Influences of arbuscular mycorrhizas on a semi-arid plant community

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Summary

Symbiotic relationships between plants and arbuscular mycorrhizal fungi are widespread in natural ecosystems. Some plant species benefit from these associations primarily through increased nutrient uptake, improved drought tolerance and resistance to pathogens. The benefits of mycorrhizal association for individual plants may have significant influences on establishment, survival, growth and reproduction, and consequently influence competition between plants, species composition, floristic diversity and succession within plant communities. The studies reported in this thesis were primarily focussed on assessing the influence of arbuscular mycorrhizas on the growth of individual plant species, competition between species and community structure in the herbaceous understorey of semi-arid open woodland in Brookfield Conservation Park, South Australia.

The mycorrhiza-responsiveness of the six most abundant plant species from the field site was assessed in two glasshouse experiments. In one experiment single plants were grown in intact soil cores from Brookfield Conservation Park, half the cores were treated with the fungicide benomyl to reduce the activity of AM fungi. In the second experiment single plants were grown in autoclaved soil with the AM fungus *Glomus mosseae* added to half the pots. The three most abundant plant species showed different growth responses to colonisation, with *Medicago minima* being highly mycorrhiza-responsive, *Salvia verbenaca* becoming colonised but exhibiting no growth response to mycorrhiza, and *Carrichtera annua* remaining uncolonised.

The contribution of arbuscular mycorrhizas to plant competition, community structure and diversity in the semi-arid herbland was determined after reducing mycorrhizal colonisation in field plots by applying the fungicide benomyl as a soil drench. Plant community structure was studied in some plots while others were used for removal experiments to examine competitive relationships between the three species *M. minima*, *C. annua* and *S. verbenaca*. In the field experiment where species were removed to study the two-way and three-way species interactions between *M. minima*, *C. annua* and *S. verbenaca*, suppression of mycorrhizas decreased the competitiveness of *M. minima*

when grown in combination with *C. annua* and/or *S. verbenaca*. Removal of the unresponsive mycorrhizal host *S. verbenaca* from plots containing *M. minima* and *C. annua* also resulted in decreased cover of *M. minima*, which suggests that *S. verbenaca* may contribute carbon to the mycorrhizal network and directly or indirectly support the growth of *M. minima*. Suppression of mycorrhizas in plots containing *C. annua* and *M. minima* also resulted in increased size and P content of *M. minima* seed, probably as a consequence of increased resource allocation to each seed when total seed numbers were reduced in the smaller plants.

In a field experiment where plant communities were not directly manipulated, floristic diversity increased in mycorrhiza-suppressed field plots due to a decrease in biomass of *M. minima* and competitive release of the non-positively responsive species *S. verbenaca* and *C. annua*. There was no change in plant species richness after fungicide treatment; all the increase in diversity being attributable to an increase in plant species evenness within the community. There was also no effect of treatment on community productivity measured as total aboveground biomass and therefore no relationship between mycorrhizal effects on diversity and productivity. Mycorrhizal responsiveness of plant species, as determined in glasshouse experiments, was not a good predictor of individual plant species response to suppression of mycorrhizal colonisation in the field. The highly mycorrhiza-responsive species *Vittadina gracilis* and *Velleia arguta* were equally abundant and productive in fully-mycorrhizal (control) and mycorrhiza-suppressed plots. This suggests that competition from the mycorrhiza-responsive dominant *M. minima* offset the benefits of mycorrhizal association for *V. gracilis* and *V. arguta*. Competitive release from *M. minima* in fungicide-treated plots resulted in differential biomass investment by the other major plant species. There was an increase in productivity of *S. verbenaca* related to increased seedling survival, while *C. annua* showed increased productivity without significant adjustment of survival. Community level influences of mycorrhiza are intimately linked to the life-strategies and mycorrhiza-responsiveness of the component plant species.

To further understand the significance of arbuscular mycorrhizas in arid and semi-arid Australia five surveys of the mycorrhizal status and infection characteristics of plants in the Simpson and Stony Deserts were undertaken. Fifty-four of the eighty-one plant species surveyed (67 %) showed at least some evidence of AM association. The

mycorrhizal status of seventy-four plant species is reported for the first time. Members of families not previously known to form AM - Frankeniaceae (*Frankenia plicata*), Myoporaceae (*Eremophila longifolia* and *E. macdonnellii*) and Marsileaceae (the aquatic fern *Marsilea drummondii*) - were all colonised. The remaining non-mycorrhizal plant species were not evenly distributed between the one fern family and twenty-four plant families represented in these surveys. Six families (Aizoaceae, Amaranthaceae, Cruciferae, Lamiaceae, Portulacaceae and Zygophyllaceae) contained only non-mycorrhizal members, while five other families (Boraginaceae, Chenopodiaceae, Compositae, Convolvulaceae and Graminae) contained some members that were not mycorrhizal. No correlation was found between plant life form or habit and the proportion of species forming AM associations. To assess whether arbuscular mycorrhizas were more common in plants occurring in dune or interdune vegetation in the Simpson Desert, surveys were conducted at three sites, two dune sites and one interdune site. No correlation was found between site type (dune or interdune) and the proportion of species forming arbuscular mycorrhizas.

Declaration

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution, and to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text.

I give consent to this copy of my thesis, when deposited at the University Library, being available for loan and photocopying.

Date

19 February 2001

Signed

Patrick James O'Connor

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Not only the cords in the net, but the air
that escapes the interstices matters:

Pablo Neruda *Those Lives*

I want now to turn to the foundations provided
by experimental science, for without experience
one cannot know anything fully.

Roger Bacon *Opus Maius* (*c.* 1260)

To my daughter Shaez

Who's wonder in the natural world finds wonder in me

Chapter 1

Introduction

The size, abundance and fecundity of individual plants and plant species in communities are the result of plant-plant and plant-environment interactions. Different plant species take advantage of different strategies for resource capture to maximise the chance of persisting in a given plant community. One strategy often shown to increase capture of phosphorus for individual plants is association with fungi in the Glomales (Zygomycotina) to form arbuscular mycorrhizas (AM). AM associations have been shown to increase plant phosphorus uptake (Smith and Read 1997), and consequently, plant growth where phosphorus availability is low. This study investigates the influence of AM on the growth of plants from a semi-arid herbland and interactions between these plants within that herbland community.

While the majority of plant species can form AM (> 80% of plant species), it is less often considered that almost all terrestrial plant ecosystems contain AM-forming glomalean fungi. The obligate nature of the symbiosis for the fungal-symbiont means that the fungus will always benefit by forming the association. However, the benefits from association are not always so obvious for the plant host. Some plants do not form any association with AM fungi, while for others there is a spectrum of response to the association extending from clear parasitism under some conditions, to obligate or near obligate dependence on the association in others (Janos 1980b; Johnson *et al.* 1997). The coexistence of plants from different parts of this spectrum suggests a role for AM associations in plant community dynamics.

Attention on the global loss of biodiversity and interest in threat abatement for vulnerable species has stimulated interest in processes that affect species richness in natural plant communities. The effects of competition (a negative interaction) and AM (in most cases a positive interaction) on plant population dynamics and plant species coexistence have rarely been studied together (Zobel and Moora 1997). Root competition has been shown to be a more important contributor to biomass reduction

than shoot competition in both greenhouse (Wilson 1988; Weiner 1990; Aerts *et al.* 1991) and field experiments (Wilson and Tilman 1993; Belcher *et al.* 1995; Gerry and Wilson 1995). Outcomes of competition between coexisting plants may, therefore, be altered by the ability of those plants to form mycorrhizas and exploit the symbiosis for increased nutrient uptake.

The primary obstacle to study of the influence of AM associations in plant communities is the difficulty of establishing adequate controls in the field. When almost all vegetated sites are inhabited by AM forming fungi, the problem is one of reducing or eliminating the activity of these fungi without introducing significant experimental artefacts. Current understanding of the functional significance of mycorrhizal associations in natural ecosystems is, therefore, largely derived from extrapolation of findings from pot and microcosm experiments. Field studies have so far produced variable results (McGonigle 1988) which implicate AM associations in plant growth enhancement (Merryweather and Fitter 1996) and ecosystem processes (Fitter 1989) but are not conclusive. This is not to say that pot and microcosm experiments cannot yield valuable information, especially in supporting and directing field experiments.

The ecology of mycorrhizas in arid and semi-arid plant communities has been little studied, especially in Australia. Some studies have included information about the mycorrhizal status of native and introduced plant species (McGee 1986; McGee 1987) in semi-arid Australia but no previous field experiments have investigated the influence of mycorrhizas on an established plant community in this bio-region. A number of studies have investigated the effects of changes in vegetation after disturbance (Brundrett and Abbott 1995) or a variety of cultural practices (McGee 1989; Michelsen 1994; Antoniolli 1999) on populations of AM fungi. Several studies have also evaluated the infectivity and effectivity of propagules (Abbott and Robson 1981; McGee 1989) from field soils in Southern Australia. However, feedbacks on plant populations from changes in the populations of mycorrhizal fungi remain difficult to quantify (Helgason *et al.* 1998; van der Heijden *et al.* 1998b) and no studies have been undertaken in Australian ecosystems. Even where whole plant communities or assemblages have been studied for mycorrhizal influences (Grime *et al.* 1987; Gange *et al.* 1990; Sanders and

(Grime *et al.* 1987; Gange *et al.* 1990; Sanders and Koide 1994; Wilson and Hartnett 1997), longitudinal studies are rare (Hartnett and Wilson 1999a).

The main objectives of this study were to examine the influence of mycorrhizal associations on the establishment, survival, growth, and fecundity of plants from a semi-arid herbland in South Australia. More specifically the study aimed to investigate;

1. the mycorrhizal status of plant species in arid and semi-arid South Australia,
2. the influence of mycorrhizal associations on the growth of major plant species from a semi-arid herbland,
3. the influence of mycorrhizal associations on competitive interactions between species from a semi-arid herbland,
4. the influence of mycorrhizal associations on the plant diversity and community structure of a semi-arid herbland.

Chapter 2

Literature Review

2.1 Introduction

The study of mutualistic associations between arbuscular mycorrhizal fungi (Endogonacaeae) and the roots of most species of vascular plant species is now more than 100 years old. While insights into the interactions between these fungi and their plant hosts have been made through experimentation under controlled conditions, the functional role of these associations in nature is far from understood. Estimates of the range of potential and actual hosts for AM fungi extend to greater than 80% of terrestrial plant species (Smith and Gianinazzi-Pearson 1988) while surveys have found the symbiosis to occur across most biogeographical zones, from the tropics (Janos 1980b) to the Tertiary sediments of the Antarctic (Phipps and Taylor 1996). Considering the pan-global occurrence of these associations, and in light of our limited knowledge of the genetic diversity and physiological plasticity of the fungal associates, it is imprudent to make generalisations about the function of mycorrhizal associations in natural or agricultural ecosystems.

At the time the field studies reported in this thesis began (February 1997), investigations into the effects of mycorrhizas at the plant population or community level were few, as were studies on natural as opposed to agricultural ecosystems. Research into the effects of mycorrhizas on the native plants or ecosystems of Australia are extremely rare. Further study in a greater range of ecosystems is essential if we are to avoid overreaching the significance of observations from pot experiments. However, results from pot experiments have often been difficult to confirm with field studies, even where control of mycorrhizas in field plots has been relatively easily achieved (McGonigle 1988). Where manipulation of vegetation, fungal populations or edaphic conditions in the field has been difficult, results have been scarce or inconclusive.

With such diversity in the soil physical and chemical environment, host plant species, fungal isolates and climatic regimes, it is difficult to make assumptions about the role of the AM associations in unstudied ecosystems. AM associations have now been implicated as altering a range of factors affecting plant growth. These include increased

phosphorus nutrition (Ross and Gilliam 1973 ; Jakobsen 1986), improved uptake of growth limiting micronutrients such as Cu, Zn, (Marschner and Dell 1994), fitness with respect to fecundity and seed viability (Koide *et al.* 1988b; Bryla and Koide 1990; Shumway and Koide 1995) and interspecific and intraspecific competition (Koide 1991a; Koide and Li 1991; Hartnett *et al.* 1993; Hetrick *et al.* 1994), tolerance of pathogens (Dugassa *et al.* 1985; Benhamou *et al.* 1994; McAllister *et al.* 1996), grazing by insects on roots (Gange *et al.* 1994; Pinochet *et al.* 1996) and increased water use efficiency and drought tolerance (Davies *et al.* 1996). Some studies have not been able to separate these different influences from one another. Other studies have ignored information about the possible range of influences mycorrhizas moderate in ecosystems, concentrating on phosphorus uptake as the primary contribution of the symbiosis to ecosystem processes.

The present study was not designed to investigate the specific mechanisms by which mycorrhizas affect plant growth and plant communities. However, some discussion of the potential effects of mycorrhizas on individual plants is necessary to understand the effect of mycorrhizas on plant growth and community structure. This chapter also reviews the literature on current understanding of the influence of mycorrhizas on plant competition and community structure.

2.2 Mycorrhizal effects on plant nutrition

2.2.1 Mycorrhizal effects on plant P uptake

Phosphorus (P) is an essential macronutrient and is involved in many metabolic processes and ultimately in the growth of plants. The supply of P to the plant is often limiting in soils of agricultural and natural systems and so plant performance can be measured as a function of P deficit. Phosphorus deficit is defined as the difference between P demand and P supply (Koide 1991b), and is affected by morphological, phenological and physiological traits which vary across plant taxa (Fohse *et al.* 1988). In mycorrhizal plants phosphorus deficit may be partially offset by increased uptake of P by extraradical hyphae associated with colonised roots (Sanders and Tinker 1973; Cooper and Tinker 1978; Pearson and Jakobsen 1993; Schweiger and Jakobsen 1998).

A number of studies have assessed the effectiveness of mycorrhizas to improve P uptake and growth in a range of plant species (Hayman and Mosse 1971; Mosse *et al.* 1973; Abbott and Robson 1977; Hall *et al.* 1977; Smith 1982; Plenchette *et al.* 1983; Hetrick *et al.* 1988; Hetrick *et al.* 1990). While most assessments of mycorrhiza-enhanced P uptake have been made in pot experiments, some studies have demonstrated mycorrhizal effects on nutrient uptake by plants under field conditions (Jakobsen and Nielsen 1983; Jakobsen 1986; Mullen and Schmidt 1993; Merryweather and Fitter 1996; Lapointe and Molard 1997; Schweiger and Jakobsen 1999). However, not all plant species form AM associations, and not all AM plant species show clear nutritional benefits from colonisation by AM fungi under all growth conditions (Tester *et al.* 1985; Fitter 1986; Francis and Read 1995).

Plant communities may often include species with varying response to the presence of AM fungi. The roots of some plant species are not colonised by AM fungi and may develop benign or possibly antagonistic interactions with the fungi (Francis and Read 1995). Other plant species are highly responsive to colonisation by AM fungi and show clear growth benefits from forming mycorrhizas (Johnson *et al.* 1997). Natural plant communities are composed of plant species from across this response spectrum, and community structure may be determined by differential contributions of the mycorrhizas to individual plant species growth and fitness.

2.2.2 Mycorrhizal effects on uptake of Zn and Cu

The most obvious and commonly studied nutritional benefit of mycorrhizas is due to increased P-uptake and growth of the host plant, however, AM have also been shown to increase the uptake of other nutrients such as Zn and Cu. Diffusion rates of both Zn and Cu in soil are low and mobility of these elements may determine uptake rates at the root surface. External hyphae of the AM fungus *Glomus mosseae* have been shown to translocate ^{65}Zn from the bulk soil into *Trifolium repens* (Cooper and Tinker 1978). Similarly, Li *et al.* (1991) showed that mycorrhizal white clover accumulated higher concentrations of Cu than non-mycorrhizal plants. A number of other studies have also reported that mycorrhizas may increase Zn and/or Cu uptake in a number of plant species (Bell *et al.* 1989; Gnekow and Marschner 1989; Faber *et al.* 1990; Kothari *et al.* 1991; Burkert and Robson 1994). Mycorrhizas may influence competition between

plants for Zn and Cu in natural plant communities where the availability of these nutrients is low.

2.3 Non-nutritional effects of AM on plant growth

Plants which form arbuscular mycorrhizas primarily benefit from the association through enhanced nutrient uptake or non-nutritional advantages such as enhanced water use efficiency or pathogen resistance. Because P is essential to plant growth, it can be difficult to discriminate between secondary benefits from improved P nutrition and other primary non-nutritional benefits. Unfortunately, few studies of non-nutritional benefits have included appropriate control treatments with non-mycorrhizal plants of equal size and phosphorus status to the mycorrhizal plants. Where adequate controls have been included, effects unrelated to P nutrition have sometimes been observed. Notable among these studies are the investigations of interactions between mycorrhizal plants and root pathogens or soil insects. Benefits to the plant derived from mycorrhizas have included increased tolerance to fungal pathogens (Dugassa *et al.* 1985; Newsham *et al.* 1995; McAllister *et al.* 1996) and reductions in the numbers of harmful soil organisms (Gange *et al.* 1994). These studies are important because they encourage thought about mycorrhizal associations to extend beyond nutritional benefits.

To obtain a complete picture of the importance of mycorrhizas in plant communities it is necessary to consider results that indicate no growth benefit resulting from mycorrhizal colonisation of host species. Dynamic processes involved in the life and growth of a plant can be masked if mycorrhizal response is only assessed as growth differences between colonised and uncolonised plants at an arbitrary stage in the life of the plants. Some plant species may acquire no apparent growth benefit from colonisation by AM fungi but may be important in maintaining propagule populations of the fungi for other plant species in subsequent seasons or sera (Janos 1985; Hetrick 1994).

2.3.1 Mycorrhizal effects on plant water use

The productivity of plants in arid and semi-arid Australia is limited by the availability of water. Where the frequency and intensity of rainfall is adequate for seed germination and early growth, subsequent availability of water will determine the rate of biomass

accumulation, lifespan and reproductive success of individual plants. Plant species vary in their capacity to exploit and efficiently use available water (Kalapos *et al.* 1996). Fertilizer application or mycorrhizal association (Michelson and Rosendahl 1990) can enhance plant water use efficiency (WUE). Differences between plant species with respect to water use characteristics may be related to differences in shoot or root morphology, stomatal conductance and transpiration rates, or competitive advantage over neighbouring plants. Enhanced phosphorus uptake by mycorrhizal plants is likely to be relatively greater under arid conditions where the diffusion of phosphorus through the soil is rate limited by low soil moisture contents (Fitter 1985). Mycorrhizal associations have been observed to affect traits influencing water use characteristics and so affect plant water use and drought resistance.

Some controversy has prevailed as to the reason for observed differences in water use by mycorrhizal and non-mycorrhizal plants. The difficulty in separating the effects of improved phosphorus nutrition from other effects of mycorrhizal colonisation on water use has only recently been overcome by conducting experiments with P-sufficient non-mycorrhizal controls (Davies *et al.* 1996). By studying the differences between mycorrhizal and P-sufficient non-mycorrhizal plants it has been possible to observe a range of metabolic and physiological responses of mycorrhizal plants to drought stress.

Davies *et al* (1996) observed an increase in the abscission of leaves and a decrease in epicuticular wax on leaves of AM roses under drought stress. This complements a number of other studies which have shown increased transpiration rates (Ruiz-Lozano *et al.* 1995), decreased stomatal resistance and increased leaf water potential (Allen and Boosalis 1983; Allen and Allen 1986) of mycorrhizal versus non-mycorrhizal plants. However, there are contradictory reports as to the effect of mycorrhizas on these parameters in some plant species; eg. there was no effect on net assimilation of CO_2, change in stomatal conductance or water use efficiency for *Citrus aurantium* (Syvertsen and Grahan 1990).

Studies of mycorrhizal effects on drought tolerance are also confounded by the functional diversity of isolates of the fungal symbiont. Ruiz-Lozano *et al.* (1995) showed that different species of fungi belonging to the genus *Glomus* could be ranked

according to their affects on drought tolerance, and that these differences were related to the different effects the endophytes had on transpiration, stomatal conductance and nutrient uptake. Results of this study are difficult to interpret because plants associated with most of the endophyte species had higher P contents than non-mycorrhizal plants, regardless of watering treatment. However, plants grown in association with efficient endophytes, *Glomus deserticola* and *G. etunicatum* (Ruiz-Lozano *et al.* 1995) and *G. deserticola* and *G. fasciculatum* (Ruiz-Lozano and Azcon 1995) exhibited differences in CO_2 exchange rates and WUE despite similar nutrient status. This is a dissimilar result to that obtained in a rhizobium-lentil association (Badareh and Ghawi 1994), where different Rhizobium strains did not alter plant WUE. This suggests that physiological processes involved in WUE are affected by the fungal symbiont in mycorrhizal associations, unlike the situation in rhizobium-plant symbioses.

2.4 Field studies

2.4.1 The importance of field studies

Busse and Ellis (1985) noted that water use in pot experiments is a poor indicator of water use in the field because of the limitation on rooting depth and volume. Controls in glasshouse experiments may not adequately mimic biotic and abiotic conditions in the field. For example, the effect of native fungal isolates on plant growth may be quite different from those recorded when 'efficient' isolates or high inoculum levels are used. Inoculum density (Carling *et al.* 1979 ; Smith and Walker 1981; Wilson 1984; Clapperton and Reid 1992) and type (Abbott and Robson 1981) may alter the extent of mycorrhizal colonisation of roots. Hence, changes in populations and/or propagule densities of AM fungi following seasonal or vegetation changes present an unpredictable colonisation potential for volunteer plants. Given that host-plant species can exert a defining influence over the species richness and abundance of AM fungi (Ezawa *et al.* 1995; Bever *et al.* 1996), and that seasonal variation can alter the levels of AM colonisation as well as the species involved (Braunberger *et al.* 1996), it would be difficult to artificially construct a community of AM fungi which reflected the seasonal and vegetation history of a plant community or soil. This is apart from the effect of altering the composition of soil and rhizosphere organisms in experiments conducted using sterilised growth media, even when soil sievings are added to non-mycorrhizal treatments (Allen *et al.* 1993). Experiments on undisturbed native soils are essential if

we are to advance our understanding of mycorrhizas and the potential influences of mycorrhizas on plant community ecology.

When field studies have been conducted by suppressing indigenous mycorrhizal fungi in fungicide-treated plots, decreases in plant phosphorus concentration (Fitter 1986), biomass production (Hartnett *et al.* 1994), fecundity (Carey *et al.* 1992) and abundance of mycotrophic plant cover (Gange *et al.* 1990) have been measured. Conversely, increases in plant phosphorus concentration (Fitter 1986), biomass production (Bentivenga and Hetrick 1991), fecundity (Carey *et al.* 1992; West *et al.* 1993b) and abundance of mycotrophic plant cover (Hartnett *et al.* 1994) have been observed in the fungicide-treated soils in the same or other studies. This inconsistency between field results and results from glasshouse experiments under 'ideal' conditions suggests a broader view of the functioning of AM fungi in ecosystems must be adopted. Some of the studies above have concluded that static or negative plant responses to reductions in mycorrhizal colonisation may be explained by altered interactions between the plants and fungal pathogens (Carey *et al.* 1992; West *et al.* 1993b; Newsham *et al.* 1994) or by altered water relations (Fitter 1986). Field experiments designed to investigate the role of mycorrhizal fungi in ecosystems need to consider the range of possible plant-plant, plant-soil and plant-microorganism interactions.

2.4.2 The problem of establishing non-mycorrhizal control treatments

Investigation into the role of mycorrhizal fungi in plant growth and ecosystem processes in the field has been restricted because of the difficulty of establishing adequate controls. Current understanding of the functional significance of mycorrhizal associations in natural ecosystems is largely derived from extrapolation of findings from pot experiments. Field studies have so far produced variable results (Mc Gonigle 1988) which implicate AM associations in plant growth enhancement (Merryweather and Fitter 1996) and ecosystem processes (Fitter 1990) but are not conclusive. A number of methods for comparing mycorrhizal and non-mycorrhizal plants in the field have been employed with varying success. Methods for suppression or eradication of AM fungi in soil have included fumigation (Stanley *et al.* 1993; Sanders and Koide 1994), fungicide application (Fitter and Nichols 1988; Gange *et al.* 1990; Carey *et al.* 1992; Newsham *et al.* 1994; Merryweather and Fitter 1995; Merryweather and Fitter 1996; Gange and

Brown 1997; Hartnett and Wilson 1999a), and stock-piling or disturbance of native soils (Allen and Allen 1986). However, all such methods can potentially affect non-target organisms and soil processes, and non-target effects should be accounted for in consideration of results from such experiments.

Given the complex interactions between soil organisms and between these organisms and vascular plants, indiscriminate eradication of soil biota may have confounding effects on the study of cost-benefit relations between the mycorrhizal associates. Studies showing mycorrhizal enhancement of host-plant pathogen resistance (Newsham *et al.* 1994; Dugassa *et al.* 1996) indicate that non-target effects of methods for the control of AM fungi may lead to an underestimate of total mycorrhizal benefit to host plants if the activity of pathogenic organisms is also suppressed by the treatment. This may be undesirable where effects are subtle or the restrictions of field studies limit the power of comparative experiments. While non-target effects of controlling the activity of AM fungi may occur, these effects may be relatively small compared to the effects of reduced activity of mycorrhizal fungi (Smith *et al.* 2000). While non-target effects from methods used to control the activity of AM fungi are unknown or difficult to measure, it remains necessary to select methods which have minimal effect on other soil organisms.

The focus of studies into the effect of biocides on AM fungi has largely been to investigate the problem of reduced mycorrhzal-benefit when control of soil insects and pathogens has been the primary objective. Reviews of the effects of general biocides and fungicides on AM formation (Menge 1982; Trappe *et al.* 1984) have helped to direct research toward treatments which will control AM colonisation. Apart from the control of the target fungi and non-target microorganisms, phytotoxic effects of some fungicides have been reported (e.g. pentachloronitrobenzine, Gnekow & Marschner (1989); Ridomil and Aliette, Sukarno *et al.* (1993)). However, no phytotoxic effects of the fungicide benomyl have been shown (Paul *et al.* 1989; Bentivenga and Hetrick 1991; Sukarno *et al.* 1993). Benzimidazole fungicides have emerged as effective inhibitors of AM-fungal spore germination (Carr and Hinkley 1985; Dodd and Jeffries 1989; Schreiner and Bethlenfalvay 1997a) and root colonisation (Pierrin and Plenchette 1993; Schreiner and Bethlenfalvay 1997a; Schreiner and Bethlenfalvay 1997b). Benomyl has often been used to successfully suppress AM activity in field experiments

(West *et al.* 1993a; Newsham *et al.* 1994; Merryweather and Fitter 1995; Hartnett and Wilson 1999b).

There are, however, conflicting reports as to the effectiveness of benomyl-application as a method for reducing root colonisation by AM fungi or mycorrhizal enhancement of plant growth (Fitter 1986; Koide *et al.* 1988a). Limitations in the efficacy of benomyl may be due to the method and timing of application, effects on the microbial community or differences in soil properties (Pedersen and Sylvia 1997). Schreiner and Bethlenfalvay (1997a) showed that benomyl was effective in significantly reducing germination of spores and early hyphal growth of *Glomus etunicatum, G. mosseae* and *Gigaspora rosea* for up to 4 weeks in both sterilised and unsterilised soils. However, this inhibition was reduced when spores were buried in the soil, probably because the fungicide can be sorbed to soil minerals and organic matter (Aharonson and Kafkafi 1975; Helweg 1977; Yarden *et al.* 1985; Liu and Hsiang 1994).

These studies do not indicate whether the fungicide kills spores or whether spore germination is simply delayed. This may be important when planning experiments, as repeated applications of the fungicide may be necessary for effective control. The effectiveness of benomyl in reducing mycorrhizal colonisation may also be limited in field soils when breakdown of the fungicide by microbes is rapid (Boatman *et al.* 1978 ; Yarden *et al.* 1985). However, mycorrhiza-mediated phosphorus uptake may be reduced by fungicide application even though colonisation levels are substantial (Bailey and Safir 1978 ; Larsen *et al.* 1996), and effects on plant community structure and productivity may still be evident (Koide *et al.* 1988a). Despite the limitations of using fungicides such as benomyl to suppress the activity of mycorrhiza fungi in the field, significant advances in understanding the importance of mycorrhizas in natural plant communities can be made through careful use of these methods.

2.5 Mycorrhizas in natural plants communities

Acknowledging the common occurrence of AM associations in nature and the large amount of data linking mycorrhizas to increased plant biomass, improved mineral nutrition and enhanced drought tolerance of selected plant species, it is easy to imagine that these symbioses play a significant role in plant-plant interactions. Where individual

plants and interactions between plants are affected by changes in the activity of mycorrhizas, community structure and the functioning of ecosystems may also be changed. Few studies have contributed to the gap between our understanding of the possible effects of AM on 'host' plants on the one hand, and plant communities on the other (Francis and Read 1994). The field data which do exist have sometimes been inconsistent (Fitter 1985) and have only recently been improved with results from controlled field studies in a limited number of ecosystems (Gange *et al.* 1990; Mullen and Schmidt 1993; Merryweather and Fitter 1996; Lapointe and Molard 1997; Hartnett and Wilson 1999a; Smith *et al.* 2000). Much of the field research has severely altered the soil conditions (Doerr *et al.* 1984; Allen and Allen 1986), impacting on the effectiveness of the symbiosis, or has not studied the indigenous mycorrhizal fungi or volunteer plants in naturally occurring assemblages (Sanders and Koide 1994). The abundance, activity and effectiveness of AM fungi in the field will vary spatially and temporally and should be considered as part of the dynamic two-way interaction which characterises the symbiosis.

2.5.2 Effects of mycorrhizas on plant competition and community structure

Competition between plants is an important determinant of the composition and dynamics of natural plant communities. Mycorrhizas may influence competition between plants, particularly where competitive interactions are due to root competition at low nutrient supply. Plant species which are more responsive to mycorrhizas, may have competitive advantages over species with low mycorrhiza-responsiveness for uptake of poorly mobile nutrients such as P. Mycorrhizal fungi may also alter competitive relationships between plant species by reducing the establishment and survival of some non-host species (Francis and Read 1994). Even where plant species show equivalent responsiveness to mycorrhizas when grown separately, it is possible for competition for the resources of the mycorrhizal mycelium to occur (Newman *et al.* 1992).

Interest in the role of mycorrhizas in determining the outcome of competition between plant species and on community structure has increased as interest in plant diversity and ecosystem function has increased in recent years. Several recent studies have illustrated that ecosystem processes such as primary production may be linked to species diversity

(Naeem *et al.* 1994; Tilman *et al.* 1996; Tilman *et al.* 1997; Hector 1999). However these results need to considered with respect to the identity and abundance of the species comprising the experimental communities (Huston 1997; Grime 1998). Ecosystem properties can be strongly correlated with the functional characteristics of the species contributing most to the biomass of the system (MacGillivray *et al.* 1995; Hooper and Vitousek 1997; Wardle *et al.* 1997). Therefore, plant communities might be most affected by changes, which most affect the dominant plant species. The influence of mycorrhizas on individual plants and on plant-plant interactions may be important not only in structuring plant communities, but also in determining the productivity and stability of the community.

2.5.2.1 Effects of mycorrhizas on plant competition

Some plant species do not form mycorrhizas, and not all AM-host species show clear nutritional benefits from colonisation by mycorrhizal fungi under all growth conditions (Tester *et al.* 1985; Fitter 1986; Francis and Read 1995). Plant communities may often include species with varying response to the presence of AM fungi. This variation may range from non-mycorrhizal plants with benign or possibly antagonistic interactions with the fungi, to highly responsive plant species with positive growth responses to AM association (Johnson *et al.* 1997). The composition of natural plant communities often includes plant species from across this response spectrum, and may be determined by differential contributions of the mycorrhizal fungi to individual plant species fitness.

A number of studies have examined competitive interactions between plant species and the influences of AM fungi on these interactions. Evidence from two-species competition experiments indicates that AM fungi can affect the competitive balance between co-occurring grasses (Fitter 1977; Hetrick *et al.* 1989; Hartnett *et al.* 1993), legumes and grasses (Crush 1974; Hall 1978), grasses and non-leguminous herbs (Allen and Allen 1984; Marler *et al.* 1999), and between different herb species (Allsopp and Stock 1992a; Moora and Zobel 1996). Where mycorrhizas influence competitive interactions between plants, plant density may alter the outcome of competition (Koide 1991a; Facelli *et al.* 1999). For example, Koide (1991a) showed that increasing the density of plantings of *Abutilon theophrasti* decreased the benefit from mycorrhizal colonisation. This work has been extended to consider interspecific competition and

results clearly show that host-plant benefits from mycorrhizas are density dependant and may be influenced by mycorrhizas even when one species has low response to mycorrhiza-formation (Hartnett *et al.* 1993). Accordingly, changes in the abundance or size of individuals from one species may influence the abundance and size of individuals of the same or different species. The influence of mycorrhizas in determining community structure will therefore depend on the relative competitiveness of the species present.

2.5.2.2 Effects of mycorrhizas on plant diversity and community structure

The presence of mycorrhizas has been shown to increase floristic diversity in experimental microcosms (Grime *et al.* 1987), and increase species richness in an early successional plant community (Gange *et al.* 1990). These increases in diversity may, however, be dependent on the identity and mycorrhizal responsiveness of the dominant plant species in the respective plant communities (Bergelson and Crawley 1988). Hartnett and Wilson (1999a) have recently shown that suppression of mycorrhizal fungi resulted in an increase in floristic diversity in a tallgrass prairie. In the tallgrass prairie, dominant C_4 grasses are highly responsive to mycorrhizal fungi and are strong competitors for subordinate species. Suppression of mycorrhizas in the tallgrass prairie has been shown to result in decreased abundance of mycorrhiza-responsive species and increased abundance of less mycorrhiza-responsive species (Wilson and Hartnett 1997; Hartnett and Wilson 1999a; Smith *et al.* 1999). The change in strength of competition can result in changes in community structure. Floristic diversity may also be changed if the dominant plant species have strong positive or negative responses to mycorrhizas. Smith *et al.* (1999) showed that plant competition and the mycorrhiza-responsiveness of each species interacted to shape a tallgrass prairie plant community.

The composition of a plant community and the mycorrhiza-responsiveness of each component species can determine the overall influence of mycorrhizas on floristic diversity within the community. The importance of mycorrhizas to community structure and diversity is then at least partially governed by the stage of succession of the plant community. Plant communities in early stages of secondary succession have been shown to support a relatively high proportion of non-mycorrhizal plant species and may not support the establishment of more mycorrhiza-responsive species (Reeves *et al.*

1979; Janos 1980a; Miller 1987). As plant communities develop from early successional stages to more stable 'protective' states (Pankow *et al.* 1991), mycorrhizas may influence the establishment success of species with differential responsiveness to colonisation and so influence the succession (Janos 1980a; Francis and Read 1994). These long-term changes in community structure may be the consequence of increased abundance or biomass of species better adapted to take advantage of mycorrhizas for nutrient uptake, and may be facilitated by increased reproductive output from these successful species.

2.5.2.3 Effects of mycorrhizas on fecundity, seed quality and offspring vigour

Plant community structure is the result of interactions between component species at all stages of the life cycles of the individual species. While competition between different plant species may be an important determinant of community structure at a given time, factors affecting reproduction and the survival of offspring will have long-term consequences for the dynamics of plant populations. The number and viability of offspring of mycorrhizal plants may be influenced by mycorrhizal colonisation. The fecundity of host plant species has been shown to increase when plants are colonised by mycorrhizal fungi (Koide *et al.* 1988b; Bryla and Koide 1990; Carey *et al.* 1992; Stanley *et al.* 1993; Newsham *et al.* 1994). Mycorrhizas have also been shown to increase the reproductive inequality within a population of *Abutilon theophrasti* (Shumway and Koide 1995). Such inequality in fecundity may result in an increase in the representation of genes of the more fecund individuals in the following generation (Heywood 1986). Increased numbers of offspring and the potential for selection of genotypes with superior competitiveness in communities where AM fungi are active may lead to substantial changes in plant community structure over time.

Just as seed number may influence the success of a plant species in establishment and persistence in a plant community, seed size and quality may also affect survival success. Seedlings with smaller seed reserves may be more susceptible to mortality under environmental stress (Westoby *et al.* 1996). Environmental stresses with the potential to reduce the establishment success of individuals with smaller seed reserves include mineral nutrient shortage (Lee and Fenner 1989; Jurado and Westoby 1992) and drought (Leishman and Westoby 1994). Mycorrhizal colonisation has been shown to

relieve both nutrient and drought stress (Sections 2.2 & 2.3.1). While phosphorus concentrations in seeds of phosphorus supplied maternal plants generally did not influence the growth of seedlings, the mycorrhizal status of maternal plants did (Lewis and Koide 1990). Some of the effect resulted from increased phosphorus content in the seed but fractionation of the stored phosphorus may also have been important (Lu and Koide 1991). A number of other studies have also shown that mycorrhizal colonisation of parent plants increased the nutrient content of seeds and the size of offspring (Koide *et al.* 1988b; Lu and Koide 1991; Koide and Lu 1992; Srivastava and Mukerji 1995). Increased offspring vigour of mycorrhizal plants has also been shown to increase competitive ability (Heppell *et al.* 1998). Thus, mycorrhizal colonisation may lead to increased fitness of host plants by increasing the chance of offspring survival and subsequent reproduction.

Indirect effects of mycorrhizas on reproduction of non-host plant species via effects on intact plant communities have rarely been reported. Sanders and Koide (1994) found no effect of mycorrhizas on the percentage of plants of the non-host species *Amaranthus retroflexus* flowering in a simple three-species plant community. However, the biomass of flowering heads and the P content of stems of *A. retroflexus* was greater in fumigated plots than in control plots where AM fungi were active (Sanders and Koide 1994). Given that some plant species have reduced growth in the presence of AM fungi (Francis and Read 1995), there may be direct effects of mycorrhizas on the reproduction of some non-host plant species. Indirect effects of mycorrhizas on the seed production of non-host species in intact communities require further research. Further study of the effects of competition on the reproduction of mycorrhizal host and non-host plant species is the first step to understanding the potential influence of mycorrhizas on long-term changes in plant community structure.

Field-based studies of competition involving mycorrhizas have been conducted in relatively few plant ecosystems. Extensive tracts of open woodland in semi-arid Australia have been overgrazed, with the understory reduced to annual herbland often dominated by introduced species. The herb and grass species comprising these disturbed communities are thought to differ in their response to mycorrhizal fungi. The influence of mycorrhiza in controlling plant diversity may be vitally important in semi-arid

ecosystems, where communities can be subjected to dramatic seasonal and inter-annual fluctuations, and may rely on high biodiversity to maintain stability (Grime 1997). This study was designed to test experimentally the relationship between mycorrhizal responsiveness of plants from a semi-arid herbland and the influence of mycorrhizas on the productivity and structure of this plant community.

2.6 Conclusion

This review presents evidence from previous studies that arbuscular mycorrhizas can influence the growth and reproduction of plants, competitive interactions between plant species, and the composition, floristic diversity and successional stage of plant communities. Plant communities in arid and semi-arid South Australia contain plant species with differential response to colonisation by AM fungi. These communities are not well studied with regard to plant competition and community dynamics. The possible role of AM associations in these communities is relatively unknown.

The aim of this project is to determine the mycorrhizal status of plant species occurring in arid and semi-arid South Australia. To investigate the potential influence of mycorrhizas on plant community structure, the project aims to assess the mycorrhiza-responsiveness of plant species occurring in the herbaceous understorey of semi-arid woodland at Brookfield Conservation Park, South Australia. This study also aims to investigate the influence of arbuscular mycorrhizas on competition between plant species, structure and floristic diversity in plant communities at Brookfield Conservation Park.

