The functioning of arbuscular mycorrhizal fungi in land under different agricultural management intensities

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Abstract

In recent decades the proportion of agricultural land in the UK and Europe under organic management, or other low-input forms of agriculture, has risen sharply. Conventional, intensive, agriculture employs techniques which have been shown to be detrimental to arbuscular mycorrhizal fungi, such as ploughing, fertilisation, pesticide application, and the growth of non-host crops. The impacts of low-input agriculture on arbuscular mycorrhiza and their functioning have been little studied, and this thesis examines the functioning of arbuscular mycorrhizal fungi (AMF) from land under different agricultural management regimes.

Using radioisotope labelling, it was found that phosphorus transfer through AMF hyphae to plants was far higher in turfs from organic pasture than from conventional pasture, integrated wheat fields or conventional wheat fields: 60 days after labelling the mean hyphal uptake of ^{33}P was over 14 times greater in shoots from the organic pasture than in any of the other management treatments. Further experiments using turfs from organic and conventional fields and their margins showed a trend of decreased AMF hyphal phosphorus transfer to plants in conventional than organic farmland, and in fields compared to field margins. This trend was reversed for the ^{14}C-carbon transfer from shoots to AMF hyphal compartments. Hyphal respiration of $^{14}CO_2$ was 25 % greater in turfs from conventional than organic fields. Using the ratio of hyphal carbon transfer to hyphal phosphorus transfer to infer the degree of mutualism of the AMF present, it was shown that AMF in land under organic management were significantly more mutualistic than those in land under conventional management, but that there was no significant effect of whether the turf was from a field or a field margin.

These results confirm the major detrimental impacts of conventional farming practices upon AMF, and that organic management has a lower impact upon AMF functioning.

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Chapter 1:
General Introduction

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1.1 Overview of agricultural intensification

The agricultural output of Britain in the 1980s was nine times that of the early 19th century (Grigg, 1989). The enormous increase in yield (Figure 1.1) and output has occurred through the development of a modern agriculture which uses fertilisers, herbicides, pesticides, and machinery (Table 1.1, Figure 1.2). Accompanying the changes in farming methods was an increase in field sizes, the removal of hedgerows and a decrease in landscape diversity. Throughout much of the 20th century, the main focus in agriculture was on increasing yields, regardless of any negative effects on the environment. Whilst these dramatic increases in British crop production were underway in the 1950s and 1960s, the functional significance of mycorrhiza, which were first recorded in the 1890s, started to be discerned. Also at this time, concern was voiced about the negative environmental impact of intensive chemical farming, famously with the publication of *Silent Spring* (Carson, 1962). These negative impacts were seen most obviously in the UK with the nationwide declines in numbers of peregrine falcons *Falco peregrinus* (Ratcliffe, 1980) and sparrowhawks *Accipiter nisus* (Newton and Haas, 1984), as well as widespread deaths of small seed-eating birds (Cramp *et al.*, 1963) caused by the use of DDT and cyclodienes such as aldrin and dieldrin as pesticides. Restrictions were therefore placed upon the use of these chemicals by the mid-1960s. However, the effects of modern farming upon below-ground ecology went largely unnoticed.

There was a "false dawn" of a role for arbuscular mycorrhizal fungi (AMF) in agriculture in the 1970s and early 1980s, with the hope that this symbiosis could be used to further increase crop production in intensive agriculture. However, it soon became clear that intensive farming practices are incompatible with the fully effective functioning of the arbuscular mycorrhizal symbiosis, and that the ubiquity of indigenous AMF in agricultural land made the introduction of new strains to the field problematic. Throughout most of the 1980s, then, there was dwindling interest shown for a role of AMF in British agriculture.

The late 1990s saw increasing consumer awareness of issues such as pesticide residues in foods and the environmental impacts of intensive farming. This, coupled with government initiatives to promote set-asides of agricultural land and conversion to organic agriculture, has seen a rapid rise of organic crop production (Figures 1.3, 1.4) and organic food consumption in the UK. Organic agriculture, and other alternatives to conventional intensive agriculture such as integrated management, seek to optimise the natural functioning of the

Table 1.1 Major events in the development of British agriculture (Grigg, 1989)

1620s – 1850s	Enclosure of open fields and common land; introduction of mixed cropping and livestock farming, incorporating two new crops, clover and turnip (for fodder)
1820s	The first reaping machines (unsuccessfully) in use
1842	The first factory for mineral fertilizer opened, dissolving bones in sulphuric acid to produce superphosphate. By 1870 Britain had over 70 superphosphate factories.
1840s	Nitrogen inputs in farmyard manure augmented by imports: guano from islands near Peru.
1856	The first pesticide dressings for wheat were used.
Late 19thC.	Ammonium sulphate used by farmers (a by-product of the gas industry) Imports from mines in Europe of potash became significant, for potassium fertilization Plant-derived pesticides in use: nicotine, derris, pyrethrum (mainly on fruit, hops, some vegetable crops).
1920s	Chemical industry developed pesticides
1924	The first certification scheme initiated for Biodynamic agriculture
Late 1930s	Over 400,000 hectares being sprayed by chemical pesticides
1946	The Soil Association established, to promote organic agriculture
Late 1940s	English farmers adopted use of selective herbicides (DNOC, MCPA, 2,4-D), initially only on cereals
1950	15 chemical ingredients used to make 352 pesticide products
From 1950s	Advance of mechanisation Almost universal use of chemical methods Organophosphorus insecticides began to replace organochlorine sprays (such as DDT)
Late 1960s	65% UK cereals treated with herbicides, also used on other crops. Increasing use of fungicides
1960s & 1970s	Some farmers adopted minimum-till cultivation (discs turn upper soil, seeds and fertilizers direct-drilled), since they were using chemical weed control, and farmers were told that ploughing decreases soil fertility.
1970s	Dramatic expansion of oilseed rape (*Brassica napus*), because European subsidies made the crop highly profitable, and also because it provided a good break crop for continuous cereal growers
1973	Common Agricultural Policy reforms guaranteed profitability to farmers, encouraging increases in output
1974	94% of UK cereals treated with herbicides
1975	200 chemical ingredients used to make over 800 pesticide products
1977	Overproduction increasingly problematic: European levies imposed for exceeding specified quotas for dairy produce
1980s	Huge surpluses, "food mountains" which by 1986 measured nearly 4 million tonnes cereals, 250,000 tonnes of butter, 53,000 tonnes beef
1982	Attempts to limit cereal output by not guaranteeing prices above a certain level
mid 1980s	Oilseed rape occupied 5% of England's arable area, second only to wheat and barley.

Table 1.2 Comparison of farm management systems in the U.K.

	Conventional agriculture	Integrated agriculture	Organic agriculture
Management intensity	Most intensive	Lower intensity	Lowest intensity, most sustainable agriculture
Ideology	Driven by profit, routinely applies agrochemicals	Aims to maintain profitability whilst being environmentally sensitive (DEFRA, 2002)	Aims to close nutrient cycling circuits, build soil fertility resources, and increase biodiversity; strictly regulated standards, consumers pay a premium price
Typical form	Specialized arable or livestock, may grow monocultures or two-crop rotations	At least 4 crops in rotations to minimize pest carry-over. May use minimum-tillage or direct drilling	Mixed arable and livestock, rotations include fertility-building and/or ley phases
Fertilizers	Synthetic, soluble fertilizers applied routinely	Applies agrochemicals when and where required, as determined by detailed testing and profitability models	Synthetic fertilizers prohibited. N-fixing crops, manures and composts, rock phosphate
N application rates[1]	186 kg N ha^{-1}	120-202 kg N ha^{-1}	Grass-clover swards grown in rotation
Pest control	Synthetic chemicals, ~430 different active ingredients permitted. May be applied preventatively	Crop rotations, synthetic chemicals applied above pest thresholds. Biological control, cultural methods (40% less pesticide spraying than conventional system[1])	Cultural methods, biological control; 7 permitted pesticides, of simple chemical form (Table 1.4), some of these chemicals restricted.
Weed control	Synthetic chemicals/ mechanical weeding	Mechanical/hand weeding	Mechanical/hand weeding, ploughing
Wheat yield[1]	7.7 t ha^{-1}	7.34 t ha^{-1}	4.31 t ha^{-1}

[1] Data from 10-year comparison of farming systems, at a site in Leicestershire, U.K. (Laegreid *et al.*, 1999)

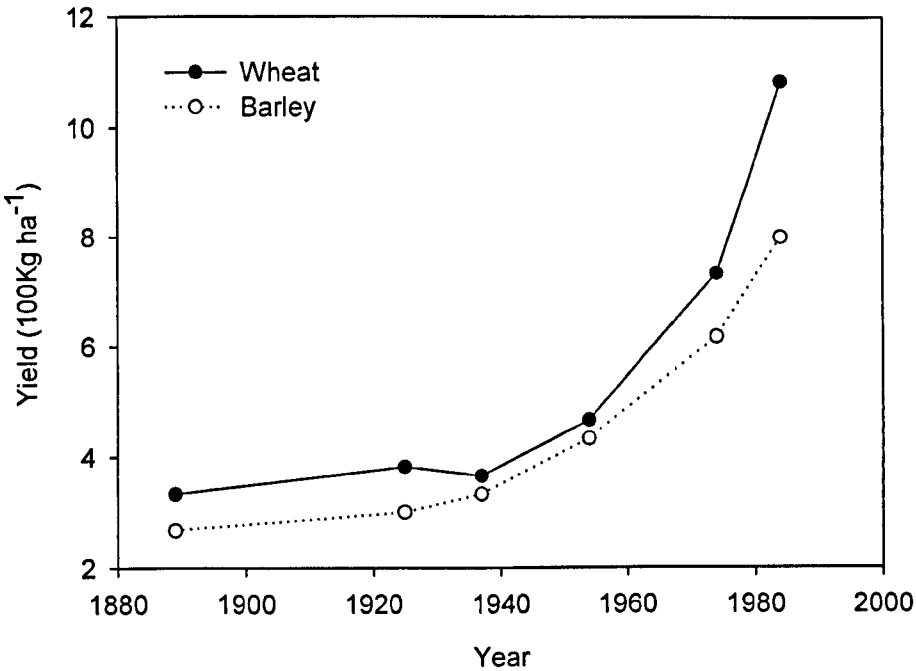

Fig. 1.1 Rising yields in wheat and barley grown in England and Wales (MAFF 1967; MAFF 1985)

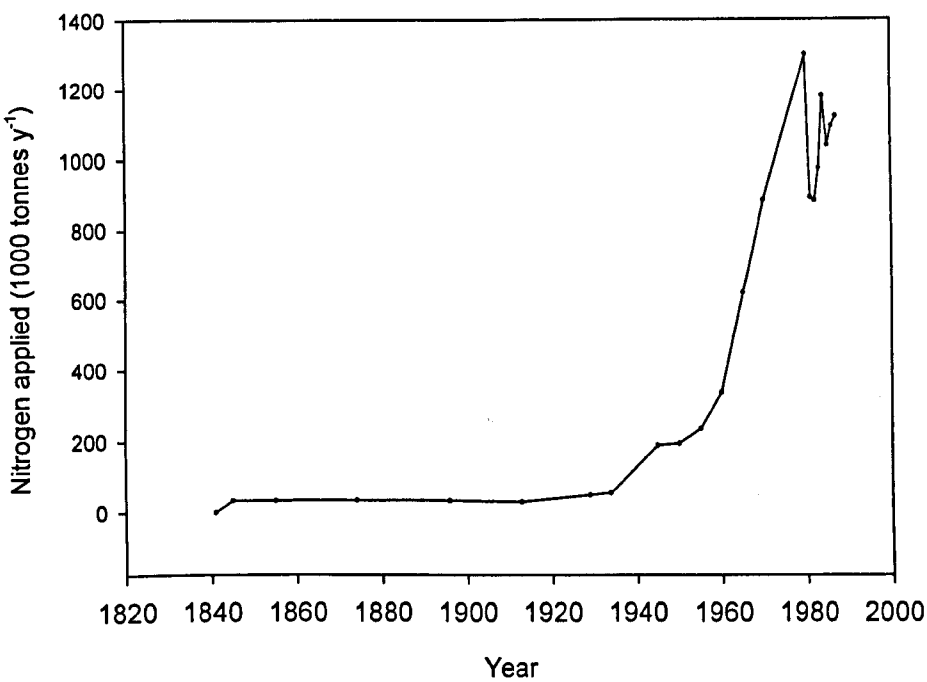

Fig. 1.2 Changing quantities of inorganic nitrogen applied in the UK. (Grigg, 1980; Marks, 1989)

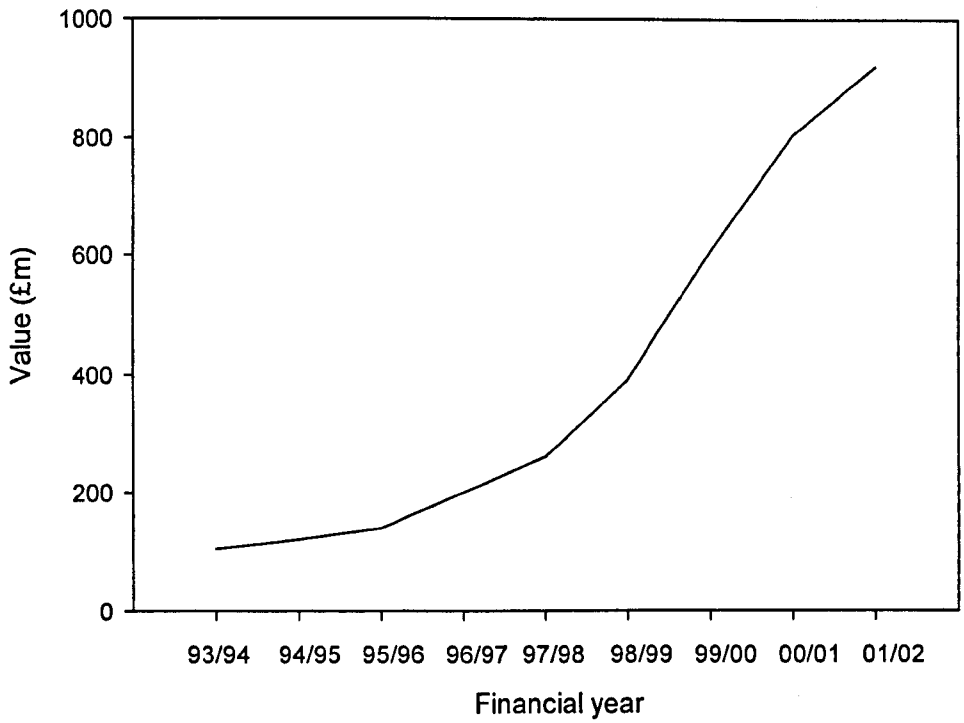

Fig. 1.3 U.K. organic retail market growth (Soil Association, 2002)

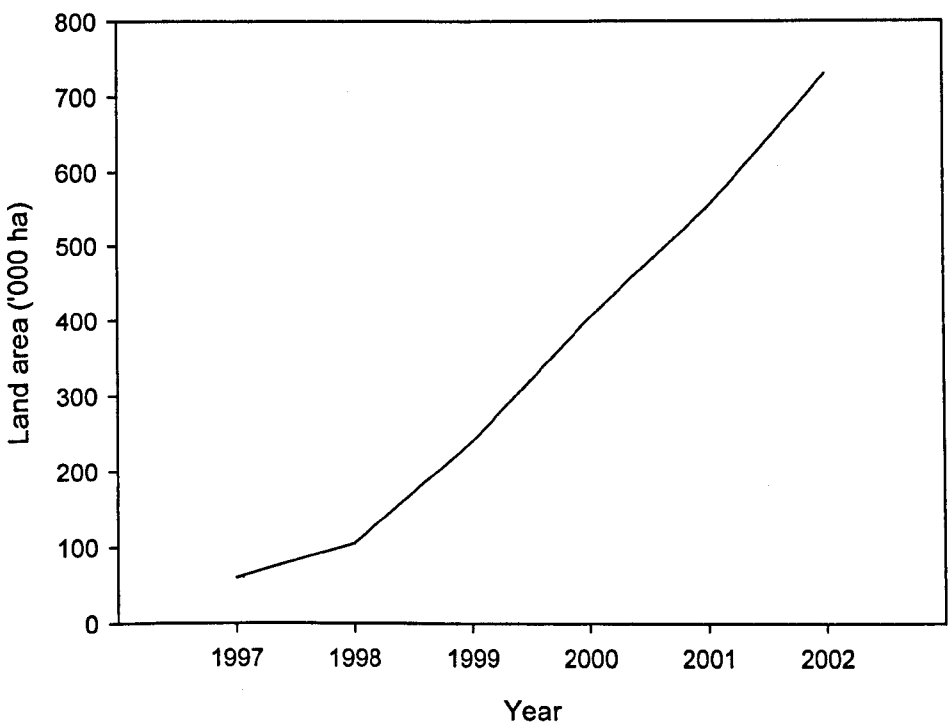

Fig. 1.4 Organically managed land in the U.K., in April of each year. (Soil Association, 2002)

environment in which the crop plants are growing, rather than governing that ecosystem by applying chemicals. Thus there has perhaps been a new dawn for mycorrhiza in agriculture, and recent research has begun examining this field.

1.2 AMF in agricultural land

It is the normal state of the majority of vascular plants to exist in symbioses with mycorrhizal fungi, and the chief organs of nutrient uptake from soil to plant are fungal hyphae, rather than plant roots (Smith and Read, 1997). Most UK crops form arbuscular mycorrhizal symbioses, the main exceptions being Brassicas such as oilseed rape, sugar beet, turnip, cabbage, broccoli and brussels sprouts (Harley and Harley, 1987). The degree to which the plant benefits from these associations varies with plant species, for example leeks are highly reliant upon AMF delivering phosphorus (P) into the roots, which is unsurprising given the very coarse and limited root system of leek plants (Oliver *et al.*, 1983). Other crops with a high mycorrhizal dependency, which may be limited when mycorrhizal inoculum levels are low, include flax (Thingstrup *et al.*, 1998), sunflower (Eason *et al.,* 1999), maize (Thompson, 1987, 1990) and sorghum (Ryan and Graham, 2002). Many plant species which have fibrous root systems, such as wheat, barley and oats, have much lower mycorrhizal dependencies (Baon *et al.*, 1992; Ryan and Graham, 2002; Thompson, 1987, 1990). These less AMF-dependent crop plants are able to produce high yields with low AMF root colonisation, given favourable conditions such as fertilised soils. Other, non-crop, plant species found in UK agricultural land are often mycorrhizal, including buttercups and common woody hedge and field margin plants such as hawthorn, sycamore and ash (Harley and Harley, 1987). There are some ruderal plants which are common weeds in agricultural fields which do not form mycorrhiza, such as dock and chickweed, cow parsley and sedges (*ibid.*).

Arbuscular mycorrhiza have long been recognised for their importance in plant nutrition, and increased growth in mycorrhizal plants has been demonstrated many times. It has been shown that plant photosynthate passes to the fungal symbiont, and it is estimated that 4 % to 20 % of total photosynthate is translocated to AMF (Smith and Read, 1997). In return, the plant receives mineral nutrients from the fungus, of which phosphorus (P) is usually of the greatest importance. Micronutrient nutrition of the plant is also improved, and benefits other than improved nutrition have been found, such as protection from fungal root pathogens (Newsham *et al.*, 1995; Azçon-Aguilar and Barea, 1996) and insect herbivores (Gange and Brown, 2001). These latter potential benefits of

AMF are pertinent to production agriculture, particularly where there is the desire to limit chemical inputs for pest control.

The extent to which mycorrhizal associations will benefit the plant evidently depends upon the conditions in which the plant is growing. The greatest benefits of mycorrhizal status to the plant tend to occur in soils of low nutrient status, particularly where P is limiting. Mycorrhizal colonisation can facilitate uptake of Zn, and in neutral to alkaline soils Zn availability can be low due to its adsorption on aluminium and iron oxides, clay minerals, organic matter and $CaCO_3$, therefore the mycorrhizal benefit may be greater in such soils.

1.2.1 Phosphorus availability to plants

Phosphorus (P) is a plant macronutrient, typically comprising 0.2 % of plant dry weight. The fraction of plant available P is often small: in arable land about 50 % soil P is in complex organic molecules, which roots cannot access directly (Mengel, 1997). In most soils, inorganic P in soil solution is very low in concentration, at 1nM - 1µM (Greenwood and Lewis, 1977). Added to this fact, P ions have very low mobility in solution, so phosphorus availability often limits plant growth. Organic P makes up 4 - 90% of total soil P, and is usually in the upper part of this range. Organic phosphates must be mineralised by phosphatases into free inorganic P before plants can utilize it (Gyaneshwar *et al.*, 2002). Roots do produce a variety of extracellular phosphatases, but much of that activity is restricted to the root surface (Koide and Zabir, 2000). Such phosphatases are possessed by AMF, bacteria, soil decomposer fungi, and plant roots (e.g. Greenwood and Lewis, 1977; Mitchell *et al.*, 1997). Fungi are well suited to P acquisition, since their narrow hyphae can penetrate well into soil aggregates, compared with plant roots.

In UK conventional agricultural systems, inorganic P is applied to soils. The P used in fertilisers is taken from phosphate rock, occurring at a limited number of sites, mainly in USA, Morocco, China, Jordan and Russia (Laegreid *et al.*, 1999). These deposits are finite, and if present consumption rates continue, known phosphate deposits will be exhausted roughly within the next 400 years (Mengel, 1997). Since ~90 % of mined phosphate is used for fertiliser, it is prudent to use it efficiently. Encouraging the presence and activity of mycorrhizal symbioses which give plants a greater ability to access soil P could be one way forward in this regard.

The proportion of plant-available soil P which enters the plant root is determined in part by root physiology and biochemistry, with variations between plant species and genotypes in affinity of the uptake system, secretion of chelators, and so on

(Marschner, 1995). Root morphology is important, since nutrients diffuse towards the root centripetally, resulting in higher nutrient concentration at the root surface as its diameter decreases (Mengel, 1985). Root hairs (~10 µm) thus have higher surface ion concentration than roots (~200 µm), while mycorrhizal hyphae (~3 µm) were observed to have hardly any surface phosphorus depletion (Silberbush and Barber, 1983). Plant P uptake will therefore be enhanced if it is in association with AMF which have a large external mycelium which effectively exploits the soil P.

AMF species differ in a variety of ways, such as the production of external mycelia, the extent of total and arbuscular colonisation of roots, and these characteristics influence their ability to acquire P and other nutrients and supply them to the plant symbiont. The length to which hyphae grow from plant roots, and the total hyphal length in the soil of arbuscular mycorrhizal external mycelia, vary with AMF species (Jakobsen, 1992a, 1992b; Smith 2000). The effectivity of P provision to plants depends upon AMF species, and even varies between ecotypes of the same AMF species (Bethlenfalvay *et al.* 1989). In a study by Schweiger *et al.* (1999), *Glomus claroideum* had hyphal P uptake rates (Bq ^{32}P m^{-1} hyphae) 4 times greater than a native AMF population, so that although it had only 63 % total external hyphal length of the native AMF, its P translocation to *Trifolium subterraneum* plants was 66 % greater than the native AMF population.

Whilst it is established that ectomycorrhizal fungi can hydrolyse organic P, there has been controversy over whether arbuscular mycorrhizal and non-mycorrhizal plants access the same P pools. Several studies have used ^{32}P-orthophosphate and found no significant differences in specific activity of ^{32}P in mycorrhizal and non-mycorrhizal plants (Sanders and Tinker, 1971; Mosse *et al.,* 1973; Powell, 1975), suggesting the plants access the same soil P pools. However, Bolan (1991) suggested that these studies may have made invalid assumptions about soil P transformations, which are complex, and Leake and Read (1997) observed that in studies such as these which use soil of low organic matter content (and hence low organic P) which is sterilised (mineralising labile organic P), most soil P may be plant-available anyway. Mycorrhiza are associated with phosphatase activity, and more recent evidence (Koide and Zabir, 2000) showed that hyphae of *Glomus intraradices* grown axenically with carrot (*Daucus carota*) were capable of hydrolysing organic P, and that they transferred significantly more P to the root in the presence of phytate than where no additional phosphorus was provided.

1.2.2 The carbon cost of the arbuscular mycorrhizal symbiosis

In contrast to most other fungi, those forming arbuscular mycorrhiza have little or no saprotrophic capabilities and are almost exclusively dependent upon their host plants for carbon, in the form of simple sugars. Current estimates of the carbon (C) costs to the plant, based mostly on pot studies, range from 4 % to 20 % (Smith and Read, 1997). However, since photosynthesis operates on a source-sink basis, this fungal drain on carbon is at least partially offset by the higher rates of photosynthesis induced by the increased rates of sugar export from shoots (Wright *et al.*, 1998a, 1998b). Where nutrients such as P are limiting to plant growth, and the AMF increase plant access to such nutrients, the resulting increases in growth more than compensate the carbon cost of the symbiosis.

Use of ^{14}C in pot studies has shown that this carbon is used for growth and metabolic activity in both intra- and extra-radical hyphae, and the transfer of recent photosynthate directly to the soil is of interest in terms of ecosystem carbon dynamics. Field studies which pulse-labelled a grassland sward with ^{13}C found that 3.9 % to 6.2 % of the C fixed passed through AMF mycelium and was respired within 21 hours (Johnson *et al.*, 2002a). Such transfer is examined more closely in Chapter 5.

1.3 Management intensity

Intensification of agriculture, with the increasing use of synthetic fertilisers and pesticides, and plant breeding, has largely ignored the biological component of soils, focusing instead upon their chemistry. Recent years have seen rising interest in sustainable agriculture, systems of management which replace or improve resource capitals of the farming unit. Consumer demand for organic produce has risen dramatically (Figure 1.3) and whilst the land farmed organically in the UK has increased in area (Figure 1.4), there is still greater demand than supply and much organic food is imported (by value, 65 % of organic food bought in the UK in 2001-2002 was imported).

The first formalization of "ecological farming" began in 1924 for Biodynamic farming (a form of organic agriculture), as a response to the rise of commercial fertilisers and intensification of agriculture, and a desire to work with the life processes giving the soil fertility (Pfeiffer, 1947). A variety of agricultural systems are now in operation, ranging from the intensive conventional farming which relies on chemical inputs, through a range of integrated management techniques, to organic farming which is strictly controlled by legislation (Table 1.2). The

different forms of agriculture provide various selective pressures on the soil biota, a theme which is explored below (Section 1.4).

Demand for organic food in the UK increased by 55 % between 1999 and 2000 and increased further by 33% between 2000 and 2001, and the UK organic market is currently worth over £920m (Soil Association, 2002b). Some consumers choose to buy organic produce believing it to be more nutritious, lower in pesticide residues, or environmentally more beneficial. In a review of 41 studies from around the world, organic crops were shown to have significantly higher levels of vitamin C, magnesium, iron and phosphorus. Spinach, lettuce, cabbage and potatoes showed particularly high levels of minerals (Worthington, 2001). Nitrate in high concentrations in vegetables have been linked to gullet cancer, and nitrate levels in organic food are on average 15 % lower (*ibid*). The UK government has provided subsidies to farmers to convert to organic production, due to the beneficial environmental effects. Because organic production has lower yields, and because organic food is perceived to be of higher value, price premiums are placed upon organic produce, such that in most cases organic farming is at least as profitable as conventional farming.

1.3.1 Conventional agriculture

Conventional agriculture remains the norm in Western industrialised countries, despite the costs (financial and ecological) of agrochemicals. Fertilisers and pesticides are applied abundantly and routinely to crops, with the result that there may be nutrient run-off and leaching from farmland into watercourses, a form of pollution which is recognised as a problem (DEFRA, 2002). However, some studies have found that where best practices are followed, such pollution is a function of the nutrient inputs rather than the type of agricultural management (Kirchmann and Bergstrom, 2001). These findings suggest that there tends to be less such pollution in organic farmland due to the fact that nutrient inputs are lower, rather than the form of nutrients used – yet the net result is still that organic farms are less polluting than their conventional counterparts. There are hidden costs of conventional agriculture, with around £120 million a year spent removing pesticides from the UK's water supply, mainly as a result of the chemicals used in conventional farming (Pretty, 2001).

Crop monocultures or two-crop rotations may be practiced, a phenomenon which was encouraged at European level throughout the 1970s (Ewald and Aebischer, 2000). Over time, the use of herbicides rids fields of most weeds, and pesticides have been held responsible for the loss of many insects and other wildlife such as birds (Cramp *et al.*, 1963; Ratcliffe, 1980; Newton and Haas, 1984). A focus

on crop yields and efficiency led to the loss of many hedgerows, although in more recent years government subsidies have encouraged the maintenance and planting of hedgerows since they are believed to be important repositories of biodiversity, which public opinion holds to be important. Conventional agriculture is considered to be the most intensive form of agriculture.

