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# Plant species composition effects on belowground properties and the resistance and resilience of the soil microflora to a drying disturbance

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**Abstract**

We hypothesised that plant species composition and richness would affect soil chemical and microbial community properties, and that these in turn would affect soil microbial resistance and resilience to an experimentally imposed drying disturbance. We performed a container experiment that manipulated the composition and species richness of 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 times over a 16-month period to determine the effect of plant community development and seasonal changes in temperature and moisture on belowground properties. Results showed that plant species composition influenced soil chemistry, soil microbial community properties and soil microbial resistance and resilience. Soil from planted treatments generally showed reduced soil microbial resistance to drying compared to unplanted control soils. Soils from under *T. repens* showed a higher resistance and resilience than the soils from under *P. lanceolata*, and a higher resistance than soils from under *L. perenne*. We suggest that differences across soils in either resource limitation or soil microbial community structure may be responsible for these results. Plant species richness rarely affected soil microbial community properties or soil microbial resistance and resilience, despite having some significant effects on plant community biomass and soil nitrogen contents in some harvests. The effect that treatments had for most variables differed between harvests, 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 affected by plant community composition, and the time of measurement, but was largely unrelated to plant species richness.

## 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). These two measures allow us to quantify the extent to which ecosystem functions are affected by a disturbance, and whether these functions are regained after the disturbance. The resistance and resilience of plant (Lepš et al., 1982; MacGillivray et al., 1995; Tilman, 1996) and aquatic (Biggs et al., 1999; Sousa, 1980) ecosystems to disturbance has been well studied, but the factors that control the resistance and resilience of soil microbes have received less attention

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(Allen-Morley and Coleman, 1989; Griffiths et al., 2001; Wardle et al., 2000). Because soil microbes are responsible for the conversion of organic matter into plant available nutrients (Wardle, 1998; Yarie and Van Cleve, 1996), 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 (De Angelis, 1992; de Ruiter, 1998; May, 1972). 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 systems, 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 (Gastine et al., 2003; Hooper and Vitousek, 1998; Tilman and Wedin, 1991). 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 species richness) is increased. Some studies have suggested that increasing plant species richness can lead to increases in net plant uptake of soil nutrients (Hooper and Vitousek, 1998; Tilman and Downing, 1994), enhanced plant productivity (Hector et al., 1999; Naeem et al., 1994; Tilman and Downing, 1994), and ultimately increases in the amount of resources returned to the soil. Increased plant species richness may also alter the soil microbial community by increasing the heterogeneity of resources (Ettema and Wardle, 2002). Plant species richness may therefore alter the effect of the plant community on soil chemical and microbial community 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 (Wardle, 2002). These changes in the plant community may be closely linked to temporal shifts in temperature, daylight hours, and moisture availability. Changes in temperature and moisture can also affect soil resource availability and soil microbial communities directly, by changing soil process rates 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 chemistry, soil microbial community properties and potentially soil microbial resistance and resilience.

We examined the effect of plant community composition, species richness and development on soil chemical and microbial community 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 community 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 richness, and may vary over time as a result of, for example, the developmental stage of the plant community and seasonal shifts in climate and light availability.

## Materials and methods

### *Experimental design*

Model grassland plant communities of varying species composition and richness were established to determine what effect plant species composition and richness (defined as the number of plant

species per container) 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 the most abundant plant species in many perennial pastures in New Zealand, and represent three different functional groups (a N-fixing, a dicotyledonous and a monocotyledonous plant). Although increasing plant species richness from 1 to 3 per container does not represent a large richness gradient, it does cover the part of the gradient where non-additive effects of increases in species richness are most likely to occur, especially given the potential complementary effect of three species from different functional groups (Hooper, 1998). 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 has been shown to be capable of distinguishing between the effects of species richness and species composition in plant communities (Hooper and Vitousek, 1998; Wardle et al., 2000). If the performance of the communities with more than one plant species 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 species richness (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 manoeuvrable) and placed outside at Lincoln. This corresponded to approximately 4.5 kg dry weight (d.w.) of soil. 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. The site of the experiment experiences a mean annual rainfall of 630 mm/year, and a mean July and January temperature of 6 and 17 °C, respectively.

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. This clipping is consistent with effects of defoliation from browsing mammals in the grassland ecosystems in which these plant species dominate, and has been regularly applied in other experiments of this type (Wardle et al., 2000). All clipped plant biomass was sorted into species, dried for 48 h at 60 °C and weighed. For each harvest, this shoot biomass was summed and added to the shoot biomass measured when each container was harvested to give an estimate of ''total shoot production'' since planting for each plant community (Wardle et al., 2000). Reproductive parts were included in this biomass. It gives some indication of average plant growth rates, and allows us to determine whether this plant community property has any effect on the belowground system. All containers were sprayed with Dichlorvos Nuvan 1000 EC Organo-Phosphate on 30 March 2000 to control an aphid infestation.

*Measurement of plant community properties*

At each harvest, dead vegetation was removed and the remaining aboveground biomass was sorted into species and dried at 60 °C for 48 h; this was termed shoot biomass. 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.

