EFFECTS OF SOIL COMPACTION ON GROWTH AND P UPTAKE BY *TRIFOLIUM SUBTERRANEUM* COLONISED BY VAM FUNGI

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SUMMARY

Although the major effect of mycorrhizal colonisation on plant growth is mediated through increased nutrient uptake, there are other ways by which plants may benefit from the presence of the fungal symbiont. Some other possible benefits are: deterred root pathogens, improved soil structure and increased tolerance of the host plant to environmental stress such as water stress.

The negative effects of soil compaction on plant growth due to soil strength and poor aeration are well known. However, the effects of mycorrhizal colonisation on growth and P uptake by mechanically impeded roots in compacted soil have received little attention and there are only a few reports on this topic (see Chapter 1). The work reported in this thesis was carried out to study the effects of vesicular-arbuscular mycorrhizal (VAM) fungi on growth and P uptake by *Trifolium subterraneum* L. at different levels of soil compaction and P application. In this study, a number of lines of investigation were conducted. The objectives and main findings of each step of this study are outlined below.

The first objective was to provide data on the effect of different levels of soil compaction and P application on growth and P uptake by *Trifolium subterraneum* L. and *Allium cepa L.* These data were also used to choose conditions and plants species for further experimentation. The results of the first experiment indicated that at higher P application, soil compaction to the bulk density of 1.6 Mg m^{-3} (penetrometer resistance = 3.5 MPa at a matric water potential of -33 kPa) had a major influence on root morphology (increased root diameter and decreased root length) and root distribution of both subterranean clover and onion plants. The change in the morphology of the plants resulted in a significant decline in P uptake per plant. Since shoot and root P concentrations of both plants were not influenced by soil compaction, the significant decrease in P uptake per plant was attributed to inhibition of root growth by increasing soil compaction.

Since it has been shown that small addition of P fertiliser to P-deficient soil increases mycorrhizal growth response, the aim of the next experiment was to study the response of *T. subterraneum* in association with *Glomus intraradices* to different levels of P fertiliser in non-compacted soil. The greatest beneficial effects of mycorrhizal colonisation, either in terms of P uptake or of plant growth, were observed when the soil was supplied with 15 mg P kg^{-1} soil. This level of P fertilisation was therefore used in the subsequent experiments.

In another experiment, which used conditions selected on the basis of previous experiments, the effect of different levels of soil compaction and P application on P uptake and growth of *T. subterraneum* in association with *Glomus intraradices*, was investigated. The results of this investigation indicated that at low P application (15 mg P kg^{-1} soil), P uptake and shoot dry weight of clover plants colonised by *Glomus intraradices* were greater than those of non-mycorrhizal plants at all levels of soil compaction, although mycorrhizal growth response decreased as soil compaction increased from a bulk density of 1.0 to 1.6 Mg m^{-3}. The results also indicated that soil compaction had no significant effect on the percentage of root length colonised, but total root length colonised significantly decreased. The decline in benefit of mycorrhizal colonisation was attributed mainly to the significant decrease in the length of clover roots due to the increase in soil strength - the penetrometer resistance of the soil was 3.5 MPa. However, a possible effect of soil compaction on external hyphae and internal colonisation could also be involved in the decline in benefit of mycorrhizal colonisation. Moreover, increased soil compaction increased the mass of P per unit volume of the soil, and this could have had a negative effect on mycorrhizal colonisation.

The next step was therefore to study the effect of soil compaction on internal colonisation and external hyphae of *G. intraradices* at two constant levels of added P (15 mg kg^{-1} and 15 mg dm^{-3} soil). The results of this experiment indicated that soil compaction

to a bulk density of 1.60 Mg m^{-3} had no significant effect on the percentage of root length containing arbuscules, vesicles or by any combination of arbuscules, vesicles and intra-radical hyphae. The air-filled porosity of highly compacted soil, which varied from 0.07 to 0.11 m^3 m^{-3} over the range of matric potentials encountered (-33 and -100 kPa), had no significant effect on the intensity of internal colonisation. No significant interaction was observed between two methods of P application in their effects on mycorrhizal growth response. Thus, the observed decline in mycorrhizal growth response with increasing soil compaction was not likely to be a consequence of the increase in mass of P per unit volume of the soil resulting from compacting the soil.

Initially, the filtration gridline method was used to estimate the amount of external hyphae, as this was the most commonly used method at the time. However, a major problem with this method is that the hyphae of non-mycorrhizal fungi often are not distinguishable from those of VAM fungi and the method was found unreliable. A new chemical method (ester-linked fatty acids analysis), which has recently been developed by other researchers, was therefore used to estimate hyphal biomass in soil and overcomes the lack of specificity encountered in measuring external hyphae with the gridline method. The results indicated that the amount of hyphal biomass (as estimated with neutral lipid fatty acids 16:1ω5 and 16:0 extracted from the soil) decreased with increasing soil compaction. However, the amounts of these two fatty acids per unit root length colonised increased with increasing soil compaction. Despite the decreased hyphal biomass per unit mass of soil in compacted soil, hyphal P inflow did not decrease with increasing soil compaction (up to a bulk density of 1.60 Mg m^{-3}). This might be due to increased hyphal biomass per unit root length colonised with increasing soil compaction. Thus, P inflow had no role on the decline in benefit of mycorrhizal colonisation. It was therefore concluded that the significant reduction in plant growth and P uptake by mycorrhizal clover plants in compacted soil was

a result of decreased total root length colonised by arbuscules and intra-radical hyphae and consequently a considerable reduction in the surface area of interface for P transfer to the host plant. However a significant reduction in hyphal biomass per pot in compacted soil might also be involved in the decline in benefit of mycorrhizal colonisation.

In the final experiment of this study, mycorrhizal growth responses of four species of VAM fungi to different levels of soil compaction and P application were compared. The results indicated that different species of VAM fungi differed in their response to soil compaction. Soil compaction decreased mycorrhizal growth response, but this decrease was more pronounced for *Glomus mosseae* and *Glomus etunicatum* than for *Glomus intraradices* and *Glomus sp.* City Beach. The reason for this might be due to different sensitivities of mycorrhizal fungi to unfavourable conditions arising from soil compaction such as poor aeration and/or to differences in the diameter of external hyphae, which may affect their abilities to penetrate small pores in compacted soil. No mycorrhizal growth response was observed when soil compaction increased to the bulk density of 1.75 Mg m^{-3}. A number of possibilities were discussed for the lack of mycorrhizal growth response in highly compacted soil, although their relative importance is not clear. From the results of the final experiment, it was concluded that the ability of mycorrhizal colonisation of *G. intraradices* and *G. sp.* City Beach to alleviate the effect of soil compaction on growth and P uptake by *T. subterraneum* was greater than the two other VAM fungi.

PUBLICATIONS FROM THE THESIS

The work has already resulted in two papers being published by internationally recognised journals (attached at the end of this thesis), one paper has been accepted for publication and the fourth paper has been submitted to New Phytologist and needs some modification for publication.

A- Journal Articles:

1. Nadian H, Smith S E, Alston A M and Murray R S 1996. The effect of soil compaction on growth and P uptake by *Trifolium subterraneum*: interactions with mycorrhizal colonisation. *Plant and Soil.* 182: 39-49. (Results of Chapter 4).

2. Nadian H, Smith S E, Alston A M and Murray R S 1997. Effects of soil compaction on plant growth, phosphorus uptake and morphological characteristics of vesicular-arbuscular mycorrhizal colonisation of *Trifolium subterraneum.* L. *New Phytologist.* 135: 303-311. (Results of Chapter 5).

3. Nadian H, Smith S E, Alston A M and Murray R S 1997. The effect of soil compaction on P inflow into mycorrhizal and non-mycorrhizal clover roots. *Australian Journal of Soil Research* (in press). (Results of Chapter 6).

4. Nadian H, Smith S E, Alston A M, Murray R S and B D Siebert 1997. Response of four species of VAM fungi to soil compaction. Submitted and needs some modification to be published in *New Phytologist.* (Results of Chapter 7).

B- Conference Articles:

1. Nadian H, Smith S E, Alston A M and Murray R S 1996. The effect of soil compaction on phosphorus uptake by *Trifolium subterraneum* in the presence of mycorrhizal colonisation. Poster presented at the Australian and New Zealand National Soil Conference, The University of Melbourne, Australia, Proceedings, Vol: 3, pp. 185-186.

2. Nadian H, Smith S E, Alston A M and Murray R S 1996. Soil compaction and mycorrhizal colonisation. Poster presented at the First International Conference on Mycorrhiza (ICOM 1), The University of California, Berkeley, U S A, Abstract, pp. 91.

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 author's 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 the thesis.

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

December, 1997

Signed

Habib Nadian Ghomsheh

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CHAPTER 1

GENERAL INTRODUCTION

Soil compaction resulting from trafficking of farm machinery may increase soil bulk density and mechanical impedance. An increase in soil bulk density may alter pore size distribution and may consequently alter the movement of air, water and nutrients. All these can affect plant growth via soil mechanical impedance (Castillo *et al.*, 1982; Misra and Gibbons, 1996) and/or via soil poor aeration (Voorhees *et al.*, 1975; Simojoki *et al.*, 1991).

Phosphorus (P) is of particular interest as a macronutrient because of its role in many important metabolic processes in plants. Diffusion is the main process in soil which brings P to the root surface where it may be taken up by the plant. The rate of movement of P in soil to plant roots is much less than the rate of absorption of P by the plant (Nye and Tinker, 1977) and in consequence, a zone of P depletion can develop in soil around roots. Because of the very low diffusion coefficient of P in soil, the characteristics of a plant root system, including its distribution, are very important to P uptake. Mechanically impeded roots, which are usually short and thick, have less ability to exploit a soil than unimpeded roots (Dolan *et al.*, 1992; Hoffmann and Jungk, 1995).

Mycorrhizal symbiosis is an intimate association which exists between plant root systems and certain groups of soil fungi. Vesicular-arbuscular mycorrhizal (VAM) fungi are among the most common and widespread soil fungi. It is now well known that VAM fungi improve mineral nutrition, particularly P nutrition, of the host plant. Some crop plants such as clover and onion absorb more P from soil when colonised by VAM fungi than when uncolonised. In mycorrhizal associations, nutrient transfer must be bidirectional in the symbiosis as a

whole. Carbohydrate movement from host plant to fungus and mineral nutrients from fungus to plant certainly takes place at the whole plant level (Smith *et al.,* 1994a). The extensive network of mycorrhizal hyphae beyond the depletion zone in soil can absorb and translocate P into host plant roots. This results in a higher rate of P uptake by mycorrhizal roots than by non-mycorrhizal roots (Sanders and Tinker, 1973; Smith, 1982; Jakobsen, *et al.*, 1992a; Smith *et al.*, 1994b).

More than 80% of plant species can be colonised by mycorrhizal fungi (Smith and Gianinazzi-Pearson, 1988), but some of species are more responsive to mycorrhizal colonisation than the other species (Smith and Read, 1997). It has been shown that mycorrhizal dependency (or responsiveness) is often a function of the morphology and characteristics of the host plant root system. Baylis (1975) suggested that plant species with coarse roots and few or short root hairs (so-called magnolioid roots) are more dependent on mycorrhizal colonisation than plant species with fine roots and long root hairs. The results reported by St John (1980) and Baon *et al.* (1994) confirm Baylis's hypothesis.

A root cannot penetrate a soil pore smaller than the diameter of the root cap if the soil is strong (Wiersum, 1957). Root growth will, therefore, be impeded by the decrease in average pore size associated with soil compaction, thereby decreasing both the volume of the soil explored by the roots and uptake of less mobile nutrients like P. Since the diameter of external hyphae of mycorrhizal fungi is much less than the diameter of impeded roots, it was proposed that external hyphae of VAM fungi may penetrate small pores and exploit a compacted soil more effectively than impeded roots.

Before this study was started, there were only two published reports on the effect of soil compaction on mycorrhizal colonisation. Mulligan *et al.* (1985), in their field experiment, found that colonisation of dry edible bean (*Phaseolus vulgaris* L.) root systems with indigenous VAM fungi decreased as the bulk density of the soil was increased from 1.1 to

1.4 Mg m^{-3} by excessive secondary tillage and traffic. In other work, Simmons and Pope (1987), in their pot experiment, found that the growth of mycorrhizal sweet gum roots *(Liquidambar styraciflua* L.) colonised by *Glomus fasciculatum* was greater than that of non-mycorrhizal roots in both uncompacted and compacted soils (bulk density = 1.25 and 1.55 Mg m^{-3}, respectively). However, the effects of soil compaction on shoot growth, P uptake and mycorrhizal colonisation were not reported.

Recently, the effects of mycorrhizal colonisation on plant growth and P uptake in compacted soil have been studied by Kothari and Singh (1996) and Li *et al.*, (1997, in press). The results reported by Kothari and Singh (1996) indicated that mycorrhizal colonisation of *Glomus intraradices* in association with citronella Java (*Cymbopagon winterianus* Jowitt) had no effect on growth and P uptake when bulk density of the soil increased from 1.2 to 1.4 Mg m^{-3}. In contrast, Li *et al.* (1997), using a compartmented mesh system, found a significant increase in hyphal contribution to P uptake when the roots of *Trifolium pratense* L. and hyphae of *Glomus mosseae* had access to outer compartments containing compacted soil (up to a bulk density of 1.8 Mg m^{-3}).

There have been no reports of how soil compaction may affect growth and P uptake by clover plants (*Trifolium subterraneum* L.) and how this effect may interact with mycorrhizal colonisation. Consequently this study was designed to answer the following questions.

1) Does soil compaction affect growth and P uptake by *Trifolium subterraneum* L.?
2) Does VA mycorrhizal colonisation interact with the effects of soil compaction to influence growth and P uptake by *T. subterraneum*?
3) How does soil compaction affect development of external hyphae and internal colonisation?
4) Do different VAM fungi cause different mycorrhizal growth response in *T. subterraneum* in compacted soil?

CHAPTER 2

REVIEW OF LITERATURE

2.1 Introduction

Soil and plant properties interact at the soil-root interface and thus determine the rate and amount of nutrients that actually enter the plant. Nutrient uptake by a plant from any soil is attributed mainly to the potential plant demand and to the ability of the soil to supply nutrients to plant roots. Boone and Veen (1994) pointed out that the demand for nutrients is determined by plant physiological and morphological characteristics. Although many characteristics of plant root systems such as length, diameter, intensity and branching are known to be genetically controlled, a number of soil properties such as soil compaction, soil aeration, water content and nutrient concentration can be involved. These characteristics of plant roots are very important in taking up nutrients, particularly those that are relatively immobile such as phosphorus.

Mycorrhiza may be considered as one of the plant root characteristics that may contribute to P nutrition of the plants. Phosphorus (P) transport across the root surface is usually faster than diffusive transport in soil, so P becomes depleted in a zone of soil around actively absorbing roots. The presence of a network of external hyphae of VAM fungi in soil increases P uptake by shortening the distance that P must diffuse through the soil to the plant. Although the greatest beneficial effects of mycorrhizal colonisation on plant growth have been shown to be due to improved P nutrition of the host plant, it has also been shown that mycorrhizal colonisation helps plants to thrive in arid conditions (Nelson and Safir,

1982), deters root pathogens (Gianinazzi-Pearson and Gianinazzi, 1983a) and increases soil aggregation in eroded soil (Koske *et al.*, 1975; Tisdall, 1991).

This chapter provides a short review of literature on general aspects of the importance of VAM fungi in increasing uptake of nutrients, particularly P, by the host plant. It will also consider the effects of soil compaction on plant growth and P uptake. To understand how soil compaction may affect mycorrhizal colonisation, we need to know how changes in soil properties arising from soil compaction may affect plant growth patterns and to what extent these changes may interact with plant growth and P uptake.

2.2 P uptake and plant growth

The amount of P and its availability in soil are important in determining the way in which mycorrhizal colonisation affects P uptake by the host plant (Bolan, 1991). To understand how mycorrhizal colonisation increases the uptake of P, firstly P transport to the plant roots and then some soil and plant properties which affect P uptake will be briefly reviewed.

2.2.1 Phosphorus in soil

The various forms of P in soils can be broadly categorised as organic and inorganic P (Dalal, 1977). One-half or more of total P in the A horizon of soils may be present as organic P, the amount depending on the organic matter in the soil. Organic P compounds are important sources of available P for plants following mineralisation. Dalal (1977) pointed out that the mineralisation of organic P in soil is largely due to the combined activities of the soil microorganisms and the free enzymes, phosphatases, present in soil. The factors that regulate the activity of microorganisms mainly govern the mineralisation of organic P.

Inorganic P can occur in soil solution, adsorbed on the soil surface or in the lattices of discrete minerals. The amount of plant-available P (principally $H_2PO_4^-$ and HPO_4^{2-}) present in the soil solution, is extremely small and in the range of 0.1-1 mg P kg^{-1} soil (Mengel and Kirkby, 1982). The greatest availability of P in soil is at around pH 6.5. At lower pH, most of the inorganic P is adsorbed onto the soil surface or precipitated as Fe and Al phosphates, whereas at higher pH (*e.g.* in calcareous soils) the availability of P is decreased by Ca and Mg (Olsen and Khasawneh, 1980; Freeman and Rowell, 1981). The availability of insoluble calcium phosphate in soil can be increased by increasing the production of chelating compounds such as citric acid in the rhizosphere (Dinkelaker *et al.*, 1989). The plant availability of P in soil depends mainly on soil pH, organic matter, soil water content and the population and activities of soil microorganisms, all of which influence the concentration of P in the soil solution.

2.2.2 Soil properties affecting P uptake

The first essential condition for the uptake of nutrients by plants is direct contact between the root surface and the nutrients in the soil solution. This can be provided either via transport of nutrients from the bulk soil to the root surface or via growth of roots to the sites where nutrients are located. P is delivered to plant roots largely by diffusion (Lewis and Quirk, 1967). Diffusion is the net movement of ions from one place to another by thermal agitation because of a concentration gradient, in the presence of a gradient, there will be a net movement from higher to lower concentrations (Lewis and Quirk, 1967; Olsen and Kemper 1968). Thus, the root of a plant, which can act as a sink for ions, can establish a concentration gradient in the water immediately surrounding the root and a net movement of ions from the soil solution to the root will occur. Nutrients may only diffuse at significant rates in the pores filled with water. Thus, soil water content has a major influence on the

effective diffusion coefficient for ion diffusion in the soil (Nye and Tinker, 1977). The effective diffusion coefficient (D_e) of P in soils can be expressed (Nye, 1966) as follows:

$$D_e = D_i \theta f/b$$

where D_e = effective diffusion coefficient in soil ($m^2\ s^{-1}$), D_i = diffusivity of the ion in water ($m^2\ s^{-1}$), θ = volumetric water content of the soil ($m^3\ m^{-3}$) which determines the fraction of the cross-section area available for diffusion, f = impedance factor and b = buffer power which can be calculated from the ratio of dD_i/dD_s, where C_i is the concentration of P in soil solution and C_s is the concentration of total diffusible P in the soil. Since diffusion is the main mechanism of P transport towards plant roots, any changes in soil properties that increase the rate of diffusive supply to the roots could increase P uptake. Many soil properties such as soil water content, soil bulk density, buffering capacity of the soil, tortuosity of the diffusion path and the diffusion coefficient of P in soil solution predominantly determine P transfer to the roots. Of these soil properties, soil water content and soil bulk density (both of which affect tortuosity) will be reviewed.

Soil water content

Soil water content is one of the most important factors which influences P diffusion in soil and consequently transfer to the plant root. Graham-Bryce (1965) in his study on the self - diffusion coefficient of rubidium found a rapid increase in the diffusion coefficient with increasing soil water content. Gahoonia *et al.* (1994) found that the water content of the soil greatly affected the depletion of inorganic P (extractable with 0.5 *M* $NaHCO_3$) in the rhizosphere. In this experiment, soil water content had no significant effect on the depletion of P in the soil solution in the immediate vicinity of maize roots, but the depletion zones extended from 1 to 2 mm when the volumetric water content of the soil was increased from 0.14 to 0.20 $m^3\ m^{-3}$, respectively. This indicates that spatial availability of soil P near the

roots is influenced by soil water content. Similarly, comparing the soils with the highest (0.40 $m^3 m^{-3}$) and lowest (0.13 $m^3 m^{-3}$) volumetric water contents, Cox and Barber (1992) observed that for maize to achieve the same total P uptake, the soil P concentrations needed to be 10 μM and 200 μM in wet and dry soil, respectively. A similar result with sugar beet was reported by Hoffmann and Jungk (1995). Such results indicate that a decrease in soil water content decreases the diffusion coefficient by decreasing the volume through which the ions can diffuse, and by increasing the tortuosity which decreases the effective concentration gradient.

Soil bulk density

An increase in soil compaction increases soil bulk density and penetrometer resistance. Increased soil bulk density alters pore size distribution and consequently alters content and movement of air and water. All these can affect nutrient dynamics at the soil-root interface. Hoffmann and Jungk (1995) found that an increase in soil bulk density from 1.30 to 1.65 Mg m^{-3} increased the effective P diffusion coefficient by increasing volumetric water content and also by increasing the amount of P per unit volume of the soil. The increase in diffusion coefficient in compacted soil in the presence of higher volumetric water content might be due to the production of a more continuous aqueous system in the soil pores, and hence a less tortuous diffusion pathway. However, Phillips and Brown (1965) showed that the buffering capacity per unit volume of soil varied linearly with bulk density, and P diffusion coefficient increased up to intermediate bulk densities (1.40 Mg m^{-3}). The effects of soil compaction on P uptake by plants will be discussed in more detail in Section 2.4.6.

2.2.3 Plant root characteristics affecting P uptake

For a relatively immobile nutrient like P, the total length and distribution of roots produced by the plant ~~is~~ are particularly important in determining total P uptake. Some morphological characteristics of roots such as length and intensity of root hairs, root diameter and root length greatly affect the ability of the roots to exploit nutrients. Roots are also able to affect chemically their immediate surroundings and hence to influence the bio-availability of P to the root.

Root hairs

Root hairs are usually between 80-1500 μm in length and 5-20 μm in diameter and are normally short-lived, collapsing after a few days (Hofer, 1991). The length of root hairs varies widely between plant species (Reid, 1981). For example, in onion plants, root hairs are virtually absent or very short, whereas the roots of rape, spinach, wheat and ryegrass plants are covered with long root hairs (Föhse *et al.*, 1991; Koide, 1991). However, the growth and intensity of root hairs of any particular plant species are affected by soil physical and chemical conditions such as soil compaction (Cornish *et al.,* 1984; Hoffmann and Jungk, 1995), soil water status (Mackay and Barber, 1985) and soil nutrient concentration (Föhse and Jungk, 1983; Ewen and Leigh, 1985; Föhse *et al.*, 1991).

Since root hairs increase the effective volume of soil explored by roots, their growth, distribution and activity are likely to affect uptake of water and nutrients. Itoh and Barber, (1983a, b) found that an increase in P uptake efficiency of plants was related to an increase in length and density of root hairs. Similarly, Föhse *et al.*, (1991) showed that in soils low in available P, the contribution of root hairs can account for up to 90% of total P uptake. In contrast, Hoffmann and Jungk (1995) reported that root hairs of sugar beet were not obviously related to P uptake efficiency, even when P availability was limited. These results

confirm those of Bole (1973) who also found that root hair development was not related to the P uptake efficiency of wheat roots. Such conflicting results might be due to the differences in length, density and diameter of the root hairs. However, differences in experimental conditions such as soil water content, soil P and soil texture might also be involved.

The length of roots and root hairs may be influenced by soil strength and this, in turn, may affect P uptake. Misra *et al.* (1988) found that large soil aggregates (19 mm) had higher mechanical resistance than small aggregates (4 mm). Total root length and the proportion of total length growing within aggregates was greater with small aggregates, whereas mean root hair length was greater with large aggregates. Misra *et al.* (1988) also reported that P uptake by cotton and sunflower decreased as soil aggregate size increased. However, increased length of root hairs in larger aggregates resulted in an increase in P uptake per unit length of root. This suggests compensation by roots in response to increased size and strength of aggregates in terms of larger root hairs, and hence increased uptake per unit length of root in stronger aggregates. Similarly, it has been shown that plants may be able to compensate when a part of the root system is subjected to stress (*e.g.* soil compaction, soil strength, soil temperature) by enhancing root growth and P uptake in an other regions where more favourable conditions exist (Russell and Goss, 1974; Crossett *et al.*, 1975; Jordan *et al.,* 1979; Shierlaw and Alston, 1984).

Root length and root diameter

Root length is one of the most important plant root characteristics which can influence the amount of P uptake from soil. Cornish *et al.* (1984) found a positive correlation between the amount of P uptake per plant and the length of ryegrass roots. Such a relationship between root length and P uptake has also been reported elsewhere (Boone and Veen,

1982; Hoffmann and Jungk, 1995). This indicates that high root length and therefore high total root surface are very effective in exploring a given volume of soil and in taking up P, due to the shorter distance that P must diffuse to the plant root surface. Plant species with thicker roots usually have higher rates of P uptake per unit root length. Hoffmann and Jungk (1995) found that an increase in root diameter in compacted soil resulted in an increase in P influx in mechanically impeded roots of sugar beet.

The relationship between the length of roots and the amount of shoots is another important factor which may affect P uptake. Föhse *et al.* (1988) reported that rape and spinach with low root:shoot ratios had higher P inflow than wheat and ryegrass with higher root-shoot ratios. Although root:shoot ratio is usually controlled by genetic factors, it may also be affected by environmental conditions such as soil compaction and nutrient availability. It has been shown that the P-deficient plants, which were grown in soil without P fertiliser application, have much higher root lengths per unit shoot weight (Hoffmann and Jungk, 1995). An increase in root surface area in deficient plants can be interpreted as a mechanism of adapting the uptake capacity of the root system to the P demand of the plant.

Root exudation

It has been shown that plant roots are able to increase the solubility of soil nutrients, including P, in the rhizosphere and thus successfully increase acquisition. Important examples of processes which can affect the availability of soil P in the rhizosphere are root-induced changes in rhizosphere pH, exudation of organic acids, phosphatase activity and microbial activity.

The plant factors that are responsible for changes in rhizosphere pH are extrusion of organic acids (Dinkelaker *et al.*, 1989; Hoffland *et al.,* 1989) and release of protons and bicarbonate ions (Gahoonia *et al.*, 1992).

Organic acids, predominantly citric but also malic and other acids, are released from the apical parts of the roots into the soil (Dinkelaker *et al.*, 1989; Grierson, 1992). Quantities of organic carbon released via the root system are estimated to be between 1-29% of the total dry matter produced by plants (Russell, 1977). Various forms of stress such as mechanical impedance, anaerobiosis, and mineral deficiency (*e.g.* of P, Zn, K, Fe) may strongly increase this carbon release (Marschner, 1991). Phosphate mobilisation by organic acids has been studied by several investigators (Fox *et al.*, 1990; Jones and Darrah, 1994). The excretion of these acids is greatly enhanced by P starvation of the plants. Rape plants were shown to excrete citric and malic acid which mobilised rock phosphate when plants were grown under conditions of P deficiency (Hoffland *et al.*, 1992). Excretion of large amounts of succinic, fumaric and citric acids from roots of leguminous crops has also been reported when the plants were grown under conditions of P starvation (Ohwaki and Hirata, 1992). The mechanisms found included the effects of protons but apparently more important is complexation of metals and ligand exchange of adsorbed P by carboxyl groups. It has been shown that citrate was much more efficient than malate in mobilising Al and Fe and to a lesser degree in mobilising P and Ca (Jones and Darrah, 1994).

P availability in the rhizosphere may be increased as a result of acidification and high cation absorption. Gahoonia *et al.* (1992) showed that with ammonium nitrogen nutrition, the soil pH around ryegrass plants was decreased by more than one unit. This resulted in a significantly greater depletion of soil P in the rhizosphere than where nitrogen was supplied as nitrate. Such a result shows the importance of proton excretion by plant roots in mobilisation of soil P in the rhizosphere.

Other root exudates such as acid phosphatases are able to hydrolyse organic P in the rhizosphere. The importance of phosphatase in P uptake by various crop plants in different soils has also been reported by Tarafdar and Jungk (1987). A significant correlation was

observed between the depletion of organic P around the root and phosphatase in the soil. The increase in inorganic P observed near the root surface by Tarafdar and Jungk (1987) and by Tarafdar and Claassen (1988) suggests that the rate of P mineralisation exceeded the influx of P into the roots. It is not yet clear whether the role of these enzymes is to release inorganic P from soil organic P, or to act as a trap for P-esters leaking out of the root. Much more work is needed to understand the reaction mechanisms of different root exudates with soil constituents and their interaction with soil organisms and with P sorption under various conditions.

In conclusion, the transfer of P from soil into plants includes plant and soil properties that interact in the rhizosphere. The amounts of P that are delivered to plant roots are determined by these properties. Attempts have been made to elucidate the nature and interactions between these properties, but it is difficult to evaluate the quantitative influence of each. However, this is a prerequisite for understanding the entire process of nutrient acquisition by plants from soil.

2.3 Mycorrhizas

One of the most important microbial effects on uptake of P by plants is due to mycorrhizas. Positive host growth responses are frequently found, particularly in soils of low nutrient status. This effect is usually attributed to enhanced nutrient uptake by mycorrhizal roots. It is commonly accepted that the fungi involved in the symbiosis are obligate symbionts.

2.3.1 Occurrence and morphology of mycorrhizal fungi

Vesicular-arbuscular mycorrhizal (VAM) fungi are the most widely distributed type of mycorrhiza. They establish their intimate association with more than 80% of plant species

(Smith and Gianinazzi-Pearson, 1988). Only a few members of Cruciferae, Proteaceae, Juncaceae, Chenopodiaceae and Cyperaceae are not able to form mycorrhizas of any sort (Harley and Smith, 1983).

VAM fungi develop a two-phase mycelial system in association with roots; an internal mycelium in the intermediate layers of the cortical parenchyma of the host plant and an external mycelium in the soil. It has been suggested that intercellular hyphae may contribute to absorption of carbon at the intercellular interface (Gianinazzi-Pearson *et al.* 1991). Vesicles and arbuscules of VAM fungi are located inside the roots of the host plant. Vesicles are apical or terminal swellings of the hyphae of VAM fungi that contain lipid. Not all VAM fungi form vesicles within roots (for example *Gigaspora margarita*). Arbuscules, the intracellular tree-like structures, are finely branched hyphae and are thought to be the main sites for metabolite and nutrient transfer between fungus and the host plant (Sanders and Tinker, 1973; Cox *et al.*, 1980; Smith and Gianinazzi-Pearson, 1988), but there is not direct proof of this as yet. External hyphae of VAM fungi are the functional organs for nutrient uptake and translocation to the host plants. External hyphae are classified as thick-walled, having diameters of more than 20 μm and thin-walled hyphae with diameters ranging from 2 to 3 μm (Friese and Allen, 1991). As will be discussed, external hyphae of different VAM fungi differ in their ability to spread into soil and absorb P and transfer it to the host roots (Jakobsen *et al.*, 1992a, b). The type of VAM fungi, the soil and the host plant may be important factors influencing the formation and spread of external hyphae in soil.

Some plant species normally become heavily colonised, whereas others are not susceptible to colonisation or have only patches of root colonised (Fitter and Merryweather, 1992). Mycorrhizal dependency might be controlled by physiological and morphological characteristics of the host roots and/or the fungus. For example, plant species with coarse

root systems and few root hairs are more responsive to mycorrhizal colonisation than plant species with fine roots and dense root hairs (Baylis, 1975; St John, 1980; Brundrett and Kendrick, 1988). An attempt was made to detect a relationship between root diameter of plant species in the British flora and mycorrhizal colonisation (Fitter, 1989). The results indicated that plant species with coarse roots were typically mycorrhizal, as predicted, but plant species with fine roots had a wide range of colonisation, suggesting that other factors may also influence mycorrhizal dependency.

2.3.2 Mycorrhizal effects on P uptake

The greatest beneficial effect of mycorrhizal colonisation on the growth of the host plant has been related to improved mineral nutrition. It is now well established that mycorrhizal plants absorb P and some other nutrients from soil more efficiently than non-mycorrhizal plants and that the external hyphae play key roles in effectively increasing the volume of soil available for acquisition of these nutrients (*e.g.* Sanders and Tinker, 1973; Cooper and Tinker, 1978; Abbott and Robson, 1982; Li *et al.*, 1991b; Smith *et al.*, 1994b). Increased uptake of the nutrients is a direct effect of colonisation and often leads to an increase in plant growth when nutrients are limiting. Much of the research on the mycorrhizal symbiosis has been concerned with the effect of mycorrhizal colonisation on P uptake and plant growth. Similar results are available for a very large number of plant species associated with different mycorrhizal fungi under different experimental conditions (*e.g.* Smith, 1982; Amijee *et al.*, 1989; Abbott *et al.*, 1984; Jakobsen *et al.*, 1992a). Data are mainly from pot experiments, but a few examples demonstrating mycorrhizal effects on nutrient supply under field conditions are also available (Jakobsen and Nielsen, 1983; Jakobsen, 1983, 1986; Merryweather and Fitter, 1996).

The increased P uptake by mycorrhizal roots might be due to more effective exploration of the volume of the soil by mycorrhizal roots than by non-mycorrhizal roots. External hyphae of VAM fungi in soil act as an extension of the root system, thereby providing a more extensive absorbing surface for uptake of P. Various mechanisms have been suggested to account for the increased efficiency of P uptake. Some of them are outlined below.

(I) The high effectiveness of fungal hyphae in improving mineral nutrition of the host plant is due not only to their small diameter and large surface area, but also to their continued development and extension in soil beyond the P depletion zones. Since the rate of movement of P by diffusion in soil is much lower than the rate of absorption of P by plant roots (Föhse *et al.*, 1988; Koide, 1991), the presence of external hyphae in soil increases P uptake by shortening the distance that P must diffuse through the soil to the roots. The fineness of external hyphae, compared with plant roots, enables the hyphae to enter very small pores that cannot be entered by most roots and thereby they may exploit a soil more effectively than roots. In compacted soil, since most large pores collapse or may be replaced by small pores, the external hyphae of VAM fungi might be expected to exploit a compacted soil more effectively than roots.

(II) It has been suggested that mycorrhizal roots might be able to exploit sources of P in soil which are not readily available to non-mycorrhizal plants. Poorly soluble P such as rock phosphate and Al and Fe phosphates (inorganic P), as well as calcium and magnesium phytate (organic P) are sources of P not normally available to plants. Murdoch *et al.* (1967) found that the increase in growth of mycorrhizal plants was larger with rock phosphate or tri-calcium phosphate than with soluble P. Similar results have also been reported following application of poorly soluble P *(e.g.* Ross and Gilliam, 1973; Powell and Daniel, 1978). It has been suggested that there is a disproportionate advantage to mycorrhizal plants when poorly soluble P has been applied. In these experiments, however, comparisons were made

at a particular level of P application between different sources of P. Pairunan *et al.* (1980), using complete P response curve for both soluble and poorly soluble P, suggested that the conclusion that mycorrhizal plants were better than non-mycorrhizal plants in getting P from poorly soluble P was not valid, because the improvement was in the same proportions as that obtained with soluble P. However, both synergistic action between mycorrhizas and P-solubilizing microorganisms (Azcón *et al.*, 1976) and possible excretion of H^+ by hyphae could increase the availability of poorly soluble P to mycorrhizal plants (Bolan, 1991).

(III) Organic P must be hydrolysed to inorganic P before it can be utilised. The hydrolysis of organic P is mediated by phosphatases that hydrolyse C-O-P ester bonds. Phosphatase activity in the rhizosphere, which may originate from either plant roots or soil microorganisms, plays an important role in the acquisition of P by plant roots. Many soil fungi produce phosphatases as extra-cellular enzymes, and *Aspergillus fumigatus* has a high capacity to produce phosphatases (Tarafdar *et al.*, 1988). The potential of VAM fungi to produce phosphatase was studied by Tarafdar and Marschner (1994). In a pot experiment, using a compartmented mesh system, they found a strong correlation between phosphatase activity and the length of hyphae of *Glomus mosseae*. Higher phosphatase activity throughout the hyphal compartment in inoculated soil, compared with non-inoculated soil, was matched by improved P nutrition and growth of mycorrhizal plants. The authors pointed out that production of phosphatase was associated with the extra-radical hyphae of the fungus. However, there was no clear evidence that mycorrhizal fungi were actually involved in the mineralisation of organic P (Jayachandran *et al.*, 1992; Joner and Jakobsen, 1994). Joner and Jakobsen (1994) pointed out that *Glomus sp.* (WUM 10) and *Glomus caledonium* were both capable of intercepting inorganic P released during mineralisation of organic P by microorganisms.

More work is needed to understand the interaction between the activity of the soil microflora in both mineralising and immobilising inorganic P and the potential capacity of mycorrhizal fungi to short-circuit this aspect of nutrient cycling.

2.3.3 Extraradical hyphae and the spatial availability of P

Differences in P content between mycorrhizal and non-mycorrhizal plants have frequently been used in the assessment of mycorrhizal effects on P supply to plants. Jakobsen (1992) pointed out this is a poor method of determining the mycorrhizal contribution, because the architecture of plant root systems is usually altered by mycorrhizal colonisation (Hetrick, 1991) and this, in turn, influences the uptake characteristics of the roots. Comparison of P inflow (P uptake per unit length of root per unit time) to mycorrhizal and non-mycorrhizal roots is more suitable for assessing the efficiency of P uptake by mycorrhizal plants (Sanders and Tinker, 1973; Jakobsen 1992). Sanders and Tinker (1973) measured P inflow to mycorrhizal and non-mycorrhizal roots of *Allium cepa* and found that the P inflow to mycorrhizal roots was much higher than that to non-mycorrhizal roots. Such increases in P inflow to mycorrhizal roots have also been reported for clover (Cooper and Tinker, 1981; Smith, 1982; Tester *et al.*, 1985; Jakobsen *et al.* 1992a), pea (Jakobsen, 1986) and onion (Sanders *et al.*, 1977; Smith *et al.*, 1994b) plants. P inflow to mycorrhizal roots is influenced by many factors such as environmental conditions, type of VAM fungi and host plant. For example, Tester *et al.* (1985) reported that the inflow of P to mycorrhizal clover (*Trifolium subterraneum*) roots was higher at high photon irradiance (450 μmol m^{-2} s^{-1}) than at low photon irradiance (100 μmol m^{-2} s^{-1}). Similarly, Son and Smith (1988) found that low irradiance (250 μmol m^{-2} s^{-1}) decreased the flow of P into mycorrhizal onion (*Allium cepa* L.) roots, even in the presence of additional P. Smith and Gianninazi-Pearson (1990) found that low irradiance had no effect on the intensity of mycorrhizal colonisation

and development of arbuscules. They pointed out that the negative effect of low irradiance on P uptake must be the result of either a more limited development of external hyphae or reduced physiological activity in terms of fungal P translocation and/or transfer to the root cells, or both.