1.3.2 Integrated agriculture

Integrated agriculture aims to "produce profitable crops and also to be environmentally sensitive" (DEFRA, 2002). The intensiveness of this type of management system lies between those of organic and conventional systems. Careful monitoring determines when it is necessary, and economic, to apply synthetic agrochemicals in a targeted way (based upon crop, variety, expected yield, soil analyses linked to global positioning satellite systems, meteorological information etc.; Laegreid *et al.,* 1999). Crop protection from pests is carried out firstly by biological and cultural methods, and pesticides are only used when economically necessary, which in field trials conducted over 10 years translated to a reduction in pesticide use of about 40 % compared to conventional management (Laegreid *et al.,* 1999). Integrated farming systems have exploited the knowledge that more than 4 crop species in a rotation decreases pest carry-over from crop to crop, and hence less pesticide is needed (Ogilvy, 1995). Use may be made of crop rotations and maintaining refuges (such as undisturbed hedgerows) for beneficial organisms which will aid pest control. Slightly fewer nutrients tend to be used than in conventional agriculture, and while paired comparisons found integrated wheat yields to be 5 % lower than conventional wheat yields, the integrated wheat was more profitable due to savings on inputs (Laegreid *et al.,* 1999). Alternatives to conventional ploughing practices may be employed, such as minimal tillage or direct drilling (*i.e.* no-till). Minimizing soil disturbance has been found to increase the numbers of earthworms, which improve soil structure and nutrition (Schmidt *et al.*, 2003). Integrated agriculture is not controlled by any regulating bodies, and therefore covers a wide range of agricultural regimes. Even on one piece of land, the treatments may vary widely from year to year, as perceived as necessary.

1.3.3 Organic agriculture

The main components of an organic farming system are the avoidance of artificial fertilisers and pesticides, the use of crop rotations, and other forms of husbandry to maintain fertility and control weeds, pests and diseases (Soil Association, 2003b). The founders of the organic movement in the UK believed that a prime

aim should be the health of all components of the agricultural system, since this would confer the health of the consumer.

> "The health of soil, plant, animal and man is one and indivisible." (Lady Eve Balfour, 1943)

Since organic agriculture avoids the use of synthetic fertilisers and pesticides, cultural and biological techniques are employed in preference to chemical treatments, for example crop rotations with N-fixing legume crops are used to build soil fertility. Organic fertilisers are based on animal manures and plant materials which have been composted together; green manures may be used. The only permitted inorganic phosphorus supply is rock phosphate from restricted sources which are low in heavy metal contamination. This is relatively unavailable to plants, but can be accessed by a consortium of bacteria and AMF (Piccini and Azçon, 1987). Pesticide use in organic farming is very restricted. In contrast to the 500 synthetic chemicals routinely used in conventional farming, few are permitted in organic farming – the exact limitations on usage vary slightly with certifying body (Table 1.3). They tend to be used as a last resort and some of these are restricted, such that permission must be given on a case by case basis by the certifying body, where all other means have failed and a plan is designed to limit potential future needs for chemical pest control (Soil Association, 2003a). In particular, organic farmers do not use herbicides, some of which (such as isoproturon) have presented particular water pollution problems (DEFRA, 2002). Pesticide pollution from organic farming is far less common than pesticide pollution from conventional agriculture (*ibid.*).

Biodynamic farming parallels organic agriculture in avoiding use of synthetic agrochemicals, instead employing biological and cultural practices. It is set apart by its aims to influence the metaphysical, and it uses special preparations added to compost in homeopathic quantities. Some studies have compared biodynamically managed sites with conventionally managed sites, and for the purposes of literature reviewing, "biodynamic" is considered here as synonymous with "organic" (Carpenter-Boggs *et al.*, 2000).

Organic agriculture has numerous certification bodies (Table 1.4) to regulate standards, which are set down in European law. The standards of these bodies vary, with UKROFS setting minimum legal standards, and the other certification bodies either following these rules or improving on them, with the Soil Association at the forefront of developing and tightening standards (as illustrated in Table 1.3). Certified organic farming and land in conversion to organic production (conversion takes 2 years) accounts for about 4.5 % of UK agricultural land today

(729,550 ha), and current Government funding plans will take it to at least 6 % in England by c. 2006 (Soil Association, 2003b) Organic agriculture is growing in popularity, with an annual growth rate of over 25 % over the last decade (Soil Association, 1999), with a 50 % rise in area farmed organically each year from 1998 to 2001, and a 91 % increase in fully organic land from 2001 to 2002 (Soil Association, 2002b). There are 3,865 UK organic farmers (including around 600 in Wales, 700 in Scotland and 100 in Northern Ireland; *ibid.*).

Table 1.3 Synthetic plant protection products allowed under Soil Association (SA) and United Kingdom Register of Organic Food Standards (UKROFS) standards for organic farming (Soil Association, 2002a). Y = yes, N = no.

Product name	**Description, conditions**	**Permitted?**	
		SA	**UKROFS**
Fatty acid potassium salt (soft soap)	Insecticide	Y, restricted	Y
Sulphur	Fungicide, acaricide, repellent	Y	Y
Copper (copper hydroxide, copper oxychloride, copper sulphate, cuprous oxide)	Fungicide. Limit of 8 Kg Cu ha^{-1} y^{-1}	Y, restricted	Y, restricted
Potassium permanganate	Fungicide, bactericide; only in fruit trees, olive trees and vines	N	Y
Paraffin oil	Insecticide, acaricide	N	Y
Mineral oils	Insecticide, fungicide; only in fruit trees, olive trees, vines and tropical crops	N	Y
Pyrethroids (only deltamethrin or lambdacyhalothrin)	Insecticide, in traps with attractants; only against *Batrocera oleae and Ceratitis capitata wied*	N	Y, restricted
Metaldehyde	Molluscicide, only in traps with repellent to higher animals	N	Y, restricted

Table 1.4 UK organic certification bodies

Soil Association (70% all certified products)
Organic Farmers and Growers Ltd.,
Biodynamic Agriculture Association
Scottish Organic Producers Association
Irish Organic Farmers and Growers Association
UK Register of Organic Food Standards (UKROFS).

Since organic produce is sold at a premium, many crops produced organically are actually more profitable than those produced conventionally. If the cost to the environment of conventional, high input agriculture were included in the price of produce, (e.g. nitrate leaching and hence eutrophication of water bodies) organic production would seem an even more attractive option (Pretty *et al.*, 2001).

1.4 Effects of management factors on AMF

In natural ecosystems, soil nutrients that are available to plants are often in low supply, and AMF play an important role in acquiring mineral nutrients from the soil and passing these to the plant, in exchange for photosynthate. In conventional, intensive agriculture, nitrogen, phosphorus and potassium are supplied in soluble forms which are readily available to the crop plants. This artificial fertilisation reduces the requirement for mycorrhizal association in order to achieve optimum plant growth. Practices of intensive agriculture often impair mycorrhizal formation: inorganic fertilisers, pesticides, tillage, and type of crop may all affect subsequent mycorrhizal infectivity, colonisation and efficiency, and influence mycorrhizal species diversity and populations (Table 1.5). In less intensive agriculture, where more complex forms of nutrients are applied in lower quantities, mycorrhizal functioning may become more important, with systems relying more on biology than chemistry.

1.4.1 Effects of fertilisation on AMF

Under different management regimes, fertiliser input may vary, both in type and quantity. Soil phosphorus is of particular interest here, since AMF are particularly involved with plant uptake of this important nutrient. Conventional farms supply phosphorus as soluble fertiliser, organic farms use more inert forms of phosphorus fertiliser, while in natural ecosystems phosphorus is usually in complex organic form and often limits plant growth.

A study of the long term (111 years) effects of animal manure and NPK fertiliser applications under different cropping systems (Motavalli and Miles, 2002) revealed significant differences in the resulting soil inorganic and organic pools, with both labile and more stable P pools, which were increased by the manure applications. The study also found that soil P pools were affected by tillage, the type of crop (continuous wheat or maize), differences in residue management (removal or incorporation of straw and stalks after cropping), and of type of manure (early use of horse manure contained a high proportion of bedding). Hence it can be seen that soil P dynamics are complex, and influenced not only by fertiliser inputs but also by other factors such as crop species and rotation.

The changing soil P status created by varying fertilisation regimes will impact upon the organisms inhabiting that soil, notably AMF.

Table 1.5 Effects of management practices on AMF (↓ decrease; ↑ increase; = no change)

Management practice	Possible effects on AMF	References
Fertilisation with soluble phosphorus	↓ root colonisation ↓ spores in soil ↓ intra- and extra-radical hyphae	Miller and Jackson, 1998 Dann *et al.*, 1996 Olsson *et al.*, 1997 Hamel *et al.*, 1996
Rock P application	No apparent effects	Dann *et al.*, 1996
Manure application	↓ or ↑ root colonisation	Douds *et al.* 1997 Tarkalson *et al.*, 1998a, 1998b Miller and Jackson, 1998
High soil N	↓ root colonisation ↓ spore populations	Hamel *et al.*, 1996 Miller and Jackson, 1998
Conventional tillage	↓ AMF nutrient uptake ↓ or = in root colonisation ↓ hyphal infectivity of roots Different AMF spore communities	Kraupfenbaur *et al.*, 1996 Gavito and Miller, 1998 Jasper *et al.*, 1989a, 1989b Hamel *et al.*, 1994 McGonigle *et al.* 1990 McGonigle and Miller, 1996
Previous crop non-mycorrhizal	Retarded AMF colonisation ↓ spore populations	Gavito and Miller, 1998 Douds *et al.*, 1997 Ryan and Ash, 1996
Many crops in rotation	↑ spore populations	Miller and Jackson, 1998
Pesticide application	↓ root colonisation ↓ hyphal P uptake	Miller and Jackson, 1998 Schweiger and Jakobsen, 1998
Soil liming	↑ root colonisation ↑ infectivity	Hamel *et al.*, 1996

Mycorrhizal fungi seem to flourish in soils of high organic matter, whilst many studies have concluded that soluble P inhibits AMF formation. For instance, high amounts of synthetic P and N fertilisers were associated with low AMF colonisation of lettuce (*Lactuca sativa* L.), while organic additions to the soil increased AMF percentage root length colonisation (Miller and Jackson, 1998). Wheat on an organic farm was found to have AMF percentage root length colonisation consistently 2 - 3 times greater than on a conventional neighbour (Ryan *et al.*, 1994) which was attributed to soluble P application, which had both immediate negative effects on colonisation and long term depressive effects on inoculum levels. The thresholds at which increased soluble soil P impact

negatively upon AMF vary with crop species, which have differing degrees of mycorrhizal dependency. For instance, leek (*Allium porrum*) has a high mycorrhizal dependency and responsiveness (Eason *et al.*, 1999) and the sensitivity of AMF in symbiosis with leek to soil P is lower than for AMF in symbiosis with less mycorrhizal-responsive crops such as wheat.

Application of the insoluble reactive rock phosphate (RRP), which is used in organic farming, had no apparent inhibitory effects on AMF: Dann *et al.* (1996) applied superphosphate or RRP to organically and conventionally grown wheat, and in the second year observed that superphosphate increased yield, biomass and grain P content, but decreased colonisation by AMF. However, high (60 – 100 %) AMF colonisation has been observed on soybean growing in soils of high P fertility (Khalil *et al.*, 1992), and moderate application of mineral fertilisers helps optimise AMF effectivity (Kraupfenbauer *et al.*, 1996). For the crop grower, then, it may be desirable to ignore the AMF and apply high rates of fertilisers, or to work towards maximising the benefits of the symbiosis by finding a balance between applying sufficient fertiliser and amounts which are inhibitory to AMF, a balance which varies with crop species. Removal of AMF from oilseed flax (*Linum usitatissimum* L.) by fumigation revealed that the effects of AMF increased as soil P ($NaHCO_3$-extractable) decreased (Thingstrup *et al.*, 1998). It was concluded that AMF are essential to flax growth below a threshold of 40 mg P Kg^{-1} soil, that is, for most flax crops.

The type of nutrient input will impact upon the communities of micro-organisms present in the soil, which in turn will mediate the effects of fertilisers on plant growth. Within the group of non-synthetic fertilisers, AMF responses vary. Spore populations of *Glomus* spp. and *Glomus etunicatus*-type were greater following fertilisation by either chicken litter or cow manure that had been composted with leaf litter, compared to applications of raw cow manure or synthetic fertiliser (in wheat and maize crops; Douds *et al.* 1997). A study by Piccini and Azçon (1987) grew alfalfa plants with or without rock phosphate, phosphate-solubilizing bacteria (PSB) and different strains of AMF. They showed that *Glomus fasciculatum* inoculation gave significantly greater shoot biomass than did *Glomus mosseae* and another *Glomus* sp., and the biomass was further increased when PSB were also present.

A study by Johnson (1993) found that 8 years of mineral fertilisation altered AMF spore populations (decreasing relative abundance of *Gigaspora gigantea, G. margarita, Scutellospora calospora* and *Glomus occultum*, whilst increasing that of *Glomus intraradix*) and that this had functional implications. Interestingly,

fertilisation seemed to select for less mutualistic fungi which exerted a higher carbon drain on the plants (big bluestem grass, *Andropogon gerardii*), resulting in smaller plants which produced fewer inflorescences. Johnson (1993) suggested that where soils are well-fertilised, plants have little to gain from the arbuscular mycorrhizal symbiosis and allocate less carbohydrates to root exudates. In response to this, aggressive strains of AMF which nevertheless are able to extract plant carbohydrates will increase at the expense of more mutualistic AMF. These traits were observed (ibid.) in the morphology of AMF inhabiting roots in the fertilised plots, which had the same numbers of vesicles but fewer hyphae and arbuscules, and therefore a decreased ability to acquire and transfer to the plant mineral nutrients from the soil.

P fertilisation may affect the fungal allocation of biomass between soil and roots, and between mycelial and storage structures. Increasing the soil P level resulted in less extra- and intra-radical AMF hyphae, as measured by both fatty acid signatures and microscopic measurements (Olsson *et al.*, 1997), and there were indications that less biomass was apportioned to storage structures at higher P levels. This could have important bearings on the many studies which use spore counts to measure mycorrhizal presence: low spore counts may indicate reallocation of fungal biomass rather than change in fungal populations.

1.4.2 Pesticide use

Pesticide use may impact upon AMF, either directly or indirectly. Negative correlations of pesticide application and AMF colonisation have been observed in lettuce (Miller and Jackson, 1998). Split root experiments have shown that the effects of pesticides depend upon individual pesticides. Hyphal P uptake was completely inhibited by the fungicide carbendazim at levels below expected field concentrations, the fungicide propiconazole had some negative effects on hyphal P uptake at 10 times higher than field concentrations, while the fungicide fenpropimorph and the insecticide dimethoate had no such negative effects even at 100x field concentrations (Schweiger and Jakobsen, 1998). Ryan *et al.* (1994) did not find any significant effects of herbicides or seed dressings on AMF.

1.4.3 Disturbance through cultivation and tillage

Regular ploughing decreases soil aggregate size, increases aeration, and increases microbial access to intra-aggregate organic matter. Hence decomposition of soil organic matter (SOM) proceeds faster following ploughing (Adu and Oades, 1978). Minimal soil disturbance, such as the use of non-inversion tillage, aids the retention of soil structure and fertility, hence encouraging beneficial soil organisms (Ogilvy, 1995). Ploughing is a practice

typical to most conventional and organic farms, whilst integrated farming may use minimal or no-till techniques combined with herbicides to control weeds.

Disturbance, such as tillage, can have large impacts on mycorrhizal fungi, and decrease their effectivity of nutrient uptake (Kraupfenbauer *et al.*, 1996). Various methods exist for tilling; conventional tillage disturbs the soil most, breaking up the hyphal network and decreasing its infectivity and effectivity of nutrient uptake (McGonigle *et al.* 1990). There is an inverse relationship between intensity of tillage and shoot P concentration at the 5 - 6 leaf stage in maize (*ibid.*). However, tillage has indirect effects upon plants through effects on physical edaphic factors. No-till treatment resulted in reduced early maize growth compared to conventional tillage, partly because shading and restricted evaporation at the soil surface lowered soil temperatures by 0.5 °C to 2 °C, and perhaps also due to the greater mycorrhizal colonisation and hence C demand on the plant (McGonigle and Miller, 1996). However, other studies have found no significant differences in mycorrhizal colonisation following tillage (Gavito and Miller, 1998), suggesting the situation is complex.

Jasper *et al.* (1989a, 1989b) examined the effects of disturbance on AMF in glasshouse experiments. When soil was mixed for one minute, subsequent AMF formation was almost eliminated, yet undisturbed controls showed rapid and extensive colonisation of bioassay plants. Jasper *et al.* (1989b) demonstrated that AMF hyphae can colonise plant roots in the absence of spores, and that soil disturbance severely decreased hyphal infectivity. It was suggested that hyphae may be of primary importance in AMF colonisation, since germination of spores seems unpredictable and may take time. If this is the case, colonisation of roots will be severely limited where soil is thoroughly disturbed.

1.4.4 Crop species

Crop species, and hence crop rotations, affect spore populations. Gavito and Miller (1998) examined tillage, P fertilisation and crop species for effects on mycorrhizal development in maize, and found the latter to be the most important factor. Where the previous crop was a "non-host" crop, mycorrhizal development in maize was delayed both in field samples and in bioassays, and spore populations were lower (Ryan and Ash, 1996; Douds *et al.* 1997). Miller and Jackson (1998) showed the number of AMF spores in soil was strongly correlated to the number of host crops in a rotation. One explanation for this is that different host species are associated with different AMF species, and fungal diversity is dependent upon plant diversity, a contradiction of the earlier view that AMF have low host specificity (Harley and Smith, 1983; Law, 1988; Helgason *et al.*, 1998).

More recent findings have shown that AMF host specificity can occur: Eom *et al.* (1999) isolated 16 AMF species from a prairie grassland, and found that 9 of these differed significantly in their spore abundance when in association with 5 host plant species, both in field samples and in pot culture. Previously, Bever *et al.* (1996) had made similar findings in laboratory microcosms, observing that association of AMF with particular host plants in the field was positively correlated with the sporulation rates observed on those hosts in the microcosm experiments. Such findings make a strong case that crop monocultures will decrease abundance of certain AMF species, and overall AMF diversity.

The effect of crop species on mycorrhiza should be taken into account when making management decisions, particularly following a drought year or other conditions which significantly deplete soil AMF inocula. The use of well-adapted crop species and cultivars can increase the effectivity of mycorrhiza (Kraupfenbauer *et al.* 1996), and the reverse seems also true. Ryan and Ash (1996) observed that drought in Australia decreased AMF inocula such that growing an AMF dependent crop became difficult, yet if a non-host crop was grown, AMF inocula levels fell still lower. In such situations intercropping may be a biologically and economically viable solution.

1.4.5 Management intensity and AMF

Just as individual factors such as fertilisation or tillage affect the presence and functioning of AMF, so different management intensities affect AMF, since the management regimes are combinations of the various agricultural practices outlined above. It is possible that factors affecting AMF may in combination act additively, synergistically, that a maximum impact has already been effected by a single factor, or that different factors act in opposite directions thus cancelling each other out. As it has previously been alluded, generalisations about management influences on AMF are difficult to make since there are so many site-specific variables. It is rare that trials comparing agricultural management systems are controlled so as to be directly comparable, and are long term enough to be realistic. Organic and conventional management systems differ in a number of ways. While experiments which examine single variables, such as fertiliser application, are useful in elucidating those given factors, it is essential to compare the overall results of different farming systems. Very few studies of AMF in different farming systems have been conducted.

In comparing farmed and unfarmed land, Helgason *et al.* (1998) showed, using molecular sequencing of partial fungal small subunit ribosomal RNA, that the diversity of AMF in arable sites is strikingly low compared with woodland sites. In

arable crops, 92 % of sequences represented *Glomus mosseae* or its close relatives, whilst the woodland sites had a much higher diversity of fungal types (Shannon-Weiner H = 0.144 in woodland samples compared to H = 0.398 in arable sites). Since a broad host range was exhibited by AMF, the authors concluded that the change in fungal sequence composition and low diversity of the fungi in arable fields was not due to the monocultures planted in agriculture, but rather reflected aspects of the agronomic regime such as ploughing, fertilisation, and fungicide application.

Organic arable farms in south-east England were found to generally have higher soil organic matter (SOM) content than similar conventional farms, which was attributed to manure application and inclusion of leys on organic farms (Armstrong Brown *et al.*, 1995). Lowered SOM is associated with lowered soil faunal populations, impoverished nutrient status, decreased water retention, and increased susceptibility to erosion. Yeates *et al.* (1997) also found organically managed grassland soils had higher organic matter content, and also greater organic C and microbial biomass C, greater respiratory and dehydrogenase activities, compared to conventionally managed grassland, but only on silt and sand soils, not loam. However, the loam site had been sown with a cereal crop 2 years previously, interrupting the long-term pasture management. Thus the type of agricultural management can impact upon a range of edaphic factors, which are likely to affect the indigenous AMF.

Addition of manures and other organic matter to agricultural soils will usually improve soil structure, aeration, water penetration, water-holding capacity, cation exchange capacity, soil buffering, microbial activity, and nutrient availability, and hence improve crop growth. However, the impact of organic inputs on AMF is not clear-cut, with some research finding it increases AMF colonisation of roots, whilst other research has found the reverse. Tarkalson *et al.* (1998b) grew corn and wheat on plots of topsoil or subsoil which were either treated with manure or treated with conventional fertiliser plus added Zn. In that study the percentage mycorrhizal colonisation was significantly higher in plots receiving conventional fertiliser plus Zn than in manured plots, which the authors suggested may be partly attributable to increased microbial competition with AMF in the rhizosphere, however, their soil analysis data shows that manured plots were far higher in soil P than the conventionally fertilised plots (95 and 142 mg P Kg^{-1} compared to 22 and 30 mg P Kg^{-1}) so it is likely that the high soil P limited AMF colonisation in the manured plots. The percentage AMF colonisation of wheat was also significantly higher in subsoil plots than topsoil plots, which Tarkalson *et al.* (1998b) saw as following the commonly held view that "lower fertility soils are benefited more

from mycorrhizae than highly fertile soils". The concentrations of Zn and Mn in the plants correlated with the percent AMF colonisation, suggesting that AMF enhanced plant Zn and Mn uptake.

General soil conditions were more strongly correlated to AMF spore populations than were management inputs (Miller and Jackson, 1998), with lower spore populations where soils were high in total P, N and C. It must be borne in mind, however, that spore numbers may not relate directly or at all to AMF colonisation or infectivity, since hyphae can perform these functions (Jasper *et al.*, 1989b).

Ryan and Ash (1999) studied whether the differing soil microbial communities of conventional and biodynamic (~17 years) dairy pastures resulted in different nutrient pathways, and concluded that there were no significant differences in mycorrhizal response to application of soluble P and N fertilisers. It would be interesting to see a follow-up study using insoluble P and N fertilisers.

A comparison of organic and conventional farms with similar edaphic and physical features showed that mycorrhizal colonisation of wheat roots was consistently over 3 times greater on organic wheat at 10 - 21 weeks (Ryan *et al.*, 1994). AMF colonisation of organic wheat rose rapidly from planting to tillering, while at the conventional site AMF infection was only initiated during the fourth week from planting. Management factors were thought responsible for differences, primarily the form of P fertiliser (insoluble or soluble); however, AMF inocula levels were significantly higher on the organic site. Ryan *et al.* (1994) point out the difficulty of separating the effects of soluble P levels from AMF inocula levels in studies comparing results of different management systems.

Arbuscular mycorrhizal colonisation and infectivity were examined during a 3-year trial cropping barley on a newly cultivated nutrient-poor and acidic soil, at different levels of soluble P fertilisation, liming and tillage (Hamel *et al* 1996). Hamel *et al.* (1996) found that AMF root colonisation of barley fell significantly over the three years under minimum tillage (5.4 % to 1.9 % RLC) and under reduced tillage (6.1 % to 1.8 % RLC) and both fell and rose under conventional moldboard tillage (1.7 % in 1988, 5.3 % in 1989, 2.6 % in 1990 RLC). The application of P fertiliser at 4 levels (0, 44, 86, and 130 Kg P ha^{-1}) decreased barley root colonisation linearly, whilst liming (also at 4 levels, 0, 3, 6, 12 T $CaCO_3$ ha^{-1}) increased colonisation in a quadratic fashion. The number of infective AMF propagules was determined under the same conditions, using the most probable number method, and was found to increase year on year under all tillage treatments, with a positive interaction with liming. However, the number of infective propagules was not affected by the P fertilisation treatments. The

authors point out that the positive effect of liming, in increasing mycorrhizal colonisation and infectivity, could either be a direct result of fungi proliferating in the soil, or an indirect result mediated by the plant response to liming. Interestingly, the number of propagules was inversely related to the mycorrhizal receptiveness of the host barley plants, as determined by percentage root length colonisation at harvest. The authors point out that they did not measure the total length of mycorrhizal roots, and that small stressed plants may develop higher percentages of internal root colonisation. Therefore the observed correlation between plant productivity and higher numbers of infective propagules may have been due to the large increase in biomass production of a host (with relatively lower root percentage colonisation levels, but not necessarily lower total length of root colonisation) leading to a net increase in overall mycorrhizal propagule production.

Hamel *et al.* (1994) tested the effects of different soil tillage, P fertilisation and liming levels on the composition of the AMF population at the same site. They recovered spores of 13 species from three genera, *Glomus, Gigaspora* and *Scutellospora.* They could not find any significant effects of the imposed treatments, but found that AMF species diversity (the number of species per plot, identified from spores) increased significantly over the three years, and the frequency of species changed with time, with *Gigaspora margarita and G. caledonium* disappearing after cultivation of the land. The absence of observed effects on species abundance in their three tillage intensities indicates that either tillage had little effect, or that their minimal tillage (working the soil over a depth of 5 - 7 cm) already maximally disturbs these fungi. Contrastingly, the relative abundance of other AMF species (*Glomus aggregatum*) increased over the experimental period, indicating that AMF species vary in their tolerance of agricultural cultivation.

Numerous studies have attempted to elucidate effects of one or two factors, such as fertilisation, tillage, or crop species, on AMF presence on roots and in the soil. Fewer studies have examined the relationship between a wide range of management factors on AMF. One such study by Miller and Jackson (1998) analysed the effects of 14 management practices (including both conventional and organic farming systems) and 14 soil and environmental conditions on AMF colonisation of lettuce, and presence of AMF spores in soil. Of the 18 fields they studied, AMF root length colonisation varied from 2 % to 60 % of root length, but only one field had over 50 % root length colonisation: this was a field cultivated organically for only a few years following being an abandoned orchard. Other fields with the highest percentage root length colonisation were also under

organic management. Spore number in the soil followed different trends from root colonisation, with these two factors being affected to differing degrees by various management practices. Interestingly, tillage had very little effect on the AMF in this study, possibly due to the fact that all fields were subject to some degree of disturbance and field traffic. It is clear from these and other such results that a wide range of factors affect the behaviour of AMF, and that relationships between AMF and their environments are very complex.

1.5 Benefits of mycorrhiza in agriculture

Given that many farmers are able to apply sufficient fertilisers to ensure optimum crop growth, should they be concerned over the mycorrhizal status of their soils? The review paper by Ryan and Graham (2002) observes that profitability may sometimes be enhanced by practices which inhibit AMF, such as P-fertilisation and tillage, since growth responses are often not observed with AMF presence. They describe results of trials in which P fertilisation (20 Kg ha^{-1}) increased shoot biomass, yield, and shoot P content of wheat and of field pea (*Pisum sativum*) more than did AMF colonisation. However, the grain zinc content was strongly correlated with AMF colonisation for both wheat (R^2 = 97 %, y = 0.25x + 11.03) and field pea (R^2 = 98 %, y = 0.39x + 18.95). Ryan and Graham (2002) state that crop micronutrient uptake may now require serious consideration in agriculture, since there is rising concern about the implications for human health of the low concentrations of zinc and other micronutrients in modern agricultural produce. The findings of Worthington (2001) that organic fruits, vegetables and grains are frequently of greater nutritive value than those which were conventionally produced, provides a strong argument for furthering organic production. This, together with the fact that more and more land is being converted to organic management, validates the need to examine the role of the arbuscular mycorrhizal symbiosis in these low-input agricultural systems.

AMF are more than facilitators of nutrient uptake: they contribute to the stability of soil aggregates (Tisdall, 1994), hence maintaining good soil structure and reducing soil erosion. Some mycorrhiza help protect plants from root pathogens (Newsham *et al.* 1995), and drought (possibly indirectly through improving plant phosphorus nutrition). From these attributes, Kling and Jakobsen (1998) conclude that both quantitative and qualitative measurements of mycorrhiza should be included in assessment of soil health. Various authors (see Penfold *et al.*, 1995) propose that soil health be included in economic assessments of farming systems, as should environmental parameters such as pollution levels of water bodies and their subsequent clean-up costs, or the biodiversity of

hedgerows. Accurate economic assessment of the overall total impact of a given farming system is difficult and controversial, yet the side effects of, for example, heavy fertilisation are paid for in some way. Organic produce is able to claim a premium price, which is required for organic production to remain economic. Some critics of conventional agriculture suggest that it should shoulder the costs of its negative effects (*e.g.* Pretty, 2001), such as pollution, loss of biodiversity, and soil erosion. Whilst this is a highly unlikely prospect, it is a valid point that the full impacts of an agricultural system should be considered, such as the maintenance of a healthy soil which is stable and resistant to erosion, and any positive impacts upon biodiversity.

1.6 Themes examined in this thesis

Given that differences have been found in the identities of AMF from different management intensities, and that AMF functionality is known to vary between species and isolates, it is to be expected that differences may occur in AMF functionality between different management systems. The following chapters describe experiments focussed upon two key aspects of the arbuscular mycorrhizal symbiosis: P transfer from AMF to plants (Chapters 3 and 4), and C transfer from plants to AMF (Chapters 5 and 6). Unlike many studies on AMF, which use known single species from culture, here the experimental systems use AMF communities from the field. This is intended to give a picture of what is actually occurring in those field sites, and therefore have a high degree of relevance to the real world.

