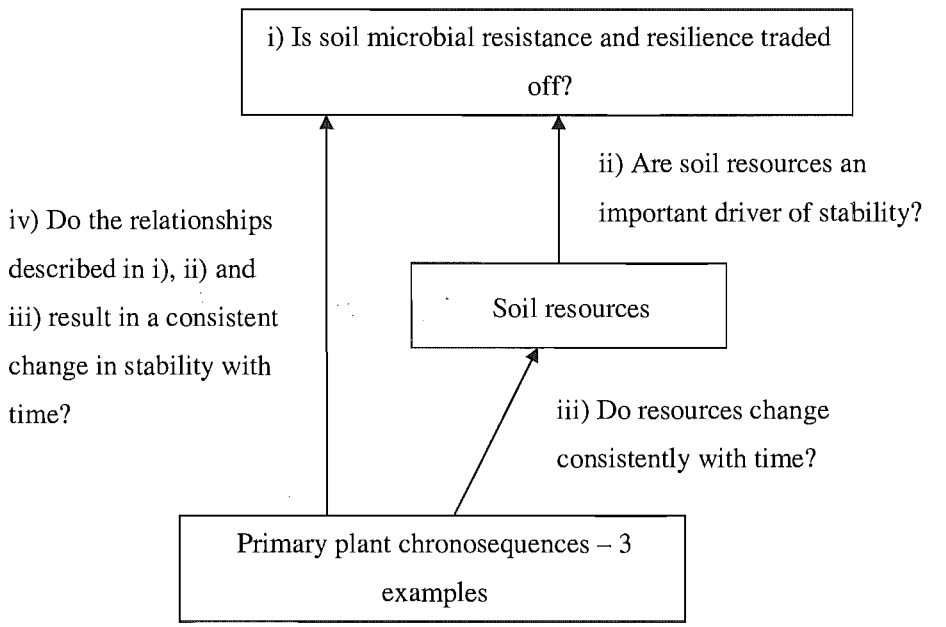


Fig. 5: Flow diagram of the relationships and hypotheses examined in Chapter 3.

3.2 Introduction

Ecological stability describes how communities respond to disturbance and can be defined as consisting of two components: resistance (the amount of change caused by a disturbance), and resilience (the rate of recovery following a disturbance) (Pimm 1984). Most research to date has focused on the stability of plant (e.g. McNaughton 1977, Tilman 1996) and aquatic (e.g. Sousa 1980, Steinman et al. 1990, Steinman et al. 1991) communities. However, soil microbes play a crucial role in ecosystem function by influencing the rate of organic matter decomposition and nutrient mineralisation, and therefore soil carbon (C) storage (Swift et al. 1979) and plant productivity (Jonasson et al. 1996a). The ability of soil microbes to resist and recover from a disturbance that disrupts these processes may therefore have consequences for ecosystem functioning. For this reason, it is important to understand how soil microbes respond to disturbance, and the factors that control this response.

A potential factor that may control soil microbial stability is the life history strategy of soil microbes within the soil microbial community. In plant communities, slow-growing species tend to be resistant but not resilient, and fast-growing species tend to be resilient but not resistant (Grime 2001). Soil microbes can also be divided into two groups with either fast or slow growth rates (Gerson and Chet 1981), suggesting that soil microbial resistance and resilience may be traded-off in a similar way to plants. One of the mechanisms that has been proposed to explain this trade-off for plants is adaptation to low belowground resources, as this tends to result in characteristics that confer high resistance, but low resilience (Lepš et al. 1982, MacGillivray et al. 1995). Theories and empirical studies also suggest that the resources in the soil before the disturbance may have an impact on soil microbial stability. For example, it has been predicted that higher amounts of soil C increases resilience (DeAngelis et al. 1989), and higher soil C and total nitrogen (N) increases resistance (Wardle 1998). The resilience of a soil system may also depend on pre-disturbance resource quality (Bosatta and Berendse 1984), and availability (Moore et al. 1993). Therefore, the amount and quality of resources in soil before a disturbance may affect the resistance and resilience of the soil microbial community.

Ecosystem development provides an ideal framework to examine the effect of pre-disturbance resources on soil microbial stability. Analysis of chronosequences, a series of soils that vary in their age but not in other soil forming factors (McLaren and Cameron 1990), shows that the amount of resources in the soil tends to increase as the system builds up towards a climax phase (Crews et al. 1995, Berendse et al. 1998, Schlesinger et al. 1998, De Kovel et al. 2000). If the system remains undisturbed for long enough, it can go into a retrogressive phase during which the amount of resources typically declines (e.g. Crews et al. 1995, Schipper et al. 2001). These changes in the amount of resources are often accompanied by changes in soil pH (Berendse et al. 1998, De Kovel et al. 2000, Merilä et al. 2002) and the quality of resources as measured by the ratios of C:N, C:phosphorus (P) and N:P (Scheu 1990, Crews et al. 1995). If the stability of soil microbes is governed by resource quantity and quality in the predictable way suggested, and if resources change in a predictable way during ecosystem development, then soil microbial resistance and resilience should show predictable trends across different chronosequences.

For the purposes of this study, a wetting-drying event on incubated soil samples was used as a model disturbance. Wetting-drying events are common disturbances in soils (Kieft et al. 1987, Fierer and Schimel 2002) and are one of the major drivers of soil microbial turnover (West et al. 1988a, Wardle and Parkinson 1990, Fierer and Schimel 2002). They generally result in the death of a portion of the soil microbial biomass and a flush of available C, N (Birch 1958, Birch 1959) and P (Turner and Haygarth 2001). This flush of C, N and P can be lost from the system by leaching (Turner and Haygarth 2001) or taken up by plants and microbes (Bottner 1985). The ability of soil microbes to resist and recover from a wetting-drying event could therefore affect the total amount of resources stored in soil, and the predictability of plant nutrient supplies.

We used soils from three primary plant chronosequences to examine whether the resistance and resilience of soil microbes to a wetting-drying event are traded-off, and whether this trade-off is related to changes in resource quantity and quality during ecosystem development. Specifically, we hypothesised that: i) soil microbial resistance and resilience to a wetting-drying event are negatively correlated along each chronosequence, ii) the trade-off in soil microbial resistance and resilience is related to changes in pre-disturbance resources during ecosystem development, iii) resource quantity and quality change

predictably during the three chronosequences in this study, and finally, iv) if there are consistent correlations between stability and resources, and resources change consistently during ecosystem development, there will also be a predictable change in soil microbial resistance and resilience with ecosystem development.

3.3 Materials and Methods

3.3.1 Soil and sites

Three primary plant chronosequences were chosen for this study, two from the Westland area of New Zealand (Kokatahi and Franz Josef), and one from the Hawaiian archipelago (Table 4). Some basic soil characteristics are given in Table 5.

The Kokatahi chronosequence consists of soils from landslides and floodplains in the Kokatahi Valley identified by Bellingham *et al.* (2001). This sequence covers early ecosystem development from colonisation to senescence of the primary coloniser *Carmichaelia odorata.* Five independent 50-m^2 plots were selected for each stage which are as follows: A) Stage 1: at least one *Carmichaelia odorata* individual per plot but less than 5% vegetative cover, B) Stage 2: 20-40 individuals of *C. odorata* per plot, but less than 50% cover, C) Stage 3: > 50 individuals of *C. odorata* per plot, and D) Stage 4: at least one senescent *C. odorata* individual per plot, < 50 total individuals and < 50% cover *C. odorata.* Five soil samples (0 - 5 cm depth) were collected from each 50-m^2 plot and pooled during March 2000. This chronosequence is relatively short, and represents the build up phase of ecosystem development only. Soil classifications were unavailable for the Kokatahi sequence.

The Franz Josef sequence consists of a series of glacial terminal moraines deposited by the Franz Josef glacier during its retreat over the last 22, 000 years. The parent material is predominantly schist (Stevens 1968). The stage names, ages (as defined by Stevens 1968), vegetation (Wardle and Ghani 1995) and New Zealand soil classification (Richardson et al.

2004) were: A) Stage IV: Hokitika Recent soil, *Carmichaelia grandiflora – Olearia avicenniaefolia* shrubland, ca. 25 years, B) Stage VII: Hokitika Recent soil, *Griselinia littoralis – O. avicenniaefolia* short forest, 130 years, C) Stage X: Ikamatua Brown soil, *Metrosideros umbellata – Weinmannia racemosa* forest, 500 years, D) Upper Wombat: Waiuta Podzol, *M. umbellata – W. racemosa – Dacrydium cupressinum – Podocarpus totara – Prumnopitys ferruginea* forest, 5000 years, E) Mapourika: Waiuta Podzol, *W. racemosa – D. cupressinum - P. ferruginea,* 12,000 years, and F) Okarito: soil classification unavailable, *Phyllocladus trichomanoides – Dacrydium colensoi – Leptospermum scoparium – P. totara – Coprosma* spp., 22,000 years. For each of the six stages, three independent 20 x 20 m plots were sampled during March 2000. Five soil samples to 5 cm depth were collected per plot and pooled. This sequence includes both the build up and retrogression phase of ecosystem development (Wardle and Ghani 1995).

The Hawaiian chronosequence is the same as that used by Crews et al. (1995), Vitousek et al. (1995), and Harrington et al. (2001), and represents ecosystem development on volcanic tephra of different ages. All stages in this sequence were dominated by *Metrosideros polymorpha.* The stage names, ages, and soil classifications (Crews et al. 1995) were as follows: A) Thurston: Hydric Dystrandept, 300 years, B) Olaa: Typic Hydranept, 2,100 years, C) Laupahoehoe: Typic Hydrandept, 20, 000 years, D) Kohola: Typic Placandept 150, 000 years, E) Molokai: Petroferric Acrohumox, 1.4 x 10^6 years and F) Kokee: Plinthic Acrudox, 4.1 x 10^6 years. Four replicate 20 x 20 m plots were set up at each stage, and five soil samples taken from each to a depth of 5 cm, during June 2000. This sequence includes the build up and retrogression phase of ecosystem development.

All soil samples were sieved to 4 mm and stored at 4°C until use. To determine changes in soil chemical properties across each chronosequence, the following was measured for each sample: pH (1:1 in water, based on the methods described by Mc Lean 1982), total C and N (using Leco, Laboratory Equipment Corporation, St Joseph, Michigan, U.S.A.), P and bicarbonate-extractable P (referred to as Olsen P) (Blackmore et al. 1987). The measurements of total C, N and P were interpreted as indicators of resource quantity, and the ratios of these variables as indicators of resource quality.

3.3.2 The disturbance and stability measure used

Stability was measured as the response (resistance and resilience) of the soil microbial community to a wetting-drying event and compared to an undisturbed control soil. Wetting-drying events involve two different disturbances: drying the soil, and then rewetting the dry soil (Kieft et al. 1987). For our purposes we have concentrated mainly on the response of the soil microbes to drying. However, we also calculated a relative measure of the resistance of soil microbes to rewetting dry soil based on measurements made in the 6 h after the soil was rewet.

To obtain a method that resulted in an equivalent drying disturbance across soils with different abilities to hold water, the water holding capacity (WHC) of each soil sample was determined, based on methods described in Saetre (1998). Water was added to soil in a tray until the soil was saturated. The soil and water were then placed in a container (8 cm in diameter, 12 cm in height) with a 1.5 cm diameter hole in the bottom covered by two layers of 2 mm mesh. The top of the container was sealed with plastic to reduce evaporation from the surface, and the soil was allowed to drain overnight in a 4°C fridge. The moisture content of the soil on a dry weight basis at this point was termed the water holding capacity (WHC) of the soil. Based on preliminary experiments, the drying soil disturbance was defined as drying the soil from 55% of its WHC to 10% of its WHC, and incubating it at 25°C in this condition overnight. The rewetting dry soil disturbance discussed above was defined as rewetting the soil from 10% WHC to 55% WHC. A relative measure of resilience to drying soil was defined as the amount of recovery that had occurred 3 days after the dried soil had been returned to 55% WHC, based on preliminary data (not presented) and the data presented in Chapter 2.

Four response variables were used to measure the resistance and resilience of the soil microbial community: basal respiration, substrate-induced respiration (SIR), glucose use and soil mineral N contents. In combination, these response variables were intended to give a summary of the soil microbial response to the wetting-drying event.

Basal respiration was measured as described by Wardle (1993). A known quantity of soil was placed in a 130-ml airtight container, and incubated at 25°C. The amount of microbial

respiration was measured by taking 1-ml subsamples of head space at 1 and 3 h after capping the container, and injecting them into an infrared gas analyser (Wardle 1993). Basal respiration measured on dried soil and on rewet dry soil was interpreted as indicating the response of soil microbial activity to the changes in resource availability that occur during wetting-drying events.

Substrate-induced respiration was measured as for basal respiration except the soil was amended with 0.02 g glucose/g dry weight before incubation (methods based on Anderson and Domsch 1978). When SIR is measured on wet soil, it gives an indication of the active microbial biomass (Anderson and Domsch 1978). Therefore, SIR measurements made on rewet dry soil were interpreted as indicating the effect of drying on the soil microbial biomass. Substrate-induced respiration was also measured on dried soil. Because the added glucose remains undissolved in dry soil, it is largely unavailable for soil microbial metabolism (West and Sparling 1986). Any measurements of resistance or resilience that included SIR measured on dry soil were therefore interpreted as indicating the effect of drying or rewetting on the ability of disturbed organisms to respond to added substrates, rather than as an indication of biomass. To distinguish between these two measures using SIR, the latter measurement will be referred to in terms of the resistance and resilience of soil microbial glucose use from here onwards, and the former in terms of the resistance and resilience of SIR.

Mineral N was measured using the methods described by Keeney and Nelson (1982). When soil is dried and rewet, there is usually a flush of mineral N. This N probably comes from two sources: the soil microbial biomass, either through the mineralisation of killed biomass or osmoregulants (Kieft et al. 1987), and from changes that can occur in organic matter, which allow increased decomposition and nutrient mineralisation (Birch 1959, Bottner 1985). Both sources are dependent on soil microbes actively mineralising organic N, and therefore mineral N measured on the dried and rewet soil was interpreted as indicating changes in soil microbial activity in response to the disturbance.

Resistance

Each soil sample was adjusted by either adding water or air-drying to 55% WHC and allowed to equilibrate in a 25°C incubator overnight. This adjustment in most of the higher organic content soils required a change in moisture content equivalent to less than 10% WHC. It is therefore likely that these soils will have recovered from this initial moisture content adjustment before our drying disturbance was imposed. The moisture content of early successional soils with a high gravel component (especially for the Kokatahi sequence) was changed by up to 50% WHC. However, given that these soils should be adapted to fast wetting-drying events, an overnight equilibration period should be sufficient to allow them to adjust to their new moisture content. We therefore consider that the soil microbes in each soil will have recovered sufficiently after overnight equilibration for the initial change in moisture content not to affect their response to the disturbance used to quantify resistance and resilience.

Subsamples of equilibrated soils were spread out on paper trays to air-dry at room temperature to 10% WHC over up to 2 days. After this, the soil that had remained at 55% WHC (the control soil (C)) and the dried soil (the disturbed soil) were each divided into two subsamples, placed in 125-ml Erlenmeyer flasks, sealed with plastic film and incubated overnight at 25°C. This time point was defined as the end of the drying disturbance and termed time 0 or t_0. One flask containing control soil and one flask containing disturbed soil were then destructively harvested for the determination of resistance to the disturbance at t_0. Basal respiration, SIR and mineral N were measured on subsamples of the control soil. The disturbed soil was divided into two portions. Subsamples from the first portion were used to measure basal respiration, SIR and mineral N contents on dry soil. Subsamples from the second portion were used to measure basal respiration and SIR immediately after the dried soil had been returned to 55% WHC. Resistance was calculated using the index described in Chapter 2.

To measure the effect of drying on the resistance of soil microbial response variables, C_0 was defined as the value of the control soil that had remained at 55% WHC throughout the

disturbance period. To measure the effect of rewetting dry soils on soil microbial response variables, C_0 was defined as the value of the dry soil before it was returned to 55% WHC.

Resilience

The remaining flask containing dry soil was returned to 55% WHC by adding the required amount of water with a syringe, incubated for a further three days (t_3) and along with the other flask containing control soil, was used to measure soil microbial resilience to drying soil at t_3. Basal respiration, SIR and mineral N contents were measured on subsamples from both flasks. Resilience was calculated as described in Chapter 2. As we only calculated resilience in relation to the drying disturbance, C_x was in all cases the soil that had remained at 55% WHC throughout the experiment.

3.3.3 Data analysis

Pearson's correlation coefficients were used to determine the relationship of soil microbial resistance and resilience to soil resources, and of resistance to resilience within each chronosequence. This was done for all response variables measured. Data were transformed as required using log and square root. Where data could not be transformed to a normal distribution, Spearman's Rank correlations were used instead. Trends during ecosystem development were analysed by regression. Normality was determined for each regression and residuals checked. Quadratic and linear models were fitted to the data, and the model of best fit was used.

3.4 Results

3.4.1 Response of soils to the wetting-drying event

Basal respiration and glucose use of soils from all three sequences were reduced by drying (Appendix I). Rewetting dry soil resulted in an increase in basal respiration in all soils. Soil microbial biomass, measured as SIR on rewet dry soil, was generally reduced by the disturbance, and the amount of mineral N in the soils was generally increased by drying. On day 3, basal respiration, glucose use and SIR had on average recovered to control levels, although there were some exceptions (Appendix I). However, the amount of mineral N in the soils had rarely recovered, and was often higher than it had been on day 0.

3.4.2 Relationships between soil microbial stability and resources

There were negative correlations between resistance and resilience for nearly all soil microbial response variables at all three sequences (Table 6). The strongest of these relationships was between the resistance and resilience of SIR for all three sequences, and the resistance and resilience of basal respiration for the Franz Josef sequence.

The nature of the relationship between the resistance of basal respiration to drying and soil resources varied across the different sequences (Table 7). The resistance of soils from the Kokatahi sequence showed a negative relationship to the ratios of C:N and C:P. In contrast, the resistance of basal respiration for the Franz Josef sequence was positively related to the ratios of C, N and P. The resistance of basal respiration to drying for the Hawaii sequence was not significantly correlated with any of the soil resources measured (data not presented). However, the resistance of soil microbial SIR to drying was more strongly related to soil resources for the Hawaii sequence than for the other two sequences: resistance increased as total C, N and P increased, but decreased as pH and the C:N ratio increased. The resistance of SIR for the Franz Josef sequence also decreased as the C:N ratio increased. The resistance of SIR for the Kokatahi sequence was negatively related to pH, but positively related to Olsen P. The relationship of the resistance of soil microbial

glucose use with resources was strongest for the Franz Josef sequence, with positive correlations between resistance and the ratios of resources, and a negative correlation with pH. In contrast to this, resistance showed a positive relationship with pH for the Hawaii sequence, as well as a positive relationship with Olsen P. There were no significant relationships between the resistance of glucose use and resources for the Kokatahi sequence (data not presented). The resistance of mineral N contents for soils from the Kokatahi sequence was largely unrelated to resources. However, soils from the Hawaii sequence showed an increase in resistance as total resources increased, and a decrease as pH increased. Soils from the Franz Josef sequence showed the opposite relationship between soil pH and the resistance of mineral N contents to that found for the Hawaii sequence. The resistance of mineral N contents was negatively related to the ratios of resources, and positively related to total P for the Franz Josef sequence.

When resistance was expressed as the amount of change in soil microbial response variables caused by rewetting dry soil, the resistance of basal respiration during the Kokatahi sequence was strongly related to soil resources (Table 8). Correlations between resistance and total resources or the ratios of those resources were negative, while the correlation between resistance and pH was positive. In contrast to this, the resistance of basal respiration to rewetting for the Franz Josef sequence was negatively related to pH and positively related to the ratios of soil resources and total C. Correlations between resistance and resources for the Hawaii sequence were generally not significant at $P < 0.05$, except for pH which showed a positive relationship to resistance. Similar relationships between resistance and soil resources were found for the resistance of glucose use to rewetting dry soil for the Franz Josef and Hawaii sequences. However, soils from the Kokatahi sequence showed no significant correlations (data not presented). The resistance of basal respiration and glucose use to drying and to rewetting dry soil showed similar relationships with resources within sequences.

The resilience of soils to drying was less strongly related to resources than resistance was (Table 9). There were no significant correlations between resources and the resilience of the soil microbial response variables for the Kokatahi sequence at $P < 0.05$ (data not presented). For the soils from the Franz Josef sequence, the resilience of basal respiration increased with total P, and decreased as the ratios of C:P and N:P increased. For the

Hawaii sequence, the resilience of this response variable increased as pH increased, but decreased as total C increased. The resilience of soil microbial SIR for the Hawaii sequence decreased as the amount of some resources increased, but increased as the ratios of the resources increased. The resilience of the soil microbial SIR for the Franz Josef sequence was only correlated with the C:N ratio. Only soil from the Hawaii sequence showed significant relationships between the resilience of glucose use and resources (Olsen P: $r = -0.4883$, $P < 0.05$, pH: $r = -0.4381$, $P < 0.05$). The resilience of mineral N contents in soils from the Franz Josef sequence were correlated with all resources except total P and the C:N ratio at $P < 0.05$. The correlations between resilience and total C, N, Olsen P or the ratios of the resources were positive, while the correlation between resilience and pH was negative. In contrast, the resilience of soils from the Kokatahi and Hawaii sequences were not significantly related to soil resources at $P < 0.05$.

3.4.3 Changes in soil microbial stability and resources during ecosystem development

Changes in total resources during ecosystem development showed several significant patterns (Table 10). Total C increased during the Kokatahi sequence, and increased and then decreased during the Franz Josef and Hawaii sequences. Total N increased and total P remained constant throughout the Kokatahi chronosequence. Total N and total P both initially increased, but then decreased during the Franz Josef and Hawaii sequences. Soil pH tended to decrease during development for all sequences, but increased slightly towards the end of the Franz Josef sequence. Despite these relatively consistent trends in total resources and pH, the remaining variables showed some different trends for different sequences. Olsen P showed no change during the Hawaii sequence, a positive hump-backed relationship during the Franz Josef sequence and an increase during the Kokatahi sequence. During both the Kokatahi and Franz Josef sequences, the ratios of C:P and N:P increased. However, these variables showed an initial decrease and then an increase during the Hawaii sequence. The C:N ratio showed different trends during development for each of the sequences, with no change for the Kokatahi sequence, an increase for the Franz Josef sequence, and a decrease followed by an increase for the Hawaii sequence.

The relationship of the resistance of soil response variables to drying with ecosystem development varied across sequences (Fig. 6). The resistance of mineral N contents to drying showed significant trends during development for all three chronosequences. However, the trend found for the Franz Josef sequence showed the opposite direction to that found for the other two sequences. The resistance of basal respiration and glucose use to drying only showed a significant trend for the Franz Josef sequence, where it increased with ecosystem development. The resistance of SIR to drying only showed a significant trend over time for the Hawaii sequence, where it initially increased and then decreased. Patterns of change over time in the resistance of soil microbial response variables were stronger for the rewetting dry soil disturbance than for the drying soil disturbance (Fig. 7). The resistance of basal respiration to rewetting dry soil decreased during the Kokatahi sequence, but there was no trend in the resistance of glucose use over time. The resistance of both basal respiration and glucose use to rewetting dry soil decreased during the Hawaii sequence. This decrease mainly occurred during the initial stages of the sequence. In contrast to this, the resistance of soil from the Franz Josef sequence for both response variables increased with time.

The resilience of soils to drying did not change significantly during the Kokatahi sequence (data not presented). Soils from the Franz Josef sequence showed a decrease over time in the resilience of basal respiration, no change in the resilience of SIR, and an increase over time in the resilience of mineral N contents (Fig. 8). Soils from the Hawaii sequence also showed a decrease in the resilience of basal respiration over time, but a hump-backed change over time for the resilience of SIR (Fig. 8).

Table 4: Characteristics of the three chronosequences

	Kokatahi sequence[1]	Franz Josef sequence[2]	Hawaii sequence[3]
Location	West Coast, South Island of New Zealand	West Coast, South Island of New Zealand	Hawaiian islands
Grid references	42°57'S, 171°35'E	43°20'S, 170°10'E	19°-22°N, 155°-160°W
Parent material	Foliated schist	Chlorite schist, biotite schist, gneiss	Basalt tephra
Mean Annual Rainfall	7000 mm	3800 - 6000mm	2500mm
Mean Annual temperature	c. 10°C	11°C	16°C
Elevation	410-500m a.s.l.	50 – 210 m a.s.l.	1100 – 1200m a.s.l.

[1] Data from Bellingham et al. (2001), [2] Data from Stevens (1968) and Wardle and Ghani (1995), [3] Data from Crews et al. (1995)

Table 5: Characteristics of the soils from each stage of each chronosequence.

Kokatahi sequence

Stage	% Carbon	% Nitrogen	pH	WHC[1]
1	0.2 (0.0)	0.01 (0.0)	5.6 (0.1)	22 (1)
2	0.2 (0.0)	0.01 (0.0)	5.4 (0.0)	24 (1)
3	14.5 (6.0)	0.8 (0.3)	4.7 (0.3)	209 (78)
4	18.5 (1.6)	1.0 (0.1)	4.4 (0.1)	222 (20)

Franz Josef sequence

Age (years)	% Carbon	% Nitrogen	pH	WHC[1]
55	3.2 (1.9)	0.2 (0.1)	5.3 (0.3)	61 (24)
130	7.6 (1.7)	0.4 (0.1)	5.0 (0.2)	142 (22)
500	33.7 (2.4)	1.3 (0.1)	3.7 (0.1)	512 (55)
5000	38.4 (1.3)	1.6 (0.1)	3.7 (0.1)	490 (24)
12 000	35.5 (6.3)	1.0 (0.1)	3.8 (0.2)	466 (95)
22 000	13 (1.6)	0.5 (0.1)	4.4 (0.1)	222 (30)

Hawaii sequence

Age (years)	% Carbon	% Nitrogen	pH	WHC[1]
300	47.5 (1.3)	1.9 (0.0)	3.4 (0.0)	506 (50)
2100	37.3 (1.2)	1.6 (0.0)	4.2 (0.1)	563 (15)
20 000	32.0 (3.9)	1.1 (0.1)	4.0 (0.1)	400 (38)
150 000	40.0 (2.1)	1.5 (0.0)	3.4 (0.1)	510 (24)
1 400 000	46.0 (1.6)	2.0 (0.0)	3.6 (0.1)	580 (35)
4 100 000	42.7 (3.5)	2.1 (0.2)	3.4 (0.1)	338 (19)

[1] Water holding capacity, measured as the amount of water retained in a soil after it has been saturated and allowed to drain overnight; expressed as gravimetric water content.

Table 6: Correlation coefficients between the resistance and resilience of soil microbial response variables to a drying disturbance for each sequence.

	Kokatahi	Franz Josef	Hawaii
Basal Respiration	-0.3352	-0.7870***	-0.1285
Substrate-induced respiration	-0.7656***	-0.8765***	-0.5881**
Glucose use	-0.5071*	-0.4952*	-0.3688†
Mineral N contents[1,2]	-0.5465*	-0.4390†	-0.5000*

[1] Spearman rank correlation for the Franz Josef sequence, [2] log transformed for the Hawaii sequence

†P < 0.1, * P < 0.05, ** P < 0.01. *** P < 0.001

Table 7: Pearson correlation coefficients between soil resources and the resistance of soil microbial response variables to drying for each of the three chronosequences.

	Basal respiration resistance[6]		SIR[2] resistance			Glucose use resistance[6]		Mineral N contents resistance		
	Kokatahi	FJ[1]	Kokatahi	FJ[1]	Hawaii[5]	FJ[1]	Hawaii	Kokatahi	FJ[1]	Hawaii
Total C[3]	-0.4453†	0.2738	0.3152	-0.3358	0.6455***	0.4416†	-0.0128	0.2964	-0.4613†	0.4964*
Total N[3]	-0.3043	0.1912	0.3137	-0.1904	0.7832***	0.3380	-0.1736	0.3342	-0.3335	0.5634**
Total P[3,5]	-0.4015†	-0.4595†	0.2165	0.2769	0.6501**	-0.2390	-0.2281	0.0992	0.7152**	0.3576†
Olsen P[3,4]	-0.3248	0.0101	0.4812*	0.1115	0.1319	0.2151	0.4368*	0.3880†	-0.0215	-0.1221
pH	-0.0802	-0.4762*	-0.4976*	0.3277	-0.4778*	-0.6173**	0.4763*	-0.3744	0.4881*	-0.8463***
C:N ratio	-0.6185**	0.5061*	0.0696	-0.5838*	-0.4748*	0.5433*	0.2509	0.1313	-0.7255**	-0.3373
C:P ratio[3]	-0.4526*	0.6833**	0.3323	-0.4525†	-0.1700	0.5841*	0.2071	0.3248	-0.8080***	-0.0055
N:P ratio[3]	-0.2977	0.7389***	0.2241	-0.3686	0.1040	0.6076**	0.1123	0.3323	-0.8679***	0.2125

[1] Franz Josef, [2] substrate-induced respiration. [3] Spearman rank transformed for the Kokatahi sequence, [4] log transformed for the Franz Josef sequence, [5] log transformed for the Hawaii sequence. [6] Correlation coefficients between the resistance of basal respiration and resources for the Hawaii sequence, and between the resistance of soil microbial glucose use and resources for the Kokatahi sequence are not presented, as no correlations were statistically significant.