*Measurement of soil chemical and microbial community properties*

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 community properties were measured. Basal respiration was measured as described by Wardle (1993), on soil that had been incubated at 25 °C for 2 days at a moisture content of 33% (dry weight basis). Ten g d.w. of soil was placed in a 130-mL airtight container, and returned to the 25 °C incubator. 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 (SIR) 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 $3 \times 1.5$ cm strip of filter paper in a Petri dish containing 30 g d.w. of soil at 33% moisture content (dry weight basis) (Wardle et al., 1999). 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.

*Measurement of soil microbial resistance and resilience*

To determine the effect of plant community composition, richness and harvest timing on the resistance and resilience of the soil microbial biomass and activity, we used a wetting–drying event as a model disturbance. Wetting–drying events are common disturbances in soils (Fierer and Schimel, 2002; Kieft et al., 1987) and are one of the major drivers of soil microbial turnover (Fierer and Schimel, 2002; West et al., 1988) 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% to 6% moisture content (dry weight basis), and the rewetting dry soil disturbance as adding water to bring soil at 6% back up to 33% moisture content (dry weight basis). 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 response of the biomass and activity of the soil microbes 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% moisture content (dry weight basis) (55% of water holding capacity) by air-drying or adding water, and allowed to equilibrate overnight in a 25 °C incubator. Subsamples of equilibrated soils from each treatment were spread out on paper trays to air-dry at room temperature to 6% moisture content (dry weight basis). Three 10 g d.w. subsamples of air-dried soil and two 10 g d.w. subsamples of the undisturbed soil at 33% moisture content (dry weight basis) 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% moisture content (dry weight basis). The latter measurement was used to determine the resistance of the soil microbes to rewetting dry soil. One flask containing undisturbed soil at 33% moisture content (dry weight basis) was used to measure these response variables at this point. Resistance (RS) was calculated as described by Orwin and Wardle (2004):

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

where $D_0$ is the difference in the value of the response variable between the control ($C_0$) and the disturbed soil at the end of the disturbance ($t_0$). This index increases monotonically with resistance, only deals with absolute differences between control and disturbed soil values, and is standardised by the control value to allow comparison between different soils. It is bounded by $-1$ and $+1$, where an index value of 1 indicates full resistance (i.e. the disturbance results in no change in the response variable), an index value of 0 indicates either a 100% decrease or increase in the response variable compared to the control (i.e. the system was not resistant to the disturbance), and a negative index value indicates a greater than 100% change in the response variable compared to the control (i.e. the system showed low resistance). This index has previously been shown to give an accurate quantification of resistance (Orwin and Wardle, 2004).

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% moisture content (dry weight basis) throughout the disturbance period. For the effect of rewetting dry soils on the resistance of soil microbial response variables, $C_0$ 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% moisture content (dry weight basis), 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 (RL) was calculated as described by Orwin and Wardle (2004):

$$\mathrm{RL}(t_3) = \frac{2 \times |D_0|}{|D_0| + |D_3|} - 1$$

where $D_0$ is as above and $D_3$ is the difference between the control and the disturbed soil on day 3 ($t_3$). This index increases monotonically as resilience increases, only deals with absolute differences between control and disturbed soil values, and is standardised by the amount of change initially caused by the disturbance ($D_0$). An index value of 1 indicates 100% recovery at the time of measurement (i.e. the value of the response variable of both the control and the disturbed soil have identical values). An index value between 0 and 1 indicates that the system has not fully recovered, with a value of 0 indicating either no recovery after the end of the disturbance (i.e. $D_0 = D_3$) or that the disturbed soil is now different to the control by the same amount, but in the opposite direction (e.g. if $D_0 = 20$, and $D_3 = 20$ or $-20$, the index will give a value of 0). A negative index value indicates that the absolute difference between the control and disturbed soil on day 3 is greater than it was at day 0, and therefore that the soil has low resilience. This index of resilience has previously been shown to give an accurate representation of resilience (Orwin and Wardle, 2004). We were only able to calculate resilience as the degree to which the rewet soil recovered from the drying disturbance. Therefore, the undisturbed control soil

was in all calculations the soil that had remained at 33% moisture content (dry weight basis) throughout the incubation.

*Data analysis*

The effect of planting treatment on plant, soil chemical and microbial community properties and soil microbial 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. ANOVA with all four harvests showed that, for nearly all response variables, there was a significant effect of treatment, harvest timing, and harvest timing × treatment. Because of this significant harvest timing × treatment effect, it was decided to analyse each harvest separately to get the most detail from the results. Where the overall treatment effect within each harvest was significant, the least significant difference (LSD) 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. Stepwise multiple regression analyses were used to determine which combinations of variables explained the most variation in soil microbial community properties 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 community properties. Explanatory variables for soil microbial community properties (basal respiration, SIR, $qCO_2$ and cellulose decomposition) included plant and soil chemical properties. All data analysis was performed using Statistix 7.0.

