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EFFECTS OF ARBUSCULAR-MYCORRHIZAL FUNGAL COLONIZATION ON MANAGEMENT OF SALINE LANDS

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SUMMARY

EFFECTS OF ARBUSCULAR-MYCORRHIZAL FUNGAL COLONIZATION ON MANAGEMENT OF SALINE LANDS

The overall aim of the research presented in this thesis was to evaluate the importance of arbuscular-mycorrhizal (AM) colonization of plants in management of saline lands. Some aspects of application of AM fungi in revegetation of saline lands are also reported.

Effects of AM pre-inoculation on mycorrhiza-responsive and non-responsive plant growth and establishment were evaluated under glasshouse conditions. The advantages of mycorrhizal fungal inoculation in increasing plant salinity tolerance and establishment in saline conditions were related to the responses of host species to AM fungi. Pre-inoculation with *Glomus intraradices* increased plant growth, nutrient uptake and establishment of mycorrhiza responsive *Trifolium subterraneum* in saline conditions, but non-mycorrhiza responsive *Festuca arundinacea* did not get growth benefits from AM in saline conditions.

The main mechanism underlying increased plant growth and establishment in saline conditions in mycorrhiza responsive plants was increased plant nutrient uptake, particularly phosphorus (P), at an early growth stage. The improvement could be explained by higher soil volume exploration by hyphae and/or roots, faster nutrient uptake and microbial changes in the soil rhizosphere.

AM inoculation and P application effects on salinity tolerance were compared in *Trifolium subterraneum*. Application of P increased plant growth and salinity tolerance in saline conditions, but AM inoculation increased nutrient uptake and plant salinity tolerance more efficiently than P application.

Effects of salinity on AM colonization of chenopods were investigated under glasshouse conditions. Salinity had no effects on AM colonization of *Atriplex nummularia*, but AM inoculation increased plant growth and nutrient uptake. The growth improvement was attributed to benefits from low AM colonization, and changes in bacterial community composition in the rhizosphere.

Roles of AM fungi in influencing P leaching from soil were investigated in experiments with repacked cores under both non-saline and saline conditions. Increased plant size via AM inoculation significantly decreased P leaching in the soil profile under both non-saline and saline conditions in low P soils. Increased root volume and extension external hyphal network were the main effects of AM fungi in increasing plant size under saline and non-saline conditions, which led to scavenging more P and depleting more soil available P, thereby decreasing P losses via leaching. Application of P increased plant size and decreased P leaching, but on the other hand increased soil available P and decreased AM colonization.

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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. 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 of this thesis.

I consent to this copy of my thesis, when deposited in the University library, being made available for loan or photocopying.

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Hamid Reza Asghari

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To my wife

CHAPER 1 - REVIEW OF LITERATURE

1.1 Introduction

Land degradation is currently recognized as one of the most important environmental problems worldwide, due to the unprecedented level of human pressure on land resources over the last several decades. This issue is particularly serious when it occurs in arid and semi-arid regions. Approximately 7% of the global land surface is covered with saline habitats and this amount is extending because of global change and human activities. Revegetation is one of the most important practices that can help the reclamation of degraded lands, but in saline soils the detrimental effects of soluble salts limit it. It is well documented that plant uptake of nutrients and soil aggregation can be assisted by mycorrhizal fungi. Use of mycorrhizal fungi could form the basis of a sustainable method to support the survival of transplanted plants in revegetation by increasing the ability of plant root systems to take up nutrients (especially phosphorus) and water. Their use might also alleviate nutrient flow-through into groundwater in saline conditions.

Plant scientists have conducted extensive studies on the influences of mycorrhizal colonization on water and nutrient uptake by different plants in many conditions. Some information is available about the effects mycorrhizal fungi on salinity tolerance of crop plant species and on the revegetation of salt-affected lands by introducing new salt tolerant plants. However, knowledge of the application of mycorrhizal fungi to rangeland plant establishment and the influence of mycorrhizal fungi on the reduction of nutrient leaching in saline conditions is more limited.

The literature review that follows begins with a discussion of global land degradation and the importance of salinization as an aspect within it. In the next section some

information about previous studies on revegetation of salt-affected land is presented, followed by reports of investigations on the effects of salinity on soil biological activities. Finally, the literature review investigates mycorrhizal symbiosis in saline soils and the effects of mycorrhizal fungi on plant growth, nutrient uptake, soil element retention and soil development in saline conditions.

1.2 Salinization

1.2.1 Definition of soil salinization

Manguet (1991) defined salinization as the accumulation of soluble salts of sodium, magnesium and calcium in soils. The concentration of salt in land and water resources can increase through natural physical and chemical processes or by human activities, processes referred to as primary and secondary salinization respectively (Ghassemi *et al.*, 1995). Primary salinization is a long-term natural process that leads to accumulation of salt in a region. This type of salinization has a wide distribution in many countries (Szabolcs, 1989). Saline regions are found in poorly drained, low-lying areas within semi-arid and arid climates in which large quantities of salts have leached from regions of higher elevation. These leached salts accumulate in the slow-flowing groundwater and are brought to the soil surface in these low-lying areas, through high evapotranspiration (ET) rates (Goudie, 1990).

Secondary salinization occurs because of human activities. In recent decades the impacts of humans on the circulation of salts in the landscape have been profound. Irrigation of cropland has led to the salinization of many soils, especially in arid and semi-arid areas. By adding more water, and inevitably more salt, to an area by irrigation, salts stored in deeper soil layers are mobilized. Another effect of irrigation is the raising of the water table, resulting

in irrigated areas becoming waterlogged and salinized (Goudie, 1990). The effects of salinization and waterlogging are highly complex and an investigation of the combined effects was beyond the scope of the work described in this thesis.

1.2.2 Importance of secondary salinization

Secondary salinization has been identified as a major process of land degradation that is caused by humans and also can be alleviated by them. There are many examples of secondary salinization that has been caused by human activities in the world (Tanji *et al.*, 1986; Mickline, 1991). In Mesopotamia between 3500 and 1700 BC human activities led to land salinization, decrease in the cultivation of wheat and replacement by barley, which is a salt-tolerant crop. Today Mesopotamia is a desert (Boyden, 1987). There is no continent free from serious occurrences of secondary salinization (Table 1.1). For example, all irrigated alluvial soils of Peru show features of salinity. In Australia 80,000 hectares of the valley of the River Murray in Northern Victoria are affected by secondary salinization (Ples & Stannard, 1977), also 1.8 million hectares of previously productive rainfed land in Western Australia are now salt-affected, and, it is forecast, will extend to 6 million hectares by 2015 (State Salinity Council, 2000). More than 40% of irrigated lands in Iran and Iraq are affected by secondary salinization (Pessarakli, 1993). Also it is well documented that British engineers caused a rise of the ground water table and therefore extensive salinization in Pakistan and India as a result of seeking increased food production to reduce risk of famine in the nineteenth century (Ghassemi *et al.*, 1995).

Table 1.1 Estimate of global secondary salinization in the world's irrigated lands (Ghassemi et al. (1995) compiled from FAO data for 1987)

Country	Total land area cropped Mha	Area irrigated		Area of irrigated land that is salt-affected	
		Mha	%	Mha	%
China	97	45	46	6.7	15
India	169	42	25	7.0	17
Soviet Union	233	21	9	3.7	18
United States	190	18	10	4.2	23
Pakistan	21	16	78	4.2	26
Iran	15	6	39	1.7	30
Thailand	20	4	20	0.4	10
Egypt	3	3	100	0.9	33
Australia	47	2	4	0.2	9
Argentina	36	2	5	0.6	34
South Africa	13	1	9	0.1	9
Subtotal	843	159	19	29.6	20
World	1,474	227	15	45.4	20

Secondary salinization will occur where there is a risk of salt accumulation due to poor quality of irrigation water, a rise of groundwater level and the appearance of deep saline materials on the surface. Good agricultural lands have become salinized by road, dam, canal and bund construction, and also by the use of saline groundwater in poor drainage lands for irrigation purposes and changing forestland into agricultural lands by removing forest (Chhabra, 1996). The degree of secondary salinization depends on climatic factors, geological factors and agricultural practices (Szabolcs, 1989).

1.2.3 Classification and significance of salt–affected soils

Salt-affected soils are classified into different groups based on the pH of water-saturated soil paste (pHs), the total soluble salt or electrical conductivity of the water-saturated soil paste extract (ECe), and the exchangeable sodium percentage (ESP). Two broad groups of salt-affected soils are classified as saline and sodic soils. This classification is on the basis of determinations made on soil samples and the effects of neutral and alkaline salts on the soil properties and plant growth (Abrol & Bhumbla, 1978; Bhumbla & Abrol, 1979; Szabolcs, 1989).

According to the US Salinity Laboratory Staff (1954) saline or solonchak soils are recognized by the presence of white salt encrustations on the surface. The common soluble salts in saline soil are chlorides and sulphates of Na, Ca and Mg. Soils with neutral soluble salts have a saturation paste pH< 8.2, and an electrical conductivity of the saturation extracts of more than 4 dS/m. Sodic or solonetz soils are defined as salt-affected soils containing a high level of exchangeable sodium and also sodium carbonates. The soluble salts in these soils mostly contain CO_3^{2-} and HCO_3^{-} of Na, which in the presence of $CaCO_3$ increases the pH of the soil and causes poor physical soil conditions. These soils have pH>8.2 and exchangeable sodium percent (ESP)>15, and the electrical conductivity is normally less than 4 dS/m (Chhabra, 1996).

When the predominant salts in the soil solution are chlorides and sulphates of Ca and Mg, the sodium absorption ratio ($SAR=Na/\{(Ca+Mg)/2\}^{½}$) is usually less than 15. When sodium salts predominate in the soil solution and the sodium absorption ratio is higher than 15, such soils are classified as saline-sodic soils (US Salinity Laboratory Staff, 1954). Saline-sodic soils can be reclaimed by using good quality water with $CaCO_3$ and gypsum (Dieleman, 1963; Leffelaar & Sharma, 1977; Jury *et al.*, 1979; Khosla *et al.*, 1979). In saline-sodic soils the effects of salinity and sodicity on plant

growth are non-additive, and growth is limited primarily by salinity effects (Largewerff & Holland, 1960; Bernstein, 1962).

Salt-affected soils (saline and sodic) are found on all continents, but Australia has the highest percentage of the salt-affected land in the world. There are some estimations of the extent of salt-affected areas in the world. About 10% of the total surfaces of dry lands are saline soils and this amount is growing continuously. It has been estimated that 2.1 billion hectares (Dregne, 1977) or 7% (Dudal & Purnell, 1986) of the world land surface is salt-affected. Table 1.2 shows that all continents are affected by salinity and sodicity problems.

Table 1.2 Regional distribution of salt-affected soils, in million hectares (http://www.fao.org/ag/agl/agll/spush/topic2.htm)

Regions	Total area	Saline soils		Sodic soils	
	Mha	Mha	%	Mha	%
Africa	1,899	39	2.0	34	1.8
Asia, the Pacific and Australia	3,107	195	6.3	249	8.0
Europe	2,011	7	0.3	73	3.6
Latin America	2,039	61	3.0	51	2.5
Near East	1,802	92	5.1	14	0.8
North America	1,924	5	0.2	15	0.8
Total	12,781	397	3.1	434	3.4

1.2.4 Effects of salinity on soil structure

Soil structure is defined as the size and arrangement of particles and pores in soil (Oades, 1984), and it reflects how the individual soil particles bind together or aggregate. Structural stability is the ability of the soil to retain the arrangement of soil particles and pores under stresses. Because of poor soil structure and soil stability in degraded lands, soil erosion caused by wind and water are important problems in these areas. Loss of surface soil is accompanied by losses in nutrients, either in runoff or through-flow to groundwater, and increased potential for surface water pollution. The role of soil stability and soil structure improvement in alleviation of soil erosion and nutrient losses is well documented for degraded lands (LeBissonnais, 1996; Barthes & Roose, 2002). There are no processes occurring in soils that are independent of soil structure, because soil structure dictates the movement of water through the system, the diffusion of gases and hence the activity of all living components in the soil profile.

Soil organic matter is responsible for aggregate stability. Microaggregates mainly form around persistent organic matter and the stability of macroaggregates depends on the amount of organic matter (Waters & Oades, 1991). Higher percentages of water-stable aggregates under cropping regimes were shown to be related to high organic matter inputs (Tisdall & Oades, 1982).

Saline soils usually provide good physical properties suitable for plant growth, when the amount of sodium does not reach very high levels (Shainberg & Letey, 1984). In saline soils due to the high electrolyte concentration, soil dispersion and swelling are minimal, but in sodic soils sodium has the opposite effect on soils to salinity. High sodium concentrations in sodic soils disperse colloidal particles, but in saline conditions colloids flocculate and improve soil aggregation. Flocculation and enhanced aggregation are beneficial in terms of soil aeration, root penetration and root growth

(Oster & Schroer, 1979), but high levels of salinity can have negative and potentially lethal effects on plants (Barbour *et al.,* 1998). Improvement impacts of salinity on soil structure and its negative effects on plant growth should be considered together.

Soil particle dispersion is the dominant mechanism causing decreases in soil hydraulic conductivity (Rhoades & Ingvalson, 1969). Dispersed clay plugs pores in the soil and decreases hydraulic conductivity. Increased sodium concentration in the soil solution increases clay dispersion and consequently decreases soil hydraulic conductivity. On the other hand swelling in saline soils is minimal because of the high salt concentration and clay dispersion is absent when the sodium concentration of the soil solution does not exceed a certain level as increased by the sodium adsorption ratio (SAR). Increased electrolytes in the soil solution cause dehydration of the clay-water system, thereby reducing the distances between particles and consequently soil particle flocculation (Rengasamy & Sumner, 1998). A threshold relation between electrolyte concentration and SAR based on clay dispersion is a useful relation to predict reduction in soil hydraulic conductivity (Yousaf *et al.*, 1987). Changes in soil hydraulic conductivity may change nutrient flow-through into the ground water.

1.2.5 Effects of salinity on plant growth

Salinity has many effects on plant growth but this section describes only those effects that may be important in the context of mycorrhizal symbiosis. An excessive amount of soluble salts in the soil influences plant growth and survival by effects on root growth, evapotranspiration (ET), photosynthesis, protein synthesis, enzyme activity and plant stunting (Staples & Toenniessen, 1984). By inhibiting root growth, salt stress decreases the volume of soil that can be explored by roots and hence the availability and uptake of

water and nutrients. When water and nutrient uptake decrease, plant growth decreases (Delane *et al.*, 1982).

When the concentration of the soluble salts in soil increases, the osmotic potential of the soil decreases (becomes more negative). In saline conditions, the plant is not able to take up water as easily as it can from a relatively non-saline soil. There is a direct and inseparable relation between salt and water stresses. When a plant is transferred from a low salt to a high salt medium, it is immediately subjected to an osmotic dehydration and a decrease in osmotic and water potential, which is called physiological drought or osmotic stress (Levitt, 1980; Chhabra, 1996). Under high levels of salinity not only may roots fail to absorb water from the soil but also, as observed in wheat, a reverse situation may arise and roots may actually lose water to soil (Blum & Johnson, 1992). Evapotranspiration is an essential process in the transportation of water and nutrients in plants. When plants are confronted by water deficiency ET decreases. This phenomenon occurs because of decreasing soil osmotic potential and low water availability, decreasing root growth and decreasing leaf area and maintenance of water in the plant to reduce the concentration of absorbed salts (Chhabra, 1996). A direct relationship between osmotic potential and ET was found by Hayward and Spurr (1994). Balba and Soliman (1978) demonstrated that yield of Sudan grass was decreased because of decreasing ET caused by salinity.

Another effect of salinity on plant growth is a reduction in photosynthesis caused by foliage scorch, tipburn and mottling necroses. Salinity and leaf area are usually inversely related; not only the total leaf area, but also net CO_2 fixation per unit leaf area may decline (McKersie & Leshem, 1994). Chatrath *et al.* (2000) demonstrated that, with an increase in the electrical conductivity (EC) of irrigation water, the net photosynthetic rate and the transpiration rate of fodder oats decreased. Reduction in

photosynthesis capacity is usually associated with a decline in carbohydrate assimilation and growth in saline conditions (Longsteth *et al.*, 1984). Stunting is another physiological effect of salinity on plant growth. Salinity can reduce cell division, cell expansion and leaf size and lead to overall plant stunting by decreasing cytokinin hormone production (McKersie & Leshem, 1994). Waisel (1991) has shown that in *Populus euphratica*, saline conditions restrict cambial activity, resulting in the formation of narrower annual growth rings.

Plant growth and production are strongly dependent on activity of proteins and enzymes. Protein synthesis in the leaves of plants growing in saline conditions will decline in response to ion toxicity and water deficiency. The effects of NaCl salinity on protein synthesis might be due to Cl toxicity or potassium/sodium (K/Na) imbalance (Marschner, 1995). To survive under saline conditions, plants must restrict Na and Cl entry, while maintaining K uptake. Potassium is the most prominent inorganic plant solute, which is essential for the plant survival in saline conditions. K contributes to reduction of stelar osmotic potential, which is a prerequisite for turgor-pressure-driven solute transport in the xylem and the water balance of plants (Marschner, 1995). Furthermore, K is necessary for enzyme activation, protein synthesis and photosynthesis, stomatal movement and maintenance of cation: anion balance in the cytosol as well as in the vacuole (Maser *et al.*, 2002). In contrast, Na is a harmful element and is not required by most glycophytes for normal growth (Niu *et al.*, 1995). Under salinity stress increased external Na not only interferes with K uptake by the root system, but also may disturb the integrity of root membranes and alter their selectivity. However, the ratio of K/Na in saline soils is often extremely low, so that Na ions can inhibit uptake of K ions. In this situation K/Na ratio in plants will decrease and enzyme functions are inhibited due to ion imbalance (Brain *et al.*, 1999). The selectivity of root

systems for K over Na in salt stressed conditions is a critical process to maintain the levels of K required for metabolic processes, for the regulation of ion transport, and for osmotic adjustment (Grattan & Grieve, 1999).

Toxicity or deficiency of one or more ions may cause growth reduction under stress. In saline conditions the concentrations of some ions increase in the plant. Some of these ions may prove toxic or cause problems in metabolism of nutrients, which are essential for normal plant growth. Antagonistic effects between Cl and H_2PO_4, Cl and NO_3, Cl and SO_4 and Na and K may disturb the normal nutrition of plants or disturb the metabolic process by effects on protein or enzyme production and enzyme activity support (Chhabra, 1996). In addition, ions that transit the soil by mass flow (such as Na and Cl) may accumulate near the root surface and compete with nutrient ions for membrane uptake sites (Kafkafi & Bernstein, 1996). In this situation soil salinity may decrease the uptake of some nutrients such as K, Ca, Cu, Fe and Mn (Hassan *et al.*, 1970).

1.2.6 Revegetation of salt-affected lands

Because of high exchangeable sodium levels, the soils of salt-affected areas are usually highly erosive. These kinds of degraded lands have a potential to be transformed from sources of erosion and desertification to areas producing valuable forage, fuel and other products. Revegetation of saline areas controls wind and water erosion, assists in using excess groundwater, provides food and cover for domestic animals or wildlife and/or provides wood for fuel.

Salt-affected soils pose hazardous conditions for plant establishment. Seeding is the least expensive method, but possibly the least likely to succeed in initiating plant

establishment. In this method germination of seeds is limited by the high concentration of salt, which may interact with other environmental factors (e.g., water availability, temperature and light) (Kigel, 1995). Seed germination is a critical stage for plant survival during the plant life cycle (Went, 1952), particularly when it occurs in saline conditions. The successful pattern of plant establishment, growth and reproduction in salt marsh and salt desert environments is significantly related to seed germination (Ungar, 1982). Therefore using the seeding method for revegetation of salt-affected areas may produce a low efficiency. An alternative method is to raise plants in a nursery and transplant them into the field, where they may become established relatively easily. Transplanting of container-raised seedlings is the most reliable method of establishing plants on saline soils. The seedlings are raised in the nursery, thus avoiding many of the hazards of germination and early growth under the direct seeding method. In comparison with direct seeding this method has higher efficiency but it is more expensive (Vlahos, 1992). However, the methods could be effectively modified to manipulate plant nutrition or to inoculate root systems with beneficial symbionts such as mycorrhizal fungi (see section 1.3.2.1). Revegetation of salt-affected lands presents a wide range of challenges with varied benefits. The next sections of this literature review will address the potential of mycorrhizas to assist in revegetation of salt-affected lands, and then mycorrhizal fungi will be introduced at section 1.3. Section 1.3.2.1 covers previous works on the effects of arbuscular-mycorrhizal (AM) fungi on salt tolerance of different plant species.

1.2.7 Potential for use of mycorrhizas

AM fungi can be very important in revegetation of disturbed lands (Reeves *et al.*, 1979). Disturbance of arid lands significantly reduces the AM inoculum potential of the

soil (i.e. propagules and/or stability of hyphal networks) (Moorman *et al.*, 1979; Reeves *et al.*, 1979), so that inoculum levels of mycorrhizal fungi may be inadequate to support establishment of transplanted seedlings in degraded soils (Moorman *et al.*, 1979; Cuenca *et al.*, 1998). Successful seedling establishment may require additional mycorrhizal inoculum (Requena *et al.*, 1996). Initial studies have shown that only about 1% of colonizing plants on a disturbed site were mycorrhizal, whereas on the adjacent, undisturbed sites about 99% were mycorrhizal (Wicklow-Howard, 1994).

It used to be thought that plants in salt marshes (naturally saline) generally had low colonization or were non-hosts. However, more recently it has been shown that salt marsh species can be highly mycorrhizal (Rozema *et al.*, 1986; van Duin *et al.*, 1989; Juniper & Abbott, 1993; Carvalho *et al.*, 2001; Hildebrandt *et al.*, 2001). Sengupta and Chaudhuri (1990) found 60-72% AM colonization in salt marsh species (*Arthrocnemum indicum, Suaeda maritima, Porteresia coarctata* and *Sesuvium portulacastrum*) that may aid in ecological adaptation and have some benefits for presence and behaviour of pioneer salt marsh plants.

Application of mycorrhizal fungi as a “biological fertilizer” during revegetation of salt degraded lands may assist plants to grow better, tolerate salt stress and increase survival of seedlings, but there is little information about this subject. Because of the importance of mycorrhizal fungi to their host plants in extreme environments (Gange *et al.*, 1990) they were selected in this study for their potential to support the seedlings during establishment stage in salt-affected soil.

1.3 Mycorrhizal fungi

1.3.1 Mycorrhizal symbiosis

Mycorrhizal associations are the most widespread symbioses between plants and microorganisms (Marschner, 1995). About 83% of dicotyledonous and 79% of monocotyledonous plants are associated with mycorrhizal fungi (Wilcox, 1991). Mycorrhizal associations are found in a very wide range of habitats, including aquatic ecosystems, deserts, lowland tropical rain forests, high altitudes, high latitudes and in canopy epiphytes (Allen, 1991). Several researchers have shown that AM fungi occur naturally in saline environments (see section 1.2.7).

Bi-directional movement of nutrients characterizes the fungus-plant symbioses, where carbon flows to the fungus and inorganic nutrients move to the plant, thereby providing a critical linkage between the plant root and soil. This association is a survival mechanism for both the fungi and plants, allowing each to survive in different environments (Gupta *et al.*, 2000). Mycorrhizal plants, in comparison with non-mycorrhizal plants, have greater nutrient uptake because they possess a network of external hyphae (Sanders & Sheikh, 1983). The hyphae are the interface between soil and plant and have a large surface area that acts as an extension of the root absorbing area (Rhodes & Gerdemann, 1975; Owusu-Bennoah & Wild, 1979; Li *et al.*, 1991). This not only increases the volume of soil from which nutrients are absorbed, but also overcomes problem of depletion of nutrients (Nurlaeny *et al.*, 1996; Smith & Read, 1997) and water (Marulanda *et al.*, 2003) depletion close to actively absorbing roots, and plays a significant role in stabilizing soil structure (section 3.2.2).

Mycorrhizal fungal associations have several advantages for their hosts, including increased growth and yield and reproductive success due to enhanced nutrient

acquisition (Mosse *et al.*, 1973; Diederichs, 1990; Lewis & Koide, 1990; Stanley *et al.*, 1993). They may also increase disease and pest resistance (Grandmaison *et al.*, 1993; Newsham *et al.*, 1995; Cordier *et al.*, 1996; Mark & Cassells, 1996), improve water relations (Allen & Allen, 1986; Davies *et al.*, 1993; Subramanian *et al.*, 1997), soil structure (Tisdall & Oades, 1979; Thomas *et al.*, 1986; Degens *et al.*, 1994; Beaden & Petersen, 2000) and tolerance of extreme pH (Sidhu & Behl, 1997; Douds *et al.*, 2000). This literature review will concentrate on structure and function of AM and their potential role in revegetating saline soils.

1.3.2 Arbuscular mycorrhizas

AM symbioses are the most widespread mycorrhizal association, with a very long evolutionary history. Fossil survey and molecular data have demonstrated that the evolutionary history of AM fungi goes back at least to the Ordovician, about 460 million years ago (Redecker, 2002). The earliest land plants have been shown to have an association with fungi that formed vesicles and arbuscules that was very similar to today's AM fungi (Nicolson, 1975; Remy *et al.*, 1994). This type of plant-fungal association is formed with 80% of angiosperms (Harley & Harley, 1987). The fungi are obligate symbionts and they need a living plant root (or equivalent structure) in order to grow and reproduce.

AM fungal development in roots of host plants starts when fungal hyphae grow from spores or from colonized roots toward the uncolonized roots. After contact of the hyphae with the root surface, the fungus is stimulated to change in morphology from an original simple branching pattern to irregularly septate pattern with reduced interhyphal spacing (Giovannetti *et al.*, 1993; Harrison, 1998). The fungus produces swollen appressoria on the root surface and spreads between and into the root cortical cells.

There are two major morphological types of AM, *Arum* and *Paris* type, they are characterised by differences in fungal development within the roots (Gallaud, 1905; Smith & Smith, 1997). In general, internal hyphae branch and make four recognizable structures, intracellular hyphae that may be coiled, intercellular hyphae, arbuscules and spherical or ovoid vesicles (Smith & Smith, 1997). In *Arum*-type associations, intercellular hyphae penetrate between the root cortical cells and spread rapidly, then lateral branch hyphae penetrate the root cortical cells and branch dichotomously and produce arbuscules (Fig 1.1,a). In *Paris*-type associations intracellular hyphae spread from cell to cell within the root cortex and form extensive intracellular hyphal coils and arbusculate coils (Fig 1.1,b) (Gallaud, 1905). The types of internal structures that develop depend on the plant/fungal combination (Smith & Smith, 1997; Cavagnaro *et al.*, 2001).

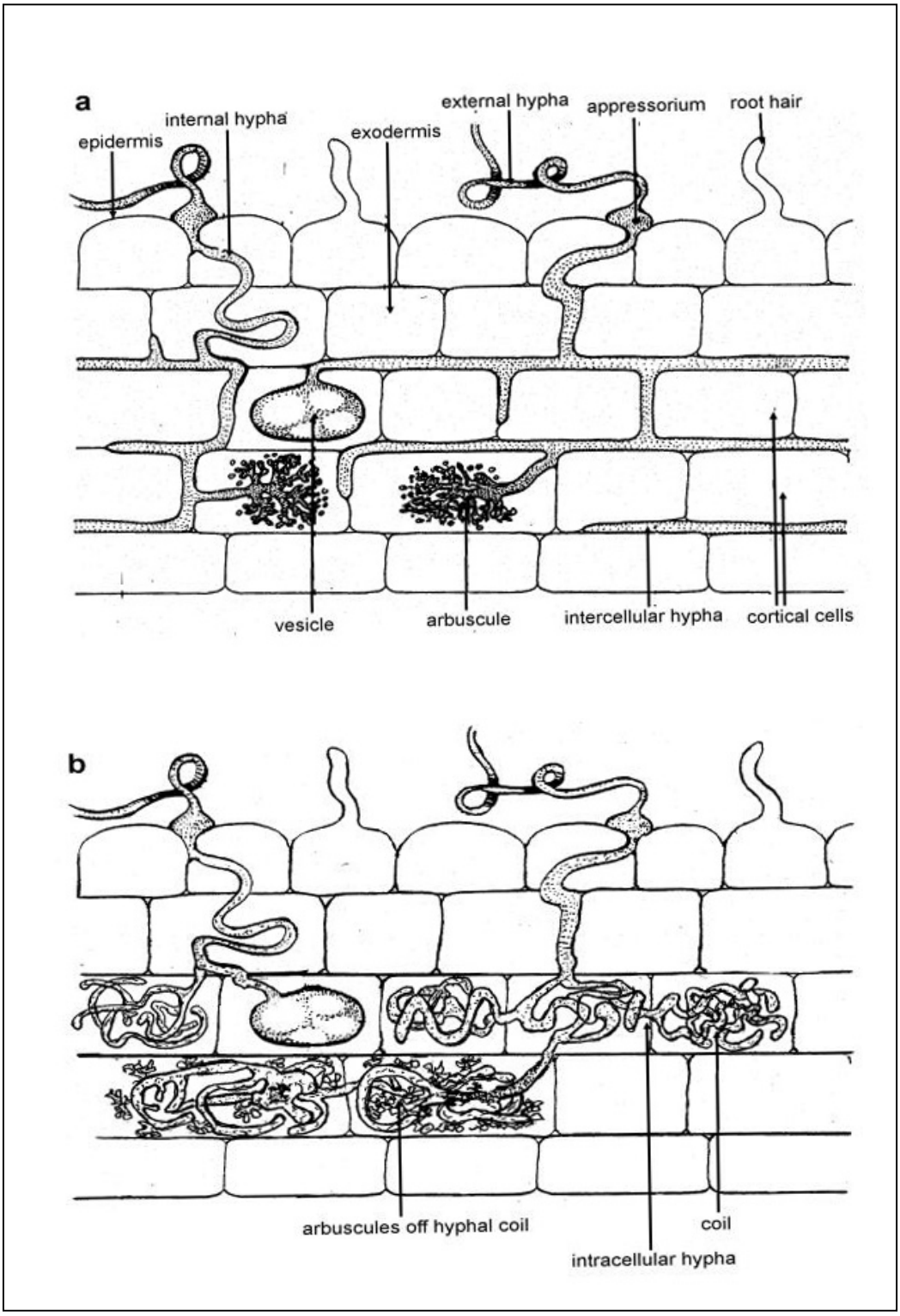


Fig 1.1 Two different morphological types of AM fungi, a) *Arum*-type arbuscular mycorrhizal structures, b) *Paris*-type arbuscular mycorrhizal structures. Diagram by Dickson (1999).

1.3.2.1 Role of AM in alleviation of salt stress in plants

AM fungi occur naturally in most plant species and different edaphic conditions and may improve the growth of many plant species under a variety of stresses (Allen & Boosalis, 1983). The fungi can reduce the impact of environmental stresses such as salinity and increase yield of crop plants such as rice, onion, bell pepper, lettuce, lucerne, tomato, cucumber and some gramineous plants (Ojala *et al.*, 1983; Rosendahl & Rosendahl, 1991; Ruiz-Lozano *et al.*, 1996; Azcón & El-Atrash, 1997; Al-Karaki & Hammad, 2001; Cantrell & Linderman, 2001). Several researchers have reported the effect of AM fungi on alleviation of salt stress, but there are different opinions about the mechanisms of salt tolerance in mycorrhizal plants. Increased tolerance to salt afforded by AM fungi is not dependent on a single mechanism but is probably the result of a combination of several mechanisms. Increase in uptake of phosphorus (P), and other mineral nutrients, K/Na ratio and some improvements in plant physiological processes are the main factors that are likely to be related to salt tolerance in AM plants growing in saline conditions; work in this area is summarized in Table 1.3.

Table 1.3 Previous reports of different effects of AM fungi on plant salinity tolerance

Author (s)	Host plant	Mycorrhizal fungi type of inoculum	Phosphorus uptake increase	Other mineral nutrient uptake increase	K/Na ratio increase	Physiological processes improvement (photosynthesis, transpiration and water use efficiency)
Al-Karaki Hammad (2001)	*Lycopersicon esculentum*	*Glomus mosseae*	NI	++	+	NI
Al-Karaki Hammad Rusan (2001)	*Lycopersicon esculentum*	*Glomus mosseae*	+	++	+	NI
Copeman Martin Stutz (1996)	*Lycopersicon esculentum*	Field soil	O	++	NI	NI
Poss Menge Jarrel (1985)	*Allium cepa* *Lycopersicon esculentum*	*Glomus mosseae* *Glomus fasciculatum* *Glomus deserticola*	++	+	+	+
Ojala Jarrel Menge (1983)	*Allium cepa*	*Glomus fasciculatum* *Glomus monosporus*	+	+	NI	+
Hirrel Gerdemann (1980)	*Allium cepa* *Capsicum annuum*	*Glomus fasciculatum* *Gigospora margarita*	++	NI	NI	NI
Ruiz-Lozano Azcon Gomes (1996)	*Lactuca sativa*	*Glomus mosseae* *Glomus fasciculatum* *Glomus deserticola*	O	NI	NI	++

Allen Cunningham (1983)	*Distichlis spicata*	*Glomus fasciculatum*	NI	NI	++	NI
Azcon El-Atrash (1997)	*Medicago sativa*	*Glomus mosseae*	+	+	+	+
Cantrell Linderman (2001)	*Lactuca sativa* *Allium cepa*	Field soil	+	+	NI	NI
Duke Johnson Koch (1986)	*Carrizo citrange*	*Glomus intraradices*	+	NI	NI	+
Pfeiffer Bloss (1987)	*Parthenium argentatum*	*Glomus intraradices*	+	NI	+	NI
Rosendahl Rosendahl (1991)	*Cucumis sativus*	*Glomus spp.*	NI	NI	NI	+

O	Indicates no effect
+	Indicates positive effect
++	Indicates more positive effect
NI	Indicates no information available

Increase in phosphorus uptake

It is well documented that as soil salinity increases plant P uptake decreases and subsequently some symptoms of P deficiency occur (Hirrel & Gerdemann, 1980; Pond *et al.*, 1984; Poss *et al.*, 1985). Plants under salinity stress may have lower H_2PO_4 availability than under low saline conditions (Sentenac & Grignon, 1985). By increasing plant P uptake, crop production was shown to be increased in saline soils (Champagnol, 1979; Hirrel & Gerdemann, 1980). The detrimental effects of salinity on sunflower seedlings were mitigated by increasing soil P (Delgado & SanchezRaya, 1997). The most important effect of AM fungi on plant nutrition is increase in P uptake, which may be the most important benefit of AM fungi to plants under salt stress. Besides direct effects of P on plant growth, P uptake may increase growth and hence dilute other plant nutrients that become concentrated because of salt stress (Hirrel & Gerdemann, 1980; Poss *et al.*, 1985). As can be seen from Table 1.3, P uptake appears to play a major role in enhanced growth and reduced salinity stress of AM plants. It has been identified as the primary mechanism, caused by the fungi, assisting the host plant to increase salt tolerance.

Mycorrhizal colonization may have effects on salinity tolerance that are not related to P nutrition. In a study on lucerne in saline conditions, AM fungi and P application both increased nitrogen (N) and P concentration, nodule formation and plant growth, but mycorrhizal inoculation protected the plants from salt stress more efficiently than any amount of plant available P in soils (Azcón & El-Atrash, 1997). This study demonstrated that the alleviation of salt effects in AM plants was not only correlated to host P increase but was based on effects other than P nutrition. These findings confirm the suggestion of other researchers that mycorrhizal fungi provide alternative

mechanisms to alleviate detrimental effects of salt stress in host plants (Azcón & Barea, 1992; Copeman *et al.*, 1996; Ruiz-Lozano *et al.*, 1996; Cantrell & Linderman, 2001).

Increase of K/Na ratio

Some previous studies on the effects of salinity on mycorrhizal plants have shown that AM roots had higher Na concentrations but also higher K concentrations and thus maintained a high K/Na compared to non-mycorrhizal plants. Allen and Cunningham (1983) have found that mycorrhizal roots of salt grass plant (*Distichlis spicata*) had higher sodium, potassium and phosphorus concentrations than non-mycorrhizal roots. In contrast, the results of other studies have shown that Na uptake decreased in AM plants compared to controls. Sodium content of shoots of mycorrhizal halophytic *Aster tripolium* plants was lower than non-mycorrhizal plants under salinity stress (Rozema *et al.*, 1986). K/Na ratio increased in mycorrhizal barley (moderate salinity tolerant plant) at high levels of salinity by decreasing Na concentration, rather than by increasing K concentration (Mohammad *et al.*, 2003).

Increased uptake of other mineral nutrients

Concentrations of Ca, Mg and Zn in onion plants inoculated with *Glomus fasciculatum* increased in saline conditions and improved the nutritional status, which was at least partially responsible for increased plant growth (Ojala *et al.*, 1983). The improved growth and nutrient acquisition (P, K, Zn, Cu and Fe) in tomato demonstrate the potential of AM fungi for protecting plants against salt stress in arid and semiarid areas (Al-Karaki, 2000; Al-Karaki & Hammad, 2001). Although effects of AM fungi in increasing some toxic elements (e.g., Na, Cl and Mn) have been reported (Allen & Cunningham, 1983; Pfeiffer & Bloss, 1988; Cantrell & Linderman, 2001), a direct

effect of AM fungi in reduction of others has also been reported. Mycorrhizal fungi decreased sodium concentration in barley (*Hordeum vulgar*) when grow in saline conditions (Mohammad *et al.*, 2003), and Mn can be reduced in mycorrhizal plants when it occurs at toxic levels compared to non-mycorrhizal plants (Sanders & Fitter, 1992; Cardoso *et al.*, 2003).

Improvement of plant physiological processes

One of the best-known responses of plants to salinity is disturbance of plant physiological processes. Reduction of photosynthesis, transpiration and water use efficiency are some physiological problems that occur under saline conditions. All three have been reported to be alleviated in mycorrhizal plants under salt stress (Ruiz-Lozano *et al.*, 1996). The same study demonstrated that the tolerance of plants under salt stress is not related to differences in the P content of treated plants, but to some improvement in physiological processes, namely increased carbon dioxide exchange rate (CER), water use efficiency (WUE), transpiration and stomatal conductance. The presence of AM fungi in the roots alters the osmotic balance and it is possible that such physiological changes in host plants may play a role in increasing tolerance to salt exposure. A study of the influence of three *Glomus* species on the response of cucumber to salt stress demonstrated greater water uptake by AM plants under saline conditions (Rosendahl & Rosendahl, 1991). A direct effect of AM fungi based on water transport by hyphae was not demonstrated in some works (George *et al.*, 1992a; George *et al.*, 1992b; Auge, 2001), but it was suggested again recently (Marulanda *et al.*, 2003). Other factors related to salt tolerance mechanisms in AM plants have also been suggested. These include compartmentalization of Na within some tissues, including the AM fungal hyphae in the roots (Cantrell & Linderman, 2001), some plant growth

hormone increases (Poss *et al.*, 1985), proline and betaine accumulation (Duke *et al.*, 1986; Ruiz-Lozano *et al.*, 1996) and leaf Na excretion (Allen and Cunningham 1983). The following sections will address the contribution of AM fungi to revegetation of saline lands and its impacts on soil structure.

1.3.2.2 Contribution of AM fungi to revegetate saline rangelands

As mentioned in the last section (1.3.2.1) AM fungi increase plant salinity tolerance and enhance plant growth in saline conditions. This contribution to crop plants is well known, but their role in establishment of seedlings of salt-affected rangeland plants has received less attention. Several plant species are well adapted to revegetate saline rangelands because of structural or physiological adaptation of roots and foliage. Depending on climate, water availability, salinity level and land use management different species of grasses or halophytes are useed to revegetate salt-affected areas. Revegetation with grasses and halophytic forage shrubs (particularly members of the Chenopodiaceae) provides many benefits including erosion control, flood mitigation, watertable lowering and habitat improvement.

Several perennial, biennial and annual grasses have been identified as suitable species for revegetation of saline areas (Rogers *et al.*, 1996). Many grasses form symbiotic association with AM fungi (Read *et al.*, 1976). In many cases the association is mutualistic in that AM fungi improve plant nutrition and growth while supporting growth of the fungi. However, in some cases there is a lower dependency on AM fungi and lower growth responses to colonization (mycorrhizal non-responsive plants) (Tawaraya, 2003). It is known that responsiveness to AM fungi, in terms of improved plant growth and/or P nutrition, varies between crop cultivars (Koide *et al.*, 1988; Hetrick *et al.*, 1992; Baon *et al.*, 1993; Zhu *et al.*, 2001), but less attention has been

devoted towards the role of AM fungi on plant growth and nutrient uptake development in non-responsive plants under salt stress.

The family Chenopodiaceae is represented in arid and halophytic plant communities worldwide. This family probably includes the largest number of halophytic members, in comparison with other plant families. Chenopods are suited to erosion control and rangeland rehabilitation on many salt-affected areas and abused lands. Early studies reported that plant species belonging to Chenopodiaceae do not form mycorrhizal associations (Stahl, 1900; Maeda, 1954). Based on failure to find any mycorrhizal association in chenopod roots among groups of plants examined in different natural environments, researchers have reported that chenopods are non-mycorrhizal (Hirrel *et al.*, 1978; Reeves *et al.*, 1979). In contrast, there are some reports of mycorrhizal associations in chenopods in field (Table 1.4) and pot culture studies. Table 1.4 shows that chenopod species that are used in revegetation programs of salt-affected lands (such as *Atriplex*) maybe highly colonized (up to 70%) with mycorrhizal fungi at different seasons in nature. Salt marsh chenopods (such as *Salicornia europaea, Suaeda maritima, and Arthrocnemum indicum*) have high levels of mycorrhizal colonization. All typical AM structures (hyphae, arbuscules, coils and vesicles) were found in chenopods; internal hyphae and vesicles are more abundant than arbuscules and coils. Glasshouse experiments have shown mycorrhizal colonization in chenopods may depend on root exudates (Ratnayake *et al.*, 1978; Graham *et al.*, 1981; Schwab *et al.*, 1982; Sacchi *et al.*, 2000), season (Allen & Cunningham, 1983; van Duin *et al.*, 1989; Siguenza *et al.*, 1996), companion plants (Hirrel *et al.*, 1978; Tester *et al.*, 1987) or environmental stresses (Schwab *et al.*, 1982; Sengupta & Chaudhuri, 1990). Results of a survey on AM fungi in pioneer salt marsh plants in India has suggested that salt stress may make chenopods more susceptible to colonization by AM fungi (Sengupta &

Chaudhuri, 1990). The effects of salt stress as an environmental factor influencing mycorrhizal colonization of chenopods and the benefits of AM association have received very little attention. Although chenopods are salt tolerant plants, induced mycorrhizal colonization of chenopods seedlings in control conditions (nursery) may further increase seedling establishment during revegetation process of salt-affected land.

Table 1.4 Observation of mycorrhizal associations in different species of the Chenopodiaceae sampled in the field

Host plant	Date / Season	Degree of colonization %	Hyphae %	Vesicle %	Arbuscule %	Coils %	Location	Author
Atriplex repanda *Atriplex atacamensis* *Atriplex mudariagae* *Atriplex mucronata* *Atriplex coquimbensis* *Atriplex deserticola* *Atriplex microphylla* *Atriplex nummularia*	Summer	4.5 34.7 21 11.8 1.1 46.6 25 30.7	NI	NI	NI	NI	Chile	(Aguilera *et al.*, 1998)
Atriplex gardneri	April-June	3-78	+	+	25	NI	Wyoming U S A	(Allen, 1983)
Atriplex canescens	June & October	NI	+	+	-	+	Colorado U S A	(Barrow & Aaltonen, 2001)
Atriplex polycarpa	September	>50	NI	NI	NI	NI	California U S A	(Bethlenfalvay *et al.*, 1984)
Atriplex barclayana	November & February	>10	NI	NI	NI	NI	Sonoran Desert Mexico	(Carrillo-Garcia *et al.*, 1999)
Atriplex halimus *Hammada scoparia*	Spring	26.1 24	17 20	16 8	2 3	NI	Negev Desert, Israel	(He *et al.*, 2002)
Salicornia europaea	August & November	5-64	1-45	1-17	2-3	NI	Central Europe	(Hildebrandt *et al.*, 2001)
Atriplex patula *Salicornia rubra*	Summer Summer and fall	70 1	+ +	28 0.1	42 0.6	NI	Manitoba Canada	(Johnson *et al.*, 1995)
Suaeda maritima *Salicornia europaea*	August & April June & April	1 2	1	-	-	NI	European soils	(Landwehr *et al.*, 2002)
Atriplex confertifolia	Spring and Summer	59	NI	NI	NI	NI	Wyoming USA	(Miller *et al.*, 1983)

Atriplex canescens *Atriplex confertifolia* *Atriplex gardneri* *Cratoeides lanata* *Grayia spinosa* *Kochia americana* *Sarcobatus vermiculatus*	June & September	NI	NI	NI	NI	NI	Wyoming U S A	(Miller, 1979)
Salsola kali *Sclerolaena diacantha*	September	>10 >10	+ +	+ +	- -	- -	South Australia	(O'Connor *et al.*, 2001)
Salicornia brachystachya *Salicornia dolichostachya*	May	1 1-30	NI	-	NI	NI	Bergen Holland	(Rozema *et al.*, 1986)
Chenopodium album *Chenopodium botrys*	Summer	65-100 50-95	NI	NI	NI	NI	North of Pakistan	(Saif & Iffat, 1976)
Arthrocnemum indicum *Suaeda maritima*	November-February	65 72	+ +	+ +	+ -	NI	Bengal India	(Sengupta & Chaudhuri, 1990)
Atriplex julacea	February-August	50	+	+	+	+	Mexico	(Siguenza *et al.*, 1996)
Atriplex nummularia	Summer	26	NI	NI	NI	NI	Chile	(Torres, 1990)
Atriplex prostrata *Halimione portulacoides* *Salicornia spp* *Suaeda maritima*	May-November	3-30	NI	NI	NI	NI	Dutch salt marsh	(van Duin *et al.*, 1989)
Atriplex canescens	NI	48	+	+	NI	NI	New Mexico	(Williams *et al.*, 1974)

NI Indicates no information available

1.3.2.3 Effects of AM fungi on soil structure

The stability of macroaggregates in soil is highly dependent on the growth and decomposition of roots and hyphae. Length of fungal hyphae was significantly correlated with aggregation in loamy soils (Tisdall & Oades, 1980). Among the fungi, AM fungi appear to be the most important mediators of soil aggregation (Rillig *et al.*, 2002). In AM associations the external hyphae provide a direct physical link between the host plant and soil resource. The external hyphae of AM fungi are effective in promoting soil aggregation and potentially have an important role in improving soil structure in degraded lands. External hyphae of AM fungi can bind the small particles into microaggregates and the resultant entanglement of microaggregates creates macroaggregates that finally leads to improved structure and aggregation stability in soils with a wide range of texture, e.g. sandy, loamy and clayey soils (Thomas *et al.*, 1986; Degens *et al.*, 1994; Beaden & Petersen, 2000). Also, because of the obligate association between roots and AM fungi, fungal hyphae have direct access to photosynthetic carbon, thus AM fungi are a direct conduit for transport of host carbon into the soil. The resultant increase in soil aggregation leads to 1) protection of organic matter within aggregates, 2) a decrease in soil nutrient losses through leaching, and 3) an increase in the ability of plants to rely on mineralization to supply future needs (Schreiner & Bethlenfalvay, 1995).

The effect of AM fungi on the stability of soil began with the studies of Tisdall and Oades (1979) who demonstrated the importance of these fungi in stabilising aggregates of pasture soils. They suggested that filamentous fungi are capable of binding the soil particles by producing mucilage on the surface of hyphae. Recent research strongly suggests that soil stability is directly related to the activity of these fungi (Wright &

Upadhyaya, 1998). These discoveries have shown that AM fungal hyphae have a major role in aggregate stabilization by producing a glycoprotein named glomalin (Wright & Upadhyaya, 1999; Franzluebbers *et al.*, 2000). The presence of glomalin and its effects on the soil structure have only recently been postulated and need confirmation.

These improvements, together with effective removal of nutrients by fungal hyphae, might be expected to reduce leaching of nutrients and elements vertically and laterally through the soil profile. However, the ability of AM fungi to control nutrient movement in soil has received almost no attention. Mycorrhizal *Liquidambar styraciflua* seedlings inoculated with *Glomus mosseae* significantly reduced the loss of NH_4 and NO_3 in percolated water, when compared to soil alone and soil with non-mycorrhizal seedlings (Haines & Best, 1976). However differences in size of the mycorrhizal and non-mycorrhizal plants confounded the interpretation of the data in terms of effects of mycorrhizal hyphae *per se*. The potential of AM fungi to improve soil structure in revegetation programs of saline lands is a very important issue, which may lead to reduce element leaching to the ground-water.