Chapter 3

General materials and methods

3.1 Study site for community experiments

3.1.1 Location

Field studies were conducted at Brookfield Conservation Park, a 5,527 ha reserve situated at the southern-most extension of the arid zone in South Australia (Lat. 34° 19′ 18″; Long. 139° 30′ 52″). The site has been protected since 1971 when it was purchased as a reserve for the Southern Hairy-nosed Wombat (*Lasiorhinus latifrons*).

3.1.2 Site description

Two study sites were chosen (Brookfield North and Brookfield South), about 7.5 km apart. The two study sites are situated in the understorey of low open woodland and tall shrubland community dominated by Sugarwood (*Myoporum platycarpum* R. Br.), Sheep Bush (*Geijera linearifolia* (DC.) J. Black) and Bullock Bush (*Alectryon oleaefolium* (Desf.) S. Reyn. ssp. *canescens* S. Reyn). The understory vegetation is characterised by annual herbs dominated by the introduced species *Medicago minima* (L.) Bartal. (Leguminosae), *Carrichtera annua* (L.) DC. (Cruciferae) and *Salvia verbenaca* L. (Labiatae) and the native species *Erodium crinitum* Carolin (Geraniaceae), *Velleia arguta* R. Br. (Goodeniaceae) and *Vittadinia gracilis* (Hook. f.) N. Burb. (Compositae). The vegetation is heavily grazed by wombats and by kangaroos (*Macropus rufus* and *Macropus fuliginosus*) and regeneration of previously dense Spear-grass (*Stipa nitida* Summerh. & C.E. Hubb.) is limited.

3.1.2.1 Experimental sites - description

Brookfield South

The understory vegatation at Brookfield South is characterised by mid-dense clumps of the perennial herb *Asphodelus fistulosus* L. (Liliaceae) over annual herbs dominated by the introduced species *Medicago minima*, *Carrichtera annua*, *Gynandriris setifolia* (L.f.) Foster (Iridaceae), *Salvia verbenaca*, *Silene apetala* Willd. (Caryophyllaceae), and *Erodium crinitum* and the introduced grass species *Bromus rubens* L. (Poaceae). This site is situated in a slight depression.

Brookfield North

The understorey vegetation at Brookfield North is dominated by the introduced herbs *Medicago minima*, *Carrichtera annua*, *Salvia verbenaca*, *Erodium crinitum* and the native species *Velleia arguta*, *Vittadinia gracilis* (Hook. f.) N. Burb (Compositae) and *Stipa nitida*.

3.1.3 Climate

Long-term temperature data are available from a nearby meteorological station at Waikerie, SA. Temperatures range from an average maximum of 32.8°C and minimum of 15.2°C in January, to lowest monthly averages of 16.2°C maximum and 5.3°C minimum in July. The highest recorded temperature for the region is 46.5°C.

Rainfall is low and irregular, averaging 248 mm but varying from 150-550 mm per annum. Total rainfall recorded at the nearby Blanchetown meteorological station (10 km from the Brookfield north site) in 1997 & 1998 was 288 mm and 353 mm respectively. Monthly rainfall data are given in Fig 3.2. Brookfield Conservation Park has a drought frequency of 78% calculated using the measure of Trumble (1948) and is characterised by extreme variation in the understorey condition between years. Rainfall during 1997 was extremely low during the growing season, falling well below the monthly average in April, June and July. The early growing season in 1998 was also a low rainfall period, with below-average rainfall in March, May and June.

3.1.4 Soil characteristics

The soils at both Brookfield sites have clay loam A horizons to a depth of 20-25 cm, overlying thick calcrete over Miocene limestone. The soils are generally low in nutrients and organic matter. Details of soil parameters are given in Table 3.1.

Figure 3.1 Map of Australia showing the location of Brookfield Conservation Park and the rainfall isohyets (mm) in arid and semi-arid Australia.

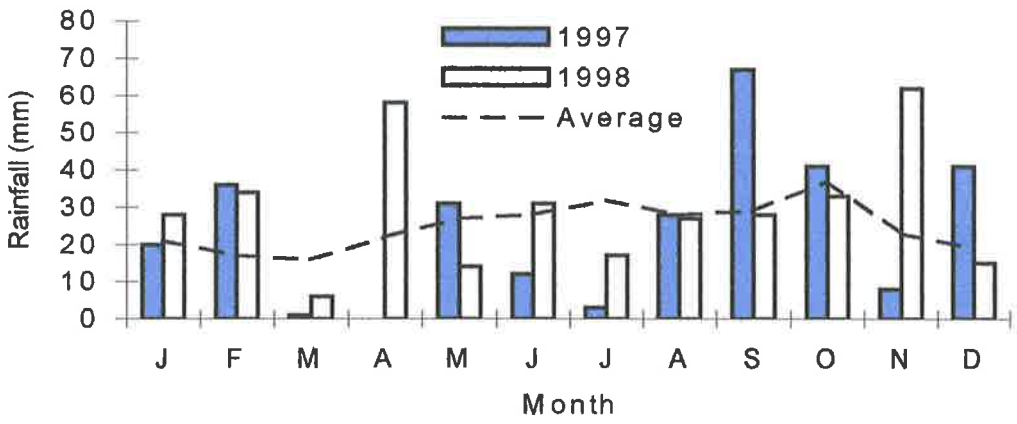

Figure 3.2 Rainfall data from Blanchetown meteorological station for 1997 and 1998, and the long-term average.

Table 3.1 Some chemical and physical characteristics of soils from field study sites, Brookfield North and Brookfield South.

Soil Parameter		Brookfield North	Brookfield South
Soil texture [A]		Clay-loam	Clay-loam
Clay	(% w/w)	27.5	26.3
Silt	(% w/w)	15.0	16.2
Sand	(% w/w)	57.5	57.5
Field Capacity	(% w/w)	22	28
pH [B]		7.85	7.93
NO_3-N [C]	($\mu g\ g^{-1}$)	8.6	NA[D]
Extractable P [E]	($\mu g\ g^{-1}$)	9.9	10.2
Organic C [F]	(%)	0.85	0.90
$CaCO_3$ [G]	(%)	4.4	8.8
CEC	(mequiv $100g^{-1}$)	24.3	21.6

[A] Particle size distribution was determined by the Bouyoucos hydrometer method (Day 1965)
[B] pH measured 1:5 soil in 0.01 M $CaCl_2$
[C] Estraction method – 1:10 soil dilution in 2M KCl (Best 1976)
[D] Not available
[E] $NaHCO_3$ extraction (Colwell 1963)
[F] Determined on a microprocessor-controlled LECO induction furnace (LECO CR 12 carbon analyser) [Merry, 1988 #198
[G] Volumetric calcimeter method (Page *et al.* 1982)

3.2 Collection of intact cores from field sites

In several experiments intact cores were collected from Brookfield North and Brookfield South. Cores were made from PVC (plumbing pipe) with diameter 10 cm and height 15 cm. Cores were collected with minimal disturbance to the soil by driving the cores into the soil surface to a depth of 12 cm. When the soil was dry, water was poured on to the soil surface to assist penetration of the core. The cores were then dug out and the bottom capped with a PVC cap with drainage holes drilled through it.

3.3 Trap plants and seed sterilisation

Where mycorrhizal colonisation potential was assessed in intact cores, *Trifolium subterraneum* L.cv. Mt Barker was used as a trap plant. Seed of *T. subterraneum* was surface sterilised in 5 % sodium hypochlorite solution containing a drop of the surfactant Tween 20. Seeds were soaked for 10 minutes in this solution and then rinsed in reverse osmosis water. Seeds were set on wet filter paper in a covered Petri-dish and germinated at 23° C in the dark.

3.4 Assessment of mycorrhizal colonisation

Levels of mycorrhizal colonisation of roots from all experiments were assessed using the modified method of (Kormanick and McGraw 1982). Roots were cut into 1 cm lengths and cleared in 10 % KOH for one week at room temperature. Roots were then washed in water, dipped in 1 N HCl and stained with 0.05% Trypan blue in lactic acid solution for one hour. Root pieces were examined for mycorrhizal colonisation at 40-100 times magnification by the grid intersect method (Giovannetti and Mosse 1980) using a light microscope.

3.5 Indices of diversity

Plant diversity was measured in plant communities using several indices. These are defined in equations 1 to 3 below.

eqn 1. Species richness S = mean number of species per plot

eqn 2. Shannon diversity $$H' = -\sum_{i=1}^{s} Pi \ln Pi$$

and

eqn 3. Shannon evenness $J = H' / \ln S$

Where Pi is the proportion of total cover or biomass contributed by the ith species.

3.6 Statistical analysis

Statistical analysis on all experiments was performed using the analysis package Genstat 5, release 4.1 (Lawes Agricultural Trust, 1998).

Chapter 4

Arbuscular Mycorrhizal Associations in the Southern Simpson and Stony Deserts of South Australia

4.1 Introduction

It is well established that arbuscular mycorrhiza (AM) are mutualistic associations which primarily assist plants in uptake of immobile nutrients such as phosphorus (P) (Smith and Read 1997). There is mounting evidence that AM can improve plant drought tolerance (Call and McKell 1984; Cui and Nobel 1992; Davies *et al.* 1993; Davies *et al.* 1996; Subramanian *et al.* 1997) also see (Sanchez-Diaz and Honrubia 1994), and that drought conditions can induce soil phosphorus deficiency (Nelsen and Safir 1982; Cox and Barber 1992). There is also evidence that AM influence plant community structure (Gange *et al.* 1990; van der Heijden *et al.* 1998b; Hartnett and Wilson 1999a), including succession in semiarid plant communities (Stevenson *et al.* 2000). These studies suggest a possible influence of AM on plant communities such as those in the arid regions of central Australia, where soil P-availability and rainfall are relatively low. In addition, the proportion of vascular plant species forming AM is commonly overestimated (Trappe 1987), probably as a result of the low proportion of species and environments surveyed (Brundrett and Abbott 1991). To develop understanding of the importance of mycorrhizas to plant establishment, survival, growth and community structure it will be necessary to collect detailed information on the mycorrhizal status of plants in a range of ecosystems.

The landscapes of northern South Australia are dominated by three main environment types (Brandle 1998):

- sandy deserts (characterized by parallel sand ridges and swales);
- stony or gibber deserts (usually associated with clay soils);
- wetlands (including creeks, floodplains and lakes).

The stony deserts were first described by European settlers in the mid 1840's. Initial impressions of the landscape were described by Captain Charles Sturt (Sturt 1849).

"....An immense plain, occupying more than half of the horizon.... A number of sandy ridges, similar to that on which we stood, abutted upon, and terminated in this plain like so many headlands projecting into the sea.
...The plain was otherwise without vegetation, and its horizon was like that of the ocean. In the direction I was about to proceed, nothing was to be seen but the gloomy stone clad plain, of an extent such as I could not possibly form any just idea. ..."

Further exploration of the gibber deserts north-west of Lake Eyre was undertaken by the Horn Natural History Expedition. A sense of the vegetation of the region is summarised by one of the participants (Spencer 1896):

"...In the foreground were white-blue saltbushes, with pale, light blue patches of low herbage and still lighter tufts of grass amongst them, standing out in strong contrast to the purple-brown gibbers."

Early survey of the Simpson Desert described the distinct feature of this landform as a cyclic pattern in the vegetation associated with the regular dune-swale sequence.

"....Although the sandhill communities are varied, they retain on the sandridge crest and inter-ridge corridor a remarkable consistency."

This pattern has been further characterized and correlated with soil texture and stability (Fatchen and Barker 1979a; Buckley 1981).

The flora of the nothern deserts of South Australia is relatively poor (Fatchen and Barker 1979a) (Brandle 1998) and characterised by a high proportion of ephemeral, or short-lived perennial or biennial species. The remote location of these deserts has meant that the vegetation has been little studied. Where study has been made it has focussed on the distribution of plant associations across the region (Wiedemann 1971; Fatchen and Barker 1979b; Buckley 1981; Brandle 1998) and on patterns in the vegetation related to the toposequence across the expansive dunefields of the Simpson Desert

(Fatchen and Barker 1979b). Beyond this, no detailed studies of the establishment, growth requirements and ecology of plant species in this region have been made.

The surveys described in this chapter were undertaken to determine the mycorrhizal status of plant species in the stony deserts and Simpson Desert. This is seen as a first step to understanding the mycorrhizal requirements of plants in arid environments and links into other studies of the influence of mycorrhizas on the ecology of plant communities in arid Australia and elsewhere. The surveys reported here also investigated relationships between the mycorrhizal status of each plant species and life-form and habit of each species. Studies in the Simpson Desert were also used to investigate the mycorrhizal status of plants in the major eco-zones of the desert and potential differences in the proportion of mycorrhizal and non-mycorrhizal species forming plant communities in these eco-zones.

4.2 Materials and methods

4.2.1 Survey sites

Survey sites are located in the Simpson Desert and Stony Desert region of arid South Australia (Fig. 4.1).

4.2.1.1 Simpson Desert

The three sites were chosen as representative of the dune and swale vegetation of three geographically separated regions within the southern desert. Sites also correspond to survey sites in the Sandy Deserts Biological Survey - Department of Environment, Heritage and Aboriginal Affairs, South Australia (in progress). The three sites are designated Macari Airstrip (MAC), 26° 16′ 57″ S, 136° 24′ 40″ E; Peera Peera Poolwanna (PEE), 26° 34′ 55″ S, 137° 43′ 35″ E; and Kallakoopah Creek (KAL), 27° 03′ 23″, 137° 32′ 32″ E. Both MAC and PEE were situated on sand dunes while KAL was situated on the sandy-loam of a broad swale. Available soil P (1N $NaHCO_3$-extractible, Colwell, 1963) was measured at 5.6 (±1.0) ppm for MAC and 8.3 (±1.4) ppm for KAL.

4.2.1.2 Stony Deserts

Collection of plant samples in the stony deserts was undertaken to determine the mycorrhizal status only of selected plant species. No attempt was made to survey different eco-zones within these deserts but rather collections were made opportunistically. Sites in the stony deserts also correspond to survey sites in the Stony Deserts Biological Survey - Department of Environment, Heritage and Aboriginal Affairs, South Australia (Brandle 1998). Surveys are reported from two stony desert sites, one northeast and one west of Lake Eyre North. The site northeast of Lake Eyre North is centred around Mirra Mitta Bore (MMB) 27° 44′ 05″ S, 138° 58′ 05″ E. The site west of Lake Eyre North is Goorikianna Creek (GOC) 27° 45′ 25″ S, 135° 14′ 51″ E. Collections at the MMB site were mostly taken from gibber plain and run-on areas with a few collections from a creek bed. The Goorikianna Creek site is a gypseous cracking clay drainage line. Available soil P (1N $NaHCO_3$-extractible, Colwell, 1963) was measured at 27.2 (±1.8) ppm for GOC (available soil-P was not measured for MMB).

4.2.2 Rainfall

Rainfall in the southern Simpson Desert is low and infrequent. While mean annual rainfall is 130-150 mm (Gaffney 1977) the total annual rainfall at Mt Dare Station on the western margin of the desert was 171 and 246 mm in 1997 and 1998, respectively. Rainfall varies considerably across the desert and survey sites were at considerable distance from all meteorological recording stations. Using rainfall records from Mt Dare Station on the western margin and Clifton Hills and Cowarie Stations on the eastern and southeastern margins respectively, Fig. 4.2 illustrates the maximum and minimum rainfall around the southern Simpson Desert for each month of 1997 and 1998. Low rainfall was recorded at all three stations in the months preceding the survey in September 1997 (a total of 6.7 mm for June, July and August at Mt Dare Station). Consequently the vegetation was sparse and plants were often in decline at time of sampling. Good winter rains in June and July 1998 (64.6 mm at Mt Dare) resulted in abundant growth with most plants flowering at the time of survey in September 1998.

Survey sites in the stony deserts were located within 40 km of meteorological stations and records from these stations give an estimate of the rainfall preceding the surveys.

Rainfall records from Mungeranie (approximately 35 km from MMB) in the twelve months before the survey was 33.8 mm. The survey took place in February 1995 and the rainfall in the preceding two months was 16.8 mm. The meteorological station at Oodnadatta airport (approximately 60 km from GOC) recorded 125.6-mm rainfall in the twelve months preceding the survey at GOC in June 1998. Rainfall in the two months preceding the GOC survey was 53 mm (as estimated from records at Oodnadatta airport).

4.2.3 Plant Cover

Plant cover at Simpson Desert sites was measured using the wheel-point method of (Griffin 1989). At each site a wheel-point with recording tynes set so that points fall at intervals of 1 m was pushed along a 600 m transect. Plant life-form was recorded for all strata above each point and converted to percent cover. Plant cover at the stony desert sites was not recorded.

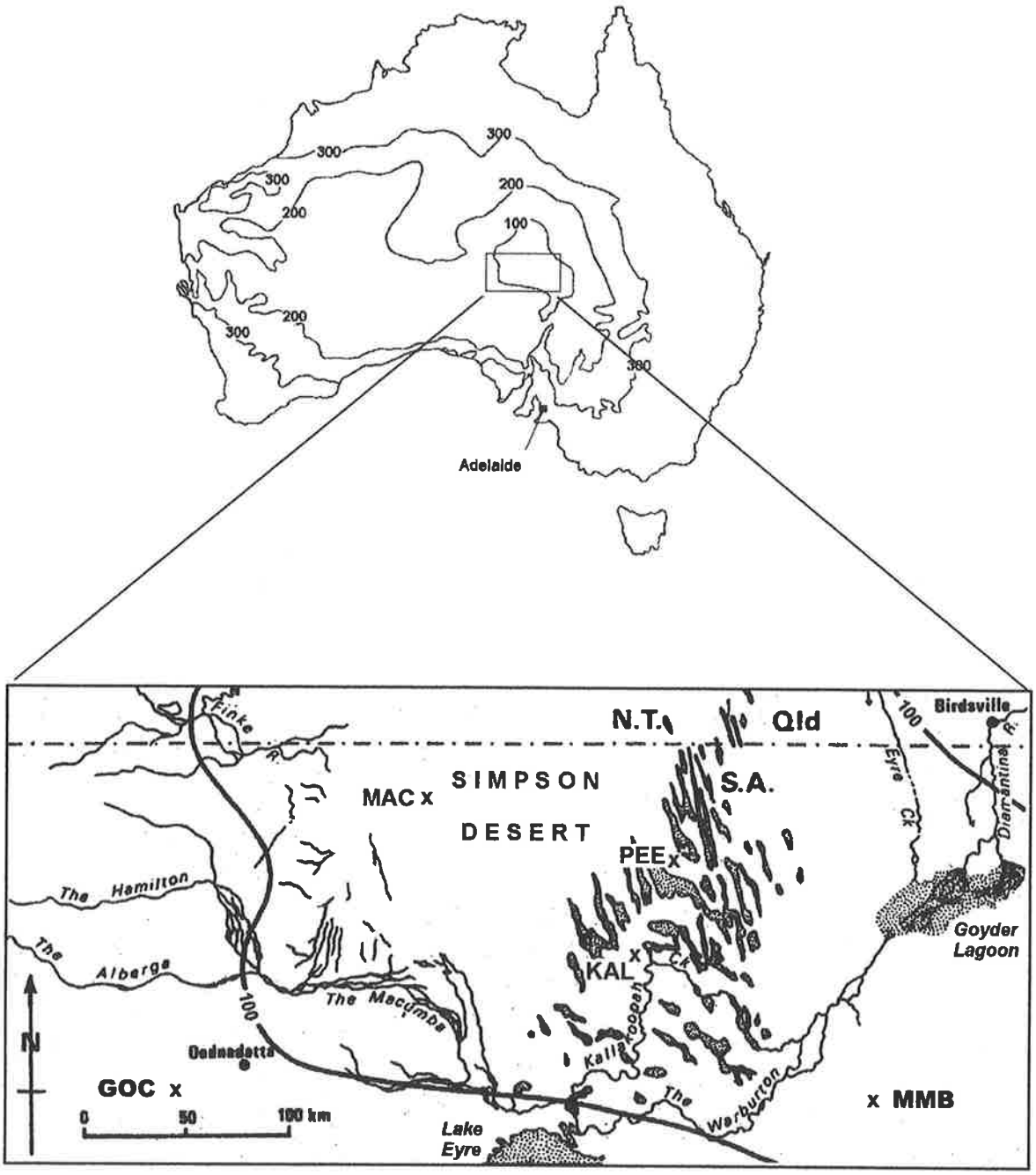

Figure 4.1 Map showing the mean annual rainfall isohyets (mm) in arid and semi-arid Australia. Inset map shows the Southern Simpson Desert and Stony Desert regions where the five survey sites are located (sites marked 'x'). KAL = Kallakoopah Creek; MAC = Macari Airstrip; PEE = Peera Peera Poolwanna; GOC = Goorikianna Creek; and MMB = Mirra Mitta Bore.

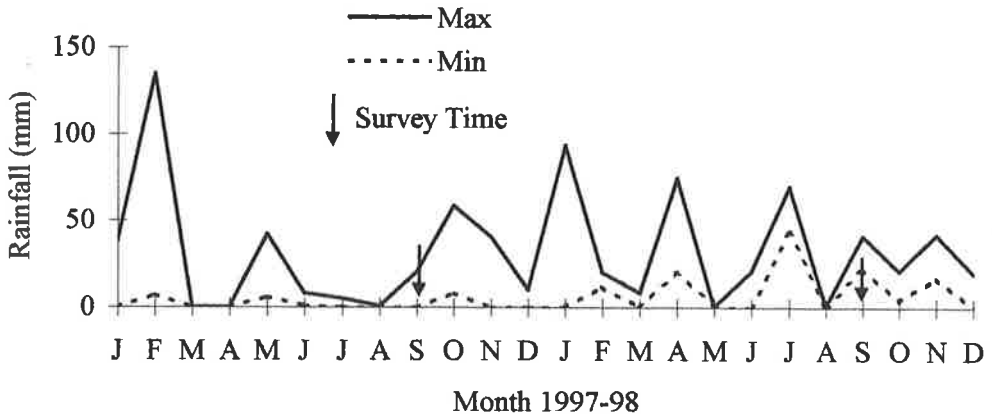

Figure 4.2 Maximum and minimum recorded rainfall around the Simpson Desert in 1997 and 1998. Approximate survey dates shown with arrows.

4.2.4 Collection of plant specimens

At each site all plants were identified and voucher specimens taken for verification. Plant names follow (Jessop and Toelken 1986) and (Jessop 1993). For collection of root material for determination of mycorrhizal colonisation, a minimum of 20 cm of fine roots was collected from at least 3 individuals of each species. Roots were washed, cut into approximately 2-cm sections and stored in 70 % ethanol. Later, ethanol was washed from the samples which were then cleared in 10 % KOH at room temperature for 6 days. Highly pigmented and woody roots were further cleared in fresh KOH solution at 80 °C for 1 h. Roots were then rinsed in water (reverse osmosis purified) and stained in 0.05 % trypan blue in lactoglycerol (modified method of (Phillips and Hayman 1970)) and mounted on microscope slides in 50 % glycerol for examination. Roots were examined using bright field microscopy at 100-400 x magnification. External and internal hyphae characteristic of Glomalean fungi (Merryweather and Fitter 1998) as well as arbuscules, vesicles (and internal spores recorded as vesicles) and hyphal coils were recorded as present or absent from each sample. Plants were not considered to be mycorrhizal if external hyphae were the only evidence of fungal colonisation. The extent of root length colonised was assessed for each sample and catagorized as: non-mycorrhizal, "0"; "low", < 10 %; "med", 10 - 30 %; or "high", > 30%.

4.3 Results

Fifty-four of the eighty-one plant species surveyed (67 %) showed at least some evidence of AM association (Tables 4.1 & 4.2). Members of families not previously known to form AM including Frankeniaceae (*Frankenia plicata*), Myoporaceae (*Eremophila longifolia* and *E. macdonnellii*) and Marsileaceae (the aquatic fern *Marsilea drummondii*) were all colonised. The remaining non-mycorrhizal plant species were not evenly distributed between the one fern family and twenty-four plant families represented in this survey (Tables 4.1 & 4.2). Six families (Aizoaceae, Amaranthaceae, Cruciferae, Lamiaceae, Portulacaceae and Zygophyllaceae) contained only non-mycorrhizal members, while five other families (Boraginaceae, Chenopodiaceae, Compositae, Convolvulaceae and Graminae) contained some members which were not mycorrhizal.

Some plant species were found to be mycorrhizal at one site but not at another. Two species present at MAC (*Sida ammophila* and *Eragrostis dielsii*) were not mycorrhizal there, but both were found to be mycorrhizal at KAL and *E. dielsii* was mycorrhizal at PEE, while *Sida ammophila* was mycorrhizal at MMB. *Arstida contorta* and *Phyllanthus lacunarius* were also found to be mycorrhizal at one site (PEE) but not at another (GOC), and *Othonna gregorii* was mycorrhizal at PEE but not at KAL. No AM colonisation was observed in the roots of 27 species, including several species found at more than one site. The roots of two species in the Chenopodiaceae (*Salsola kali* and *Sclerolaena diacantha*) contained internal hyphae and vesicles but no arbuscules. All other mycorrhizal plants formed arbuscules and/or vesicles except four species, *Eremophila longifolia, Gnephosis eriocarpa, Mimulus prostratus* and *Solanum ellipticum*, which formed coils but no arbuscules or vesicles.

Six of the plants assessed for AM colonisation had conservation ratings (Brandle 1998). Ratings for the conservation importance of all plant species were determined by Lang and Kraehenbuehl (1997) and Briggs and Leigh (1995). Four of these species formed AM - *Frankenia plicata, Mimulus prostratus, Stemmodia haegii* and *Nicotiana truncata* - while two were non-mycorrhizal - *Eragrostis tenellula* and *Teucrium* sp. nov.

Table 4.3 shows that the proportion of plant species forming AM did not differ significantly between different life forms ($\chi^2 = 1.74$; 2 d.f. = 2; $P > 0.3$). Plant habit did not influence the formation of AM because the proportion of annual plants forming AM (Table 4.3) was not significantly different from that of perennials ($\chi^2 = 1.00$; d.f. = 1; $P > 0.3$).

Due to differences in rainfall between the survey years 1997 and 1998, plant cover differed between survey sites in the Simpson Desert. The sites surveyed in 1998, KAL and PEE, had significantly greater herb cover (56.2% and 39.6% respectively) than MAC (1.5%) which was surveyed in 1997 ($\chi^2 = 48.5$; 2 d.f.; $P < 0.001$). Shrub and grass cover were not significantly different between the sites (shrub $\chi^2 = 3.4$ and grass $\chi^2 = 1.7$ on 2 d.f.; $P > 0.05$).

There was no significant difference between the swale site (KAL) and the combined dune sites (PEE and MAC) with respect to the number of species forming mycorrhizas ($\chi^2 = 1.12$; d.f. = 1; $P < 0.2$; Table 4.4). However, more of the species at the dune site PEE were mycorrhizal (89%) than at the other two sites (Table 4.4).

Table 4.1 Arbuscular mycorrhizal status of plant species from the dunes and swales of the southern Simpson Desert, South Australia

[a] New record of mycorrhizal status, [b] Colonisation; 0 = no mycorrhiza found; low is <10%; med is 10 - 30%; high is >30%; + denotes presence, - denotes absence

Family	Species	Colonisation[b]	External Hyphae	Internal Hyphae	Coils	Arbuscules	Vesicles
	Macari Airstrip						
Amaranthaceae	Ptilotus latifolius [a]	0	-	-	-	-	-
	Ptilotus obovatus var. obovatus [a]	0	-	-	-	-	-
Compositae	Polycalymma stuartii	high	+	+	-	+	-
Cucurbitaceae	Citrullus lanatus	med	+	+	-	+	+
Goodeniaceae	Goodenia cycloptera [a]	med	+	+	-	+	+
Graminae	Aristida holathera [a]	0	+	-	-	-	-
	Eragrostis dielsii [a]	0	-	-	-	-	-
	Eriachne aristidea [a]	low	+	+	-	+	+
	Triodia basedowii [a]	low	+	+	+	-	-
Malvaceae	Sida ammophila [a]	0	-	-	-	-	-
Papilionaceae	Crotelaria eremaea ssp.eremaea [a]	med	+	+	+	+	+
	Indigofera brevidens [a]	low	+	+	-	-	+
	Cullen pallida [a]	med	-	+	-	+	+
	Tephrosia sphaerospora [a]	high	+	+	+	+	+
Portulaceae	Calandrinia sp. [a]	0	+	-	-	-	-
Solanaceae	Solanum elipticum [a]	med	+	+	+	-	-
	Peera Peera Poolwanna						
Amaranthaceae	Ptilotus sessilifolius [a]	0	-	-	-	-	-
Apiaceae	Trachymene glaucifolia [a]	med	+	+	+	+	-
Boraginaceae	Trichodesma zeylanicum [a]	high	+	+	+	+	+
Chenopodiaceae	Salsola kali	low	+	+	-	-	+
	Sclerolaena diacantha [a]	low	+	+	-	-	+
Compositae	Polycalymma stuartii [a]	low	+	+	-	-	+
	Othonna gregorii [a]	med	+	+	+	+	-
Euphorbiaceae	Phyllanthus lacunarius [a]	high	+	+	+	+	+

Table 4.1 (continued)

Family	Species	Colonisation[b]	External Hyphae	Internal Hyphae	Coils	Arbuscules	Vesicles
	Peera Peera Poolwanna						
Goodeniaceae	Goodenia cycloptera [a]	high	-	+	+	+	-
	Scaevola parvibarbata [a]	med	+	+	+	+	-
Graminae	Aristida contorta [a]	low	+	+	-	-	+
	Paractaenum novae-hollandiae [a]	0	-	-	-	-	-
	Triraphis mollis [a]	low	-	+	-	+	-
	Eragrostis dielsi [a]	low	+	+	+	+	+
Papilionaceae	Tephrosia sphaerospora [a]	high	+	+	-	+	+
	Swainsona phacoides [a]	high	+	+	+	+	+
	Cullen discolor [a]	high	+	+	+	+	+
Solanaceae	Nicotiana velutina [a]	low	-	+	+	+	+
	Kallakoopah Creek						
Amaranthaceae	Ptilotus sessilifolius [a]	0	+	-	-	-	-
Boraginaceae	Omphalalappula concava [a]	0	+	-	-	-	-
Chenopodiaceae	Atriplex limbata [a]	0	-	-	-	-	-
	Enchylaena tomentosa	0	+	-	-	-	-
	Salsola kali	0	-	-	-	-	-
	Sclerolaena holtiana [a]	0	-	-	-	-	-
Compositae	Gnephosis eriocarpa [a]	low	-	+	+	-	-
	Othonna gregorii [a]	0	-	-	-	-	-
	Polycalymma stuartii	med	+	+	+	+	-
	Rhodanthe moschata [a]	med	+	+	+	+	+
	Rhodanthe floribunda [a]	high	+	+	+	+	+
Convolvulaceae	Convolvulus eyreanus [a]	high	+	+	+	+	-
Cruciferae	Blennodia pterosperma [a]	0	-	-	-	-	-
	Lepidium phlebopetalum [a]	0	-	-	-	-	-
Euphorbiaceae	Euphorbia tannensis	high	+	+	+	+	+
Frankeniaceae	Frankenia plicata [aE]	low	+	+	-	-	+
Geraniaceae	Erodium crinitum [a]	med	+	+	+	+	-

Table 4.1 (continued)

Family	Species	Colonisation[b]	External Hyphae	Internal Hyphae	Coils	Arbuscules	Vesicles
	Kallakoopah Creek						
Goodeniaceae	Goodenia lunata [a]	med	+	+	+	+	-
	Scaevola parvibarbata [a]	low	+	+	+	+	-
Graminae	Enneapogon avenaceus [a]	med	+	+	-	-	+
	Eragrostis dielsii [a]	med	+	+	-	+	-
Liliaceae	Bulbine alata [a]	high	+	+	+	+	+
Malvaceae	Abutilon otocarpum [a]	high	+	+	-	-	+
	Sida ammophila [a]	low	+	+	+	+	-
Myoporaceae	Eremophila longifolia [a]	low	+	+	+	-	-
	Eremophila macdonnellii [a]	low	+	+	+	+	+
Papilionaceae	Swainsona phacoides [a]	high	+	+	+	+	+
Plantaginaceae	Plantago drummondii	high	+	+	+	+	+
Portulaceae	Portulaca intraterranea [a]	0	-	-	-	-	-
Solanaceae	Nicotiana velutina [a]	med	+	+	-	+	-
Zygophyllaceae	Zygophyllum howittii [a]	0	+	-	-	-	-

[E] endangered [Briggs, 1995 #1660]

Table 4.2 Arbuscular mycorrhizal status of plant species from the stony deserts, South Australia

[a] New record of mycorrhizal status, [b] Colonisation; 0 = no mycorrhiza found; low is <10%; med is 10 - 30%; high is >30%; + denotes presence, - denotes absence, [c] Specimen not identified

Family	Species	Colonisation[b]	External Hyphae	Internal Hyphae	Coils	Arbuscules	Vesicles
	Goorikianna Creek						
Chenopodiaceae	Atriplex holocarpa [a]	0	-	-	-	-	-
	Salsola kali	0	+	-	-	-	-
Compositae	yellow daisy [ac]	high	+	+	+	+	-
Goodeniaceae	Goodenia fascicularis [a]	low	+	+	-	-	-
Graminae	Dactylateneum radulans [a]	med	+	+	+	+	-
Lamiaceae	Teucrium sp. *nov* [aE]	0	-	-	-	-	-
Marsileaceae	Marsilea drummondii [a]	med	+	+	+	-	+
Papilionaceae	Swainsona campylantha [a]	high	+	+	+	+	-
	Trigonella suavissima [a]	med	+	+	+	+	-
Scrophulariaceae	Stemmodia haegii [aE]	low	+	+	-	+	-
Solanaceae	Nicotiana truncata [aE]	low	-	+	-	+	-
Zygophyllaceae	Zygophyllum compressum [a]	0	+	-	-	-	-
	Mirra Mitta Bore						
Aizoaceae	Trianthema triquetra [a]	0	-	-	-	-	-
Boraginaceae	Trichodesma zeylanicum [a]	high	+	+	+	+	+
Chenopodiaceae	Atriplex spongiosa [a]	0	-	-	-	-	-
	Atriplex vesicaria	0	-	-	-	-	-
	Salsola kali	0	+	+	-	-	-
Compositae	Calotis ancyrocarpa [a]	med	+	+	-	-	+
	Gnephosis eriocarpa [a]	low	-	+	+	-	-
	Ixiolaena brevicompta [a]	low	+	+	+	+	+
	Rutidosis helichrysoides [a]	0	-	-	-	-	-
Convolvulaceae	Evolvulus alsinoides [a]	0	-	-	-	-	-
Euphorbiaceae	Euphorbia wheeleri [a]	low	+	+	-	+	+
	Phyllanthus lacunarius [a]	0	-	-	-	-	-

Table 4.2 (continued)

Family	Species	Colonisation[b]	External Hyphae	Internal Hyphae	Coils	Arbuscules	Vesicles
	Mirra Mitta Bore						
Goodeniaceae	Scaevola humilis [a]	med	+	+	-	+	+
Graminae	Aristida contorta [a]	0	-	-	-	-	-
	Enniapogon polyphyllus [a]	0	-	-	-	-	-
	Eragrostis leptocarpa [a]	0	-	-	-	-	-
	Eragrostis setifolia [a]	0	-	-	-	-	-
	Eragrostis tenellula [aQ]	0	-	-	-	-	-
	Panicum decompositum [a]	med	-	+	-	+	+
Malvaceae	Sida ammophila [a]	low	+	+	-	+	+
Papilionaceae	Cullen cineria [a]	high	+	+	-	+	+
	Cullen patens [a]	high	-	+	+	+	+
Portulaceae	Portulaca oleracea	0	-	-	-	-	-
Scrophulariaceae	Mimulus prostratus [aR]	low	+	+	+	-	-

[E] endangered, [R] rare, [Q] possibly significant – not yet assessed [Briggs, 1995 #1660]

Table 4.3 Distribution of arbuscular mycorrhiza forming plant species among life forms and growth habits of plants of the southern Simpson and stony deserts, South Australia.

Total number of species = 81 though some species occurred at more than one site.

		Number of species		Total number of species	Percentage of spp. with AM
		mycorrhizal	non-mycorrhizal		
Habit					
	Annual	26	17	43	60
	Perennial	27	11	38	71
Life form					
	Grass	8	7	15	53
	Herb	34	14	48	71
	Shrub	11	7	18	65

Table 4.4 Distribution of arbuscular mycorrhiza forming plant species at different topographic sites within the Simpson Desert.

Total number of species = 52 though some species occurred at more than one site.

Sites	Number of species		Total no. of species	Percentage of species with AM
	mycorrhizal	non-mycorrhizal		
MAC	10	6	16	63
KAL	20	11	31	65
PEE	16	2	18	89

4.4 Discussion

The five families in these surveys that contained only non-mycorrhizal members (Aizoaceae, Amaranthaceae, Cruciferae, Portulacaceae and Zygophyllaceae) have all been considered largely non-mycorrhizal families (Malloch *et al.* 1980; Newman and Reddell 1987; Tester *et al.* 1987). Of the plant genera surveyed in these families, *Lepidium*, *Portulaca*, *Calandrinia* and *Zygophyllum* have previously been found to include species that form mycorrhizas (listed in Tester *et al.* 1987). Species in these genera may be less likely to form mycorrhizas where the growing season is short and inoculum potential of the fungi is low.

In a harsh and seasonal environment such as the northern deserts of South Australia, AM fungi are likely to spread from resistant propagules such as spores and/or from established hyphal networks. The high cover of predominantly mycorrhizal ephemerals at the Simpson Desert sites surveyed after rain in 1998 infers that AM propagules were ubiquitously abundant in these soils. Some AM colonisation of ephemerals may have arisen from *de novo* spread of hyphae from the roots of perennials, though shrubs were sparse and perennials generally possess root systems which are more active and abundant in the sub-surface layers (especially at the sand dune sites). The spread of mycorrhizas from established nurse plants may be more important after disturbance (Carrillo-Garcia *et al.* 1999). Threats to populations of plant species with conservation ratings include soil disturbance from grazing by introduced animals in heavier clay-loams and cracking clay soils. The presence of perennial nurse plants may be important in maintaining levels of AM propagules for recolonisation of these rare and endangered plants.

The occurrence of mycorrhizal species in the Chenopodiaceae has occasionally been reported by other authors [Allen, 1983 ; Bethlenfalway, 1984 ; Aguilera, 1998] including in *Salsola kali* (Allen *et al.* 1989) despite numerous early reports that members of this family were not mycorrhizal (Hirrel *et al.* 1978; and references in Tester *et al.* 1987). The presence of vesicles in the absence of arbuscules in the Chenopodiaceae has been observed previously (McGee 1986). In the absence of arbuscules the association may not contribute significantly to the uptake to P by these plants (Smith and Read 1997). However, seasonal patterns have been shown to

influence the formation of arbuscules in coastal sand dune plants (Siguenza *et al.* 1996) and arbuscules in chenopodiacean plants have been observed to be short lived (Allen 1983; Allen *et al.* 1989). Arbuscules may occur in the roots of the chenopod species found to contain internal hyphae in the surveys reported here but were not detected due to the limitations of sampling in remote regions of South Australia.

Because the opportunities for sampling were restricted to five short surveys and the quantity of roots collected was small, absence of colonisation is not conclusive evidence that a species is non-mycorrhizal. The fact that no colonisation was found in root samples of several species at one site (*Aistida contorta, Eragrostis dielsii, Othonna gregorii, Phyllanthus lacunarius* and *Sida ammophila*), but that these species were colonised at other sites suggests (1) differences in the activity of mycorrhizal roots due to differences in rainfall preceding the surveys, or (2) inadequate collection of roots from one or more of the sites, leading to biased estimates of the proportion of mycorrhizal plants. Several studies of plant species growing on sand-dunes have shown a high proportion of these species to be mycorrhizal (Koske 1975; Koske and Halvorson 1981; Logan *et al.* 1989) and mycorrhizal fungi have been implicated as agents for sand stabilization on dunes (Koske *et al.* 1975; Jehne and Thompson 1981; Sylvia 1986). Given the low fertility of dune sands in the Simpson Desert, it is likely that AM association improves the P-uptake capacity of many of these species. The benefits of AM association to plants growing in the heavier, more fertile soils of the stony deserts are less clear. However, the influence of mycorrhiza after soil disturbance and on establishment, growth, interspecific competition and fecundity may affect the survival success of plants at these sites.