## Results

*Characteristics of plant species in monoculture and mixture*

Plant species differed in their biomass for all harvests, but planting species in 2- and 3-way mixtures had only occasional non-additive effects on plant community properties (Table 1). In general, clover plants showed a high aboveground biomass at each harvest time (and a corresponding high total shoot production), but a low belowground biomass (Table 1). Ryegrass and plantain plants showed the opposite trend. Many of the mixtures containing clover showed a similar shoot biomass to clover in monoculture in some harvests, despite lower clover densities. A similar trend was found for the root biomass of mixtures containing ryegrass or plantain in some harvests. Harvest timing 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 increasing the number of plant species from 1 to 3 in the third harvest, where the biomass in the three-species mixture was significantly higher than that of all the three corresponding monocultures. Total shoot production in two mixtures in the third harvest (the clover + ryegrass and the all three species treatment) also responded positively to plant species richness.

*Effect of plants on soil chemical and microbial community properties*

Plant species differed in their effects on the soil chemical and microbial community properties measured, but increasing plant species richness from 1 to 3 rarely had a significant effect, and then only for soil chemical measurements (Tables 2 and 3). Soil planted with clover in monoculture had the highest concentrations of ammonium and nitrate, followed by soil planted with ryegrass and then by soil planted with plantain (Table 2). The clover + ryegrass treatment showed a non-additive effect on soil nitrogen (N) contents in the third (nitrate concentration decreased), and fourth harvest (ammonium concentration increased), compared to monocultures of these species. Soil from under most mixtures containing plantain showed a very low available N concentration, regardless of whether clover was present. 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

*Table 1.* Effect of planting treatment on the means of plant shoot and root mass, the shoot:root ratio and total shoot production for each harvest, as assessed by ANOVA with block and treatment as explanatory variables

| Variable | Harvest timing (months) | Clover | Ryegrass | Plantain | Clover + Ryegrass | Clover + Plantain | Ryegrass + Plantain | All three plant species | *F*-statistic | *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|
| Shoot mass (g d.w.) | 3 | 12.61c | 15.27bc | 19.35a | 17.77ab | 19.59a | 18.14ab | 17.61ab | 3.37 | 0.0117 |
| | 6[1] | 22.65ab | 9.96d | 16.22c | 20.52bc | 26.19a | 12.83e | 25.04ab | 18.52 | < 0.0001 |
| | 11 | 37.91b | 16.90c | 16.97c | 43.01ab | 41.24ab | 16.70c | **45.61a** | 48.14 | < 0.0001 |
| | 16 | 23.72a | 11.03c | 12.31c | 20.45ab | 18.68b | 10.84c | 19.84ab | 11.23 | < 0.0001 |
| Root mass (g d.w.) | 3[2] | 3.46c | 16.12ab | 17.33a | 12.05b | 13.72ab | 16.79ab | 16.05ab | 12.81 | < 0.0001 |
| | 6[1] | 6.82d | 16.67bc | 29.50a | 14.56c | 25.98a | 20.91ab | 25.78ab | 18.09 | < 0.0001 |
| | 11[1] | 12.10d | 20.78c | 32.30ab | 25.30bc | 35.43a | 30.87ab | 27.64abc | 8.57 | < 0.0001 |
| | 16 | 10.13c | 19.81ab | 24.49a | 17.83b | 18.37b | 21.92ab | 24.55a | 6.41 | 0.0002 |
| Shoot:root ratio | 3[1] | 3.88a | 1.02d | 1.16cd | 1.48bc | 1.95b | 1.15cd | 1.16cd | 17.68 | < 0.0001 |
| | 6[1] | 3.56a | 0.60c | 0.59c | 1.44b | 1.03b | 0.63c | 1.14b | 33.02 | < 0.0001 |
| | 11[1] | 3.40a | 0.84d | 0.59e | 1.89b | 1.20c | 0.57e | 1.79b | 31.46 | < 0.0001 |
| | 16[1] | 2.51a | 0.56d | 0.47d | 1.20b | 1.03bc | 0.53d | 0.82c | 24.06 | < 0.0001 |
| Total Shoot Production (g d.w.) | 3 | 12.61b | 17.68a | 21.06a | 19.88a | 21.31a | 20.43a | 17.97a | 3.83 | 0.0059 |
| | 6[1] | 25.51ab | 11.87d | 18.85bc | 24.37ab | 30.31a | 15.15cd | 27.34a | 11.39 | < 0.0001 |
| | 11[1] | 72.20b | 23.71c | 27.33c | **98.13a** | 83.08ab | 27.46c | **93.82a** | 64.03 | < 0.0001 |
| | 16[1] | 60.66a | 23.56b | 25.44b | 77.90a | 63.66a | 29.27b | 80.37a | 28.13 | < 0.0001 |

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 species richness.
[1]Log transformed.
[2]Square root transformed.