†$P < 0.1$, * $P < 0.05$, ** $P < 0.01$. *** $P < 0.001$

Table 8: Pearson correlation coefficients between soil resources and the resistance of soil microbial response variables to rewetting dry soil for the three chronosequences

	Basal respiration resistance			Glucose use resistance[5]	
	Kokatahi[2]	Franz Josef	Hawaii	Franz Josef	Hawaii[4]
Total C[1]	-0.8661***	0.4973*	-0.1071	0.5077*	-0.2492
Total N[1]	-0.8170***	0.3856	0.2983	0.3494	-0.3666†
Total P[1,4]	-0.5889**	-0.1795	-0.3659†	-0.2032	-0.3694†
Olsen P[1,3]	-0.8026***	0.2542	0.1695	0.2016	0.2968
pH	0.5845**	-0.6537**	0.4372*	-0.6270**	0.6179**
C:N ratio	-0.6692**	0.6511**	0.3963†	0.6392**	0.2948
C:P ratio[1]	-0.8830***	0.6652**	0.2813	0.6040**	0.1691
N:P ratio[1]	-0.8101***	0.6652**	0.1168	0.5587*	0.0400

[1] Spearman rank correlation for the Kokatahi sequence, [2] log transformed for the Kokatahi sequence, [3] log transformed for the Franz Josef sequence, [4] log transformed for the Hawaii sequence. [5] Correlation coefficients between the resistance of soil microbial glucose use to rewetting dry soil for the Kokatahi sequence are not presented, as no correlations were statistically significant.
†P < 0.1, * P < 0.05, ** P < 0.01, *** P < 0.001

Table 9: Pearson correlation coefficients between soil resources and the resilience of soil microbial response variables to drying for the three chronosequences[3]

	Basal respiration resilience		SIR resilience		Mineral N contents resilience	
	Franz Josef	Hawaii	Franz Josef	Hawaii	Franz Josef	Hawaii
Total C	-0.0889	-0.4644*	0.3231	-0.2648	0.5825*	0.2485
Total N	-0.0384	-0.3832†	0.1855	-0.4413*	0.5614*	0.2411
Total P[2]	0.4864*	-0.1493	-0.2092	-0.7031***	0.1136	0.0610
Olsen P[1]	0.2326	-0.1298	-0.0890	-0.2828	0.4745*	0.3834†
pH	0.2886	0.4822*	-0.2915	-0.0417	-0.6156**	0.3307
C:N ratio	-0.3273	0.0927	0.5219*	0.4332*	0.4113†	-0.0789
C:P ratio	-0.5017*	-0.1685	0.3763	0.5232**	0.5476*	0.0568
N:P ratio	-0.5681*	-0.2543	0.2773	0.3954†	0.5846*	0.1877

[1] Log transformed for the Franz Josef sequence, [2] log transformed for the Hawaii sequence. [3] Correlations coefficients for the Kokatahi sequence are not presented, as no correlations were statistically significant at $P < 0.05$.
†P < 0.1, * P < 0.05, ** P < 0.01. *** P < 0.001

Table 10: Patterns in the change in soil resources over time for the three chronosequences

		Kokatahi	Franz Josef	Hawaii
Total C[1]	R^2	0.6324***	0.7729***	0.5279***
	Relationship	+ve linear	-ve quadratic	-ve quadratic
Total N[2]	R^2	0.8112***	0.8130***	0.7006***
	Relationship	+ve log linear	-ve quadratic	-ve quadratic
Total P[2]	R^2	0.2380*	0.6499***	0.8957***
	Relationship	+ve log linear	-ve quadratic	-ve quadratic
Olsen P[2]	R^2	0.6991***	[1]0.6376***	0.0392
	Relationship	+ve log linear	-ve quadratic	not significant
pH	R^2	0.5737***	0.8252***	0.5366***
	Relationship	-ve linear	+ve quadratic	-ve linear
C:N ratio	R^2	0.1353	0.4957**	0.5874***
	Relationship	not significant	+ve linear	+ve quadratic
C:P ratio[1]	R^2	0.6753***	0.7055***	0.4522**
	Relationship	+ve linear	+ve linear	+ve quadratic
N:P ratio[1]	R^2	0.6995***	0.7854***	0.1359†
	Relationship	+ve linear	+ve linear	+ve quadratic

+ve = positive, -ve = negative, [1] rank or [2] log transformed at Kokatahi
†P < 0.1, * P < 0.05, ** P < 0.01. *** P < 0.00

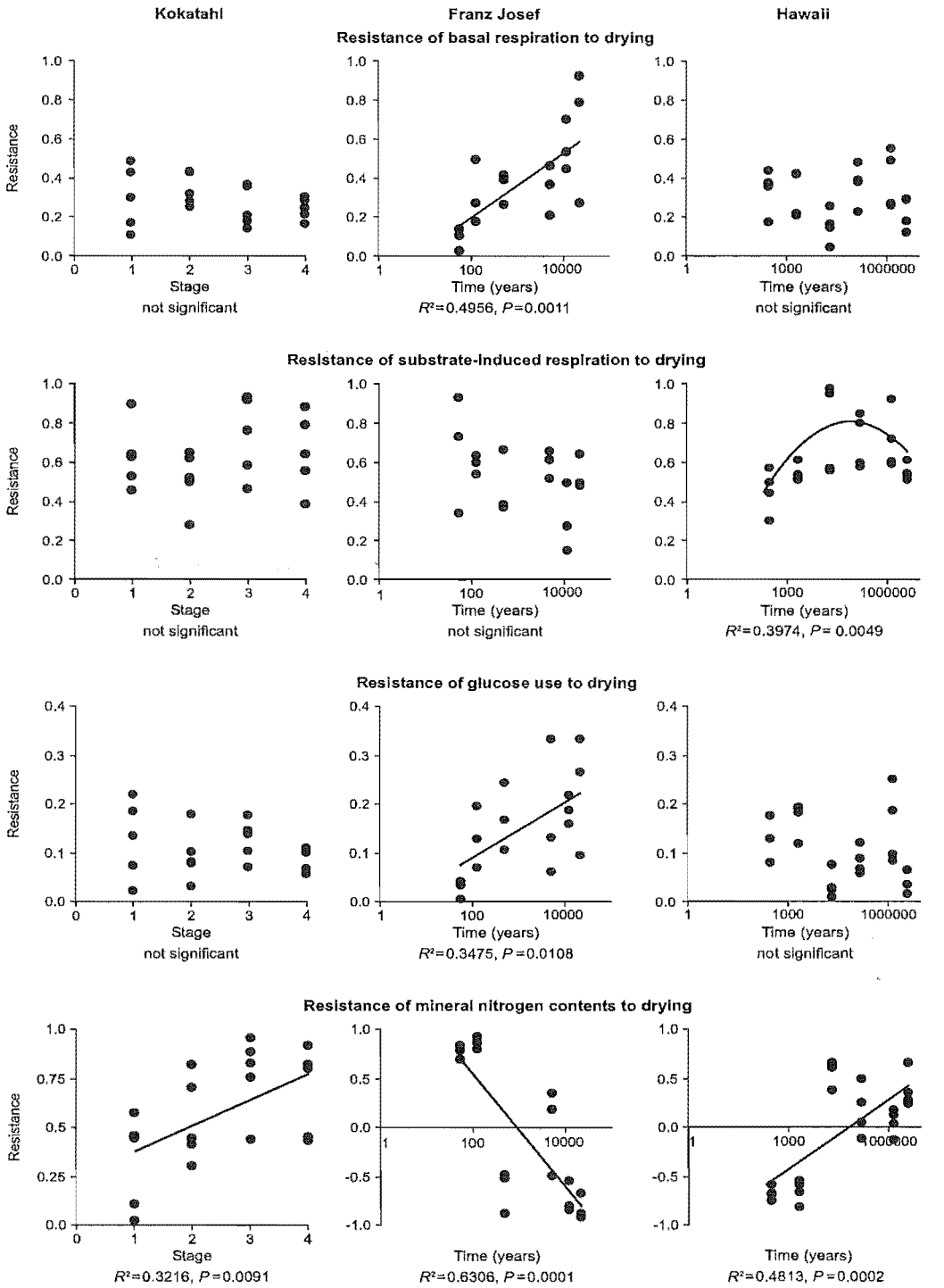


Fig. 6: Changes in the resistance of soil microbial response variables to drying during ecosystem development for the three chronosequences. The best linear or quadratic regression describing patterns over time is presented.

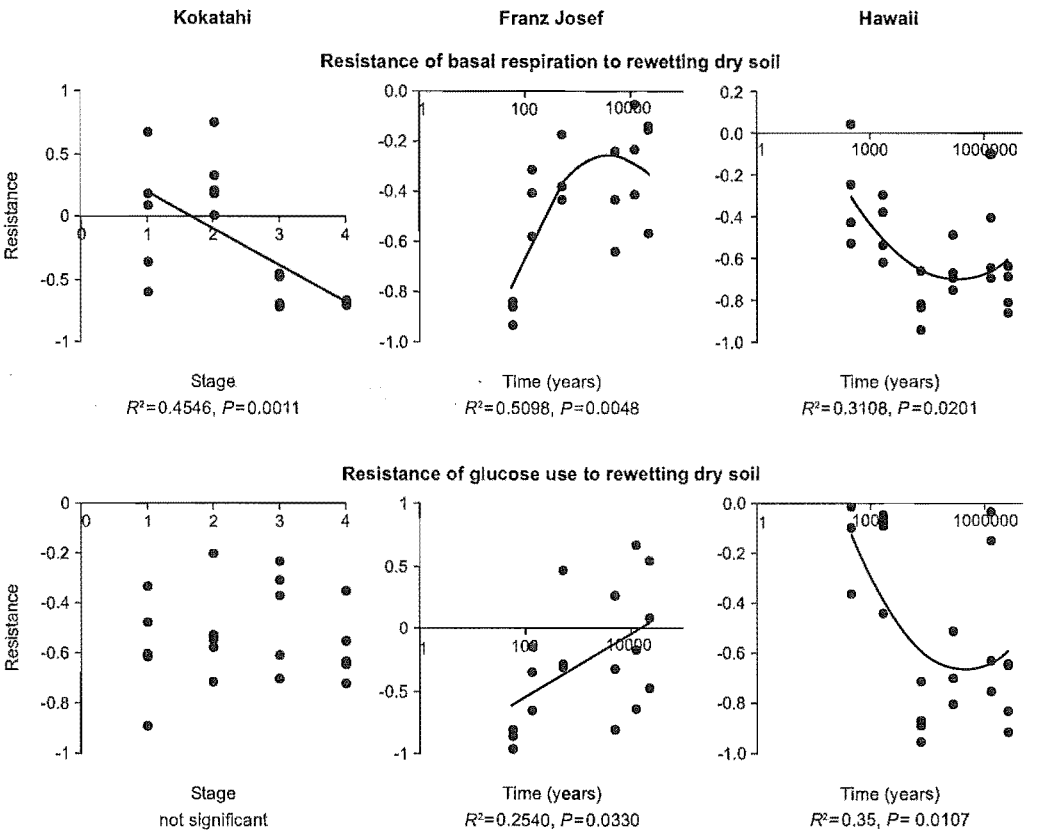


Fig. 7: Changes in the resistance of soil microbial response variables to rewetting dry soil during ecosystem development for the three sequences. The best linear or quadratic regression describing patterns over time is presented.

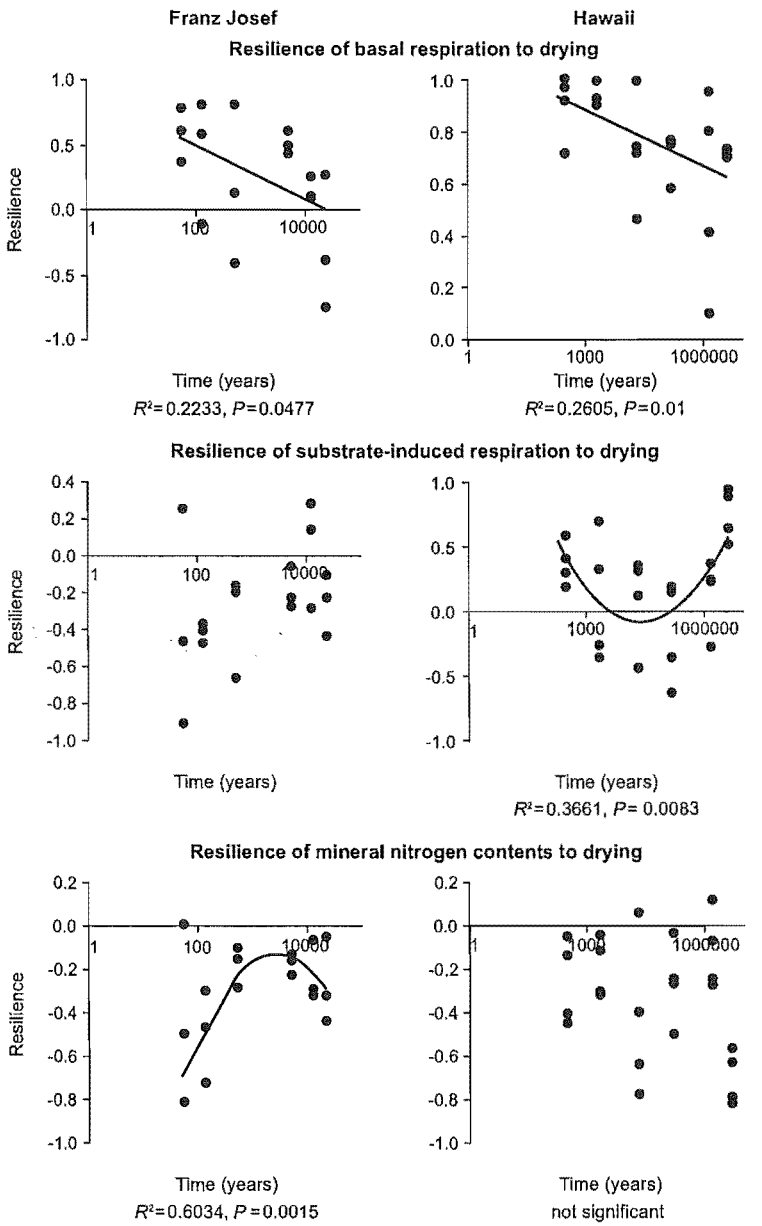


Fig. 8: Changes in the resilience of soil microbial response variables to drying soil during ecosystem development. The best linear or quadratic regression describing patterns over time is presented. The resilience of soils from the Kokatahi sequence are not presented as no significant patterns were found over time at $P < 0.05$.

3.5 Discussion

3.5.1 Relationships between soil microbial stability and soil resources

The stability of soil microbial activity, SIR and mineral N contents showed some consistent trends across the three sequences. Consistent with our first hypothesis, resistance and resilience were negatively correlated for nearly all response variables, for all three sequences (Table 6). This suggests that there may be subsets of the soil microbial community that have different life history strategies (Gerson and Chet 1981), and characteristics associated with resistance or resilience that are traded-off in a similar manner to that found in plant (Lepš et al. 1982, Huston and Smith 1987, Herbert et al. 1999) and aquatic (Sousa 1980) systems. This trade-off may be related to the active and dormant fraction of the soil microbial biomass. Bottner (1985) suggested that the active fraction is killed by drying, and that the dormant fraction survives. The active fraction may represent the fast-growing, r-selected part of the soil microbial biomass, which is less resistant to disturbances, but more resilient because of its fast growth rate. The dormant fraction may represent the slow-growing, K-selected part of the soil microbial biomass, which is resistant to disturbance but recovers slowly.

We hypothesised that the trade-off in the resistance and resilience of the soil microbial community would be related to soil resources. Consistent with this, resources which showed a positive correlation with the resistance of a particular response variable often showed a negative correlation with the resilience of that variable, and vice versa (Table 7, 9). However, the main mechanism underlying these relationships appeared to vary both within and between sequences. Firstly, the importance of resource quantity versus resource quality in driving resistance and resilience varied across the different sequences. Secondly, the stability of a given soil microbial response variable often showed a positive correlation with a resource for one sequence, but a negative correlation with the same resource for another sequence. Thirdly, the stability of several response variables did not show significant relationships to any of the resources measured (Table 7 – 9). Finally, the relationship between the resistance of different response variables to resources within sequences varied, with the resistance of SIR and mineral N contents to drying showing the

opposite relationship to resources to that of the resistance of basal respiration and glucose use to drying and rewetting.

The different relationships of the resistance of SIR and mineral N contents and the resistance of basal respiration and glucose use with resources can be explained by examining what these variables mean in more detail. One of the sources of mineral N released by drying soil is probably from the N contained in the soil microbial biomass killed by the disturbance (Bottner 1985). Therefore, the more biomass killed by the disturbance, the more N released, and the lower the resistance of SIR and mineral N contents. The resistance of basal respiration and glucose use measure different aspects of the activity of the soil microbial community in response to the disturbance, and were therefore related to resources in the same direction. It is logical that these two groups of response variables were related to resources in opposite directions. If a large proportion of the biomass is killed during a disturbance, more mineral N will be released, resulting in activity rates in dry soil that are closer to undisturbed soil rates. When soil is rewet, there is usually a large increase in resource availability (Birch 1959, Skopp et al. 1990). If most of the biomass survives the disturbance, it may be able to increase its activity to a greater extent in response to this increase in resources, than in soils where more of the biomass was killed. In combination, this suggests that the resistance of basal respiration and glucose use will be high when the resistance of SIR and mineral N contents is low in both dry and rewet dry soil, and vice versa, resulting in opposite correlations with resources. Our results are consistent with both aquatic (Steinman et al. 1990, Biggs et al. 1999) and plant (Herbert et al. 1999) studies, which have found that the type of relationship between resources and stability can depend on the response variable measured.

None of the theoretical relationships of resistance and resilience to resources proposed by previous studies (e.g. Bosatta and Berendse 1984, Moore et al. 1993, MacGillivray et al. 1995, Wardle 1998) were supported for all three sequences, and many were directly contradicted for at least one sequence. For example, it has been suggested that higher soil C and N contents should result in a soil microbial biomass with a higher resistance to disturbance (Wardle 1998). However, total C and N were significantly correlated with the resistance of SIR for the Hawaii sequence only, suggesting that these resources are only important for stability in some contexts. Overall, the nature of relationships between

stability and resources appeared to be highly variable, and to depend on the soil microbial response variable and sequence measured.

3.5.2 Changes in soil resources and microbial stability during ecosystem development

The second aspect of this study looked at how soil resources and soil microbial resistance and resilience change during ecosystem development, as represented by the three chronosequences. Although the direction of correlations between soil microbial stability and resources varied among the different sequences, there were consistent patterns within them and some consistent trends across them. Therefore, if resources change in a similar way during each sequence, there may be some consistent patterns of change in resistance and resilience during ecosystem development, as hypothesised. The changes in total C and N generally followed the expected patterns of a build up during the relatively short Kokatahi sequence, and a build up and decline phase during the longer Hawaii and Franz Josef sequences. During all three sequences pH declined over time, although it began to increase again at the end of the Franz Josef sequence. These trends are consistent with results found by other studies at these and other sites (Crews et al. 1995, Berendse et al. 1998, Ohtonen et al. 1999, Bellingham et al. 2001). Despite the consistent changes in total resources during development across sequences, changes in the ratios of C, N and P often changed differently during the development of different sequences (Table 10). This may be because different processes were operating within each sequence, or because they were operating at different rates. Climate, initial fertility, plant species traits, and parent material characteristics are likely to alter the rate of development of different soils (Walker and del Moral 2003), and potentially the rate of change of C, N and P relative to each other. For example, the Kokatahi and Franz Josef sequences were colonised by N-fixing shrubs at the beginning of succession (Stevens 1968, Wardle and Ghani 1995, Bellingham et al. 2001), rather than in mid-succession as occurred at the Hawaii sequence. This would have affected the rate of change of N relative to other soil resources. It is interesting to note that the C:N ratio of soils from the Hawaii sequence changed in the opposite direction during development to that found by Crews et al. (1995) for the same sequence. This may be because the plots sampled by Crews et al. (1995) in mid-succession did not contain *Acacia*

koa (a N-fixing tree), whereas ours did. This again highlights the impact that plants can have on soil resources, as well as the potential problem of spatial variation within stages of the same age during chronosequences (Walker and del Moral 2003).

Given that the relationship of stability to resources and the change in resource quality during development was context-dependent, it is not surprising that there were few consistent relationships between stability and ecosystem development across sequences. Stability was occasionally related to the same resource in the same direction in more than one sequence. However, because these resources often changed differently during each sequence, the way that stability changed over time was also often different between sequences. For example, the C:N ratio showed a positive relationship to the resistance of basal respiration to rewetting dry soil, and the resilience of SIR for both the Hawaii and Franz Josef sequences, but the relationship between these stability variables and time were different for the two sequences. This is presumably because the C:N ratio showed a different relationship with time during the two sequences. However, some trends were still evident within sequences. Firstly, the trade-off between the resistance and resilience of some response variables was still apparent over time (Figs. 6, 8). Consistent with the variable correlations of resources with stability at each sequence, the pattern of stability did not always follow the theoretical increase in resistance (Odum 1969) and decline in resilience with time that has been proposed in other empirical studies (McNaughton 1977, Sousa 1980, Grime et al. 2000). Secondly, the difference in the response of biomass- and activity-related response variables was still evident for the Franz Josef sequence; biomass-related response variables decreased in resistance over time and activity-related response variables increased. The opposite pattern was found for resilience. Although the relationship of stability to resources and resources to time was often sufficient to predict how stability changed with time within sequences, there were also instances where there were correlations with resources but not with time and vice versa, suggesting that other factors may influence soil microbial stability.

3.5.3 Conclusion

The role of resources in determining resistance and resilience depended on the sequence and response variables considered. The way that resources changed relative to each other over time also depended on the sequence. These two trends meant that there were few consistent patterns of resistance and resilience over time between the three sequences. There are many other factors that may influence the stability of soil microbes and which may have differed across the sequences. These include soil microbial community composition (Allen-Morley and Coleman 1989, Cortez 1989, Whitford 1989, Orchard et al. 1992), plant composition (Wardle et al. 2000), adaptation to disturbance (West et al. 1988a, West et al. 1988b), soil texture and clay content (West et al. 1988a), substrate diversity (Harrison 1979, Loreau 2001) and food web structure (De Angelis 1992, de Ruiter 1998). Overall, our data show that the interactions amongst soil microbial resistance, resilience, resources and time are complex, and depend on the specific situation examined, suggesting that soil microbial stability is not consistently driven by any single, dominant factor.

Chapter 4: Plant species composition, but not diversity, affects soil microbial resistance and resilience to a drying disturbance.

4.1 Abstract

Despite the strong link between the function of plant and soil microbial communities, few studies have looked at the effect of plant species composition and diversity on soil microbial resistance and resilience to disturbance. We hypothesised that plant species composition would affect soil chemistry and soil microbial response variables, and that these in turn would affect soil microbial resistance and resilience to an experimentally imposed drying disturbance. The diversity of plant communities, the stage of community development and seasonal changes in moisture and temperatures can alter the effect of plants on the soil environment and therefore may also affect soil microbial resistance and resilience. We performed a glasshouse experiment that manipulated the composition and diversity of three common pasture plant species (*Trifolium repens, Lolium perenne,* and *Plantago lanceolata*) by growing them in monoculture, and in all the possible two and three-way combinations, along with an unplanted control soil. Experimental units were harvested at four different points over a 16-month period to determine the effect of plant community development and seasonal changes in temperature and moisture on treatment effects. Results showed that plant composition influenced the soil chemical variables measured, soil microbial response variables and soil microbial stability. The presence of plants generally reduced the resistance of soil microbes to the drying disturbance. Soils planted with *T. repens* showed a higher resistance and resilience than the soils planted with *P. lanceolata*, and a higher resistance than soils planted with *L. perenne*. We suggest that either differences in resource limitation or soil microbial community structure may be responsible for these results. Plant species diversity rarely affected soil microbial response

variables or their stability, despite some significant diversity effects on plant community biomass and soil nitrogen contents. The time of harvest also influenced treatment effects for most variables, suggesting that results can be altered by the stage of plant community development or by extrinsic environmental factors that varied with harvest timing. These results in combination show that soil microbial resistance and resilience was altered by the composition of the plant species present and the time of measurement, but was largely unrelated to plant species diversity. This supports the view that soil organisms, and their resistance and resilience to disturbance, are driven by the traits of the dominant plant species in a community, and therefore by plant community composition rather than species diversity.

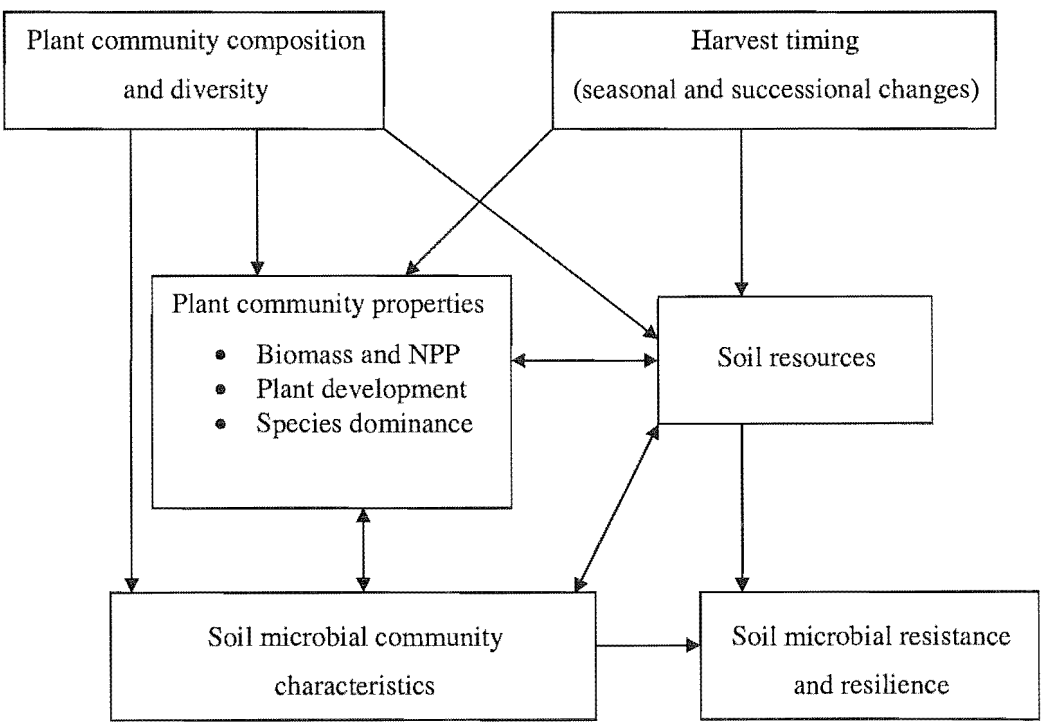


Fig. 9: Flow diagram of the relationships examined in Chapter 4

4.2 Introduction

The stability (resistance and resilience) of ecosystems has become an important topic in current ecology. The resistance and resilience of plant (Lepš et al. 1982, MacGillivray et al. 1995, Tilman 1996) and aquatic ecosystems (Sousa 1980, McGrady-Steed et al. 1997, Biggs et al. 1999) in response to disturbance has been well studied, but the factors that control the resistance and resilience of soil microbes have received less attention (Allen-Morley and Coleman 1989, Wardle et al. 2000, Degens et al. 2001, Griffiths et al. 2001b). Because soil microbes are responsible for the conversion of organic matter into plant available nutrients (Yarie and Van Cleve 1996, Wardle 1998), their ability to resist and recover from disturbances may affect plant nutrient supplies and therefore have an influence on plant productivity. Their resistance and resilience may even regulate whole ecosystem stability (O'Neill 1976).

Resistance and resilience may be driven by biotic factors such as species composition (Lepš et al. 1982, MacGillivray et al. 1995), diversity (May 1972, McNaughton 1977) and food web structure (May 1972, De Angelis 1992, de Ruiter 1998). Extrinsic factors such as nutrient availability (Bosatta and Berendse 1984, De Angelis 1992, Moore et al. 1993), pH (Wardle 1998) and the amount of detritus in a system (De Angelis et al. 1989) may also drive resistance and resilience. In soil, many of these potential driving factors can be altered by plant community composition, including the composition and structure of the soil microbial community (Griffiths et al. 1992, Wardle and Nicholson 1996), and the chemical properties of the soil (Tilman and Wedin 1991, Hooper and Vitousek 1998, Gastine et al. 2003). It is therefore reasonable to predict that the presence of different plant species may result in soil communities with different abilities to resist and recover from disturbances.

The effect of plant communities on soil properties may change when the number of plant species within them (i.e. their diversity) is increased. Some studies have suggested that increasing plant diversity can lead to increases in net plant uptake of soil nutrients (Tilman and Downing 1994, Tilman et al. 1997a, Hooper and Vitousek 1998), enhanced plant productivity (Naeem et al. 1994, Tilman and Downing 1994, Hector et al. 1999), and

ultimately increases in the amount of resources returned to the soil. Increased plant diversity may also alter the soil microbial community, by increasing the heterogeneity of resources (Ettema and Wardle 2002). Plant species diversity may therefore alter the effect of the plant community on soil properties and have an impact on soil microbial resistance and resilience.

Interactions between different plant species, and the effect of species and communities on soil properties are unlikely to be static over time. As plant communities develop, the total biomass of the plant community will change, different plant species within a community may become dominant, and plants of the same species may invest energy into different tissues (e.g. reproductive vs. vegetative) and chemical constituents (e.g. defence compounds, phenolics, lignin). These will all result in variation over time in the amount and types of resources returned to the soil. These changes in the plant community may be closely linked to changes in season, and temporal shifts in temperatures and moisture availability. Changes in temperature and moisture can also affect soil resource availability and soil microbial communities directly, by changing soil process rates (Lomander et al. 1998, Nedwell 1999) and the movement of dissolved organic matter and nutrients. Therefore, both the developmental stage of a plant community and fluctuations in environmental conditions may have an effect on soil chemical properties, the soil microbial community and potentially soil microbial resistance and resilience.

We examined the effect of plant community composition, diversity and development on soil biotic and abiotic properties, and soil microbial resistance and resilience, using three common pasture plants (*Trifolium repens, Lolium perenne,* and *Plantago lanceolata*) planted in all possible combinations. Specifically, we hypothesised that different plant species will create soils with different soil chemical and microbial properties, and that these differences would in turn affect soil microbial resistance and resilience to an experimentally imposed drying disturbance. We also hypothesised that the nature of these relationships may be altered by changes in plant species diversity, and may vary over time as a result of, for example, the developmental stage of the plant community and temporal variability in temperature and rainfall.

4.3 Materials and Methods

4.3.1 Experimental design

Model grassland plant communities of varying species composition and diversity were established to determine what effect plant species composition and diversity has on plant, soil and microbial properties and whether this effect varies over time as the plant communities develop and seasons change. We planted 192 square containers (height 32 cm, width 21 cm) with 24 replicates of eight planting treatments. These consisted of: an unplanted control treatment (bare soil), *Trifolium repens* (clover), *Lolium perenne* (ryegrass), and *Plantago lanceolata* (plantain) in monoculture, these three plant species in all possible pair-wise combinations (clover + ryegrass, clover + plantain, plantain + ryegrass), and all three plant species together. These three plant species are commonly found growing together in pasture systems, and represent three different functional groups (a N-fixer, a dicot and a monocot). Six replicates of each treatment were harvested at each of 3, 6, 11 and 16 months after planting to determine how the stage of plant community development and temporal variation in temperature and moisture affected soil properties. This design is based on a replacement series approach and has been shown to be capable of distinguishing between diversity and composition effects in plant communities (Hooper and Vitousek 1998, Wardle et al. 2000). If the performance of the more diverse communities was either significantly higher or significantly lower than the performance of all of the component species in monoculture, this was interpreted as an effect of diversity (Garnier et al. 1997).

Soil was collected from under pasture at Lincoln, New Zealand (43° 30' S) and homogenised by mechanical sieving to 4 mm. The soil contained 4% C, 0.32% N, and had a pH of 5.1. Containers were filled with soil to 17 cm depth (sufficient depth for unimpeded plant growth for the required time, but still easily manouverable) and placed outside at Lincoln. Clover, ryegrass and plantain seedlings were grown in vermiculite for 5 weeks, and then planted out into designated containers on 19 February 2000. Six plants were planted in each container, giving three plants of each species for the pair-wise combinations and two plants of each species for the three-species treatment. Containers

were placed outside so that they would be exposed to natural variation in temperature, rainfall and daylight hours.

Containers were weeded and watered as required. Plants were regularly clipped to 10 cm in height to maintain the desired plant species composition and to avoid containers becoming overgrown. One such clipping was performed 1 month before each harvest to standardise any changes in plant effects. All clipped plant biomass was sorted into species, dried for 48 h at 60°C and weighed. For each harvest, this biomass was summed and added to the total biomass measured when each container was harvested to give an estimate of net primary productivity (NPP) for each plant community (Wardle et al. 2000). Reproductive parts were included in this biomass. All containers were sprayed with Dichlorvos Nuvan 1000 EC Organo-Phosphate on 30 March 2000 to control an aphid infestation.