1.4 Summary

On a global basis, salt-affected soils occupy an estimated >7% of the earth's land surface and nearly 33% of the potential arable lands of the world. The pressure of food shortage is compelling developing countries to bring new lands under crop production. Reclamation of salt-affect soils will provide important opportunities for increasing bio-production and alleviating pressure on traditionally cultivated lands. Biological activity of soils, including mycorrhizal symbiosis, has been shown to be an important consideration in effective revegetation programs.

However, little consideration has been given to the potential of pre-inoculation of different rangeland plants with mycorrhizal fungi to increase survival of seedlings

transplanted into saline soils, to increase retention of P and other elements in the soil profile, and to decrease pollution of ground water or runoff by the reduction of element flow-through. Mycorrhizal fungi may increase rangeland seedling survival during transplantation into saline conditions and act as a biological fertilizer during revegetation of salt degraded lands. Application of mycorrhizal fungi may decrease the element flow-through into the ground-water by improving soil structure and enhance retention of elements in soil profile and finally reduce water pollution.

1.5 Aims of study

1. To study the role of mycorrhizal fungi in growth and survival of transplanted seedlings in saline conditions in pot experiments, by determining**:**

- Growth, vitality and survival of transplanted seedlings
- Nutrient and toxic element uptake

2. To compare the effects of AM fungi and P in seedling establishment and nutrient uptake and hence to explore the natural mechanisms underling mycorrhizal effects

3. To study the effects of AM on plant salinity tolerance in a rangeland plant that is not responsive to mycorrhizal inoculation under non-saline soil

4. To study the effects of salinity stress on mycorrhizal colonization in a chenopodiaceous plant species

5. To study the influence of mycorrhizal fungi on the P losses via leaching process in soil profile

To achieve the above aims, this study began by finding a suitable soil and investigating the effects of the AM fungus *Glomus intraradices* on plant establishment at different salinity levels in a mycorrhizally responsive plant species (*Trifolium subterraneum*),

then comparing the effects of AM fungi and P on that species (Chapter 3). To understand the effects of AM fungi on salinity tolerance in a mycorrhizally non-responsive plant, the study continued by investigating the effects of the same AM fungus on a rangeland grass (*Festuca arundinacea*) (Chapter 4). The effects of salt stress on mycorrhizal colonization in a chenopod (*Atriplex nummularia*) and influence of AM fungal inoculation on plant growth were investigated (Chapter 5). Finally AM fungi influences on phosphorus leaching in the soil profile were studied in cores experiments with *T. subterraneum* seedlings in non-saline and saline conditions (Chapter 6). All results are discussed in Chapter 7 to draw conclusions and suggestions for future work.

CHAPTER 2 - GENERAL MATERIALS AND METHODS

In the experiments described in this thesis, three soil types from the Monarto area of South Australia (Mount Lofty Ranges) and three different plant species were used. The general materials and methods are explained in this chapter and the detailed materials and methods and modifications for individual experiments are provided in appropriate chapters.

2.1 Soils

For the purpose of this study three different soils were selected from 6 sites in the Monarto area, 60 km south-east of Adelaide on the eastern flank of the Mount Lofty Ranges (Chittleborough *et al.*, 1976). Samples were collected from different horizons, air-dried, passed through a 2 mm mesh sieve and mixed carefully. In experiments that needed autoclaved soil, the mixed soil was autoclaved at 110 °C and 240 kPa pressure for 1 hour on two occasions over a two-day period to ensure all possible mycorrhizal propagules had been destroyed.

2.2 AM inoculum sources

Most of the AM inocula that were used in this study were from the collection of the Discipline of Soil and Land Systems, School of Earth and Environmental Science, University of Adelaide, and kindly supplied by Ms Debbie Miller. None of the AM fungi used were isolated from saline environments.

Glomus intraradices Schenk and Smith (DAOM 181602) was used in all experiments except one described in Chapter 5, in which a mixture of *Glomus intraradices* and the following five *Glomus* species were used as a combined source of inoculum;

Glomus etunicatum Becker and Gerdemann (UT 316A-2), originally obtained from Dr Joe Morton, INVAM, University of West Virginia, USA,

Glomus mosseae (Nicolson and Gerdemann) Gerdemann and Trappe (NBR4-1), originally obtained from Dr P. McGee, University of Sydney, NSW, Australia,

Glomus fasciculatum (Thaxter) Gerd, and Trappe emend. Walker and Koske (LPA7), originally obtained from the Turin Botanic Garden, Italy,

Glomus geosporum (Nicolson and Gerdemann) Walker, obtained from Dr J. Jansa Institute of Plant Sciences, Swiss Federal Institute of Technology Zurich (BEG 154)

Glomus caledonium (Nicolson and Gerdemann) Trappe and Gerdemann, obtained from Dr J. Jansa Institute of Plant Sciences, Swiss Federal Institute of Technology Zurich (BEG 162).

2.3 Seed sources

Subterranean clover (*Trifolium subterraneum* L. cv. Mt Barker) was supplied by Mt. Barker Agricultural Seeds, South Australia. Tall fescue (*Festuca arundinacea* Schreb. cv. Vulcan) and Italian ryegrass (*Lolium multiflorum* Lam. cv. Tatila) were obtained from Adelaide Seed Company and old man saltbush (*Atriplex nummularia* Lindl.) seeds were collected from the field site in the Monarto area in March 2002.

2.4 Surface sterilization of seeds

T. subterraneum, *F. arundinacea* and *L. multiflorum* seeds were sterilized with a mixture of 1 part sodium hypochlorite (12.5 % w/v) and 2 parts of reverse-osmosis

(R.O.) water. Seeds were soaked for 10 minutes in diluted sodium hypochlorite and then rinsed three times with R.O. water. *Atriplex* seeds were surface-sterilized by the same method after acid washing (section 2.5).

2.5 Seed germination

After surface-sterilization, seeds of *T. subterraneum*, *F. arundinacea* and *L. multiflorum* were germinated on moist filter paper at 23 °C in a germinator. *Atriplex* seeds present dormancy at the pericarp level and needed a different method to facilitate germination. Pericarps of dried seeds were removed by thrashing, then seeds were submerged in concentrated sulphuric acid (H_2SO_4). After 25 minutes the seeds were rinsed with R.O. water to remove acid (Campbell & Matthewson, 1992). Acid-treated seeds were soaked for 48 h in R.O. water to remove acid completely, and then surface sterilized with sodium hypochlorite (as mentioned before). Thereafter, they were planted in a tray with 5 cm deep quartz sand, and covered by a thin layer of sand. R.O. water was added regularly to keep the seeds moist. The seed container was placed in a constant temperature room at 23°C for two weeks.

2.6 Seedling production and transplantation

For the purpose of plant establishment in saline conditions, and for phosphorus (P) leaching experiments (Chapters 3, 4 and 6), seedlings were grown in small plastic bags and later transplanted into the main pots or cores. Seedling bags were made from black plastic rolls, cut and sealed by a plastic heat sealer. The size of each bag was 4×10 cm and its soil capacity was 80 g of inoculated or non-inoculated soil. To promote nodule development, subterranean clover seeds were coated in a suspension of *Rhizobium leguminosarum* biovar *trifolii* prior to planting. Pre-germinated seeds were sown in the soil in each bag to produce mycorrhizal or non-mycorrhizal seedlings. R.O. water was

added by weight to keep the soil in the bags at 80% field capacity. Long Ashton nutrient solution (Hewitt, 1966) without P (10 ml per pot) was added twice during pre-transplantation growth. After 2 to 3 weeks, depending on season, growth conditions and colonization, seedlings were transplanted from nursery bags into the main pots with different experimental conditions (see individual chapters).

2.7 Growth conditions

The plants were grown in a glasshouse under controlled environmental conditions of approximately 22°C/14°C day/night temperature and 250 to 1100 μmol m^{-2} s^{-1} irradiance, depending on season and weather conditions. Plants were watered with R.O. water three times weekly to keep soil at 80% field capacity.

2.8 Harvesting

At harvest, shoot and root fresh weight, plant height and leaf numbers were recorded. Soil containing plant roots was put into a 2 mm sieve and washed with R.O. water. Roots were carefully collected from the sieve and a sub sample (0.2 g) of root was cleared and stained for examination of mycorrhizal colonization (section 2.9). Plant shoots and the remaining roots were dried at 80 °C for 48 hours and then shoot and root dry weights were measured. Dried material was used for nutrient analyses when appropriate.

2.9 Root clearing and staining

Mycorrhizal colonization was determined after clearing and staining plant roots by a modification of the method of Phillips and Hayman (1970): phenol was omitted from the reagents. Root sub-samples were cut into pieces approximately 1-2 cm long, placed

into 10% KOH and left to clear at room temperature for several days, the length of time depending on the plant species (clover for 5 days and *Atriplex* and tall fescue for 3 days). Cleared roots were washed with running R.O. water, soaked in 0.1 M HCl for 1 minute and washed with R.O. water. The roots were stained with 0.01% trypan blue in lactoglycerol for 1 hour at room temperature. Finally the roots were rinsed with R.O. water and placed into a lactic acid: glycerol (1:1) solution and stored at room temperature.

2.10 Assessment of colonization

Percentage of root length with mycorrhizal colonization was evaluated under a microscope using the gridline method of Giovannetti and Mosse (1980). The samples of stained roots were spread randomly on a grid-marked Petri dish. Roots were observed at × 40 magnifications and total intersects and intersects containing any mycorrhizal structure (arbuscules, vesicles or hyphae) were scored. The ratio of the two intersect values was used to obtain the percentage of colonization of the roots.

2.11 Measurement of external hyphae

External AM hyphae were extracted from the soil and measured using a modification of the method of Jakobsen (1992). Duplicate 2 g sub samples of the soil were added to a small amount of water and stirred to break up aggregates. Then the material was gently washed on a 38 μm sieve using R.O. water to remove clay particles. Sieve contents were transferred to a Waring blender filled to 250 ml with R.O. water and blended for 15 seconds at high speed. The soil solution was transferred quickly in to a 250 ml wide-necked Erlenmeyer flask, shaken vigorously for 5 seconds by hand and left on the bench for 60 seconds. Duplicate 3 ml aliquots were pipetted into a filtration device (Carbon 14 Centralen, Denmark) holding 10 filters. Filters were 25 mm diameter

Millipore filter membranes of pore size 8 µm. After pulling down the supernatant by vacuum, filters were covered with 2 ml of trypan blue staining solution (above) and left for 10 minutes. After removing the remaining stain, filters were placed on slides and mounted in glycerol with a cover slip and viewed at 200 × magnification under a compound microscope. Hyphal lengths on each filter were measured using the intercept method (10 × 10 eyepiece grid graticule) and calculated using the method of Tennant (1975) on each filter. There were 2 filters in each sample and twenty measurements were made for each filter.

2.12 Plant tissue phosphorus (P) determination

The Hanson (1950) method was used to determine the concentration of P in plant tissues. Up to 50 mg of oven-dried plant root or shoot samples were ground and digested with 7 ml nitric-perchloric acid (6:1) mixture in a 50 ml digestion tube overnight. Then the tubes were heated on a digestion block (Tecator Model 1016, Höganäs, Sweden) programmed as shown in Table 2.1. Dried digested materials were diluted to 50 ml with deionised water and left to stand overnight. Depending on solution P concentration, 5 or 10 ml aliquots of the diluted digest were added to 4 ml of colour reagent (containing 1 part nitric acid, 1 part 0.25% ammonium vanadate and 1 part of 5% ammonium molybdate) and diluted to 50 ml with deionized water. After 30 minutes absorbances were read on a UV-VIS Shimadzu spectrophotometer at 390 nm using a blue filter. P content of plant tissues was calculated from tissue P concentration and tissue weight.

Table 2.1 Digestion steps in programmed Tractor digestion block

Step	Temperature (°C)	Ramp (min)	Time (h)
1	150	10	2
2	200	10	1
3	165	10	2

2.13 Assessment of soil available phosphorus

Phosphorus was extracted from the soil with $NaHCO_3$ at pH 8.5 by a modified Colwell (1963) method. One gram of air-dried soil was added to 100 ml 0.5 M $NaHCO_3$ and incubated for 16 h at 20 °C in an over-end shaker. Five ml of the extract was transferred to a centrifuge tube and neutralised with 5 ml 0.5 M HCl. The suspension was incubated at room temperature overnight until effervescence stoped. The neutralised solution was mixed with reagent in the autoanalyser using the method described by Rayment and Higginson (1992). The reagent used for this method was made as follows: 4.5 g of ascorbic acid was dissolved in a mixture of 107 ml 5N H_2SO_4, 293 ml deionized water, 75 ml ammonium molybdate and 25 ml potassium antimony tartrate, following which 0.1 g of SLS (sodium lauryl sulphate) was added to the solution. The autoanalyser consisted of a Burkard sampler, Technicon peristaltic pump, Chemlab spectrophotometer and Omniscribe Chart recorder.

2.14 Assessment of total phosphorus in soils

Total soil P content was determined by modification of the method of Jackson (1958). 0.1 g of ground soil was digested with 7 ml nitric-perchloric acid (6:1) mixture in a 50 ml digestion tube for 18 h at 160 °C. The dried digested material was diluted with 50 ml deionised water and P was assessed colorimetrically using an autoanalyser (section 2.13).

2.15 Statistical analysis

Preliminary t-test were used to analyse homogeneity of variances (results not shown). Data were normally distributed and had hemogenous variances. Data were analysed statistically using ANOVA, GenStat 6 Release 6.1 (2002), Lawes Agricultural Trust (IACR Rothamsted). Probabilities of significance among treatments and interactions and LSDs ($P<0.05$) were used to compare means within and among treatments. Standards errors of means were represent as vertical bars in graphs.

CHAPTER 3 - MYCORRHIZAL POTENTIAL IN SEEDLING ESTABLISHMENT OF *TRIFOLIUM SUBTERRANEUM* UNDER SALINE CONDITIONS

This chapter describes four experiments. Experiments 1 and 2 were designed to produce some preliminary knowledge which was necessary for Experiments 3 and 4. Because of the important role of soil properties in influencing mycorrhizal fungal colonization and plant growth, the first experiment was done to select an appropriate soil for the rest of the work described in this chapter and in Chapters 4 and 5. Improved plant growth and nutrient uptake in mycorrhizal seedlings compared to non-mycorrhizal seedlings could have effects on plant salinity tolerance and plant establishment under saline conditions, so the second experiment was carried out to explore the possibility of producing matched *T. subterraneum* clover seedlings (mycorrhizal and non-mycorrhizal), which had the same size and same P content, and which could then be used in transplantation experiments. Growth mycorrhizal and non-mycorrhizal seedlings in saline conditions was investigated in the third experiment. Finally the effects of AM fungi and P fertilizer on plant establishment were compared in the fourth experiment.

3.1 Introduction

Many salt-affected areas remain unproductive for many years because of plant establishment problems. Despite using salt-tolerant plants and other techniques for plant establishment in these habitats, revegetation can be very difficult (Chapter 1.2.6). In conjunction with breeding and using salt-tolerant plants, it has been reported that mycorrhizal fungal colonization can further enhance tolerance (Al-Karaki *et al.*, 2001; Cantrell & Linderman, 2001) and might play a significant role in establishment of plants in salt-stressed soils. Contributions of AM fungi to agriculture are well known,

but their role in helping plants to establish in saline conditions has received less attention (Giri *et al.,* 2002). No matched seedlings have been used in previous studies. Mycorrhizal seedlings of neem (*Azadirachta indica*) have been shown to have more dry matter accumulation as compared to non-mycorrhizal seedlings over a range of salinity levels (Pande & Tarafdar, 2002). Mycorrhizal wild bean plants (*Strophostyles helvola*) had greater vigour and enhanced growth because of increased chlorophyll contents, shoot dry weight, root-available water and number of root nodules compared to non-mycorrhizal plants in saline conditions (Tsang & Maun, 1999). Increased plant growth and vigour via mycorrhizal symbiosis under saline conditions may be important in revegetation of salt-affected lands.

Decreased P uptake as a result of low osmotic potential of the soil solution under saline conditions was identified as a detrimental effect of salt stress in plants (Poss *et al.*, 1985; Sentenac & Grignon, 1985; Delgado & SanchezRaya, 1997). On the other hand it was shown that non-mycorrhizal plants grow similarly to mycorrhizal plants if they receive additional P fertilization under salt stress (Hirrel & Gerdemann, 1980), which suggests that the mechanism of salinity tolerance in mycorrhizal plants may involve only improvement in P nutrition. The effects of AM fungi and P fertilizer on some aspects of plant salinity tolerance have been compared previously in alfalfa (*Medicago sativa*) (Azcón & El-Atrash, 1997), guayule (*Parthenium argetatum*) (Pfeiffer & Bloss, 1988), tomato and onion (Poss *et al.*, 1985), but their effects on plant establishment and nutrient concentrations have not received much attention.

Most previous research has used conditions in which plants were established into the soil and salt concentrations were increased gradually or suddenly, followed by assessment of mycorrhizal development and function (Chapter 2.3.2.1), but in this

study pre-existing saline conditions were used to simulate natural conditions, and pre-inoculated mycorrhizal transplants were used, so that effects of salinity on initial AM colonization were minimized. This method can be of the practical importance in field conditions for saline land revegetation. Seedlings could be grown in nursery conditions under non-saline conditions and sufficient time allowed to elapse between inoculation and imposition of salinity stress (or transplantation) to allow colonization to occur. The objective of this chapter was to evaluate potential of AM fungi in *T. subterraneum* seedling establishment under saline conditions, and then compare it with P application.

3.2 Materials and Methods

3.2.1 Experiment 1. Soil selection

Six sandy-textured soils were collected from different sites in the Monarto area (Table 3.1). Soil texture, organic matter, pH, electrical conductivity (EC_e), total and available P, total cation exchange capacity (CEC), exchangeable sodium percentage (ESP), and water content at field capacity were measured.

For the purpose of studying the effects of AM fungi on plant establishment at different salinity levels in mycorrhizally responsive and non-responsive plants (Chapters 3 and 4), a soil with following characteristics was required:

- Sufficient P to allow some growth of AM plants, but low enough to enable study of P addition on responses to salinity
- Properties suitable for the growth of AM fungi
- Sandy texture for easy root washing
- Low salinity level to allow addition of different levels of salt within the range required for study.

There was some information about collected soils, including soil series descriptions and classification (Chittleborough *et al.*, 1976), but there was no information about their ability to support mycorrhizal colonization. Therefore the chemical and physical properties of soils, which influence the rate of colonization in host plants, were investigated in soils collected from this region

T. subterraneum was chosen as the host plant because of its responsiveness to AM fungi and the considerable amount of previous work on AM colonization and plant growth and nutrition (Abbott & Robson, 1977; 1978; Smith *et al.*, 1979; Smith & Smith, 1981; Smith *et al.*, 1981). *Glomus intraradices* Schenk & Smith (DAOM 181602) was chosen because of its positive effects on plant growth and nutrient contents in association with *T. subterraneum* (Nadian *et al.*, 1997 and see references above), its ability to increase salinity tolerance in different plants (Duke *et al.*, 1986; Pfeiffer & Bloss, 1988; Jalaluddin, 1993; Mohammad *et al.*, 2003) and its occurrence in saline conditions in nature (Brown & Bledsoe, 1996; Aliasgharzadeh *et al.*, 2001).

After seed sterilization of *T. subterraneum* with NaOCl and germination in a germinator, seeds were coated in a suspension of *Rhizobium leguminosarum* biovar *trifolii* to promote nodule development prior to planting (Chapter 2.4 and 2.6). Two pre-germinated seeds were sown in each of six different non-autoclaved soils in 500 cm^3 pots. Soils were not autoclaved, to show the infectivity potential of indigenous fungi or inoculum (any mycorrhizal infection could result from inoculum or combination of inoculum and indigenous fungi which shows infectivity potential of the soil). There were four replicate pots per soil and harvest. All pots were inoculated with dried pot culture material of *Glomus intraradices*, consisting of a soil/sand mix plus colonized root fragments, spores and external hyphae, mixed with the soils in the ratio

10% inoculum to 90% soil. There were no control (non-mycorrhizal) treatments in this experiment, therefore the individual effect of indigenous fungi on host plant colonization was not assessed, but the next experiment (Chapter 3.3.2) showed that the same batch of inoculum produced the same colonization rate in autoclaved non-calcareous Ferries McDonald soil. Plants were thinned after emergence to one per pot. Plants were grown under glasshouse conditions in August 2001 (Winter), and watered with R.O. water thrice weekly to maintain soil moisture at 80% field capacity. No additional nutrients were added. Plants were harvested 2, 4 and 6 weeks after sowing. Plants were removed from the soils and a sub-sample of each root system was used for assessment of AM colonization (Chapter 2.10). Shoots and remaining roots were dried at 80ºC for 48 hours to determine dry weight.

3.2.2 Experiment 2. Production of matched seedlings for transplantation

The aim was to determine at what age inoculated and non-inoculated seedlings had similar growth and P content. The *T. subterraneum* seeds were surface sterilized and coated with a suspension of *Rhizobium leguminosarum* biovar *trifolii* (Chapter 2.4). One pre-germinated seed was sown in each plastic bag containing 60g autoclaved Ferries McDonald soil inoculated or not with *Glomus intraradices*. The inoculum was dried pot culture material, consisting of soil/sand mix plus colonized root fragments, spores and external hyphae. This was mixed with autoclaved Ferries McDonald soil in the ratio 10% inoculum to 90% soil (6g inoculum in each bag). Non-inoculated pots received an additional 6g autoclaved Ferries McDonald soil. Plants were grown in a glasshouse with natural light in October 2001 (Spring). R.O. water was added to maintain soil moisture at 80% field capacity. Three millilitres of Long Ashton nutrient

solution (Hewitt, 1966) without P were added to each seedling to help develop plant growth. Eight seedlings were harvested 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 days after planting from each AM inoculated (M) and non-inoculated (NM) treatment. Seedlings were washed carefully and leaf number and SDW of each seedling were then determined. Because of the low root weight at the early stages of plant growth, the whole of the roots were used for assessment of AM colonization. Also because of the low amount of dried shoot for P analyses, shoots of the 8 replicate samples were pooled and shoot P concentration determined in M and NM plant at 6-12 days by the method of Hanson (1950) (Chapter 2.12). Individual shoots were used to determine shoot P concentration between 14 and 24 days. Roots of harvested seedlings were cut to 1 cm segments then stained by trypan blue and mycorrhizal colonization was determined (Chapters 2.9 and 2.10).

3.2.3 Experiment 3. Effects of *Glomus intraradices* on *Trifolium subterraneum* seedling growth after transplanting at different salinity levels

This experiment had a randomized complete block design with three replications per treatment and harvest. The treatments in this experiment were 2 levels of AM inoculation (inoculated and non-inoculated), 5 levels of salinity (2.2, 3.5, 5, 7.5 and 12 dS/m) and 3 harvest times (10, 20 and 30 days after transplanting). Five levels of soil salinity were produced by adding 0, 0.25, 0.5, 1 and 2g NaCl per kg of the soil. Soils were incubated for one week, and then pots were filled with 1400g of the soil. Seedlings were grown for 2 weeks in a glasshouse, and then one pre-inoculated or non-inoculated matched seedling was transplanted into each pot. The plants were grown in a glasshouse with natural light in February 2002 (Summer). Plants were watered thrice

weekly with R.O. water to maintain soil moisture at 80% field capacity. No additional nutrients were added to the pots during the growth period. Plants were harvested 10, 20 and 30 days after transplanting. They were washed thoroughly and leaf number, shoot and root fresh and dry weights, shoot and root P, Na and K concentrations and root mycorrhizal colonization were measured at each harvest. After plant tissue digestion, P concentration was determined colorimetrically (Chapter 2.12), K and Na were determined using flame photometry (the same digestion method as for P was used). Sub-samples of roots for AM colonization were washed carefully and were stained by trypan blue and mycorrhizal colonization was evaluated in each sample (Chapter 2.8-10). Salinity response in terms of total dry weight was calculated in M or NM plants as follows:

$$\%\,\text{Salinity response} = \frac{\text{DW (+salt) - DW (-salt)}}{\text{DW (-salt)}} \times 100 \qquad \text{Eqn 1}$$

Mycorrhizal growth response (MGR) was calculated using the individual total plant dry weight (DW) of M and the mean dry weight of NM plants at each harvest as follows:

$$\%\,\text{MGR} = \frac{\text{DW(M) - mean DW (NM)}}{\text{mean DW (NM)}} \times 100 \qquad \text{Eqn 2}$$

Mycorrhizal P response (MPR) was calculated as follows:

$$\%\,\text{MPR} = \frac{\text{P content (M) - mean P content (NM)}}{\text{mean P content (NM)}} \times 100 \qquad \text{Eqn 3}$$

Mycorrhizal potassium (K) response (MKR) was calculated similarly.

Probabilities of significance among treatments and interactions and LSDs (P<0.05) were used to compare means within and among treatments.

3.2.4 Experiment 4. Effects of *Glomus intraradices* and P application on *Trifolium subterraneum* seedling growth after transplanting to different salinity levels

This experiment had a randomized complete block design with three replications. The treatments were 2 levels of AM inoculation (inoculated and non-inoculated), 2 levels of P (0 and 60 mg/kg soil), 3 levels of salinity (2.2, 12 and 15 dS/m) and 2 harvest times (20 and 40 days after transplanting). Pre-inoculated and non-inoculated *T. subterraneum* seedlings were produced as described in Experiment 2 (Chapter 3.2.2). The highest salinity level was made by addition of 3g NaCl per kg soil. To use the same nutrient conditions and the same bacterial population in inoculated and non-inoculated seedlings, the same amount of mycorrhizal inoculum used to inoculate seedlings was stirred in R.O. water and filtered through Whatman no: 1 (11 μm) filter paper and 5 ml of filtrate were added to each non-inoculated seedling during the pre-transplant stage. A preliminary experiment (results not shown) had investigated the effects of additional P (10-100 mg P per kg soil) vis-a-vis mycorrhizal inoculation on growth and P uptake of *T. subterraneum* (direct seeding, not seedling transplantation was used). The experiment had shown that non-inoculated *T. subterraneum* had the same growth and P content as plants inoculated with *Glomus intraradices* in Ferries McDonald soil at 6 weeks, when 60 mg P was added per kg of the soil.

The soil was thoroughly mixed with 60 mg/kg P fertilizer as NaH_2PO_4 and/or different levels of NaCl. Preliminary analyses (results not shown) indicated this addition of NaH_2PO_4 increased soil available P to 45 mg/kg (Colwell, 1963) and had no effects on soil electrical conductivity. Soils were incubated for one week, and then pots were filled with 1400 g. One pre-inoculated or ..

non-inoculated seedling was transplanted into pots with different soil salinity and P levels. Transplanted seedlings were grown in a glasshouse in October 2003 (Spring) and were harvested 20 and 40 days after transplanting. Leaf number, shoot and root dry weights were determined at each harvest. After plant tissue digestion (Chapter 2.12) nutrient concentrations were determined by Inductively Coupled Plasma emission spectroscopy (ICP). Plant roots were washed carefully and stained by trypan blue, and mycorrhizal colonization was evaluated in each sample (Chapter 2.9 and 2.10).

3.3 Results

3.3.1 Results of Experiment 1. Soil selection

Chemical and physical characteristics of the six soils are given in Tables 3.1 and 3.2. Overall the results of soil analysis show that all the soils had sandy texture, with minimum 65% sand in Premimma Bk horizon (P1) and maximum 92.5% sand in Camel Hill (CH). Premimma C horizon (P2) had the highest salinity level and CH the lowest (5.4 and 1.3 dS/m respectively). The highest available P was 41.2 mg/kg in Kalibar (K) soil, and the lowest was 1.8 in P2.

The colonization percentages and average shoot dry weights of *T. subterraneum* at 2, 4 and 6 weeks after sowing in the six different soils are shown in Figs. 3.1 and 3.2 respectively. At 2 weeks after planting plants growing in K soil and F1 soil had 2 and 9% colonization, respectively, but there was no colonization in other soils. Mycorrhizal colonization rapidly increased in K and F1 soils up to 53% and 44%, respectively, at 4 weeks. At the same time low colonization was found in CH, P1 and P2 soils. Mycorrhizal colonization was at a low level in P2 and calcareous Ferries McDonald (F2), moderate in CH and P1 and high in K and F1 soils, 6 weeks after planting (Fig.

3.1). As time increased shoot dry weight of plants in all soils increased. Plants growing in K soil had the highest SDW at the first harvest, and F2 soil had the lowest SDW. After six weeks plants in K soil had the highest growth, followed by F1, but plants in P1 and P2 soils had the lowest level of growth (Fig. 3.2).

In summary, the results of mycorrhizal colonization and plant growth show that Kalibar and non-calcareous Ferries McDonald soils had a reasonable potential for mycorrhizal colonization and plant growth. The reasons for low mycorrhizal colonization in CH, P1, P2 and F2 soils were not investigated further. Because of the lower level of salinity and adequate available P for germination and the higher sandy texture of non-calcareous Ferries McDonald compared with Kalibar soil, Ferries McDonald soil (F1) was selected for plant establishment studies. No further discussion of these results is presented.

Table 3.1 Experiment 1. Some physical characteristics of six soils collected from Monarto area (Chittleborough *et al.*, 1976)

Soil Series	**Plant cover**	**Texture**	**Sand** (%)	**Silt** (%)	**Clay** (%)	**W.C (F.C)** [A]
Camel Hill (CH)	Annual grasses and forbs	Sandy	92.5	2.5	5.0	6.1
Kalibar (K)	Perennials (*Atriplex nummularia*)	Loamy sand	67.5	20	12.5	24.9
Ferries McDonald non- calcareous (F1)	Annual grasses and forbs	Sandy loam	82.5	7.5	10.0	15.1
Premimma (P1)	Bare soil	Sandy loam	65.0	10.0	25.0	19.5
Premimma (P2)	Bare soil	Sandy loam	82.5	2.5	15.0	15.6
Ferries McDonald calcareous (F2)	Annuals	Sandy loam	80.0	5.0	15.0	13.5

[A] Water content at field capacity

Table 3.2 Experiment 1. Some chemical characteristics of six soils collected from Monarto area (Chittleborough *et al.*, 1976)

Soil Series	EC[A] dS/m	pH (H_2O) [B]	pH ($CaCl_2$)[C]	OM [D] (%)	P avai [E] (mg/kg)	P total [F] (mg/kg)	Total CEC [G] (meq/100g)	ESP [I]
Camel Hill (CH)	1.3	7.0	7.0	1.0	9.4	145	5	4
Kalibar (K)	4.8	9.0	8.0	1.1	41.2	465	9.1	8.7
Ferries McDonald non-calcareous (F1)	2.2	8.3	7.6	1.3	15.3	245	9.7	6.1
Premimma (P1)	4.4	9.0	8.0	0	3.5	120	8.5	16.4
Premimma (P2)	5.4	8.9	7.8	0	1.8	50	8.2	17
Ferries McDonald calcareous (F2)	2.1	8.3	7.8	1.9	10.6	125	16.1	4.9

[A] Electrical conductivity
[B] pH in water
[C] pH in $CaCl_2$
[D] Organic matter
[E] Available phosphorus (Colwell, 1963)
[F] Total phosphorus
[G] Cation exchangeable capacity
[I] Exchangeable sodium percentage

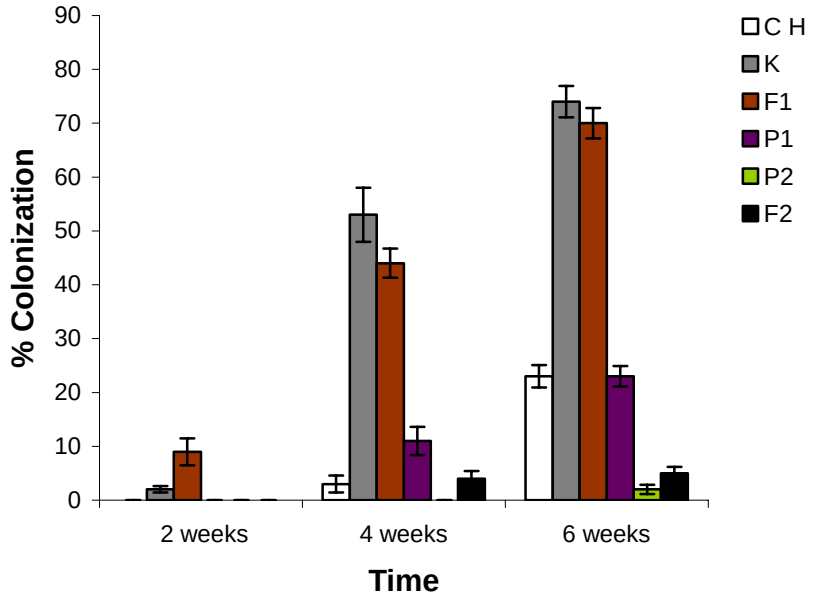


Fig 3.1 Experiment 1. AM colonization of *Trifolium subterraneum* after 2, 4 and 6 weeks planted in six soils collected from Monarto area. CH= Camel Hill soil, K= Kalibar soil, F1= Non-calcareous Ferries McDonald soil, P1= Premimma BK horizon soil, P2= Premimma C horizon soil, F2= Calcareous Ferries McDonald soil. Vertical bars represent standard error of the means, n=4.

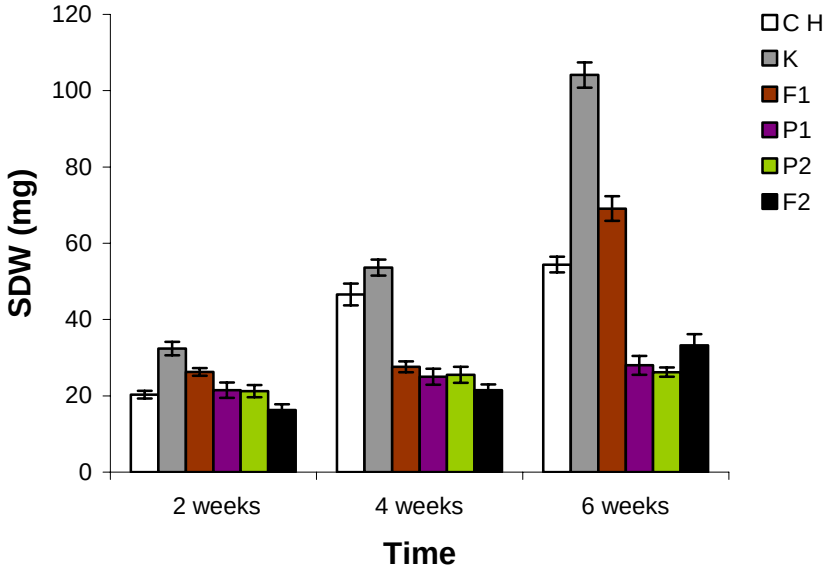


Fig 3.2 Experiment 1. Shoot dry weight (SDW) of *Trifolium subterraneum* after 2, 4 and 6 weeks planted in six soils collected from Monarto area. Vertical bars represent standard error of the means, n=4.

3.3.2 Results of Experiment 2. Production of matched seedlings for transplantation

No mycorrhizal colonization was found in roots of NM seedlings. Roots of M seedlings were 5% colonized at 12 days, and increased up to 29% at 24 days after planting (Fig. 3.3). There were no significant differences in SDW between M and NM seedlings at 6 and 8 days, but at 10 and 12 days NM seedlings had higher SDW than M seedlings ($P<0.05$) (Fig. 3.4). Mycorrhizal and NM seedlings had the same SDW at 14-24 days after planting. Shoot P concentration decreased with increasing seedling age in both M and NM seedlings (Fig. 3.5). Shoot P concentration in M was the same as NM seedlings up to 16 days, but NM seedlings had lower shoot P concentration between 18-24 days after planting. The cotyledon leaves of NM seedlings started to become yellow at 16 days, but all of the M seedlings kept their cotyledons green up to 24 days after planting. It was concluded that at 14-16 days after planting, M and NM seedlings have the same SDW and P content. This was determined as the best time for transplanting to minimize confounding effects of differences between M and NM plants in the second stage of transplanting experiments. No further discussion of these results is presented.

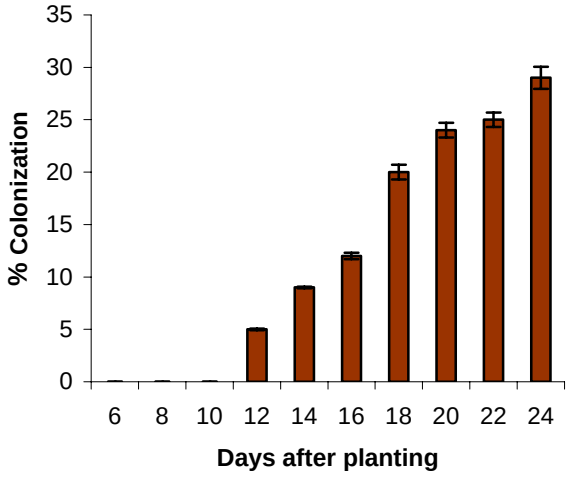


Fig 3.3 Experiment 2. AM colonization of *Trifolium subterraneum* seedlings at 6-24 days after planting. Vertical bars represent standard error of the means, n=8.

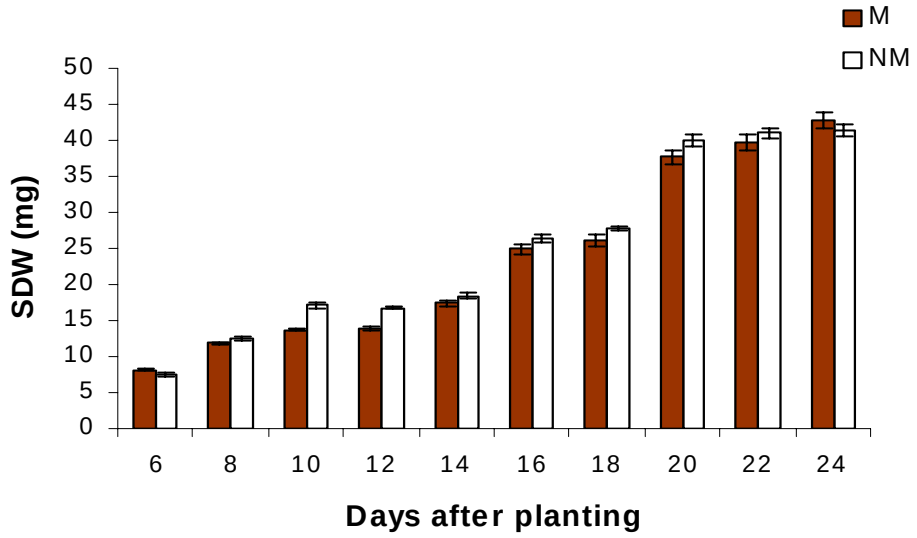


Fig 3.4 Experiment 2. Shoot dry weights of mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum* seedlings at 6-24 days after planting. Vertical bars represent standard error of the means, n=8.

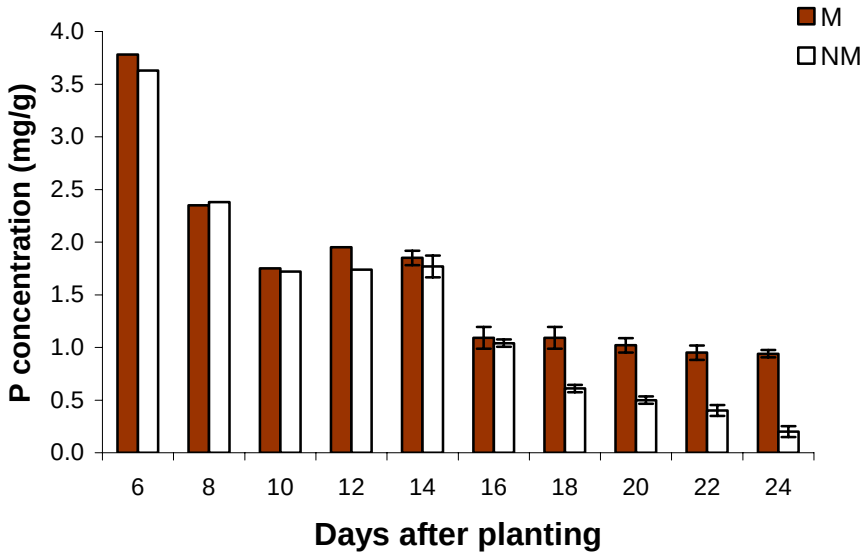


Fig 3.5 Experiment 2. Shoot P concentrations of mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum* seedlings at 6-24 days after planting. Vertical bars represent standard error of the means, n=8. Replicate plants harvested at times from 6 to 12 days were pooled for analyses.

3.3.3 Results of Experiment 3. Effects of *Glomus intraradices* on *Trifolium subterraneum* seedling growth after transplanting at different salinity levels

Colonization and plant growth

No AM colonization was found in NM treatments (results not shown). At 10 days after transplanting to different salinity treatments colonization had increased to between 50 and 65% (Fig. 3.6). There were no significant differences in percent root length colonized between salinity treatments at any harvest. However, there was a trend toward lower percent colonization as salinity increased at harvest 3.

Mycorrhizal inoculation increased plant dry weight (Fig. 3.7). At 10 days after transplanting, total dry weights of M plants were greater than NM plants at the three lower salinity levels (Fig. 3.7 A). Increased salinity had no significant effects on total dry weight of either M or NM plants at 10 days. Differences of total dry weight between M and NM plants were significant at 20 and 30 days at all salinity levels (Fig. 3.7 B and C). The effects of salinity on total dry weight were significant at 20 and 30 days, with increased salinity decreasing total dry weight in both M and NM plants. Mycorrhizal plants had higher S/R ratios than NM plants (results not shown).

The effects of different levels of salinity on total dry weight are shown in terms of salinity responses (Calculated by Eqn 1) at the third harvest (Fig. 3.8). M plants showed smaller decreases in total dry weight in response to salinity than NM plants. Non-mycorrhizal plants responded negatively to salinity at all salinity levels. In contrast, in M plants a negative response to salinity was only observed at 5 dS/m and above.

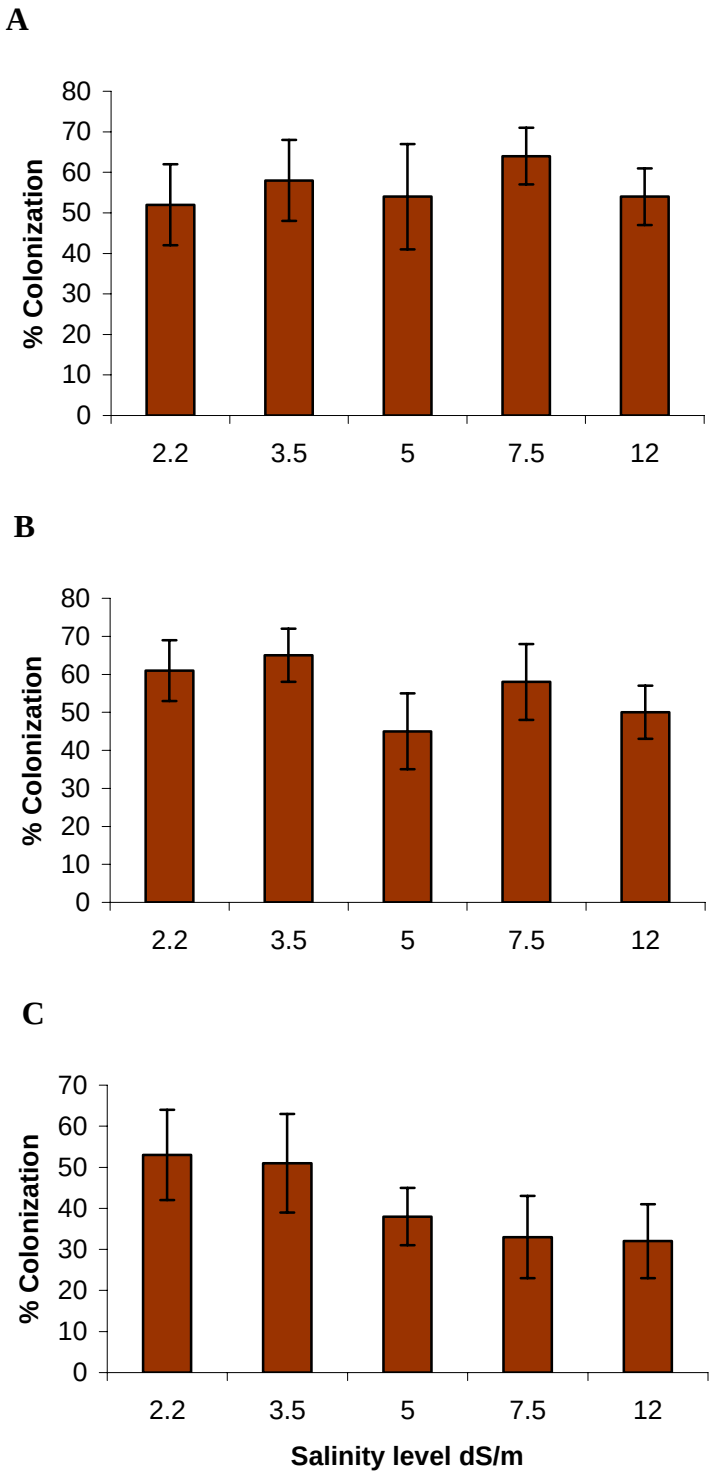


Fig 3.6 Experiment 3. Colonization of roots of *Trifolium subterraneum* grown in different levels of salinity at 10 (A), 20 (B) and 30 (C) days after transplanting. Vertical bars represent standard error of the means, n=3.

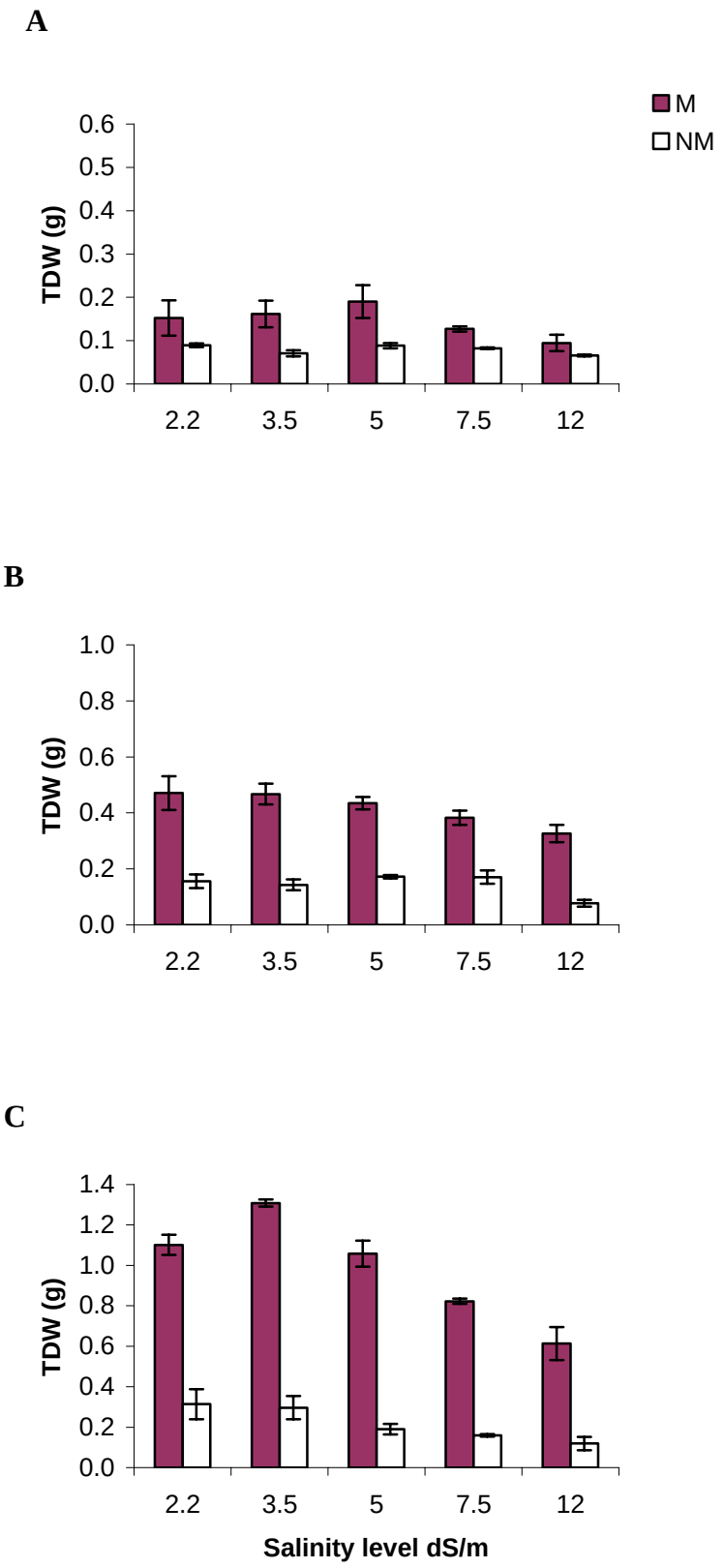


Fig 3.7 Experiment 3. Total dry weights (TDW) of mycorrhizal and non-mycorrhizal *Trifolium subterraneum*, at 10, 20 and 30 days (A, B and C respectively) after transplanting to different salinity levels. Vertical bars represent standard errors of the means, n=3.