1.7 Aims and hypotheses

This research project aims to examine mycorrhizal functioning in soils under different management intensities, with particular focus on the functioning of the external AMF mycelium which is likely to be most susceptible to certain agricultural management practices. Certain basic questions need to be elucidated, such as whether there are differences in phosphorus translocation from AMF to plant hosts, and whether the carbon transport from plants to their mycorrhizal fungal partners varies under different management systems. Related issues also need to be investigated, such as any relationships of mycorrhizal phosphorus and carbon transfer with AMF colonisation, plant phosphorus status or soil phosphorus availability.

It is hypothesised that the importance of mycorrhizal functioning increases with decreasing management intensity, and that this will result in increased AMF functioning. Accordingly, both phosphorus uptake by AMF and carbon

translocation from the host plant to the fungal symbionts are hypothesised to be greater where there is less intensive management, and greatest in "undisturbed" environments. The degree of mutualism, in terms of the relative proportions of P and C, may also vary, with less mutualistic fungi being selected for in high intensity management systems. This would manifest as greater quantities of C per unit of P being transferred under higher management intensities, such that the plant receives less benefit from the AMF.

1.8 Summary

Arbuscular mycorrhiza occur widely and often confer benefits to plants, particularly by aiding plant phosphorus nutrition. The occurrence and functioning of mycorrhiza is variable, and evidence suggests that their importance may be very different in natural ecosystems than in land under organic or conventional management. It is clear that mycorrhiza are affected by certain management practices, and mycorrhizal functioning probably varies with management intensity. The following chapters describe experiments designed to elucidate the functioning of AMF in systems under varying management intensities. If mycorrhizal functioning was more fully understood, it may be possible to apply that knowledge to improve plant nutrition in low-input farming systems, and to optimise phosphorus uptake efficiency.

Chapter 2:
Description of field sites, general materials and methods

Chapter 2: Description of field sites, general materials and methods

2.1 Introduction

This chapter details field sites and methods used for work described in the following four chapters. More specific details, or modifications of the methods detailed here, are given in the relevant chapters.

2.2 Site descriptions

Each of the following chapters uses turfs originating in various field sites (Table 2.1). The living material used in Chapter 3 originated in a series of fields belonging to CWS Agriculture, sited near Stoughton and Quenby, Leicestershire. These fields were chosen to represent a variety of management systems, ranging from permanent organic pasture to integrated and conventional cropping systems. The experiments related in Chapters 4 and 6 use a series of turfs from the same sites, which were chosen from across England to have organic and conventional fields under winter wheat at the time of sampling. A field margin was also sampled from each of the fields studied in Chapters 4 and 6, to provide a relatively undisturbed comparison paired to the fields, as explained in Section 4.1. Photographs of these fields and their margins are given in Figures 2.1 and 2.2, and their management histories are given in Tables 2.2 and 2.3. The experiment described in Chapter 5 took turfs from a calcareous semi-natural grassland site in Wardlow Hay Cop, Derbyshire (Ames, 2003). The site was chosen because it has a broad range of mycorrhizal species and has been pasture since records began.

2.2.1 Nutrient status of field sites

Basic measurements of soil nutrient status were taken from the field sites. Using methods described below, extractable phosphorus, nitrate and ammonium, loss on ignition, and pH in water and in CaCl were determined (Tables 2.4 and 2.5).

2.3 Materials and methods

2.3.1 Soil sampling and preparation

When sampling from fields, care was taken to avoid land within approximately 50 m of the edges of the field, ground over which tractors or other vehicles were known to have driven, and any other areas deemed to be unrepresentative of the field. Where several turfs were taken from one field, they were taken in an irregular pattern, such that no two samples were taken from the same furrow. The

Table 2.1 Location and soil types (according to Soil Survey of England and Wales, 1984) for field sites used in Chapters 3 – 6.

Site	Location		Soil type
High Mowthorpe, North Yorkshire	N 54:06:17	W 0:38:53	Typical brown calcareous earth
Loddington, Leicestershire	N 52:36:53	W 0:50:31	Reddish silty clay loam
Quenby, Leicestershire	N 52:38:35	W 0:58:14	Typical brown earth
Stoughton, Leicestershire	N 52:37:09	E 1:03:45	Clayey pelo-stagnogley soil
Terrington St Clement, Norfolk	N 52:44:38	E 0:17:22	Calcareous alluvial gley soil
Wardlow, Derbyshire	N 53:16:02	W 1:43:27	Shallow rendzina

Table 2.2 Cropping history of fields used in Chapters 4 and 6 (Org = organic, Conv = conventional, Int = integrated management regime)

Site	Field	Crop history								
Quenby	Org pasture 1 - 4	Permanent pasture for many decades								
Stoughton	Conv pasture 1, 2	Permanent pasture since 1945								
Stoughton	Conv wheat 1 – 4 and Int wheat 1 - 4	Winter wheat 2001	Italian ryegrass silage ley 2000	Italian ryegrass silage ley 1999	Winter wheat 1998	Winter beans 1997	Winter wheat 1996	Set-aside 1995	Winter wheat 1994	Winter rape 1993
Wardlow	Pasture	Permanent semi-natural grassland, lightly grazed in Summer								

Fig. 2.1 Photographs of organic (left) and conventional (right) fields from Stoughton (A), Loddington (B), Terrington (C) and High Mowthorpe (D), sown with winter wheat. Shown at time of sampling.

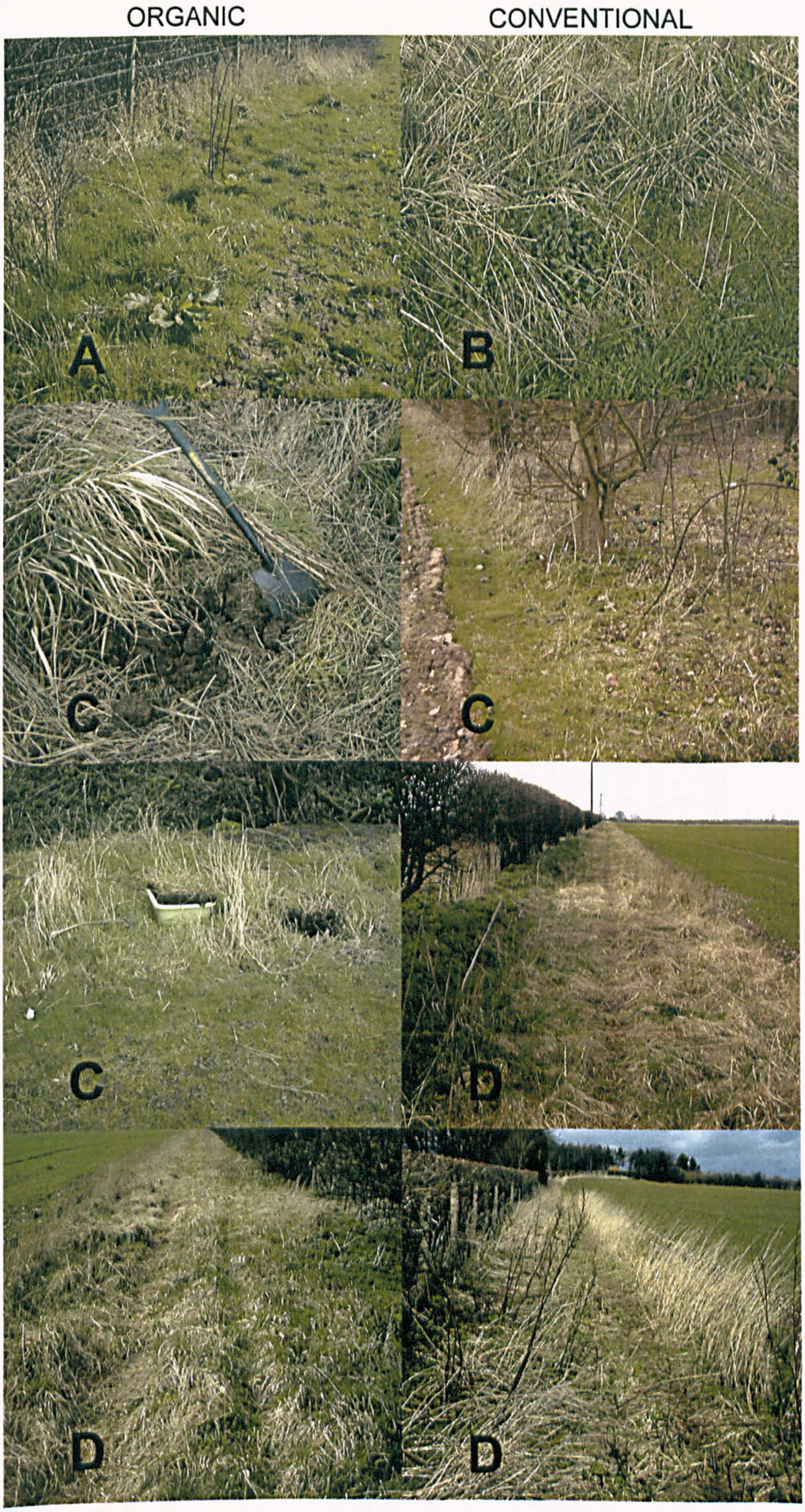

Fig. 2.2 Photographs showing field margins of organic (left) and conventional (right) fields at Stoughton (A), Loddington (B), Terrington (C) and High Mowthorpe (D).

Table 2.3 Cropping history of fields used in Chapters 4 and 6. (Org = organic, Conv = conventional, FM = field margin).

Site	Field	Crop and planting date					
High Mowthorpe, North Yorkshire	Conv wheat 1	Winter wheat 2001	Potato 2001	Spring barley 1999	Winter wheat 1997		
	Conv wheat 2	Winter wheat 2001	Potato 2001	Spring barley 1999	Winter wheat 1997		
	Org wheat	Winter wheat 2001	Potato 2001	Spring Wheat 1999	Spring wheat 1998		
Loddington, Leicestershire	Conv wheat	Winter wheat 2001	Winter wheat 2000	Winter beans 1999	Winter wheat 1998		
Stoughton, Leicestershire	Org wheat	Winter wheat 2001	Winter rye/potatoes 2000	Winter beans 1999	Winter oats 1998	Winter wheat 1997	Grass/clover ley 1994-1996
Terrington, Norfolk	Conv wheat	Winter wheat cv. Consort 31/10/01	Winter wheat cv. Claire 13/10/00	Winter beans cv. Clipper 17/11/99	Winter wheat cv. Madrigal 01/10/98	Winter wheat cv. Madrigal 15/10/97	Winter wheat cv. Riband 17/12/96
	Org wheat 1	Winter wheat cv. Hereward 17/10/01	Potato cv. Sante 07/05/01 – 16/10/01	White clover cv. Aran & Lucerne cv. Vertus 10/04/99	Spring Barley cv. Derkado 23/02/99	Turnip cv. Appin27/08/98	Spring beans cv. Alfred 19/02/98
	Org wheat 2	Winter wheat cv. Hereward 19/10/01	Potato cv. Sante 05/05/01 – 16/10/01	White clover cv. Aran & Lucerne cv. Vertus 09/04/99	Spring wheat cv. Chablis 11/02/99	Spring wheat cv. Imp19/02/98	Winter wheat cv. Riband 15/10/96

Table 2.4 Soil nutrient data for fields used in experiments described in Chapters 3 and 5. (Org = organic, Conv = conventional, Int = integrated management regime). Data are given as mean (standard error), expressed on a dry weight basis, except for pH data which are median (range).

Site	Field	Bicarbonate extractable P ($\mu g\ P.g^{-1}$ soil)	KCl extractable NH_4-N ($\mu g\ N\ g^{-1}$ soil)		KCl-extractable NO_3-N ($\mu g\ N\ g^{-1}$ soil)		Loss-on-ignition (%)		pH in water		pH in CaCl	
Quenby	Org pasture 1	15.42	0.94	(0.341)	106.9	(3.29)	11.3	(0.31)	6.3	(0.08)	5.5	(1.11)
Quenby	Org pasture 2	10.28	0.55	(0.406)	94.75	(30.8)	12.0	(0.76)	5.5	(1.53)	6.2	(0.03)
Quenby	Org pasture 3	3.63	9.92	(0.239)	44.64	(1.25)	10.7	(0.38)	4.3	(1.69)	4.9	(0.11)
Quenby	Org pasture 4	2.28	0.45	(0.423)	61.96	(1.76)	11.4	(0.14)	6.7	(1.63)	5.9	(0.09)
Stoughton	Conv pasture 1	28.13	1.08	(0.670)	145.5	(48.2)	7.5	(0.08)	8.0	(0.03)	7.4	(0.07)
Stoughton	Conv pasture 2	35.80	0.01	(0.005)	59.95	(6.82)	6.3	(0.04)	6.5	(0.03)	5.6	(0.04)
Stoughton	Conv wheat 1	17.31	0.56	(0.279)	34.08	(7.48)	5.0	(0.02)	7.0	(0.10)	5.8	(0.06)
Stoughton	Conv wheat 2	16.10	0.82	(0.280)	47.86	(5.85)	5.9	(0.11)	7.1	(0.25)	6.2	(0.21)
Stoughton	Conv wheat 3	5.34	1.19	(0.239)	71.27	(9.77)	5.9	(0.03)	6.6	(0.03)	4.6	(0.95)
Stoughton	Conv wheat 4	10.51	0.02	(0.001)	16.98	(1.84)	6.1	(0.03)	7.7	(1.67)	6.8	(0.17)
Stoughton	Int wheat 1	31.39	0.57	(0.430)	33.46	(4.51)	9.9	(4.42)	7.1	(1.57)	6.5	(0.20)
Stoughton	Int wheat 2	33.44	0.02	(0.016)	49.78	(8.40)	6.2	(0.09)	8.1	(0.09)	7.3	(0.95)
Stoughton	Int wheat 3	10.32	0.59	(0.086)	58.81	(7.61)	7.8	(0.02)	8.3	(0.12)	7.6	(0.01)
Stoughton	Int wheat 4	11.62	0.38	(0.120)	53.47	(9.87)	6.9	(0.07)	7.8	(0.04)	6.8	(1.02)
Wardlow	Pasture, National Nature Reserve	15.42	1.58	(0.310)	29.49	(6.89)	12.0	(0.82)	6.8	(0.87)	5.9	(0.18)

Table 2.5 Soil nutrient data for fields used in experiments described in Chapters 4 and 6. (Org = organic, Conv = conventional, FM = field margin). Data are given as mean (standard error), expressed on a dry weight basis, except for pH data which are median (range).

Site	Field	Bicarbonate extractable P ($\mu g\ P\ g^{-1}$ soil)		KCl extractable NH_4-N ($\mu g\ N\ g^{-1}$ soil)		KCl-extractable NO_3-N ($\mu g\ N\ g^{-1}$ soil)		Loss-on-ignition (%)		pH in water		pH in CaCl	
High Mowthorpe, North Yorkshire	Conv wheat 1	36.44	(3.7)	1.40	(0.52)	2.87	(0.50)	6	(0.21)	7.8	(0.05)	7.2	(0.13)
	Conv FM 1	38.00	(2.6)	3.51	(1.09)	6.81	(0.24)	7.1	(0.36)	5.7	(0.03)	5.0	(0.02)
	Conv wheat 2	38.68	(0.3)	2.73	(0.78)	3.29	(1.31)	7.2	(0.13)	7.2	(0.09)	6.6	(0.15)
	Conv FM 2	33.30	(2.0)	1.53	(0.26)	1.39	(0.63)	7	(0.28)	6.5	(0.17)	5.8	(0.26)
	Org wheat	34.41	(11.7)	2.17	(0.53)	1.07	(0.58)	7.5	(0.14)	7.7	(0.15)	7.1	(0.02)
	Org FM	27.47	(11.3)	1.98	(0.50)	1.51	(0.40)	26	(17.7)	7.8	(0.34)	7.2	(0.05)
Loddington, Leicestershire	Conv wheat	39.84	(0.5)	1.66	(0.44)	0.83	(0.31)	6.5	(0.06)	7.1	(0.13)	6.2	(0.02)
	Conv FM	40.73	(4.6)	2.67	(0.53)	4.30	(0.03)	6.7	(0.50)	6.6	(0.37)	5.8	(0.35)
Stoughton, Leicestershire	Org wheat	6.34	(0.9)	2.68	(0.15)	0.94	(0.01)	5.9	(0.20)	6.8	(0.07)	6.2	(0.08)
	Org FM	14.67	(2.8)	5.77	(0.15)	0.98	(0.43)	9.1	(0.31)	6.3	(0.17)	5.5	(0.04)
Terrington, Norfolk	Conv wheat	54.53	(13.4)	2.16	(0.09)	0.73	(0.27)	3.8	(0.11)	8.1	(0.29)	7.2	(0.44)
	Conv FM	84.29	(5.4)	2.03	(0.13)	0.78	(0.22)	5.1	(0.17)	7.8	(0.11)	6.9	(0.08)
	Org wheat 1	1.5	(3.4)	1.94	(0.16)	0.64	(0.36)	4.5	(0.01)	8.3	(0.07)	7.5	(0.03)
	Org FM 1	13.67	(0.9)	2.54	(0.66)	0.66	(0.37)	4.4	(0.08)	8.2	(0.15)	7.5	(0.08)
	Org wheat 2	22.28	(1.6)	1.50	(0.03)	2.83	(0.85)	3.5	(0.10)	8.1	(0.12)	7.3	(0.07)
	Org FM 2	81.99	(7.6)	2.60	(0.10)	0.87	(0.36)	6.6	(0.01)	7.9	(0.14)	7.2	(0.18)

aim of all these measures was to take samples which were representative of the field as a whole, and not unduly affected by soil compression caused by vehicles, or other possible gradients in chemical applications, soil water drainage, or the congregation of farm animals.

Turf samples were removed by cutting a block of turf, using a spade, and placing it directly into a plastic tub for transportation. At the same time as removal of the turf, soil was sampled adjacent to the hole from which each turf was removed, using a soil corer (32 mm diameter) to a depth of 15 cm, and sealed inside polythene bags. These soil cores were refrigerated at 4 °C until the various nutrients were measured, which was always within 7 days from sampling for extractable phosphorus, nitrate and ammonium. Soil pH was measured within 14 days. Prior to nutrient analysis, each soil core was sieved to 2 mm, removing large stones and roots, and mixed thoroughly. The analysis for each core was carried out separately, and the values for the cores within the same field were meaned and shown in Tables 2.4 and 2.5.

After being transported from the field, the turf monoliths were maintained in controlled environment rooms as described in Chapters 3 – 6. The monoliths were carefully cut, using a sharp knife, into the shape of the pot into which they were to be placed. These pieces of turf were then inserted into their pots, ensuring that no pockets of air remained between the soil and the walls of the pot. The pots were placed in drip trays, and watered by filling the drip trays with distilled water (d.H_2O).

2.3.2 Extractable soil phosphorus

The extractable soil phosphorus (P) was measured using the method of Olsen *et al.* (1954). Firstly 2.5 g soil (+/- 0.02 g, weighed to 0.0001 g) was placed into a 250 ml plastic bottle. To this was added 50 ml 0.5 M $NaHCO_3$ (adjusted to pH 8.5), and 0.8 ml of charcoal suspension (15 g charcoal in 90 ml d.H_2O). The bottle was capped and shaken for 30 minutes in an end-over-end soil shaker. The soil suspensions were then filtered (Whatman No. 40) and analysed by a modification of the Murphy-Riley method (Murphy and Riley, 1962). Blanks were carried throughout the procedure, and the $NaHCO_3$-charcoal suspension from these blanks used for the standards, which contained known volumes of di-sodium hydrogen orthophosphate.

2.3.3 Colorimetric determination of P concentration

Phosphorus concentration was determined colorimetrically following formation of a blue molybdo-phosphate complex (Murphy and Riley, 1962). In the modified

method used here, 0.5 ml aliquots of each filtered sample were placed in two plastic 4.5 ml cuvettes (one of these for colour development, and one as a standard, without ascorbic acid which catalyses the reaction). To each of these was added 0.5 ml of colour developing solution (Table 2.6) and either (a) 0.1 ml 0.5 M ascorbic acid and 2.4 ml d.H_2O, or (b) 2.5 ml d.H_2O. After 15 minutes for colour development, the samples were tapped sharply to remove any air bubbles, and measured on a spectrophotometer (Cecil CE 1020, Spectronic, Leeds, U.K.) at 882 nm. This high wavelength reduces posssible interference from organic matter, as does the addition of charcoal.

Table 2.6 Composition of colour developing solution used for colorimetric determination of phosphorus concentration.

Ingredient	Strength	Volume in 500 ml
Sulphuric acid	2.5 M	250 ml
Ammonium molybdate	4 % w/v	74 ml
Antimony potassium tartrate	0.2908 % w/v	25 ml
Distilled water		151 ml

The cuvettes which had no ascorbic acid added served as sample blanks to control for any light absorption by organic matter in the sample, rather than light absorbed by the developed blue colour of the solution. The absorbance values for each of these was subtracted from those for the cuvettes containing ascorbic acid, which had developed blue colouration. In this way the phosphorus concentration was determined for each extract, without the varying organic matter content of the different soils affecting the values obtained. Results were defined using a standard curve obtained using a standard P solution containing known amounts of sodium hydrogen orthophosphate ($Na_2HPO_4.2H_2O$).

Colour development was complete at 15 minutes from the solution in the cuvettes being mixed, and remained stable for many hours. This was verified by re-reading the absorbance of some samples several times at different time intervals.

Blanks were carried through the extraction and colorimetry procedures both with and without the addition of charcoal, to ensure that the charcoal did not contain enough phosphorus to affect the results obtained. The amount of phosphorus within the charcoal was found to be negligible.

Values obtained were converted to a soil dry weight basis, using data for soil moisture obtained by weighing approximately 15 g fresh soil, drying at 80 °C for at least 48 h, and weighing this dried soil. All samples were measured for soil

moisture in triplicate, while 5 subsamples of each soil core were measured for extractable P.

2.3.4 Soil nitrate and ammonium

The soil nitrate and ammonium were determined by potassium chloride (KCl) extraction and flow injection analysis (FIA) (Tecator 5012, Foss UK Ltd, Didcot, Oxon). Sieved soils were weighed to 5 g subsamples (+/- 0.02 g, weighed to 0.0001 g) into a plastic bottle with 250 ml capacity. To each bottle was added 50 ml 2 M KCl. The bottles were capped and shaken for 1 hour in an end-over-end soil shaker. The soil suspensions were then filtered (Whatman No. 42) into clean plastic vials, which were capped and frozen until they could be analysed, using standard FIA protocols. Blanks were carried throughout the procedure. Values obtained were converted to a soil dry weight basis, using data for soil moisture obtained by weighing approximately 15 g fresh soil, drying at 80 °C for > 48 h, and weighing this dried soil. All samples were measured for soil moisture in triplicate, while five subsamples were taken from each soil core to use for soil nitrate and ammonium determination.

2.3.5 Soil pH

Soil pH was determined both in water and in calcium chloride ($CaCl_2$), to elucidate any variation in readily-exchangeable ions. Samples of sieved soil were measured in triplicate using 5 g soil (fresh weight) to 10 ml d.H_2O, thoroughly mixed to a slurry. After measuring the pH in these samples in water using a pH meter (Jenway PHM6, Spectronic, Leeds, U.K.), 200 µl of a $CaCl_2$ solution was mixed into each sample to bring it to a molarity of 0.01 M. Readings were taken once the pH value had stabilised. The data for soil pH are given as the median and range of each triplicate set (rather than the mean and standard error as most data presented here) since pH is not a linear scale.

2.3.6 Soil loss on ignition

Loss on ignition (LOI) provides a crude measure of the organic carbon in a soil, and an approximation of the organic carbon in many soils can be calculated by multiplying the loss on ignition by 1.72 (Allen *et al.*, 1974). Soils of course vary in their proportion of organic carbon, and different conversion factors may be appropriate for different soil types, ranging from 1.77 – 1.93 for a mor soil to 1.97 – 2.07 for a mull soil (*ibid.*), additionally the length and temperature of combustion may affect these conversion factors, hence the LOI data are expressed without transformation. These values of LOI allow rough comparisons of the organic carbon contents to be drawn between the soils, even if their exact values of

organic carbon are unknown. This method was chosen for its simplicity, which made it preferable to other methods for determining organic carbon content.

To determine the loss on ignition, as a measure of organic carbon, approximately 5 g of fresh soil was weighed (to 0.0001 g) into a ceramic beaker, heated at 105 °C for 24 h and re-weighed to determine dry weight (dwt), then combusted at 450 °C for 4 hours (after Allen *et al.*, 1974), allowed to cool slightly, and stored in a dessicator until re-weighed. The loss on ignition was expressed as a percentage of the dry weight.

2.3.7 Plant tissue total nitrogen and phosphorus

The total nitrogen (N) and phosphorus (P) of plant shoots or roots were determined by wet-ashing, and analysing by FIA. After the plant tissue had been dried at 80 °C for >48 h, the dried samples were weighed into glass digest tubes. A salycilic-sulphuric acid mix (33 g in 1 L) was added to the tubes, using 4 ml acid to <150 μg plant tissue dry weight. A small amount of catalyst (1:10 copper sulphate to lithium sulphate) was added to each tube. The tubes were placed in a heating block, with marbles fitted over the tubes to act as condensers. They were heated thus for 7 h at 370 °C. After they had cooled they were diluted and measured by FIA.

2.3.8 Choice of bioassays

Where the infection of roots by AMF was to be measured, bioassays were used. This was to ensure that when comparing root colonization between different turfs, the plant species and age were controlled. AMF may colonize different plant species to differing extents and with differing morphology, so by standardizing the plants in which the AMF colonization is measured, fair comparisons can be made.

The species chosen for use as a bioassay was *Plantago lanceolata* (from Emorsgate Seeds), since it is known to be infected by a wide range of AMF (INVAM 1995), and has roots which clear well. It is a plant native to the UK, which can be found in environments such as those being sampled here, and is therefore an appropriate choice. *Plantago lanceolata* is known to be a successful host to a range of AMF under conditions of low light intensity, at a range of temperatures, in high humidity and in constrained space (INVAM 1995). Prior to use, the seeds were surface sterilized and germinated, as in Section 2.3.9.

2.3.9 Sterilizing and germinating seeds

Surface-sterilizing seeds prior to germination reduces pathogens of the seeds, and by increasing scarification of the seeds may hasten germination. The procedure was carried out on all seeds used in the experiments described below (unless stated otherwise). A universal vial was one-third filled with the seeds, then sufficient calcium hypochlorite (5 g in 70 ml d.H_2O, filtered until clear) was added to the vial to bring the volume to two-thirds full. The vial was capped and then shaken in a vortex for 10 minutes. After this the liquid was flitered off using a vacuum flask, and thoroughly rinsed using distilled water. The seeds were then spread over moist filter paper in clear dishes with lids, and sealed using Nescofilm (Nippon Shoji Kaisha Ltd., Osaka, Japan) to retain moisture. They were kept under lights in growth rooms until both shoot and root had emerged to approximately 1 cm. At this stage the germinated seedlings were transplanted as required.

2.3.10 Clearing and staining procedure for light microscopy of roots

Trypan Blue was the stain used for light microscopy of AMF colonised roots (Brundrett *et al.,* 1996). The concentration of this stain was 0.75 g Trypan Blue in 1 litre liquid, which was a 1:1:1 mixture of lactic acid, glycerol, and distilled water.

To examine AMF colonisation, the excised roots were first gently washed in water, either in a sieve or in a shallow dish using a small paintbrush to clean away adhering soil particles. They were cleared by submerging the roots in 10 % KOH at 80 °C for 1 hour. Following clearing, the roots were rinsed in water and placed into 10 % HCl for 30 minutes, before being immersed in the Trypan Blue stain for 1 hour. Subsequent to staining, the roots were destained by placing either in 50 % glycerol, or lactoglycerol destaining solution (14:1:1 lactic acid: glycerol: distilled water) for at least 24 hours.

2.3.11 Estimation of AMF colonization

Roots which had been cleared and stained using Trypan blue were laid on glass slides in 50 % glycerol, covered with a glass cover-slip, and examined by light microscopy for AMF infection. Starting in one corner, the slide was scanned, under x 200 magnification, in one direction until a piece of root was encountered. With the root section filling the view, presence of AMF in the vertical transect across the centre of the field of view was scored. This presence of AMF was scored in terms of presence of hyphae, arbuscules and vesicles, examining the section at x 400 where necessary. The slide was then moved in the same direction until the field of view contained an entirely new section of the slide, and

presence of AMF again scored crossing the central transect. Using such a method, at least 100 measurements are required for reliable estimation of AMF colonization (Giovanetti and Mosse, 1980), however it was not always practicable to take this number of measurements (when dealing with large numbers of slides), so fewer than 100 measurements were usually recorded. The number of transects observed on each root is detailed in the relevant chapters, and was never less than 36 observations per slide. This method will therefore provide a rough estimate of the degree of AMF colonization.