*Table 2.* Effect of planting treatment on the means of soil chemical properties in each harvest as assessed by ANOVA with block and treatment as explanatory variables

| Variable | Harvest timing (months) | Bare Soil | Clover | Ryegrass | Plantain | Clover + Ryegrass | Clover + Plantain | Ryegrass + Plantain | All three plant species | *F*-statistic | *P*- value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ammonium ($\mu$g $NH_4^+$ g d.w.$^{-1}$) | 3 | 0.11 | 0.66 | 0.64 | 0.25 | 1.02 | 0.67 | 0.58 | 0.62 | 1.57 | 0.1770 |
| | 6 | 0.08c | 2.14a | 0.38bc | 0.15bc | 0.55b | 0.08c | 0.16bc | 0.08c | 19.58 | < 0.0001 |
| | 11[1] | 1.22c | 6.79a | 4.42ab | 0.84c | 2.92b | 0.71c | 0.63c | 1.31c | 14.93 | < 0.0001 |
| | 16 | 0.03f | 4.80bc | 2.80de | 0.17f | **11.00a** | 3.17cd | 1.10ef | 6.02b | 37.71 | < 0.0001 |
| Nitrate ($\mu$g $NO_3^-$ g d.w.$^{-1}$) | 3[1] | 8.66bc | 16.00a | 7.35cd | 0.96g | 12.19ab | 4.13ef | 1.91fg | 5.26de | 20.74 | < 0.0001 |
| | 6[1] | 8.73a | 11.04a | 4.04b | 0.74c | 8.08a | 0.62c | 0.55c | 0.67c | 39.98 | < 0.0001 |
| | 11[1] | 8.40a | 7.45a | 6.83a | 0.24d | **3.60b** | 0.48cd | 0.24d | 0.95c | 51.37 | < 0.0001 |
| | 16[1] | 11.08c | 33.39a | 10.80c | 0.74e | 21.08b | 3.98d | 1.27e | 7.95c | 37.13 | < 0.0001 |
| pH | 3 | 5.43d | 5.30e | 5.57abc | 5.57abc | 5.47cd | 5.62a | 5.58ab | 5.50bcd | 7.23 | < 0.0001 |
| | 6 | 5.22cd | 4.98e | 5.33bc | 5.47a | 5.10de | 5.25c | 5.38ab | 5.27bc | 11.17 | < 0.0001 |
| | 11 | 5.27b | 4.90e | 5.45a | 5.47a | 5.07d | 5.10cd | 5.47a | 5.17c | 48.77 | < 0.0001 |
| | 16 | 5.27b | 4.90d | 5.50a | 5.50a | 5.07c | 5.27b | 5.55a | 5.20b | 27.96 | < 0.0001 |

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 species richness.
[1]Log transformed.

*Table 3.* Effect of planting treatment on the means of soil microbial community properties in each harvest as assessed by ANOVA with block and treatment as explanatory variables

| Variable | Harvest timing (months) | Bare Soil | Clover | Ryegrass | Plantain | Clover + Ryegrass | Clover + Plantain | Ryegrass + Plantain | All three plant species | *F*-statistic | *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Basal Respiration ($\mu$g $CO_2$–C g d.w.$^{-1}$ h$^{-1}$) | 3[1] | 1.05d | 1.39c | 1.65b | 1.92a | 1.56bc | 2.01a | 1.86a | 1.94a | 22.72 | < 0.0001 |
| | 6 | 0.94d | 1.41c | 1.76ab | 1.91a | 1.56bc | 1.84a | 1.83ab | 1.75ab | 11.30 | < 0.0001 |
| | 11 | 1.27d | 2.33c | 2.25c | 3.13a | 2.61bc | 3.15a | 3.07ab | 3.05ab | 16.84 | < 0.0001 |
| | 16 | 1.10c | 1.70b | 1.95ab | 2.25a | 2.02ab | 2.37a | 2.34a | 2.15a | 7.88 | < 0.0001 |
| SIR ($\mu$g $CO_2$-C g d.w.$^{-1}$ h$^{-1}$) | 3[1] | 3.61e | 4.96d | 6.04bc | 6.36ab | 5.62c | 7.06a | 6.57ab | 7.04a | 27.88 | < 0.0001 |
| | 6 | 3.47d | 6.08c | 6.54bc | 6.98abc | 6.54bc | 7.49ab | 7.55a | 6.81abc | 14.25 | < 0.0001 |
| | 11 | 4.29c | 11.33a | 9.24b | 12.90a | 11.41a | 12.32a | 12.04a | 12.30a | 20.59 | < 0.0001 |
| | 16[1] | 3.67f | 10.70cd | 8.15e | 12.48abc | 10.26d | 13.51a | 11.58bcd | 12.59ab | 64.83 | < 0.0001 |
| $qCO_2$ | 3 | 0.30 | 0.28 | 0.27 | 0.30 | 0.28 | 0.28 | 0.28 | 0.27 | 1.01 | 0.4392 |
| | 6 | 0.27 | 0.23 | 0.27 | 0.27 | 0.24 | 0.25 | 0.24 | 0.26 | 2.00 | 0.0832 |
| | 11 | 0.30a | 0.20c | 0.25b | 0.24b | 0.23bc | 0.26b | 0.25b | 0.22bc | 5.07 | 0.0005 |
| | 16[2] | 0.30a | 0.16c | 0.24ab | 0.18abc | 0.20abc | 0.18bc | 0.20abc | 0.17bc | 8.65 | < 0.0001 |

Means within a row followed by the same letter are not significantly different from each other at $P < 0.05$.
[1]Log transformed; [2]rank transformed.

the mixtures. Soil pH was also affected by plant species composition, with soils planted with clover having a lower pH than soils planted with ryegrass or plantain (Table 2). Soils from planted treatments showed enhanced soil microbial basal respiration and SIR compared to the bare soil (Table 3). In general, soils from under clover monocultures showed a lower basal respiration than 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. Increasing the number of plant species from 1 to 3 had no non-additive effects on soil microbial community properties. However, in the third harvest, mixtures containing plantain had a basal respiration that was the same as that of the plantain monoculture, despite a lower density of plantain plants. Planting either clover or plantain with ryegrass resulted in soils with a SIR the same as that of the clover and plantain monocultures. Basal respiration and SIR tended to increase with time, but in some treatments declined by the fourth harvest.