The primary advantage of P uptake by mycorrhizal hyphae compared with roots is their ability to extend beyond the P-depletion zone surrounding the root. Rhodes and Gerdemann (1975) showed that mycorrhizal hyphae could absorb ^{32}P at up to 7 cm from the roots and translocate it to the host plant roots. However, they provided no information on actual depletion of P in the soil. Owusu-Bennoah and Wild (1979) found that most P taken up by mycorrhizal and non-mycorrhizal onion (*Allium cepa*) roots came from within 2 and 1 mm respectively of the root surface. They suggested that the effect of mycorrhizal colonisation was similar to that of a cylinder of root hairs 1 mm long. In contrast with this small effect, Li *et al.* (1991a), using a compartmented mesh system, found that in mycorrhizal white clover (*Trifolium repens* L.) the depletion of $NaHCO_3$-extractable P extended up to 11.7 cm (the length of the hyphal compartment) from the root surface, but in the non-mycorrhizal plants, the P depletion zone extended only about 1 cm from the root surface. From the data produced by these two experiments, it is not possible to know whether the observed differences in the abilities of VAM fungi to extend depletion zones of P are due to the differences in the hyphal characteristics or due to the differences in soil type, host plants and experimental conditions. The results reported with different species of VAM fungi under same experimental conditions indicated that mycorrhizal fungi differ in their ability to spread into the soil and absorb P beyond the P depletion zones around the roots (see below).

Abbott and Robson (1985a) found that *Glomus fasciculatum* produced less external hyphae per unit length of colonised root than did *Gigaspora calospora.* The results are in

agreement with those of Graham *et al.* (1982) but not with those of Sanders *et al.* (1977) who found that the weights of external hyphae per cm root colonised by *Glomus mosseae, Glomus macrocarpum, Glomus microcarpum* and *G. calospora* were similar. Abbott and Robson (1985a) observed that *G. fasciculatum* increased plant growth in spite of the relative paucity of external hyphae it formed compared with *G. calospora.* They pointed out that perhaps the hyphae formed by *G. fasciculatum* are better distributed for enhancing P uptake than are those formed by *G. calospora.* The results reported by Jakobsen *et al* (1992a) clearly indicated that the ability of VAM fungal hyphae to spread into soil is an important factor in improving mineral nutrition of the host plant. They compared the hyphal spread of *Acaulospora laevis, Glomus sp.* and *Scutellospora calospora* in association with *Trifolium subterraneum* in two-compartment systems. The results of this study indicated that hyphal density of *S. calospora* declined with increasing distance from the roots, whereas *A. laevis* had a constant hyphal density up to a distance of 11 cm from the roots at the final harvest. The superior ability of *A. laevis* to spread into soil beyond the P depletion zones resulted in a higher P inflow to mycorrhizal roots compared with the two other fungi. In an another experiment, Jakobsen *et al.* (1992b) found that the ability of *A. laevis* to transport ^{32}P over soil-root distances longer than 1 cm (up to 7 cm) was greater than *Glomus sp.* and *Scutellospora calospora.* Despite the low hyphal length density of *S. calospora* in soil, this fungus accumulated more ^{32}P in its hyphae and failed to transport it to the host plant compared with the two other fungi. The reasons for the poor P transfer by *S. calospora* have not been determined. However, they may include: 1) poor arbuscular development within the host roots, so that the total surface area available for P transfer was low and/or 2) the presence of a limiting factor in the hyphae for P transfer through the hyphae to the intra-radical hyphae or arbuscules within the roots.

Burkert and Robson (1994) found that the ability of plants colonised by *A. laevis* to absorb ^{65}Zn from soil 40 mm from *Trifolium subterraneam* roots was greater than that for *G. sp.* and *S. calospora*. Higher ^{65}Zn uptake by *A. laevis* beyond the roots was positively correlated with the hyphal length. However, *G. sp.* had a greater length of hyphae than *A. laevis* immediately adjacent to the roots. Hyphal length of *S. calospora* was much lower than that of the two other mycorrhizal fungi at all distances from the roots. These results once again emphasise that there are great differences in the abilities of the hyphae of VAM fungi to spread into soil and consequently to absorb nutrients and transport them to the host plants.

The results reported by Jakobsen *et al.* (1992a, b) and Burkert and Robson (1994) indicate that the faster spread of external hyphae of *A. laevis* in the soil, compared with that of the other two fungi, enables this fungus to exploit a larger volume of the soil and consequently increase the rate of uptake by shortening the distance that nutrients must diffuse through the soil to the host plant. This indicates that hyphal spread is an important factor affecting mineral nutrition of the host plant, although the capacity of external hyphae to absorb nutrients and transfer them to the host plant may also be involved.

2.3.4 Translocation of P within hyphae

Smith *et al.* (1994b) identified three steps in the transfer of inorganic P (P_i) between the mycorrhizal fungus and the host plant: 1) uptake of P_i from soil by extra-radical fungal hyphae, 2) translocation through the hyphae to the intra-radical hyphae or arbuscules within the root and 3) transfer from fungus to plant root across the fungus-root interface. The latter step involves two sequential membrane transport steps: efflux from the fungus (passive step) and uptake by the plant root cells (active step). Uptake of P_i from the soil by external hyphae must also be an active step in transfer of P to the host plant roots

(Woolhouse, 1975, Smith and Smith 1990). Following P uptake, much of the P_i in vacuoles of external hyphae is converted into polyphosphate via polyphosphate kinase (Cox and Tinker, 1976; White and Brown, 1979; Cox *et al.*, 1980). Transport of P along hyphae is clearly of great importance of this whole process. It has been suggested that the transfer of P along hyphae occurs mainly by transport of orthophosphate, either by diffusion, mass flow, or cytoplasmic streaming (Thompson *et al.*, 1987; Jennings 1987, 1989) and also via movement of polyphosphate granules (*e.g.* Cox *et al.*, 1980). Recently, it has been shown that polyphosphate is in soluble form in the vacuole of living hyphae of the ecto-mycorrhizal fungus *Pisolithus tinctorius*, and probably also in many other fungi (Orlovich and Ashford, 1993). They suggested that polyphosphate is transported by the motile tubule and vacuole system present in this fungus and the movement of P along hyphae is independent of the cytoplasmic streams . Transfer of P by this mechanism over the long distances which would be required in external hyphae of VAM fungi is still an open question (Ashford *et al.*, 1994). In VAM fungi, polyphosphate is broken down by alkaline polyphosphatase activity in the vacuoles in the arbuscules (Gianinazzi *et al.*, 1983b) and the activity of endo- and exo-polyphosphatase (Capaccio and Callow, 1982).

The role of alkaline phosphatase in fungal P metabolism is still not understood. The activity of alkaline polyphosphatase has been shown to be located exclusively in the arbuscules and internal hyphae (Capaccio and Callow, 1982). In contrast, it has been suggested that the activity of this enzyme might be an effective vital marker for the external hyphae (Dodd, 1994). Furthermore, Larsen *et al.*, (1996) have shown that while the fungicide benomyl inhibits P uptake and transfer to plants via fungal hyphae, it does not affect alkaline phosphatase activity, so that the activity of this enzyme was not a suitable physiological marker in this instance. More work is needed to understand the actual role of this enzyme in fungal P metabolism.

2.3.5 Uptake of other nutrients

Although the greatest beneficial effect of VAM fungi on the growth of the host plant has been related to improved P nutrition, it has also been shown that mycorrhizal colonisation can increase the uptake of some other nutrients such as Zn and Cu from soil. The mobility of these two nutrients in soil is low and diffusion is the rate-limiting step in the transport of these elements to the root surface. Thus Zn and Cu concentration gradients and depletion zones in the rhizosphere may be similar to those of P.

It has been shown that mycorrhizal *Araucaria cunninghamii* plants had higher concentrations of ^{65}Zn than non-mycorrhizal plants (Bowen *et al.*, 1974). The results of Cooper and Tinker (1978) showed that external hyphae of *G. mosseae* were able to translocate ^{65}Zn from soil into *Trifolium repens* roots. Subsequent reports indicated that mycorrhizal colonisation increased concentration of ^{65}Zn in maize (Faber *et al.*, 1990; Kothari *et al.*, 1991), peanut (Bell *et al.*, 1989), apple (Gnekow and Marschner, 1989), wheat (Thompson, 1990) and in subterranean clover (Burkert and Robson, 1994).

There is some evidence that concentrations of Cu in mycorrhizal plants are higher than those in non-mycorrhizal plants (Daft *et al.*, 1975; Kucey and Janzen, 1987; Gnekow and Marschner, 1989). Li *et al.* (1991b) showed that the concentration of Cu was significantly higher in mycorrhizal plants than in non-mycorrhizal plants and that the quantity of Cu transported was not directly proportional to the quantity of P being transported at the same time. With low P application, the concentration of Cu in roots was maintained high, whereas with high P application the concentration of Cu in roots decreased due to its translocation to the shoots. This may be due to the antagonistic effects between Cu and P in uptake and translocation by plant roots and/or, as Li *et al.* (1991b) suggested, due to the presence of a mechanism in VAM roots regulating the release and transport of Cu from

roots according to the shoot demand. The mechanisms leading to better uptake of Zn and Cu by VAM fungi might be similar to those of P.

2.3.6 Quantity of hyphae produced in soil

The amounts of VAM fungal hyphae in soil varies greatly. In some cases, fungal hyphae in soil could be 80 to 200 times the length of colonised root (Sanders and Tinker, 1973; Tisdall and Oades, 1979; Jakobsen *et al.*, 1992a), which explains why hyphae may efficiently acquire a large amount of P beyond the depletion zone around the roots. The range of maximum values reported for soil hyphae range between 1 and 27 m g^{-1} of soil (Tisdall and Oades, 1979; Abbott *et al.*, 1984; Abbott and Robson, 1985a; Sylvia, 1988). The biomass of external hyphae has also been estimated by severals workers (see below). Bethlenfalvay and Ames (1987) found 10-40 µg chitin g^{-1} soil. Olsson *et al.*, (1995) found that the amounts of neutral and polar fatty acids (another estimate of hyphal biomass in soil: see below) were between 0.1-5.0 and 0.3-31.0 nmol g^{-1} soil respectively, while Sanders *et al.* (1977) found 3.6 µg of hyphae per cm of colonised root. The relationship between the amount of external hyphae and the rate of root colonisation might be influenced by the type of soil, plants and fungi.

2.3.7 Soil properties influencing development of external hyphae

Despite the importance of external hyphae of VAM fungi in taking up nutrients from soil, little is known about the effect of soil properties on hyphal development. However, there is some information on the effect of soil P content, which is negatively correlated with hyphal growth. Phosphate addition to plants with adequate P for growth decreased four-fold the length of external hyphae per cm colonised root (Abbott *et al.*, 1984). Similarly, Schwab *et al.* (1983) found that high P application reduced the proliferation of external hyphae.

However, it has been reported that a small addition of P to P-deficient soil increased the amount of external hyphae (Abbott *et al.*, 1984).

Formation and development of external hyphae of VAM fungi may be influenced by soil pH. Abbott and Robson (1985b) found a significant increase in the length of external hyphae of *Glomus sp.* (WUM 16) when soil pH was increased from 5.0 to 7.4 by adding $CaCO_3$. The poor formation of the external hyphae of *G. sp.* (WUM 16) at low pH might be due to the toxicity of Al and Mn ions and/or to the deficiency of Ca and Mg which can occur in acid soils. However, the ability of some species of VAM fungi to form external hyphae in acid soils is greater than in alkaline soils. For example, Graham *et al.* (1982) found that isolates of *Glomus fasciculatum* from acid soils produced less external hyphae when grown at a higher soil pH (7.3) than did isolates from more alkaline soils. This suggests that VAM fungi differ in their sensitivity to soil pH.

Carbon dioxide is known to have an effect on the metabolism and growth of fungi (Tabac and Cooke, 1968; Griffin, 1981). Bécard and Piché (1989) found that the fungal growth *in vitro* of *Gigaspora margarita* was stimulated greatly when the concentration of CO_2 was increased from 3×10^{-4} to 5×10^{-3} m^3 m^{-3} in the air. The importance of CO_2 during symbiosis had also been investigated by Saif (1984). In three host plants grown on soil maintained at 0.16 m^3 m^{-3} O_2, the percentage of root length colonised by *Glomus macrocarpum* and the number of vesicles increased as the concentration of CO_2 in the soil air was increased to 4×10^{-2} m^3 m^{-3}. He did not measure the amount of external hyphae in the soil, however he pointed out that the effect of CO_2 on the mycorrhizal plants was an indirect one on the extra-radical phase of the fungus. Such increase in hyphal growth may not occur in compacted soils, because the increase in the concentration of CO_2 which results indirectly from soil compaction is accompanied by a decrease in the concentration of O_2 in the soil air.

2.3.8 Measurement of external hyphae

Several different methods have been used to assess the amount of external hyphae of VAM fungi in soil. The filtration-gridline method has been widely used, but it is difficult to distinguish hyphae of the inoculant VA mycorrhizal fungi from those of the fungi present in the control treatment (Abbott and Robson, 1985a; Sylvia, 1992; Sukarno *et al.*, 1993). Attempts have been made to use hyphal diameter larger than 5μm as a criterion to identify VAM fungi hyphae (Graham *et al.*, 1986; Bethlenfalvay and Ames, 1987). However, it has been shown that most hyphae in pot cultures inoculated with VAM fungi had diameters of less than 5 μm (Abbott and Robson, 1985a). Both Nicolson (1959) and Friese and Allen (1991) have emphasised the striking variation in diameter of mycorrhizal hyphae from 2 to 20 μm. Thus it is inappropriate to use diameter as a criterion for identification. As Abbott and Robson (1985a) emphasised, it is not easy to distinguish hyphae of the inoculant VAM fungi from those of fungi present in control treatments by the filtration-gridline method.

Chitin determination is an indirect method that has been used to estimate hyphal biomass in soil under controlled experimental conditions (Pacovsky and Bethlenfalvay, 1982; Bethlenfalvay and Ames, 1987). Since the cell walls of most fungi and many other organisms contain chitin, its utility for estimating VAM fungi is limited (Sylvia, 1992).

Attempts have also been made to estimate mycorrhizal biomass by analysing ester-linked phospholipid fatty acids (PLFA). Several fatty acids have been reported as common in lipids from VAM structures (Beilby and Kidby, 1980; Pacovsky and Fuller, 1988: Pacovsky, 1989) and have been used as indicators of fungal biomass. Olsson *et al.* (1995) found that the amounts of both phospholipid and neutral lipid fatty acids 16:1ω5, 16:0 and 20:5 were greater in inoculated soil than in uninoculated soil. These fatty acids have therefore been suggested as indicators of fungal biomass (Jakobsen. 1991).

Although external hyphae of VAM fungi play key roles in the absorption of nutrients, in the spread of colonisation and in the formation and stabilisation of soil aggregates, quantification of external hyphae in soil is still subject to limitations.

2.4 Soil compaction, plant growth and P uptake

Plant growth is restricted when the uptake of water, oxygen and nutrients is less than the demand of the plant. Soil compaction may cause such restrictions. Compaction can arise in a number of ways: natural pedogenic processes, compaction by heavy farm machinery and by stock grazing, and by hard-setting of soil on drying. The functioning of roots in compacted soil can be restricted by mechanical impedance (Tsegaye and Mullins, 1994; Materechera *et al.*, 1991), and/or by poor aeration caused by low air-filled porosity and discontinuity of pores (Agnew and Carrow, 1985; Hoffmann and Jungk, 1995). An impeded root system may greatly restrict plant uptake of less mobile nutrients like P and Zn, particularly in soils with low concentrations of these nutrients.

The effects of mechanical impedance on root growth have been reviewed extensively by Barley and Greacen (1967); Taylor *et al.* (1972); Russell (1977); Bennie and Krynauw (1985) and Bengough and Mullins (1990). However, the effects of soil compaction on nutrient uptake by roots have received much less attention than the effects on growth itself.

2.4.1 Soil mechanical impedance

Mechanical impedance refers to the resistance offered by the soil matrix against deformation by a growing root: it permits root elongation only to the extent that root pressure exceeds the mechanical impedance (Bennie, 1991). Mechanical impedance increases with increasing soil compaction and it also increases as the matric potential of soil water decreases during

drying. Mechanical impedance to root growth is one of the most common factors determining root elongation and proliferation within a soil profile (Bengough and Mullins, 1990). Penetrometers are widely used to estimate resistance of soil to root growth.

2.4.2 Comparison of root resistance with penetrometer resistance

The best way to determine the relationship between penetrometer resistance and root resistance is to measure both independently in the same soil. However, because of experimental difficulties in measuring root resistance, relatively few such studies have been made (Misra *et al,* 1986a; Bengough and Mullins, 1991).

Indirect evidence for the difference between root and penetrometer resistance comes from comparing studies of root elongation rate and penetrometer resistance with measurements of the maximum pressure that roots can exert. The maximum axial root growth pressure that a root can exert is between 0.24 and 1.45 MPa (Misra *et al.*, 1986b) depending on plant species. Critical values of penetrometer resistance at which root elongation ceases are between 0.8 and 5.0 MPa (Greacen *et al.,* 1969). The results are variable because of differences between type of plant and soil (Greacen, 1986). It is clear that penetrometers experience greater resistance than plant roots when penetrating the same soil. The reason for this difference must be physical differences in the way in which plant roots and metal probes penetrate soil (Bengough and Mullins, 1990). In spite of this difference between penetrometer and root resistance, penetrometers have been widely used to provide estimates of resistance to root growth in soil.

2.4.3 Effect of soil compaction on plant growth

The ability of plant roots to penetrate a compacted soil is usually decreased by high mechanical impedance and collapse of most large pores and there are numerous reports

which indicate root growth of different plant species decreases with increasing mechanical impedance. For example restricted root growth of barley (Russell and Goss, 1974; Goss, 1977), maize (Barley, 1962; Boone and Veen, 1982; Shierlaw and Alston, 1984; Materechera *et al.,* 1991), pea (Castillo *et al.*, 1982; Tsegaye and Mullins, 1994), ryegrass (Cornish, *et al.*, 1984; Shierlaw and Alston, 1984), sugar beet (Draycott *et al.*, 1970; Hoffmann and Jungk, 1995) and white clover (Cook *et al.*, 1996) have been reported. Effects of soil compaction on the growth of roots and shoots of clover (*Trifolium subterraneum* L.) and onion (*Allium cepa* L.) plants have not been investigated, as far as I am aware.

When sufficient continuous pores larger than the root tip are available in soils, the root will grow through these pores following a path of low mechanical resistance. In most soils, the root tips may be thicker than the pores: in this case, the soil particles will be moved aside by the advancing roots. Roots must overcome mechanical impedance of the soil to penetrate pores of diameters smaller than themselves. Aubertin and Kardos (1965) found that maize roots were able to widen the narrow pore necks by plastic deformation and subsequently to enlarge into pore cavities.

As soil compaction decreases the number of large pores, the fine roots might be expected to penetrate the compacted soil more easily than the thicker ones (Edwards *et al.*, 1964). Richards and Greacen (1986) and Greacen (1986) developed a theoretical model of cavity expansion in granular media, and pointed out that thin roots may deform the soil elastically, thereby encountering less resistance than thicker roots which cause plastic deformation. However, such a response does not always occur (Gooderham, 1977) and interpretation of various responses is difficult as differences in root diameters are confounded with species, which for other reasons may vary in response to compacted soil (see below).

It has been shown that plant species have different abilities to penetrate a compacted soil. Elkins *et al.* (1977) indicated that roots of Pensacola bahia grass (*Paspalum notatum* cv Flugge) were able to penetrate compacted soil layers that restricted growth of cotton roots. They suggested that the fibrous sheath beneath the epidermis in the roots of bahia grass gave them rigidity and enabled them to penetrate a compacted soil. Taylor and Ratliff (1969) found that elongation rate of cotton roots (*Gossypium hirsutum* cv Empire) decreased by 62% when penetrometer resistance of the soil was increased from 0.40 to 1.0 MPa, whereas a similar increase in penetrometer resistance decreased elongation rate of groundnut roots (*Arachis hypogea* cv Virginia Bunch) by only 29%.

Among root morphological characteristics, root diameter is an important factor influencing root penetrability. Materechera *et al.* (1991) found that in strong soil, dicotyledonous species generally had larger root elongation and root thickness than those of monocotyledons. This suggests that the roots of most dicotyledonous species could have exerted more axial growth pressure on the soil than those of monocotyledons, because of differences in root diameters. Earlier, Misra *et al.* (1986b) had reported that the maximum growth pressures exerted by the roots increased with increasing root diameter. In contrast, Taylor and Gardner (1960) could not find differences between penetrating ability of legumes (root diameter 1.80 mm) and that of non-legumes (root diameter 0.55 mm). Bennie and Burger (1981) found that the relative decrease in root length, rooting density, or the number of roots entering a compacted layer was the same for maize, cotton, wheat and groundnuts. They suggested that the differences observed among plant species are merely a function of differences in their abilities to produce roots in an uncompacted soil. Accordingly, plants with many roots will have a higher probability of finding sites of lower mechanical impedance than plants with few roots. However, differences in abilities of roots

to penetrate compacted soil might also be due to the genetic differences and/or physiological differences which exist among plant species.

Changes in mechanical impedance due to soil compaction through the rooting zone can modify root growth and root distribution. Russell and Goss (1974) showed that when elongation of root axes of barley was limited by mechanical impedance but there was sufficient opportunity for the lateral roots to grow, there was no significant difference in P uptake and plant dry weight between plants with impeded and unimpeded roots. Shierlaw and Alston (1984) grew maize and ryegrass in pots containing three layers of soil. The top and bottom layers had a bulk density of 1.20 Mg m^{-3}, while the bulk density of the central layer of soil was compacted to one of the 12 bulk densities between 1.20 and 1.75 Mg m^{-3}. They found that total dry weight of the plant tops and total root length were only slightly affected by compaction of the soil, whereas root distribution was greatly altered. In fact, the plants were able to compensate for the lack of rooting depth caused by compaction by producing more roots in the surface layer of the soil. The importance of these observations for evaluating practical situations is obvious - an appreciable restriction in root extension caused by mechanical impedance may not necessarily decrease the yield of crops.

2.4.4 Effect of soil compaction on root morphology

Mechanical impedance arising from soil compaction can affect plant morphological characteristics such as root thickness, root shape, root branching and leaf area. Mechanically impeded roots are usually shorter, thicker and more irregularly shaped than the thinner fibrous roots that develop under low-strength conditions (Goss and Russell, 1980; Materechera, *et al.*, 1991). The increase in diameter (Materechera, *et al.*, 1991; Hoffmann, and Junk 1995; Misra and Gibbons, 1996) of mechanically impeded roots is due

to an increase in thickness of the cortex, in which cells become shorter and wider, while the cell volume is unaffected (Barley, 1976; Atwell, 1988).

It has been shown that mechanical impedance in compacted soil affects the pattern of root branching differently. A number of authors reported that increased mechanical impedance increased lateral branching (Russell and Goss, 1974; Goss, 1977), while others (Schuurman, 1965; Boone and Veen, 1982) found that the number of lateral roots per main axis decreased with increasing soil compaction. An increase in root branching at increased mechanical impedance has generally been found in artificial substrates (ballotini) when existing pores are large enough to allow unimpeded growth of lateral roots. In a strong soil, however, outgrowth of lateral roots may be obstructed because most pores are smaller than the diameter of the laterals roots. Another phenomenon obscuring the influence of a high mechanical impedance on root branching is the fact that lateral roots emerge much closer to the apex of the main axes than at low mechanical impedance (Goss, 1977). For the study of the influence of the mechanical impedance on root branching, basal segments, on which lateral roots have fully developed, should be compared.

Some anatomical changes in plant roots may occur due to mechanical impedance. Thickening of the casparian strips and cell walls in the xylem vessels observed by Baligar *et al.* (1975) were a result of increased soil bulk density. Thickening of the casparian strips and cell cortex had no effect on the movement of P, which follows a symplastic pathway (Baligar *et al.*, 1980).

Mechanical impedance of roots may also affect growth and morphology of plant leaves. Cook *et al.* (1996) found that both the leaf area and relative expansion rates of leaves decreased as soil compaction increased. The decrease in leaf area might be due to insufficient supply of water and nutrients to the leaves: the reduction in leaf area may be a mechanism of adaptation to decrease transpiration.

It has been shown that ethylene can occur in anaerobic or partially anaerobic soils at concentrations which can be injurious to plant roots (Smith and Restall, 1971; Dowdell *et al.*, 1972; Jackson *et al.*, 1981). Smith and Robertson (1971) reported that concentrations of ethylene higher than 1 cm^3 m^{-3} (up to 10 cm^3 m^{-3}) decreased the growth of roots of rice, rye and barley, although the sensitivity of different species varied widely.

It has also been shown that mechanically impeded roots produce more ethylene than unimpeded roots and high concentrations of ethylene may affect the pattern of root growth. Kays *et al.*, (1974) found that when the axial growth of bean roots was impeded by a barrier, the rate of ethylene evolution increased by as much as six times that of unimpeded controls. The increase in the concentration of ethylene resulted in radial expansion of the roots. Similarly, Moss *et al.* (1988) who studied the production of ethylene by the roots of maize grown in glass chambers containing small glass spheres (ballotini), found that mechanical impedance decreased the root length of maize by up to 40%, while ethylene evolution from roots was increased 2-3.5 fold. These observations show that there is a correlation between the production of ethylene and the change in the pattern of root growth cause by mechanical impedance. The role of enhanced ethylene evolution in the response of roots to mechanical impedance is not clear. However, Moss *et al.* (1988) suggested that the principal cause of the faster ethylene production by impeded roots was not a direct effect of mechanical impedance, but was instead a result of the radial expansion of the roots into rigid media, resulting in some physical wounding. However, this suggestion does not entirely explain why the production of ethylene observed by Kays *et al.* (1974) started to increase within 1 hour when the axial growth of bean roots was impeded by a solid barrier. Further work is needed to understand better of the role of ethylene in the response of roots to mechanical impedance.

2.4.5 Soil aeration and plant growth

As the process of soil compaction leads primarily to the collapse of the large pores, that is, those pores responsible for effective aeration, the net effect of compaction is to increase soil water at the expense of soil air. During compaction, the largest air-filled pores disappear and are replaced by smaller, mainly water-filled pores. Such changes in soil pore size distributions restrict the rate of gas diffusion which leads to a considerable change in the concentrations of O_2 and CO_2 in the soil atmosphere (Grable, 1966; Cannell, 1977). An increase in the concentration of CO_2 up to 0.10 m^3 m^{-3} and a decrease in the concentration of O_2 up to 0.06 m^3 m^{-3} in the air of compacted soil have been reported (Shierlaw and Alston, 1984; Simojoki *et al.*, 1991). The concentrations of O_2 and CO_2 in the soil atmosphere are influenced by a number of factors such as soil water content, biological activities, root density, soil texture and soil temperature.

Meek and Stolzy (1978) noted that restriction of soil aeration for 24 hours can decrease growth, while longer periods may result in cell death. Trought and Drew (1980a) measured the concentration of a wide range of nutrients in waterlogged wheat seedlings and found that there was a considerable decline in uptake of all ions shortly after flooding of the soil. This is consistent with a general impairment of root function due to O_2 deficiency in and around the roots. Similarly, Atwell and Steer, (1990) found that uptake of N, P, K and Ca by maize from nutrient solution was impaired due to poor aeration. The primary mechanism responsible for the inhibition of shoot growth is still not clear. Trought and Drew (1980b) investigated some of the main soil factors which could impair plant growth and concluded that O_2 deficiency in the root tissues, rather than changes in soil chemistry, caused shoot growth to be impaired. Jackson and Drew (1984) discussed 'positive' and 'negative' messages emanating from roots as potential growth-controlling factors; some of these could be hormonal, *e.g.* ethylene or abscisic acid.

In compacted soil, the effect of O_2 deficiency on plant growth is usually combined with the effect of mechanical impedance (Blackwell *et al.*, 1985). Eavis (1972) attempted to separate these two factors by defining an 'aeration deficiency index' which required measuring root elongation rate at a specified physical resistance without an aeration factor. Voorhees *et al.* (1975) found that at mechanical resistances > 1 MPa, the root elongation rate of pea decreased with increasing air-filled porosity of the soil, whereas at mechanical resistances ≤ 1.0 MPa root elongation rate decreased with decreasing air-filled porosity. This suggests that the sensitivity of the roots to poor aeration decreased with increasing mechanical resistance.

Since soil aeration depends on a range of both plant and soil properties, no single property can describe soil aeration completely. However, air permeability, oxygen diffusion coefficient and carbon dioxide and oxygen content in the soil atmosphere are presented as indicators of soil aeration (Gliński and Lipiec, 1990). Air-filled porosity is the simplest indicator of soil aeration status and has been widely used (Stepniewski *et al.*, 1994).

Lower limits of air-filled porosity for maintaining satisfactory aeration depend on the soil properties and plant species. Blackwell *et al.* (1985) found that in soils subjected to wheel traffic, soil aeration was poor enough to impair crop establishment, growth and yield when air-filled porosity was ≤ 0.05 m^3 m^{-3}. In contrast, Grable and Siemer (1968) presented a safe limit of 0.12-0.15 m^3 m^{-3} air porosity for root growth of maize. A tentative lower limit of 0.10 m^3 m^{-3} air porosity in soils for growth of plant was suggested by Wesseling and van Wijk, (1957).

2.4.6 Effect of soil compaction on P uptake

The effects of soil compaction on nutrient uptake by roots have been studied much less than the effects on plant growth itself. Soil compaction decreases the larger pore fractions and

increases the smaller. Such changes in soil pore size distributions affect capacity and movement of air, water and nutrients. All these affect the supply of nutrients, particularly relatively immobile ions such as P, to plant roots and consequently may affect plant growth. In addition to soil physical properties, root morphological characteristics such as root diameter, root length, root branching, length and density of root hairs may have a major influence on uptake of P (see Section 2.2.3).

It has been shown that soil compaction had no significant effect on the concentration of P in the shoots of pea (Castillo *et al.*, 1982); ryegrass (Cornish *et al.*, 1984) and sugar beet (Hoffmann and Jungk, 1995). However, the uptake of P per plant has been reported to be impaired by soil compaction. Boone and Veen (1982) showed that at low P, soil compaction to a penetrometer resistance of 3.0 MPa had no effect on P uptake, whereas with high P application, P uptake significantly decreased as soil compaction was increased. Such declines in P uptake from compacted soil have also been reported elsewhere (Cornish *et al.* 1984; Dolan *et al.*, 1992; Hoffmann and Jungk, 1995). With respect to the results of these experiments it seems there is a relationship between root development and P uptake in soil: decreased P uptake per plant appears to be a consequence of the restriction in the length of roots in compacted soil.

Change in the pattern of root growth (decreased root length and increased root diameter) may affect total P uptake per unit root length. Cornish *et al.*, (1984) found that an increase in the bulk density of the soil from 1.0 to 1.54 Mg m^{-3} increased total P uptake per unit length of ryegrass roots. Similarly, Peterson and Barber (1981) found that increasing root diameter from 0.34 to 0.44 mm increased maximal influx from 4.6 to 7.1 pmol cm^{-2} s^{-1}. Barber (1984) suggested this may be due to the greater area of plasma membrane within the cortex per unit area of the root surface. Shierlaw and Alston (1984) studied the effect of soil compaction on growth and P uptake by maize and ryegrass plants and showed that total

P uptake per unit length of ryegrass roots increased up to intermediate bulk densities (1.35-1.45 Mg m^{-3}), but such increase was not observed in maize plants with increasing soil compaction, although the length of maize roots responded to the compaction treatments in a manner similar to those of ryegrass.

Change in root:shoot ratios resulting from soil compaction may have an influence on P uptake. Hoffmann and Jungk (1995) found that an increase in soil compaction from a bulk density of 1.3 to 1.65 Mg m^{-3} decreased root: shoot ratio of sugar beet plants in terms of root length per unit shoot weight. The decrease in this ratio was accompanied with an increase in P inflow to the roots with increasing soil compaction. It is not clear whether decreased root:shoot ratio was a consequence of the improved P nutrition of the plant or due to a greater effect of soil compaction on root length than on shoot growth.

2.5 Summary and conclusions

In VA mycorrhizal plants, it is widely accepted that the enhancement of plant growth which arises from fungal colonisation is attributed to the improved soil exploitation provided by the external hyphae. Increased rates of P uptake and inflow to plant roots have generally been considered to be the major consequence of mycorrhizal colonisation, although it has been shown that VAM fungi can also significantly enhance absorption of some other nutrients such as Zn and Cu.

The presence of a network of external hyphae in soil beyond the P depletion zones around the roots provides a large absorbing surface for uptake of relatively immobile ions like P which are usually delivered to plants by slow diffusion processes. Although external hyphae of VAM fungi play key roles in the formation and functioning of mycorrhiza in ecosystems, the nature of the relationship between the growth of hyphae in soil and within the root is not well known. Sanders *et al.* (1977) found a positive correlation between the

amount of external hyphae and the extent of internal colonisation of four species of VAM fungi, but such relationships have not been found for other species (Graham *et al.*, 1982; Abbott and Robson, 1985a; Jakobsen *et al.*, 1992a). Soil properties, morphological and physiological characteristics of mycorrhizal fungi and the host plants may affect this relationship. Further study is needed with a wider range of VAM species to understand the relationship between the amount of external hyphae and mycorrhizal roots. Our knowledge about the interactions between external hyphae and soil properties is poor. We do not know why species of VAM fungi differ in their ability to absorb P and transfer it to the host plant. If there is a positive correlation between the ability of a fungus to absorb P and its ability to spread into soil, then which mechanisms are responsible for hyphal spread? Despite the importance of external hyphae in improving the mineral nutrition of the host plant, there are no reliable methods to quantify the amount of external hyphae of VAM fungi from those of non-mycorrhizal fungi in soil. Improved methods are essential to further our understanding of the functioning of the hyphal system.

It has been shown that there is a correlation between mycorrhizal dependency and root morphology of the host plant (Baylis, 1975; St John, 1980; Baon *et al.* 1994). This review has also shown that an increase in soil bulk density due to soil compaction may alter soil pore size distribution, aeration and movement of water and nutrients. All of these can affect the morphological characteristics of root systems and this, in turn, may affect the mineral nutrition of the plant. Changes in the morphological characteristics of the plant roots resulting from soil compaction may affect mycorrhizal colonisation, but little is known about the effect of colonisation on growth and P uptake when a host plant is subjected to soil compaction. There have been no reports of how soil compaction may affect P uptake and the pattern of the growth of subterranean clover and how these changes may interact with mycorrhizal colonisation. Since the diameter of external hyphae is much less than the

diameter of clover roots, soil compaction may affect the growth of external hyphae and the growth of roots differently and this, in turn, may affect the relationship between external hyphae and root colonisation differently. A change in this relationship may influence the rate of P uptake by clover plants.

The aim of this project, therefore, is to investigate the effects of mycorrhizal colonisation on growth and P uptake by *T. subterraneum* at different levels of soil compaction. How soil compaction may change the morphology of clover roots and to what extent this change may affect the development of external hyphae and internal colonisation.

CHAPTER 3

GENERAL METHODS AND MATERIALS

This chapter describes the experimental procedures and measurement techniques used frequently in this study. Further details and modifications related to individual experiments are given in subsequent chapters.

3.1 Choice of soil

For the purpose of this study, a soil with high compatibility and low P content was required. A fine-textured red brown earth with low P content (Table 3.1) was collected from the 0 - 20 cm layer at Adelaide in South Australia. Compacting the soil to a bulk density of 1.6 Mg m^{-3} resulted in some relatively fine cracks in the soil because of its high silt content (see Table 3.1 for the distribution of particle size). To alleviate this problem and to have better aeration in compacted soil, the percentage of the coarse particles (sand) in the soil was increased by mixing 4 parts of the fine-textured red brown earth and with 1 parts of a coarse-textured solonised brown soil. (Table 3.1). The solonised brown soil was collected from the 0-20 cm layer at Avon in South Australia. Both solonised brown soil and red brown earth had been used in previous experiments to study the effect of mycorrhizal colonisation on P uptake by Baon *et al.* (1992) and E. Facelli (unpublished), respectively. Some chemical and physical properties of the soils and the soil mix used in this study are given in Table 3.1.

3.2 Soil sterilisation

The soil mix was autoclaved at 121°C and 300 kPa pressure for 1 hour on two consecutive days to kill propagules of VAM fungi. The thickness of the soil layer in autoclave plastic bags was kept at about 7 cm to achieve maximum air flow throughout the soil during sterilisation.

Table 3.1 Some chemical and physical characteristics of the red brown earth, solonised brown soil and soil mix used in this study of the interactions between soil compaction, mycorrhizal colonisation and P nutrition in *Trifolium subterraneum* L.

Soil characteristics	Red brown earth	Solonised brown soil	Soil mix
EC[A] (1:5, soil:water) (ds m^{-1})	0.48	0.31	0.41
pH (1:5, soil:water)	6.3	8.3	6.6
Extractable P[B] (mg kg^{-1} soil)	9.9	6.8	9.2
Organic C (g kg^{-1} soil)	34.3	6.8	29.1
Sand (% w/w)	13.7	76.2	25.1
Silt (% w/w)	62.4	14.6	52.5
Clay (% w/w)	23.90	9.2	22.4
Soil texture[C]	Silt loam	Sandy loam	Silt loam

[A] Electrical conductivity.
[B] $NaHCO_3$ extraction (Colwell, 1963).
[C] Particle size distribution was determined by the Bouyoucos hydrometer method (Day, 1965).

3.3 Nutrients applied to soil

In pot experiments with *Trifolium subterraneum* (cv. Mt. Barker), basal nutrients at the following concentrations (Smith and Smith, 1981) were mixed throughout the soil before the soil was compacted. Each pot was supplied with macronutrients containing (mg kg^{-1} air dry soil) 13.9 K_2SO_4, 7.2 $MgSO_4.7H_2O$, 17.8 $CaCl_2.2H_2O$ and with micronutrients

containing (μg kg^{-1} air dry soil) 114 H_3BO_3, 72 $MnCl_2.4H_2O$, 8.8 $ZnSO_4.7H_2O$, 3.2 $CuSO_4.5H_2O$, 1.0 $NaMoO_4.2H_2O$ and Fe-EDTA to give 200 μg Fe kg^{-1} air dry soil.

3.4 Soil compaction

In these experiments, the soil mix was passed through a 2 mm mesh sieve and was thoroughly mixed with a basal fertiliser solution (see above). The desired amounts of P fertiliser as NaH_2PO_4 and sufficient distilled water to bring the water content of the soil to 0.2 kg kg^{-1} soil were mixed throughout the soil. The soil was double wrapped in plastic and held for 2 days at room temperature before packing into pots. The PVC pots were 90 mm in diameter and between 100-150 mm in length (depending on the extent of soil compaction). The pots were filled with soil in successive layers of 3 cm, and each layer was compacted to the desired bulk density with a hydraulic ram. Between each compaction, the soil surface was roughened to obtain a homogenous soil structure. Compacting the soil in successive layers of 3 cm resulted in an uniform soil compaction through the soil core (Fig. 3.1).