2.3.12 Scintillation counting

Samples of ^{14}C and ^{33}P were measured by liquid scintillation using a Packard Scintillation Counter (Packard Bioscience, Meriden, USA) according to the manufacturer's instructions (details of sample preparation are in Chapters 3 - 6). In order to avoid the possibility that sample readings may be affected by chemiluminesence, all samples were counted twice, with a delay of several hours before the second reading. The data used for analysis was taken from the latter of these two readings. Counts of the two readings were compared, and in the majority of cases any differences were negligible. The vials used to contain samples were plastic scintillation vials from Packard.

2.3.13 Statistical analyses

Collated results were analysed, where appropriate, by either one-way or general linear model Analysis of Variance (ANOVA), using Minitab 13 (Minitab Inc.). Where ANOVA tests were used, normal distributions of the data were verified using the Anderson-Darling test for normality, and equal distributions were confirmed using Bartlett's test. Statistical significance was inferred where $p < 0.05$.

Percentage data (measurements of AMF root colonisation) were arcsine square root ($\sin^{-1}\sqrt{x}$) transformed. Where raw data did not conform to the assumptions of ANOVA, they were either $\log_e$ transformed, or Box-Cox transformed, as detailed where relevant, to conform to the assumptions of the statistical tests being employed. In some cases the data could not be transformed to meet the assumptions of ANOVA, in which case the non-parametric Kruskal-Wallace test was used.

Chapter 3:
Arbuscular mycorrhizal hyphal translocation of P to plants in turfs from different management systems: changes over time

Chapter 3: Arbuscular mycorrhizal hyphal translocation of P to plants in turfs from different management systems: changes over time

3.1 Introduction

Arbuscular mycorrhizas are to be found across the UK in agricultural land, yet they can be negatively affected by agricultural management treatments such as tillage, fertilisation, pesticide use, and species of plant (Chapter 1). Given the different environments created by various management treatments, there may be selection pressures acting to produce different AMF communities, which will inevitably function differently. It may take many years following cessation of inhibitory management practices before AMF functioning in low input agriculture or set-aside land becomes comparable to their functioning in ecosystems which have existed for millennia and to which AMF have presumably adapted: grazed meadows and early agriculture which was established in the Neolithic period. This study seeks to find any differences in the functioning of the symbiosis under different management regimes, by looking at a key aspect of the symbiosis: mycorrhizal transfer of phosphorus from the soil to the plant. This is examined over time, by taking several subsequent shoot harvests.

Arbuscular mycorrhizal fungi are present in most terrestrial ecosystems, and different environmental conditions will impact upon the populations of these fungi. Helgason *et al.* (1998) showed, using molecular sequencing of partial fungal small subunit ribosomal RNA, that the diversity of AMF in arable sites is strikingly low compared with woodland sites. In arable crops, 92 % of sequences represented *Glomus mosseae* or its close relatives, whilst the woodland sites had a much higher diversity of fungal types (Shannon-Weiner $H' = 0.144$ in woodland samples compared to $H' = 0.398$ in arable sites). Since certain AMF types were found in both crop plants and woodland species, the authors inferred that the AMF had a broad host range, and concluded that the change in fungal sequence composition and the low diversity of the fungi in arable fields was not due to the monocultures planted in agriculture, but rather reflected aspects of the agronomic regime such as ploughing, fertilisation, and fungicide application. Various studies have shown that AMF can be influenced by tillage (Jasper *et al.*,1989a, 1989b; McGonigle *et al.*,1990; Hamel *et al.*,1996; McGonigle and Miller, 1996; Gavito and Miller, 1998), fertilisation (Ryan *et al.*, 1994; Dann *et al.*,1996; Kraupfenbauer *et al.*, 1996; Douds *et al.*,1997; Olsson *et al.*, 1997; Miller and Jackson, 1998;

Thingstrup *et al.*, 1998), chemical applications (Miller and Jackson, 1998; Schweiger and Jakobsen, 1998), and by growing non-mycorrhizal crops (Kraupfenbauer *et al.*,1996; Ryan and Ash, 1996; Douds *et al.*,1997; Gavito and Miller, 1998; Miller and Jackson, 1998; Chapter 1). Conventional agriculture liberally employs such practices, whilst other forms of lower input agriculture, such as organic agriculture, limit the types of inputs and management practices (Soil Association, 2002a). It is therefore possible that different forms of agriculture will impact differently upon AMF.

Some studies have examined the impacts of different agricultural regimes on mycorrhizal colonisation of roots and presence of AMF spores in the soil (Ryan *et al.*, 1994; Miller and Jackson, 1998). However, little is known of the relationships between these factors and the net results of the symbiosis in terms of impacts on the plant hosts or changes in nutrient cycling. It has been shown that the length of root colonised by AMF can relate directly to the amount of phosphorus, zinc, and other nutrients taken up by the plant (*e.g.* Hamel *et al.*,1996; Tarkalson *et al.*,1998a, 1989b; Ryan and Graham, 2002), but it has also been found that different strains of AMF vary in the amounts of P they transfer to their hosts (Jakobsen *et al.*,1992; van der Heijden *et al.*,1998a, 1998b; Eason *et al.*, 1999). Hence in a field situation, where many different strains of AMF are present, the results of a change in the AMF population may be highly complex and impossible to infer by simply measuring the presence of the fungi or their resting structures. Koide and Kabir (2000) explored the idea of selection for functional complementarity in AMF, whereby species of AM fungi are selected for which complement each other in their abilities to spatially exploit soil resources. However, as Koide and Kabir (2000) pointed out, "we are still a long way from understanding the control of both the composition of such AMF communities and their temporal stability." If the plant were capable of wholly controlling which AMF colonise their roots, and to what extent, this could explain the lower AMF colonisation of roots observed in conventional crops compared to organic crops (Ryan *et al.*, 1994) where the benefits which AMF would confer on the plant (*e.g.* high P availability) are already provided by the farmer in synthetic form.

Sustained yields in low intensity, organic management, where soluble phosphate fertilisers are not permitted, are dependent on greater efficiency of nutrient use than in conventional agriculture. AM fungi are thought to play a significant role in these processes - but little is known about the responses of mycorrhizal functioning to different management intensities in the UK. A study by Eason *et al.* (1999) took AMF spore inocula from organic and conventional farms (from three

soil series) and inoculated plants (*Trifolium repens* and *Allium ameloprasum*) growing in irradiated soil. They found a greater shoot productivity response to organic than conventional AMF inocula in the soil of lowest P status (extractable P: 0.10 mg Kg^{-1} and 0.40 mg Kg^{-1} after irradiation), in which plants also had greater root length and greater root length colonisation. However, in another soil with higher P status (extractable P: 2.91 mg Kg^{-1} and 7.65 mg Kg^{-1} after irradiation), no differences were observed between organic and conventional AMF inocula. This type of study is useful in bringing to light some of the differences in functioning of different AMF communities from the field. However, it cannot fully explain AMF functioning in the field, since the method of extracting spores from soil will produce different inocula than a root would be exposed to in an intact field soil.

This chapter presents an experiment which is designed specifically to overcome the limitation of previous studies by using intact turfs with an undisturbed hyphal network (Jasper *et al.*, 1989a,b) and colonised roots, in addition to spores in the soil. Since different species of AMF sporulate to differing extents and may rely to varying degrees upon hyphal networks for colonisation, this approach is less selective of AMF and therefore more relevant and applicable to the field situation.

The intact turfs removed from the field were provided with a ^{33}P source in an adjacent hyphal (root-free) compartment, and the shoots were harvested to quantify shoot ^{33}P uptake. Subsequent to the labelling experiment, bioassay seedlings were sown into the turfs and later examined for AMF infection. This use of bioassays enabled comparisons to be made of AMF infection across the management systems, which contained different indigenous plant species, and AMF may colonise different plant species to differing extents (INVAM, 1995). The aim here was to compare the potential of the AMF communities present to colonise plant roots, rather than to compare the lengths of roots colonised in the different plant species present, since the identity of the plants present is, in itself, a function of the management system.

3.1.1 Aims and hypotheses

This study aims to quantify AMF hyphal P uptake in turfs from different management systems (conventional, conventional pasture, integrated and organic), to determine whether the AMF from these different environments function differently in terms of P uptake. The experiment involved taking several subsequent harvests, such that hyphal P uptake over time can be examined. It was hypothesised that (a) under organic management, the AMF phosphorus

uptake and translocation to plants is greater than in integrated and conventionally managed farmland, and that (b) there is a systematic increase in quantity of hyphal P transfer as management intensity decreases. At the end of the P-labelling experiment, a bioassay for AMF colonisation was performed, to test the null hypothesis (c) the root-colonising ability of AMF present in the turfs is the same across all management treatments.

3.2 Materials and methods

3.2.1 Experimental design

Turfs were removed from CWS Agriculture at Stoughton Estate, Leicestershire, UK. Long-term trials involving different farming methods are taking place there, offering a variety of sites including long-term organic, integrated and conventional land management. There were 4 management treatments; each treatment had 2 or 4 replicate fields, with 3 (pseudo)replicate turfs taken from each field (in order of decreasing management intensity):

- Conventional land sown with wheat (4 fields)
- Integrated land sown with wheat (2 fields)
- Conventional permanent pasture (2 fields)
- Permanent pasture under long-term (>10 years) organic management (4 fields)

These management treatments were chosen to span a wide range of agricultural management intensities, with conventional levels of soluble P fertilisation in the conventional treatments and manuring of organic treatments, and with varying degrees of disturbance. The permanent pastures had not been ploughed for many years (at least 50 years). The integrated wheat fields had been direct drilled for the preceding 12 years, so had experienced relatively low disturbance, whilst the conventional wheat fields were ploughed conventionally. The floristic composition also varied, from wheat and ryegrass monocultures to the diverse organic meadows which contained many mycorrhizal species. (Chapter 2 provides data on soils.)

A radioisotope tracer ($^{33}PO_4$) was used to elucidate P movement within the systems (plant, AMF, soil). To enable quantification of fungal hyphal translocation, as distinct from total translocation of nutrients by hyphae and roots, a compartmentalised system was adopted. This entailed the use of 35 µm pore-sized nylon mesh, which allows hyphae to grow freely but excludes plant roots,

enabling the creation of hyphal (root-free) compartments in specially constructed pots.

The turfs were placed in the outer compartments of split-pots (Figure 3.1), which were separated, by a 35 μm pore sized nylon mesh barrier, from a central root-free compartment, into which AMF hyphae could grow freely. Each cylindrical pot was divided in two by a solid Perspex barrier, creating two separate systems. An AMF hyphae-free control was created by cutting every 5 days along the mesh interface in the central portion of one side of each cylindrical pot, to sever AMF hyphae growing from the plants into this compartment (indicated by red lines in Figure 3.1). In the turfs which contained wheat, the vegetation was originally very sparse, so the existing wheat plants were supplemented by sowing pre-germinated (Section 2.3.9) *Trifolium repens* seedlings onto the bare soil. This species was chosen since it is mycorrhizal, had been used successfully in other preceding experimental work and has a relatively fast growth rate making it suitable for subsequent shoot harvests.

These split-pot systems were grown for 3 months under controlled environment conditions 20 °C, 18 h day, 18 °C, 6 h night, light intensity 280 μmol m^{-2} s^{-1}. A ^{33}P source was then provided in the root-free hyphal compartments (Section 3.2.3), and the plant shoots harvested at several time points (Section 3.2.5).

3.2.2 Hyphal compartment substrate

The root-free hyphal compartments contained a standard sand-soil mixture. This mixture was a 1:1 ratio of silica sand and soil taken from the site under long-term permanent organic pasture, which was known to have a relatively low P status (Section 2.2.1, Quenby 4 in Table 2.4). It was deemed necessary to provide a substrate for the hyphal compartments which was low in P, since many experiments have shown the presence of high levels of soil P to inhibit AMF (Dann *et al.*, 1996; Hamel *et al.*, 1996; Olsson *et al.*, 1997; Miller and Jackson, 1998). By mixing the sand and soil together, a substrate was created which was low in P, but containing low amounts of many nutrients, and freely draining whilst containing some organic matter. The soil had been sieved to 2 mm, mixed thoroughly with the sand, air-dried, and left for over 3 months to reduce the AMF inoculum potential. The silica sand was soaked in 1 % v/v hydrochloric acid for 24 h and rinsed thoroughly with distilled water. The addition of sand ensured good drainage and a further decrease in the P status, whilst the soil provided structure and a range of nutrients as found in the field. Preliminary experiments had found

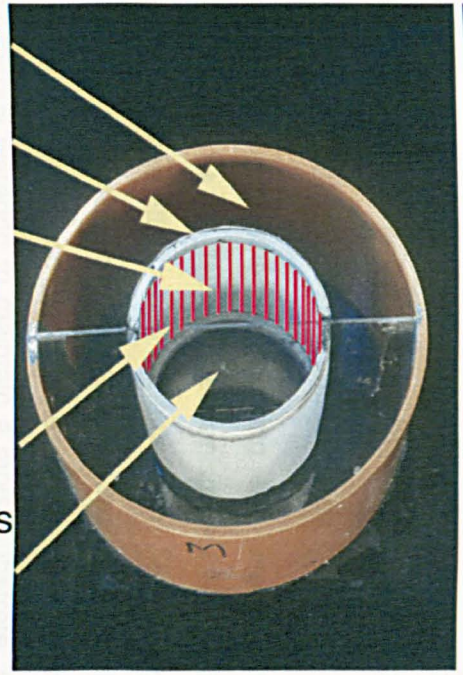

Fig. 3.1a Split-pot
(8cm deep, 4cm inner diameter, 8 cm outer diameter)

Fig. 3.1b Split-pot containing organic turf

of sand and soil was more suitable than pure sand for the labelling method using membranes, increasing the proportion of ^{33}P leaving the membranes.

3.2.3 Anion exchange resin membranes

The ^{33}P was provided by means of anion-exchange resin membranes (BDH Laboratory Supplies, Dorset, UK), each measuring 31 mm x 41 mm, which the ^{33}P was bound to, thus introducing the isotope to the system in a known location and with a known surface area. Prior to use, and prior to cutting to size, the membrane sheets were pre-charged by immersion in three subsequent solutions (over 36 hours) of 0.5 M $NaHCO_3$, then loaded with ^{33}P (as $H_3{}^{33}PO_4$, specific activity 5775.7 GBq/mg, 37 MBq ml^{-1}, from ICN Biomedicals, Inc., Basingstoke), by placing them into shallow trays, each containing 100 ml distilled water and 6 MBq ^{33}P for 2 hours. This loading solution also contained 20 μg P to increase the value of the membrane as a P source for foraging AMF hyphae. Each membrane sheet was cut into 12 smaller membrane pieces after loading, giving approximately 0.5 MBq per membrane piece.

These membranes were imaged (Packard Instant Imager, Meriden, USA) prior to insertion into the hyphal compartments, and also at the end of the experiment, to quantify the amount of radioactivity on each membrane before and after the experiment. After ^{33}P loading and imaging, the membranes were inserted into the root-free compartments (one membrane per hyphal compartment).

3.2.4 Validation of use of membranes

Preliminary experiments exploring the use of these anion-exchange resin membranes as ^{33}P delivery mechanisms had found that the soil moisture content did have some effect upon the amount of ^{33}P coming off the membranes. These experiments inserted membranes loaded with ^{33}P into pots containing the sand-soil substrate, but no plants. These pots were given three watering treatments: (1) normal watering to maintain a moist substrate, (2) wet, where the substrate was saturated, and (3) very wet, where the pots were sitting in water. Over a period of 49 days, 17 – 51 % of the ^{33}P came off the membranes under the normal watering regime, whilst 84 - 89 % came off under the wet treatment and 93 – 94 % in the very wet treatment (calculated such that isotope decay was not included in these figures). These results demonstrated the importance of maintaining a standardized watering regime across all treatments when using these membranes.

3.2.5 Harvest of shoots

To trace uptake of ^{33}P from the membrane into plants, shoots were harvested at 7, 14, 21 and 60 days after insertion of the membranes. This harvesting was carried out by clipping an equal proportion of each plant, whilst leaving 2 cm (above the soil) of living shoots to re-grow for the next harvest. These shoots were oven dried at 80 °C for over 48 hours, homogenised, wet-ashed (Section 2.3.7), and the ^{33}P concentration measured by liquid scintillation (4 ml acid digest diluted to 24 ml, a 1 ml aliquot of this mixed with 10 ml Emulsifier Safe E (Packard); Packard Scintillation Counter). The total P concentration in the digested shoot material was determined by a colorimetric method (Section 2.3.3).

3.2.6 Harvest of roots and soil

Following the final shoot harvest, 60 days after labelling, a 32 mm diameter core was removed from the soil of each split-pot, and sieved to separate out roots from soil. The roots were washed then dried, and the soil was mixed to homogenise it, then dried (80 °C for 48 hours). Two duplicate subsamples of roots and of soil were wet-ashed for each split-pot, and the concentrations of ^{33}P and total P determined (Section 3.2.3).

3.2.7 AMF colonisation

To determine the potential of the indigenous AMF in each of the systems to colonise roots, a standardized bioassay was employed. Subsequent to the final harvest, seedlings of *Trifolium repens* were sown into the turf of each split-pot, and left to grow for 8 weeks. The roots of five bioassay plants from each split-pot were then removed from the turf, washed, cleared and stained (Section 2.3.10). The stained roots were cut from the very thick main roots, and examined for AMF colonisation at x 200 and x 400 magnification (Section 2.3.11). The excised roots from the five bioassay plants from each split-pot were mixed together and examined on one slide, from which 36 measurements of colonisation status were taken. The results for each slide were combined with those for the other split-pots within each treatment, for analysis of effects of management.

3.2.8 Statistical analyses

Subsamples of dried shoots, roots and soils were weighed and wet-ashed in duplicate. After determining the ^{33}P content of these subsamples, the mean of each duplicate was taken and used in subsequent statistical analyses. Blank acid digests were scintillation counted, and their mean values subtracted from each of the sample values, such that where results are reported below, zero (fg ^{33}P g^{-1}) represents the mean background activity. To allow comparison of ^{33}P quantified

at different points in time, data were corrected for decay such that their activities are standardized to the time when the membranes were inserted. Since loading of membranes and membrane insertion were carried out over two days, and measurement of ^{33}P by scintillation counting took place throughout day and night, decay was calculated to the nearest 6 hours. These calculations were based upon the equation

$$I_t = I_0 e^{-\lambda t}$$

where I_t is the activity at time t, I_0 is the activity at time 0, and λ is 0.693 divided by the half-life of the isotope, which was 25.3 days.

Where Analysis of Variance (ANOVA) tests were used, the data was first $\log_e$ transformed, or Box-Cox transformed, since the raw data had unequal variances. Since some data still did not fit the assumptions of ANOVA after transformation, non-parametric tests were then used. Percentage data (of root length colonisation) was arcsine-square root transformed prior to statistical analysis.

3.3 Results

3.3.1 Hyphal uptake of ^{33}P translocated to shoots

After 7 days, there was a significant effect of management, with more ^{33}P in the shoots of mycorrhizal organic turfs, compared to the other systems (2-way ANOVA on $\log_e$ transformed data: $F = 7.42$, d.f. = 3, 63, $p < 0.001$; Figure 3.2). At the 7 day harvest there were no significant differences in uptake of ^{33}P by plants with intact or severed (control treatment) hyphae (2-way ANOVA: $F = 0.03$, d.f. = 1,63, $p = 0.874$; Figure 3.2).

At the 14 day harvest the organic intact mycorrhizal systems had a mean ^{33}P uptake 7 times greater than the conventional and integrated systems and the organic non-mycorrhizal control (Figure 3.3). After 14 days there was a significant effect of severing hyphae (2-way ANOVA on Box-Cox transformed data: $F = 9.47$, d.f. = 1,64, $p = 0.003$), of management system ($F = 32.61$, d.f. = 3,64, $p < 0.001$), and there was a significant interaction between severing hyphae and management system ($F = 6.62$, d.f. = 3, 64, $p = 0.001$; Figure 3.3). No significant variation was detected in uptake of ^{33}P at 14 days between mycorrhizal conventional crop, conventional pasture, integrated crop or severed controls (Tukey test, $p > 0.05$; Figure 3.3).

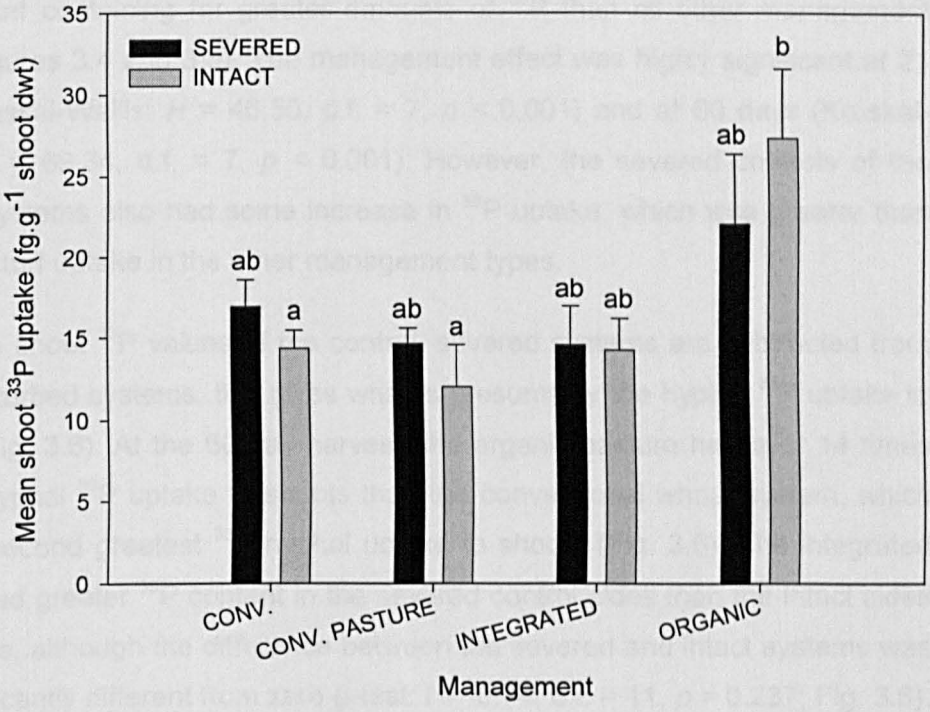

Fig. 3.2 Mean shoot ^{33}P, at 7 days after insertion of membranes, in intact and severed turfs from different management systems, calculated for decay. Error bars are one standard error. Bars with the same letter are not significantly different (Tukey test $p > 0.05$)

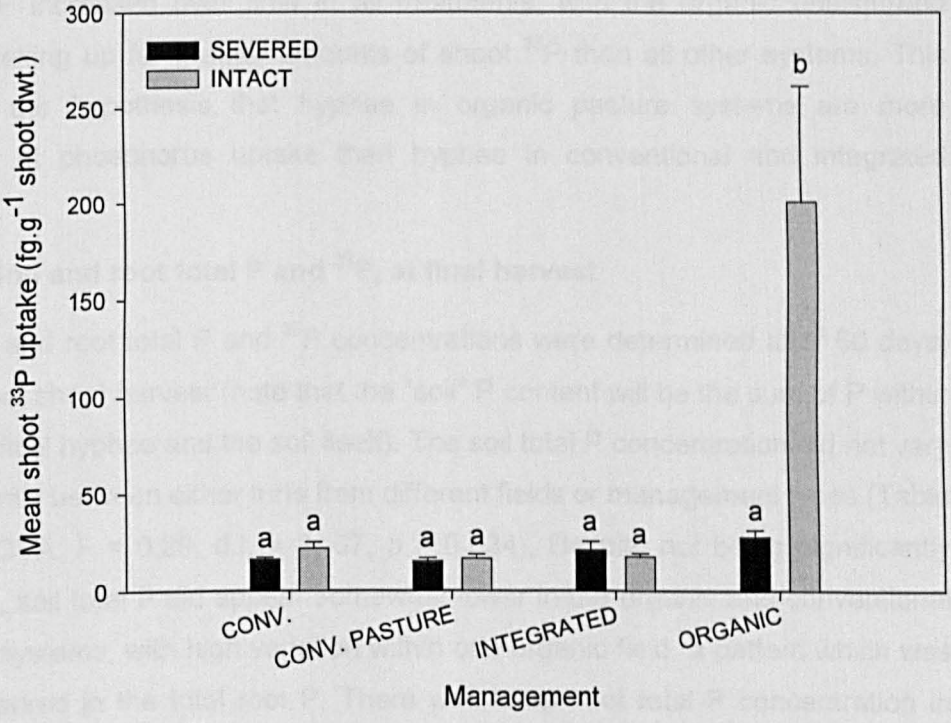

Fig. 3.3 Mean shoot ^{33}P, at 14 days after insertion of membranes, in intact and severed turfs from different management systems, calculated for decay. Error bars are one standard error. Bars with the same letter are not significantly different (Tukey test, $p > 0.05$).

At the 21 and 60 day harvests the trend continues, with shoots from the intact organic turf containing far greater amounts of ^{33}P than all other management types (Figures 3.4 and 3.5). The management effect was highly significant at 21 days (Kruskal-Wallis: $H = 46.50$, d.f. = 7, $p < 0.001$) and at 60 days (Kruskal-Wallis: $H = 69.34$, d.f. = 7, $p < 0.001$). However, the severed controls of the organic systems also had some increase in ^{33}P uptake, which was greater than the intact turf uptake in the other management types.

When the shoot ^{33}P values of the control, severed systems are subtracted from the undisturbed systems, this gives what is presumably the hyphal ^{33}P uptake to shoots (Fig. 3.6). At the 60 day harvest, the organic pasture had over 14 times greater hyphal ^{33}P uptake to shoots than the conventional wheat system, which had the second greatest ^{33}P hyphal uptake to shoots (Fig. 3.6). The integrated system had greater ^{33}P content in the severed control sides than the intact sides at 60 days, although the difference between the severed and intact systems was not significantly different from zero (*t*-test: $t = -0.74$, d.f. = 11, $p = 0.237$; Fig. 3.6).

Overall a trend can be seen for each of the systems to exhibit increased shoot ^{33}P content over time, for the intact systems to take up greater amounts of ^{33}P than severed systems, and for the organic systems to take up significantly more ^{33}P than turfs from all other management types (Figures 3.2 – 3.6). Uptake of shoot ^{33}P increased over time in all treatments, with the organic undisturbed system taking up far greater amounts of shoot ^{33}P than all other systems. This upholds the hypothesis that hyphae in organic pasture systems are more effective at phosphorus uptake than hyphae in conventional and integrated systems.

3.3.2 Soil and root total P and ^{33}P, at final harvest

The soil and root total P and ^{33}P concentrations were determined after 60 days, at the final shoot harvest (note that the "soil" P content will be the sum of P within extra-radical hyphae and the soil itself). The soil total P concentration did not vary significantly between either turfs from different fields or management types (Table 3.1, ANOVA: F = 0.29, d.f. = 3, 67, $p = 0.834$). Despite not being significantly different, soil total P did appear somewhat lower in the organic and conventional pasture systems, with high variation within one organic field, a pattern which was more marked in the total root P. There was lower root total P concentration in organic and conventional pasture turfs (Table 3.1), although some fields within management treatments varied widely, notably within the conventional treatment.

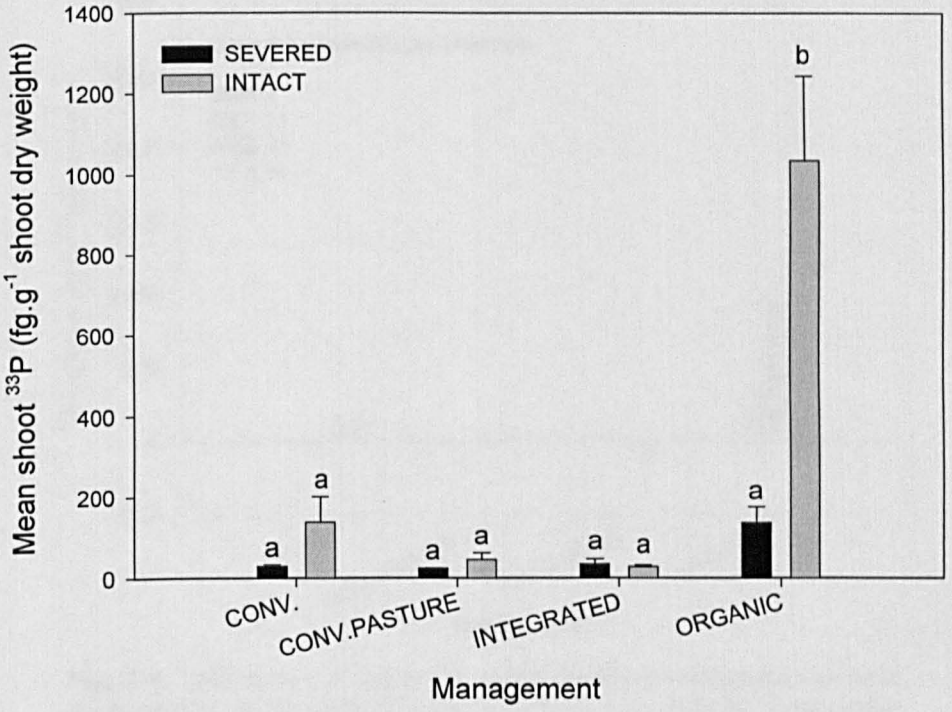

Fig. 3.4 Mean shoot ^{33}P at 21 days from labelling, in intact and severed turfs from different management systems, calculated for decay. Error bars are one standard error. Bars with the same letter are not significantly different (Tukey test, $p > 0.05$).