4.3.2 Measurement of plant variables

At each harvest, dead vegetation was removed and the remaining aboveground biomass was sorted into species and dried at 60°C for 48 h. The soil was homogenised, and 10 – 15% was removed for the extraction of roots, which were cleaned and dried at 60°C for 48 h for determination of dry weight. The total weight of soil, its moisture content and the weight of the subsample were measured so that both the total amount of roots per container and roots per unit weight of soil could be calculated. The remaining soil was then sieved to 4 mm and stored at 4°C for the remaining measurements.

4.3.3 Measurement of baseline soil chemical and soil microbial variables

For each soil sample, pH (1:1 in water, based on methods described by Mc Lean (1982)), the concentration of ammonium and nitrate (Keeney and Nelson 1982), and various soil microbial properties were measured. Basal respiration and substrate-induced respiration (SIR) was measured on each soil after it had been adjusted to 33% moisture content on a dry weight basis (MC), and allowed to equilibrate in a 25°C incubator for 2 days. Basal respiration was measured as described by Wardle (1993). Ten g dry weight (d.w.) of soil

was placed in a 130-ml airtight container, and incubated at 25°C. The rate of soil microbial respiration was measured by taking 1-ml subsamples of headspace gas at 1 and 3 h after the container was capped, and injecting them into an infrared gas analyser (Wardle 1993). Substrate-induced respiration was measured based on the method described by Anderson and Domsch (1978). CO_2-C was measured as above, except that the soil was amended with 0.02 g glucose/g d.w. before capping the airtight container. A relative measure of the metabolic quotient (qCO_2) was calculated as basal respiration divided by SIR (Anderson and Domsch 1985). The ability of soil microbes in each soil sample to decompose cellulose was measured by burying a weighed 3 x 1.5 cm strip of Whatman filter paper in a Petri dish containing 30 g d.w. of soil at 33% MC. The Petri dish was sealed and incubated at 25°C for 10 days, after which the cellulose paper was removed, cleaned, oven dried and weighed to determine mass loss.

4.3.4 Soil microbial resistance and resilience

To determine the effect of plant community composition, diversity and development on the resistance and resilience of the soil microbes, we used a wetting-drying event as a model disturbance. Wetting-drying events are common disturbances in soils (Kieft et al. 1987, Fierer and Schimel 2002) and are one of the major drivers of soil microbial turnover (West et al. 1988a, Fierer and Schimel 2002) and therefore nutrient availability. Wetting-drying events involve two disturbances: drying, and rewetting of the dried soil (Kieft et al. 1987). For this study we concentrated mainly on the response of the soil microbes to drying. However, a relative measure of the resistance of dry soil to rewetting was also calculated, based on measurements made in the 6 h immediately after rewetting. Based on preliminary experiments, the drying disturbance was defined as drying the soil from 33% MC to 6% MC, and the rewetting dry soil disturbance as adding water to bring soil at 6% MC back up to 33% MC. These moisture contents corresponded to 55% and 10% of water-holding capacity, where 100% water-holding capacity was measured as the amount of water retained in a soil following saturation and 18 h of drainage (Saetre 1998).

Three response variables were used to measure the resistance and resilience of the soil microbial community: basal respiration, SIR, and glucose use. In combination, these

response variables were intended to give a summary of the soil microbial response to the wetting-drying event. Basal respiration measured on dried soil and on rewet dry soil was interpreted as indicating the response of soil microbial activity to the changes in resource availability that occur during wetting-drying events. Substrate-induced respiration measured on wet soil gives an indication of the active microbial biomass (Anderson and Domsch 1978). Therefore, SIR measurements made on rewet dry soil were interpreted as indicating the effect of drying on the soil microbial biomass. Substrate-induced respiration was also measured on dried soil. Because the added glucose remains undissolved in dry soil, it is largely unavailable for soil microbial metabolism (West and Sparling 1986). Any measures of resistance or resilience that included SIR measured on dry soil were therefore interpreted as indicating the effect of drying or rewetting on the ability of disturbed organisms to respond to added substrates, rather than as an indication of biomass. To distinguish between these two measures using SIR, the latter measurement will be referred to in terms of the resistance and resilience of soil microbial glucose use from here onwards, and the former in terms of the resistance and resilience of SIR.

Each soil sample was adjusted to 33% MC (55% of water holding capacity) by air-drying or adding water, and allowed to equilibrate overnight in a 25°C incubator. The MC of unadjusted soils ranged from 13% to 40%, with approximately 75% being between 25 and 35%. For most soils the change in moisture content will therefore have been relatively minor, and the time allowed for equilibration sufficient. For the soils with a comparatively low initial MC (about 10% of the samples), this adjustment may have resulted in a stronger microbial response. However, the strongest part of this response will take place within the time allowed for equilibration. As these soils were from a range of treatments and harvests, it is unlikely that they skew the overall results. We therefore consider that the soil microbes in each soil will have recovered sufficiently after overnight equilibration for the initial change in moisture content not to affect their response to the disturbance used to quantify resistance and resilience.

Subsamples of equilibrated soils from each treatment were spread out on paper trays to air-dry at room temperature to 6% MC. Three 10 g d.w. subsamples of air-dried soil and two 10 g d.w. subsamples of the undisturbed soil at 33% MC were then placed in 125-ml Erlenmeyer flasks, sealed with plastic and incubated at 25°C overnight. Resistance to

drying was determined after this incubation (time 0 or t_0); one flask containing dry soil was used to measure basal respiration and SIR in dry soil, and a further flask containing dry soil was used to measure these response variables immediately after the soil had been returned to 33% MC. The latter measurement was used to determine the resistance of the soil microbes to rewetting dry soil. One flask containing undisturbed soil at 33% MC was used to measure these response variables at this point. Resistance was calculated using the index described in Chapter 2. For the effect of drying on the resistance of soil microbial response variables, C_0 was defined as the value of the undisturbed soil for the appropriate response variable that had remained at 33% MC throughout the disturbance period. For the effect of rewetting dry soils, C_0 was defined as the value of the dry soil for the appropriate response variable.

For resilience, the remaining flask with dry soil was rewet to 33% MC by adding the required amount of water with a syringe, and incubated for a further 3 days (t_3) to allow some recovery. Basal respiration and SIR were measured on the control and disturbed soil samples as for resistance. Resilience was calculated using the index described in Chapter 2. We were only able to calculate resilience as the degree to which the rewetted soil recovered from the drying disturbance. Therefore, the undisturbed soil was in all calculations the soil that had remained at 33% MC throughout the incubation.

4.3.5 Data analysis

The effect of planting treatment on plant, soil chemical and microbial response variables and resistance and resilience was assessed using ANOVA with block and treatment as factors. As the addition or removal of the bare soil control treatment only made small differences to results of data analyses, we have presented the full ANOVA with all 8 treatments. Where the overall treatment effect was significant, the least significant difference statistic was used to determine which treatments were significantly different to each other. Data were transformed as necessary to meet the assumptions of normality and homogeneity of variances. Potential mechanisms behind changes in soil abiotic and biotic properties were assessed by correlation analysis across all experimental units within each harvest. Pearson's correlation coefficient was used when the data were normal or could be

transformed to a normal distribution, and Spearman Rank correlations were used when they could not. We performed stepwise multiple regression analyses to determine which combinations of variables explained the most variation in soil microbial response variables and resistance and resilience. Only variables that remained significant at $P < 0.05$ were retained. Initial explanatory variables for resistance and resilience included plant, soil chemical and microbial variables, and the ability of soil microbes to decompose cellulose. Explanatory variables for soil microbial basal respiration, SIR, qCO_2 and cellulose decomposition included plant and soil chemical variables.

4.4 Results

4.4.1 Characteristics of the different plant species in monoculture and in mixture

For all harvests, plant species composition had an effect on plant biomass, in contrast to the rare effects of plant diversity (Table 11). In general, clover plants showed a high aboveground biomass (and corresponding high NPP), but a low belowground biomass (Table 11). Ryegrass and plantain plants showed the opposite trend. The timing of the harvest also had an influence on plant biomass, with root biomass peaking in the third harvest for all three plant species, but trends in shoot biomass showing variable trends over time for each plant species. Shoot biomass only responded to diversity in the third harvest, where the biomass in the three species mixture was significantly higher than that of all the three corresponding monocultures. NPP in two mixtures in harvest 3 (the clover + ryegrass and the all three species treatment) also responded positively to plant diversity.

4.4.2 Effect of plants on soil chemical and microbial variables

Plant species composition had an effect on the soil chemical and microbial variables measured, but plant diversity rarely had a significant effect, and then only for

measurements of soil chemistry (Table 12, 13). Soil planted with clover in monoculture showed the highest concentrations of ammonium and nitrate, followed by soil planted with ryegrass and then by soil planted with plantain (Table 12). Plant diversity had an effect on soil nitrogen (N) contents in the clover + ryegrass treatment in harvest 3 (nitrate concentration decreased), and the clover + ryegrass treatment in harvest 4 (ammonium concentration increased). The timing of the harvest also affected soil chemistry, with a peak in ammonium in the third harvest for the bare soil and monoculture treatments, but a peak in the fourth harvest for the mixtures. Soil pH was also affected by plant species composition, with soils planted with clover showing a lower pH than soils planted with ryegrass or plantain (Table 12). The presence of plants enhanced soil microbial basal respiration and SIR in all treatments (Table 13). In general, soils from under clover monocultures showed a lower basal respiration than that in soils from under ryegrass and plantain. Soils from under clover also tended to have a lower microbial metabolic quotient (qCO_2) than that under the monocultures of the other two plant species. There was no effect of plant diversity on soil microbial properties. Basal respiration and SIR tended to increase with time, but in some treatments declined in harvest 4.

The presence of different plant species had positive, negative or neutral effects on the ability of soil microbes to decompose cellulose, but diversity had no significant impact (Fig. 10). For the first two harvests the soil from the clover monoculture supported a higher decomposition rate than the bare soil and soil from the other monocultures. In the last two harvests, the decomposition rate in soils planted with clover was the same as that of the bare soil. The presence of ryegrass had no effect on decomposition rate, while soils planted with plantain tended to show a low decomposition rate.

4.4.3 The effect of plants on soil microbial resistance and resilience

Soil microbial basal respiration and glucose use were reduced by the drying disturbance (Appendix II). Soil microbial biomass (measured as SIR on rewet dry soil) was also reduced by the disturbance. Rewetting dry soil resulted in a large increase in basal respiration and glucose use. The basal respiration of most soils had recovered to close to control levels by day 3, but some were still higher than the control soil. Soil microbial

biomass and glucose use showed varying levels of recovery on day 3. In particular, soils that had been planted with plantain often showed a higher SIR on day 3 than the control soil, indicating an over-compensatory response (Appendix II).

The resistance of the soil microbes to both soil drying and rewetting dry soil showed no significant treatment x harvest interactions, so data from all harvests were analysed together to test for overall effects of planting treatments (Fig. 11). In all treatments, soil microbial basal respiration and glucose use in dry soil was near to zero, and the index of resistance gave similar results for both response variables. Therefore, we have not presented the results for the resistance of glucose use to drying. The strength of plant composition effects on resistance depended on which soil microbial response variable was measured. In general, the presence of plants reduced the resistance of soil microbes compared to the bare soil, with the exception of the resistance of soil microbial SIR, which showed no treatment effects. The resistance of basal respiration to drying and rewetting dry soil was also the same in the clover monoculture and bare soil treatments. There was one significant diversity effect; the resistance of basal respiration to rewetting dry soil was lower in soil from the clover + ryegrass treatment then in either of the corresponding monocultures. The effect of harvest timing on resistance depended on the microbial response variable measured. The resistance of basal respiration to either disturbance decreased in harvest 2, compared to the resistance of SIR to drying, which showed lower resistance in harvest 4, and the resistance of glucose use to rewetting dry soil, which showed higher resistance in harvests 2 and 3.

The resilience of soil microbial basal respiration to drying did not respond to treatment or harvest timing (data not presented). The resilience of SIR to drying showed a transient effect of treatment – only the first two harvests showed significant effects at $P < 0.05$ (Fig. 12). The presence of different plant species had positive, negative or neutral effects on the resilience of this response variable, with soils from the clover and ryegrass monocultures showing the highest resilience. In harvest 1, soils from treatments containing plantain showed either the same or reduced resilience compared to the bare soil. In harvest 2, results were similar but stronger, with all treatments containing plantain having a low resilience and the clover, ryegrass and clover + ryegrass treatment having a relatively high resilience.

The resilience of soil microbial glucose use to drying responded to the presence and composition of plant species, but not to plant diversity (Fig. 13). Plant composition effects strengthened with time. Soil from the plantain monoculture showed a low resilience compared to the other monocultures in all harvests. Soils from under the clover and ryegrass monocultures had a similar resilience to the bare soil for most harvests, except for the third harvest where soil from the clover monoculture had a lower resilience. Resilience of the soil from the mixtures containing plantain tended to become more similar to the bare soils with time.

4.4.4 Relationships of plant and soil chemical variables with soil microbial variables

Plant and soil chemical variables were correlated with soil microbial variables and their resistance and resilience across treatments, but within harvest dates, to evaluate some of the potential drivers behind treatment effects. Soil microbial variables were related to both plant and soil variables (Table 14). Increases in the concentration of mineral N and decreases in pH were correlated with lower soil microbial basal respiration. The effects of plant shoot biomass were transient, but root mass (expressed on a per unit soil weight basis) had a positive relationship and the shoot:root ratio a negative relationship with basal respiration across all harvests. SIR was negatively related to nitrate concentration, but showed fewer significant relationships with ammonium concentration and pH than did basal respiration. Plant variables were only significantly correlated with SIR in the first two harvests. The metabolic quotient showed some significant relationships with plant and soil variables, but these varied across harvests.

The ability of soil microbes to decompose cellulose showed some consistent relationships with plant and soil properties (Table 15). The initial relationship of NPP and shoot biomass with decomposition was negative, but became positive by the last two harvests. A low root mass (expressed on a per unit soil weight basis) and a high shoot:root ratio were associated with a faster decomposition rate. Higher concentrations of mineral N and a lower pH were also correlated with faster decomposition rates (Table 15).

The resistance of soil microbial response variables to drying and rewetting and the resilience of basal respiration were rarely significantly correlated with more than one soil or plant variable, so we have only presented multiple regression analyses for this data. However, the resilience of both SIR and glucose use to drying showed many significant correlations with plant, soil and microbial response variables (Table 16). The patterns found were similar for both of these measures of resilience. Relationships with plant variables were negative except for the shoot:root ratio, but the variables that were significant varied with time. Where significant, increases in mineral N were associated with an increase in resilience. In contrast, pH showed a negative relationship to resilience for both harvest 2 and 4. Basal respiration showed a negative correlation with resilience for harvest 1 and 4. The ability of the soil microbes to decompose cellulose also had a positive relationship with resilience.

Despite the many significant correlations, multiple regression analyses showed that only one or two of these variables were important drivers (Table 17). The drivers of basal respiration varied among harvests, with shoot biomass and pH initially being important, followed by nitrate concentration for the later harvests. Multiple regressions explaining variation in SIR were more consistent across harvests, with root mass (expressed on a per unit soil weight basis) and pH showing a positive relationship with SIR in the first harvest and nitrate concentration explaining the most variation in the remaining three harvests. The metabolic quotient was also related most strongly to different variables in different harvests, but the shoot:root ratio became a more consistent explanatory variable towards the end of the experiment. Variation in the decomposition of cellulose was explained primarily by nitrate concentration, but plant variables were also important in some harvests. Multiple regressions rarely explained much of the variation in the resistance of soil microbial response variables, except for the last harvest where root mass (expressed on a per unit soil weight basis) and SIR appeared to be important drivers of the resistance of basal respiration to drying and rewetting dry soil (Table 17). The variation in the resilience of basal respiration to drying was also largely unaccounted for by our explanatory

Table 11: Effect of planting treatment on the means of plant shoot and root mass, the shoot:root ratio and NPP for each harvest, as assessed by ANOVA with block and treatment as explanatory variables. C, R, and P = clover, ryegrass and plantain in monoculture respectively, CR = clover + ryegrass, CP = clover + plantain, RP = ryegrass + plantain and CRP = all three plant species together. Means within a row followed by the same letter are not significantly different from each other at $P = 0.05$. Numbers in bold indicate a significant effect of plant diversity.

VARIABLE	HARVEST	C	R	P	CR	CP	RP	CRP	*F*-statistic	*P*-value
Shoot mass	1	12.61c	15.27bc	19.35a	17.77ab	19.59a	18.14ab	17.61ab	3.37	0.0117
(g d.w.)	2[1]	22.65ab	9.96d	16.22c	20.52bc	26.19a	12.83e	25.04ab	18.52	< 0.0001
	3	37.91b	16.90c	16.97c	43.01ab	41.24ab	16.70c	**45.61a**	48.14	< 0.0001
	4	23.72a	11.03c	12.31c	20.45ab	18.68b	10.84c	19.84ab	11.23	< 0.0001
Root mass	1[2]	3.46c	16.12ab	17.33a	12.05b	13.72ab	16.79ab	16.05ab	12.81	< 0.0001
(g d.w.)	2[1]	6.82d	16.67bc	29.50a	14.56c	25.98a	20.91ab	25.78ab	18.09	< 0.0001
	3[1]	12.10d	20.78c	32.30ab	25.30bc	35.43a	30.87ab	27.64abc	8.57	< 0.0001
	4	10.13c	19.81ab	24.49a	17.83b	18.37b	21.92ab	24.55a	6.41	0.0002
Shoot:root ratio	1[1]	3.88a	1.02d	1.16cd	1.48bc	1.95b	1.15cd	1.16cd	17.68	< 0.0001
	2[1]	3.56a	0.60c	0.59c	1.44b	1.03b	0.63c	1.14b	33.02	< 0.0001
	3[1]	3.40a	0.84d	0.59e	1.89b	1.20c	0.57e	1.79b	31.46	< 0.0001
	4[1]	2.51a	0.56d	0.47d	1.20b	1.03bc	0.53d	0.82c	24.06	< 0.0001
Shoot NPP	1	12.61b	17.68a	21.06a	19.88a	21.31a	20.43a	17.97a	3.83	0.0059
(g d.w.)	2[1]	25.51ab	11.87d	18.85bc	24.37ab	30.31a	15.15cd	27.34a	11.39	< 0.0001
	3[1]	72.20b	23.71c	27.33c	**98.13a**	83.08ab	27.46c	**93.82a**	64.03	< 0.0001
	4[1]	60.66a	23.56b	25.44b	77.90a	63.66a	29.27b	80.37a	28.13	< 0.0001

[1] Log transformed, [2] square root transformed

Table 12: Effect of planting treatment on the means of soil chemical variables in each harvest as assessed by ANOVA with block and treatment as explanatory variables. Codes as in Table 11, B = bare soil. Means within a row followed by the same letter are not significantly different from each other at $P = 0.05$. Numbers in bold indicate a significant effect of plant diversity.

VARIABLE	HARVEST	B	C	R	P	CR	CP	RP	CRP	*F*-statistic	*P*-value
Ammonium	1	0.11	0.66	0.64	0.25	1.02	0.67	0.58	0.62	1.57	0.1770
(μg NH_4^+ g d.w.$^{-1}$)	2	0.08c	2.14a	0.38bc	0.15bc	0.55b	0.08c	0.16bc	0.08c	19.58	< 0.0001
	3[1]	1.22c	6.79a	4.42ab	0.84c	2.92b	0.71c	0.63c	1.31c	14.93	< 0.0001
	4	0.03f	4.80bc	2.80de	0.17f	**11.00a**	3.17cd	1.10ef	6.02b	37.71	< 0.0001
Nitrate	1[1]	8.66bc	16.00a	7.35cd	0.96g	12.19ab	4.13ef	1.91fg	5.26de	20.74	< 0.0001
(μg NO_3^- g d.w.$^{-1}$)	2[1]	8.73a	11.04a	4.04b	0.74c	8.08a	0.62c	0.55c	0.67c	39.98	< 0.0001
	3[1]	8.40a	7.45a	6.83a	0.24d	**3.60b**	0.48cd	0.24d	0.95c	51.37	< 0.0001
	4[1]	11.08c	33.39a	10.80c	0.74e	21.08b	3.98d	1.27e	7.95c	37.13	< 0.0001
pH	1	5.43d	5.30e	5.57abc	5.57abc	5.47cd	5.62a	5.58ab	5.50bcd	7.23	< 0.0001
	2	5.22cd	4.98e	5.33bc	5.47a	5.10de	5.25c	5.38ab	5.27bc	11.17	< 0.0001
	3	5.27b	4.90e	5.45a	5.47a	5.07d	5.10cd	5.47a	5.17c	48.77	< 0.0001
	4	5.27b	4.90d	5.50a	5.50a	5.07c	5.27b	5.55a	5.20b	27.96	< 0.0001

[1]Log transformed

Table 13: Effect of planting treatment on the means of soil microbial variables in each harvest as assessed by ANOVA with block and treatment as explanatory variables. Codes as in Table 11, B = bare soil. Means within a row followed by the same letter are not significantly different from each other at $P = 0.05$.

VARIABLE	Harvest	B	C	R	P	CR	CP	RP	CPR	*F*-statistic	*P*-value
Basal Respiration	1[1]	1.05d	1.39c	1.65b	1.92a	1.56bc	2.01a	1.86a	1.94a	22.72	0.0000
(μg CO_2-C g d.w.$^{-1}$ h^{-1})	2	0.94d	1.41c	1.76ab	1.91a	1.56bc	1.84a	1.83ab	1.75ab	11.30	0.0000
	3	1.27d	2.33c	2.25c	3.13a	2.61bc	3.15a	3.07ab	3.05ab	16.84	0.0000
	4	1.10c	1.70b	1.95ab	2.25a	2.02ab	2.37a	2.34a	2.15a	7.88	0.0000
SIR	1[1]	3.61e	4.96d	6.04bc	6.36ab	5.62c	7.06a	6.57ab	7.04a	27.88	0.0000
(μg CO_2-C g d.w.$^{-1}$ h^{-1})	2	3.47d	6.08c	6.54bc	6.98abc	6.54bc	7.49ab	7.55a	6.81abc	14.25	0.0000
	3	4.29c	11.33a	9.24b	12.90a	11.41a	12.32a	12.04a	12.30a	20.59	0.0000
	4[1]	3.67f	10.70cd	8.15e	12.48abc	10.26d	13.51a	11.58bcd	12.59ab	64.83	0.0000
qCO_2	1	0.30	0.28	0.27	0.30	0.28	0.28	0.28	0.27	1.01	0.4392
	2	0.27a	0.23b	0.27ab	0.27a	0.24bc	0.25abc	0.24abc	0.26abc	2.00	0.0832
	3	0.30a	0.20c	0.25b	0.24b	0.23bc	0.26b	0.25b	0.22bc	5.07	0.0005
	4[2]	0.30a	0.16c	0.24ab	0.18abc	0.20abc	0.18bc	0.20abc	0.17bc	8.65	0.0000

[1] Log transformed, [2] rank transformed.

Table 14: Correlation coefficients of soil microbial variables with plant and soil chemical variables across all experimental units for each harvest (n = 42 and excludes the bare soil treatment).

	Basal Respiration (μg CO_2-C g d.w.$^{-1}$ h^{-1})				Substrate-induced respiration (μg CO_2-C g d.w.$^{-1}$ h^{-1})				Metabolic quotient (qCO_2)			
	H1	H2	H3	H4	H1	H2	H3	H4	H1	H2	H3	H4
NPP[1] (g d.w.)	0.5520***	-0.0088	0.0763	-0.0600	0.5409***	0.1229	0.0683	0.0831	0.2502	-0.1814	-0.2216	-0.1870
Shoot (g d.w.)	0.5862***	-0.0647	-0.0850	-0.1772	0.5298***	0.0520	0.0635	-0.0499	0.3176*	-0.1723	-0.2269	-0.1494
Roots[6] (g per g soil d.w.)	0.5662***	0.4735**	0.4018**	0.4195**	0.5854***	0.3437*	0.2255	0.0588	0.2344	0.2540	0.3599*	0.3733**
S:R[2, 3-6]	-0.3611*	-0.4752**	-0.3230*	-0.3883*	-0.4618**	-0.2726†	-0.0770	-0.0183	-0.0509	-0.3493*	-0.4284**	-0.3902*
Ammonium[3,5-7] (μg g d.w.$^{-1}$)	-0.1508	-0.4311**	-0.5802***	-0.2122	-0.0979	-0.1778	-0.4647**	-0.1396	-0.0983	-0.3505*	-0.3612*	0.0810
Nitrate[3-6] (μg g d.w.$^{-1}$)	-0.4650**	-0.4704**	-0.6218***	-0.4801**	-0.4628**	-0.4271**	-0.4792**	-0.4077**	-0.2134	-0.1525	-0.3800**	-0.0695
pH	0.3425*	0.4702**	0.2136	0.3861*	0.3862*	0.3204*	-0.0270	0.0810	0.0959	0.2884†	0.3932*	0.3258*

[1] Net primary productivity, [2] shoot:root ratio. Log transformed for [3] harvest 1 (H1), [4] for harvest 2 (H2), [5] for harvest 3 (H3) and [6] for harvest 4 (H4). [7] Spearman Rank Correlation Coefficient for harvest 2 .

†$P < 0.1$, * $P < 0.05$, ** $P < 0.01$. *** $P < 0.001$

Table 15: Correlation coefficients of decomposition with plant and soil chemical variables across all experimental units for each harvest (n = 42 and excludes the bare soil treatment).

	Decomposition of cellulose (% mass loss)			
	H1	H2[4]	H3[5]	H4
NPP[1] (g d.w.)	-0.4316**	0.0243	0.3160*	0.3608*
Shoot (g d.w.)	-0.4301**	0.0627	0.3800*	0.3961**
Roots[6] (g per g soil d.w. $^{-1}$)	-0.6803***	-0.6728***	-0.2522	-0.4449**
S:R[2, 3-6]	0.6389***	0.6202***	0.4470**	0.5613***
Ammonium[3,5-7] (μg g d.w.$^{-1}$)	0.2468	0.6365***	0.4669**	0.6603***
Nitrate[3-6] (μg g d.w.$^{-1}$)	0.8265***	0.7347***	0.4483**	0.7541***
pH	-0.3943**	-0.6498***	-0.4057***	-0.5829***

[1] Net primary productivity, [2] shoot:root ratio. Log transformed for [3] harvest 1 (H1), [4] for harvest 2 (H2), [5] for harvest 3 (H3) and [6] for harvest 4 (H4). [7] Spearman Rank Correlation Coefficient for harvest 2.

* $P < 0.05$, ** $P < 0.01$. *** $P < 0.001$

Table 16: Correlation coefficients of the resilience of soil microbial response variables with soil, plant and microbial variables across all experimental units (n = 42 and excludes the bare soil treatment).

	Resilience of substrate-induced respiration to drying				Resilience of glucose use to drying			
	H1	H2	H3	H4	H1	H2	H3	H4[11]
NPP[1] (g d.w.)	-0.4091**	-0.1103	0.0795	0.1245	-0.3789*	-0.0469	0.0049	0.2686†
Shoot (g d.w.)	-0.3805*	-0.1532	-0.0114	0.1681	-0.3606*	-0.1283	-0.0416	0.2515
Roots[9] (g per g soil d.w. $^{-1}$)	-0.2295	-0.5909***	-0.2179	-0.5180***	-0.1248	-0.4972***	-0.1120	-0.5556***
S:R[2, 6-9]	0.0638	0.3856*	0.0957	0.4959***	-0.0773	0.3320*	0.0522	0.5852***
Ammonium[6,8-10] (μg g d.w.$^{-1}$)	0.0404	0.4392**	0.1131	0.1805	0.0606	0.4579**	0.1588	0.2816†
Nitrate[6-9] (μg g d.w.$^{-1}$)	0.4477**	0.6622***	0.1348	0.2935†	0.3915*	0.6690***	0.1874	0.4622**
pH	-0.2050	-0.4431**	-0.0486	-0.3337*	-0.1179	-0.4259**	0.0162	-0.5410***
BR[3] (μg CO_2-C g d.w.$^{-1}$ h^{-1})	-0.3931*	-0.1721	0.0651	-0.3407*	-0.3152*	-0.1711	-0.0097	-0.3314*
SIR[4] (μg CO_2-C g d.w.$^{-1}$ h^{-1})	-0.3526*	-0.1474	0.0988	0.1071	-0.2741†	-0.1428	-0.0558	0.0076
qCO_2	-0.2324	-0.0926	-0.0125	-0.3950**	-0.2157	-0.0966	0.0909	-0.4610**
Decomposition ability[5,7,8]	0.4853**	0.6726***	-0.0526	0.4826**	0.4299**	0.6976***	0.1333	0.5062***

[1] Net primary productivity, [2] shoot:root ratio, [3] basal respiration, [4] substrate-induced respiration, [5] % cellulose mass loss over 10 days. Log transformed [6] for harvest 1 (H1), [7] for harvest 2 (H2), [8] for harvest 3 (H3) and [9] for harvest 4 (H4). [10] Spearman Rank Correlation Coefficient for harvest 2 and [11] for harvest 4.

* $P < 0.05$, ** $P < 0.01$. *** $P < 0.001$

Table 17: Relationships between soil microbial response variables and driving variables as assessed by stepwise multiple regression. The model presented represents the combination of variables that maximised R^2. Only variables that remained significant in the model at $P < 0.05$ are included.