3.5 Measurement of penetrometer resistance

As discussed in Chapter 2, penetrometers have been widely used and provide the best estimates of resistance to root growth. In this study, penetrometer resistance of the soil for each bulk density was measured at a matric potential of -33 kPa, with a 2 mm diameter cone with a 30° semi-angle, driven at a penetration rate of 5 mm min^{-1}. The penetrometer resistance (Q_p) was calculated from the following equation (Bengough and Mullins, 1990):

$$Q_p = \frac{4F}{\pi d^2}$$

where d was the diameter of cone and F was the force required to penetrate the soil.

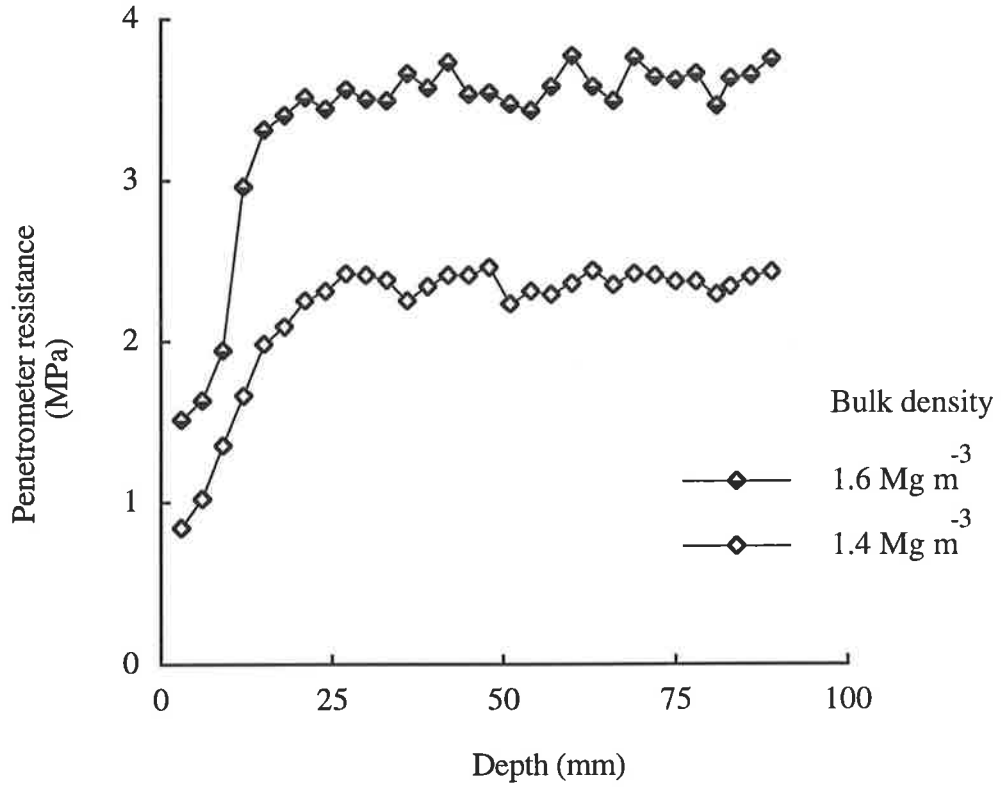

Figure 3.1 Penetrometer resistance of the soil (MPa) with depth (mm) at bulk densities of 1.4 and 1.6 Mg m^{-3}. Each value is the mean of four replicates.

3.6 Inoculation procedure

VAM inoculum was obtained from pot cultures of clover (*T. subterraneum*) grown in a soil:sand mix (1:9) containing 10% of a dry inoculum from pot cultures of *Glomus intraradices* (Schenck & Smith). Roots from the pot culture were harvested at least two months after transplanting and washed with deionized water. The roots were chopped into segments about 1 cm long, thoroughly mixed and used as fresh inoculum. For the mycorrhizal treatments, each seedling was inoculated by placing 0.25 g fresh inoculum in each planting hole. Seedlings for the non-mycorrhizal treatments received 0.25 g non-colonised clover roots to ensure that the microflora and nutrient status were similar.

3.7 Plant growth

Seeds of *T. subterraneum* (clover) were sterilised with NaOCl solution (5 g dm^{-3}) for 20 min and then rinsed three times with sterile water. The seeds were germinated on filter paper wetted with sterile water at 23°C. Clover seedlings, uniform in size, were transplanted into each pot containing 620 g oven-dry soil. In all experiments, each clover seedling received a 0.5 cm^{-3} dense suspension of *Rhizobium leguminosarum* biovar *trifolii*. The surface of the soil was covered with a 10 mm deep layer of white polythene beads to minimise evaporation and control the growth of algae. The water content of the soil was brought to field capacity (matric potential, -33 kPa) by watering to weight. The plants were grown in a glasshouse, or in a growth room under controlled conditions (irradiance and photoperiod) as specified in the relevant chapters.

3.8 Harvest, drying plant materials and separation roots from soil

At harvest, shoots and roots of clover plants were separated. After the fresh weight of the shoots and roots were recorded, the plant materials were dried at 70°C for 48 hours to determine dry weight. Soil containing plant roots was put onto a 2 mm sieve layered with a piece of 0.5 mm sieve. The sieve with the soil was soaked in a bucket filled with water for at least 20 minutes. When the soil became soft, soil particles were carefully separated from roots by washing with tap water. The roots were placed in a plastic tray containing deionised water to remove the remaining soil particles and organic debris. The fresh roots were weighed and cut into segments about 1 cm long and thoroughly mixed. Sub-samples of the roots were taken for the measurement of the percentage of root length colonised, root length and for the determination of P concentration.

3.9 Root staining

A modification of the method of Phillips and Hayman (1970) was used to clear and stain the root samples. Root segments of each sample were kept in sufficient 10% KOH solution at room temperature for 4 to 6 days (depending on age of the roots and air temperature). After clearing, roots were collected onto a fine-mesh sieve, and washed with running water. The roots were soaked in 1 *M* HNO_3 and again washed with tap water. The roots were stained with 0.01% trypan blue in lactoglycerol for about 45 minutes at air temperature. The samples of stained roots were covered with 50% glycerol and stored in vials.

3.10 Measurement of mycorrhizal colonisation and root length

The percentage of root length colonised was measured with a grid line intersect method as described by Giovannetti and Mosse (1980). The samples of stained roots were evenly spread on a petri dish under which a grid of lines was marked to form squares of 5 mm sides. Intersections between stained root (colonised and non-colonised) and vertical and horizontal lines were observed through a dissecting microscope with × 16 and × 40 magnifications. A compound microscope with × 100 and × 400 magnifications was used sometimes to confirm the structures of the fungi. Total root length was calculated according to Tennant (1975), and the proportion of root length that was mycorrhizal was assessed simultaneously.

3.11 Determination of P concentration in plant tissues

Samples of dried shoots and roots were ground and used to determine the concentration of P in plant tissues with the phosphovanado-molybdate method (Hanson, 1950), using a two step procedure as follow:

3.11.1 Digestion

Dried ground roots and shoots (100 mg when sufficient plant materials were available) were placed in a 50 cm^3 digest tube and 4 cm^3 of acid mixture (concentrated nitric acid and 70-72% perchloric acid, 6:1) was added. The tubes were placed in a digestion block and kept in the fume hood overnight. The time and temperature needed for digestion were programmed as follows.

Step	Time (minute)	Temperature (°C)	Ramp (minute)
1	10	100	10
2	20	150	10
3	40	200	10
4	70	250	10
5	15	280	10

3.11.2 Spectrophotometric analysis

Under the above conditions, the digestion of plant materials was completed when 0.5 cm^3 remained in the digest tubes. After finishing the digestion, the remaining materials were diluted with distilled water to 50 cm^3. A 20 cm^3 aliquot of diluted digest was transferred to a 50 cm^3 volumetric flask, and 4 cm^3 mixed reagent (concentrated nitric acid, ammonium vanadate and ammonium molybdate) was added as described by Hanson (1950). The solution was diluted to volume with distilled water and mixed for 2 minutes. The concentrations of P in the plant samples were measured with a spectrophotometer (LKB Biochorom-Ultrospect 4050) at 390 μm, 30 minutes after mixing. Standard curves of P were obtained by preparing standard solutions over the concentration range 0-10 μg P cm^{-3}.

3.12 Calculation of mycorrhizal growth response

The percentage of mycorrhizal growth response was calculated from the following equation (Baon *et al.*, 1993):

$$\frac{\textit{shoot dry weight of mycorrhizal plants} - \textit{shoot dry weight of non-mycorrhizal plants}}{\textit{shoot dry weight of non-mycorrhizal plants}} \times 100$$

3.13 Measurement of O_2 and CO_2 in soil air

In this study, two days prior to harvest of the plants, samples of soil air were collected with syringes from the centre of the pots. Carbon dioxide was measured by injecting samples into an air stream passing through an infra-red gas analyser (Atkins and Pate, 1977). Oxygen was measured by means of a gas chromatograph fitted with a thermal conductivity detector and a coaxial column containing 5A molecular sieves. The carrier gas was helium at a flow rate of 60 cm^3 per minute.

3.14 Determination of pore size distribution

To determine the pore size distributions of soil, the soil at a matric potential of -33 kPa was compacted into PVC rings, 90 mm in diameter and 30 mm in length, to one of these bulk densities: 1.0, 1.1, 1.2, 1.4, 1.6 and 1.75 Mg m^{-3}. For each bulk density, the soil cores were saturated with distilled water in sintered glass funnels connected to a flexible tube. Water contents of the soil were determined at different water matric potentials using pressure plates for low potentials (-1500, -300, -100, -33 kPa) and sintered glass funnels for high potentials (-10, -3, -1, 0 kPa). The amount of water remaining in the soil at equilibrium is a function of the sizes and volumes of the water-filled pores, and hence it is a function of the matric potential. The radii of pores were calculated from the following equation:

$$\Psi = \frac{-2\gamma \cos\theta}{r}$$

where Ψ is matric potential, r is the radius of the pore, γ is surface tension of water, and θ is the angle of contact of liquid/solid interface (usually assumed to be zero). Volumetric water contents of the soil at different levels of bulk density and matric potential which were used to calculate pore size are given in Table 3.2.

Table 3.2 Soil volumetric water content (m^3 m^{-3}) at different levels of water matric potential (kPa) and bulk density (Mg m^{-3}).

Matric potential (kPa)	Soil bulk density (Mg m^{-3})					
	1.0	1.10	1.20	1.40	1.60	1.75
0	0.55 (± 0.02)[A]	0.52 (± 0.03)	0.49 (± 0.04)	0.45 (± 0.02)	0.41 (± 0.02)	0.40 (± 0.02)
-1	0.43 (± 0.03)	0.42 (± 0.02)	0.42 (± 0.04)	0.41 (± 0.01)	0.40 (± 0.02)	0.39 (± 0.02)
-3	0.33 (± 0.02)	0.33 (± 0.02	0.35 (± 0.03)	0.36 (± 0.02)	0.38 (± 0.03)	0.37 (± 0.01)
-10	0.24 (± 0.02)	0.26 (± 0.02)	0.29 (± 0.02)	0.32 (± 0.02)	0.36 (± 0.02)	0.35 (± 0.01)
-33	0.19 (± 0.01)	0.21 (±0.02)	0.24 (± 0.01)	0.28 (± 0.03)	0.33 (± 0.02)	0.34 (± 0.02)
-100	0.16 (± 0.01)	0.18 (± 0.01)	0.20 (± 0.01)	0.26 (± 0.02)	0.29 (± 0.02)	0.32 (± 0.02)
-300	0.13 (± 0.01)	0.16 (± 0.01)	0.18 (± 0.01)	0.21 (± 0.01)	0.26 (± 0.03)	0.28 (± 0.02)
- 1500	0.11 (± 0.01)	0.13 (± 0.01)	0.14 (± 0.01)	0.16 (± 0.01)	0.19 (± 0.01)	0.20 (± 0.01)

[A] Standard error of the mean. $n=3$

3.15 Soil pore continuity

Pore continuity is one of the most important soil structural characteristics. It influences soil physical properties such as the movement of air and water as well as root growth and

biological activity in general. Since measurement of pore continuity in soil is very difficult, the saturated hydraulic conductivity of the soil which is a function of pore continuity was used to assess pore continuity.

To measure the saturated hydraulic conductivity of the soil, the soil at a water matric potential of -33 kPa was compacted into PVC pots as described in Section 3.4 to bulk densities of 1.20, 1.40, 1.60, 1.75 Mg m^{-3} and the saturated hydraulic conductivities of the soil at these bulk densities were measured by using pots as simple permeameter and measuring water flux at low a pressure head (Marshall and Holmes, 1988).

As expected, soil compaction to a bulk density of 1.75 Mg m^{-3} has a dramatic influence on saturated hydraulic conductivity (Table 3.3); this change is a direct result of reduced pore size and pore continuity.

Table 3.3 Saturated hydraulic conductivity of the soil as affected by different levels of soil compaction.

Bulk density (Mg m^{-3})	Saturated hydraulic conductivity (m sec^{-1})
1.20	8.7×10^{-6}
1.40	3.8×10^{-6}
1.60	1.5×10^{-6}
1.75	7.0×10^{-7}

CHAPTER 4

THE EFFECT OF SOIL COMPACTION ON GROWTH AND P UPTAKE BY *TRIFOLIUM SUBTERRANEUM* AND *ALLIUM CEPA*

4.1 Introduction

It has been shown that root growth of plants is restricted as penetrometer resistance increases due to soil compaction (Boone and Veen, 1982; Cornish *et al.*, 1984; Agnew and Carrow, 1985; Dolan *et al.*, 1992). The ability of plant species to penetrate a compacted soil depends mainly on soil physical characteristics, such as mechanical impedance, water content and pore size distribution, and root morphological characteristics such as root diameter and root proliferation.

Since major root functions are the supply of the shoot with water and nutrients, an impeded root system may decrease the capacity of the plant to take up nutrients, particularly less mobile ions like P (Boone and Veen, 1982; Shierlaw and Alston, 1984; Hoffmann and Jungk, 1995). As the diameters of external hyphae of VAM fungi are less than the diameters of impeded roots, external hyphae of VAM fungi may penetrate smaller pores and exploit a compacted soil more effectively than impeded roots.

This chapter describes three experiments. Experiment 1 was designed to provide data on the effects of soil compaction on P uptake and growth of clover (*Trifolium subterraneum* L. cv. Mt. Barker) and onion (*Allium cepa* L.) plants in the absence of mycorrhizal colonisation. These data were also required to decide which species to use for subsequent experiments in this project. These two plants were selected because of their good response

to mycorrhizal colonisation. There have been no reports of how soil compaction may affect growth and P uptake by either species.

Although mycorrhizal growth response is usually observed in soils low in P, it has been shown that a small addition of P fertiliser to P-deficient soil increases mycorrhizal growth response (Abbott *et al.*, 1984). Experiment 2 was therefore conducted to study the mycorrhizal response to P of *T. subterraneum,* the species selected from Experiment 1. The aim of Experiment 2 was to determine the amount of P fertiliser required to achieve the greatest mycorrhizal growth response.

Experiment 3, which used conditions selected on the basis of Experiments 1 and 2, was carried out to study the effects of mycorrhizal colonisation on plant growth and P uptake by *T. subterraneum* in compacted soil.

4.2 Materials and Methods

The experiments described in this chapter had randomised complete block designs with treatments arranged in factorial combination. The treatments and the experimental conditions used in each experiment are given in Table 4.1.

4.2.1 Experiment 1

The soil was thoroughly mixed with a basal fertiliser (see Chapter 3) and phosphorus fertiliser as NaH_2PO_4 at rates of 0, 25 or 45 mg P kg^{-1} soil. For onion plants, nitrogen fertiliser was added to soil as $NaNO_3$ at the rate of 27 mg kg^{-1} soil (Smith *et al.*, 1986). The soil was compacted into PVC pots to bulk densities of 1.0, 1.2, 1.4 and 1.6 Mg m^{-3}. At a matric potential of -33 kPa, penetrometer resistances of the soil at these bulk densities were: 0.4, 1.1, 2.3 and 3.5 MPa, respectively. Seedlings of clover plants (2 days old) were inoculated with *Rhizobium leguminosarum* before transplanting. Four plants (either clover

Table 4.1 Treatment combinations and conditions used in experiments on the interactions between soil compaction, mycorrhizal and non-mycorrhizal plants and P applied to the soil.

	Experiment 1	Experiment 2	Experiment 3
Treatments			
Plant species	*Trifolium subterraneum* and *Allium cepa*	*Trifolium subterraneum*	*Trifolium subterraneum*
Bulk density (Mg m^{-3})	1.0, 1.2, 1.4, 1.6	1.0	1.0, 1.4, 1.6
P applied (mg kg^{-1})	0, 25, 45	0, 15, 25, 35, 45, 60, 80	0, 15, 60
Mycorrhiza (M)	–	M	M
Non-mycorrhiza (NM)	NM	NM	NM
No. of treatments	24	14	18
No. of replicates	3	3	4
Growth conditions			
Duration of experiment (weeks)	7	7	7
Mycorrhizal fungus	–	*Glomus intraradices*	*Glomus intraradices*
No. of plants (pot^{-1})	4	2	4
Soil dry mass (g pot^{-1})	620	620	620
Environmental conditions			
Plants were grown in	Growth room	Growth room	Glasshouse
Photoperiod (h)	16	16	–
Irradiance (µmol m^{-2} s^{-1})	360	360	–
Temperature (day/night) °C	21/15	21/15	–

or onion) were transplanted into each pot containing 620 g soil and grown in a growth room where the photoperiod was 16 h and the irradiance was 360 µ mol m^{-2} s^{-1}. The day and night air temperatures were 20 and 15°C, respectively. The water content of the soil

was brought to field capacity (-33 kPa) by watering to weight. Plants were harvested after 7 weeks. For the treatment with 25 mg P kg^{-1} soil, the soil in each pot was divided into layers of 2 and 2.5 cm (in highly and slightly compacted soil respectively) and root fresh weights of the roots of *T. subterraneum* and *A. cepa* were recorded for each soil depth. Diameters of the root axes and first order lateral roots were measured at 3 mm behind the root apex with a binocular microscope fitted with an eyepiece micrometer. In clover plants, the values of root diameters for the main axes and the first lateral roots are the means of 12 and 24 measurements, respectively. In onion plants, the values are the means of 24 measurements of the diameters of lateral roots.

4.2.2 Experiment 2

In Experiment 2, the response of mycorrhizal and non-mycorrhizal clover plants to different levels of P fertiliser was determined in non-compacted soil (bulk density = 1.0 Mg m^{-3}). P fertiliser was used at rates of 0, 15, 25, 35, 45, 60, 80 mg P kg^{-1} soil. For mycorrhizal treatments, each seedling was inoculated with 0.25 g of fresh roots of *T. subterraneum* colonised by *Glomus intraradices* (originally provided by NPI, Utah). For non-mycorrhizal treatments, each seedling received 0.25 g of non-colonised clover roots. Clover plants were grown in a growth room where growth conditions were similar to Experiment 1.

4.2.3 Experiment 3

This experiment was a combination of Experiment 1 and 2. The soil was compacted to bulk densities of 1.0, 1.4 and 1.6 Mg m^{-3}. P fertiliser as NaH_2PO_4 was added at rates of 0, 15 and 60 mg P kg^{-1} soil. Four two-day old seedlings were transplanted into each pot and inoculated by 0.25 g fresh roots of clover plants colonised by *Glomus intraradices*. The plants were grown in a glasshouse for 7 weeks during mid-October to early December

where the mean minimum and maximum daily temperatures were 19.4 and 24.1°C respectively. The plants were harvested 7 weeks after planting. Root colonisation, mycorrhizal growth response, P concentrations in shoots and roots, root length, root diameter, O_2 and CO_2 concentrations in the soil atmosphere (in mycorrhizal treatments) were measured as described in Chapter 3.

In these experiments, data were analysed with Genstat (Genstat 5 Committee, 1987). Multiple linear comparisons between means were made with Tukey's honestly significant difference statistic (Zar, 1984). In Experiment 1, data for clover and onion plants were analysed separately.

4.3 Results

4.3.1 Results of Experiment 1

Increasing soil compaction from a bulk density of 1.0 to 1.6 Mg m^{-3} increased the penetrometer resistance of the soil (Fig. 4.1). This resulted in a significant reduction in shoot and root dry weights of both *T. subterraneum* and *A. cepa* when bulk density of the soil exceeded 1.2 Mg m^{-3} with P addition of either 25 and 45 mg P kg^{-1} soil (Table 4.2).

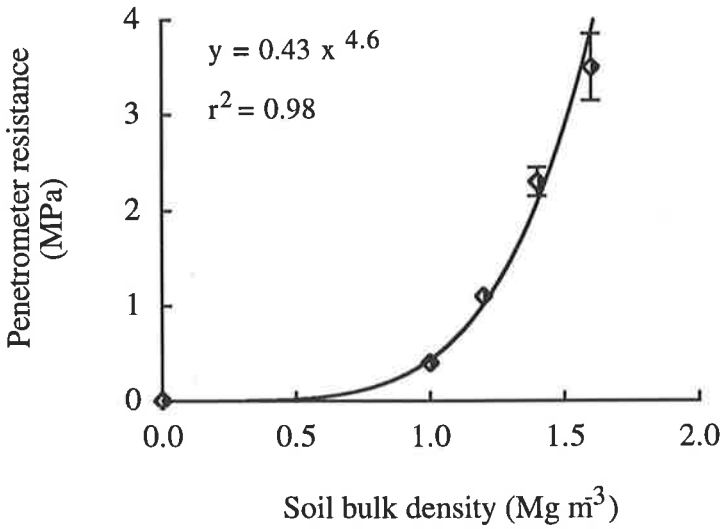

Figure 4.1 Experiment 1 Relationship between bulk density and penetrometer resistance of the soil at matric potential of -33 kPa. Vertical bars represent standard errors of the means.

Table 4.2 Experiment 1 Shoot and root dry weights of *Trifolium subterraneum* and *Allium cepa* as affected by soil compaction and P applied to the soil.

Bulk density ($Mg\ m^{-3}$)	P applied ($mg\ kg^{-1}$)	Shoot dry weight ($g\ pot^{-1}$)	Root dry weight ($g\ pot^{-1}$)
		Trifolium subterraneum L.	
1.0	0	0.19 (± 0.02)[A]	0.09 (±0.01)
	25	0.66 (± 0.07)	0.40 (±0.05)
	45	1.49 (± 0.09)	0.82 (±0.06)
1.2	0	0.19 (± 0.02)	0.08 (±0.01)
	25	0.63 (± 0.07)	0.38 (±0.05)
	45	1.50 (± 0.08)	0.79 (±0.05)
1.4	0	0.15 (± 0.01)	0.07 (±0.09)
	25	0.40 (± 0.03)	0.24 (±0.02)
	45	0.92 (± 0.06)	0.50 (±0.05)
1.6	0	0.12 (± 0.02)	0 06 (±0.01)
	25	0.32 (± 0.02)	0.19 (±0.02)
	45	0.71 (± 0.06)	0.33 (±0.04)
Tukey's HSD (p = 0.05)		*0.25*	*0.18*
		Allium cepa L.	
1.0	0	0.10 (±0.01)	0.07 (±0.01)
	25	0.42 (±0.04)	0.27 (±0.04)
	45	0.89 (±0.07)	0.55 (±0.05)
1.2	0	0.10 (±0.01)	0.06 (±0.01)
	25	0.39 (±0.04)	0.19 (±0.08)
	45	0.76 (±0.09)	0.52 (±0.05)
1.4	0	0.06 (±0.00)	0.04 (±0.00)
	25	0.20 (±0.04)	0.13 (±0.02)
	45	0.42 (±0.05)	0.27 (±0.04)
1.6	0	0.05 (±0.00)	0 03 (±0.00)
	25	0.15 (±0.02)	0.09 (±0.01)
	45	0.30 (±0.04)	0.19 (±0.02)
Tukey's HSD (p = 0.05)		*0.22*	*0.14*

[A] Standard error of the mean.

Soil compaction decreased root length of both clover and onion plants (results not shown), but increased the diameter of their roots (Table 4.3). At a low soil bulk density (bulk density = 1.0 Mg m^{-3}), there was a fairly even distribution of the roots (both clover and onion) with depth, but at high penetrometer resistances roots accumulated in the upper soil layers and most roots failed to penetrate into deeper layers, particularly in the case of onion plants (Fig. 4.2).

Table 4.3 Experiment 1 The effect of soil compaction and P applied on the diameter of the roots of *Trifolium subterraneum* and *Allium cepa.*

P applied	Bulk density (Mg m^{-3})			
(mg kg^{-1})	1.0	1.2	1.4	1.6
	Diameter, main axes of clover roots (mm)			
0	0.48 (± 0.02)[A]	0.50 (± 0.05)	0.61 (± 0.04)	0.76 (± 0.03)
25	0.50 (± 0.04)	0.52 (± 0.05)	0.60 (± 0.07)	0.78 (± 0.06)
	Diameter, first order lateral roots of clover plant (mm)			
0	0.39 (± 0.04)	0.40 (± 0.03)	0.45 (± 0.03)	0.52 (± 0.04)
25	0.41 (± 0.02)	0.42 (± 0.05)	0.47 (± 0.04)	0.52 (± 0.06)
	Diameter, lateral roots of onion plant (mm)			
0	0.50 (± 0.05)	0.51 (± 0.05)	0.59 (± 0.04)	0.68 (± 0.05)
25	0.53 (± 0.05)	0.52 (± 0.02)	0.62 (± 0.05)	0.70 (± 0.05)

[A] Standard error of the mean.

There was no interaction between soil compaction and P application in their effect on the concentration of P in the shoots and roots of clover and onion plants. Irrespective of soil compaction, the concentration of P in the shoots and roots of the both plants species increased as P applied to soil was increased (Table 4.4 and 4.5). Although soil compaction had no significant effect on P concentration in clover and onion plants, total P uptake decreased as soil compaction increased to a bulk density of 1.6 Mg m^{-3} when the soil was supplied with 25 and 45 mg P kg^{-1} soil (Table 4.4 and 4.5).

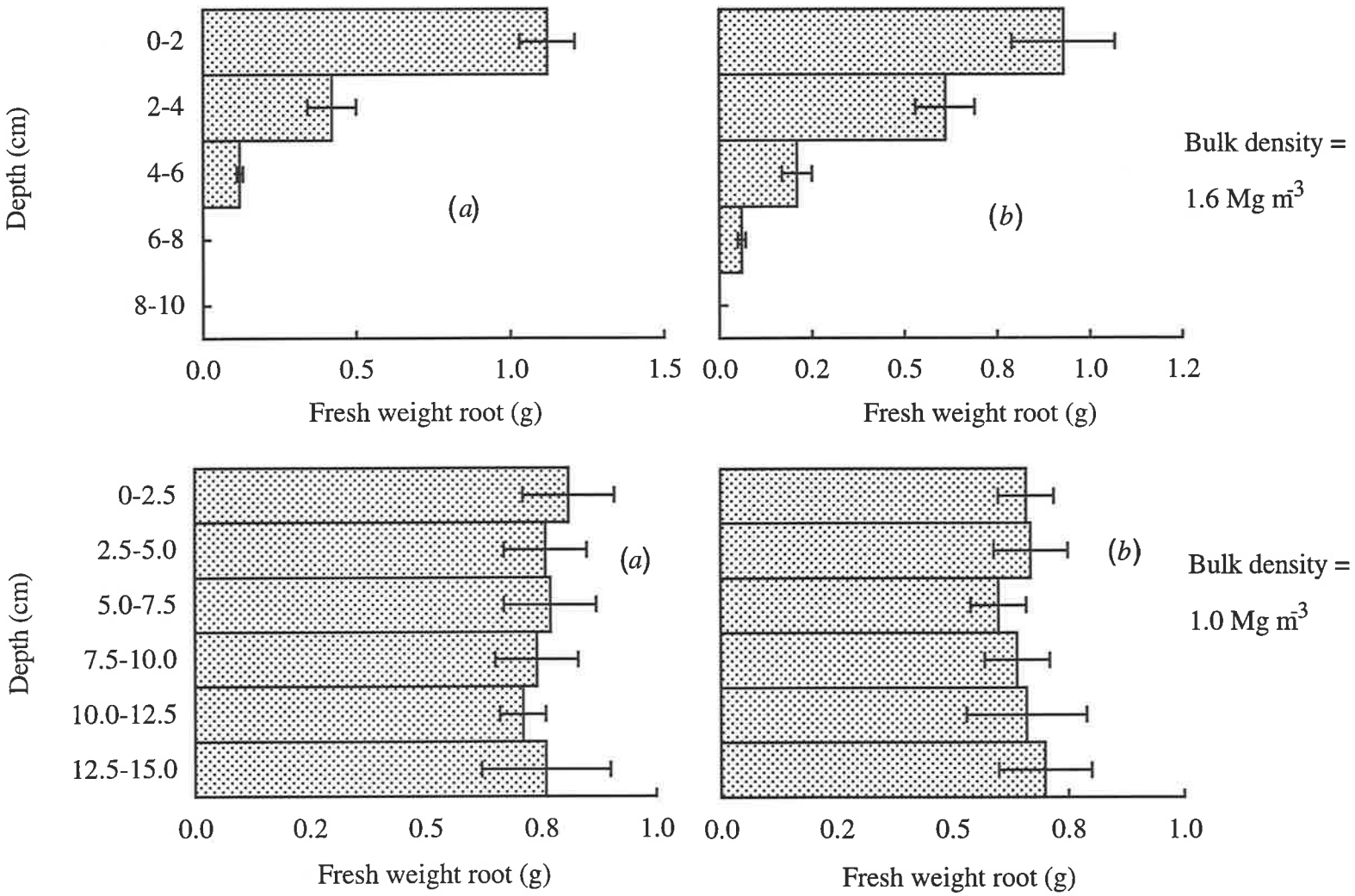

Fig. 4.2 Experiment 1 Root distribution of *Allium cepa* (*a*) and *Trifolium subterraneum* (*b*) with depth in slightly and highly compacted soil (bulk density = 1.0 and 1.6 Mg m^{-3}, respectively), when the soil was supplied with 25 mg P kg^{-1} soil.

Table 4.4 Experiment 1 The concentration of P in the shoots and roots and total P uptake by *Trifolium subterraneum* as affected by soil compaction and P applied to the soil.

P applied	Bulk density (Mg m^{-3})			
(mg kg^{-1})	1.0	1.2	1.4	1.6
	Shoot P concentration (µg P mg^{-1} dry wt.)[A]			
0	0.85 (± 0.03)[B]	0.90 (± 0.03)	0.80 (± 0.03)	0.76 (± 0.05)
25	1.28 (± 0.02)	1.31 (± 0.06)	1.22 (± 0.09)	1.17 (± 0.07)
45	1.53 (± 0.11)	1.58 (± 0.07)	1.42 (± 0.09)	1.41 (± 0.10)
	Root P concentration (µg P mg^{-1} dry wt.)[A]			
0	0.90 (± 0.03)	0.96 (± 0.09)	0.88 (± 0.02)	0.80 (± 0.05)
25	1.31 (± 0.10)	1.36 (± 0.06)	1.32 (± 0.08)	1.18 (± 0.09)
45	1.66 (± 0.07)	1.71 (± 0.06)	1.60 (± 0.07)	1.54 (± 0.06)
	Total P uptake (mg pot^{-1})			
0	0.23 (± 0.03)	0.25 (± 0.02)	0.18 (± 0.01)	0.14 (± 0.02)
25	1.37 (± 0.12)	1.35 (± 0.21)	0.81 (± 0.02)	0.59 (± 0.04)
45	3.62 (± 0.22)	3.74 (± 0.23)	2.11 (± 0.23)	1.49 (± 0.15)
Tukey's HSD (p = 0.05)		*0.68*		

[A] For shoot and root P concentration, no interaction was observed between soil compaction and P application.
[B] Standard error of the mean.

The results of the first experiment indicated that at higher P applications, the growth (roots and shoots) and P uptake by clover and onion plants decreased as soil compaction was increased. Both plants exhibited similar responses to soil compaction and *T. subterraneum* was selected for subsequent experiments. This was done because clover plants grown in compacted soil appeared healthier than onion plants and it was much easier to transplant clover seedlings than onion seedlings into compacted soil. Moreover, amounts of material for analysis were higher for clover than for onion plants.

Table 4.5 Experiment 1 The concentration of P in the shoots and roots and total P uptake by *Allium cepa* as affected by soil compaction and P applied to the soil.

P applied	Bulk density (Mg m^{-3})			
(mg kg^{-1} soil)	1.0	1.2	1.4	1.6
	Shoot P concentration (µg P mg^{-1} dry wt.)[A]			
0	0.65 (± 0.03)[B]	0.70 (± 0.03)	0.62 (± 0.04)	0.62 (± 0.07)
25	1.02 (± 0.07)	1.03 (± 0.03)	0.97 (± 0.03)	0.92 (± 0.04)
45	1.32 (± 0.07)	1.32 (± 0.04)	1.26 (± 0.09)	1.22 (± 0.09)
	Root P concentration (µg P mg^{-1} dry wt.)[A]			
0	0.73 (± 0.06)	0.70 (± 0.05)	0.68 (± 0.03)	0.62 (± 0.04)
25	1.10 (± 0.08)	1.15 (± 0.09)	1.05 (± 0.08)	1.00 (± 0.03)
45	1.42 (± 0.09)	1.44 (± 0.08)	1.35 (± 0.05)	1.30 (± 0.09)
	Total P uptake (mg pot^{-1})			
0	0.12 (± 0.02)	0.10 (± 0.01)	0.06 (± 0.00)	0.05 (± 0.00)
25	0.72 (± 0.05)	0.69 (± 0.04)	0.34 (± 0.04)	0.24 (± 0.00)
45	1.95 (± 0.07)	1.76 (± 0.16)	0.89 (± 0.12)	0.62 (± 0.06)
Tukey's HSD (p = 0.05)		*0.32*		

[A] No interaction was observed between soil compaction and P application in their effects on shoot and root P concentrations.

[B] Standard error of the mean.

4.3.2 Results of Experiment 2

At lower P applications (15, 25, and 35 mg P kg^{-1} soil), shoot dry weight of mycorrhizal clover was greater than that of non-mycorrhizal clover, whereas at higher P applications (45, 60 and 80 mg P kg^{-1} soil) the growth of inoculated and non-inoculated plants was not different (Fig. 4.3*a*). A similar trend to that of shoot dry weight was observed for total P uptake by clover plants (Fig. 4.3*b*). The greatest mycorrhizal growth response (Fig. 4.4*a*) and the greatest percentage of root length colonised (Fig. 4.4*b*) were observed when 15 mg P kg^{-1} soil was applied. This level of P fertilisation was therefore used in Experiment 3.

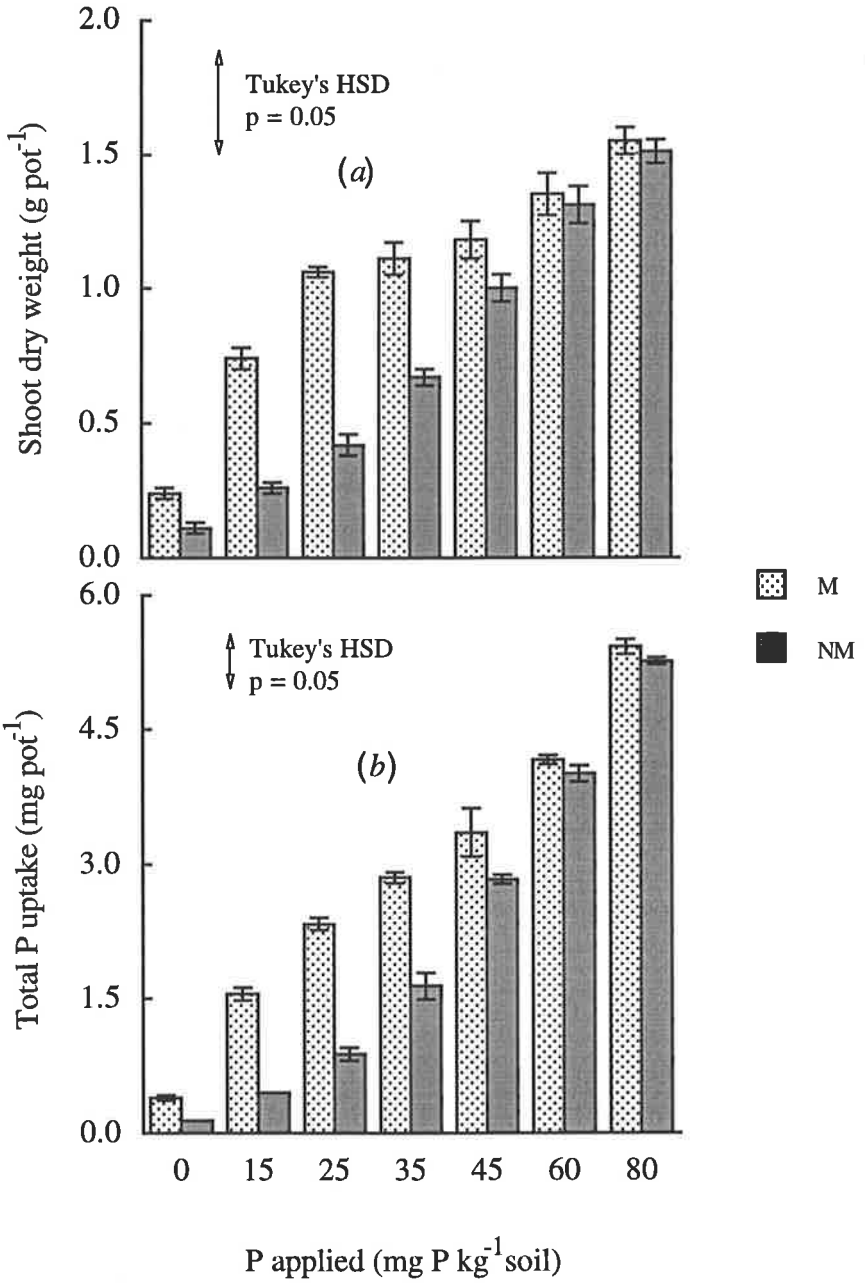

Figure 4.3 Experiment 2 Shoot dry weight (*a*) and total P uptake (*b*) by mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum* as affected by P applied to the soil. Vertical bars represent standard errors of the means, n=3.