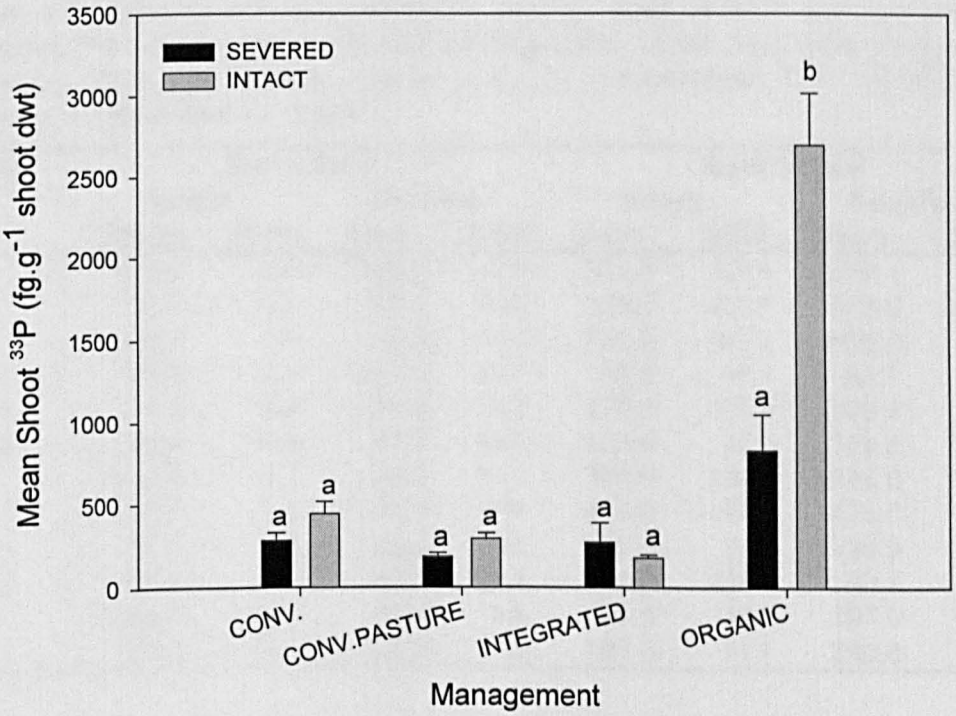

Fig. 3.5 Mean shoot ^{33}P at 60 days from labelling, in intact and severed turfs from different management systems, calculated for decay. Error bars are one standard error. Bars with the same letter are not significantly different (Tukey test, $p > 0.05$).

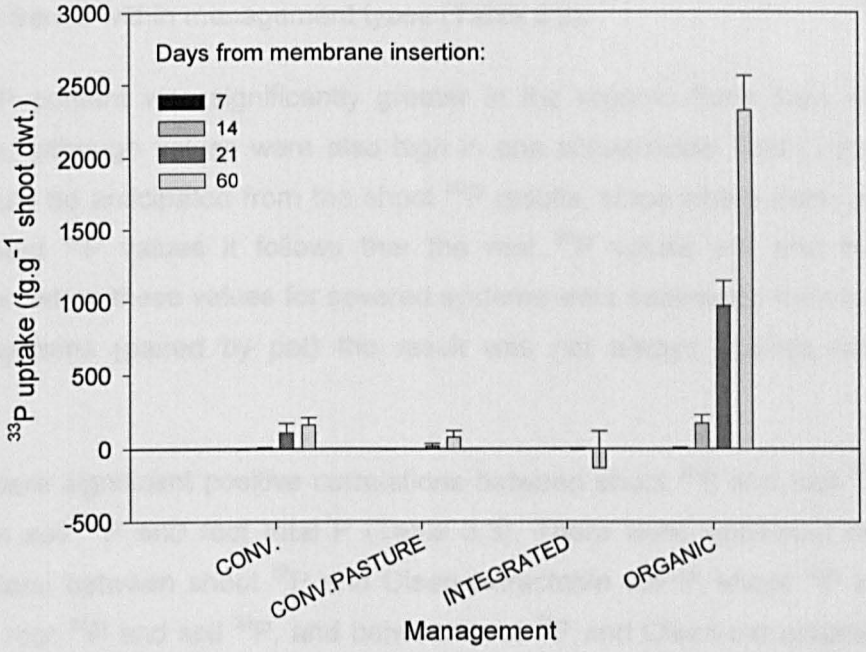

Fig. 3.6 Differences in shoot ^{33}P uptake between undisturbed and cut sides of pots, at harvests of 7, 14, 21 and 60 days. Activity is calculated for decay. Error bars are one standard error.

Table 3.1 Data for soil and root total P, μg P g^{-1} (dwt), in intact and severed turf systems from fields under different management, at 60 days after membrane insertion. Data are means, standard errors. C, conventional; CP, conventional pasture; I, integrated; O, organic.

Field	Soil total P				Root total P			
	Intact		Severed		Intact		Severed	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
C1	113.0	20.8	108.9	15.4	341.0	189.0	219.1	65.7
C2	19.1	32.1	86.9	58.5	349.0	233.0	568.0	478.0
C3	108.7	9.9	92.5	11.4	730.0	340.0	638.0	520.0
C4	99.0	12.0	137.0	80.7	68.5	38.4	84.7	39.7
CP1	71.2	13.8	97.9	16.2	170.0	133.0	269.4	73.8
CP2	95.4	10.8	81.3	18.9	125.9	72.2	194.0	207.0
I1	110.9	47.1	96.6	64.1	384.0	249.0	535.0	243.0
I2	142.7	5.4	98.9	49.8	473.0	249.0	428.0	117.0
O1	58.7	9.4	88.3	23.1	157.8	37.7	195.5	36.8
O2	63.0	14.7	47.5	39.3	304.0	237.0	99.7	73.8
O3	221.0	136.0	45.4	8.5	76.6	15.9	197.0	138.0
O4	54.6	27.5	64.8	5.3	183.8	51.7	240.8	26.2

Soil total ^{33}P had high variation between samples and it was therefore difficult to find any trends within management types (Table 3.2).

Root ^{33}P content was significantly greater in the organic fields than all other systems, although values were also high in one conventional field (Table 3.2). This could be anticipated from the shoot ^{33}P results, since where there are very high shoot ^{33}P values it follows that the root ^{33}P values will also be high. However, when these values for severed systems were subtracted from those for intact systems (paired by pot) the result was not always positive (data not shown).

There were significant positive correlations between shoot ^{33}P and root ^{33}P, and between soil ^{33}P and root total P (Table 3.3). There were significant negative correlations between shoot ^{33}P and Olsen-extractable soil P, shoot ^{33}P and soil total P, root ^{33}P and soil ^{33}P, and between root ^{33}P and Olsen-extractable soil P (Table 3.3, Spearman's rank correlation $p < 0.05$).

3.3.3 Resin membranes

The membranes were imaged before insertion and after the experiment, and their activities calculated for the latter date, enabling comparison of the values. The difference in membrane radioactivity between before and after insertion was calculated for each membrane to give the amount of ^{33}P moving off the membranes into the system (Figure 3.7). These values reflect the amounts of shoot ^{33}P recorded, with significantly greater amounts moving into the systems from organic farmland than conventional pasture and integrated turfs, while conventional cropping systems had intermediate amounts of ^{33}P moving into the system (Kruskal-Wallis test, $p < 0.05$). This shows that movement of ^{33}P off the anion-exchange resin membranes is affected at the membrane surface by factors which vary under the different management treatments, which could be AMF hyphae growing from the turfs to the membrane surface, growing faster (with hyphae possibly reconnecting following severing) in the organic pasture system. It could also be due to the lower soil P in the organic pasture turfs creating a gradient for P diffusion away from the membrane. Since there was continuity of soil water between the compartments, some ^{33}P may have moved by mass flow, and the possible role of root hairs which could protrude through the mesh into the hyphal compartment could not be determined here.

Interestingly, there were no significant differences in the amounts of ^{33}P coming off the membranes between the intact and severed treatments, despite the significant differences in shoot uptake for this treatment. This suggests that

Table 3.2 Data for root and soil ^{33}P (fg ^{33}P g^{-1}), in intact and severed turf systems from fields under different management, at 60 days after membrane insertion. Data are means standard errors. C, conventional; CP, conventional pasture; I, integrated; O, organic.

Field	Soil ^{33}P				Root ^{33}P			
	Intact		Severed		Intact		Severed	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
C1	27.4	9.1	42.7	20.1	89.7	63.6	5.5	4.6
C2	64.3	17.6	59.5	19.2	129.5	13.9	76.0	24.4
C3	65.5	0.2	31.4	4.7	12.6	10.4	26.8	13.8
C4	18.1	9.9	49.1	19.4	847.7	471.2	108.6	49.8
CP1	34.8	17.5	56.6	13.4	2.4	1.1	24.6	20.4
CP2	72.3	26.9	45.7	15.7	31.0	13.1	42.6	17.7
I1	64.7	17.2	84.9	14.2	42.2	19.2	79.2	5.8
I2	39.7	39.7	60.2	22.7	16.0	13.2	73.4	41.5
O1	21.9	9.8	83.2	13.4	1550.1	430.1	0.2	0.2
O2	26.4	22.5	9.6	8.4	949.1	402.1	843.8	697.6
O3	84.2	22.5	49.5	1.0	157.7	80.9	2.1	0.9
O4	18.4	13.9	54.8	31.6	949.0	22.3	995.1	332.0

Table 3.3 Relationships between variables measured in intact turf systems, results of Spearman's rank correlation tests. All ^{33}P data is from intact turf systems.

Variable 1	Variable 2	r_s	d.f.	p
Shoot ^{33}P	Root ^{33}P	0.697	31	**0.001**
Shoot ^{33}P	Soil ^{33}P	-0.289	28	0.122
Shoot ^{33}P	Olsen extractable P	-0.772	34	**0.001**
Shoot ^{33}P	Root total P	-0.067	31	0.712
Shoot ^{33}P	Soil total P	-0.356	31	**0.046**
Root ^{33}P	Soil ^{33}P	-0.612	22	**0.004**
Root ^{33}P	Olsen extractable P	-0.533	22	**0.007**
Root ^{33}P	Root total P	-0.083	22	0.705
Root ^{33}P	Soil total P	-0.048	22	0.840
Soil ^{33}P	Olsen extractable P	0.232	28	0.218
Soil ^{33}P	Soil total P	0.103	28	0.593
Soil ^{33}P	Root total P	0.417	28	**0.030**
Olsen extractable P	Soil total P	0.316	30	0.078
Olsen extractable P	Root total P	0.058	31	0.749
Soil total P	Root total P	0.147	31	0.447

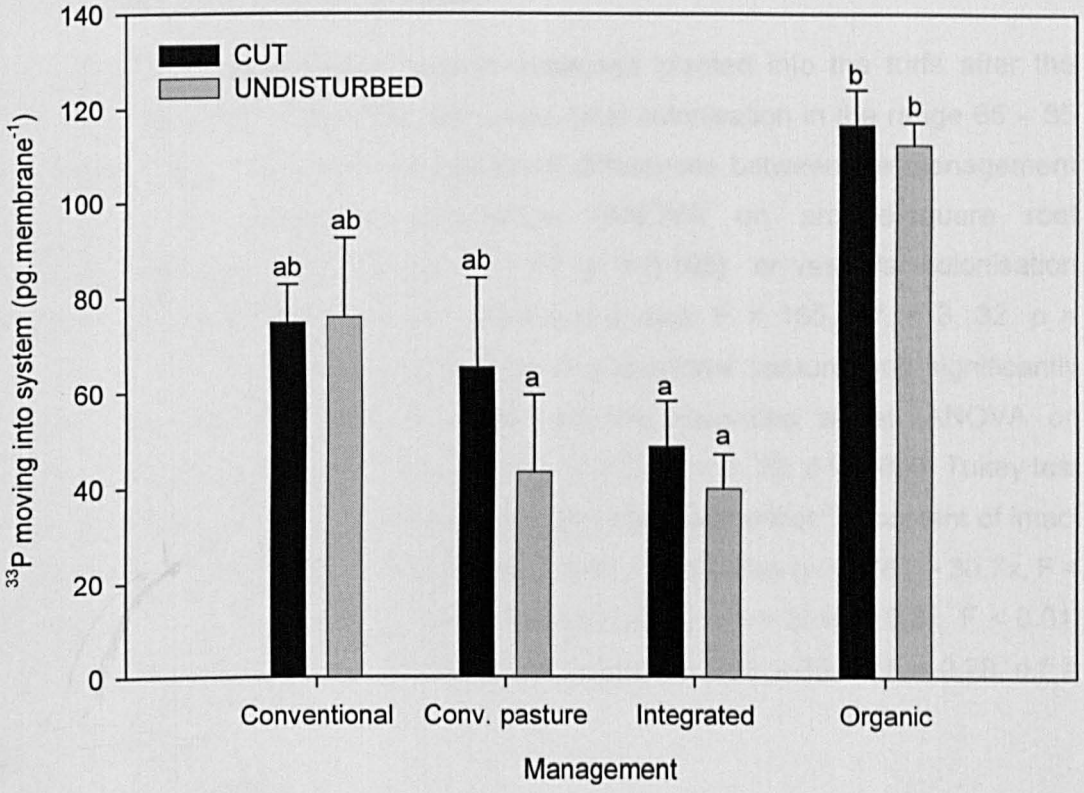

Fig. 3.7 Amount of ^{33}P which came off membranes into system, during 60 days; activity calculated at standard date. Bars with the same letter are not significantly different (Kruskal-Wallis test, $p>0.05$).

passive diffusion and chemical exchange may be the driving forces in the ^{33}P leaving the membranes.

3.3.4 AMF colonisation of roots

The AMF colonisation of *T. repens* bioassays planted into the turfs after the labelling experiment was high, with mean total colonisation in the range 65 – 85 % (Figure 3.8). There were no significant differences between the management treatments in arbuscular colonisation (ANOVA on arcsine-square root transformed data: $F = 2.22$, d.f. = 3, 32, $p = 0.105$) or vesicular colonisation (ANOVA on arcsine-square root transformed data: $F = 165$, d.f. = 3, 32, $p = 0.198$), but for the total colonisation the conventional pasture was significantly higher than the conventional wheat and the integrated wheat (ANOVA on arcsine-square root transformed data: $F = 5.18$, d.f. = 3, 32, $p = 0.005$; Tukey test $p < 0.05$). There was no significant correlation between shoot ^{33}P content of intact turfs (at 60 days from labelling) and total AMF colonisation ($y = 4781 - 30.7x$, $F = 0.20$, d.f. = 1,34, $p = 0.660$), arbuscular colonisation ($y = 2519 - 0.8x$, $F < 0.01$, d.f. = 1, 34, $p = 0.985$) or vesicular colonisation ($y = 331 - 35.1x$, $F = 0.28$, d.f. = 1,34, $p = 0.603$).

3.4 Discussion

3.4.1 *Shoot ^{33}P uptake*

The evidence gathered in this experiment clearly shows that in this case, arbuscular mycorrhizal hyphae in organic pasture are more effective at acquiring P and translocating it to plants than those in conventional and integrated farmland. This upholds the hypothesis that under organic management, the AMF phosphorus uptake and translocation to plants is greater than in integrated and conventionally managed farmland. However, the second hypothesis, that there is a systematic increase in quantity of hyphal P transfer as management intensity decreases, does not seem to hold, since there was no significant effect of AM hyphal networks on uptake of ^{33}P from root-free soil compartments in turfs under conventional cropping, conventional pasture or integrated cropping systems. This may suggest that these management treatments are all equally severe in inhibiting AMF or discouraging the development of an AMF community which translocates large amounts of P to plants.

The trend for shoot ^{33}P concentration to increase with length of time from labelling is unsurprising, firstly since the hyphae may take some time to locate the ^{33}P source, proliferate in its vicinity, and translocate the tracer to the shoots, and

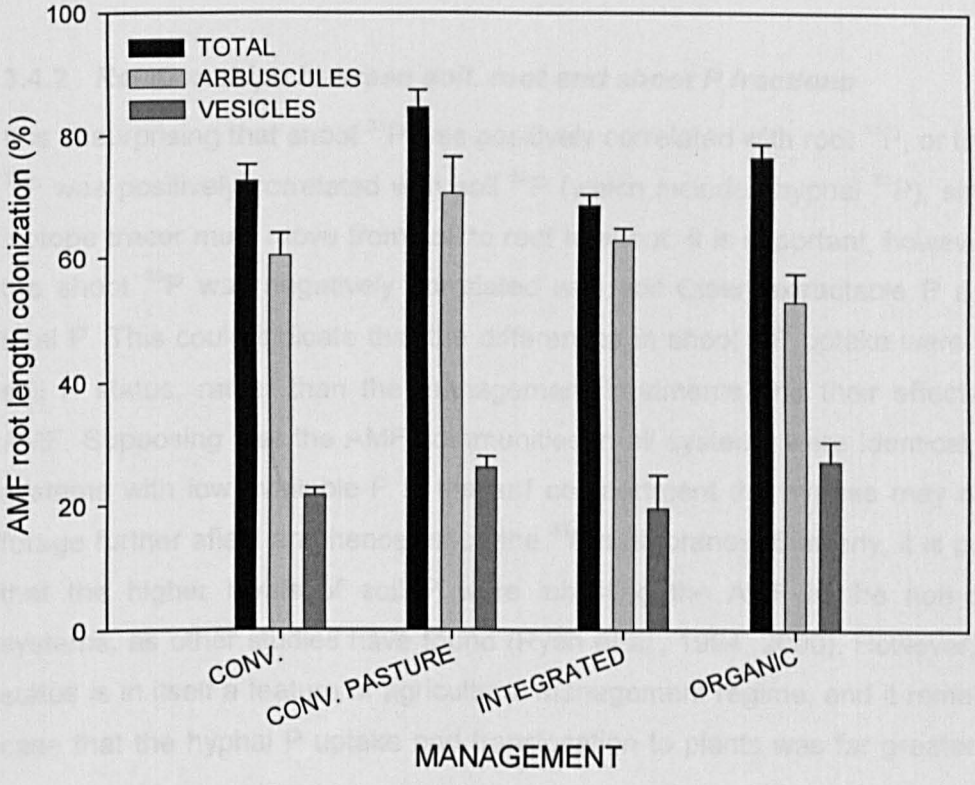

Fig. 3.8 Estimated AMF root length colonization under different management systems. Error bars are one standard error.

secondly since the harvesting and subsequent re-growth of the plant shoots creates a sink for the ^{33}P.

3.4.2 *Relationships between soil, root and shoot P fractions*

It is unsurprising that shoot ^{33}P was positively correlated with root ^{33}P, or that root ^{33}P was positively correlated with soil ^{33}P (which includes hyphal ^{33}P), since the isotope tracer must move from soil to root to shoot. It is important, however, that the shoot ^{33}P was negatively correlated with soil Olsen-extractable P and soil total P. This could indicate that the differences in shoot ^{33}P uptake were due to soil P status, rather than the management treatments and their effects upon AMF. Supposing that the AMF communities in all systems were identical, in the systems with low available P in the turf compartment the hyphae may need to forage further afield and hence reach the ^{33}P membranes. Similarly, it is possible that the higher levels of soil P were inhibiting the AMF in the non-organic systems, as other studies have found (Ryan *et al.*, 1994, 2000). However, soil P status is in itself a feature of agricultural management regime, and it remains the case that the hyphal P uptake and translocation to plants was far greater in the organic pasture systems.

3.4.3 *Root AMF colonisation*

The fact that there was no significant relationship between AMF percentage root length colonisation (% RLC) and shoot ^{33}P of intact systems strongly supports the hypothesis that there is a functional difference between the AMF present in the different systems. A similar conclusion was drawn by Ryan *et al.* (2002), who state that "the importance of AMF for crop growth and nutrition varies between regions for reasons not yet known." Their paper suggested that there is little role for AMF in the growth and nutrition of wheat in the south-eastern wheat-belt of Australia (on alkaline clay soils), in contrast with similar experiments in the northern Australian wheat-belt, although in one experiment there was a significant positive relationship between AMF % RLC and grain P and Zn concentrations, (*ibid.*)

It is perhaps surprising that there was such high colonisation in the disturbed and high P status turfs, relative to the organic pasture, a finding contrary to those of Ryan *et al.* (1994), who found that organic wheat had three times the % RLC by AMF of conventional wheat (over 60 % mean RLC in organic, approximately 20 % mean RLC in conventional wheat). The mean percentage root length AMF

colonisation in wheat in soils of comparable bicarbonate-extractable P status ranged from 0 % (following non-host crops) to 79 % (Ryan *et al.*, 2002).

The high AMF colonisation could be due to the fact that the bioassay plant species used is highly mycorrhiza-susceptible, and this suggests that the indigenous AMF do have the capacity to colonise a large proportion of plant roots. Whether the roots of the actual plants harvested in this experiment were colonised to a similar degree as the bioassays is of course unknown, and it is possible that this was indeed correlated to the ^{33}P uptake of the intact turf systems. However, since the plant species varied with management treatment, and mycorrhizal colonisation varies with plant identity, a direct comparison of the AMF colonisation of the roots of these different plant species would have been unfair. Also, the identity of plants growing in the different management systems is itself a function of management, and the AMF indigenous to each turf may exhibit specificity (in patterns of colonisation, and in function) with regard to the plants present (Helgason *et al.*, 2002). It remains the case that, as measured by the bioassay study, the AMF in the organic pasture (which had vastly greater shoot ^{33}P uptake) did not have any greater capacity to colonise roots than the AMF in the other systems. The differences in shoot ^{33}P concentration were therefore not due to the inherent ability of the indigenous AMF to colonise roots in those soils.

A note of caution should be sounded in interpreting the results of this bioassay colonisation study, since the numbers of roots sampled and the number of measurements of colonisation taken were both small. Hence it is plausible that these results are not detailed enough to give a reliable picture of the colonisation. However, it is likely that any marked trends would be apparent here.

3.4.4 *Anion exchange resin membranes*

This novel use of anion exchange resin membranes showed them to provide a successful method for supplying a ^{33}P source, in a defined location and a known amount. The different management treatments resulted in differing amounts of ^{33}P being removed from the membranes, despite the fact that the membranes were in standardized substrates and at standard moisture conditions, signifying that the movement of ^{33}P off the membranes is affected by the turfs, possibly by the hyphae growing from them. Other possibilities include movement of ^{33}P via mass flow of the soil solution, which may have been affected by the lower soil P status of the organic pasture turfs.

Direct comparison of these results with those of other researchers who employed different labelling techniques cannot be made, due to differing experimental systems. A very rough comparison is given here: if the mean amount of ^{33}P in the plant shoots of each pot (at 60 days from labelling) is expressed as a percentage of the amount of ^{33}P which came off the membrane, the value for the organic pasture is approximately 3.9 %. This is less than the published percentage of 18.75 % ^{32}P, of that provided in a soil compartment which was in plant shoots at 35 days from labelling by Schweiger *et al.*, (1999). This comparison suggests that the anion-exchange resin membrane method may bind more tightly to the P and hence be slightly less efficient in the use of the radioisotope than the method of mixing the isotope through the soil, however, the fact that the methods employed here were practicable and gave meaningful results validates this method.

3.4.5 *Severing treatment*

In most cases, the hyphal severing treatment resulted in lower ^{33}P uptake than in the adjacent intact system. This is clearly illustrated by Figure 3.6, which shows the differences in shoot ^{33}P uptake (severed systems subtracted from intact systems), where the values are all positive, with the exception of one value, which had a very high standard error and was not significantly different from zero.

Within the organic pasture systems, the hyphal severing treatment did reduce ^{33}P uptake by shoots, and these severed systems had higher uptake than those of the other management treatments. It could be that here the severing treatment was not frequent enough to prevent all re-growth of hyphae. Thus, in Chapter 4, where severing treatments are also employed, the severing occurred more frequently (at 3 day intervals), resulting in no apparent ^{33}P uptake in severed controls.

The method of severing hyphal connections at a nylon mesh surface has been applied successfully in other work (Hetrick, 1996; Johnson *et al.*, 2002), and has advantages over other methods of maintaining "non-mycorrhizal" controls. Many pot experiments inoculate previously sterile substrates with AMF inocula (e.g. Jakobsen, 1992; Smith, 2000; Joner and Leyval, 2001) or use fungicides to inhibit AMF activity (Allison, 2002; Schweiger, 1999). Inoculation with cultured isolates may not be comparable to the complex AMF communities which occur in the field. Such experiments usually use one or a few AMF species. Even where a number of AMF species are used as mixed inocula, root infection and subsequent activity of these fungi may be different from that which occurs in the field. Certain fungi may naturally colonise new roots from an existing colonised

living root, and methods which use inocula consisting of spores or pieces of excised roots will select against such species. Also, the relative abundance of each AMF species is very unlikely to equate to that which would be found naturally, and about which little is known. Fungicides may limit the growth of AMF, and reduce colonisation, but there still tends to be some residual existence and growth of fungi (O'Connor *et al.*, 2002). Fungicides may impact upon variables other than fungi, and have been shown to reduce shoot dry mass and seed yield, to be less effective against AMF communities than single AMF species (Schreiner, 1997), and to increase soil N availability (by 13 mg N kg^{-1}; Khaliq and Sanders, 2000). Another side effect of fungicide application is to decrease effects of fungal root pathogens (Newsham *et al.*, 1995), further confounding interpretation of experimental results. Overall, the method of hyphal severing appears a superior method for creating control treatments.

3.4.6 *Overview*

Very large differences were found in shoot ^{33}P uptake between the management systems. This reflects the fact that these environments are themselves very different, with the organic pasture being low in P and having high plant diversity, whilst the other systems were subject to varying degrees of P fertilisation and disturbance. One might expect that these different environments, and perhaps the plants inhabiting them, will select for different communities of AMF.

The experiment related in this Chapter set out to compare very different agricultural systems, and found huge differences in hyphal uptake of ^{33}P to shoots, which at 60 days from labelling was over 14 times greater in the organic pasture than any of the other management treatments. However, in attempting to remain as close as possible to the actual field situations, some variables were not controlled for: soil nutrient status, plant species and biomass. The following chapter aims to control these latter two variables (plant species and biomass), whilst still using intact turfs from the field, such that results will be indicative of the real life field situation.

Chapter 4:
Effects of agricultural management on uptake of ^{33}P over different distances from plant roots by arbuscular mycorrhizal hyphae

Chapter 4: Effects of agricultural management on uptake of ^{33}P over different distances from plant roots by arbuscular mycorrhizal hyphae

4.1 Introduction

The data presented in Chapter 3 showed that large differences can occur in mycorrhizal hyphal uptake of ^{33}P and its translocation to plant shoots, in response to agricultural management treatments. Various factors may be responsible for these observations, such as the ability of AMF present to reach the source of ^{33}P (their hyphal extensions), AMF hyphal ability to access the ^{33}P, and the degree to which these symbiotic fungi transfer the ^{33}P which they have accessed into the plant hosts. It is possible that management systems may select different AMF, as suggested by the work of Johnson (1993), Helgason *et al.* (1998), and Oehl *et al.*, (2003), and consequently the P uptake to plant shoots could differ with management regime.

The experiment described in Chapter 3 trialled the method of supplying ^{33}P on anion exchange resin membranes, and examined the shoot uptake, over time, of ^{33}P in turfs from different management systems. Having found that the supply method worked well, in that the membrane-bound ^{33}P was accessed and translocated from a hyphal compartment to plant shoots, with significant differences in the final shoot ^{33}P concentrations of plants in turfs from different management regimes, a further experiment was designed to study ^{33}P uptake in a wider range of turfs. This chapter describes a study designed to elucidate differences in hyphal P uptake and translocation to plant shoots in organic and conventional farmland, and in the "undisturbed" margins of the organic and conventional fields. This experiment seeks build upon the earlier work, going further than the study in Chapter 3 firstly by sampling turfs from a wider range of sites across England (Section 2.2), secondly by including turfs from "undisturbed" field margins, thirdly by quantifying ^{33}P uptake over different distances, and lastly by controlling plant species, and to some extent, plant biomass.

As in the previous work, it is hypothesized that the ^{33}P uptake to plant shoots will be greatest where the management is least intensive, since the management practices employed have been found to be inhibitory to AMF (Chapters 1 and 2, and references therein). Thus the relatively undisturbed and unmanaged field margins are expected to have greatest ^{33}P uptake by shoots, and the conventional fields the least.

By sampling turfs from a range of sites, undue influence on results by site-specific variables (such as soil type) was eliminated. The very marked effect of the organic pasture treatment on AMF ^{33}P uptake in Chapter 3 could have resulted partially from influences specific to that site, so by using turfs from geographically disparate sites there will be greater support for drawing conclusions with wider relevance to agricultural management across the UK.

Plant species was controlled among the organic and conventional fields during turf sampling, and among all turfs during set-up of the experimental system by sowing in standardized plant species. Closely comparable fields were selected which were all under the same crop at the time of sampling (Winter wheat). Ideally, each field would have had the same management treatments for a number of years prior to the sampling date; however, it was practically impossible to attain such close comparability. The aim of selecting fields under the same current crop was that any selective effect exerted by plant species upon the identity of AMF colonising roots would be controlled, since it is known that the presence of different crops can affect AMF populations (Ryan *et al.*, 2002; Daniell *et al.* 2001; Douds *et al.*, 1997). The field margins could not be controlled for plant species at the outset, due to the natural variability in such systems, but subsequent to sampling the turfs, the indigenous vegetation of all turfs was removed and new standard vegetation grown from seed. This seed mixture was a meadow mix, selected as it contained a range of native mycorrhizal plant species. By containing a range of species, it was intended that normal populations of AMF would be supported by the system.