	Harvest 1		Harvest 2		Harvest 3		Harvest 4	
	Model	R^2	Model	R^2	Model	R^2	Model	R^2
BR[2]	St (+); pH (+)	0.4620***	Amm[1] (-); pH (+)	0.3075***	Nit[1] (-)	0.3866***	Nit[1] (-)	0.2305**
SIR[2]	Rt (+); pH (+)	0.4371*	Nit[1] (-)	0.1600**	Nit[1] (-)	0.2296*	Nit[1] (-)	0.1662**
qCO_2	St (+)	0.1009*	Amm[1] (-)	0.1760**	S:R[1] (-);	0.1835***	S:R[1] (+)	0.1522*
Decomposition[4]	Nit[7] (+); Rt (-)	0.7400***	Nit[1] (+); S:R[1] (+)	0.7360***	St (+); Amm[1] (+)	0.4178***	Nit[1] (+)	0.5687***
Resistance to drying of:								
BR[4,6]	n.s.		n.s.		qCO_2 (-)	0.1407*	Rt[1] (-), SIR (-)	0.3054***
SIR	n.s.		Rt (+)	0.1581**	n.s.		n.s.	
Resistance to rewetting dry soil of:								
BR[3]	qCO_2 (+); NPP (-)	0.2761**	Amm[7] (+)	0.1007*	S:R[1] (+)	0.1101*	Rt[1] (-); SIR (-)	0.4261***
Glucose use[6]	n.s.		n.s.		n.s.		n.s.	
Resilience to drying of:								
BR	NPP (-)	0.0944*	n.s.		n.s.		n.s.	
SIR[5]	Lst (+)	0.2355**	Nit[1] (+); Rt (-)	0.5184***	n.s.		Rt[1] (-); Lst (+)	0.3476***
Glucose use[5]	Lst (+)	0.1848**	Nit[1](+)	0.4643***	n.s.		pH (-)	0.2145***

BR = basal respiration (μg CO_2-C g d.w.$^{-1}$ h^{-1}), SIR = substrate-induced respiration (μg CO_2-C g d.w.$^{-1}$ h^{-1}), Lst = % cellulose mass lost, St = shoot biomass (g dry weight), Rt = roots (per unit soil weight), S:R = shoot:root ratio, Nit = nitrate concentration (μg NO_3^- g d.w.$^{-1}$), Amm = ammonium concentration (μg NH_4^+ g d.w.$^{-1}$). Log transformed[1] in Harvest 1[2], Harvest 2[3], Harvest 3[4], square root transformed in Harvest 2[5], in Harvest 4[6]; n = 41[7].
†$P < 0.1$, * $P < 0.05$, ** $P < 0.01$. *** $P < 0.001$

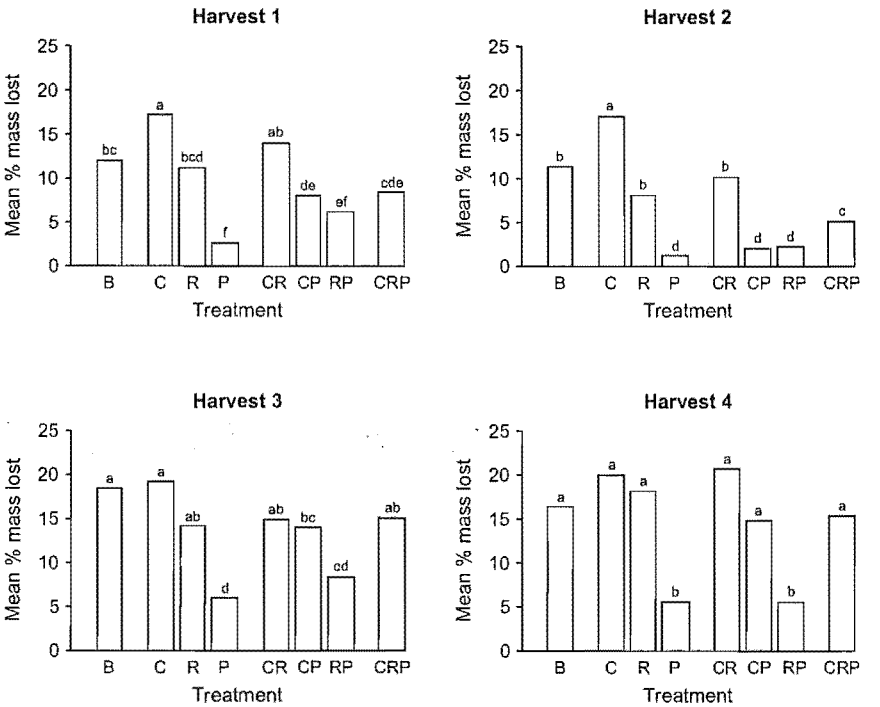


Fig. 10: Effect of treatment and harvest timing on the ability of soil microbes to decompose a strip of cellulose paper over a 10-day period, as analysed by ANOVA. Data for harvest 2 were square root transformed for analysis, and data for harvest 3 were log transformed. Within each panel, bars topped with the same letter are not significantly different from each other at $P < 0.05$. B = bare soil, C = clover in monoculture, R = ryegrass in monoculture, P = plantain in monoculture, CR = clover + ryegrass, CP = clover + plantain, RP = ryegrass + plantain, CRP = clover + ryegrass + plantain.

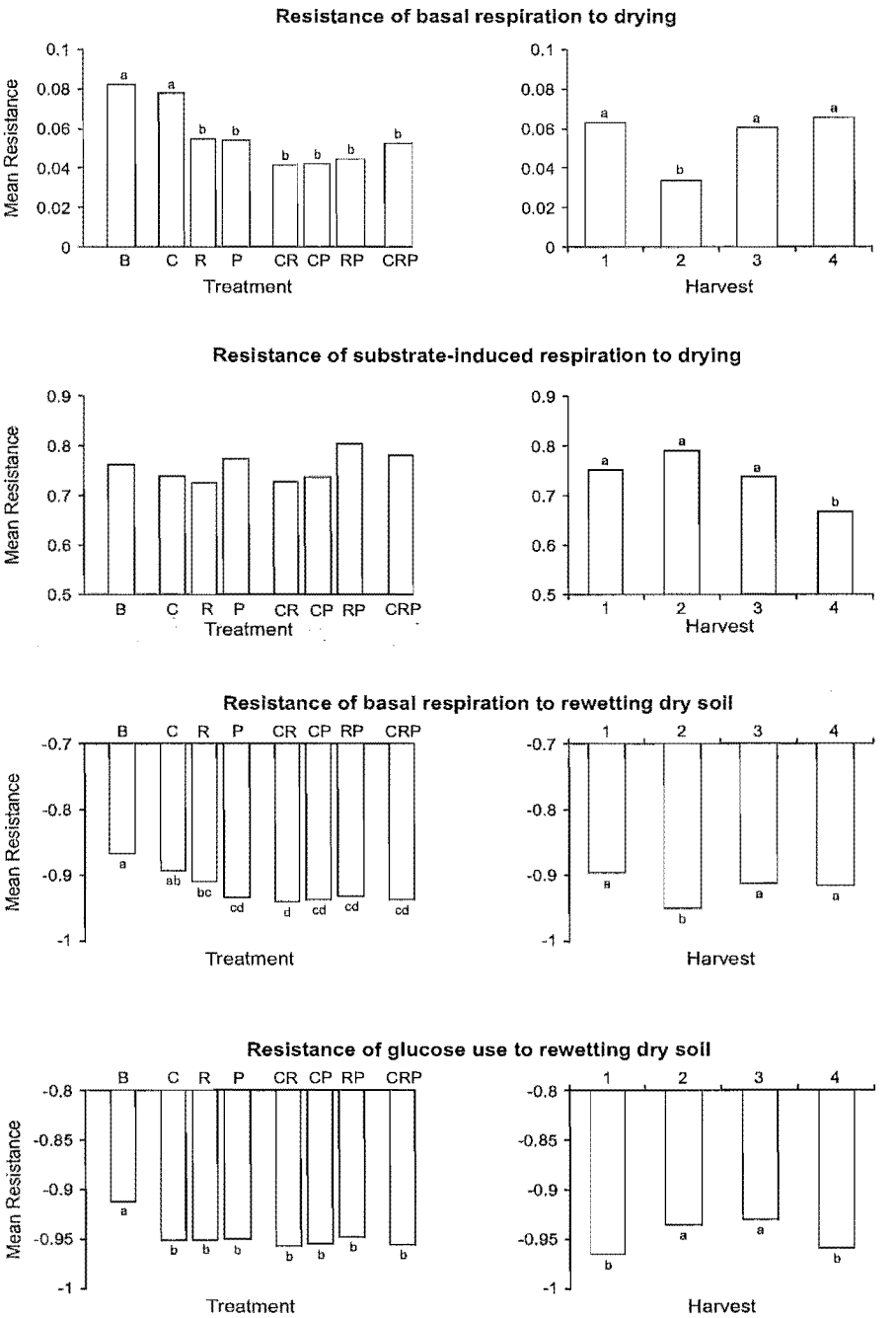


Fig. 11: Effect of treatment and harvest on the resistance of soil microbial parameters, as analysed by ANOVA. As there was no significant treatment x harvest interaction, data for all harvests were pooled. All resistance variables apart from the resistance of SIR were log transformed before analysis. Bars within each panel topped with the same lower-case letter are not significantly different from each other at $P < 0.05$. Codes as in Fig. 10.

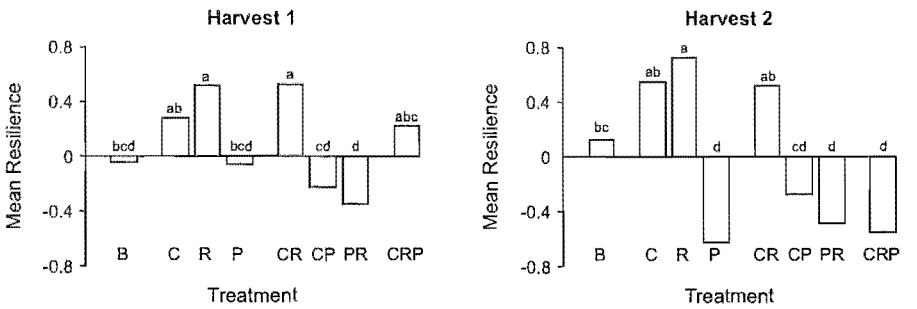


Fig. 12: Effect of treatment and harvest timing on the resilience of SIR for harvests 1 and 2 (harvests 3 and 4 did not show any significant responses to treatment (data not presented)) as analysed by ANOVA. Within each panel, bars with the same lower-case letter are not significantly different from each other at $P < 0.05$. Codes as in Fig. 10.

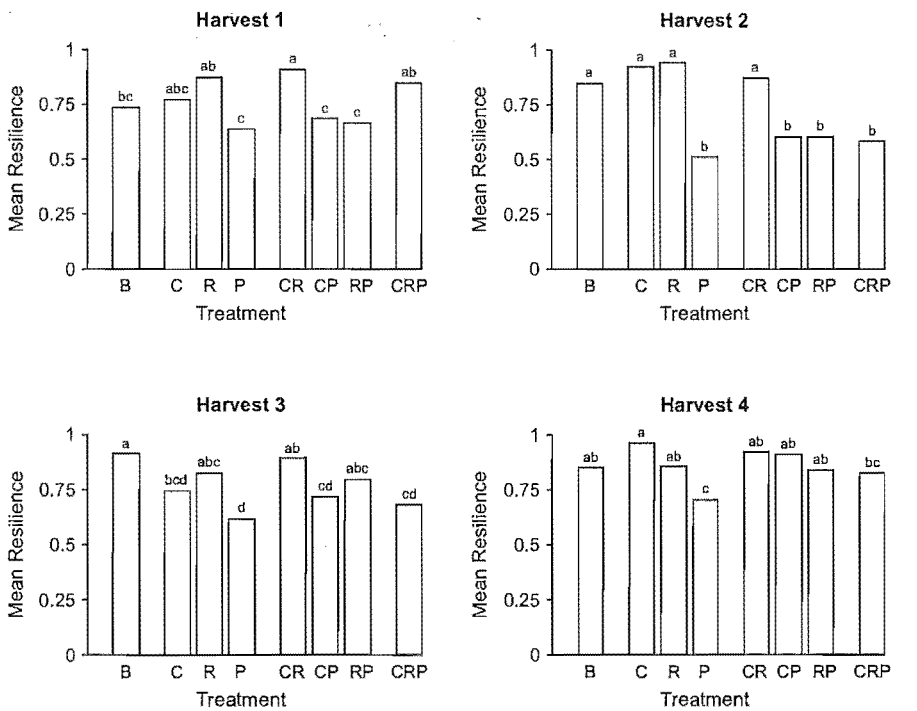


Fig. 13: Effect of treatment and harvest timing on the resilience of glucose use for each of the four harvests as analysed by ANOVA. Within each panel, bars topped with the same lower-case letter are not significantly different from each other at $P < 0.05$. Codes as in Fig. 10.

variables. For the resilience of SIR and glucose use, however, nitrate concentration and root mass (expressed on a per unit soil weight basis), and decomposition ability appeared to be important drivers.

4.5 Discussion

Plant species composition had significant effects on soil chemical and soil microbial properties, and on the resistance and resilience of the soil microbial community. Although increasing plant diversity altered plant and soil chemical properties in some harvests and for some treatments, it did not have an impact on soil microbial properties. In contrast, the timing of the harvest did influence the effect of different plant species on soil and microbial properties, and the occurrence of plant diversity effects.

4.5.1 Effect of plants on soil chemical and microbial properties

Each plant species in monoculture produced a soil with distinctive chemical and soil microbial properties, supporting our first and second hypotheses. In comparison with the unplanted control, plant species were capable of exerting positive, negative or neutral effects on the soil chemical variables measured, depending on the context. This is consistent with the findings of previous studies (Tilman and Wedin 1991, Bardgett et al. 1999b, Gastine et al. 2003). Although the presence of plant species enhanced soil microbial activity and SIR, the magnitude of this effect depended on the plant species. Similar results have been found in other studies (Bardgett et al. 1999b, Spehn et al. 2000a, Stephan et al. 2000). The differences in soil biotic and abiotic properties between soils under different plant species were probably a result of differences in the amount and quality of resources that each plant species adds to (Porazinska et al. 2003) and removes from (Tilman and Wedin 1991) the soil. The differences in soil microbial response variables across different plant treatments appeared to be driven by both plant and soil chemical properties (Tables 13, 14) (Swift et al. 1979, Wardle et al. 1999).

4.5.2 Effect of plants on soil microbial resistance and resilience

Despite the strong effect of plant species on soil microbial and chemical properties, the effects of plant species composition on soil microbial resistance and resilience were variable, and depended on which microbial response variable was considered. Studies in aquatic (Steinman et al. 1990, Biggs et al. 1999, Herbert et al. 1999) and forest (Herbert et al. 1999) systems have also found that the stability of different response variables can respond to the same variables or treatments in different ways depending on the context. However, there were some consistent trends. The composition of plant species had a significant effect on soil microbial resistance, and resulted in either no change or a decrease in resistance compared to the unplanted control soil. This is consistent with other studies (Wardle et al. 1999, Wardle et al. 2000). Most soil microbes in the unplanted soil treatment were probably inactive or growing only slowly because of a lack of resource input. It has been suggested that slower-growing organisms in soil survive drying while the actively growing ones are killed (Bottner 1985), which may explain the higher resistance found for the unplanted soils. Soils planted with clover consistently showed a higher resistance and resilience than soils planted with plantain, and a higher resistance than soils planted with ryegrass. This suggests that the different effects of each plant species on the soil environment and soil microbial community were sufficient to cause significant differences in soil microbial resistance and resilience.

4.5.3 Potential drivers behind trends in resistance and resilience

The differences in resistance and resilience in soils from the different treatments may be the result of some soils being N limited, and others being carbon (C) limited. Our study provides several lines of evidence for this. Firstly, although the resistance of the soil microbial community was rarely strongly related to soil variables, SIR and root biomass were negatively correlated with the resistance of basal respiration for the final harvest (Table 17). Roots supply a large portion of the C used by soil microbes (Wheatley et al. 1990), and the amount of soil microbial biomass is also generally related to the amount of C in the system (Wardle 1998). The negative correlation between these variables and the resistance of basal respiration may therefore indicate that low C availability (i.e. C

limitation) can lead to higher resistance. Secondly, the amount of mineral N in soils planted with clover and ryegrass indicated that these soils were probably more C limited than N limited, whereas in soils planted with plantain the reverse was true. This difference was also evident for soil microbial resilience, and sometimes resistance, with soils planted with clover or ryegrass showing a higher stability than soils planted with plantain. This suggests that soils that are C limited may be more resilient, and sometimes more resistant, than soils that are N limited. This interpretation was supported further by a positive correlation between soil nitrate concentrations and the resilience of SIR and glucose use (Table 17). These results are consistent with studies that suggest that higher inputs of nutrients (in our case N) can increase resilience (De Angelis 1992, Moore et al. 1993, Herbert et al. 1999), and that communities limited by different resources may respond differently to the same disturbance (Bosatta and Berendse 1984, Huston 1997, Biggs et al. 1999).

The presence of different plant species may have resulted in soil microbial communities that differed in their responses to the drying disturbance. Although we did not directly measure soil microbial community composition, there were several indications that the soil biota did differ across treatments. The activity, SIR and qCO_2 of the different soils varied (Table 13), and the ability of the different soil communities to decompose cellulose differed across treatments. The subset of the soil microflora that are capable of decomposing cellulose tend to be slower-growing K-selected organisms (Swift et al. 1979). As a result, the rate of cellulose mass loss may give an indication of the potential activity of organisms that are not measured by the short-term response measured by SIR. All of these indicators of soil microbial community composition were related to measures of soil microbial resistance and resilience at various times during the experiment (Table 17), supporting the suggestion that differences in soil microbial community composition may have had an impact on stability. Other studies involving plant (Lepš et al. 1982, MacGillivray et al. 1995), aquatic (Sousa 1980), and soil (Allen-Morley and Coleman 1989, de Ruiter 1998) communities have also suggested that the characteristics of the organisms within a community, and therefore community composition, will determine its resistance and resilience.

4.5.4 Effect of plant diversity on soil microbial properties

Although the effect of plant communities on shoot biomass, NPP and soil N values was altered by increasing plant diversity in some treatments and harvests, these changes rarely flowed through to soil microbial response variables or to resistance and resilience. In the one harvest where there was a significant effect, increased plant species diversity had a negative impact on the resistance of basal respiration (Fig. 11). It is possible that the soil microbial community was slow to respond to changes in plant diversity because the aboveground and belowground systems are only weakly coupled (Van der Putten et al. 2000, Raffaelli et al. 2002), or simply because the magnitude of the effect of plant diversity on the soil environment was not sufficient to influence the soil microbial community. Plant diversity effects may also become stronger with time (Tilman et al. 2001).

4.5.5 Effect of harvest timing on soil chemical and microbial properties

Harvest timing significantly affected nearly all variables measured. Different variables explained the most variation in microbial response variables in different harvests (Table 17). This suggests that the developmental stage of the plant community and/or external variation in temperature and moisture may serve as determinants of the nature of plant community effects on soil properties, and therefore on the resistance and resilience of the soil microbial community. This is consistent with other studies that have found both the direction and magnitude of soil responses to plant community characteristics to vary with time (Wedin and Tilman 1990, Wardle and Nicholson 1996). The one exception to this trend was the resistance of the soil microbes, which did not show any significant interactions between harvest and treatment, possibly because resistance was not strongly related to soil properties. Overall, this suggests that the results gained from studies on the effects of species composition and diversity will be context-dependent, and may vary according to temporal factors such as time of year or stage of plant community development.

4.5.6 Conclusion

The presence of different plant species results in soils with different abiotic and biotic characteristics. These different characteristics in turn influence the ability of the soil microbes to resist and recover from disturbances. It seems likely that resource limitation or changes in soil microbial community structure in response to variation in the inputs of different plant species may be responsible for across-treatment differences. Increases in plant diversity rarely had any consequences for soil microbial properties, even when there was an increase in aboveground biomass and a change in mineral N in the soil due to diversity effects. However, the time of harvest did have a significant interactive effect with planting treatment for most of our response variables, indicating that there may be variation in results across studies simply because of differences in the timing of measurements. It thus appears that plant composition, rather than plant diversity, was the primary driver of soil microbial community characteristics and their resistance and resilience within harvests, but that the strength and direction of these relationships show significant temporal variation.

Chapter 5: Carbon substrate composition and diversity affect ecosystem functions and soil microbial stability

5.1 Abstract

Despite many studies suggesting that substrate quality is a major driver of litter decomposition, very few studies have examined the ecological role of specific substrates or combinations of substrates. Because carbon (C) substrate composition and diversity may affect their decomposition rate by altering soil chemistry and soil microbial community structure, they have the potential to affect other aspects of ecosystem function, such as the decomposition rate of other substrates, soil microbial stability (resistance and resilience) and plant growth. Eight C substrates that varied in their chemical complexity were added to a base soil on their own, in pairs, in fours and with all other substrates. Treatments were organised in such a way as to vary in the number of types of substrates (analogous to plant functional groups) as well as the number of C substrates. Carbon substrates were added to a base soil every 4 days for 92 days, and then the soil was analysed for changes in soil chemistry, soil microbial community structure and several aspects of ecosystem function. The decomposition rate of the added C substrates was affected by which combination of C substrates was added, but not by their diversity. Carbon substrate composition also affected the soil chemical variables measured, and soil microbial community structure and activity. These changes in soil and microbial characteristics resulted in differences in the ability of the soil microbes to decompose cellulose paper, soil microbial resistance and resilience to a drying disturbance, and plant growth. Carbon substrate diversity had variable effects, with a stronger influence on some aspects of soil chemistry and soil microbial community structure than others. Carbon substrate diversity also had variable effects on different measures of ecosystem function, with strong effects on plant growth, fewer effects on the decomposition of cellulose paper and rare effects on soil microbial stability. Where significant, these diversity effects saturated at low levels, were more common when at least

two substrate functional groups were added, and were idiosyncratic in nature, depending strongly on which combination of substrates was added. Overall, C substrate composition, and sometimes diversity, affected soil chemical variables and soil microbial community composition. These changes had flow-on effects to the decomposition of cellulose paper, soil microbial resistance and resilience, and plant growth, suggesting that the composition and diversity of substrates can be an important driver of ecosystem function.

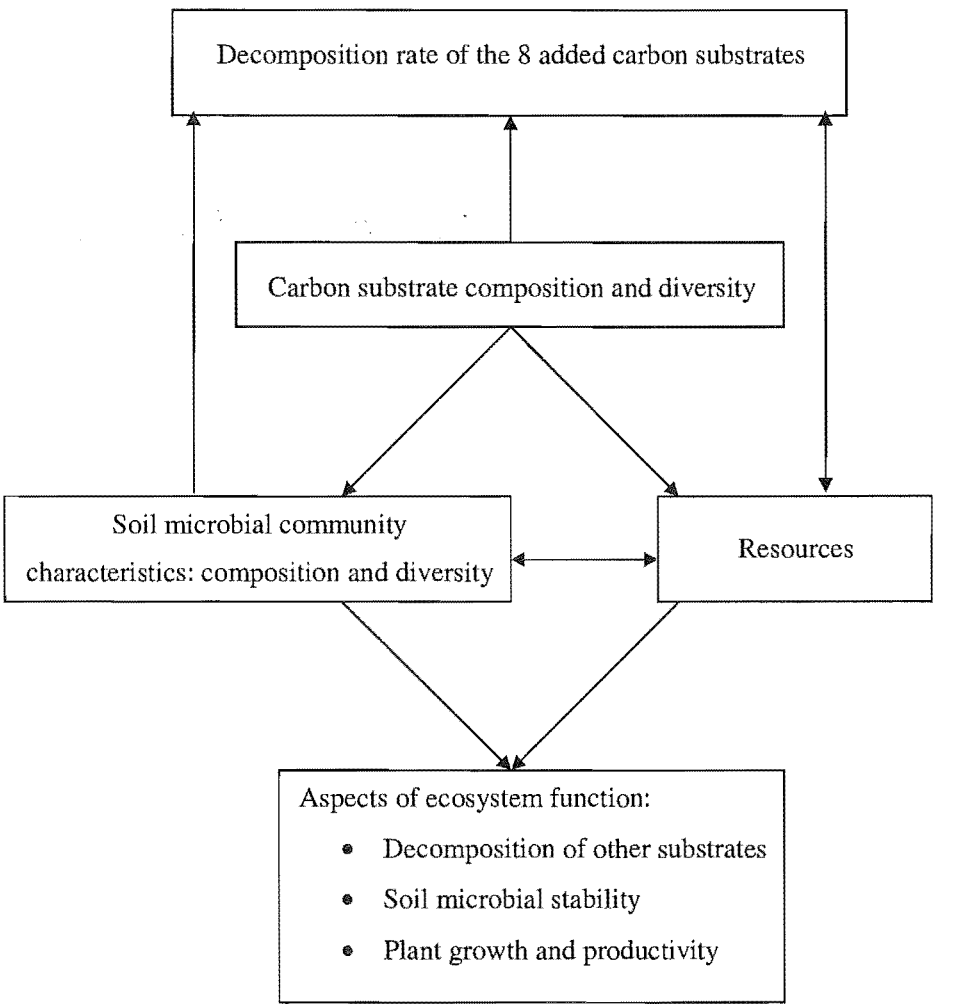


Fig. 14: Flow diagram of hypotheses and interactions examined in Chapter 5

5.2 Introduction

The decomposition of carbon (C) substrates by soil microbes is an important process that potentially affects nutrient mineralisation, plant productivity (Grayston et al. 1996), and C storage in soils (Catovsky et al. 2002). Decomposition may be driven by many factors, including temperature, moisture, nitrogen (N) contents and the chemical nature of the C substrates (Hector et al. 2000, Gartner and Cardon 2004). Of these, the effect of the latter are the least understood, despite many studies invoking this factor as a possible driver of litter decomposition rates (Bardgett and Shine 1999, Nilsson et al. 1999). Carbon substrates may have important effects on soil chemistry (Hector et al. 2000, Magill and Aber 2000) and the soil community (Degens 1998b, Schutter and Dick 2001), and therefore may indirectly affect other aspects of ecosystem functioning. For example, the decomposition of substrates not previously encountered may be dictated by the soil environment and the activity and composition of the soil microbial community already present (Kourtev et al. 2002). These factors may also alter the ability of the soil system to resist and recover from disturbances, and therefore their ability to maintain some level of function regardless of changes in the environment (De Angelis 1992, MacGillivray et al. 1995). Different C substrates can also alter the balance of N mineralisation and immobilisation, potentially affecting the availability of plant nutrients, and therefore plant nutrient acquisition and productivity (Loreau 2001).

Although individual C substrates can undoubtedly affect the soil environment, the microbial community and therefore some aspects of ecosystem functioning, most natural C inputs, such as plant litter and rhizosphere exudates, contain more than one substrate. There is a high potential for C substrates to interact with each other, raising the possibility that mixing substrates (i.e. increasing substrate diversity) may have a non-additive effect on soil chemistry and microbial communities, and therefore on other aspects of ecosystem function driven by soil microbes. Studies to date have manipulated C substrate diversity indirectly only, by altering plant or litter diversity. Their results suggest that plant and litter diversity can have non-additive effects on litter decomposition (Wardle et al. 1997a, Hector et al. 2000), soil chemistry (Briones and Ineson 1996, Hooper and Vitousek 1998), and soil microbial activity and biomass (Christie et al. 1974, Bardgett and Shine 1999).

Carbon substrate diversity has the potential to affect other aspects of soil microbial community structure and ecosystem functioning. Increases in substrate diversity may create new niches for microbial growth, and therefore support a more diverse microbial community (Beare et al. 1995, Grayston et al. 1998), which in turn could influence organic matter decomposition, nutrient mineralisation and plant growth (Loreau 2001). Alternatively, the addition of a greater number of substrates may decrease overall decomposition rates, due to the increased chance of including substrates that inhibit decomposition (Loreau 2001). Substrate diversity has also been predicted to result in higher soil microbial resistance to disturbance, but to have no effect on recovery rates (Harrison 1979). This suggests that C substrates, and their diversity, may have a much greater role to play in ecosystem functioning than previously recognised. However, this has not been experimentally tested.

We conducted an experiment in which C substrates were directly manipulated. Eight C substrates were added to a base soil on their own and in mixtures of varying diversity over a 3-month period. Soils were analysed for changes in the soil environment, microbial activity and community characteristics, and several aspects of ecosystem function. We proposed the following questions: is C substrate composition and diversity an important driver of C substrate decomposition rates? Does C substrate composition and diversity affect soil chemistry or soil microbial community structure? If so, does this then have implications for other aspects of ecosystem function such as decomposition, stability and plant growth?

5.3 Materials and Methods

5.3.1 Experimental design

For this experiment eight C substrates were used, representing four types of chemicals that vary in their complexity and characteristics: simple sugars, polysaccharides, tannins and

fatty acids. Substrates within each chemical type should have similar effects on soil chemistry, soil microbial communities and be metabolised in a similar way by soil microbes. These chemical types are therefore analogous to the functional group concept used in plant systems, and will be called this from here onwards. Each substrate contained only C, hydrogen and oxygen to avoid the confounding effect of varying amounts of nutrients, such as N and phosphorus. A substrate diversity experiment was set up in which each C substrate was added to a base soil on its own, in two pair-wise combinations, in two four-way combinations and with all other C substrates (Table 18). An additional treatment remained unamended to serve as a control (termed the blank soil from here onwards). Treatments containing a mixture of substrates were organised in such a way that the number of functional groups as well as the number of substrates within them differed. Within this framework, combinations were randomly assigned without replacement, with the criterion that the four-way combinations contained the same substrates as two of the pair-wise combinations. Substrates were added so that the amount of C added to each soil was the same for each treatment, and mixtures contained equal proportions of C from each substrate. This design is conceptually analogous to experiments that have looked at plant and litter diversity in which species or functional groups are represented as monocultures and as multiple species mixtures (Hooper and Vitousek 1998, Wardle et al. 2000).

Mineral soil was collected from beneath pasture at Lincoln, New Zealand (43° 30' S), mechanically sieved to 4 mm, and stored at 4°C until used. The soil contained 4% C, 0.32% N and had a pH of 5.1. The experiment was set up with five replicate blocks of the 22 treatments. Soil for each block was adjusted to 33% moisture content on a dry weight basis (MC) (equivalent to 55% water holding capacity) and incubated at 25°C for 4 days in the dark, re-sieved to 4 mm and then incubated for a further day before beginning the experiment. For each experimental unit, we placed 1100 g dry weight (d.w.) of pre-incubated soil into rectangular containers (235mm x 375mm x 55mm), which were then enclosed in clear plastic bags to reduce moisture loss. Based on a preliminary experiment to determine the optimum amount and frequency of C addition, 0.0007 g C g soil d.w.$^{-1}$, was added to the base soil every 4 days over 92 days. Carbon substrates were added in powder form by shaking them through 0.05 mm sieves onto the soil surface, and then gently mixing the soil. The soils were incubated at 25°C throughout the experiment, and the moisture content of the soil in each container was maintained at 33%.

Table 18: Description of carbon substrate composition and the number of functional groups and substrates involved in each of the 22 treatments.

Treatment	C substrates added	Number of functional groups of C substrates	Number of C substrates
Blank	No substrates	0	0
Simple sugars			
A	Glucose (Glu)	1	1
B	Sucrose (Suc)	1	1
Polysaccharides			
C	Cellulose (Cell)	1	1
D	Starch (Star)	1	1
Tannins			
E	Gallic acid (Gall)	1	1
F	Tannic acid (Tan)	1	1
Fatty acids			
G	Stearic acid (Stear)	1	1
H	Palmitic acid (Pal)	1	1
AB	Glu + Suc	1	2
CD	Cell + Star	1	2
EF	Gall + Tan	1	2
GH	Stear + Pal	1	2
AC	Glu + Cell	2	2
BG	Suc + Stear	2	2
DE	Star + Gall	2	2
FH	Tan + Pal	2	2
ABGH	Glu + Suc + Stear + Pal	2	4
CDEF	Cell + Star + Gall + Tan	2	4
ACFH	Glu + Cell + Tan + Pal	4	4
BGDE	Suc + Stear + Star + Gall	4	4
ALL	All substrates	4	8

The preliminary experiment involved adding three different amounts of glucose at three different frequencies to the pasture soil used in the current experiment, over a 3-month period (Table 19). Within each amount treatment, the mass of glucose added at each time point depended on the frequency of addition, but was the same over every 8-day cycle. For example, in treatment 4 (Table 19), 0.027 g was added every 2 days to make a total of 0.108 g glucose per 8-day cycle, and in treatment 7 (Table 19), 0.54 g glucose was added every 4 days to also make a total of 0.108 g glucose per 8-day cycle. The basal respiration and substrate-induced respiration (SIR) of the soil microbes were measured in each treatment during one 8-day cycle after 1 (data not presented) and 3 months, and the coefficient of variation (CV) calculated. Results showed that the amount and frequency of addition did not alter the CV of SIR, but did have an effect on the CV of basal respiration (Fig. 15). Adding glucose every 2 days resulted in the most stable basal respiration, followed by treatments where glucose was added every 4 days at high and medium amounts, and then by the medium amount every 8 days treatment. Adding substrates every

Table 19: Amounts and frequency of glucose addition used in the preliminary experiment designed to determine optimal C substrate addition rates. Amounts in brackets = g of glucose added at the frequency indicated in the second column.