The proportion of plant species not forming AM was high (27%) relative to other surveys of arid ecosystems (Bethlenfalvay *et al.* 1984; Dhillion *et al.* 1995). However, the occurrence of AM associations in plant families often cited as non-mycorrhizal recommends a cautious approach to categorisation based on comprehensive survey of plants in a wide range of ecosystems. The co-occurrence of mycorrhizal and non-mycorrhizal species also warrants further investigation into niche separation for nutrient acquisition in the stony and Simpson Deserts. Potential influences of mycorrhizas on the structure of arid plant communities may depend on the mix of species and the relative

responsiveness of each species to mycorrhizal colonisation. The following chapters explore some aspects of the importance of mycorrhizas to plant species and plant communities found in semi-arid South Australia.

Chapter 5

Mycorrhizal effects on growth, reproductive output and community composition in intact microcosms

5.1 Introduction

Associations between plants and arbuscular mycorrhizal (AM) fungi are common in natural and agricultural ecosystems (Smith and Read 1997). Many plant species show positive growth responses to colonisation by AM fungi (Hayman and Mosse 1971; Mosse *et al.* 1973; Abbott and Robson 1977; Hall *et al.* 1977; Plenchette *et al.* 1983; Hetrick *et al.* 1988; Hetrick *et al.* 1990). However, not all plant species form AM associations, and not all AM plant species show clear nutritional benefits from colonisation by mycorrhizal fungi under all growth conditions (Tester *et al.* 1985; Fitter 1986; Francis and Read 1995). Plant communities often include species with varying response to the presence of AM fungi. This variation may range from non-mycorrhizal plants with benign or possibly antagonistic interactions with the fungi (Francis and Read 1995), to highly responsive plant species with positive growth responses to AM association (Johnson *et al.* 1997). The composition of natural plant communities often includes plant species from across this response spectrum, and may be determined by differential contributions of the AM fungi to individual plant species fitness.

Evidence from a number of two-species and multi-species experiments indicates that AM fungi can affect competition between co-occurring plant species. Crush (1974) and Hall (1978) showed that the competitive ability of legumes in legume-grass mixtures was improved by AM fungi. Similarly, the competitive balance between different grass species (Fitter 1977; Hartnett *et al.* 1993), between grasses and non-leguminous herbs (Allen and Allen 1984; Marler *et al.* 1999) and between different herb species (Allsopp and Stock 1992a; Moora and Zobel 1996) can be altered by AM fungi. In more complex and species-rich plant communities, mycorrhizas may have strong effects on competitive hierarchies of co-occurring species and therefore influence community structure. Several studies in experimental microcosms have demonstrated that community structure can be significantly altered by mycorrhizal activity (Grime *et al.*

(Grime *et al.* 1987; Wilson and Hartnett 1997). The presence of mycorrhizas in these experimental microcosms has been shown to either increase (Grime *et al.* 1987) or decrease (Wilson and Hartnett 1997) floristic diversity depending on the identity and mycorrhizal responsiveness of the dominant plant species (Bergelson and Crawley 1988). Field studies have also shown that the presence of mycorrhizas can increase species richness (Gange *et al.* 1990) and diversity (Hartnett and Wilson 1999b), though the mycorrhizal dependency of the dominant plant species in these communities may have had a strong influence on these measures of community structure.

Several experiments were designed to investigate the influence of AM fungi on individual plant species, interspecific competition and community structure at the semi-arid herbland site – Brookfield Conservation Park. These experiments were designed to examine responses of individual plant species and interactions between species growing in intact microcosms taken from Brookfield Conservation Park at the beginning of the growing season. Intact cores were used to ensure that any established mycelium of AM fungi remained undisturbed, and that the community of AM fungi present at the site was sampled without bias. This method also allowed for any effects of AM fungi and intact soil on seed germination to be maintained, and for natural densities of plants to develop in community structure experiments.

The experiments were designed to:

1. determine the response to AM colonisation of the most abundant species in the semi-arid herbland,
2. investigate the effects of AM colonisation and competition on growth and reproduction of the dominant host and non-host plant species in the semi-arid herbland,
3. determine the influence of AM colonisation on growth and reproductive output of plants in intact microcosms taken from the semi-arid herbland.

5.2 Methods

5.2.1 Site descriptions

Microcosms and seeds for these experiments were collected from Brookfield Conservation Park in 1997 and 1998. Site and soil descriptions are given in Chapter 3.

5.2.2 Collection and treatment of intact cores from Brookfield Conservation Park

Intact soil cores (PVC cylinders) were collected from areas adjacent to the experimental plots at Brookfield North and Brookfield South, as described in Section 3.2. At the time of collection seedling emergence was just beginning. Each core contained several seedlings at the cotyledon, or first leaf stage of growth. Cores were transferred to a glasshouse and manipulated for experiments as set out below. At the end of the first and third weeks in the glasshouse half the cores for each plant species were dipped in a solution of 1.5 g L^{-1} benomyl (a.i.) for five minutes.

5.2.3 Mycorrhizal responsiveness of individual plant species

Mycorrhizal responsiveness of the most common plants in field plots (*Medicago minima, Carrichtera annua, Salvia verbenaca, Velleia arguta, Vittadinia gracilis, Erodium crinitum, Asphodelus fistulosus and Gynandriris setifolia*) was assessed in two different ways as follows. At the start of the growing season in 1998, intact soil cores (PVC cylinders) were collected from areas adjacent to the experimental plot at Brookfield North and Brookfield South, as described in Sections 3.2. During collection, cylinders were positioned in such a way that 10 cores were collected containing each of the major plant species (*V. gracilis* was not collected due to late germination of this species). Cores were transferred to a glasshouse and weeded to leave only one individual of the target species in each core. Cores were randomly positioned in the glasshouse and maintained weed-free and watered (three times per week) for 12 weeks. Cores receiving benomyl (Section 5.2.2) were designated the mycorrhiza-suppressed treatment.

After 12 weeks growth, roots were washed free of soil and a sub-sample taken for determination of the extent of mycorrhizal colonisation (Section 3.4). Shoots were dried and weighed. Because of the heavy textured soils, the presence of fragments of calcrete in the topsoil and the fragile roots systems of many of the plant species present, it was not possible to collect all root material from all species. Determinations of the effect of mycorrhizas on plant growth are therefore based on differences in shoot growth between treatments.

The second method for testing the mycorrhizal responsiveness of major plant species involved growing one individual in 400g closed pots of Mallala soil:sand mix (Dickson

et al. 1999). *E. crinitum* was not tested as seeds were unavailable. Five replicate pots for each species were designated as the mycorrhizal treatment and contained 10% soil and root inoculum from *Glomus mosseae* (isolate NBR 4-1) cultured on *Trifolium subterraneum* cv. Mt Barker in the glasshouse. The other five pots for each species received an equal amount of non-mycorrhizal inoculum from pot cultures grown at the same time without mycorrhizal propagules. Pots were randomised in a glasshouse and watered three times per week. All pots received 10 ml of Long Ashton solution (without P) once a week. Plants were harvested after 6 weeks growth. Roots were washed free of soil and a sub-sample taken for determination of the extent of mycorrhizal colonisation (Section 3.4). Shoots were dried and weighed and used for determination mycorrhiza-responsiveness to maintain consistency between the two assessment methods.

Mycorrhizal responsiveness was calculated using mean shoot dry weights for each species following both assays. Mycorrhizal responsiveness = (mycorrhizal$_{\text{shoot dry weight}}$ – non-mycorrhizal $_{\text{shoot dry weight}}$) / non-mycorrhizal $_{\text{shoot dry weight}}$ x 100. Shoot dry weights were $\log_{10}$ (dwt +1) transformed and percent root length colonised were arcsine-squareroot transformed to improve normality (Zar 1999). Differences in shoot dry weight and colonisation of plants in field-collected cores and inoculated pots were tested using two-way ANOVA with treatment and species as factors. Treatment differences were separated by Tukey's HSD test for significantly different means in both core and pot experiments.

5.2.4 AM effects on competition between host and non-host plant species

Effects of AM fungi on competition between the mycorrhizal host species *M. minima* and the co-occurring non-host *C. annua* were determined experimentally in intact cores. At the start of the growing season in 1998, 40 intact soil cores (PVC cylinders) were collected from areas adjacent to the experimental plot at Brookfield North (Section 3.2). Cores were transferred to a glasshouse and plants thinned to leave only one individual of the target species (either *M. minima* or *C. annua*) and 10 individuals of the neighbor species in each core. This resulted in 20 cores containing 1:10, *M. minima*:*C. annua* and 20 cores containing 1:10, *C. annua*:*M. minima*. Cores were randomly positioned in the glasshouse and half the cores were treated with benomyl to suppress the activity of AM fungi (Section 5.2.2). All cores were subsequently maintained weed-free and watered (three times per week) for 12 weeks.

At the end of 12 weeks growth, half the cores in each treatment were harvested for shoot dry weight of target and neighbor plants. In each core, roots of *M. minima* were washed free of soil and a sub-sample taken for determination of the extent of mycorrhizal colonisation (Section 3.4). Watering of the remaining cores was stopped at 12 weeks and at 16 weeks the number of mature fruits on each target plant was counted as a measure of reproductive output.

For determination of the effects of competition between *M. minima* and *C. annua*, shoot dry weights of target plants of these species without neighbors were included from Experiment 5.2.3. Percent root length colonised was transformed (arcsine-squareroot), as was shoot dry weight of target and neighbor plants ($\log_{10}$ (dwt +1)), to improve normality before analysis (Zar 1999). The effects of benomyl application and neighbor competition were used as factors in a two-way ANOVA on target plant shoot weight. Effects of benomyl on reproductive output of target plants in cores with 10 neighbors were determined by *t*-test for both plant species.

5.2.5 Mycorrhizal effects on plant communities in intact microcosms

The following experiments were designed to investigate the effects of AM colonisation and plant-plant interactions on growth and reproduction of the dominant host and non-host plant species in the semi-arid herbland at Brookfield Conservation Park. Two sites were chosen where the plant species composition was slightly different but the plant community was in a similar stage of early secondary succession. Results from studies of plant communities at the two sites could then be compared to determine the effects of mycorrhizas on plant community structure independent of species identity. The two sites chosen were Brookfield North and Brookfield South about 7.5 km apart in the herb-dominated understorey of a low open woodland (see Chapter 3).

5.2.5.1 Microcosms from Brookfield South

Twenty intact soil microcosms (PVC cores) were collected (Section 3.2) from an area adjacent to the experimental plots at Brookfield South. Microcosms were transferred to a glasshouse and randomly positioned on a bench. Half the microcosms were treated with benomyl to suppress the activity of AM fungi (Section 5.2.2) and all microcosms

were subsequently maintained with volunteer plants by watering three times per week for 12 weeks.

After 12 weeks growth, roots were washed free of soil and a sub-sample taken from each species for determination of the extent of mycorrhizal colonisation (Section 3.4). Shoots were dried and weighed. Because of the heavy textured soils, the presence of fragments of calcrete in the topsoil and the fragile roots systems of many of the plant species present, it was not possible to collect all root material from all species. Determinations of the effect of mycorrhizas on plant growth are therefore based on differences in shoot growth between treatments.

Effects of suppression of mycorrhizal colonisation (benomyl addition) on community structure in microcosms from Brookfield South were analysed by MANOVA on shoot dry weights. Microcosms were used as replicates and fungicide application was used as the treatment. Plant species were included as variates but only the nine most abundant species were included, as all other species were present in less than 30% of pots in at least one treatment. Where MANOVA indicated a significant treatment effect on shoot dry weight, protected ANOVA was performed on species-by-shoot dry weight, species-by-relative biomass and species-by-percent root length colonised to assess the influence of treatment on individual species. Shoot dry weights and relative shoot dry weights were $\log_{10}$ (dry weight + 1) transformed and percent root lengths colonised were arcsine-squareroot transformed to improve normality (Zar 1999). Significant treatment effects on shoot dry weight, relative shoot dry weight and percent root length colonised by AM fungi, were separated by least significant difference (LSD) for each species. Significant effects of suppression of AM colonisation on species richness, species diversity and evenness (calculated using equations 1, 2 & 3 Chapter 3) were compared using *t*-tests.

5.2.5.2 Microcosms from Brookfield North

Microcosms from Brookfield North were collected to investigate the effects of AM colonisation on community structure and the reproductive output of the dominant mycorrhizal host and non-host plant species in this semi-arid herbland community. Forty intact soil microcosms (PVC cores) were collected (Section 3.2) from an area

adjacent to the experimental plots at Brookfield North. Microcosms were transferred to a glasshouse and randomly positioned on a bench. Half the microcosms were treated with benomyl to suppress the activity of AM fungi (Section 5.2.2) and all the microcosms were subsequently maintained with volunteer plants by watering three times per week for 12 weeks.

After 12 weeks growth, the above ground biomass was removed from half of the microcosms in each treatment and the dry weight of shoots of each species was determined. Intact cores were planted with one germinated seedling of the mycorrhizal trap plant *Trifolium subterrraneum* and watered three times per week for six weeks. At the end of six weeks, the roots of *T. subterraneum* were washed free of soil and a sub-sample taken for determination of the extent of mycorrhizal colonisation (Section 3.4). The remaining 20 microcosms were left unwatered until 16 weeks when the reproductive output of *M. minima*, *C. annua* (fruit number) and *S. verbenaca* (inflorescence nodes) was determined for each core.

Effects of suppression of mycorrhizas (benomyl addition) on plant species richness, evenness and diversity (calculated using equations 1, 2 & 3 Chapter 3) on community structure in microcosms from Brookfield North were analysed in the same way as those in microcosms from Brookfield South (Section 5.2.5.1). Effects of suppression of mycorrhiza on species richness, species diversity and evenness were compared using *t*-tests. Significant effects of benomyl treatment on the percent root length colonisation of the trap plant *T. subterraneum* by AM fungi were determined using a *t*-test. Effects of benomyl on the reproductive output of *M. minima*, *C. annua* (fruit number) and *S. verbenaca* (number of inflorescence nodes) in microcosms were tested using *t*-tests. Simple linear regression was used to test correlations between reproductive output and shoot dry weight for these three plant species.

5.3 Results

5.3.1 Mycorrhizal responsiveness of individual plant species

In the host plant responsiveness tests in field cores, average percent root length colonised was reduced to approximately 70 % of controls when cores were treated with benomyl (Table 5.1). Root colonisation was significantly reduced ($P < 0.05$) by

benomyl addition in *M. minima, S. verbenaca, V. arguta* and *A. fistulosus*. Colonisation was also reduced in *E. crinitum* and *G. setifolia*, though this was not signficant ($P > 0.05$). *C. annua* roots were not colonised in any intact cores. Growth of the mycorrhizal host plants *M. minima, V. arguta, A. fistulosus* and *G. setifolia* was significantly reduced in intact cores when benomyl was applied. Growth of the host plants *S. verbenaca* and *E. crinitum* was not significantly influenced by benomyl treatment, while the non-host *C. annua* showed a significant ($P < 0.01$) growth increase when benomyl was applied (Table 5.1). No fungi were observed in the roots of *C. annua* in intact cores.

In the host plant responsiveness tests in inoculated pots (Table 5.2), there was no colonisation of roots from any species in uninoculated pots (except *G. setifolia,* which was contaminated with an unknown AM fungus). The growth of *M. minima, V. arguta, V. gracilis* and *A. fistulosus* was enhanced by inoculation with the mycorrhizal fungus *G. mosseae* (NBR 4-1). *Salvia verbenaca* showed no response to inoculation and *C. annua* and *G. setifolia* showed significant ($P < 0.05$) growth depression in inoculated pots (Table 5.2).

Table 5.1 Growth response of single plant species in intact cores treated with or without benomyl. Cores were taken from both field sites at Brookfield Conservation Park. in 1998. Each value represents the mean (SE) of five replicates.

Plant species	Brookfield collection site	Shoot dry weight (g plant^{-1})		% Colonisation		Mycorrhizal response[1]
		Control	Fungicide	Control	Fungicide	
M. minima	North	2.90 (0.24)*	0.53 (0.23)	80.8 (1.6)*	55.0 (3.0)	448
C. annua	North	1.32 (0.16)	2.01 (0.05)*	0.0 (0.0)	0.0 (0.0)	-34
S. verbenaca	North	0.98 (0.07)	1.04 (0.18)	80.9 (3.2)*	57.1 (4.0)	-6
V. arguta	North	0.89 (0.17)*	0.26 (0.15)	75.1 (2.3)*	54.4 (8.6)	245
E. crinitum	North	1.20 (0.14)	0.98 (0.25)	61.3 (8.1)	43.2 (5.1)	22
A. fistulosus	South	0.49 (0.19)*	0.10 (0.04)	65.0 (2.6)*	43.1 (5.0)	416
G. setifolia	South	0.21 (0.01)*	0.18 (0.03)	44.8 (9.4)	27.1 (9.7)	16

[1]Mycorrhizal response calculated as (M-NM)/NM*100

*Different letters indicate significant difference ($P < 0.05$; Tukey's HSD) between treatments for each plant species.

Table 5.2. Growth response of single plant species to mycorrhizal colonisation in pots inoculated with the AM fungus *Glomus mosseae* NBR 4-1. Each value represents the mean (SE) of five replicates.

Plant species	Shoot dry weight (g plant^{-1})		% Colonisation		Mycorrhizal response[1]
	Non-mycorrhizal control	Mycorrhizal	Non-mycorrhizal control	Mycorrhizal	
M. minima	0.04 (0.01)	0.07 (0.01)*	0.0 (0.0)	48.8 (12.3)*	96
C. annua	0.18 (0.00)*	0.14 (0.02)	0.0 (0.0)	0.0 (0.0)	-20
S. verbenaca	0.12 (0.03)	0.10 (0.01)	0.0 (0.0)	79.5 (2.7)*	-13
V. arguta	0.01 (0.00)	0.04 (0.01)*	0.0 (0.0)	47.5 (7.0)*	386
V. gracilis	0.01 (0.00)	0.12 (0.02)*	0.0 (0.0)	56.0 (4.3)*	729
A. fistulosus	0.02 (0.00)	0.07 (0.01)*	0.0 (0.0)	59.8 (6.7)*	264
G. setifolia	0.10 (0.01)*	0.09 (0.00)	13.0 (8.9)[3]	17.8 (4.8)	-11

[1]Mycorrhizal response calculated as (M-NM)/NM*100
*Different letters indicate significant difference ($P < 0.05$; Tukey's HSD) between treatments for each plant species.
[3]Control pots contaminated with unknown AM fungus

5.3.2 Effects of benomyl on competition between a host and a non-host plant species

5.3.2.1 Plant growth and mycorrhizal colonisation

The interspecific competition effects of *M. minima* and *C. annua* on each other were not significantly altered by benomyl application (Table 5.3; $P > 0.05$ for both species). There were strong negative effects of benomyl addition on the growth of *M. minima* with and without neighbors (Fig. 5.1A). There was also a strong main effect of competition from *C. annua* on the growth of *M. minima* target plants with and without benomyl (Table 5.3; $P = 0.017$). Similarly, there was a strong main effect of competition from *M. minima* on the growth of *C. annua* target plants with and without benomyl (Table 5.3; $P < 0.001$). However, the effect of benomyl addition on the growth of *C. annua* was positive regardless of the presence or absence of neighbours (Fig. 5.1B; Table 5.3; $P < 0.001$).

Treatment of intact cores with benomyl significantly ($P < 0.001$; Table 5.3) reduced colonisation of *M. minima* roots by AM fungi, independent of competition effects (Fig. 5.1C).

Table 5.3 Results of ANOVA on the effects of interspecific competition and suppression of mycorrhizal colonisation on the shoot dry weight and root colonisation of target species *M. minima* and *C. annua* in intact cores from Brookfield North, n = 5.

ANOVA		Shoot dry weight of *M. minima* target plant			Shoot dry weight of *C. annua* target plant			Percent colonisation (*M. minima* roots)		
Transformation		Log_{10} (dry weight +1)			Log_{10} (dry weight +1)			arcsine ($\sqrt{}$)		
Factors	df	MS	F-value	$P > F$	MS	F-value	$P > F$	MS	F-value	$P > F$
Fungicide	1	0.714	43.28	< 0.001	0.160	27.28	< 0.001	0.425	41.08	< 0.001
Competition	1	0.122	7.37	0.017	0.380	64.84	< 0.001	0.013	1.22	0.286
C x F	1	0.007	0.40	0.535	0.024	4.05	0.063	0.000	0.04	0.852

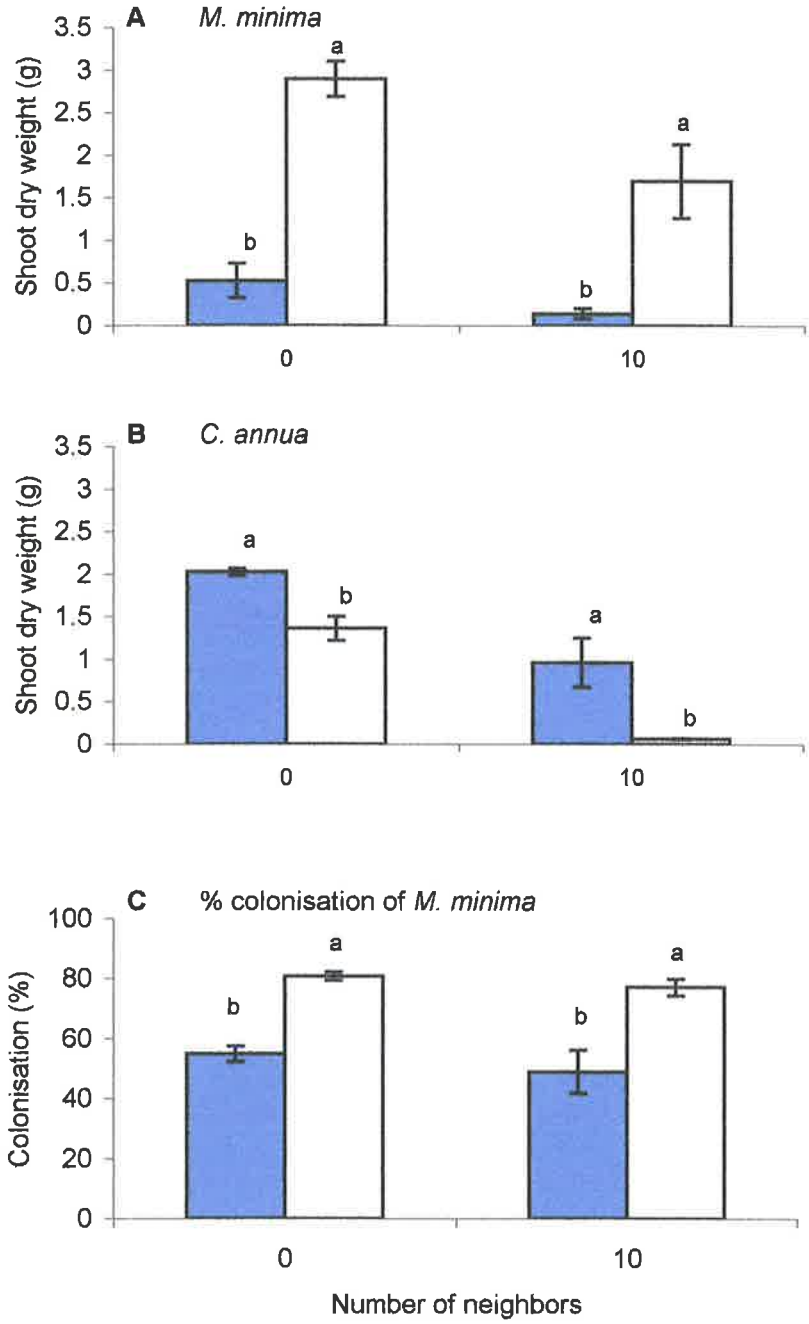

Figure 5.1 Effects of interspecific competition (0 & 10 neighbors) and suppression of AM fungi (benomyl, blue bars; control, unshaded bars) on growth of target plants (A) *M. minima*, and (B) *C. annua* and the percent colonisation of *M. minima* roots in intact cores (C). Treatment means followed by different letters within each plant density are significantly different (Tukey's HSD, $P < 0.05$), n = 5.

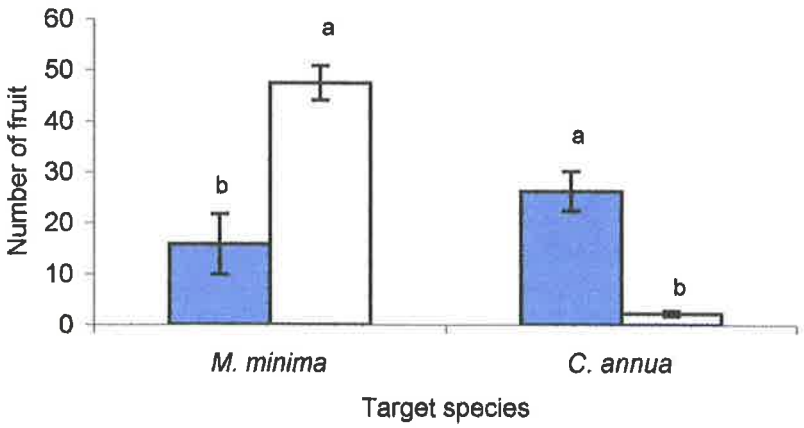

Figure 5.2. Effect of benomyl treatment (blue bars) compared to controls (unshaded bars) on reproductive output (number of fruit) of the target plants *M. minima* (with 10 *C. annua* competitors) and *C. annua* (with 10 *M. minima* competitors) in intact cores. Different letters indicate significant difference between means for each target plant species (*t*-test; $P \leq 0.002$), n = 5.

5.3.2.2 Reproductive output

Reproductive output of *M. minima* and *C. annua* under interspecific competition (10 neighbors) was influenced by benomyl treatment. *C. annua* target plants produced significantly ($t = 6.10$, df = 8, $P < 0.001$) more mature siliques under competition from *M. minima* when AM fungi were suppressed by benomyl addition (Fig. 5.2). Conversely, production of mature pods by *M. minima* target plants was significantly ($t = -4.64$, df = 8, $P = 0.002$) reduced under competition from *C. annua* when the activity of AM fungi was suppressed by benomyl (Fig. 5.2).

5.3.3 Effects of benomyl on plant communities in microcosms from Brookfield South

5.3.3.1 Plant growth and mycorrhizal colonisation

Eighteen plant species grew in intact microcosms from Brookfield South; nine of these species were present in at least 30% of microcosms in each treatment. Results of the MANOVAs on total shoot dry weight (Wilk's Lambda $F = 2.98$; df = 17,315; $P < 0.05$) of major plant species showed that there was a significant interaction between species and treatment. There was a significant effect of benomyl treatment on colonisation of some species ($P < 0.001$; Table 5.4). Treatment with benomyl significantly reduced the AM colonisation of roots of seven of the nine major species (Table 5.5). *B. rubens* and *C. annua* were the only species which did not show a significant reduction in colonisation due to benomyl treatment, although *B. rubens* did show a large non-significant reduction. AM colonisation in *S. apetala* was significantly reduced by benomyl treatment but colonisation in this species was not high in untreated microcosms (Table 5.5).

There was a significant ($P = 0.015$) effect of benomyl treatment on total dry weight of some plant species in these microcosms (Table 5.4). Both *S. apetala* and *B. rubens* had increased total shoot weight in benomyl-treated microcosms (Table 5.5). No plant species showed a significant negative growth response to benomyl treatment, although, *M. minima, G. setifolia* and *A. fistulosus* all showed large non-significant decreases. The combined shoot dry weight of *M. minima,* and *A. fistulosus* was reduced from 11.3% of total shoot dry weight in control microcosms to only 2.1% in benomyl-treated microcosms. Both *C. annua* and *S. verbenaca* showed large non-significant increases in

shoot dry weight in benomyl-treated microcosms. There was also a significant (P = 0.002) effect of benomyl treatment on the relative shoot dry weight of some species in these microcosms (Table 5.4). The relative dry weight of *G. setifolia* was reduced while the relative dry weight of *S. apetala* was increased in benomyl-treated microcosms (Fig 5.3). *B. rubens* and *C. annua* showed large non-significant increases in relative shoot dry weight in benomyl treated microcosms while *M. mimima* and *A. fistulosus* showed non-significant decreases in relative shoot dry weight in benomyl treated microcosms relative to controls.

5.3.3.2 Species richness, diversity and eveness

Community structure was not measurably altered by addition of benomyl to intact microcosms from Brookfield South (Table 5.6). Richness, diversity and evenness values were not significantly different ($P > 0.05$) in benomyl-treated microcosms from values in control microcosms.

Table 5.4 Results of ANOVA of plant shoot dry weight, relative dry weight and percent root length colonised in microcosms from Brookfield South, n = 5.

ANOVA		Dry weight per intact core			Relative dry weight per intact core			Percent root length colonized		
Transformation		Log_{10} (dry weight +1)			Log_{10} (relative dry weight +1)			arcsine ($\sqrt{}$)		
Factors	df	MS	F-value	$P > F$	MS	F-value	$P > F$	MS	F-value	$P > F$
Plant species	9	0.035	28.93	< 0.001	0.164	28.22	< 0.001	0.713	13.73	< 0.001
Fungicide	1	0.000	0.03	0.861	0.027	4.67	0.032	5.691	109.69	< 0.001
P x F	9	0.003	2.37	0.015	0.018	3.07	0.002	0.233	4.49	< 0.001

Table 5.5. Total shoot dry weight and percentage root length colonised by AM fungi for each major species in benomyl-treated and control microcosms from Brookfield South, n = 5.

Plant species	Shoot dry weight (g)		Percent root length colonised			
	Control	Benomyl	Control	n[1]	Benomyl	n
G. setifolia	0.826 (0.092)	0.655 (0.057)	41.3 (3.3)	9	11.9 (3.1)**	10
S. apetala	0.591 (0.142)	1.059 (0.187)**	8.1 (2.4)	10	0.2 (0.2)*	10
M. minima	0.195 (0.067)	0.049 (0.018)	84.0 (3.0)	7	23.4 (4.1)***	8
B. rubens	0.170 (0.080)	0.732 (0.282)***	50.1 (1.8)	5	23.4 (0.9)	8
L. pumila	0.090 (0.039)	0.121 (0.057)	47.6 (2.7)	7	6.8 (2.3)***	5
A. fistulosus	0.043 (0.021)	0.022 (0.008)	50.7 (8.5)	7	5.6 (2.7)***	7
C. annua	0.027 (0.015)	0.116 (0.066)	0.0 (0.0)	3	0.0 (0.0)	5
S. verbenaca	0.022 (0.011)	0.255 (0.014)	87.3 (4.4)	4	5.7 (2.9)***	5
M. vulgare	0.009 (0.005)	0.012 (0.006)	63.9 (3.9)	4	2.5 (1.8)***	3
Others	0.138 (0.062)	0.285 (0.106)	NA	-	NA[2]	-

[1]Number of microcosms occupied by each species from a maximum of 10.
Mean shoot dry weight or percent root length colonised of each species in benomyl-treated microcosms is significantly different (P<0.05, **P<0.01, ***P<0.001) from controls as determined by LSD.
[2]NA = not applicable (shoot dry weight, percentage root length colonised and number of microcosms occupied for each minor species given in Appendix 1).

Table 5.6. Mean (SE) plant species richness, diversity and evenness in microcosms from Brookfield South. Effects of fungicide (benomyl) compared to watered controls, n = 10.

Treatment	Community indices		
	Richness (*S*)	Diversity (*H'*)	Evenness (*J*)
Control	7.60 (0.27)	1.50 (0.06)	0.75 (0.04)
Benomyl	7.40 (0.37)	1.36 (0.09)	0.68 (0.04)
t-test			
P-value	0.667	0.177	0.195

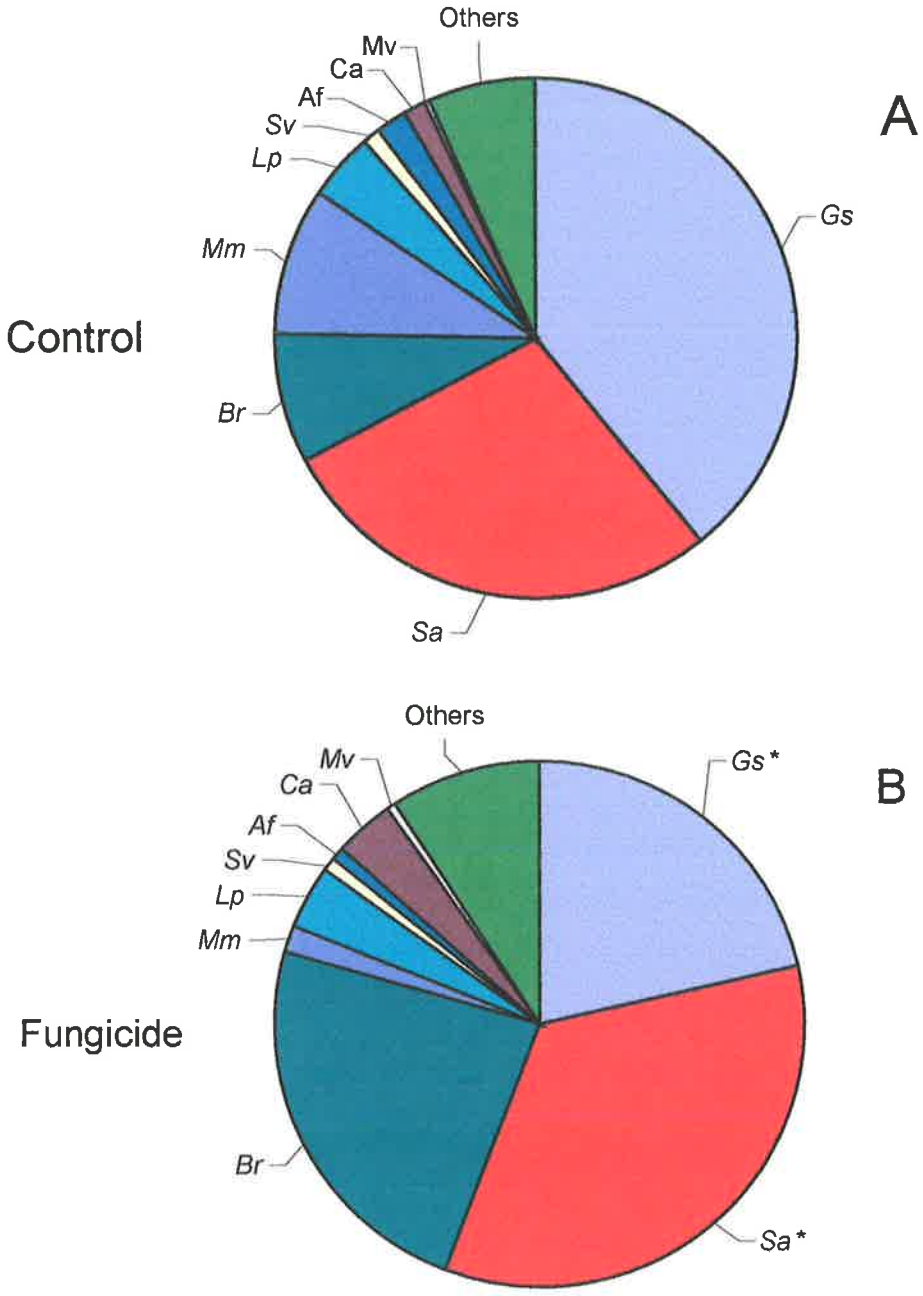

Figure 5.3 Species composition (relative shoot dry weight of each species) in microcosms from Brookfield South with AM fungi active (A) or suppressed by benomyl (B). Species surveyed were *Gs* = *Gynandriris setifolia; Sa* = *Silene apetala; Br* = *Bromus rubens; Mm* = *Medicago minima; Lp* = *Lophochloa pumila; Sv* = *Salvia verbenaca; Af* = *Asphodelus fistulosus; Ca* = *Carrichtera annua; Mv* = *Marrubium vulgare*; Others = 9 species listed in Appendix 1. Asterisks indicate species with significantly (*$P < 0.05$, LSD) different response to benomyl treatment, n =10.

5.3.4 Effects of benomyl on plant communities in microcosms from Brookfield North

5.3.4.1 Plant growth and mycorrhizal activity

Twelve plant species grew in intact microcosms from Brookfield North; only four of these species were present in at least 30% of microcosms in each treatment. Results of the MANOVAs on total shoot dry weight (Wilk's Lambda $F = 3.99$; df = 4,90; $P = 0.01$) of major plant species showed that there was a significant interaction between species and treatment. There was a significant ($P = 0.045$; Table 5.7) negative effect of benomyl treatment on the activity of AM fungi in these microcosms. Treatment with benomyl had significantly reduced the AM colonisation potential of soils in intact microcosms by the end of the experiment.

There was a significant ($P = 0.005$; Table 5.8) positive effect of benomyl treatment on total shoot dry weight of *C. annua* in these microcosms (Table 5.9). There was also a significant ($P = 0.032$; Table 5.8) effect of benomyl treatment on the relative shoot dry weight of some species in these microcosms. The relative shoot dry weight of *S. verbenaca* was reduced while the relative shoot dry weight of *C. annua* was increased in benomyl-treated microcosms (Fig 5.4). *M. minima* and *V. gracilis* both showed large but non-significant reduction in total shoot dry weight (Table 5.9) and relative shoot dry weight (Fig 5.4) in benomyl-treated microcosms compared to control microcosms. The combined shoot dry weight of *M. minima* and *V. gracilis* was reduced from 13.5% of the total in control microcosms to only 6.1% of the total in benomyl-treated microcosms.

5.3.4.2 Species richness, diversity and evenness

Plant species richness, diversity and evenness were not significantly ($P > 0.05$) altered by addition of benomyl to intact microcosms from Brookfield North (Table 5.10) compared with controls.

5.3.4.3 Reproductive output of major species

The reproductive output the most abundant species in intact microcosms from Brookfield North was differentially effected by benomyl treatment (Fig. 5.5). The number of pods of *M. minima* was not significantly ($t = -0.66$, df = 14, $P = 0.519$)

altered by benomyl treatment. Benomyl treatment significantly (t = 2.98, df = 18, P = 0.008) increased the number of siliques produced by *C. annua*, and reduced (t = -2.65, df = 18, P = 0.016) the number of inflorescence nodes on *S. verbenaca*.

Reproductive outputs of *C. annua* and *S. verbenaca* were signifiantly correlated (*C. annua*, r^2 = 95.4, $P < 0.001$; *S. verbenaca*, r^2 = 45.2, $P < 0.001$) with shoot dry weight of those species. The number of pods produced by *M. minima* was not significantly correlated ($r^2 < 1.0$, P = 0.339) with shoot dry weight of *M. minima* in microcosms.

Table 5.7. Mycorrhizal colonisation of *Trifolium subterraneum* trap plants in cores from Brookfield North treated with fungicide (benomyl) or untreated (control). Mycorrhizal colonisation potential measured as mean (SE) percentage of root length colonized, n = 10.

	% Root length colonised
Control	82.3 (3.0)
Benomyl	74.0 (2.7)
t-test	
P–value	0.045

Table 5.8. Results of ANOVA of dry weight of plants in cores treated with benomyl from Brookfield North, n = 10.