Turfs were taken from fields under conventional and organic management, and from the field margins of these fields. The margins provided a relatively "undisturbed" environment, which may be closer to the original environment of the fields prior to cultivation. Inevitably, some management practices aimed at the cultivated fields will have impacted upon the field margins, for example chemicals sprayed onto the fields may drift onto the field margins, or leach through the soil. Heavy agricultural vehicles may have been driven over the field margins, which may also have been periodically mown. However, these habitats are nonetheless subject to far less intensive management practices than the cultivated fields. Importantly, these field margins have not been recently ploughed, so the hyphal networks should be better established and those species that spread mainly by root-hyphal-root contact rather than spores are likely to be better represented than in the ploughed field environment. The fields and their margins share the same original soil type and weather conditions, although the microclimate of hedges is likely to be different to that at the centre of fields due to

denser vegetation. Hence the field margins provide a good baseline with which to compare the mycorrhizal functioning in the fields.

This experiment supplied ^{33}P at two distances from the root compartment, to elucidate any differences in uptake related to distance (*i.e.* variation in hyphal extensions). It is possible that the low ^{33}P uptake by some turfs observed in Chapter 3 was due to the inability of the AMF hyphae present to reach the ^{33}P source. If this were the case, then when the distance to the ^{33}P source is increased, those AMF which have shorter-growing hyphae should exhibit lower ^{33}P uptake, and any management-specific differences should become apparent. A further examination of the question of whether the distances AMF hyphae are able to reach in the different systems is provided by a bioassay study. Bioassay seedlings were planted into hyphal compartments at two distances from the turf compartment, and later examined for infection. If the AMF hyphae in some systems grow further, then the infection of bioassays at the further distance ought to be colonised to a greater extent. The selection pressures in regularly ploughed land will be against AMF species which rely upon an extensive mycelium and root-hyphal-root colonisation, favouring instead those species which colonise primarily by spores and hence less affected by soil disturbance. It is therefore hypothesized that the relatively undisturbed field margins will favour fungi with longer hyphae, and hence these systems will exhibit greater ^{33}P translocation to shoots over the longer distance.

4.1.1 Aims and hypotheses

This study has three aims:

i) To quantify AMF hyphal P translocation to plants in a range of soil types, under standardised conditions, over different distances (1 cm and 3 cm)

ii) To compare AMF hyphal P translocation to plants growing in soil monoliths taken from land under organic and conventional management, and from relatively undisturbed field margins

iii) To compare AMF colonisation of bioassay seedlings over different distances from turfs taken from land under organic and conventional management, and from relatively undisturbed field margins

It is hypothesized that there will be greater AMF hyphal P translocation to plants (a) at the shortest distance from the P source, (b) in the organic turfs than the conventional turfs, (c) in margin turfs than in field turfs. A null hypothesis is also proposed: that

there will be no differences in length of roots colonised in bioassay seedlings across all the treatments.

4.2 Materials and methods

4.2.1 Turfs and split-pots

Turfs were taken from fields under Winter wheat (*Triticum aestivum*), from 4 organic and 4 conventional fields, plus from the margin of each field. These were placed into rectangular split-pots measuring 12 x 21 x 15 cm (width, length, depth), with a turf compartment of 12 x 12 x 15 cm (width, length, depth) (Figure 4.1). Each split-pot contained three barriers of nylon mesh with pore size of 35 μm, a size chosen to exclude roots but allow free growth of hyphae. The turf compartment was separated, by 35 μm pore sized mesh barrier, from an autoclaved sand-soil hyphal compartment. Within the hyphal compartment was a further divided "^{33}P source compartment" of 1 cm, at either 1 cm or 3 cm from the turf. Adjacent to the turf compartment, the hyphal compartment (B in Figure 4.1) of either 1 cm or 3 cm length formed the distance across which the AMF hyphae must grow to access the ^{33}P source, or to colonise the bioassay seedlings, which were situated in a 1 cm compartment surrounded on two sides by mesh (C in Figure 4.1). The mesh barrier to this compartment (C) on both sides, it was ensured that the positioning of the ^{33}P membranes was accurate and standardised, and that the membranes would not move (due to pressures from watering, or disturbance of the sand-soil matrix in the severed control pots). Bioassay seedlings were planted into this compartment, and the mesh ensured that the roots of these seedlings remained at the determined distance from the turf compartment, such that any infection of these roots must take place over a distance of either 1 cm or 3 cm, depending upon the pot.

The final compartment (D) was of variable length, such that all the split-pots were of the same total length, and the total hyphal compartment (B + C + D) was of the same size in all the pots. Since the size of the hyphal compartment will impact upon the moisture status of the turf compartment, by acting as a reservoir, and the transpiration of plants in the turf will create mass flow in the soil solution from the hyphal compartment to the turf compartment, it is important here to have a standard size of hyphal compartments across both the 1 cm- and the 3 cm-distanced pots. This ensures that the results from the 1 cm and the 3 cm pots are comparable.

The different distances were chosen to elucidate any differences in P uptake by the AMF communities in the different turf systems. It has been found that AMF hyphal length density was related to ^{32}P uptake (Jakobsen *et al.*, 2001) and that hyphal length

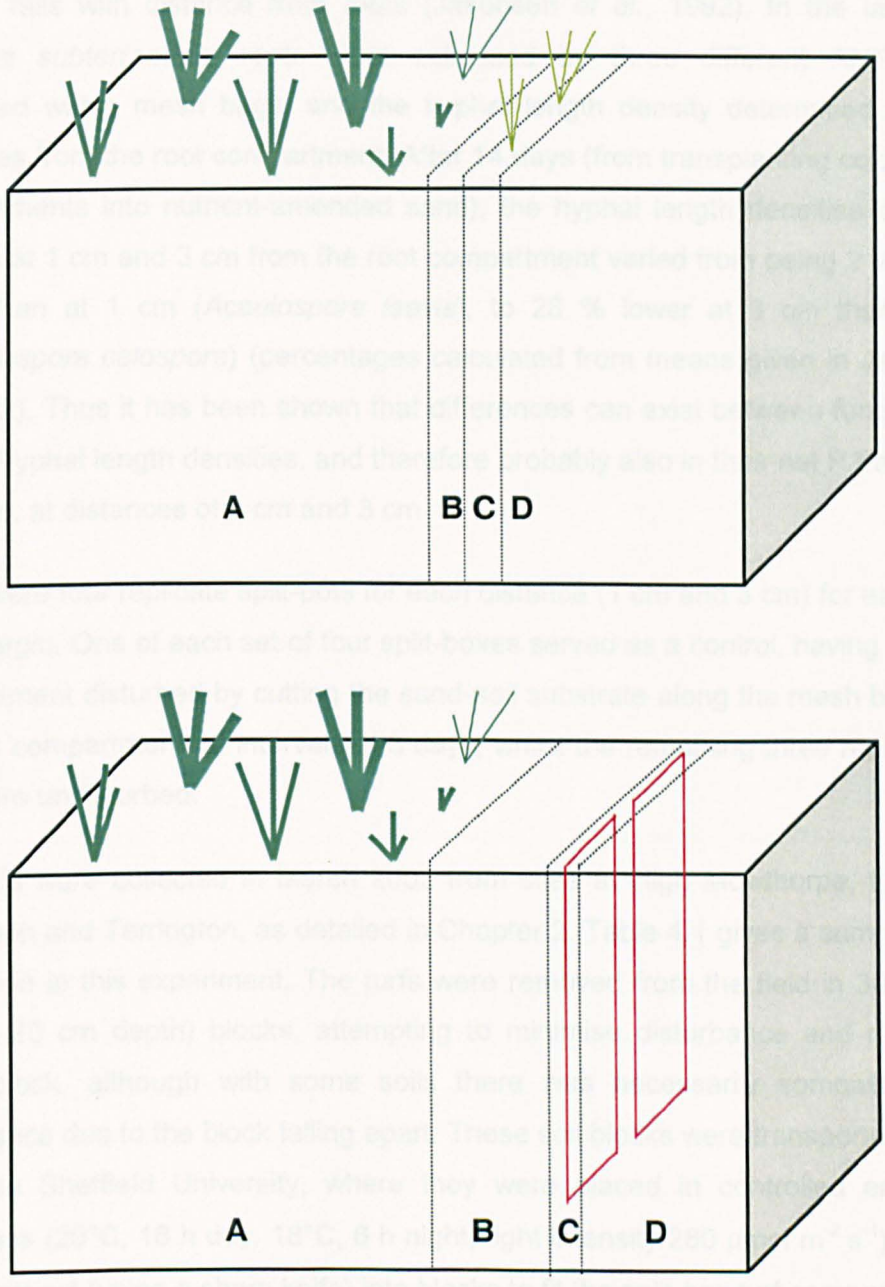

Figure 4.1 Diagrams of split-pots. The box dimensions were 12 x 21 x 15cm (width, length, depth); the turf compartment **A** measured 12 x 12 x 15cm (width, length, depth). Compartment **B** was either 1 cm or 3 cm long; compartment **C** contained either bioassay seedlings or membranes. These split-pots were specially constructed using PVC plastic and Tangit™ All Pressure glue (ASTM D-2564 from Henkel KGaA, Germany).

A – Turf compartment

B, C, D – Hyphal compartments, separated by 35µm pore sized mesh, containing autoclaved sand-soil mix

C – ^{33}P source compartment, situated either at 1cm or 3 cm from A

density falls with distance from roots (Jakobsen *et al.*, 1992). In the latter study, *Trifolium subterraneum* roots were colonised by three different AMF isolates, contained within mesh bags, and the hyphal length density determined at varying distances from the root compartment. After 14 days (from transplanting colonised root compartments into nutrient-amended sand), the hyphal length densities of different isolates at 1 cm and 3 cm from the root compartment varied from being 2 % higher at 3 cm than at 1 cm (*Acaulospora laevis*), to 28 % lower at 3 cm than at 1 cm (*Scutellospora calospora*) (percentages calculated from means given in Jakobsen *et al.*, 1992). Thus it has been shown that differences can exist between fungal species in their hyphal length densities, and therefore probably also in their net P translocation to plants, at distances of 1 cm and 3 cm.

There were four replicate split-pots for each distance (1 cm and 3 cm) for each field or field margin. One of each set of four split-boxes served as a control, having the hyphal compartment disturbed by cutting the sand-soil substrate along the mesh barrier near the root compartment, at intervals of 3 days, whilst the remaining three replicate split-pots were undisturbed.

The turfs were collected in March 2002 from sites at High Mowthorpe, Loddington, Stoughton and Terrington, as detailed in Chapter 2. Table 4.1 gives a summary of the replication in this experiment. The turfs were removed from the field in 30 cm by 34 cm (by 20 cm depth) blocks, attempting to minimise disturbance and maintain an intact block, although with some soils there was necessarily somewhat greater disturbance due to the block falling apart. These soil blocks were transported in plastic boxes to Sheffield University, where they were placed in controlled environment conditions (20°C, 18 h day, 18°C, 6 h night, light intensity 280 μmol m^{-2} s^{-1}). The turfs were then cut (using a sharp knife) into blocks to fit the split-box turf compartments, 12 cm by 12 cm and 15 cm deep. Once the turfs were in the split-boxes and the remaining compartments of the box filled with the autoclaved sand-soil mix, they were maintained in controlled environment conditions, as above, for 2 weeks, then transported to a glasshouse at Sheffield University Experimental Gardens. The temperature in the glasshouse ranged from 18 °C to 34 °C, the lighting was natural until the autumn equinox, after which the natural daylight was supplemented with fluorescent bulbs for a 16 h day. Membranes (see Section 4.2.6) were inserted on the 21st October 2002 and final harvest was 30 days later. The turfs were watered throughout with distilled water to maintain moist soil.

Table 4.1 Distribution of sampling sites across field sites. Each field and field margin had 3 turfs in intact boxes and 1 turf in a severed (control) box, for each type of split-pot (^{33}P source compartment at either 1 cm or 3 cm from root compartment). Total 128 boxes.

Field site	Conventional winter wheat	Margin to Conventional winter wheat	Organic winter wheat	Margin to Organic winter wheat
High Mowthorpe, N. Yorkshire	Field 1 Field 2	Field 1 Field 2	Field 3	Field 3
Loddington, Leicestershire	Field 4	Field 4		
Stoughton, Leicestershire			Field 5	Field 5
Terrington, Norfolk	Field 6	Field 6	Field 7 Field 8	Field 7 Field 8

4.2.2 Standardisation of vegetation

After time for the existing plants (detailed in Table 4.2) to grow and the AMF to further establish throughout the soil (6 weeks), the shoots were treated with herbicide (Bio™ Organic Weed Control, contains 2.4 % natural fatty acids; applied according to manufacturer's instructions) to kill the native flora, allowing the vegetation to be standardised. Several applications were necessary to remove the deeper-rooting species, and the turfs were left barren for 4 weeks (without watering) to allow for decomposition of the herbicide (double the length of time recommended by the manufacturer before re-sowing). The pots were then re-sown with a 1:1 mixture of *Basic general purpose wild flowers* (EM1(F), Emorsgate Seeds) and *General purpose meadow grass mixture* (EG1). Table 4.3 details the species contained in these mixtures.

Table 4.3 Species sown into turf compartment.

Wild flower mix Latin name	Common name	% of final mix	Mycorrhizal Status[1]
Achillea millefolium	Yarrow	0.5	AM
Daucus carota	Wild Carrot	5	AM
Galium verum	Lady's Bedstraw	2.5	AM
Leucanthemum vulgare	Oxeye daisy	6.25	AM
Plantago lanceolata	Ribwort plantain	7	AM
Ranunculus acris	Meadow Buttercup	10	AM
Rhinanthus minor	Yellow Rattle	8.75	NON
Silene dioica	Red Campion	10	NON
Agrostis capillaris	Common Bent	3	AM
Cynosurus cristatus	Crested Dogstail	22	AM
Festuca rubra ssp. commutata	Red Fescue	9	AM
Festuca rubra ssp. juncea	Red Fescue	16	AM

[1]As indicated in Harley and Harley, 1987

Table 4.2 Plant species observed to be indigenous to turfs sampled from each site. Species below are known to form arbuscular mycorrhizas unless indicated: [1]AMF infection sometimes absent, [2]infection absent or slight; [3]mycorrhizal status uncertain, as described in Harley and Harley (1987).

Field	Management	Vegetation	Site	Observed species
1	Conventional	Wheat	High Mowthorpe	*Triticum aestivum* (wheat), *Convolvulus arvensis* (field bindweed)
1	Conventional	Field margin	High Mowthorpe	Coarse grasses, *Hieracium anglicum* (hawkweed), *Ranunculus sp.* (buttercup)
2	Conventional	Wheat	High Mowthorpe	*Triticum aestivum*, *Convolvulus arvensis*, *Plantago lanceolata* (ribwort plantain), *Stellaria media* [2] (chickweed)
2	Conventional	Field margin	High Mowthorpe	Fine and coarse grasses, *Anthriscus sylvestris*[2] (cow parsley), *Lamium purpureum* (red dead nettle)
3	Organic	Wheat	High Mowthorpe	*Triticum aestivum*, *Stellaria media*[2], *Trifolium praetense* (red clover), *Senecio vulgaris*[3] (groundsel)
3	Organic	Field margin	High Mowthorpe	Medium grasses, *Anthriscus caucalis*[3] (bur chervil), *Glechoma hederacea*[1] (ground ivy)
4	Conventional	Wheat	Loddington	*Triticum aestivum*
4	Conventional	Field margin	Loddington	Coarse and medium grasses, *Plantago lanceolata*, *Leucanthemum vulgare* (ox-eye daisy), *Lotus corniculatus*[1](bird's foot trefoil)
5	Organic	Wheat	Stoughton	*Triticum aestivum* , fine creeping grass
5	Organic	Field margin	Stoughton	Medium grasses, *Ranunculus acris*, *Anthriscus caucalis*[3](bur chervil), *Glechoma hederacea*[1] (ground ivy)
6	Conventional	Wheat	Terrington	*Triticum aestivum*, *Stellaria media*[2], *Polygonum persicaria*[2] (redshank)
6	Conventional	Field margin	Terrington	Medium grasses, *Plantago lanceolata*, *Stellaria media*[2]
7	Organic	Wheat	Terrington	*Triticum aestivum*, *Veronica persica* (speedwell)
7	Organic	Field margin	Terrington	Coarse grasses, *Anthemis arvensis*[3] (corn chamomile), *Rumex obtusifolius*[2] (dock), *Carlina vulgaris* (thistle)
8	Organic	Wheat	Terrington	*Triticum aestivum*, *Carlina vulgaris*, *Convolvulus arvensis*, *Polygonum persicaria*[2]
8	Organic	Field margin	Terrington	Fine grasses, *Acer psuedoplatanus* (sycamore), *Glechoma hederacea*[1]

The bags of mixed seed were emptied into shallow trays, one for the wild flowers and one for the meadow grasses, and sown onto the surface of the turfs by evenly distributing a standard volume (2 ml) of each seed mixture, taken at random from different places in the trays of seeds. This procedure was instigated in an attempt to reduce possible stratification of seed types (according to size) within the bag. According to close inspection of the seeds as they were being sown, the species composition was indeed standardised between the pots. The density of seeds sown was greater than recommended for sowing in the field: by having greater plant biomass and competition between plants it was hoped that greater use of the P source would be made by the AMF and plants, and that thus the experimental results would be more marked.

Once the seeds were sown onto the soil surface, the pots were covered with cling-film (Somerfield Ltd., UK) which was pierced with small holes, to keep the seeds moist whilst allowing exchange of air. This covering was removed once the seedlings had established. The plants grew to fill the turf compartment (Figure 4.2).

4.2.3 Hyphal compartment substrate

It was necessary to provide a standard substrate for the non-turf compartments of the split-pots, such that it would not act as AMF inoculum, and have a low phosphate status. The sand-soil mix used to fill the compartments used soil taken from mixed woodland at Stoughton. This soil had a relatively high organic matter content due to high inputs of leaf litter and was therefore suitable for mixing with sand to increase the total organic matter content of the sand-soil mixture, and provide a variety of nutrients. A 1:5 ratio of soil to sand was used, which was based partly on the need to maintain low P status, partly on the results of preliminary experiments, and was partly determined by availability of the materials. A greater proportion of sand increases the homogeneity of the substrate, since the soil is likely to be spatially variable in nutrients whilst the washed silica sand is much more homogenous.

The soil was sieved to 2 mm, steam sterilised at 95 °C for two 1-hour periods on consecutive days, subjected to 3 cycles of thorough wetting and drying and left for any remaining soil biota to re-equilibrate towards a climax community composition. This soil was mixed in a 1:5 ratio with washed and autoclaved (121 °C for 20 minutes) silica sand. Subsequent to this, the mixture was again steam sterilised at 95 °C for 1 hour, and mixed with washed and autoclaved (121 °C for 20 minutes) Arnold sand to give a final ratio of 1:6 soil to sand. This mixture was sieved to 2 mm, wetted and

Figure 4.2 Split-pots shown from above, after being re-sown with a standardised plant mixture.

dried, and then packed into the split-boxes. These sterilisation and wetting-drying protocols are as recommended by INVAM (1994) (see also Section 3.2.2).

4.2.4 Toxicity test on autoclaved soil-sand substrate

The process of autoclaving organic soils is known to create the possibility of manganese (Mn) toxicity, which could inhibit plant growth. The soil which was sterilised to be used in these experiments was only treated at 95°C rather than the standard autoclave temperature of 121°C, and subjected to two cycles of thorough wetting and drying, (as recommended by INVAM, 1994), with the aim of decreasing possible Mn toxicity.

To test whether the autoclaved soil-sand substrate had any toxic effects, a small bioassay experiment was undertaken. Seeds of *Plantago lanceolata* were germinated on filter paper, and after 7 days the seedlings were transferred into a seed tray containing either the autoclaved soil-sand substrate or washed and autoclaved Arnold sand. Thirty seedlings were placed into each tray, all of a similar size, and they were watered with distilled water and left to grow in controlled environment plant growth rooms for 30 days. During this time it was evident that the seedlings in the autoclaved sand-soil mixture had greater biomass (mean 18.38 +/- 3.0 mg root dwt, mean 33.19 +/- 18 mg shoot dwt) than the seedlings grown in the autoclaved sand (mean 4.76 +/- 0.2 mg root dwt, mean 3.85 +/- 0.2 mg shoot dwt).

4.2.5 Seedling bioassay

After the split-pot systems were set up, with the re-sown meadow mixture plants growing and the sand-soil substrate in place, a bioassay of AMF in the hyphal compartments was carried out. This was done by surface sterilising and germinating seeds of *Plantago lanceolata* (Section 2.3.9), then planting three of these seedlings (at 10 days from sterilising) into the compartments where the ^{33}P-membranes would later be inserted. These seedlings were left to grow for 4 weeks, then carefully removed, causing minimal disturbance to the system. The roots were washed in water, cleared and stained (Section 2.3.10), then roots were examined at a magnification of x 200 and x 400, and scored for infection by AMF (Section 2.3.11).

4.2.6 Anion exchange resin membranes

As in Chapter 3, anion exchange resin membranes (BDH Laboratory Supplies) were employed as a means of supplying known quantities of ^{33}P to a hyphal compartment. Here, the membranes were cut into pieces of 9 cm x 3 cm and, after loading with ^{33}P, two of these pieces were inserted into the membrane compartment of each pot.

Membranes were pre-charged by immersion in several solutions of 0.5 M $NaHCO_3$, then loaded with ^{33}P (as $H_3{}^{33}PO_4$, specific activity >148 TBq.mmol^{-1}, from ICN Biomedicals, Inc.) by placing them into shallow trays containing 50 ml distilled water and 0.26 MBq ^{33}P. Two pieces of membrane were placed into each tray, and these same pairs were inserted into each pot. The membranes were left in the ^{33}P solution for 2 hours, with the liquid being gently agitated approximately every 15 minutes to ensure even distribution of phosphorus within the liquid. One hour after immersion, 20 μg of non-radioactive P (as sodium hydrogen orthophosphate, $Na_2HPO_4.2H_2O$) was added to each tray, to increase the total P on the membranes. This was intended to make the membranes into a greater resource and hence be more likely to encourage hyphal proliferation in that area to access the membrane-bound P. After the 2 hours, the membranes were removed, laid to dry in trays lined with tissue paper, then imaged (Packard Instant Imager) to measure the radioactivity of each membrane. Membranes were then inserted into the membrane compartment of the split-pot systems with minimal disturbance, by making an appropriately sized slit in the sand-soil matrix and sliding the membrane into the slit next to the flat side of a knife, using forceps to adjust the position of the membrane. The sand-soil substrate was re-packed to its original density over the membranes. The control pots had the sand-soil substrate disturbed every 3 days by cutting through the sand-soil substrate adjacent to the turf compartment (hyphal compartment B in Figure 4.1) several times, using a knife.

4.2.7 Harvesting

The ^{33}P-membranes were left in the split-pots for 30 days, then a complete harvest of plant shoots was taken by cutting at the soil surface. These shoots were dried in paper envelopes at 80 °C for >48 hours. The shoots from each split-pot were homogenised by cutting them finely using scissors and mixing thoroughly, then triplicate samples were weighed and wet-ashed (approximately 100 mg dry shoot in 4 ml acid mix, as in Section 2.3.7, heated to 370 °C for 7 hours). The digests were diluted to 24 ml, and a 1 ml aliquot was added to 10 ml Emulsifier Safe E™ (Packard) in a plastic scintillation vial and the radioactivity measured on a scintillation counter (Packard). For each split-pot, 3 subsamples of the homogenised shoots were wet-ashed and measured; the mean value of these three sub-samples (taken after conversion to ng$^{33}P.g^{-1}$ shoot dry weight) was used in subsequent calculations of results.

4.3 Results

4.3.1 Severed control treatment

The severing treatment of control split-pots was highly effective. The amount of ^{33}P in control shoots, with average background counts subtracted, was not significantly different from zero (*t*-test: $p = 0.476$, $t = 0.06$, d.f. = 15). There were no significant differences between shoot ^{33}P values of controls grouped by management ($F = 1.26$, d.f. = 3, 12, $p = 0.333$), sampling site ($F = 0.57$, d.f. = 3, 12, $p = 0.643$, or distance of ^{33}P source from turf ($F = 0.07$, d.f. = 1, 14, $p = 0.797$). Henceforth in this chapter the values of ^{33}P uptake in intact split-pots are not compared to the control treatments, since the latter are not significantly different from background counts as measured in blank acid digest samples.

4.3.2 ^{33}P uptake to shoots

The results are shown both as the shoot concentration of ^{33}P (amount of ^{33}P per unit shoot dry weight (dwt), and as the total shoot ^{33}P per split-pot. When the values for shoot ^{33}P are grouped by management type, the means for the 1 cm distance split-pots increase thus: *conventional field* < *conventional margin* < *organic field* < *organic margin*. This relationship is more marked where the results are expressed per unit shoot dry weight (Figures 4.3a, 4.3b). However, although there is a gradient apparent which corresponds to management intensity, and the 3 cm distances are usually lower than the 1 cm distances, the differences are not significant (Table 4.4).

When data are analysed at the field level, some significant differences can be found. Over both distances, an organic margin from High Mowthorpe had significantly higher shoot ^{33}P concentration (ng $^{33}P.g^{-1}$ shoot dwt) than an organic field at High Mowthorpe, a conventional field at High Mowthorpe, a conventional margin at Loddington and an organic margin at Stoughton (two-way ANOVA on distance and field, see Table 4.4; Figure 4.4). Similarly, the pots with 3 cm distance from turf to ^{33}P source had significantly higher shoot ^{33}P concentration (ng $^{33}P.g^{-1}$ shoot dwt), in the same organic margin from High Mowthorpe than in organic wheat at High Mowthorpe, conventional margin at Loddington and organic margin at Stoughton (one-way ANOVA, see Table 4.4). However, when these last data were tested as ng^{33}P per pot, the differences were not significant.

There was only a slight difference between the mean shoot ^{33}P concentrations for all 1 cm (15.0 ng^{33}P g^{-1}, +/- 1.9) and all 3 cm (14.0 ng^{33}P g^{-1}, +/- 1.9) distance split-pots, with the 1 cm systems having slightly higher shoot uptakes of ^{33}P, but this was not

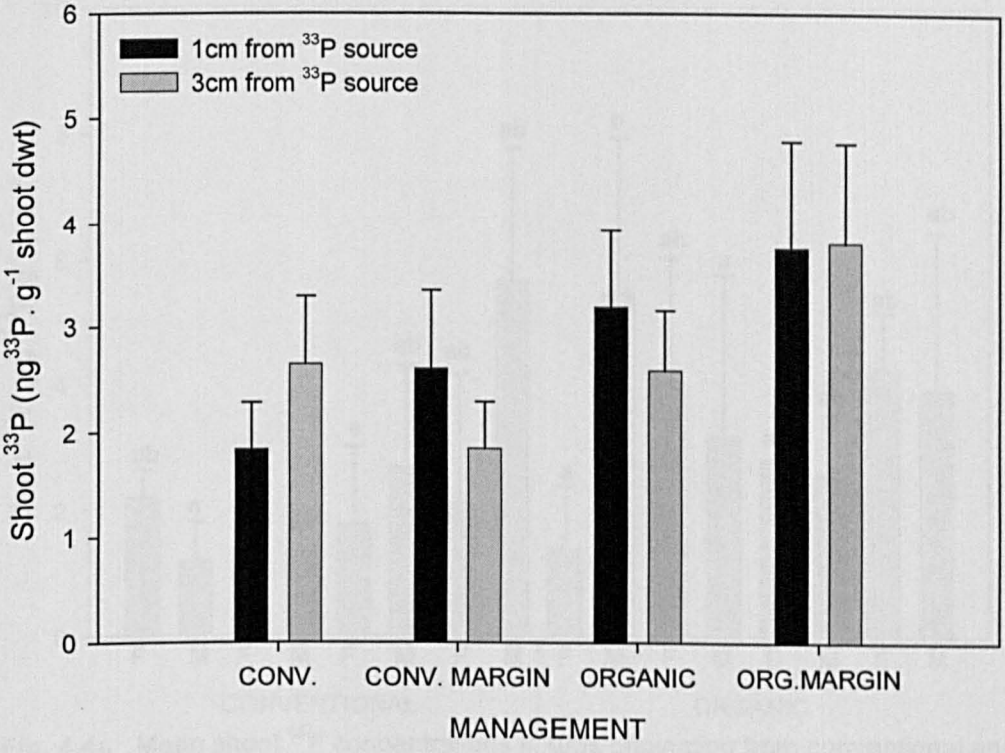

Fig. 4.3a Mean shoot ^{33}P concentration in turfs, at 1cm or 3cm from ^{33}P source, which originated from conventional (CONV.) and organic (ORG.) fields and their margins. Error bars are one standard error.