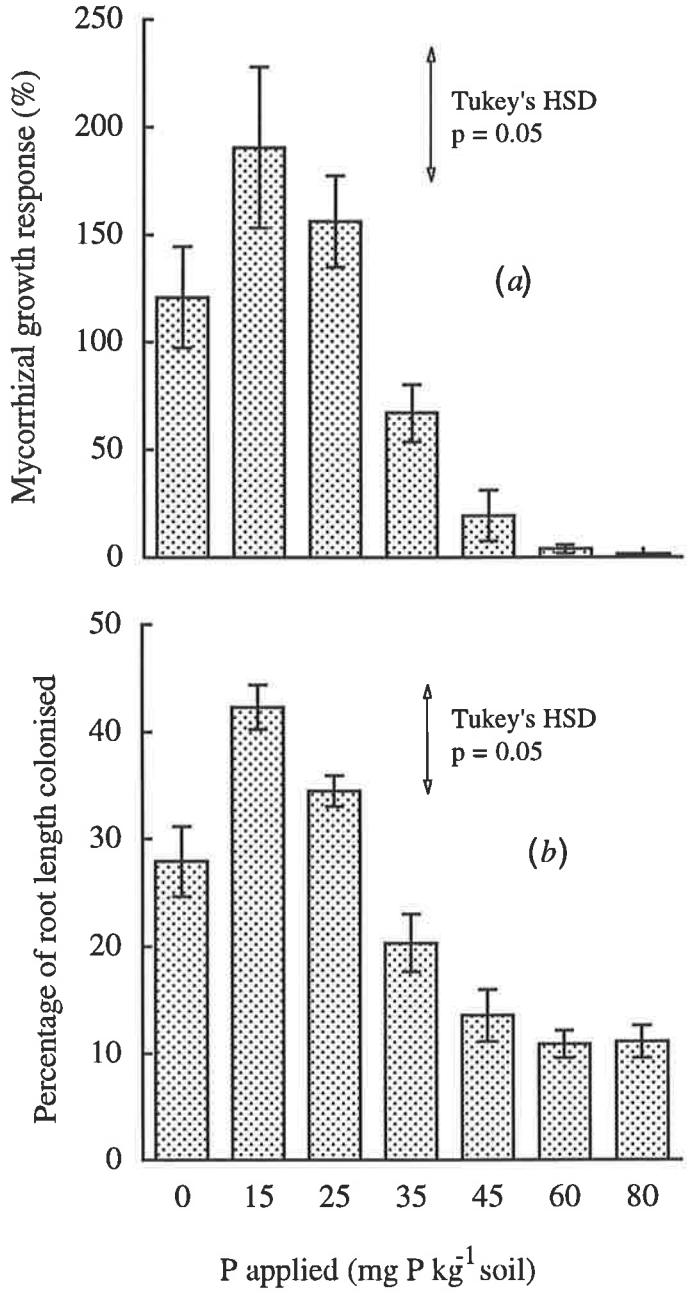

Figure 4.4 Experiment 2 **The effect of different levels of P application on the growth response of *Trifolium subterraneum* to mycorrhizal colonisation (*a*) and on the percentage of root length colonised (*b*). Vertical bars represent standard errors of the means, n=3.**

4.3.3 Results of Experiment 3

There was an interaction between soil compaction and mycorrhizal colonisation in their effects on plant growth and P uptake. With no added P, mycorrhizal colonisation increased shoot dry weights only in slightly compacted soil (Fig. 4.5), a result reflected in mycorrhizal growth response (Fig. 4.6). With 15 mg P kg^{-1} soil, shoot dry weight of mycorrhizal plants was greater than that of non-mycorrhizal plants at all levels of compaction of the soil. However, mycorrhizal growth response decreased from 130% to 104% as soil compaction was increased to the bulk density of 1.6 Mg m^{-3} (Fig. 4.6). No significant difference was observed between shoot dry weight of mycorrhizal and non-mycorrhizal plants at 60 mg P kg^{-1} at any level of soil compaction (Fig. 4.5), which confirms the results of Experiment 1 (Fig. 4.3a).

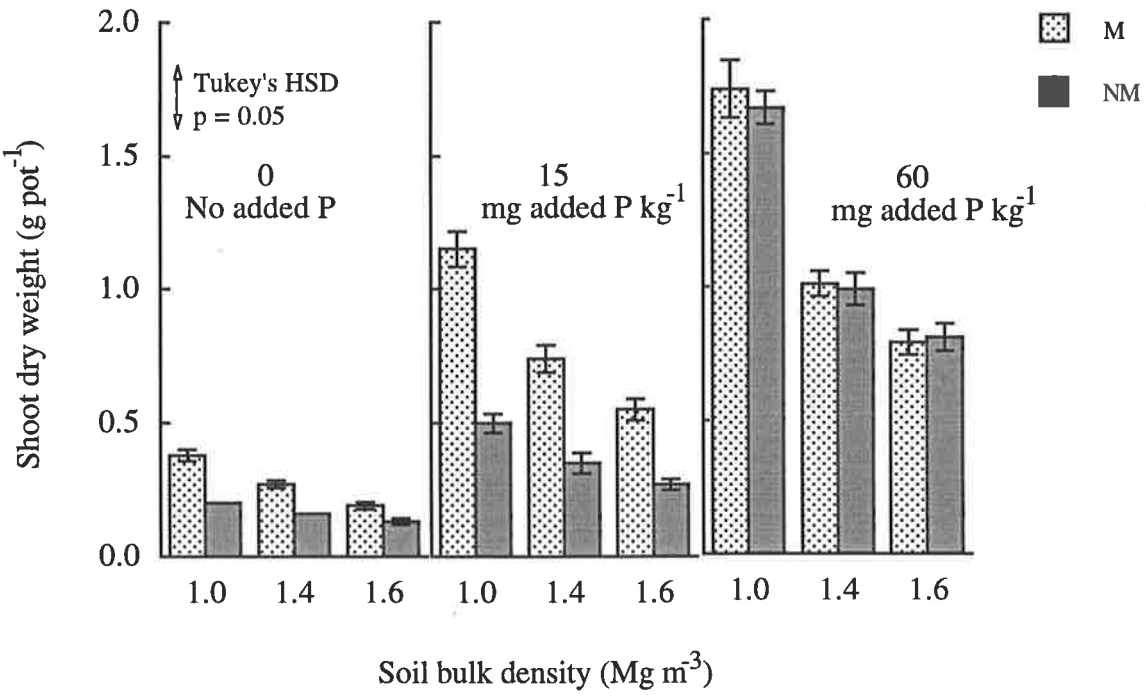

Figure 4.5 Experiment 3 The effect of soil compaction on shoot dry weight of mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum* with no added P, 15 and 60 mg added P kg^{-1} soil. Vertical bars represent standard errors of the means, n=4.

With added P, soil compaction decreased both root length (Table 4.6) and root dry weight (result not shown) and confirmed the results of Experiment 1. With 15 mg P kg^{-1} soil, there was a significant difference between root length of mycorrhizal and non-mycorrhizal clover up to a bulk density of 1.4 Mg m^{-3} (Table 4.6).

No interaction was observed between P applied and mycorrhiza in their effect on the diameter of roots of *T. subterraneum*. However, irrespective of P applied and mycorrhizal colonisation, the diameters of the main axes and first order lateral roots increased with increasing soil compaction (Table 4.6).

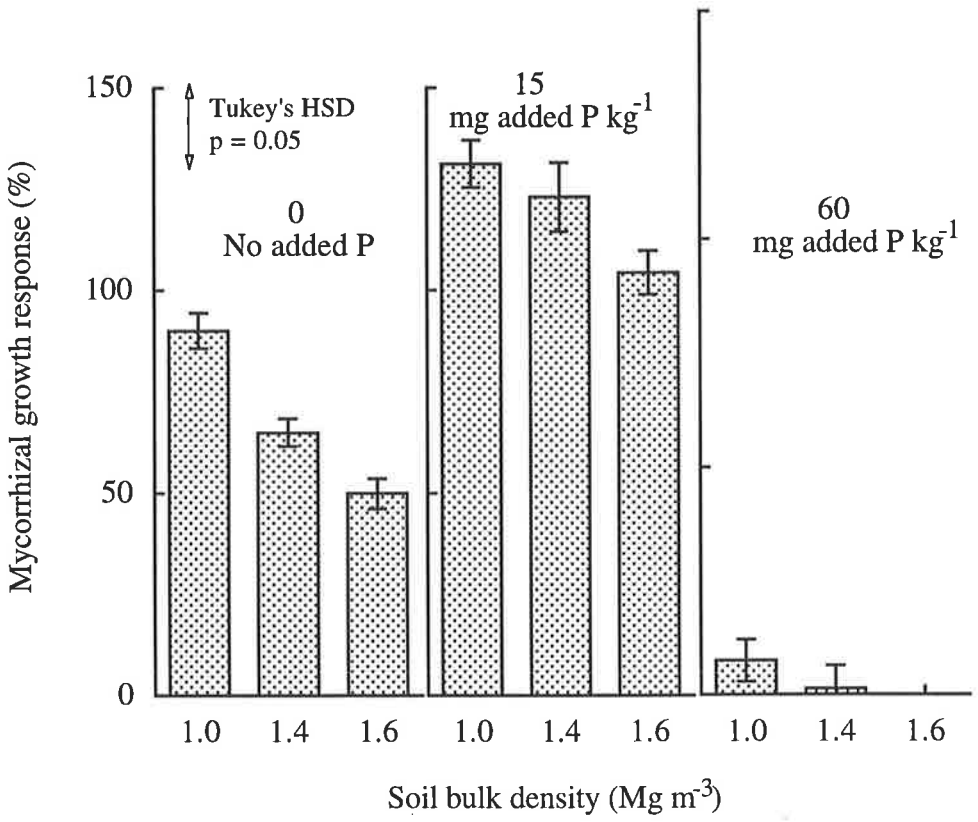

Figure 4.6 Experiment 3 The effect of soil compaction on the response of *Trifolium subterraneum* to mycorrhizal colonisation with no added P, 15 and 60 mg P kg^{-1} soil. Vertical bars represent standard errors of the means, n=4.

Table 4.6 Experiment 3. The effect of soil compaction and P applied on the diameter of the main and first order lateral roots and root length of mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum*.

P applied (mg kg^{-1})	Mycorrhiza	Bulk density (Mg m^{-3}) 1.0	1.4	1.6
		Diameter, main axes (mm)[A]		
0	M	0.51 (± 0.02)[B]	0.63 (± 0.03)	0.79 (± 0.06)
	NM	0.50 (± 0.02)	0.64 (± 0.04)	0.81 (± 0.04)
15	M	0.54 (± 0.03)	0.66 (± 0.06)	0.84 (± 0.06)
	NM	0.49 (± 0.04)	0.69 (± 0.07)	0.83 (± 0.05)
60	M	0.55 (± 0.03)	0.68 (± 0.05)	0.85 (± 0.05)
	NM	0.54 (± 0.04)	0.68 (± 0.04)	0.86 (± 0.05)
		Diameter, first order lateral roots (mm)[A]		
0	M	0.41 (± 0.02)	0.43 (± 0.03)	0.48 (± 0.03)
	NM	0.44 (± 0.03)	0.44 (± 0.04)	0.52 (± 0.06)
15	M	0.42 (± 0.03)	0.41 (± 0.04)	0.53 (± 0.06)
	NM	0.39 (± 0.05)	0.42 (± 0.05)	0.50 (± 0.04)
60	M	0.45 (± 0.04)	0.47 (± 0.03)	0.52 (± 0.03)
	NM	0.44 (± 0.03)	0.47 (± 0.03)	0.51 (± 0.03)
		Root length (m pot^{-1})		
0	M	31.1 (± 3.3)	17.3 (± 2.2)	7.3 (± 0.7)
	NM	19.5 (± 2.4)	11.1 (± 1.5)	5.1 (± 0.5)
15	M	73.7 (± 4.3)	35.8 (± 2.9)	13.5 (± 1.5)
	NM	38.1 (± 3.8)	17.3 (± 2.7)	6.9 (± 0.7)
60	M	168.1 (± 4.5)	69.5 (± 3.5)	29.4 (± 2.5)
	NM	168.2 (± 4.4)	71.6 (± 3.1)	27.9 (± 2.9)
Tukey's HSD (p = 0.05)			*15.3*	

[A] There was no interaction between P applied and mycorrhiza in their effect on the diameter of *Trifolium subterraneum* roots.

[B] Standard error of the mean.

Irrespective of soil compaction, mycorrhizal colonisation increased shoot and root P concentrations (results not shown). Total P uptake by mycorrhizal plants exceeded that of non-mycorrhizal plants with 15 mg P kg^{-1} soil at all levels of soil compaction, although the absolute difference became smaller as soil compaction was increased from a bulk density of 1.0 to 1.6 Mg m^{-3} (Table 4.7).

Table 4.7 Experiment 3. The effect of soil compaction and P applied on total P uptake by mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum.*

P applied	Mycorrhiza	Bulk density (Mg m^{-3})		
(mg kg^{-1})		1.0	1.4	1.6
		Total P uptake (mg pot^{-1})		
0	M	0.54 (± 0.04)[A]	0.39 (± 0.03)	0.27 (± 0.02)
	NM	0.24 (± 0.02)	0.18 (± 0.01)	0.14 (± 0.04)
15	M	2.31 (± 0.15)	1.45 (± 0.08)	1.04 (± 0.06)
	NM	0.71 (± 0.04)	0.44 (± 0.03)	0.35 (± 0.03)
60	M	5.22 (±0 11)	2.98 (± 0.05)	2.13 (± 0.07)
	NM	4.93 (±0 12)	2.92 (± 0.19)	2.15 (± 0.08)
Tukey's HSD (p = 0.05)			*0.33*	

[A] Standard error of the mean.

Soil compaction had no significant effect on the percentage of root length colonised (Fig. 4.7), but total root length colonised decreased as soil compaction increased when the soil was supplied with 15 mg P kg^{-1} soil (Fig. 4.8).

Soil compaction decreased the oxygen content of the soil atmosphere from 0.18 m^3 m^{-3} in slightly compacted soil to 0.10 m^3 m^{-3} in highly compacted soil at low P application (Fig. 4.9), but increased the carbon dioxide content of the soil atmosphere from 0.04 m^3 m^{-3} to 0.09 m^3 m^{-3}. The concentrations of oxygen in the soil atmosphere are probably close to the minimum concentrations that occurred, since the samples were collected during the day

(when temperature and presumably respiration were high) and shortly before harvesting when plant growth was at its maximum. The effects of P application were small compared with the effects of compaction, but there was a trend towards lower concentration of oxygen and higher concentration of carbon dioxide with increasing P, again presumably reflecting increased plant growth and respiration.

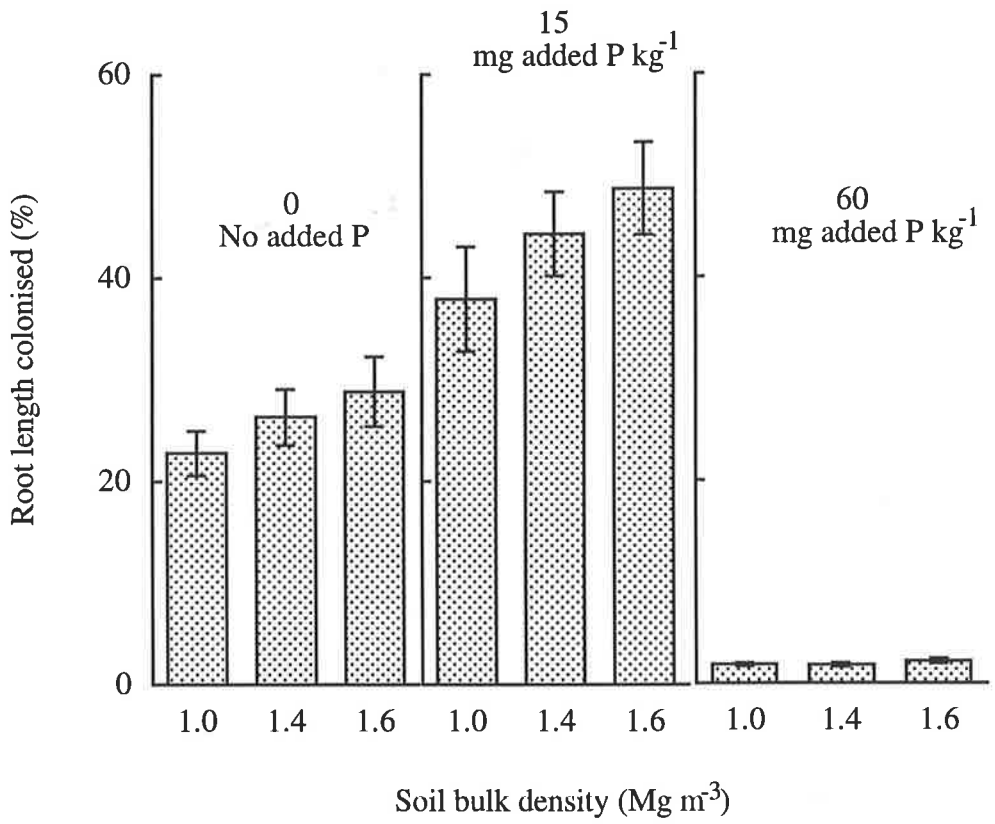

Figure 4.7 Experiment 3 The percentage of root length of *Trifolium subterraneum* colonised by *Glomus intraradices* as affected by soil compaction with no added P, 15 and 60 mg added P kg^{-1} soil. Vertical bars represent standard errors of the means, n=4.

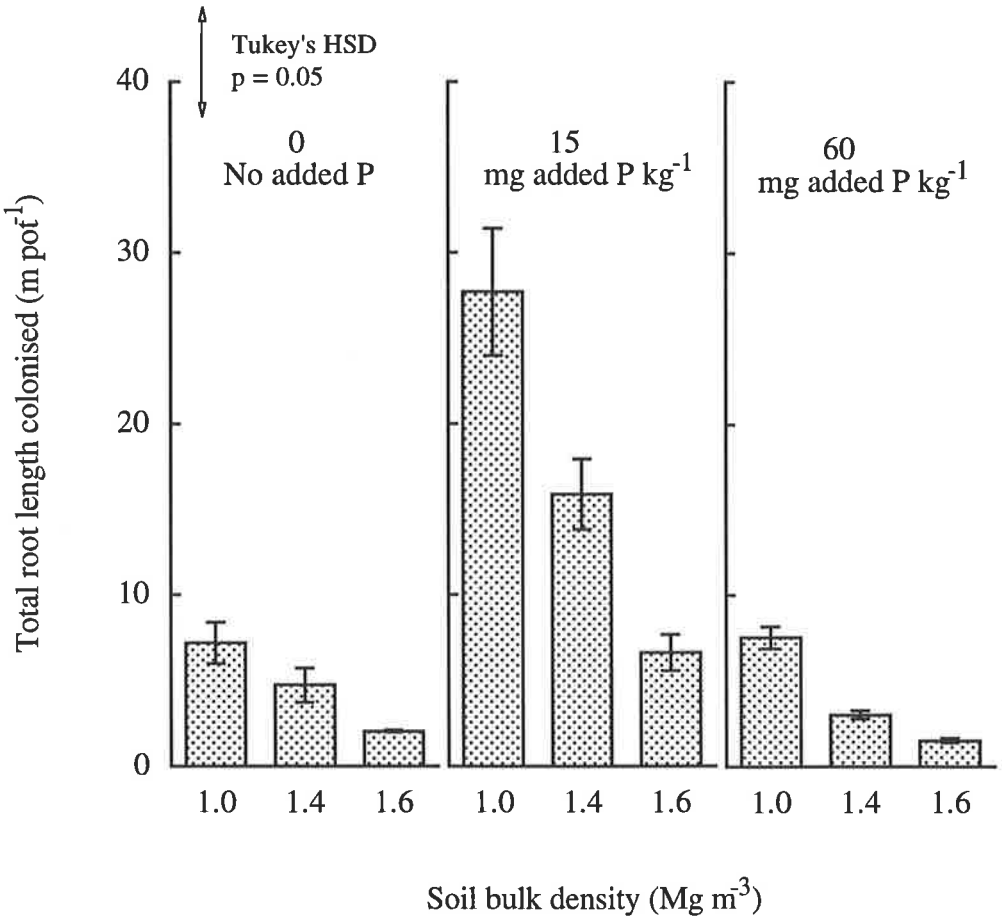

Figure 4.8 Experiment 3 The effect of soil compaction on total root length of *Trifolium subterraneum* colonised by *Glomus intraradices* with no added P, 15 and 60 mg P kg^{-1} soil. Vertical bars represent standard errors of the means, n=4.

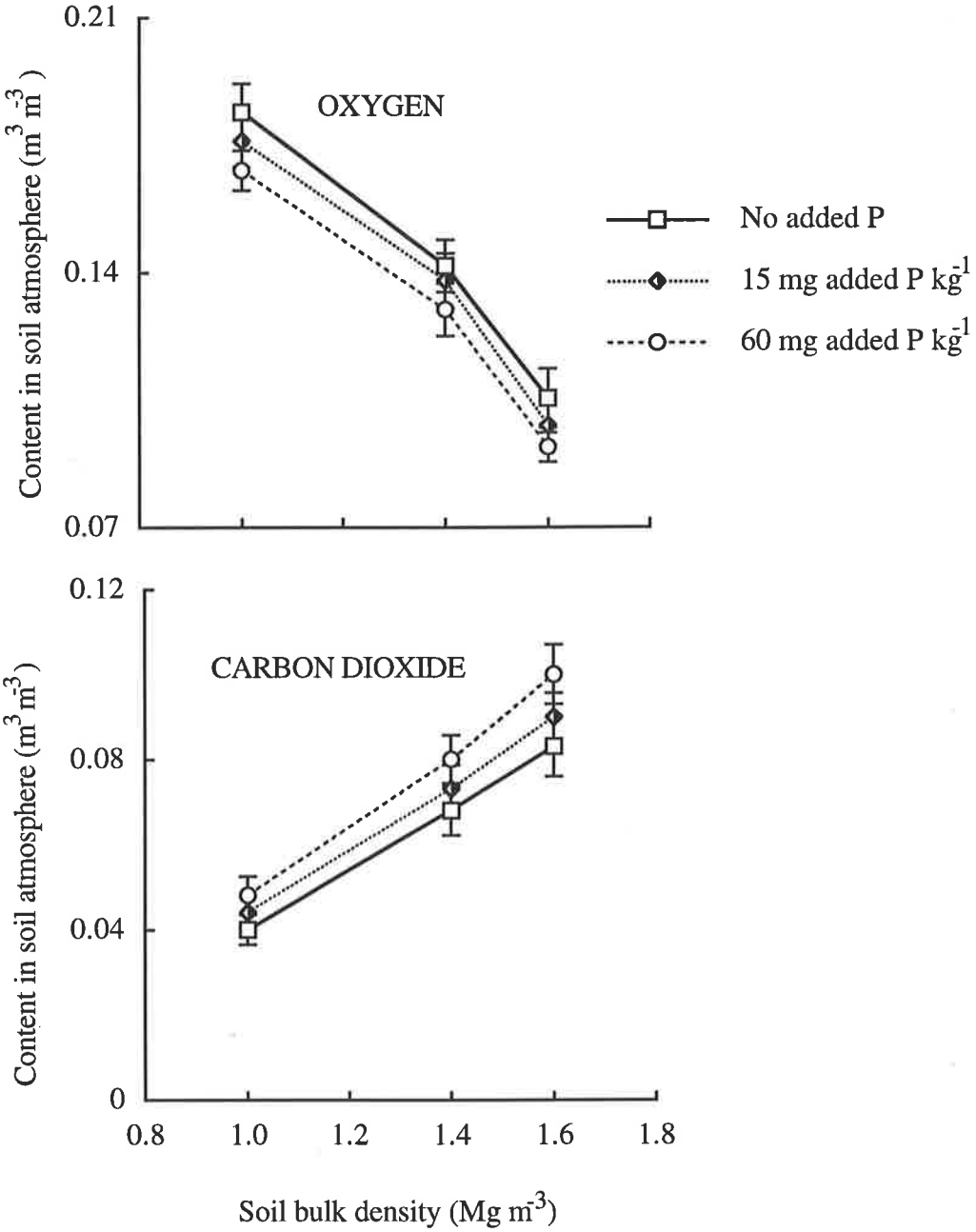

Figure 4.9 Experiment 3 The effect of soil compaction and P applied on the concentrations of oxygen and carbon dioxide in the air of soil inoculated with *Glomus intraradices* in association with *Trifolium subterraneum*. Vertical bars represent standard errors of the means, n=4.

4.4 Discussion

In broad terms, the results of these experiments confirm previous findings on the effects of soil compaction on root growth, P uptake and growth of non-mycorrhizal plants, and show the interaction of these processes with mycorrhizal colonisation. The most important finding was that although there was a mycorrhizal growth response in highly compacted soil of low or moderate P status, the response was not proportionately greater than in slightly compacted soil. Mycorrhizal colonisation did not therefore completely compensate for the poor root growth. Reasons for this, in terms of the function of external hyphae or development of intra-radical interfaces for P transfer, have not yet been elucidated.

The results from Experiment 1 indicate that with no added P, different levels of soil compaction did not significantly affect shoot dry weight of clover or onion plants, suggesting that the low P concentration of the soil (Table 3.1) might be sub-optimal for plant growth (Jones, 1967). The largest effect of compaction on plant growth was observed at the highest P application. A similar trend was observed by Boone and Veen (1982) with maize. Increasing soil compaction increased the diameter of both main axes and first order lateral roots. The increase in root diameter behind the apex has, as yet, not been adequately explained. However, it has been suggested that the increase in root diameter in mechanically impeded roots might be due to enlargement of the cortex in which the cells become shorter in the longitudinal direction but remain wide in transverse (Barley, 1976; Atwell, 1988). The enlargement of the cortex is thought to be a consequence of both the increase in the diameter of the outer cells, and an increase in the number of cells per unit length of root (Bengough and Mullins, 1990). The results for *T. subterraneum* and *A. cepa* thus confirm much previous work with other species (Goss, 1977; Shierlaw and Alston, 1984; Materechera *et al.*, 1991; Hoffmann and Jungk, 1995). Since soil compaction had no significant effect on the concentration of P in the roots and shoots of either onion or clover

(Table 4.3 and 4.4), the decreased total P uptake with increasing soil compaction was mainly a direct consequence of the decrease in root growth and probably in the diffusion coefficient of P, due to high tortuosity.

The main new finding of this study was that at low P application (when plants were severely P limited), mycorrhizal colonisation increased both total P uptake and shoot dry weight over the range of compaction studied, but this increase was less in highly compacted soil than that in slightly compacted soil. Decreased total P uptake by mycorrhizal *T. subterraneum* with increasing soil compaction was due, at least in part, to the extreme decrease in the root length and the resultant decrease in total root length colonised per plant by the fungi from 27.8 to 6.6 m pot^{-1} in slightly and highly compacted soil respectively. That there was no mycorrhizal growth response to treatment with 60 mg P kg^{-1} soil at any level of soil compaction clearly concurs with the general observation that the positive effects of mycorrhizal colonisation on plant growth occur in soils that have a low P content or imbalanced nutrient supplies (Smith, 1980).

Although an increase in soil bulk density from 1.0 to 1.6 Mg m^{-3} had no significant effect on the percentage of the root length colonised, there was a trend towards greater percentage of root length colonised with compaction of the soil (Fig. 4.7). This slight increase observed in the compacted soil may be due to a decline in the rate of root growth, being larger than any decrease in the rate of colonisation. This needs further investigation by methods such as those used by Bruce *et al.* (1994) to evaluate the effects of P on colonisation. Poor aeration (decreased O_2 and increased CO_2) observed in the highly compacted soil had no effect on the percentage of root length colonised. Saif (1983) found that the influence of soil O_2 on the percentage of root length colonised was very small compared with that on the number of arbuscules, vesicles and entry points. The intensities

of arbuscule, vesicle and intra-radical hyphae were not measured in this experiment and it is not clear how poor aeration resulting from soil compaction may affect them.

4.5 Conclusion

This study indicated that soil compaction decreased P uptake and plant growth, whereas in the presence of mycorrhizal colonisation P uptake and plant dry weight increased and compensated, in part, for the effect of the compaction. Decline in the benefit of mycorrhizal colonisation in compacted soil was attributed to the significant decrease in the length of roots. However, some other possibilities may also be involved in this decline. One of these posibilities is the increased mass of P applied per unit volume of the soil when it is compacted; this may have a relatively negative effect on mycorrhizal development. Another possibility may be a decline in the growth and development of external hyphae due to collapse of most large and medium pores in compacted soil. The observed decrease in the concentration of O_2 in the atmosphere of compacted soil may also negatively influence development of internal colonisation including arbuscules, vesicles and intra-radical hyphae. The change in the morphology of clover roots (decreased root length and increased root diameter) caused by soil compaction in this experiment may change the proportion of root length colonised to the amount of external hyphae and this, in turn, may affect P inflow to mycorrhizal roots.

CHAPTER 5

EFFECTS OF SOIL COMPACTION AND TWO P FERTILISATION REGIMES ON THE INTERNAL COLONISATION AND DEVELOPMENT OF EXTERNAL HYPHAE OF *GLOMUS INTRARADICES* IN ASSOCIATION WITH *TRIFOLIUM SUBTERRANEUM*

5.1 Introduction

In the previous chapter, a significant difference was observed between P uptake and plant growth of mycorrhizal and non-mycorrhizal *Trifolium subterraneum* in both slightly and highly compacted soil, but mycorrhizal growth response decreased with increasing soil compaction. The observed decline in benefit of mycorrhizal colonisation with increasing soil compaction was attributed to the significant decrease in total root length colonised by *Glomus intraradices*. However, some other possibilities which may involve in the decline in benefit of mycorrhizal colonisation in compacted soil were presented in Chapter 4. One possible reason for the decline was the increase in mass of P per unit volume of soil caused by compaction. Therefore, any changes in mycorrhizal colonisation in slightly and highly compacted soil might be due to either the physical effect of compaction or to differences in the concentration of P. One of the aims of the experiment described in this chapter was therefore to compare the effect on mycorrhizal colonisation of holding constant either the mass concentration of P (mg P kg^{-1} soil) or the volume concentration of P (mg P dm^{-3} soil) in the immediate vicinity of the roots over the range of compaction levels studied.

There have been no studies of how changes in root growth in compacted soil may affect the development of arbuscules, vesicles and intra-radical hyphae. Increased root diameter in mechanically impeded roots, and increased CO_2 and decreased O_2 contents of the soil atmosphere arising from compaction of the soil, might affect the frequency of arbuscules, vesicles and intra-radical hyphae. Changes in these frequencies, particularly arbuscules, with increasing soil compaction might also contribute to the relatively low mycorrhizal growth response and P uptake observed. Moreover, change in soil pore size distribution and poor aeration in compacted soil may also affect the development of external hyphae. Thus, a second aim of the work described in this chapter was to investigate the effect of soil compaction on the intensity of mycorrhizal colonisation including arbuscules and vesicles and the development of external hyphae.

5.2 Materials and Methods

The experiment had a randomised complete block design with three replications. The treatments (2 × mycorrhiza × 2 regimes of P application × 3 compaction levels) were arranged in factorial combination.

5.2.1 P applied to soil

The effect on mycorrhizal colonisation of holding either the mass concentration of P constant at 15 mg P kg^{-1} soil or the volume concentration of P constant at 15 mg P dm^{-3} soil at different levels of soil compaction was compared. For the treatment with 15 mg P kg^{-1}, the pots contained 620 g soil (oven dry) and received 9.3 mg P. Thus, phosphorus concentration (mg P kg^{-1} soil) was constant, whereas the mass of P per unit volume of soil increased with increasing soil compaction. For the treatment with 15 mg P dm^{-3}, the pots (all containing 620 g oven dry soil) with bulk densities of 1.1, 1.4 and 1.6 Mg m^{-3} soil

received 8.45, 6.64 and 5.81 mg P, respectively. Thus, the mass of P per unit volume of soil was the same for all levels of soil compaction with this P treatment, but concentration per unit soil mass varied.

5.2.2 Soil compaction, plant material and growth conditions

The soil was autoclaved and compacted (see Chapter 3) into PVC pots to bulk densities of 1.1, 1.4 and 1.6 Mg m^{-3}. For the mycorrhizal treatments, each seedling was inoculated with 0.25 g fresh inoculum of *Glomus intraradices* and for the non-mycorrhizal treatments each seedling received 0.25 g non-colonised clover roots as described in Chapter 3. Three *T. subterraneum* plants were grown in each pot. The experiment was conducted in a growth room where the photoperiod was 16 h and the irradiance was 380 μmol m^{-2} s^{-1}. The day and night air temperatures were 20 and 16°C, respectively.

5.2.3 Measurements

Plants were harvested 7 weeks after planting. The shoots and roots were cleaned and washed, and their fresh and dry weights were recorded. Root samples were stained and the percentage of root length colonised was determined. The concentrations of P in the shoots and roots of clover plants and the concentrations of O_2 and CO_2 in the soil atmosphere were measured as described in Chapter 3.

Assessment of colonisation by VA mycorrhizal fungi followed the method of McGonigle *et al.* (1990). This method determines the fraction of the root length containing arbuscules, vesicles and either arbuscules, vesicles or intra-radical hyphae. In this experiment, percentage colonisation was used instead of fraction of colonisation to retain uniformity with the other chapters. Sub-samples of roots were randomly selected and mounted on glass slides. At least 150 intersects were assessed from each root sample.

The lengths of total external hyphae and of living hyphae were determined after staining them with acid fuchsin and FDA (fluorescein diacetate) respectively. The method for extraction of hyphae from the soil was modified from Abbott *et al.* (1984). Four soil cores (13 mm in diameter) were taken from each pot just before the plants were harvested. The soil was mixed, and a 2 g sub-sample was added to 300 cm^3 of sodium hexametaphosphate (35 g dm^{-3} in distilled water) and stirred with a magnetic stirrer for 10 min. A 5 cm^3 aliquot was removed and filtered through a 8 μm Millipore filter. To measure the length of living external hyphae, the retained material was stained in 0.5 cm^3 of a freshly prepared solution of FDA in 60 mM sodium phosphate buffer at pH 7.4 with a final concentration of 0.01 g dm^{-3} FDA (Schubert *et al.*, 1987). All procedures, from filtering the soil suspension and addition of FDA, were completed within 30 min to overcome problems of loss of enzyme activity and fading of fluorescence. A similar procedure was used to measure the length of total external hyphae, but the material retained from filtering of a 5 cm^3 aliquot was stained with 0.5 cm^3 of 0.1g dm^{-3} acid fuchsin solution for 30 min. The length of total external hyphae and living hyphae were determined by counting the number of intersections between hyphae and a grid eyepiece micrometer at × 160 magnification on a Zeiss Standard Lab 16 equipped for epifluorescence microscopy. The excitation filter was BP 450-490 and the barrier filter was LP 520

The soil porosity for different levels of soil compaction was calculated from the following equation:

$$Soil\ porosity = 100\ (1-\frac{\rho_b}{\rho_s})$$

where ρ_b and ρ_s are bulk and particle densities of the soil, respectively. Soil particle density is usually taken to be 2.65 Mg m^{-3}, and this value was therefore used to calculate the soil porosity.

For all analyses, the F-test or analysis of variance was used to test the significance of the main factors using Genstat (Genstat 5 Committee, 1987).

5.3 Results

No interaction was observed between two methods of P application and soil compaction in their effects on the shoot dry weights of mycorrhizal and non-mycorrhizal clover plants (see Table 5.1 for interactions). Irrespective of P application, mycorrhizal colonisation increased shoot dry weight of clover plants at all levels of compaction of the soil, although this increase was smaller with increasing soil compaction, and confirmed the results of the previous experiments (Fig. 5.1). Roots exhibited a similar response (Fig. 5.2). No interaction was observed between the two regimes of P fertilisation and soil compaction in their effects on mycorrhizal growth response. However, irrespective of P application, mycorrhizal growth response decreased as soil compaction increased (Fig. 5.3).

Although P uptake decreased with increasing soil compaction (Table 5.2), P uptake per unit root length by both mycorrhizal and non-mycorrhizal plants increased with increasing soil compaction. There was a significant difference in total P uptake per unit root length between mycorrhizal and non-mycorrhizal plants at all levels of soil compaction (Fig. 5.4).

No significant difference was observed in the length of total external hyphae and of living external hyphae between inoculated and non-inoculated soils. However, irrespective of P application, soil compaction decreased the length of living external hyphae (Fig. 5.5).

The percentage of the root length colonised by arbuscules, by vesicles and by either arbuscules, vesicles or hyphae in slightly compacted soil were 27, 11 and 45, respectively and were not affected by soil compaction. However, the total root length colonised by arbuscules, by vesicles or by either arbuscules, vesicles or hyphae decreased with increasing soil compaction, irrespective of P application (Fig. 5.6).

Table 5.1 Levels of significance for shoot and root dry weights of *Trifolium subterraneum*, P uptake, mycorrhizal growth response, mycorrhizal colonisation, external hyphae and soil air oxygen and carbon dioxide.

Source of variance	Compaction (C)	Mycorrhiza (M)	Phosphorus (P)	C × M	C × P	M × P	C × M × P
df	1	1	1	2	2	1	2
Shoot dry weight	***[A]	***	***	***	n.s.[B]	**	n.s.
Root dry weight	***[A]	***	***	***	n.s.	n.s.	n.s.
Shoot P concentration	n.s.	***	*	n.s.	n.s.	n.s.	n.s.
Root P concentration	n.s.	***	*	*	n.s.	n.s.	n.s.
Total P uptake	***	***	***	**	n.s.	**	n.s.
Total P uptake per unit length of root	***	***	n.s.	**	n.s.	n.s.	n.s.
Mycorrhizal growth response (%)	***	-[c]	n.s.	-	n.s.	-	-
Arbuscular colonisation (%)	n.s.	-	n.s.	-	n.s.	-	-
Vesicular colonisation (%)	n.s.	-	n.s.	-	n.s.	-	-
Arbuscular colonisation (m pot^{-1})	***	-	n.s.	-	n.s.	-	-
Vesicular colonisation (m pot^{-1})	***	-	n.s.	-	n.s.	-	-
Length of living external hyphae	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Soil air O_2 and CO_2	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[A] *, **, *** significant at the 0.05, 0.01, 0.001 probability level, respectively.
[B] n.s. = not significant at p = 0.05.
[c] Mycorrhizal was not considered as source of variance in calculating the ANOVA of colonisation.