ANOVA		Dry weight per intact core			Relative dry weight per intact core		
Transformation		Log_{10} (dry weight +1)			Log_{10} (relative dry weight +1)		
Factors	df	MS	F-value	$P > F$	MS	F-value	$P > F$
Plant species	4	0.392	50.16	< 0.001	0.112	48.18	< 0.001
Fungicide	1	0.014	1.83	0.180	0.000	0.05	0.828
P x F	4	0.031	3.99	0.005	0.006	2.78	0.032

Table 5.9 Total shoot dry weight of each major species in benomyl-treated and control microcosms from Brookfield North.

Plant species	Shoot dry weight (g)			
	Control	n	Benomyl	n[1]
S. verbenaca	0.820 (0.137)	10	0.732 (0.153)	10
C. annua	0.813 (0.237)	10	1.724 (0.230)***	10
M. minima	0.220 (0.044)	7	0.143 (0.041)	9
V. gracilis	0.050 (0.013)	6	0.023 (0.013)	4
Others[2]	0.103 (0.082)	-	0.116 (0.048)	-

[1]Number of microcosms occupied by each species from a maximum of 10.
[2]Shoot dry weight and number of microcosms occupied by each minor species given in Appendix 2).
***Mean shoot dry mass of plant in benomyl-treated microcosms significantly different (LSD, $P<0.001$) from controls.

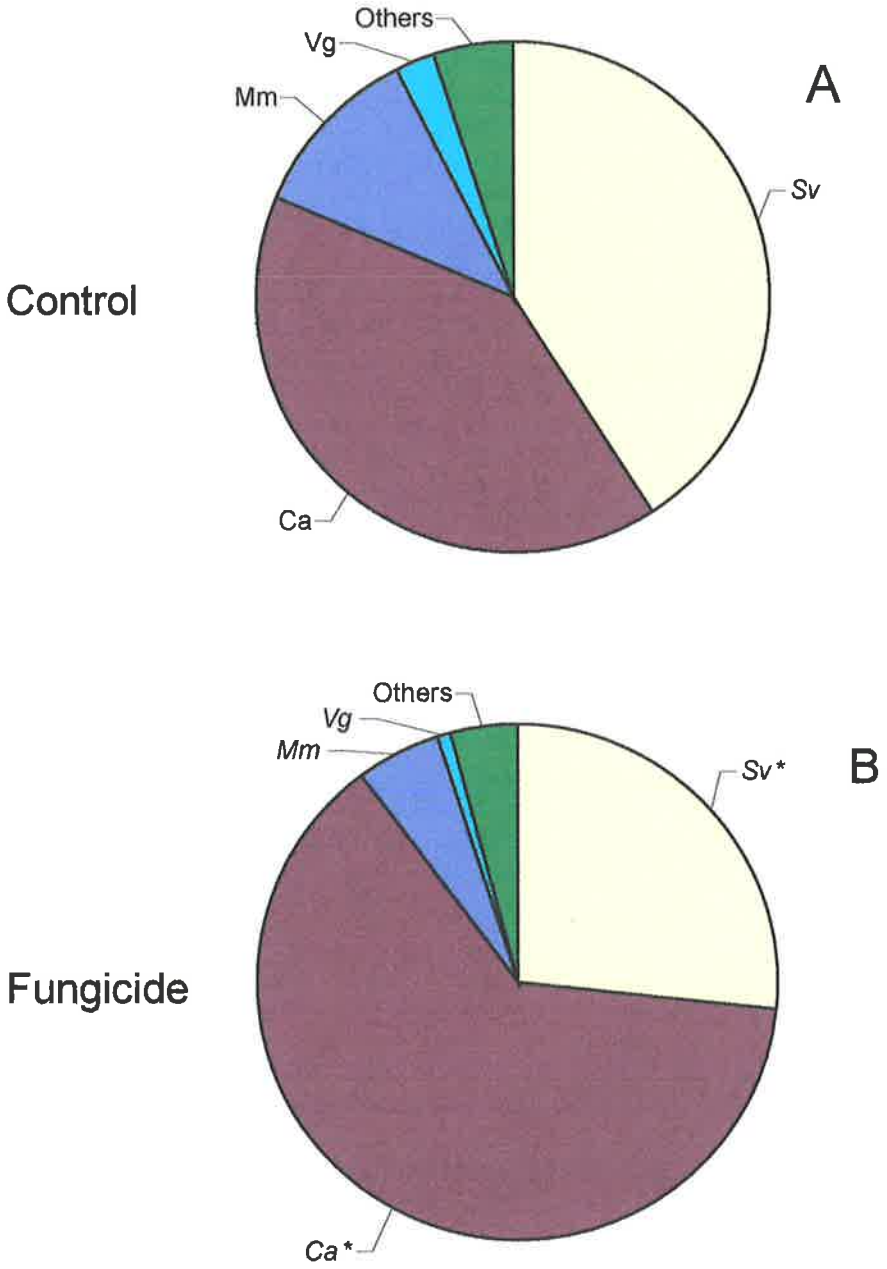

Figure 5.4 Species composition (relative shoot dry weight of each species) in microcosms from Brookfield North with AM fungi active (A) or suppressed by benomyl (B).). Species surveyed were *; Sv = Salvia verbenaca; Ca = Carrichtera annua ; Mm = Medicago minima; Vi = Vittadinia gracilis*; Others = 7 species listed in Appendix 2. Asterisks indicate species with significantly (*$P < 0.05$, LSD) different response to benomyl treatment, n =10.

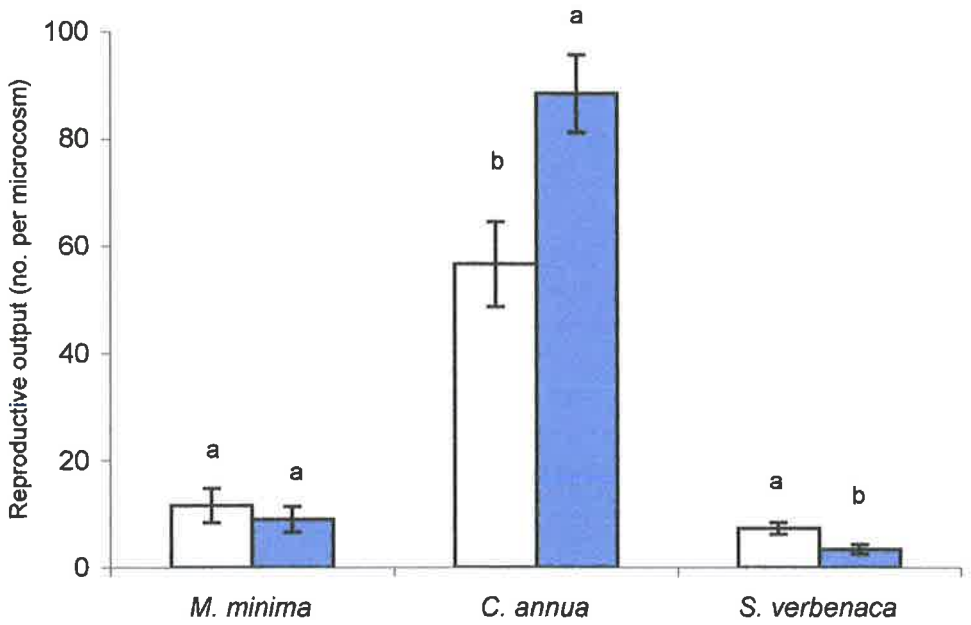

Figure 5.5 Effect of benomyl treatment (blue bars) compared controls (unshaded bars) on mean (SE) reproductive output (number of fruit - *M. minima* and *C. annua*; number of inflorescence nodes - *S. verbenaca*) of the three most abundant plant species in microcosms from Brookfield North. Different letters indicate significant difference between means for each plant species (*t*-test, $P < 0.02$), n = 10.

Table 5.10 Mean (SE) plant species richness, diversity and evenness in microcosms from Brookfield North. Effects of fungicide (benomyl) compared to watered controls, n = 10.

	Community indices		
	Richness (*S*)	Diversity (*H'*)	Eveness (*J*)
Control	4.10 (0.35)	0.93 (0.08)	0.70 (0.06)
Benomyl	4.30 (0.37)	0.81 (0.08)	0.58 (0.05)
t-test			
P-value	0.697	0.280	0.148

5.4 Discussion

Benomyl successfully reduced the mycorrhizal colonisation of the roots of host plants. Suppression of the activity of AM fungi did not significantly alter diversity, species richness or evenness of plant communities in intact microcosms from either site. This occurred despite reduction in AM colonisation of a number of species in these microcosms and significant changes in the productivity of several plant species in these communities. Changes in diversity might be expected when the productivity of the dominant plant species displays different response to mycorrhizal colonisation than subordinate species (Bergelson and Crawley 1988; Wilson and Hartnett 1997; Hartnett and Wilson 1999a). However, plant communities that contain relatively even proportions of mycorrhiza-responsive and non-responsive species may respond equivocally to a reduction in mycorrhizal activity as shown here.

Tests for individual responsiveness to AM fungi showed that plant species varied in their ability to form mycorrhizas and to show growth responses when mycorrhizal (Table 5.1 & 5.2). In both microcosm experiments, only two plant species contributed more than 50 % of total shoot weight. In microcosms from Brookfield South, *G. setifolia* (a host plant with low responsiveness) and *S. apetala* (very low colonisation, almost a non-mycorrhizal plant) were the most abundant species, while in microcosms from Brookfield North, *S. verbenaca* (non-responsive host) and *C. annua* (non-host) were the most abundant species. In both cases, the relative abundance of these species increased and the other species decreased when mycorrhizal activity was suppressed. While effects of mycorrhiza-suppression on the total aboveground biomass in microcosms were slightly different to relative biomass responses, diversity and evenness indices are both calculated from the relative (not total) contribution of each species to community structure. The large number of species with non-significant but variable response to suppression of mycorrhizal activity, resulted in low net effects on diversity and evenness.

Interestingly, one of the dominant species in each of the microcosm experiments formed no association (*C. annua*, Brookfield North) or only a weak association (*S. apetala*, Brookfield South) with AM fungi. The other dominant species in these microcosms forms high levels of root colonisation (*S. verbenaca*, Brookfield North; and *G. setifolia*,

Brookfield South) in undisturbed soils from these sites (Table 5.1). The two strongly mycorrhizal species (high % root length colonised in control microcosms) showed no response (*S. verbenaca*) or only a weak, equivocal response (*G. setifolia*) to colonisation in single species responsiveness tests. Such facultative response to mycorrhizal association is thought to be characteristic of early-successional species, while many pioneer species do not form mycorrhiza at all (Janos 1980b; Janos 1980a). The dominance of these non-responsive species in this early successional community is consistent with theory about the role of arbuscular mycorrhizas in community succession (Janos 1980a; Janos 1985; Reeves 1985; Pankow *et al.* 1991).

Despite the low responsiveness of the mycorrhizal host plants *S. verbenaca* and *G. setifolia*, the relative aboveground biomass of both these species was reduced in benomyl-treated microcosms. Therefore, loss of mycorrhizal function does not explain the decline in biomass of these species. Indirect effects of reduced mycorrhizal activity may have resulted in increased competition from the weakly or non-mycorrhizal co-dominant in these experiments. The non-host species *C. annua* showed a negative growth response to the presence of AM fungi in single species tests. Suppression of the activity of AM fungi in microcosms from Brookfield North would have reduced mycorrhizal-antagonism of this species and would consequently explain the increase in biomass of *C. annua* in benomyl-treated microcosms. Increased competition from *C. annua* in benomyl-treated microcosms would explain the relative decline in biomass of *S. verbenaca* in this experiment. Similarly, reduced mycorrhizal activity in benomyl-treated microcosms from Brookfield South may have released *S. apetala* from antagonistic effects of the fungi and resulted in increased competition for *G. setifolia*. While the mycorrhizal responsiveness of *S. apetala* was not tested in these experiments, the low amount of colonisation in roots from control microcosms suggests that this species is likely to receive minimal growth benefits, if any, from the symbiosis.

A second potential explanation for the increase in biomass of *S. apetala* in benomyl-treated microcosms from Brookfield South is that competition from mycorrhiza-responsive host-plants was reduced by fungicide application. While the reduction in individual biomass of *M. minima* and *A. fistulosus* was not statistically significant, both these species are highly mycorrhiza-responsive and their combined biomass was

reduced from 11.3% in control microcosms to 2.1% in benomyl-treated microcosms. A similar effect in the experiment on microcosms from Brookfield North may have contributed to competitive release of *C. annua* from the highly mycorrhiza-responsive host-plants *M. minima* and *V. gracilis*. In this experiment the combined biomass of these two most abundant mycorrhiza-responsive species was reduced from 13.5% in control microcosms to 6.1% in benomyl-treated microcosms. Effects of AM fungi on competition between pairs of plant species with different mycorrhiza-responsiveness are well known (Allen and Allen 1984; Koide and Li 1991; Allsopp and Stock 1992b; Hartnett *et al.* 1993; Sanders and Koide 1994) but are more difficult to determine in complex species mixes. Direct competition between all species pairs was not determined in these experiments, however, increases in biomass of non-host and non-responsive host plants when mycorrhizas were suppressed with benomyl suggest that mycorrhizas mediate competition between species in these communities.

Influences of mycorrhiza on species richness in microcosms are inherently difficult to assess. Species richness was not altered by benomyl-treatment in either microcosm experiment reported here. If mycorrhizas had significant influence on survivorship and/or competitiveness of individual species in these plant communities species richness might be affected. One problem for determining mycorrhizal influence on species richness was the variation in species composition between replicate microcosms. Spatial heterogeneity and the low frequency of some species in these semi-arid herblands resulted in a number of species being represented in less than 30% of microcosms. If survivorship of some species was affected by mycorrhiza it would be difficult to detect in the low number of samples used here. In microcosms constructed by planting seedlings at approximately natural relative abundance and densities, Wilson *et al.* (1997) found that five out of eight species planted had survivorship in untreated soils of less than 75% after 42 days. Where low survivorship is not the result of mycorrhizal effects, determination of mycorrhizal influence on species richness and diversity may be confounded by other factors affecting survival.

While variation in species composition between microcosms from Brookfield Conservation Park reduced the power to analyse effects of mycorrhiza on floristic diversity, benefits in obtaining information from realistic species relative abundances,

density and proximity in these cores may be some compensation. The relative abundance and density of neighbors can affect the competitiveness of target plants (Goldberg 1987; Miller and Werner 1987; Wilson and Tilman 1991). Constructed plant communities are at risk of including 'hidden treatments' (*sensu* Huston, 1997). The risk of 'hidden treatments' in constructed communities is non-random selection of species with particular attributes (e.g. high or low mycorrhizal responsiveness), potentially leading to changes in diversity related directly to inherent species properties not community level interactions. For example, if an experiment were designed to include seven non-mycorrhizal plant species and one obligate-host species, the obligate-host species is unlikely to survive in treatments where AM fungi were absent. Differences in plant species richness between mycorrhizal treatments would reflect species selection, not necessarily community interactions. Constructed microcosms may produce results potentially biased by the species present, a situation which potentially explains the conflicting results of Grime *et al.* (1987) and Wilson and Hartnett (1997) with respect to the effects of AM fungi on floristic diversity. In the current study, species presence and abundance in microcosms was randomly determined at the point where intact cores were collected in a uniform grid within the natural plant community. That there was no significant effect of AM fungi on floristic diversity within these experiments may be due to a third possible effect. Reductions in the abundance of some species were offset by increases in the abundance of others. The different effects of AM fungi on the growth of different plant species compensated for each other and resulted in no net change on floristic diversity. A second benefit of using intact microcosms from field sites is that the soil was only slightly disturbed and mycorrhizal fungi and other fungi and microorganisms were at approximately natural densities and diversity at the beginning of the experiments.

Treatment of microcosms from Brookfield North with benomyl did alter the reproductive output of two of the dominant species. The reproductive output of *C. annua* was increased and the reproductive output of *S. verbenaca* was decreased when mycorrhizas were suppressed. The strong correlation between vegetative biomass and reproductive output for these two species indicates that effects of mycorrhiza on fecundity result from effects on vegetative competitiveness and are not due to direct effects on reproduction. Mycorrhizal effects on the reproduction of *S. verbenaca* and *C.*

annua may be explained by the phenology of these early successional species. Both *S. verbenaca* and *C. annua* produce large numbers of seeds relative to their total biomass and can flower and set seed rapidly in response drought conditions. If mycorrhiza were permanently suppressed in this plant community, increased seed production from non-host and non-responsive plants may lead to changes in community structure. Such changes were not examined in this study of one generation in a plant community.

Indirect effects of mycorrhiza on reproduction of non-host plant species via effects on intact plant communities have rarely been reported. Carey *et al.* (1992) observed potential effects of mycorrhiza on the fecundity of the mycorrhizal host *Vulpia ciliata* through mycorrhizal protection from pathogenic fungi. Other studies have shown that mycorrhizal effects on maternal plants may translate into effects on the vigor of offspring plants (Lewis and Koide 1990; Lu and Koide 1991; Koide and Lu 1992) and the structure of plant populations (Shumway and Koide 1995). Sanders & Koide (1994) found no effect of mycorrhiza on the percentage of plants of the non-host species *Amaranthus retroflexus* flowering in a simple three-species plant community. However, the biomass of flowering heads and the P content of stems of *A. retroflexus* was greater in fumigated plots than in control plots where AM fungi were active (Sanders and Koide 1994). Indirect effects of mycorrhiza on the seed production of non-host species in intact communities require further research.

The effects of interspecific competition on the growth of *M. minima* and *C. annua* were not altered by suppression of AM fungi by benomyl. In this experiment mycorrhiza did enhance the growth of *M. minima* but did not enhance the competitive ability of this plant in combination with the non-host *C. annua*. Conversely, the negative impact of mycorrhiza on the growth of *C. annua* did not reduce the ability of this plant to compete with *M. minima*. Reproductive output was also affected by fungicide treatment in the cores with interspecific competitors. These results were consistent with effects of fungicide treatment on shoot biomass. A number of studies have shown that competitive outcomes between plants with different mycorrhiza-responsiveness can be altered in the presence of AM fungi (Allen and Allen 1984; Koide and Li 1991; Hartnett *et al.* 1993; Moora and Zobel 1996; West 1996; Watkinson and Freckleton 1997). However, these relationships may be density dependent (Hartnett *et al.* 1993; Watkinson and

Freckleton 1997). Since the effects of mycorrhizas may be reduced at high plant densities (Koide 1991a; Facelli *et al.* 1999), the high density of competitors in these experiments may have obscured differential effects of mycorrhizas on competition between the plant species. While this is undesirable with respect to investigations of plant-plant interactions, realistic densities decrease the likelihood of density acting as a 'hidden treatment' (*sensu* Huston, 1997) when changes in floristic diversity are being related to the presence or absence of AM fungi.

Suppression of AM fungi altered community structure in intact microcosms taken from this semi-arid herbland. Change in the abundance of component species was partially explained by species response to AM fungi, but competitive interactions between species were also apparent. The reduced growth of non-responsive host species *S. verbenaca* and *G. setifolia* in benomyl-treated microcosms provides evidence for strong influences of AM fungi on interspecific competition in these systems. Mycorrhizas also indirectly influence the reproductive output of the dominant plant species, *M. minima* and *C. annua*, in Brookfield North microcosms. The combined mycorrhizal influence on vegetative and reproductive successes of dominant plant species suggests that AM fungi may play a significant role in structuring these plant communities over time. The semi-arid herbland at Brookfield Conservation Park is grassland degraded by overgrazing and disturbance of the cryptogamic surface crust. The loss of perennial grasses and invasion by ruderal species reflects a shift to pioneer species less dependent on the maintenance of stable mycorrhizal activity. While mycorrhizas clearly alter community interactions, the lack of a net effect of these interactions on floristic diversity may be due to the successional state of the community in which mycorrhizas influence several weakly-responsive dominant species through strong indirect effects on responsive competitors. Further investigation of the effects of arbuscular mycorrhizas on the structure and floristic diversity of this semi-arid plant community under field conditions are described in the following chapters.

Chapter 6

Mycorrhizas influence plant community structure, richness, evenness and diversity in a semi-arid herbland

6.1 Introduction

Plant communities often include species with varying response to the presence of AM fungi. This variation may range from non-mycorrhizal plants with benign or possibly antagonistic interactions with the fungi (Francis and Read 1995), to highly responsive plant species with positive growth responses to AM association (Johnson *et al.* 1997). The composition of natural plant communities often includes plant species from across this response spectrum, and may be determined by differential contributions of the mycorrhizal fungi to individual plant species fitness (Sanders and Koide 1994; Sanders *et al.* 1999). Few investigations of the effects of the AM symbiosis on the composition natural plant communities have been carried out under field conditions (Sanders *et al.* 1999). Field studies are necessary to determine the influence of mycorrhizas on whole plant community structure and diversity. Plant species occurring in the semi-arid herblands at Brookfield Conservation Park displayed differential responsiveness to mycorrhizas and some effects of mycorrhizas on plant-plant interactions in microcosms (Chapter 5). To understand these plant-plant interactions more fully and to determine the effects of mycorrhizas on the structure in these semi-arid plant communities, studies need to be conducted in the field with natural species assemblages and at natural plant densities.

In plant communities, the influence of mycorrhizas on the growth of individual species is affected by plant-plant interactions. An established mycorrhizal mycelium is a resource and co-occurring plant species may differ in their ability to compete for this resource despite showing individual responsiveness to mycorrhizal association when grown separately in pots (Newman *et al.* 1992). The extent of host-plant benefit from mycorrhizas is also density dependent (Koide and Li 1991; Facelli *et al.* 1999) and may be influenced by neighbour competition (Hartnett *et al.* 1993). Accordingly, changes in the abundance or size of individuals from one species may influence the abundance and size of individuals of the same or different species. The role of mycorrhizas in

regulating community structure will therefore depend on the identity and functional characteristics of the plant species present and can only be satisfactorily examined in intact communities.

Several studies have found that ecosystem processes such as primary productivity are linked to species diversity (Naeem *et al.* 1994; Tilman *et al.* 1996; Tilman *et al.* 1997; Hector 1999). While increasing species richness has been shown to increase primary productivity, species identity is also an important factor controlling productivity (Hooper and Vitousek 1997; Huston 1997; Tilman *et al.* 1997; Symstad *et al.* 1998; Wilsey and Potvin 2000). Some resolution to the question of whether increased productivity is a function of species identity or diversity has been offered by Wilsey and Potvin (2000). These authors suggest that diversity, measured as evenness, i.e. the distribution of abundance or biomass among species in a community, can have a direct effect on plant productivity. However, these results come from experiments where evenness was manipulated in non-natural plots with very low species richness.

Grime *et al.* (1987) found that community structure could be significantly altered by mycorrhizal activity in species-rich mixtures of plants in experimental microcosms. The presence of mycorrhizas can increase floristic diversity (Grime *et al.* 1987) and species richness (Gange *et al.* 1990); however this may depend on the identity and mycorrhizal responsiveness of the dominant plant species (Bergelson and Crawley 1988). Hartnett and Wilson (1999) have recently shown that suppression of mycorrhizal fungi resulted in an increase in floristic diversity in a tallgrass prairie, probably because the dominant C_4 grasses in that system are more strongly responsive to mycorrhizal colonisation than the other species present.

Field-based studies of competition involving mycorrhizas have been conducted in relatively few plant ecosystems. Edaphic and climatic conditions, floristic composition and stage of succession all contribute to structuring plant communities and all may vary between different communities. To fully appreciate the importance of mycorrhizas in community structure and plant diversity it is necessary to examine the contribution of mycorrhizas to a range of ecosystems. Extensive tracts of open woodland in semi-arid Australia have been overgrazed, with the understory reduced to annual herbland often

dominated by introduced species. The herb and grass species comprising these disturbed communities differ in their response to mycorrhizal fungi (Chapter 4). The influence of mycorrhiza in controlling plant diversity may be vitally important in semi-arid ecosystems, where communities can be subjected to dramatic seasonal and inter-annual fluctuations, and may rely on high biodiversity to maintain stability (Grime 1997). This study was designed to test experimentally the relationship between mycorrhizal responsiveness of plants from a semi-arid herbland and the influence of mycorrhizas on the productivity and structure of this plant community.

I hypothesise that the strength of the mycorrhizal responsiveness of the subordinate species in a multi-species community is not as important in determining species diversity as the strength of competition from the dominant species. To test this hypothesis experimentally, changes in the community structure of two similar semi-arid herblands were measured after formation of mycorrhizas had been suppressed by fungicide application. The investigations were designed to answer the following questions.

1. Does suppression of the AM fungi influence growth of the plant species present in these semi-arid herblands?
2. Are interactions between the different plant species in these communities altered by suppression of AM fungi?
3. Does suppression of AM fungi change plant species richness, diversity and evenness in these semi-arid herblands?
4. Do these closely related herblands differ in their response to suppression of AM fungi?

Experiments were carried out over two years, 1997 and 1998. Results from the different years are described and discussed separately.

6.2 Materials and Methods

6.2.1 Site descriptions

These experiments were conducted at Brookfield Conservation Park in 1997 and 1998.. Two study sites were selected to account for variation in plant species composition in the Park. Site and soil descriptions are given in the Chapter 3.

6.3 Plant community structure 1997

6.3.1 Introduction

To investigate the influence of AM fungi on community structure in semi-arid herblands, two experimental sites were established at Brookfield Conservation Park, South Australia in 1997. The two sites were chosen to represent slightly different plant communities. These sites were used to establish techniques for the study of mycorrhizal associations under field conditions.

6.3.2 Materials and Methods

6.3.2.1 Field plot establishment

In March 1997, thirty-nine experimental plots were established at Brookfield Conservation Park. These plots were 1.5 m^2 and were established in open herbland (>10 m to the nearest tree or large shrub) by covering them with a wire cage (5 cm grid mesh, 1.5 m x 1.0 m x 0.5 m) to prevent grazing by large herbivores. Fifteen plots were established in five blocks at Brookfield North and twenty-four plots in eight blocks were established at Brookfield South. Each plot was separated from the others by a 1.5 m spacing, to ensure adequate buffering of any plot effects. Each plot was divided into a central zone 1 m^2 and a surrounding buffer zone to reduce edge effects from surrounding untreated vegetation. Plots in each block were randomly assigned one of the three treatments (mycorrhiza-suppressed, watered and unwatered control).

6.3.2.2 Benomyl and water applications

Mycorrhiza-suppressed plots received the fungicide benomyl as a soil drench (Benlate® Du Pont, 6 g a.i. in 15 L of water per plot). Watered plots received 15 litres of water per plot and control plots received no amendments. Treatments began immediately after the first rains in April, before any germination had occurred and were repeated every three weeks for a total of 10 applications of 15 L each, ending in early November. Previous experiments had shown that benomyl was an effective fungicide for the suppression of colonisation of plant roots by Glomalean fungi in field plots (Merryweather and Fitter 1996). The use of benomyl is thought to be a conservative approach to studying AM effects on plant communities (Wilson and Hartnett 1997). It was necessary to include a treatment receiving only water as well as an unwatered

control treatment to determine effects of additional water on the vegetation at these low rainfall sites.

6.3.2.3 Assessment of mycorrhizal activity

Assessment of the AM status of field plots was made in the middle of the growing season and again late in the growing season, before annual plants had begun to senesce. Assessment in mid-season, after six benomyl applications, was made by collecting roots from the surface 10 cm of soil in the central zone of each plot using a 14 mm diameter cork-borer. Five cores were removed from randomly selected points in each plot and bulked. Live roots were washed free of soil and debris and stored in 50 % Ethanol for later examination.

Late in the season, after 10 treatment cycles, the AM status of field plots was assessed by measuring the mycorrhizal colonisation potential of treated soils using a trap-plant bioassay. One soil core was collected from each plot using the method described in Chapter 3. PVC cores (10 cm x 15 cm) of intact soil were taken to a glasshouse where they were kept free of weeds and planted with germinated seeds of *Trifolium subterraneum* L. cv. Mt Barker. Cores were watered three times per week and harvested after 6 weeks growth. A sub-sample of root material from each core was taken for estimation of mycorrhizal colonisation.

For estimation of root length colonised after both collection procedures above, roots were cut into approximately 1-cm lengths and cleared and stained according to the procedure set out in Chapter 3.

6.3.2.4 Measurement of plant cover and community structure

The vegetation in each plot was assessed using a point-quadrat method early and late in the growing season. For point quadrat assessment of the vegetation, a vertical frame consisting of 5 50 cm long pins set 10 cm apart was positioned at 10 points (10 cm apart) within the central zone of each plot, for a total of 50 points. All plants touching each pin were recorded to yield a cover frequency for each plant species (number of touches per species ÷ 50 pins x 100), and a relative cover estimate (number of touches per species ÷ total number of touches of all species x 100). Cover frequency of each

species was used to calculate species richness (*S*), diversity (Shannon *H'*) and evenness (Shannon *J*), using equations 1, 2 & 3 in Chapter 3.

6.3.2.5 Statistical analysis

Changes in community structure were analysed by conducting a MANOVA on relative cover and on total cover. Cover estimates were arcsine transformed to improve multivariate normality before analysis. Plots were used as replicates and treatments as factors in the analysis. Plant species were included as variables (in each case only the most abundant species were included in analysis, ie. species accounting for more than 4 % of total cover in any of the treatments). Where the MANOVAs indicated a significant treatment effect, two-way ANOVA was performed on percent cover to assess the influence of treatment on individual species. Effects of suppression of mycorrhiza on species richness, diversity and evenness were analysed using one-way ANOVA.

All percentage colonisation data were arcsine squareroot transformed before analysis to improve normality (Zar 1999). Differences between treatments with respect to mycorrhizal status and activity in field plots were tested by one-way ANOVA. Treatment effects on cover estimates for a given species were significant when mean cover between treatments differed by more than the Least Significant Difference (LSD) calculated from the data. Root colonisation levels and indices of diversity were separated by Tukey's HSD test for significantly different means at both experimental sites.

6.3.3 Results

6.3.3.1 Rainfall

Monthly rainfall records from the Blanchetown meteorological station (10 km from the Brookfield North site) in 1997 are shown in Fig. 3.2. Total rainfall for 1997 was 288 mm and rainfall during the growing season (March – September) was extremely low. Rainfall was well below the long-term monthly average for March, April, June and July 1997 (Fig. 3.2).

6.3.3.2 Suppression of AM fungi

In the experiments in 1997, the application of benomyl at the rate of 4-g m^{-2} did not significantly reduce the colonisation potential of AM fungi in field plots at either site (Table 6.1). Colonisation of the mixed root samples from field plots at Brookfield North was not significantly different in the benomyl treated plots from the watered control plots. Colonisation of *T. subterraneum* roots in cores taken from the field after ten treatment cycles was not significantly different in benomyl treated plots than in watered or unwatered control plots at either site (Table 6.1).

6.3.3.3 Relative cover

There was no significant ($P > 0.05$) interaction between treatment and relative cover of any of the nine most common species or on all other species combined at Brookfield South (Table 6.2). Relative cover of plant species present at Brookfield South is shown in Appendix 3.

There was a significant ($P < 0.01$; Table 6.3) effect of treatment on relative cover of species at Brookfield North. Nested two-way ANOVA showed that there was a species-by-treatment interaction ($P < 0.001$; Table 6.4). *Post hoc* tests revealed that the four most abundant plant species were differentially affected by treatment (Fig. 6.1). Relative cover of *C. annua* was lower and relative cover of *M. minima* was higher in water treated plots than in unwatered or benomyl-treated plots. Relative cover of *S. verbenaca* was lower in watered and benomyl-treated plots than in controls. In watered plots *V. arguta* had higher relative cover than in unwatered plots but was not significantly different to benomyl-treated plots.

6.3.3.4 Total cover of each species

Results of the MANOVA on total cover of major plant species showed that there was a significant interaction between species and treatment ($P < 0.05$; Table 6.2) at the Brookfield South site. There was also a significant ($P < 0.01$) main effect of treatment on relative cover (Table 6.2). Nested two-way ANOVA (Table 6.5) with *post hoc* tests showed significantly (LSD, $P < 0.05$) greater cover of *S. apetala* in benomyl-treated plots than in watered or unwatered control plots (Appendix 4). The only other species to show a significant response to treatment was *G. setifolia,* which had increased cover in

water and benomyl-treated plots relative to untreated controls (Appendix 4). There was a trend toward greater productivity in plots treated with water or benomyl, which was supported by the main effect of treatment on total cover (Table 6.2).

The MANOVA on total percentage cover of major species at the Brookfield North site showed a significant interaction between species and treatment at this site ($P < 0.01$; Table 6.3). Subsequent analysis by two-way ANOVA and *post hoc* testing (Tukey's HSD), revealed significant ($P < 0.05$) changes in the percent cover of two species due to treatment (Fig. 6.2). The cover of *C. annua* was significantly greater in benomyl-treated plots than in watered plots (Fig. 6.2). Cover of *C. annua* in control plots was intermediate and not significantly different from either of the other treatments. Cover of *M. minima* was significantly ($P < 0.05$) greater in watered plots than in benomyl-treated or unwatered control plots (Fig. 6.2).

6.3.3.5 Richness, diversity and evenness

There was a significant ($P = 0.013$) difference in species richness between treatments at the Brookfield South site in 1997 (Table 6.6). Application of water, with or without benomyl, increased species richness relative to unwatered controls. There was, however, no significant ($P = 0.60$) difference in diversity (H') or evenness (J) between treatments at this site (Table 6.6). Treatment with benomyl or water had no significant effect on richness ($P = 0.634$), diversity ($P = 0.337$) or evenness ($P = 0.316$) in the plant community at Brookfield North in 1997 (Table 6.7).

Table 6.1 Effect of benomyl (fungicide) on mycorrhizal colonisation potential compared to watered and unwatered controls at Brookfield North and Brookfield South 1997. Mycorrhizal colonisation potential measured as mean (±SE) percentage of root length colonised.

	% Root length colonized		
	Field roots	Bioassay on field cores	
	Brookfield North[1]	Brookfield North	Brookfield South[2]
Control	NA[3]	63.8 (16.3)	76.5 (5.8)
Water	54.4 (3.2)	71.2 (12.8)	73.5 (14.6)
Fungicide	57.5 (9.3)	57.8 (5.8)	70.1 (11.4)
		Analysis of variance	
P – value	0.643	0.199	0.631

[1]n = 5, [2]n = 8, [3]NA = not available

Table 6.2 Results of MANOVA testing the effect of benomyl and water addition on total and relative plant cover for each species in Brookfield South field plots 1997. Treatment was used as a factor and the nine major species plus all other species combined were used as variables within blocks, n = 8. * $P < 0.05$, ** $P < 0.01$, ns = not significant

	Brookfield South																	
MANOVA	Relative cover									Total Cover								
Transformations	arcsin									arcsin								
Factor	Treatment			Species			T*S			Treatment			Species			T*S		
Test statistics	df	test	*P*	df	test	*P*	df	test	*P*	df	test	*P*	df	test	*P*	df	test	*P*
Approx. F test	2,203	0.00	ns	9,203	72.33	**	18,203	1.30	ns.	2,203	5.45	**	9,203	59.75	**	18,203	1.72	**

Table 6.3 Results of MANOVA testing the effect of benomyl and water addition on total and relative plant cover for each species in Brookfield North field plots 1997. Treatment was used as a factor and the eight major species as well as all other species combined were used as variables within blocks, n = 5. * $P < 0.05$, ** $P < 0.01$, ns = not significant

	Brookfield North																	
MANOVA	Relative cover									Total Cover								
Transformations	arcsin									arcsin								
Factor	Treatment			Species			T*S			Treatment			Species			T*S		
Test statistics	df	test	*P*	df	test	*P*	df	test	*P*	df	test	*P*	df	test	*P*	df	test	*P*
Approx. F test	2,104	0.00	ns	8,104	51.99	**	16,104	4.30	**	2,104	1.07	ns	8,104	30.38	*	16,104	2.10	*

Table 6.4 Results of ANOVA testing the effect of treatment of field plots with fungicide, water or unwatered controls on total cover and relative cover for the main species Brookfield North plots 1997, n = 5.

ANOVA		Total cover			Relative cover		
Transformation		arcsin (%)			arcsin (%)		
Factors	df	MS	F-value	$P > F$	MS	F-value	$P > F$
Fungicide treatment	2	0.0125	1.08	0.343	0.0000	0.00	0.996
Species	7	0.3367	29.09	< 0.001	0.2209	46.91	< 0.001
F * S	14	0.0247	2.13	0.017	0.2096	4.45	< 0.001

Table 6.5 Results of ANOVA testing the effect of treatment of field plots with fungicide, water or unwatered controls on total cover for the main species in Brookfield South plots 1997, n = 8.

ANOVA		Total cover		
Transformation		arcsin (%)		
Factors	df	MS	F-value	$P > F$
Fungicide treatment	2	0.0966	5.45	0.005
Species	9	1.0591	59.75	< 0.001
F * S	18	0.0304	1.72	0.039

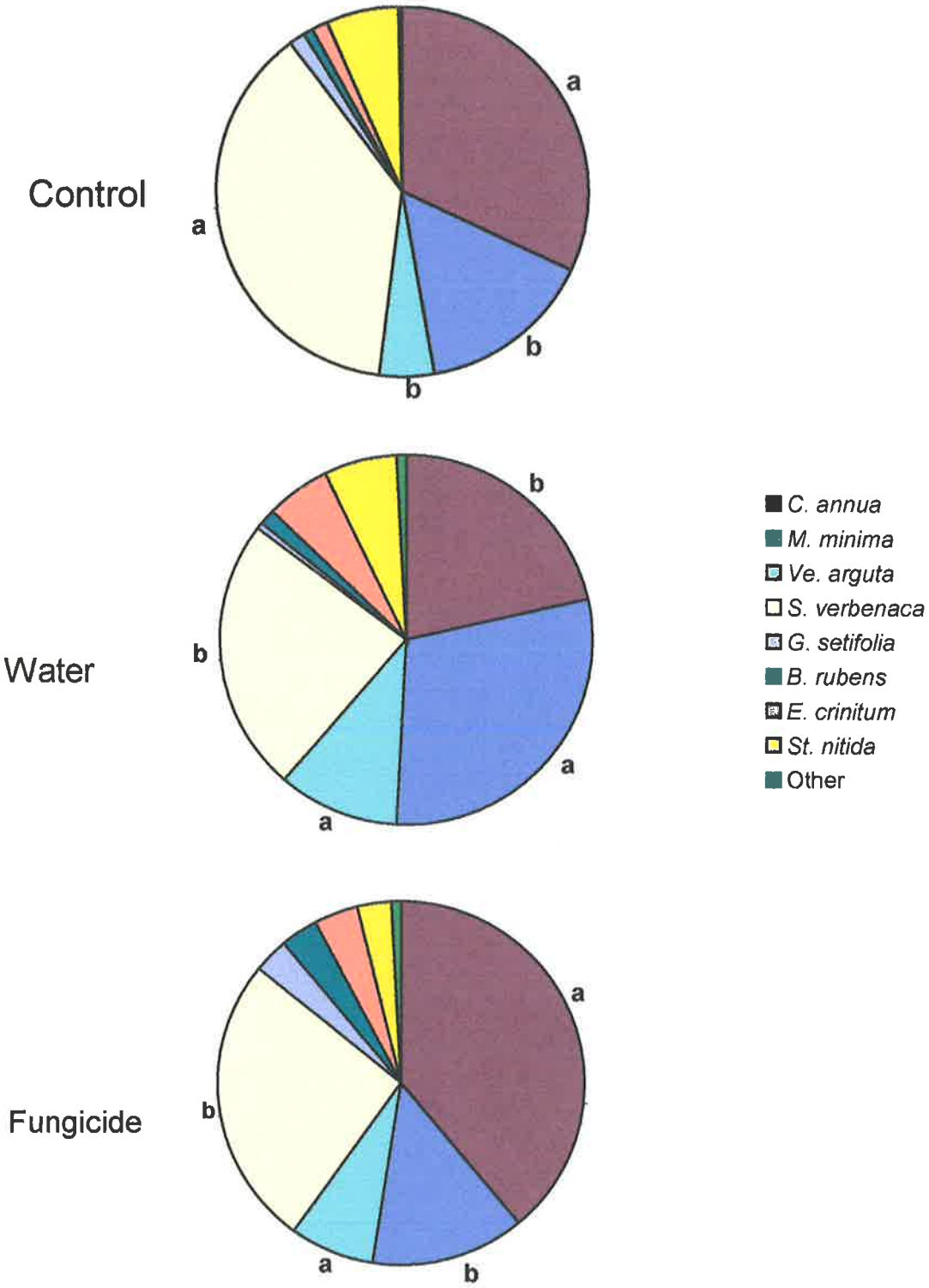

Figure 6.1 Effects of fungicide (benomyl) treatment on relative cover of major plant species compared to watered and unwatered control plots at Brookfield North 1997. Species with significantly (LSD, $P < 0.05$) different response to treatments are marked with different letters in those treatments, n = 5.