Treatment	Frequency (days)	g glucose added/ 8 d cycle
1	0	0.000 (0)
2	2	0.012 (0.003)
3	2	0.036 (0.009)
4	2	0.108 (0.027)
5	4	0.012 (0.006)
6	4	0.036 (0.018)
7	4	0.108 (0.054)
8	8	0.012 (0.012)
9	8	0.036 (0.036)
10	8	0.108 (0.108)

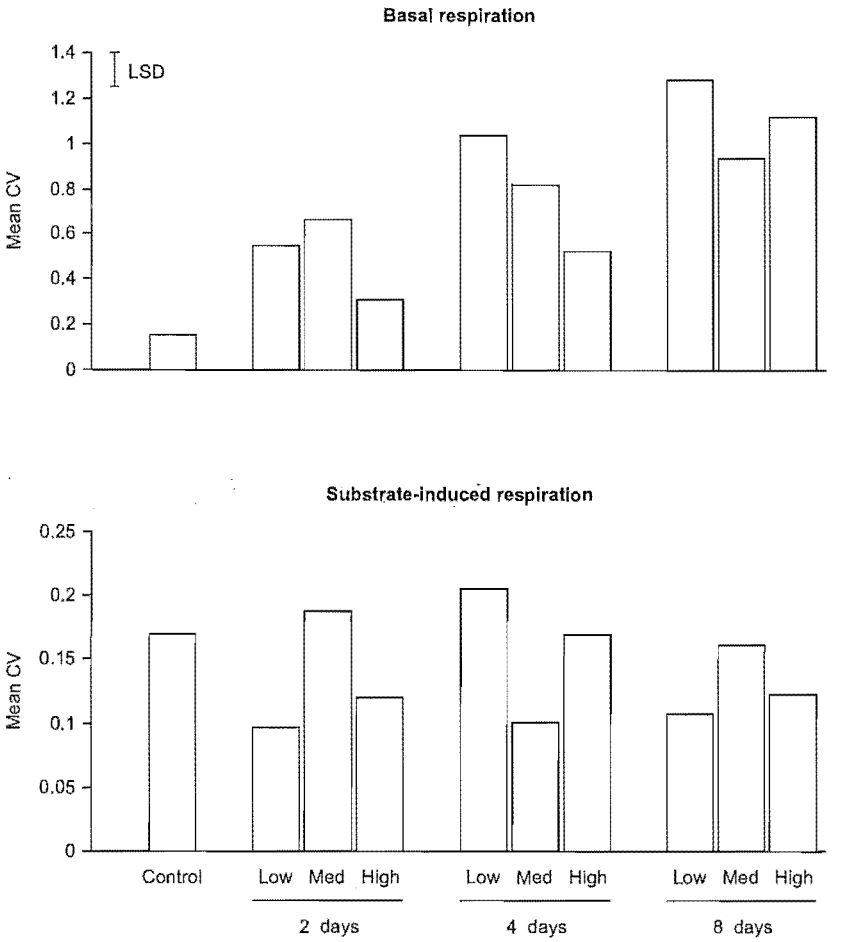


Fig. 15: Effect of the addition of three different amounts of glucose at three different frequencies to a base soil on the coefficient of variation of soil microbial response variables over one 8-day cycle of addition. Low, medium and high correspond to the amount of glucose added, and 2, 4, and 8 days refers to the frequency of addition. See Table 19 for more details.

2 days was impractical for the current experiment, as this did not leave enough time to measure the soil microbial response to that C addition. Therefore, adding substrates every 4 days was chosen as the optimal frequency of C addition. We chose to add the medium amount of substrate, as this resulted in a reasonably stable basal respiration, and, although not significantly different to the other treatments, a more stable SIR on average (Fig. 15). This should reduce the potential for variation in results caused by fluctuations in the activity and biomass of the soil microbial community.

5.3.2 Soil chemical and microbial properties

Basic soil and microbial measurements

Each experimental unit was harvested 4 days after the final substrate addition. For each unit, total C, total N (Leco, Laboratory Equipment Corporation, St Joseph, Michigan, U.S.A), the C:N ratio, pH (Blackmore et al. 1987) and various soil microbial properties were measured. The percentage of added C respired from the soil during the experiment was calculated by comparing the difference between the expected total amount of carbon if none of the added carbon had been respired (initial soil C plus the 18.22 g C added per container over the 92 days) with the final amount of soil carbon, and then dividing by the total amount of C added. This gives an indication of the decomposition rate of the C substrates. Baseline basal respiration and substrate-induced respiration (SIR) measurements were made on soils at 33% MC following 2 days incubation at 25°C. Basal respiration was measured as described by Wardle (1993). Ten g d.w. of soil was placed in a 130-ml airtight container, and incubated at 25°C. The rate of soil microbial respiration was measured by taking 1-ml subsamples of headspace 1 and 4 h after capping the airtight container, and injecting them into an infrared gas analyser (Wardle 1993). Substrate-induced respiration (SIR) was measured based on the method described by Anderson and Domsch (1978). This involved the same measurement as for basal respiration, except the soil was amended with 0.01 g glucose g soil $d.w^{-1}$. before capping the airtight container. A relative measure of the soil microbial metabolic quotient (qCO_2) was calculated as basal respiration divided by SIR (Anderson and Domsch 1985).

Soil microbial catabolic activity and community structure

The catabolic response profile (CRP) and phospholipid fatty acid (PLFA) methods were used to determine treatment effects on soil microbial community catabolic activity and structure.

CRPs were measured for each experimental unit as described by Degens and Harris (1997) and Degens (1998b). Twenty-six organic compounds were used, added at 0.3 M for amino acids, amines and aromatics, 1.5 M for carbohydrates, and 2.0 M for carboxylic acids. These correspond to the same amount of compound added per g of soil as used by Degens and Harris (1997). The CRP compounds consisted of L-asparagine, L-arginine, L-cysteine, L-glutamic acid, DL-histidine, DL-tyrosine, N-methyl-D-glucamine, L-serine, D-glucosamine, L-glutamine, D-glucose, DL-mannose, L-ascorbic acid, citric acid, urocanic acid, Na-formate, D-gluconic acid, a-keto-glutaric acid, a-ketobutyric acid, DL-malic acid, quinic acid, malonic acid, oxalic acid, pantothenic acid, L-tartaric acid and tween-80. Each CRP compound was made up in solution, and the pH adjusted to 7 with the addition of either concentrated sulphuric acid or NaOH. Soil microbial respiration in response to each of these compounds was measured as for basal respiration, except that 1 ml of a CRP compound and 0.5 ml of water was added before the airtight container was capped. The control consisted of adding 1.5 ml of water. Respiration in response to the added compound over and above the response to added water was calculated by taking the difference between the respiration from samples amended with water only (CRP control soil) and samples amended with CRP compounds. These respiration values were then summed to form a measure of total CRP. The proportion of total CRP that was due to respiration in response to amino acids and amides, and to carboxylic acids was also calculated. These two measures will be termed proportional amino acid use and proportional carboxylic acid use from here onwards.

PLFAs were measured for each experimental unit as described by Bligh and Dyer (1959), and as modified by White et al. (1979) and Bardgett et al. (1996). Lipids were extracted from 1.5 g of fresh soil using a mix of chloroform, methanol and citrate buffer (1:2:0.8 by volume). The supernatant from this was split into two phases by adding chloroform and

citrate buffer. The lower chloroform phase containing the lipids was recovered and evaporated under a stream of N gas. These lipids were re-suspended in chloroform, and then separated into neutral lipids (eluted with chloroform), glycolipids (eluted with acetone), and phospholipids (eluted with methanol) by fractionation on silicic acid columns (Isolute; 500 mg silicic acid in 6-ml reservoirs). The phospholipids were retained and evaporated under a stream of N gas, and then mild alkaline methanolysis was performed to create methyl esters. These samples were also evaporated under N gas and stored at –20°C until analysis by gas chromatography (GC).

After GC analysis, peaks were identified by calculating retention times relative to two added internal standards (C13 and C19) and comparing these with peaks from a bacterial methyl ester standard (Supeloc Bacterial Acid Methyl Esters CP Mix 47080-U). The abundance of individual fatty acids was calculated as relative nmoles per g of dry soil, and characterised using standard nomenclature (Tunlid et al. 1989). PLFAs used to represent bacteria were: cyclic fatty acids (cy-17:0, cy-19:0), branched fatty acids (i-15:0, a-15:0, i-16:0, i-17:0) and 15:0. A relative measure of the fungal:bacterial ratio was calculated by dividing fungal PLFA (18:2ω9,12) by bacterial PLFA. All identified peaks were summed to form a measure of total PLFA.

5.3.3 Aspects of ecosystem function

For each harvested experimental unit, we considered three aspects of ecosystem function: decomposition, plant growth and soil microbial stability (resistance and resilience to a drying disturbance).

Decomposition of cellulose paper

The ability of soil microbes in each soil sample to decompose cellulose paper was measured by burying a weighed circle of Whatman filter paper (47-mm diameter) in a Petri dish containing 30 g d.w. of soil at 33% MC. The Petri dish was sealed and incubated at

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25°C for 16 days, after which the filter paper was carefully removed, cleaned, oven dried, and weighed to determine mass loss.

Soil microbial stability

To determine the effect of C substrate composition and diversity on the resistance and resilience of the soil microflora, we used a wetting-drying event as a model disturbance based on the approach described in Chapter 2. Wetting-drying events are common disturbances in soils (Kieft et al. 1987, Fierer and Schimel 2002), and are one of the major drivers of soil microbial turnover (West et al. 1988a, Fierer and Schimel 2002), and therefore have an effect on nutrient availability. Wetting-drying events involve two disturbances: drying, and rewetting of the dried soil (Kieft et al. 1987). For our purposes we have concentrated mainly on the response of the soil microbes to drying. However, a relative measure of the resistance of dry soil to rewetting was also calculated based on measurements made in the 4 h immediately after rewetting. Based on preliminary experiments, the drying disturbance was defined as drying the soil from 33% MC to 6% MC, and the rewetting dry soil disturbance as adding water to bring soil at 6% MC back up to 33% MC. These moisture contents corresponded to 55% and 10% of the water-holding capacity of the base soil before the substrates were added, where 100% water-holding capacity was measured as the amount of water retained in the soil following saturation and 18 h of drainage (Saetre 1998).

Three response variables were used to measure the resistance and resilience of the soil microbial community: basal respiration, SIR, and glucose use. In combination, these response variables were intended to give a summary of the soil microbial response to the wetting-drying event. Basal respiration measured on dried soil and on rewet dry soil was interpreted as indicating the response of soil microbial activity to the changes in resource availability that occur during wetting-drying events. Substrate-induced respiration measured on wet soil gives an indication of the active microbial biomass (Anderson and Domsch 1978). Therefore, SIR measurements made on rewet dry soil were interpreted as indicating the effect of drying on the soil microbial biomass. Substrate-induced respiration was also measured on dried soil. Because the added glucose remains undissolved in dry

soil, it is largely unavailable for soil microbial metabolism (West and Sparling 1986). Any measures of resistance or resilience that included SIR measured on dry soil were therefore interpreted as indicating the effect of drying or rewetting on the ability of disturbed organisms to respond to added substrates, rather than as an indication of biomass. To distinguish between these two measures using SIR, the latter measurement will be referred to in terms of the resistance and resilience of soil microbial glucose use from here onwards, and the former in terms of the resistance and resilience of SIR.

Soil from each experimental unit was adjusted to 33% MC and allowed to equilibrate overnight in a 25°C incubator. This adjustment was very minor as all soils were very close to 33% MC. After the soils were equilibrated, a 60-g d.w. subsample of each soil was spread out on paper trays to air-dry at room temperature to 6% MC. Six 10-g d.w. subsamples of air-dried soil and four 10-g d.w. subsamples of the non-dried soil were then placed in 130-ml Schott glass bottles, sealed with plastic and incubated at 25°C overnight. Resistance to drying was determined after this incubation (time 0 or t_0); two bottles containing dry soil were used to measure basal respiration and SIR in dry soil, and a further two bottles containing dry soil were used to measure these response variables immediately after the soil had been returned to 33% MC. The latter measurement was used to determine soil microbial resistance to rewetting dry soil. Two bottles containing undisturbed soil were used to measure basal respiration and SIR at this point. Resistance was calculated using the index presented in Chapter 2.

To quantify the effect of drying on the resistance of soil microbial response variables, C_0 was defined as the value of the soil for the appropriate response variable that had remained undisturbed at 33% MC. To quantify the effect of rewetting dry soils on soil microbial response variables, C_0 was defined as the value of the dry soil for the appropriate response variable.

The remaining two bottles with dry soil were rewet to 33% MC by adding the required amount of water with a syringe, and incubated for a further 3 days (t_3) to allow some recovery; this gives a relative measure of resilience. Basal respiration and SIR were measured on the control and disturbed soil samples as for resistance. Resilience was calculated using the index described in Chapter 2. We were able to calculate resilience as

the degree to which the rewetted soil recovered from the drying disturbance only. Therefore, the undisturbed soil was the soil that had remained at 33% MC throughout the experiment.

Plant growth

To assess potential aboveground effects of treatments, seeds of mustard (Yates Mustard Quick Salad) were germinated and grown on wet filter paper for 6 days, and then three seedlings were planted into a 150-g d.w. subsample of soil from each experimental unit. Planted seedlings were maintained at 18°C with 16 h of daylight for 3 weeks, and were watered as required. At the end of the growth period, shoots were harvested and roots extracted from the soil. Both shoot and root biomass were dried at 60°C for 48 h and weighed.

5.3.4 Data analysis

The effect of treatments on soil chemical, soil microbial and ecosystem properties were determined using ANOVA with block and treatment as factors. The least significant difference test at $P < 0.05$ was used to determine differences between treatments where ANOVA indicated an overall treatment effect. Data were transformed as necessary to meet the assumptions of normality and homogeneity of variances. Principal component analysis was performed on the CRP and PLFA data to determine the effect of treatment on soil microbial community catabolism and structure. The proportion that each substrate or PLFA made up of total CRP or PLFA was used for this analysis to avoid confounding results with differences in biomass. The Shannon-Weiner index (H') was also calculated for each experimental unit for both PLFA and CRP data, to obtain relative measures of microbial diversity. For each treatment involving mixtures of substrates, we calculated expected values for each response variable based on the values obtained from the component substrates when added alone, i.e.,

$$\text{Expected value (E)} = \left[\sum_{i=1}^{S} M_i\right] / S$$

where M_i is the value of the response variable when component substrates were added alone, and S is the number of substrates in the mixture. For each of the 13 multiple substrate treatments, this value was calculated separately for each replicate block. Wherever a response variable involved a calculation that included measurements of more than one component (resistance and resilience, qCO_2, the C:N ratio, shoot:root ratio, catabolic and PLFA diversity, proportional amino or carboxylic acid use), expected values were determined for each component and then the calculation applied to those values. For example, to calculate expected resistance and resilience values, the expected values for each response variable for the control and disturbed soils at t_0 and t_3 were determined first, and then these values were put into the resistance and resilience indices. For pH, values were transformed to give the concentration of H^+, the expected value formula applied and then logged to give an expected pH. Paired *t*-tests were used to determine whether expected and observed values for each mixed substrate treatment differed significantly at $P < 0.05$. To determine whether there were any overall patterns of change in the magnitude or direction of mixing effects between treatments as C substrate diversity increased, we performed ANOVA on the (O-E)/E ratio across all the mixed substrate treatments, where O is the observed value and E is the expected value. This ratio was not calculated for resistance and resilience, or for H', because the nature of these indices mean that dividing by E skews the results. For pH, (O-E) was used rather than (O-E)/E as pH is already on a log scale and so takes changes in magnitude into account. Effects of substrate diversity on CRP and PLFA principal component scores was analysed using ANOVA. If the principal component score of a mixture was significantly higher or lower than the score of all of its component substrates, this was interpreted as an effect of diversity. To evaluate whether the aspects of ecosystem function measured may be related to soil chemistry and soil microbial diversity and composition, we used Pearson Correlations, or Spearman Rank correlations where data were inherently non-normal.

5.4 Results

Carbon substrate treatments had significant effects on all soil chemical, microbial community characteristics, and the aspects of ecosystem functioning measured (Table 20), except for the fungal:bacterial ratio, branched PLFA content, and catabolic and PLFA diversity (data not presented). The magnitude and direction of C substrate mixing effects were often different between treatments, but only for some response variables (Table 21).

5.4.1 Soil chemical and microbial properties

Carbon substrate composition and occasionally diversity affected the amount of added C respired, as well as soil chemical and microbial variables. The amount of added C respired varied depending on which substrate was added (Table 22). Soils amended with fatty acids respired the least C, and those amended with sucrose, starch, or gallic acid respired the most. Carbon substrate diversity did not affect the amount of C respired, except in one treatment; the observed amount of added C respired from the BGDE treatment was lower than expected ($P < 0.05$).

Total C and the C:N ratio were higher in all C substrate-amended treatments than the blank soil, and varied with C substrate composition (Table 22). The addition of C substrates increased soil pH, but to a lesser extent in the soils amended with a tannin than the other amended soils. The only soil chemical variable that responded to C substrate diversity was pH, with all mixtures containing a tannin showing a lower pH in the substrate mixtures than expected (Fig. 16). However, the magnitude of mixing effects did not show any obvious trends as diversity increased.

Adding C substrates singly enhanced basal respiration and SIR compared to the blank soil, but the extent of this differed among substrates (Table 22). Adding C substrates from within the same functional group usually resulted in similar increases in both basal respiration and SIR, but there were some exceptions. Stearic acid-amended soils showed a higher basal respiration than palmitic acid-amended soils, glucose-amended soils showed a

higher SIR than sucrose-amended soils, and gallic acid-amended soils showed a higher SIR than tannic acid-amended soils. Simple sugars stimulated SIR to the greatest extent. These differences in basal respiration and SIR resulted in soils with different qCO_2 values (Table 22). Substrate diversity had variable effects on soil microbial variables. Basal respiration was unaffected by C substrate diversity (data not presented), but SIR of some treatments did respond to diversity, with two positive and one negative response (Fig. 17). Mixing substrates also had one significant effect on qCO_2, which was lower than expected in the EF treatment at $P < 0.05$. The magnitude and direction of differences between observed and expected values did not vary in any consistent way between treatments for SIR.

Carbon substrate composition and diversity had an effect on CRPs and PLFAs. Adding each C substrate alone enhanced the use of all groups of CRP compounds, compared to the blank soil (Table 23). However, proportional carboxylic acid use was lower and proportional amino acid use was higher in amended soils than in the blank soil. Soils amended with gallic acid or a fatty acid showed the highest proportional amino acid use, and soils amended with a sugar or a tannic acid showed the highest proportional carboxylic acid use. These results were reflected in the PCA, which separated all amended treatments from the blank soil, and several single C substrate treatments from each other (Fig. 18a). The first axis explained 30.6% of the variation, and second axis explained 18.4%. Total PLFAs increased in response to C substrate addition, but there were few effects of C substrate composition (Table 23). Soils amended with gallic and tannic acid showed a higher cyclic PLFA content than the other pure C substrate treatments. This was reflected in the PCA, which distinguished the tannin-amended soils from the other treatments (Fig. 18b). The PCA scores of soils amended with single C substrates from the same functional group were not significantly different to each other, expect for glucose and sucrose on PC axis 1. Several treatments were not significantly different from the blank soil. PC axis 1 and 2 explained 33.9 and 17.8% of the variation respectively.

C substrate diversity altered CRPs in several treatments, and generally increased total use more than was expected based on the values of single substrate treatments (Fig. 19). Carbon substrate diversity resulted in several treatments showing a higher than expected proportional carboxylic acid use (Fig. 19). Proportional amino acid use was also lower than expected in several treatments (Treatments EF, ABGH, CDEF, and ALL). There were no

obvious trends in the magnitude of the difference between expected and observed values for total CRP or proportional carboxylic acid use. Catabolic diversity was generally unresponsive to increased C substrate diversity, except in two treatments (EF and ABGH), which had a lower catabolic diversity than expected. There were no significant effects of C substrate diversity on CRP PC scores (Fig. 18c). There were very few significant effects of C substrate diversity on PLFA data and none on PC scores (Fig. 18d). Total PLFA in treatment AC and branched PLFA contents in treatments FH and ACFH showed higher than expected values. In contrast, PLFA diversity in treatments EF and BG, and cyclic PLFA content in EF were lower than expected.

5.4.2 Aspects of ecosystem function

The addition of single C substrates generally reduced the decomposition of cellulose paper compared to the blank soil (Table 24). Soils amended with a simple sugar or polysaccharide tended to show a higher rate of cellulose paper decomposition than those amended with a tannin or fatty acid. Soils amended with C substrates from within the same functional group showed similar rates of cellulose paper decomposition, except that soils that had been amended with starch had a greater stimulatory effect than those that had been amended with cellulose. Substrate mixing reduced cellulose paper decomposition in some treatments (Fig. 20), although the magnitude and direction of mixing effects did not show any consistent trends as substrate diversity increased. Correlations between cellulose paper decomposition and soil microbial community activity and structure rarely explained more than 10% of the variation, except for proportional amino acid use ($r = 0.2784$, $P < 0.001$). Correlations with C ($r = -0.5231$, $P < 0.001$), and C:N ratios ($r = -0.4868$, $P < 0.001$) showed stronger relationships with cellulose paper decomposition.

Basal respiration and glucose use were reduced by the drying disturbance (Appendix III). Soil microbial biomass, measured as SIR on rewet dry soil, was also reduced by the disturbance. Basal respiration and glucose use increased when the soil was rewet. Basal respiration was still higher than the control soil on day 3 for all soils, indicating that it had not fully recovered. The SIR and glucose use of soil from some treatments had recovered to control levels by day 3, but several others showed higher values than the control soils by

day 3, indicating an over-compensatory response. When these results were expressed as resistance and resilience, several treatment effects were apparent.

The resistance and resilience of soil microbes to drying and rewetting soil was affected by C substrate composition and sometimes by C substrate mixing, but there were few consistent trends. Soils amended with C substrates from within the same functional group showed similar responses to the disturbance for most response variables (Table 24). Although the resistance and resilience of most soils amended with single C substrates were the same as for the blank soil, there was usually at least one treatment that was not. For example, the sucrose-amended soil had a higher resistance of basal respiration to drying, and glucose and gallic acid-amended soils showed a higher resilience of SIR than the blank soil (Table 24). There were also some differences between amended treatments. For example, the resistance of basal respiration to drying of sucrose-amended soil was higher than that of soils amended with polysaccharides or fatty acids, and soils amended with cellulose or palmitic acid tended to have lower resilience than some of the other treatments.

Some treatments showed a significant difference between observed and expected values for some measures of soil microbial stability, indicating that mixing substrates can have a non-additive effect on stability. Treatment AB showed a lower resistance of glucose use to drying and rewetting than expected, and treatment AC showed a higher resistance of glucose use to drying than expected. Treatment CDEF showed a higher resistance of basal respiration, SIR, and glucose use to drying, and a lower resilience of basal respiration and SIR than expected ($P < 0.05$).

There were several significant correlations between stability and soil chemical and microbial properties across experimental units, but most explained less than 10% of the variation. However, some measures of stability were more strongly correlated with community variables. The resistance of basal respiration to drying was correlated with basal respiration ($r = -0.4124$, $P < 0.001$), qCO_2 ($r = -0.3953$, $P < 0.001$), and the first PC axis of CRPs ($r = 0.4165$, $P < 0.001$). The resistance of basal respiration to drying was also related to the first PC axis of CRPs ($r = 0.3764$, $P < 0.001$), and the resilience of SIR and glucose use to drying were significantly correlated with qCO_2 ($r = -0.4336$, $P < 0.001$, and

r = -0.3730, $P < 0.001$ respectively). The resilience of SIR was also correlated with SIR (r = 0.4009, $P < 0.001$).

Carbon substrate composition and diversity had strong effects on plant growth. Adding C substrates reduced shoot and root growth in all amended treatments, and trends within chemical functional groups were similar (Table 24). Soils amended with gallic or tannic acid showed higher shoot and root growth than the soils amended with other substrates, but were still much lower than the blank soil. The shoot:root ratio was high in the blank, gallic acid-, and tannic acid- amended soils, but low in the other treatments. Increasing the diversity of added C substrates resulted in a lower shoot and root biomass, and shoot:root ratio than expected in several treatments (Fig. 21). All mixture treatments that resulted in non-additive effects on plant growth contained either gallic or tannic acid. The magnitude and direction of effects were not related to increasing C substrate diversity.

Shoot and root growth were strongly correlated with pH (r = -0.5351, $P < 0.001$; r = -0.5297, $P < 0.001$ respectively), and basal respiration (r = -0.4973, $P < 0.001$, and r = -0.5438, $P < 0.001$ respectively). Shoot and root growth were also correlated with the first (r = 0.3676, $P < 0.001$ and r = 0.4802, $P < 0.001$ respectively) and second (r = 0.3977, $P < 0.001$ and r = 0.3145, $P < 0.001$ respectively) axes of the CRP PCA. Although other variables were significantly correlated with plant growth, they explained less than 10% of the variation.

Table 20: Effect of C substrate treatment and blocking on soil response variables for data from all 22 treatments, as shown by ANOVA

	Treatment		Block	
Response Variable	*F*	*P*	*F*	*P*
% added C respired[3]	6.64	0.0000	8.45	0.0000
Carbon (%)	11.25	0.0000	1.84	0.1288
C:N ratio	8.15	0.0000	23.11	0.0000
pH	254.77	0.0000	6.14	0.0002
Basal respiration[4] ($\mu g\ CO_2$–C $g^{-1}\ h^{-1}$)	15.67	0.0000	2.7	0.0361
Substrate induced respiration[4] ($\mu g\ CO_2$–C $g^{-1}\ h^{-1}$)	78.96	0.0000	4.95	0.0012
qCO_2[5]	25.48	0.0000	0.75	0.5619
Total CRPs[1] ($\mu g\ CO_2$–C $g^{-1}\ h^{-1}$)[4]	130.15	0.0000	11.68	0.0000
Proportional amino acid use	27.45	0.0000	1.66	0.1674
Proportional carboxylic acid use	37.75	0.0000	1.96	0.1076
PC axis 1 (CRP[1])	36.53	0.0000	0.95	0.4400
PC axis 2 (CRP[1])	94.63	0.0000	3.57	0.0097
Total PLFA[2] content (relative nmoles)	1.92	0.0198	2.77	0.0327
Cyclic PLFA[2] content (relative nmoles)[4]	2.17	0.0071	0.65	0.6255
PC axis 1 (PLFA[2])[6]	2.44	0.0022	2.39	0.0577
PC axis 2 (PLFA[2])[6]	2.15	0.0076	3.23	0.0162
Cellulose paper decomposition (% mass loss)	8.61	0.0000	2.05	0.0945
Resistance to drying of:				
Basal respiration[6]	2.15	0.0074	4.32	0.0032
Substrate-induced respiration	1.8	0.0318	1.5	0.2091
Glucose use[5]	1.75	0.0388	2.31	0.0644
Resistance to rewetting of:				
Basal respiration[6]	1.82	0.0295	3.06	0.0295
Glucose use[6]	2.03	0.0122	2.36	0.0597
Resilience to drying of:				
Basal respiration	2.1	0.0094	1.56	0.1914
Substrate-induced respiration	2.38	0.0028	1.93	0.1132
Glucose Use	2.41	0.0025	0.85	0.4965
Shoot biomass[4] (g d.w.)	139.01	0.0000	7.86	0.0000
Root biomass[4] (g d.w.)	30.54	0.0000	4.76	0.0016
Shoot:root ratio[4]	5.85	0.0000	4.64	0.0020

[1] Catabolic response profile, [2] phospholipid fatty acid. [3] Does not include blank in analysis, [4] log transformed, [5] square root transformed, [6] rank transformed.

Table 21: Effect of treatment and block on the response of variables to C substrate mixing for the 13 treatments containing more than one C substrate, as shown by ANOVA. Substrate mixing effects are expressed as (O-E)/E where O = observed values, and E = expected values based on the value of response variables when component C substrates were added singly to the soil. The effect of mixing on soil pH is expressed as (O-E).

	Treatment		Block	
Response Variable	*F*	*P*	*F*	*P*
% added C respired	1.74	0.0884	2.57	0.0498
Carbon	1.86	0.0653	3.07	0.0249
C:N ratio	1.29	0.2548	13.07	0.0000
pH	22.80	0.0000	12.88	0.0000
Basal respiration	0.38	0.9631	1.04	0.3961
Substrate-induced respiration[1]	2.48	0.0132	2.90	0.0315
qCO_2[1]	1.13	0.3565	0.47	0.7565
Total catabolic response profile	5.63	0.0000	0.19	0.9428
Proportional amino acid use	1.64	0.1130	2.88	0.0323
Proportional carboxylic acid use[2]	3.48	0.0010	3.86	0.0084
Total phospholipid fatty acid content	1.87	0.0631	4.11	0.0061
Cyclic fatty acid content[1]	1.28	0.2637	1.89	0.1271
Branched fatty acid content	1.38	0.2109	7.00	0.0002
Fungal:bacterial ratio	0.89	0.5619	1.32	0.2754
Cellulose decomposition	2.59	0.0100	2.25	0.0780
Shoot biomass[1]	58.64	0.0000	3.92	0.0079
Root biomass[1]	10.51	0.0000	3.00	0.0275
Shoot:root ratio[1]	5.27	0.0000	5.54	0.0009

[1]Log transformed, [2] rank transformed.

Table 22: Effects of pure C substrate treatments on soil chemical and microbial variables. Numbers within columns followed by the same letter are not significantly different at $P < 0.05$. Lower-case letters are derived from LSD analysis on all 22 treatments where ANOVA showed a significant effect of treatment. Treatment codes are given in Table 18.

		Soil chemical variables			Soil microbial variables		
	C respired[1]	Total C	C:N ratio	pH	BR[2,4]	SIR[3,4]	qCO_2[5]
Treatment	(%)	(%)			($\mu g\ CO_2\text{-}C\ g^{-1}\ h^{-1}$)	($\mu g\ CO_2\text{-}C\ g^{-1}\ h^{-1}$)	
A	72.4bc	4.5cd	13.3b	5.80ab	2.9cd	42.1a	0.07e
B	86.3a	4.2e	13.4b	5.79b	2.3de	40.9b	0.07e
C	64.6c	4.6bc	14.0a	5.82ab	3.9ab	15.1de	0.26bc
D	81.1ab	4.3de	13.2b	5.83ab	3.9ab	17.5d	0.22c
E	84.5a	4.2e	13.5b	5.53c	1.4f	27.5c	0.05e
F	65.5c	4.6bc	14.1a	5.15d	1.6ef	13.1ef	0.13d
G	60.0de	4.7ab	14.6a	5.81ab	4.7a	12.2f	0.38a
H	52.9e	4.8a	14.5a	5.84a	3.4bc	11.3f	0.30b
Blank	N/A	3.88f	11.7c	4.96e	0.3g	1.9g	0.23c

[1] The percentage of the C added over the duration of the experiment that was respired from the soil (does not include blank in the analysis), [2] basal respiration, [3] substrate-induced respiration. [4] Log transformed, [5] square root transformed.