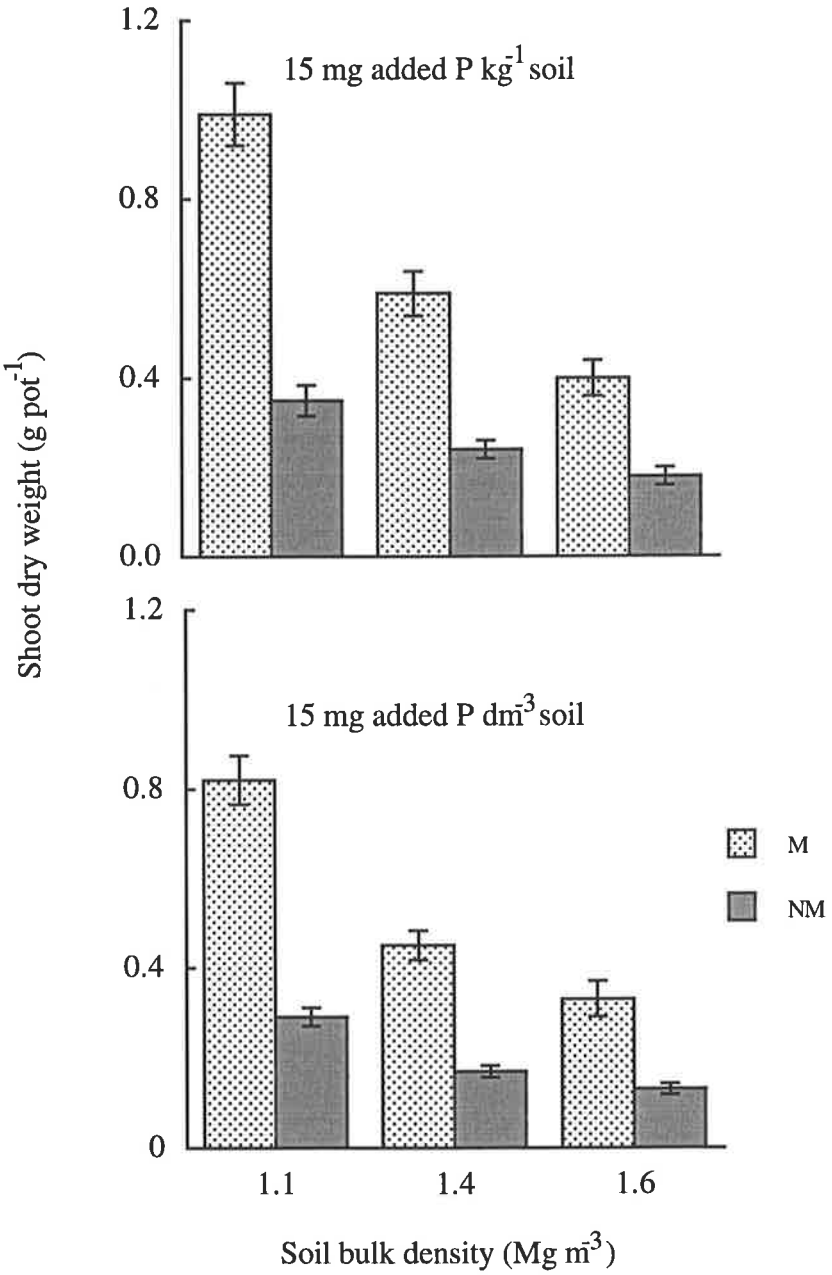

Figure 5.1 **The effect of soil compaction on shoot dry weight of mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum* at two levels of P application. Vertical bars represent standard errors of the means, n=4.**

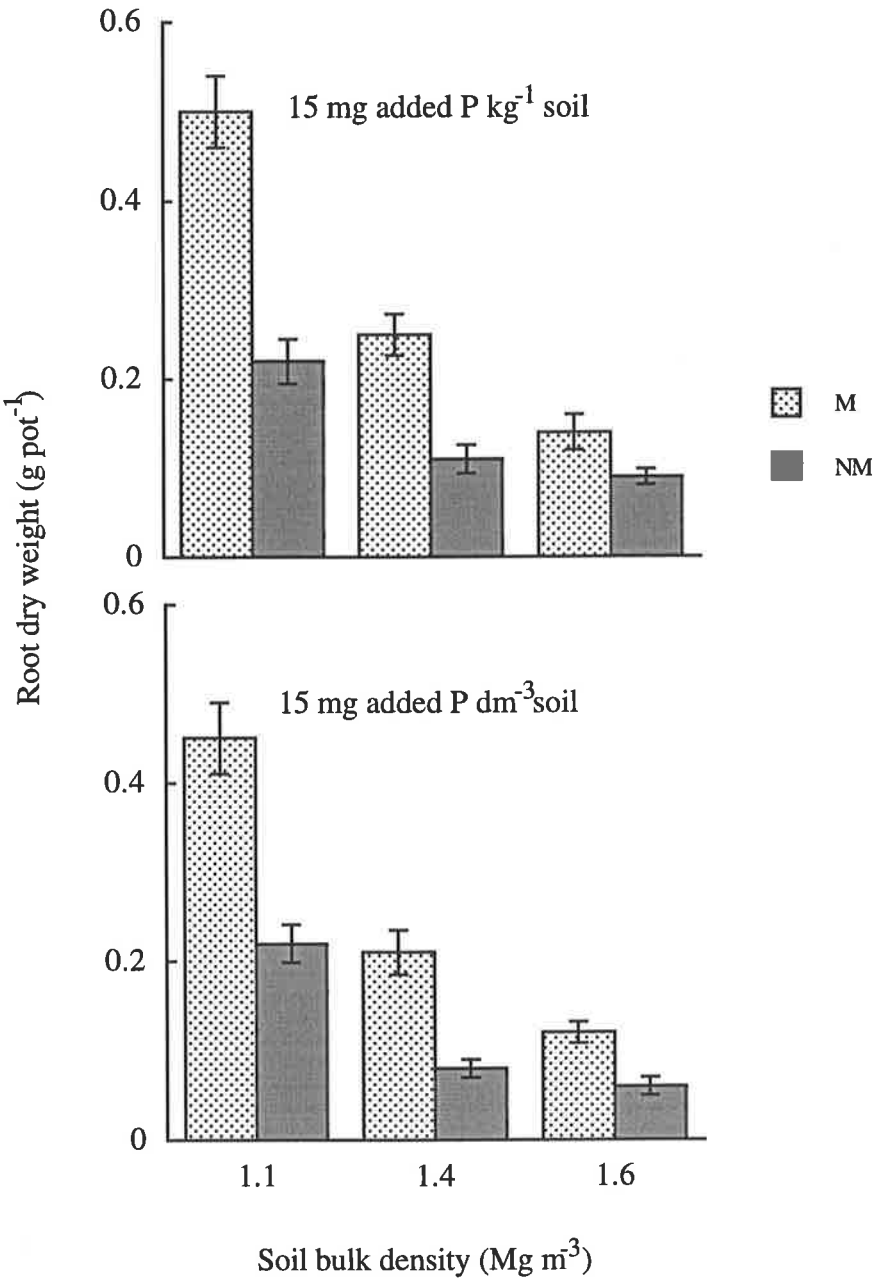

Figure 5.2 **The effect of soil compaction on root dry weight of mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum* at two levels of P application. Vertical bars represent standard errors of the means, n=4.**

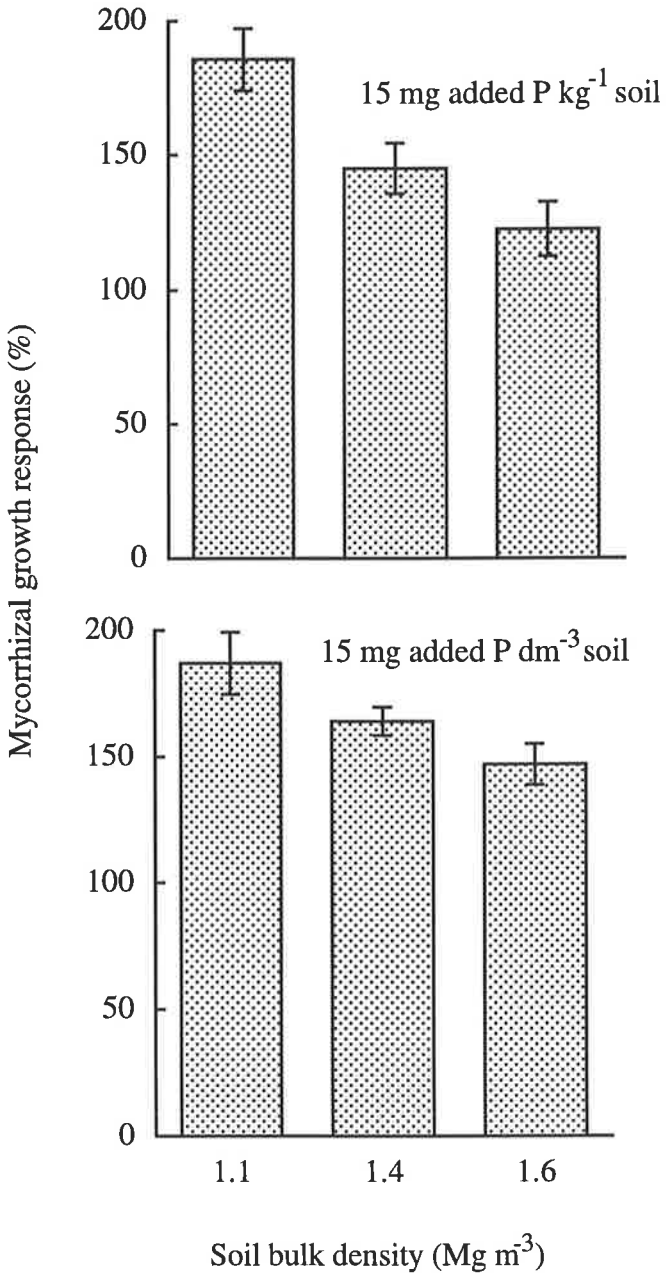

Figure 5.3 **Mycorrhizal growth response of *Trifolium subterraneum* as affected by soil compaction and P application. Vertical bars represent standard errors of the means, n=4.**

Table 5.2 The effects of soil compaction on total P uptake and the concentration of P in the shoots and roots of mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum.*

Bulk density	P applied[A]	Mycorrhiza	Total P uptake	P concentration	
				Shoot	Root
(Mg m^{-3})			(mg pot^{-1})	(μg mg^{-1} dry weight)	
1.1	P1	M	1.56 (±0.11)[B]	1.25 (± 0.04)	1.20 (± 0.07)
		NM	0.46 (±0.05)	0.85 (± 0.03)	0.95 (± 0.05)
	P2	M	1.86 (±0.15)	1.26 (± 0.05)	1.21 (± 0.05)
		NM	0.54 (±0.05)	0.92 (± 0.06)	1.02 (± 0.03)
1.4	P1	M	0.81 (±0.08)	1.23 (± 0.03)	1.20 (± 0.05)
		NM	0.24 (±0.01)	0.94 (±0.05)	0.98 (± 0.08)
	P2	M	1.10 (±0.07)	1.31 (± 0.04)	1.31 (± 0.03)
		NM	0.37 (±0.04)	1.02 (± 0.02)	1.05 (± 0.02)
1.6	P1	M	0.54 (±0.04)	1.25 (± 0.06)	1.24 (± 0.06)
		NM	0.16 (±0.01)	0.81 (± 0.03)	0.82 (± 0.03)
	P2	M	0.70 (±0.06)	1.31 (± 0.06)	1.27 (± 0.03)
		NM	0.23 (±0.02)	0.85 (± 0.03)	1.09 (± 0.07)

[A] P1 = 15 mg P dm^{-3} soil.; P2 = 15 mg P kg^{-1} soil.

[B] Standard error of the mean.

No interaction was observed between two regimes of P application and soil compaction in their effects on root colonisation (Table 5.1 for interactions). At a matric potential of -33 kPa, the air-filled porosity of the soil decreased from 0.38 to 0.07 m^3 m^{-3} when soil compaction was increased from a bulk density of 1.1 to 1.6 Mg m^{-3} (see Table 5.3). The air-filled porosity in highly compacted soil, which varied from 0.07-0.11 m^3 m^{-3} over the range of matric potentials encountered (-33 and -100 kPa, Table 5.3), had no significant effect on the percentage of root colonisation.

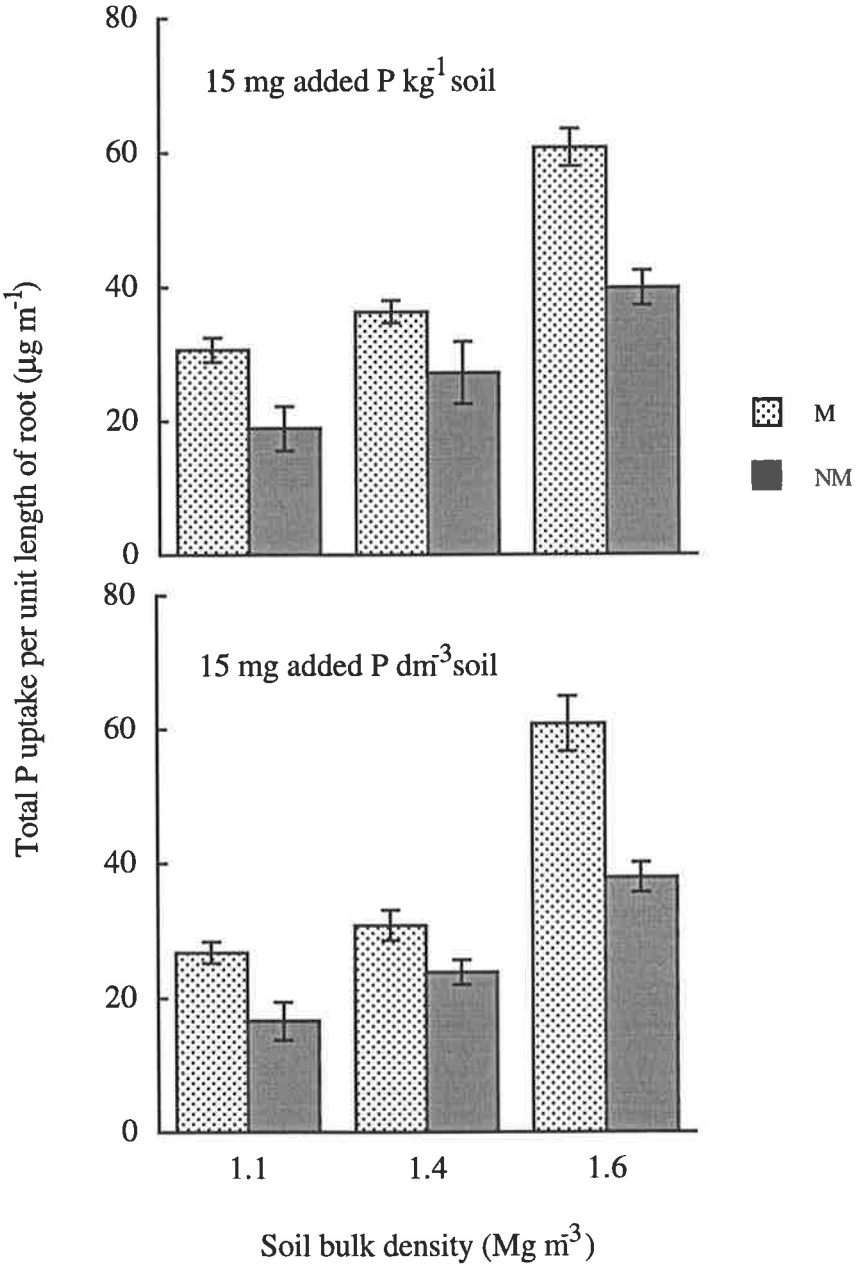

Figure 5.4 **The effect of soil compaction on total P uptake per unit length of root by mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum* at two levels of P application. Vertical bars represent standard errors of the means, n-4.**

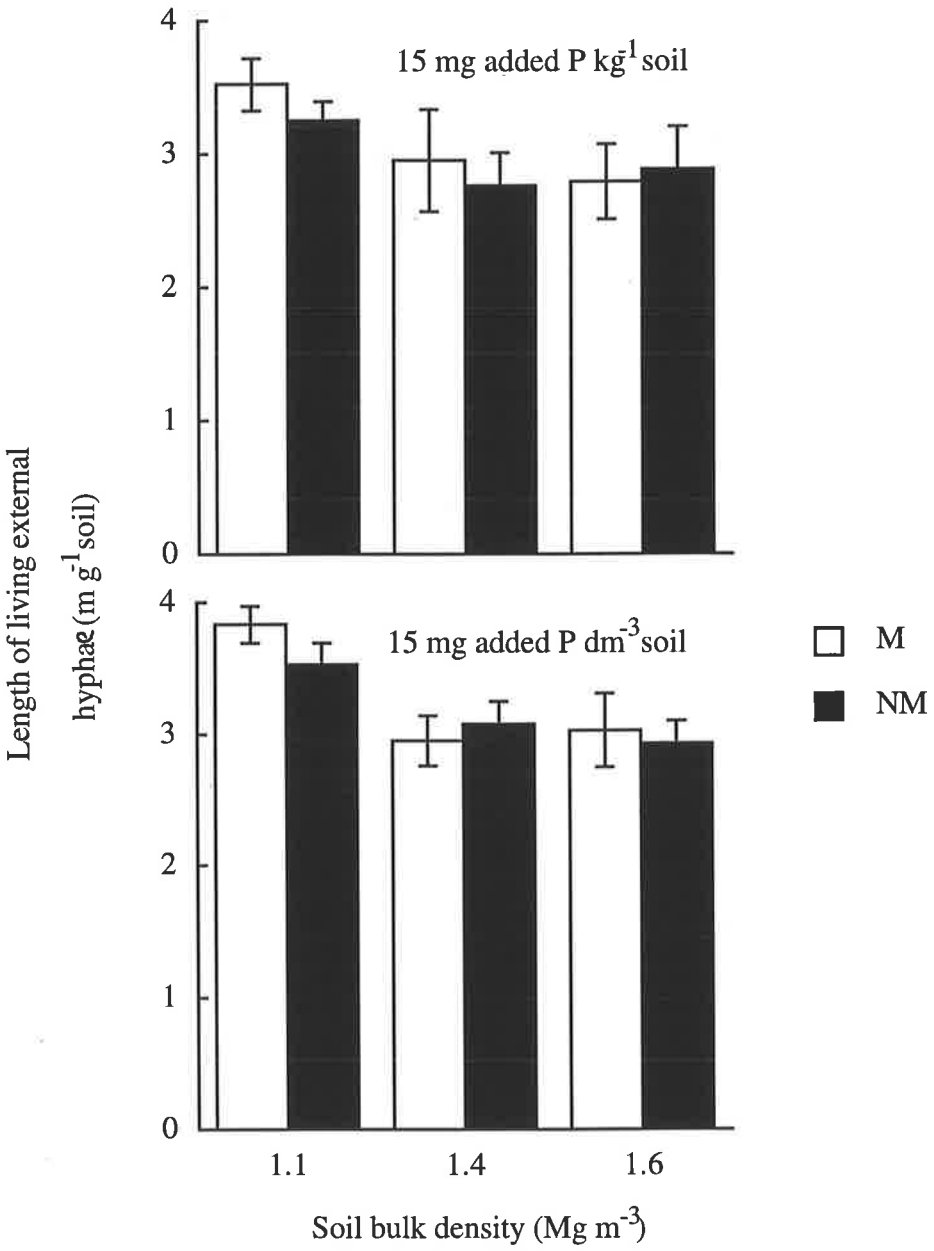

Figure 5.5 The length of living external hyphae of *Glomus intraradices* in inoculated (M) and non-inoculated soil (NM) as affected by soil compaction and P application. Vertical bars represent standard errors of the means, n=4.

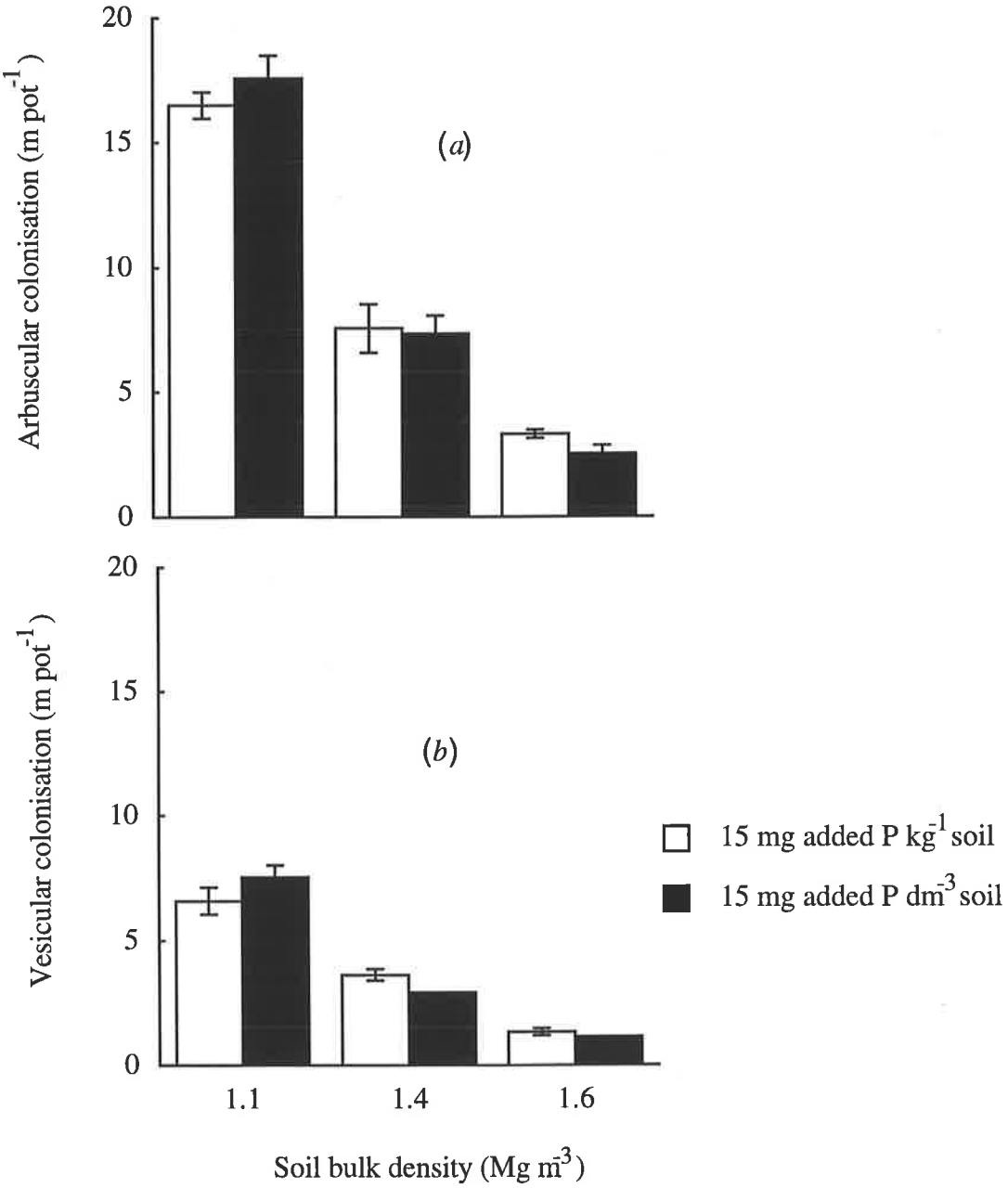

Figure 5.6 **The effect of soil compaction on total root length of *Trifolium subterraneum* colonised by arbuscules (*a*) and vesicles (*b*) at two levels of P application. Vertical bars represent standard errors of the means, n=4.**

In this experiment, the concentrations of O_2 and CO_2 in the soil air were measured in both inoculated and non-inoculated soils . The results indicated that there was no significant difference in the concentration of O_2 and CO_2 in the soil atmosphere between inoculated and non-inoculated soils (results not shown).

Table 5.3 The effects of soil compaction on air-filled porosity, water content and total porosity of the soil used in this study at two levels of soil matric potentials.

Bulk density ($Mg\ m^{-3}$)	Penetrometer resistance (MPa)	Volumetric water content ($m^{-3}\ m^{-3}$)	Air-filled porosity ($m^{-3}\ m^{-3}$)	Total porosity ($m^{-3}\ m^{-3}$)
		Matric potential (-33 kPa)		
1.1	0.8	0.21	0.38	0.59
1.4	2.3	0.28	0.19	0.47
1.6	3.5	0.33	0.07	0.40
		Matric potential (-100 kPa)		
1.1	0.8	0.18	0.41	0.59
1.4	2.3	0.26	0.21	0.47
1.6	3.5	0.29	0.11	0.40

5.4 Discussion

In this experiment, no significant difference was observed in the percentage of root colonisation between 15 mg P kg^{-1} soil and 15 mg P dm^{-3} soil, although a decrease in intensity of internal colonisation has been reported with increasing soil P concentration (Amijee *et al.*, 1989; Smith and Gianinazzi-Pearson, 1990; Bruce *et al.,* 1994). This may be related to the small difference in concentration of P between 15 mg P kg^{-1} soil and 15 mg P dm^{-3} soil and suggests mycorrhizal growth response is not very sensitive to P concentration in this range. Thus, decrease in mycorrhizal growth response with increasing soil

compaction observed in this and in the previous experiment is not likely to be a consequence of the increase in mass of P per unit volume of the soil, resulting from soil compaction.

The higher P uptake per unit length of root by mycorrhizal plants with increasing soil compaction compared with non-mycorrhizal roots, indicates that the efficiency of mycorrhizal roots in absorbing P was greater in highly compacted soil than in slightly compacted soil (Fig. 5.4) and also that the factor limiting growth of mycorrhizal plants was not the efficiency of the roots *per se*.

There have been no previous reports of how the effects of changes in root growth in compacted soil may interact with the development of characteristic mycorrhizal structures. The results of this experiment show that compaction had no significant effect on the percentage of root colonised by arbuscules, vesicles or by any combination of arbuscules, vesicles and intra-radical hyphae. The simplest explanation is that internal colonisation is not influenced by compaction. However, there are two opposing effects operating: (1) increase in root diameter and consequently in root cortex (Atwell, 1988) resulting from soil compaction might increase the surface area per unit length of root available for colonisation, and (2) poor aeration (increased CO_2 and decreased O_2) might decrease the intensity of internal colonisation. This suggestion is supported by the results of Saif (1981) who found that the number of vesicles per unit root length decreased with decreasing O_2 concentration in the soil air from 0.21 to 0.02 m^3 m^{-3}. The number of vesicles also decreased with increasing CO_2 concentration from 0.04 to 0.08 m^3 m^{-3}, when O_2 concentration was maintained at 0.16 m^3 m^{-3} in the soil air (Saif, 1984). A significant decrease in the number of entry points with decreasing O_2 concentration from 0.16 to 0.02 m^3 m^{-3} in the soil air was also observed (Saif 1983). Accordingly, low air-filled porosity and decreased O_2 in the soil air in highly compacted soil may have negative effects on development of internal

colonisation. Moreover, the air-filled porosity of highly compacted soil varied from 0.07 to 0.11 m^3 m^{-3} over the range of matric potentials encountered (-33 and -100 kPa). The latter value is close to the air-filled porosity of 0.12 which has been suggested as inadequate to provide sufficiently rapid diffusion of oxygen in soil for plant growth (Grable and Siemer, 1968).

The significant decrease in the total root length colonised by arbuscules, by vesicles or by any combinations of arbuscules, vesicles or hyphae with increasing soil compaction can be attributed to the low total length of roots in compacted soil. There seems, therefore, to be no evidence that the decreased mycorrhizal growth response in compacted soil was due to a decrease in arbuscule formation per unit root length colonised.

It was impossible to compare the length of external hyphae in inoculated and non-inoculated soil because of difficulties in differentiating between hyphae of VA mycorrhizal fungi and those of non-VA mycorrhizal fungi (saprophytic Zygomycetes) when the hyphae were fragmented into small pieces (Abbott and Robson, 1985a; Jakobsen *et al.*, 1994). It is difficult to know to what extent soil compaction may have affected the length of external hyphae of VAM fungi compared with those of non-VAM fungi. However, changes in physical properties of the soil such as pore size distribution and aeration may influence the development of external hyphae.

5.5 Conclusion

The results of this experiment demonstrated that the decline in benefit of mycorrhizal colonisation with increasing soil compaction observed in this and the previous experiment could not be a consequence of the increase in the mass of P per unit volume of the soil resulting from soil compaction. On the other hand, since poor aeration had no effect on the intensity of internal colonisation, it was concluded that the observed decline in benefit of

mycorrhizal colonisation in compacted soil was due, at least in part, to inhibition of root growth and consequently to the considerable decrease in total root length colonised by arbuscules and intra-radical hyphae. However, a possible decrease in the length of external hyphae might also be involved.

CHAPTER 6

PHOSPHORUS INFLOW TO MYCORRHIZAL AND NON-MYCORRHIZAL PLANTS OF *TRIFOLIUM SUBTERRANEUM* AS AFFECTED BY SOIL COMPACTION AND P APPLICATION

6.1 Introduction

It has been shown that mycorrhizal colonisation increases P inflow to mycorrhizal roots (Sanders and Tinker, 1973; Sanders *et al.*, 1977; Smith *et al.*, 1979; Smith, 1982; Tester *et al.*, 1985; Jakobsen *et al.*, 1992a; Smith *et al.*, 1994b). However, the effect of mycorrhizal colonisation on P inflow to mycorrhizal roots in compacted soil has not been studied. Changes in pore size distributions resulting from soil compaction may alter movement of air, water and nutrients. All these can affect growth and morphology of plants (Shierlaw and Alston, 1984; Hoffmann and Jungk, 1995). Morphological changes of plants in compacted soil such as increases in root diameter and decreased root length, which were observed in the experiments reported in Chapter 4, may also affect P inflow. Moreover, an increase in volumetric water content of the soil with increasing soil compaction may affect the diffusion coefficient of P and this, in turn, may affect P inflow (Hoffmann and Jungk, 1995). Hence, one objective of the work described in this chapter was to study how soil compaction influences P inflow to mycorrhizal and non-mycorrhizal roots. Was the observed decline in benefit of mycorrhizal colonisation in the previous experiments due to a possible decline in P inflow?

The filtration gridline method is the most common method used to assess the length of external hyphae in soil. However, a major problem is that the external hyphae of VAM fungi

are often not distinguishable from those of non-VAM fungi (*e.g.* saprophytic Zygomycetes) when hyphae are fragmented (Abbott and Robson, 1985a; Sylvia, 1992; Jakobsen *et al.*, 1994). It is also difficult to extract hyphae effectively from many soils, further restricting the application of this technique. Probably because of these problems, no significant difference was observed in the length of external hyphae between inoculated and non-inoculated soil (Chapter 5). Attempts have also been made to estimate the amount of external hyphae by chitin extraction (Bethlenfalvay and Ames, 1987) and by an immunofluorescence assay (Kough and Linderman, 1986), but quantification of external hyphae by these two methods is not completely satisfactory. For example, Schmitz *et al.* (1991) were unable to obtain a good correlation between chitin content and VAM colonisation in roots, because chitin is present in other organisms (Sylvia, 1992). More recently, analysis of ester-linked phospholipid fatty acids (PLFA) has been applied, and this method appears to offer considerable improvements on previous methods with respect to both sensitivity and specificity for different groups of fungi. Lipids are the major storage compounds in mycorrhizal fungi and are present in large amounts in vesicles, hyphae and spores. Fatty acids 16:1, 16:0 and 18:1 were found to be dominant in lipids from spores of *Glomus caledonium* (Beilby and Kidby, 1980). The fatty acids 16:1ω5, 18:ω7c, 18:3, 20:4 and 20:5 have also been found in higher amounts in the plant roots colonised by several species of VAM fungi compared with non-mycorrhizal roots (Pacovsky and Fuller, 1988; Pacovsky, 1989). Graham *et al.* (1995) found that production in citrus of fatty acid 16:1ω5 by *Glomus intraradices* and *Glomus rosea* was correlated with the development of mycorrhizal colonisation. Olsson *et al.* (1995) have also shown that the amounts of both neutral lipid fatty acids (NLFA) and PLFA 16:1ω5 and 16:0 extracted from the soil were greater in inoculated soil than in non-inoculated soil. These fatty acids have therefore been suggested as indicators of VA fungal biomass (Jakobsen, 1991). However, some types of fatty acids

such as 16:1ω5 and 20:5 have also been reported in bacteria and non-VAM fungi (Nichols *et al.*, 1986; Amano *et al.*, 1992), so that some 'background' values are to be expected.

Since the filtration gridline method was not successful in assessment of external hyphae in the work described in Chapter 5, this experiment also included estimates of the amount of external hyphae in soil using ester-linked fatty acid analysis to study the effect of soil compaction on external hyphae and to determine whether P inflow was related to biomass of VAM fungi hyphae in soil.

6.2 Materials and Methods

The experiment had a randomised complete block design with 36 treatments (2 P levels, 2 mycorrhiza, 3 compaction levels, 3 harvests) arranged in factorial combination with three replicates.

The soil was autoclaved and basal nutrients (see Chapter 3) and P fertiliser as NaH_2PO_4 at rates of 0 and 15 mg P dm^{-3} were mixed throughout the soil. The soil was then compacted into PVC pots to bulk densities of 1.2, 1.4 and 1.6 Mg m^{-3} as described in Chapter 3.

6.2.1 Plant material and growth conditions

Seeds of *T. subterraneum* were germinated and six 2-day old seedlings were transplanted into each pot. Each clover seedling was inoculated with 0.25 g fresh root of *T. subterraneum* colonised with *G. intraradices*. The plants were grown in a growth room with photon flux density of 410 µmol m^{-2} s^{-1} during a 16 h photoperiod. The air temperatures at day and night were 21 and 15°C, respectively. The water content of the soil was brought to field capacity (matric potential = -33 kPa) by watering to weight.

6.2.2 Measurements

Plants were harvested 20, 40 and 60 days after planting. At each harvest, roots from each pot were carefully washed free of soil and cut into 1 cm segments. Fresh weight of roots and shoots were recorded. Plant dry weights, shoot and root P concentrations, root length and percentage of root length colonised were measured as described in Chapter 3.

P inflow (uptake per unit length per unit time) to the roots during the three harvest periods was calculated by the formula:

$$I = [(P_2 - P_1)\ ln\ (L_2 L_1^{-1})][(T_2 - T_1)(L_2 - L_1)]^{-1}$$

following the method of Brewster and Tinker (1972), where P refers to plant phosphorus content (mol), T to plant age (second) and L to root length (m). The initial P content per plant was 0.82 μmol, based on the average of P content in seeds. The value for initial root length was assumed to be 10 μm.

Mycorrhizal responses in terms of P inflow (*a*) and P uptake per plant (*b*) were calculated by the following equations:

(a)

$$\frac{P\ inflow\ to\ mycorrhizal\ roots - P\ inflow\ to\ non\text{-}mycorrhizal\ roots}{P\ inflow\ to\ non\text{-}mycorrhizal\ roots} \times 100$$

(b)

$$\frac{P\ uptake\ per\ plant\ by\ mycorrhizal\ roots - P\ uptake\ per\ plant\ by\ non\text{-}mycorrhizal\ roots}{P\ uptake\ per\ plant\ by\ non\text{-}mycorrhizal\ roots} \times 100$$

Lipid extraction and fatty acid analysis were carried out using a modified method of Frostegård *et al.* (1991) based on the procedure of Bligh and Dyer (1959). Four soil cores (13 mm in diameter) were taken from each pot just before the plants were harvested. The

soil was mixed, and a 5 g sub-sample (wet weight) was extracted into 10 cm^3 of one-phase chloroform:methanol:citrate buffer (1:2:0.8, v/v/v) by shaking the suspension for 3 h. After centrifugation (2500 rpm for 10 min) the pellets were washed with 5 cm^3 of the one-phase mixture. The extracts were separated into two phases by adding 3.1 cm^3 chloroform and 3.1 cm^3 citrate buffer (pH 4). The extracted lipids were fractionated into neutral lipids (triacylglycerols), glycolipids and polar lipids (phospholipids) on silicic acid columns (Unisel 100-200 mesh) by elution with 5 cm^3 methanol, 10 cm^3 acetone and 5 cm^3 methanol, respectively. The neutral and polar lipid fractions were dried under a stream of nitrogen. Fatty acid methyl esters, nonadecanoate and tridecanoate, were added as internal standards (100 µl of each) to the dried lipids. All samples were then subjected to a mild alkaline methanolysis (Dowling *et al.,* 1986). The fatty acid methyl esters were separated and quantified by gas chromatography using a Hewlett Packard (model 5890A) fitted with a flame ionisation detector and a capillary column (BP20, 50 m (1), 0.32 (i. d.): SGE, Melbourne, Australia). Hydrogen was used as the carrier gas (column head pressure, 65 kPa). Major fatty acid peaks were identified by comparison of retention times of a standard fatty acid mixture (Nu-Check, USA No. 68B or NIH, USA specifically analysed fish oil). The nomenclature of the fatty acids followed that used by Tunlid and White (1992).

6.3 Results

Irrespective of mycorrhizal colonisation, the uptake of P per unit length of root per unit time (P inflow) increased as soil compaction increased (Table 6.1). Although no significant interaction was observed in P inflow between soil compaction and mycorrhizal colonisation (see Table 6.2 for interactions), there was a trend towards higher P inflow via hyphae in highly compacted soil (bulk density = 1.6 Mg m^{-3}) than in slightly compacted soil (1.2 Mg m^{-3}), particularly with 15 mg P dm^{-3} soil. However, calculation of the mycorrhizal responses

in terms of P inflow or P uptake per plant indicated that soil compaction did not have much effect on mycorrhizal P responses at the first and second harvests, but the responses decreased with increasing soil compaction at the third harvest with both levels of soil P, results shown only for treatments with 15 mg P dm^{-3} (Table 6.3).

Table 6.1 P inflow (pmol × 10^{-1} m^{-1} s^{-1}) to mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum* roots as affected by soil compaction and P application for three harvest periods.

Bulk density ($Mg\ m^{-3}$)	Harvest period (days) 0-20 M	0-20 NM	20-40 M	20-40 NM	40-60 M	40-60 NM
			No added P			
1.2	26.4 (±4.2)[a]	15.9 (±3.7)	3.6 (±0.2)	1.1 (±0.1)	1.2 (±0.3)	0.2 (±0.2)
1.4	33.1 (±6.8)	20.7 (±3.2)	4.1 (±0.9)	1.4 (±0.9)	1.8 (±0.2)	0.5 (±0.4)
1.6	49.6 (±4.6)	29.3 (±6.3)	5.5 (±1.3)	2.2 (±1.1)	1.9 (±0.3)	0.5 (±0.3)
			15 mg added P dm^{-3} soil			
1.2	58.5 (±5.3)	34.7 (±6.1)	9.2 (±0.9)	4.7 (±0.6)	3.4 (±0.7)	0.7 (±0.1)
1.4	73.2 (±6.44)	45.7 (±13.5)	12.7 (±0.7)	6.1 (±0.3)	3.6 (±0.2)	1.2 (±0.4)
1.6	112.3 (±11.7)	71.6 (±18.0)	15.1 (±0.2)	7.6 (±0.4)	5.3 (±0.9)	1.9 (±0.1)

[a] Standard error of the mean.

The highest P inflow was observed during the first harvest period (0-20 days), particularly when the soil was supplied with 15 mg P dm^{-3}. In highly compacted soil and when the soil was supplied with 15 mg P dm^{-3}, P inflows to non-mycorrhizal and mycorrhizal roots were 7.2 and 11.2 pmol m^{-1} s^{-1}, respectively (see Table 6.1).