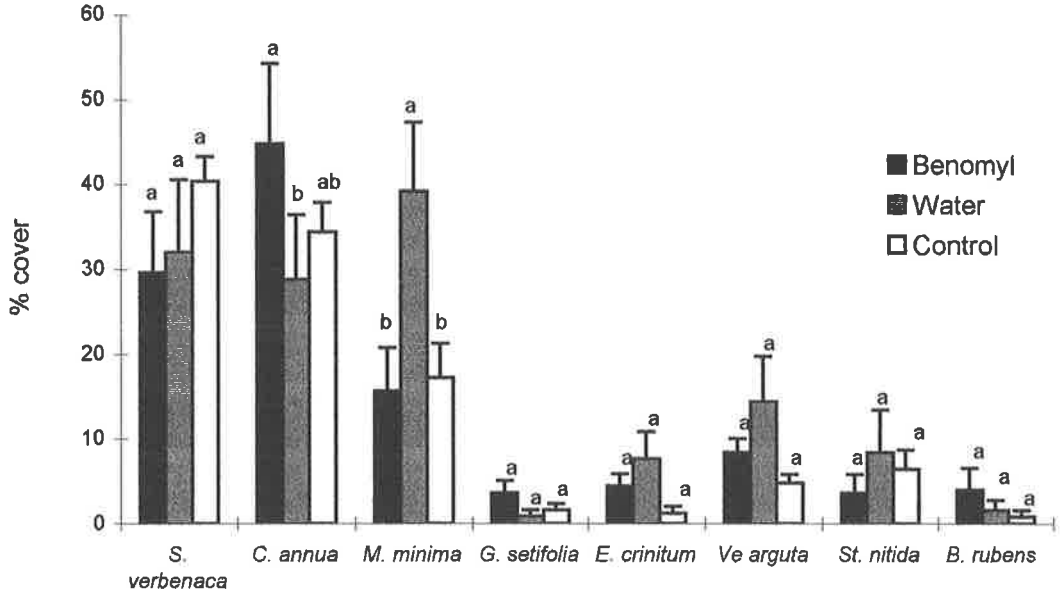

Figure 6.2. Percentage cover of each major species in plots treated with benomyl compared to watered control and unwatered control plots at Brookfield North 1997. Different letters above the bar indivates a significant difference (LSD, $P > 0.05$) between treatments for that species. Minor species (5) each represent less than 1% of total cover in each of the treatments, n = 5.

Table 6.6 Mean (SE) plant species richness, diversity and evenness in Brookfield South plots 1997. Effects of fungicide (benomyl) compared to watered and unwatered controls. Means within a parameter followed by the same letter are not significantly different ($P < 0.05$; Tukey's HSD), $n = 8$.

Treatment	Richness (*S*)	Diversity (*H'*)	Evenness (*J*)
Control	6.6 (1.4)[a]	1.41 (0.26)[a]	0.75 (0.07)[a]
Water	8.6 (1.6)[b]	1.65 (0.17)[a]	0.77 (0.08)[a]
Fungicide	8.5 (1.2)[b]	1.64 (0.16)[a]	0.77 (0.05)[a]
		Analysis of variance	
P - value	0.013	0.060	0.738

Table 6.7 Mean (SE) plant species richness, diversity and evenness in Brookfield North plots 1997. Effects of fungicide (benomyl) compared to watered and unwatered controls. Means within a parameter followed by the same letter are not significantly different ($P < 0.05$; Tukey's HSD), n = 5.

Treatment	Richness (S)	Diversity (H')	Evenness (J)
Control	5.2 (0.37)[a]	1.42 (0.05)[a]	0.87 (0.02)[a]
Water	5.6 (0.51)[a]	1.57 (0.10)[a]	0.92 (0.04)[a]
Fungicide	5.8 (0.58)[a]	1.51 (0.09)[a]	0.87 (0,01)[a]
		Analysis of variance	
P - value	0.634	0.337	0.316

6.3.4 Discussion

Plant communities at both sites in Brookfield Conservation Park were affected by applications of benomyl and water. Results from these field experiments support the findings from studies of these plant communities in microcosms (Chapter 5). Application of benomyl tended to increase the cover of the non-mycorrhizal host *C. annua* (at Brookfield North) and the almost non-host *S. apetala* (at Brookfield South). At Brookfield North this change in abundance of *C. annua* was offset by an increase in the abundance of the strongly mycorrhiza-responsive host plant *M. minima*. These changes were not apparent at Brookfield South where the most abundant mycorrhiza-forming plant species was the non-responsive host *G. setifolia*. Changes in community structure were larger at Brookfield North than at Brookfield South, although species richness, diversity and evenness were not different in fungicide-treated plots relative to watered control plots at either site.

The results of this experiment show that the activity of AM fungi was not significantly suppressed in field plots at either site when benomyl was applied at the rate of 4 g m^{-2}. This was an unexpected result as numerous studies have successfully suppressed AM colonisation with this or a lower rate of benomyl-application (Jalali and Domsch 1975; Fitter and Nichols 1988; Merryweather and Fitter 1996; Pedersen and Sylvia 1997; Hartnett and Wilson 1999b). Some attempts to suppress AM fungi with benomyl have not been successful (Koide *et al.* 1988a; Cade-menun and Berch 1997; Pedersen and Sylvia 1997), suggesting that there may be site specific factors which interfere with the effectiveness of benomyl.

The method of detecting change in the activity of AM fungi used in this experiment was conservative and may not have detected dynamic changes throughout the growing season. The effect of benomyl on AM formation can be dose dependant (Schreiner and Bethlenfalvay 1997a) and may depend on the timing of application and site conditions (Pedersen and Sylvia 1997). The use of root colonisation levels as a surrogate measure of mycorrhizal activity may also result in overestimation of the capacity of the AM association to enhance P-uptake. (Larsen *et al.* 1996). Larsen *et al.* (Larsen *et al.* 1996) showed that hyphal P-uptake was inhibited when benomyl was applied to an established

hyphal network in soil. Benomyl has also been shown to decrease spore germination and hyphal growth of several isolates of AM fungi (Schreiner and Bethlenfalvay 1997a). The extent and activity of the extra-radical mycelium may be significantly lowered in benomyl-treated soils without large reductions in intra-radical colonisation levels. This is even more likely when colonisation levels are measured as totals (living and dead hyphae) and not as living only, because previously poisoned infection units may remain visible until the fungal structures degenerate. Mycorrhizal effects on plant growth may also have occurred early in the growth of plants, before AM activity was assessed.

Colonisation levels measured in field collected roots from benomyl-treated plots at Brookfield North may also have been inflated by the method of assessment. The only plant species to show significant increase in total cover in benomyl-treated plots was *C. annua*. While *C. annua* is a non-mycorrhizal plant, it has a fine root system, which is easily damaged during collection and clearing and staining. If such damage did occur, root samples from field plots may have underrepresented the uncolonised roots in samples from all plots, but more so where non-mycorrhizal roots were more abundant. The second method of assessing AM activity in these soils was also conservative. While benomyl is known to suppress the germination of AM fungal spores (Schreiner and Bethlenfalvay 1997a), the spores may not be killed and may germinate at a later stage when benomyl concentrations have declined. The bioassay method used also assumes that *Trifolium subterraneum* roots will be colonized in proportion to the activity of AM fungi in the soil. Over a six week growth period in a low nutrient soil, plant growth may slow, presenting less root for depleted inoculum to colonise. As infection units grow and the root is more completely colonized by the AM fungi, the relative rate of extension of infection units may slow (Bruce *et al.* 1994), resulting in an equalisation of colonisation between inoculum sources of varied size or activity.

While the methods of assessing the activity of AM fungi in the field may have been conservative, it remains possible that the activity of the fungicide was not sufficient to suppress the fungi significantly. The soil at the two field sites was a clay-loam with approximately 1 % organic matter and high $CaCO_3$. Benomyl is known to sorb to soil particles (Helweg 1977; Liu and Hsiang 1994) and can be degraded by soil organisms

(Yarden *et al.* 1985). The application of benomyl in these experiments may have been too infrequent, allowing partial degradation of the fungicide and possibly selection and growth of benomyl-degrading organisms, facilitating more rapid degradation after subsequent applications. The concentration of benomyl used may also have been insufficient to overcome the sorptive capacity of the experimental soil, rendering some of the fungicide biologically unavailable. This possibility was investigated at the Brookfield South site (Chapter 7).

Responses of individual plant species to treatment of soils with benomyl were weak. The greater percentage cover of *S. apetala* in Brookfield South plots treated with benomyl than in watered and unwatered control plots suggests an effect of benomyl on direct or indirect constraints on the growth of this species. The lower percentage cover of *G. setifolia* in unwatered control plots than in benomyl or water-treated plots is probably due to increased productivity in plots receiving water in this dry year. Plots at Brookfield South were invaded by the white snail, *Cernuella virgata*, which preferentially clustered in plots receiving water - with or without benomyl (Appendix 5). Due to the remoteness of the field sites and the desire to leave the plots as exposed to environmental processes as possible, the impact of the snails was minimised by baiting. Visual inspection of plants in all plots found no obvious effect of the snail infestation on the aboveground parts of the nine most abundant species. If the snails preferred to feed on some species rather than others, measurements of the cover of each species may have underestimated the production of species palatable to the snails. However, the minimal differences between treatments in the relative cover of each species suggest that snail damage was minimal or minimal and indiscriminate across treatments.

Plant species at Brookfield North showed responses to benomyl application consistent with loss of mycorrhizal function in the system. Benomyl-induced changes in the total cover of a species indicates that that species has shown an absolute net increase or decrease in cover due to treatment. A more subtle effect of treatment may be change in the cover of a species relative to the other species present in the community. Changes in relative cover may occur even where no change in the total cover of that species is apparent. Increase in the relative cover of a species in benomyl-treated plots may indicate either a direct effect of benomyl treatment on that species or effects of benomyl

on other species within the community. The increase in the relative cover of the mycorrhiza-responsive species *M. minima*, concomitant with the decrease in relative cover of the non-mycorrhizal species *C. annua* in watered plots compared to benomyl-treated and control plots indicates potential loss of mycorrhizal function of the highly mycorrhiza-responsive *M. minima* due to benomyl addition. This effect is also reflected in the absolute cover of these two species, which varied with treatment in direct relationship to mycorrhizal-responsiveness. The unresponsive mycorrhiza-forming plant *S. verbenaca*, showed a decrease in relative cover when productivity of the system was increased by the addition of water (with or without benomyl). This response to reduced abiotic stress supports the idea of an inverse relationship between stress tolerance and competitive ability (Grime 1979; Grime and Hodgson 1987). The total cover of *S. verbenaca* was not significantly altered by increase in the availability of water, possibly because net resource supply to *S. verbenaca* was not significantly different between plots (Davis *et al.* 1998).

Without direct evidence that the activity of mycorrhizal fungi was reduced by benomyl addition, it is speculative to suggest that mycorrhizal activity was an important influence on community structure or diversity at either site. The increase in species richness in plots receiving regular water (with or without benomyl) at Brookfield South was more likely to have been due to increased germination and survival of species which were in low abundance or require high soil moisture content to germinate or survive. The low rainfall in the early part of the growing season may have resulted in unfavourable conditions for some species in the dry control plots. The absence of any other effects of treatment on diversity at either site could be expected when mycorrhizal activity was not significantly different between treatments. Differential responses of the most abundant species could compensate for each other and changes in community structure would be masked in indices such as H' and J. Further investigation of these semi-arid herblands was warranted, and more effective control and measurement of the effects of benomyl on AM fungi required. Experiments were repeated with some modification in the subsequent winter growing season in 1998.

6.4 Plant community structure 1998

6.4.1 Introduction

The most notable characteristic of semi-arid Australia is the unpredictability of rainfall. The low rainfall in early months at Brookfield Conservation Park in 1997 resulted in relatively slow growth of plants and potentially influenced the outcome of experiments detailed in Section 6.3. To investigate the possible influence of mycorrhiza on these plant communities further, the experimental design was modified and repeated in 1998 at the Brookfield North site. Brookfield North was chosen because the changes in community structure had been more obvious at this site than at Brookfield South in 1997. The problem of snail infestation at Brookfield South also made this a less desirable site for further experiments. While the experimental design was similar to that used at Brookfield North in 1997, the aims were modified to overcome problems identified in the first year.

The aims of this experiment were;

1. to test the effects of a higher concentration of benomyl on AM fungi at Brookfield North,
2. to determine the effects of suppression of AM fungi on the growth of the plant species in the herb community at Brookfield North,
3. to determine whether interactions between the different species in this plant communities are altered by suppression of AM fungi,
4. to determine whether suppression of AM fungi changes plant species richness, diversity and evenness in this herb community,
5. to test the effects of benomyl on non-target soil fungi and soil nutrient availability.

6.4.2 Materials and methods

6.4.2.1 Field plot establishment

In March 1998, twenty-one new experimental plots were established at the Brookfield North site. These 1.5 m^2 plots were established in the same way as in 1997 (Section 6.3.2.1). Plots in each block were randomly assigned one of the three treatments (mycorrhiza suppressed, watered and unwatered control). Mycorrhiza-suppressed plots received the fungicide benomyl as a soil drench (Benlate® Du Pont, 9 g a.i. in 15 L of water per plot). The concentration of fungicide was increased from that used in 1997

because it had been shown that benomyl is sorbed to particles in the surface layer of this soil (see Chapter 7) and that the lower application rate was not effective in suppressing the capacity of AM fungi to colonise roots. Adequate suppression of the AM fungi requires percolation of the fungicide to more than 5 cm below the soil surface. Watered plots received 15 L of water per plot and control plots received no amendments. Treatments began after the first rains in April and were repeated every two weeks until mid-September, for a total of ten applications.

6.4.2.2 Assessment of mycorrhizal activity

Assessment of the effectiveness of benomyl for suppression of the activity of AM fungi was made by collecting roots from field plots on 6th June 1998 (after three applications of benomyl) and 8th August 1998 (after seven applications of benomyl). Roots were collected from the buffer zone of each plot by excavating the whole root system. On 6th June roots were collected from the dominant species *M. minima*, while on the 8th August roots were collected from each of the most abundant species *M. minima*, *C. annua*, *S. verbenaca* and *V. arguta.* Roots from each species in each plot were cut into approximately 1-cm lengths and cleared, stained and examined as described in Chapter 3.

6.4.2.3 Non-target effects of benomyl

To test for any effects of benomyl application on non-target soil fungi, three soil samples (40 g) were collected on 8th August from the surface 10 cm of the buffer zone of each of the plots. Sub-samples from each plot within a treatment were combined to produce a composite sample for assessing the population of fungi The remaining soil was used to determine the NO_3-N concentrations of soil after benomyl or water application. Extraction of fungal propagules and dilution plating methods followed a modified method of Alef (1995). Fungi were cultured at 10^{-2} - 10^{-5} dilutions.

6.4.2.3.1 Effects of benomyl on non-target soil fungi populations

To extract fungal propagules 5 g of moist soil was mixed in 0.1 % (45 ml) for 1 hr on an end-over-end shaker. One millilitre of this solution was diluted 1:10 in 0.1 % (autoclaved) $Na_2H_2P_2O_7$. Serial dilutions were made using this method to produce dilutions 10^{-2} - 10^{-5} for solution plating. Plates were made using half-strength PDA +

7.5 g agar L^{-1} (autoclaved). Antibiotics were added before pouring at rates of Streptomycin 25 μg g^{-1} and Ampicillin 37.5 μg g^{-1}. Three replicate plates of each soil dilution from plots in each treatment were made by applying 100 μL of soil dilutions 10^{-2} – 10^{-5} to the sterile plates. Plates were incubated at 25°C and observed after 1 week. Fungal colonies were counted on each plate and colonies were described using morphological features. Five colonies were sub-cultured and identified to genus or species. Only one fungus isolate, *Fusarium* sp. 'Brookfield' was absent from fungicide treated soils. This isolate along with another *Fusarium* isolate (*Fusarium* sp. 'Cambrai') known to be pathogenic to *Medicago sativum* L. was tested against *M. minima* and *C. annua* for pathogenicity in a controlled bioassay following a modified method of Keijer *et al.* (1997). *Salvia verbenaca* was not included in this test as seeds proved difficult to germinate unless buried in non-sterile soil.

6.4.2.3.2 Bioassay to determine in vitro *pathogenicity of* Fusarium *sp. 'Brookfield' on host plants*

Round Petri dishes (9 cm diameter) provided sterile growing conditions for seedlings and permitted continuous observation of plants and *Fusarium* isolates. Phytogel was used as the basal growth medium. Each plant and fungal isolate combination was replicated five times. Seeds of *M. minima* (pre-scoured on sandpaper) and *C. annua* were surface sterilised in 4 % NaOCl solution for 10 minutes and rinsed in distilled water. Seeds were placed on wet filter paper in sterile Petri dishes in an incubator at 22°C for 4 days. Four germinated seedlings of one of the plant species were placed at equal intervals on the Phytogel plates and secured with a drop of 1 % (w/v) water agar (see Figure 6.3). Square plugs 0.25 cm^2 of one of the *Fusarium* isolates were placed between the first and second and third and fourth seedling (Figure 6.3). Controls were established by inoculation with plugs of growth medium agar (Section 6.4.2.3.1). Petri dishes were sealed with Parafilm to prevent contamination and drying and the lower half of each plate was wrapped in aluminium foil to protect the roots from light. Plates were placed in an upright position at an angle of 75° and incubated in the dark at 25°C for three days. Plates were then transferred to a controlled growth chamber with day/night conditions of 22°C/16°C respectively, and a 14 hr light period (450 μE m^{-2}). Plates were observed every two days for development of the fungi and assessed for

symptoms of pathogen effects after 14 days. Pathogenicity scores for the four plants in each Petri dish were averaged before analysis.

Roots of all seedlings were observed under a light microscope at 5x magnification for symptoms of disease development. A disease severity index from 0 to 3 was developed to evaluate the extent of root discolouration on each plant. The absence of discolouration was rated 0. Where less than 25 % of the root showed symptoms of discolouration a rating of 1 was used. Roots showing between 25 % and 75 % discolouration were rated 2. Roots with more than 75 % of their length showing signs of discolouration were given a rating of 3.

6.4.2.3.3 Effects of benomyl and water addition on N and P concentrations in field soil

Soil samples were taken from field plots for assessment of soil N and P on two separate occasions. Samples were first taken on 8th August 1998, 18 hrs after the eighth application of benomyl. Samples were again taken on 18th November 1998 eleven weeks after the final application of fungicide, and after most annual plants had seeded. On both occasions three samples totaling approximately 100 g soil were collected from the surface 10 cm of the buffer zone of each plot using a cork-borer. The three samples from each plot were combined, sieved through a 1 mm sieve and stored at 4°C until extraction. On the second collection date plots in the unwatered control treatment were not sampled.

Available-P

Approximately 1 g of air-dry soil was weighed into acid-washed plastic bottles and 100 mL of 0.5 M $NaHCO_3$ added. Bottles were shaken end-over-end overnight and left to settle for 1 hr. A 2.5 mL sub-sample of the supernatent was added to 2.5 mL of 0.5 M H_2SO_4. Available-P was determined by the method of Colwell (1963).

Nitrate-N

Approximately 5 g of air-dry soil was weighed into plastic bottles and 50 mL of 2 M KCl was added. Bottles were shaken end-over-end for 1 hr and left to settle at 4°C

overnight. A 5 mL subsample of the supernatent was added to 5 mL deionised water. Nitrate-N was determined by the method of Best (1976).

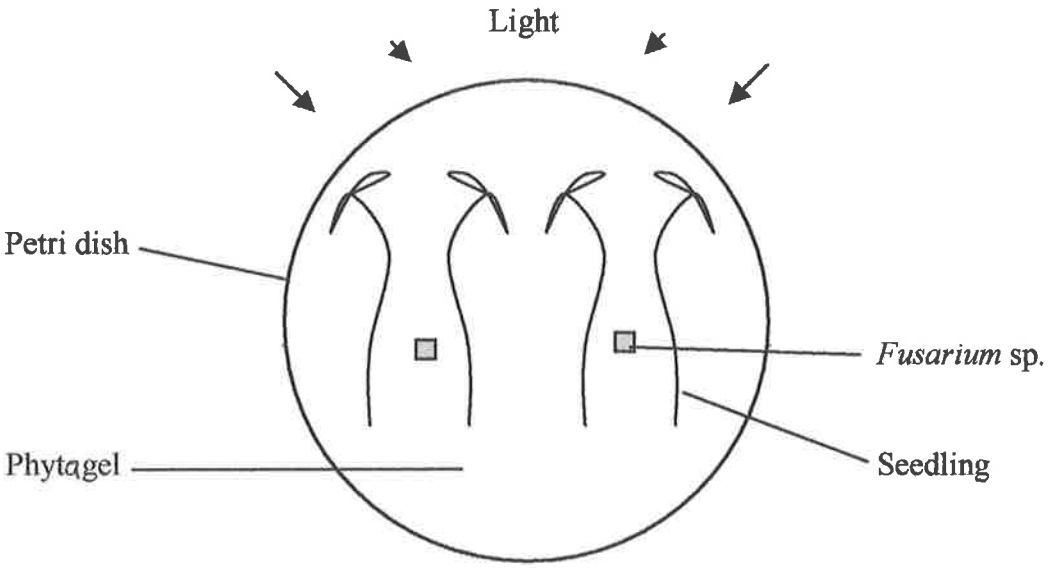

Figure 6.3 Schematic representation of the bioassay for *in vitro* determination of pathogenicity of *Fusarium* isolates on *M. minima* and *C. annua*.

6.4.2.4 Measurement of plant cover, number and biomass

The vegetation in each plot was assessed using a point-quadrat method (Section 6.3.2.4) early (6th June) and late in the season (8th August), and by collection of plants for biomass measurements at the peak of vegetative growth just before the onset of flowering. On 9th August 1998, a 20 x 20 cm sub-plot within each plot was sampled for number and standing biomass of all plant species. The plants were removed by cutting at the soil surface, separated into different species and were later dried overnight at 80°C in an oven before weighing for total dry weights. Cover frequency, plant number and biomass per species were used to calculate species richness (S, mean number of species per plot) diversity (Shannon's H') and evenness (J) using equations 1, 2 & 3 (Section 3.5).

6.4.2.5 Statistical analysis

Changes in community structure were analysed by conducting a MANOVA on plant number and biomass for each species. Plots were used as replicates and fungicide application was used as the treatment. Plant species were included as variables (only the six most abundant species were included as all other species represented less than 2% each of the total plot biomass and were irregularly distributed between the replicates). Because the MANOVAs indicated a significant treatment effect, two-way ANOVA was performed on species-by-number and species-by-biomass to assess the influence of treatment on individual species. Relative biomass and relative number data were arcsin transformed before analysis to improve normality (Zar 1999). Treatment effects on biomass and abundance for a given species were significant when mean biomass or number differed between treatments by more than the Least Significant Difference (LSD) calculated from the data. Effects of suppression of mycorrhiza on species richness, diversity and evenness were analysed using one-way ANOVA.

All percentage colonisation data were arcsine squareroot transformed before analysis (Zar 1999). Differences between treatments with respect to mycorrhizal colonisation of plants from field plots were tested by two-way ANOVA with species and treatment as factors. The number of colony forming units of fungi from field plots was analysed for differences between treatments by one-way ANOVA. Pathogenicity scores for the *Fusarium* isolates on *M. minima* and *C. annua* roots were tested for differences using a

Kruskal-Wallis test (Zar 1999) and scores for each inoculation treatment and plant species were tested for difference from zero using one-sample *t*-tests. Except for plant biomass and abundance data, treatment differences were separated by Tukey's HSD test for significantly different means in all experiments

6.4.3 Results

6.4.3.1 Rainfall

Monthly rainfall records from the Blanchetown meteorological station (10 km from the Brookfield North site) in 1998 are shown in Fig. 3.2. Total rainfall for 1998 was 353 mm and rainfall during the growing season (March – September) was low. Rainfall during the early part of the growing season in 1998 was low, with below-average rainfall in March, May and June (Fig. 3.2).

6.4.3.2 Suppression of mycorrhizal colonisation

The application of benomyl as a soil drench successfully suppressed mycorrhizal colonisation with respect to watered and unwatered control treatments throughout the growth period (Table 6.8). By early June colonisation of *M. minima* roots had been significantly reduced ($P < 0.001$, 6.8) in benomyl treated plots (50%) compared with both watered (75%) and control plots (74%). Total colonisation of the roots of host plant species just prior to the onset of flowering was reduced to ~50% that in watered and control plots. Root of *C. annua* displayed no signs of colonisation by AM fungi.

6.4.3.3 Growth and survival responses of plant species

The structure of this semi-arid herbland community was altered in benomyl-treated plots over the period of one growing season. There was a significant increase in total plot biomass in plots receiving water (with or without benomyl) relative to unwatered control plots ($P > 0.05$; Table 6.9). Treatment with water had no effect on the proportional distribution of biomass or abundance between species. Effects of mycorrhizal suppression on the relative contribution of each species to the biomass and abundance of each species in the field plots are shown in Figures 6.4 and 6.5, respectively. Benomyl applications resulted in a 62 % reduction in the proportion of total plot biomass made up by the mycorrhizal plant *M. minima* relative to watered plots (Figure 6.4). Benomyl treatment also resulted in an increase in the proportion of plot

biomass made up by the non-mycorrhizal plant *C. annua* (159 %) and the facultatively mycorrhizal species *S. verbenaca* (1,540 %) relative to watered plots. There was no significant ($P > 0.05$) change due to treatment in the relative contribution to plot biomass of any of the minor species (Figure 6.4).

Results from cover estimates were consistent with biomass and abundance results and are therefore not shown. The response of individual plant species to suppression of AM fungi in field plots was only partially explained by mycorrhizal responsiveness as determined in pot experiments (see Chapter 5). Results of MANOVAs on the interaction between treatment and species significant for both the total and relative biomass ($P < 0.01$; Table 6.10) and the total and relative plant number ($P < 0.01$; Table 6.11). The dominant species *M. minima* showed a significant ($P < 0.001$) reduction in aboveground biomass in plots treated with benomyl relative to watered and unwatered control plots (Figure 6.6). *C. annua* and *S. verbenaca* both showed significant ($P < 0.001$) increase in total aboveground biomass when benomyl was added to field plots (Figure 6.6). The only species to show significant ($P < 0.05$) change in plant abundance due to treatment were *S. verbenaca* and *C. annua*. The number of *S. verbenaca* seedlings surviving was greater in benomyl-treated plots than in watered or unwatered control plots (Figure 6.7). The number of individuals of *C. annua* was also greater in benomyl-treated plots than in unwatered control plots (Figure 6.7). Total plant density also increased in benomyl treated plots (Table 6.9). None of the other species present at the site showed any significant alteration ($P > 0.05$) in abundance or biomass due to benomyl treatment.

The relative abundance of individual species was also altered by application of benomyl (Figure 6.5). The relative abundance of *M. minima* was reduced in benomyl-treated plots compared to watered and unwatered controls. The relative abundance of *C. annua* remained approximately the same in different treatments, while *S. verbenaca* showed an increase in relative abundance in benomyl-treated plots with respect to watered (710 %) and unwatered control plots (Figure 6.5). Differences in the relative abundance of minor species were not significantly affected by treatment ($P > 0.05$).

6.4.3.4 Richness, diversity and evenness

Alterations in the abundance and biomass of individual plant species due to suppression of AM fungi did not translate to a net change in species richness (Table 6.9). Plant species diversity increased in mycorrhiza-suppressed plots relative to watered and unwatered control plots (Table 6.10). Species evenness also increased significantly ($P < 0.05$) in benomyl-treated plots compared to watered plots and unwatered control plots were not significantly different than either of the more productive treatments. Diversity increased by approximately 29 % and evenness by approximately 32 % relative to controls. The minor species (all species excluding *M. minima*, *C. annua* and *S. verbenaca*) contributed equally ($H' = 0.41$) to the diversity of both watered and fungicide-treated plots. Differences in diversity between the treatments came from changes in the contribution of the three most abundant species. These species contributed 71 % of H' in benomyl-treated plots but only 63 % in watered plots.

6.4.3.5 Non-target effects of benomyl

6.4.3.5.1 Effects on soil nutrient status

Benomyl-addition to field plots did not result in significant increases in available-P immediately after fungicide-treatment in the middle of the growing season ($P = 0.175$; Table 6.12), or at the end of the growing season after treatments had ceased ($P = 0.301$; Table 6.13). Available-P was higher in field soils in the middle of the growing season (August 1998) than in early summer (November 1998) when annual plants were already dead. Soil NO_3-N was significantly ($P < 0.001$) higher in fungicide-treated plots than watered and unwatered control plots within eighteen hours of the eighth application of benomyl (Table 6.12). The increase in available nitrogen in benomyl-treated plots relative to watered plots was still apparent at the end of the growing season ($P = 0.018$; Table 6.13).

6.4.3.5.2 Effects on soil fungi

In vitro tests on the population of culturable fungi in field soils revealed that benomyl-addition had a minor effect. The number of colony forming units of culturable fungi was not significantly different ($P = 0.354$) between control plots and watered or benomyl-treated plots (Table 6.14). Thirteen species of fungi were cultured from field soils in this experiment. The isolated fungi were characterised by morphological features and

identified to genus where possible. Only one isolate present in the control and watered plots was absent in the benomyl-treated soil. This was identified as an isolate of *Fusarium* sp. (*Fusarium* sp. 'Brookfield'). However, no fungi were observed on the roots of *C. annua* in any treatment in field plots at any time during this experiment.

In *in vitro* pathogenicity tests on the two dominant plant species from the site, *Fusarium* sp. 'Brookfield' was pathogenic on *C. annua* but was not pathogenic on *M. minima* (Table 6.15). The known pathogenic isolate *Fusarium* sp 'Cambrai' was more pathogenic on the roots of *M. minima* than the *Fusarium* sp. 'Brookfield' isolate but the pathogenicity of the two isolates was not significantly different on *C. annua* (Table 6.15). Pathogenicity scores for both isolates on both plant species were significantly different to zero, while scores in control treatments were not significantly different from zero ($P < 0.05$; Table 6.15)

Table 6.8 Mean (SE) percentage root length colonised with AM fungi in field plots at Brookfield North 1998 treated with benomyl (fungicide) compared to watered or unwatered controls. Means within a species and sampling time followed by the same letter are not significantly different (Tukey's HSD, $P < 0.05$), n = 7.

	Root length colonised (%)			
	June 1998	August 1998		
	M. minima	*M. minima*	*S. verbenaca*	*Ve. arguta*
Control	74.4 (2.4)[a]	80.5 (3.0)[a]	75.8 (2.8)[a]	62.1 (6.7)[a]
Water	75.4 (3.1)[a]	88.0 (2.0)[a]	80.2 (5.6)[a]	51.1 (7.5)[a]
Fungicide	49.5 (4.9)[b]	46.7 (5.1)[b]	45.2 (7.9)[b]	24.7 (2.6)[b]
		Analysis of variance		
P – value				
Treatment	< 0.001		< 0.001	
Species	NA[1]		< 0.001	
T*S	NA		ns[2]	

[1]NA = not applicable
[2]ns = not significant ($P > 0.05$)

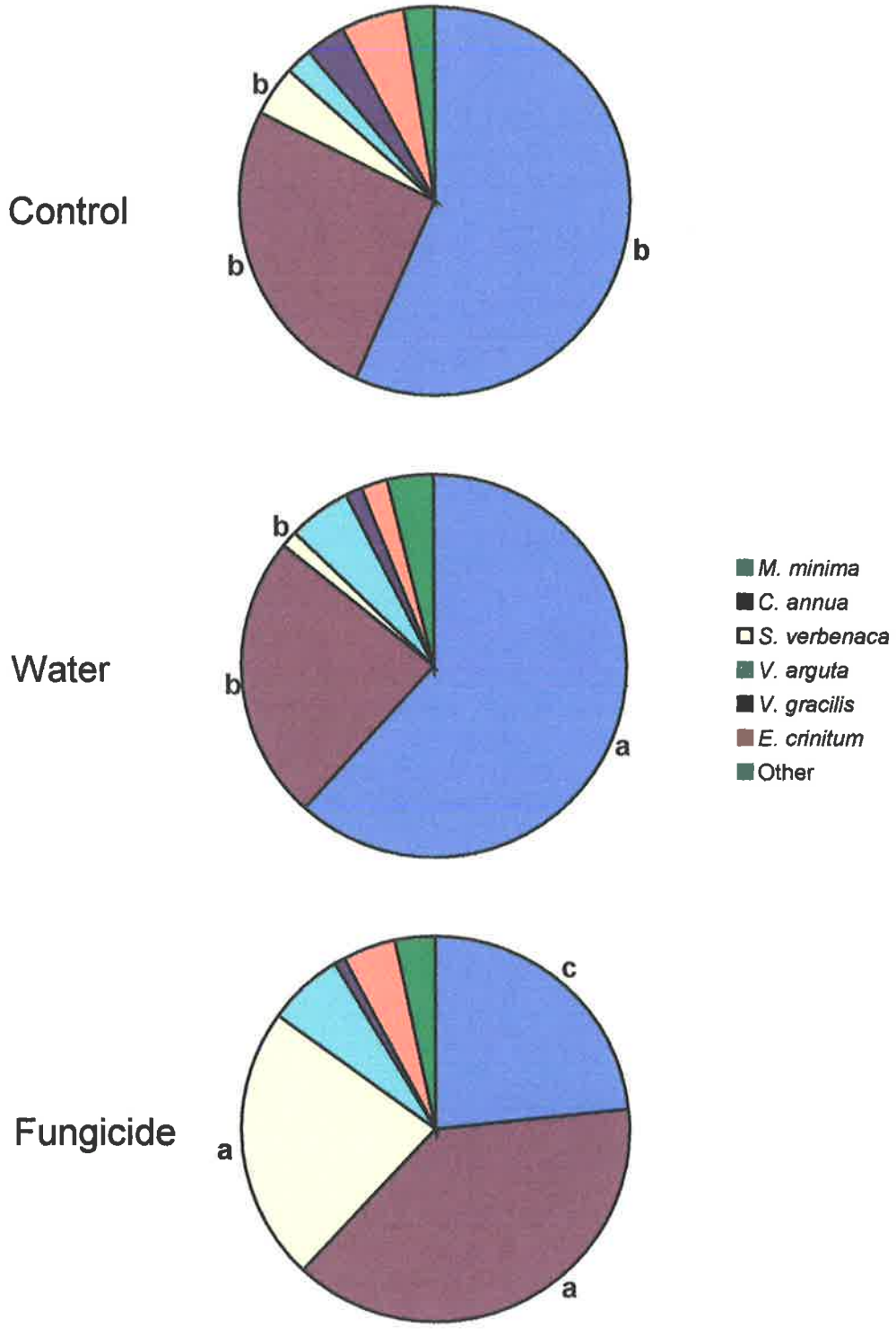

Figure 6.4 Species composition (relative aboveground harvestable biomass of each species) in plots with AM fungi suppressed by benomyl-addition (fungicide) compared to watered and unwatered control plots at Brookfield North 1998. Species with significantly ($P < 0.05$, LSD) different response to treatments are marked with different letters in those treatments, n =7.

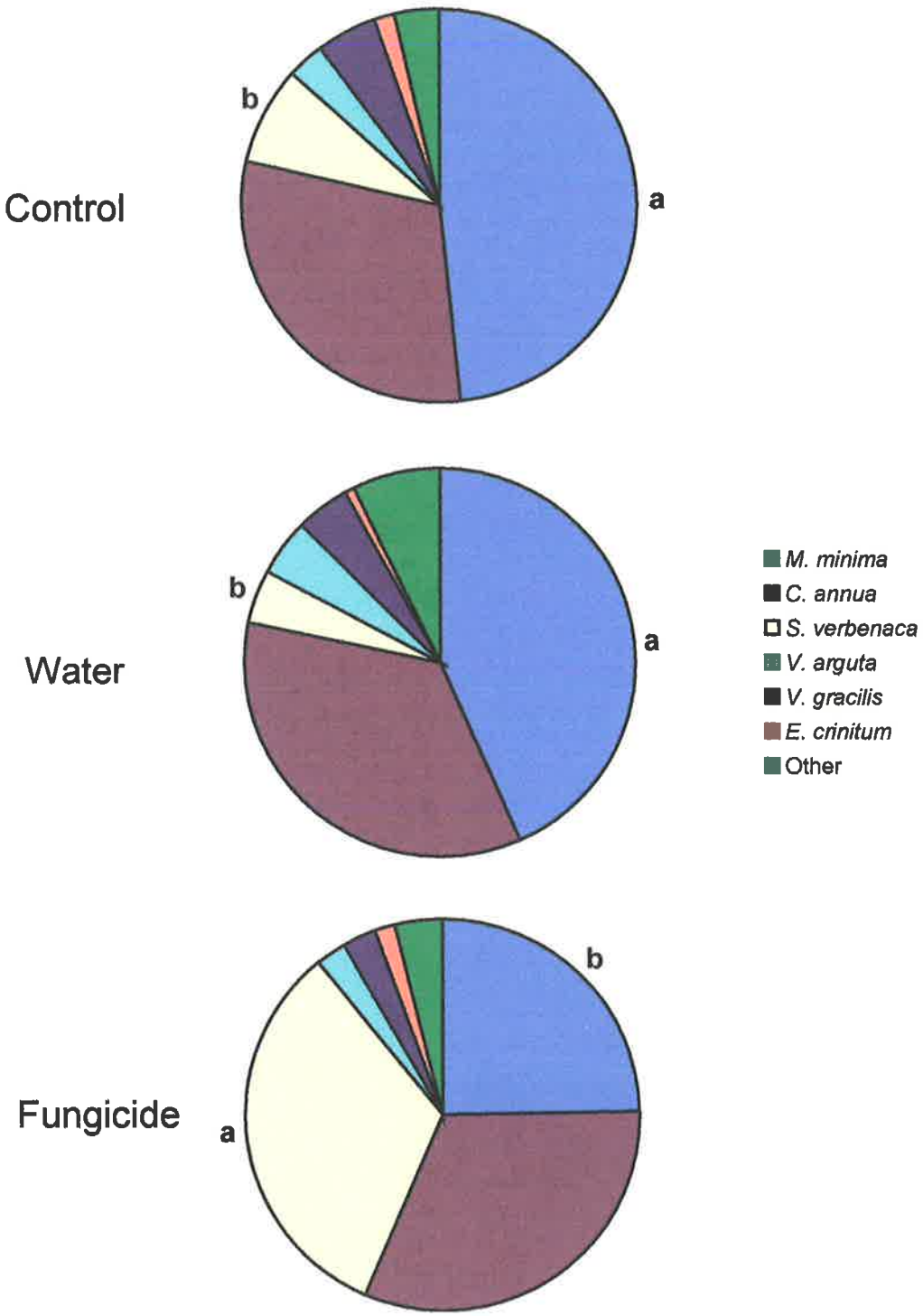

Figure 6.5 Species composition (relative abundance of each species) in plots with AM fungi suppressed by benomyl-addition (fungicide) compared to watered and unwatered control plots at Brookfield North 1998. Species with significantly ($P < 0.05$, LSD) different response to treatments are marked with different letters in those treatments, n =7.