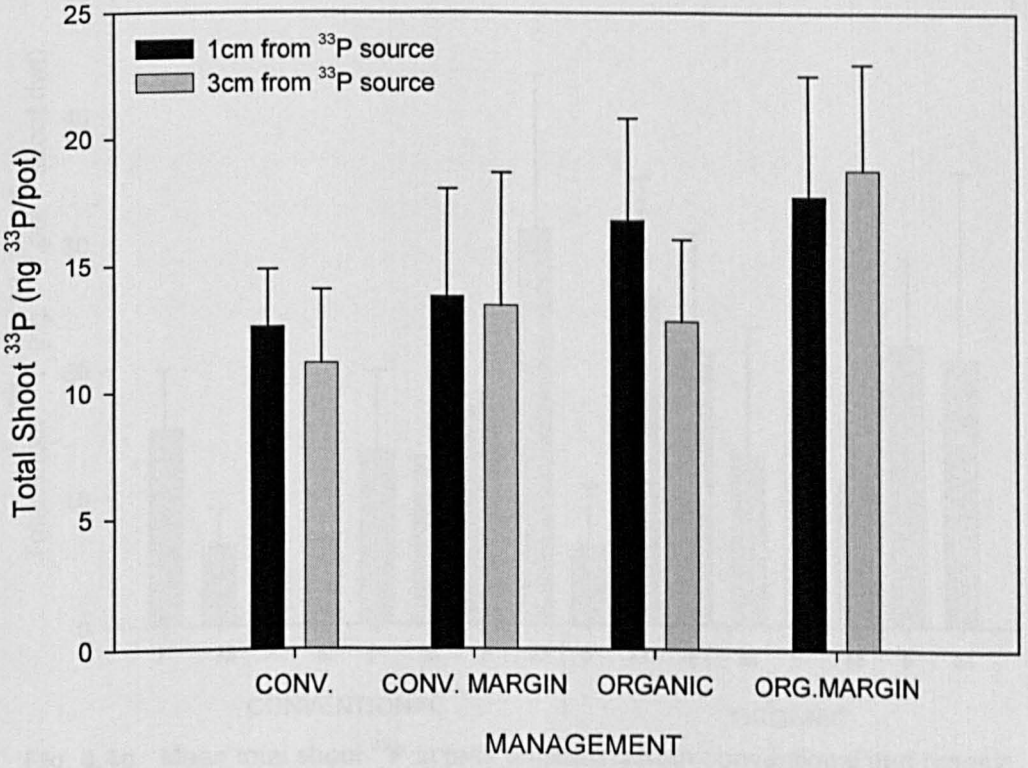

Fig. 4.3b Mean total shoot ^{33}P in turfs at 1cm or 3cm from ^{33}P source, which originated from conventional (CONV.) and organic (ORG.) fields and their margins. Error bars are one standard error.

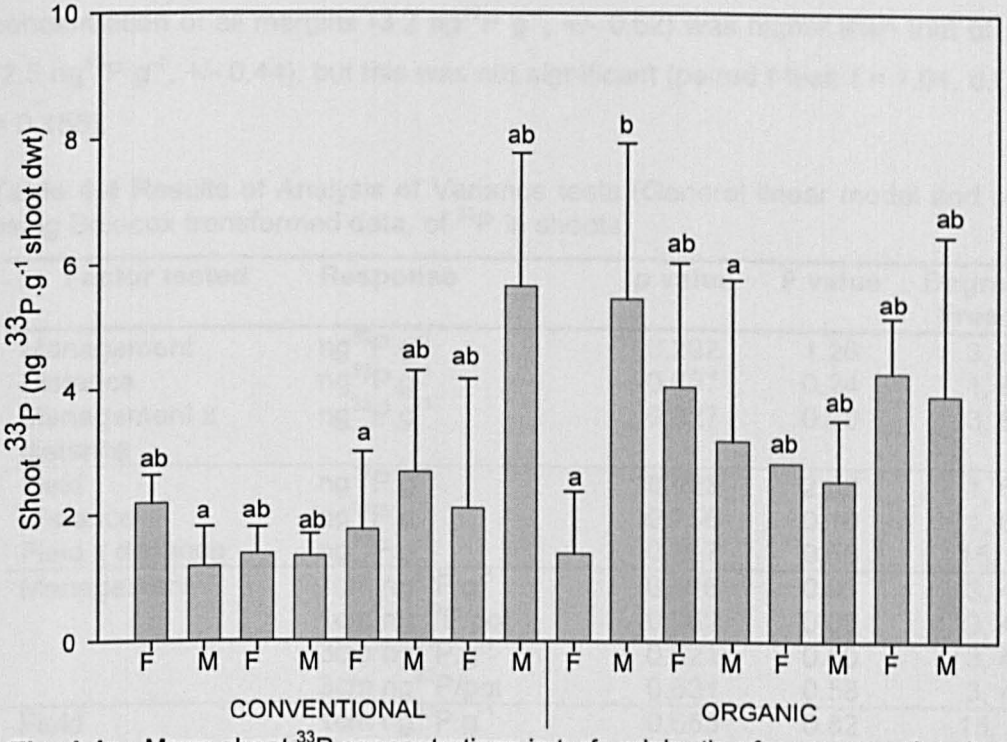

Fig. 4.4a Mean shoot ^{33}P concentrations in turfs originating from conventional and organic fields (F) and their margins (M). Bars for adjacent fields and margins are adjacent above. Bars with the same letter are not significantly different. Error bars are one standard error.

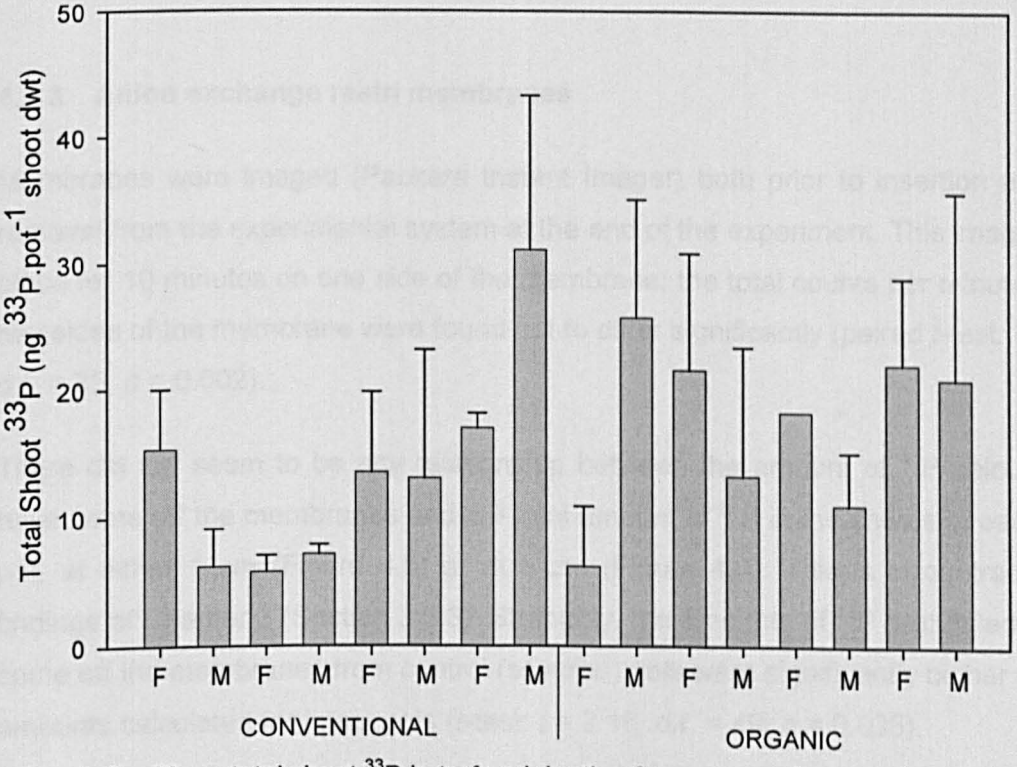

Fig. 4.4b Mean total shoot ^{33}P in turfs originating from conventional and organic fields (F) and their margins (M). Bars for adjacent fields and margins are adjacent above. Bars are not significantly different (Tukey test: $p < 0.05$). Error bars are one standard error.

significant (Table 4.4). In comparing the margins with the fields, the mean shoot ^{33}P concentration of all margins (3.2 $ng^{33}P\ g^{-1}$, +/- 0.62) was higher than that of all fields (2.5 $ng^{33}P\ g^{-1}$, +/- 0.44), but this was not significant (paired *t*-test: $t = 1.04$, d.f. = 21, $p = 0.155$).

Table 4.4 Results of Analysis of Variance tests (General linear model and one-way) using Box-cox transformed data, of ^{33}P in shoots.

Factor tested	Response	*p* value	F value	Degrees of Freedom
Management	$ng^{33}P.g^{-1}$	0.292	1.26	3, 84
Distance	$ng^{33}P.g^{-1}$	0.627	0.24	1, 84
Management x distance	$ng^{33}P.g^{-1}$	0.827	0.30	3, 84
Field	$ng^{33}P.g^{-1}$	**0.023**	2.08	1, 60
Distance	$ng^{33}P.g^{-1}$	0.756	0.10	1, 60
Field x distance	$ng^{33}P.g^{-1}$	0.897	0.56	15, 60
Management	1cm $ng^{33}P.g^{-1}$	0.446	0.91	3, 42
	1cm ng ^{33}P/pot	0.962	0.09	3, 42
	3cm $ng^{33}P.g^{-1}$	0.621	0.60	3, 42
	3cm $ng^{33}P$/pot	0.631	0.58	3, 42
Field	1cm $ng^{33}P.g^{-1}$	0.653	0.82	15, 30
	1cm ng ^{33}P/pot	0.359	1.15	15, 30
	3cm $ng^{33}P.g^{-1}$	**0.024**	2.33	15, 30
	3cm $ng^{33}P$/pot	0.138	1.58	15, 30
Distance	$ng^{33}P.g^{-1}$	0.648	0.21	1, 90

4.3.3 Anion exchange resin membranes

Membranes were imaged (Packard Instant Imager) both prior to insertion and after removal from the experimental system at the end of the experiment. This imaging took place for 10 minutes on one side of the membrane: the total counts per minute for the two sides of the membrane were found not to differ significantly (paired *t*-test: $t = 3.38$, d.f. = 35, $p = 0.002$).

There did not seem to be any relationship between the amount of ^{33}P calculated to have come off the membranes and the total amount of ^{33}P in the shoots of each intact pot, at either 1 cm (Figure 4.5) or at 3 cm (Figure 4.6). This is in contrast to the findings of Chapter 3 (Section 3.3.3). Strangely, the amounts of ^{33}P calculated to have come off the membranes from control (severed) pots were significantly higher than the amounts calculated for intact pots (*t*-test: $t = 2.16$, d.f. = 49, $p = 0.035$).

4.3.4 Mycorrhizal colonisation

The total AMF root length colonisation of the bioassays was similar across all treatments, which were not significantly different at either 1 cm (ANOVA: F = 0.31, d.f

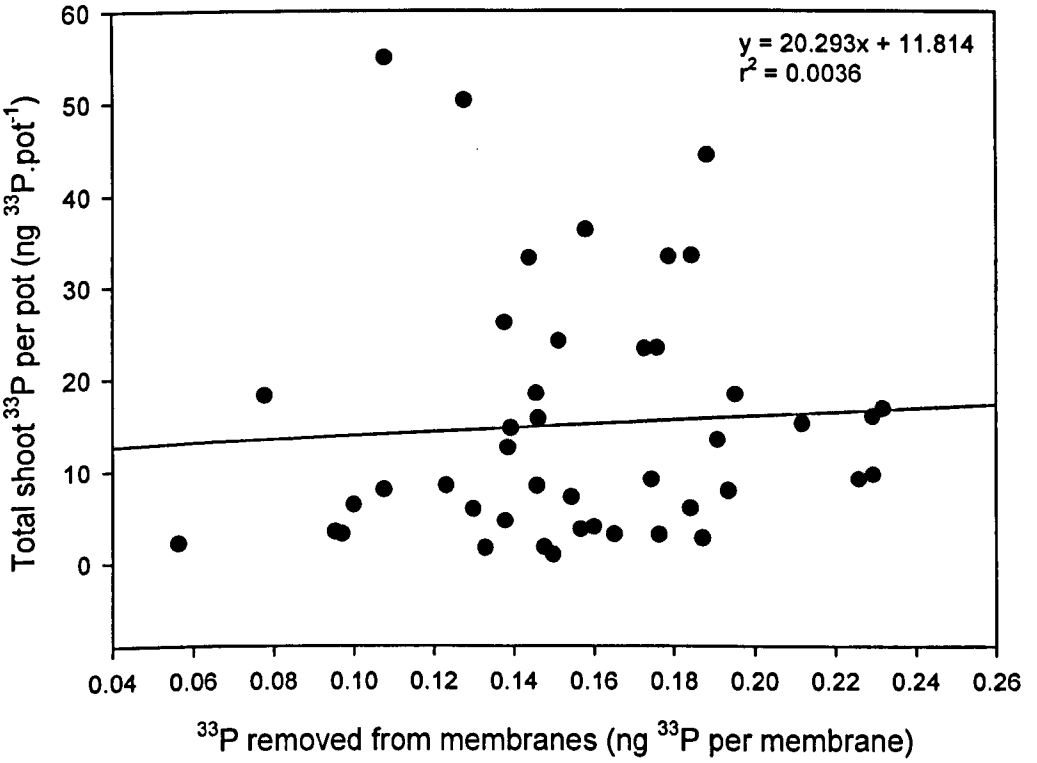

Fig. 4.5 Relationship between amount of ^{33}P removed from membranes and total shoot ^{33}P per pot, in split-pots with **1 cm** between turf and membrane.

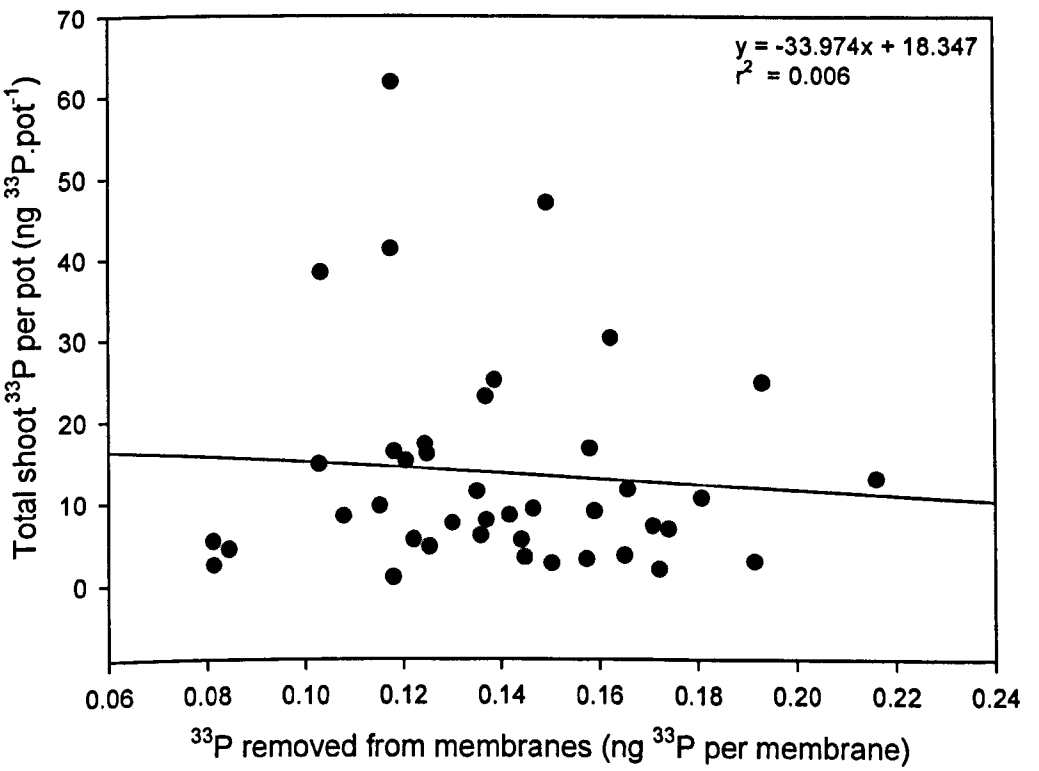

Fig. 4.6 Relationship between amount of ^{33}P removed from membranes and total shoot ^{33}P per pot, in split-pots with **3 cm** between turf and membrane.

= 3, 44, p = 0.819) or 3 cm (ANOVA: F = 0.05, d.f = 3, 44, p = 0.987) from the turf compartment (Figure 4.7).

4.3.5 Relationships between shoot ^{33}P uptake, mycorrhizal colonisation, and soil P status

There were no significant relationships between the variables measured in this experiment, shoot ^{33}P at 1 cm and 3 cm from the membrane compartments, AMF root length colonisation, and Olsen-extractable P (Chapter 2) in the turf compartments (Table 4.5).

Table 4.5 Relationships between variables measured in intact turf systems, results of correlation tests. All ^{33}P data is from intact turf systems and is in ng^{33}P g^{-1} (d.f. = 14).

Variable 1	Variable 2	*r*	*p*
Shoot ^{33}P at 1 cm	Olsen-extractable P	0.040	0.883
Shoot ^{33}P at 1 cm	AMF root length colonisation at 1 cm	0.145	0.593
Shoot ^{33}P at 3 cm	Olsen-extractable P	-0.221	0.411
Shoot ^{33}P at 3 cm	AMF root length colonisation at 3 cm	-0.433	0.094
Olsen-extractable P	AMF root length colonisation at 1 cm	0.026	0.924
Olsen-extractable P	AMF root length colonisation at 3 cm	-0.219	0.415

4.4 Discussion

4.4.1 *Effect of management treatments on shoot ^{33}P uptake*

Whilst the differences in shoot ^{33}P concentrations between the different management treatments are not significant, it is apparent from Figure 4.3 that there is a relationship between management intensity and shoot ^{33}P uptake. That this is not statistically significant is perhaps not too surprising given the many variables and confounding factors: a wide range of soils, varying past management histories, varying indigenous plant species and presumably AMF species, inherently variable radioisotope counts, and a relatively low level of replication given these variables. The experiment builds upon that of Chapter 3, which had very markedly different results between management treatments, and the methods and ideology of this experiment have as their foundation the work described in Chapter 3. It is therefore considered here that these results are biologically meaningful.

The trend seen for increasing shoot ^{33}P concentration with decreasing management intensity supports the hypothesis that AMF hyphae have a greater role in P translocation to plants in systems under low management. The hypotheses that there

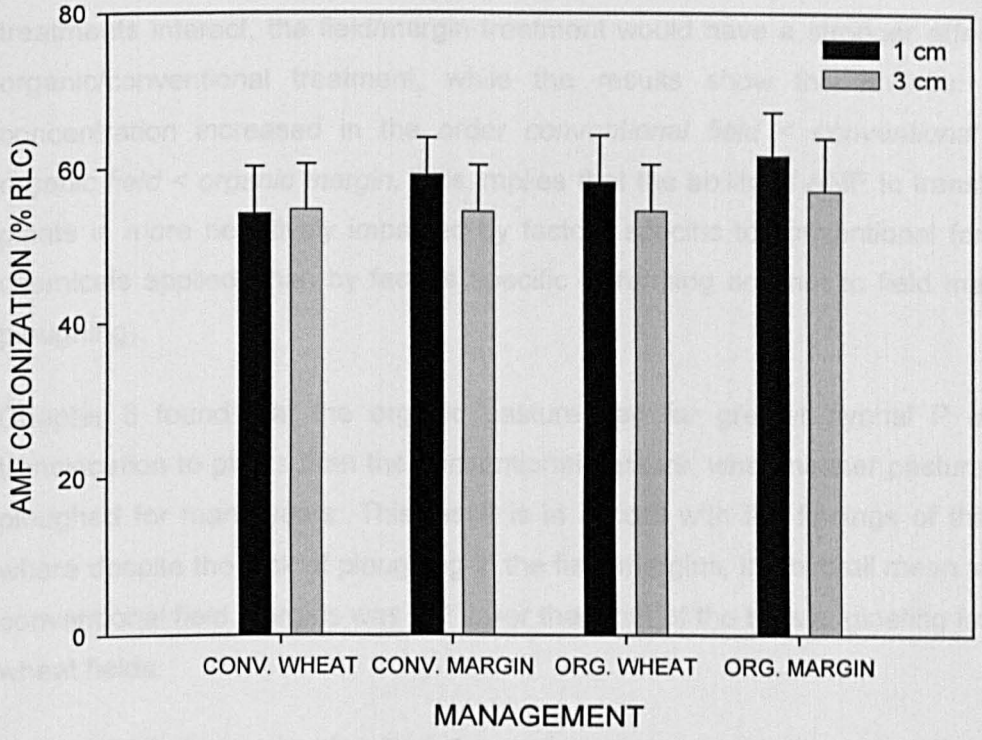

Fig. 4.7 AMF colonization of bioassays in hyphal compartments at 1 cm and 3 cm from turf compartment. Error bars are one standard error.

would be greater hyphal ^{33}P translocation to plants in (a) organic than conventional, and (b) margins than fields, are upheld. However, it was expected that where these treatments interact, the field/margin treatment would have a stronger effect than the organic/conventional treatment, while the results show the reverse: shoot ^{33}P concentration increased in the order *conventional field* < *conventional margin* < *organic field* < *organic margin*. This implies that the ability of AMF to translocate P to plants is more negatively impacted by factors specific to conventional farming (e.g. chemicals applied) than by factors specific to farming and not to field margins (e.g. ploughing).

Chapter 3 found that the organic pasture had far greater hyphal P uptake and translocation to plants than the conventional pasture, when neither pasture had been ploughed for many years. This result is in accord with the findings of this Chapter, where despite the lack of ploughing in the field margins, the overall mean shoot ^{33}P of conventional field margins was still lower than that of the turfs originating from organic wheat fields.

4.4.2 *Hyphal severing treatment*

The hyphal severing treatment, which took place at 3 day intervals, appears highly effective, since there was no detectable shoot ^{33}P uptake under this treatment. Comparing this finding to the results of hyphal severing in Chapter 3, where there was discernible shoot ^{33}P uptake in severed systems, suggests that any future work involving this method should implement the severing at 3 day intervals.

4.4.3 *Membranes*

It is of interest that there was not any apparent relationship between the amount of ^{33}P remaining on the membrane and the shoot ^{33}P concentration, in contrast to the findings of Chapter 3. Further study into the mechanisms governing the process by which the ^{33}P comes away from the anion-exchange resin membrane is required to understand this phenomenon.

4.4.4 *Mycorrhizal colonisation*

The use of bioassay seedlings situated in the hyphal compartments at 1 cm or 3 cm from the turf compartment demonstrated that the AMF present in all systems were able to colonise roots at those distances from existing colonised roots. This use of bioassays was useful, since it established that the split-pot system and the substrate within it were indeed conducive to the effective growth of AMF through the hyphal compartments, as was expected from previous work (Chapter 3; *e.g.* Jakobsen *et al.*,

1992a, 1992b, 2001). Hence the AMF hyphae in all systems would also be able to access the ^{33}P sources. However, the bioassays did not reveal any differences in colonisation patterns across the management treatments.

The absence of observed relationships between management intensity and the AMF colonisation of *Plantago* bioassays has various possible explanations. Most obviously, there may be no difference in the ability of the AMF in each system to colonise roots at those distances. Alternatively, it could be that the relatively small number of intersects examined on each slide and the relatively small number of replicates masked any weak patterns which did, in fact, exist. Another explanation is that the conditions (bioassay species of high mycorrhizal dependency in low nutrient substrate) favoured colonisation and that once hyphae had reached the bioassay roots, they proliferated abundantly within the roots. In this case, it could be useful to use a range of bioassay species, of varying mycorrhizal dependencies, and over a greater range of distances from the turf compartment.

Given that there was no statistical significance here of the effects of management intensity on the ability of indigenous AMF to deliver P to the plant, it is unsurprising that neither were there apparent effects of management treatments in the bioassay study. The fact that there was not a very strong relationship between management intensity and shoot ^{33}P makes it difficult to make strong inferences about the function of the AMF in the different systems. It is entirely possible, however, that the infectivity and root length colonisation of different AMF may be identical, whilst their abilities to provide plants with soil-derived nutrients (and to confer other benefits such as protection from fungal root pathogens) may differ markedly.

Some studies have observed differences in AMF % root length colonisation (RLC) between agricultural management systems. Mäder *et al.* (2002) found 40 % higher RLC in organic than conventional farming systems in Switzerland; Ryan (1999) measured mean RLC to be 48 % in conventional dairy farms versus 71 % in paired biodynamic dairy farms in Australia. In the latter study, it was concluded that the AMF colonisation was a function of the soil P status, and in this chapter the sand-soil matrix in the hyphal compartments was standardized across all treatments, such that the substrate P status was the same for all bioassay seedlings. This could have led to the similar levels of colonisation observed here. However, whilst the levels of mycorrhizal colonisation are pertinent, what is of greater interest here is the functioning of the mycorrhiza in the various systems. Scullion *et al.* (1998) did find evidence that AMF spore inocula from organic farmland were more effective at delivering P to *Allium ameloprasum* plants growing in soil of low fertility (total shoot P content 5.08 mg for

organic versus 1.09 mg for conventional AMF inocula). It is studies such as this, which examine the functional aspects of mycorrhiza, which are required to understand the importance of AMF in differently managed systems.

4.4.5 *Relationships between shoot ^{33}P uptake, mycorrhizal colonisation, and soil P status*

The fact that there were no apparent relationships between shoot ^{33}P uptake, mycorrhizal colonisation, and soil extractable P could be seen as a vindication of the hypothesis that there are functional differences in the AMF indigenous to different systems, since this could indicate that hyphal P acquisition and translocation to plants is independent of the degree of root colonisation or soil nutrient status. However, the effects of management treatment on shoot ^{33}P concentration are simply not strong enough to draw any definite conclusions, and the data could even be interpreted to suggest that there are no differences whatsoever between the AMF indigenous to the different turfs.

4.4.6 *Overview*

Chapter 3 showed a strong management effect on AMF functioning in a few, very different, agricultural systems. This Chapter sought to find functional differences between mycorrhizal communities across a wide range of sites under similar management regimes. A trend was found here, of increasing hyphal P translocation to plants with decreasing management, although this trend was weak.

This is an under-researched area which is of great relevance to today's agriculture, where land is increasingly being converted to organic management. These experiments examining mycorrhizal functioning under different management intensities are novel, and have uncovered the likelihood that AMF in certain low-intensity management systems translocate greater amounts of P to plants than those under intensive agriculture. More research is needed in this important and topical area, using carefully-controlled long-term experimental manipulations such as the "DOK" trials comparing bio-dynamic, organic and conventional agriculture in Switzerland over 25 years (Mäder *et al.*, 2002). If the work presented here is indeed representative of what occurs in the field, it could have implications for farming practice: particularly when land is converted to a lower intensity of management.

Chapter 5:
Carbon flux from plant shoots through soil to double-walled hyphal in-growth cores: temporal changes

Chapter 5: Carbon flux from plant shoots through soil to double-walled hyphal in-growth cores: temporal changes

5.1 Introduction

Various studies have examined carbon (C) transfer from plants to AMF, usually in pot studies using single isolates of AMF, and have estimated that 1 - 20 % of photosynthate is allocated to AMF (Paul and Kucey, 1981; Jakobsen and Rosendahl, 1990). Some studies have used natural AMF communities to trace AMF external hyphal carbon uptake (Gavito and Olsson, 2003) and hyphal respiration (Johnson *et al.*, 2001, 2002b), but very little work has been done on AMF carbon dynamics in the field (Johnson *et al.*, 2002a). The quantities of carbon which AMF receive from their plant hosts vary with AMF species (Pearson and Jakobsen, 1993), and depend upon environmental conditions such as light intensity (Son and Smith, 1988), defoliation (Allsopp, 1998), and soil liming (Johnson *et al.*, 2002a).

The use of hyphal in-growth cores as a means to control hyphal growth has been used successfully by Johnson *et al.*, (2001, 2002a, 2002b) as an alternative to sterilizing control soil using heat or chemical methods, which have drawbacks since this may affect the chemistry and biology of the soil system. These mesh-bound cores are left *in situ* for hyphae to grow into, whilst excluding roots. By rotating the cores, hyphal connections are severed, creating control cores. Johnson *et al.* (2001) found that by rotating control cores weekly, the length of extractable hyphae within the cores significantly decreased compared to static cores (mean hyphal length 2.4 (+/- 0.6) cm g^{-1}dwt in rotated control cores, and 9.1 (+/- 1.5) cm g^{-1}dwt in static cores). Later work by Johnson *et al.* (2002a) using $^{13}CO_2$ pulse labelling of turfs containing hyphal in-growth cores found significant differences in $^{13}CO_2$ evolved from static and rotated cores after removal of cores from the turfs. In a similar study using ^{14}C pulse-labelling (Johnson *et al.*, 2002b), core rotation decreased the ^{14}C concentration in the soil within the core by 90% compared to static cores. This was attributed to the exclusion of hyphal pathways for ^{14}C movement into the cores, since tests found no significant effect of core rotation on passive diffusion of ^{14}C from bulk soil into the cores (*ibid.*).