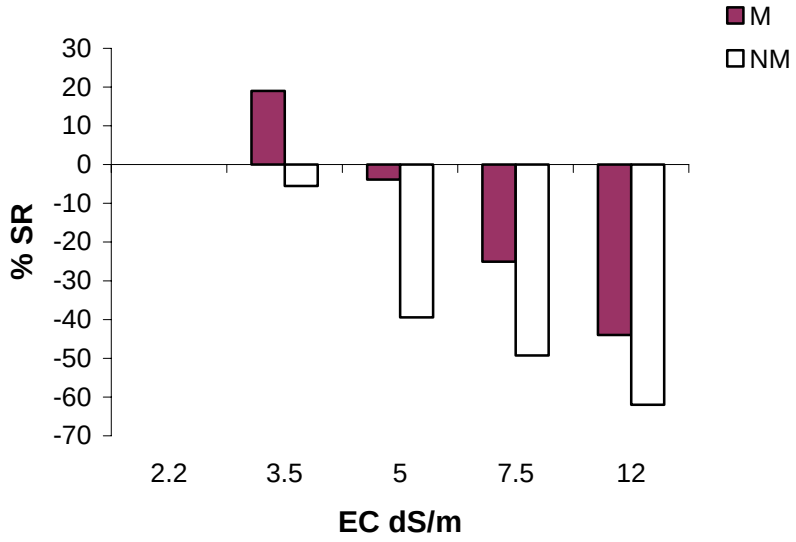


Fig 3.8 Experiment 3. Percentage salinity responses (SR) in terms of total dry weight in mycorrhizal and non-mycorrhizal *Trifolium subterraneum* at the third harvest (30 days). Calculations as in Equation 1.

Mycorrhizal growth responses (MGR) (Eqn 2) in terms of total dry weight at different salt levels and different harvests are shown in Fig 3.9. Mycorrhizal growth responses increased with time in all salinity treatments. The biggest MGR was at 3.5 dS/m at 30 days. There was a decrease in MGR above 5 dS/m at all harvests.

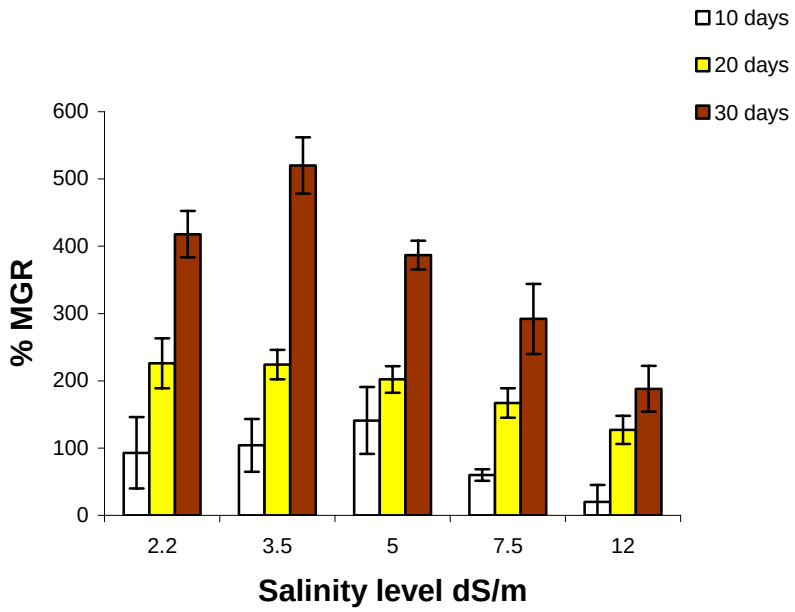


Fig. 3.9 Experiment 3. Mycorrhizal growth response (MGR) of *Trifolium subterraneum* in terms of total dry weight at different salinity levels and different harvests. Vertical bars represent standard errors of the means, n=3. Calculations as in Equation 2.

Nutrient concentrations

Phosphorus

Phosphorus concentrations in shoots and roots of M plants were much higher than NM plants at 10 days, and there was no effect of increasing salinity at that time (Table 3.3). The differences between shoots of M and NM plants decreased at 20 days compared to 10 days, due to decreasing P concentrations in M plants and increases in NM plants, the latter especially at low salinity. There was a similar trend in P concentrations in roots at 20 days (Table 3.4). No significant effects of salinity on root and shoot P concentrations were found at 20 days. At 30 days M plants still had higher root P

concentrations even at high salinity, due to an increase in P in M roots compared with those at low salinity (Table 3.5). There were no significant differences in shoot P concentrations between M and NM plants at that time. Salinity had no effects on P concentration in shoots and roots at 30 days. Phosphorus content of the shoots and roots were used to calculate MPR (Fig. 3.10), using Eqn 3. MPRs in roots and shoots were significantly higher at first harvest than second and third harvests at nearly all salinity levels. Differences between harvests were small at high salinity.

Potassium

Inoculated plants had higher root K concentrations than NM plants at 10 days, but not at 20 and 30 days (Tables 3.3-3.5). Shoot K concentrations were not affected by mycorrhizal inoculation at any harvests. Salinity had no effects on root or shoot K concentrations. The MKR for shoots and roots calculated from plant K content (Fig. 3.11), shows that mycorrhizal effects on total K uptake were generally higher at 30 days than 10 days. MKRs showed a decreasing trend with increasing salinity at all salinity treatments.

Table 3.3 Experiment 3. Shoot and root nutrient concentrations of mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum* at 10 days after transplanting to different salinity levels. Means of 3 replicates ± standard error

Treatments	Salinity (dS/m)	Root (mg/g)			Shoot (mg/g)		
		P	K	Na	P	K	Na
M	2.2	3.3±0.2	32.1±0.1	18.9±1.2	2.7±0.0	14.1±3.5	6.3±1.3
	3.5	4.1±0.1	35.2±0.9	12.8±1.1	2.4±0.2	16.5±2.3	6.9±0.7
	5	3.9±0.3	37.8±2.3	17.2±0.4	3.1±0.4	12.6±1.8	8.2±1.2
	7.5	4.5±0.3	37.4±3.5	14.9±0.8	2.9±0.2	10.6±1.7	11.0±1.1
	12	3.7±0.4	46.4±0.3	13.0±1.6	2.5±0.3	15.0±2.3	16.5±2.2
NM	2.2	0.9±0.1	34.6±1.2	10.7±0.8	0.5±0.1	16.4±2.9	5.8±0.3
	3.5	0.5±0.1	37.6±1.8	13.4±0.6	0.7±0.1	17.3±0.6	4.6±0.2
	5	0.6±0.2	32.0±1.8	12.0±0.1	0.6±0.1	17.3±1.4	6.1±0.4
	7.5	0.4±0.1	33.4±1.1	17.2±1.4	0.5±0.1	12.0±1.3	6.3±1.1
	12	0.2±0.0	28.2±2.0	19.3±2.4	0.4±0.1	14.9±2.2	11.7±1.2
Inoculation		***	**	ns	***	ns	***
Salinity		ns	ns	ns	ns	ns	***

,* significant at the 0.01, 0.001 probability levels, respectively
ns not significant at p=0.05

Table 3.4 Experiment 3. Shoot and root nutrient concentrations of mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum* at 20 days after transplanting to different salinity levels. Means of 3 replicates ± standard error

Treatments	Salinity (dS/m)	Root (mg/g)			Shoot (mg/g)		
		P	K	Na	P	K	Na
M	2.2	2.8±0.1	19.5±1.8	15.0±1.3	2.4±0.0	12.7±0.9	5.1±0.2
	3.5	2.8±0.3	23.2±5.8	14.9±1.9	2.3±0.2	10.1±1.5	5.9±0.6
	5	2.8±0.4	16.9±0.8	15.2±1.5	2.6±0.2	12.3±3.1	8.1±0.6
	7.5	2.4±0.4	20.7±2.6	14.8±0.3	2.2±0.3	9.9±0.3	13.6±0.2
	12	2.5±0.3	16.5±0.4	14.5±1.1	2.5±0.4	9.4±0.7	17.3±5.8
NM	2.2	1.9±0.5	21.7±5.4	8.6±1.1	1.7±0.4	12.5±2.3	2.9±0.5
	3.5	2.3±0.2	22.2±2.0	11.2±1.1	1.0±0.3	15.4±2.0	3.3±0.3
	5	2.0±0.7	22.7±2.9	11.1±2.2	1.6±0.5	11.5±1.0	5.1±1.3
	7.5	1.5±0.3	20.8±2.6	12.0±1.0	1.2±0.2	10.4±0.6	5.8±0.9
	12	1.5±0.1	16.7±3.4	16.2±2.2	1.2±0.1	11.3±6.2	13.0±2.5
Inoculation		**	ns	**	***	ns	**
Salinity		ns	ns	ns	ns	ns	***

,* significant at the 0.01, 0.001 probability levels, respectively
ns not significant at p=0.05

Table 3.5 Experiment 3. Shoot and root nutrient concentrations of mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum* at 30 days after transplanting to different salinity levels. Means of 3 replicates ± standard error

Treatments	Salinity (dS/m)	**Root** (mg/g)			**Shoot** (mg/g)		
		P	**K**	**Na**	**P**	**K**	**Na**
M	**2.2**	2.1±0.7	17.2±2.4	8.4±.0.9	2.2±0.3	16.1±5.9	4.7±1.3
	3.5	2.1±0.7	23.7±3.4	10.5±1.1	1.9±0.4	18.8±1.8	8.1±0.7
	5	2.7±0.2	21.1±1.9	9.5±0.8	1.8±0.4	21.2±1.4	8.5±2.4
	7.5	3.3±0.0	28.8±1.7	10.3±0.7	1.4±0.4	10.7±0.9	10.9±1.7
	12	3.4±0.2	26.4±1.1	12.1±0.6	1.8±0.1	8.8±1.4	17.6±2.3
NM	**2.2**	1.8±0.3	27.4±1.3	8.1±0.4	1.7±0.4	32.4±12.1	4.1±0.6
	3.5	1.6±0.1	28.4±2.2	10.0±0.4	1.8±0.4	29.2±10.9	5.3±1.0
	5	1.7±0.2	29.8±1.3	10.5±0.1	1.2±0.1	16.2±6.0	10.3±2.2
	7.5	1.2±0.0	22.6±1.4	9.8±0.1	1.6±0.0	14.8±3.7	9.9±1.5
	12	1.6±0.1	26.4±5.1	16.5±1.5	1.8±0.1	14.0±3.7	23.1±2.2
Inoculation		***	*	ns	ns	ns	ns
Salinity		ns	ns	***	ns	ns	***

*, *** significant at the 0.05, 0.001 probability levels, respectively
ns not significant at p=0.05

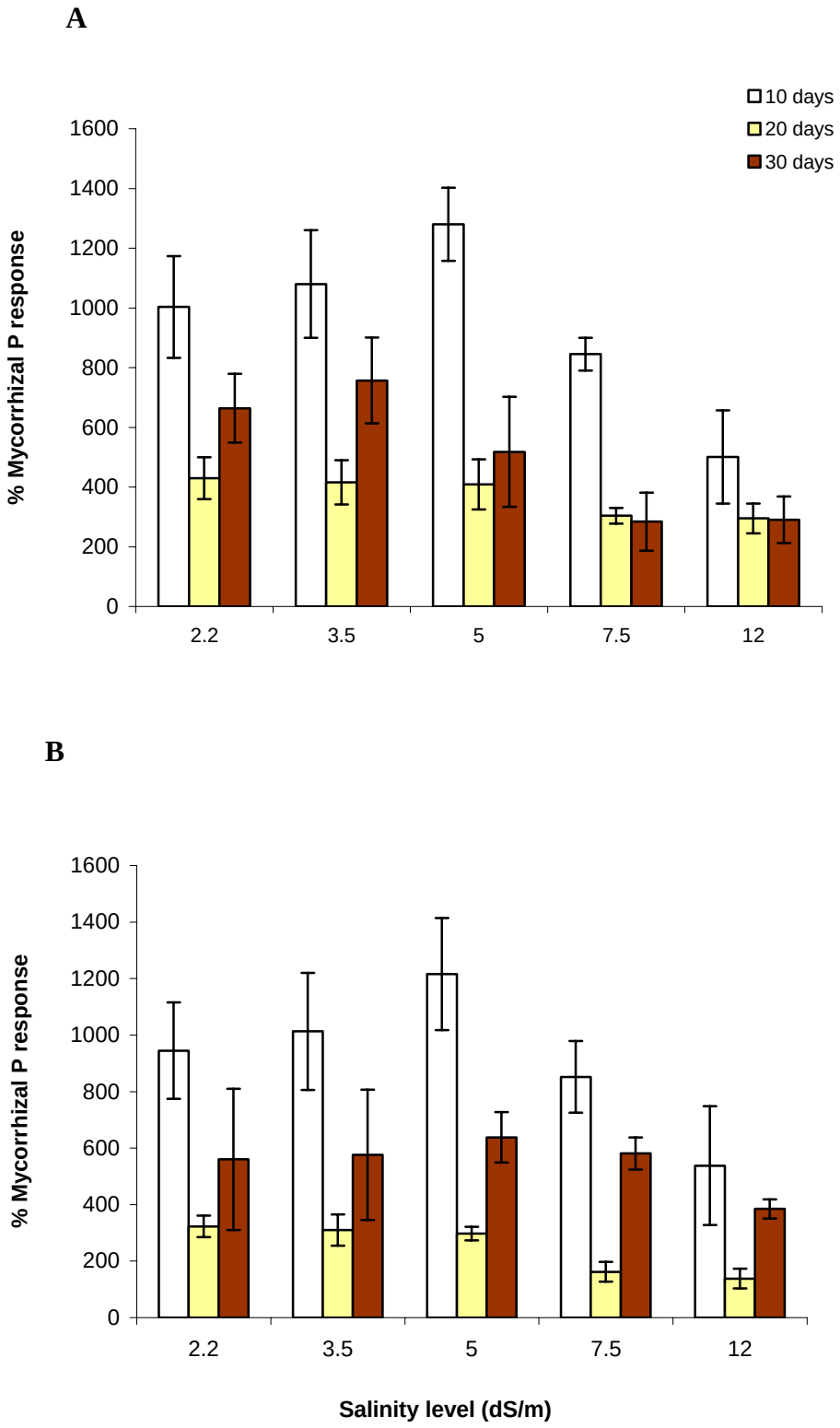


Fig. 3.10 Experiment 3. Mycorrhizal P response (MPR) of *Trifolium subterraneum* shoots (A) and roots (B) at different salinity levels and different harvests. Vertical bars represent standard errors of the means, n=3. Calculations as in Equation 3.

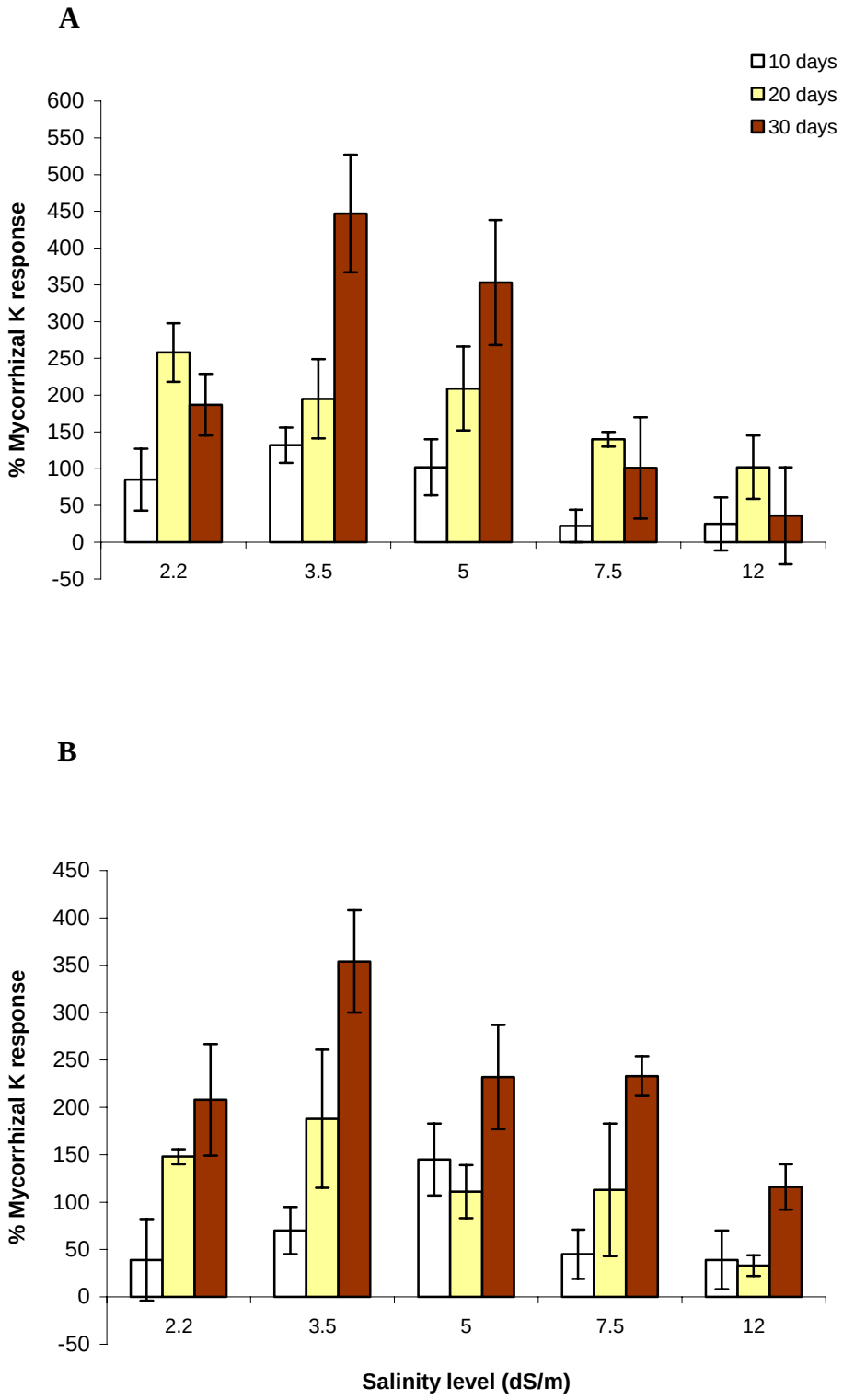


Fig. 3.11 Mycorrhizal K response (MKR) of *Trifolium subterraneum* shoots (A) and roots (B) at different salinity in three harvests. Vertical bars represent standard errors of the means, n=3.

Sodium

With increasing salinity shoot Na concentration was significantly increased in both M and NM plants at all harvests (Tables 3.3-3.5). Mycorrhizal inoculation increased shoot Na concentrations at 10 and 20 days, but no effects were found at 30 days. Increased salinity had no effects on root Na concentrations at 10 and 20 days, but increased root Na concentrations at 30 days. Mycorrhizal plants had higher root Na concentrations than NM plants at 20 days only. No mycorrhizal effects were found at 10 and 30 days.

Potassium / Sodium ratio

K/Na ratios (based on concentrations) are shown in Table 3.6. At 10 days NM plants had higher root K/Na ratio at the lowest salinity, but M plants showed higher K/Na ratio at the highest salinity. At the same harvest shoot K/Na ratio was higher in NM than M plants at moderate salinity levels (3.5, 5 and 7.5 dS/m). At 20 days root and shoot K/Na ratios in NM plants were higher than NM plant at low salinity levels (2.2 and 3.5 dS/m), but no differences were found at high salinity levels. Nearly the same trend was found at 30 days.

In summary, the results of Experiment 3 showed that improved growth and nutrient uptake (particularly P) in inoculated *T. subterraneum* may explain the potential of AM fungi to increased plant establishment in saline conditions. Improved P uptake via the AM pathway demonstrates an important mechanism to increase plant establishment in saline conditions at the early stage of transplanting. So further work with AM non-responsive plants in terms of P uptake is needed to show AM effects on plant salinity tolerance, when there is no effects of AM on P nutrition (Chapter 4).

Table 3.6 K/Na ratio in shoots and roots of mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum* at 10, 20 and 30 days after transplanting to different salinity levels. Means of 3 replicates ± standard error

Treatments	Salinity	K/Na	
		Root	Shoot
10 days			
M	**2.2**	1.7±0.1	2.2±0.2
	3.5	2.8±0.3	2.4±0.2
	5	2.2±0.2	1.5±0.1
	7.5	2.5±0.1	1.0±0.1
	12	3.7±0.5	1.0±0.3
NM	**2.2**	3.3±0.3	2.8±0.3
	3.5	2.8±0.2	3.7±0.1
	5	2.5±0.1	2.9±0.4
	7.5	2.0±0.2	2.1±0.2
	12	1.5±0.1	1.3±0.3
LSD (*P*<0.05)		0.7	0.7
20 days			
M	**2.2**	1.3±0.2	2.5±0.2
	3.5	1.5±0.2	1.8±0.4
	5	1.1±0.1	1.5±0.4
	7.5	1.4±0.2	0.7±0.0
	12	1.2±0.1	0.8±0.4
NM	**2.2**	2.4±0.4	4.4±0.8
	3.5	2.0±0.2	4.8±1.8
	5	2.1±0.1	2.5±0.6
	7.5	1.6±0.1	1.9±0.4
	12	1.1±0.3	1.1±0.8
LSD (*P*<0.05)		0.5	1.8
30 days			
M	**2.2**	2.0±0.1	3.5±1.2
	3.5	2.3±0.2	2.3±0.2
	5	2.3±0.3	3.0±0.1
	7.5	2.9±0.3	1.0±0.2
	12	2.2±0.1	0.5±0.0
NM	**2.2**	3.4±0.2	7.3±2.2
	3.5	2.8±0.1	5.1±1.2
	5	2.8±0.1	1.4±0.3
	7.5	2.3±0.1	1.4±0.2
	12	1.7±0.5	0.7±0.2
LSD (*P*<0.05)		0.7	2.5

3.3.4 Results of Experiment 4. Effects of *Glomus intraradices* and P application on *Trifolium subterraneum* seedling growth after transplanting to different salinity levels

Survival, colonization and plant growth

AM inoculated plants had better vigour than non-inoculated plants after transplanting to different salinity levels. Inoculated plants kept their cotyledon leaves until three weeks after transplanting, but non-inoculated plants lost them after one week. Also primary (spade) leaves in inoculated plants remained up to four weeks, but no primary leaves were alive in non-inoculated plants after two weeks, particularly at high salinity levels. Many non-inoculated plants transplanted to the highest salinity level (15 dS/m) had died (particularly NM-P plants) at both harvests, but some inoculated plants survived (Fig. 3.12). Because of low numbers of replicates (3) and mortality at high salinity in this experiment, results at 15 dS/m could not be statistically analyzed. So the highest level of salinity (15 dS/m) in this experiment was removed and only results of low and high (2.2 and 12 dS/m) are presented.

Non-inoculated plants with and without P (NM+P and NM-P respectively) did not become colonized by AM fungi (results not shown). Salinity had no significant effects on root colonization in the different mycorrhizal treatments at 20 and 40 days after transplanting (Fig. 3.13). At 20 days AM inoculated plants (M-P and M+P) had the same percentage of root length colonized at both salinity levels, but at 40 days M-P plants had slightly but significantly higher colonization than M+P plants at both salinity levels.

Mycorrhizal inoculation had significant effects in increasing total dry weight at both salinity levels and harvests (Fig. 3.14). At 20 days inoculated plants had significantly higher total dry weight than non-inoculated plants with or without P at the low salinity

level (Fig. 3.14 A). At the same harvest at high salinity the effects of M+P treatment were significant compared to NM+P and NM-P, but not M-P treatments. Non-mycorrhizal plants with P added (NM+P) had higher total dry weight than NM-P plants at the low salinity level but not at the high salinity level.

At 40 days plants inoculated with AM fungi showed significantly higher total dry weight than non-inoculated plants with or without P at both salinity levels (Fig. 3.14 B). There were no differences in total dry weight between M-P and M+P plants at both salinity levels. Non-mycorrhizal plants with P (NM+P) had higher total dry weight than NM-P plants at the low salinity level. However, at the high salinity level growth of both NM treatments was extremely poor.

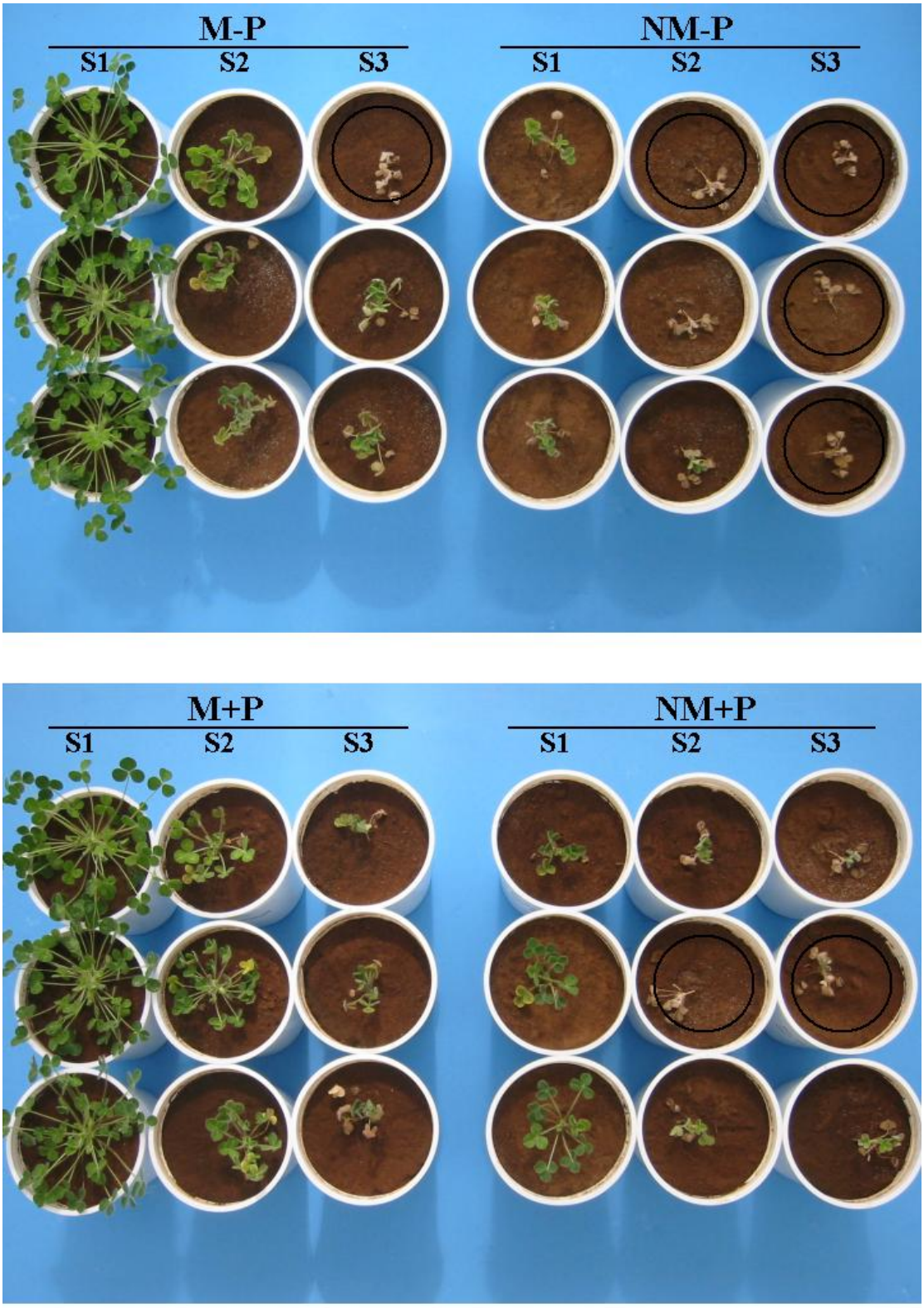


Fig 3.12 Experiment 4. Survival of *Trifolium subterraneum* with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal without P added (NM-P) and non-mycorrhizal with P added (NM+P), grown 40 days after transplanting in soil with 2.2 (S1), 12 (S2) and 15 (S3) dS/m salinity in 3 replicates (dead plants are highlighted by circles).

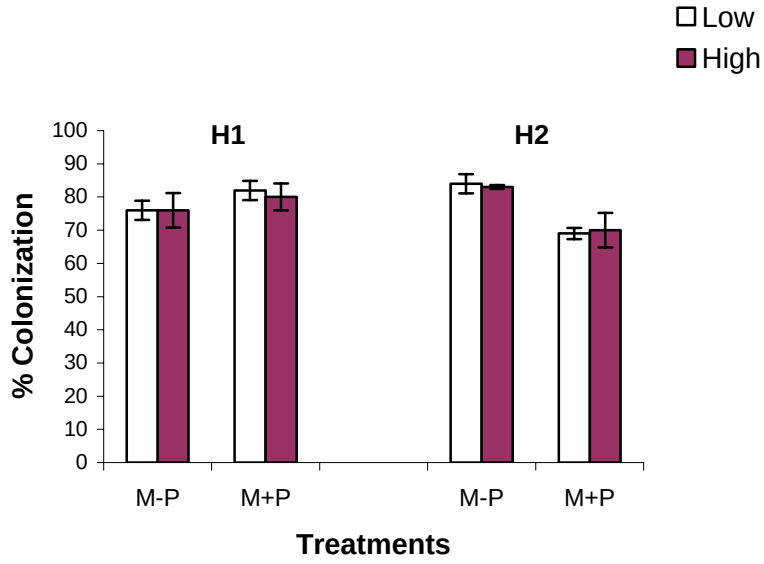


Fig 3.13 Experiment 4. Colonization of roots of *Trifolium subterraneum* with different treatments; mycorrhizal (M-P) and mycorrhizal with P added (M+P), grown at low and high salinity levels (2.2 and 12 dS/m, respectively) at 20 (H1) and 40 (H2) days after transplanting. Vertical bars represent standard error of the means, n=3.

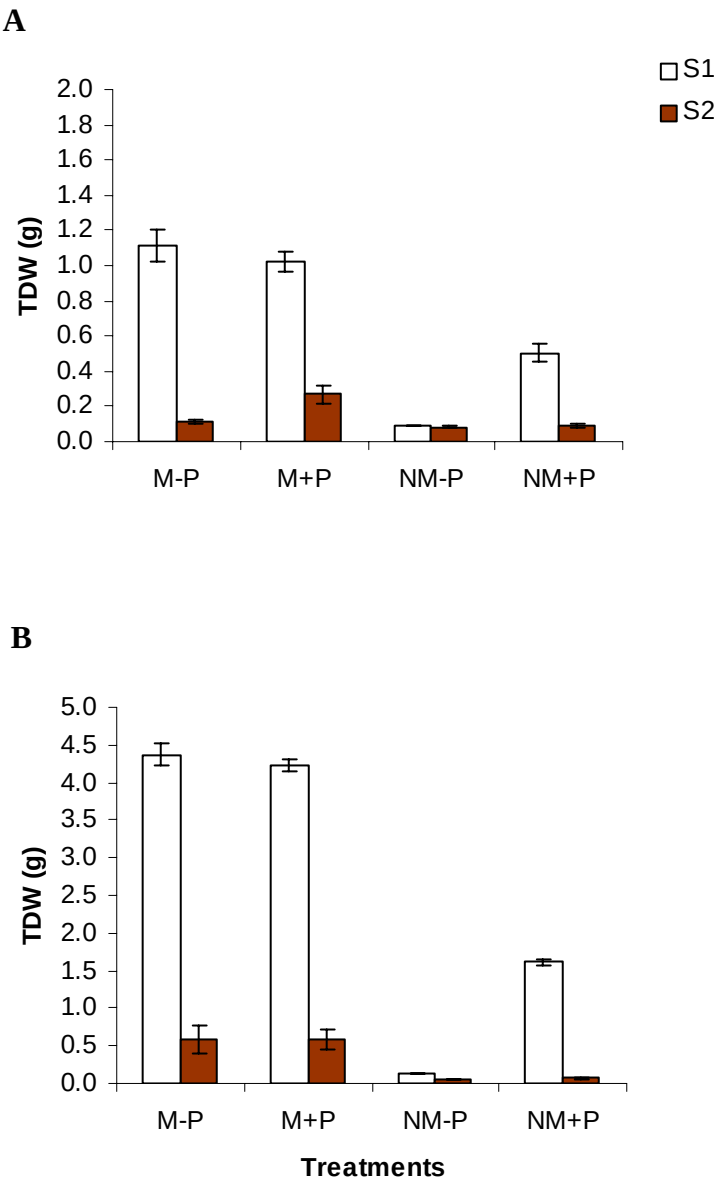


Fig 3.14 Experiment 4. Total dry weights (TDW) of *Trifolium subterraneum* with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal with P added (NM+P) and non-mycorrhizal without P added (NM-P), at 20 (A) and 40 (B) days after transplanting in low (S1) and high (S2) salinity levels. Vertical bars represent standard error of the means, n=3.

Nutrient concentrations

Phosphorus

The effects of different treatments on mineral concentrations in *T. subterraneum* shoots and roots at 20 and 40 days after transplanting are shown in Tables 3.7 and 3.8. Concentrations were greatly influenced by AM inoculation and P application. Overall the results show that mycorrhizal plants (M-P and M+P) had higher root and shoot P concentrations at both salinity levels and harvests when compared to non- mycorrhizal plants (NM-P and NM+P). At 20 days M-P and M+P plants had higher shoot P concentrations compared to NM-P and NM+P plants at both salinity levels (Table 3.7). At the same harvest there were no differences in shoot concentrations between NM+P and NM-P at low and high salinity levels, but M+P plants had higher P concentrations in their shoots than M-P plants. The same trend was found in the roots. At 40 days M-P and M+P plants had higher shoot P concentrations than NM-P at both salinity levels (Table 3.8). Mycorrhizal plants with P (M+P) had higher shoot P concentrations than NM+P at both salinity levels, but M-P plants had higher shoot P concentrations only at the high salinity level when compared with NM+P. At the same harvest the same trend was found in roots. The highest P concentrations in shoots and roots were found in M+P at low salinity.

Potassium

In this experiment AM inoculated plants with or without P had higher shoot K concentrations at 20 days than NM+P and NM-P plants at low salinity. No differences were found between NM+P and NM-P plants (Table 3.7). There were no differences in shoot K concentrations between mycorrhizal and P treatments at high salinity. Root K

concentrations were greater in M-P and M+P than in NM-P plants at both salinity levels. NM+P plants had higher root K concentrations compared with NM-P plants at low but not at high salinity. At 40 days shoot K concentrations in M-P plants were significantly higher than NM-P at low and high salinity levels, but M+P and NM+P had higher shoot K concentration than NM-P plants only at low salinity (Table 3.8). Roots of M-P and M+P plants had greater K concentrations than NM-P at both salinity levels, but NM+P plants had higher K concentrations than NM-P plants only at low salinity. Calculation of MKR showed an increasing trend with plant age (results not shown).

Sodium

At 20 days, increased salinity increased Na concentrations in shoots of plants in all treatments. There were no differences in shoot Na concentrations between mycorrhizal and/or P treatments within each salinity treatment (Table 3.7). Roots of M-P and M+P plants had consistently high Na concentrations, regardless of salinity treatment. Values were similar to both NM treatments at high salinity and significantly higher than NM treatments at low salinity. At 40 days no significant differences were found in shoot and root concentrations of Na between mycorrhizal and P treatments within each salinity treatment, although high salinity itself increased Na concentrations, with the effect particularly marked in shoots (Table 3.8).

Potassium / Sodium ratio

K/Na ratios (based on concentrations) are shown in Table 3.9. At 20 days there were no differences in root K/Na ratio between mycorrhizal and P treatments at low salinity. As salinity increased, M-P and M+P plants showed higher root K/Na ratios than NM+P or NM-P roots. At the same harvest the K/Na ratio in shoots of M-P plants was higher than

NM-P plants at low salinity level, but no differences were found between mycorrhizal and P treatments at high salinity; all ratios were low. At 40 days, M+P and NM+P plants at low salinity both had higher root K/Na ratios than NM-P plants but were similar to NM+P. At high salinity M-P and M+P plants had higher K/Na ratio in roots than both NM+P and NM-P plants. At the same harvest M-P plants had significantly higher K/Na ratio in shoots than other mycorrhizal or P treatments at both salinity levels; K/Na ratios were lowest in NM-P plants. Mycorrhizal plants with P added and NM+P plants had higher shoot K/Na ratios compared to NM-P at low, but not at high salinity.

Concentrations of other plant nutrients (Mn, B, Mg, Ca, S, Al, Fe and Zn) were not consistently different between treatments at either salinity levels or harvests (Appendices 1 and 2).

In summary, although M-P plants in this experiment had higher concentration values of P and K compared with Experiment 3, these results confirm the results of Experiment 3 in showing that AM inoculated *T. subterraneum* had better nutrient uptake, growth and establishment than non-inoculated plants in saline conditions. AM inoculated seedlings had potential to take up more P than non-inoculated plants (with or without P) at the early stage of transplantation to saline conditions. The benefits of AM inoculation to increase K uptake were more obvious at high salinity and later in growth (40 days) when compared with non-inoculated plants (with or without P). The increased K/Na ratio (via increased K uptake) in AM inoculated plants at high salinity may be another mechanism to increase *T. subterraneum* salinity tolerance in saline conditions.

Table 3.7 Experiment 4. Shoot and root nutrient concentrations of *Trifolium subterraneum* with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal without P added (NM-P) and non-mycorrhizal with P (NM+P), at 20 days after transplanting to different salinity levels. Means of 3 replicates ± standard error

Treatments	Salinity	P	K	Na
		(mg /g)		
Shoot				
M-P	**Low salinity**	3.8±0.2	22.1±2.6	7.1±0.8
	High salinity	1.7±0.3	8.8±0.8	29.0±1.5
M+P	**Low salinity**	5.1±0.3	20.7±0.8	9.7±0.2
	High salinity	2.7±0.4	10.3±0.4	29.0±0.6
NM-P	**Low salinity**	0.5±0.0	7.7±1.6	5.9±0.9
	High salinity	0.7±0.0	10.4±0.5	32.7±2.9
NM+P	**Low salinity**	0.9±0.2	12.1±5.1	5.4±0.8
	High salinity	0.7±0.1	8.5±1.3	36.7±4.3
LSD (*P*<0.05)		0.8	6.9	5.9
Root				
M-P	**Low salinity**	4.7±0.4	25.7±1.8	16.0±1.6
	High salinity	2.5±0.4	22.0±1.0	14.7±1.4
M+P	**Low salinity**	6.2±0.3	26.3±1.7	19.1±1.5
	High salinity	4.0±0.6	32.0±3.8	20.7±0.8
NM-P	**Low salinity**	0.9±0.1	8.2±0.6	5.4±0.0
	High salinity	1.2±0.4	6.4±1.5	15.3±1.2
NM+P	**Low salinity**	1.2±0.1	19.0±5.5	9.2±0.7
	High salinity	1.0±0.0	5.3±0.9	13.4±2.6
LSD (*P*<0.05)		1.1	7.4	4.5

Table 3.8 Experiment 4. Shoot and root nutrient concentrations of *Trifolium subterraneum* with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal without P added (NM-P) and non-mycorrhizal with P (NM+P), at 40 days after transplanting to different salinity levels. Means of 3 replicates ± standard error

Treatments	Salinity	P	K	Na
		(mg /g)		
Shoot				
M-P	**Low salinity**	2.3±0.1	27.3±1.8	4.4±0.1
	High salinity	2.1±0.2	16.8±4.7	20.2±9.9
M+P	**Low salinity**	4.3±0.3	28.7±0.9	6.3±0.2
	High salinity	2.5±0.3	12.5±1.6	34.3±4.9
NM-P	**Low salinity**	0.4±0.0	6.0±0.6	4.9±1.1
	High salinity	0.7±0.2	5.4±1.9	23.4±6.2
NM+P	**Low salinity**	1.1±0.5	22.0±4.5	5.2±0.6
	High salinity	0.7±0.1	7.8±2.2	31.7±7.1
LSD (*P*<0.05)		0.8	8.7	14.2
Root				
M-P	**Low salinity**	3.1±0.2	26.0±1.5	12.5±1.7
	High salinity	2.7±0.3	30.3±1.8	17.4±0.7
M+P	**Low salinity**	4.5±0.3	30.3±1.7	14.3±0.8
	High salinity	3.0±0.6	35.0±1.5	20.7±1.8
NM-P	**Low salinity**	0.9±0.1	14.8±2.6	8.4±0.1
	High salinity	1.3±0.3	11.8±2.5	16.2±0.1
NM+P	**Low salinity**	2.3±0.6	31.0±2.0	13.5±1.7
	High salinity	1.2±0.0	7.7±1.8	15.4±3.9
LSD (*P*<0.05)		1.0	6.6	6.0

Table 3.9 Experiment 4. K/Na ratio in shoots and roots of *Trifolium subterraneum* with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal without P added (NM-P) and non-mycorrhizal with P (NM+P), at 40 days after transplanting to different salinity levels. Means of 3 replicates ± standard error

Treatments	Salinity	K/Na	
		Root	Shoot
20 days			
M-P	**Low salinity**	1.6±0.2	3.3±0.7
	High salinity	1.5±0.1	0.3±0.0
M+P	**Low salinity**	1.4±0.1	2.1±0.1
	High salinity	1.5±0.1	0.4±0.0
NM-P	**Low salinity**	1.5±0.1	1.4±0.4
	High salinity	0.4±0.1	0.3±0.0
NM+P	**Low salinity**	2.0±0.5	2.6±1.4
	High salinity	0.4±0.1	0.2±0.1
LSD (*P*<0.05)		0.5	1.8
40 days			
M-P	**Low salinity**	2.2±0.3	6.3±0.5
	High salinity	1.7±0.0	1.6±0.5
M+P	**Low salinity**	2.7±1.0	4.5±0.2
	High salinity	1.7±0.2	0.4±0.1
NM-P	**Low salinity**	1.7±0.3	1.5±0.5
	High salinity	0.7±0.1	0.2±0.0
NM+P	**Low salinity**	2.5±0.2	4.2±0.5
	High salinity	0.5±0.1	0.2±0.0
LSD (*P*<0.05)		0.7	1.2

3.4 Discussion

Overall the results of Experiments 3 and 4 show that seedling growth and hence establishment was increased by pre-inoculation of *T. subterraneum* with *G. intraradices* in saline conditions, compared with non-inoculated conditions. Pre-inoculation increased mycorrhizal effectiveness and ensured that roots were well colonized under salt stress. There was no significant effect of salt on root colonization at early stages after transplanting (Figs 3.6 and 3.13). Initial mycorrhizal colonization depends on germination of spores or other fungal propagules in the soil, growth of hyphae through the soil and finally hyphal entry to the roots (Bowen, 1987). Previous studies in alfalfa and tomato showed that mycorrhizal colonization was negatively affected by increased soil salinity, possibly via the effects of salinity on initial colonization (Azcón & El-Atrash, 1997; Al-Karaki, 2000; Al-Karaki & Hammad, 2001; Al-Karaki *et al.*, 2001). In this investigation seedling pre-inoculation allowed the fungi to become established before salinity stress was imposed, and the percentage of mycorrhizal colonization was not significantly affected by salt level (Experiment 4). It is likely that pre-inoculation in the nursery would increase mycorrhizal colonization and consequently improve ability of seedlings to benefit from any mycorrhizal effects and potentially tolerate salinity stress after transplanting. Field testing would be an essential prerequisite to large-scale application.

The results of Experiments 3 and 4 show that AM inoculated plants had higher growth and P concentration than non-inoculated plants. These results are in agreement with previous work showing that plant growth in saline soils is increased by AM inoculation, an effect which is at least partly related to enhanced P concentrations in shoots and roots (Hirrel & Gerdemann, 1980; Ojala *et al.*, 1983; Poss *et al.*, 1985). Mycorrhizal pre-inoculated plants in Experiments 3 and 4 had significantly higher P

concentrations in shoots and roots than non-inoculated plants at early stages after transplanting to low or high salinity levels (Tables 3.3 and 3.7). Although in Experiment 2, M plants had 3.3 times higher shoot P concentration than NM plants at 24 days in low salinity without transplanting, AM inoculated plants in Experiment 3 had 5.4 times higher shoot P concentration than non-inoculated plants at 10 days after transplanting (25 days old). Perhaps after transplanting, nutrient uptake via roots of inoculated and non-inoculated plants were depressed and the roots were not taking up P at early stage of transplanting, but inoculation had the potential to facilitate absorption of a massive amount of new P via hyphae soon after transplanting (Fig. 3.10). Results of Experiment 4 showed that despite a high level of added P (60 mg per kg soil) in NM+P treatment, NM plants could not take up the additional P from the soil, but AM inoculated plants could absorb the additional P at both low and high salinities. This suggests that AM inoculation is more efficient than P application in increasing plant P concentrations at early growth stages at low and high salinity (Table 3.7). Calculation of MPR in Experiment 3 and comparison of P concentration in M and NM plants in Tables 3.3 and 3.7 showed the efficiency of mycorrhizal fungi to increase P uptake at early stages after transplanting. Although a previous study showed that AM inoculation did not increase P uptake at an early stage in *T. subterraneum* (Abbott & Robson, 1978), results of this study are consistent with some other previous observations that mycorrhizal colonization increased P inflow at an early stage of plant growth, compared to non-mycorrhizal plants, and declined with increasing plant age, in *T. subterraneum* (Jakobsen *et al.*, 1992) and *Allium cepa* (Smith *et al.*, 1986). The critical role of P in early crop plant growth has been demonstrated previously (Grant *et al.*, 2001).

Despite little consistent effect of AM inoculation in increasing K concentrations in roots in Experiment 3 (Table 3.3), Experiment 4 showed that inoculation increased K concentration in roots and shoots at both salinity levels at 40 days, and there was a salinity-induced reduction in K concentration in shoots (Tables 3.7 and 3.8). Some studies have reported that AM fungal colonization had no significant effects on plant K contents or concentrations (Poss *et al.*, 1985; Rozema *et al.*, 1986; Pfeiffer & Bloss, 1988; Al-Karaki, 2000; Cantrell & Linderman, 2001; Mohammad *et al.*, 2003), but results of this study indicate that root K concentrations increased even at high salinity. Application of P did not change K concentrations in non-inoculated plants at high salinity, but AM inoculation increased K concentration at both salinity levels (particularly at 40 days) in Experiment 4 (Tables 3.7 and 3.8). Increased K concentration in NM plants by P application at low salinity at 40 days, suggests that increased K concentration in AM plants could be an indirect effect, due to better P nutrition in AM plants. So further investigations are needed to determine the direct effects of AM fungi in K uptake at saline conditions. It is possible that increased K concentrations (directly or indirectly due to AM fungi) may be another salinity tolerance mechanism in AM inoculated *T. subterraneum*.

The results of Experiment 3 showed that shoot Na concentrations increased with increasing salinity and that, overall, concentration in shoots of AM inoculated plants were higher than those in non-inoculated plants at 10 and 20 days. This difference decreased with time. At 30 days shoots and roots of AM inoculated plants grown at 12 dS/m (the highest salinity applied) had a lower Na concentration than NM plants (Table 3.5). In Experiment 4 roots but not shoots of M-P and M+P plants had consistently higher Na concentrations than NM-P or NM+P plants at low salinity levels at 20 days, but at 40 days no significant differences were found in shoots and

roots at either salinity level. Previous reports about the effects of AM fungi on Na uptake have been inconsistent. Some researchers found that following addition of NaCl to the soil concentrations of Na in shoot tissue of non-halophytic plants were higher in mycorrhizal plants compared to non-mycorrhizal plants (Allen & Cunningham, 1983; Pfeiffer & Bloss, 1988; Cantrell & Linderman, 2001). In contrast, mycorrhizal plants of the halophytic *Aster tripolium* had less Na in shoots than non-mycorrhizal plants when grown in saline conditions (Rozema *et al.*, 1986). Mycorrhizal inoculation also decreased shoot Na concentration in tomato and barley plants grown in a soil with a high level of salinity, but had no effect when plants were grown in low salinity (Al-Karaki, 2000; Mohammad *et al.*, 2003). In Experiments 3 and 4 AM inoculated plants accumulated more Na in their roots than shoots at low salinity, but when salinity increased shoots of AM inoculated plants had higher Na concentrations than roots (Tables 3.3-3.5, 3.7 and 3.8). Cantrell and Linderman (2001), suggested root Na compartmentalization as salinity tolerance mechanism in AM onion plants, such that AM plants compartmentalize Na in intraradical hyphae or root cell vacuoles and hence do not translocate it to the shoots. However, the results of this study do not confirm root Na compartmentalization as a salinity tolerance mechanism in AM *T. subterraneum.*

Although the results of Experiment 3 showed a small effect of AM inoculation on K/Na ratio only at high salinity at 10 days, results of Experiment 4 showed a larger effect particularly in shoots at 40 days. Experiment 4 showed that K/Na ratio was higher in shoots of AM inoculated plants without P addition than in non-inoculated plants without P addition (apart from high salinity at 20 days) at both salinity levels and harvests (Table 3.9). The addition of P alone or in combination with AM inoculation had no significant effects on shoot K/Na ratio at high salinity at 40 days.