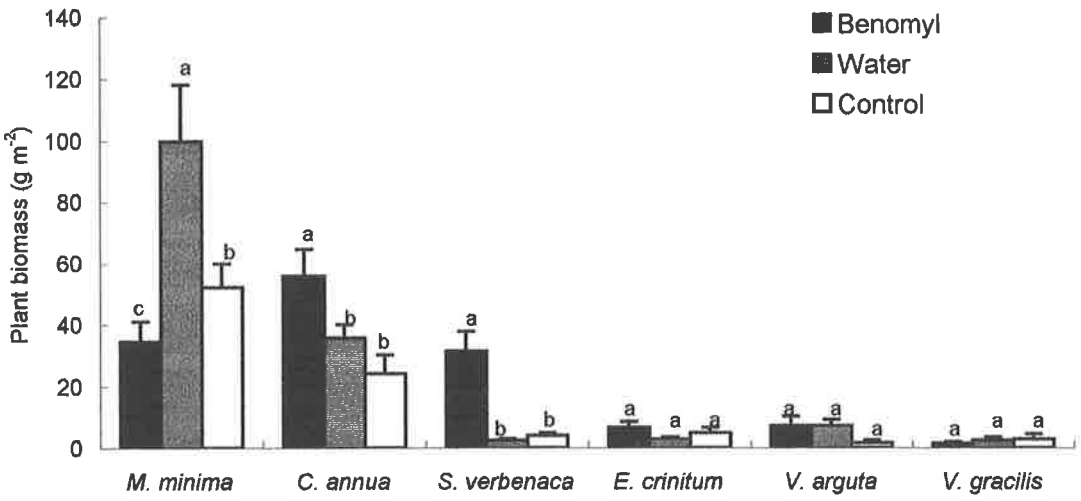

Figure 6.6 Aboveground biomass (mean ±SE) response of major plant species in field plots at Brookfield North 1998 to suppression of AM fungi by benomyl application compared to watered and unwatered control plots. Different letters above the bar indicates a significant difference ($P < 0.05$; LSD) between treatments for that species. Species shown accounted for > 90% of plot biomass, n = 7.

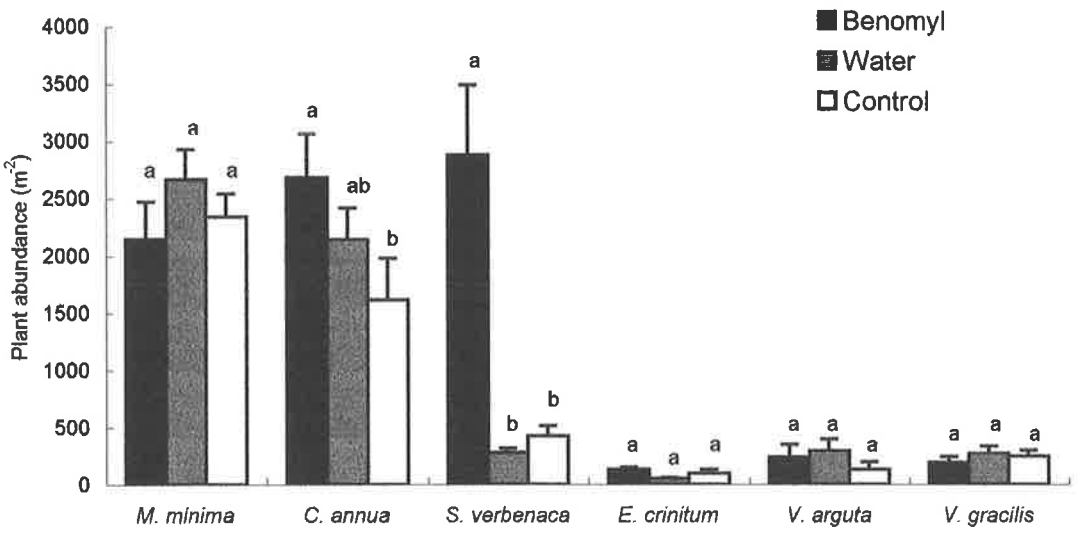

Figure 6.7 Abundance response (mean ±SE) of major plant species in field plots at Brookfield North 1998 to suppression of AM fungi by benomyl application compared to watered and unwatered control plots. Different letters above the bar indicates a significant difference ($P < 0.05$; LSD) between the treatments for that species. Species shown accounted for > 90% of plot biomass; n = 7.

Table 6.9 Mean (SE) aboveground dry matter production, plant density and plant species richness, diversity and evenness in field plots treated with benomyl (fungicide), water or unwatered controls, n = 7.

			Community indices		
	Aboveground dry matter ($g\ m^{-2}$)	Plant density (m^{-2})	Plant species richness (species m^{-2})	Plant species diversity (Shannon H')	Plant species evenness (Shannon J)
Control	91.3 (11.8)[a]*	5057 (571)[a]	6.71 (0.75)[a]	1.12 (0.10)[a]	0.60 (0.05)[ab]
Water	157.3 (22.3)[b]	6165 (436)[a]	8.29 (0.52)[a]	1.10 (0.04)[a]	0.53 (0.03)[a]
Fungicide	142.3 (17.5)[b]	8860 (1003)[b]	8.29 (0.84)[a]	1.42 (0.03)[b]	0.70 (0.05)[b]

* Different letters indicate a significant ($P < 0.05$; Tukey's HSD) difference between treatments for that parameter.

Table 6.10 Results of MANOVAs testing the effect of benomyl and water addition on total and relative plant biomass for each species in Brookfield North field plots 1998. Treatment was used as a factor and the six major species plus all other species combined were used as variables within blocks, n = 7. * $P < 0.05$, ** $P < 0.01$, ns = not significant.

MANOVA	Total aboveground biomass									Relative aboveground biomass								
Transformations	arcsin									arcsin								
Factor	Treatment			Species			T*S			Treatment			Species			T*S		
Test statistics	df	test	*P*	df	test	*P*	df	test	*P*	df	test	*P*	df	test	*P*	df	test	*P*
Approx. F test	2,120	4.20	**	6,120	52.96	**	12,120	8.74	**	2,120	0.00	ns	6,120	124.8	**	12,120	16.56	**

Table 6.11 Results of MANOVAs testing the effect of benomyl and water addition on total and relative plant abundance for each species in Brookfield North field plots 1998. Treatment was used as a factor and the six major species as well as all other species combined were used as variables within blocks, n = 7. * $P < 0.05$, ** $P < 0.01$, ns = not significant.

MANOVA	Total number of plants									Relative number of plants								
Transformations	arcsin									arcsin								
Factor	Treatment			Species			T*S			Treatment			Species			T*S		
Test statistics	df	test	*P*	df	test	*P*	df	test	*P*	df	test	*P*	df	test	*P*	df	test	*P*
Approx. F test	2,120	9.21	**	6,120	57.57	**	12,120	6.98	**	2,120	0.00	ns	6,120	87.93	**	12,120	8.81	**

Table 6.12 Mean (±SE) NO_3-N and available-P concentrations in Brookfield North plots on 8th August 1998 (18 hrs after the eighth fungicide and water application), n = 7.

Treatment	NO_3-N concentration ($\mu g\ N\ g^{-1}$ soil)	Available P concentration ($\mu g\ P\ g^{-1}$ soil)
Control	8.6 (0.8)[a]	32.6 (1.2)[a]
Water	7.2 (0.6)[a]	35.6 (2.6)[a]
Fungicide	14.7 (1.2)[b]	38.6 (4.3)[a]

Means within each parameter followed by the same letter were not significantly different ($P < 0.05$; Tukey's HSD)

Table 6.13 Mean (±SE) NO_3-N and available-P concentrations in Brookfield North plots on 18th November 1998 (11 weeks after the tenth fungicide and water application), n = 7.

Treatment	NO_3-N concentration ($\mu g\ N\ g^{-1}$ soil)	Available P concentration ($\mu g\ P\ g^{-1}$ soil)
Water	23.4 (2.9)[a]	9.9 (1.5)[a]
Fungicide	35.1 (4.8)[b]	14.5 (3.9)[a]

Means within each parameter followed by the same letter were not significantly different (one-way ANOVA, $P < 0.05$).

Table 6.14 Mean (SE) number of colony forming units of fungi from Brookfield North plots treated with benomyl (fungicide) or water. Fungi cultured *in vitro* by dilution plating, n = 6.

Plot treatment	Colony forming units (cfu g^{-1} soil)
Control	6881 (2592)
Water	8119 (2126)
Fungicide	3750 (1549)
Analysis of variance	
P-value	0.354

Table 6.15 Pathogenicity tests of *Fusarium* sp. 'Cambrai' and *Fusarium* sp. 'Brookfield' on the roots of *M. minima* and *C. annua*.

Fusarium isolate	Disease severity index	
	M. minima[1]	*C. annua*
Control (none)	0.10 (0.22)[a*2]	0.20 (0.21)[a*]
'Brookfield'	0.55 (0.11)[a]	1.95 (0.45)[b]
'Cambrai'	1.80 (0.54)[b]	2.35 (0.82)[b]
Kruskal-Wallis test *P*-value	< 0.001	<0.001

[1]Means for each plant species followed by the same letter were not significantly different ($P < 0.05$; Tukey's HSD)
[2]Mean not significantly different to zero ($P < 0.05$; one-sample *t*-test)

6.4.4 Discussion

The concentration of benomyl and the frequency of benomyl-application at Brookfield North in 1998 resulted in satisfactory suppression of AM colonisation of host plants. The effect of benomyl on AM formation can be dose dependent and may depend on the timing of application and site conditions (see Section 6.3.4 for further discussion). Several field studies have shown the effects of mycorrhizas can be significantly affected when levels of mycorrhizal root colonisation are reduced by small amounts. Carey *et al.* (1992) showed that effects of mycorrhizas were altered even when mycorrhizal root colonisation was only reduced to an average of approximately 70% of that in untreated controls. Smith *et al.* (1999) measured changes in individual plant growth, community structure and diversity when mycorrhizal root colonisation was reduced in benomyl-treated plots to approximately 40% of that in untreated controls. Suppression of AM colonisation in benomyl-treated plots to approximately 50% of the levels in control plots at Brookfield North is therefore considered adequate for investigation of the influence of AM fungi in this community.

While benomyl-addition reduced the activity of AM fungi in host plants throughout this experiment, potential side-effects of benomyl on soil nutrients and non-target fungi were also observed. Benomyl has been commonly used as a soil drench, but there has been little discussion of its effects on soil nutrient availability or plant nutrition (Cade-menun and Berch 1997). In this experiment there was an increase in NO_3-N concentration in benomyl-treated soils, potentially leading to increased plant growth. Several workers have reported improved plant growth and increased foliar N concentrations in benomyl-treated plants (Verkade and Hamilton 1983; Fitter 1986; Cade-menun and Berch 1997). There are several explanations for the enrichment of NO_3-N in these soils.

One explanation is that the nitrogen is released from the fungicide molecule during degradation. The fungicide benomyl, active constituent carbendazim, contains a benzimidazole group; N making up 19.3% of the whole benomyl molecule. The availability of this N to plants depends on degradation and immobilisation rates. Degradation rates are significantly enhanced by microbial activity (Helweg 1977; Yarden *et al.* 1985) and more rapid when the soil has previously been treated with the

fungicide (Helweg 1977). While species of fungi previously reported to be capable of degrading benomyl (*Alternaria alternata* and *Bipolaris tetramera*; Yarden *et al.*, 1985) were not found in the Brookfield North soils, degradation of the fungicide was apparent (results not shown), and release of N was assumed.

A second explanation for increase NO_3-N in benomyl-treated soils is that the fungicide caused a release of nutrients from microorganisms killed by the treatment. Populations of soil fungi were not significantly altered by benomyl treatment (except colonisation of AM fungi in the roots of host plants), though the method of culturing these organisms may not detect dynamic changes in active populations. Bacterial populations can also be altered by benomyl application (von Fassen 1974; Wainwright and Pugh 1974; Foster and McQueen 1977; Somda *et al.* 1991) and release of N may result from these changes. However, release of N from microbial biomass should be accompanied by release of other nutrients such as P from the decaying microbial cells. There was no significant increase in available-P in the benomyl-treated soils 18 hrs after fungicide was applied or after the growing season was over. While P released from microorganisms may have been more rapidly immobilised than N, it seems unlikely that the increased NO_3-N concentrations in benomyl-treated soils were the result of nutrient flush from decaying microbes.

A third possible explanation for increased NO_3-N concentrations in benomyl-treated soils is that benomyl affects transformation of N and rates of nitrification. Only NO_3-N was measured in this experiment, with concentrations of other forms of nitrogen unknown. It was assumed that most available N would be in the NO_3 form in this slightly alkaline soil (pH 7.85), however, nitrification which might naturally occur in these soils could be inhibited by the effects of benomyl on soil bacteria (von Fassen 1974; Foster and McQueen 1977; Somda *et al.* 1991). If this were the case differences in N concentrations between soils under different treatments may be less important than suggested by NO_3-N concentrations, and depend on the capacity of individual plant species to take up different forms of N. If N was more available in benomyl-treated soils, non-leguminous plants may have been advantaged over legumes in these plots.

In this study approximately 12 g N m^{-2} was added as part of the fungicide over the growing season. Eightly percent of this had been added by the time of the first

measurement of soil NO_3-N. This is a significant nitrogen source in soil with naturally low fertility but did not result in increased productivity in the benomyl treated soils relative to the watered controls. Plant communities on infertile soils have been shown to be unresponsive to nutrient amendment (Chapin *et al.* 1986; Koide *et al.* 1988a). Potential effects of increased soil N availability on the productivity of individual species are difficult to examine independent of benomyl effects on mycorrhizal associations and plant community changes flowing from these effects. However it is thought that while the application rate of benomyl in this experiment was higher than in similar experiments (Koide *et al.* 1988a; Carey *et al.* 1992; West *et al.* 1993a; West *et al.* 1993b; Newsham *et al.* 1994; Hartnett and Wilson 1999b), the winter growing season was short and the soils at this site have been shown to adsorb benomyl in the surface layers (Chapter 7). Total benomyl-N addition was less than 30% of that added by Cade-menun and Berch (1997) who reported significant increases in foliar N concentrations and plant growth in treatments where benomyl was added. No positive effects of benomyl on the growth of any of the major plant species from this site were recorded in the mycorrhiza-responsiveness assays (Chapter 5), except *C. annua* where effects were due to mycorrhizal suppression and not nutrition.

Another confounding effect of benomyl treatment was the potential effect on non-target fungi, which may have direct effects on plant health in the community. Benomyl has been shown to reduce populations of soil fungi in field studies (West *et al.* 1993a; Shukla and Mishra 1996), or have no effect after long-term field applications (Hart and Brookes 1996; Smith *et al.* 2000). Effects of benomyl on the total population of soil fungi may be due to dramatic effects on only a few abundant species (West *et al.* 1993a). Other indicators of non-target effects of benomyl on soil microbial populations such as changes in total bacterial biomass, numbers of fungal-feeding and predatory nematodes, microbial biomass carbon and substrate induced respiration have been observed (Smith *et al.* 2000). Many of these indicators are indirectly affected by benomyl through effects of the fungicide on AM fungi. However, there may also be direct effects of benomyl on soil microbes and microbial ecology. The size of non-target effects of benomyl must be considered with respect to the magnitude of changes to mycorrhizal root colonisation (Smith *et al.* 2000) and the likely consequences of these changes for the plant community.

While no significant changes in total number of soil fungi were observed in *in vitro* assays in the present study, one isolate of *Fusarium* sp. was absent from benomyl-treated plots. This isolate was shown to be pathogenic to *C. annua* and possibly pathogenic to *M. minima* (the disease rating on *M. minima* was not different to controls but was significantly greater than zero) in *in vitro* tests. No symptoms of Fusarium attack were present on the roots of *M. minima*, *C. annua*, *S. verbenaca* or *V. arguta* in any of the field plots. Although tests showed *Fusarium* sp 'Brookfield' was potentially pathogenic to some of these plant species, this may have had no importance in the field. However, pathogenic fungi can play a role in plant performance that may be mediated by AM fungi, even where the pathogenic fungi produce asymptomatic infections (Newsham *et al.* 1994). If *Fusarium* sp Brookfield does play a significant role in plant productivity, it does not discriminate between the two plant species tested. Potential negative effects of *Fusarium* sp. 'Brookfield' on plants in this experiment may have been ameliorated by AM fungi in control treatments (Newsham *et al.* 1994), though this may not necessarily account for all the benefits of mycorrhizal association for host plants. The non-host plant *C. annua*, was not significantly advantaged by benomyl-suppression of AM and pathogenic fungi in intact cores, relative to benefits of suppression of AM fungi alone (Chapter 5).

Community structure change was associated with suppression of mycorrhizal activity in this semi-arid herbland. While total plot biomass was greater in watered plots (with or without benomyl-addition) the relative proportions of species in the community was unchanged by water alone. The addition of benomyl had an effect on community structure independent of any effect due to increased productivity. The decrease in mycorrhizal activity resulting from fungicide application caused significant changes in the abundance and productivity of the dominant species and in the relative contributions of a number of species to community composition. The two most abundant species in control plots, *M. minima* and *C. annua*, were affected differently by the reduction in mycorrhizal activity. The dominant, highly mycorrhiza-responsive plant *M. minima*, had reduced productivity, while the non-mycorrhizal *C. annua* increased in biomass in mycorrhiza-suppressed plots. These results are readily explained as direct consequences of loss of mycorrhizal function in the system. The strongly mycorrhiza-responsive *M.*

minima showed yield decline while the non-mycorrhizal *C. annua* was released from any antagonism with the AM fungi (see Chapter 5). At the same time, competition from *M. minima* was relieved. The increase in biomass and abundance of *S. verbenaca* is largely due to increased survivorship in mycorrhiza-suppressed plots. This is presumably due to reduced competition from the highly mycorrhiza-responsive *M. minima* on the mycorrhizal but poorly responsive *S. verbenaca.*

Negative growth response to interaction with AM fungi has been previously observed for non-mycorrhizal plants (Francis and Read 1995). The antagonism between *C. annua* and AM fungi is not large (> -34% mycorrhizal response, Chapter 5) and is unlikely to account for all the increased biomass of this species in fungicide-treated plots. (Sanders and Koide 1994) found a similar relationship between the decline in biomass of the mycorrhiza-responsive plant *Abutilon theophrasti* and the concomitant increase in biomass of the non-mycorrhizal species *Amaranthus retroflexus.* The non-mycorrhizal species *C. annua* and the poorly mycorrhiza-responsive species *S. verbenaca* behave in the same way in the present study as the poorly mycorrhiza-responsive plant species in the study of Hartnett and Wilson (1999a). The productivity of species with low mycorrhizal dependency in the present study was not restricted by loss of mycorrhizal function after competitive release from the highly mycorrhiza-responsive *M. minima.*

Change in biomass of the three most abundant species in this semi-arid herbland accounted for all the increase in diversity (29 %) in mycorrhiza-suppressed plots. However, equal contributions of the minor species to diversity in mycorrhiza-suppressed or control plots indicates that loss of mycorrhizal activity did not result in significant decline in the highly mycorrhiza-responsive species *V. gracilis* and *V. arguta.* As neither of these minor species showed significant reduction in aboveground biomass or number in response to mycorrhiza-suppression it is concluded that release from competition with the highly mycorrhiza-responsive *M. minima* offset the cost of losing mycorrhizal function. This indicates that while some of the minor plant species at this site were highly responsive to mycorrhiza, they may not have been as competitive as *M. minima* in acquiring resources from the external mycorrhizal mycelium. The high root density of the dominant plant, *M. minima*, in control plots may also have reduced

mycorrhizal benefit to subordinate species. The restricted growth of minor host-plant species could have resulted from reduced mycorrhizal benefit at high plant densities (Koide 1991a; Koide and Li 1991). Competition for nutrients in this plant community may extend from competition between plants along the mutualism-parasitism continuum (Johnson *et al.* 1997) to competition between plants with equivalent mycorrhizal responsiveness but different capacities to exploit the symbiosis for nutrient uptake or alleviation of water stress.

Reduction in the activity of mycorrhizal fungi resulted in an increase in plant species diversity relative to watered control plots. This was due to an increase in species evenness in this semi-arid plant community. The intermediate species evenness in unwatered control plots may be a function of lower competition due to water stress (Grime 1979; Grime and Hodgson 1987). The increased diversity in mycorrhiza-suppressed plots supports the findings of Hartnett and Wilson (1999a), that diversity (both richness and evenness) increased when mycorrhizal fungi were suppressed over several growing seasons in a tallgrass prairie. Results presented here conflict with the microcosm experiment of Grime *et al.* (1987) who showed that mycorrhizal association increased plant species diversity (due to increased evenness). The field experiment of Gange *et al.* (1990) also showed that reduction in mycorrhizal activity was correlated to a decrease in plant species richness. Mycorrhizal associations were also shown to increase plant species diversity in macrocosms simulating North American old-field ecosystems and increase evenness in microcosms simulating a European calcareous grassland (van der Heijden *et al.* 1998b). Results from this semi-arid herbland support the hypothesis that mycorrhizal effects on plant species diversity are not absolute but depend on the mycorrhiza-responsiveness of the component plant species, especially the dominant species (Bergelson and Crawley 1988; Hartnett and Wilson 1999b).

Further support for this hypothesis comes from the present study, where species richness was unchanged by suppression of AM fungi, as was functional group richness. Decreased mycorrhizal activity resulted in a redistribution of biomass within the community, ie. an increase in evenness, but no change in aboveground biomass of the community as a whole. This was also true in the studies of European calcareous grassland species in microcosms (van der Heijden *et al.* 1998b), and tallgrass prairie in

the field (Hartnett and Wilson 1999b). The fact that increasing diversity in my experiment did not coincide with increasing productivity suggests that productivity may be linked to species or functional group richness (Naeem *et al.* 1994; Tilman *et al.* 1996; Tilman *et al.* 1997; Symstad *et al.* 1998; Hector 1999; Wilsey and Potvin 2000), neither of which was changed in the current study. There is no evidence to suggest that the effect of increasing diversity on plant productivity found in other studies (Tilman *et al.* 1997; Hector 1999) can be explained by increasing evenness (Wilsey and Potvin 2000). Further study of the importance of functional group richness in plant community productivity and stability, should consider mycorrhizal responsiveness as a key functional characteristic of plant species, particularly in disturbed and early successional communities.

The behavior of individual plant species was a strong determinant of community structure. Changes in species composition following fungicide treatment of field plots altered plant density. Changes in plant density were largely attributable to increased survivorship of seedlings of *S. verbenaca* in mycorrhiza-suppressed plots. This highlights the different response of species to competitive release. There was an increase in aboveground biomass of *S. verbenaca* related to increased seedling survival, while *C. annua* showed increased plot biomass without significant adjustment of survival. These differences may result in long-term effects on community structure not seen in one growing season, especially as *S. verbenaca* is a short-lived perennial.

6.5 Overall discussion of findings from 1997 and 1998

Experiments at both study sites in Brookfield Conservation Park showed significant effects of benomyl-application on growth of some plant species and on community structure as a whole. While results from 1997 were inconclusive due to uncertainty about the effect of benomyl on the activity of AM fungi, the repeated experiment in 1998 was consistent with the previous result and correlations between community structure and mycorrhizal influences were substantially stronger. While the two study sites did differ with respect to the composition of the communities present, trends in response to benomyl addition were similar. Combined with the results from studies of mycorrhizal influences on individual plant species and simple combinations of species

(Chapter 5) there is considerable evidence that mycorrhizas actively influence community structure in these semi-arid herblands.

The seasonal fluctuations in aridity and levels of standing biomass may influence mycorrhizal activity in these semi-arid herblands. The difference in precipitation between the years at Brookfield North influenced productivity in the system and may consequently have influenced the levels of intra- and interspecific competition in the different years. Severe grazing pressure may also have affected belowground allocation of carbon and hence interactions between soil organisms and the plant community. Grazing can significantly reduce levels of mycorrhizal root colonisation and spore densities by decreasing the leaf area and increasing the root:shoot ratio, thereby decreasing source capacity below that required by the demands of the AM fungi sink (Bethlenfalvay and Dakessian 1984; Bethlenfalvay *et al.* 1985). The present plant community reflects previous grazing disturbance by large populations of kangaroos. Overgrazing has facilitated the establishment of several ruderal species including the non-mycorrhizal *C. annua* and the unresponsive host plant *S. verbenaca*, probably as a result of the decline of native herbs and grasses and the associated soil flora. With the recognition of some specificity between isolates of AM fungi and plant species from a grassland ecosystem (van der Heijden *et al.* 1998a), it is possible to ascribe AM fungal communities a determinate role in plant community structure (van der Heijden *et al.* 1998b). Restoration of the semi-arid grasslands of southern Australia will require ecological approaches to re-establishment of some plant species, and greater understanding of soil processes in disturbed and undisturbed systems.

Chapter 7

The fate and efficacy of benomyl applied to soils to suppress the activity of AM fungi.

7.1 Introduction

Most research on the response of plants to mycorrhizal association has involved growing single plants or simple intra- or interspecific mixtures of plants in pots. Study of the influence of AM in intact plant communities and natural plant populations has been limited by the difficulty of establishing appropriate non-mycorrhizal controls when most ecosystems contain plant species which would be naturally colonized by the indigenous AM fungi. Soil fumigation or fungicides have been used to suppress the activity of indigenous AM fungi. The systemic fungicide benomyl has been commonly used to suppress AM activity in field experiments (West *et al.* 1993a; Newsham *et al.* 1994; Merryweather and Fitter 1995; Hartnett and Wilson 1999b). Benomyl has been preferred over other fungicides because it has proved more effective in some studies (West *et al.* 1993a; Schreiner and Bethlenfalvay 1997a) and has fewer direct effects on plants than other fungicides (Paul *et al.* 1989; Sukarno *et al.* 1993). However, benomyl has not always been effective in reducing colonisation or biomass of mycorrhizal plants (Fitter 1986; Koide *et al.* 1988a) see also section 6.3.3). Limitations in the efficacy of benomyl are likely due to the method and timing of application, effects on the microbial community or differences in soil properties (Pedersen and Sylvia 1997).

The effectiveness of a fungicide in reducing the activity of AM fungi in soil is determined by the persistence, adsorption and biological-availability of that fungicide. Control of AM fungi may also be a function of depth of penetration of the fungicide and the activity of AM-fungi at depth. Benomyl and its primary degradation product carbendazim (methyl 2-benzimidazole carbamate) are known to adsorb to soil particles (Liu and Hsiang 1994). It was hypothesised that the limited effectiveness of benomyl in suppressing the activity of AM fungi at Brookfield South 1997 may have been due to reduced biological availability of benomyl in this clayey soil.

To test this hypothesis it was necessary to:

1. Develop a bioassay to assess the activity of benomyl in soil
2. Determine the activity and persistence of benomyl in field soils at different depths within the root zone
3. Determine the effect of repeated benomyl application on the activity of AM fungi at different depths within the root zone

7.2 Materials and Methods

7.2.1 Field Site

The Brookfield South field site is described in Section 3.1.2.1. The soil at this site is a clay-loam and is described in Section 3.1.4. For investigations into the fate and efficacy of benomyl at this site, watered and benomyl-treated plots from blocks 1-5 in the community structure experiment were selected. These plots had already received nine applications (one every three weeks) of benomyl or water in the preceding months (see Section 6.3.2.2).

7.2.2 Fungicide and water applications

Four treatments were established at Brookfield South to determine the persistence and activity of benomyl and effects on the activity of AM fungi at this site. Benomyl-treated and watered plots had received 15 L of fungicide or water respectively every three weeks (for a total of nine applications) until three weeks prior to the commencement of these experiments. Each of the five watered and five benomyl-treated plots was divided into two sub-plots (0.75 m^2). On 27th November 1997 one of the sub-plots in each plot was randomly chosen to receive 7.5 L of benomyl solution (Benlate® Du Pont, 6 g a.i. in 15 L of water per plot) as a soil drench. The other half of each plot received the same volume of water (7.5 L water).

These applications of fungicide and water resulted in four soil treatment combinations;

Benomyl application	Total benomyl (g m^2)	Treatment regime
Continuous (CS)	40	10 consecutive applications of benomyl
Residual (RE)	36	9 consecutive applications of benomyl followed by one application of water
Single (SI)	4	9 consecutive applications of water followed by one application of benomyl
Control (CN)	0	10 consecutive applications of water

After treatment plots were left for 24 h before soil cores and samples were collected.

7.2.3 Soil sampling

7.2.3.1 Soil cores for assessment of mycorrhizal activity

Cores were taken from field plots which had received 10x benomyl and 10x water by driving a PVC corer (22 cm high, 15 cm diameter) into the soil to a depth of 20 cm. Each core was taken intact and divided into five sections each 4 cm high. Each section was capped from below with a tight-fitting PVC cap with drainage holes in it and planted with one seedling of the trap plant *Trifolium subterraneum* L. cv. Mt Barker. Trap plants were harvested after 21 days for determination of AM colonisation of the roots. Mycorrhizal colonisation was assessed by the method described in Section 3.4.

7.2.3.2 Soil samples for benomyl bioassay

Soil samples were taken from 0-10 cm below the soil surface using a metal soil corer (10 cm diameter) inserted vertically into the soil to a depth of 12 cm. Three replicate cores were collected from each of the five sub-plots of each of the four fungicide treatments. Cores were cut into 2 cm thick sections and samples from the 0-2, 4-6 and 8-10 cm sections were stored in plastic bags and removed to the laboratory for analysis by bioassay.

7.2.4 Bioassay for benomyl adsorption and persistence in field soils

The bioassay for assessing the activity of benomyl in field soils was a modified version of that described by Liu and Huang (1994). The soil-agar pellet bioassay assessed the toxicity of benomyl-treated field soils to the test fungus *Penicillium* sp. 320. The fungal isolate *Penicillium* sp. 320 was obtained from the Undergraduate Teaching Unit, Faculty of Agricultural and Natural Resource Sciences, Adelaide University.

Ten millilitres of autoclaved potato dextrose agar (PDA) medium (19.5 g L^{-1}) were poured into plastic Petri dishes and allowed to solidify. An aluminium ring (22 mm diameter x 10 mm height) was placed into the centre of each agar plate and filled with 1 g (wet weight) of soil from field samples. The soil pellet was spread evenly around the ring and 1 ml of molten PDA agar (~ 50° C) was added dropwise to the soil pellet to improve contact between the soil pellet and the agar plate. A 1 ml suspension of spores of *Penicillium* sp. 320 (15 x 10^6 spores ml^{-1}) was poured onto the agar plates and swirled around to ensure even coverage of the plates. Plates were incubated in the dark for 48 h. The area of the zone of inhibition (area between the outside of the aluminium ring and the inside edge of the *Penicillium* sp. mycelium) was recorded for each plate. There were three replicate plates for each soil sample from each replicate core collected in the field.

7.2.5 Statistical analysis

The mycorrhizal colonisation bioassay was analysed using a two-way analysis of variance on a split-plot design using treatments as the main plot and depth as the sub-plot. Percent colonisation data were arcsine transformed before analysis to improve normality (Zar 1999).

The bioassay for persistence of benomyl was analysed using general analysis of variance with a treatment structure of treatment*depth and a block structure of block*cores. The results from replicate plates for each sample were averaged before analysis. Data from the zero benomyl treatment were removed before analysis, as there were no fungitoxic effects from soils at any depth in this treatment. Data from the fungicide bioassay were square root transformed before analysis. Tukey's Honestly Significant Difference tests were used to compare means in both bioassays.

7.3 Results

7.3.1 AM colonisation down the soil profile

Colonisation of trap plants was significantly (P = 0.031, Table 7.1) reduced in the 0-4 cm zone of soils treated with benomyl compared to soils treated with water only (Fig. 7.1). There was no significant (P > 0.05) reduction in colonisation of trap plants in any of the soil depths below 4 cm (Fig. 7.1).

7.3.2 Benomyl adsorption and persistence in field soils

There was no background suppressive effect of soil from any depth in the watered (control) plots on the growth of *Penicillium* sp. 300 in the soil toxicity bioassay. Surface soils from all treatments where benomyl had been applied produced fungitoxic effects on the *Penicillium* sp. in the bioassay. There was a reduction in the fungitoxic potential of soils treated with benomyl with increasing soil depth in all treatments (Figure 7.2).

The number and timing of benomyl applications did have a significant (Table 7.2, P = 0.027) effect on fungitoxic activity in soils at different depths. While all benomyl treatment regimes resulted in significantly higher concentrations of fungitoxins in the surface soil (0-2 cm) than at subsequent depths (4-10 cm), not all treatment regimes were the same. Where benomyl had been added continuously throughout the growing season, fungitoxic activity in the soil was not different between depths of 4-6 cm and 8-10 cm below the surface. The other benomyl treatments (Single and Residual) resulted in significantly higher concentrations of fungitoxins at soil depths of 4-6 cm than at 8-10 cm (Figure 7.2).

Table 7.1 Results of ANOVA testing the effect of soil depth and treatment of field plots with fungicide (benomyl) on mycorrhizal colonisation of *Trifolium subterrraneum* trap plants in intact cores from Brookfield South 1997, n = 5.

ANOVA		Percent mycorrhizal root length		
Transformation		arcsin (%)		
Factors	df	MS	F-value	$P > F$
Fungicide treatment	1	0.0959	6.59	0.015
Depth	4	0.4550	31.26	<0.001
F * D	4	0.0439	3.01	0.031

Table 7.2 Results of ANOVA testing the effect of soil depth and fungicide treatment on the persistence and activity of benomyl (measured in bioassay by suppression of *Penicillium* sp.) in field plots at Brookfield South 1997.

ANOVA		Area of zone of suppression		
Transformation		square root		
Factors	df	MS	F-value	$P > F$
Fungicide treatment	2	4.334	4.00	0.021
Depth	2	261.9	241.49	<0.001
F * D	4	3.099	2.86	0.027

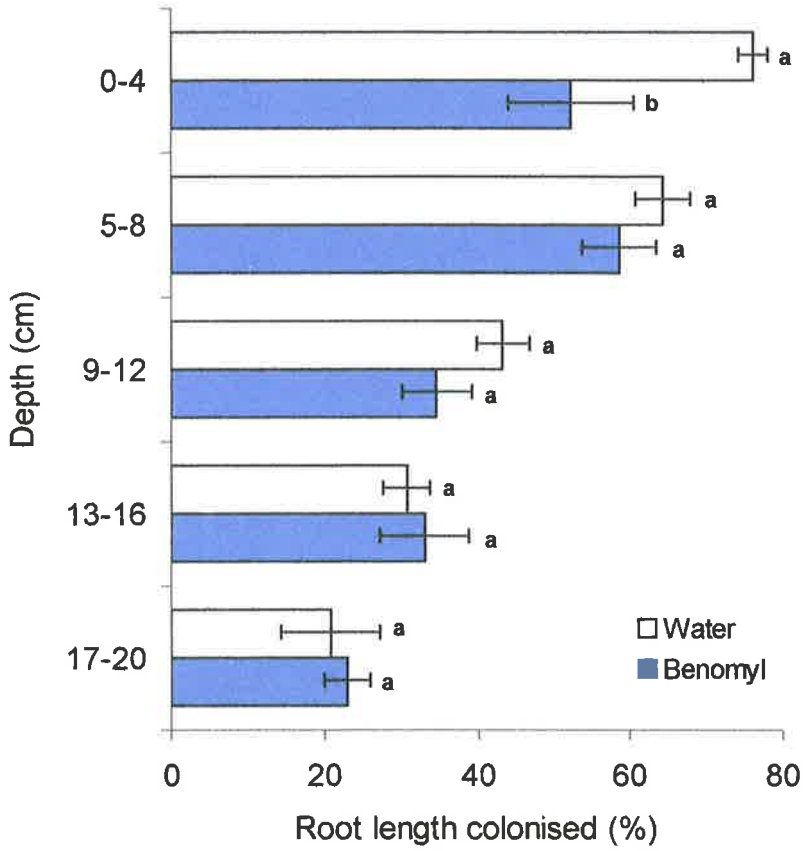

Figure 7.1. Mean (±SE) mycorrhizal colonisation in roots of *Trifolium subterraneum* trap plants in intact soil cores taken from 0-20 cm depth in field plots. Treatment means within a depth followed by different letters are significantly different (Tukey's HSD, $P < 0.05$), n=5.

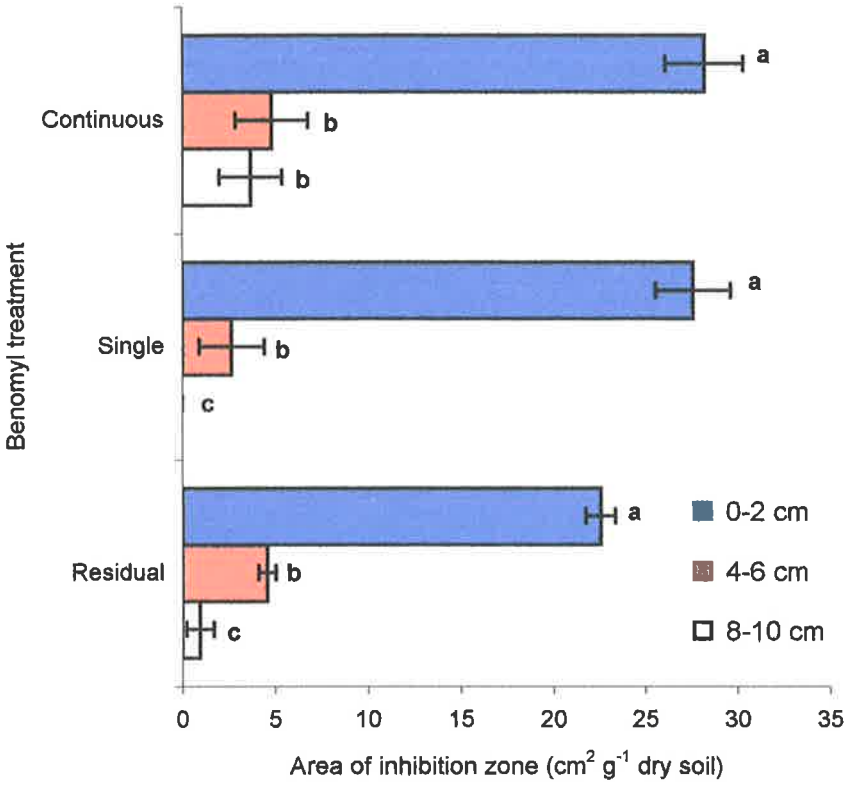

Figure 7.2 Mean (±SE) area of zone of suppression of *Penicillium* sp. in the bioassay for benomyl persistence at different soil depths (0-10 cm) after different treatment regimes in field plots at Brookfield South 1997. Means within a treatment followed by different letters are significantly different (Tukey's HSD, $P<0.05$), n=15.

7.4 Discussion

The reduction in mycorrhizal colonisation of trap plants in surface soil treated with benomyl indicates suppression of AM colonisation potential by the fungicide. However, a trap-plant bioassay on intact cores from 0-12 cm did not detect any effect of benomyl on the colonising potential of AM fungi in the same plot experiment (Section 6.3.3.2). Significantly lower concentrations of fungitoxins in benomyl-treated soil from depths below 2 cm was correlated with a decline in the suppression of AM colonisation potential in these deeper soils. This suggests that the activity of AM fungi below the top few centimetres was less affected by surface applications of benomyl than was the surface soil.