Different plant species had positive, negative or neutral effects on the ability of soil microbes to decompose cellulose, but increasing the number of plant species from 1 to 2 or 3 had no significant non-additive impacts (Figure 1). 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. For the final two harvests, the decomposition rate in soils that had been planted with clover was the same as that of the bare soil. Soil from under ryegrass showed no change in decomposition rates compared to the bare soil, while soils from under plantain tended to show a low cellulose decomposition rate. Mixtures containing plantain often showed a cellulose decomposition rate that was similar to that of the plantain monocultures, despite the lower density of plant species in mixtures.

### *Effect of plants on soil microbial resistance and resilience*

The resistance of the soil microbes to both drying and rewetting dry soil showed no significant harvest timing × treatment interactions, so data from all harvests were analysed together to test for overall effects of planting treatments (Figure 2). 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, soil from the planted treatments showed reduced soil microbial resistance compared to the bare soil, with the exception of the resistance of soil microbial SIR, which showed no responses to treatments. The resistance of basal respiration to drying and rewetting dry soil was also the same in soil from the clover monoculture and bare soil treatments. There was one significant non-additive effect of increasing the number of plant species; the resistance of basal respiration to rewetting dry soil was lower in soil from the clover + ryegrass treatment than in either of the corresponding monocultures. However, mixtures of ryegrass or plantain with clover showed a resistance of basal respiration to drying and rewetting that was similar to these species in monoculture, despite the presence of clover. The effect of harvest timing on resistance depended on the microbial response variable measured. The resistance of basal respiration to either disturbance decreased in the second harvest, compared to the resistance of SIR to drying, which showed lower resistance in the fourth harvest, and the resistance of glucose use to rewetting dry soil, which showed higher resistance in the second and third harvests.

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 planting treatment – only the first two harvests showed significant effects at $P < 0.05$ (Figure 3). Different plant species had positive, negative or neutral effects on the resilience of this response variable compared to the bare soil, with soils from the clover and ryegrass monocultures showing the highest resilience. In the first harvest, soils from treatments containing plantain showed either the same or reduced resilience compared to the bare soil. In the second harvest, results were similar but stronger, with soil from all treatments

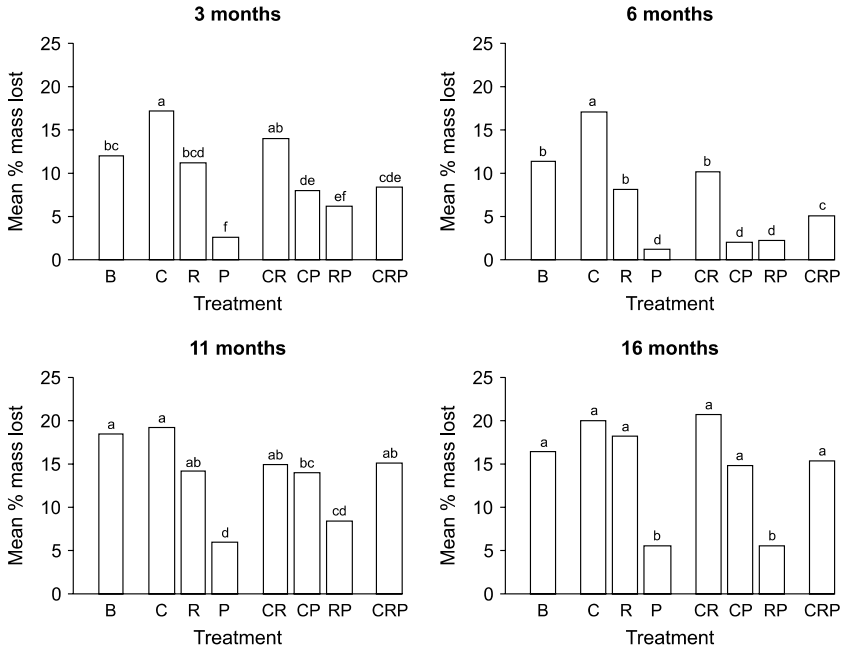


*Figure 1.* Effect of treatment and harvest timing on the ability of soil microbes to decompose a strip of cellulose paper over a 10-day period. Data for the second harvest were square root transformed for analysis, and data for the third harvest were log transformed. Within each panel, bars topped with the same lower-case 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.

containing plantain showing a low resilience and soil from the clover, ryegrass and clover + ryegrass treatments showing a relatively high resilience.