These results indicate that increased K concentration was more effective than decreases in Na concentration in increasing K/Na ratio in AM plants. In contrast, decreased Na concentration with no changes in K concentrations at high salinity has been reported as a salinity tolerance mechanism in AM tomato and barley (Al-Karaki, 2000; Mohammad *et al.*, 2003). Calculation of MKR showed that the efficiency of AM fungi in K uptake increased with time. Increased MKR and K/Na ratio in AM plants with time, suggest that again increased K uptake could be a mechanism to increase plant salinity tolerance in *T. subterraneum* that operates relatively late in growth.

In summary, the results indicate that pre-inoculation with AM fungi had a significant role in promoting seedling growth and establishment of *Trifolium subterraneum* in saline conditions. Improved nutrient uptake (e.g. P) is probably the major mechanism involved in increasing seedling salinity tolerance and this agrees with previous work (Marschner & Dell, 1994; Al-Karaki & Al-Raddad, 1997; Al-Karaki, 2000). Increased salinity tolerance in AM *T. subterraneum* plants appears to be based on additional effects (partly effects on K uptake) other than increased P uptake, which has been reported previously (Poss *et al.*, 1985; Ruiz-Lozano *et al.*, 1996; Azcón & El-Atrash, 1997; Cantrell & Linderman, 2001). In this study, increased P uptake in AM plants may have played the main role in tolerance of salt stress at an early stages due to efficient operation of the AM pathway and increased K uptake at the later stages after transplanting (Figs 3.10 and 3.15 respectively). AM inoculation was more efficient than P application to increase P at early and K (particularly at high salinity) at the late stage of *T. subterraneum* transplantation in saline conditions. These results may be of practical importance as they highlight the potential of using AM pre-inoculated seedlings of mycorrhizal responsive plants to revegetate saline lands.

Results of this chapter showed that increased salinity tolerance and plant establishment in AM plants are mainly due to increased P uptake. Investigation of salinity tolerance in a non-responsive plant might show other mechanisms of AM on plant salinity tolerance, when there are no effects of AM on P nutrition. Chapter 4 reports investigation of AM effects on establishment and nutrient uptake in a non-responsive plant.

CHAPTER 4 - EFFECTS OF MYCORRHIZAL FUNGI ON NUTRIENT UPTAKE AND ESTABLISHMENT OF A NON-RESPONSIVE MYCORRHIZAL PLANT IN SALINE CONDITIONS

The effects of mycorrhizal fungi on establishment and nutrient uptake in a responsive mycorrhizal plant under saline conditions were studied in the last chapter. Results of Chapter 3 showed that increased salinity tolerance and plant establishment in AM plants are mainly due to increased P uptake. It was suggested that the effects of plant salinity tolerance in AM plants could be an indirect effect, due to better P nutrition in AM plants. To study the influences of AM fungi on nutrient uptake and plant establishment in saline conditions when there is no effect of AM on P nutrition, non-responsive mycorrhizal plant species were selected to provide M and NM matched plants which may distinguish nutritional (P) and growth effects from other possible effects on salinity tolerance. Two experiments are described in this chapter. The first was done to select an appropriate plant for this study by comparing the growth and P uptake responses of *Festuca arundinacea* and *Lolium multiflorum* to *Glomus intraradices* in Ferries McDonald soil, and the second was done to investigate the effects of *G. intraradices* on nutrient uptake and seedling establishment in the selected plant (*F. arundinacea*) at different salinity levels.

4.1 Introduction

Even though it has been shown that AM fungi increase plant salinity tolerance in some plant species, neither mechanisms underlying benefits from AM association nor the factors responsible for different degrees of the benefits in different plants are well defined and understood. Increased mineral nutrient uptake as a major salinity tolerance factor in AM plants under saline conditions has been reported in AM responsive plants

previously (Table 1.3) and confirmed in the work described in Chapter 3. Among mineral nutrients, improved P uptake in AM plants was suggested as the main nutrient that increases plant salinity tolerance in some responsive AM plants (Chapter 1.3.2.1). In saline conditions, plant growth may be restricted by the lack of P (Hirrel & Gerdemann, 1980; Pond *et al.*, 1984; Poss *et al.*, 1985), even though there may be sufficient quantities of all other essential nutrients, so increased P uptake by AM fungi may indirectly increase the efficiency of uptake of other nutrients.

Variation in mycorrhizal responsiveness among species is an important issue in revegetation programs. There is little evidence related to the effects of mycorrhizal fungi on plant nutrient uptake and establishment in non-responsive plants such as grasses. The symbiotic associations between plants and AM fungi in grassland associations may be of great importance in grass establishment in saline conditions, but information on AM susceptibility, host dependence, and host responsiveness to the fungi is scarce. This chapter aimed to study the effects of AM fungi in plant nutrient uptake and establishment of a non-responsive AM plant in saline conditions, when there is no effect of AM on P nutrition. This approach is capable of revealing direct effects of AM fungi on uptake of nutrients other than P in AM plants in saline conditions, and also may indicate the potential of AM-inoculated grasses in revegetating of saline lands.

4.2 Materials and Methods

4.2.1 Experiment 1. AM responsiveness of *Festuca arundinacea* and *Lolium multiflorum*

The aim of this experiment was to select an appropriate non-responsive AM plant for Experiment 2 by comparing the growth and P uptake responses of *F. arundinacea* and

L. multiflorum to *G. intraradices* in Ferries McDonald soil. *F. arundinacea* and *L. multiflorum* were selected for this experiment because of their susceptibility to colonization by AM fungi (Harley & Harley, 1987; Wilson & Hartnett, 1998), and the likelihood that they may be unresponsive to AM fungi. *F. arundinacea* and *L. multiflorum* seeds were sterilized and germinated on moist filter paper at 23°C in a germinator (Chapter 2.4 and 2.5). Two pre-germinated seeds were sown in autoclaved Ferries McDonald soil (1400g soil per pot) inoculated (M) or not (NM) with *G. intraradices*. The inoculum was dried pot culture material, consisting of soil/sand mix plus colonized root fragments, spores and external hyphae. This was mixed with Ferries McDonald soil in the ratio 10% inoculum to 90% soil. Non-inoculated pots received an additional 10% Ferries McDonald soil. R.O. water was added to keep soil at 80% field capacity. Plants were thinned to one seedling per pot after one week. Plants were grown in a glasshouse in February 2002 (late summer), and harvested 40 and 60 days after planting. Plant shoot and root fresh weights were recorded and shoot and root dry matter were determined after drying at 80 °C for 48 hours. Sub-samples of roots were washed carefully, and cut to 1 cm segments, then stained by trypan blue and mycorrhizal colonization was determined (Chapters 2.9 and 2.10). Extraradical hyphae were not measured. The experiment had a randomised complete block design with 4 treatments (2 plants and 2 mycorrhiza). Four replicate pots were harvested at each time. Probabilities of significance among treatments and interactions and LSDs (P<0.05) were used to compare means within and among treatments.

4.2.2 Experiment 2. Effects of *Glomus intraradices* on *Festuca arundinacea* nutrient uptake and seedling establishment at different salinity levels

Seeds were surface sterilized (Chapter 2.4). Single pre-germinated seeds were sown in plastic bags containing 60g autoclaved Ferries McDonald soil, inoculated or not with

G. intraradices (Chapter 2.6). The inoculum was dried pot culture material, consisting of soil/sand mix plus colonized root fragments, spores and external hyphae. This was mixed with Ferries McDonald soil in the ratio 10% inoculum to 90% soil (6g inoculum in each bag). NM pots received an additional 6g Ferries McDonald soil. To use the same nutrient conditions and provide the same bacterial population in M and NM seedlings, the same amount of mycorrhizal inoculum used to inoculate seedlings was stirred in R.O. water and filtered through Whatman no: 1 (11μm) filter paper and 5 ml of filtrate were added to each NM seedling during the pre-transplant stage. Three ml Long Ashton nutrient solution (Hewitt, 1966) without P were added to each seedling. Seedlings were grown in a glasshouse with natural light in November 2003 (early summer). R.O. water was added to maintain soil moisture at 80% field capacity. After 3 weeks (20% root length colonized in M plants) inoculated and non-inoculated seedlings of the same size were transplanted to different levels of salinity.

Three levels of soil salinity (2.2, 7.5 and 12 dS/m) were produced after adding 0, 1 and 2g NaCl per kg of the soil as solution. Soils were incubated for one week, and then pots were filled with 1400g of the soil. One pre-inoculated or non-inoculated seedling was transplanted into the pots with different salinity levels. There were four replicate pots per treatment and harvest. The plants were grown in a glasshouse with natural light. Plants were watered thrice weekly with R.O. water to maintain soil moisture at 80% field capacity. No additional nutrients were added to the pots during the growth period. Plants were harvested 20 and 40 days after transplanting. They were washed thoroughly and leaf and tiller number, shoot and root dry weights were determined. Nutrient concentrations were determined by ICP. Sub-samples of plant roots were washed carefully and stained by trypan blue, and mycorrhizal colonization was evaluated in each sample (Chapter 2.9 and 2.10). The experiment had a randomised complete block

design with 6 treatments (2 mycorrhiza and 3 salinity levels). Probabilities of significance among treatments and interactions and LSDs ($P<0.05$) were used to compare means within and among treatments.

4.3 Results

4.3.1 Experiment 1. AM responsiveness of *Festuca arundinacea* and *Lolium multiflorum*

Total dry weights of M or NM *F. arundinacea* and *L. multiflorum* and percentage AM colonization in M plants are shown in Fig 4.1. In *F. arundinacea* AM colonization decreased from 41% at 40 days to 36% at 60 days. In *L. multiflorum* colonization was 21% at 40 days and very similar at 60 days. *F. arundinacea* AM inoculated and NM plants had a similar total dry weight at 40 and 60 days after planting. However AM inoculated *L. multiflorum* plants had slightly lower total dry weights than NM at the same harvests. The effect of AM inoculation on shoot / root ratio was significant in *F. arundinacea* only at 40 days, but no differences were found in *L. multiflorum* at either harvest (Table 4.1). Analysis of shoot P concentrations showed that M and NM *F. arundinacea* had the same values at both harvests, but M plants in *L. multiflorum* had significantly higher shoot P concentration than NM plants at the same harvests (Fig. 4.2). These results indicate that *F. arundinacea* does not display biomass or shoot P concentration responses to symbiosis with *G. intraradices* in Ferries McDonald soil. *L. multiflorum* showed a small growth depression in AM plants, accompanied by an increase in P concentration. *F arundinacea* was therefore chosen as an appropriate non-responsive plant for experiment 2.

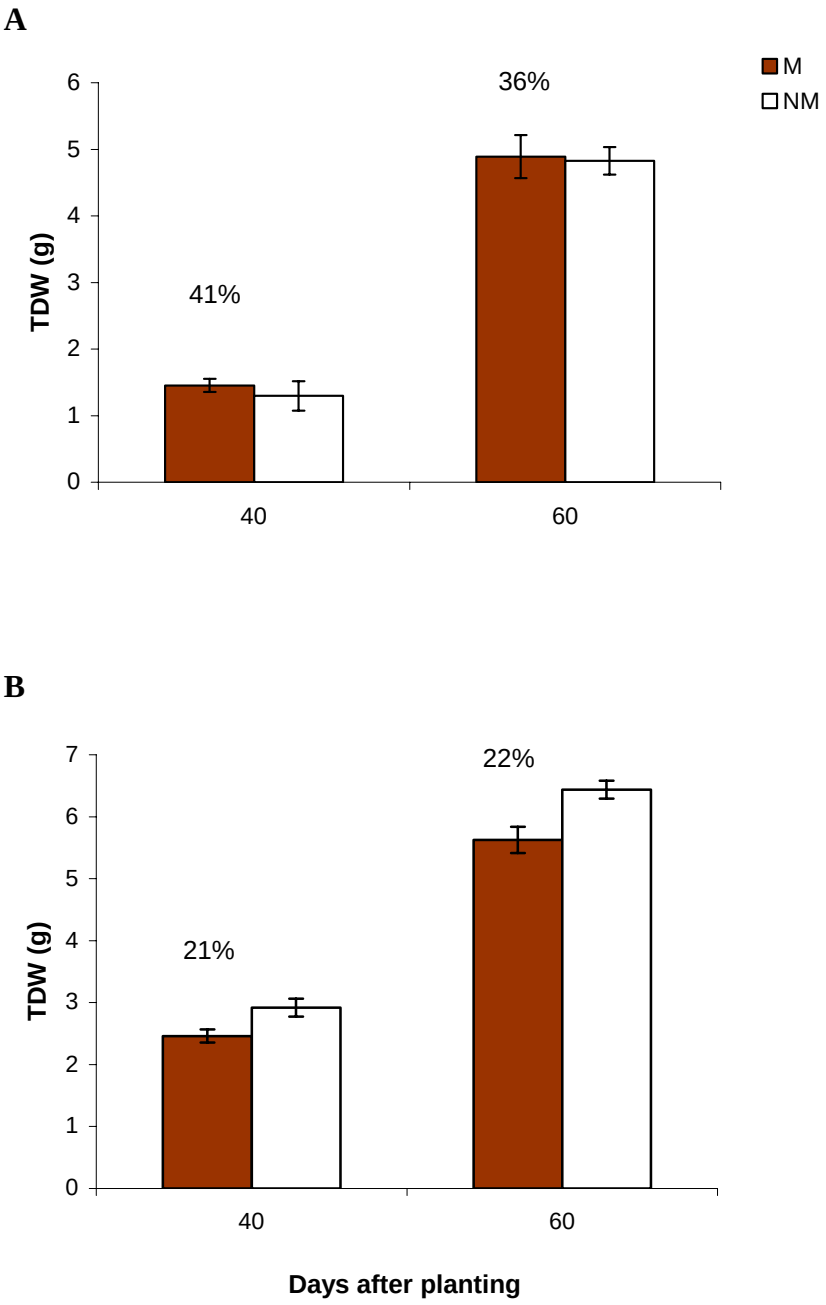


Fig 4.1 Experiment 1. Total dry weights in AM inoculated and non-inoculated *Festuca arundinacea* (A) and *Lolium multiflorum* (B) after 40 and 60 days. Numbers at top of the M bars show percentages AM colonization. Vertical bars represent standard error of the means, n=4.

Table 4.1 Experiment 1. Shoot/root ratio in mycorrhizal (M) and non-mycorrhizal (NM) *Festuca arundinacea* and *Lolium multiflorum* at 40 and 60 days after planting. Means of 4 replicates ± standard error

Treatments	*F. arundinacea*	*L. multiflorum*
40 days		
M	2.7±0.1	1.9±0.1
NM	3.1±0.2	2.0±0.3
AM inoculation	**	ns
60 days		
M	1.6±0.2	1.7±0.1
NM	1.6±0.1	1.3±0.1
AM inoculation	ns	ns

** significant at the 0.01 probability levels, respectively
ns not significant at p=0.05

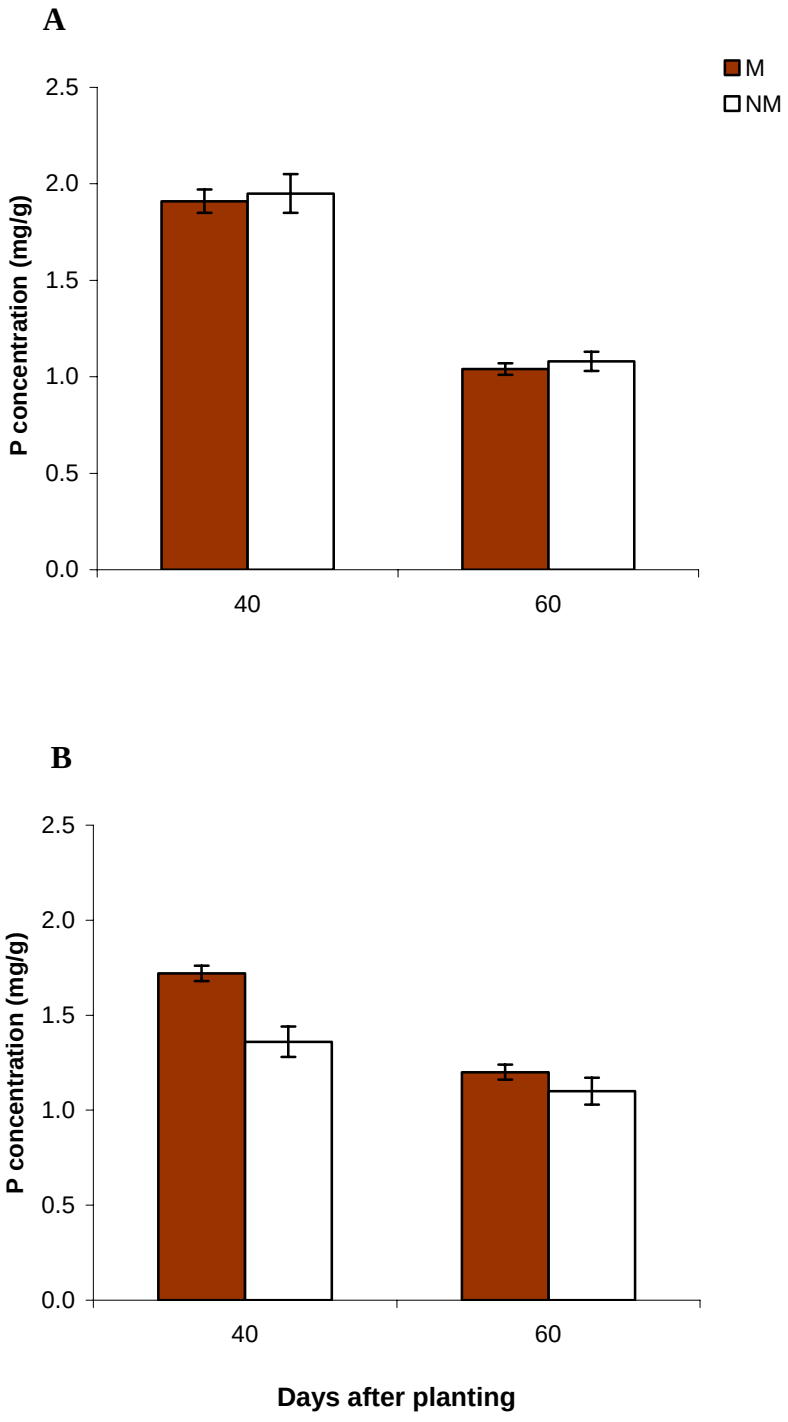


Fig 4.2 Experiment 1. Shoot P concentrations in AM inoculated and non-inoculated *Festuca arundinacea* (A) and *Lolium multiflorum* (B) after 40 and 60 days. Vertical bars represent standard error of the means, n=4.

4.3.2 Experiment 2. Effects of *Glomus intraradices* on *Festuca arundinacea* nutrient uptake and seedling establishment at different salinity levels

No AM colonization was found in NM treatments (results not shown). Salinity and time decreased the percent of root length colonized. At 20 days after transplanting colonization decreased from 43% in 2.2 dS/m to 5% in 12 dS/m (Fig. 4.3). At 40 days 26% colonization was found at 2.2 dS/m and 5% at 12 dS/m.

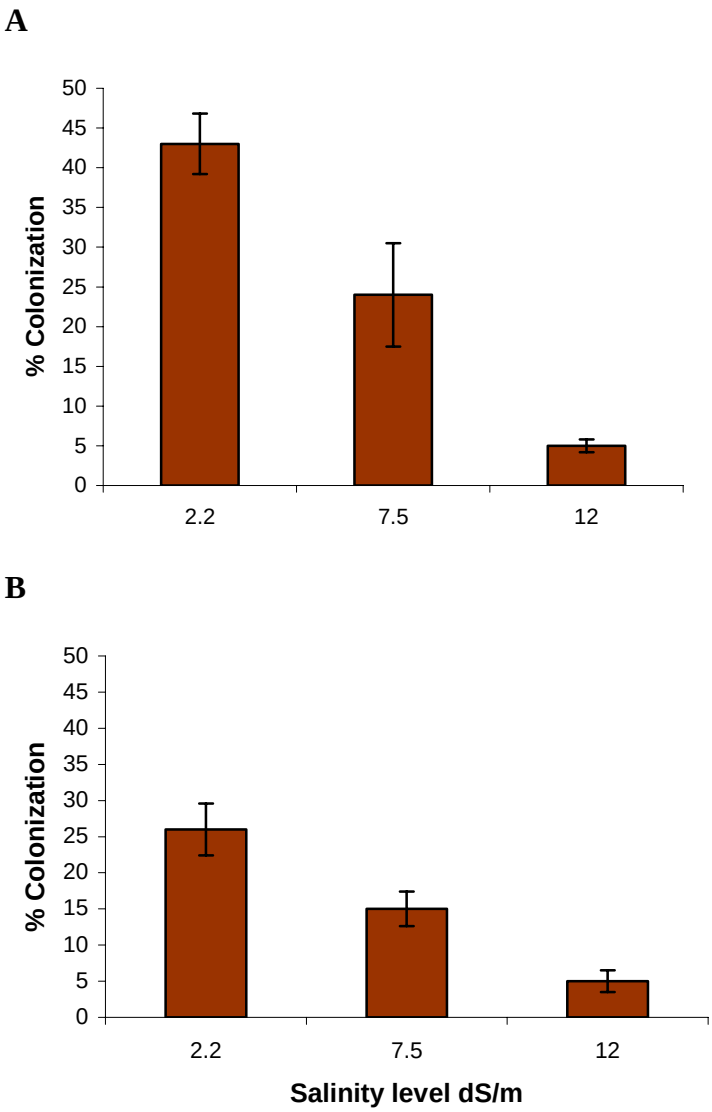


Fig 4.3 Experiment 2. Colonization in roots of *Festuca arundinacea* grown in different levels of salinity at 20 (A) and 40 (B) days after transplanting. Vertical bars represent standard error of the means, n=4.

There were no significant differences in plant height between M and NM plants, but height was significantly decreased by increased salinity at either harvests. The tiller and leaf number in M plants were higher than NM plants particularly at low salinity levels, again at both harvests (results not shown).

Increasing salinity significantly decreased plant growth of both M and NM plants at both harvests (Fig 4.4). At 20 days after transplanting, M plants grown at 2.2 dS/m had higher total dry weight than NM plants, but no significant differences were found at 7.5 dS/m and 12 dS/m. At 40 days M and NM plants had the same total dry weight at all salinity levels. Although Fig 4.4 shows some differences between M and NM plant at 12 dS/m the differences were not significant. Calculation of mycorrhizal growth responses (MGR) in terms of total dry weight at different salinity levels and different harvests showed that MGR decreased with increased salinity at both harvests, and in AM plants growing at 20 days it was higher than 40 days at 2.2 dS/m salinity, but no differences were found at higher salinity levels. AM plants had lower shoot / root ratio at 7.5 and 12 dS/m at 20 days, but no significant differences were found at 40 days (Table 4.2).

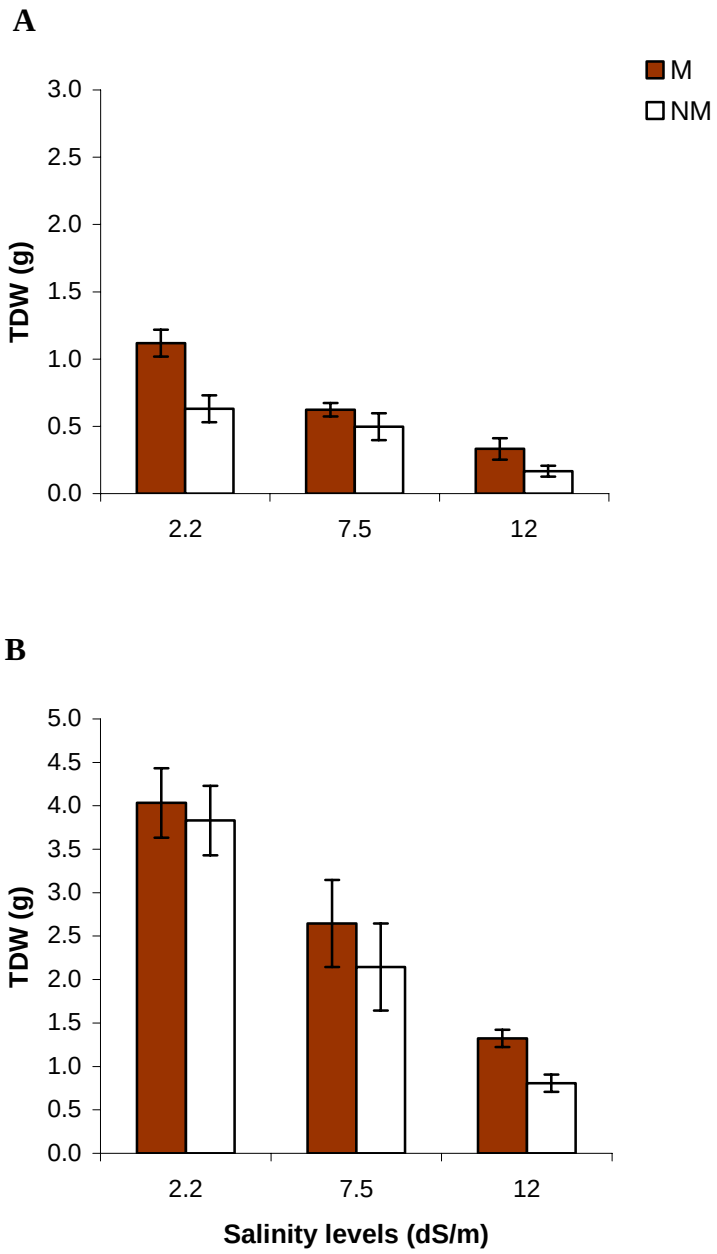


Fig 4.4 Experiment 2. Total dry weights of mycorrhizal and non-mycorrhizal *Festuca arundinacea* at 20 (A) and 40 (B) days after transplanting in different salinity levels. Vertical bars represent standard errors of the means, n=4.

Table 4.2 Experiment 2. Shoot/root ratio in mycorrhizal (M) and non-mycorrhizal (NM) *Festuca arundinacea* at 20 and 40 days after transplanting to different salinity levels. Means of 4 replicates ± standard error

Treatments	Salinity (dS/m)	Shoot/root
20 days		
M	**2.2**	2.6±0.3
	7.5	2.1±0.2
	12	2.0±0.2
NM	**2.2**	3.0±0.5
	7.5	3.7±0.4
	12	3.8±0.8
LSD (*P*<0.05)		1.4
40 days		
M	**2.2**	1.5±0.1
	7.5	2.0±0.2
	12	3.9±0.8
NM	**2.2**	1.8±0.1
	7.5	3.0±0.5
	12	5.5±2.1
LSD (*P*<0.05)		3.0

Shoot and root nutrient concentrations of M and NM plants at 20 and 40 days after transplanting are shown in Tables 4.3 and 4.4. Overall the results indicate that pre-inoculation with *G. intraradices* increased plant nutrient concentrations (particularly in roots) at an early stage after transplanting. At 20 days root Fe, Mg, Na, K, P, Al, and Zn concentrations of M plants were higher than NM plants particularly at the low salinity level. Smaller effects of AM inoculation were found in shoots at the same harvest. The

influences of AM on plant nutrient concentrations decreased with time. At 40 days M plants had higher root Cu, Mg and Al than NM plants. Increased salinity significantly increased Na concentration in roots and shoots of M and NM plants at both harvests, but K concentration significantly decreased in shoot and root of M and NM plants at 20 days but not in 40 days.

Effects of AM on P concentration were significant in roots at 20 days and no significant effects were found in shoot at both harvests. Phosphorus contents of the shoots and roots were used to calculate MPR (Fig. 4.5), using Eqn 3 (Chapter 3.2.3). As salinity increased MPR in shoots and roots decreased at both harvests. MPRs in shoot were significantly higher at 20 days than 40 days at 2.2 and 7.5 dS/m, but in roots differences were significant only at 2.2 dS/m. MKRs (Chapter 3.2.3) in shoots and roots are shown in Fig. 4.6. Increased salinity decreased MKR in shoot and roots at both harvests. In shoots MKR was significantly higher at 20 days than 40 days only at 2.2 dS/m, but in roots it was significant at all salinity levels.

K/Na ratios (based on concentrations) are shown in Table 4.5. As salinity increased K/Na ratios decreased in shoots and roots at both harvests. At 20 days K/Na in roots was higher in M plants than NM plants only at 2.2 dS/m. At the same harvest NM plants had higher shoot K/Na than M plants at 2.2 dS/m. No significant differences were found at 40 days.

Table 4.3 Experiment 2. Shoot and root nutrient concentrations of mycorrhizal (M) and non-mycorrhizal (NM) *Festuca arundinacea* at 20 days after transplanting to different salinity levels. Means of 4 replicates ± standard error

Treatments	Salinity level dS/m)	Fe	Mg	Na	K	P	Al	Cu	Zn
		(mg /g)						(µg/g)	
Shoot									
M	**2.2**	0.3±0.1	4.9±0.2	2.7±0.5	37.5±2.2	3.2±0.1	0.3±0.1	26.5±1.0	39.0±1.4
	7.5	0.2±0.0	4.5±0.2	3.5±0.8	29.8±3.1	2.8±0.2	0.2±0.1	22.8±1.3	54.7±4.8
	12	0.1±0.0	4.2±0.2	8.4±2.6	25.3±2.4	2.6±0.1	0.1±0.0	26.0±2.6	62.6±2.1
NM	**2.2**	0.1±0.0	4.0±0.1	1.3±0.1	33.5±1.0	2.4±0.3	0.1±0.0	22.9±1.6	46.4±1.8
	7.5	0.1±0.0	4.3±0.4	5.5±1.0	25.7±2.4	2.8±0.1	0.1±0.0	26.0±1.3	62.5±1.6
	12	0.1±0.0	4.7±0.3	10.1±1.6	25.0±2.9	2.6±0.2	0.1±0.0	34.4±2.2	80.3±4.0
AM inoculation		*	ns	ns	ns	ns	*	ns	***
Salinity		ns	ns	***	**	ns	ns	**	***
Root									
M	**2.2**	1.0±0.1	3.1±0.4	5.6±0.8	22.1±1.4	2.2±0.3	2.1±0.4	53.2±1.8	39.1±1.5
	7.5	1.2±0.2	4.1±0.3	12.1±0.8	17.5±1.5	1.9±0.2	1.9±0.4	49.9±2.7	37.6±2.9
	12	1.7±0.2	3.0±0.3	16.0±0.8	12.0±1.3	1.4±0.1	2.5±0.4	62.9±7.9	35.0±0.7
NM	**2.2**	0.4±0.1	3.1±0.4	5.1±0.4	14.6±1.6	1.3±0.1	0.6±0.1	36.1±1.3	20.0±0.4
	7.5	0.3±0.0	2.5±0.2	10.6±1.2	11.2±1.9	1.4±0.1	0.4±0.1	40.6±1.4	21.2±1.5
	12	0.2±0.0	2.6±0.3	13.1±0.6	6.2±0.8	1.2±0.1	0.4±0.0	66.2±8.0	27.0±2.8
AM inoculation		***	*	*	***	**	***	ns	***
Salinity		ns	ns	***	***	ns	ns	***	ns

*, **, *** significant at the 0.05, 0.01, 0.001 probability levels, respectively. ns not significant at p = 0.05

Table 4.4 Experiment 2. Shoot and root nutrient concentrations of mycorrhizal (M) and non-mycorrhizal (NM) *Festuca arundinacea* at 40 days after transplanting to different salinity levels. Means of 4 replicates ± standard error

Treatments	Salinity level (dS/m)	Fe	Mg	Na	K	P	Al	Cu	Zn
		(mg /g)						(µg/g)	
Shoot									
M	**2.2**	0.1±0.0	4.6±0.3	1.7±0.5	26.3±2.1	2.2±0.0	0.1±0.0	16.4±1.1	29.6±1.5
	7.5	0.2±0.0	5.1±0.7	2.3±0.5	26.8±2.2	2.6±0.1	0.3±0.0	19.8±2.2	54.5±5.8
	12	0.2±0.0	4.1±0.1	6.4±0.5	30.5±2.0	2.9±0.1	0.2±0.0	21.8±0.9	66.6±4.6
NM	**2.2**	0.3±0.1	4.2±0.3	1.9±0.1	26.3±0.6	2.2±0.2	0.2±0.0	16.7±0.5	35.8±2.5
	7.5	0.1±0.0	3.6±0.3	3.7±1.1	27.0±1.5	2.5±0.2	0.3±0.1	19.7±1.9	49.1±6.4
	12	0.2±0.0	3.9±0.3	5.5±1.1	30.5±2.8	2.9±0.2	0.2±0.0	23.6±1.2	63.9±2.5
AM inoculation		ns	ns	ns	ns	ns	ns	ns	ns
Salinity		ns	ns	***	ns	**	ns	**	***
Root									
M	**2.2**	0.4±0.1	2.6±0.2	6.1±2.8	14.4±0.3	1.3±0.1	1.0±0.1	27.9±0.7	17.7±0.8
	7.5	0.5±0.1	3.4±0.3	8.8±2.0	16.1±1.5	1.5±0.1	0.8±0.2	33.4±2.4	21.2±1.3
	12	0.4±0.0	3.1±0.4	13.9±1.0	12.3±1.9	1.6±0.1	0.5±0.0	44.2±8.6	34.0±9.7
NM	**2.2**	0.5±0.0	1.9±0.2	3.4±0.5	13.9±1.5	1.3±0.1	0.8±0.0	26.0±2.1	15.0±1.3
	7.5	0.3±0.1	2.8±0.2	10.5±1.3	14.5±0.9	1.3±0.1	0.4±0.1	21.5±1.8	18.1±2.0
	12	0.3±0.1	2.9±0.3	11.0±0.8	11.8±2.0	1.4±0.2	0.5±0.1	23.1±2.5	23.2±3.0
AM inoculation		ns	*	ns	ns	ns	*	**	ns
Salinity		ns	*	***	ns	ns	**	ns	*

*, **, *** significant at the 0.05, 0.01, 0.001 probability levels, respectively. ns not significant at p = 0.05

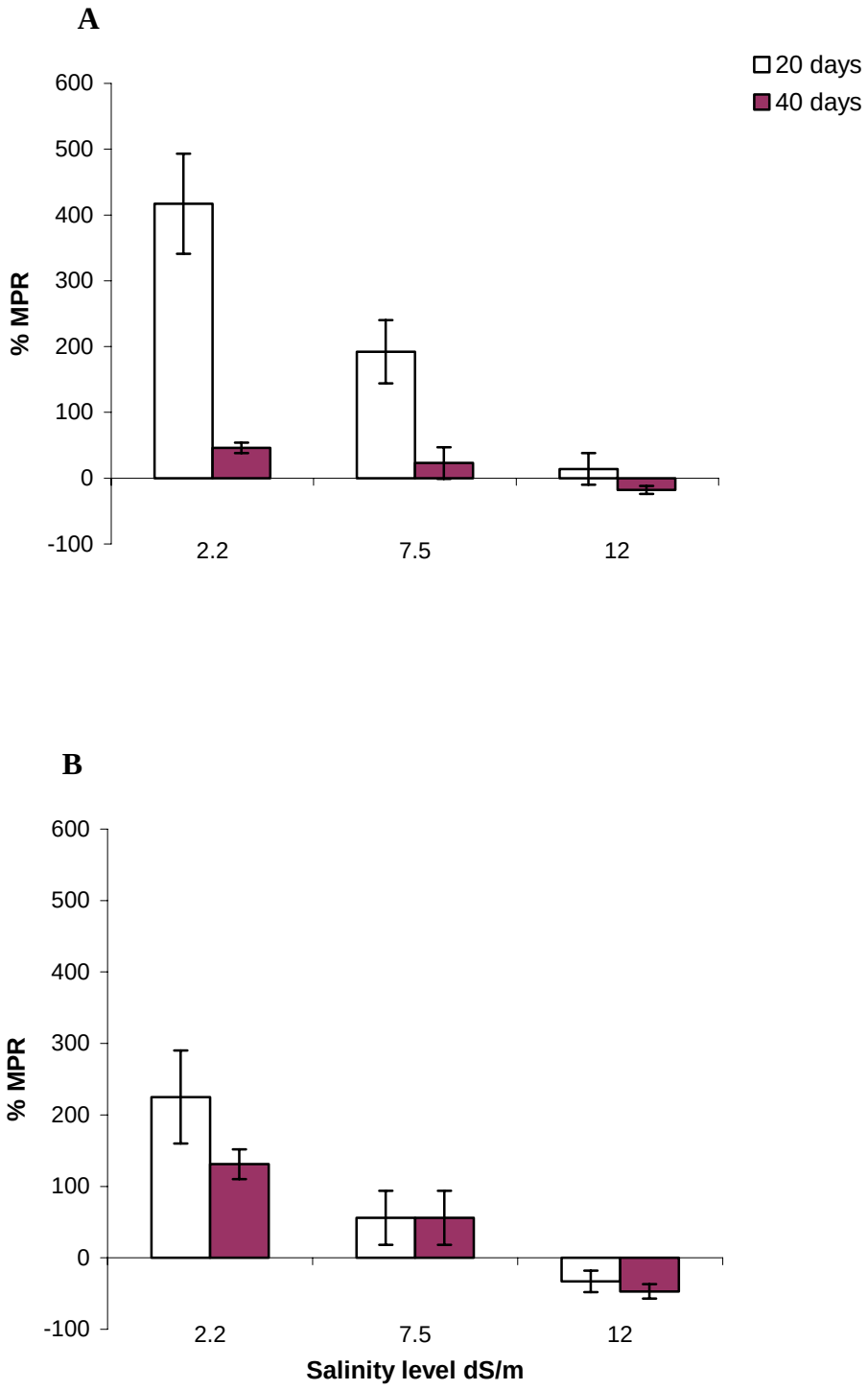


Fig. 4.5 Experiment 2. Mycorrhizal P response (MPR) of *Festuca arundinacea* shoots (A) and roots (B) at different salinity levels and different harvests. Vertical bars represent standard errors of the means, n=4.

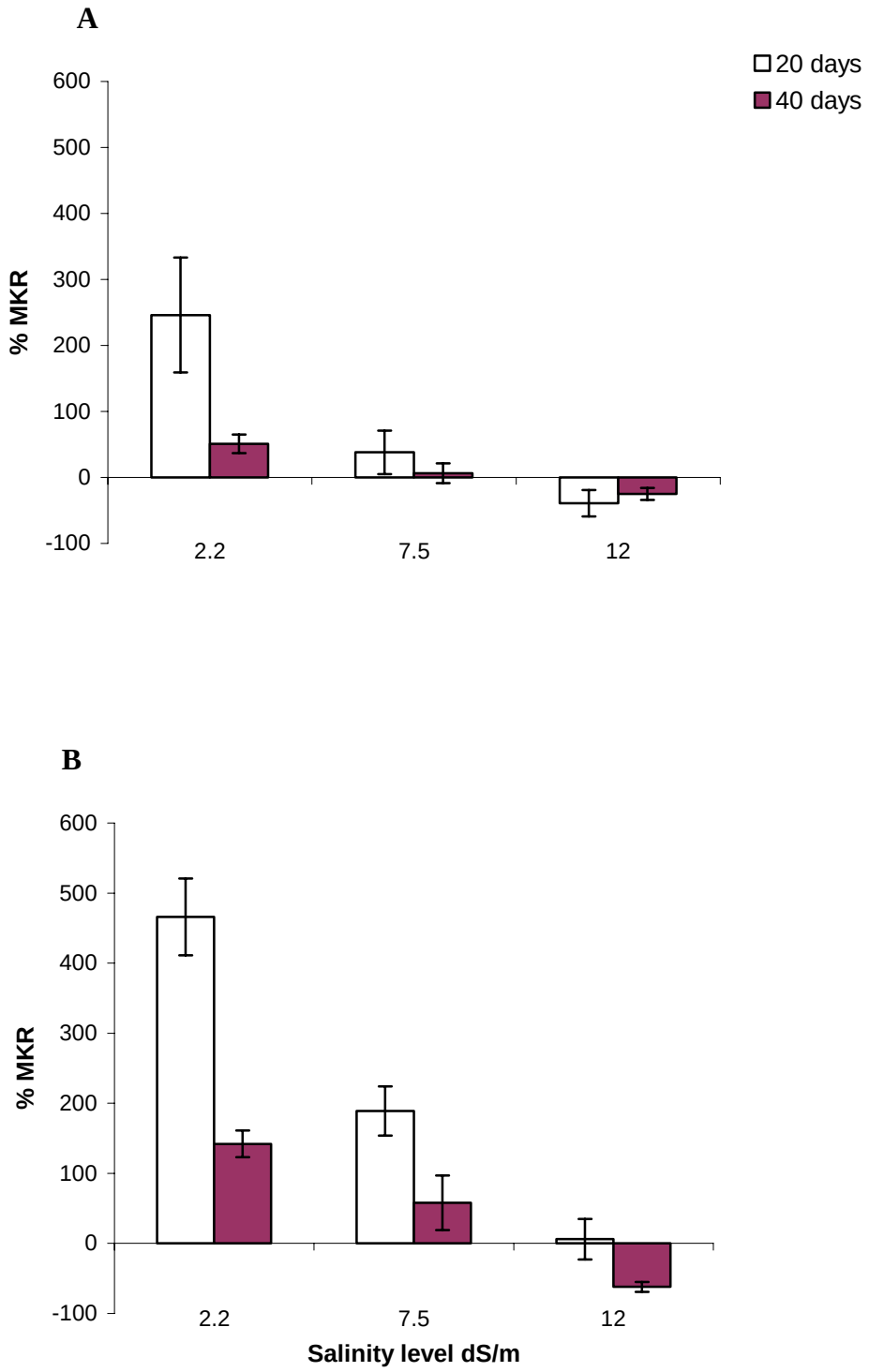


Fig. 4.6 Experiment 2. Mycorrhizal K response (MKR) of *Festuca arundinacea* shoots (A) and roots (B) at different salinity levels and different harvests. Vertical bars represent standard errors of the means, n=4.

Table 4.5 Experiment 2. K/Na ratios in shoots and roots of mycorrhizal (M) and non-mycorrhizal (NM) *Festuca arundinacea* at 20 and 40 days after transplanting to different salinity levels. Means of 4 replicates ± standard error

Treatments	Salinity (dS/m)	K/Na	
		Root	Shoot
20 days			
M	**2.2**	4.1±0.5	15.6±3.8
	7.5	1.5±0.2	9.3±1.4
	12	0.8±0.1	3.9±1.1
NM	**2.2**	2.8±0.1	26.6±2.4
	7.5	1.1±0.3	5.2±1.0
	12	0.5±0.1	2.8±0.7
LSD (*P*<0.05)		0.8	6.0
40 days			
M	**2.2**	3.7±1.1	24.1±8.8
	7.5	2.5±1.0	14.0±3.3
	12	0.9±0.1	4.9±0.6
NM	**2.2**	4.2±0.3	13.9±1.4
	7.5	1.4±0.1	10.3±3.5
	12	1.1±0.1	6.6±1.6
LSD (*P*<0.05)		1.9	12.6

4.4 Discussion

Results of Experiment 1 indicate that *Festuca arundinacea* and *Lolium multiflorum* had different responsiveness to *Glomus intraradices*. *F. arundinacea* did not show a responsiveness, but *L. multiflorum* showed a negative responsiveness to AM fungi. Under the same growth conditions AM *F. arundinacea* plants had higher colonization than *L. multiflorum* with no AM effects on total dry weight and shoot P concentration. Plant nutrient uptake and growth response to AM fungi depend on several inherent morphological and physiological and also environmental factors; different plant species showed different responses to different AM species (Khalil *et al.*, 1994; Saggin-Junior & Siqueira, 1995; Hetrick *et al.*, 1996; van der Heijden *et al.*, 1998), different soil available P (Siqueira & Saggin-Junior, 2001; Zhu *et al.*, 2001) and different environment (Brundrett, 1991; Janos, 1995). Cool-season grasses, *F. arundinacea* and *L. multiflorum*, have highly fibrous root systems and are weakly or non-dependent on mycorrhizal symbiosis (Wilson *et al.*, 1989; Hetrick *et al.*, 1991). However, their AM susceptibility, host dependence, and host responsiveness could vary in different growth conditions. Results of Experiment 1 suggest that *F. arundinacea*, with no responsiveness to AM fungi in terms of growth and P uptake in Ferries McDonald soil, was an appropriate plant to test the influence of *G. intraradices* on nutrient uptake, and seedling establishment at different salinity levels, in the absence of any effect on growth or P uptake.

In Experiment 2, increased salinity significantly decreased mycorrhizal colonization in *F. arundinacea.* The reduced root length colonized under saline conditions could be related to lack of mycorrhizal responsiveness in this plant. Mycorrhizal responsiveness was reported as an intrinsic property of plant species (Janos, 1988). Plant root architecture and plant P requirement were defined as factors related to plant

mycorrhizal dependency (Baylis, 1975). Plants with finely branched and hairy roots or requiring low P supply are expected to have low responsiveness to mycorrhizal associations (Baylis, 1975). *F. arundinacea* with highly fibrous roots (Gibson & Newman, 2001) was reported as a facultatively mycorrhizal plant (Stahl, 1900; Janos, 1980). It possible that because *F. arundinacea* has low responsiveness to AM fungi, salinity stress could easily decrease mycorrhizal colonization.

In the first experiment, *F. arundinacea* did not show a positive growth or P response to AM fungi at 40 or 60 days after planting. However, significant positive responses were found in pre-inoculated M plants in Experiment 2 at low salinity level at the same time (20 days in the nursery + 20 days after transplanting). Nevertheless by 60 days (20+40 days) any growth and P response had disappeared (Fig. 4.4). The results of Experiment 2 showed that AM pre-inoculation increased plant nutrient concentration and plant vigour (tiller and leaf number and total dry weight) at an early stage after transplanting in low salinity conditions and had no significant effect at high salinity. The influences of AM fungi on plant growth and nutrient uptake at high salinity were demonstrated in Chapter 3 and have been reported previously (Rosendahl & Rosendahl, 1991; Mohammad *et al.*, 2003) in cucumber and barley respectively. Results of Chapter 3 together with results of Rosendahl and Rosendahl (1991) and Mohammad *et al* (2003) suggested that the influences of AM fungi are more important under salinity stress conditions, to enhance plant salinity tolerance, but the results of this chapter do not support the suggestion in *F. arundinacea* (a mycorrhizal non-responsive plant). It seems that non-responsive species get no net benefits from AM with respect to increased plant salinity tolerance.

The results of Experiment 2 indicate that when seedlings of a mycorrhizal non-responsive plant were pre-inoculated with AM fungi, the plant showed a greater

response to AM inoculation in terms of growth and nutrient content than non-inoculated plants at an early stage after transplanting (Figs. 4.5 and 4.6). These results confirm the results of Chapter 3, which shows the critical role of plant AM pre-inoculation to improve plant nutrient and establishment at the early stage of transplanting in saline conditions.

Overall the results of MPR and MKR in shoots and roots showed that M plants took up more P and K at 20 days than 40 days after transplanting at low salinity level. As salinity increased, plants showed nearly similar values of P and K uptake at both harvests (apart from differences between shoots and roots) (Figs. 4.5 and 4.6). The reduction of P and K uptake in M plants with increased time and salinity could be related to decreased AM colonization. Positive correlation between plant growth responsiveness and AM root colonization in cool-season grasses were reported previously (Wilson & Hartnett, 1998).

Increased Cu, Mg and Al concentrations in roots of M plants where there is no P increase at 40 days, and trends in MPRs and MKRs in shoots and roots at 7.5 and 12 dS/m at 20 and 40 days, may show the direct effects of AM fungi on uptake of other nutrient apart from P effects. Further studies on use of extra P in *F. arundinacea* are needed for better understanding of direct effects of AM and P on the uptake of other nutrients.