Table 6.2 **Probability of F for plant growth, P uptake, mycorrhizal growth response of *Trifolium subterraneum* and NLFA extracted from the soil as indicator of hyphal biomass.**

Source of variance	df	Shoot dry weight (mg plant^{-1})	Mycorrhizal growth respose (%)	Root length colonised (%)	Root length colonised (m pot^{-1})	P uptake (µg plant^{-1})	P inflow (pmol × 10^{-1} m^{-1} s^{-1})	NLFA 16:1ω5 (nmol g^{-1} soil)	NLFA 16:1ω5 (µmol m^{-1} root length colonised)
Phosphorus (P)	1	***[A]	*	**	***	***	***	***	**
Mycorrhiza (M)	1	***	-[B]	-	-	***	***	***	-
Compaction (C)	2	***	*	n.s.	***	***	***	***	***
Harvest (H)	2	***	***	***	***	***	***	***	***
P × M	1	***	-	-	-	***	*	***	-
P × C	2	***	n.s.[C]	n.s.	***	***	*	*	n.s.
M × C	2	***	-	-	-	***	n.s.	***	-
P × H	2	***	n.s.	n.s.	***	***	***	**	*
M × H	2	***	-	-	-	***	***	***	-
C × H	4	***	n.s.	n.s.	***	***	***	*	n.s.
P × M × C	2	*	-	-	-	n.s.	n.s.	n.s.	-
P × M × H	2	***	-	-	-	***	n.s.	*	-
P × C × H	4	***	n.s.	n.s.	***	***	*	n.s.	n.s.
M × C × H	4	**	-	-	-	*	n.s.	n.s.	-
P × M × C × H	4	n.s.	-	-	-	n.s.	n.s.	n.s.	-

[A] *, **, ***, significant at p = 0.05, 0.01 and 0.001, respectively.

[B] Mycorrhiza was not considered as source of variance in calculating the ANOVA of colonisation.

[C] n.s., not significant at p = 0.05.

A positive correlation was observed between P inflow via hyphae and the amounts of neutral lipid fatty acids (NLFA) 16:1ω5 and 16:0 extracted from soil per unit length of colonised root (Fig. 6.1). Irrespective of soil compaction, the P concentrations in the shoots and roots of mycorrhizal plants were greater than those of non-mycorrhizal plants, particularly at the first harvest (results not shown). The difference between total P uptake by mycorrhizal and non-mycorrhizal plants increased with time and it became significant at the second and third harvest in slightly and highly compacted soil, respectively (Table 6.4).

Table 6.3 The effect of soil compaction on mycorrhizal P response in terms of P inflow (pmol $\times 10^{-1}$ m^{-1} s^{-1}) and P uptake per plant (μg $plant^{-1}$) at 20, 40 and 60 days from planting when the soil was supplied with 15 mg P dm^{-3}.

Bulk density (Mg m^{-3})	Days from planting 20	40	60
	Mycorrhizal P response (P inflow) %		
1.2	73 (± 13)[A]	95 (± 22)	552 (± 249)
1.4	81 (± 40)	110 (± 32)	337 (± 180)
1.6	67 (± 20)	100 (± 13)	184 (± 52)
	Mycorrhizal P response (P uptake) %		
1.2	32 (± 6)	90 (± 8)	195 (± 50)
1.4	39 (± 12)	112 (± 14)	155 (± 10)
1.6	29 (± 4)	88 (± 4)	134 (± 11)

[A] Standard error of the mean.

Irrespective of P application, shoot dry weights of mycorrhizal and non-mycorrhizal clover plants followed a similar pattern to that of total P uptake over the harvest periods (Table 6.4). Mycorrhizal growth response increased as the plants aged, although this increase was smaller with increasing soil compaction (Fig. 6.2). Addition of 15 mg P dm^{-3} to the P-deficient soil increased mycorrhizal growth response (Fig. 6.2).

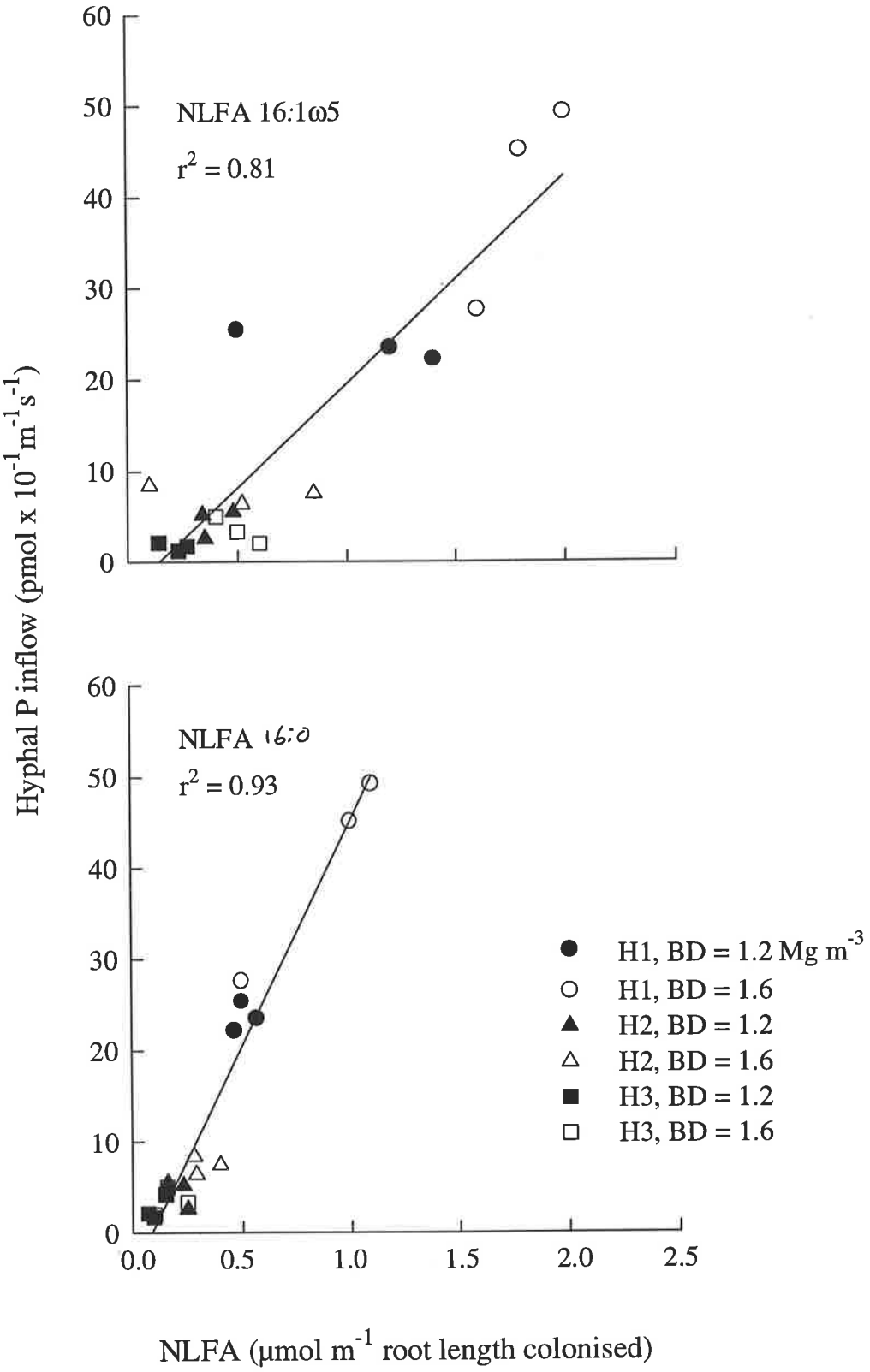

Figure 6.1 Relationship between hyphal biomass (as estimated with NLFA 16:1ω5 and 16:0) per unit root length colonised and hyphal P inflow at three harvests (H1, H1, H3) in slightly and highly compacted soil (BD = 1.2 and 1.6 Mg m^{-3} respectively), when the soil was supplied with 15 mg P dm^{-3} soil. Values were corrected for background NLFA. Values all three replicates are shown.

Root:shoot dry weight ratios of clover plants decreased as soil compaction increased at both levels of P application, irrespective of mycorrhizal colonisation (Table 6.5). No interaction was observed in root:shoot dry weight ratio between mycorrhizal and non-mycorrhizal plants.

Table 6.4 The effect of soil compaction on shoot dry weight and total P uptake by mycorrhizal (M) and non-mycorrhizal (NM) *Trifolium subterraneum* at 20, 40 and 60 days from planting.

Bulk density ($Mg\ m^{-3}$)	Mycorrhiza	Days from planting		
		20	40	60
		Shoot dry weight (mg plant^{-1})[A]		
1.2	M	20.0	88.0	193.2
	NM	21.0	57.2	93.5
1.4	M	18.0	62.5	131.7
	NM	19.3	43.5	70.2
1.6	M	17.2	53.2	100.5
	NM	18.8	38.8	56.5
Tukey's HSD (p = 0.05)		*24.8*		
		Total P uptake (μg plant^{-1})[A]		
1.2	M	66.1	228.0	384.8
	NM	52.1	118.7	137.7
1.4	M	54.5	159.2	159.2
	NM	42.2	79.4	79.4
1.6	M	55.1	130.2	187.7
	NM	44.3	71.5	80.0
Tukey's HSD (p = 0.05)		*74.7*		

[A] No interaction was observed between soil compaction and two levels of P application in their effects on shoot dry weight and total P uptake per plant at three harvest times, (see Table 6.2).

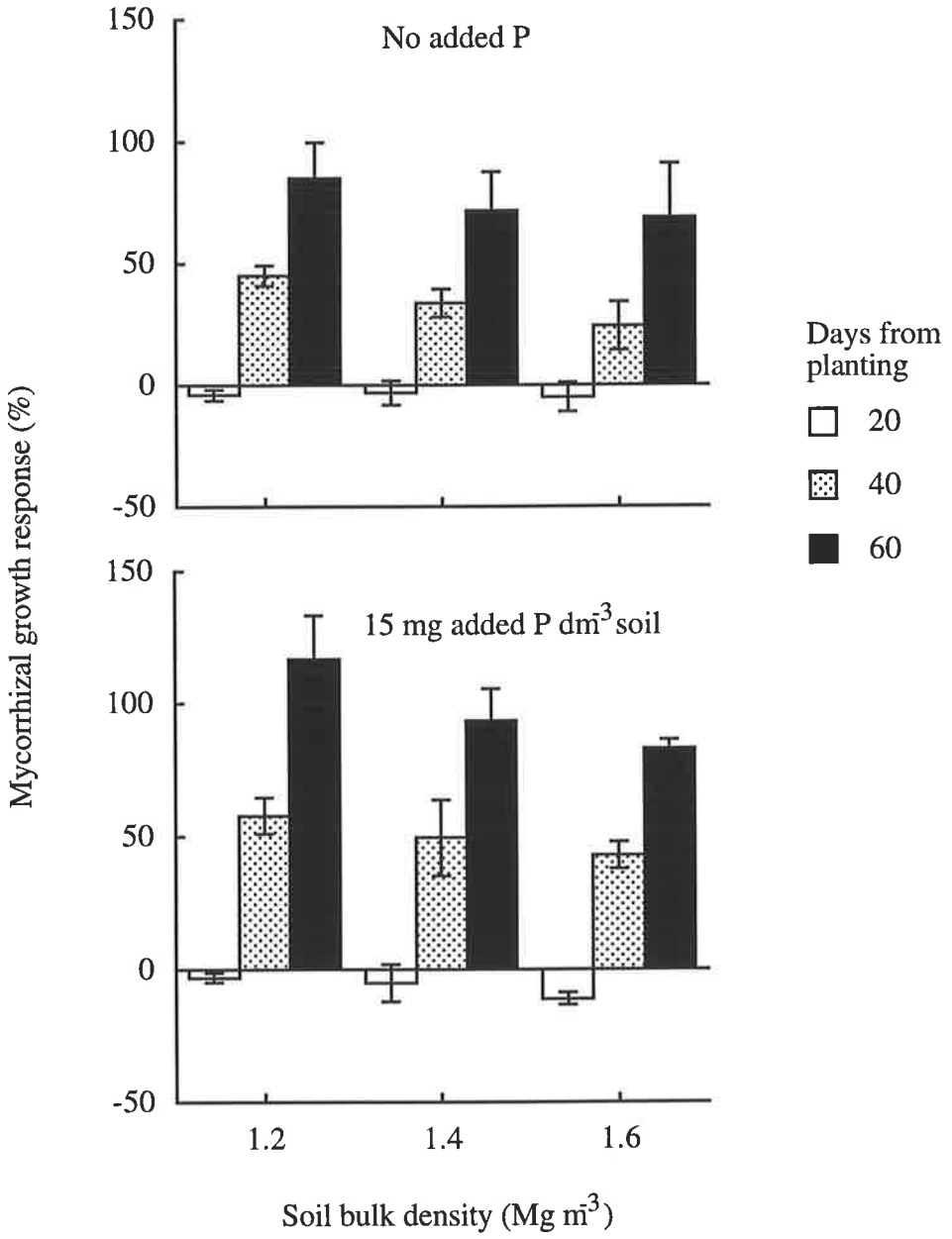

Figure 6.2 **Mycorrhizal growth response of *Trifolium subterraneum* as affected by soil compaction and P application at 20, 40 and 60 days from planting. Vertical bars represent standard errors of the means, n=3.**

The amounts of neutral lipid fatty acids (NLFA) 16:1ω5 and 16:0 (used as an estimate of hyphal biomass) per g soil were greater in inoculated soil than in non-inoculated soil (Table 6.6). The amounts of these two fatty acids decreased with increasing soil compaction, but increased with harvesting times. Despite the lower values of NLFA 16:1ω5 and 16:0 in compacted soil, the amounts of these two fatty acids per unit length of colonised root increased with increasing soil compaction (Fig. 6.3 and 6.4). Addition of 15 mg P dm^{-3} to the P-deficient soil resulted in an increase in hyphal biomass in soil (both NLFA 16:1ω5 and 16:0). The greatest amounts of NLFA 16:1ω5 and 16:0 per m colonised root were observed at the first harvest and they significantly decreased over the second and third harvests (Fig. 6.3 and 6.4). Note that the amount of NLFA presented in these two figures have been corrected for background by subtracting the amounts of NLFA in the non-inoculated control pots from those in the inoculated pots, assuming that background is the same in both inoculated and non- inoculated soils. A positive correlation was observed between NLFA 16:1ω5 and 16:0 extracted from the soil and total root length colonised over the harvest periods in slightly and highly compacted soil (Fig. 6.5).

Table 6.5 The effect of soil compaction on root:shoot dry weight ratios of *Trifolium subterraneum* at two levels of P application at 20, 40 and 60 days from planting[A].

Bulk density	Days from planting					
(Mg m^{-3})	20	40	60	20	40	60
	No added P			*15 mg added P dm^{-3}*		
1.2	0.56	0.61	0.62	0.55	0.57	0.60
1.4	0.31	0.59	0.56	0.36	0.42	0.41
1.6	0.30	0.46	0.49	0.33	0.35	0.41
Tukey's HSD (p = 0.05)			*0.12*			

[A] No interaction was observed between root:shoot dry weight ratios of mycorrhizal and non-mycorrhizal *Trifolium subterraneum* at different levels of soil compaction, P application and three harvest times.

Root colonisation can be expressed either as the total length of colonised root per plant (m mycorrhizal root) or the percentage of root length colonised by mycorrhizal fungi. Soil compaction had no significant effect on the percentage of root length colonised, whereas total root length colonised decreased with increasing soil compaction at 40 and 60 days from planting with 15 mg P dm^{-3} and at 60 days from planting with no added P (Table 6.7). Irrespective of soil compaction, fertilisation with $Na_2H_2PO_4$ increased the percentage of root length colonised (Table 6.7 and see Table 6.2 for interactions).

Table 6.6 The effect of soil compaction and P applied on neutral lipid fatty acids (NLFA) 16:1ω5 and 16:0 in inoculated (M) and non-inoculated (NM) soils at 20, 40 and 60 days from planting of *Trifolium subterraneum*.

Bulk density	Mycorrhiza	NLFA 16:1ω5 (nmol g^{-1} soil)			NLFA 16:0 (nmol g^{-1} soil)		
		Days from planting			Days from planting		
(Mg m^{-3})		20	40	60	20	40	60
				No added P			
1.2	M	6.4 (±0.8)[A]	8.3 (±0.8)	14.2 (±2.6)	4.2 (±0.4)	6.3 (±1.1)	10.2 (±1.7)
	NM	2.4 (±0.4)	3.5 (±0.5)	5.2 (±0.5)	2.0 (±0.2)	3.2 (±0.3)	5.5 (±0.6)
1.6	M	3.1 (±0.5)	5.5 (±0.7)	7.7 (±0.7)	2.2 (±0.3)	3.8 (±0.5)	5.3 (±0.7)
	NM	1.0 (±0.2)	2.7 (±0.4)	3.7 (±0.4)	1.1 (±0.2)	2.2 (±0.2)	3.1 (±0.5)
				15 mg added P dm^{-3}			
1.2	M	11.0 (±1.9)	19.9 (±2.6)	28.8 (±4.2)	6.1 (±0.4)	11.9 (±1.1)	16.3 (±0.9)
	NM	3.9 (±0.5)	5.2 (±0.4)	8.4 (±0.4)	2.9 (±0.1)	4.0 (±0.2)	6.7 (±0.5)
1.6	M	6.6 (±0.8)	11.3 (±1.2)	16.1 (±2.1)	3.7 (±0.4)	5.7 (±0.5)	7.2 (±1.1)
	NM	2.1 (±0.3)	3.5 (±0.3)	5.3 (±0.7)	1.5 (±0.1)	2.5 (±0.3)	3.5 (±0.1)

[A] Standard errors of the mean.

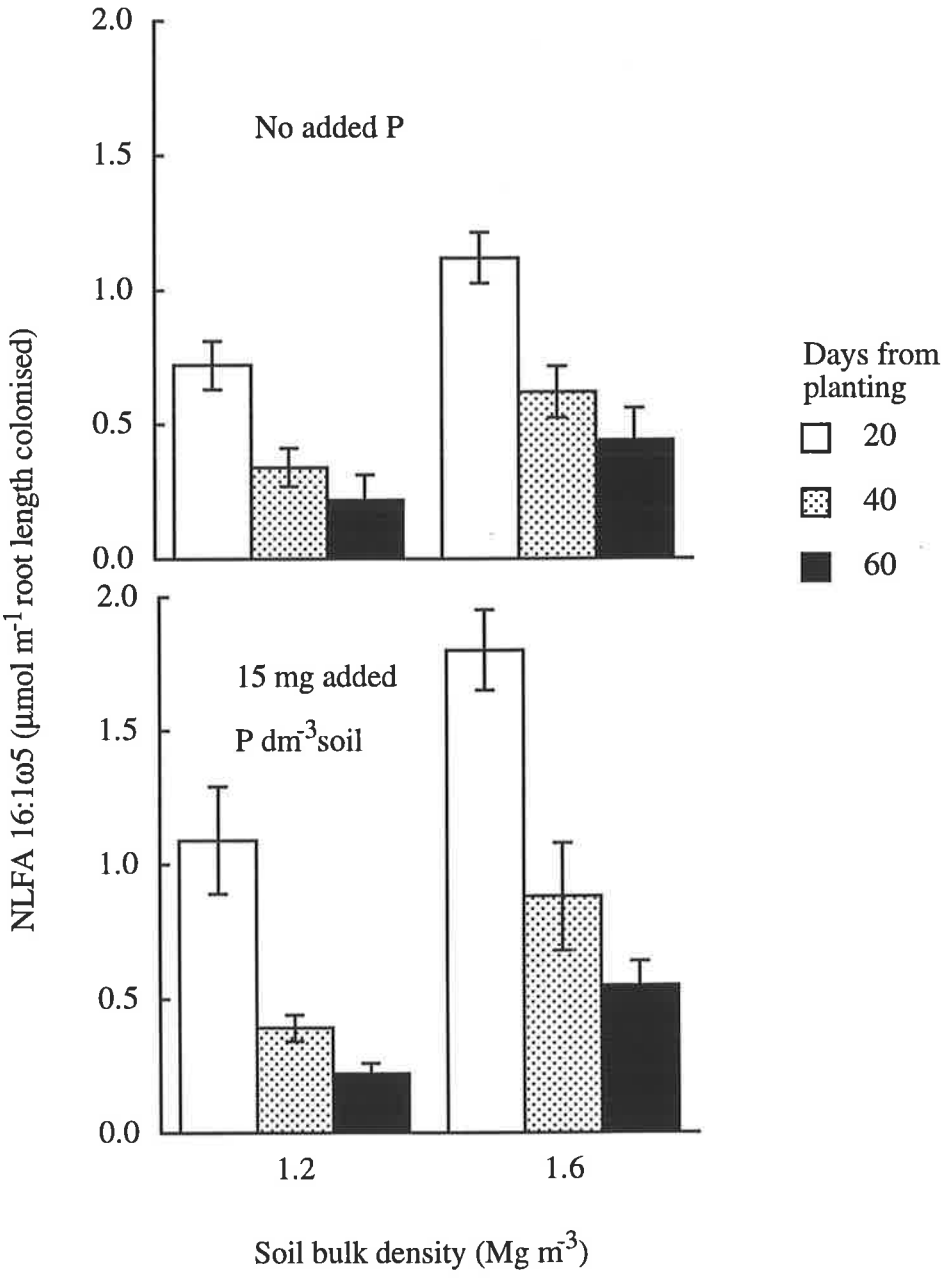

Figure 6.3 The amount of hyphal biomass (as estimated with NLFA 16:1ω5) per unit length of colonised root of *Trifolium subterraneum* as affected by soil compaction and P applied at 20, 40 and 60 days from planting. Values were corrected for background NLFA. Vertical bars represent standard errors of the means, n=3.

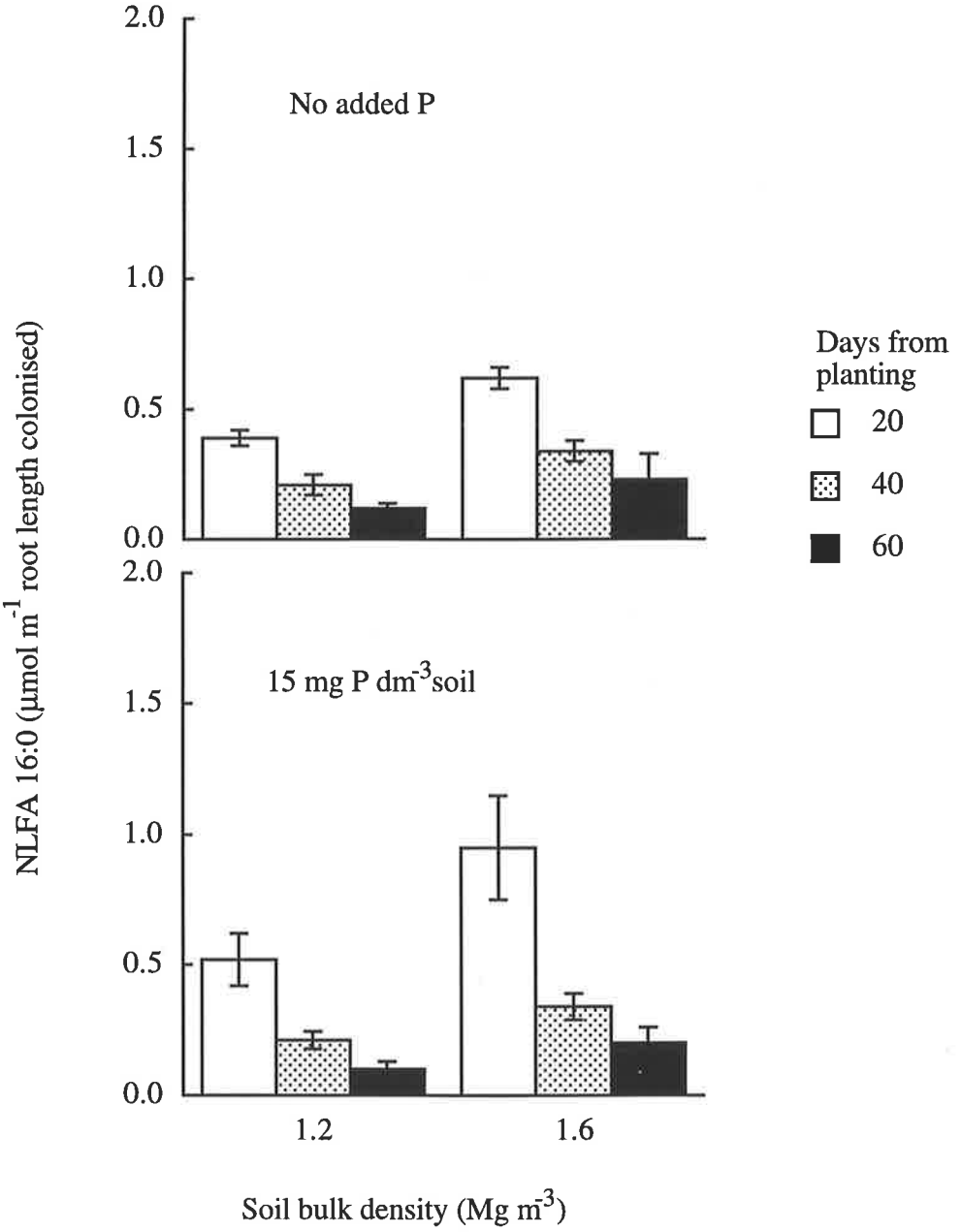

Figure 6.4 **The amount of hyphal biomass (as estimated with NLFA 16:0) per unit length of colonised root of *Trifolium subterraneum* as affected by soil compaction and P applied at 20, 40 and 60 days from planting. Values were corrected for background NLFA. Vertical bars represent standard errors of the means, n=3.**

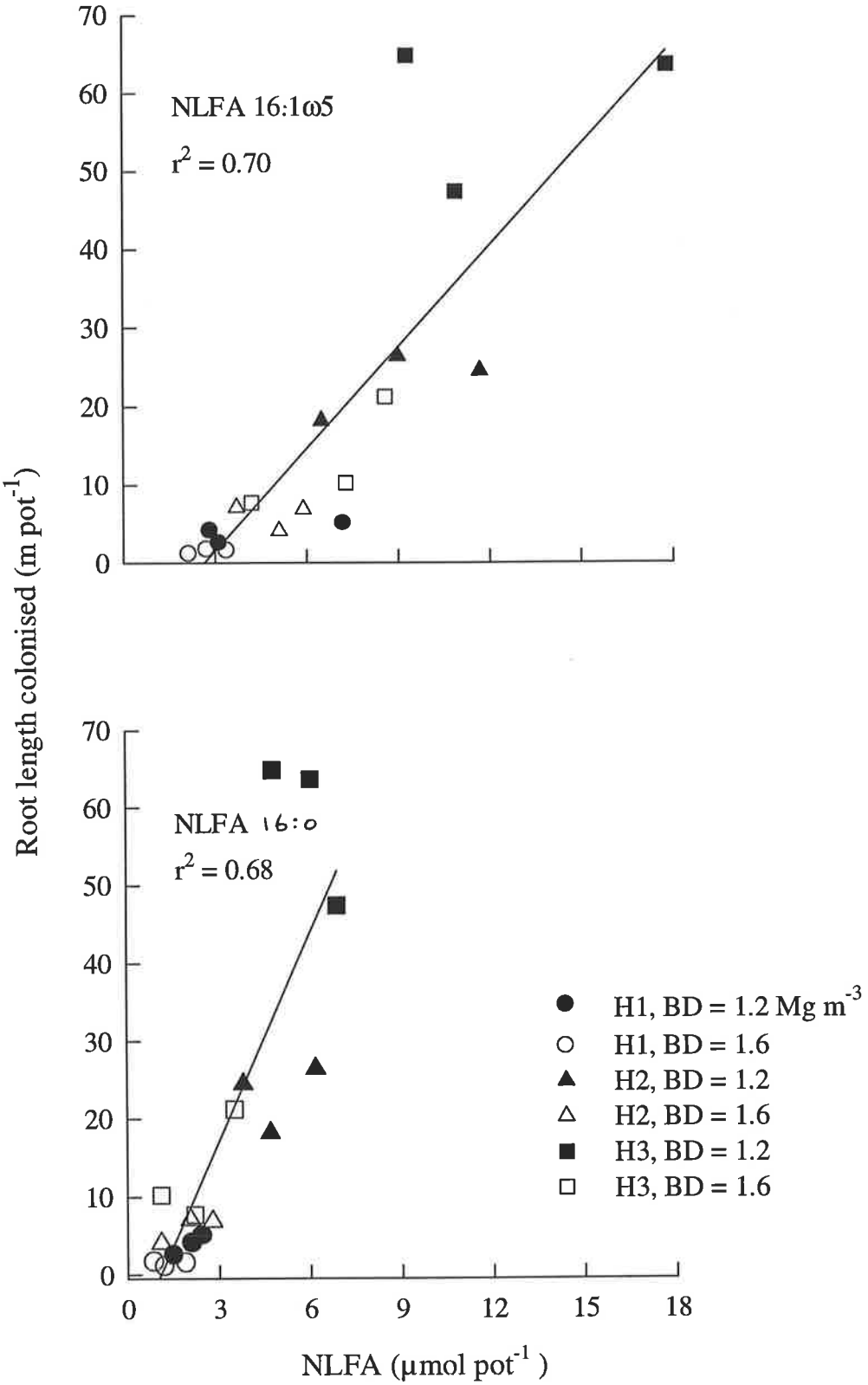

Figure 6.5 Relationship between hyphal biomass (as estimated with NLFA 16:1ω5 and 16:0) per pot and total root length colonised of *Trifolium subterraneum* at three harvests in slightly and highly compacted soil as described in Fig. 6.1. Values were corrected for background NLFA. Values all three replicates are shown.

Fig. 6.6 indicates volumetric water contents of the soil at matric potentials between -33 and -100 kPa over the range of soil compactions encountered.

Soil compaction led to the collapse of most large pores (Fig. 6.7). In slightly compacted soil (bulk density = 1.2 Mg m^{-3}), 15% of the total porosity resided in pores of 100-300 μm diameters, whereas in highly compacted soil (bulk density = 1.6 Mg m^{-3}) this percentage decreased to 5.4%. In highly compacted soil, 28% of the total pore space comprised pores larger than 3 μm diameter, whereas the value was 68% in slightly compacted soil.

Table 6.7 Percentage of root colonised and total root length colonised as affected by soil compaction and P application at 20, 40 and 60 days from planting.

P applied (mg dm^{-3})	Bulk density (Mg m^{-3})	Days from planting 20	40	60
		Root length colonised (m $plant^{-1}$)		
0	1.2	0.59 (± 0.12)[A]	1.51 (± 0.13)	4.40 (± 0.49)
	1.4	0.32 (± 0.07)	0.87 (± 0.07)	2.21 (± 0.40)
	1.6	0.19 (± 0.02)	0.46 (± 0.07)	1.00 (± 0.15)
15	1.2	0.66 (± 0.12)	3.39 (± 0.43)	9.77 (± 0.93)
	1.4	0.44 (± 0.12)	1.87 (± 0.34)	4.58 (± 0.69)
	1.6	0.25 (± 0.03)	0.89 (± 0.15)	2.17 (± 0.69)
Tukey's HSD (p = 0.05)			*1.87*	
		Root length colonised (%)		
0	1.2	28.1 (± 2.8)[A]	33.0 (± 1.7)	40.7 (± 3.7)
	1.4	29.3 (± 3.7)	34.2 (± 4.6)	43.1 (± 5.1)
	1.4	29.7 (± 3.6)	33.4 (± 5.9)	39.6 (± 2.7)
15	1.2	29.8 (± 3.1)	37.7 (± 4.1)	49.0 (± 3.7)
	1.4	35.6 (± 3.4)	39.6 (± 4.3)	54.7 (± 7.7)
	1.6	32.1 (± 3.3)	40.9 (± 6.7)	51.7 (± 9.4)

[A] Standard error of the mean.

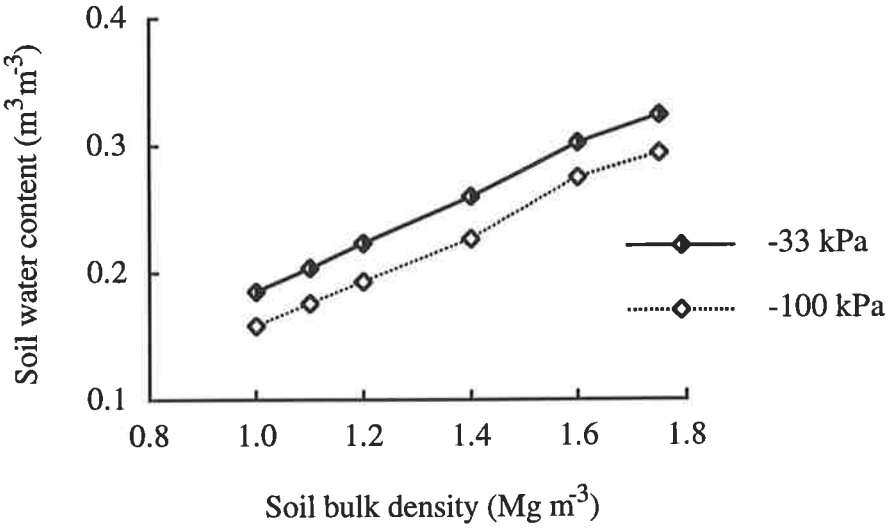

Figure 6.6 Relation of volumetric water content to the bulk density of the soil at water potentials of -33 and -100 kPa.

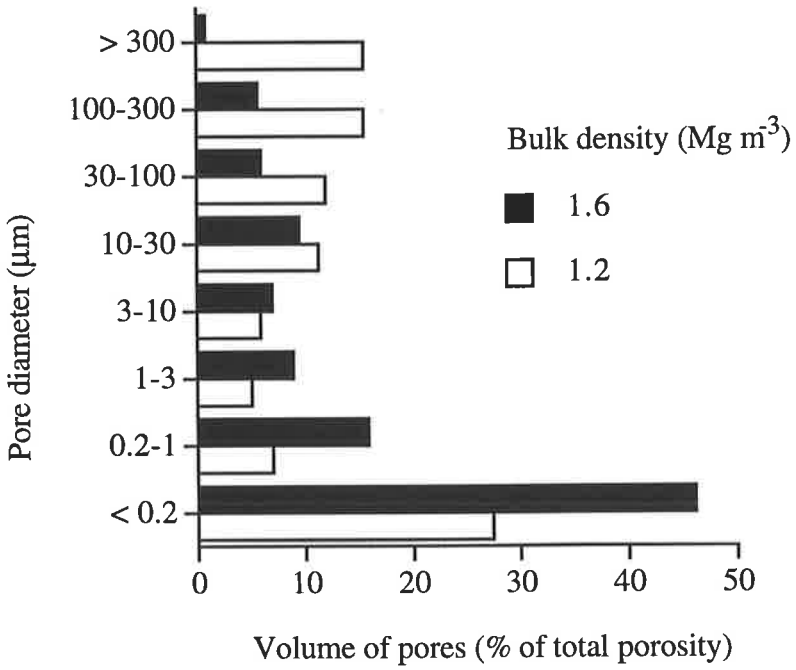

Figure 6.7 Pore size distribution in slightly and highly compacted soil (bulk density = 1.2 and 1.6 Mg m^{-3}, respectively).

6.4 Discussion

The effect of soil compaction on increasing root diameter and decreasing root length (reported in Chapter 4 and 5) increased P inflow per unit length of root in clover plants. Soil compaction has also been shown (this chapter) to increase the amount of hyphal biomass (as estimated with NLFA 16:1ω5 and 16:0 extracted from the soil) produced per unit root length colonised (Fig 6.3 and 6.4). and this, in turn, may affect the inflow of P to mycorrhizal clover roots.

Results of the effects of soil compaction on P inflow to non-mycorrhizal clover in this investigation confirm previous work. Irrespective of mycorrhizal colonisation, increased P inflow to clover roots in compacted soil may be attributed, in part, to the increase in volumetric water content (Fig. 6.6) which would increase P diffusion coefficient (Cox and Barber, 1992). Hoffmann and Jungk (1995) reported that compaction of the soil from a bulk density of 1.30 to 1.65 Mg m^{-3} increased the effective P diffusion coefficient by increasing volumetric water content.

P inflow would also be expected to increase with soil compaction due to the increase in root diameter of *Trifolium subterraneum*, as observed in the previous work under similar experimental conditions (Chapter 4). Thick roots present a greater surface area per unit length and have a greater area of plasma membrane for absorption within the cortex per unit area of root surface than thin roots. Hoffmann and Jungk (1995) found that P influx to mechanically impeded roots of sugar beet (*Beta vulgaris* cv Bravo), with bigger root diameters, was higher than P influx to unimpeded roots, and Peterson and Barber (1981) found that influx increased with increasing root diameter. An increase in P uptake per unit length of ryegrass roots due to the increase in root diameter in compacted soil has also been reported (Cornish *et al.*, 1984).

Irrespective of mycorrhizal colonisation, increased P inflow with increasing soil compaction might be associated with a decline in root:shoot dry weight ratios (Table 6.5). Decreases in such ratios of subterranean clover with improved P nutrition have been reported previously (Sanders *et al.*, 1977; Smith, 1982).

Irrespective of soil compaction, mycorrhizal colonisation increased P inflow, particularly when the P deficient soil was supplied with 15 mg P dm^{-3} soil. The values of P inflow to non-mycorrhizal and mycorrhizal plants during the first harvest period (0-20 days) in slightly compacted soil (bulk density = 1.2 Mg m^{-3}) with 15 mg P dm^{-3} soil (3.5 and 5.8 pmol m^{-1} s^{-1}, respectively) are comparable with the P inflows to control and mycorrhizal *T. subterraneum* (2.7 and 4.6 pmol m^{-1} s^{-1}, respectively) observed by Smith (1982) when the plants were harvested 22 days after planting.

Despite the low percentage of root length colonised, the inflow of P was higher at the first harvest than at the second and third harvest at both soil P contents. Similar declines in P inflow with plant age have been reported elsewhere (Smith, 1982; Jakobsen *et al.*, 1992a; Pearson and Schweiger, 1993; Smith *et al.*, 1994b; Sukarno *et al.*, 1996). Several factors may have contributed to these observations, although their relative importance is not clear. One explanation for the higher P inflow at the first harvest might be due to high formation of external hyphae (as estimated with NLFA 16:1ω5 and 16:0 extracted from the soil) per unit length of colonised root (see below) and hence more effective exploitation of soil P by the external mycelium. Another possible explanation is that the depletion of P from the soil is relatively small during early growth when the density of young roots is low and external hyphae may confer a greater advantage in terms of P inflow to colonised roots than at later time when rooting density and inter-root competition is greater. Moreover, it has been shown that in both *Trifolium subterraneum* and *Allium cepa*, the proportion of the root

colonised by arbuscules declined as mycorrhizal roots aged (Smith and Dickson, 1991) and this may influence the transfer of P from fungus to plant.