Table 23: Effects of pure C substrate treatments on soil microbial catabolic response profiles and phospholipid fatty acid contents (PLFA). Numbers within columns followed by the same letter are not significantly different at $P < 0.05$. Lower case letters are derived from LSD analysis on all 22 treatments where ANOVA showed a significant effect of treatment. Treatment codes are given in Table 18.

	Catabolic Response Profile			PLFA	
Treatment	Total[2] ($\mu g\ CO_2\text{-}C\ g^{-1}\ h^{-1}$)	Proportional amino acid use	Proportional COOH[1] use	Total (rel. nmoles)	Cyclic[2] (rel. nmoles)
A	257.5a	0.28cd	0.56c	136.5bc	18.9b
B	257.1a	0.28d	0.57c	170.2ab	18.7b
C	107.2e	0.35b	0.51d	142.6ab	16.7b
D	139.6cd	0.37bc	0.50de	156.8ab	18.4b
E	228.8b	0.3a	0.56c	141.9ab	34.3a
F	143.8c	0.31c	0.61b	153.6ab	37.3a
G	106.0e	0.42a	0.47f	184.3a	17.9b
H	128.1d	0.42a	0.48ef	168.0ab	15.6b
Blank	44.3f	0.16e	0.77a	97.4c	16.2b

[1] COOH = carboxylic acid. [2] Log transformed.

Table 24: Effects of pure C substrate treatments on measures of ecosystem function. Numbers within columns followed by the same letter are not significantly different at $P < 0.05$. Lower case letters are derived from LSD analysis on all 22 treatments where ANOVA showed a significant effect of treatment. Treatment codes are given in Table 18.

		Resistance to drying			Resistance to rewetting		Resilience to drying			Plant growth		
Treatment	Decomp[1] (% mass loss)	BR[2,6]	SIR[3]	Glucose Use[5]	BR[2,6]	Glucose Use[6]	BR[2]	SIR[3]	Glucose Use	Shoot[4] (g d.w.)	Roots[4] (g d.w.)	Shoot: root[4] ratio
A	35.8bc	0.05abc	0.54d	0.003cd	-0.94abc	-0.98bcd	0.49ab	0.34a	0.75abc	0.04d	0.006ef	6.4b
B	28.6cd	0.06a	0.56cd	0.004bcd	-0.94ab	-0.98bcd	0.53ab	0.04abcde	0.71abc	0.04d	0.007def	5.3b
C	30.6cd	0.02cde	0.68abcd	0.004bcd	-0.97cdef	-0.98bcd	-0.08c	-0.19bcde	0.54cde	0.03d	0.005f	6.6b
D	49.2ab	0.01de	0.77ab	0.002d	-0.99f	-0.99d	0.63a	-0.26de	0.55bcde	0.04d	0.007ef	7.2b
E	17.2de	0.04abcd	0.66abcd	0.003cd	-0.96bcdef	-0.99cd	0.46ab	0.34a	0.78ab	0.29c	0.025c	11.7a
F	10.0ef	0.05abc	0.62abcd	0.005bcd	-0.95abcdef	-0.98abc	0.68a	0.27abc	0.79ab	0.53b	0.046b	11.9a
G	0.6f	0.01e	0.58bcd	0.003abcd	-0.98ef	-0.98abcd	0.57a	-0.05abcde	0.59bcde	0.04d	0.008def	5.5b
H	7.7ef	0.02cde	0.62abcd	0.005bcd	-0.98def	-0.98bcd	-0.04bc	-0.30e	0.37e	0.04d	0.009d	4.8b
Blank	55.0a	0.04bcde	0.62abcd	0.011a	-0.89abcde	-0.94ab	0.42ab	-0.21cd	0.47de	1.38a	0.121a	11.9a

[1]Decomposition of cellulose paper, [2]basal respiration, [3]substrate-induced respiration. [4]Log transformed, [5]square root transformed, [6]rank transformed. Resistance and resilience values are calculated using the indices given in Chapter 2.

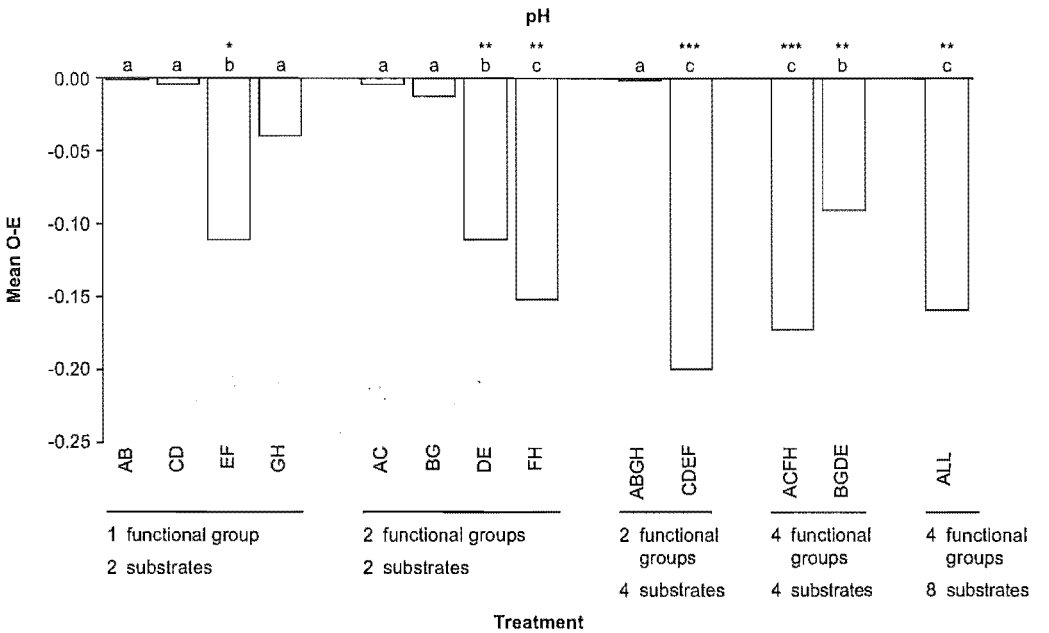

Fig. 16: Effect of carbon substrate mixtures on soil pH, as calculated by (O–E) where O = observed values and E = expected values based on the effects of component substrates when added alone. Bars topped with the same lower-case letter are not significantly different at $P < 0.05$ (Least significant differences test following ANOVA). Capital letters indicate substrates present in mixture (Table 18). Stars indicate that the observed values were different to the expected values, according to paired *t*-tests. *$P < 0.05$; **$P < 0.01$; *** $P < 0.001$.

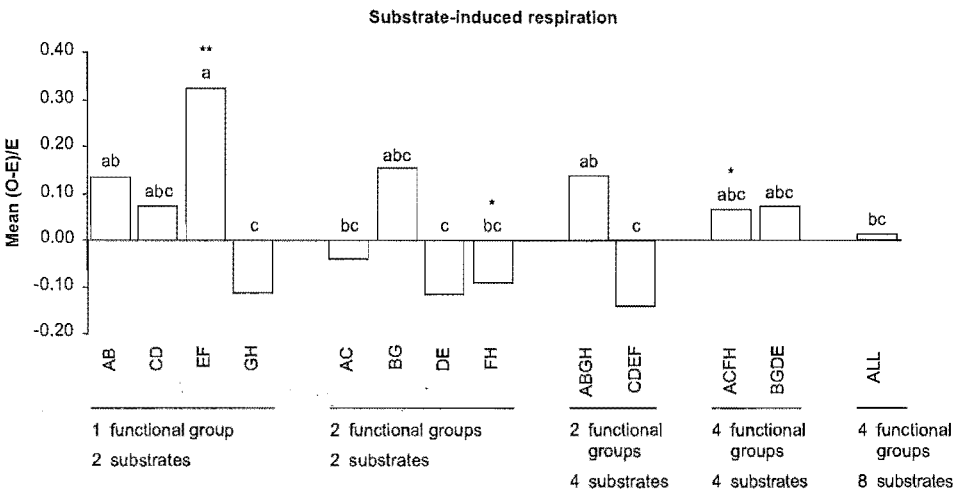

Fig. 17: Effect of carbon substrate mixtures on soil microbial SIR, as calculated by (O–E)/E where O = observed values and E = expected values based on the effects of component substrates when added alone. Bars topped with the same lower-case letter are not significantly different at $P < 0.05$ (Least significant differences test following ANOVA). Capital letters indicate substrates present in mixture (Table 18). Stars indicate that the observed values were different to the expected values, according to paired *t*-tests. *$P < 0.05$; **$P < 0.01$; *** $P < 0.001$.

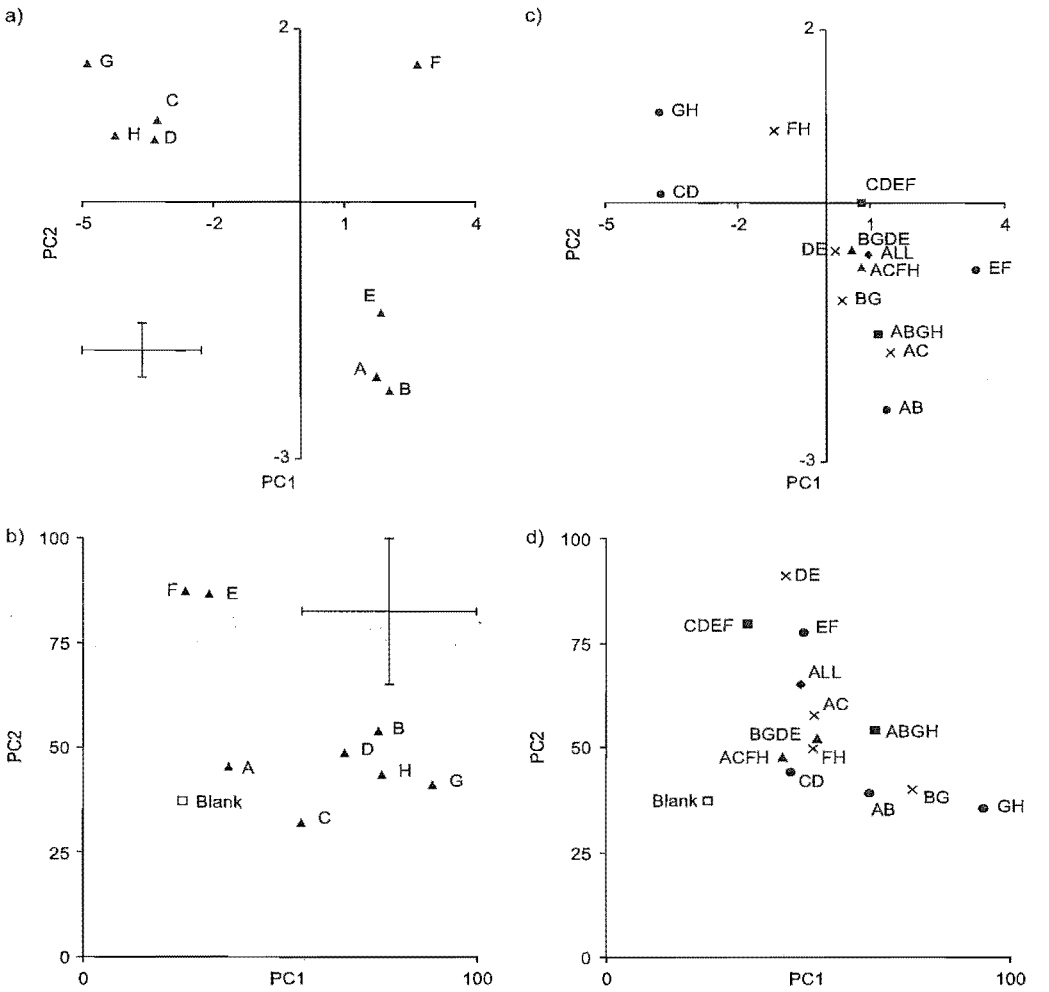

Fig. 18: Effect of C substrate identity and diversity on the principal component (PC) scores of catabolic response profiles (CRP) and phospholipid fatty acids (PLFA). a) and b) show PC scores for the pure C substrate treatments for CRP and PLFA respectively, and c) and d) show the scores for the 13 mixture treatments for CRP and PLFA respectively. PC axes for PLFA were rank transformed. The PC scores for the CRP of the blank soil (panel a) and c)) was (5.01, 8.09). Error bars are LSD ($P < 0.05$) for each axis, error bars in panel a) also apply to panel c); and error bars in panel b) also apply to panel d). In panel c) and d): circles = 1 functional group, two C substrates; crosses = two functional groups, two C substrates; squares = two functional groups, four C substrates; triangles in c) and d) = four functional groups, four C substrates; diamond = all eight substrates. Capital letters indicate substrates present in treatments (Table 18).

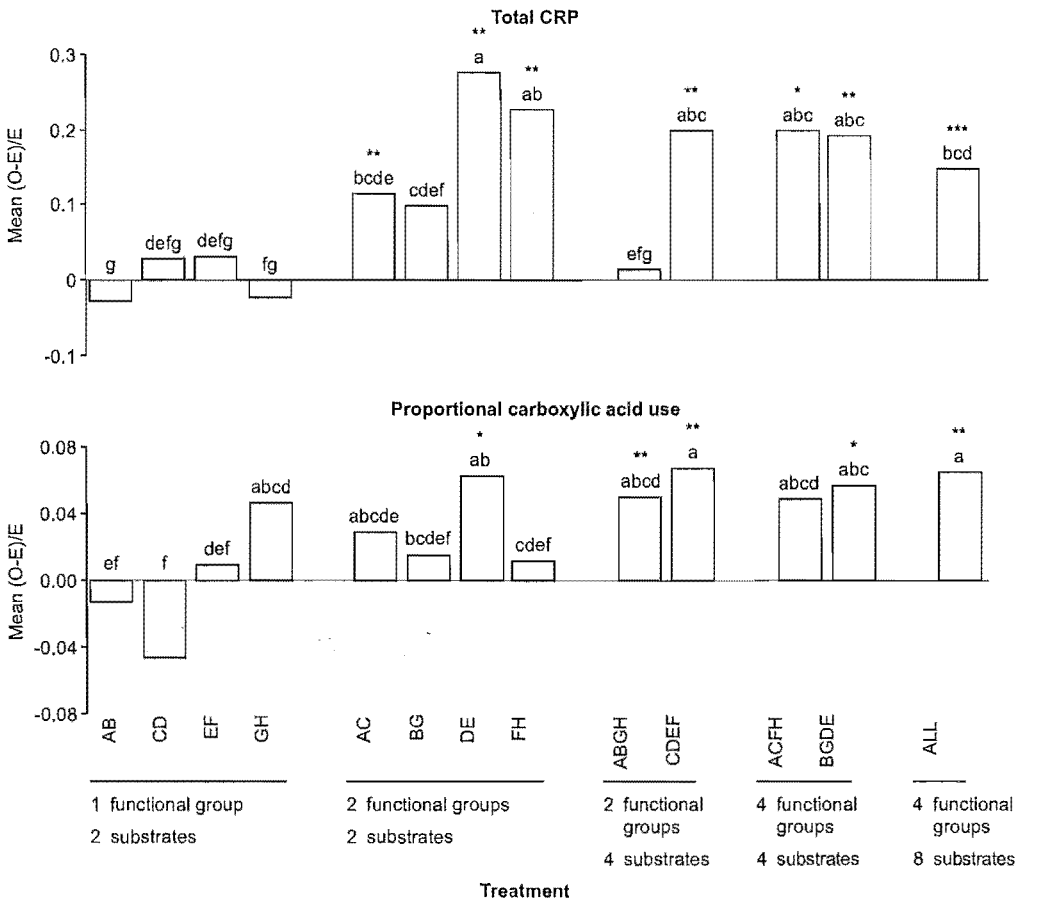

Fig. 19: Effect of carbon substrate mixtures on total catabolic response profile (Total CRP = sum of all responses to all added CRP compounds) and proportional carboxylic acid use (total respiration in response to carboxylic acids/total CRP), as calculated by (O–E)/E where O = observed values and E = expected values based on the effects of component substrates when added alone. Bars topped with the same letter are not significantly different at $P < 0.05$ (Least significant differences test following ANOVA). Capital letters indicate substrates present in mixture (Table 18). Stars indicate that the observed values were different to the expected values, according to paired *t*-tests. *$P < 0.05$; **$P < 0.01$; *** $P < 0.001$.

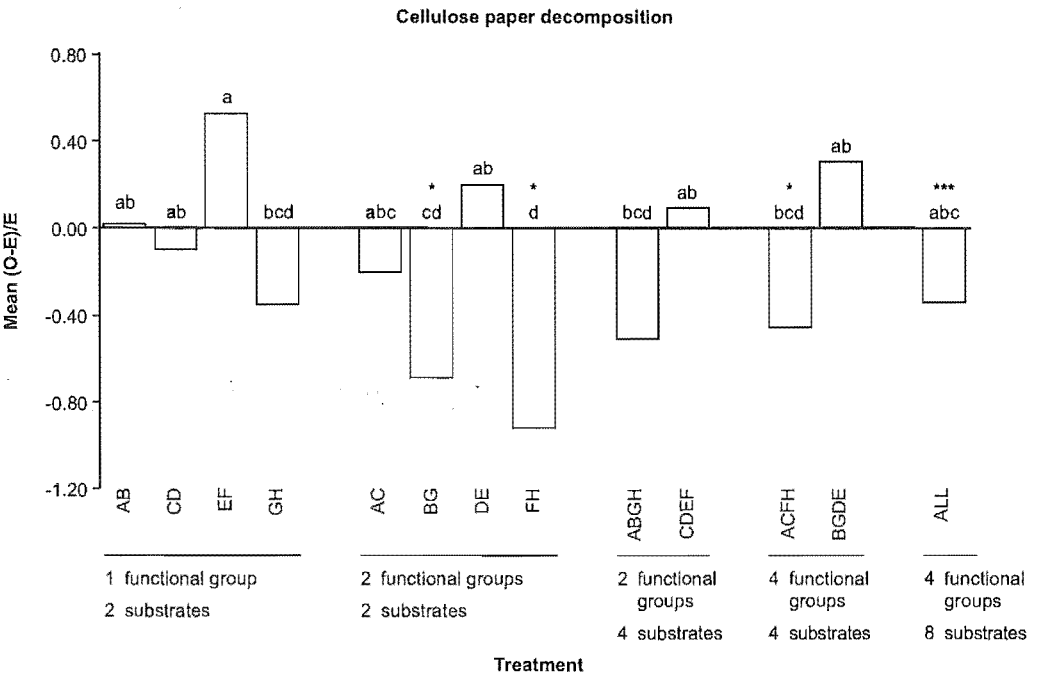

Fig. 20: Effect of carbon substrate mixtures on the decomposition of cellulose, as calculated by (O–E)/E where O = observed values and E = expected values based on the effects of component substrates when added alone. Bars topped with the same lower-case letter are not significantly different at $P < 0.05$ (Least significant differences test following ANOVA). Capital letters indicate substrates present in mixture (Table 18). Stars indicate that the observed values were different to the expected values, according to paired *t*-tests. *$P < 0.05$; **$P < 0.01$; *** $P < 0.001$.

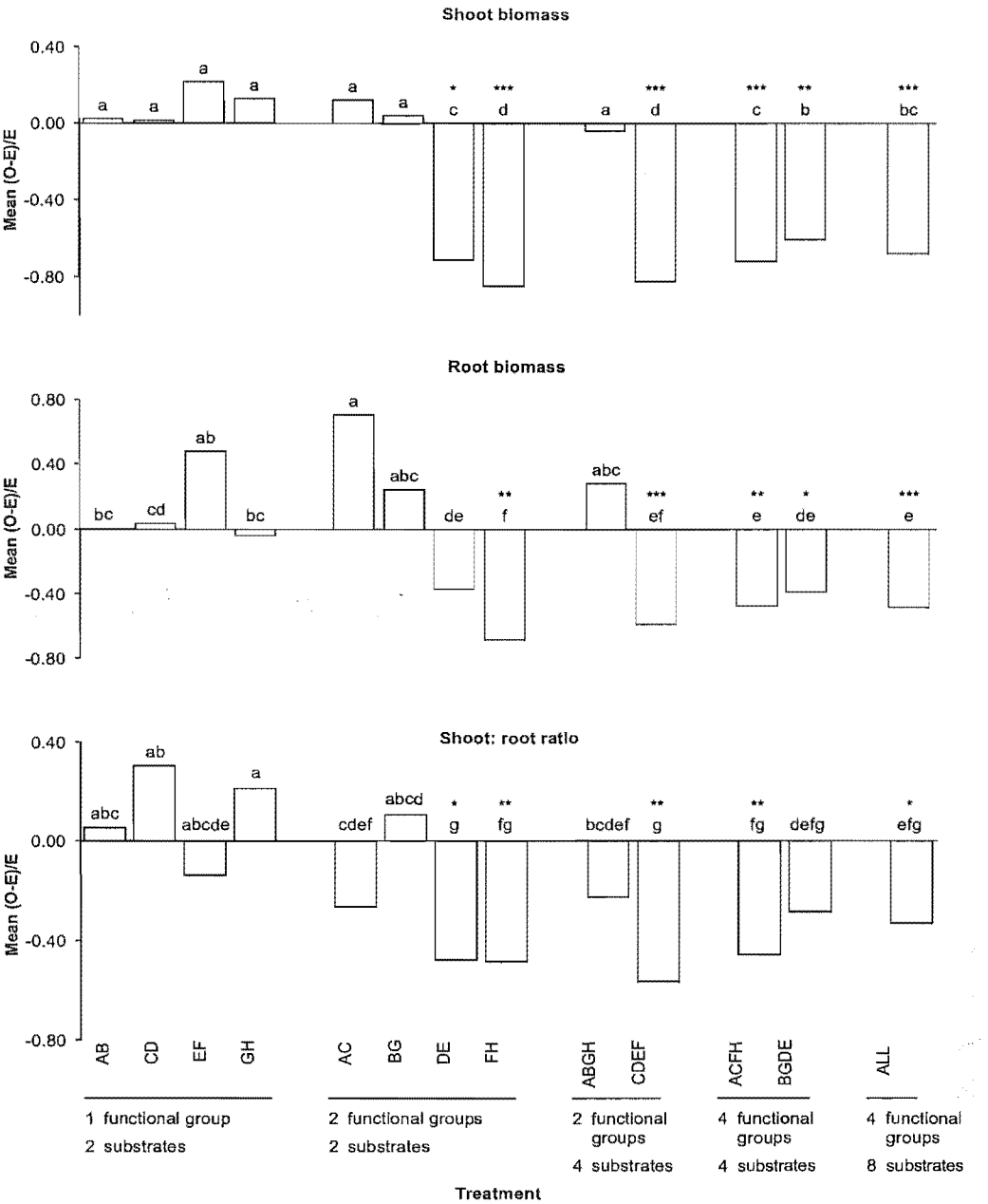

Fig. 21: Effect of carbon substrate mixtures on plant variables, as calculated by (O–E)/E where O = observed values and E = expected values based on the effects of component substrates when added alone. Bars topped with the same lower-case letter are not significantly different at $P < 0.05$ (Least significant differences test following ANOVA). Capital letters indicate substrates present in mixture (Table 18). Stars indicate that the observed values were different to the expected values, according to paired *t*-tests. *$P <$ 0.05; **$P <$ 0.01; *** $P < 0.001$.

5.5 Discussion

5.5.1 Carbon substrate composition: soil chemical and microbial properties

Carbon substrate composition affected the amount of added C respired over the duration of the experiment, indicating that the chemical characteristics of substrates can be an important driver of decomposition rates. This variability in the amount of C respired resulted in different amounts of C remaining in the soil (Table 22), and suggests that C substrate composition may affect C storage in soils, at least in the short term (Catovsky et al. 2002).

Carbon substrate composition affected some aspects of soil chemistry, such as pH and the C:N ratio, and soil microbial activity, SIR and the microbial metabolic quotient (qCO_2) (Table 22). These results are consistent with other studies that have manipulated the types of substrate inputs to the soil by adding different chemicals (Degens 1998b), or by growing different plant species (Bardgett et al. 1999b, Wardle et al. 2003). We predicted that adding different C substrates would alter soil microbial community structure and catabolism. Both CRPs and soil PLFA contents were altered by C addition (Table 23), consistent with other studies (e.g. Degens 1998b, Grayston et al. 1998). However, C substrate composition had a much stronger effect on CRPs than on PLFAs. This suggests that adding C substrates can alter the catabolic activity of the soil microbial community without large changes in microbial composition occurring, and that these two aspects of the soil community may be only weakly linked.

5.5.2 Carbon substrate composition: aspects of ecosystem function

The effect of C substrate composition on the aspects of ecosystem function measured was variable (Table 24). Decomposition of cellulose paper responded to C substrate composition. This is consistent with some studies that have examined how plant species, and therefore types of C inputs, affect decomposition (Spehn et al. 2000a), but not others (Wardle et al. 1999). Cellulose paper decomposition results did not appear to be strongly

correlated with components of the microbial community discriminated using CRPs or PLFAs, or to measures of microbial diversity. This is consistent with the study of Degens (1998a), which found that the decomposition of straw was not always altered by differences in soil microbial community composition and diversity. However, this may be because the methods used in our study are biased towards bacteria (Øvreås 2000), and so may not reliably measure the primary decomposers of cellulose, which are fungi (Hu and van Bruggen 1997). Cellulose paper decomposition was correlated with proportional amino acid use, and to C and the C:N ratio. This suggests that changes in soil microbial catabolic activity, N (Magill and Aber 2000), C, and energy availability (Clarholm 1985, Saetre 1998) caused by adding the C substrates may have been the primary drivers behind our results.

Soil microbial stability was affected by C substrate composition but in a complex way. There were three relatively consistent trends (Table 24). Firstly, resistance was either the same or reduced in amended soils compared to the blank soil. Secondly, the stability of soils amended with a simple sugar were often different to soils amended with a polysaccharide, and thirdly, soils amended with palmitic acid tended to have comparatively low resilience. The complexity of the results suggests that different mechanisms were operating in different treatments, and for different response variables, consistent with other studies (Wardle et al. 1999, Griffiths et al. 2000).

The resistance and resilience of most response variables were not strongly related to soil chemical variables, or to measures of CRPs, PLFAs and their diversity. Griffiths et al. (2000, 2001) also found few consistent relationships between microbial diversity and stability. However, the stability of some response variables in our study were correlated with components of the microbial community discriminated using PCA of CRPs, and to basal respiration and SIR. This suggests that the catabolic activity of soil microbes and their SIR may have an impact on the stability of some response variables, and is consistent with results found by Degens et al. (2001). Other potential drivers that may explain the differences in stability include multi-trophic interactions among soil biota (de Ruiter 1998), and nutrient availability (De Angelis 1992, MacGillivray et al. 1995).

Plant growth was negatively affected by C substrate addition compared to the unamended soil (Table 24), suggesting that the addition of C substrates enhanced nutrient immobilisation and therefore reduced plant growth (Jonasson et al. 1996b, Baudoin et al. 2003). This effect was less obvious for soils amended with gallic or tannic acid than for those amended with the other substrates. This may have been because tannins inhibited the growth of micro organisms that would otherwise have immobilised nutrients (Swift et al. 1979), enhanced the availability of some nutrients, or reduced the activity of phytotoxic compounds (Hättenschwiler and Vitousek 2000). It is also possible that the addition of tannins resulted in a stronger priming effect on soil organic matter than the other substrates, enhancing nutrient mineralisation. Plant growth was also correlated with the ordination score values derived from PCA of CRPs, and to basal respiration. This suggests that differences among treatments in the catabolic ability and activity of soil microbes may have affected nutrient mineralisation, and therefore plant growth.

5.5.3 Carbon substrate diversity

Carbon substrate diversity had variable effects on soil properties and the aspects of ecosystem functioning measured. However, there were several consistent trends. Firstly, non-additive effects of mixing C substrates generally required the presence of at least two chemical functional groups, with the exception of the EF treatment. Studies that have examined the effect of litter diversity on litter decomposition have also found greater effects when species belong to different, rather than the same, functional groups (Wardle et al. 1997a). Secondly, increasing diversity beyond two substrates (or functional groups of substrates) did not increase the effect of diversity. This supports several studies that have shown that diversity effects saturate at relatively low levels (Wardle et al. 1997a, Smith and Bradford 2003). Thirdly, the impacts of mixing C substrates on ecosystem functions were often neutral or negative, consistent with the predictions of the theoretical model of Loreau (2001). Fourthly, the occurrence of a significant diversity effect depended strongly on which substrates were mixed, suggesting that a particular combination of C substrates was required before non-additive effects were found. This supports both the idiosyncratic hypothesis of how diversity affects ecosystem function (Lawton 1994), and the view that

composition is of greater importance than diversity *per se* for soil microbes (Bardgett and Shine 1999, Wardle et al. 2003).

Different effects of diversity were found for different response variables and functions. Carbon substrate diversity did not affect the amount of added C that was respired, with the exception of one treatment. This suggests that C substrate diversity may not affect C storage in soils, in contrast to the prediction of Catovsky et al. (2002) that plant diversity, and therefore probably substrate diversity, may have positive effects on soil C storage. It also suggests that differences in C substrate quality may not be an important factor influencing the non-additive effects of litter diversity on processes such as decomposition. The measured soil chemical variables, soil microbial activity, SIR and qCO_2 were also largely unaffected by C substrate diversity, consistent with several other studies that have considered the effects of plant diversity on these properties (Gastine et al. 2003, Wardle et al. 2003). The pH of soils that had been amended with a mixture containing a tannin were up to 0.2 units lower than expected (Fig. 16), although this may not be large enough to have an important biological impact.

Carbon substrate diversity had a more consistent impact on CRPs. Treatments containing mixtures of substrates often had a higher than expected value of total CRP, a higher than expected proportional carboxylic acid use (Fig. 19) and a lower than expected proportional amino acid use. This suggests that mixing C substrates resulted in greater use of some CRP compounds than would have occurred when these C substrates were added singly. This could occur, for example, if adding a mix of substrates to soil enhanced the decomposition of substrates already in the soil (e.g. Wu et al. 1993), potentially enhancing overall catabolic activity. In contrast to this, PLFAs rarely responded to substrate diversity. C substrate diversity also rarely affected catabolic or PLFA diversity, and where it did, it had a negative effect. This contradicts the hypothesis that substrate diversity should increase microbial diversity (Grayston et al. 1998). Other studies that have manipulated substrate diversity by altering plant diversity have also found few effects of diversity on soil microbial composition (Wardle et al. 2003, Hedlund et al. 2003, but see Schutter and Dick 2001 with regard to substrate diversity). Overall, this suggests that mixing C substrates may have no effect on soil microbial community composition and diversity, but may still

have an effect on soil microbial catabolic activity and the types of C substrates metabolised.