In summary the results of this chapter show that although *F. arundinacea* was identified as a mycorrhizal non-responsive plant, AM seedling pre-inoculation did increase its responsiveness to mycorrhizal fungi at an early stage after transplanting. Despite this, pre-inoculation had no effect in increasing salinity tolerance. This substantiates the finding described in Chapter 3, which indicated that increased capacity to absorb P was the main mechanism underling increased salinity tolerance.

Apart from P uptake, AM fungi may have direct effects on uptake of other nutrients. Responsiveness of *F. arundinacea* to AM fungi after transplanting occurred only at low salinity, and increased salinity removed the benefits of AM pre-inoculation. More investigations with different grass species are suggested in the future to figure out the role of AM fungi on grass salinity tolerance.

CHAPTER 5 - EFFECTS OF MYCORRHIZAL INOCULATION ON COLONIZATION AND GROWTH RESPONSES OF *ATRIPLEX NUMMULARIA* IN SALINE CONDITIONS

This chapter describes a field survey and four pot experiments. Results of the field survey showed a relatively high AM colonization in *A. nummularia* in saline Kalibar soil at Monarto area. Experiment 1 was designed to study the effects of salinity stress on mycorrhizal colonization by one fungus in *A. nummularia* using autoclaved Ferries McDonald soil. Results of Experiment 1 showed a low level of colonization, with a growth response in inoculated mycorrhizal plants. To confirm these results Experiment 2 was conducted with some modifications in fungal species and inoculation methods. Experiment 3 evaluated the mycorrhizal inoculum potential (MIP) of Kalibar soil and Experiment 4 investigated the effects of salinity on mycorrhizal colonization by indigenous AM fungi in *T. subterraneum* and *A. nummularia* in non-autoclaved Kalibar soil.

5.1 Introduction

The Chenopodiaceae is an economically important family that probably contains most of the halophytic plants that are very important in revegetation of saline areas. Chenopods are generally regarded as non-host plants but have shown different levels of mycorrhizal colonization in the field at a range of different growth conditions (Table. 1.4). Variation of plant growth conditions may improve mycorrhizal colonization in chenopods (Schwab *et al.*, 1982). Sengupta and Chaudhuri (1990) found high levels of colonization in two chenopods, *Arthrocnemum indicum* and *Suaeda maritima*, in salt marshes of the Ganges delta in India. They found 60% AM colonization with the presence of typical vesicles and arbuscules in *A. indicum* and 48% colonization with

vesicles in *S. maritima*. They hypothesized that salt stress might make the chenopods susceptible to colonization by AM fungi in saline habitats. The primary aim of the work discussed in this chapter was to investigate this hypothesis.

In many plant species the presence of AM fungi confers ecophysiological benefits, particularly in saline soils with low levels of nutrients such as P (Hirrel & Gerdemann, 1980; Ojala *et al.*, 1983; Poss *et al.*, 1985 and see Chapter 3). The contribution of AM fungi to enhance nutrient uptake and plant growth in saline conditions in crop plants is well known (Ojala *et al.*, 1983; Copeman *et al.*, 1996; Al-Karaki *et al.*, 2001), but their role in nutrient uptake, growth and establishment in chenopods has received less attention and is generally believed to be low. However inoculation of four-wing saltbush (*Atriplex canescens*) with *Glomus mosseae* increased plant growth and survival of plants in coal mine spoils in the field (Aldon, 1975), and abundant intracellular and intercellular hyphae were found in inoculated plants. The same plant species was also heavily colonized with *G. mosseae* and colonization increased plant growth under glasshouse conditions (Williams *et al.*, 1974).

Mycorrhizal associations affect plant nutrient uptake and nutrient cycling and the bacterial community composition in the rhizosphere (Beare *et al.*, 1997; Pare *et al.*, 2000). Changes in soil bacterial community composition may have effects on soil organic matter decomposition and mineralization and plant nutrient supply. However, there are no published reports on the effects of mycorrhizal fungi on the bacterial community composition in the rhizosphere of chenopods. The aim of the work discussed in this chapter was to determine the effect of salt stress on AM colonization of *A. nummularia,* and the effects of AM fungal inoculation on growth, bacterial rhizosphere community composition and nutrient uptake in this plant at low and high

salinity. The work on the bacterial rhizosphere community was carried out in Experiment 2, in order to explore reasons for the effects of AM inoculation, despite low colonization. It was hypothesized that the inoculum might have changed bacterial community composition and hence nutrient mobilization and availability.

5.2 Materials and Methods

5.2.1 Field survey. The occurrence of mycorrhizal colonization in *Atriplex nummularia* grown in Kalibar soil (Monarto)

Root samples of the perennial *A. nummularia* growing in Kalibar soil were collected from Monarto area in three different seasons, August 2002, November 2002 and February 2003. Kalibar soil properties are shown in Tables. 3.1 and 3.2. At each sampling time, two root samples and about 400 g of the soil were collected from 0-20 cm topsoil near four individual *A. nummularia* plants that had no adjacent plants within approximately 1 m. The samples of each plant were washed carefully with water, cut into 2 cm segments and stored in 70% ethanol. Three sub-samples were taken from each sample. The root samples were stained with trypan blue; then mycorrhizal colonization was evaluated in each sample (Chapter 2.9 and 2.10).

5.2.2 Experiment 1. Effects of salinity stress on mycorrhizal colonization (one fungus) in *Atriplex nummularia* in autoclaved Ferries McDonald soil

Because of appropriate soil chemical and physical proprieties and reasonable potential for mycorrhizal colonization and plant growth and also for consistency with experiments reported in Chapters 3 and 4, Ferries McDonald soil was selected in this experiment. Soil was collected from the top 15 cm from Monarto area in August 2002. The soil was dried, sieved, mixed thoroughly, and then autoclaved (Chapter 2.1) (Soil properties are shown in Tables. 3.1 and 3.2). Two levels of salinity (2.2 and 12 dS/m)

were produced by adding 0 or 2 g NaCl per kg of the soil as solution, and then soils were incubated for one week. Three pre-germinated *A. nummularia* seeds (Chapter 2.5) were sown in autoclaved soil (400g soil per pot) inoculated or not with *Glomus fasciculatum* Thaxter (LPA7). The inoculum was dried pot culture material, consisting of soil/sand mix plus colonized root fragments, spores and external hyphae. This was mixed with Ferries McDonald soil in the ratio 10% inoculum to 90% soil. Non-inoculated pots received an additional 10% Ferries McDonald soil. R.O. water was added regularly to maintain the soil at 80% field capacity. After one week, plants were thinned to one seedling per pot. There were three replicate pots per soil and harvest. Plants were grown in a glasshouse in November 2002 (spring) and harvested at 3, 6 and 9 weeks after planting. At each harvest plant leaf number, plant height, shoot and root fresh weight were recorded and shoot dry matter was determined after drying at 80 °C for 2 days. Shoot P concentration was determined by the method of Hanson (1950) (Chapter 2.12). Roots of harvested plants were washed carefully, and cut into 1 cm segments, then stained and mycorrhizal colonization determined (Chapters 2.9 and 2.10). Percentage of soil organic matter (OM%) in inoculum, and inoculated and non-inoculated soil was determined by the method of Walkley and Black (1934). Rhizosphere soil for DNA extraction was obtained by removing the soil adhering to the roots with a brush. The rhizosphere soil was stored at –20°C until analysed.

DNA extraction from soil

All solutions and plastic-ware used for DNA extraction and amplification were sterilized before use. Total DNA was isolated from the rhizosphere soil samples by a bead beater (Marschner *et al.*, 2002). Briefly, 200 mM phosphate buffer and 10% SDS were added to 300 mg of soil sample, which was then homogenized in a bead beater (Fast-Prep, Model FP120, Bio101, Vista CA) at 5.5 m/s for 30 s. After proteins were removed with a protein precipitation solution (PPS®, Bio101, USA, Vista, CA), the

DNA was bound to a silica matrix (Binding matrix®, Bio101), washed twice with an ethanol-salt solution (per liter: 10 mM Tris-HCl, 0.5 mM EDTA, 5 mM NaCl and 500 ml ethanol). After evaporating the remaining ethanol at room temperature for 20 min, the pellet was resuspended in 150 µl of ultra pure water. The samples were centrifuged at 14000 RCF (relative centrifugal force) for 2 minutes and 100 µl of the supernatant containing the extracted DNA were transferred into a fresh tube. The DNA samples were stored at –20°C for further analysis.

Polymerase chain reaction (PCR)

The bacterial rhizosphere community composition was determined by ribosomal intergenic spacer amplification (RISA) (Yin *et al.*, 2000). The ribosomal intergenic spacer region was amplified with the primers 1405F (TGYACACACCGCCCGT) and 23R (GGGTTBCCCCATTCRG) (Yin et al. 2000). The 25 µl PCR mix had the following composition: PCR buffer (10×) 2.5 µl, dNTPs (2 nmol/µl) 3 µl, each primer (5 pmol/µl) 1 µl, Taq polymerase (5U/µl) 0.25 µl, $MgCl_2$ (2.5 mM) 2.5 µl, H_2O 12.75 µl and 2 µl of a DNA extract diluted 100 fold. Bacterial 16S-23S rRNA intergenic fragments were amplified in a Mastercycler Gradient thermocycler (Eppendorf, Hamburg, Germany) with the following program: 35 cycles of 1 min denaturation at 94°C, 1 min at 52°C for primer annealing and 2 min at 72°C for primer extension. In the first cycle, the denaturation phase was extended to 5 min at 94°C to prevent annealing of the primers to non-target DNA. The 35 cycles were followed by a final step of 10 min at 72°C and cooling at 10°C.

Gel electrophoresis

The DNA fragments were resolved by electrophoresis on agarose gels. Agarose gel (2%) was prepared with adding 1.5 g high-purity agarose into 75 ml 1 × TBE buffer (Tris, Boric acid and EDTA). PCR products (25 µl) were mixed with 8 µl PCR dye containing SYBR® and the entire volume was loaded on the gel. An amplified mix of pure cultures of *Pseudomonas fluorescens*, *Bacillus amyloliquefaciens* and *B. subtilis* was added to gel and their bands were used as standards. The gel was electrophoresed in 1 × TBE buffer for 3 hours at 80 V. DNA bands were visualised and photographed under UV light with a video imaging system. Bacterial community composition was not analysed in this experiment.

5.2.3 Experiment 2. Effects of salinity stress on mycorrhizal colonization (mixture of six fungi) in *Atriplex nummularia* in autoclaved Ferries McDonald soil

A low colonization rate in Experiment 1 (5.3.2 below), may have been related to using an inappropriate fungus to colonize with *A. nummularia* roots. In Experiment 2 a mixture of different *Glomus* species was used as the inoculum to increase the chances of colonization by different AM fungi. The species of AM fungi most commonly found in saline soils are member of the genus *Glomus* (Allen & Cunningham, 1983; Ho, 1987; Hildebrandt *et al.*, 2001; Landwehr *et al.*, 2002), so six different *Glomus* species were selected. Equal amounts of dried pot culture inoculum of *G. mosseae* Nicolson and Gerdemann, *G. intraradices* Schenk and Smith, *G. etunicatum* Backer and Gerdemann, *G. geosporum* Nicolson and Gerdemann, *G. caledonium* Nicolson and Gerdemann and *G. fasciculatum* Thaxter, consisting of colonized root fragments and soil/sand containing spores and external hyphae were mixed and added into the soil at a 1:9 ratio (w/w). The same amount of autoclaved mycorrhizal inoculum was added to mock-inoculated (control) pots in order to provide approximately the same nutrient levels as

inoculated pots. To establish a similar bacterial community in both mycorrhizal and non-mycorrhizal plants, 100 g of pot culture inoculum was suspended in 500 ml water and shaken for 5 minutes, then filtered through a Whatman no. 1 (11μm) filter paper and added to non-mycorrhizal pots. Soils were incubated for one week to allow the bacterial community to equilibrate, and then pots were filled with 400 g of inoculated or non-inoculated soil. Plants were grown in a glasshouse in April 2003 (Autumn) and were harvested 6 and 10 weeks after planting. There were four replicate pots per soil and harvest. Shoot nutrient concentrations were determined by ICP. To avoid confusing roots from the inoculum with those of *A. nummularia* roots, the intact roots from each plant were carefully removed from the soil then washed with R.O. water, and only roots that were clearly attached to the main root were examined for colonization.

Gel electrophoresis

In this experiment PCR fragments of products were separated by two methods; 2% agarose gel (same as Experiment 1) and a 5% polyacrylamide gel and visualized with SYBR® Gold Stain. Acrylamide gel electrophoresis was performed with 5% (wt/vol) acrylamide gels with a 37.5:1 acrylamide / bisacrylamide ratio and containing 7 M urea. The gel was allowed to polymerize overnight. DNA samples containing 10 μl of the PCR products were electrophoresed in 1 × TBE buffer at 60°C at a constant voltage of 200 V for 5 h (BIO-RAD D-Gene™ Systems, Germany, Munich). After electrophoresis, the gel was stained for 30 min with SYBR® and photographed under UV light with a video imaging system. Bacterial community composition was analysed in this gel.

Bacterial community composition analyses

TotalLab Non-linear Dynamics Ltd (Newcastle upon Tyne, UK) software was used to digitise banding patterns in the polyacrylamide gel. Both number of species (band position) and the abundance (band intensity) of species were used to represent community composition. Community composition based on relative band intensity and position was analyzed by performing principal component analyses and canonical correspondence analyses with Monte Carlo permutation tests (CANOCO 4.0, Microcomputer Power, Ithaca, USA). Community similarities were graphed by using ordination plots with scaling focused on inter-sample differences (Jongman *et al.*, 1995). Factors potentially affecting community composition such as presence or absence of mycorrhizal fungi, salinity, SDW and shoot nutrient contents were used as environmental data (Marschner & Baumann, 2003).

5.2.4 Experiment 3. Mycorrhizal inoculum potential in Kalibar soil

A bioassay experiment was designed to determine the mycorrhizal inoculum potential (MIP) of Kalibar soil (Moorman *et al.*, 1979). *Trifolium subterraneum* was selected as bioassay plant because of its ability to serve as a host plant and in order to link the results with Chapters 3 and 6. Pre-germinated *T. subterraneum* seedlings were planted in 10 pots (1400g soil per pot) of non-autoclaved Kalibar soil and were grown in a glasshouse in February 2003 (Summer). Plants were harvested 6 and 8 weeks after planting, and percentage of root length colonized was determined. At 8 weeks after planting spores were separated from the soils by a wet sieving method (Brundrett *et al.*, 1996) and identified microscopically to the genus level.

5.2.5 Experiment 4. Effects of salt stress on mycorrhizal colonization in *Atriplex nummularia* and *Trifolium subterraneum* in Kalibar soil

Because of the high level of colonization in *A. nummularia* in Kalibar soil in field conditions, Experiment 4 was designed to investigate the effects of salt stress on mycorrhizal colonization in *A. nummularia* and compare it with *T. subterraneum* in this soil. In this experiment pre-germinated seeds of *A. nummularia* and *T. subterraneum* were sown in non-autoclaved Kalibar soil (400g soil per pot) with different salinity levels (4.8 and 12 dS/m, by adding 0 and 1.5 g NaCl per kg soil respectively). Plants were grown in a glasshouse in June 2003 (Winter) and harvested at 10 weeks after planting. SDW, P concentration and AM colonization were determined as before.

5.3 Results

5.3.1 Field survey. The occurrence of mycorrhizal colonization in *Atriplex nummularia* grown in Kalibar soil (Monarto)

Roots of perennial *A. nummularia* collected from the field at the Kalibar soil site showed a relatively high level (10-30%) of mycorrhizal colonization in the different seasons. Different mycorrhizal structures were found in the root samples (Fig. 5.1). The variability between the different plants harvested at each sampling date was high (results not shown). There were no differences in percentages of colonization between different sampling times. Vesicles and hyphae were observed at all samples, but arbuscules were more often observed in November and February in young roots.

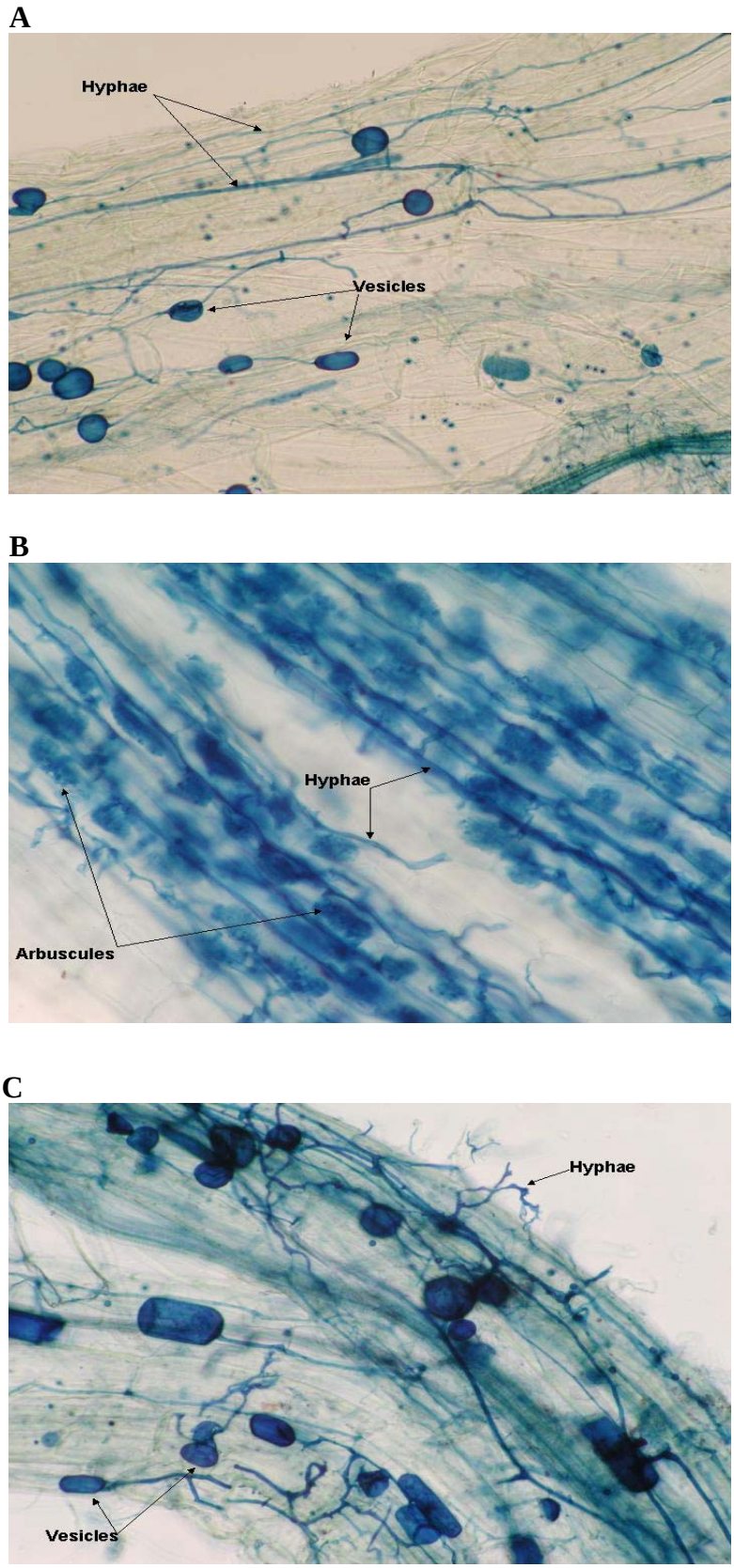


Fig 5.1. Field survey. Roots of *Atriplex nummularia* showing AM colonization in August (A), November (B) and February (C) collected from the field at Kalibar, in Monarto area.

5.3.2 Experiment 1. Effects of salinity stress on mycorrhizal colonization (one fungus) in *Atriplex nummularia* in autoclaved Ferries McDonald soil

There was no mycorrhizal colonization in non-inoculated plants. Some AM colonization was found in inoculated pots, but it was difficult to distinguish the *A. nummularia* root segments from highly colonized roots originating from the inoculum. There was no effect of increasing salinity on % root length colonized. Mycorrhizal inoculation increased plant SDW. This increase was more obvious at low salinity and was significant (P <0.05) at 6 and 9 weeks but not at 3 weeks after planting (Fig. 5.2). Increased salinity decreased SDW in inoculated and non-inoculated plant at all harvests. There were no differences in P concentration and content between inoculated and non-inoculated plants at any harvest or salinity level (Table. 5.1). Salinity decreased shoot P content in inoculated and non-inoculated plants. Percentage of organic material (OM) in inoculum was very low (0.1%) and there were no significant differences between inoculated and non-inoculated pots (1.25 and 1.20 respectively).

The RISA banding patterns of the bacterial rhizosphere community of the third harvest showed some differences between low and high salinity levels and inoculated and non-inoculated treatments (Fig. 5.3). DGGE banding patterns at 9 weeks did not show reasonable band numbers for statistical analysis (gel not shown), so banding patterns were not analyzed statistically.

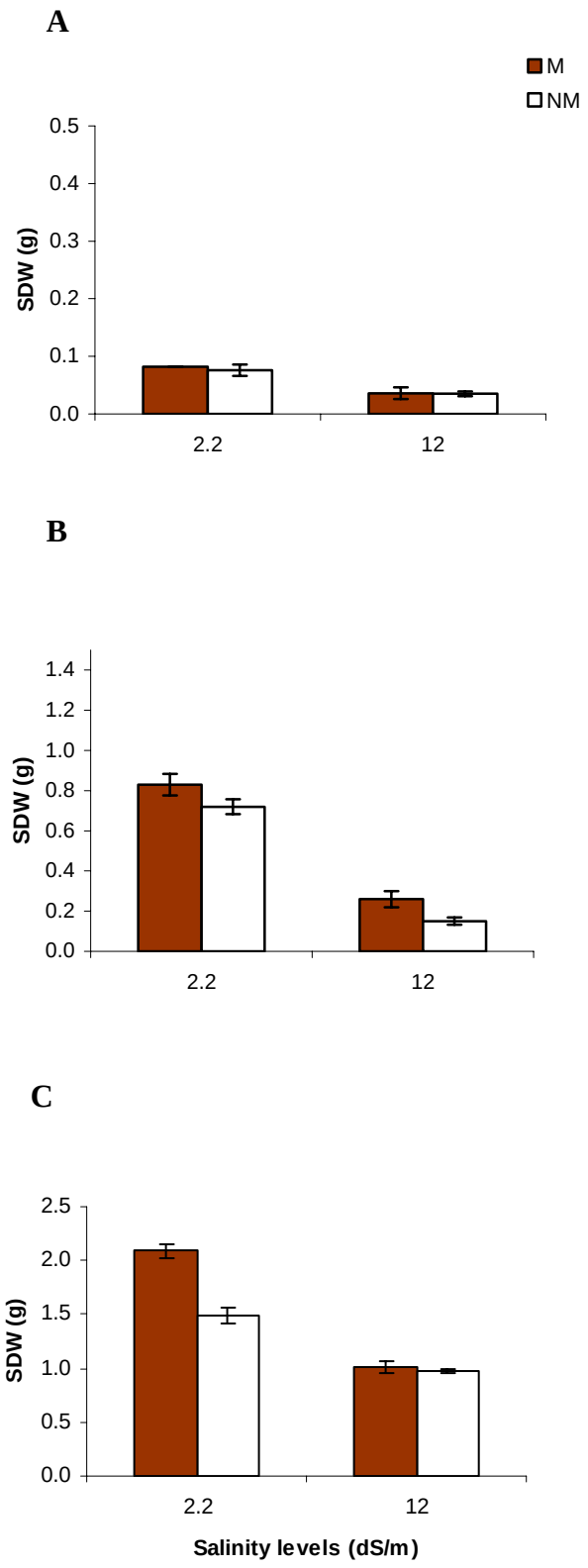


Fig 5.2 Experiment 1. Shoot dry weight of AM inoculated (M) and non-inoculated (NM) *Atriplex nummularia* at high and low levels of salinity at 3, 6 and 9 weeks (A, B and C respectively). Vertical bars represent standard error of the means, n=3.

Table 5.1 Experiment 1. Shoot P concentration and content of AM inoculated and non-inoculated *Atriplex nummularia* at low or high salinity levels at 3, 6 and 9 weeks after planting. Means of 3 replicates ± standard error

Treatments	Salinity	P concentration	P content
		mg/g	mg/plant
3 weeks			
Inoculated	**Low salinity**	3.1±0.2	0.3±0.0
	High salinity	3.5±0.2	0.1±0.0
Non-inoculated	**Low salinity**	3.5±0.2	0.3±0.1
	High salinity	2.5±0.1	0.1±0.0
AM inoculation		ns	ns
Salinity		ns	**
6 weeks			
Inoculated	**Low salinity**	3.0±0.2	2.5±0.1
	High salinity	3.5±0.2	0.9±0.1
Non-inoculated	**Low salinity**	3.7±0.8	2.6±0.4
	High salinity	3.6±0.2	0.5±0.1
AM inoculation		ns	ns
Salinity		ns	***
9 weeks			
Inoculated	**Low salinity**	1.8±0.3	3.7±0.3
	High salinity	2.9±0.7	2.9±0.3
Non-inoculated	**Low salinity**	2.8±0.4	4.2±0.5
	High salinity	2.4±0.4	2.3±0.2
AM inoculation		ns	ns
Salinity		ns	**

, * significant at the 0.01, 0.001 probability level, respectively
ns not significant at p=0.05

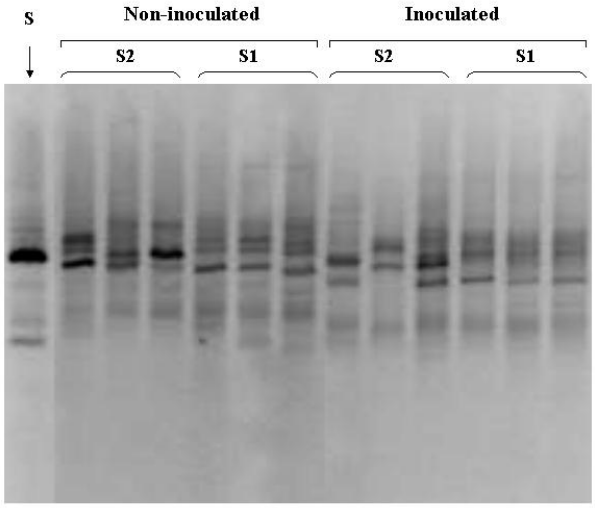
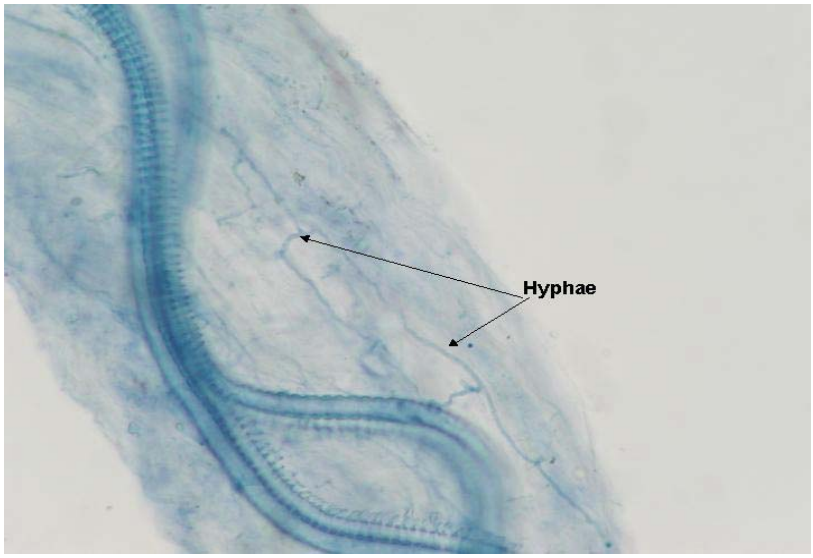


Fig 5.3 Experiment 1. Ribosomal intergenic spacer amplification (RISA) agarose gel of rhizosphere communities of AM inoculated and non-inoculated in *Atriplex nummularia* grown at low (S1) and high (S2) salinity levels. S = Bacterial standard mix (Pure cultures of *Pseudomonas fluorescens, Bacillus amyloliquefaciens* and *B. subtilis*).

5.3.3 Experiment 2. Effects of salinity stress on mycorrhizal colonization (mixture of six fungi) in *Atriplex nummularia* in autoclaved Ferries McDonald soil

No mycorrhizal colonization was found in mock-inoculated plants. Colonization was very low (1-2% of the root length) and patchy in inoculated plants. After 6 weeks, mycorrhizal hyphae had penetrated the roots and were observed on the surface and within the root cortical cells of young and old roots, but no arbuscules or vesicles were found (Fig. 5.4 A). After 10 weeks all typical AM structures (hyphae, arbuscules and vesicles) were found, but the distribution remained patchy and the percentage of root length colonized did not increase compared to 6 weeks. There was no effect of salinity on % root length colonized.

A



B

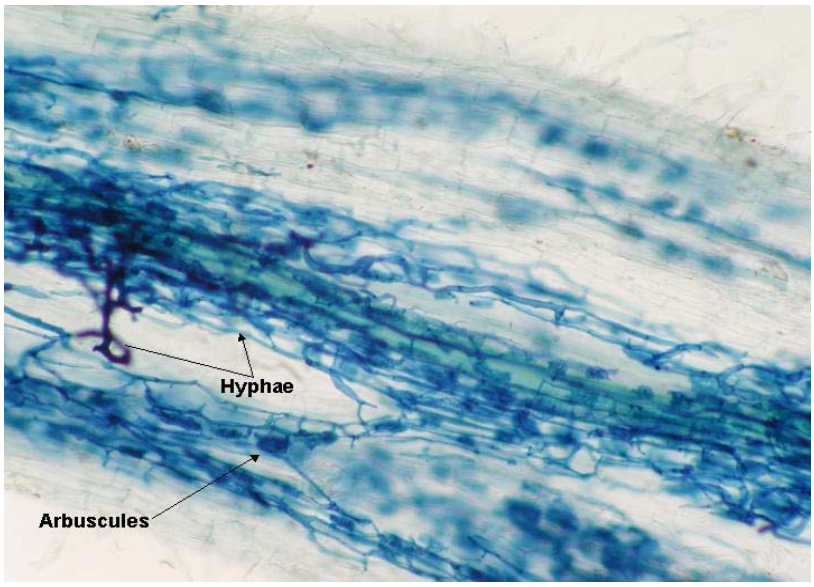


Fig 5.4 Experiment 2. Root of inoculated *Atriplex nummularia* showing internal hyphae at 6 weeks and arbuscules 10 weeks after planting.

Mycorrhizal inoculation significantly increased plant SDW at both salinity levels at 6 weeks but not at 10 weeks (Fig. 5.5). After 6 weeks SDW of inoculated plants was increased by 78% at low and 175% at high salinity, compared to the mock-inoculated plants. High salinity significantly decreased SDW in inoculated and mock-inoculated

plants at both harvests. This decrease was more obvious in the mock-inoculated plants than in inoculated plants after 6 weeks.

The effects of AM inoculation and salinity on shoot nutrient concentrations and contents are shown in Tables. 5.2 and 5.3. At 6 weeks AM inoculation decreased P, K, Fe and Zn concentrations, but had no effects on Na and Mg shoot concentrations. Inoculated plants had significantly higher P and Mg concentrations at 10 weeks, but there were no effects on the concentrations of other nutrients. Salinity increased Na concentration at 6 weeks and Na and Zn concentrations at 10 weeks. AM inoculation and salinity had stronger effects on plant nutrient contents at 6 weeks than 10 weeks. After 6 weeks the inoculated plants had significantly higher P, K, Mg and Na contents than the mock-inoculated plants, but inoculation had no effect on Zn and Fe content. At 10 weeks after planting mycorrhizal inoculation significantly increased P and Mg concentration and content, but did not affect the concentration and content of the other nutrients. Compared to the low salinity level, high salinity significantly decreased the content of all nutrients investigated except Na and Fe at 6 weeks. After 10 weeks salinity affected only trace element uptake; at high salinity, Fe content was decreased while Zn content was increased.

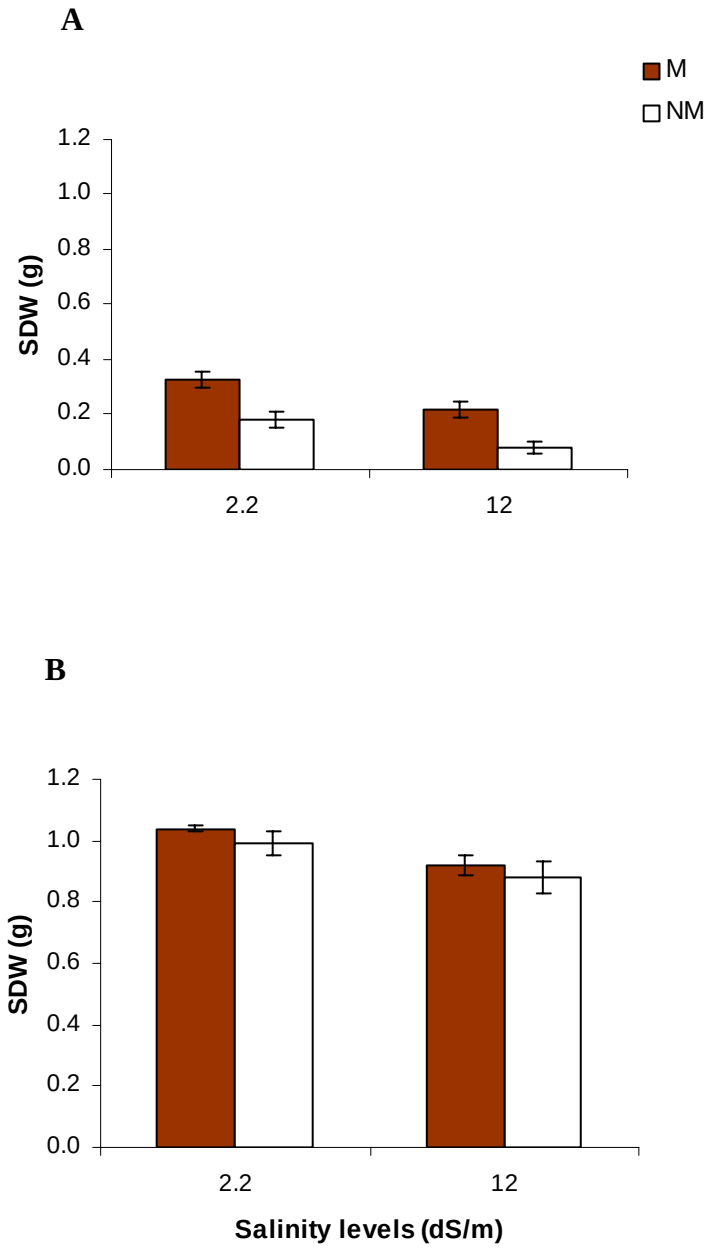


Fig 5.5 Experiment 2. Shoot dry weight of AM inoculated (M) and mock-inoculated (NM) *Atriplex nummularia* at low or high salinity at 6 (A) and 10 (B) weeks after planting. Vertical bars represent standard errors of the means, n=4.

Table 5.2 Experiment 2. Shoot nutrient concentration of AM inoculated and mock-inoculated *Atriplex nummularia* at low or high salinity levels at 6 and 10 weeks after planting. Means of 4 replicates ± standard error

Treatments	Salinity	P	K	Mg	Na	Fe	Zn
		(mg/g)				(µg/g)	
6 weeks							
Inoculated	**Low salinity**	3.4±0.3	61.3±4.6	7.0±0.9	42.5±6.5	65.0±10.0	60.0±5.0
	High salinity	3.4±0.2	58.3±3.6	6.5±0.3	53.5±2.5	66.0±5.0	63.0±10.0
Mock-inoculated	**Low salinity**	4.7±0.3	82.0±2.6	6.2±0.4	39.3±2.5	98.0±5.0	119.0±15.0
	High salinity	4.4±0.5	75.0±8.1	5.5±0.5	63.5±2.1	221.0±55.0	87.0±5.0
Inoculation		*	**	ns	ns	**	**
Salinity		ns	ns	ns	**	ns	ns
10 weeks							
Inoculated	**Low salinity**	3.1±0.2	46.0±4.3	5.3±8.1	20.8±1.8	32.0±2.0	32.0±5.0
	High salinity	3.0±0.2	42.8±3.4	3.5±0.3	24.6±4.2	32.0±2.0	41.0±5.0
Mock-inoculated	**Low salinity**	2.3±0.1	43.5±2.0	2.9±0.1	19.9±0.8	44.0±10.0	34.0±0.5
	High salinity	2.8±0.1	44.8±2.2	3.3±0.4	30.5±2.8	31.0±2.5	59.0±10.0
Inoculation		**	ns	*	ns	ns	ns
Salinity		ns	ns	ns	*	ns	**

*, ** significant at the 0.05, 0.01 probability level, respectively
ns not significant at p=0.05

Table 5.3 Experiment 2. Shoot nutrient content of AM inoculated and mock-inoculated *Atriplex nummularia* at low or high salinity levels at 6 and 10 weeks after planting. Means of 4 replicates ± standard error

Treatments	Salinity	P	K	Mg	Na	Fe	Zn
		(mg/plant)				(µg/plant)	
6 weeks							
Inoculated	**Low salinity**	1.1±0.1	20.0±2.3	2.2±0.1	13.5±2.0	20.0±1.5	19.0±1.5
	High salinity	0.7±0.1	12.4±0.8	1.4±0.2	11.5±1.2	14.0±1.5	14.0±2.5
Mock-inoculated	**Low salinity**	0.8±0.1	14.2±2.0	1.1±0.2	6.7±0.6	18.0±3.5	22.0±6.0
	High salinity	0.3±0.1	5.7±1.1	0.4±0.1	4.9±1.1	17.0±4.0	6.0±1.0
Inoculation		***	**	***	***	ns	ns
Salinity		***	**	**	ns	ns	*
10 weeks							
Inoculated	**Low salinity**	3.2±0.1	47.9±4.5	5.6±1.0	21.6±1.7	33.0±1.5	33.0±4.0
	High salinity	2.7±0.2	39.2±4.1	3.2±0.2	22.4±3.6	29.0±1.0	37.0±2.5
Mock-inoculated	**Low salinity**	2.3±0.1	43.1±2.7	2.9±0.2	19.8±1.4	43.0±8.0	34.0±2.0
	High salinity	2.4±0.2	39.5±3.7	2.9±0.4	26.4±2.0	27.0±1.5	51.0±6.0
Inoculation		**	ns	*	ns	ns	ns
Salinity		ns	ns	ns	ns	*	*

*, **, *** significant at the 0.05, 0.01, 0.001 probability level, respectively
ns not significant at p=0.05

Bacterial community composition

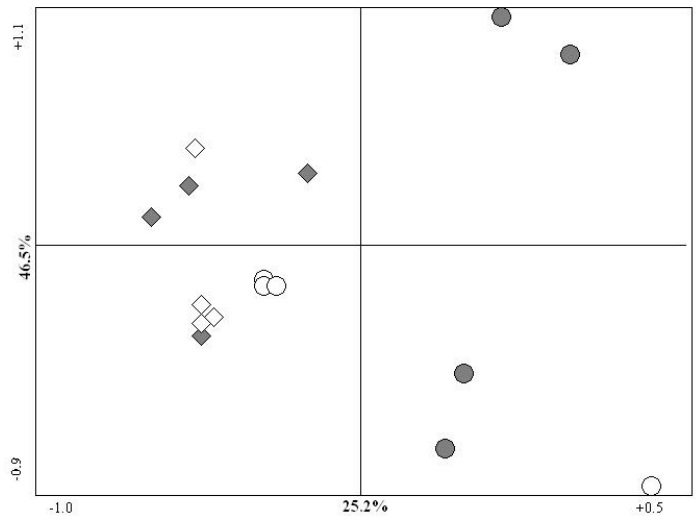
The bacterial community composition in the rhizosphere of *A. nummularia* was significantly affected by mycorrhizal inoculation and salinity (Table. 5.4, Fig. 5.6). The PCA showed that salinity affected the bacterial community composition in the inoculated plants but not in mock-inoculated plants at both 6 and 10 weeks. After 6 weeks the replicates within the mock-inoculated treatments had similar bacterial community composition, whereas more variation was found in inoculated treatments. After 10 weeks, the variability of the bacterial community composition was similar in inoculated and mock-inoculated plants (Fig. 5.6). The bacterial community composition in the rhizosphere was analyzed with canonical correspondence analyses (CCA) and Monte Carlo Permutation Test using salinity levels, mycorrhizal inoculation, SDW and plant nutrient contents and concentrations as environmental data (Table 5.4). After 6 weeks, the bacterial community composition in the rhizosphere was significantly correlated with (in order of decreasing importance): salinity > SDW > Na concentration > K content > P concentration > P content > mycorrhizal inoculation. At 10 weeks, the bacterial community composition was significantly correlated with (in order of decreasing importance): Mg content > salinity > Mg concentration > mycorrhizal inoculation > P content > SDW > K content and > Na concentration. Other environmental factors such as Fe, Zn content and concentration had no significant effect at either harvest.

Table 5.4 Levels of significance of correlation between rhizosphere bacterial community composition and environmental variables in decreasing order of importance (eigenvalue) at 6 and 10 weeks in AM inoculated and mock-inoculated *Atriplex nummularia* at low or high salinity levels, generated by Monte Carlo Permutation test

6 weeks		10 weeks	
Variable	**Significance**	**Variable**	**Significance**
Salinity	**	Mg content	**
SDW	*	Salinity	*
Na concentration	*	Mg concentration	*
K content	*	Inoculation	*
P concentration	*	P content	**
P content	*	SDW	*
Inoculation	*	K content	*
		Na concentration	*

*, ** significant at the 0.05, 0.01 probability level, respectively

A



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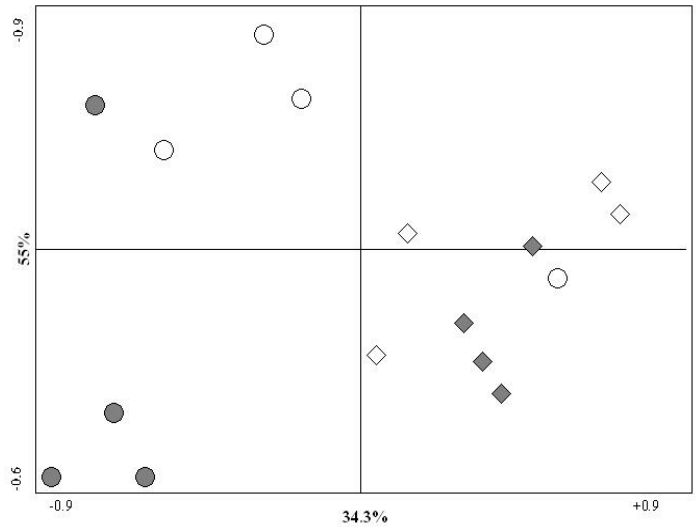

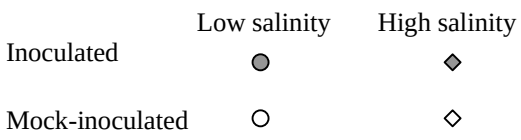

Fig 5.6. Experiment 2. Ordination plot of bacterial rhizosphere communities of inoculated and mock-inoculated *Atriplex nummularia* at low or high salinity generated by principal component analysis of 16S rDNA RISA banding patterns at 6 weeks (A) and 10 weeks (B) weeks after planting.

5.3.4 Experiment 3. Mycorrhizal inoculum potential in Kalibar soil

This bioassay experiment showed that the roots of *T. subterraneum* were colonized by AM fungi at 57 and 75% respectively 6 and 8 weeks after planting in non-autoclaved Kalibar soil (Fig. 5.7). All typical AM structures (hyphae, arbuscules and vesicles) were found at both harvests. The most commonly identified AM fungi were *Glomus* species (Fig. 5.8).

5.3.4 Experiment 4. Effects of salt stress on mycorrhizal colonization in *Atriplex nummularia* and *Trifolium subterraneum* in Kalibar soil

Ten weeks after planting *T. subterraneum* roots were highly colonized by AM fungi, whereas only 4% colonization with a patchy pattern was found in *A. nummularia* roots. All AM structures were found in *T. subterraneum* roots at both salinity levels, but only vesicles and hyphae were detected in *A. nummularia* roots (Fig. 5.9). The mycorrhizal structures in *A. nummularia* roots were similar to those found in the field survey (see Chapter 5.3.1). Increased salinity significantly decreased SDW, shoot P concentration, P content and colonization in *T. subterraneum*. In *A. nummularia* salinity decreased SDW, but had no significant effects on P content and concentrations (Table. 5.5 and Fig. 5.10).

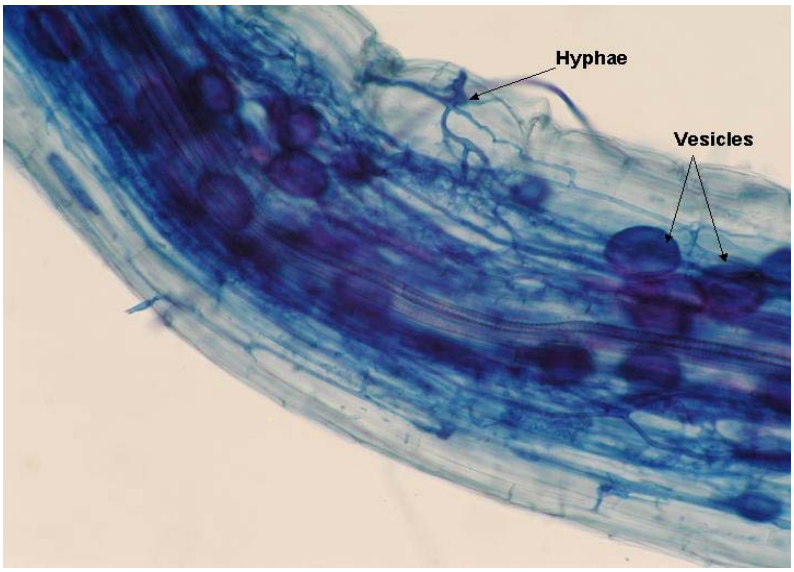

Fig 5.7 Experiment 3. Roots of *Trifolium subterraneum* colonized by AM fungi grown in non-autoclaved Kalibar soil pots at 8 weeks after planting.

Fig 5.8 Experiment 3. A single spore of AM fungi (*Glomus sp*.), trapped in a pot culture of *Trifolium subterraneum* in Kalibar soil at 8 weeks.

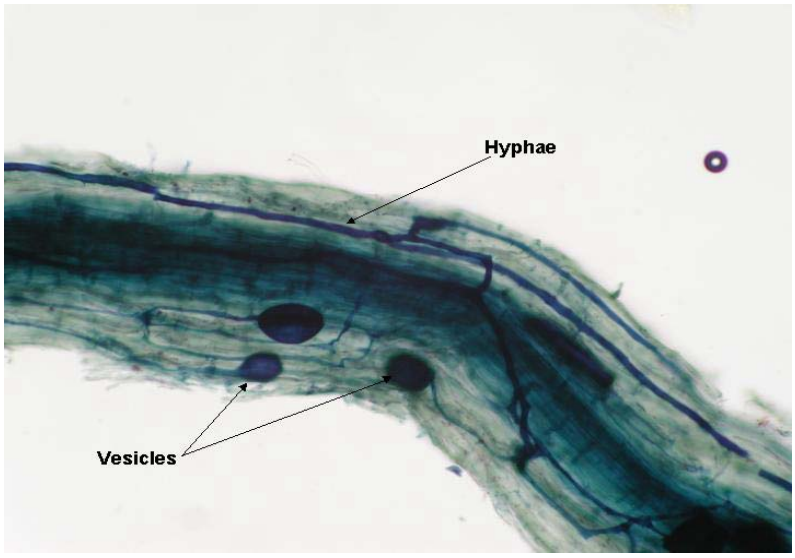

Fig 5.9 Experiment 4. Vesicles and hyphae in roots of *Atriplex nummularia* planted in non-autoclaved Kalibar soil at high salinity level.