It is assumed that the fungitoxic potential in benomyl-treated soils is due to the activity of residual benomyl or degradation products of benomyl such as carbendazim. While absolute concentrations of benomyl and carbendazim in soil were not measured in these experiments, the bioassay used to assess activity of these compounds has been shown to provide a good surrogate measure of the concentration of toxic compounds (Liu and Hsiang 1994). Despite considerable differences between treatments in the timing and amount of benomyl applied to the soil, large differences in fungitoxin concentrations at different depths in the soil were evident in all benomyl-treated plots. This suggests that benomyl (or toxic breakdown products such as carbendazim (Kling and Jakobsen 1997)) are either more concentrated or more available at the top of the soil profile. The availability of benomyl and its breakdown products in soil is determined by rates of immobilisation on soil particles and is affected by the texture and organic matter content of the soil (Aharonson and Kafkafi 1975; Helweg 1977). As all measurements of fungitoxin activity were taken in the top 10 cm of the soil profile (A horizon) where the soil texture is relatively homogeneous and organic matter content was low (Section 3.1.4), activity is unlikely to have been affected by soil depth. Therefore differences in activity of benomyl-derived fungitoxins at different depths in the soil are likely to reflect differences in the concentration and not the availability of these toxins. Higher concentrations of benomyl-derived fungitoxins in the surface soil may be due to preferential adsorption to soil minerals and organic matter before they can percolate down through the soil profile.

Given the ten-fold difference in total benomyl added to CI-plots relative to SI-plots, and the similar fungitoxic activity of soils from these two treatments, it must be assumed that breakdown of benomyl and carbendazim did occur in these soils. Carbendazim can be rapidly degraded by soil organisms (Yarden *et al.* 1985) and is more rapidly degraded in soils with a history of prior treatment with carbendazim (Yarden *et al.* 1985). Therefore, persistence of all the added fungicide would have been unlikely if it was available to effective degrading microorganisms. Protection from degradation of at least a portion of the benomyl-derived toxins may have been due to adsorption in the surface soil and would explain the persistence of relatively high concentrations of fungicide in the RS treatment. The RS soil had not received any benomyl in the three weeks preceding sampling but apparently contained concentrations of fungitoxin similar to the other benomyl treatments. One explanation for the apparently high residual concentrations of benomyl-derived toxins in the RS treatment is that addition of agar to the soil pellets in the benomyl detection bioassay may have increased desorption of the fungicide bound to soil particles, rendering it more readily detectable (Liu and Hsiang 1994).

Concentrations of fungitoxins in soil were not cumulative over time. Ten and nine times the benomyl had been added to soils in the CS and RE treatments respectively, compared to the SI treatment, yet total concentrations of toxins in the SI soil accounted for approximately 82 % and 108 % of the activity in CS and RE soils respectively. Some of the fungicide may have percolated to below 10 cm depth and cannot be accounted for in all treatments. However, the relatively high concentrations of fungitoxins in SI soils may be due to the short time between benomyl application and sample collection or the fact that the SI soils had no prior history of exposure to benomyl. The most likely explanation for the non-additive effect of benomyl addition over time is that benomyl was rapidly degraded to non-toxic compounds in soils which had a recent history of exposure, but that a percentage of the fungitoxins became sorbed to soil particles and unavailable to degrading microorganisms.

Several previous studies have indicated more rapid degradation of carbendazim in non-sterile than in sterilised soils (Aharonson and Kafkafi 1975; Helweg 1977; Yarden *et al.* 1985). Degradation rates for carbendazim in soil vary according to initial

concentration, soil type and environmental conditions but have been measured to be as rapid as 50% in 4 weeks (Liu and Hsiang 1994). In our experiment toxicity of the added fungicide was able to persist for 3 weeks or more. The inhibition of spore germination and germ tube growth of AM fungi can occur at concentrations down 10 mg benomyl kg^{-1} soil (Schreiner and Bethlenfalvay 1997a). The fact that colonisation of clover roots was not reduced in soils below 4 cm depth indicates that the toxicity in these soils measured by suppression of *Penicillium* sp. was not sufficient to suppress the activity of AM fungi below this depth. Shreiner and Bethlenfalvay (1997) showed that benomyl was much less effective at reducing the germination of spores of the AM fungi *Glomus etunicatum* (Becker & Gerd.), *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe and *Gigaspora rosea* (Nicol. & Schenck) when the spores were buried than when they were exposed at the soil surface. Sorption of the fungicide to soil particles may result in significant decrease in the effective concentration of the fungicide at depth and fungicide application rates should be adjusted accordingly.

Many studies have observed negative correlation between mycorrhiza formation and increasing soil depth (Schwab and Reeves 1981; Jakobsen and Nielsen 1983; Al- Agely and Reeves 1995; Kabir *et al.* 1998; Nehl *et al.* 1999). However, the abundance and activity of mycorrhizal fungi at different depths may depend on seasonal and site factors (Sparling and Tinker 1978; Nehl *et al.* 1999) and different species of AM fungi may have different vertical distributions in the soil profile (Douds *et al.* 1995). Most studies have measured changes in root colonisation and/or fungal propagules over large depth intervals, and have ignored the effect of secondary spread of colonisation down the profile (Nehl *et al.* 1999). The potential for vertical spread of colonisation in Brookfield soils may account for the failure of the trap-plant bioassay on 12 cm deep intact cores (Chapter 6) to detect any effect of benomyl on the colonising potential of AM fungi in the same plot experiment. Colonisation of trap-plant roots in the surface soil may have occurred through spread of infection units or secondary colonisation up the soil profile (Nehl *et al.* 1999). However, given the relatively small effect of benomyl treatment on root length colonised in the top 4 cm of soil, it is probable that differences in colonisation of the whole root system in treatments in the 12 cm intact cores were too small to be detected using this bioassay.

While uncertainties about the concentration of benomyl required to effectively suppress AM activity remain, it is clear that benomyl can be used as a soil drench to reduce AM colonisation in field situations. Methods for detecting the effectiveness of benomyl application need to consider the confounding effects of transient suppression and recovery of the fungi and the relationship between root colonisation and nutrient uptake capacity. Rooting depth may be significant in determining the effectiveness of benomyl as roots reaching below the surface soils where benomyl is suppressed may become colonised by AM fungi. Fungicide may slow the rate of colonisation but may not kill the fungi, leaving open the possibility that colonisation may recover as the toxic activity of the fungicide diminishes over time. Timing of fungicide application, soil texture and microbial activity may all combine to determine the effective toxicity of the fungicide to the target fungi. Recognition of these interacting factors is essential for optimisation of fungicide use.

The bioassay used in this study was a useful tool for measuring the activity of benomyl and its breakdown products at different depths in field soils. While the concentration of toxins was not determined, the relative activity of toxins declined as soil depth increased. The decline in fungicide activity at depth was correlated with a decline in the suppressive effect of the fungicide on the activity of AM fungi. Repeated applications of the fungicide only slightly increased the levels of toxicity in the soils, probably because bio-degradation of the fungicide was more rapid in soils with a recent history of exposure to the fungicide.

Chapter 8

Mycorrhizal effects on plant species interactions in the field

8.1 Introduction

Several multi-species experiments have shown that AM fungi can affect competition between co-occurring plant species. These include increased competitive ability of legumes in legume-grass mixtures (Crush 1974; Hall 1978) and shifts in the competitive balance between different grass species (Fitter 1977; Hartnett *et al.* 1993), between grasses and non-leguminous herbs (Allen and Allen 1984; Marler *et al.* 1999) and between different herb species (Allsopp and Stock 1992a; Moora and Zobel 1996). Sanders and Koide (1994) established experimental communities containing the mycorrhizal-host species *Abutilon theophrasti* and *Setaria lutescens* and the non-mycorrhizal species *Amaranthus retroflexus*. Each of these species responded differently to inoculation with AM fungi and community structure was subsequently altered. Mycorrhizal colonisation also affected the reproduction of the two host species, potentially impacting on the community in ensuing years. The effects of mycorrhiza on more complex and species-rich plant communities and competitive hierarchies of co-occurring species have also been studied (Grime *et al.* 1987; Gange *et al.* 1990; Wilson and Hartnett 1997; Hartnett and Wilson 1999b). However, it is not always simple to evaluate competitive interactions in species-rich mixtures. Most of these studies have been conducted in pots or microcosms at set plant densities, with controlled and often synchronous timing of germination of component species, and sometimes in disturbed soil where the mycelial network of AM fungi is not established before germination. Field studies are necessary to test many of these results in natural ecosystems (Sanders *et al.* 1999).

Competition between different plant species can potentially occur at all stages in the plant's life cycle, and successive generations of plants must be able to establish, survive and reproduce to maintain viable populations. Mycorrhizal colonisation has been shown to influence host-plant fecundity (Koide *et al.* 1988b; Bryla and Koide 1990; Carey *et al.* 1992; Stanley *et al.* 1993; Newsham *et al.* 1994) and seed size (Shumway and Koide 1995). Mycorrhizas have also been shown to improve the vigour of offspring plants

(Lewis and Koide 1990; Lu and Koide 1991; Koide and Lu 1992; Shumway and Koide 1994; Srivastava and Mukerji 1995; Heppell *et al.* 1998). Differences in these traits may all contribute to increased fitness of a plant species in subsequent generations. Competition between plant species will also impact on species fitness. Study of the effects of mycorrhizal fungi on plant ecology must account for possible interactions between co-occurring plant species, especially where they have differential responsiveness to AM colonisation.

The three most abundant plant species in the semi-arid herbland at Brookfield North are *Medicago minima, Carrichtera annua* and *Salvia verbenaca. M. minima* has been shown to be highly mycorrhiza-responsive, while *S. verbenaca* is a non-responsive mycorrhizal host and *C. annua* does not form any association with AM fungi (Chapter 5). Natural plant communities often include plant species with differential responses to the presence of AM fungi, and the structure of plant communities may be determined by differential contributions of the mycorrhizal fungi to individual plant species fitness (Sanders and Koide 1994; Sanders *et al.* 1999). In previous experiments described in this thesis, the influence of mycorrhizas on interactions between co-occurring plant species have been studied in a semi-arid plant community without manipulations of the species present or the densities at which species occur Chapters 5 & 6). The experiments described in this chapter were designed to investigate interactions between the mycorrhiza-responsiveness and competitive strength of the three most abundant plant species in the understorey at Brookfield North (described in Chapter 3) by removing all minor species and each of the three most abundant species in turn. Removal experiments have long been used to study the mechanisms, importance, and consequences of species interactions within natural plant communities (Aarssen 1990). Factors affecting the competitiveness of the dominant plant species may influence species coexistence (Aarssen 1990; Goldberg and Barton 1992). By removing each of the dominant plant species from plots at Brookfield North, the effect of mycorrhizas on competitive interactions between these plants could be studied.

The purpose of this study was to;

1. study the interactions between the three dominant plant species at Brookfield North, showing differential response to AM association under natural conditions of germination, plant growth and mycorrhizal establishment,
2. observe any effects of AM on resource allocation to seeds of *M. minima* in competition with *C. annua*,
3. investigate the effects of AM colonisation of parent plants on the vigour of offspring from *M. minima* grown in competition with the non-mycorrhizal *C. annua*.

8.2 Materials and methods

8.2.1 Competition between plant species with different mycorrhiza-responsiveness

A field experiment was established to examine the effects of AM on competition between plant species with different levels of mycorrhiza-responsiveness.

8.2.1.1 Field plot establishment

In March 1998, eighteen experimental plots were established at the Brookfield North site (described in section 3.1.2.1). These plots were 1.5 m^2 and were established in open herbland (>10 m to the nearest tree or large shrub) by covering them with a wire cage (5 cm grid mesh, 1.25 m x 1.2 m x 0.5 m) to prevent grazing by large herbivores. The eighteen plots were established in six blocks with one plot in each block randomly assigned to one of the three treatments (mycorrhiza suppressed, watered and unwatered control). Each plot was separated from the others by a 1.5 m spacing, to ensure adequate buffering of any plot effects. Aboveground biomass of *S. verbenaca* plants that had survived from the previous year was removed from the 1.5 m^2 plot area by cutting at ground level. *S. verbenaca* did not re-grow after cutting and all *S. verbenaca* seedlings subsequently recorded in these plots had germinated from seed in 1998. Each plot was divided into a central zone 1 m^2 and a surrounding buffer zone to reduce edge effects from surrounding untreated vegetation. Each 1 m^2 central zone was further divided into four 40 cm x 40 cm sub-plots separated from each other by a 10 cm buffer zone.

In April 1998, the four sub-plots within each plot were randomly allocated to one of each of four species-interaction treatments. Treatments were established by removal of

all seedlings of plant species other than *M. minima, S. verbenaca* and *C. annua* by cutting at ground level. One sub-plot in each plot was dedicated as a three-way interaction treatment (*M. minima* vs *C. annua* vs *S. verbenaca*). In each of the other three sub-plots, one species was removed to establish three, two-way interaction treatments (*M. minima* vs *C. annua*; *M. minima* vs *S. verbenaca*; and *C. annua* vs *S. verbenaca*). The density of remaining plants was not manipulated.

8.2.1.2 Benomyl and water applications

Fungicide and water were applied at the same times and rates as in the concurrent experiment on community structure at the same field site (Section 6.4.2.1). Mycorrhiza-suppressed plots received the fungicide benomyl as a soil drench (Benlate® Du Pont, 9 g a.i. in 15 L of water per plot). Watered plots received 15 L of water per plot and control plots received no amendments. Treatments began after the first rains in April and were repeated every two weeks until mid-September, for a total of ten applications. It was necessary to include a treatment receiving only water as well as an unwatered control treatment to determine effects of additional water on plant-interactions at these low rainfall sites.

8.2.1.3 Assessment of mycorrhizal activity

Assessment of the AM status of field plots was made by collecting roots of the mycorrhizal host-plant *M. minima* from field plots on 6th June and 8th August 1998. Roots were collected from the buffer zone between sub-plots in each plot by excavating the whole root system. Roots were cut, cleared and stained and examined for mycorrhizal colonisation using the methods described in Section 3.4.

Percentage colonisation data were arcsine-squareroot transformed before analysis to improve normality (Zar 1999). Differences between treatments with respect to mycorrhizal activity in field plots at the two sampling times were tested by two-way ANOVA. Root colonisation levels were separated by Tukey's HSD test for significantly different means.

8.2.1.4 Measurement of plant cover and competition between plant species

The vegetation in each plot was assessed using a point-quadrat method early and late in the growing season (6th June and 8th August 1998). For point quadrat assessment of the vegetation, a vertical frame consisting of 5 50 cm long pins set 10 cm apart was positioned at 5 points (5 cm apart) within the central zone of each sub-plot, for a total of 25 points. All plants touching each pin were recorded to yield a cover frequency for each plant species (number of touches per species ÷ 25 pins x 100). Cover frequency of each species was used to estimate the probability of touching that species in a given sub-plot.

The classical distribution for the number of touches is binomial with $n = 25$ trials and an unknown probability p that any one trial results in a touch. The probability p may vary with species, combination of species in competition and with whole plot treatment (benomyl, water or control). In addition, the blocking and sub-plot randomisation introduce variation that should be accounted for in the analysis. To account for these fixed and random factors, the data were analysed by modelling the probability of plants touching each random point using a generalised linear mixed model assuming a binomial distribution with a logit link. This approach allowed all three species to be included in one analysis, enabling some interpretation of competition effects between species. In sub-plots where a particular species did not occur, cover for this species was included as a zero response from 25 touches. Handling the data in this way produced results equivalent to those obtained when these plots were ignored. Standard errors of predicted cover were not calculated as it would be inappropriate to back-transform the data used in the model. Wald Statistics (approximate chi-squared statistics under the hypothesis of non-effect) were employed to determine significance of fixed effects after random effects had been accounted for (Cox and Hinkley 1974).

In addition to the analysis of all treatments described above, an analysis of the cover data in *M. mimina* vs *C. annua* sub-plots was also undertaken. Plant species were used as variates, main plot treatment as a fixed factor and blocks as a random factor in a MANOVA of cover estimates (proportion of 25 points touching each species (arcsine-transformed)) for *M. minima* and *C. annua.* Nested one-way ANOVAs were subsequently performed on cover estimates of *M. minima* and *C. annua.*

8.2.1.5 Non-target effects of benomyl

Effects of benomyl application on non-target soil fungi and on soil N and P were assessed in the concurrent experiment on plant community structure at the same field site (Section 6.4.3.5).

8.2.2 Offspring vigour

To further examine the effects of competition between an AM host and non-host plant species, an experiment was designed to determine the impacts of AM fungi on the seed size and offspring competitiveness of *M. minima* plants grown in competition with *C. annua* in the field plots described above.

8.2.2.1 Seed collection and measurement

Seed of *M. minima* and *C annua* was collected from all *M. minima* vs *C. annua* sub-plots of the benomyl and water-treated plots of the Removal Experiment (Section 8.2.1) on 18th November 1998. Seed of both species was also collected from the central-buffer zone of benomyl and water treated plots. Fifty pods of *M. minima* plus one hundred seeds of both *M. minima* and *C. annua* from each field sub-plot were air-dried, weighed and stored in the laboratory. Seed samples of *M. minima* were digested in nitric acid and analysed for nutrient content by Inductively Coupled Plasma Atomic Emission Spectrometry. Nitrogen content was analysed using a combustion technique using a Carlo Erba Instrument.

Effects of treatment on pod and seed weight of *M. minima*, seed weight of *C. annua* and number of seeds per pod of *M. minima* were analysed for significant differences by one-way ANOVA with blocks included as a random effect.

8.2.2.2 Offspring competitiveness

Seed of *M. minima* from each *M. minima* vs *C. annua* sub-plot of the benomyl and water-treated plots was pre-treated (scoured on sand-paper and surface sterilised in 5 % sodium hypochlorite solution containing a drop of the surfactant Tween 20. Seeds were soaked for 10 minutes in this solution and then rinsed in reverse osmosis water) and germinated on wet filter paper in a covered Petri-dish at 24° C. Germinated seeds were

planted into boxes (24 cm x 24 cm x 20 cm) containing eight kilograms of unsterilised Brookfield North soil (Section 3.1.4). Seeds were planted at 2-cm intervals on a 12 x 12 grid pattern. The two outside rows of seeds were from the central buffer zone of the watered plots from Brookfield North. The central grid (8 x 8) was planted with seed from the benomyl-treated and water-treated plots in alternate positions. Seed from the six replicate field plots were planted into two corresponding sets of six boxes. Plants were grown in a temperature-controlled glasshouse (mean daily temperature, minimum 19.4°C, maximum 24.1°C) and watered to field capacity three times per week.

M. minima plants in one set of six boxes were harvested after four weeks, while those from the other set were harvested after six weeks. Plants were harvested from the central grid (6 x 6) by cutting the individual stems at the soil surface. Each plant was weighed for fresh weight, dried at 80° C for 24 hrs and re-weighed for dry weight. A total of 18 offspring plants from parents from both benomyl- and water-treated field plots were harvested from each of the six boxes at each harvest.

To examine the effects of maternal treatment on offspring vigour, plant dry weight was analysed by ANOVA. Only results from the final harvest are reported, as results were similar at four and six weeks. Average seed weight, average seedpod weight and average seed P-content were all used as covariates in ANCOVA of offspring shoot dry weight. None of these variables was a significant covariate and so ANCOVA tables are not shown. Competitive effects on equality amongst seedlings with different maternal treatment was examined using ANOVA on Gini coefficients derived from Lorenz Curves for the respective maternal treatments.

8.3 Results

8.3.1 Plant cover and competition between plant species

8.3.1.1 AM colonisation in field plots

There was an increase ($P < 0.001$; Table 8.1) in AM colonisation of the roots of *M. minima* in field plots between sampling times (Table 8.2). Benomyl treatment resulted in suppression of mycorrhizal colonisation ($P < 0.001$; Table 8.1). Colonisation in benomyl-treated plots was reduced to approximately 49 % and 63 % of that in watered plots in June and August, respectively (Table 8.2)..

8.3.1.2 Effects of species competition and mycorrhizas on plant cover

The picture is not simple because a number of interactions between species, species combination, treatment and survey time were evident in the analysis. Results of Wald tests for fixed effects in the generalised linear mixed model are presented in Table 8.3. The four-way interaction could not be estimated due to the experimental design. There was a significant interaction between treatment, species and survey time (Table 8.3). Fig. 8.1 shows that cover of all species increased between survey times. This increase was particularly noticeable for *M. minima*. It is clear from Fig. 8.1 that addition of water increased the cover of *M. minima* relative to unwatered controls, while benomyl treatment counteracted this effect. The cover of *S. verbenaca* and *C. annua* was greater in benomyl-treated plots relative to both watered and unwatered control plots (Fig. 8.1).

The interaction between plant species and species combination was significant ($P < 0.001$; Table 8.3). There appears to be no significant difference in the proportion of touches of *M. minima* in sub-plots with all three species present or when *C. annua* is removed (Table 8.4). However, the proportion of touches of *M. minima* did decrease slightly when *S. verbenaca* was removed (Table 8.4). The proportion of touches of both *S. verbenaca* and *C. annua* was not significantly different in any of the species combinations (Table 8.4).

There was also an interaction between survey time, treatment and species combination ($P < 0.025$; Table 8.3). The highest cover occurred in sub-plots where all three species were competing, regardless of survey time or treatment.

In sub-plots where *S. verbenaca* had been removed there was a significant treatment effect on cover of *M. minima* and *C. annua* (MANOVA: Wilk's Lambda = 0.1080; df = 4, 18; $P < 0.001$). Results from the one-way ANOVA's on cover of *M. minima* and *C. annua* indicate that there was a significant effect of treatment on the cover of each species (Table 8.5). Fungicide (benomyl) treatment resulted in an increase in cover of *C. annua* relative to controls, whereas cover of *M. minima* was higher in watered plots than benomyl-treated and unwatered control plots (Fig. 8.2).

Table 8.1 Results of ANOVA testing the effect of treatment of field plots with fungicide, water or unwatered controls on percent root length of *M. minima* colonized with AM fungi in field plots on 6th June and 8th August 1998, n = 6.

ANOVA		Root length colonised (%)		
Transformation		arcsin (√)		
Factors	df	MS	F-value	$P > F$
Fungicide treatment	2	0.5793	60.83	< 0.001
Sample date	1	0.3294	34.59	< 0.001
F x H	2	0.0127	1.33	0.282

Table 8.2 Mean (SE) percent root length of *M. minima* colonised with AM fungi in field plots treated with benomyl (fungicide) compared to watered or unwatered controls, n = 6.

	Root length colonised (%)	
	June 1998	August 1998
Control	67.5 (3.1)[a1]	88.2 (2.6)[a]
Water	77.6 (2.7)[a]	87.1 (3.6)[a]
Fungicide	38.0 (3.1)[b]	54.7 (4.7)[b]

[1]Means within a sampling time followed by the same letter are not significantly different (Tukey's HSD, $P < 0.05$)

Table 8.3 Wald Statistics on fixed effects in ANOVA on cover of *M. minima*, *C. annua* and *S. verbenaca* in field plots treated with fungicide, water or unwatered controls.

Fixed Effects	df	Wald Statistic	*P* – value
Treatment	2	12.1	ns[1]
Species	2	332.9	ns
Species combination	3	47.1	ns
Survey time	1	59.0	ns
Treatment x Combination	6	15.2	ns
Treatment x Species	4	163.4	ns
Combination x Species	6	2 4.4	< 0.001
Treatment x Survey	2	5.8	ns
Combination x Survey	3	19.5	ns
Species x Survey	2	12.9	ns
Treatment x Combination x Species	12	9.0	ns
Treatment x Combination x Survey	6	14.5	< 0.025
Treatment x Species x Survey	4	18.4	< 0.001
Combination x Species x Survey	6	3.8	ns
Treatment x Species x Combination x Survey[2]	12	NA	NA

[1]ns = not significant
[2]Four-way interaction cannot be estimated due to the nature of the experimental design

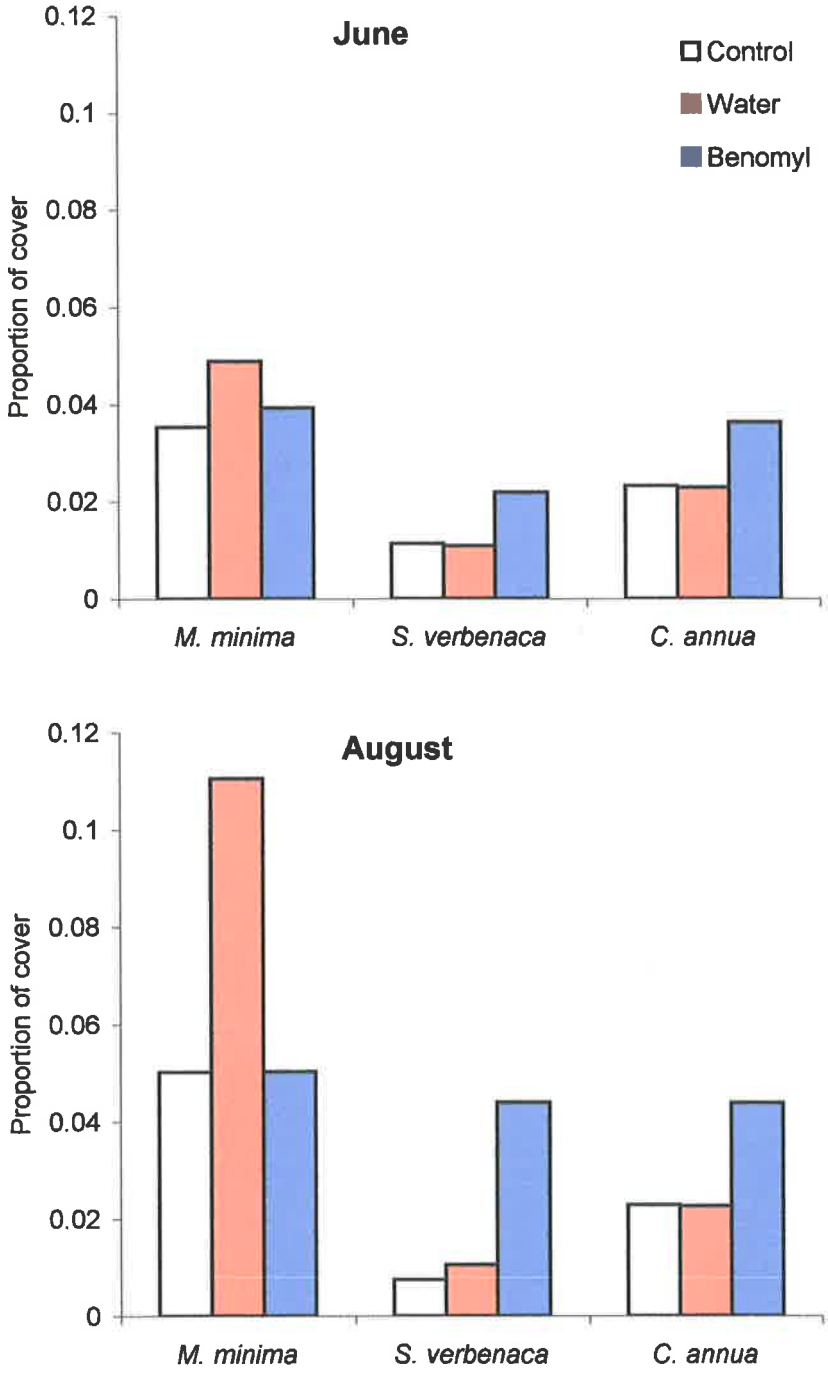

Figure 8.1 Proportion of cover of *M. minima*, *S. verbenaca* or *C. annua* in field-plots treated with benomyl or water compared to untreated control plots surveyed on 6th June and 8th August, n = 6.

Table 8.4 Probability of plant species covering a random point in sub-plots with different combinations of *M. minima*, *S. verbenaca* and *C. annua*, n = 6

Plant species	Species combination			
	All species	*M. minima* versus *S. verbenaca*	*M. minima* versus *C. annua*	*S. verbenaca* versus *C. annua*
M. minima	0.4338	0.4364	0.3601	-
S. verbenaca	0.0959	0.1093	-	0.1245
C. annua	0.2358	-[1]	0.2188	0.2196

[1]Plant species not present in this species combination

Table 8.5 Results of ANOVA testing the effect of treatment of field plots with fungicide, water or unwatered controls on plant cover in the *M. minima* vs *C. annua* sub-plots, n = 6.

ANOVA		Plant cover					
		M. minima			*C. annua*		
Transformation		arcsin (%)			arcsin (%)		
Factors	df	MS	F-value	*P* > F	MS	F-value	*P* > F
Fungicide treatment	2	0.2941	13.75	0.001	0.0436	6.48	0.016

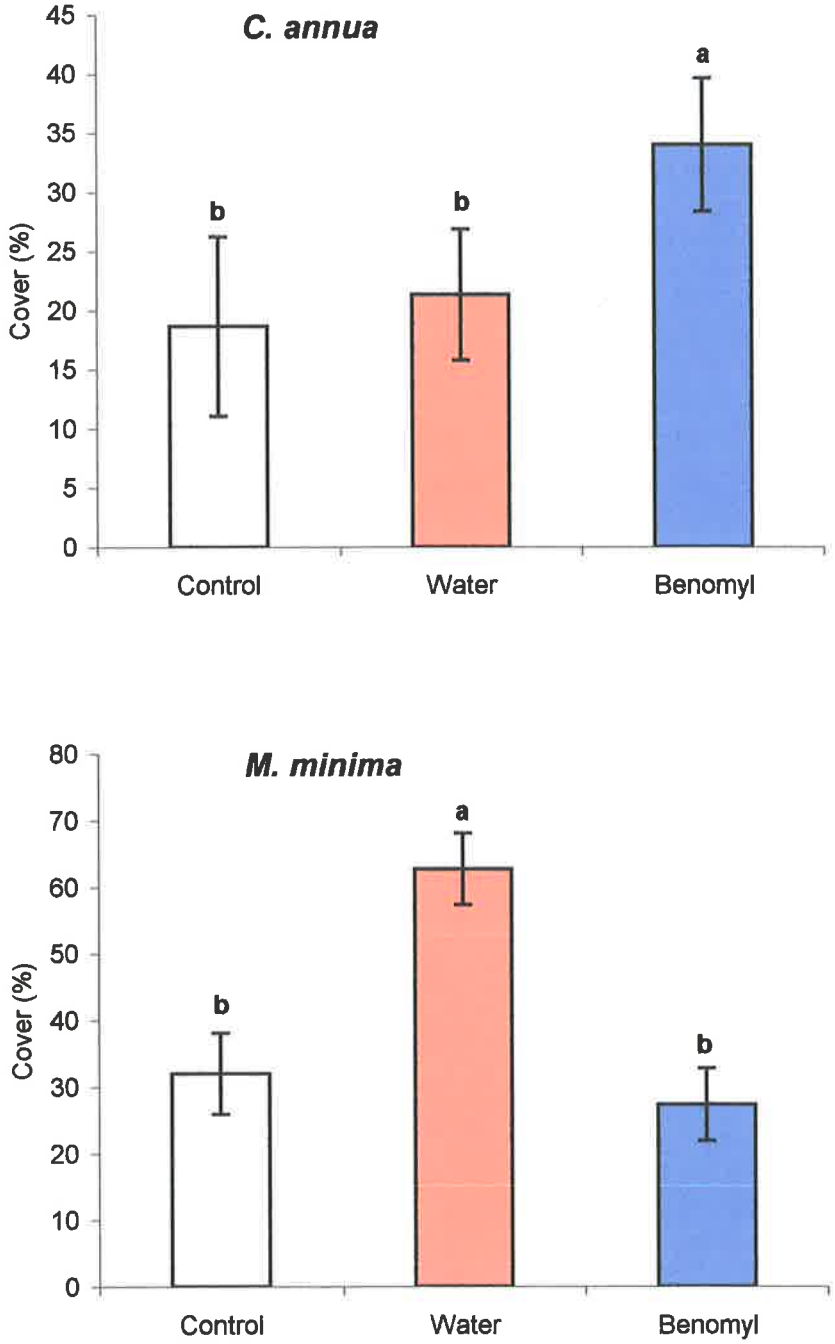

Figure 8.2 Cover of *C. annua* and *M. minima* in *C. annua* v *M. minima* sub-plots treated with benomyl or water, compared to untreated controls. Bars represent standard error of the mean. Letters above the bar indicate a significant difference ($P < 0.05$; Tukey's HSD) between the treatments, n = 6.

8.3.2 Offspring vigour

8.3.2.1 Allocation of seed resources

The concentrations of N, S, Zn and Cu were higher, and the concentration of Ca lower, in *M. minima* seeds from plots treated with fungicide (benomyl) than in seeds from plots treated with water (Table 8.6). While seed P concentration was not different between treatments, total seed P content was significantly higher in fungicide treated plots than watered plots because seed mass was greater in fungicide treated plots (Table 8.7).

The relative frequency distribution of *M. minima* seedpod weights (Figure 8.3) showed a slight positive skew for seedpods from benomyl-treated plants. However, the distribution of seedpod weights did not correspond in significantly different seedpod population inequality between parental treatments (measured by Gini coefficients; $P = 0.793$; Table 8.7).

There was no seedling mortality in any of the boxes for offspring from either parental treatment. After six weeks, seedlings from fungicide-treated parent plants were significantly larger than seedlings from water-treated control plants ($P < 0.051$; Table 8.8). This maternal treatment effect was not apparent after only four weeks. The relative frequency distribution of offspring dry weights after four and six weeks (Figure 8.4) showed a close-to-normal distribution for seedlings from both parental treatments. Hence, there was no significantly different population inequality, measured by Gini coefficients, in the distribution of seedling weights from different parental treatments ($P = 0.875$; Table 8.8).

Table 8.6 Nutrient concentrations in seed from *M. minima* plants treated with fungicide (benomyl) or water in *M. minima* vs *C. annua* sub-plots, n = 6

Nutrient	Seed nutrient concentration	
	Water	Fungicide
P (mg g^{-1})	4.23 (0.22)	4.10 (0.14)
N (mg g^{-1})	74.01 (1.08)**[1]	76.88 (0.53)
K (mg g^{-1})	7.90 (2.86)	7.73 (0.19)
Ca (mg g^{-1})	2.33 (0.10)**	1.85 (0.06)
S (mg g^{-1})	3.03 (0.09)*	3.43 (0.10)
Mg (mg g^{-1})	1.76 (0.07)	1.63 (0.05)
Zn (μg g^{-1})	48.18 (1.53)*	60.03 (1.94)
Cu (μg g^{-1})	15.05 (0.77)*	18.18 (0.80)
Fe (μg g^{-1})	73.18 (11.07)	89.52 (4.96)
B (μg g^{-1})	15.82 (0.38)	15.95 (0.65)
Mn (μg g^{-1})	18.89 (0.74)	17.82 (0.36)

[1]nutrient concentrations in seed from watered plots are significantly different (*$P < 0.05$, **$P < 0.01$; ANOVA) to those in fungicide treated plots

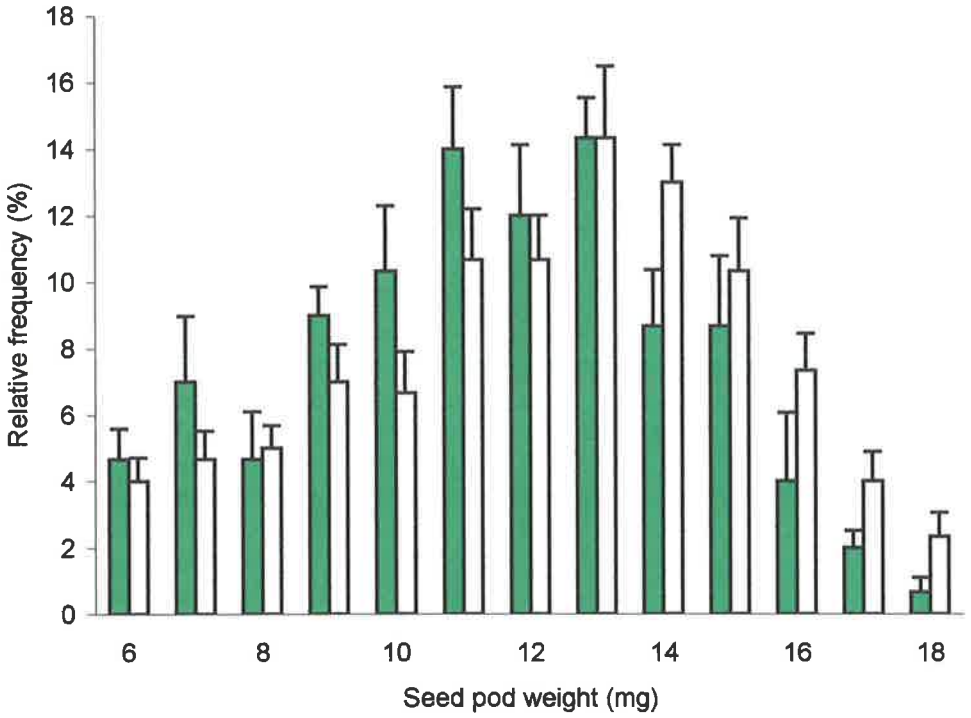

Figure 8.3 Mean relative frequency of *M. minima* seed pod weights from plants treated with benomyl (unshaded bars) or water (green bars), n = 300.

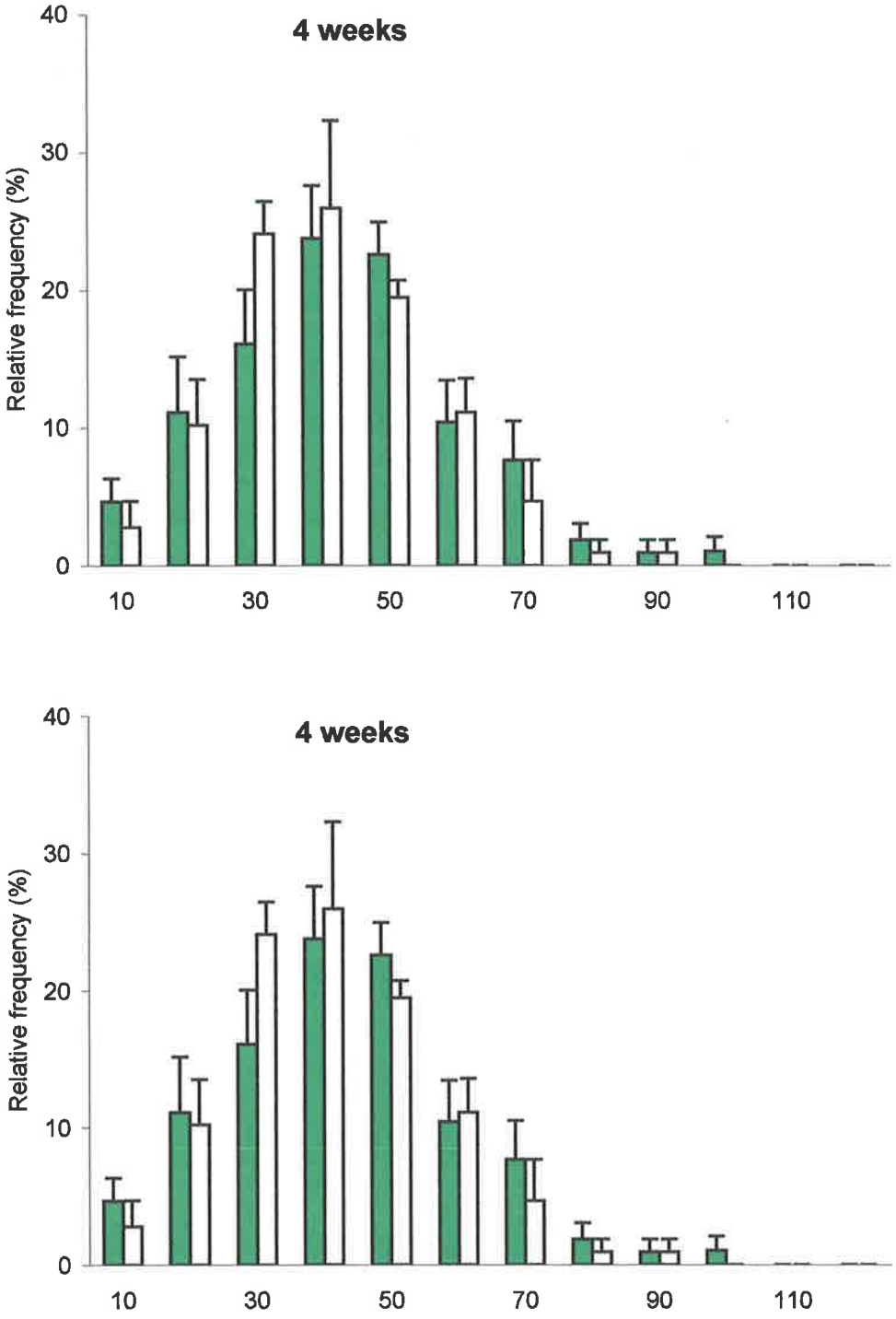

Figure 8.4 Mean relative frequency of shoot dry weights of *M. minima* offspring from maternal plants treated with benomyl (unshaded bars) or water (green bars) after four or six weeks growth. Bars represent standard error of the mean for each size category, n = 108.