The resilience of soil microbial glucose use to drying responded to the species composition of the plant community, but not to increasing the number of plant species from 1 to 2 or 3 (Figure 4). 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 in mixtures appeared to be particularly affected by the inclusion of plantain, with these soils showing a low resilience, especially in the second harvest. Resilience of the soil from the mixtures containing plantain tended to become more similar to the bare soils with time.

### *Relationships of plant and soil chemical variables with soil microbial variables*

Stepwise multiple regression was performed to assess which variables were the most likely drivers of soil microbial community properties and soil microbial resistance and resilience across treatments within each harvest date (Table 4). The variables that explained the most variation in basal respiration varied among harvests, with shoot biomass and pH initially being important, followed by nitrate concentration for the later harvests. Multiple regression relationships 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 primarily explained by different variables in different harvests, but the shoot:root ratio became a more consistent explanatory variable towards the end of the experiment. Variation in

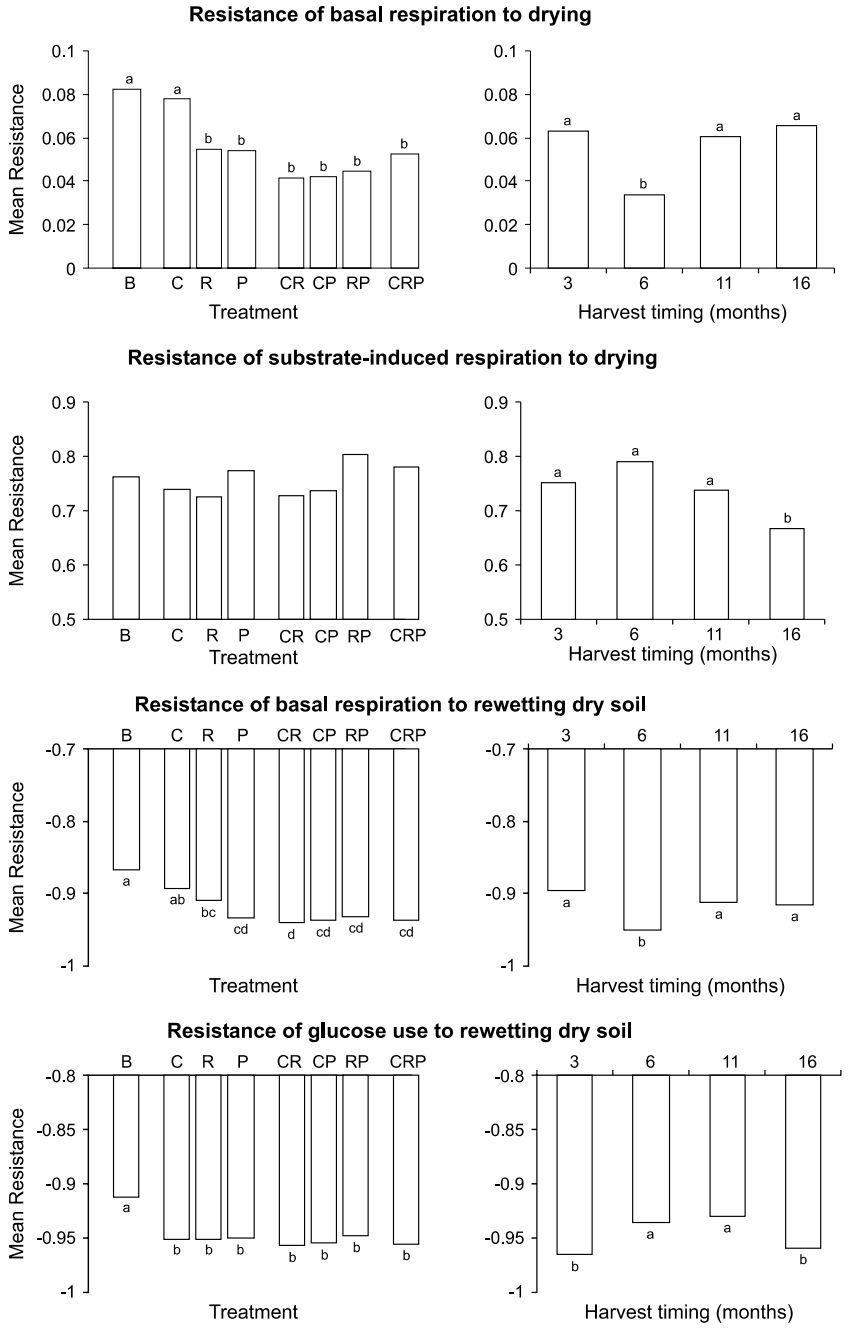


*Figure 2.* Effect of treatment and harvest timing on the resistance of soil microbial parameters. As there was no significant treatment × harvest timing interaction, data for all harvests were pooled. All resistance variables apart from the resistance of SIR were log transformed before analysis. Bars within each panel topped with the same lower-case letter are not significantly different from each other at $P < 0.05$. Treatment codes as for Figure 1.