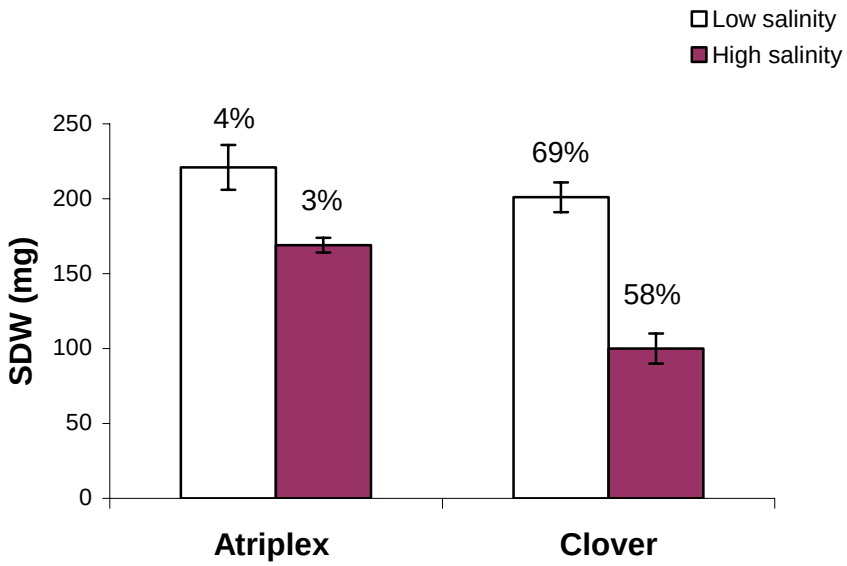

Fig 5.10 Experiment 4. Shoot dry weight of *Atriplex nummularia* and *Trifolium subterraneum* at low and high levels of salinity at 10 weeks. Numbers at top of the bars shows percentages of AM colonization. Vertical bars represent standard errors of the means, n=5.

Table 5.5 Experiment 4. Shoot P concentration and content of *Atriplex nummularia* and *Trifolium subterraneum* planted in non-autoclaved Kalibar soil at low or high salinity levels 10 weeks after planting. Means of 5 replicates ± standard error

Plant species		P concentration	P content
		mg/g	mg/plant
Atriplex nummularia			
	Low salinity	2.0±0.2	0.45±0.1
	High salinity	1.9±0.1	0.32±0.0
Salinity		ns	ns
Trifolium subterraneum			
	Low salinity	6.3±0.6	1.22±0.1
	High salinity	2.7±0.4	0.26±0.1
Salinity		**	**

** significant at the 0.01 probability level
ns non significant

5.4 Discussion

Although a relatively high level of AM colonization was found in *A. nummularia* in the field survey in naturally saline Kalibar soil, increased salt stress did not increase AM colonization following inoculation under glasshouse conditions. The results from Experiments 1 and 2 indicate that despite using *Glomus* species that naturally occur in saline soils (Allen & Cunningham, 1983; Ho, 1987; Hildebrandt *et al.*, 2001; Landwehr *et al.*, 2002), salinity did not increase AM colonization of *A. nummularia* in autoclaved Ferries McDonald soil. Results of Experiments 3 and 4 show that although Kalibar soil had an intrinsic AM infectivity with respect to *T. subterraneum*, colonization was very

low and not dependent on salinity in *A. nummularia*. Changing the soil from Ferries McDonald to Kalibar did not help to show the salinity effects on AM colonization in *A. nummularia*. Similar levels of AM colonization in the same plant species grown in a non-saline soil in the Chilean arid zone (Aguilera *et al.*, 1998) confirm that improved AM colonization in chenopods is not generally related to salt stress. My results, together with the findings of Aguilera et al. (1998) suggested that salinity had no effect on AM colonization at least in *A. nummularia* in glasshouse conditions. Thus the hypothesis that AM colonization in chenopods in general is increased with salinity stress (Sengupta and Chaudhuri 1990) is not substantiated.

The reasons for high AM colonization in *A. nummularia* in the field compared with pot experiments are not clear. The environmental conditions such as light, temperature, water availability and microorganisms may affect AM colonization of *A. nummularia* under field conditions. Additionally, the plants sampled in the field were older than those used in the pot experiments. It is possible that colonization of the roots of *A. nummularia* require more than 3 months to become established or that the older roots are more susceptible to colonization that young roots. Another reason for high percentage of colonization in field and low colonization of *A. nummularia* in pot experiments may related to differences between fungal strains that were used for inoculation and were exist in field conditions. AM fungi identification in roots of *A. nummularia* from the field by molecular methods is needed to identify species that colonize the roots.

The results of this study suggest that salinity may not be the only factor that influences occurrence of AM colonization of chenopods in saline conditions and other environmental factors and their interactions could influence colonization. For example under field conditions microorganisms in the rhizosphere may produce hydrolytic

enzymes, which dilate the plant root cortical cell and provide a better situation for penetration and ramification of mycorrhizal hyphae in the root (Caroline & Bagyaraj, 1995; Mamatha *et al.*, 2002). Environmental conditions may also influence root exudates. Increased exudation of sugars and amino acids has been shown to increase colonization of chenopods (Schwab *et al.*, 1982). So further, long-term investigations are needed to identify other factors and their interactions that contribute to mycorrhizal colonization of chenopods in saline conditions.

In Experiments 1 and 2 a low level of colonization was found. However, despite these low colonization levels, mycorrhizal inoculation increased plant growth and influenced nutrient uptake. Results of Experiments 1 and 2 showed that AM inoculation increased SDW of *A. nummularia* particularly at 6 weeks. In Experiment 1, shoot P concentration in AM inoculated plants increased at the high salinity in early stages of plant growth. In Experiment 2 AM inoculation increased nutrient uptake, particularly at 6 weeks and the higher salinity level. Increased plant growth at very low AM colonization is in agreement with other studies with *Salix repens* (less than 5% colonization) and *Chrysanthemum morifolium* (3.9% colonization) (van der Heijden, 2001; Sohn *et al.*, 2003; van der Heijden & Kuyper, 2003). The influence of AM inoculation on the growth of chenopods has been reported previously. In a pot experiment, colonization by *Glomus mosseae* increased plant growth of *Atriplex canescens* (percentage of colonization was not reported) (Williams *et al.*, 1974). AM colonization of *Atriplex canescens* (only intracellular and intercellular hyphae) increased dry weight, P content and survival in mine overburden under field conditions (Aldon, 1975). AM inoculation increased *A. nummularia* plant growth and nutrient uptake (particularly an the early stage) in the present study and, together with the findings of Williams *et al.* (1974) and Aldon (1975), shows the beneficial effect of AM inoculation in chenopods. Carbon flow

from the roots of *Atriplex gardneri* and *Salsola kali* for AM spore production has also been demonstrated previously (Allen & Allen, 1988). Further works with radioactive tracers are needed to investigate nutrient flow from fungi into the roots to determine if bi-directional movement of nutrients and direct effects of AM fungi in nutrient uptake occurs in chenopods.

The improved growth at a low level of colonization may also be due to changes in the rhizosphere bacterial community which might be expected to alter nutrient mineralisation from organic matter. Results of Experiment 2 showed that mycorrhizal colonization and salinity affected the bacterial community composition in the rhizosphere. The effect of mycorrhizal inoculation on bacterial community composition is in agreement with previous studies (Paulitz & Linderman, 1989; Marschner *et al.*, 2001; Marschner *et al.*, 2003; Wamberg *et al.*, 2003). The effects of mycorrhizal colonization on the soil microbial community may be due to changes in root carbon exudation (Graham *et al.*, 1981; Dixon *et al.*, 1989), rhizosphere pH changes (Bago & Azcon-Aguilar, 1997; Marschner & Baumann, 2003) and/or exudates of the mycorrhizal fungi (Koide & Kabir, 2000). It could be also speculated that the higher variability in bacterial community composition in inoculated plants is induced by small-scale differences in the viability of the AM hyphae (Olsson *et al.*, 1996) and the patchy AM root colonization.

In Experiment 2 changes in rhizosphere bacterial community composition were correlated with salinity, particularly in inoculated plants at 6 weeks (Fig. 5.6). Changes in soil bacterial community composition by increased salinity is in agreement with the results of other studies. The total number of culturable bacteria has previously been shown to decrease with increased soluble salt concentration (Ragab, 1993). In a study by Polonenko *et al.* (1986) salt stress (1000 and 1500 kPa) caused a significant

reduction of the number of culturable soil bacteria after 7 days, but after more than 7 days of salt stress the number of viable bacteria significantly increased indicating an adaptation to salinity. Salt tolerant bacteria and bacterial adaptation to salinity have been reviewed previously (Zahran, 1997).

Microbial functions are affected by numerous factors such as plant species, growth stage, and soil type and most functions are carried out by a large number of microbial species acting together (Zak *et al.*, 1994). Therefore changes in the abundance of a single species often do not lead to changes in a specific function (Nannipieri *et al.*, 2002; Avrahami *et al.*, 2003; Miethling *et al.*, 2003). On the other hand, it has been shown that changes in microbial community composition can affect a specific function (e.g. enzyme activity) (Kandeler *et al.*, 2002; Avrahami *et al.*, 2003; Marschner & Baumann, 2003). While many functions such as mineralization are carried out by a large number of different soil microorganisms there are certain functions such as N_2 fixation and nitrification that are only performed by a very limited number of species. It may be speculated that changes in rhizosphere bacterial community composition of *A. nummularia* induced by AM fungi inoculation may improve availability of nutrients and then plant growth.

In summary, the results indicate that salt stress had no effects on AM colonization in *A. nummularia* and inoculation with mycorrhizal fungi increased plant growth and nutrient contents of young seedlings in saline conditions. The improved growth and nutrient uptake could be due to direct effects of AM on plant nutrient uptake and also indirect effects via AM-induced changes in the bacterial community composition. These results may be of practical importance as they highlight the potential of using mycorrhizal inoculation to revegetate saline lands.

CHAPTER 6 - EFFECTS OF MYCORRHIZAL FUNGI ON MOBILITY OF PHOSPHORUS DURING LEACHING OF REPACKED COLUMNS OF A SOIL WITH LOAMY SAND TEXTURE IN SALINE CONDITIONS

Previous chapters have considered the effects of AM colonization on plant responses to salinity: this chapter discusses effects of AM colonization on soil properties. Two experiments are described. The first investigates the effects of *T. subterraneum* inoculated or not inoculated with AM fungi on P leaching through a repacked core from localized P sources under non-saline conditions. The second experiment investigates the effect of saline conditions and mycorrhizal inoculation on the mobility of P in cores planted with *T. subterraneum*. Phosphorus leaching is an environmental issue, particularly in coarse textured soils, where P has to be added to stimulate or maintain plant growth (e.g. in some parts of Iran). In these situations there is a risk of P leaching both laterally and vertically. Hence the effects of mycorrhizas both in stimulating plant growth and potentially reducing leaching could be important.

The main hypothesis in this chapter is that AM plants scavenge P more effectively than NM plants and thereby reduce leaching.

6.1 Introduction

Excessive applications of P fertilizer on high P soils or soils with poor P-fixing capacity may result in significant water degradation if P leaves the site and contaminates surface and ground water. Vertical leaching of P has been reported in very sandy soils (Ozanne *et al.*, 1961; Bolland & Gilkes, 1998). High P fertilizer use in crop production has resulted in total P contents in many agricultural lands that are more than twice those of 50 years ago (Tunney, 1992). Only 10-30% of applied P fertilizer is used by plants during the first year (McLaughlin *et al.*, 1991) and a large proportion of applied P is

fixed by soil or lost via flow through the soil profile. Phosphorus may be lost from agricultural lands by three different processes; surface runoff, erosion and leaching. Because of the high P fixation capacity of agricultural soils, it has long been considered that leaching of P from the soil profile to water is negligible (Brookes *et al.*, 1997). Although the impacts of such P losses are relatively small they are environmentally significant and receiving increasing attention (Sharpley *et al.*, 2001; Brye *et al.*, 2002; Toor *et al.*, 2003).

The application of P fertilizer above 60 mg/kg (Olsen-P) has been shown to result in a rapid increase in total P concentration in leachate from an experimental wheat field at Rothamsted (Heckrath *et al.*, 1995), which indicates the importance of P flow-through in one soil profile. Even a small quantity of P movement from the soil to the aquatic environment could significantly change the quality of fresh water, particularly as an extremely low level of P (0.02-0.035 mg/l) triggers eutrophication processes in fresh water (Vollenweider, 1975; Sharpley & Rekolainen, 1997).

Increased growth of AM plants compared with non-mycorrhizal plants has largely been related to increased P uptake and better P nutrition. Exploration of a large soil volume (Tinker, 1978), faster movement of P into AM hyphae (Sanders & Tinker, 1971; Cress *et al.*, 1979; Bolan *et al.*, 1987b) and soil P solubilization (Hetrick, 1989) are reported as the mechanisms for increased P uptake in AM plants. AM fungal hyphae contribute to absorption and translocation of P from distances and soil pores that are not accessible to plant roots (Sanders & Tinker, 1971). Furthermore, AM hyphae reduce levels of available P up to 12 cm away from roots, compared with a depletion zone around non-mycorrhizal plants of a few millimetres (Li *et al.*, 1991; George *et al.*, 1992a). The contribution of AM hyphae to P uptake can be at least 13-20% of total plant P in *Zea mays* (Kothari *et al.*, 1990) and more than 75% in *Trifolium repens* (Li *et al.*, 1991).

Recently it has been shown that 100% of plant P in *Linum usitatissimum*, *Lycopersicon esculentum* and *Medicago truncatula* can delivered via AM hyphae (Smith *et al.*, 2003). These effects on P capture might reduce P flow-through in soil profiles. However, this has not been investigated directly. The only related work is the effect of AM plants in reducing leaching of nitrate (Haines & Best, 1976), but in this case the roles of AM fungi themselves could not be evaluated because M and NM plants were of markedly different size.

The potential of AM fungi to improve soil aggregation and stability, and hence decrease soil nutrient losses through leaching, has been explained in Chapter 1. These improvements, together with effective removal of nutrients by fungal hyphae, might be expected to reduce leaching vertically and laterally through the soil profile. However the ability of AM fungi to control nutrient movement in soil has received almost no attention. The experiments described in this chapter investigated the effects of mycorrhizal fungi on mobility of P under leaching of repacked columns of a loamy sand soil in both non-saline and saline conditions.

Preliminary experiments were undertaken to optimise setting up of cores with non-calcareous Ferries McDonald loamy sand used in previous experiments (results not shown). The results showed that the leachate contained a brown colour that interfered with P analyses by the autoanalyzer. Different methods (freezing, centrifuging and filtering) were tested to remove the colour without affecting P content in leachate, but the results were not satisfactory. As a consequence, three different soils were collected and their structure, water content, electrical conductivity, pH, organic matter, P absorption capacity and available P were analysed (results not shown). A loamy sand soil from Monarto area was selected for the work described in this chapter.

Because of the potential differences in fixation and leaching of different P fertilizers (rock phosphate, superphosphate and NaH_2PO_4) in the core profile a preliminary investigation was carried out to compare their fixation and leaching in cores without plants. This experiment showed how much P fertilizer was fixed in the soil, how much was available for plant uptake and how much appeared in leachate, under different levels of application of each fertilizer. After selection of an appropriate P fertilizer, a preliminary experiment was carried out to compare the effects of different levels and methods of fertilizer application on soil available P and dissolved P in leachate. The methods of application tested were (1) addition as solution with irrigation water or (2) mixing with soil. In the second case P was either mixed in the soil at the top of the core or applied as a thin layer in the middle.

Because of different infectivity of AM fungi in different soils (Chapter 3.3), the infectivity of *G. intraradices* on *T. subterraneum* in the selected Monarto soil was investigated in another preliminary experiment. The results are not presented in detail. The findings are briefly summarised;

- NaH_2PO_4 was selected as the P fertilizer to be used, because of its solubility and movement in the soil profile.
- A thin layer of soil well-mixed with P (as NaH_2PO_4 solution) in the middle of the P treated core was selected as the method of P application.
- *T. subterraneum* was well colonized (75% root length) by *G. intraradices* 10 weeks after transplanting. This fungus was therefore selected.
- Filtering the leachate compared with freezing or centrifuging produced a reliable result for dissolved P measurement by the autoanalyzer.

6.2 Materials and methods

6.2.1 Soil properties

The soil (not previously described) was collected from Monarto area of South Australia (Chapter 2.1) and had a loamy sand surface texture. It was collected near a stream channel and is mapped and assessed as minor alluvial of recent origin (Chittleborough *et al.*, 1976). It was prepared for the experiments as explained in Chapter 2.1. Soil was composed of 80% sand, 7.5% silt, 12.5% clay, and 0.63% organic matter, and had a pH 7.69; electrical conductivity (EC_e) 0.25 dS/m; 69.3% P absorption capacity; 11.4 mg/kg P (Colwell, 1963) and 14.5% water content at field capacity.

6.2.2 Experiment 1. Effects of mycorrhizal fungi on mobility of P under leaching of repacked columns of a loamy sand soil in non-saline conditions

This experiment compared the effects of pre-inoculated and non-inoculated *T. subterraneum* seedlings on P leaching from soil cores. Matched M and NM seedlings of *T. subterraneum* were produced as explained in Chapter 2.6. Three seedlings were transplanted into each core. The 150 mm x 400 mm PVC tubes for the cores were constructed in three sections: irrigation system, column and cap, which were packed and set up according to the method developed by Kirkby (1997) (Fig.6.1) and modified as follows;

1. A cap with a hole, which was placed at the bottom of the column to collect the leachate, was used to cap the core.
2. A 2 mm plastic mesh sieve with a thin layer of acid-washed coarse sand (approximately 2 cm) was placed at the bottom of the cores to facilitate collection of small soil particles and prevent movement with the leachate (section D).
3. 5 kg of autoclaved soil was packed up to 20 cm height by shaking the core (section C).

4. In P-treated cores, 0.5 kg (approximately 3 cm) of autoclaved soil was well mixed with P fertilizer (see below) and added at the top of section C (section B). The same amount of soil without P was used in non-P treated cores.
5. Seedlings were transplanted at the top of the P layer and 1.5 kg autoclaved soil was added into the core to bed the seedlings (approximately 10 cm) (section A).
6. Each core was fitted with an irrigation system composed of a head with hypodermic needles.

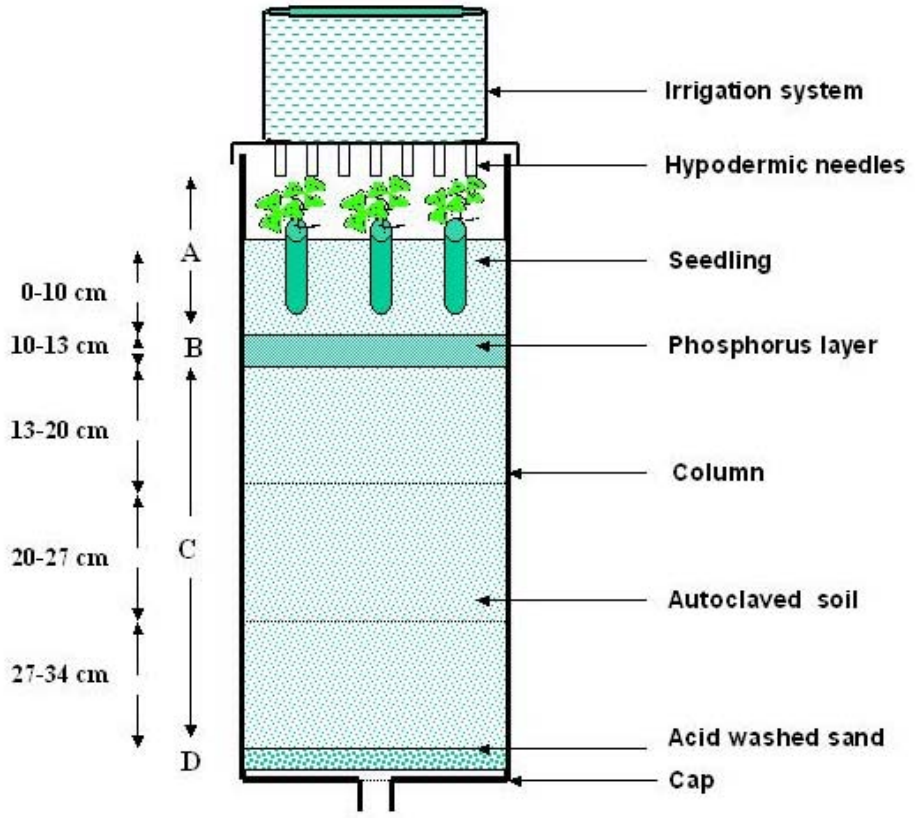

Fig 6.1 Diagram of core set-up (see text for extended description of layers and method of construction).

Layer B was prepared by thorough mixing of 1.5 g NaH_2PO_4 (as solution) with one kg soil (300 mg P per kg soil) and incubated for 2 weeks before use. After seedlings were transplanted, cores were irrigated with R.O. water to 80% of field capacity of the top layer (A) for three weeks to allow the plant roots to penetrate the soil profile (B and C) with minimum P leaching from the B layer. Cores were then irrigated at 80% of field capacity of the whole soil for 10 weeks. The plants were grown in a glasshouse with

natural light in May 2003 (late autumn). Leachate was collected at the end of the experiment by adding 2500 ml R.O. water in three steps (850, 650, and 1000 ml) during 12 hours. During final irrigation, the tops of the cores were completely covered by the flat bottom of the irrigation system. Because of the design of the irrigation system, no water was lost via evapotranspiration from shoots and soil surface during irrigation.

The leachate was filtered (0.45 μm) and the amounts of dissolved P were measured by autoanalyzer. Ten weeks after transplanting, cores were cut into 5 sections (0-10, 10-13, 13-20, 20-27, 27-34 cm), then after separation of the roots by sieving, available and total P were measured (Chapter 2.13) in different sections of each core. Sub-samples of plant roots were washed carefully and stained with trypan blue, and mycorrhizal colonization was evaluated for each core section (Chapter 2.9 and 2.10). Harvested shoots and roots were dried at 80°C for 48 hours and weighed, and shoot and root P concentration and content were then determined (Chapter 2.12). The experiment had a randomised complete block design with 4 treatments; inoculated seedlings in cores without P layer (M-P), inoculated seedlings in cores with P layer (M+P), non-inoculated seedlings in cores without P layer (NM-P) and non-inoculated seedlings in cores with P layer (NM+P), with three replicates. Probabilities of significance among treatments and interactions and LSDs ($P<0.05$) were used to compare means within and among treatments.

6.2.3 Experiment 2. Effects of mycorrhizal fungi on mobility of P under leaching of repacked columns of a loamy sand soil in saline conditions

This experiment compared the effects of pre-inoculated and non-inoculated *T. subterraneum* seedlings on P leaching in an experiment with soil cores in saline

compared with non-saline conditions. The same materials and methods as Experiment 1 were used in this experiment with the following modifications:

- Saline conditions were produced by thorough mixing of 0.5g NaCl per kg of the soil (as solution) and incubated for 2 weeks before use.
- In P- treated cores, 1 kg of soil containing 400 mg P (2 g NaH_2PO_4 per kg soil) was used as the P layer (B-see fig.6.1).
- 2 seedlings were transplanted into each core.
- External hyphal length was measured in each soil layer (Chapter 2.11).
- Because of a leaf disease, plants were harvested at 8 weeks.
- The plants were grown in December 2003 (Summer).

The experiment had a randomised complete block design with 8 treatments; inoculated seedlings in cores without P layer (M-P), inoculated seedlings in cores with P layer (M+P), non-inoculated seedlings in cores without P layer (NM-P) and non-inoculated seedlings in cores with P layer (NM+P), in 2 levels of salinity (0 and 0.5 g NaCl per kg of soil) with four replicates.

6.3 Results

6.3.1 Results of Experiment 1

Colonization

No mycorrhizal colonization was found in non-mycorrhizal plants (results not shown). Mycorrhizal colonization decreased with increasing soil depth in both M-P and M+P plants (Fig. 6.2) M-P plants had significantly higher colonization than M+P plants to 20 cm, but no significant differences were found below 20 cm.

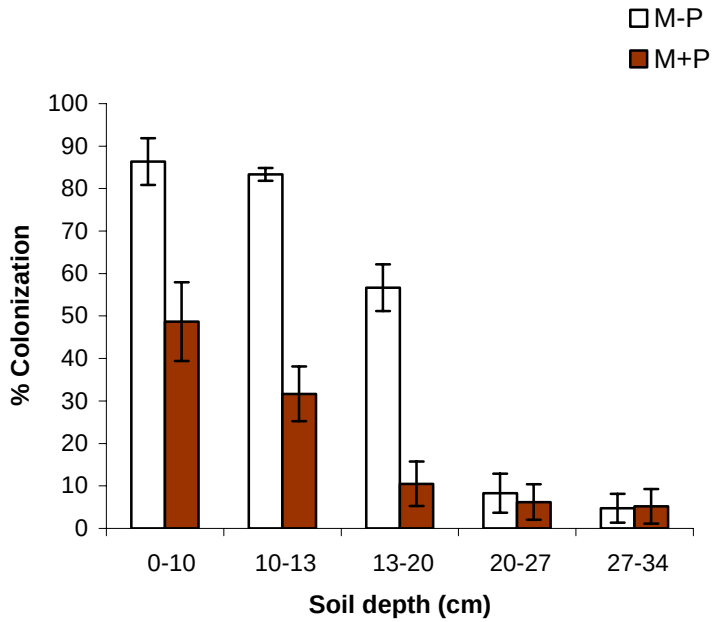

Fig 6.2 Experiment 1. Colonization of roots of *Trifolium subterraneum* at different depths grown in cores with different treatments; mycorrhizal (M-P) and mycorrhizal with P added (M+P), 10 weeks after transplanting. Vertical bars represent standard error of the means, n=3.

Plant growth

Shoot (SDW) and root dry weights (RDW) of plants in different treatments at 10 weeks are shown in Fig. 6.3. Both P application and AM inoculation significantly increased shoot and root dry weight compared with NM-P treatment. SDW and RDW of M+P and NM+P plants had the same values and were higher than M-P plants; M-P plants were larger than NM-P plants. Root distribution in different soil layers in different treatments is shown in Fig. 6.4. Although root distribution varied in 0-10, 10-13 and 13-20 layers, individual treatments showed similar trends in total RDW, NM+P > M+P > M-P > NM-P. Below 20 cm NM+P and M+P RDW tended to decrease, and M-P did not change, so that in the lowest layer M-P had significantly higher root dry weight than other treatments.

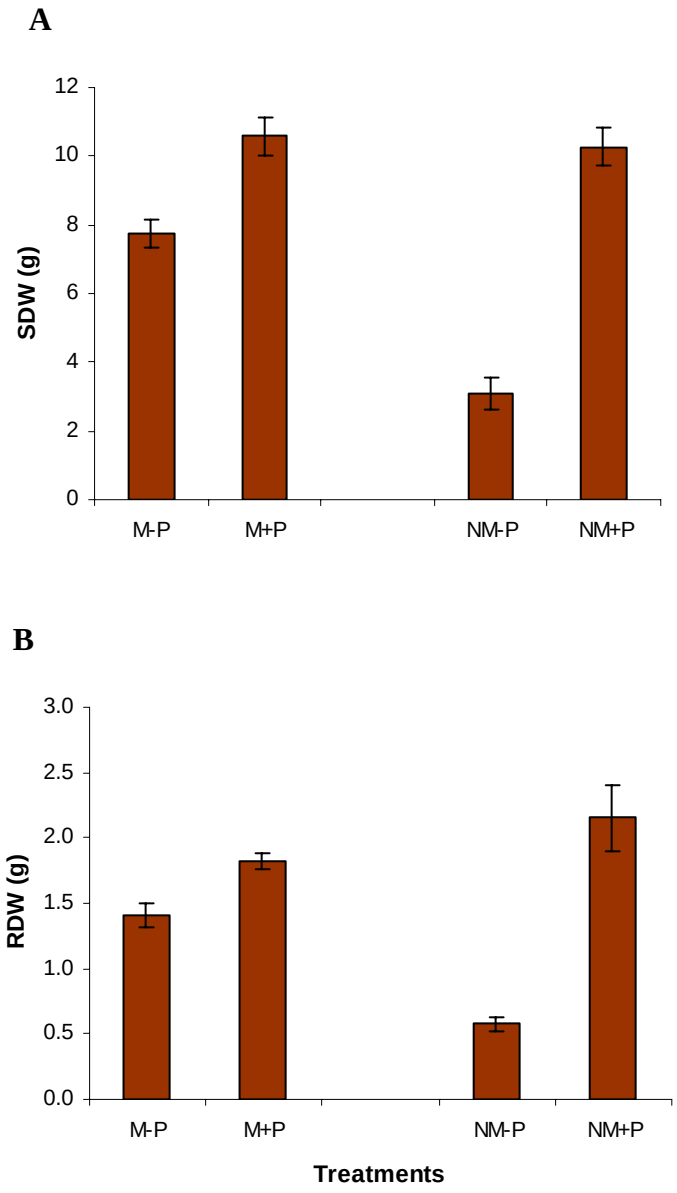

Fig 6.3 Experiment 1. Shoot (A) and root (B) dry weights of *Trifolium subterraneum* grown in cores with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal without P added (NM-P) and non-mycorrhizal with P added (NM+P), 10 weeks after transplanting. Vertical bars represent standard error of the means, n=3.

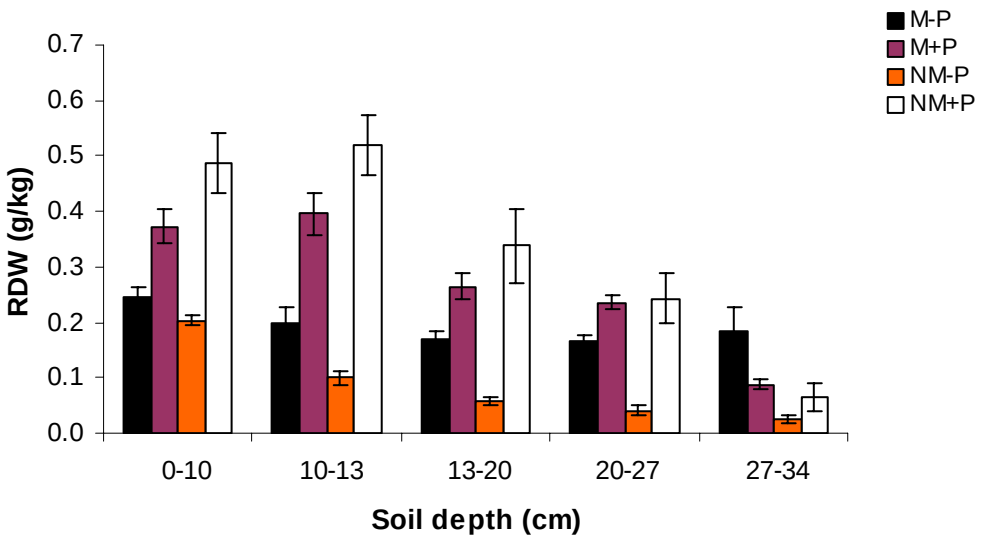

Fig. 6.4 Experiment 1. Root distribution of *Trifolium subterraneum* at different soil depths with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal without P added (NM-P) and non-mycorrhizal with P added (NM+P). Vertical bars represent standard error of the means, n=3.

Plant phosphorus concentration and content

Shoot and root P contents of *T. subterraneum* plants at 10 weeks after transplanting to cores are shown in Fig. 6.5. Overall the M+P and NM+P plants had significantly higher P contents than M-P and NM-P plants in both shoots and roots, and M-P plants took up more P than NM-P plants. Shoots of NM+P plants had higher P content than M+P, but no significant differences were found in their roots.

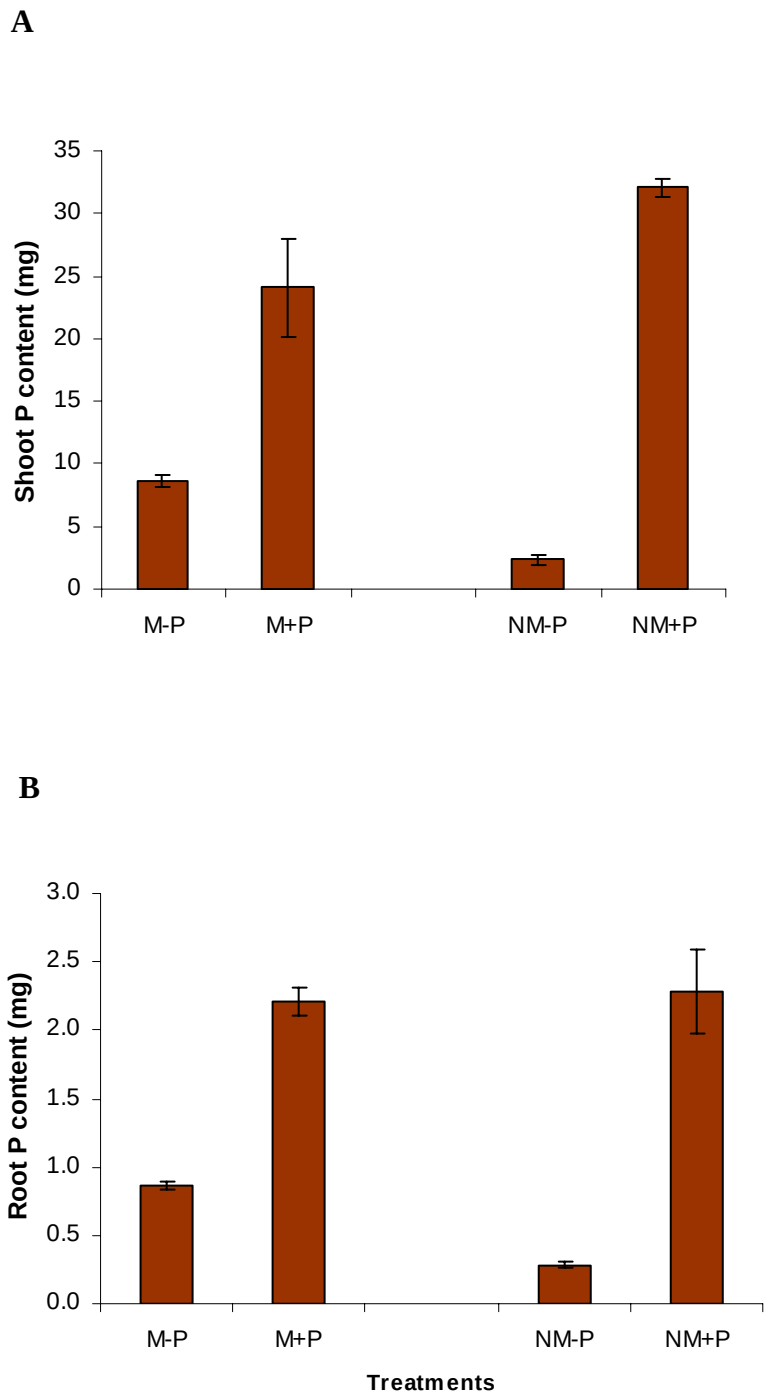

Fig 6.5 Experiment 1. Shoot (A) and root (B) P content of *Trifolium subterraneum* grown in cores with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal without P added (NM-P) and non-mycorrhizal without P added (NM+P), 10 weeks after transplanting. Vertical bars represent standard error of the means, n=3.

Volume of leachate

The amounts of leachate collected after irrigation with 2500 ml of R.O. water are shown in Fig. 6.6. There were marked differences between treatments at the different stages; NM-P cores started to leach after adding 850 ml of water, but no leachate was collected from the other treatments at this stage. After addition of 1500 ml water, M-P cores started to leach, and leachate collected from NM-P cores was twice the amount of that from M-P cores. After adding 2500 ml of water all cores yielded some leachate. By the end of the irrigation NM-P cores had yielded highest volume of leachate, M-P cores were intermediate and both NM+P and M+P, were low and produced the same volume of leachate.

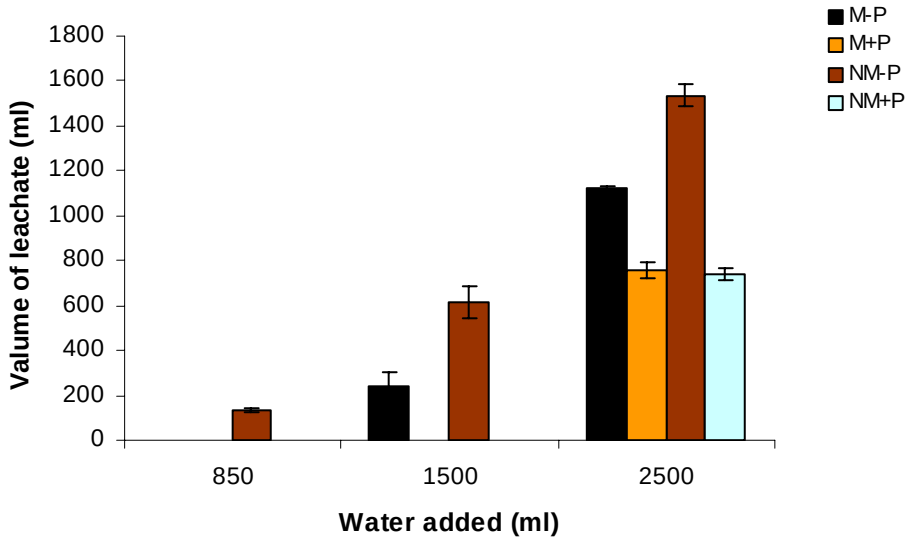

Fig 6.6 Experiment 1. Volume of leachate collected from cores after 10 weeks growth of *Trifolium subterraneum* with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal without P added (NM-P) and non-mycorrhizal with P added (NM+P), after irrigation with 2500 ml R.O. water in three steps (850, 1500 and 2500 ml) during 12 hours. Vertical bars represent standard error of the means, n=3.

Phosphorus in leachate

Leachate collected from M-P treatment had the lowest P concentrations (151 µg/l), and NM-P treatment the highest (302 µg/l); the concentrations of P in leachates from M+P and NM+P treatment were intermediate and similar (193 and 220 µg/l respectively). Total dissolved P in leachates is shown in Fig. 6.7. Cores with NM-P treatments had the highest total P in the leachate, and there were no differences between the other treatments.

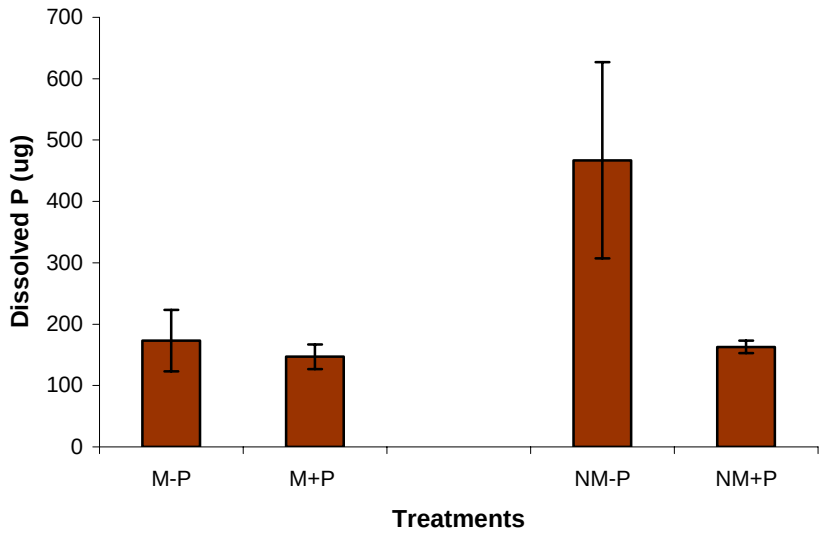

Fig 6.7 Experiment 1. Total dissolved P in leachate from cores after 10 weeks growth of *Trifolium subterraneum* with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal without P added (NM-P) and non-mycorrhizal with P added (NM+P), after irrigation with 2500 ml R.O. water during 12 hours. Vertical bars represent standard error of the means, n=3.

Phosphorus in soil

Available and total P in different soil layers in different treatments at the end of the experiment are shown in Fig. 6.8. Soils from M-P treatments showed the lowest levels of available P in the different layers. The highest available P was in the P layer between 10-13 cm in all P-treated cores. In this layer available P in NM+P treatment was 60 mg/kg and in M+P treatment 50 mg/kg. The average of amounts of available P in sum of different layers of each core of M-P, M+P, NM-P and NM+P treatments were 5.4, 19.2, 11.1 and 25.8 mg/kg respectively. Analyses of total P in different soil layers in different treatments did not show a consistent trend, but higher P in the P-treated layer was found in M+P and NM+P treatments (Fig. 6.8).

Phosphorus budget

Available P budget in M-P and NM-P cores was calculated to investigate the effects of AM inoculation on available P status in the soil (Table 1). M-P plants removed more available P than NM-P plants from the cores, and available P remaining in soil after leaching in M-P was lower than NM-P. Furthermore, the percentage of the P removed by leachate in M-P cores was lower than NM-P.

Table 6.1 Experiment 1. Available P budget in cores after 10 weeks growth of *Trifolium subterraneum* with different treatments; mycorrhizal without P (M-P) and non-mycorrhizal without P added (NM-P), after irrigation with 2500 ml R.O. water during 12 hours.

	M-P	NM-P
P removed by plant (%)	11.1	2.8
P removed by leachate (%)	0.20	0.55
P remaining available in soil (%)	47.0	96.0

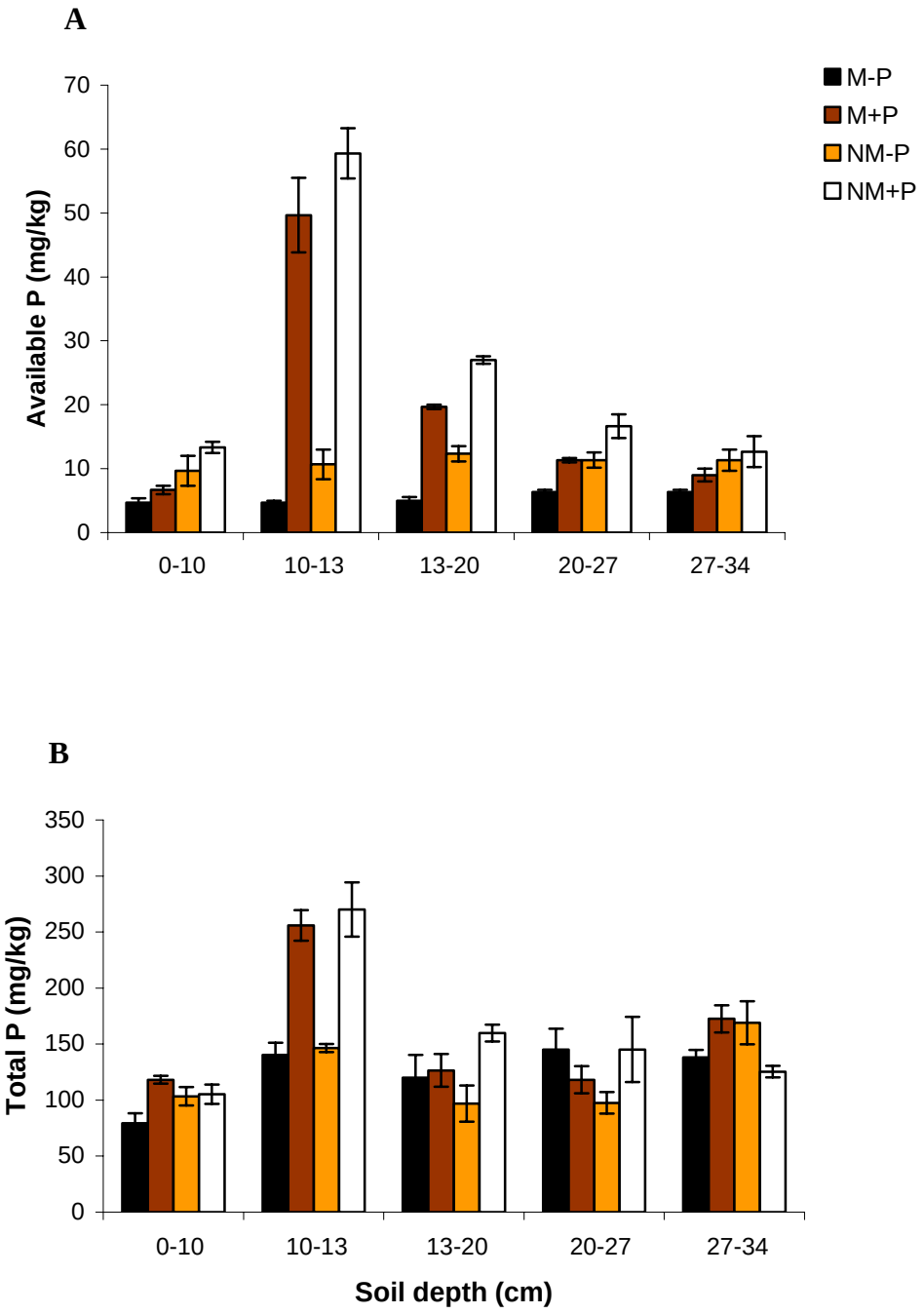

Fig 6.8 Experiment 1. Soil available (A) and total (B) P in different soil layers from cores after 10 weeks growth of *Trifolium subterraneum* with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal without P added (NM-P) and non-mycorrhizal with P added (NM+P), after irrigation with 2500 ml R.O. water. Vertical bars represent standard error of the means, n=3. In P added treatments, P was added in the 10-13 cm layer.

In summary, the results showed that AM inoculation and/or P application increased plant size. As expected leachate collected from cores treated with P and/or AM fungi (M-P, M+P and NM+P) had significantly less dissolved P than untreated (NM-P) cores regardless of P application. M+P and NM+P plants had similar dry weights and P contents (i.e. they could be considered as matched plants), but available P in different layers of the soil (except the lower layer) in M+P cores was significantly lower than NM+P cores. No significant differences were found in P removal from the cores treated by P addition (M and NM). However in the absence of P addition, AM inoculation significantly decreased total volume and total dissolved P in leachate, compared with non-treated cores.

6.3.2 Results of Experiment 2

Colonization and external hyphae

Percentage of root length of colonized and length of external hyphae in different treatments and at different depths are shown in Figs. 6.9 and 6.10 respectively. No AM colonization was found in non-mycorrhizal plants (results not shown). Increased soil depth decreased AM colonization in both M-P and M+P plants and at both salinity levels. Salinity did not decrease colonization in either M-P or M+P plants. M-P plants had significantly higher colonization than M+P plants at 10-16 and 16-22 cm layers in low salinity and 0-10, 10-16 and 16-22 cm layers in high salinity (Fig. 6.9).

With low salinity, length of external hyphae was significantly higher in the M-P treatment than those the non-mycorrhizal ‘background’ hyphae in the NM-P treatment particularly at 10-16 and 16-22 cm layers. M+P treatment had very similar length densities of external hyphae to non-inoculated treatments. Hyphal length density

decreased with depth and in the 10-16 and 16-22 cm layers, the length of external hyphae in non-inoculated cores was generally less than 20% of external hyphal length in M-P cores (Fig. 6.10).

Increased salinity decreased length of external hyphae in M-P treatment, but it still had significantly higher hyphal length density than NM-P treatment. An unexpectedly high length density of external hyphae was found in M+P below 22 cm in high salinity compared to the same treatment at the same depth in low salinity.