Table 8.7 Mean (SE) values for seed weight, total seed P and seedpod inequality for *M. minima* plants grown in *M. minima* v *C. annua* field plots treated with benomyl (fungicide) or water

Maternal treatment	Seed weight (mg)	Total seed P (μg)	Gini coefficient (seedpod weight)
Water	0.84 (0.03)	3.54 (0.19)	0.137 (0.008)
Fungicide	1.01 (0.01)	4.13 (0.14)	0.140 (0.009)
ANOVA			
P- values	0.002	0.012	0.793

Table 8.8 Mean (SE) shoot dry weight and Gini coefficients for competing offspring from benomyl and water treated parent plants, n = 108

Maternal treatment	Shoot dry weight (μg)		Gini coefficient (shoot dry weight)
	4 weeks	6 weeks	6 weeks
Water	39.0 (1.8)	81.7 (3.8)	0.198 (0.017)
Fungicide	38.5 (1.6)	90.8 (3.6)	0.195 (0.014)
ANOVA			
Parent	0.238		0.875
Harvest	< 0.001		-
P x H	0.051		-

8.4 Discussion

By the end of the growing season there were obvious but opposite effects of benomyl treatment on *M. minima*, compared to *S. verbenaca* and *C. annua*. The picture is not simple because the four-way interaction term could not be calculated. However, it is clear that additional water increased the cover of *M. minima*, while benomyl-addition counteracted this effect. Conversely, additional water did not significantly increase the cover of *S. verbenaca* or *C. annua*, whereas the addition of benomyl did increase the cover of these two species. Thus it is evident that there was competition between the three plant species and that processes affected by benomyl mediated this competition.

The mycorrhiza-responsiveness of the three plant species in this experiment, the effective suppression of AM colonisation in this experiment, and the minimal side-effects of benomyl on non-target organisms and soil nutrients at this site (Chapter 6), implicate AM fungi as an agent responsible for at least part of the interspecific competition in this system. The reduced cover of *M. minima* in plots where *S. verbenaca* had been removed (relative to plots where all three species were present) adds extra support to the idea that competition between plant species is mediated by mycorrhizal activity. When *S. verbenaca* was removed *C. annua* cover did not increase significantly, suggesting that the reduction in *M. minima* growth was not entirely due to direct competition but that *M. minima* may derive some benefit from the presence of *S. verbenaca*.

S. verbenaca has been shown to support a high level of AM colonisation but derives little growth benefit from the mycorrhizal association (Table 5.1 & 5.2). While direct measurements of the extraradical mycelium of AM fungi were not made, any AM-hyphal network supported by *S. verbenaca* would be a potential resource for the mycorrhiza-responsive *M. minima*. Loss of *S. verbenaca* as a host for this network by removing the aboveground, photosynthesising shoots might result in higher maintenance costs for *M. minima*, thus rendering it less competitive against a non-host plant such as *C. annua*. The increased growth of *S. verbenaca* and *C. annua* in benomyl-treated plots is also likely to be the consequence of release from competition with *M. minima* due to reduction in mycorrhizal function.

While it is possible that mycorrhizal connections between *M. minima* and *S. verbenaca* could result in net carbon transfer between the species, transfer of this type has not yet been shown to be biologically significant for the host plant (Fitter *et al.* 1998). It has been shown previously that one plant species may derive more nutritional (and so growth) benefit from a mycorrhizal network than other host plants on the same network (Newman *et al.* 1992; Marler *et al.* 1999). A non-host or a facultatively mycorrhizal plant such as *S. verbenaca* may be able to colonise disturbed sites where mycorrhizal propagules are in low abundance. However, as succession proceeds and more obligately mycorrhizal plants become more competitive, through more effective utilisation of the mycorrhizal network, non-mycorrhizal and facultatively mycorrhizal plants may be disadvantaged (Janos 1980a; Allen and Allen 1984). In this experiment the mycorrhiza-responsive host *M. minima* was relatively more productive than the non-host *C. annua* when AM activity was not suppressed. The development and maintenance of a community of AM fungi capable of forming mutualistic associations with *M. minima* contributes to the competitive successes and dominance of that species in this semi-arid herbland. *S. verbenaca* may contribute to the maintenance of the AM fungus community and thereby contribute to succession in the plant community as conditions become more favourable for mycorrhiza-responsive plant species such as *M. minima.* Differential growth response in the presence of AM fungi has been previously been observed in co-occurring plant species and may be an important determinant of community structure (Grime *et al.* 1987; Hetrick *et al.* 1989; Koide and Li 1991; Sanders and Koide 1994; Hartnett and Wilson 1999a).

Another determinant of plant community structure, which may be influenced by mycorrhiza, is fecundity. Arbuscular mycorrhiza have been shown to significantly increase fecundity of host plant species by directly increasing seed quality (Sanders and Koide 1994) or seed production (Stanley *et al.* 1993) via enhanced nutrient uptake, or indirectly by reducing the negative effects of pathogenic fungi (Carey *et al.* 1992; Newsham *et al.* 1994). Mycorrhizas have also been shown to improve the vigour of offspring plants (Lewis and Koide 1990; Lu and Koide 1991; Koide and Lu 1992; Shumway and Koide 1994; Srivastava and Mukerji 1995; Heppell *et al.* 1998). However, the reproductive response of a host plant to mycorrhizal colonisation will be influenced by competition from other plant species differently affected by the

mycorrhizal fungi. In this experiment, a reduction in mycorrhizal activity resulted in increased competition for the mycorrhiza-responsive *M. minima* from the non-host *C. annua*.

While benomyl-suppression of mycorrhizal colonisation decreased the competitiveness of *M. minima*, the seeds of *M. minima* from benomyl-treated plots were larger and contained more P than *M. minima* seeds from watered plots. Unfortunately, seed number was not counted in this experiment and total reproductive output cannot be calculated. However, reduced *M. minima* seed size and quality in watered control (mycorrhizal) plots is unexpected, because vegetative growth was greater in these plots than in fungicide-treated (mycorrhiza-suppressed) plots. There are several possible explanations for this result, as follows.

Mycorrhizal colonisation of *M. minima* in field plots was reduced by benomyl addition to approximately 43 % of watered controls in June. This large reduction in the activity of AM fungi and increased competition from *C. annua* may have resulted in a decrease in uptake of P by the mycorrhizal network and increased mortality of the highly mycorrhiza-responsive *M. minima* plants. Seedlings that have smaller seed reserves are more susceptible to mortality when environmental hazards reduce establishment success (Westoby *et al.* 1996). Environmental hazards resulting in reduced establishment of individuals with smaller seed reserves include mineral nutrient shortage (Lee and Fenner 1989; Jurado and Westoby 1992) and drought (Leishman and Westoby 1994). Hence, the larger seed of *M. minima* plants from benomyl-treated plots may be the result of selection pressures on the early stages of growth of the parent plants. This selection pressure may have resulted in survival and sexual reproduction of individuals with a genotypic advantage over small seeded individuals in the population. If large seed size is genetically determined in *M. minima*, then seeds from the surviving plants in benomyl-treated plots would be expected to be larger than seeds from a population where nutrient stress was not such a severe selection pressure (watered plots).

Alternatively, the difference in *M. minima* seed size between treatments may be the result of the compromise in reproductive allocation between more small seeds and fewer large seeds (Leishman *et al.* 1995). Seeds with more resources are thought to have

improved chance of establishment and survival (Salisbury 1942; Grime *et al.* 1988; Kidson and Westoby 2000). In an environment where resources are scarce or competition for resources is strong, plants may adopt a strategy of investing in fewer seeds with greater resources and consequently greater chance of surviving. In water-treated plots, *M. minima* plants had access to P through mycorrhizal networks and consequently, were more competitive against *C. annua*. When mycorrhizal activity was suppressed by benomyl, the competitiveness of *M. minima* was reduced. Survival of *M. minima* offspring in plots with low activity of AM fungi would be increased if offspring had larger seed reserves to sustain them through early environmental stresses. Nutrient stress in circumstances where mycorrhizal activity is low may stimulate *M.minima* to reduce reproductive output and increase resource allocation to each seed.

The hypothesis that greater seed reserves results in more competitive offspring was borne out in the study of offspring competition. There was no significant difference in the offspring size after four weeks, but by six weeks offspring of plants grown under mycorrhiza-suppressed conditions were larger. The differences in average seed size and seed-P (used as covariates) between maternal treatments did not explain the superior size of offspring from maternal plants with low mycorrhizal colonisation. This may be due to the use of average seed size, seeds from each block were weighed in groups of 100 because individual seeds weighed $\leq$ 1 mg. The effect of averaging may have reduced the power to discriminate between treatments. Another possible explanation is that differences in offspring size were not large enough to be explained by seed weight or P-content. Because the soil used to assess offspring competitiveness was unsterilised field soil, which has been shown to produce a mycorrhizal growth response *in M. minima* (Chapter 5), colonisation of offspring plants may have allowed seedlings with small P-reserves to grow as rapidly as seedlings with larger seed reserves until seedlings were large enough to compete with each other.

Despite differences in the mean size of offspring from different parental treatments, there was no significant effect of parental treatment on size inequality between offspring. This may also be due to unimpeded growth of all seedlings in the unsterilised soil until seedling size resulted in competition late in the experiment (between four and six weeks). The fact that there was no mortality in offspring from either parental

treatment also suggests that competition between individual seedlings may not have been strong in the early stages of growth. There was little difference in the inequality between seedpods or between offspring from different parental treatments. The low inequality in offspring size suggests that competition was not very intense. Where competition is intense, asymmetric competition is likely (Weiner 1990) and inequality within the population is likely to increase. Arbuscular mycorrhiza have been shown to increase the competitiveness of single responsive plant species (Koide and Shumway 1995; Heppell *et al.* 1998) but little consideration has been given to the additional effects of interspecific plant competition in more complex plant communities. Mortality has been shown to be higher amongst classes of smaller individuals (Schmitt *et al.* 1987; Heppell *et al.* 1998) and long-term changes in community structure could be expected if mortality rates changed due to changes in mycorrhizal activity. While it seems mortality rates of *M. minima* offspring may be increased by suppression of mycorrhiza in the parent generation, it is likely that this increased seed size and subsequent seedling survival is a trade-off for high seed numbers when mycorrhizal activity is high.

These experiments show that AM fungi can increase the vegetative growth and competitiveness of the mycorrhiza-responsive *M. minima* at the expense of the non-mycorrhizal species *C. annua*. *M. minima* also competes effectively with the mycorrhiza-nonresponsive species *S. verbenaca*, and derives some additional benefit from the presence of this species by drawing on the mycorrhizal network partially supported by *S. verbenaca*. Offspring of *M. minima* plants grown in field plots where the activity of AM fungi had been reduced by fungicide application were more vigourous than offspring from mycorrhizal control plots. The increased vigour of offspring from mycorrhiza-suppressed plots may lead to increased survival if the mycorrhizal inoculum level at this site remained low or if seed were dispersed to a disturbed site where competition from non-mycorrhizal ruderals such as *C. annua* was more intense. *M. minima* may modify seed production, and seed size and nutrient content, to optimise its colonisation success at sites in varying stages of development of a robust mycorrhizal network.

Changes in the activity of the AM network due to soil disturbance or reduction in the amount of carbon allocated belowground as a consequence of overgrazing, or prolonged drought, may lead to changes in community structure at this semi-arid site. The consequences of interspecific plant competition, mediated by mycorrhizal association of some species, will result in interannual variability in the competitiveness of annual plants, and in fluctuations in community structure.

Chapter 9

General Discussion and Future Research

9.1 Introduction

The main findings of this study show that the activity of mycorrhizal fungi influences plant growth and reproduction, plant competition, and community structure in a semi-arid herbland in South Australia. This supports the important role the soil community plays in determining the structure and diversity of plant communities (Bever *et al.* 1997), which has become more apparent as ecological studies have focussed on how biodiversity influences the functioning of ecosystems (Tilman 2000). Account must be taken of the potential role of microorganisms such as AM fungi in structuring plant communities (Watkinson 1998; Clay and Holah 1999). While factors such as climate, resource availability, disturbance frequency and plant-plant and plant-herbivore interactions have been considered major determinants of plant biodiversity (Tilman and Pacala 1993), symbiotic associations between plants and AM fungi may affect biodiversity and influence plant communities differently as other factors vary. For a comprehensive understanding of the factors influencing plant biodiversity it is necessary to study the effects of AM fungi on a number of plant communities differing in composition and controlling influences. Arid environments have received less attention than temperate ones. One important outcome of this study is that it has established the mycorrhizal status of 74 plant species in arid and semi-arid Australia and the influence of arbuscular mycorrhizas in a semi-arid herbland at Brookfield Conservation Park, South Australia.

9.2 Effects of mycorrhizas on plant growth, community structure and diversity

The effect of mycorrhizas on plant community structure and diversity was shown to be dependent on the responsiveness to mycorrhizal colonisation and the dominance of individual species in the community. The growth of a number of host-plant species was significantly increased by mycorrhizal colonisation in individual species responsiveness tests (Chapter 5); however, the biomass of several of these species was not significantly different in benomyl-treated or untreated field plots (Chapter 6). The benefit that a plant species derives from mycorrhizal association is thus shown to depend on the competitiveness of neighbouring species. *Vittadinia gracilis* and *Velleia arguta* both

showed large growth responses to colonisation by mycorrhizal fungi. While the reduction in activity of mycorrhizas in benomyl-treated field plots would be predicted to result in reduced growth of these species, competitive release from the highly mycorrhiza-responsive *Medicago minima* resulted in no net change in their biomass. In this way the mycorrhiza-dependency of each species interacts with the competitiveness of that species relative to other species present in the community to determine the effects of mycorrhizas on community structure. It also follows that effects of mycorrhizas on plant diversity result from the interaction of mycorrhiza-responsiveness and relative competitiveness. While a reduction in the activity of mycorrhizal fungi may result in loss of highly mycorrhiza-dependent species from the community (a reduction in species richness), an increase in species evenness will tend to increase diversity.

At the time these field studies began (February 1997), results from previous investigations had indicated that the activity of mycorrhizas could increase plant diversity, however, a limited number of experimental plant communities had been investigated (Grime *et al.* 1987; Gange *et al.* 1990). It was not clear whether mycorrhizas would increase plant biodiversity in all plant communities. Bergelson and Crawley (1988) recognised that the mycorrhiza-responsiveness of the dominant plant species could influence diversity within the community and Hartnett and Wilson (1999a) have recently shown this in a tallgrass prairie community. In the main experiment reported here (Chapter 6), floristic diversity increased, whereas, species richness was not affected when mycorrhizal activity was reduced. Thus emphasising the importance of changes in species evenness in the determination of plant diversity.

Competition between the three dominant species in the understorey at Brookfield North (*M. minima, C. annua* and *S. verbenaca*) is affected by the activity of AM fungi (Chapter 5 & 7). Reduction in the activity of AM fungi resulted in reduced growth of the mycorrhiza-responsive species *M. minima* and increased growth of the non-mycorrhizal *C. annua* (Chapter 6 & 7). Removal of the non-responsive mycorrhizal host *S. verbenaca* had a negative effect on *M. minima*, similar to the effect resulting from suppression of AM fungi (Chapter 6). This effect is probably because *S. verbenaca* is a mycorrhizal plant and supports the persistence and growth of the mycorrhizal mycelium without showing any growth response. Reduction in the activity of AM fungi resulted in

a decrease in growth of *M. minima* and an increase in the relative competitiveness of *C. annua* and *S. verbenaca.* Reduced competition from M. minima in mycorrhiza-suppressed plots also compensated for the reduction in growth of the mycorrhiza-responsive species *V. gracilis* and *V. arguta.* The combined effect of reduced competitive dominance of *M. minima* in mycorrhiza-suppressed plots was an increase in species evenness and diversity (*H'*). Recently, Smith *et al.* (1999) have also shown that mycorrhizal fungi can indirectly affect diversity in plant communities by influencing the pattern and strength of competitive interactions between the plants (in a tallgrass prairie). The mycorrhiza-responsiveness of component plant species, especially dominant species, can be an important determinant of diversity and structure in plant communities.

Where plant diversity is measured as species richness alone, careful attention should be given to species selection and planting densities (ie. in experimental microcosms), and to factors other than treatment which influence the survival of species occurring in low abundance. In microcosms constructed by planting seedlings at approximately natural relative abundance and densities Wilson *et al.* (1997) found that five out of eight species had survivorship in fully-mycorrhizal microcosms of less than 75% after 42 days. One species, *Liatris aspera*, was absent from three of the 16 fully-mycorrhizal microcosms and one of the 16 mycorrhiza-suppressed microcosms. Where low survivorship is not the result of mycorrhizal affects, determination of mycorrhizal influence on species richness and diversity may be confounded by other factors affecting survival. The non-treatment effects influencing species survival in the experiment of Wilson *et al.* (1997) can be considered to be a hidden treatment(s) (*sensu* Huston, 1997), potentially resulting in "diversity" differences unrelated to mycorrhizal treatments. The creation of a plant community in microcosms from a subset of naturally co-occurring species also carries a probability that a species highly responsive to treatment will be included. In this case, the effect of mycorrhizas on diversity could be predetermined by the inclusion of one or more highly mycorrhiza-responsive dominant species. The selection of species for experimental microcosms is probably the cause of the conflicting results of (Grime *et al.* 1987) and (Wilson and Hartnett 1997) with respect to the effect of mycorrhizas on plant biodiversity.

In the experiments described in Chapter 5, microcosms were constructed by collecting intact cores from the field. The limited size and number of these microcosms necessarily resulted in the absence of minor species in many of the replicates. Although occurrence of species in each microcosm was randomly determined, the low number and small size of replicates may have obscured the effects of treatment on diversity. Significant changes were observed in the relative contribution of each species to total microcosm biomass but the balance of species richness and relative biomass was not altered enough to significantly affect diversity, richness and evenness in mycorrhiza-suppressed microcosms from either Brookfield North or Brookfield South (Section 5.3.3 & 5.3.4). Species richness was lower in microcosms than in field plots (Chapter 6) because minor species were absent from many replicates. Results showed that reduced dominance of the unresponsive mycorrhiza-forming species in the mycorrhiza-suppressed treatments were offset by increases in the biomass of non-mycorrhizal species. The combined effect of changes in biomass of all species was no net change in species evenness. In field plots where mycorrhizal activity was adequately suppressed (Section 6.4), diversity increased due to increased evenness – ie. the dominant mycorrhiza-responsive plant had reduced abundance. While results from the microcosm experiments may be an artefact of the experimental design, they suggest an effect of mycorrhizas on plant diversity other than an increase (Grime *et al.* 1987) or decrease (Wilson and Hartnett 1997; Hartnett and Wilson 1999a). No net effect of mycorrhizas on plant diversity might be expected when changes in the abundance or biomass of mycorrhiza-responsive and unresponsive plants compensate for each other; or when the dominant species are unresponsive to mycorrhizas and suppression of mycorrhizas has little effect on community structure.

Succession

Facultative responsiveness to mycorrhizal colonisation is thought to be characteristic of early-successional species, while many pioneer species do not form mycorrhiza at all (Janos 1980b; Janos 1980a). The presence of non-mycorrhizal and mycorrhiza-unresponsive species in the early successional semi-arid understorey communities at Brookfield Conservation Park is consistent with theory about the role of mycorrhiza in community succession (Janos 1980a; Janos 1985; Reeves 1985; Pankow *et al.* 1991). Disturbed sites with low numbers of propagules of arbuscular mycorrhizas will not

support obligately mycorrhizal plant species and will not favour highly mycorrhiza-responsive species. Pioneer species such as the annuals *C. annua*, *Si. apetala*, and the short lived *S. verbencaca* will establish and grow. As the soil stabilises and propagule numbers of AM fungi increase, mycorrhiza-responsive species such as *M. minima* will become more competitive. Changes in the Brookfield grassland due to overgrazing may have changed the quantity and/or quality of resources for the soil microbial community, including the AM fungi. Grazing has been shown to significantly reduce levels of mycorrhizal root colonisation and spore densities by reducing the leaf area and increasing the root:shoot ratio, thus reducing the source capacity required to meet the demands of the AM fungi sink (Bethlenfalvay and Dakessian 1984; Bethlenfalvay *et al.* 1985). As the grazing impact on the vegetation is ongoing, reestablishment of perennial grasses and other species characteristic of the climax community may not be possible in the prevailing conditions, and succession may be stalled at the current pioneer sera.

Semi-arid grasslands in South Australia are characteristically open in structure with perennial tussock grasses interspersed with predominantly annual herbs (Specht 1972). The density of cover is typically low and gaps are common. This is in contrast to the closed turf-forming systems most studied in the temperate regions of Northern Hemisphere (Francis and Read 1994). However, some results from studies in temperate northern ecosystems may be usefully applied to the grassy understorey at Brookfield Conservation Park. Francis and Read (1994) have shown that AM fungi can have adverse effects on 'r' selected plant species while at the same time having beneficial effects on 'K' selected species. The potential creation of 'gaps' through reduction in the density of tussock grasses in a semi-arid grassland may result in invasion by ruderal herb and grass species which might otherwise be uncompetitive in the 'closed' grassland. This may be facilitated by a shift in the composition of the AM fungal community away from perennial 'K' selected species in symbiosis with the perennial grasses, and toward 'r' selected species which form associations with annual plant species during the winter growing season. The lack of host specificity of AM fungi (mostly shown in experiments with single fungal species) does not necessary mean that the fungi do not have different abilities to compete for host resources, form mycelial networks, persist in the community (Ezawa *et al.* 1995) and antagonise non-host plant species. However, current knowledge of the ecology of different species of AM fungi is

limited and methods for identification, detection and quantification of AM fungi are not yet refined enough to study functioning fungal communities. Molecular techniques for detecting different isolates of AM fungi in the soil are developing and may offer possibilities for more detailed study of the fungi (Kjoller and Rosendahl 2000).

9.4 Mycorrhizal effects on plant interactions, reproduction and offspring vigour

In a plant community dominated by short-lived species, such as those in the semi-arid herbland studied here, the reproductive output of individual species will play a large part in determining community structure in successive years. Effects of mycorrhizas on reproduction, either directly on seed number or quality or indirectly by altering competitive outcomes between parent plants, will influence the populations of those plant species in successive years. Competition between the three most abundant plant species in the semi-arid herbland was mediated by mycorrhizas. Competition between plants impacted on both the growth and the reproduction of individual species. Suppression of mycorrhizas in field plots resulted in reduced growth of the mycorrhiza-responsive species *M. minima*, and competitive release of the mycorrhiza-unresponsive species *C. annua* and *S. verbenaca* (Chapter 8). The effect of mycorrhizas on competition between these species is also supported by results from experiments in intact cores (Chapter 5).

In field plots where *M. minima* was competing with *C. annua*, suppression of mycorrhizas resulted in decreased growth of the mycorrhiza-responsive *M. minima*, but seed size and quality of this species increased (Chapter 8). Offspring of *M. minima* plants from mycorrhiza-suppressed plots also showed greater vigour than offspring from control plants when offspring from both control and mycorrhiza-suppressed parents were grown in competition with each other (Chapter 8). The increased seed size and quality of *M. minima* from mycorrhiza-suppressed plots may be the result of a compromise in reproductive allocation between more small seeds and fewer large seeds. The decreased competitiveness of *M. minima* in mycorrhiza-suppressed plots (compared to *C. annua*) may have stimulated *M. minima* to reduce reproductive output and increase resource allocation to each seed to optimise the competitiveness of offspring in the subsequent generation. Further work is necessary to fully understand the effects of

mycorrhizas on reproduction in these species and on long-term effects on plant community dynamics.

9.5 Non-target effects of suppressing AM fungi with the fungicide benomyl

Research into the effects of AM fungi in natural plant communities has been limited by the difficulty of obtaining appropriate non-mycorrhizal controls. Soils supporting plant communities will ordinarily contain AM fungi. The use of the systematic fungicide benomyl to suppress the activity of AM fungi has proved successful in a number of studies (Pedersen and Sylvia 1997; Hartnett and Wilson 1999b; Smith *et al.* 2000). Benomyl application successfully reduced mycorrhizal colonisation in the current field experiments (Chapter 6, 7 & 8) and resulted in minimal impact on the fungal community in experimental soils (Chapter 6). However, treatment of field soils with benomyl did result in elevated soil NO_3-N concentrations and treatment with water (with or without benomyl) resulted in increased aboveground biomass relative to unwatered control plots (Section 6.4). Increased availability of water and/or nitrogen could have increased competition in these treatments due to increased net resource supply. It is difficult to determine whether changes in soil nitrogen were due to direct effects of benomyl (ie. hydrolysis of carbendazim) or indirect effects of benomyl on soil organisms. Smith *et al.* (2000) have shown that compared to the effect of benomyl on a range of soil organisms and soil resources the relative effects on mycorrhizal colonisation was large. Further work would be necessary to track the effects of benomyl on soil and microbial properties.

The current study did not examine any possible differential effects of treatment on different species of AM fungi in the field soils. Benomyl applications can effect the sporulation and spore production of different AM fungal species differently (Schreiner and Bethlenfalvay 1997b; Schreiner and Bethlenfalvay 1997a). As different AM fungal species may affect plant community structure differently (van der Heijden *et al.* 1998a), impacts of benomyl treatment on the composition of the AM-fungal community may result in effects on the plant community beyond the effects of reduced total mycorrhizal activity. These more subtle effects of the AM fungi on the semi-arid plant community in this study may be included as an intrinsic effect of suppressing the activity of at least some of the AM fungi. In experiments where the soil is disturbed by

mixing, similar changes in the abundance and composition of propagules of AM fungi may potentially occur (Jasper *et al.* 1991). In experiments on plant community structure where the composition of the plant community is non-randomly determined (e.g. Grime (1987; Wilson and Hartnett 1997), soil disturbance in mycorrhizal treatments may lead to changes in the composition of the AM-fungal community. This may potentially lead to changes in the structure and diversity of the plant community (van der Heijden *et al.* 1998b). An additional "hidden treatment" (*sensu* Huston, 1997) effect may then occur if changes in the fungal community result in changes in the number or degree of compatible mycorrhizal associations leading to increased nutrient uptake by some plants.

Suppression of the activity of AM fungi with benomyl resulted in a growth depression in some mycorrhiza-responsive species in cores (Chapter 5). AM fungi were also shown to have some antagonistic effects on the growth of the non-mycorrhizal plant *C. annua*. This antagonistic effect was alleviated when the soils were treated with benomyl. The mechanism of antagonism of non-mycorrhizal plants by AM fungi is not clear but has been observed in other studies (Francis and Read 1995). This effect of AM fungi cannot be discounted in consideration of the factors determining plant community structure. Mycorrhizal antagonism of *C. annua* may be particularly important in shaping the semi-arid plant community studied here as the fecundity of *C. annua* was highly correlated with the size of parent plants. Long-term effects of mycorrhizal antagonism of *C. annua* may be important in plant succession at this site.

9.6 The mycorrhizal status of plants in the Simpson Desert and Stony deserts

The AM status of 74 plant species from arid Australia is reported for the first time. Fifty-four of the eighty-one plant species surveyed (67 %) in the Simpson and stoney deserts showed at least some evidence of AM association (Chapter 3). Members of families not previously known to form AM - Frankeniaceae (*Frankenia plicata*), Myoporaceae (*Eremophila longifolia* and *E. macdonnellii*) and Marsileaceae (the aquatic fern *Marsilea drummondii*) - were all colonised. The remaining non-mycorrhizal plant species were not evenly distributed between the one fern family and twenty-four vascular plant families represented in this survey. Six families (Aizoaceae, Amaranthaceae, Cruciferae, Lamiaceae, Portulacaceae and Zygophyllaceae) contained

only non-mycorrhizal members, while five other families (Boraginaceae, Chenopodaceae, Compositae, Convolvulaceae and Graminae) contained some members which were not mycorrhizal. No significant difference in the proportion of species forming mycorrhizas was observed between different topographic sites or between different plant habits or growth forms. Further research is required to adequately test whether different plant communities in arid South Australia differ in their dependence on mycorrhizal associations.

9.7 Future Research

This study showed that plants in arid and semi-arid Australia commonly form arbuscular mycorrhizal associations and that these associations can be important in structuring plant communities. However, study of the effects of mycorrhizas on natural plant communities is limited by the difficulty of obtaining appropriate non-mycorrhizal controls. In the studies reported here the activity of mycorrhizal fungi was suppressed by the use of the fungicide benomyl, however, potential non-target effects of fungicides on soil organisms and soil resources places limitations on the use of these chemicals. Investigations into the character and significance of mycorrhizas to plants in arid Australia are also limited because of the remoteness of many ecosystems and the variability and unpredictability of seasonal conditions.

Further work is needed to:

1. determine the mycorrhizal status of more of the plant species of arid and semi-arid Australia,
2. assess the importance of mycorrhizal associations in the ecology of plants in a wider range of ecosystems and in different seasons in the Simpson Desert and Stony deserts,
3. develop methods of establishing non-mycorrhizal controls which have minimal impact on non-target organisms and soil resources in field studies,
4. develop methods of detecting the presence, abundance and activity of different species and isolates of AM fungi in field experiments,
5. understand the antagonistic effects of AM fungi on some non-mycorrhizal plant species, especially in the context of competition, reproduction and succession,

6. investigate the effects of competition on the reproductive investment of mycorrhizal plant species, including effects on long-term community dynamics.

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Appendices

Appendix 1

Shoot dry weight and percent root length colonised of minor species (present in less than 30 % of microcosms in either treatment) in microcosms from Brookfield South.

Plant species	Shoot dry weight (g)		Percent root length colonised			
	Benomyl	Control	Benomyl	n[1]	Control	n
Medicago polymorpha	0	0.054 (0.054)	-	0	88.6	1
Vulpia myuros	0.051 (0.034)	0.035 (0.017)	0.8 (0.8)	2	53.1 (1.6)	5
Anagallus arvensis	0	0.023 (0.016)	-	0	37.9 (12.5)	5
Crassula colorata	0.007 (0.005)	0.011 (0.011)	2.6 (2.6)	3	44.7	1
Bromus sp.	0.050 (0.036)	0.010 (0.010)	0 (0)	2	0	1
Spergularia diandra	0.002 (0.002)	0.006 (0.004)	27.8 (13.9)	2	90 (10)	2
Oxalis pes-caprae	0.001 (0.001)	0 (0)	35.7	1	-	0
Phyllanthus lacunarius	0.001 (0.001)	0 (0)	0	1	-	0
Stipa nitida	0.174 (0.102)	0 (0)	0 (0)	2	-	0

[1]Number of microcosms occupied by each species from a maximum of 10.

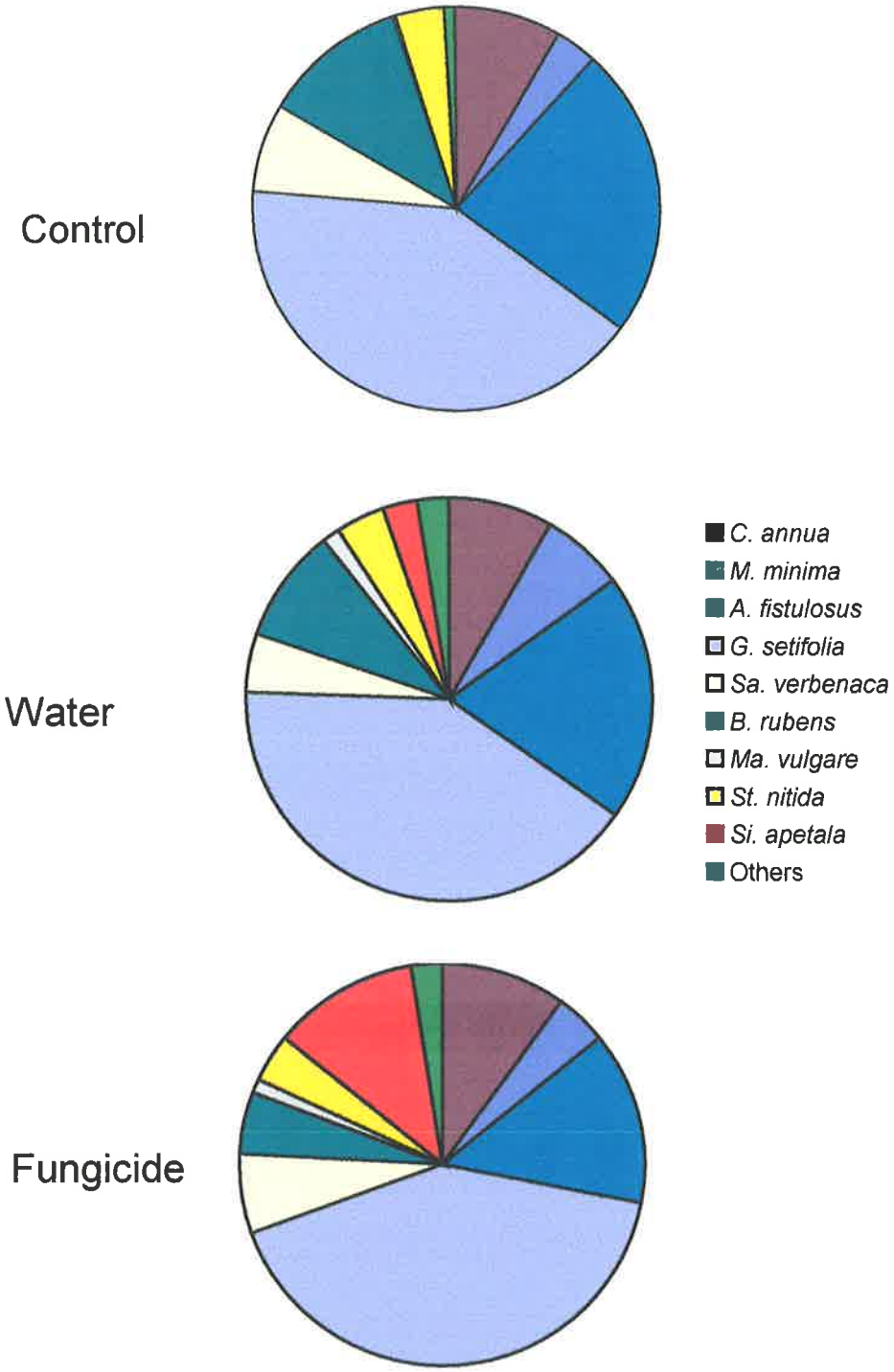
Appendix 2

Shoot dry weight of minor species (present in less than 30 % of microcosms) in Brookfield North microcosms.

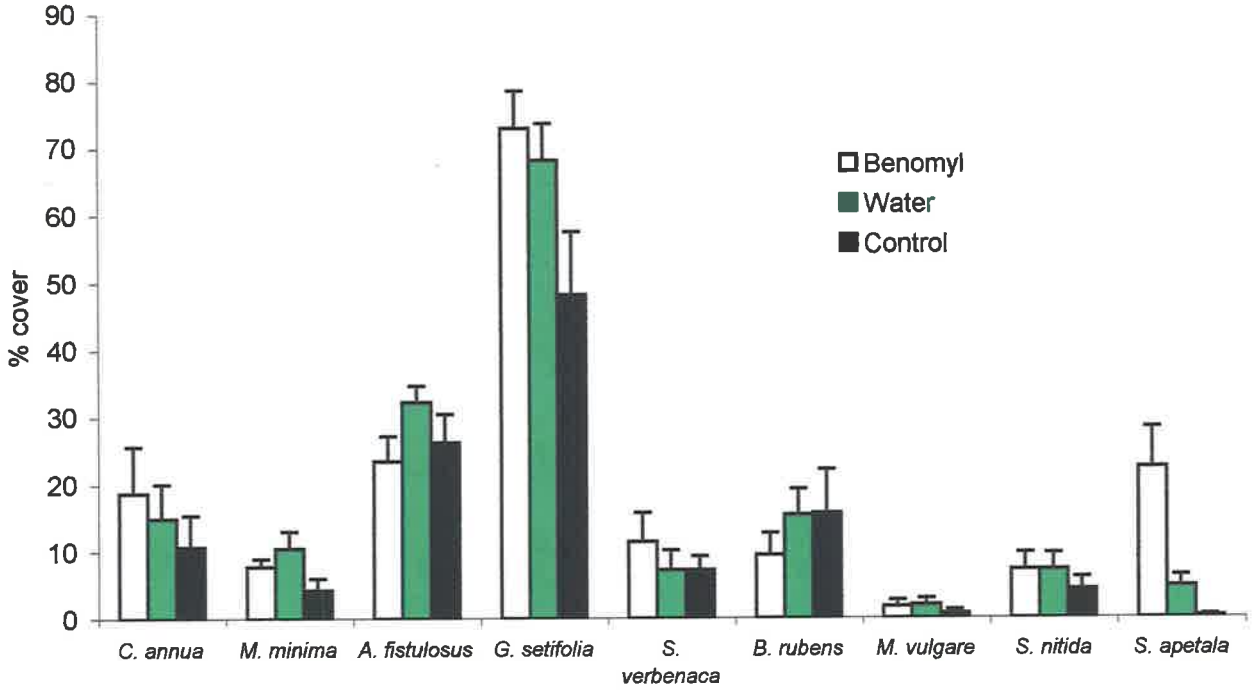
Plant species	Shoot dry weight (g)			
	benomyl	n[1]	Control	n
Gynandriris setifolia	0.003	1	0	0
Erodium crinitum	0.011 (0.009)	2	0.007 (0.005)	3
Velleia arguta	0.041 (0.037)	3	0.005	1
Silene apetala	0.004	1	0.005 (0.004)	2
Spergularia diandra	0	0	0.002	1
Phyllanthus lacunarius	0.006	1	0	0
Stipa nitida	0.051 (0.039)	2	0.084	1

[1]Number of microcosms occupied by each species from a maximum of 10.

Appendix 3



Comparison of community structure differences in the Brookfield South community after fungicide application (benomyl) and water addition in 1997, n = 8.



Percentage cover of each major species in plots treated with benomyl compared to watered and unwatered control plots at Brookfield South 1997. Different letters above the bar indicates a significant difference (LSD, $P > 0.05$) between treatments for that species. Minor species (9) each represent less than 1% of total cover in each of the treatments, n = 8.

Appendix 5

Mean (SE) number of white snails (*Cernuella virgata*) in Brookfield South plots 1997, n = 6.

Treatment	Number of *C. virgata* (m^{-2})
Control	400 (104)[a]
Water	837 (196)[b]
Fungicide	926 (86)[b]
P - value	0.036

Means followed by the same letter are not significantly different ($P < 0.05$) according to Tukey's HSD.