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

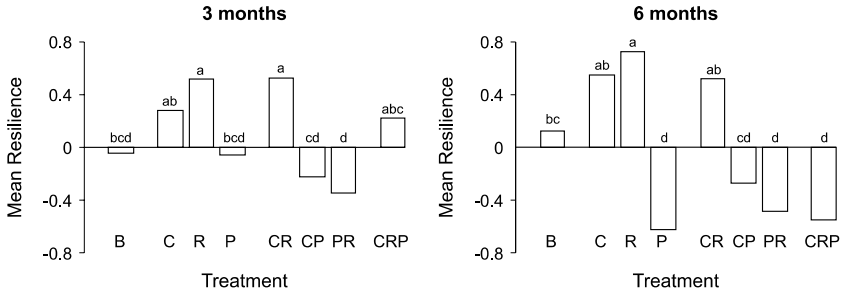


*Figure 3*. Effect of treatment and harvest timing on the resilience of SIR for the first and second harvests (the third and fourth harvests did not show any significant responses to treatment (data not presented)). Within each panel, bars with the same lower-case letter are not significantly different from each other at $P < 0.05$. Treatment codes as for Figure 1.

root mass (expressed on a per unit soil weight basis) and SIR appeared to have an important influence on the resistance of basal respiration to drying and rewetting dry soil (Table 4). The variation in the resilience of basal respiration to drying was also largely unaccounted for by the explanatory variables used. For the resilience of SIR and glucose use, however, nitrate concentration, root mass (expressed on a per unit soil weight basis), and ability to decompose cellulose appeared to be important variables.

## Discussion

The species composition of the plant communities had significant effects on soil chemical and soil microbial community properties, and on soil microbial resistance and resilience. Although increasing plant species richness altered plant and soil chemical properties in some harvests and for some treatments, it had no impact on soil microbial community properties and had an effect on soil microbial resistance

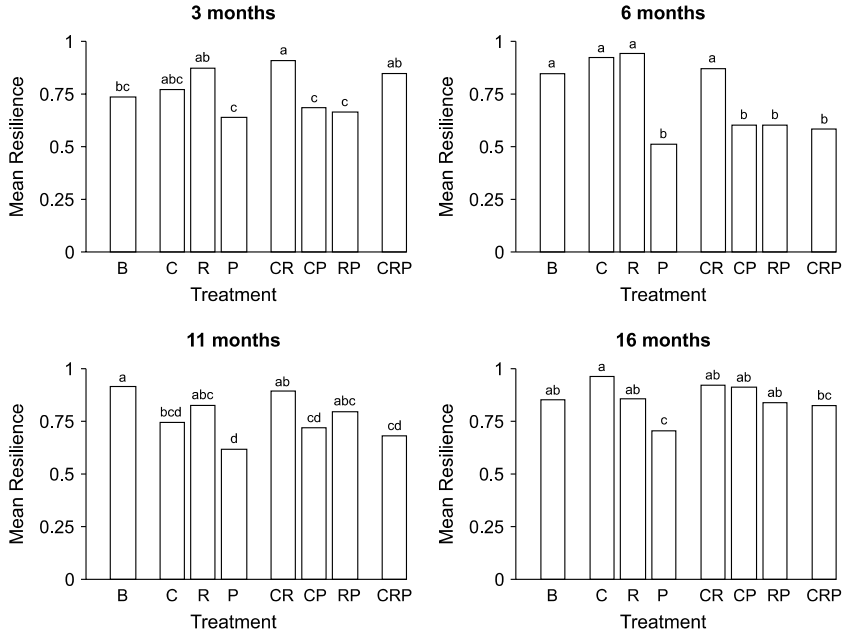


*Figure 4*. Effect of treatment and harvest timing on the resilience of glucose use for each of the four harvests as analysed by ANOVA. Within each panel, bars topped with the same lower-case letter are not significantly different from each other at $P < 0.05$. Treatment codes as for Figure 1.

and resilience in one instance only. In contrast, the timing of the harvest did influence the effect of different plant species on soil and microbial properties, and whether there were any significant plant species richness effects.

*Effect of plants on soil chemical and microbial community properties*

Each plant species in monoculture produced a soil with distinctive chemical and soil microbial community properties. 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 (Bardgett et al., 1999; Gastine et al., 2003; Tilman and Wedin, 1991). Although all soils from planted treatments showed enhanced soil microbial activity and SIR compared to the bare soil, the magnitude of this effect depended on which plant species was present. Similar results have been found in other studies (Bardgett et al., 1999; 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 planted treatments appeared to be influenced by both plant and soil chemical properties (Table 4) (Swift et al., 1979; Wardle et al., 1999).

*Effect of plants on soil microbial resistance and resilience*

Despite the strong effect of plant species on soil microbial community and chemical properties, their effects on soil microbial resistance and resilience were variable, and depended on which microbial response variable was considered. Studies in aquatic (Biggs et al., 1999; Herbert et al. 1999; Steinman et al., 1990) 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 species composition of the plant community 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, 2000). Soils that had been planted with clover consistently showed a higher resistance and resilience than soils that had been planted with plantain, and a higher resistance than soils that had been planted with ryegrass. This suggests that the different effects of each plant species on the soil environment and microbial community were sufficient to cause significant differences in soil microbial resistance and resilience.