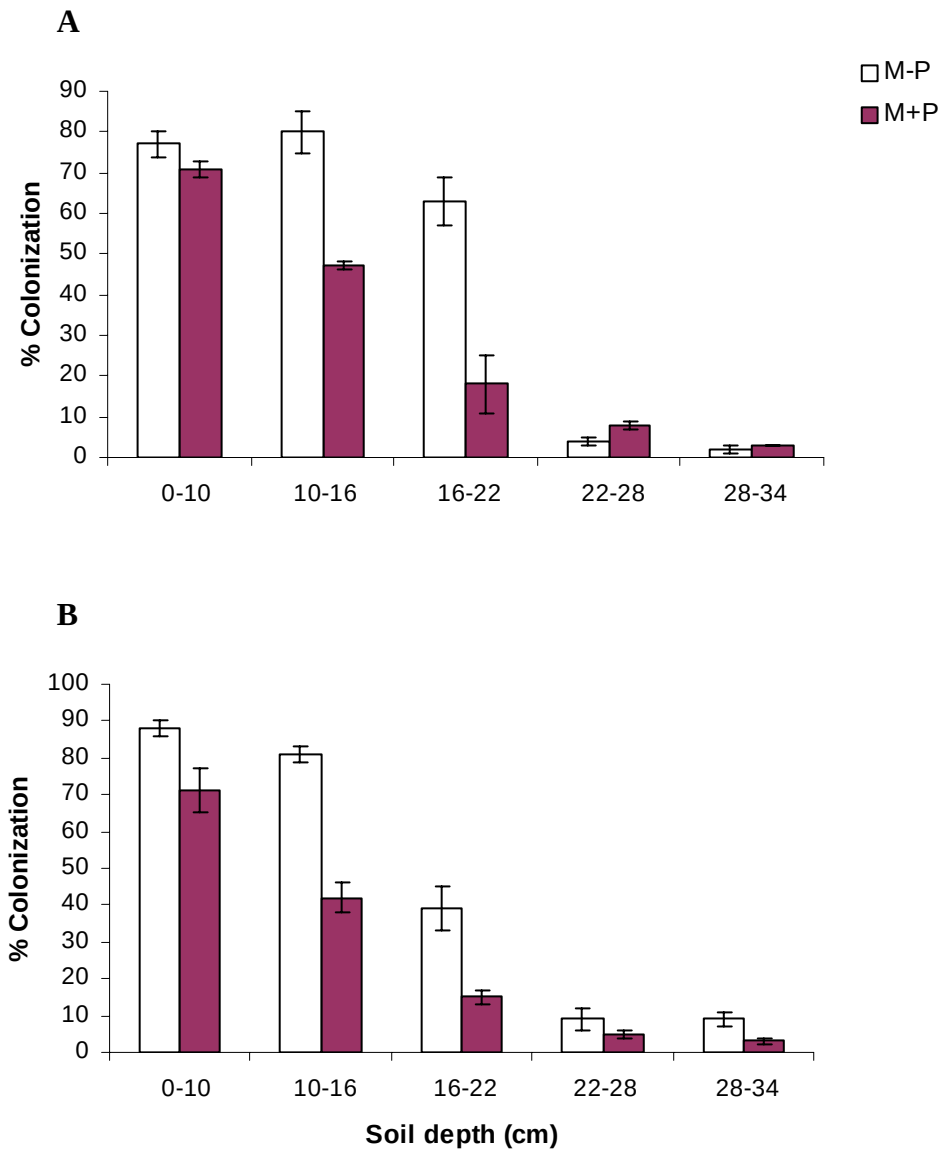

Fig 6.9 Experiment 2. Percentage of root length colonized of *Trifolium subterraneum* at different depths grown in cores with different treatments; mycorrhizal (M) and mycorrhizal with P added (M+P), 8 weeks after transplanting at low (A) and high (B) salinity levels. Vertical bars represent standard error of the means, n=4.

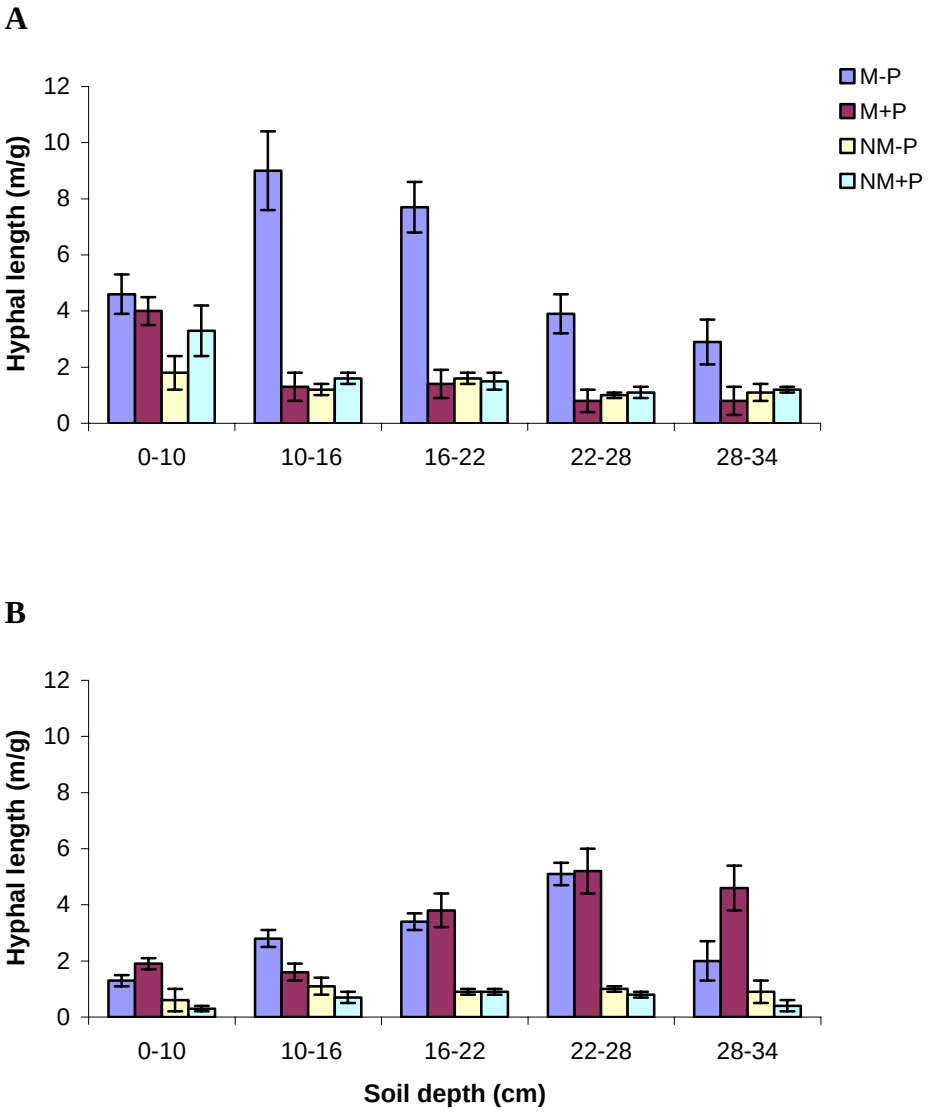

Fig 6.10 Experiment 2. Length density of external hyphae associated with *Trifolium subterraneum* at different depth grown in cores with different treatments; mycorrhizal (M) and mycorrhizal with P added (M+P), 8 weeks after transplanting at low (A) and high (B) salinity levels. Vertical bars represent standard error of the means, n=4. In P added treatments, P was added in the 10-13 cm layer.

Plant growth

At low salinity M-P plants had higher SDW than NM-P plants, but M+P and NM+P plants were similar and had higher SDW (Fig. 6.11). At the same salinity level, the same trend was found in RDW for M-P and NM-P, but NM+P had higher RDW than M+P plants. Increased salinity decreased growth of M-P and NM-P plants, but no significant effect was found in M+P and NM+P. However, M-P plants had higher SDW and RDW than NM-P at high salinity; no differences were found between M+P and NM+P plants.

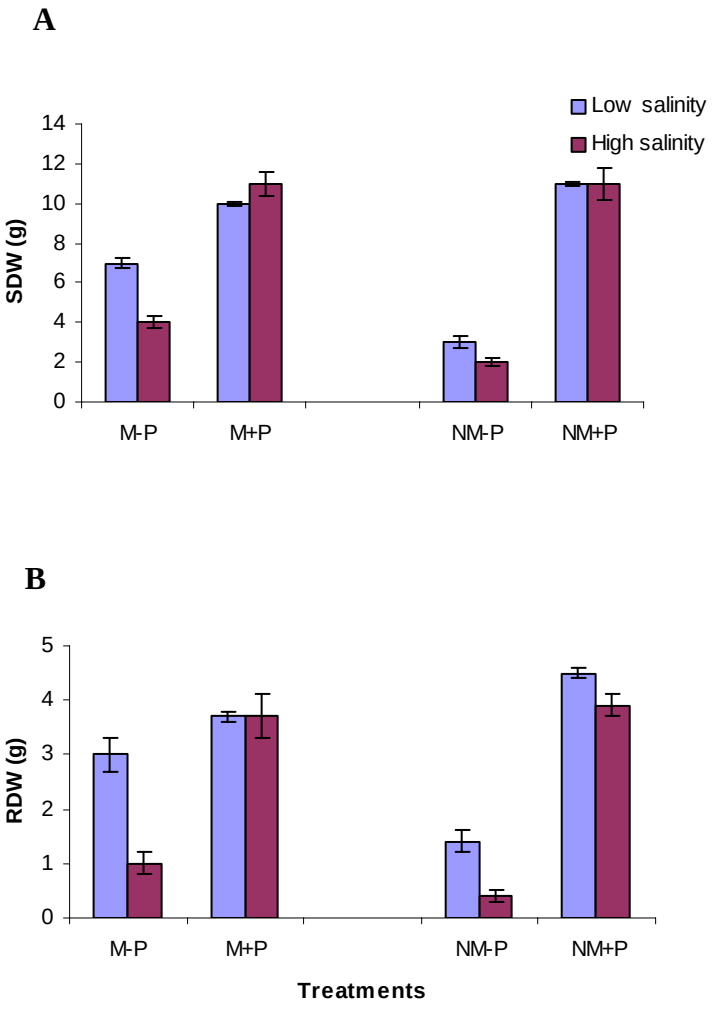

Fig 6.11 Experiment 2. Shoot (A) and root (B) dry weights of *Trifolium subterraneum* grown in cores with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal without P added (NM-P) and non-mycorrhizal with P added (NM+P), at low and high salinity levels, 10 weeks after transplanting. Vertical bars represent standard error of the means, n=4.

Shoot phosphorus content

Shoot P contents of *T. subterraneum* plants at 8 weeks after transplanting to cores in high and low salinity are shown in Fig. 6.12. Overall the shoots of M+P and NM+P plants had significantly higher P contents than those M-P and NM-P plants at both salinities. M-P plants had significantly higher shoot P content than NM-P plants at both salinities. M+P plants had higher shoot P content than NM+P plants at low salinity, but had the same values at high salinity. Increased salinity significantly decreased shoot P content in M+P and NM+P plants.

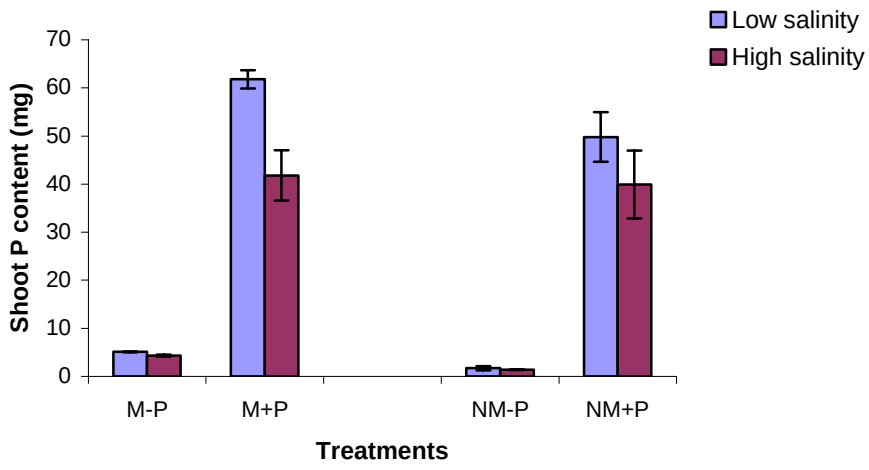

Fig 6.12 Experiment 2. Shoot P content of *Trifolium subterraneum* grown in cores with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal without P added (NM-P) and non-mycorrhizal with P added (NM+P), at low and high salinity levels, 10 weeks after transplanting. Vertical bars represent standard error of the means, n=4.

Volume of leachate

Volumes of the leachate collected from cores are shown in Fig. 6.13. Salinity increased the volume of leachate in all treatments, apart from M+P for which there was no change. NM-P treatments had the highest volume of leachate, M+P and NM+P had similar values and the lowest, and M-P treatment was intermediate at both salinity levels.

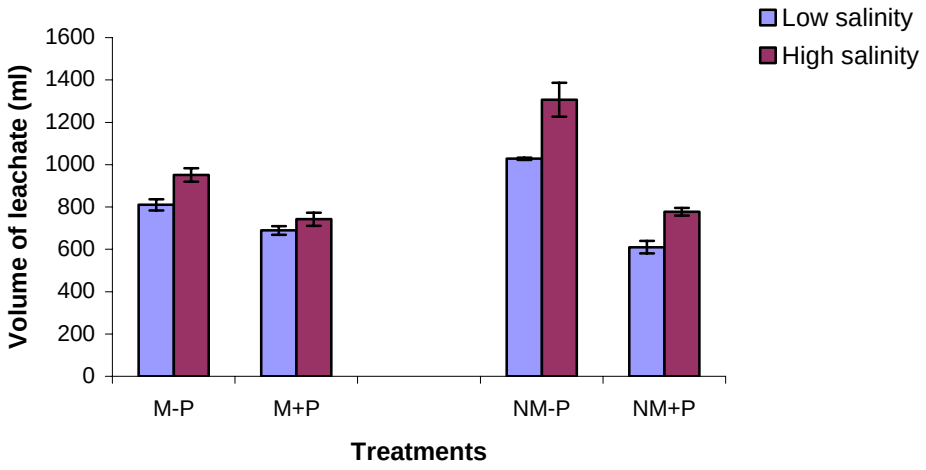

Fig 6.13 Experiment 2. Total volume of leachate collected from cores after 8 weeks growth of *Trifolium subterraneum* with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal without P added (NM-P) and non-mycorrhizal with P added (NM+P), in low and high salinity, irrigated with 2500 ml R.O. water during 12 hours. Vertical bars represent standard error of the means, n=4.

Phosphorus in leachate

Amounts of dissolved P in leachate (Fig. 6.14) were highest in NM-P cores compared with other treatments at both salinity levels. The concentrations of P in leachate collected from NM-P cores were higher than other treatments in low salinity, but no significant differences were found in high salinity (results not shown). Leachate of

M+P and NM+P treatments had similar and lower P content than M-P and NM-P at both salinity levels. Salinity increased P content in leachate of NM+P treatment but had no effects on other treatments.

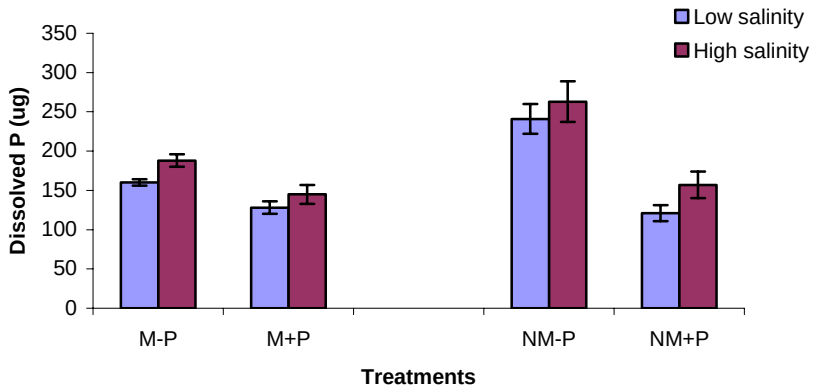

Fig 6.14 Experiment 2. Total dissolved P in leachate collected from cores after 8 weeks growth of *Trifolium subterraneum* with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal without P added (NM-P) and non-mycorrhizal with P added (NM+P) at low and high salinity, irrigated with 2500 ml R.O. water during 12 hours. Vertical bars represent standard error of the means, n=4.

Available phosphorus in soil

Soils without P addition had significantly lower available P in layer B (10-16cm) and below than when P was added (Fig. 6.15). The M-P treatment had lower available P than NM-P in all layers at both salinity levels. Differences were particularly large at 10-16, 16-22 and 22-28 cm layers at low salinity level, but differences were smaller at high salinity. Available P in soil treated with M+P was lower than NM+P in the 10-16 cm layer in low salinity and all layers (apart from 0-10 cm) in high salinity. The average of amounts of available P in sum of different layers of cores of M-P, M+P, NM-P and NM+P in low salinity were 6.4, 34.1, 11 and 38.6 mg/kg, and in high salinity 9.4, 44.9,

13 and 53.8 mg/kg respectively. Salinity had no significant effects on available P in different treatments.

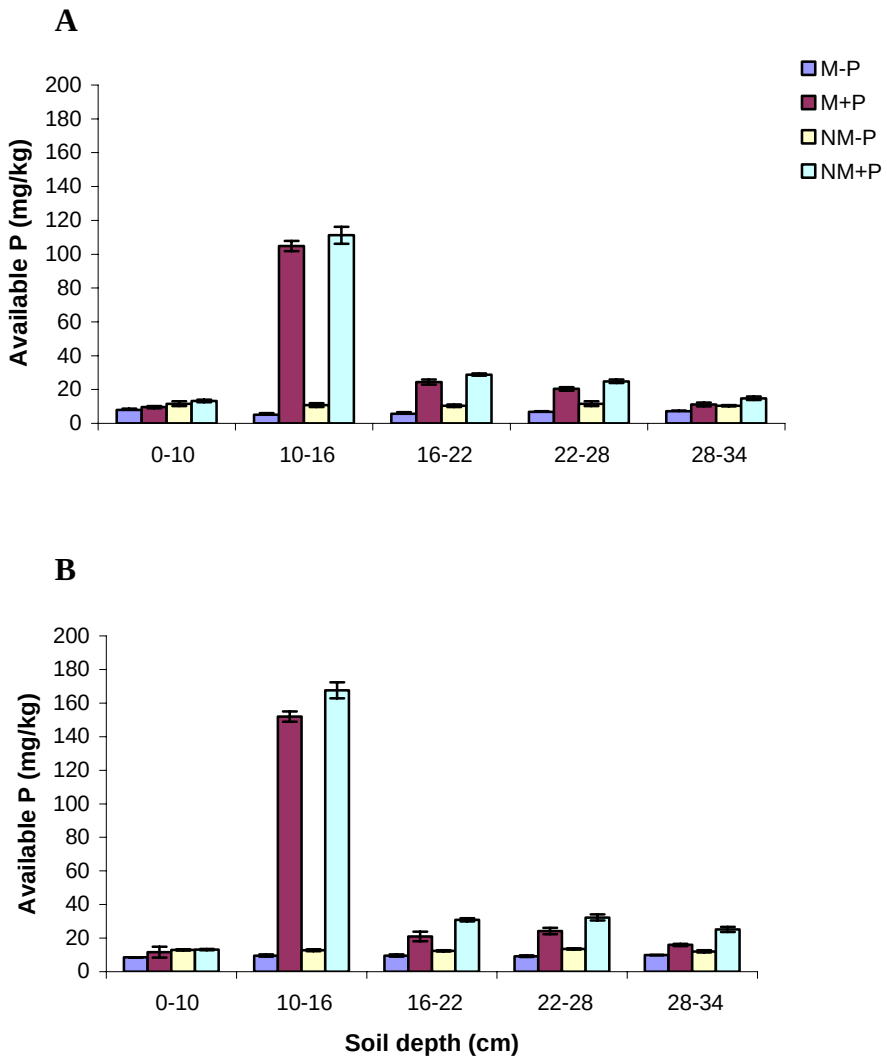

Fig 6.15 Experiment 2. Available P in soil at different depths in cores after 8 weeks growth of *Trifolium subterraneum* with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal without P added (NM-P) and non-mycorrhizal with P added (NM+P), in low (A) and high (B) salinity, after irrigation with 2500 ml R.O. water. Vertical bars represent standard error of the means, n=4. In P added treatments, P was added in the 10-13 cm layer.

In summary, the results of Experiment 2 showed that mycorrhizal inoculation increased plant growth in the absence of P addition, but when P was added inoculated and non-inoculated plants had nearly the same growth. These trends were similar at both salinity levels. Salinity decreased plant growth in both inoculated and non-inoculated plants without P addition, but no differences were found after P addition. Non-inoculated plants without P had the highest amount of leachate and total P removal from the cores in leachate. Inoculation and P addition decreased the volume of leachate and P removal. Salinity increased the volume of leachate in all treatments except M+P. Salinity had the effect of increasing the volume of leachate from non-inoculated compared to inoculated plants regardless of P addition. Salinity increased P content in leachate of NM+P treatment but had no effects on other treatments. Available P in cores containing inoculated plants was significantly lower than non-inoculated plants at both salinity levels. In M-P cores available P was significantly lower in the layers that had higher external hyphae.

6.4 Discussion

Both AM fungi and P increased growth of *T. subterraneum* in low and high salinity, results which are consistent with much previous work on effects of AM and P on growth of clover, including results in Chapter 3. Effects of AM inoculation and P are not discussed further here. Results of both experiments show that, as expected, plants with larger growth (M-P, M+P and NM+P) had higher P content than plants with smaller growth (NM-P). Despite 8 weeks growth in plants of Experiment 2 compared with 10 weeks in Experiment 1, plants in Experiment 2 had higher dry weight than in Experiment 1, which may be related to growth in different seasons. Plants in

Experiment 1 were grown in winter, but in Experiment 2 were grown in summer in glasshouse conditions without additional light. M+P and NM+P plants had the same root and shoot weight (matched plants), and had nearly the same root distribution in the profile (apart from 0-10 cm layer). Larger plants were associated with more effective removal of P into biomass, reduced volume of leachate and reduced total P in leachate. Increased root biomass and extension of roots to deeper soil layers in M plants at low and high salinity may have enabled plants to access available P resources otherwise capable of being removed by leaching, hence reducing the dissolved P in leachate. Previous work has shown that plants with higher root length densities and deeper root distribution could capture more nitrate and decrease nitrate leaching from the soil profile than plants with lower root densities and shallow distribution (Bowman *et al.*, 1998; Dunbabin *et al.*, 2003). In *T. subterraneum* increased surface and volume of the roots induced by AM fungi may lead to increased P uptake, but no effects were found when the P level supplied was sufficient for maximum growth (Bolan *et al.*, 1987a). These results highlighted the role of AM fungi in low P conditions in increasing plant growth and scavenging more P than NM plants, which may contribute to decreasing available P and P loss through leaching. In high P conditions, no clear effect of AM fungi in additional scavenging was found between (matched) M and NM plants.

In Experiment 2 in low salinity, M-P treatment had the highest density of external hyphae (particularly in 10-16, 16-22 and 22-28 cm layers) compared with NM-P treatment. Increased salinity decreased density of external hyphae in M-P treatment. An unexpectedly high density of external hyphae was found in M+P treatment at high salinity. Because of the low level of AM colonization in M+P plants at the middle soil depths it seems that the external hyphae in this treatment could be from other fungi than AM. The external hyphae in non-inoculated cores in Experiment 2 are likely to be from

dead or saprophytic fungi (Drew, 2002; Smith *et al.*, 2004), as there was no mycorrhizal colonization. It is presumed that these hyphae were also present in mycorrhizal treatments. Comparison of Figs. 6.10 and 6.15 show that lower amounts of available P were found at the layers that had higher densities of external hyphae. Increased external hyphal growth in soil enhanced depletion of P from soil by different AM species (Li *et al.*, 1991; Jakobsen *et al.*, 1992; Johansen *et al.*, 1993; Nurlaeny *et al.*, 1996; Liu *et al.*, 2003). In Experiment 2, increased salinity and addition of P decreased external hyphae length density. The negative effects of salinity on AM external hyphae growth have been demonstrated previously (Juniper & Abbott, 1991; McMillen *et al.*, 1998), which could be due to specific ion toxicity or to osmotic stress. These findings may indicate that in low P soil, AM external hyphae could decrease soil available P by scavenging, thereby decreasing the potential for P removal from upper horizons by leaching.

For soils high in P, regardless of the level of salinity, there is no evidence for an effect of external hyphae in scavenging P. This conclusion is supported by reduction of AM colonization and low hyphal densities in P-treated plants (Figs. 6.9 and 6.10). However, this conclusion assumes that roots of M plants have the same uptake capacity as NM plants. In other words, the assumption is that the hyphal pathway and root pathway are additive. However, recent results (Smith *et al.*, 2003; 2004) suggest that P uptake at the root surface can be reduced in mycorrhizal plants and that much of the P enters via the AM pathway. If this is the case, then hyphae (at least) in high salinity treatment (M+P) may have played a role. Clarification would require further work.

In Experiment 1, calculations of the available P in cores with no P applied shows that in M-P cores less P was available at the end of the experiment than in NM-P cores (Table 1). Regardless of differences in root biomass, higher P depletion by uptake by M-P

plants may have been caused by roots and/or external hyphae, but in NM-P plants was caused only by roots. It is very possible that a large amount of available P was taken up by external hyphae in the AM system and stored in external hyphae. This P would not be detectable by bicarbonate extraction nor available for leaching. Storage of a large proportion of absorbed P in external hyphae has been reported previously (Solaiman *et al.*, 1999; Solaiman & Saito, 2001). Lower available P in M-P than NM cores in both experiments and higher proportions of external hyphae in Experiment 2 supports the case for uptake and retention of high amounts of P in AM external hyphae as well as transfer to plants.

In Experiment 2, although salinity decreased root and shoot biomass in M and NM plants in absence of P addition, M-P plants had higher biomass than NM-P plants (Fig. 6.11) and lower amounts of P were lost as leachate (Fig. 6.14). When P was added to the soil there were no differences in the volume of leachate and dissolved P removal between M and NM treatments. Salinity significantly increased dissolved P in leachate of NM-P cores, but had no effects on M-P cores (Figs. 6.13 and 6.14).

Besides the effects of AM fungi on reduction of available P in different layers of the cores due to increased exploration of the soil by roots and/or external hyphae, reduction of water transport through the soil and subsoil seems also to decrease P loss by leaching. In both experiments M plants without P had higher root biomasses than NM and lower volumes of leachate and dissolved P in leachate. Also, NM cores with P addition had higher root biomass, and lower volumes of leachate and dissolved P in leachate than NM-P cores (Figs. 6.6, 6.7, 6.13 and 6.14). Previous work has shown that large consumption of P and water associated with intensive root crop-grain reduced P in the leachate (Leinweber *et al.*, 1999). In this study effects of P (due to plant growth and hence increased evapotranspiration) on reduction of leachate volume was greater

than the reduction of leachate volume through the AM inoculation. However, addition of P increased available P immediately under the P application layer, which is capable of being leached through the cores. The high P adsorption capacity of the soil in lower layers may masked P removal in the leachate. Shorter cores are suggested for the future work to show a clear effect of different treatments.

In summary, AM fungi significantly decreased P leaching from the cores under low P conditions by mechanisms that include increased plant size, spread of roots and spread of hyphae beyond the P depletion zone (the latter process delivering more P to the roots). Addition of P also increased plant growth and decreased P leaching from the soil, but significantly increased soil available P which is capable of being leached later. AM fungi and addition of P increased plant growth in saline conditions and decreased the volume of leachate and dissolved P in leachate. The effects of P were higher than AM inoculation in reducing P loss from the cores at both salinity levels in this experiment. The enhancement of plant growth by P appears to be the reason for lower P loss from the cores. In soil low in P, AM fungi will reduce leaching via (1) enhancement of plant growth, and (2) scavenging and removing P from the soil by roots and/or hyphae (regardless of salinity levels). These effects were not observed in soils high in P. However, there is no evidence for a hyphal effect on soil structure that might affect leaching independently of growth. Testing this hypothesis in the physical effects of AM fungi on P leaching will require the use of undisturbed soil cores and possibly longer growth period, rather than repacked cores in which the structure was largely been destroyed.

CHAPTER 7 - GENERAL DISCUSSION AND FUTURE RESEARCH

7.1 Introduction

The main aim of this study was to evaluate the importance of AM colonization of plants in management of saline soils. The findings presented in Chapter 3-6 will be discussed in this chapter, which will particularly focus on three aspects of AM application in saline soils:

1) Potential of inoculation with AM fungi to improve establishment of non-halophytic plants in saline soils and mechanisms underlying any improvement.
2) Investigation of reports that increased salinity resulted in relatively high AM colonization of the halophytic chenopod *Atriplex nummularia* and potential consequences of this for plant establishment.
3) Roles of plants and AM fungi in influencing P leaching through soil and potential losses to ground water, under both non-saline and saline conditions.

7.2 Discussion

7.2.1 Potential of inoculation with AM fungi to improve establishment of non-halophytic plants in saline soils and mechanisms underlying any improvement

Pre-inoculation of responsive (*T. subterraneum*) and non-responsive (*F. arundinacea*) plants with AM fungi showed different levels of salinity tolerance affected by AM colonization. Overall the results presented in Chapter 3 show that AM pre-inoculation of *T. subterraneum* increased seedling growth and survival in saline conditions.

Although AM pre-inoculation of *F. arundinacea* increased plant growth in low salinity (Chapter 4, Experiment 2), the plant did not get growth or nutrient uptake benefits from AM in saline conditions (Chapter 4). Comparison of the results in Chapters 3 and 4 shows that differences in responsiveness to mycorrhizal colonization (at least in the species used) influence plant growth and establishment in saline conditions. Preinoculation with AM fungi had significant effects on plant salinity tolerance in mycorrhizal responsive *T. subterraneum,* but no increased salinity tolerance was found in the non-mycorrhizal responsive plant *F. arundinacea*. This was partly because of the general lack of responsiveness of *F. arundinacea* and partly because AM colonization was decreased at high salinity in this species. As salinity increased, *F. arundinacea* significantly decreased its association with AM fungi, but no reduction in association was found in *T. subterraneum* (Table 7.1). The mechanisms controlling the extent of AM colonization are not yet understood. If the present results can be generalized, with respect to salinity tolerance, plants with high responsiveness to AM fungi seem to get more benefits from AM association than plants with low responsiveness. More work with a range of plant species would be required to confirm this.

Table 7.1 Mycorrhizal colonization in AM responsive (*T. subterraneum*) and non-responsive (*F. arundinacea*) species at low and high salinity levels in Ferries McDonald soil 60 days after planting

Plant species	Salinity (dS/m)	Colonization (%)
T. subterraneum	**2.2**	84
	12	83
F. arundinacea	**2.2**	26
	12	5

Effects of AM colonization on plant salinity tolerance were explained mainly by increased nutrient uptake, particularly P. The probable reasons for increased nutrient uptake in mycorrhizal plants compared to non-mycorrhizal plants, in saline conditions, are higher soil volume exploration by hyphae and/or roots (Chapter 6), faster nutrient uptake (particularly at an early stage of plant growth) (Chapter 3) and microbial changes in the rhizosphere (Chapter 5). In the AM-responsive plant (*T. subterraneum*) colonization significantly increased P uptake in high salinity, but in the non-responsive plant (*F. arundinacea*) no differences were found between mycorrhizal and non-mycorrhizal plants (Chapters 3 and 4). This finding is consistent with the main mechanism of tolerance being increased plant growth as a result of improved P nutrition. It will be important to explore effects of inoculation and of P supply on the salinity tolerance and establishment of species that are naturally more tolerant than the two investigated in the work described in this thesis.

It has been reported that increased P uptake via AM is equivalent to increased application of P fertilizers with respect to plant growth (abstract reported in Hirrel & Gerdemann, 1978). Both AM inoculation and P application increased plant growth in saline conditions (Chapter 3 and 6), but higher root K/Na ratios at high salinity in mycorrhizal *T. subterraneum* plants (with or without P) than non-mycorrhizal plants (with P addition) and also higher shoot K/Na ratio in M-P plants than in either NM-P or NM+P plants (particularly at late growth stage) (Chapter 3) suggested another possible beneficial effect of AM inoculation compared to P application Furthermore, nutrients other than P also increased in AM *F. arundinacea* compared to NM plants when both plants had the same internal P concentration (Chapter 4). These results tend to confirm previous reports that AM inoculation increases plant salinity tolerance more efficiently

than P application (Poss *et al.*, 1985; Azcón & El-Atrash, 1997). However, again more work is required with a range of species to clarify the apparently conflicting reports of the mechanism(s) underlying AM effects on salinity tolerance. It may well be that the mechanisms vary with plant and fungal species.

7.2.2 Investigation of reports that increased salinity resulted in relatively high AM colonization of the halophytic chenopod *Atriplex nummularia* and potential consequences of this for plant establishment

Overall the results of Chapter 5 showed that despite using different AM fungi, different soils and different growing seasons, salinity had no significant effects on colonization of *A. nummularia* in glasshouse conditions. The low levels of AM colonization in glasshouse conditions compared with the high levels of colonization in field conditions indicate that some specific factors in the field allow the higher levels of AM colonization. The high level of AM colonization in *T. subterraneum* in Ferries McDonald and Kalibar soils under glasshouse conditions show the potential of these soils to support AM colonization. The environmental factors, plant age, plant phenology (Wilson & Hartnett, 1998), soil hydrolytic enzymes (Caroline & Bagyaraj, 1995; Mamatha *et al.*, 2002) and soil microbial community composition are likely affect AM colonization of *A. nummularia* under field conditions. Although the short-term glasshouse experiment did not show any effects of salinity on AM colonization in chenopods, long-term field studies may provide an opportunity to show some effect of salinity on AM colonization in chenopods.

Although only a low level of AM colonization was found in *A. nummularia* under glasshouse conditions in Chapter 5, inoculated plants had higher growth than non-inoculated plants. It is possible that addition of inoculum in AM treatments increased soil fertility and thereby caused higher growth. However, soil analysis of P, N and OM

in inoculated and non-inoculated soils before planting did not show any significant differences between the two soils. This, together with the results of previous studies (Williams *et al.*, 1974; Aldon, 1975; Schmidt & Reeves, 1984), suggests that increased plant growth is caused by AM fungal inoculation. These effects can be attributed to either (1) benefits of the low level of AM colonization (direct effect on plant nutrition) or (2) changes in bacterial community composition (indirect effect), in the rhizosphere of *A. nummularia* as shown in Chapter 5. Such changes could be related to changes in nutrient mineralisation and hence availability. Again, this would need to be examined in additional experiments targeted to different bacterial functional groups. The role of the (low) colonization in directly increasing nutrient uptake could be examined using ^{32}P- labelled phosphate in a compartmented pot system such that ^{32}P was only available to AM fungal hyphae in soil. Such methods have been applied successfully by Jakobsen's group (see, e.g., Smith *et al.*, 2004). From a practical perspective, AM inoculated chenopod seedlings may show improved plant growth and salinity tolerance in revegetation programs of saline soils.

7.2.3 Roles of plants and AM fungi in influencing P leaching in soil and potential losses to ground water, under both non-saline and saline conditions

The results presented in Chapter 6 showed that increased plant size via AM inoculation or P application significantly decreased P leaching from profiles of low P soils. Increased plant size has two effects in reducing P losses vertically and laterally: 1) to increase rooting volume and extend the external hyphal network (only in inoculated plants) and 2) to increase shoot growth and deplete available P from soil. The first effect causes a large soil volume to be explored and thereby aids in the scavenging of P, and reducing P losses vertically. The second effect causes the depletion of more P from

the soil. Increased plant growth (following increased P uptake from the soil) will increase plant cover and thereby decrease surface runoff, and associated P losses. The effects of plant cover (shoot growth) on reduction of soil erosion and P losses in runoff in agricultural and pasture systems have been reported previously (Sharpley & Withers, 1994; Sharpley & Rekolainen, 1997; Bundy *et al.*, 2001; Sharpley *et al.*, 2001). This could be important in saline conditions, where plant cover and vigour are attenuated.

High levels of P addition also increased plant growth but significantly increased soil available P, an affect that then increases risk of P losses via leaching vertically and laterally, especially in coarse textured soils with low sorption capacity. The increased soil P had an inhibitory effect on AM colonization. The negative correlation between high levels of available P and activity of AM fungi has been reported by Hayman (1987) and subsequently by other workers. The results highlight the potential (positive) contribution of AM fungi in the management of saline and non-saline lands with low P levels, as previously suggested for non-P fertilized crop lands (Bethlenfalvay, 1992; Schreiner & Bethlenfalvay, 1995).

The external hyphae of AM fungi in inoculated plants have two main functions in reducing P leaching: a) decreasing soil available P (by retaining P in external hyphae or translocating it into the plants) and 2) improving the soil structure. Chapter 6 produced some results to support the first function, but because of the use of repacked soil cores and short time of plant growth in cores (8-10 weeks), the effects of AM fungi on soil stability and soil structural improvement could not be investigated. The role of external hyphae of AM fungi in promoting soil aggregation and potentially improving soil structure have been documented (reviewed by Miller and Jastrow, 2000) but there has been no work directly investigating the possible improvement of soil structure

following addition of P fertilizer. However, effective removal of P by external hyphae (Kothari *et al.*, 1991; Li *et al.*, 1991; Liu *et al.*, 2003) and results in Chapter 6, together with potential improvement of soil structure and structural stability are expected to reduce P leaching vertically through the soil profile. However, more work with intact cores over a longer time period is suggested to show the effects of AM fungi on soil structure and water conductivity in the soil profile.

In Chapter 6 the effects of AM fungi and salinity on root architecture and soil structure were not investigated. Although no significant differences were found in S/R ratio between different treatments (results not shown), AM inoculated plants had different root distributions than non-inoculated plants in soil profiles (Fig. 6.4). The influence on of root architecture to the extent of capture of nitrate in the soil profile has been reported previously (Bowman *et al.*, 1998; Dunbabin *et al.*, 2003). Using 3D imaging of plant roots and tracer dye techniques could show the effects of AM on root architecture and water transmission and consequent potential effects on nutrient flow-through. AM inoculation in land revegetation programs may increase root growth and distribution in soil profile, both of which are important in reducing nutrient losses via leaching.

7.3 Potential advantages and constraints for application of AM fungi to revegetation of saline environments.

This study showed some advantages of AM inoculation for revegetation of saline lands. To achieve better results in use of AM fungi in revegetation programs of saline lands, the following aspects need to be considered:

Soils:

- The soils must be able to support AM colonisation of the chosen species (see below).

- It will be an additional advantage if the soils have high infectivity, again with respect to the chosen plant.
- Advantages of AM inoculation will be greatest in low P soils.
- Where P losses by erosion or leaching are a problem, AM fungi are likely to provide benefits in increasing plant cover (in low P soils) and removal or immobilisation of available P from/in the profile.

Plants:

- Plants will need to be chosen taking into account the purposes of the revegetation program, such as erosion control, grazing and native vegetation restoration. Selection of native plants may be important in maintaining natural biodiversity and minimizing spread of weeds.
- Benefits of AM fungi are likely to be greatest in responsive plants
- Preinoculation of seedlings may be advantageous and may help to overcome deficiencies in soil infectivity (see above) and salinity damage at early growth stages.
- Chenopods are likely to be valuable in revegetation of saline environments, regardless of possible mycorrhizal effects on growth.

Fungi:

- Selection of indigenous AM species will also maintain biodiversity.
- Selection of locally adapted fungi should be considered.
- Selection of salinity tolerant AM species could be an appropriate route for future research.

Differences in local conditions will mean that locally relevant research may be needed on the soils and the responsiveness of significant plant species.

APPENDICES

Appendix 1. Shoot and root nutrient concentrations of *Trifolium subterraneum* with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal with P added (NM+P) and non-mycorrhizal without P (NM-P), at 20 days after transplanting to different salinity levels. Means of 3 replicates ± standard error

Treatments	Salinity level	Mn	B	Mg	Ca	S	Al	Fe	Zn
		(mg /g)						(µg/g)	
Shoot									
M-P	**Low salinity**	0.2±0.0	0.1±0.0	5.2±0.4	18.7±1.3	4.2±0.5	0.29±0.02	0.2±0.0	21.0±2.3
	High salinity	0.1±0.0	0.1±0.0	5.9±0.0	23.3±1.0	2.8±0.5	0.44±0.03	0.3±0.0	16.0±1.1
M+P	**Low salinity**	0.1±0.0	0.1±0.0	5.6±0.2	17.1±1.0	3.3±0.4	0.57±0.11	0.4±0.1	13.0±0.6
	High salinity	0.1±0.0	0.1±0.0	6.4±0.5	23.3±1.8	2.6±0.3	0.53±0.14	0.4±0.1	18.0±1.7
NM+P	**Low salinity**	0.2±0.0	0.1±0.1	7.9±0.5	20.3±1.5	2.1±0.3	0.63±0.16	0.4±0.1	12.0±0.0
	High salinity	0.1±0.0	0.3±0.0	6.7±0.5	24.2±2.5	1.6±0.2	0.48±0.17	0.3±0.1	14.0±1.1
NM-P	**Low salinity**	0.1±0.0	0.3±0.0	5.7±0.4	15.8±1.0	1.3±0.1	1.49±0.65	1.1±0.4	17.0±6.9
	High salinity	0.1±0.0	0.3±0.0	7.3±0.7	28.3±2.3	1.5±0.0	1.25±0.27	0.8±0.2	13.0±1.7
LSD (*P*<0.05)		0.04	0.1	1.4	4.9	1.0	0.9	0.6	9.0
Root									
M-P	**Low salinity**	0.6±0.1	0.1±0.0	4.9±0.2	5.7±0.3	7.8±0.3	4.60±0.53	2.3±0.3	34.0±3.5
	High salinity	0.4±0.1	0.6±0.1	3.8±0.4	6.3±0.6	5.8±0.9	4.63±0.67	3.3±0.5	31.0±1.7
M+P	**Low salinity**	0.7±0.1	0.1±0.0	5.1±0.3	5.1±0.2	9.2±0.4	4.67±0.60	2.1±0.3	26.0±1.1
	High salinity	0.8±0.2	0.3±0.0	3.7±0.1	5.4±0.4	7.5±1.1	4.60±0.46	2.8±0.4	27.0±1.6
NM+P	**Low salinity**	0.5±0.0	0.2±0.1	7.4±0.7	6.2±0.2	3.6±0.1	7.27±0.03	4.8±0.2	27.0±3.5
	High salinity	0.3±0.0	0.6±0.1	5.0±0.5	12.2±3.6	2.6±0.4	5.47±1.13	3.9±0.8	32.0±10.4
NM-P	**Low salinity**	0.3±0.0	0.7±0.1	4.5±0.3	7.5±0.1	2.1±0.1	5.73±1.32	4.0±0.9	56.0±30.6
	High salinity	0.2±0.0	0.9±0.1	4.7±0.6	14.4±2.3	2.4±0.3	5.37±0.92	3.7±0.7	42.0±10.4
LSD (*P*<0.05)		0.3	0.3	1.3	4.7	1.6	2.6	1.8	38.8

Appendix 2. Shoot and root nutrient concentrations of *Trifolium subterraneum* with different treatments; mycorrhizal (M-P), mycorrhizal with P added (M+P), non-mycorrhizal with P added (NM+P) and non-mycorrhizal without P (NM-P), 40 days after transplanting to different salinity levels. Means of 3 replicates ± standard error

Treatments	Salinity level	Mn	B	Mg	Ca	S	Al	Fe	Zn
		(mg /g)						(µg/g)	
Shoot									
M-P	**Low salinity**	0.1±0.0	0.2±0.0	4.9±0.4	17.8±0.7	1.6±0.1	0.3±0.0	0.2±0.0	19.0±1.7
	High salinity	0.1±0.0	0.2±0.0	6.5±0.4	25.3±1.8	2.7±0.6	0.3±0.0	0.2±0.0	23.0±3.4
M+P	**Low salinity**	0.2±0.0	0.1±0.0	5.7±0.3	20.0±0.0	1.8±0.1	0.3±0.0	0.2±0.0	16.0±1.1
	High salinity	0.2±0.0	0.2±0.0	7.7±0.6	26.7±1.5	3.5±0.2	0.3±0.0	0.2±0.0	15.0±2.3
NM+P	**Low salinity**	0.2±0.0	0.1±0.0	9.9±0.8	23.0±2.0	2.7±0.2	0.5±0.1	0.4±0.0	11.0±1.7
	High salinity	0.2±0.0	0.2±0.0	6.4±0.2	23.7±1.5	1.4±0.2	3.7±1.1	2.7±0.1	21.0±2.3
NM-P	**Low salinity**	0.1±0.0	0.3±0.1	6.3±0.3	19.3±0.4	1.5±0.1	1.6±0.3	1.0±0.2	13.0±1.7
	High salinity	0.2±0.0	0.8±0.2	6.8±0.6	27.7±2.9	1.6±0.2	3.9±1.0	2.7±0.6	18.0±4.0
LSD (*P*<0.05)		0.05	0.2	1.5	5.0	0.8	1.6	1.1	7.4
Root									
M-P	**Low salinity**	0.4±0.1	0.1±0.0	5.4±0.3	5.4±0.1	3.9±0.2	2.5±0.3	1.8±0.2	20.0±0.0
	High salinity	0.5±0.1	0.1±0.0	5.07±0.4	6.1±0.2	5.9±0.2	4.1±1.0	2.8±0.7	26.0±0.6
M+P	**Low salinity**	0.6±0.1	0.1±0.0	6.0±0.3	7.2±1.3	3.8±0.5	3.2±0.2	1.5±0.3	19.0±0.6
	High salinity	0.7±0.2	0.1±0.0	4.8±1.0	7.3±1.0	5.8±0.6	3.6±0.2	1.7±0.7	20.0±1.1
NM+P	**Low salinity**	0.4±0.0	0.1±0.0	8.6±0.7	5.8±0.1	4.7±0.2	3.1±0.7	2.2±0.5	22.0±1.7
	High salinity	0.3±0.0	1.7±0.4	6.0±0.8	19.0±2.5	2.7±0.1	13.5±2.3	9.4±1.3	48.0±5.7
NM-P	**Low salinity**	0.2±0.0	0.3±0.1	7.0±0.7	8.4±0.1	3.9±0.6	3.3±0.6	2.2±0.5	32.0±1.0
	High salinity	0.3±0.0	1.9±0.1	6.0±0.5	26.4±3.6	3.4±0.8	12.4±1.3	8.7±0.1	63.0±14.4
LSD (*P*<0.05)		0.3	0.5	1.6	5.4	1.4	3.8	2.6	19.6

REFERENCES

Abbott LK, Robson AD. 1977. Growth stimulation of subterranean clover with vesicular arbuscular mycorrhizas. *Australian Journal of Agricultural Research* **28**: 639-649.

Abbott LK, Robson AD. 1978. Growth of subterranean clover in relation to the formation of endomycorrhizas by introduced and indigenous fungi in a field soil. *New Phytologist* **81**: 575-585.

Abrol IP, Bhumbla DR. 1978. Some comments on terminology relating to salt-affected soils. In: *Dryland-saline-seep Control*. Edmonton, Canada: 6-19 to 16-27.

Aguilera LE, Gutierrez JR, Moreno RJ. 1998. Vesicular arbuscular mycorrhizae associated with saltbushes *Atriplex* spp. (Chenopodiaceae) in the Chilean arid zone. *Revista Chilena De Historia Natural* **71**: 291-302.

Aldon EF. 1975. Endomycorrhizae enhance survival and growth of fourwing saltbush on coal mine spoils. United States Dept. of Agriculture Forest Service research note Pacific Southwest, RM-294, 2p.

Aliasgharzadeh N, Rastin NS, Towfighi H, Alizadeh A. 2001. Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil. *Mycorrhiza* **11**: 119-122.

Al-Karaki GN. 2000. Growth of mycorrhizal tomato and mineral acquisition under salt stress. *Mycorrhiza* **10**: 51-54.

Al-Karaki GN, Al-Raddad A. 1997. Effects of arbuscular mycorrhizal fungi and drought stress on growth and nutrient uptake of wheat genotypes differing in drought resistance. *Mycorrhiza* **7**: 83-88.

Al-Karaki GN, Hammad R. 2001. Mycorrhizal influence on fruit yield and mineral content of tomato grown under salt stress. *Journal of Plant Nutrition* **24**: 1311-1323.

Al-Karaki GN, Hammad R, Rusan M. 2001. Response of two tomato cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress. *Mycorrhiza* **11**: 43-47.

Allen EB, Allen MF. 1986. Water relations of xeric grasses in the field: interactions of mycorrhizas and competition. *New Phytologist* **104**: 559-571.

Allen EB, Allen MF. 1988. Facilitation of succession by the nonmycotrophic coloniser *Salsola kali* (Chenopodiaceae) on a harsh site: effects of mycorrhizal fungi. *American Journal of Botany* **75**: 257-266.

Allen EB, Cunningham GL. 1983. Effects of vesicular-arbuscular mycorrhizae on *Distichlis spicata* under three salinity levels. *New Phytologist* **93**: 227-236.

Allen MF. 1983. Formation of vesicular-arbuscular mycorrhizae in *Atriplex gardneri* (Chenopodiaceae): seasonal response in a cold desert. *Mycologia* **75**: 773-776.

Allen MF. 1991. *The Ecology of Mycorrhizae.* Cambridge: Cambridge University Press.

Allen MF, Boosalis MG. 1983. Effects of two species of VA mycorrhizal fungi on drought tolerance of winter wheat. *New Phytologist* **93**: 67-76.

Auge RM. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* **11**: 3-42.

Avrahami S, Liesack W, Conrad R. 2003. Effects of temperature and fertilizer on activity and community structure of soil ammonia oxidizers. *Environmental Microbiology* **5**: 691-705.

Azcón R, Barea JM. 1992. The effect of vesicular-arbuscular mycorrhizae in decreasing calcium acquisition by alfalfa plants in calcareous soils. *Biology and Fertility of Soils* **13**: 155-159.

Azcón R, El-Atrash F. 1997. Influence of arbuscular mycorrhizae and phosphorus fertilization on growth, nodulation and N_2 fixation (N^{15}) in *Medicago sativa* at four salinity levels. *Biology and Fertility of Soils* **24**: 81-86.