Carbon substrate diversity had occasional effects on soil microbial stability, more frequent effects on the decomposition of cellulose paper (Fig. 20), and large impacts on plant growth (Fig. 21). These results are partially in agreement with several other studies that have found that increasing plant diversity has little or no effect on decomposition (Hector et al. 2000, Spehn et al. 2000a) or soil microbial stability (Wardle et al. 1999, Wardle et al. 2000). However, where C substrate diversity effects were significant, they often had large effects on both the decomposition of cellulose paper and measures of soil microbial stability, suggesting that C substrate diversity may have important consequences for ecosystem processes in some contexts. Catabolic diversity was not related to cellulose paper decomposition, suggesting that high catabolic diversity does not necessarily increase the decomposition of all added substrates. Several of the significant effects of substrate diversity on microbial stability contradicted the predictions of Harrison (1979), who suggested that substrate diversity should increase the resistance of biomass, but have no effect on resilience. Our results showed both positive and negative effects of C substrate diversity on soil microbial resistance, and several significant effects on resilience.

The effect of C substrate diversity on plant growth was context-dependent, with a negative effect on growth in some treatments. However, this only occurred when the soils had been amended with mixtures containing a tannin, suggesting that the sampling effect may have been the primary mechanism behind these diversity effects (Hooper and Vitousek 1997, Huston 1997). We predicted that C substrate diversity could have two contradictory effects. If C substrate diversity increases microbial diversity, and if this in turn increases decomposition and nutrient mineralisation, plant growth may increase (Loreau 2001). Alternatively, increasing substrate diversity increases the chance of including recalcitrant substrates that reduce decomposition, nutrient mineralisation and therefore plant growth (Loreau 2001). Our results do not support the first of these explanations. Carbon substrate diversity did not increase catabolic or PLFA diversity, had either no effect on or reduced the decomposition of other substrates as indicated by cellulose paper decomposition, and resulted in reduced rather than increased plant growth. The second of these explanations therefore seems more likely. Alternatively, the concentration of the tannins in mixture may

simply not have been sufficient to have the relatively positive effect they had in monoculture.

5.5.4 Conclusion

Carbon substrate composition, and sometimes diversity, was found to have a significant impact on the soil environment, and soil microbial activity and community structure. This in turn had flow-on effects to some aspects of ecosystem functioning. The eight C substrates showed different rates of decomposition, suggesting that differences in resource quality among substrates can play an important role in determining litter decomposition rates. Carbon substrate composition affected soil chemical properties and microbial community structure, which in turn strongly affected several aspects of ecosystem functioning. However, C substrate diversity had variable effects on the soil environment and microbial communities, with a strong impact on some response variables but not others. This was reflected in the various measures of ecosystem functioning, which were also sometimes responsive to C substrate diversity. Overall, C substrates, and mixtures of C substrates, were shown to be a significant driver of soil chemical properties, and soil microbial community structure and activity, and therefore of aboveground and belowground function.

Chapter 6: Assessing diversity, composition, and resources as drivers of ecosystem function and stability

The primary goal of the research described in this thesis was to determine the factors that drive ecosystem function and ecological stability, with an emphasis on soil microbial stability and the interaction between plant and microbial function. This required the development of a method that could quantify the stability of the soil microbial community to a disturbance and the derivation of indices that could summarise this information. The approach used was presented in Chapter 2. The research presented in the middle chapters (3 – 5) of this thesis aimed to determine the role of three potential drivers of ecosystem function and stability: diversity, composition and soil resources. This final chapter will discuss the likely importance of these factors, and how successful my approach was in assessing this.

6.1 Success of the stability measure

6.1.1 Choice of disturbance

To test how soil microbial stability responded to diversity, composition and resources, an assay capable of measuring the response of the soil microbes to a model disturbance was required. Theory and empirical evidence suggest that the type of disturbance will affect the response of the organisms measured (Steinman et al. 1990, Sankaran and McNaughton 1999, Joergensen and Raubuch 2003). Disturbances can operate on both long and short-term time scales. My research focused on a short-term disturbance and the short-term response to that disturbance. Short-term disturbances occur frequently in the soil environment, for example, through wet-dry and freeze-thaw cycles, or the input of labile organic matter. All of these disturbances affect soil microbial processes, including the

decomposition of organic matter and nutrient mineralisation (Birch 1964, Alon and Steinberger 1999, Herrmann and Witter 2002). These processes can have an impact on the amount of carbon (C) stored in the soil (Catovsky et al. 2002), and on plant growth and productivity (Grayston et al. 1996, Jonasson et al. 1996a). Therefore, any disruption to soil microbial processes caused by short-term disturbances may have downstream effects on other ecosystem functions. The response of soil microbes to longer-term disturbances, such as those that occur at the whole plant level, may be a fruitful area for future research.

The response of a system to a disturbance will depend on whether that disturbance is negative or positive. The wetting-drying event used as a disturbance here encompasses the soil microbial response to both negative and positive disturbances. Drying reduces the availability of water as well as the C and nutrients dissolved in that water (Lundquist et al. 1999, Ilstedt et al. 2000, Liu et al. 2000). At the same time, demand for C and nutrients is greater (Schimel 1995, Liu et al. 2000), as soil microbes produce compatible solutes to mediate the effects of low water availability (Kieft et al. 1987). The response of the soil microbes to drying therefore quantifies the effect of a negative disturbance. When water is added to dry soil there is a positive effect of an increase in resource and water availability (Birch 1958, Skopp et al. 1990, Turner and Haygarth 2001), as well as a potentially negative effect of a rapid change in osmotic pressure (Kieft et al. 1987). The response of soil microbes to rewetting dry soil therefore represents the effect of a disturbance that can have positive and/or negative effects.

The magnitude of a disturbance can have a fundamental effect on the response of the system (Connell 1978, Zhang and Zak 1995). It is therefore important to choose a disturbance that can be standardised across a range of soils. Wetting-drying events are ideal disturbances for this. Two approaches to drying could have been used in this research. Firstly, the soil could have been dried from a particular moisture content to a lower one, for example, from 100% to 10% moisture content on a dry weight basis (MC). However, the soils from the chronosequences (Chapter 3) varied considerably in their organic matter content, and therefore their water-holding capacity (WHC). At 100% MC, water may already be limiting in a soil with a high WHC, but in a soil with a low WHC, the same MC may result in an excess of water and anaerobic conditions. Drying soils down to 10% from 100% MC could therefore result in quite a different disturbance depending on

the soil; a high-WHC soil would experience drying from a relatively dry state to an even drier one, and a low-WHC soil would experience drying from a wet state to a dry one. This method therefore only works when the experiment deals with soils with the same or similar WHC, as in Chapters 4 and 5. A second approach is to dry the soil from a known WHC to a lower WHC. This allows differences in organic matter content and WHC to be taken into account, effectively standardising the disturbance across different soils. This approach was used in Chapter 3, and was successful in allowing the comparison of soil microbial responses from different soils.

Defining the disturbance as a change in WHC means that it encompasses differences between soils in the time they take to dry and their water potential at 10% WHC. Although these factors will have been the same, or very similar, across treatments for the soil used in Chapter 4 and 5, they may have had an impact on the disturbance experienced by the soil microbes in the soils used in Chapter 3. Depending on WHC, soils took between several hours to 2 days to dry from 55% WHC to 10% WHC. This may have resulted in some soils retaining an active microbial community for longer during drying than other soils, and therefore potentially using up more of the labile C as it was released by the drying process. This may explain some of the variation found in resistance and resilience across soils. The water potential of different soils at the same percentage of WHC can be different, resulting in varying availability of soil water to soil microbes (McLaren and Cameron 1990). Although this was unlikely to be important when the soils were wet, it is possible that the water in some soils was less available at 10% WHC than in others, effectively resulting in a harsher disturbance in terms of water availability. This may have been one of the reasons why soil microbes from different soils responded differently to the disturbance, and would be interesting to explore further in future research.

6.1.2 Measurements of stability

The way that ecological stability is defined and the response variables measured will determine the types of measurements made and their relevance to particular hypotheses. Stability has been defined in numerous different ways, including variability, persistence, constancy, resistance, and resilience (Pimm 1984, Grimm and Wissel 1997). It has also

been measured at numerous different levels, including for species composition (e.g. Biggs et al. 1999, Sankaran and McNaughton 1999), diversity (e.g. Degens et al. 2001), community biomass (e.g. Tilman 1996), and process rates (e.g. Joergensen and Raubach 2003). Because different mechanisms can operate depending on the type of stability and response variables measured (e.g. Tilman 1996), many studies on stability are not comparable. This has lead to a great deal of confusion and the publication of earlier (Pimm 1984), and more recent (Grimm and Wissel 1997) articles urging researchers to use consistent definitions and to specify the type and scale of the disturbance, and the reference state to which responses are compared.

The definitions and response variables used to describe stability should be relevant to the primary goal of a study. The primary goal in this research was to determine the role of several factors in driving belowground ecosystem functions and stability. Stability was therefore defined in terms of resistance and resilience, and was measured as the response of a combination of community aggregate properties and indicators of process rates to a wetting-drying event. Resistance and resilience give a detailed description of stability as they describe both the amount of change caused by the disturbance and the rate of recovery after the disturbance (Pimm 1984). Resistance and resilience have also been associated with trade-offs in life history strategies (Lepš et al. 1982, MacGillivray et al. 1995), and therefore give some insight into the potential role of biotic composition in stability. The response variables used to quantify resistance and resilience measure community properties and processes that can affect ecosystem function, and are therefore relevant to the primary goals of this thesis. If the focus of this research had been to determine the mechanisms behind soil microbial stability, other factors such as trophic interactions and population fluctuations may have been useful measures. These factors have been shown to have an effect on community stability in other studies (e.g. McNaughton 1977, Tilman 1996) and may be interesting to investigate further in future research.

The ways that the response of variables to a disturbance are summarised will determine how results are interpreted. Several different indices have been used in the literature, many of which are unable to quantify resistance and resilience accurately in all possible scenarios (Tables 1, 2). The indices that were developed in Chapter 2 successfully overcame these problems. They also have an advantage over other indices in that they give

higher values for higher resistance or resilience, and so are easy to interpret. The three experiments in which the indices were used show that they were frequently capable of identifying differences between treatments (Chapter 4 and 5) and trends over environmental gradients (Chapter 3). If these indices become commonly used, they will contribute to increasing the ease with which studies can be compared, and they should help ensure the use of consistent terminology and precise descriptions of what is being measured.

6.1.3 The timing of measurements

The accuracy of measurements of resistance and resilience will be determined by their timing. If resistance is defined as the amount of change caused by a disturbance (Pimm 1984), an accurate measurement of resistance requires knowing the point at which the difference between the value of the disturbed and undisturbed system (D_0) is the highest. It is much easier to measure this when the disturbance has a negative effect on the system measured. Once the factor causing a decrease in the response variable has been removed, the system should begin to recover. The maximum D_0 should therefore be at the point where the disturbance ends. When a disturbance has a positive effect on a system, the maximum D_0 could occur at any point after the disturbance. For example, when glucose is added to soil, it may take several hours or days before the maximum respiration point is reached, and this may vary across different soils.

The timing of most measurements of resistance in the research presented here dealt with the effect of drying, i.e. a negative disturbance, on soil microbial response variables. Due to time constraints, the resistance of basal respiration and glucose use to rewetting dry soil was measured as the response of the soil microbes only within the first few hours after rewetting. This measure may not have included the maximum deviation away from the control soil, and should therefore be viewed as a relative measure of the short-term response to rewetting dry soil rather than an absolute measure of resistance. The measurement of the resistance of SIR to drying should also be re-defined slightly. To obtain a reliable estimate of soil microbial biomass using SIR, the soil must be wet so that the added glucose is available (West and Sparling 1986). SIR was therefore measured after

the dry soil had been rewet, so that the measurement of the resistance of SIR to drying included the negative osmotic effect of rewetting dry soil, and any increase in biomass that occurred after this, but before SIR was measured. These changes are likely to be small and consistent across different soils, and therefore should not significantly alter the interpretation of the results.

Measurements of resilience require some understanding of how soil is likely to respond to a disturbance. Resilience can be measured as the relative amount of recovery at a particular time point, or the curve of recovery can be followed in more detail with multiple measurements. In the research presented here, resilience was measured as the amount of recovery at a particular time point (3 days after the dry soil was returned to 55% WHC). This approach was chosen because it was not practical to measure a detailed recovery curve in the multi-treatment replicated experiments presented here, and because it allows the resilience of multiple soils to be compared easily. The timing of this measurement was based on preliminary experiments (not presented) and the data presented in Chapter 2. The experiment presented in Chapter 2 indicated three potential curves of recovery (Fig. 3, Chapter 2). The SIR of the clover soil had nearly recovered fully by day 3, the SIR of the humus soil took much longer to recover, and the SIR of the plantain soil showed an increase above the control soil at around day 3, followed by a decline back to control levels. Three days of recovery therefore gave the best representation of all of these recovery rates. Although this approach worked and was capable of distinguishing between the resilience of different soils, it may be worth exploring the shape of the recovery curve in more detail in future research.

A similar argument can be applied to the measurement of control soils over time – Fig. 3, and in particular Fig. 3c, showed that there can be some variation in control values across time. If time had allowed, it may have been more accurate to use an average control value measured at several time points to quantify C_0 and C_x. However, the data presented in Fig. 3 showed that variability among replicates is generally low, suggesting that this is unlikely to have made much difference to the research presented here.

Overall, the approach used to determine the resistance and resilience of the soil microbial community was successful in identifying differences between treatments and trends over

time. As with any methodology, there were some limitations, especially in the timing of the measurements. However, as these limitations were taken into account when examining the data, the general interpretation of the results should be correct.

6.2 Diversity as a driver of ecosystem function and stability

Diversity has been proposed as an important driver of ecosystem function and stability (MacArthur 1955, May 1972). Although there have been several studies on the role of plant and litter diversity in driving some belowground processes (e.g. Hector et al. 2000, Porazinska et al. 2003), little is understood about how diversity affects soil microbial stability. Two different approaches were used in the research presented here to determine the effect of diversity on soil microbial function and stability. Firstly, the diversity of factors that are likely to affect soil microbial function (plant species and C substrates) were manipulated. Secondly, the diversity of the soil microbial community itself was measured in the C substrate experiment and correlated with ecosystem functions and stability. This is not a direct measure of the effect of soil microbial diversity and therefore should be interpreted with caution, but can nevertheless give some indication of whether soil microbial diversity might be an important driver.

6.2.1 Plant species and C substrate diversity

The results presented in Chapters 4 and 5 showed that plant species and C substrate diversity effects were context-dependent. Plant diversity only had an impact on plant community biomass and mineral N contents, and then only in some harvests (Tables 11, 12) and scarcely affected soil microbial properties (Table 13, Figs. 10 - 13). In contrast, C substrate diversity affected soil microbial community structure and several aspects of ecosystem function and stability (Figs. 17, 19 – 21). However, non-additive mixing effects only occurred in some treatments, and the extent to which this occurred varied with the

response variable measured. This suggests that plant diversity does not affect soil microbial function and stability, but that C substrate diversity can in some contexts.

The plant diversity results were consistent with some studies, but not others. Plant diversity has been found to have a positive effect on plant biomass and nutrient retention in a number of studies (Tilman et al. 1997a, Symstad et al. 1998, Spehn et al. 2000b), but not others. Many other studies have found no or few effects of plant diversity on soil microbial community structure (Hedlund et al. 2003, Wardle et al. 2003), function (Spehn et al. 2000a, Porazinska et al. 2003), and stability (Wardle et al. 1999, Wardle et al. 2000). My results deviate from some other studies in two ways. The increase in productivity due to increased diversity did not appear to be due to the facilitative effect that N-fixers (in this experiment, clover) often have on the biomass of other plant species (e.g. Hooper and Vitousek 1998, Hooper and Dukes 2004), as the biomass of ryegrass and plantain were reduced by competition with clover, but clover biomass was unaffected by competition with the other plant species. Soil mineral nitrogen (N) contents increased in harvest 4 rather than decreasing, as usually reported in plant diversity experiments (Tilman et al. 1997a, Hooper and Vitousek 1998). The lack of relationship between plant productivity and the soil microbial biomass is consistent with some studies (Groffman et al. 1996, Wardle et al. 1999), but not with others (Zak et al. 1994, Broughton and Gross 2000, Spehn et al. 2000a). It is possible that there was a lag effect, and that if the plant community experiment had continued for longer there would have been some effect of aboveground diversity on belowground properties, as suggested by some studies (Van der Putten et al. 2000, Raffaelli et al. 2002). The C substrate diversity results were largely consistent with other experiments that have mixed substrates. For example, litter-mixing studies often find idiosyncratic effects of diversity on the soil microbial biomass (Wardle et al. 1997a, Bardgett and Shine 1999) and plant growth (Nilsson et al. 1999). My results differ from some litter mixing studies (Briones and Ineson 1996, Wardle et al. 1997a, Bardgett and Shine 1999, Smith and Bradford 2003) in that the diversity of the C substrates did not affect their decomposition rate, with the exception of one treatment (Section 5.4.1).

The main mechanism by which plants can affect soil microbes and their function is by the types and amounts of resources they return to the soil. Therefore, combining the results from the C substrate and plant species diversity study may suggest some ecological

explanations as to why there were no effects of plant species diversity on soil microbial response variables. Firstly, the C substrate experiment showed that the response of the soil microbes and ecosystem functions to substrates from within the same functional group was often similar. This suggests that the substrates deposited by each of the three plant species used in the plant community experiment may have been from within the same functional group, and were therefore too similar to induce a non-additive effect when the different plant species were grown together. Secondly, the C substrate experiment showed that non-additive mixing effects occurred only for particular combinations of substrates. This suggests that the organic matter inputs of the three plant species to the soil may not have included substrates that interacted non-additively, that the concentration of those substrates were insufficient to have an effect, or that those substrates never came into contact with each other. Thirdly, the effect of C substrate mixing reached saturation at low diversity levels. Each plant species may have already deposited substrates into the soil that had a non-additive effect on soil microbial function and stability, but adding more substrates through the addition of more plant species did not increase the effect of diversity beyond that.

There are two methodological reasons why diversity effects may have been more common in the C substrate experiment than in the plant community experiment. Firstly, it may be related to differences in scale between experiments. The C substrate experiment involved mixing substrates evenly throughout the soil, and measurements were then made on this soil. The primary selective force was C substrate quality, because most other driving factors, such as temperature, moisture, and C quantity, were kept constant. The soil measurements therefore quantify direct effects of one factor only, and that factor should have had an even effect on all parts of the soil. In contrast, the plant community experiment focused on the effect of the whole plant community on all of the soil in the containers in which the plants were grown. Plants can affect the oxygenation (McLaren and Cameron 1990), moisture content (Hooper and Vitousek 1998), and structure (Tisdall and Oades 1982) of the soil environment, and provide soil microbes with C substrates of different quality and quantity (Vinton and Burke 1995). These plant effects on soil microbes may be positive, neutral, or negative depending on context (van Veen et al. 1989, Wardle 2002). Because all of the soil was used, and homogenised by sieving, the average effect of all of these processes is measured, resulting in an increase in the chance of a net

neutral effect of plant diversity. Further, the C substrates added to the soil by plants are deposited in a highly localised way: litter is deposited on the soil surface, and root exudates are deposited primarily at root tips (Wardle 1992). Although litter is likely to be mixed in a natural system, an interaction between root exudates from different plant species requires their rhizospheres to interact in some way. It has been shown that the biomass of bacteria and fungi on the root surfaces of ryegrass and plantain are higher when these two plant species are grown together than when they are grown separately (Christie et al. 1974), suggesting that the rhizospheres of different plant species can interact. However, because all of the soil was used in my experiment, any significant effect of diversity caused by these localised interactions between multiple species may have been diluted by soil that has not been in direct contact with litter, leachate from that litter, or root exudates from more than one species.

It seems likely from the evidence provided by the C substrate experiment and previous litter mixing studies that there can be non-additive effects of plant diversity at the microsites where those substrates are deposited, but that once the impact of plants is scaled up to the whole plant level, other factors can dilute or override these effects. This does not mean that non-additive effects due to substrate or litter mixing are unimportant; both of these have been shown to affect plant growth (Fig. 21, Nilsson et al. 1999), and this may have implications for competition and therefore plant community structure.

The second potential methodological reason behind differences in the frequency of plant and C substrate diversity effects is the criteria used to determine whether or not there was a significant effect of diversity. The experimental design used in my research allows comparison between monocultures and mixtures of either plants or C substrates. This design allows discrimination of the sampling effect from complementarity and facilitation, and is therefore one of the more robust designs used in diversity studies (Hooper 1998). However, because the effect of plant intraspecific competition is not quantified in this design, the researcher has to assume that it is not occurring, and therefore that the biomass of the monoculture represents the total biomass that species would attain regardless of the number of plants. An effect of diversity therefore requires the value of the mixture to be either significantly higher or significantly lower than all component species in monoculture (Huston et al. 2000, Hooper and Dukes 2004). This principle is based on transgressive

overyielding, and is the most stringent test of diversity effects (Garnier et al. 1997a, Hector 1998, Huston and McBride 2002, Hooper and Dukes 2004). In contrast to plants, litter and C substrates are inert and so there is no competition between them. This means that an expected value can be calculated based on the value of the single substrate treatments, and the proportion of each substrate in the mixture. Any deviation away from the expected value can be considered an effect of increasing diversity. It is therefore much easier to get a significant effect of diversity. For example, imagine a system where the value of a mixture containing species A and B in equal proportions was 10, and that A and B in monoculture have a value of 6 and 20 respectively. If this were a plant experiment, the researcher would conclude, that there was no diversity effect based on the criteria of Huston et al. (2000) – the value of the mixture falls between the values of the monocultures. In contrast, if this were a litter diversity experiment, the researcher could legitimately conclude that there was a diversity effect, as the observed value is lower than the expected value of 13 ((½ x 6) + (½ x 20) = 13). Both approaches are perfectly valid, but can result in different conclusions.

6.2.2 Microbial diversity

Soil microbial diversity, as measured by Shannon-Weiner indices calculated for the CRPs and PLFAs in the C substrate experiment, did not appear to be strongly related to ecosystem function. Catabolic diversity was significantly related to the decomposition of cellulose paper (r = -0.2225, $P < 0.05$), the resistance of SIR (r = 0.1925, $P < 0.05$), and to the shoot:root ratio (r = -0.2636, $P < 0.01$), but PLFA diversity was unrelated to any measure of ecosystem function at $P < 0.05$. The soil microbial community is extremely diverse, and a wide range of microbial species can decompose the same substrates. It has therefore been suggested that most soil microbes are redundant, and that soil microbial diversity may only be important for processes that are performed by a few microbial species (so-called narrow processes), as more diverse communities are more likely to contain the species that can perform those processes (Schimel 1995). Cellulose can be decomposed only by a subset of the microbial community (Swift et al. 1979, Hu and van Bruggen 1997), and therefore measures a narrower process than stability. However, increased microbial diversity was related to lower, rather than higher, decomposition rates.

Other studies that have measured the effect of soil microbial diversity on function have found variable results. Some studies have found no effect on soil processes (Griffiths et al. 2001b), effects on some but not all soil processes (Griffiths et al. 2000), or effects on decomposition (Degens 1998a) and stability (Degens et al. 2001) in some contexts only. Where soil microbial species diversity can be controlled more easily, as in mycorrhizal studies, results have shown idiosyncratic (Jonsson et al. 2001) or positive effects on plant biomass (van der Heijden et al. 1998, but see Wardle 1999 on van der Heijden et al.'s interpretation).

Overall, the results presented in this thesis and other studies suggest that diversity can affect plant community properties, but that it is not a strong driver of soil microbial function and stability. Plant diversity only had one significant effect on soil microbial properties. C substrate diversity effects were idiosyncratic, depended strongly on which combination of substrates were added, and saturated at low diversity levels. Microbial diversity was largely unrelated to function. These results therefore supported the idiosyncratic model of diversity – function relationships, where the effect of an increase or decrease in species number depends on which component of a system is added or removed, rather than the number of components *per se* (Lawton 1994). For example, the removal of clover from a clover – ryegrass mixture would not change the resistance of soil microbial basal respiration to drying, but the removal of ryegrass from that mixture would (Fig. 11). My results also supported the redundant taxa hypothesis, where some components of a system can be lost without any change in function, but others cannot (Walker 1992). For example, adding substrates from within the same functional group in the C substrate experiment often resulted in similar soil microbial responses (Table 22 - 24). The loss of one substrate from within a functional group may therefore have no effect on ecosystem function. In combination, this means that the effects of species loss are unpredictable, especially in the absence of knowledge on how each and every species contributes to ecosystem function and stability and how each species interacts with other components of the ecosystem (Mikola and Setälä 1998c). Obtaining this knowledge is an enormous task, suggesting that conservationists should focus on conserving the whole community rather than individual populations to ensure the maintenance of ecosystem function. The idiosyncratic nature of diversity effects on soil systems, and the presence of redundant components within the system, is consistent with many other studies that have concluded

that diversity *per se* is not a strong driver of soil microbial properties (Hooper and Vitousek 1997, Wardle et al. 1997b, Bardgett and Shine 1999, Korthals et al. 2001), and suggests that other factors may be more important.

6.3 Composition as a driver of ecosystem function and stability

The composition of communities has been suggested as an important driver of ecosystem function, especially the traits of the dominant species. As soil microbial and plant function are strongly linked, it seems likely that plant species composition may have an important impact on soil microbial function. One of the main mechanisms by which plants may affect soil microbes is by the composition of the substrates they deposit into the soil. Therefore, I manipulated both plant and C substrate composition to determine what effect these had on soil microbial function and stability, and in the C substrate experiment, whether this change in composition had an effect on plant growth. Although soil microbial composition was not directly manipulated, correlations between indicators of soil microbial community structure and ecosystem functions may show whether it is likely to be an important driver of function.

6.3.1 Plant species and C substrate composition

Both plant composition and C substrate composition had a strong effect on soil microbial community structure, function and stability. This supports the suggestion that there is a strong link between plant and microbial function, and that substrate quality is an important mechanism by which plants can affect soil processes. This is consistent with numerous other studies that have found strong effects of plants or substrates on soil microbial community structure (Bardgett et al. 1999b, Bardgett and Shine 1999), function (Spehn et al. 2000a), and stability (Wardle et al. 1999, Wardle et al. 2000). For example, many studies show that plant and litter composition can affect the soil microbial biomass

(Bardgett and Shine 1999, Wardle et al. 2003), and the decomposition of added substrates (Nilsson et al. 1999, Spehn et al. 2000a).

The characteristics of the dominant plant(s) have been suggested as the primary driver of ecosystem function (Grime 1998). This was supported to some extent by the plant community experiment presented in Chapter 4, as plant traits and their effect on soil chemistry were found to be related to soil microbial properties and function (Table 17), consistent with other studies (e.g. Grayston et al. 1998, Wardle et al. 1998, Flachini et al. 2003). However, this was not always related to how dominant that species was, as measured by aboveground biomass. For example, most of the biomass (68%) in the ryegrass + plantain treatment in harvest 3 was produced by plantain plants. However, the resilience of glucose use was much closer to that of the soil from the ryegrass monoculture, than to the resilience of soil from the plantain monoculture. This suggests that the dominant plant from a microbial perspective may be different to the dominant plant from an aboveground perspective. This may be worth investigating further – which characteristics of plants are the primary drivers of soil microbial properties, and do dominant plants in a mixed species community have the greatest effect on soil microbial function?

6.3.2 Soil microbial composition

One of the main mechanisms by which plant species or substrate composition can affect soil microbes and their function is by altering soil microbial community structure. Soil microbial composition was measured directly in the C substrate diversity experiment (Chapter 5) and indirectly in the plant community experiment (Chapter 4).

In the plant community experiment, the ability of soil microbes to decompose cellulose paper was used as an indirect measure of the slower-growing, K-selected portion of the soil microbial community. In that experiment, decomposition rate was strongly correlated with several measures of soil microbial stability. However, in the C substrate experiment, cellulose paper decomposition was not related to any measure of soil microbial stability or community structure. This suggests that cellulose paper decomposition is not driven by

species composition in all contexts, or that it is not a good indicator of species composition. It also suggests that at least part of the relationship between decomposition and stability in the plant community experiment may have been due to the relationship of decomposition with N availability (Table 15).

When relationships between soil microbial composition and ecosystem functioning were evaluated, as was done in the C substrate diversity experiment (Chapter 5), results were variable. There were many significant correlations between variables derived from CRP analysis and measures of ecosystem function, but the variables derived from PLFA analysis were only occasionally related to ecosystem function, and then often weakly (Table 25). This suggests that the composition of the soil microbial community (PLFA data) may not have a large effect on function, but that its catabolic ability and activity may. The lack of relationship between composition and function may partly be because many species of soil microbes are capable of performing the same function (Schimel 1995). The metabolic state and catabolic ability of the soil microbial community may, however, affect their ability to decompose cellulose paper and cope with disturbances as both of these alter the substrates available in the soil. It also seems likely that the catabolic ability of the soil microbial community will affect nutrient mineralisation and immobilisation, and therefore plant growth. There may have been a stronger effect of composition on soil microbial function if the C substrate treatments had altered the fungal biomass or the fungal:bacterial ratio, as bacteria and fungi are often controlled by different factors (Mikola and Setälä 1998b) and function differently (Allen et al. 1999, Ohtonen et al. 1999, Ley and Schmidt 2002). Other studies have found that soil microbial species composition can be important for stability (Allen-Morley and Coleman 1989), decomposition (Bärlocher and Corkum 2003), enzyme activities (Waldrop et al. 2000), and plant growth (van Der Heijden et al. 1998, Jonsson et al. 2001). However, the studies of Allen-Morley and Coleman (1989), and Bärlocher and Corkum (2003) used communities with unrealistically low diversity levels, and the studies of van der Heijden et al. (1998) and Jonsson et al. (2001) dealt with the more direct effect of mycorrhizal species composition on plant growth, which may explain this difference.

Table 25: Correlation coefficients between soil microbial community variables, resources and ecosystem functions, using data from Chapter 5.

		Resistance to drying of:			Resistance to rewetting of:		Resilience to drying of:			Plant growth		
	Decomp[1]	BR[2]	SIR	Glucose Use[1]	BR[2]	Glucose Use[1]	BR	SIR	Glucose Use	Shoot[3]	Roots[3]	Shoot: root[2]
CRP PC1	0.2834**	0.4165***	-0.2487**	-0.0183	0.3764***	0.0197	0.1772†	0.2835**	0.2054*	0.3676***	0.4802***	0.2931**
CRP PC2	0.0653	-0.1955*	0.1533	0.2583**	-0.1114	0.2646**	-0.0766	-0.2812**	-0.2586**	0.3977***	0.3145***	0.3228**
P(AA use)	-0.4082***	-0.3393***	0.1493	-0.0319	-0.2945**	-0.0677	-0.0939	-0.1264	-0.0734	-0.1769†	-0.2983**	-0.1887*
P(COOH)[2]	0.3371***	0.2996**	-0.0346	0.1415	0.2772**	0.1628t	0.0898	0.0174	0.0043	0.3610***	0.4656***	0.2333*
PLFA PC1	-0.1684†	-0.0513	-0.0408	0.0315	0.0498	0.0443	0.0540	-0.0658	-0.1665	-0.2609**	-0.1420	-0.3064***
PLFA PC2	-0.0683	0.2399*	0.1012	-0.0326	0.2046*	-0.0106	-0.1785†	-0.0206	0.1171	0.1209	0.2527**	-0.0398
Cyclic	-0.0694	0.1280	0.0846	-0.0396	0.0830	-0.0141	-0.1847†	0.0609	0.2037*	0.1896*	0.2229*	0.0809
Branched	-0.0292	-0.1050	-0.0968	-0.0899	-0.0203	-0.0496	-0.1289	0.1852†	0.1576	-0.0419	-0.1544	-0.0824
C	-0.5231***	-0.0832	0.0479	-0.0367	-0.0591	-0.0678	-0.1175	-0.0050	0.0643	-0.2239*	-0.1638t	-0.3226***
C:N ratio	-0.4868***	0.1342	-0.0649	0.0075	0.1591t	0.0094	0.0006	0.1127	0.1155	-0.2656**	-0.0841	-0.2540**
N	0.0552	-0.2649**	0.1105	-0.0524	-0.2717**	-0.0836	-0.1145	-0.1495	-0.0989	0.0891	-0.0655	-0.0467
pH[3]	-0.0254	-0.2816**	-0.0412	-0.1474	-0.3079**	-0.1327	-0.1066	-0.0890	-0.1950*	-0.5351***	-0.5297***	-0.3545***

BR = basal respiration, SIR = substrate-induced respiration, CRP PC1 And PC2= Axis 1 and 2 from principal component analysis of catabolic response profiles, PLFA PC1 and PC2 = axis 1 and 2 from principal component analysis of phospholipid fatty acids, P(AA use) = proportional amino acid use, P(COOH) = proportional carboxylic acid use, cyclic and branched = relative nmoles of cyclic and branched PLFA respectively.