*Drivers of 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 dichotomy. Firstly, although the resistance of the soil microbial community was rarely strongly related to soil variables, SIR and root biomass were negatively related to the resistance of basal respiration for the final harvest (Table 4). Roots supply a large portion of the C used by soil microbes, and the amount of soil microbial biomass is also generally positively related to the amount of C in the system (Wardle, 1998). The negative correlation between the resistance of basal respiration and SIR and root biomass 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 the microbial biomass in these soils were probably limited more by C than by N, whereas in soils planted with plantain the reverse was true. Soils that had been planted with clover or ryegrass showed a higher stability than soils that had been planted with plantain. This suggests that the microbial community in soils that are C limited may be more resilient, and sometimes more resistant, than soils that are N limited. This explanation is supported further by the observation that soil nitrate concentrations were positively related with the resilience of

*Table 4*. Relationships between soil microbial response variables and driving variables as assessed by stepwise multiple regression

| Harvest timing | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Variable | 3 months | | 6 months | | 11 months | | 16 months | |
| | 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] | $qCO_2$ (+); TSt (–) | 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 | TSt (–) | 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*** |

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. $n = 42$ and excludes the bare soil treatment.
BR = basal respiration ($\mu$g $CO_2$-C g d.w.$^{-1}$ h$^{-1}$), SIR = substrate-induced respiration ($\mu$g $CO_2$-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 ($\mu$g $NO_3^-$ g d.w.$^{-1}$), Amm = ammonium concentration ($\mu$g $NH_4^+$ g d.w.$^{-1}$), TSt = total shoot production from planting to harvest. Log transformed[1] in the first[2], second[3], and third harvests[4]; square root transformed in the second[5] and fourth harvests[6]; [7]$n = 41$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

SIR and glucose use in the second harvest (Table 4). These results are consistent with theoretical studies by De Angelis (1992), which suggest that higher inputs of nutrients can increase resilience because recovery rates are not limited by these nutrients. Thus, the higher N content of the C limited soils may have resulted in higher resilience. The higher resistance of C limited soils, including the unplanted soil treatment, may be explained by the physiology of the soil microbes occupying these soils. Because of the low C availability, most microbes in these soils were probably inactive or growing only slowly. Slower-growing organisms in soil may survive drying while the actively growing ones are killed (Bottner, 1985), resulting in higher resistance in C limited soils. These results are consistent with the hypothesis that communities limited by different resources may respond differently to the same disturbance (Biggs et al., 1999; Bosatta and Berendse, 1984; Huston, 1997).

Different plant species may have created soils with microbial communities that differed in their responses to the drying disturbance. Several studies have shown that different plant species grown in the same soil can result in soils that vary in their community composition (Grayston et al., 1998; Stephan et al., 2000; Wardle et al., 1999). Further, studies involving soil (Allen-Morley and Coleman, 1989; de Ruiter, 1998), plant (Lepš et al., 1982; MacGillivray et al., 1995), and aquatic (Sousa, 1980) systems have suggested that community composition is an important driver of resistance and resilience. Although we did not directly measure soil microbial community composition, there were several indications that the soil biota did differ across treatments. The basal respiration, SIR and $qCO_2$ of the different soils varied (Table 3), and the ability of the 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. Most 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 4), supporting the suggestion that differences in soil microbial community composition may have had an impact on stability.

*Effect of plant species richness on soil microbes*

Although the effect of plant communities on shoot biomass, total shoot production and soil N values was altered by increasing plant species richness from 1 to 2 or 3 in some treatments and harvests, these changes rarely flowed through to soil microbial community properties or to resistance and resilience. In the one harvest for which there was a significant effect, increasing plant species richness from one to two had a negative impact on the resistance of basal respiration to rewetting dry soil (Figure 2). It is possible that the soil microbial community was unresponsive to changes in plant species richness because the magnitude of the effect of plant species richness on the soil environment was insufficient to influence the soil microbial community. Although there were few effects of species richness *per se*, several results from the mixtures did suggest that individual species had particularly strong effects on the variables measured. For example, mixtures that included plantain showed soil N values, decomposition rates, and (for some variables) resistance and resilience values that were the same or similar to those of the plantain monocultures, but were significantly different to that of the monocultures of the other component species. This again emphasises the importance of plant community composition in determining the characteristics and function of the soil system.

*Effect of harvest timing on soil chemistry and soil microbes*

Harvest timing significantly affected nearly all variables measured. The variables that explained the greatest proportion of variation across treatments in soil microbial community properties and resistance and resilience were often different for different harvest dates (Table 4). This suggests that the developmental stage of the plant community and/or external variation in climate due to seasonal changes may alter the nature of plant community effects on soil properties, and therefore 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 (Wardle and Nicholson, 1996; Wedin and Tilman, 1990). The one exception to this trend was the resistance of the soil microbes, which did not show any significant interactions between harvest timing and treatment, possibly because resistance was not strongly related to soil chemical or microbial community properties. Overall, this suggests that the results gained from studies on the effects of species composition and species richness will be context-dependent, and may vary according to factors that vary temporally. It thus appears while plant species composition was the primary driver of soil microbial community properties and their resistance and resilience within harvests, the strength and direction of these effects show significant temporal variation.

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