Bago B, Azcon-Aguilar C. 1997. Changes in rhizosphere pH induced by arbuscular mycorrhiza formation in onion (*Allium cepa* L). *Zeitschrift fur Pflanzenernahrung und Bodenkunde* **160**: 333-339.

Balba AM, Soliman MF. 1978. Quantative evaluation of the relative specific effect of cations on the plant growth and nutrient absorption. Proc. 11th international Soil Science Society Congress, Edmonton, 1: 319-320.

Baon JB, Smith SE, Alston AM. 1993. Mycorrhizal responses of barley cultivars differing in P efficiency. *Plant and Soil* **157**: 97-105.

Barbour MG, Burk JH, Pitts WD, Gillian FS, Schwartz MN. 1998. *Terrestrial Plant Ecology.* Menlo Park, California: Benjamin Cummings.

Barrow JR, Aaltonen RE. 2001. Evaluation of the internal colonization of *Atriplex canescens* (Pursh) Nutt. roots by dark septate fungi and the influence of host physiological activity. *Mycorrhiza* **11**: 199-205.

Barthes B, Roose E. 2002. Aggregate stability as an indicator of soil susceptibility to runoff and erosion; validation at several levels. *Catena* **47**: 133-149.

Baylis GTS. 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. In: Sanders FE, Mosse B, Tinker PB, *Endomycorrhizas*. London, UK: Academic Press. 373-389.

Beaden BN, Petersen L. 2000. Influence of arbuscular mycorrhizal fungi on soil structure and aggregate stability of a vertisol. *Plant and Soil* **218**: 173-183.

Beare MH, Hus S, Coleman DC, Hendrix PF. 1997. Influences of mycelial fungi on soil aggregation and organic matter storage in conventional and no-tillage soils. *Applied Soil Ecology* **5**: 211-219.

Bernstein L. 1962. Salt-affected soils and plants. Problems of the arid zones symposium, Paris, Unesco, 139-147.

Bethlenfalvay GJ. 1992. Mycorrhizae and crop production. In: *Proceedings of a symposium on Mycorrhizae in Sustainable Agriculture. Special publication No. 54*. Asa. 1-27.

Bethlenfalvay GJ, Dakessian GJ, Pakovsky RS. 1984. Mycorrhizae in a southern Californian desert. Ecological implications. *Canadian Journal of Botany* **62**: 519-524.

Bhumbla DR, Abrol IP. 1979. Saline and sodic soils. In: *Proceedings of Soils and Rice Symposium*. IRRI, Los Banos, Languna, Philippines,

Blum A, Johnson JW. 1992. Transfer of water from roots into dry soil and the effect on wheat water relations and growth. *Plant and Soil* **145**: 141-149.

Bolan NS, Robson AD, Barrow NJ. 1987a. Effects of phosphorus application and mycorrhizal inoculation on root characteristics of subterranean clover and ryegrass in relation to phosphorus uptake. *Plant and Soil* **104**: 294-298.

Bolan NS, Robson AD, Barrow NJ. 1987b. Effects of vesicular-arbuscular mycorrhiza on the availability of iron phosphates to plants. *Plant and Soil* **99**: 401-410.

Bolland M, Gilkes R. 1998. The relative effectiveness of superphosphate and rock phosphate for soils where vertical and lateral leaching of phosphate occurs. *Nutrient Cycling in Agroecosystems* **51**: 139-153.

Bowen GD. 1987. The biology and physiology of infection and its development. In: Safir, G R, *Ecophysiology of VA Mycorrhizal Plants*. Boca Raton Florida.: CRC Press. 27-70.

Bowman DC, Devitt DA, Engelke MC, Rufty TW. 1998. Root architecture affects nitrate leaching from bentgrass turf. *Crop Science* **38**: 1633-1639.

Boyden S. 1987. *Western civilization in biological perspective: Pattern in Biohistory.* Oxford: Oxford University Press.

Brain J, Atwell B, Paul E, Kriedemann P, Collin GN. 1999. *Plants in Action: adaptation in nature, performance in cultivation.* South Yarra: Macmillan Education Australia.

Brookes PC, Heckrath G, De Smet J, Hofman G, Vanderdeelen J. 1997. Losses of phosphorous in drainage water. In: Tunney H, Carton OT, Brookes PC, Johnston AE, *Phosphorus Loss from Soil to Water*. Wallingford: CAB International. 253-271.

Brown AM, Bledsoe C. 1996. Spatial and temporal dynamics of mycorrhizas in *Jaumea carnosa*, a tidal saltmarsh halophyte. *Journal of Ecology* **84**: 703-715.

Brundrett M. 1991. Mycorrhizas in natural ecosystems. *Advances in Ecological Research* **21**: 171-313.

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N. 1996. *Working with Mycorrhizas in Forestry and Agriculture.* Canberra, Australia: Australian Centre for International Agricultural Research.

Brye KR, Andraski TW, Jarrell WM, Bundy LG, Norman JM. 2002. Phosphorus leaching under a restored tallgrass prairie and corn agroecosystems. *Journal of Environmental Quality* **31**: 769-781.

Bundy LG, Andraski TW, Powell JM. 2001. Management practice effects on phosphorus losses in runoff in corn production systems. *Journal of Environmental Quality* **30**: 1822-1828.

Campbell EE, Matthewson WJ. 1992. Optimizing germination in *Atriplex nummularia* (Lind) for commercial cultivation. *South African Journal of Botany* **58**: 478-481.

Cantrell IC, Linderman RG. 2001. Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity. *Plant and Soil* **233**: 269-281.

Cardoso E, Navarro RB, Nogueira MA. 2003. Changes in manganese uptake and translocation by mycorrhizal soybean under increasing Mn doses. *Revista Brasileira De Ciencia Do Solo* **27**: 415-423.

Caroline M, Bagyaraj DJ. 1995. Mycorrhization helper bacteria and their influence on growth of cowpea. In: Adholeya A, Singh S, *Mycorrhizae: biofertilizers for the future*. New Delhi: TERI. 192-196.

Carrillo-Garcia A, de la Luz JLL, Bashan Y, Bethlenfalvay GJ. 1999. Nurse plants, mycorrhizae, and plant establishment in a disturbed area of the Sonoran Desert. *Restoration Ecology* **7**: 321-335.

Carvalho LM, Cacador I, Martins-Loucao MA. 2001. Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal). *Mycorrhiza* **11**: 303-309.

Cavagnaro TR, Gao LL, Smith FA, Smith SE. 2001. Morphology of arbuscular mycorrhizas is influenced by fungal identity. *New Phytologist* **151**: 469-475.

Champagnol F. 1979. Relationship between phosphate nutrition of plants and salt toxicity. *Phosphorus in Agriculture* **76**: 255-264.

Chatrath A, Mandal PK, Anuradha M. 2000. Effect of secondary salinization on photosynthesis in fodder Oat (*Avena sativa*) genotypes. *J Agronomy and Crop Science* **184**: 13-16.

Chhabra R. 1996. *Soil Salinity and Water Quality.* USA: Balkema Publisher.

Chittleborough DJ, Maschmedt DJ, Wright MJ. 1976. Soils of the Monarto Town Site. CSIRO Division of Soils, Adelaide. 58.

Colwell JD. 1963. The estimation of the phosphorus fertilizer requirements of wheat in southern New South Wales. *Australian Journal of Experimental Agricultural and Animal Husbandry* **3**: 190-197.

Copeman RH, Martin CA, Stutz JC. 1996. Tomato growth in response to salinity and mycorrhizal fungi from saline or non-saline soils. *Hortscience* **31**: 341-344.

Cordier C, Gianinazzi S, Gianinazzi-Pearson V. 1996. Colonization patterns of root tissues by *Phytophthora nicotianae* var *parasitica* related to reduced disease in mycorrhizal tomato. *Plant and Soil* **185**: 223-232.

Cress WA, Throneberry GO, Lindsey DL. 1979. Kinetics of phosphorus absorption by mycorrhizal and nonmycorrhizal tomato roots. *Plant Physiology* **64**: 484-487.

Cuenca G, De Andrade Z, Escalante G. 1998. Arbuscular mycorrhizae in the rehabilitation of fragile degraded tropical lands. *Biology and Fertility of Soils* **26**: 107-111.

Davies FT, Jr., Potter JR, Linderman RG. 1993. Drought resistance of mycorrhizal pepper plants independent of leaf P concentration-response in gas exchange and water relations. *Physiologia Plantarum* **87**: 45-53.

Degens BP, Sparling GP, Abbott LK. 1994. The contribution from hyphae, roots and organic carbon constituents to the aggregation of a sandy loam under long-term clover-based and grass pastures. *European Journal of soil science* **45**: 459-468.

Delane R, Greenway HJ, Manns R, Gibbs J. 1982. Ion concentration and carbohydrate status of the elongating leaf tissue of *Hourdeum vulgare* growing at high external NaCl. *Journal of Experimental Botany* **33**: 557-573.

Delgado IC, SanchezRaya AJ. 1997. Influence of phosphorus on growth and nutrient transport in sunflower seedlings under saline conditions. *Phyton-International Journal of Experimental Botany* **61**: 117-125.

Dickson S. 1999. *Phosphate transfer efficiency of two arbuscular mycorrhizal fungi*. PhD Thesis, University of Adelaide, Adelaide.

Diederichs C. 1990. Improved growth of *Cajanus cajan* (L.) Millsp. in an unsterile tropical soil by three mycorrhizal fungi. *Plant and Soil* **123**: 261-266.

Dieleman PJ. 1963. Reclamation of salt-affected soils in Iraq. ILRI Pub., Wageningen. *11:* 175.

Dixon RK, Garrett HE, Cox GS. 1989. Boron fertilization, vesicular-arbuscular mycorrhizal colonization and growth of *Citrus Jambhiri* Lush. *Journal of Plant Nutrition* **12**: 687-700.

Douds DD, Gadkar V, Adholeya A. 2000. Mass production of VAM fungus biofertilizer. In: Mukerji KG, Chamola BP, Singh J, *Mycorrhizal Biology*. New York: Kulwer Academic Publisher.

Dregne HE. 1977. Managing saline water for irrigation. Management Saline Water for Irrigation: Planning for Future, Texas Tech Uni, Lubbock, TX, USA., Intern Center for Arid and Semi-arid, pp 618.

Drew E. 2002. *External AM hyphae: their growth and function in media of varying pore size*. Ph.D Thesis, University of Adelaide, Adelaide.

Dudal R, Purnell MF. 1986. Land resources: salt-affected soils. *Reclamation and Revegetation Research* **5**: 1-9.

Duke ER, Johnson CR, Koch KE. 1986. Accumulation of phosphorus, dry matter and betaine during NaCl stress of split-root citrus seedlings colonised with vesicular-arbuscular mycorrhizal fungi on zero, one or two halves. *New Phytologist* **104**: 583-590.

Dunbabin V, Diggle A, Rengel Z. 2003. Is there optimal root architecture for nitrate capture in leaching environments? *Plant Cell and Environment* **26**: 835-844.

Franzluebbers AJ, Wright SF, Stuedemann JA. 2000. Soil aggregation and glomalin under pastures in the Southern Piedmont USA. *Soil Science Society of America, Journal* **64**: 1018-1026.

Gallaud I. 1905. Etudes sur les mycorrhizes endotrophes. *Revue Générale de Botanique* **17**: 5-48, 66-83, 123-135; 223-239; 313-325; 425-433; 479-500.

Gange AC, Brown VK, Farmer LM. 1990. A test of mycorrhizal benefit in an early successional plant community. *New Phytologist* **115**: 85-91.

George E, Häussler K, Kothari SK, Li X-L, Marschner H. 1992a. Contribution of mycorrhizal hyphae to nutrient and water uptake of plants. In: Read DJ, Lewis DH, Fitter AH, Alexander IJ, *Mycorrhizas in Ecosystems*. Cambridge: C.A.B International. 42-48.

George E, Häussler K, Vetterlein G, Gorgus E, Marschner H. 1992b. Water and nutrient translocation by hyphae of *Glomus mosseae*. *Canadian Journal of Botany* **70**: 2130-2137.

Ghassemi F, Jakeman AJ, Nix HA. 1995. *Salinization of Land and Water Resources.* Sydney, Australia: New South Wales University Press.

Gibson DJ, Newman JA. 2001. *Festuca arundinacea* Schreber (*F. elatior* L. ssp *arundinacea* (Schreber) Hackel). *Journal of Ecology* **89**: 304-324.

Giovannetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist* **84**: 489-500.

Giovannetti M, Sbrana C, Avio L, Citernesi AS, Logi C. 1993. Differential hyphal morphogenesis in arbuscular mycorrhizal fungi during pre-infection stages. *New Phytologist* **125**: 587-593.

Giri B, Kapoor R, Mukerji KG. 2002. VA Mycorrhizal techniques/VAM technology in establishment of plants under salinity stress conditions. In: Mukerji KG, Manoharachary C, Chamola BP, *Techniques in Mycorrhizal Studies*. Netherlands: Kulwer. 313-327.

Goudie AS. 1990. Soil salinity-causes and controls. In: *Techniques for Desert Reclamination*. England: John Wiley and Sons. 110-111.

Graham JH, Leonard RT, Menge JA. 1981. Membrane-mediated decrease in root exudation responsible for inhibition of vesicular-arbuscular mycorrhiza formation. *Plant Physiology* **68**: 548-552.

Grandmaison J, Olah GM, Van Calsteren MR, Furlan V. 1993. Characterization and localization of plant phenolics likely involved in the pathogen resistance expressed by endomycorrhizal roots. *Mycorrhiza* **3**: 155-164.

Grant CA, Flaten DN, Tomasiewicz DJ, Sheppard SC. 2001. The importance of early season phosphorus nutrition. *Canadian Journal of Plant Science* **81**: 211-224.

Grattan SR, Grieve CM. 1999. Salinity- mineral nutrient relations in horticultural crops. *Scientia Horticulturae* **78**: 127-157.

Gupta V, Satynarayana T, Sandeep G. 2000. General aspects of mycorrhiza. In: Mukerji KG, Chamola BP, Singh J, *Mycorrhizal Biology*. New York: Kluwer.

Haines BL, Best GR. 1976. *Glomus mosseae*, endomycorrhizal with *Liquidambar styraciflua* L. seedlings retards NO_3, NO_2 and NH_4 nitrogen loss from a temperate forest soil. *Plant and Soil* **45**: 257-261.

Hanson WC. 1950. The photometric determination of phosphorus in fertilizers using the phosphovanado-molybdate complex. *Journal of the Science of Food and Agriculture* **1**: 172-173.

Harley JL, Harley EL. 1987. A check-list of mycorrhiza in the British flora. *New Phytologist* **105**: 1-102.

Harrison MJ. 1998. Development of the arbuscular mycorrhizal symbiosis. *Current Opinion in Plant Biology* **1**: 360-365.

Hassan NAK, Drew JV, Kundsen D, Olsen R. 1970. Influence of soil salinity on production of dry matter and uptake and distribution of nutrients in barley and corn. *Agronomy Journal* **62**: 43-45.

Hayman DS. 1987. VA mycorrhizas in field crop systems. In: Safir GR, *Ecophysiology of VA Mycorrhizal Plants*. Boca Raton: CRC Press. 172-192.

Hayward HA, Spurr WA. 1994. Effects of iso-osmotic concentrations of inorganic and organic substances on the entry of water in corn roots. *Botanical Gazette* **106**: 131-139.

He XL, Mouratov S, Steinberger Y. 2002. Spatial distribution and colonization of arbuscular mycorrhizal fungi under the canopies of desert halophytes. *Arid Land Research and Management* **16**: 149-160.

Heckrath G, Brookes PC, Poulton PR, Goulding KWT. 1995. Phosphorus leaching from soils containing different phosphorus concentrations in the Broadbalk experiment. *Journal of Environmental Quality* **24**: 904-910.

Hetrick BAD. 1989. Acquisition of phosphorus by VA mycorrhizal fungi and the growth responses of their host plants. In: Boddy L, Marchant R, Read DJ, *Nitrogen, Phosphorus and Sulphur Utilization by Fungi*. Cambridge: Cambridge University Press. 205-226.

Hetrick BAD, Wilson GWT, Leslie JF. 1991. Root Architecture of Warm-Season and Cool-Season Grasses - Relationship to Mycorrhizal Dependence. *Canadian Journal of Botany* **69**: 112-118.

Hetrick BAD, Wilson GWT, Todd TC. 1992. Relationships of mycorrhizal symbiosis, rooting strategy, and phenology among tallgrass prairie forbs. *Canadian Journal of Botany* **70**: 1521-1528.

Hetrick BAD, Wilson GWT, Todd TC. 1996. Mycorrhizal response in wheat cultivars: Relationship to phosphorus. *Canadian Journal of Botany* **74**: 19-25.

Hewitt EJ. 1966. *Sand and Water Culture Methods Used in the Study of Plant Nutrition.* Farnham Royal, United Kingdom,: Commonwealth Agric. Bureaux.

Hildebrandt U, Janetta K, Ouziad F, Renne B, Nawrath K, Bothe H. 2001. Arbuscular mycorrhizal colonization of halophytes in Central European salt marshes. *Mycorrhiza* **10**: 175-183.

Hirrel MC, Gerdemann JW. 1978. Improved salt tolerance in bell pepper by two vesicular-arbuscular (VA) mycorrhizal fungi. *Agronomy Abstract* 140-141.

Hirrel MC, Gerdemann JW. 1980. Improved growth of onion and bell pepper in saline soils by two vesicular-arbuscular mycorrhizal fungi. *Soil Science Society of America, Journal* **44**: 654-658.

Hirrel MC, Mehravaran H, Gerdemann JW. 1978. Vesicular-arbuscular mycorrhiza in Chenopodiaceae and Cruciferae: do they occur ?. *Canadian Journal of Botany* **56**: 2813-2817.

Ho I. 1987. Vesicular arbuscular mycorrhizae of halophytic grasses in Alvard desert of Oregon. *Northwest Sciences* **61**: 148-151.

Jackson ML. 1958. *Soil Chemical Analysis.* Prentice Hall. 659 p.

Jakobsen I, Abbott LK, Robson AD. 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. *New Phytologist* **120**: 371-380.

Jalaluddin M. 1993. Effect of VAM fungus (*Glomus intraradices*) on the growth of sorghum, maize, cotton and pennisetum under salt stress. *Pakistan Journal of Botany* **25**: 215-218.

Janos DP. 1980. Mycorrhizae influence tropical succession. *Biotropica* **12**: 56-64 and 83-95.

Janos DP. 1988. Mycorrhiza applications in tropical forestry: are temperate-zone approaches appropriate? The Asian Seminar, Kuala Lumpur, Malaysia, 133-188.

Janos DP. 1995. Mycorrhizas, succession and the rehabilitation of deforested lands in the humid tropics. In: Frankland JC, Magan N, Gadd GM, *Fungi and Environmental Change*. Cambridge: Cambridge University Press.

Johansen A, Jakobsen I, Jensen ES. 1993. External hyphae of Vesicular-Arbuscular Mycorrhizal fungi associated with *Trifolium-Subterraneum* L .3. Hyphal transport of P ^{32} and N ^{15}. *New Phytologist* **124**: 61-68.

Johnson G-PC, Kenkel NC, Booth T. 1995. The distribution and phenology of arbuscular mycorrhizae along an inland salinity gradient. *Canadian Journal of Botany* **73**: 1318-1327.

Jongman RH, ter Braak CJF, Van Tongeren OFR. 1995. *Data Analysis in Community and Landscape Ecology.* Cambrige, UK: Cambridge University Press.

Juniper S, Abbott LK. 1991. The effects of salinity on spore germination and hyphal extension of some VA mycorrhizal fungi. 3rd European Symposium on Mycorrhizas, University of Sheffield, Sheffield, UK.

Juniper S, Abbott LK. 1993. Vesicular-arbuscular mycorrhizas and soil salinity. *Mycorrhiza* **4**: 45-57.

Jury WA, Jarrel WW, Devitt D. 1979. Reclamation of saline-sodic soils by leaching. *Soil Science Society of America, Journal* **43**: 1100-1106.

Kafkafi U, Bernstein N. 1996. Root growth under salinity stress. In: Waisel Y, Eshel A,Kafkafi U, *Plant Roots, The Hidden Half*. New York: Marcel Dekker.

Kandeler E, Marschner P, Tscherko D, Gahoonia TS, Nielsen NE. 2002. Microbial community composition and functional diversity in the rhizosphere of maize. *Plant and Soil* **238**: 301-312.

Khalil S, Loynachan TE, Tabatabai MA. 1994. Mycorrhizal dependency and nutrient uptake by improved and unimproved corn and soybean cultivars. *Agronomy Journal* **86**: 949-958.

Khosla BH, Gupta RK, Abrol IP. 1979. Salt leaching and effect of gypsum application in a saline-sodic soil. *Agricutural Water Management* **2**: 193-212.

Kigel J. 1995. *Seed Germination in Arid and Semiarid Regions.* New York: Marcel Dekker, Inc.

Kirkby CA, Smythe LJ, Cox JW, Chittleborough DJ. 1997. Phosphorus movement down a toposequence from a landscape with texture contrast soils. *Australian Journal of Soil Research* **35**: 399-417.

Koide R, Li M, Lewis J, Irby C. 1988. Role of mycorrhizal infection in the growth and reproduction of wild vs cultivated plants. 1. Wild vs. cultivated oats. *Oecologia* **77**: 537-542.

Koide RT, Kabir Z. 2000. Extraradical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate. *New Phytologist* **148**: 511-517.

Kothari SK, Marschner H, Romheld V. 1991. Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil. *Plant and Soil* **131**: 177-185.

Kothari SK, Marschner H, Römheld V. 1990. Direct and indirect effects of VA mycorrhizal fungi and rhizosphere microorganisms on acquisition of mineral nutrients by maize (*Zea mays* L.) in a calcareous soil. *New Phytologist* **116**: 637-645.

Landwehr M, Hildebrandt U, Wilde P, Nawrath K, Toth T, Biro B, Bothe H. 2002. The arbuscular mycorrhizal fungus *Glomus geosporum* in European saline, sodic and gypsum soils. *Mycorrhiza* **12**: 199-211.

Largewerff JV, Holland JP. 1960. Growth and mineral content of carrots and beans as related to varying osmotic and ionic composition effects in saline-sodic sand culture. *Agronomy Journal* **52**: 603-608.

LeBissonnais Y. 1996. Aggregate stability and assessment of soil crustability and erodibility .1. Theory and methodology. *European Journal of Soil Science* **47**: 425-437.

Leffelaar PA, Sharma R. 1977. Leaching of a highly saline-sodic soil. *Journal of Hydrology* **32**: 203-218.

Leinweber P, Meissner R, Eckhardt KU, Seeger J. 1999. Management effects on forms of phosphorus in soil and leaching losses. *European Journal of Soil Science* **50**: 413-424.

Levitt J. 1980. *Responses of Plants to Environmental Stress.* Academic Press.

Lewis JD, Koide RT. 1990. Phosphorus supply, mycorrhizal infection and plant offspring vigour. *Functional Ecology* **4**: 695-702.

Li X-L, George E, Marschner H. 1991. Extension of the phosphorus depletion zone in VA-mycorrhizal white clover in a calcareous soil. *Plant and Soil* **136**: 41-48.

Liu A, Hamel C, Begna SH, Ma BL, Smith DL. 2003. Soil phosphorus depletion capacity of arbuscular mycorrhizae formed by maize hybrids. *Canadian Journal of Soil Science* **83**: 337-342.

Longsteth D, Bolanos JA, Smith JE. 1984. Salinity effects on photosynthesis and growth in *Alternanthera philoxeroides* (Mart) Griseb. *Plant Physiology* **75**: 1044-1047.

Maeda M. 1954. The meaning of mycorrhiza in regard to systematic botany. *Kumamoto Journal of Science. Biology* **3**: 57-84.

Mamatha G, Bagyaraj DJ, Jaganath S. 2002. Inoculation of field-established mulberry and papaya with arbuscular mycorrhizal fungi and a mycorrhiza helper bacterium. *Mycorrhiza* **12**: 313-316.

Manguet M. 1991. Some solutions to improve dryland agriculture. In: *Desertification: Natural Background and Human Mismanagement*. Germany: Springer Verlag. 217-224.

Mark GL, Cassells AC. 1996. Genotype-dependence in the interaction between *Glomus fistulosum*, *Phytophthora fragariae* and the wild strawberry (*Fragaria vesca*). *Plant and Soil* **185**: 233-239.

Marschner H. 1995. *Mineral Nutrition of Higher Plants.* London San Diego: Academic Press.

Marschner H, Dell B. 1994. Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil* **159**: 89-102.

Marschner P, Baumann K. 2003. Changes in bacterial community structure induced by mycorrhizal colonisation in split-root maize. *Plant and Soil* **251**: 279-289.

Marschner P, Crowley DE, Lieberei R. 2001. Arbuscular mycorrhizal infection changes the bacterial 16 S rDNA community composition in the rhizosphere of maize. *Mycorrhiza* **11**: 297-302.

Marschner P, Kandeler E, Marschner B. 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. *Soil Biology & Biochemistry* **35**: 453-461.

Marschner P, Neumann G, Kania A, Weiskopf L, Lieberei R. 2002. Spatial and temporal dynamics of the microbial community structure in the rhizosphere of cluster roots of white lupin (*Lupinus albus* L.). *Plant and Soil* **246**: 167-174.

Marulanda A, Azcon R, Ruiz-Lozano JM. 2003. Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought stress. *Physiologia Plantarum* **119**: 1-8.

Maser P, Gierth M, Schroeder JI. 2002. Molecular mechanisms of potassium and sodium uptake in plants. *Plant and Soil* **247**: 43-54.

McKersie BD, Leshem YY. 1994. *Stress and Stress Coping in Cultivated Plants.* Kluwer Academic Publishers.

McLaughlin MJ, Fillery IR, Till AR. 1991. Operation of the phosphorus, sulphur and nitrogen cycles. In: Gifford RM, Barson MM, *Australia's Renewable Resources: Sustainability and Global Change*. Canberra, Australia: Bureau of Rural Resources. 67-116.

McMillen BG, Juniper S, Abbott LK. 1998. Inhibition of hyphal growth of a vesicular-arbuscular mycorrhizal fungus in soil containing sodium chloride limits the spread of infection from spores. *Soil Biology & Biochemistry* **30**: 1639-1646.

Mickline PP. 1991. *The Water Management Crisis in Soviet Central Asia.* Center for Russian and East European Studies. University of Pittsburgh, Pittsburgh.

Miethling R, Ahrends K, Tebbe CC. 2003. Structural differences in the rhizosphere communities of legumes are not equally reflected in community-level physiological profiles. *Soil Biology & Biochemistry* **35**: 1405-1410.

Miller RM. 1979. Some occurrences of vesicular-arbuscular mycorrhiza in natural and disturbed ecosystems of Red Desert. *Canadian Journal of Botany* **57**: 619-623.

Miller RM, Jastrow JD. 2000. Mycorrhizal fungi influence soil structure. In: Kapulink Y, Douds DD, *Arbuscular Mycorrhizas: Physiology and Function*. Dordrecht: Kluwer Academic Publishers. 3-19.

Miller RM, Moorman TB, Schmidt SK. 1983. Interspecific plant association effects on vesicular-arbuscular mycorrhiza occurrence in *Atriplex confertifolia*. *New Phytologist* **95**: 241-246.

Mohammad MJ, Malkawi HI, Shibli R. 2003. Effects of arbuscular mycorrhizal fungi and phosphorus fertilization on growth and nutrient uptake of barley grown on soils with different levels of salts. *Journal of Plant Nutrition* **26**: 125-137.

Moorman T, Reeves FB, Good CW. 1979. The role of endomycorrhizae on revegetation practices in the semi-arid west. II. A bioassay to determine the effect of land disturbance on endomycorrhizal populations. *American Journal of Botany* **66**: 14-18.

Mosse B, Hayman DS, Arnold DJ. 1973. Plant growth responses to vesicular-arbuscular mycorrhiza. V. Phosphate uptake by three plant species from P-deficient soils labelled with P^{32}. *New Phytologist* **72**: 809-815.

Nadian H, Smith SE, Alston AM, Murray RS. 1997. Effects of soil compaction on plant growth, phosphorus uptake and morphological characteristics of vesicular-arbuscular mycorrhizal colonization of *Trifolium subterraneum*. *New Phytologist* **135**: 303-311.

Nannipieri P, Kandeler E, Ruggiero P. 2002. Enzyme activities and microbiological and biochemical processes in soil. In: Burns R, Dick R, *Enzymes in the Environment. Activity, Ecology and Application*. USA: Marcel Dekker. 1-34.

Newsham KK, Fitter AH, Watkinson AR. 1995. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *Journal of Ecology* **83**: 991-1000.

Nicolson TH. 1975. Evolution of vesicular-arbuscular mycorrhizas. In: Sanders FE, Mosse B, Tinker PB, *Endomycorrhizas*. London: Academic Press. 25-34.

Niu XM, Bressan RA, Hasegawa PM, Pardo JM. 1995. Ion homeostasis in NaCl stress environments. *Plant Physiology* **109**: 735-742.

Nurlaeny N, Marschner H, George E. 1996. Effects of liming and mycorrhizal colonization on soil phosphate depletion and phosphate uptake by maize (*Zea mays* L.) and soybean (*Glycine max* L.) grown in two tropical acid soils. *Plant and Soil* **181**: 275-285.

Oades JM. 1984. Soil organic matter and structural stability: mechanisms and implications for management. *Plant and Soil* **76**: 319-337.

O'Connor PJ, Smith SE, Smith FA. 2001. Arbuscular mycorrhizal associations in the southern Simpson Desert. *Australian Journal of Botany* **49**: 493-499.

Ojala JC, Jarrell WM, Menge JA, Johnson ELV. 1983. Influence of mycorrhizal fungi on the mineral nutrition and yield of onion in saline soil. *Agronomy Journal* **75**: 255-259.

Olsson PA, Bååth E, Jakobsen I, Söderström B. 1996. Soil bacteria respond to presence of roots but not to mycelium of arbuscular mycorrhizal fungi. *Soil Biology & Biochemistry* **28**: 463-470.

Oster JD, Schroer W. 1979. Infiltration as influenced by irrigation water quality. *Soil Science of America, Journal* **43**: 444-447.

Owusu-Bennoah E, Wild A. 1979. Autoradiography of the depletion zone of phosphate around onion roots in the presence of vesicular-arbuscular mycorrhiza. *New Phytologist* **82**: 133-140.

Ozanne P, Kirton D, Shaw T. 1961. The loss phosphorus from sandy soils. *Australian Journal of Agricaltural Ressearch* **12**: 409-413.

Pande M, Tarafdar JC. 2002. Effect of phosphorus, salinity and moisture on VAM fungal association in neem (*Azadirachta indica* Linn.). *Symbiosis* **32**: 195-209.

Pare T, Gregorich EG, Nelson SD. 2000. Mineralization of nitrogen from crop residues and N recovery by maize inoculated with vesicular-arbuscular mycorrhizal fungi. *Plant and Soil* **218**: 11-20.

Paulitz TC, Linderman RG. 1989. Interactions between fluorescent pseudomonads and VA mycorrhizal fungi. *New Phytologist* **113**: 37-45.

Pessarakli M. 1993. *Handbook of Plant and Crop Stress.* New York: Marcel Dekker, Inc.

Pfeiffer CM, Bloss HE. 1988. Growth and nutrition of guayule (*Parthenium argentatum*) in a saline soil as influenced by vesicular-arbuscular mycorrhiza and phosphorus fertilization. *New Phytologist* **108**: 315-321.

Phillips JM, Hayman DS. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**: 158-161.

Ples S, Stannard ME. 1977. Environmental changes due to irrigation development in semi-arid parts of New South Wales, Australia. In: Worthington EB, *Arid Land Irrigation in Developing Countries. Environmental Problems and Effects*. New York: Pergamon Press. 181.

Polonenko DR, Mayfield CI, Dumbroff EB. 1986. Microbial responses to salt-Induced osmotic-stress .5. Effects of salinity on growth and displacement of soil bacteria. *Plant and Soil* **92**: 417-425.

Pond EC, Menge JA, Jarrell WM. 1984. Improved growth of tomato in salinized soil by vesicular-arbuscular mycorrhizal fungi collected from saline soils. *Mycologia* **76**: 74-84.

Poss JA, Pond E, Menge JA, Jarrell WM. 1985. Effect of salinity on mycorrhizal onion and tomato in soil with and without additional phosphate. *Plant and Soil* **88**: 307-319.

Ragab M. 1993. Distribution pattern of soil microbial population in salt-affected soils. In: Lieth H, Al-Masoom AA, *Towards the Rational Use of High Salinity Tolerance Plants, vol 1, Deliberations about High Salinity Tolerance Plants and Ecosystems*. Dordrecht, Netherlands: Kluwer Academic Publishers. 467-472.

Ratnayake RT, Leonard RT, Menge JA. 1978. Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation. *New Phytologist* **81**: 543-552.

Rayment GE, Higginson FR. 1992. *Australian Laboratory Handbook of Soil and Water Chemical Methods.* Melbourne: Inkata Press.

Read DJ, Koucheki HK, Hodgson J. 1976. Vesicular-arbuscular mycorrhiza in natural vegetation systems. I. The occurrence of infection. *New Phytologist* **77**: 641-653.

Redecker D. 2002. Molecular identification and phylogeny of arbuscular mycorrhizal fungi. *Plant and Soil* **244**: 67-73.

Reeves FB, Wagner D, Moorman T, Kiel J. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid west. I. A comparison of incidence of mycorrhizae in severely disturbed vs. natural environments. *American Journal of Botany* **66**: 6-13.

Remy W, Taylor TN, Hass H, Kerp H. 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proceedings of the National Academy of Sciences, USA* **91**: 11841-11843.

Rengasamy P, Sumner ME. 1998. Processes Involved in Sodic Behaviour. In: Sumner ME, Ravendra N, *Sodic Soils, Distribution, Properties, Managements and Environmental Consequences*. New York: Oxford University Press. 35-50.

Requena N, Jeffries P, Barea JM. 1996. Assessment of natural mycorrhizal potential in a desertified semiarid ecosystem. *Applied and Environmental Microbiology* **62**: 842-847.

Rhoades JD, Ingvalson RD. 1969. Macroscopic swelling and hydraulic conductivity properties of four vermiculitic soils. *Soil Society of America, Journal* **33**: 364-369.

Rhodes LH, Gerdemann JW. 1975. Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. *New Phytologist* **75**: 555-561.

Rillig MC, Wright SF, Eviner VT. 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. *Plant and Soil* **238**: 325-333.

Rogers ME, Noble CL, Pederick RJ. 1996. Identifying suitable grass species for saline areas. *Australian Journal of Experimental Agriculture* **36**: 197-202.

Rosendahl CN, Rosendahl S. 1991. Influence of vesicular-arbuscular mycorrhizal fungus (*Glomus spp*.) on the response of cucumber (*Cucumis sativus* L.) to salt stress. *Environmental and Experimental Botany* **31**: 313-318.

Rozema J, Arp W, Vandiggelen J, Vanesbroek M, Broekman R, Punte H. 1986. Occurrence and ecological significance of vesicular arbuscular mycorrhiza in the salt-Marsh environment. *Acta Botanica Neerlandica* **35**: 457-467.

Ruiz-Lozano JM, Azcón R, Gomez M. 1996. Alleviation of salt stress by arbuscular mycorrhizal *Glomus* species in *Lactuca sativa* plants. *Physiologia Plantarum* **98**: 767-772.

Sacchi GA, Abruzzese A, Lucchini G, Fiorani F, Cocucci S. 2000. Efflux and active re-absorption of glucose in roots of cotton plants grown under saline conditions. *Plant and Soil* **220**: 1-11.

Saggin-Junior OJ, Siqueira JO. 1995. Evaluation of the symbiotic effectiveness of endomycorrhizal fungi for coffe tree. *Revista Brasileira De Ciencia Do Solo* **19**: 221-228.

Saif SR, Iffat N. 1976. Vesicular arbuscular mycorrhizae in plants and endogonaceous spores in the soil of northern areas of Pakistan. *Pakistan Journal of Botany* **8**: 163-179.

Sanders FE, Sheikh NA. 1983. The development of vesicular-arbuscular mycorrhizal infection in plant root systems. *Plant and Soil* **71**: 223-246.

Sanders FE, Tinker PB. 1971. Mechanism of absorption of phosphate from soil by *Endogone* mycorrhizas. *Nature* **233**: 278-279.

Sanders IR, Fitter AH. 1992. The ecology and functioning of vesicular-arbuscular mycorrhizas in coexisting grassland species I. Seasonal patterns of mycorrhizal occurrence and morphology. *New Phytologist* **120**: 517-524.

Schmidt SK, Reeves FB. 1984. Effect of the non-mycorrhizal pioneer plant *Salsola kali* L. (Chenopodiaceae) on vesicular-arbuscular mycorrhizal (VAM) fungi. *American Journal of Botany* **71**: 1035-1039.

Schreiner RP, Bethlenfalvay GJ. 1995. Mycorrhizal interactions in sustainable agriculture. *Critical Reviews in Biotechnology* **15**: 271-285.

Schwab SM, Johnson ELV, Menge JA. 1982. The influence of Simazine on formation of vesicular-arbuscular mycorrhizae in *Chenopodium quinona* Willd. *Plant and Soil* **64**: 283-287.

Sengupta A, Chaudhuri S. 1990. Vesicular arbuscular mycorrhiza (VAM) in pioneer salt marsh plants of the Ganges river delta in West Bengal (India). *Plant and Soil* **122**: 111-113.

Sentenac H, Grignon C. 1985. Effect of pH on ortho-phosphate uptake by corn roots. *Plant Physiology* **77**: 136-141.

Shainberg I, Letey J. 1984. Response of soil to sodic and saline conditions. *Hilgardia* **52**: 1-57.

Sharpley AN, Kleinman P, McDowell R. 2001. Innovative management of agricultural phosphorus to protect soil and water resources. *Communications in Soil Science and Plant Analysis* **32**: 1071-1100.

Sharpley AN, Rekolainen S. 1997. Phosphorous in Agriculture and its Environmental implications. In: Tunney H, Carton OT, Brookes PC, Johnston AE, *Phosphorus Loss from Soil to Water*. Wallingford: CAB International. 1-53.

Sharpley AN, Withers PJA. 1994. The environmentally-sound management of agricultural phosphorus. *Fertilizer Research* **39**: 133-146.

Sidhu OP, Behl HM. 1997. Response of three *Glomus* species on growth of *Prosopis juliflora* Swartz at high pH levels. *Symbiosis* **23**: 23-34.

Siguenza C, Espejel I, Allen B. 1996. Seasonality of mycorrhizae in coastal sand dunes of Baja California. *Mycorrhiza* **6**: 151-157.

Siqueira JO, Saggin-Junior OJ. 2001. Dependency on arbuscular mycorrhizal fungi and responsiveness of some Brazilian native woody species. *Mycorrhiza* **11**: 245-255.

Smith FA, Smith SE. 1981. Mycorrhizal infection and growth of *Trifolium subterraneum*: use of sterilized soil as a control treatment. *New Phytologist* **88**: 299-309.

Smith FA, Smith SE. 1997. Structural diversity in (vesicular)-arbuscular mycorrhizal symbiosis. *New Phytologist* **137**: 373-388.

Smith SE, Nicholas DJD, Smith FA. 1979. Effect of early mycorrhizal infection on nodulation and nitrogen fixation in *Trifolium subterraneum* L. *Australian Journal of Plant Physiology* **6**: 305-316.

Smith SE, Read DJ. 1997. *Mycorrhizal Symbiosis.* Cambridge, UK: Academic Press Ltd.

Smith SE, Smith FA, Jakobsen I. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology* **133**: 16-20.

Smith SE, Smith FA, Jakobsen I. 2004. Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytologist* **162**: 511-524.

Smith SE, Smith FA, Nicholas DJD. 1981. Effects of endomycorrhizal infection on phosphate and cation uptake by *Trifolium subterraneum*. *Plant and Soil* **63**: 57-64.

Smith SE, St John BJ, Smith FA, Bromley JL. 1986. Effects of mycorrhizal infection on plant growth, nitrogen and phosphorus nutrition of glasshouse-grown *Allium cepa* L. *New Phytologist* **103**: 359-373.

Sohn BK, Kim KY, Chung SJ, Kim WS, Park SM, Kang JG, Rim YS, Cho JS, Kim TH, Lee JH. 2003. Effect of the different timing of AMF inoculation on plant growth and flower quality of chrysanthemum. *Scientia Horticulturae* **98**: 173-183.

Solaiman MZ, Ezawa T, Kojima T, Saito M. 1999. Polyphosphates in intraradical and extraradical hyphae of an arbuscular mycorrhizal fungus, *Gigaspora margarita*. *Applied and Environmental Microbiology* **65**: 5604-5606.

Solaiman MZ, Saito M. 2001. Phosphate efflux from intraradical hyphae of *Gigaspora margarita in vitro* and its implication for phosphorus translocation. *New Phytologist* **151**: 525-533.

Stahl E. 1900. Der sinn der mycorhizenbildung. *Jahrbuch fur Wissenschaftliche Botanik* **34**: 539-668.

Stanley MR, Koide RT, Shumway DL. 1993. Mycorrhizal symbiosis increases growth reproduction and recruitment of *Abutilon theophrasti* Medic in the field. *Oecologia* **94**: 30-35.

Staples RC, Toenniessen GH. 1984. *Salinity Tolerance in Plants: Strategies for Crop Improvement.* John Wiley and Sons.

State Salinity Council. 2000. *Natural Resources Management in Western Australia, The State Salinity Strategy*. Government of Western Australia, 72 pp, 72 pp.

Subramanian KS, Charest C, Dwyer LM, Hamilton RI. 1997. Effects of arbuscular mycorrhizae on leaf water potential, sugar content, and P content during drought and recovery of maize. *Canadian Journal of Botany* **75**: 1582-1591.

Szabolcs I. 1989. *Salt-affected Soils.* Boca Raton, Florida: CRC Press.

Tanji KK, Lauchi A, Meyer J. 1986. Selenium in the San Joaquin Valley. *Environment* **28**: 6-11 and 34-39.

Tawaraya K. 2003. Arbuscular mycorrhizal dependency of different plant species and cultivars. *Soil Science and Plant Nutrition* **49**: 655-668.

Tennant D. 1975. A test of a modified line intersect method of estimating root length. *Journal of Ecology* **63**: 995-1001.

Tester M, Smith FA, Smith SE. 1987. The phenomenon of 'nonmycorrhizal' plants. *Canadian Journal of Botany* **65**: 419-431.

Thomas RS, Dakessian S, Ames RN, Brown MS, Bethlenfalvay GJ. 1986. Aggregation of a silty clay loam soil by mycorrhizal onion roots. *Soil Society of America, Journal* **50**: 1494-1499.

Tinker PB. 1978. Effects of vesicular-arbuscular mycorrhizas on plant nutrition and plant growth. *Physiologie Végetalé* **16**: 743-751.

Tisdall JM, Oades JM. 1979. Stabilization of soil aggregates by the root systems ryegrass. *Australian Journal of Soil Research* **17**: 429-441.

Tisdall JM, Oades JM. 1980. The effect of crop rotation on aggregation in a red-brown earth. *Australian Journal of Soil Research* **18**: 423-433.

Tisdall JM, Oades JM. 1982. Organic matter and water-stable aggregates in soils. *Journal of Soil Science* **33**: 141-163.

Toor GS, Condron LM, Di HJ, Cameron KC, Cade-Menun BJ. 2003. Characterization of organic phosphorus in leachate from a grassland soil. *Soil Biology & Biochemistry* **35**: 1317-1323.

Torres J. 1990. *Determinacion e identificacion de micorrizas vesiculo arbusculares (MVA) en plantas lenosas de vivero de interes para la forestacion de zonas aridas.* Memoria de Titulo Ingeniero Forestal: Universidad de Chile, Chile.

Tsang A, Maun MA. 1999. Mycorrhizal fungi increase salt tolerance of *Strophostyles helvola* in coastal foredunes. *Plant Ecology* **144**: 159-166.

Tunney H. 1992. Some environmental implications of phosphorus use in the European Comminity. Proceedings of the 4th International IMPHOS Conference, Ghent, Belgium, World Phosphate Institute, Casablanca, Morocco, pp 347-359.

Ungar IA. 1982. Germination ecology of halophytes. In: Sen DN, Rajpurohit KS, *Contribution to the Ecology of Halophytes*. Junk, the Hague. 143-154.

US Salinity Laboratory Staff. 1954. Diagnosis and improvement of saline and alkali soils. Government printing office, Handbook 60, Washington, DC.

van der Heijden EW. 2001. Differential benefits of arbuscular mycorrhizal and ectomycorrhizal infection of *Salix repens*. *Mycorrhiza* **10**: 185-193.

van der Heijden EW, Kuyper TW. 2003. Ecological strategies of ectomycorrhizal fungi of *Salix repens*: root manipulation versus root replacement. *Oikos* **103**: 668-680.

van der Heijden MGA, Boller T, Wiemken A, Sanders IR. 1998. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology* **79**: 2082-2091.

van Duin WE, Rozema J, Ernst WHO. 1989. Seasonal and spatial variation in the occurrence of vesicular- arbuscular (VA) mycorrhiza in salt-marsh plants. *Agriculture Ecosystems & Environment* **29**: 107-110.

Vlahos S. 1992. Nitch seeding for the establishment of saltbush on saline soils in western Australia. Productive use of saline land., Workshop of Australian Centre for International Agricultural Research, Adelaide, The Waite Agricultural Research Institute, 59-66.

Vollenweider RA. 1975. Input-output models with special reference to the phosphorus loading concept in limnology. *Schweizerische Zeitschrift fur Hydrologie* **35**: 53-48.

Waisel Y. 1991. Adaptation to salinity. In: Rayhavendra AS, *Physiology of Trees*. New York: John Wiley and Sons. 359-383.

Walkley A, Black IA. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science* **37**: 29-37.

Wamberg C, Christensen S, Jakobsen I, Muller AK, Sorensen SJ. 2003. The mycorrhizal fungus (*Glomus intraradices*) affects microbial activity in the rhizosphere of pea plants (*Pisum sativum*). *Soil Biology & Biochemistry* **35**: 1349-1357.

Waters AG, Oades JM. 1991. Organic matter in water-stable aggregates. In: Wilson WS, *Advances in Organic Matter Research*. Cambridge: Royal Society of Chemistry. pp163-174.

Went FW. 1952. The effects of rain and temperature on plant distribution in the desert. Proceedings of the International Symposium on Desert Research, Research Council of Israel Special Publication, 230-240.

Wicklow-Howard MC. 1994. Vesicular-arbuscular mycorrhizae from sagebrush steppe habitat in Western Idaho and parts of eastern and Centran Oregon. Eastside Ecosystem Management Project, Biose, Idaho.

Wilcox HE. 1991. Mycorrhizae. In: Waisel Y, Eshel A, Kafkafi U, *The Plant Root, the Hidden Half*. New York: Marcel Dekker. 731-765.

Williams SE, Wollum AG, Aldon EF. 1974. Growth of *Atriplex canescens* (Pursh) Nutt. improved by formation of vesicular-arbuscular mycorrhizae. *Proceedings of the Soil Science Society of America* **38**: 962-965.

Wilson GWT, Hartnett DC. 1998. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. *American Journal of Botany* **85**: 1732-1738.

Wilson GWT, Hetrick BAD, Kitt DG. 1989. Suppression of vesicular-arbuscular mycorrhizal fungus spore germination by non sterile soil. *Canadian Journal of Botany* **67**: 18-23.

Wright SF, Upadhyaya A. 1998. A survey of soil for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil* **198**: 97-107.

Wright SF, Upadhyaya A. 1999. Quantification of arbuscular mycorrhizal fungi activity by the glomalin concentration on hyphal traps. *Mycorrhiza* **8**: 283-285.

Yin B, Crowley D, Sparovek G, De Melo WJ, Borneman J. 2000. Bacterial functional redundancy along a soil reclamation gradient. *Applied and Environmental Microbiology* **66**: 4361-4365.

Yousaf M, Ali OM, Rhoades JD. 1987. Clay dispersion and hydraulic conductivity of some salt-affected arid land soils. *Soil Science Society of America, Journal* **51**: 905-907.

Zahran HH. 1997. Diversity, adaptation and activity of the bacterial flora in saline environments. *Biology and Fertility of Soils* **25**: 211-223.

Zak JC, Willig MR, Moorhead DL, Wildman HG. 1994. Functional diversity of microbial communities - a quantitative approach. *Soil Biology & Biochemistry* **26**: 1101-1108.

Zhu YG, Smith SE, Barritt AR, Smith FA. 2001. Phosphorus (P) efficiencies and mycorrhizal responsiveness of old and modern wheat cultivars. *Plant and Soil* **237**: 249-255.