Although soil compaction significantly decreased hyphal biomass (as estimated with NLFA 16:1ω5 and 16:0) per unit mass of soil, P inflow did not decreased with increasing soil compaction. This might be due to the increase in the amount of hyphal biomass per unit root length colonised with increasing soil compaction, particularly at the first harvest. The positive correlation observed between the hyphal biomass per unit length of colonised root and P inflow at different harvest times and soil bulk densities (Fig. 6.1), suggests that the ratio of hyphal biomass to root length colonised may be more important for P inflow than the total amount of external hyphae or total root length colonised. Sanders *et al.*, (1977) found that the similarity of hyphal inflows of three different VAM mycorrhizal fungi was matched by the equal amounts of external hyphae produced per unit length of colonised root. However, interpretation of data on the basis of the relationship between NLFA and root colonisation needs consideration of the methods used. NLFA methods may give estimates of living fungal biomass in soil, whereas, trypan blue stains all mycelium whether living and dead. It is likely that progressive death of internal fungal structures occurred during the experiment (Smith and Dickson, 1991; Tisserant *et al.*, 1993) so that the amount of living intra-radical fungus would have been overestimated in older plants.

P inflow via hyphae was calculated by subtracting P inflow to mycorrhizal roots from P inflow to non-mycorrhizal roots (Sukarno *et al.*, 1996). This method of calculating inflow assumes that the roots themselves, whether colonised or not, absorb P at the same rate (Jakobsen, 1994). This assumption is difficult to test. Pearson and Jakobsen (1993) used a compartmented system to investigate the relative contribution of hyphae and roots to P uptake by mycorrhizal plants. Some of their results suggested that presence of the fungus altered the ability of the root to absorb P, but the results may have been influenced by the

size of the soil compartments and hence total amount of P available. Ability of roots to absorb P might also be affected by the P status of the plant. Setting aside these problems, the method used here might provide a satisfactory method of comparing the effects of soil compaction on P inflow via hyphae with different P treatments.

Diminished mycorrhizal growth response with increasing soil compaction observed in this experiment at the third harvest and in previous experiments might be due, at least in part, to the decline in mycorrhizal response in terms of P inflow with increasing soil compaction which was apparent at the third harvest (Table 6.3). This decline is matched by a significant decline in hyphal biomass (as estimated with NLFA) per unit root length colonised with increasing time (Fig. 6.3 and 6.4).

Despite the importance of external hyphae in improving mineral nutrition of the host plant, reliable methods for the assessment of external hyphae in soil have not been available (see Chapter 2). In this study, the amounts of fatty acids 16:1ω5 and 16:0, which have been used as indicators of fungal biomass of VAM fungi (Pacovsky and Fuller, 1988; Pacovsky, 1989; Olsson *et al.*, 1995) were measured at two P applications. The amounts of phospholipid fatty acids (PLFA) 16:1ω5 and 16:0 were either undetectable or present in very small and inconsistent amounts in inoculated soil. Olsson *et al.* (1995) found that both PLFA and NLFA 16:1ω5 and 16:0 were higher in soil inoculated with *Glomus caledonium* than in non-inoculated soil, although the amount of NLFA 16:1ω5 extracted from inoculated soil was 25 times that of PLFA 16:1ω5. Since neutral lipids are the major storage compounds in mycorrhizal fungi, NLFA 16:1ω5 and 16:0 extracted from the soil includes neutral lipids present in both hyphae and spores. In this study the amounts of neutral lipids in spores were not measured, nor was the production of spores monitored. Therefore, although the amounts of NLFA 16:1ω5 and 16:0 have been used here as

estimates of hyphal biomass, future work should determine the possible contribution of lipids in spores to the values obtained.

The amounts of NLFA 16:1ω5 and 16:0 in inoculated soil were considerably greater than in non-inoculated soil (Table 6.6). The increased NLFA 16:1ω5 and 16:0 in both slightly and highly compacted soil as plants aged is matched by the increase in the total root length colonised over the harvest periods and resulted to a positive correlation between the amount of external hyphae and root length colonised (Fig. 6.5). An increase in the amount of external hyphae (measured using the membrane filter technique and trypan blue staining) with increasing time over the harvest periods has also been reported (Abbott and Robson, 1985a; Jakobsen *et al.*, 1992a).

Decreases in the amount of external hyphae per g soil (see Table 6.6) in compacted soil might be due to a significant decrease in the percentage of pores larger than 3 μm from 68% in slightly compacted soil to 28% in highly compacted soil (Fig. 6.7). These are pores that may be able to be colonised by hyphae. Moreover, poor aeration (decreased O_2 and increased CO_2 in the soil air) reported in Chapter 4 may also be involved in the decline in the amount of external hyphae in the soil. A similar decrease in the length of living external hyphae of VAM and non-VAM fungi (measured with acid fuchsin and fluorescein diacetate) with increasing soil compaction was also observed in the previous work (Chapter 5).

6.5 Conclusion

The results of this experiment indicated that P inflow was higher to impeded roots than to unimpeded roots, and this observation is consistent with the increased P uptake per unit root length of both mycorrhizal and non-mycorrhizal plants with increasing soil compaction observed in Chapter 5. Since mycorrhizal fungi tended to increase the efficiency of roots in P uptake per unit root length per unit time, it was concluded that P inflow had no role in the

decline in benefit of mycorrhizal colonisation in terms of plant growth and total P uptake per plant with increasing soil compaction. With respect to the results presented in this and previous chapters, the decline in mycorrhizal response in terms of plant growth and P uptake per plant with increasing soil compaction was attributed mainly to the significant decrease in total root length colonised and also to the decrease in the amount of hyphal biomass (NLFA 16:1ω5 and 16:0) per pot observed in compacted soil.

CHAPTER 7

EFFECT OF SOIL COMPACTION ON P UPTAKE AND GROWTH OF *TRIFOLIUM SUBTERRANEUM* COLONISED BY FOUR SPECIES OF VAM FUNGI

7.1 Introduction

The results presented in previous chapters indicated that *Glomus intraradices* Schenck & Smith significantly increased P uptake and shoot dry weight of *Trifolium subterraneum* in compacted soil, although mycorrhizal growth response decreased with increasing soil compaction. The amount of external hyphae in soil was assessed using ester-linked fatty acid analysis. The amount of hyphal biomass in soil, estimated in terms of the amount of neutral lipid fatty acids 16:1ω5 and 16:0 per g soil, also decreased with increasing soil compaction.

Changes in soil physical properties caused by soil compaction, such as poor soil aeration resulting from collapse of most large pores may differently affect the development of mycorrhizal colonisation by different species of VAM fungi. For example, Saif (1983) found different species of VAM fungi were different in their responses to changing concentrations of O_2 in the air of non-compacted soil. Furthermore, since species of VAM fungi have different hyphal diameters, their ability to penetrate small pores which occur in compacted soil may vary considerably. There have been no reports of how soil compaction may differently affect P uptake and the development of mycorrhizal colonisation of different species of VAM fungi. Hence, the objective of this chapter was to compare the mycorrhizal growth responses of four species of VAM fungi to different levels of soil compaction.

It has been shown that mycorrhizal colonisation also increases uptake of some micro-nutrients such as Zn and Cu (*e.g.* Li *et al.*, 1991b; Burkert and Robson, 1994). In this experiment, the effect of mycorrhizal colonisation on uptake of Zn, Cu and Mn by *T. subterraneum* in compacted and uncompacted soil was also considered.

7.2 Materials and Methods

This experiment had a randomised complete block design with 40 treatments (5 mycorrhiza, 4 compaction levels and 2 P levels) in factorial combination and three replicates.

7.2.1 Adjustment of soil pH

Results of a preliminary experiment indicated that the percentage of clover roots colonised by *Glomus sp.* City Beach and *G. mosseae* and the mycorrhizal growth response of *T. subterraneum* were increased by increasing soil pH from 6.6 (initial pH of the soil, see Table 3.1) to 7.3 by adding lime. In this experiment, finely powdered $CaCO_3$ was added and mixed throughout the soil at a rate of 1.5 g kg^{-1} soil, giving a soil pH of 7.3 (1:5 soil:water).

7.2.2 Soil compaction

The soil mix was autoclaved and thoroughly mixed with basal nutrients (see Chapter 3) and P fertiliser at rates of 0 or 15 mg P dm^{-3}. The soil was compacted into PVC pots to bulk densities of 1.20, 1.40, 1.60 and 1.75 Mg m^{-3}. Penetrometer resistances of the soil at these bulk densities were 1.3, 2.3, 3.5 and 4.1 MPa, respectively.

7.2.3 Plant material and growth conditions

Seeds of clover (*T. subterraneum*) were sterilised and germinated on filter paper wetted with sterile water. Inoculum was obtained from pot cultures of *T. subterraneum* grown for

2 months in a soil:sand mix (1:9) containing 10% of a dry inoculum from pot cultures of *Glomus intraradices* (originally provided by NPI, Utah), *Glomus sp.* City Beach WUM 16, *Glomus etunicatum* UT 316 A-2 and *Glomus mosseae* NBR 4-1. The roots were washed with deionised water, cut into segments about 1 cm long, thoroughly mixed and used as fresh inoculum. The percentages of root length of pot-culture plants colonised by *G. intraradices, Glomus sp.* City Beach, *G. etunicatum* and *G. mosseae* were 86, 78, 91 and 61, respectively. An attempt was made to standardise inoculum in the experiment. For the mycorrhizal treatments except *G. mosseae*, each seedling was inoculated by placing 0.25 g fresh inoculum in each planting hole. For *G. mosseae* 0.35 g fresh inoculum was used because of the lower percentage of colonisation than the other three fungi. Thus the weights of colonised root used as inoculum for the four fungi were 0.22, 0.20, 0.23 and 0.21 g, respectively. Seedlings for the non-mycorrhizal treatments received equivalent weights of non-colonised clover roots. Each seedling received 0.5 cm^3 of a dense suspension of *Rhizobium leguminosarum* biovar *trifolii*. Five clover seedlings were transplanted into each pot and grown in a growth room where the photoperiod was 16 hours and the irradiance was 350 $\mu mol\ m^{-2}\ s^{-1}$. The day and night air temperatures were 20 and 15°C, respectively. The surface of the soil was covered with a 10 mm deep layer of white polythene beads to minimise evaporation and control the growth of algae.

7.2.4 Measurements

Shoots were harvested after 8 weeks and weighed after drying at 70°C for 2 days. The roots were carefully washed with deionised water to remove soil, cut into 1 cm segments and thoroughly mixed. Sub-samples of roots were taken for determination of root length, mycorrhizal colonisation and dry weight. Dried ground shoots and roots were digested in HNO_3 based on the method of Zarcinas *et al.* (1987) for analysis with an Inductively

Coupled Plasma (ICP) Spectrometer for P and the micro-nutrients Zn, Cu and Mn. Lipid extraction and fatty acid analysis were carried to estimate the amount of external hyphae in soil as described in Chapter 6.

Mycorrhizal response in terms of P uptake per plant was calculated by the equation (*b*) presented in Chapter 6 (Section 6.2.2).

Data were analysed with Genstat (Genstat 5 Committee, 1987). Multiple linear comparisons between means were made with Tukey's honestly significant difference statistic (Zar, 1984).

7.3 Results

There was no interaction between soil compaction and P application in their effect on the percentage of root length colonised (see Table 7.1 for interactions). Irrespective of P application, no significant difference was observed in the percentage of root length colonised by the four species of VAM fungi in slightly compacted soil (bulk density = 1.20 Mg m^{-3}). However, soil compaction up to the bulk density of 1.60 Mg m^{-3} differently affected the percentage of root length colonised by the different mycorrhizal fungi (Fig. 7.1). Soil compaction had no significant effect on the percentage of root length colonised by *G. sp.* City Beach and *G. intraradices* up to a bulk density of 1.60 Mg m^{-3}, whereas percentage of colonisation by *G. etunicatum* and *G. mosseae* decreased as soil compaction increased (Fig. 7.1). At a bulk density of 1.75 Mg m^{-3}, the percentage of root length colonised decreased to about 15% for all four fungi at both levels of soil P. This resulted in the lack of mycorrhizal growth response to all the four species of fungi (Fig. 7.2).

Table 7.1 Levels of significance for shoot and root dry weights of *Trifolium subterraneum*, tissue P, Zn, Cu and Mn concentrations, mycorrhizal colonisation and NLFA 16:1ω5 and 16:0 extracted from soil, used as estimate of hyphal biomass in soil.

Source of variance	Compaction (C)	Mycorrhiza (M)	Phosphorus (P)	C × M	C × P	M × P	C × M × P
df	3	4	1	12	3	4	12
Shoot dry weight	***[A]	***	***	***	***	***	n.s.[B]
Root dry weight	***	***	***	***	***	***	n.s.
Mycorrhizal growth response (%)	***	***[C]	n.s.	**	n.s.	**	n.s.
Mycorrhizal P response (%)	***	***	n.s.	***	n.s.	*	n.s.
Root length colonised (%)	***	***	n.s.	**	n.s.	**	n.s.
Shoot P concentration	***	***	***	***	***	n.s.	**
Root P concentration	***	***	***	***	*	**	n.s.
Shoot Zn concentration	***	***	-	*	-	-	-
Root Zn concentration	***	***	-	***	-	-	-
Shoot Cu concentration	n.s.	n.s.	-	n.s.	-	-	-
Root Cu concentration	***	***	-	***	-	-	-
Shoot Mn concentration	***	***	-	***	-	-	-
Root Mn concentration	***	***	-	***	-	-	-
NLFA 16:1ω5 nmol g^{-1} soil	***	***	–	***	-	-	-
NLFA 16:0 nmol g^{-1} soil	***	***	–	***	-	-	-

[A] *, **, *** significant at the 0.05, 0.01, 0.001 probability level, respectively.
[B] n.s. = not significant at p = 0.05.

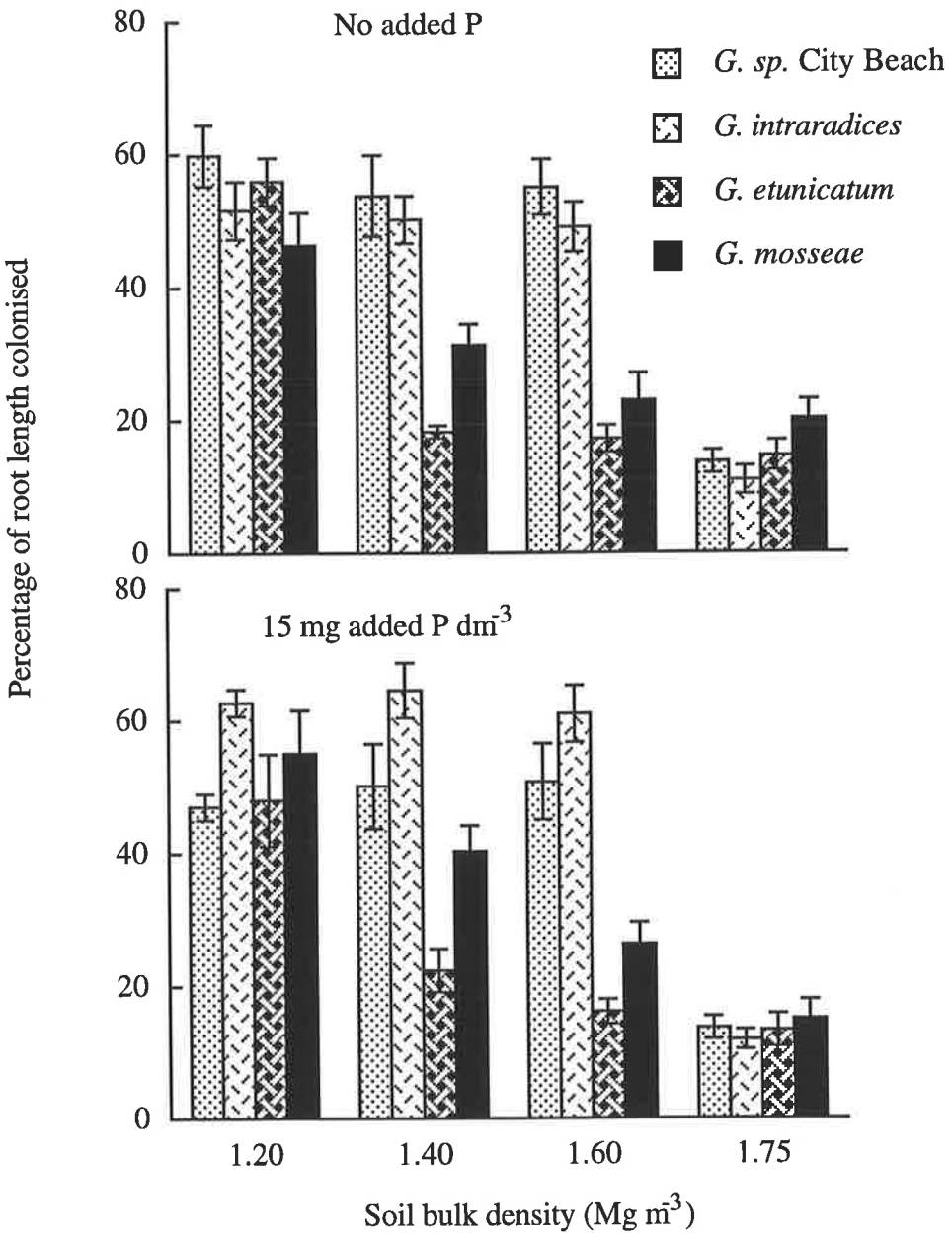

Figure 7.1 The percentage of root length colonised of *Trifolium subterraneum* by four species of VAM fungi as affected by soil compaction and P application. Vertical bars represent standard errors of the means, n=3.

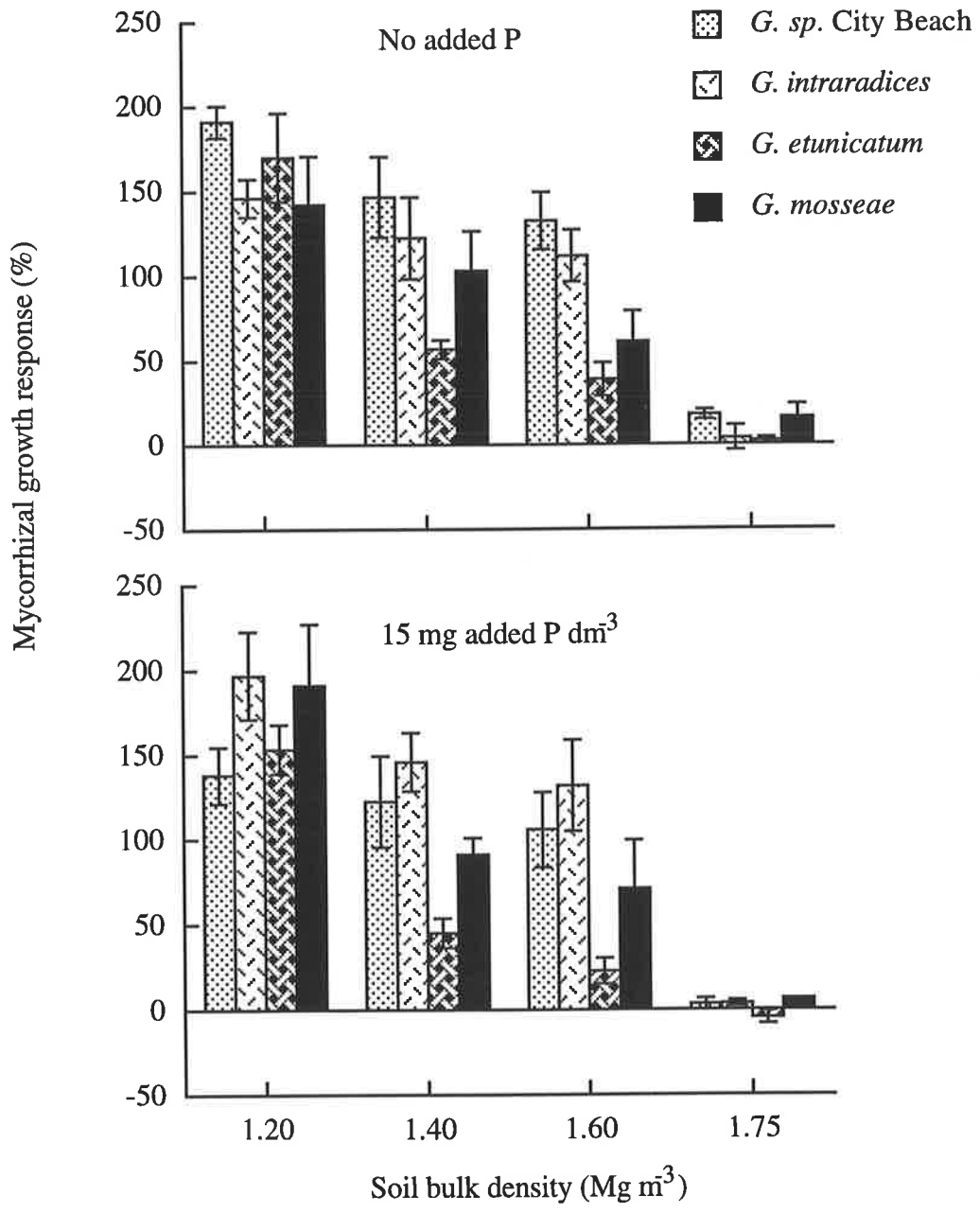

Figure 7.2 Mycorrhizal growth response of *Trifolium subterraneum* in association with four species of VAM fungi as affected by soil compaction and P application. Vertical bars represent standard errors of the means, n=3.

Irrespective of P application, shoot dry weights of clover plants colonised by *G. sp.* City Beach and *G. intraradices* were greater than those of non-mycorrhizal clover plants up to the bulk density of 1.60 Mg m^{-3}, whereas colonisation by *G. etunicatum* and *G. mosseae* increased shoot dry weight of clover plants only at bulk densities of 1.20 and 1.40 Mg m^{-3}, respectively (Table 7.2).

Root dry weights of both mycorrhizal and non-mycorrhizal clover plants were also decreased by soil compaction, although root dry weights of clover plants colonised by *G. sp.* City Beach, *G. intraradices* and *G. mosseae* were greater than those of non-mycorrhizal roots only up to a bulk density of 1.40 Mg m^{-3} (Table 7.2).

Table 7.2 Shoot and root dry weights of *Trifolium subterraneum* with non-mycorrhizal (NM) and mycorrhizal fungi as affected by soil compaction.

Bulk density (Mg m^{-3})	NM	*G. sp.* City Beach	*G. intraradices*	*G. etunicatum*	*G. mosseae*
			Shoot dry weight (g pot^{-1})		
1.20	0.36	0.91	1.01	0.92	0.99
1.40	0.31	0.70	0.73	0.45	0.60
1.60	0.22	0.48	0.50	0.28	0.37
1.75	0.21	0.22	0.22	0.21	0.23
Tukey's HSD (p = 0.05)			*0.24*		
			Root dry weight (g pot^{-1})		
1.20	0.24	0.47	0.53	0.46	0.53
1.40	0.15	0.27	0.31	0.23	0.25
1.60	0.10	0.18	0.19	0.14	0.17
1.75	0.09	0.10	0.09	0.09	0.09
Tukey's HSD (p = 0.05)			*0.10*		

[A] No interaction was observed between soil compaction, P application and mycorrhiza (see Table 7.1).

The amounts of NLFA 16:1ω5 and 16:0 extracted from the soil, which were used as indicators of hyphal biomass, were measured in the soil supplied with 15 mg P dm^{-3}. In slightly compacted soil, there was a significant difference in the amounts of NLFA 16:1ω5 and 16:0 between pots containing inoculated plants and non-inoculated plants (Table 7.3). The amounts of these two fatty acids in the soil inoculated with *G. etunicatum* either per unit mass of soil (Table 7.3) or per unit root length colonised (Fig. 7.3) were much higher than in the soil inoculated with the three other mycorrhizal fungi used in this experiment. Soil compaction decreased the amounts of NLFA 16:1ω5 and 16:0 extracted from the soil. The decreases were more pronounced in the soil inoculated with *G. etunicatum* and *G. mosseae* than in the soil inoculated with *G. sp.* City Beach and *G. intraradices* (Table 7.3).

Table 7.3 The amounts of NLFA 16:1ω5 and 16:0 in soil inoculated by four species of VAM fungi and in non-inoculated soil (NM) in association with *Trifolium subterraneum* as affected by soil compaction when the soil was supplied with 15 mg P dm^{-3}.

Bulk density ($Mg\ m^{-3}$)	NM	*G. sp* City Beach	*G. intraradices*	*G. etunicatum*	*G. mosseae*
			NLFA 16:1ω5 (nmol g^{-1} soil)		
1.20	7.3 (±0.6)	25.8 (±2.7)	33.0 (±3.4)	46.5 (±2.5)	29.9 (±3.9)
1.40	ND	ND[A]	ND	ND	ND
1.60	5.0 (±0.6)	14.4 (±1.4)	17.4 (±2.9)	6.7 (±0.6)	8.7 (±0.7)
1.75	4.2 (±0.7)	4.4 (±0.8)	4.0 (±0.6)	3.9 (±0.6)	4.0 (±0.6)
Tukey's HSD (p = 0.05)			*8.23*		
			NLFA 16:0 (nmol g^{-1} soil)		
1.20	3.3 (±0.4)	8.3 (±0.6)	9.9 (±0.9)	14.4 (±1.3)	8.3 (±0.7)
1.40	ND	ND	ND	ND	ND
1.60	2.6 (±0.4)	4.6 (±0.4)	6.1 (±0.7)	3.0 (±0.5)	3.8 (±0.6)
1.75	0.9 (±0.1)	0.9 (±.0.1)	0.9 (±0.1)	0.8 (±0.0)	0.9 (±0.1)
Tukey's HSD (p = 0.05)			*3.01*		

[A] ND, not determined.

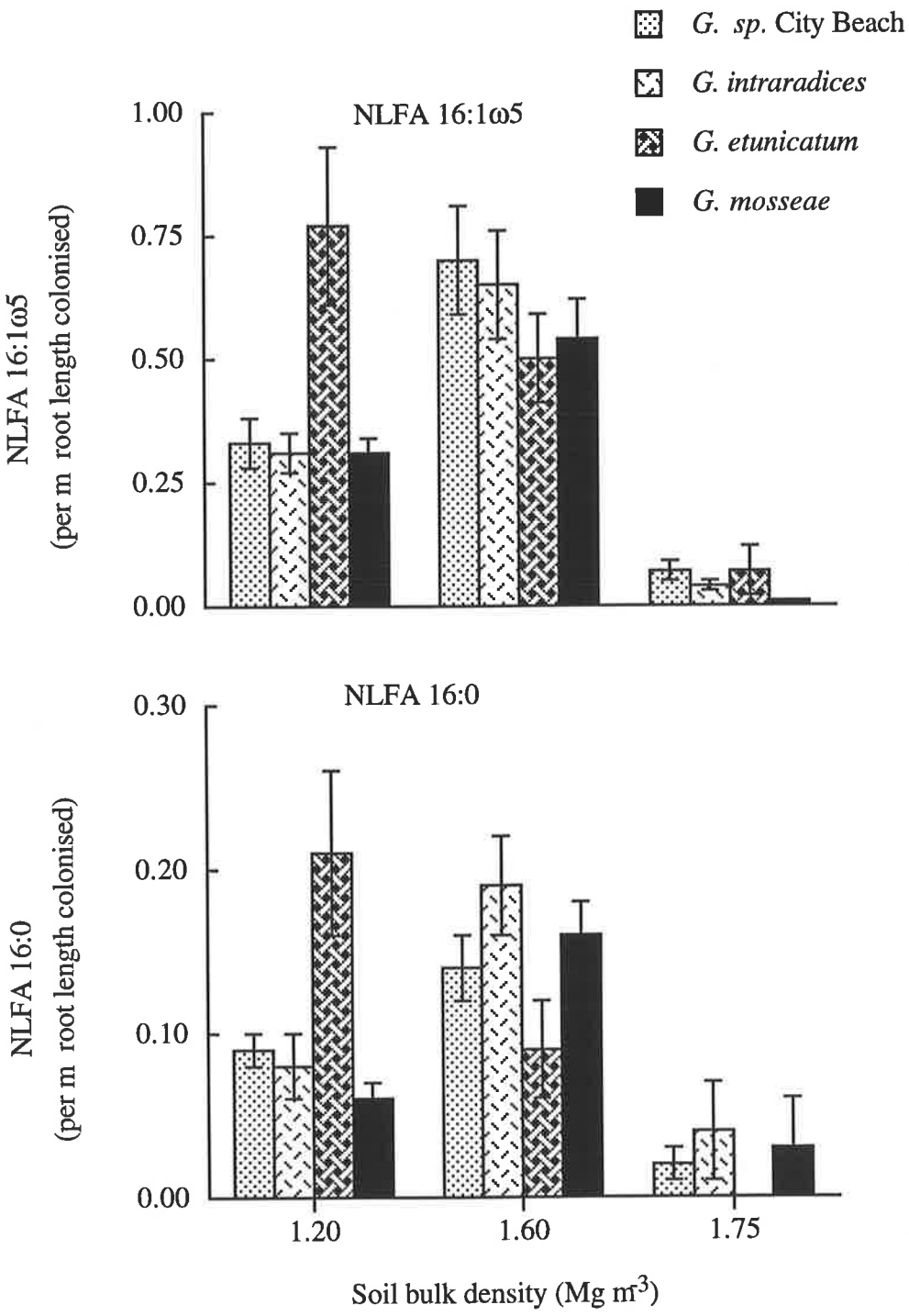

Figure 7.3 The effect of soil compaction on hyphal biomass in soil (as estimated with NLFA 16:1ω5 and 16:0) per unit length of colonised root. Values were corrected for background NLFA 16:1ω5 and 16:0. Vertical bars represent standard errors of the means, n=3.

At bulk density of 1.20 Mg m^{-3}, the concentration of P in the shoots and roots of mycorrhizal clover plants was higher than that in the shoots and roots of non-mycorrhizal plants (Table. 7.4). Shoot and root P concentrations of clover plants were increased by *G. sp.* City Beach and *G. intraradices* up to a bulk density of 1.60 Mg m^{-3}, by *G. mosseae* up to the bulk density of 1.40 Mg m^{-3} and by *G. etunicatum* only at a bulk density of 1.20 Mg m^{-3} (Table 7.4). This resulted in greater mycorrhizal growth responses to *G. sp.* City Beach and *G. intraradices* than to the two other fungi in compacted soil (Fig. 7.2). There was no significant interaction between soil compaction and P application in their effect on mycorrhizal response in terms of P uptake per plant between four species of VAM fungi, however, irrespective of P application, soil compaction decreased mycorrhizal P responses and the decreases were more pronounced for *G. etunicatum* and *G. mosseae* than for the two other fungi (Fig. 7.4).

Table 7.4 The concentration of P in the shoots and roots of non-mycorrhizal (NM) and mycorrhizal *Trifolium subterraneum* as affected by soil compaction.

Bulk density (Mg m^{-3})	NM	*G. sp.* City Beach	*G. intraradices*	*G. etunicatum*	*G. mosseae*
		Shoot P concentration (μg mg^{-1} dry weight)			
1.20	0.85	1.41	1.36	1.30	1.30
1.40	0.79	1.30	1.33	0.97	1.21
1.60	0.89	1.32	1.34	0.87	1.00
1.75	0.63	0.65	0.63	0.65	0.65
Tukey's HSD (p = 0.05)			*0.28*		
		Root P concentration (μg mg^{-1} dry weight)			
1.20	0.90	1.46	1.51	1.34	1.45
1.40	0.85	1.35	1.48	1.05	1.32
1.60	0.90	1.46	1.47	0.95	1.17
1.75	0.67	0.70	0.81	0.75	0.74
Tukey's HSD (p = 0.05)			*0.29*		

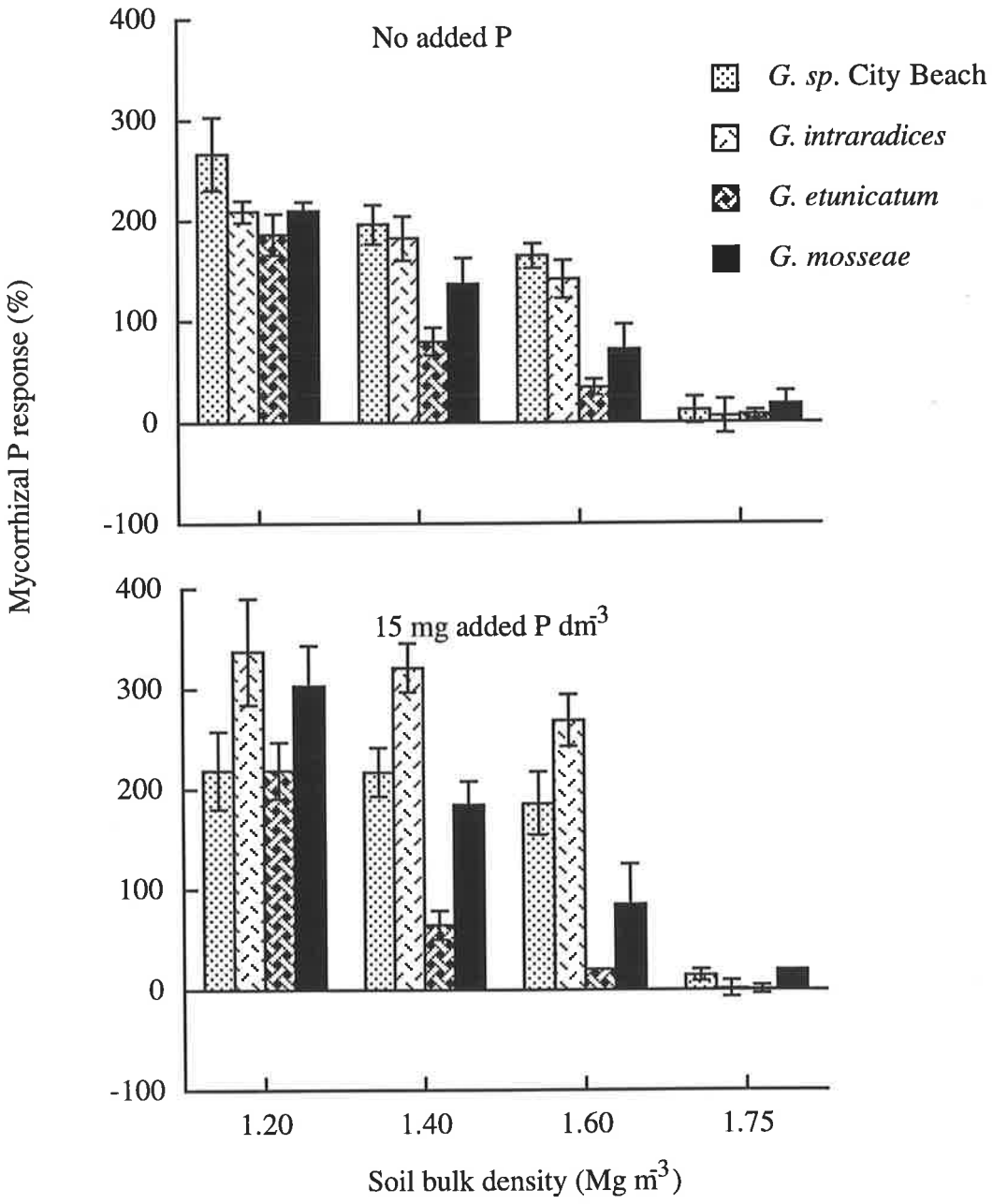

Figure 7.4 The effect of soil compaction on mycorrhizal P response in terms of P uptake per plant by *Trifolium subterraneum* as affected by soil compaction and P application. Vertical bars represent standard errors of the means, n=3.

The concentrations of Zn, Cu and Mn in the shoots and roots of mycorrhizal and non-mycorrhizal clover plants were measured in the soil supplied with 15 mg P dm^{-3} soil. The concentration of Zn in the shoots and roots of mycorrhizal and non-mycorrhizal clover plants at all levels of soil compaction followed a similar pattern to that of the tissue P concentration (Fig. 7.5).

Mycorrhizal colonisation had no effect on the concentrations of Cu in the shoots at any level of soil compaction (Fig. 7.6). However, the concentration of Cu in the root of clover plants was increased by *G. sp.* City Beach, *G. intraradices* and *G. mosseae* up to a bulk density of 1.60 Mg m^{-3} and by *G. etunicatum* up to a bulk density of 1.40 Mg m^{-3}.

The concentration of Mn in the roots and shoots of clover plants colonised by *G. sp.* City Beach, *G. intraradices* (up to the bulk density of 1.60 Mg m^{-3}), *G. etunicatum* (only in shoots) and *G. mosseae* (up to the bulk density of 1.40 Mg m^{-3}) was significantly lower than that in the roots and shoots of non-mycorrhizal clover (Fig. 7.7).

Soil compaction decreased the O_2 content of the soil atmosphere from 0.16 m^3 m^{-3} in slightly compacted soil to 0.05 m^3m^{-3} in highly compacted soil, whereas the concentration of CO_2 in the atmosphere of the soil increased with increasing soil compaction (Fig. 7.8).

The results reported in Chapter 6 indicated that soil compaction to the bulk density of 1.6 Mg m^{-3} led to the collapse of most large pores and consequently changed pore size distribution of the soil. In this experiment, compacting the soil to a bulk density of 1.75 Mg m^{-3} caused a greater decrease in the percentage of pores larger than 3 μm diameter (decreased from 28% at the bulk density of 1.6 Mg m^{-3} to 19% at bulk density of 1.75 Mg m^{-3}).