[1] Square root transformed, [2] log transformed, [3] rank transformed.

† $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

There is, however, some indirect evidence that the composition of soil microbial communities at a broad scale may be important for soil microbial stability. Resistance and resilience are often traded-off, with resistant organisms showing low resilience and vice versa. This trade-off has been attributed to differences in life-history strategies in plant systems (Lepš et al. 1982, MacGillivray et al. 1995). In particular, it has been proposed that plants with a ruderal life-history strategy tend to be resilient, whereas those with a stress-tolerant life-history strategy tend to be resistant (MacGillivray et al. 1995). This can be tested indirectly for soil microbes, using the data collected here, by correlating resistance and resilience. In all experiments, the resistance and resilience of the soil microbial SIR were negatively correlated (Tables 6, 26). This strongly suggests that the attributes of the soil community that result in high SIR resistance also result in low resilience and vice versa, and therefore that composition may be an important driver of soil microbial stability. Identifying what these characteristics are, and the role of disturbance, competition, and stress in selecting for them, may be an interesting topic for future research.

Overall, these results suggest that soil microbial composition, along with the composition and traits of plant species, may play an important role in ecosystem function and stability. These results have several implications for management practices. The fact that C substrates can have important impacts on soil microbial function and stability suggests that the addition of other substrates such as fertilisers, pesticides or biocontrol agents may have downstream effects on soil microbial function and stability. Growing different plants, as in crop rotation systems, can enhance or reduce soil microbial function and stability (Vandermeer 1990). These changes in belowground function may have implications for future plant growth and productivity and C storage in soils, as shown in Chapter 5 (Table 24, Fig. 21). It is also likely that the composition of higher trophic levels in both aboveground and belowground systems will affect soil microbial community structure, function and stability (e.g. Bardgett et al. 1998, Mikola 1998, Mikola and Setälä 1998a, Wardle et al. 2003). The stability of different components of a food web may have effects on other components of that foodweb (O'Neill 1976). These interactions may be worth further investigation and would increase our understanding of the role of composition in ecosystem function and stability.

Table 26: Correlation coefficients between the resistance and resilience of soil microbial response variables to a drying disturbance in the different experiments. BR = basal respiration, SIR = substrate-induced respiration.

Response variable	Plant community expt				C substrate expt
	Harvest 1	Harvest 2	Harvest 3	Harvest 4	
BR[1, 3-5]	0.1093	-0.2410	-0.3759*	0.2822†	0.0397
SIR[2]	-0.5498***	-0.6234***	-0.6408***	-0.5687***	-0.6400***
Glucose use[6,7]	-0.1606	0.0285	0.0500	-0.0876	-0.1300

[1] Basal respiration, [2] substrate-induced respiration. [3] Rank transformed in harvest 2, [4] log transformed in harvest 3 and in the C substrate experiment, [5] square root transformed in harvest 4, [6] square root transformed in harvest 3 and in the C substrate experiment, and [7] rank transformed in harvest 4.
† $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

6.4 Soil resources as a driver of ecosystem function and stability

Soil resources are known to have a strong impact on both aboveground and belowground function. However, understanding of how resources affect stability is scarce, despite many models suggesting that it may be important. In Chapters 3 – 5, some aspects of soil resources were measured. In Chapter 5, it was clearly shown that C substrate quality, independently of nutrient contents, can have a strong influence on soil microbial function, and therefore may be one of the major drivers of decomposition rates and stability. Most theories on the relationship between resources and stability, however, focus on nutrient availability and the amount of resources, rather than C quality *per se*. Although the amount of C and nutrients was not directly manipulated in the research presented here, correlation analysis can be used to determine whether these resources may have been an important driver of soil microbial stability. Three of the main models on the relationship between resources and stability that can be tested here will be discussed in the following section.

Trade-offs in the resistance and resilience of plant biomass may be due to trade-offs in characteristics associated with nutrient-stress tolerance (MacGillivray et al. 1995). Given

that there was a consistent trade-off in the resistance and resilience of the soil microbial biomass, nutrient-stress tolerance may be an important factor for soil microbial stability. Two of the measures of resources used in the research presented here can be used as indicators of nutrient stress. An increase in the C:N ratio of soil can be interpreted as a decrease in the availability of N relative to C, and therefore as an indication of increasing nutrient stress. The same reasoning applies to C:phosphorus (P) and N:P ratios (Sterner and Elser 2002). A second potential measure of nutrient stress is the amount of mineral N and P in the system. The higher the concentration of available nutrients, the less limited by that nutrient a system should be. There was some evidence that nutrient stress as indicated by these two measures was related to the resistance and resilience of the soil microbial biomass in the predicted direction (Tables 7 – 9, 16). However, there were also several relationships between these indicators and the stability of SIR that were in the opposite direction to that predicted, or that showed no significant trends (Table 7 – 9, 25). Overall, this suggests that the traits associated with nutrient stress tolerance may have important impacts on soil microbial stability, but only in some contexts.

Increases in organic C, N and pH may contribute to increasing the resistance of the soil microbial biomass (Wardle 1998), and increases in organic C may increase resilience (O'Neill 1976, DeAngelis et al. 1989, De Angelis 1992). However, the resilience of the soil microbial biomass was unrelated to total C (Tables 9, 25). The suggested relationship between the resistance of SIR and resources was supported by some results. For example, the resistance of SIR from the Hawaii sequence was correlated positively to total C and N (Table 7). Other results, however, contradicted it. For example, a negative rather than a positive relationship was found between pH and resistance at the Kokatahi and Hawaii sequences (Table 7). Wardle (1998) based his meta-analysis on the seasonal dynamics of the soil microbial biomass, which reflects changes in moisture and temperature over months or years. This suggests that the factors that control long and short-term changes in the soil microbial biomass may be different, or that C, N and pH are only important drivers in some contexts, and only for resistance.

One last model on the relationship between resources and stability focused on the resilience of process rates, rather than the stability of biomass. The resilience of C and N mineralisation of soils in response to the addition of C or N may vary depending on

whether the soil is C or N limited (Bosatta and Berendse 1984). Carbon-limited soils were predicted to be stable under all circumstances. However, N-limited soils were predicted to be stable under certain circumstances only and may oscillate during recovery (Bosatta and Berendse 1984). My results can be used to test these hypotheses to some extent, as the resilience of basal respiration and mineral N contents should give an indication of the resilience of C and N mineralisation to the drying disturbance, and the disturbance results in the addition of C and N. Once again, there was some evidence that supported the model, and some that did not. The soil from the C substrate diversity experiment may have been primarily N limited by the end of the experiment, because the main C source (the C substrates that were added) contained no N. In contrast, the soils from the plant community experiment may have been more C limited, as the main source of C for the soil microbes was from plant inputs, at least some of which will have contained N. The average resilience of basal respiration in the plant community experiment (using the index described in Chapter 2) ranged between 0.65 and 0.74 across the four harvests, compared to 0.41 in the substrate diversity experiment, suggesting that the C substrate experiment soils did have a lower overall resilience than soils from the plant community experiment. However, correlations between C:N ratios and the resilience of basal respiration in this experiment and others, were not significant, or in the opposite direction to that predicted (Table 9, 25). As Bosatta and Berendse's model predicts that N-limited systems can be stable in some circumstances, these contradictory results may still fit their model. It appears that C and N limitation may play a role in the resilience of C and N mineralisation, but in some contexts only.

These results suggest that soil resources may play an important role in soil microbial community properties and function. However, exactly what this role is depends on context, as has been suggested in previous studies (Moore et al. 1993, Biggs et al. 1999, Herbert et al. 1999). The context-dependent nature of resource effects on stability may be partly because soil resources co-vary with many other factors that may have a greater influence on function, resulting in significant correlations between resources and ecosystem function that do not correspond with cause and effect. Alternatively, resources may be important only in some situations. For example, bacteria are thought to be primarily top-down controlled, and fungi bottom-up controlled (Mikola and Setälä 1998b), suggesting that resources may be an important driver only where the system is fungi-dominated. To

understand the role of soil resources in soil microbial function more fully requires experiments that directly manipulate the amount and quality of soil resources.

Soil microbial stability and its relationship to resources and ecosystem development during the three chronosequences examined were highly context-dependent. From the results presented in Chapters 4 and 5, it seems that the most likely driver of this context-dependency was the different composition of each chronosequence's plant and/or soil microbial communities, or the different C substrates that those communities deposited. Plant and microbial diversity rarely had any impact on soil microbial function or stability, suggesting that these factors are not important. Carbon substrate diversity did have an impact on ecosystem function in several treatments, but this depended on which combination of substrates was added, suggesting that composition was again an important driving force. Resources may also be an important driver of soil microbial function and stability, but more experiments are required to determine exactly what their role is. It was clear that plants can affect soil microbial function and stability, and that soil microbial properties can also affect plant growth.

6.5 Summary of important findings

1. The resistance and resilience of the soil microbial SIR was negatively correlated
2. Soil microbial function and stability was influenced by:
   - Soil resources
   - Plant species composition
   - Carbon substrate composition and diversity
   - Microbial community characteristics, especially its catabolic ability
3. Soil microbial function and stability was not (or was rarely) affected by:
   - Plant diversity
   - Soil microbial diversity

4. Plant growth can be affected by the type and diversity of C substrates added to a soil

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Appendix I: Averages and standard errors (SE) of control and disturbed values for soil microbial and soil chemical variables at the end of the drying and rewetting disturbance (day 0), and after three days of recovery (day 3), for the a) Kokatahi, b) Franz Josef and c) Hawaii chronosequences used in Chapter 3. Control refers to measurements made on soil that remained at 55% water-holding capacity (WHC) throughout the measured time period, dry refers to measurements made on soil that had been dried from 55% WHC to 10% WHC, and rewet refers to measurements made on dry soil rewet from 10% WHC to 55% WHC.

a) Kokatahi chronosequence

Basal Respiration (μg CO_2-C g^{-1} h^{-1})

Stage	Day 0						Day 3			
	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
1	0.31	0.07	0.13	0.03	0.23	0.08	0.33	0.09	0.28	0.05
2	0.37	0.05	0.19	0.03	0.30	0.05	0.42	0.08	0.33	0.06
3	9.03	3.29	3.10	0.70	18.00	5.11	7.75	2.17	9.02	2.78
4	9.32	1.20	3.56	0.45	21.96	2.39	9.46	1.28	9.37	1.34

Substrate-induced respiration (μg CO_2-C g^{-1} h^{-1})

Stage	Day 0						Day 3			
	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
1	0.47	0.11	0.10	0.03	0.48	0.15	0.52	0.10	0.58	0.18
2	0.80	0.12	0.13	0.02	0.53	0.10	0.90	0.14	0.77	0.17
3	44.51	15.68	10.07	4.21	36.46	11.18	36.91	14.42	38.63	9.33
4	47.55	9.27	7.74	1.88	35.17	2.77	44.40	2.41	43.35	5.94

Kokatahi chronosequence cont'd

Soil mineral nitrogen contents

Stage	Day 0				Day 3			
	Control	SE	Dry	SE	Control	SE	Rewet	SE
1	0.99	0.22	1.32	0.43	1.09	0.41	1.29	0.54
2	1.34	0.43	1.29	0.67	1.44	0.58	1.33	0.39
3	209.70	73.17	252.87	103.79	238.12	81.55	285.96	98.52
4	153.21	19.03	180.56	17.14	176.27	26.98	252.59	25.93

b) Franz Josef chronosequence

Basal Respiration (μg CO_2-C g^{-1} h^{-1})

Site age	Day 0						Day 3			
(years)	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
55	2.83	1.14	0.63	0.34	8.84	4.81	2.72	1.20	2.99	1.34
130	7.60	1.45	3.34	0.41	12.19	2.09	5.97	1.37	6.27	0.88
500	14.79	2.67	9.04	2.21	26.30	3.26	13.84	0.32	19.80	2.60
5,000	16.19	0.99	9.29	1.70	32.88	2.00	13.84	0.48	13.64	1.65
12,000	12.16	2.16	9.70	1.45	25.64	3.39	10.35	1.23	12.87	1.88
22,000	5.00	0.38	4.28	1.00	11.82	0.79	4.25	0.41	4.98	1.11

Franz Josef chronosequence cont'd

Substrate-induced respiration (μg CO_2-C g^{-1} h^{-1})

Site age	Day 0						Day 3			
(years)	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
55	17.83	10.02	1.45	0.94	16.80	9.48	18.04	9.37	15.56	7.59
130	40.84	3.82	9.79	2.75	30.71	2.81	38.89	8.44	32.51	4.44
500	65.64	12.34	23.09	8.78	45.67	5.02	74.92	9.17	51.87	9.03
5,000	56.27	8.38	19.76	9.22	53.98	2.10	54.16	1.41	37.13	6.25
12,000	58.14	8.41	21.74	5.59	57.87	20.97	52.81	1.98	45.49	6.12
22,000	25.10	1.33	9.53	2.56	18.33	2.33	24.63	2.19	20.67	4.80

Soil mineral nitrogen contents

Site age	Day 0				Day 3			
(years)	Control	SE	Dry	SE	Control	SE	Rewet	SE
55	48.76	23.84	46.65	21.89	51.99	24.98	63.32	25.84
130	71.24	22.40	75.81	22.47	79.95	35.93	96.64	42.28
500	18.09	7.23	90.10	12.14	11.97	7.15	118.16	24.21
5,000	116.44	41.77	212.22	38.26	125.64	38.37	260.06	39.02
12,000	8.31	3.90	57.36	10.56	11.52	8.21	95.12	28.74
22,000	1.67	0.53	21.98	3.37	2.11	2.17	39.24	10.99

c) **Hawaii chronosequence**

Basal Respiration (μg CO_2-C g^{-1} h^{-1})

Site age	Day 0						Day 3			
(years)	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
300	17.43369	2.2	9.03	2.11	24.82	3.55	12.94	1.65	13.15	1.39
2100	19.25619	0.893	8.90	1.02	33.37	2.32	16.09	1.43	15.86	1.21
20000	17.60725	2.333	4.88	1.59	49.70	6.77	13.57	0.60	14.71	1.78
150000	16.73177	2.743	8.85	1.73	49.81	1.63	16.26	1.79	15.95	2.15
1400000	19.88239	1.539	11.07	1.99	43.05	3.37	16.20	0.97	16.09	2.11
4100000	11.54106	0.326	4.11	0.70	33.55	2.30	9.68	1.10	10.20	1.08

Substrate-induced respiration (μg CO_2-C g^{-1} h^{-1})

Site age	Day 0						Day 3			
(years)	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
300	66.20	6.08	16.74	3.68	41.06	6.01	64.03	6.96	52.72	5.65
2100	82.37	1.27	23.99	2.17	57.82	1.79	75.02	13.00	49.73	3.86
20000	87.18	16.63	5.72	2.35	72.78	10.95	56.75	5.87	49.70	6.49
150000	94.18	14.44	14.48	3.59	88.42	5.19	101.08	4.23	77.23	9.34
1400000	90.76	9.95	24.76	6.81	82.62	2.44	71.13	9.42	64.41	7.08
4100000	69.21	3.47	5.70	1.51	48.58	1.97	44.49	7.37	42.53	7.67

Hawaii chronosequence cont'd

Soil mineral nitrogen contents

Site age	Day 0				Day 3			
(years)	Control	SE	Dry	SE	Control	SE	Rewet	SE
300	6.13	1.46	37.76	6.31	1.30	0.57	54.49	3.87
2100	10.47	1.58	62.52	3.56	5.77	1.04	83.02	7.27
20000	138.62	20.34	180.47	33.34	157.52	24.82	275.16	38.02
150000	95.78	18.25	161.14	22.85	104.47	19.95	213.96	28.42
1400000	85.44	7.25	163.19	8.99	94.51	6.30	194.45	19.16
4100000	35.79	6.75	51.07	6.76	33.31	8.30	123.99	15.46

Appendix II: Averages and standard errors (SE) of control and disturbed values for soil microbial variables at the end of the drying and rewetting disturbance (day 0), and after three days of recovery (day 3), for a) Harvest 1, b) Harvest 2, c) Harvest 3 and d) Harvest 4 (Chapter 4). Control refers to measurements made on soil that remained at 55% water-holding capacity (WHC) throughout the measured time period, dry refers to measurements made on soil that had been dried from 55% WHC to 10% WHC, and rewet refers to measurements made on dry soil rewet from 10% WHC to 55% WHC.

a) Harvest 1

Basal Respiration ($\mu g\ CO_2\text{-}C\ g^{-1}\ h^{-1}$)

Treatment	Day 0						Day 3			
	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
Bare	1.05	0.04	0.20	0.02	2.36	0.09	0.95	0.09	1.07	0.12
C	1.39	0.08	0.16	0.05	2.86	0.16	1.25	0.12	1.44	0.10
R	1.65	0.16	0.21	0.04	3.70	0.19	1.53	0.12	1.39	0.14
P	1.92	0.15	0.23	0.06	4.24	0.46	1.65	0.14	1.97	0.14
CR	1.56	0.17	0.13	0.04	3.34	0.16	1.49	0.17	1.48	0.13
CP	2.01	0.16	0.17	0.04	4.47	0.32	1.43	0.11	1.84	0.13
RP	1.86	0.12	0.22	0.06	4.43	0.25	1.47	0.08	1.78	0.15
CRP	1.94	0.19	0.21	0.06	4.43	0.54	1.46	0.15	1.61	0.12

Harvest 1 cont'd

Substrate-induced respiration (μg CO_2-C g^{-1} h^{-1})

Treatment	Day 0						Day 3			
	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
Bare	3.61	0.25	0.11	0.02	3.29	0.10	3.69	0.20	3.33	0.26
C	4.96	0.18	0.05	0.02	4.14	0.18	4.59	0.25	4.50	0.38
R	6.04	0.41	0.08	0.03	4.92	0.24	6.11	0.22	5.60	0.29
P	6.36	0.12	0.09	0.04	5.13	0.18	6.40	0.06	7.83	0.31
CR	5.62	0.24	0.07	0.04	4.58	0.39	5.50	0.08	5.54	0.22
CP	7.06	0.47	0.09	0.02	6.21	0.49	6.88	0.56	7.96	0.83
RP	6.57	0.16	0.12	0.02	5.90	0.26	6.84	0.31	8.18	0.43
CRP	7.04	0.48	0.06	0.03	6.21	0.51	6.80	0.63	7.25	0.82

b) Harvest 2

Basal Respiration (μg CO_2-C g^{-1} h^{-1})

Treatment	Day 0						Day 3			
	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
Bare	0.94	0.07	0.05	0.02	2.12	0.12	0.64	0.15	0.92	0.06
C	1.41	0.10	0.20	0.05	3.69	0.22	1.15	0.09	1.14	0.14
R	1.76	0.10	0.15	0.05	3.54	0.20	1.28	0.17	1.49	0.18
P	1.91	0.06	0.15	0.05	5.60	0.24	1.41	0.22	2.00	0.06
CR	1.56	0.10	0.00	0.00	3.60	0.15	1.36	0.09	1.33	0.15
CP	1.84	0.16	0.09	0.04	5.84	0.32	1.74	0.12	1.80	0.13
RP	1.83	0.08	0.09	0.05	5.19	0.26	1.26	0.12	1.53	0.17
CRP	1.75	0.07	0.07	0.02	4.73	0.20	1.35	0.11	1.42	0.26

Harvest 2 cont'd

Substrate-induced respiration ($\mu g\ CO_2\text{-}C\ g^{-1}\ h^{-1}$)

Treatment	Day 0						Day 3			
	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
Bare	3.47	0.19	0.18	0.07	3.27	0.05	3.41	0.23	3.65	0.14
C	6.08	0.34	0.16	0.05	5.48	0.27	5.66	0.18	5.40	0.17
R	6.54	0.23	0.15	0.05	5.23	0.26	6.62	0.31	6.70	0.34
P	6.98	0.28	0.16	0.08	6.42	0.36	6.87	0.30	9.17	0.39
CR	6.54	0.30	0.18	0.05	5.31	0.24	6.40	0.24	6.19	0.36
CP	7.49	0.61	0.16	0.07	7.18	0.24	7.88	0.47	9.62	0.32
RP	7.55	0.33	0.22	0.04	6.83	0.24	7.27	0.68	9.11	0.79
CRP	6.81	0.26	0.20	0.04	6.35	0.20	6.82	0.38	8.27	0.49

c) Harvest 3

Basal Respiration (μg CO_2-C g^{-1} h^{-1})

Treatment	Day 0						Day 3			
	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
Bare	1.27	0.07	0.18	0.04	2.70	0.13	1.07	0.12	1.26	0.08
C	2.33	0.24	0.31	0.07	5.70	0.23	2.14	0.10	1.86	0.23
R	2.25	0.12	0.18	0.07	5.10	0.48	1.98	0.16	1.88	0.16
P	3.13	0.25	0.31	0.06	9.14	0.57	2.83	0.16	3.44	0.13
CR	2.61	0.05	0.27	0.05	6.44	0.32	2.41	0.10	2.38	0.08
CP	3.15	0.16	0.38	0.06	8.11	1.05	2.59	0.14	3.08	0.18
RP	3.07	0.18	0.24	0.07	7.35	0.29	2.87	0.19	2.77	0.16
CRP	2.74	0.26	0.32	0.08	8.10	0.62	2.51	0.22	2.84	0.26

Harvest 3 cont'd

Substrate-induced respiration ($\mu g\ CO_2\text{-}C\ g^{-1}\ h^{-1}$)

Treatment	Day 0						Day 3			
	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
Bare	4.29	0.14	0.18	0.05	3.86	0.15	4.10	0.10	4.08	0.12
C	11.33	0.68	0.42	0.09	9.39	0.52	10.98	0.63	9.37	0.80
R	9.24	0.54	0.27	0.06	7.98	0.48	9.02	0.56	8.86	0.68
P	12.90	0.51	0.35	0.13	10.48	0.24	13.74	0.26	16.82	0.65
CR	11.41	0.23	0.20	0.04	9.60	0.18	11.32	0.30	10.71	0.39
CP	12.32	0.37	0.37	0.14	10.14	1.04	12.29	0.41	14.30	0.54
RP	12.04	0.63	0.28	0.07	10.06	0.48	11.57	0.75	12.52	1.16
CRP	12.30	1.25	0.45	0.13	11.45	0.72	12.88	1.47	15.16	1.59

d) Harvest 4

Basal Respiration (μg CO_2-C g^{-1} h^{-1})

Treatment	Day 0						Day 3			
	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
Bare	1.10	0.17	0.18	0.04	2.13	0.12	0.92	0.06	0.97	0.06
C	1.70	0.12	0.27	0.04	5.32	0.38	1.58	0.07	1.71	0.03
R	1.95	0.17	0.24	0.03	4.82	0.26	1.57	0.22	1.95	0.12
P	2.25	0.29	0.20	0.05	8.05	0.52	2.42	0.18	2.75	0.29
CR	2.02	0.08	0.25	0.07	5.85	0.27	1.90	0.11	2.09	0.12
CP	2.37	0.11	0.16	0.05	6.70	0.31	1.94	0.07	2.28	0.12
RP	2.34	0.05	0.22	0.06	6.35	0.12	1.96	0.05	2.39	0.09
CRP	2.15	0.10	0.15	0.06	6.05	0.28	1.94	0.08	1.77	0.23

Harvest 4 cont'd

Substrate-induced respiration ($\mu g\ CO_2\text{-}C\ g^{-1}\ h^{-1}$)

Treatment	Day 0						Day 3			
	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
Bare	3.67	0.12	0.11	0.05	3.04	0.18	3.52	0.15	3.48	0.26
C	10.70	0.39	0.13	0.05	8.53	0.60	9.54	0.46	9.58	0.41
R	8.15	0.40	0.13	0.07	6.57	0.29	7.37	0.64	8.41	0.40
P	12.48	0.87	0.24	0.04	9.47	0.75	12.58	0.66	14.93	1.43
CR	10.26	0.79	0.15	0.02	9.05	0.33	10.64	0.15	10.86	0.20
CP	13.51	0.49	0.18	0.06	11.01	0.48	12.86	0.45	12.81	0.59
RP	11.58	0.36	0.25	0.10	9.23	0.28	11.02	0.31	12.05	0.49
CRP	12.59	0.77	0.09	0.03	9.53	0.62	11.26	0.49	10.17	0.70

Appendix III: Averages and standard errors (SE) of control and disturbed values for a) basal respiration and b) substrate-induced respiration at the end of the drying and rewetting disturbance (day 0), and after three days of recovery (day 3), for the soils from the carbon substrate experiment (Chapter 5). Control refers to measurements made on soil that remained at 55% water-holding capacity (WHC) throughout the measured time period, dry refers to measurements made on soil that had been dried from 55% WHC to 10% WHC, and rewet refers to measurements made on dry soil rewet from 10% WHC to 55% WHC. See Table 18 for treatment codes.

a) Basal Respiration ($\mu g\ CO_2\text{-}C\ g^{-1}\ h^{-1}$)

Treatment	Day 0						Day 3			
	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
A	2.85	0.18	0.24	0.07	8.83	0.38	1.89	0.42	2.90	0.23
B	2.30	0.41	0.21	0.01	8.21	1.12	2.07	0.11	2.76	0.20
C	3.89	0.49	0.13	0.03	9.96	0.52	3.15	0.45	7.62	0.50
D	3.92	0.40	0.08	0.03	11.29	1.06	2.73	0.27	3.79	0.58
E	1.43	0.12	0.10	0.04	5.62	0.32	1.21	0.08	1.72	0.10
F	1.63	0.23	0.13	0.05	5.85	0.25	1.71	0.05	1.98	0.10
G	4.66	0.74	0.08	0.02	8.58	0.62	4.58	0.88	5.80	0.76
H	3.38	0.37	0.11	0.04	9.55	0.68	3.10	0.27	5.37	0.66
AB	2.71	0.10	0.21	0.08	10.33	0.46	1.98	0.17	2.73	0.20
CD	3.87	0.17	0.06	0.01	11.82	0.82	3.12	0.37	6.45	0.69
EF	1.67	0.02	0.18	0.03	4.37	0.65	1.30	0.17	1.71	0.11
GH	3.77	0.38	0.18	0.05	8.98	1.27	3.51	0.36	5.46	0.54

Basal respiration cont'd

Treatment	Day 0						Day 3			
	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
AC	3.68	0.43	0.20	0.03	8.89	0.85	3.19	0.26	4.91	0.50
BG	3.34	0.21	0.19	0.04	8.84	0.78	3.08	0.24	4.59	0.35
DE	2.84	0.22	0.20	0.07	9.81	0.91	2.18	0.20	3.28	0.44
FH	2.36	0.26	0.17	0.07	8.11	0.52	2.32	0.11	3.12	0.33
ABGH	3.00	0.62	0.25	0.05	9.67	0.81	2.98	0.40	3.72	0.30
CDEF	2.53	0.14	0.17	0.04	7.87	0.49	2.01	0.25	4.19	0.29
ACFH	2.75	0.12	0.23	0.07	7.74	0.66	2.45	0.19	3.43	0.28
BGDE	3.27	0.14	0.15	0.05	10.03	0.18	2.77	0.26	3.49	0.49
ABCDEFGH	2.75	0.10	0.20	0.07	8.89	0.40	2.46	0.35	3.14	0.57
0	0.34	0.10	0.04	0.02	1.05	0.19	0.33	0.03	0.47	0.05

b) Substrate-induced respiration (μg CO_2-C g^{-1} h^{-1})

Treatment	Day 0						Day 3			
	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
A	42.06	1.17	0.26	0.05	29.15	1.27	39.91	2.31	45.94	2.25
B	35.91	6.10	0.27	0.02	31.25	1.86	37.58	4.99	46.99	3.10
C	15.10	0.93	0.11	0.02	11.86	0.52	14.18	1.11	18.72	0.56
D	17.48	0.53	0.07	0.03	15.86	1.13	20.70	3.62	20.34	1.56
E	27.48	2.52	0.15	0.05	21.60	2.24	27.70	3.00	23.75	2.41
F	13.07	1.14	0.12	0.03	9.64	0.59	12.85	1.07	13.58	0.71
G	12.15	1.63	0.17	0.10	8.08	0.61	11.58	1.84	14.63	1.97
H	11.25	0.66	0.11	0.05	10.07	1.64	9.31	1.04	14.73	1.04
AB	43.45	2.67	0.17	0.03	28.21	1.83	35.17	3.28	38.77	7.45
CD	17.51	1.47	0.07	0.02	15.18	1.39	15.73	0.83	17.54	0.96
EF	26.60	1.89	0.14	0.04	18.35	1.56	23.37	2.02	21.50	1.16
GH	10.22	0.82	0.15	0.04	8.02	0.96	10.39	0.70	15.50	1.60
AC	27.43	1.59	0.31	0.05	22.90	0.93	28.22	0.84	35.69	1.69
BG	24.58	1.80	0.15	0.03	18.29	1.40	26.38	2.95	30.11	3.31
DE	19.55	1.36	0.14	0.02	17.84	1.13	19.43	1.77	25.40	1.60
FH	10.99	0.54	0.26	0.04	10.54	0.44	10.90	0.85	13.37	0.84

Substrate-induced respiration cont'd

Treatment	Day 0						Day 3			
	Control	SE	Dry	SE	Rewet	SE	Control	SE	Rewet	SE
ABGH	28.20	1.55	0.18	0.08	20.40	0.91	27.65	2.67	32.57	3.19
CDEF	15.58	0.92	0.18	0.03	14.82	0.21	14.85	0.77	19.05	1.66
ACFH	21.68	0.41	0.23	0.10	22.52	5.07	21.59	1.08	24.69	1.05
BGDE	24.50	1.19	0.16	0.05	21.24	0.99	22.82	1.75	28.34	2.90
ABCDEFGH	21.72	1.61	0.08	0.03	18.65	1.41	20.67	1.47	27.40	1.29
0	1.93	0.10	0.04	0.01	1.47	0.17	1.95	0.17	2.66	0.16