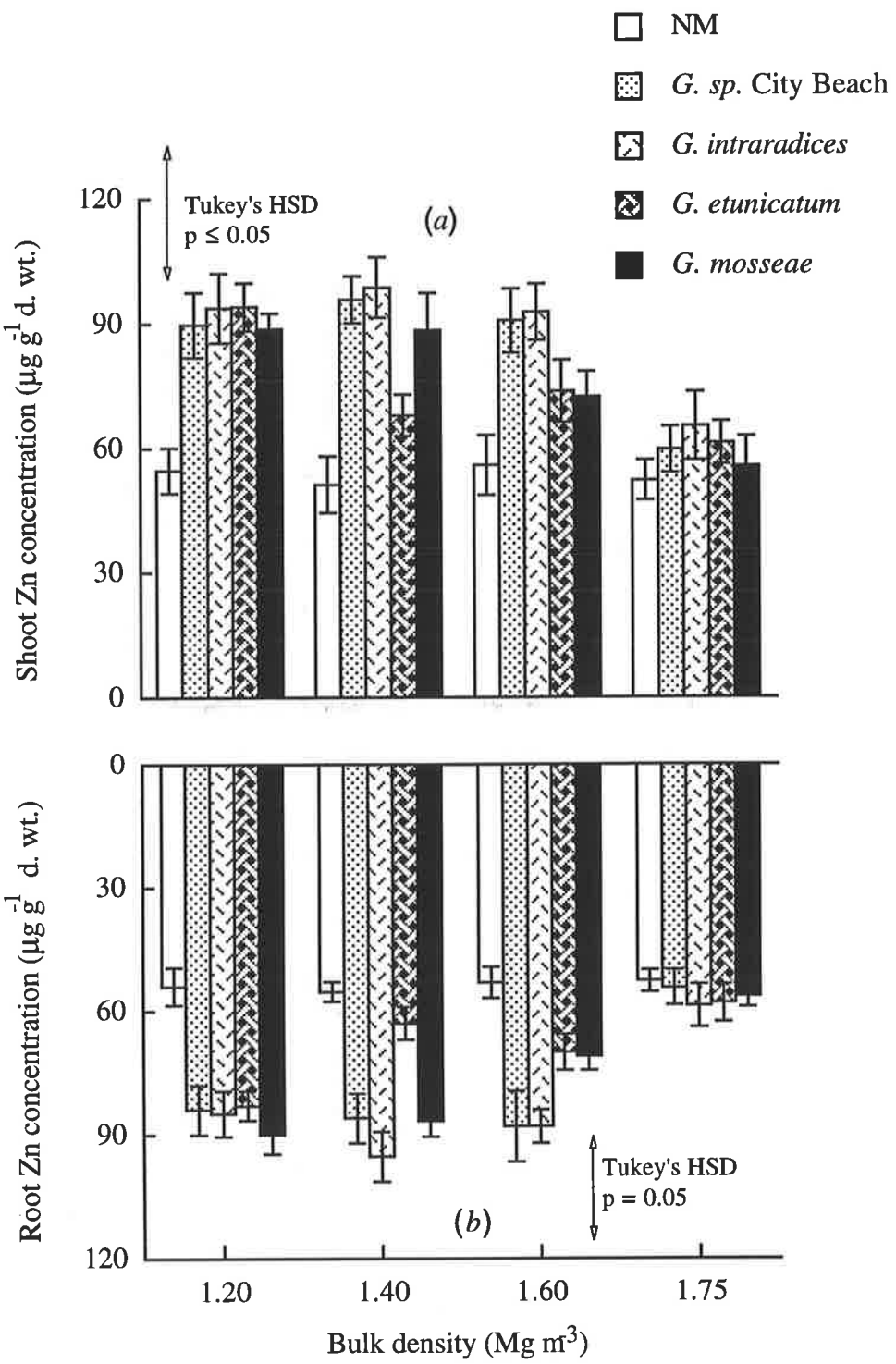

Figure 7.5 The effect of soil compaction on shoot (*a*) and root (*b*) Zn concentrations of non-mycorrhizal (NM) and mycorrhizal *Trifolium subterraneum* when the soil was supplied with 15 mg P dm^{-3}. Vertical bars represent standard errors of the means, n=3.

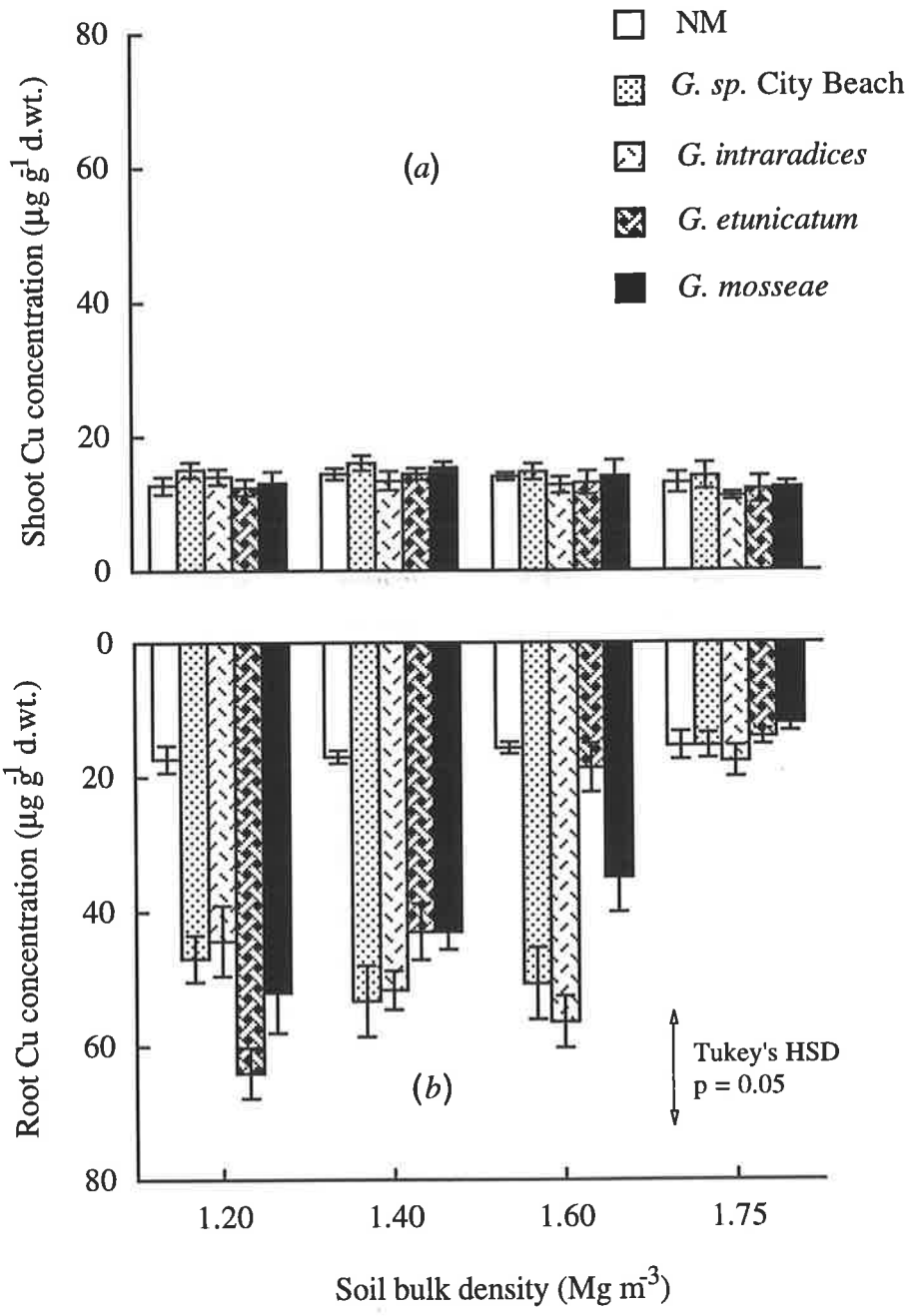

Figure 7.6 The effect of soil compaction on the concentration of Cu in the shoots (*a*) and roots (*b*) of non-mycorrhizal (NM) and mycorrhizal *Trifolium subterraneum* when the soil was supplied with 15 mg P dm^{-3}. Vertical bars represent standard errors of the means, n=3.

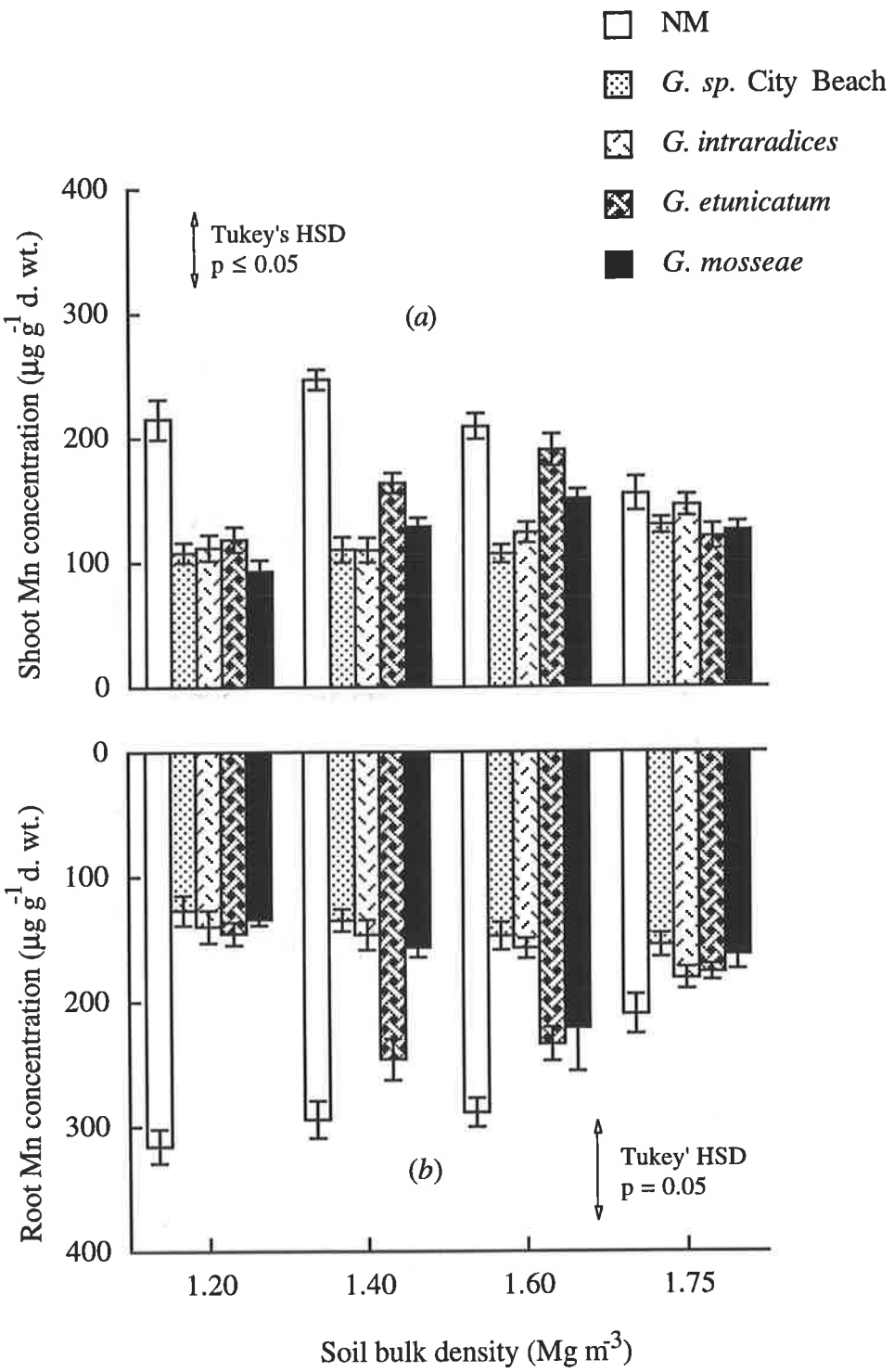

Figure 7.7 The effect of soil compaction on the concentration of MN in the shoots (*a*) and roots (*b*) of non-mycorrhizal (NM) and mycorrhizal *Trifolium subterraneum* when the soil was supplied with 15 mg P dm^{-3}. Vertical bars represent standard errors of the means, n=3.

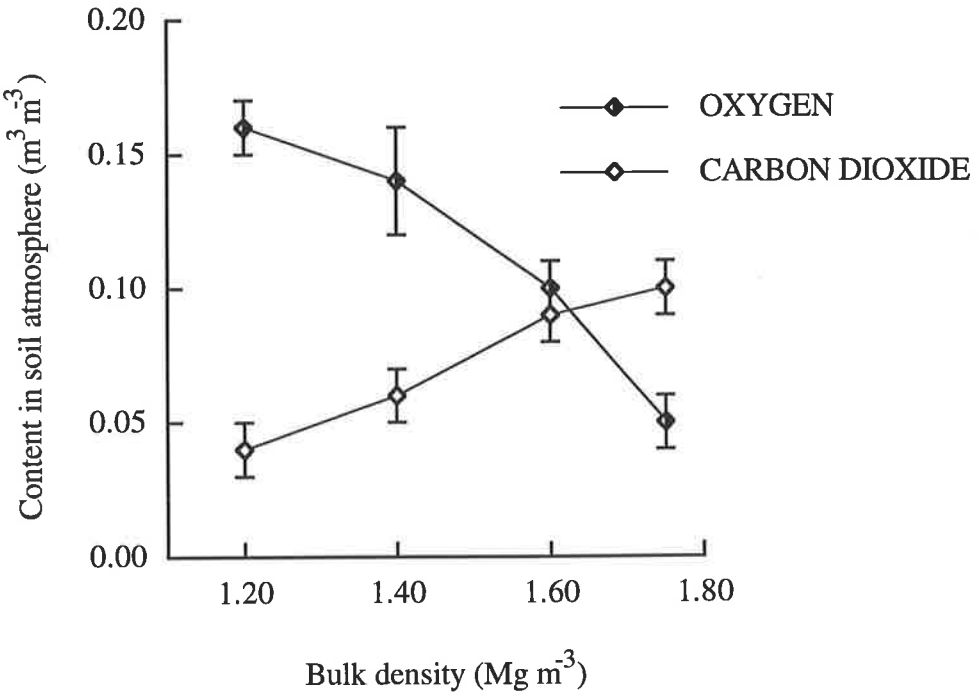

Figure 7.8 The effect of soil compaction on the concentration of oxygen and carbon dioxide in the soil atmosphere when the soil was supplied with 15 mg P dm^{-3}. Vertical bars represent standard errors of the means, n=3.

7.4 Discussion

Although no significant difference was observed in the percentage of root length colonised by the four species of VAM fungi in slightly compacted soil (irrespective of P application), soil compaction differently affected the percentage of root length colonised by the different fungi up to a bulk density of 1.60 Mg m^{-3}. The mechanisms by which soil compaction decreased colonisation of *T. subterraneum* roots by *G. etunicatum* and *G. mosseae* are not clear. However, the simplest explanation is that these two fungi are more sensitive than the other species to the poor aeration encountered in compacted soil (Fig. 7.8). Saif (1983)

found that *G. mosseae*, *Glomus macrocarpus* and 'white reticulate' differed in their responses to changing concentrations of O_2 in the air of non-compacted soil. The decreases in the percentage of root length colonised by *G. etunicatum* and *G. mosseae* with increasing soil compaction led to a greater decline in mycorrhizal responses in terms of plant growth and P uptake per plant than the two other fungi.

The greater effect of compaction on decreasing hyphal biomass in soil (*i.e.* the amounts of NLFA 16:1ω5 and 16:0) in *G. etunicatum* and *G. mosseae* than in *G. sp.* City Beach and *G. intraradices* might be due to differences in the diameter of the external hyphae, which can affect their ability to penetrate small pores in compacted soil. Abbott and Robson (1985a) observed large differences in the diameter of hyphae produced in soil by different species of VAM fungi (see below).

In slightly compacted soil (bulk density = 1.20 Mg m^{-3}), *G. etunicatum* produced more external hyphae than did the other three species examined here, assuming that NLFA 16:1ω5 and 16:0 indicate the amount of hyphal biomass in soil. The high formation of external hyphae in slightly compacted soil by *G. etunicatum* contrasts with the length of root colonised by this fungus. The results are in agreement with those of Abbott and Robson (1985a), who found that species of VAM fungi differed in the length of external hyphae produced per unit of colonised root. Similarly, Graham *et al.* (1982) indicated that different isolates of *Glomus fasciculatum* had different abilities to produce external hyphae in soil, although root colonisation was similar for all the isolates. In contrast, a strong correlation between the weight of external hyphae and the length of mycorrhizal roots has been shown by Sanders *et al.* (1977). The extent to which a root may be colonised by VAM fungi, the ability of fungi to produce external hyphae and the relation between these two can be modified by a variety of environmental factors, many of which apparently exert their influence through effects on the physiology of the plant. Among environmental factors,

Abbott and Robson (1984) pointed out that soil pH and soil P status may affect the relationship between the amount of external hyphae and the extent of internal colonisation. The high amount of external hyphae in slightly compacted soil produced by *G. etunicatum* relative to the root length colonised might therefore be due to the suitability of the soil for the development fungal hyphae and/or to the morphological and physiological characteristics of the fungus. The diameters of the external hyphae produced by the four species may differ from each other, particularly in compacted soil. Since the relationship between hyphal biomass and hyphal length is affected by the diameter of external hyphae, the conclusion made regarding the relationship between root colonisation and external hyphae based on the amount of hyphal biomass may change if it is made on the basis of actual length of external hyphae. In this study, because of the difficulties in differentiating between hyphae of VAM fungi and those of non-VAM fungi using the filtration gridline method (see Chapter 5 and 6) measurement of the diameters of external hyphae of VAM fungi was impossible. Further work should include development of methods to assess the diameters of hyphae and the effect of compaction on them.

Soil compaction to a bulk density of 1.60 Mg m^{-3} significantly decreased the hyphal biomass formed by *G. etunicatum* (Table 7.1) and changed the relationship between root colonisation and hyphal biomass observed in slightly compacted soil (bulk density = 1.20 Mg m^{-3}). The observed change might be due to a greater sensitivity of this fungus to unfavourable conditions arising from soil compaction. This clearly shows the effect of environmental conditions (here soil compaction) on the relationship between root colonisation and the amount of external hyphae.

There was no mycorrhizal growth response at the soil bulk density of 1.75 Mg m^{-3}. A large reduction in the concentration of O_2 in the soil atmosphere (0.05 m^3 m^{-3}) which has been suggested is insufficient for plant growth (Boone and Veen, 1994) and increased the

concentration of CO_2 in the soil air might be responsible, at least in part, for the lack of mycorrhizal growth response. Saif (1983) found that mycorrhizal growth response and P uptake by mycorrhizal plants significantly decreased when the concentration of O_2 in the soil atmosphere was lowered from 0.16 to 0.02 m^3 m^{-3}. The lack of mycorrhizal growth response observed in highly compacted soil might also be indirectly due to a significant decrease in the percentage of pores larger than 3 µm diameter (19% in highly compacted soil, compared with 60% in slightly compacted soil) and to a significant decrease in the continuity of pores (see Chapter 3, Section 3.15). This led to a significant decrease in hyphal biomass in highly compacted soil.

Another possible explanation for the lack of mycorrhizal growth response in highly compacted soil may be due to production of ethylene by mechanically impeded roots (Kays *et al.,* 1974; Moss *et al.,* 1988). Azcón-Aguilar *et al.* (1981) found that ethrel, a compound which can readily release ethylene, depressed VAM formation when it was either applied to the rooting medium or sprayed on the foliage. Similarly, Ishii *et al.* (1996) reported that hyphal growth of *Glomus mosseae* and *Gigaspora ramispophora* were severely inhibited as the concentration of ethylene was increased. These findings agree with previous papers indicating that ethylene could be an inhibitor of the development of fungal propagules in soil (*e.g.* Primrose, 1979).

The superior ability of *G. sp.* City Beach and *G. intraradices* to increase the concentration of Zn in clover plants and Cu in clover roots, but not in the shoots, compared with *G. etunicatum* and to some extent *G. mosseae* might be due to their higher formation of external hyphae in compacted soil. A similar increase in the concentration of Cu in the roots upon mycorrhizal colonisation has been shown for *Zea mays* L. (Kothari *et al.,* 1990). The reason for the increase in Cu being observed only in the roots is not clear, but might be due to sequestration of Cu in the fungus, for example, via a chemical reaction between Cu

and fungal polyphosphate granules, as has been shown for Ca and Fe by White and Brown (1979). Similarly, Bradley *et al.* (1982) found that the concentration of Cu in roots was higher than in shoots of plants with ericoid mycorrhizas. They suggested that internal proliferation of the hyphal complexes that occurs within cortical cells of the host roots increases the area of wall material available for complexing by several orders of magnitude. Turnau *et al.* (1993) used electron energy loss spectroscopy to investigate the possibility that heavy metals are sequestered in the hyphae and not transferred to the host plant. They found a greater accumulation of Cd, Ba and Ti in the fungal structures than in the root cells themselves. They suggested that sequestration of the metals by polyphosphate in the hyphae might have been a mechanism facilitating exclusion of metals from the shoot, and thus avoiding metal toxicity. However, further work is needed to confirm these suggestions. If such a mechanism is to operate, the absence of any significant difference in the concentration of Cu between shoots of clover plants colonised by all the four fungi in highly compacted soil (bulk density = 1.75 Mg m^{-3}) might therefore be due to the lack of formation of hyphae by these fungi in highly compacted soil.

The concentration of Mn in mycorrhizal plants (shoots and roots) was significantly lower than that in non-mycorrhizal plants up to the bulk density of 1.60 Mg m^{-3} (Fig. 7.7). This might be due to tissue dilution of Mn in larger plants or to a significant decline in the proportion of Mn-reducers of the total microbial population in the rhizosphere of mycorrhizal plants. The second suggestion is supported by the results of Kothari *et al.* (1991), who found that the number of Mn-reducers in the rhizosphere of non-mycorrhizal plants was 20 to 30 times that in the rhizosphere of mycorrhizal plants, and this led to a higher Mn concentrations in the non-mycorrhizal plants.

7.5 Conclusion

The results of this experiment showed that the fungal isolates differed in their ability to increase P uptake and the growth of the host plant. The greater decrease in mycorrhizal growth response with *Glomus etunicatum* and to some extent with *Glomus mosseae* under increasing soil compaction (up to a bulk density of 1.60 Mg m^{-3}) compared with *G. sp.* City Beach and *G. intraradices* might be due, at least in part, to the greater sensitivity of these two mycorrhizal fungi to the poor aeration arising from soil compaction. However, possible differences in hyphal diameters, which may affect hyphal penetration of small pores, could also be involved.

The lack of mycorrhizal growth response observed in highly compacted soil (bulk density = 1.75 Mg m^{-3}) was attributed to a number of possible causalities: poor aeration, reduction in the size and continuity of the pores, ethylene production by impeded roots, physical impairment of hyphal growth in compacted soil or by a combination of these factors. Since all these possibilities may affect mycorrhizal growth response simultaneously, it is difficult to separate the effect of each factor from the effect of the others upon mycorrhizal growth response, with respect to the experimental system used in this study.

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSION

8.1 Introduction

The findings of this study show how soil compaction affects growth and P uptake by *Trifolium subterraneum* and how these effects interact with mycorrhizal colonisation. In this final chapter, the findings presented in Chapters 4 to 7 of the thesis are integrated and discussed in the light of questions raised in Chapter 1. The discussion covers four main aspects of the work: 1) the effect of soil compaction on plant growth, 2) phosphorus uptake as affected by soil compaction, 3) the effect of mycorrhizal colonisation on growth and P uptake by *T. subterraneum* in compacted soil and comparison of mycorrhizal growth responses of four species of VAM fungi to soil compaction, and 4) growth and development of external and internal colonisation over the range of soil compaction.

8.2 Effects of soil compaction on the growth of non-mycorrhizal plants

Soil compaction, usually caused by passage of vehicular traffic, has adverse effects on crop performance. These effects can be linked to morphological and physiological changes in plants where roots are subjected to mechanical impedance. Morphological changes include restriction of root extension and shoot growth, and modification of the rooting pattern and root diameter. The results presented in Chapter 4 showed that increased penetrometer resistance of the soil from 0.4 to 3.5 MPa decreased root dry weight of clover (*Trifolium subterraneum* L.) and onion (*Allium cepa* L.) plants, although the ability of clover plants to penetrate compacted soil was greater than that of onion plants. From the data presented in

Chapter 4, the exact mechanism(s) responsible for the difference in the ability of the roots of onion and clover plants to penetrate compacted soil is not clear. However, it has been suggested that root diameter may have large influence on the penetration of roots into strong soil. Some authors have shown that the ability of roots to penetrate a compact soil increases with increasing root diameter (Whiteley and Dexter, 1981; Materechera *et al.*, 1991), whereas Taylor and Gardner (1960) could not find differences between the penetrating ability of legumes and non-legumes with different diameters. Bennie and Burger (1981) did not find any differences in penetration by cotton, maize, wheat and peanuts, into compacted soil. Although the diameter of clover roots was smaller than that of onion roots (Chapter 4), their abilities to penetrate compacted soil were greater than those of onion roots. This suggests that some other morphological and physiological characteristics of the roots may influence penetrability of roots into strong soil. It has been shown that the plastic and elastic properties of the walls of root cells which influence the extensibility of the cell may play a major role in controlling the growth of roots (Cleland, 1971; Pritchard *et al.*, 1987). Thus, the superior ability of clover roots compared with onion roots to penetrate compacted soil might be due to differences in the elastic properties of their roots. Moreover, it seems the roots of onion are very fragile and this may influence the rigidity of the root tips and hence their ability to penetrate strong soil.

The observed decrease in plant growth in this study might be attributed to the increased penetrometer resistance of the soil and poor aeration due to soil compaction. In a heterogeneous soil, the influence of restricted aeration is difficult to distinguish from that of restriction to plant growth caused by mechanical impedance. Although there are variations in the reported values of O_2 concentration in the soil atmosphere below which anoxia may occur, root growth generally appears to be unrestricted by poor aeration when O_2 concentration in the soil atmosphere is greater than 0.10 m^3 m^{-3} (Boone and Veen, 1994).

Accordingly, the decreased plant growth at a bulk density of 1.4 Mg m^{-3} with an O_2 concentration of about 0.14 m^3 m^{-3} in the soil air (Chapter 4, Table 4.2 and Fig. 4.9) can attributed mainly to mechanical impedance (the penetrometer resistance of the soil was 2.3 MPa at a matric potential of -33 kPa), whereas decreased plant growth at a bulk density of 1.75 Mg m^{-3} with an O_2 concentration of about 0.05 m^3 m^{-3} in the soil air (Chapter 7) can be attributed to the combined effects of poor aeration and mechanical impedance.

8.3 Effects of soil compaction on P uptake by non-mycorrhizal plants

The effect of soil compaction on P uptake per plant is similar to the effect of soil compaction on plant growth. To understand the influence of soil compaction on P uptake a number of factors have to be taken into account. Increased soil compaction, which decreased the proportion of large pores (Chapter 4), significantly decreased the growth of clover roots (both root length and root dry weight) and resulted in a significant decrease in P uptake per plant (Chapters 4-7). However, soil compaction to a bulk density of 1.60 Mg m^{-3} did not decrease plant tissue P concentration (Chapter 4), suggesting that P transfer to the roots may not be restricted by soil compaction. This is supported by the results presented in Chapter 6 which indicated that the rate of P uptake per unit time per unit length (P inflow) by clover roots increased with increasing soil compaction. The increased P inflow to impeded roots in compacted soil was attributed to the change in soil physical properties (volumetric water content and possibly diffusion coefficient of P) and change in root morphology (increased root diameter). The effects of these changes on P inflow were discussed in detail in Chapter 6. In fact, an increase in P inflow to impeded roots can be interpreted as a mechanism of adapting the uptake capacity of the root system to P demand of the plant, and also of compensating for the effect of soil compaction on root growth.

8.4 Effects of mycorrhizal colonisation on growth and P uptake in compacted soil

The results of this study indicate that the effect of soil compaction on plant growth and P uptake by clover plants reported in Chapter 4 interacted with the effect of mycorrhizal colonisation. The results presented in Chapters 4-7 clearly show that *Glomus intraradices* increased plant growth and P uptake by clover plants, although this increase diminished with increasing soil compaction. It has been shown that there is a relationship between mycorrhizal dependency and the morphology of the host roots. Baylis (1975) suggested that plant species with coarse roots and few root hairs are more responsive to mycorrhizal colonisation than plants with finely branched roots and long or numerous root hairs, and this suggestion has received some experimental support (St John, 1980; Brundrett and Kendrick, 1988; Baon *et al.*, 1994). In this study, increased root diameter of *T. subterraneum* due to soil compaction (Chapter 4) did not coincide with increased mycorrhizal dependency (Table 8.1). Poor aeration, discontinuity of pores and presumably ethylene production by impeded roots may explain the differences between effects seen with plants having genetically determined differences in rooting characteristics and effects resulting from soil compaction. Similarly, Simmons and Pope (1987) found that mycorrhizal dependency of sweet gum (*Liquidambar styraciflua* L.) and yellow poplar (*Liriodendron tulipifera* L.) in association with *Glomus macrocarpum* and *Glomus fasciculatum* decreased as soil bulk density was increased from a bulk density of 1.25 to 1.55 Mg m^{-3}, although the effect of soil compaction on root diameter was not reported.

Table 8.1 Mycorrhizal dependency of *Trifolium subterraneum* in association with four species of VAM fungi at different levels of soil compaction[A].

Mycorrhizal fungi	Soil bulk density (Mg m^{-3})			
	1.20	1.40	1.60	1.75
G. sp. City Beach	214	210	196	104
G. intraradices	255	233	221	106
G. etunicatum	186	147	125	95
G. mosseae	237	181	173	103

[A] Mycorrhizal dependency was calculated by the percentage of dry weight of mycorrhizal plants over the dry weight of non-mycorrhizal plants (Menge *et al.*, 1978).

The results presented in Chapter 7 (see also Table 8.1) clearly indicate that the effects of soil compaction on symbiotic performance differ between the mycorrhizal fungi. The decreased mycorrhizal growth response at higher levels of soil compaction, particularly for *G. mosseae* and *G. etunicatum*, and at the highest level soil compaction (bulk density = 1.75 Mg m^{-3}) for all the four mycorrhizal fungi, could have been caused by the change in the concentration of O_2 and CO_2 in the soil air, by restriction of hyphal growth due to the changed pore size distribution, and probably by ethylene production from impeded roots or by any combination of these factors. Since all these unfavourable conditions arising from soil compaction may affect mycorrhizal colonisation simultaneously, it is difficult to separate the effect of each factor from the effect of the others upon mycorrhizal growth response. From the data presented in Chapter 7 and with respect to the experimental system used in this study, it is therefore impossible to draw any final conclusions on the relative importance of these possibilities on mycorrhizal growth response. According to the literature, it is reasonable to expect a combination of unfavourable conditions arising from soil compaction to affect mycorrhizal colonisation, although their relative importance is not clear. Since poor aeration and mechanical impedance resulting from soil compaction may have different effects on the morphology and physiology of clover plants, these may

differently affect mycorrhizal colonisation. For example, the type and intensity of effects of ethylene production by mechanically impeded roots (Kays *et al.*, 1974; Moss *et al.*, 1988) on mycorrhizal colonisation (Azcón-Agular *et al.*, 1981; Ishii *et al.*, 1996) may be different from the effects of poor aeration on mycorrhizal development (Saif, 1981, 1983, 1984). Hence, it will be important to separate the effect of poor aeration and mechanical impedance on mycorrhizal development by using an experimental system which restricts only root growth by increasing soil strength but not restrict soil aeration (uncompactable soil) as used by Materechera *et al.* (1991).

Another possible explanation for the greater mycorrhizal growth response to *G. intraradices* and *G. sp.* City Beach than to *G. etunicatum* and *G. mosseae* in compacted soil is possible differences in hyphal diameters, which may give rise to different abilities to penetrate small pores in compacted soil. There are few reports of the diameters of mycorrhizal hyphae in soil, but they have been shown to range from 27 μm (for main runner hyphae) to 2 μm for finer branches (*e.g.* Nicolson, 1959; Friese and Allen, 1991 and see Smith and Read, 1997). It would be interesting 1) to investigate the range of diameters of external hyphae produced by each species and 2) to examine the effects of changes in pore size distribution on penetration of hyphae with different diameters.

The results of this study once again confirm that small additions of P to P-deficient soil stimulate growth and P uptake by clover plants (Abbott and Robson, 1984) even in compacted soil up to the bulk density of 1.6 Mg m^{-3}. Since soil compaction had no significant effect on internal colonisation (Chapter 5), the observed decline in P uptake per plant by increasing soil compaction was attributed mainly to the significant decrease in total root length colonised, although a significant decrease in hyphal biomass (NLFA 16:1ω5 and 16:0) per unit mass of soil can also be involved. Despite the significant decrease in hyphal biomass per unit mass of soil, hyphal P inflow was not decreased by soil compaction. This

might be due to the increased hyphal biomass per unit root length colonised. However, a considerable decrease in mycorrhizal P response (*i.e.* in terms of P inflow) was observed with increasing soil compaction at the third harvest, but not at the first and second harvest (Table 6.3). This is matched by the effect of soil compaction on mycorrhizal growth response observed in Chapters 4-7. In fact, during the harvest period of 40-60 days, when P inflow had decreased (probably due to the decreased hyphal biomass per unit root length colonised), the effect of soil compaction in decreasing mycorrhizal growth response was more pronounced than that at the first and second harvests.

The results presented in Chapter 7 show that there was no difference in tissue P concentration and consequently P uptake per plant between non-mycorrhizal and mycorrhizal plants colonised by the four fungi when the soil was compacted to a bulk density of 1.75 Mg m^{-3}. In contrast, Li *et al.* (1997), using a compartmented mesh system, found a significant increase in hyphal contribution to P uptake when the roots of red clover (*Trifolium pratense* L.) and hyphae of *Glomus mosseae* had access to outer compartments containing compacted soil (up to the bulk density of 1.80 Mg m^{-3}). The observed benefit in mycorrhizal colonisation in compacted soil to a bulk density of 1.80 Mg m^{-3} might be due to the fact that red clover was planted in a central compartment (uncompacted soil with a bulk density of 1.3 Mg m^{-3}) where poor aeration and presumably ethylene production would not be expected to be a problem for plant growth and mycorrhizal colonisation.

8.5 Effects of soil compaction on the growth of external hyphae

The results of this study indicate that soil compaction decreased the amount of external hyphae whether this was measured as the length of living external hyphae (Chapter 5) or as the neutral lipid fatty acids 16:1ω5 and 16:0 extracted from the soil (Chapters 6 and 7). Although Li *et al.*, (1997) did not measure the amount of external hyphae in the soil, they

found that the P depletion zone in the hyphal compartments extended to about 30 and 50 mm from the root surface in highly and slightly compacted soil respectively, suggesting soil compaction restricted hyphal spread. Similarly, Kothari and Sing (1996) found that increasing soil compaction from a bulk density of 1.2 to 1.4 Mg m^{-3} decreased the length of external hyphae. The decline in the amount of external hyphae reported in Chapters 6 and 7 might be due to direct and/or indirect effects of soil compaction. Collapse of most large and medium pores, discontinuity of pores and consequently poor aeration arising from soil compaction (Chapters 4 and 7) might be responsible for direct effects of soil compaction on the growth of external hyphae. However, a possible indirect effect of soil compaction on the amount of external hyphae per pot may be due to the significant decrease in total root length colonised (Chapters 4-7). It has been shown that the length of external hyphae can be 80-200 times that of the colonised root length (Sanders and Tinker 1973; Tisdall and Oades 1979; Jakobsen *et al.*, 1992a). Therefore, if development of external hyphae in soil is a function of the extent of internal colonisation, then it can be concluded that the observed decrease in the amount of external hyphae might also be due to the indirect effect of soil compaction on root growth. The experimental system used by Materechera *et al.* (1991) may be useful to distinguish between the direct and indirect effects of soil compaction on the development of external hyphae. In this system, root growth can be restricted by soil strength (by an external pressure), but external hyphae may penetrate a remoulded (uncompacted) soil without restriction.

The results presented in Chapter 6 indicate that the amounts of NLFA 16:1ω5 and 16:0, as indicators of hyphal biomass, produced per unit length of colonised root changed with time. In both compacted and uncompacted soil, hyphal biomass per unit root length colonised decreased as plants aged. This confirms the results of Abbott and Robson (1985a) who found that the length of hyphae per cm root colonised by *Glomus fasciculatum* in

association with *T. subterraneum* declined continuously throughout the experiment. Similarly, Bethlenfalvay *et al.* (1982) found that the ratio of fungal biomass, estimated by chitin assay, outside the root to that within the root of soybean colonised with *Glomus fasciculatum* decreased with time. However, Abbott and Robson (1985a) found that the length of hyphae per unit length of root colonised by *Glomus calospora* increased initially before subsequently declining. In contrast with these results, the weight of external hyphae per unit length of root colonised by several species of VAM mycorrhizal fungi did not appear to change with time in the period of 17 to 49 days from sowing in the work of Sanders *et al.* (1977). Such variations in the results might be due to differences in the formation of external hyphae in soil and/or in the differences in the spread of colonisation within roots with time (Jakobsen *et al.*, 1992a, b; Abbott and Robson, 1985a), as well as to differences in the techniques used.

Soil compaction differently affects root length of clover plants and the amount of hyphal biomass in the soil. An increase in soil compaction from a bulk density of 1.20 to 1.60 Mg m^{-3} decreased the colonised root length more than 4 times, whereas similar levels of soil compaction decreased the amounts of NLFA 16:1ω5 and 16:0 only about 2 times (Chapter 6). This led to an increase in hyphal biomass per unit length of colonised root with increasing soil compaction. Since the diameter of external hyphae is much less than the diameter of mechanically impeded roots the greater effect of soil compaction on the growth of clover roots than on external hyphae would be expected, as was proposed in Chapter 1.

The results presented in Chapter 7 indicate that the amount of NLFA 16:1ω5 and 16:0 extracted from soil varied with fungal isolates in slightly compacted soil (bulk density = 1.2 Mg m^{-3}). The nature of the relationship between the growth of hyphae in soil and within a root is not well known. It seems this relationship is controlled by the type of fungus, soil characteristics and morphological characteristics of the host roots (see Chapter 2 for more

details). Basically, our knowledge about growth, spread and ability of external hyphae to absorb P and translocate it to the host plant is sparse and information available in this aspect is limited only to very few species of VAM fungi. Study of the effects of different properties of soil and environmental conditions on the development of external hyphae of different species of VAM fungi should allow significant advances to be made in our knowledge of the relationship between external hyphae and internal colonisation and ecology of external hyphae in the future.

8.6 General conclusions

1. Although there was a mycorrhizal response in terms of plant growth and P uptake (pre plant) up to a bulk density of 1.6 Mg m^{-3}, the response decreased with increasing soil compaction. Mycorrhizal colonisation did not therefore compensate for the effect of soil compaction on root growth. This was attributed mainly to the significant decrease in total root length colonised by arbuscules and a considerable decrease in hyphal biomass per pot with increasing soil compaction.

2. A positive correlation observed between P inflow and hyphal biomass in soil produced per m root length colonised throughout the experiment led to the conclusion that the amount of external hyphae per unit length of colonised root is an important factor influencing P inflow to mycorrhizal roots.

3. Increased P inflow to impeded roots with increasing soil compaction can compensate, at least in part, for the adverse effect of soil compaction on root length.

4. The lack of mycorrhizal growth response observed at the bulk density of 1.75 Mg m^{-3} might be due to poor aeration, collapse of most large and medium pores, poor continuity of pores and presumably production of ethylene, although their relative importance is not clear.

6. The ability of mycorrhizal colonisation of *Glomus intraradices* and *Glomus sp.* City Beach to alleviate the effect of soil compaction on growth of *T. subterraneum* was greater than the two other VAM fungi used in this study.

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