The majority of C allocated to AMF is respired. Johnson *et al.* (2002a) calculated that within 21 h of pulse-labelling, between 3.9 and 6.2 % of C fixed by the plants was respired by AMF mycelium. The patterns of CO_2 evolution from the cores were related to the photoperiod, peaking during the dark period at 10 – 20 hours

following pulse-labelling with ^{13}C, falling during the following photoperiod, to rise again at 34 hours from labelling, in the next dark period (*ibid.*). After 7 days, the $^{13}CO_2$ evolving from hyphal in-growth cores had dropped to control levels, and no ^{13}C-enrichment was detected remaining in the cores at that time, although the authors acknowledge that may have been due to difficulty with detecting ^{13}C in the organically-rich soil. Even the hyphal carbon which is not respired has a high turnover rate: Staddon *et al.* (2003) recently used pulse-labelling with fossil CO_2 (free of ^{14}C) to measure turnover of hyphal carbon (in several *Glomus* species) in pot cultures with *Plantago lanceolata*. By collecting AMF hyphae near the plant roots and measuring their ^{14}C content, they found the hyphal ^{14}C content returned to near control levels 5 – 6 days after labelling, although some ^{14}C depletion was still evident 30 days after labelling.

Chapters 3 and 4 described experimental systems which used nylon mesh barriers to create root-free hyphal in-growth compartments. The size of the mesh pores excludes plant roots. However, it is possible that root hairs may protrude through the mesh pores, since the radius of root hairs varies from 5 to 20 μm, and their length varies from 0.1 to 1.5 mm (Nielsen, 2002). This possibility of root hairs protruding into "hyphal" compartments mars an experimental system which has advantages over the use of fungicides to create non-hyphal controls. Hence a novel system was designed, that of double-walled hyphal in-growth cores, which have an outer mesh-bounded compartment where it is possible that root hairs may protrude, and an inner compartment which is too distant from the bulk soil for root hairs to penetrate. When measuring below-ground carbon allocation to AMF hyphae, it is thus possible to measure carbon in two zones, both of which exclude roots and will be colonised by AMF mycelium, and one of which it is certain that no root hairs will penetrate. Further, if it were to be assumed that root hairs are not responsible for any significant input of carbon to these hyphal in-growth cores, then double-walled cores would enable measurement of ^{14}C fractions in an outer core compartment, (0 – 5 mm from the bulk soil), and in an inner core compartment (5 – 15 mm from the bulk soil). This may indicate in which of these zones the most active AMF hyphae are concentrated.

This novel use of a doubly-compartmented system for measuring ^{14}C translocated to hyphae at different distances from the soil-root phase may be of use in comparing ^{14}C translocation to, and respiration from, AMF hyphae indigenous to different management systems, as explored in Chapter 6. For the piloting of this experimental design, turfs were sampled from land known to support a range of mycorrhizal species. Little work has been published describing

flow of recent photosynthate to AMF external mycelium over a time series, so this was examined here, such that an appropriate time for harvesting the following experiment (Chapter 6) could be determined.

5.1.1 Aims and hypotheses

This experiment therefore aimed (i) to pilot the use of double-walled hyphal in-growth cores, (ii) to examine flow of carbon into double-walled hyphal in-growth cores over time, and (iii) to examine $^{14}CO_2$ evolution from the cores over time, this being a reflection of hyphal respiration rates. It was hypothesised that (a) values of ^{14}C measured from rotated (severed hyphae) cores would always be lower than for cores left static prior to labelling, (b) the $^{14}CO_2$ evolution from cores would decrease with time, and (c) the ^{14}C content of soil (containing hyphae) within cores would decline with time after labelling. In relation to root hairs, it was hypothesised that if root hairs are penetrating the outer core mesh, there will be much higher ^{14}C in the soil of the outer core compartment than in the inner core compartment.

5.2 Materials and Methods

5.2.1 Material and growth conditions

The turfs in this study originated from Wardlow Hay Cop, Derbyshire, a mixed species calcareous grassland subjected to light grazing in summer (Section 2.2; Rodwell, 1992; Pigott, 1962). The most common plant species extant here are given in Table 5.1. In January 2002, thirty-six turfs were cut from the grassland and placed directly into pots measuring 13 cm x 13 cm x 13 cm (width, depth, height; tapered to base of 11 cm x 11 cm) with the soil fitting closely into the pots. These turfs were placed in controlled environment conditions (20 °C, 18 h day, 18 °C, 6 h night, light intensity 280 μmol m^{-2} s^{-1}). The pots were kept moist with distilled water and the shoots were periodically trimmed to simulate grazing. During their time in the growth room, the pots were re-distributed every month in an attempt to nullify any edge effects due to possible environmental gradients.

Table 5.1 Plant species common to the turfs used (Rodwell, 1992; Ames, 2003), and their mycorrhizal associations (Harley and Harley, 1987).

Form arbuscular mycorrhiza	Usually not mycorrhizal
Avenula pratensis	*Carex flacca*
Briza media	
Festuca ovina	
Hieracium pilosella	
Koeleria macrantha	
Leontodon hispidus	
Linum catharticum	
Lotus corniculatus	
Plantago lanceolata	
Sanguisorba minor	
Scabiosa columbaria	
Thymus praecox	

5.2.2 Double walled cores

Double walled cores (32 mm diameter, 120 mm tall) were designed to create hyphal compartments in two zones: an outer compartment (0 – 5 mm from bulk soil) and a central inner compartment (> 5 mm from bulk soil, 21 mm in diameter). These cores were made from a frame of PVC piping with four windows cut away, with 35 μm pore sized nylon mesh (Plastok Ltd., Birkenhead, U.K.) attached to the sides and base (Figure 5.1). The mesh was adhered to the frame using Polypipe glue (Polypipe Gap Filling Cement, Substance Identification no. 1133; Polypipe Plc., Doncaster, U.K.), which was cured at 80 °C for at least 24 hours. The inner cores were assembled and fixed inside the outer cores using plastic spacers stuck with Polypipe glue.

The cores were filled with a 1:1 sand-soil mixture, using autoclaved acid-washed silica sand, and soil as described in Section 3.2.2. The soil had been sieved to 2 mm, thoroughly mixed with the sand and homogenised, air dried, then stored in the dark for 9 months. This process ensured that no viable AMF inoculum remained in the soil, a fact verified by growing 36 bioassay seedlings of *Plantago lanceolata* in seed trays filled with the medium for 2 months, kept moist with distilled water, in controlled environment conditions (20 °C, 18 h day, 18 °C, 6 h night, light intensity 280 μmol $m^{-2}s^{-1}$). The excised roots of these seedlings were cleared, stained, and examined at x 100 magnification (as described in Section 2.3.11), and no AMF infection was observed.

In January 2002, two double-walled cores were inserted into each of the micro-turfs, using a soil corer to make an appropriately sized hole in the soil. They were

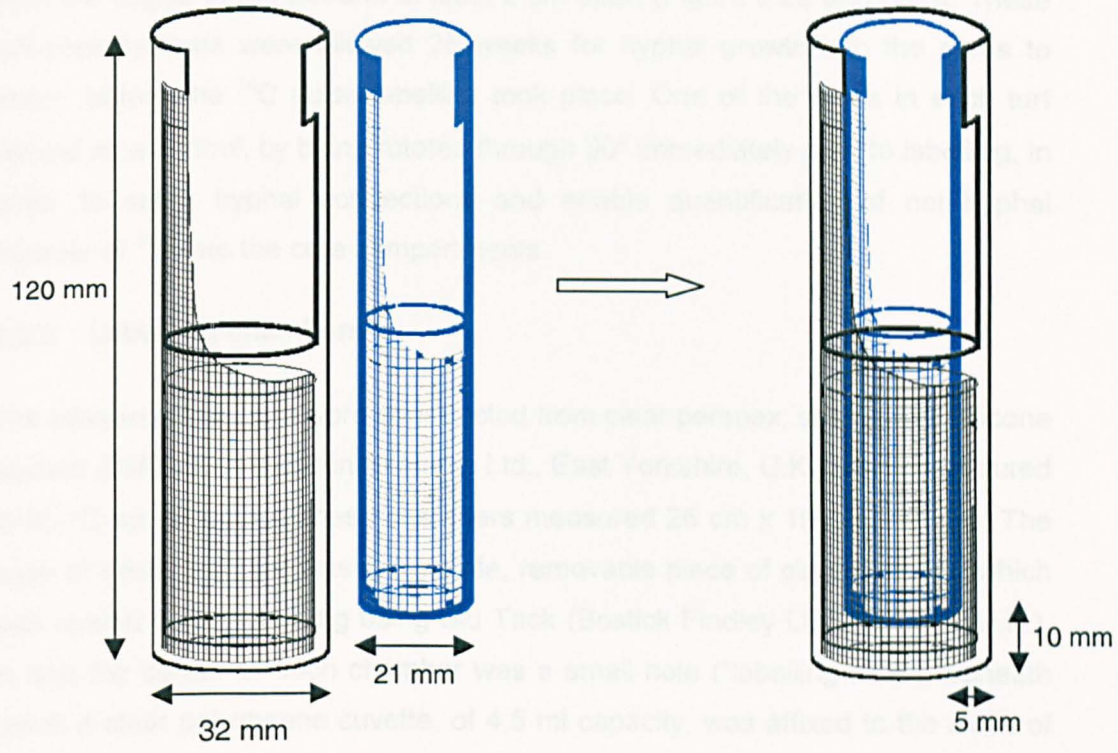

Fig. 5.1 Diagrams of double-walled hyphal in-growth cores. Two cores (left) made from plastic tubing with windows cut, with sides and base covered by 35 μm nylon mesh, were fixed together to form a core (right) with inner and outer compartments. Cores are shown here with mesh cut away. There was 5 mm between the sides of the inner and outer cores, and 10 mm between their bases.

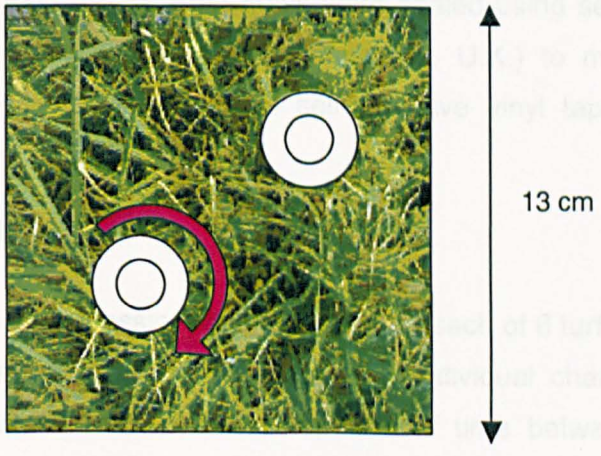

Fig. 5.2a Plan-view diagram of micro-turf with two double-walled mesh cores inserted, one of which was rotated, prior to labelling, to sever hyphal connections.

situated in diagonally opposite corners of the turfs, approximately 2 cm away from the edges of the pot and at least 2 cm apart (Figure 5.2a and 5.2b). These turf-core systems were allowed 25 weeks for hyphal growth into the cores to occur, before the ^{14}C pulse labelling took place. One of the cores in each turf served as a control, by being rotated through 90° immediately prior to labelling, in order to sever hyphal connections and enable quantification of non-hyphal transfer of ^{14}C into the core compartments.

5.2.3 Labelling chambers

The labelling chambers were constructed from clear perspex, sealed with silicone sealant (Silfix HM, Hodgson Sealants Ltd., East Yorkshire, U.K.) which was cured at 50 °C for 48 hours. These chambers measured 26 cm x 19 cm x 19 cm. The base of each chamber was a separate, removable piece of clear perspex, which was sealed during labelling using Blu Tack (Bostick Findley Ltd., Stafford, U.K.). In one top corner of each chamber was a small hole ("labelling hole"), beneath which a clear polystyrene cuvette, of 4.5 ml capacity, was affixed to the sides of the chamber using silicone sealant. The ^{14}C tracer ($NaH^{14}CO_3$; Section 5.2.5) was injected through this hole, using a syringe, into the cuvette. Prior to each labelling event, these cuvettes were pre-rinsed in a saturated solution of $NaHCO_3$ followed by rinsing with distilled water. The aim of this was to prevent binding of the labelling solutions onto the surface of the plastic cuvette. In another side of the chamber (adjacent to that on which the cuvette was affixed) was a second hole for gas sampling (Figure 5.3). Both holes were sealed using self-adhesive vinyl tape (Scientific Laboratory Supplies, Nottingham, U.K.) to maintain the chamber as gas tight, and re-sealed with self-adhesive vinyl tape following labelling or gas sampling.

5.2.4 Experimental design

The micro-turfs were randomly assigned to five cohorts, each of 6 turfs. The turfs within each cohort were labelled simultaneously, in individual chambers, and each cohort of turfs was left for a different period of time between the ^{14}C labelling event and the destructive harvesting of the system. These time periods were 8 h, 10 h, 25 h, 36 h, and 7 days.

5.2.5 ^{14}C labelling

The turf-core system was labelled with ^{14}C by providing $^{14}CO_2$ in the atmosphere surrounding the plants for a period of 1 hour. To do this, each turf was sealed

Fig. 5.2b Mixed micro-turf from Wardlow, with two double walled hyphal ingrowth cores inserted. Core A left undisturbed, core B rotated prior to labelling to sever hyphae.

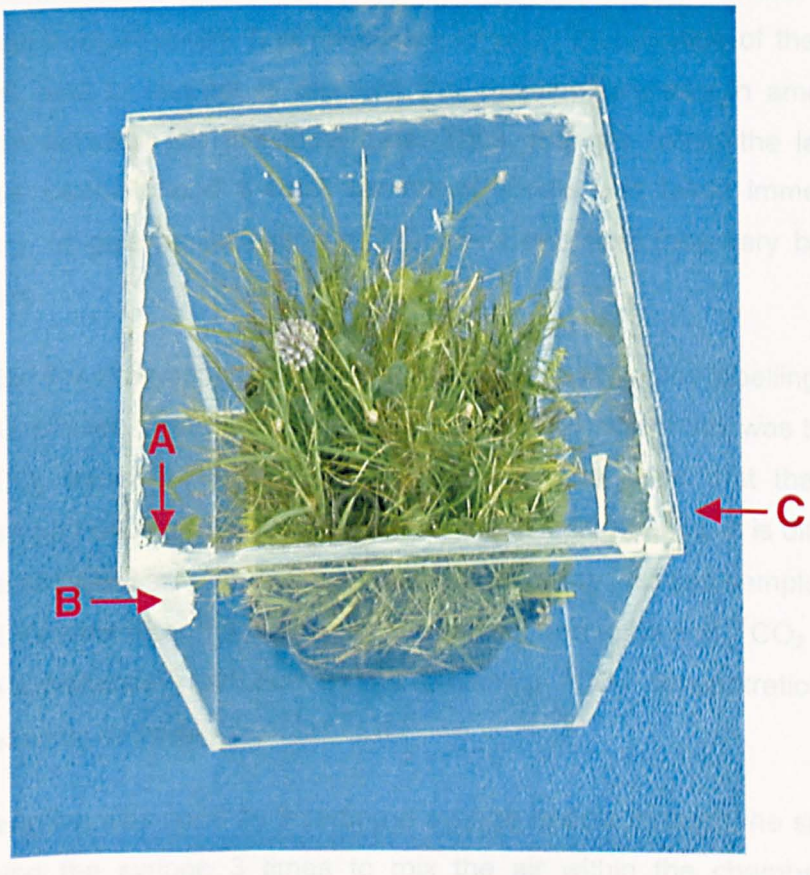

Fig. 5.3 Micro-turf shown inside labelling chamber. Arrow A shows labelling hole, arrow B shows cuvette inside chamber, arrow C shows gas sampling hole.

inside a perspex labelling chamber, as described above. The labelling and sampling holes in the chambers were sealed with self-adhesive vinyl tape. The chambers were placed under lights (340 μmol m^2 s^{-1} PAR) in a fume cupboard. To provide a labelled atmosphere, 0.6 MBq of $NaH^{14}CO_3$ (*i.e.* 8.1 μl, at specific activity 2.1 GBq.mMol^{-1}, obtained from ICN Pharmaceuticals Inc., Basingstoke, U.K.) was injected through the labelling hole into the chamber cuvette. The labelling hole was re-sealed with self-adhesive vinyl tape to form a gas-tight septum for injecting lactic acid. To release the ^{14}C as CO_2, approximately 3 ml of 10 % lactic acid was injected into the cuvette containing the ^{14}C-bicarbonate, and the labelling hole quickly re-sealed with tape to make the chamber gas-tight. The chambers were left for 1 hour before being opened to allow ambient air to contact the turf.

5.2.6 Gas sampling and shoot sampling

Assessing how much $^{14}CO_2$ has been fixed by the plants is problematic, since the aim of this experiment is to trace the isotope through to AMF hyphae, and thus by the time of final harvest the $^{14}CO_2$ initially fixed has moved around the system and some has been respired. It is necessary to verify that fixation of the $^{14}CO_2$ has occurred, and to attempt to discount that major differences in amount of $^{14}CO_2$ fixation between the individual turfs. Thus, the gas within the labelling chamber was sampled, and a small sample of shoots was taken immediately after labelling, to give an indication of ^{14}C levels and how they vary between individual turfs.

The gas within the chamber was sampled twice during each pulse labelling event: at 10 and 60 minutes after the $^{14}CO_2$ was released. The aim of this was to verify that the $^{14}CO_2$ was indeed present within the chamber, and that the $^{14}CO_2$ concentration had declined towards the end of the labelling period. It is difficult to compare the two gas samples, since the exact time of the first gas sample varied from 5 to 11 minutes after the isotope was released, and uptake of $^{14}CO_2$ is likely to decrease exponentially with time as the remaining $^{14}CO_2$ concentration in the chamber becomes diluted.

This gas sampling was done by inserting a syringe needle through the sampling hole, pumping the syringe 3 times to mix the air within the chamber, then removing 10 ml of air from the chamber. This gas sample was then injected through a Suba seal into a plastic scintillation vial containing 7 ml Carbosorb (Packard Biosystems) and 7 ml Permafluor E+ (Packard Biosystems), within

which a vacuum had been created by syringing out 20 ml of air from the vial. The syringe was pumped several times with the needle extending through the Suba seal into the vial headspace, over at least 15 minutes. The syringe was then finally evacuated and removed, and the vials were quickly capped. These samples were counted directly on a liquid scintillation counter (Tri-Carb 3100 TR, Packard).

Following the 1-hour labelling, the chambers were opened and ambient air allowed to circulate around the plants. A small sub-sample of representative vegetation was removed from each turf, immediately post-labelling, and dried in a paper envelope at 80 °C for > 48 hours. This vegetation sub-sample was removed from a part of the turf equidistant from both cores, at one corner of the pot. Following drying, vegetation samples were cut with scissors into a small pieces, and mixed, to homogenise them. They were then oxidised (see below). Should the ^{14}C content of these shoot sub-samples have varied widely, other results would need to be treated with caution since it could indicate disparate amounts of ^{14}C have entered the system and hence the amounts of ^{14}C in the various compartments of the system could not be compared between individual turfs.

5.2.7 Harvest of shoots and bulk soil

The turfs remained under lights, with a 16-hour photoperiod and an 8-hour night when appropriate, for the time periods of (i) 8 h, (ii) 10 h, (iii) 25 h, (iv) 36 h, and (v) 7 days. The turfs were kept moist by watering with distilled water. After the allotted time interval, the cores were removed from the turfs and incubated (see below, Section 5.2.9), the plant shoots were cut at the level of the soil surface (and dried in a paper envelope at 80 °C for > 48 hours), and bulk soil samples were taken. Prior to drying, the shoots were sorted into species which are typically mycorrhizal or non-mycorrhizal (Table 5.1), since only the mycorrhizal shoots should exhibit a strong relationship between foliar ^{14}C uptake and ^{14}C within (and respired from) the hyphal in-growth cores.

The bulk soil was sampled by taking a 5 mm vertical slice through the middle of the pot, equidistant from where the two cores were situated. This slice of soil was dried in a paper envelope at 80 °C for at least 48 hours, stored in a dessicator, and subsequently homogenised by grinding with a pestle and mortar.

5.2.8 Analysis of ^{14}C content

The vegetation and soil samples were oxidized on a Packard Sample Oxidizer (model 307), following the manufacturer's instructions. A recovery test was performed at the start of each day, prior to samples being run, using non-active SpecCheck (Packard) and ^{14}C-SpecCheck (Packard). The percentage of ^{14}C recovery varied slightly from day to day, and each value was used to calculate the sample ^{14}C oxidised on that same day. The samples were oxidized in Combusto-cones (Packard) wrapped in tissue, adding 200 µl Combustaid (Packard) to moisten each soil sample, immediately prior to burning. Three sub-samples were oxidized from each sample to ensure reliability, and the mean of these three values was used in subsequent statistical analysis. A blank was oxidized between each set of three sub-samples to check for carry-over of activity. With soil samples, the carry-over was negligible, but with the highly radioactive vegetation samples there was some carry-over of radioactivity after the sample. Therefore the values obtained for ^{14}C in vegetation samples had the value measured in each preceeding blank subtracted from them, to reduce error caused by variable carry-over from samples. The ^{14}C was collected in a mixture of 10 ml Carbosorb with 10 ml Permafluor E+, in plastic scintillation vials, and measured using a liquid scintillation counter (Packard Scintillation Counter).

Results for this experiment are given as mass of ^{14}C, calculated (using the specific activity of the isotope) from output from the liquid scintillation counter given as disintegrations per minute.

5.2.9 Incubation of cores

5.2.9.1 Rationale

Incubation of cores, in a sealed container containing a KOH trap, enabled the $^{14}CO_2$ which evolved from the cores to be quantified. Some of this $^{14}CO_2$ will be the product of AMF hyphal respiration, whilst some may have other origins (such as other soil micro-organisms respiring $^{14}CO_2$ originating in root exudates). What portion of $^{14}CO_2$ evolved from the cores is likely to be from hyphal respiration may be calculated by subtracting the $^{14}CO_2$ evolution of rotated cores from that of undisturbed cores. Since some of the $^{14}CO_2$ trapped from the cores may have moved through chemical rather than biological pathways, and may passively diffuse out from the cores, several trapping periods were used. This also enabled calculation of changes in rate of $^{14}CO_2$ evolution over time, since it may be

expected that this rate will decline with time after cores were removed from the turfs.

Several subsequent traps were used for each core, three for the intact cores, and two for the soil-sand from each core compartment. The first trap was 90 minutes, a slightly longer time period than the second and third traps which were each of 60 minutes. The longer time period of the first trap was chosen since there may be greater passive diffusion of $^{14}CO_2$ at the start of the incubation, and if the rate of $^{14}CO_2$ evolution were very much greater during this time period, this could perhaps be attributed to non-hyphal pathways. Once the cores were cut open and the soil-sand from each compartment incubated separately, the time period of 60 minutes for the fourth trap allowed initial rate of $^{14}CO_2$ evolution in the soil-sand to be quantified, following the severe disturbance of mixing the soil-sand which could induce greater $^{14}CO_2$ evolution caused by breaking up hyphae. The 14 hour time period for the fifth trap was mainly chosen for convenience. However, a longer trap could enable small differences to become apparent, since by this stage the hyphal respiration rate may have fallen (due to length of time from cores being removed from turfs, and to the disturbance created after cutting the cores open).

5.2.9.2 Method

Following removal from the micro-turfs, the cores were incubated and $^{14}CO_2$ released was trapped and measured. Each core was placed into a 500 ml conical flask, which was sealed with a rubber bung, and placed in a water bath in a controlled temperature room at 18 °C. Within each flask was a plastic scintillation vial containing 3 ml 0.1 M KOH (potassium hydroxide), to absorb ^{14}C escaping from the core. The cores were incubated thus for 90 minutes, then the flasks were unsealed, and the vial of KOH removed and replaced with a fresh vial also containing 3 ml 0.1 M KOH. The flasks were then resealed, and after a further 1 hour incubation these second vials were removed and again replaced with a third set of vials, which were incubated for a further hour (Figure 5.4). The cores were then split open and the sand-soil in each compartment was removed and mixed. Sub-samples were taken and dried (in paper envelopes at 80 °C for at least 48 h, measured by sample oxidation as detailed above in Section 5.2.5), whilst the remainder of the sand-soil mix from each compartment was placed into plastic tubes (32 mm diameter, 200 mm high, solid base), and sealed with a Suba seal. Into each of these tubes was placed a plastic eppendorf vial containing 1 ml 0.1 M KOH. The soils were further incubated thus for 90 minutes, at which point the

vials of KOH were removed and replaced, then there was a final incubation period of 14 hours (Figure 5.4).

From each of the 3 ml KOH traps, a 1 ml aliquot was taken and placed into 10 ml Emulsifier Safe (Packard Biosystems), and the radioactivity measured on a liquid scintillation counter. From each of the 1 ml KOH traps the aliquot taken was 0.5 ml, which was also placed into 10ml Emulsifier Safe and analysed as above.

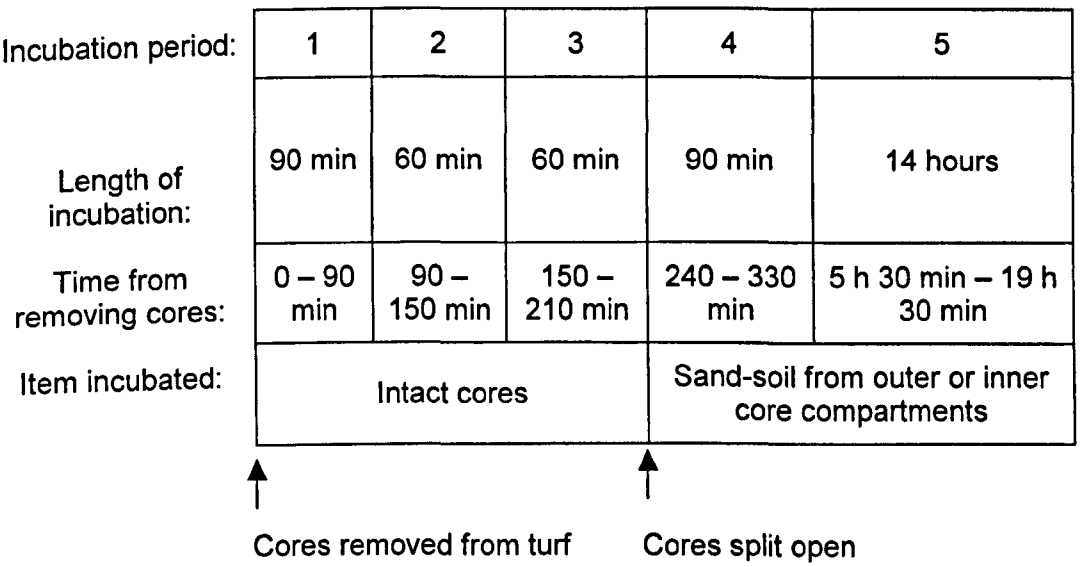

Fig. 5.4 Incubation times of intact cores and sand-soil from core compartments with different KOH traps.

5.3 Results

Results are given firstly for concentrations of ^{14}C in the labelling chambers at the start and at the end of the pulse labelling, and uptake by plant shoots. The second section of results pertains to the $^{14}CO_2$ trapped from intact cores following removal of cores from turfs. The data for $^{14}CO_2$ trapped from intact cores by three subsequent KOH traps is followed by that for $^{14}CO_2$ trapped during incubation of soil from each core compartment. The final section of results contains data for ^{14}C in the soil within the cores.

5.3.1 ^{14}C content of gas samples and initial shoot samples

The gas samples taken from the labelling chambers give an indication of the amounts of ^{14}C in the labelling chambers early in the labelling period, and at 60 minutes from labelling. Early gas samples were not significantly different from each other (ANOVA on $\log_e$ transformed data: $F = 0.93$, d.f. = 3,20, $p = 0.443$;

Figure 5.5). Of the 60 minute gas samples, the cohort of turfs which were subsequently left for 25 hours until harvesting had significantly lower ^{14}C in the labelling chamber than those cohorts left for 8 h and 10 h to harvest (ANOVA on $\log_e$ transformed data: $F = 3.89$, d.f. = 4,25, $p = 0.014$; Tukey multiple comparison test, $p < 0.05$; Figure 5.5). This could imply that more ^{14}C was taken up by the 25-hour turfs; however when the values of ^{14}C in gas samples taken early in the labelling period are subtracted from the values in samples taken 60 minutes after labelling, for each turf, there is some variation between labelling cohorts but the differences are not significant since the individual values vary greatly, and are not all positive (ANOVA: $F = 0.92$, d.f. = 3,20, $p = 0.451$).

Different sets of plants labelled together did vary in quantities of ^{14}C found in shoot samples taken immediately post-labelling, with shoots from the 8-hour turfs containing significantly higher concentrations of ^{14}C than the 10-hour and 30-hour turf cohorts (ANOVA on $\log_e$ transformed data: $F = 6.4$, d.f. = 3, 20, $p = 0.003$). No samples were taken from the 7-day turfs (Figure 5.6). Some of the variability in ^{14}C content of shoots between turfs may have been due to differences in shoot composition, which were inevitable in such a small subsample.

There was no significant relationship between ^{14}C concentration of shoot samples taken immediately after labelling ($ng^{14}C.g^{-1}$ shoot dry weight, mean of three replicates) and the difference in ^{14}C between early and late gas samples (CPM ml^{-1}) for all the turfs ($y = 162 - 0.0004x$, $F < 0.00$, d.f. = 1, 16, $p = 0.983$). However, the shoot samples taken were rather small, and how representative the gas samples are may be questionable, since for the labelling of two individual turfs, the early gas samples were smaller than the later samples. This could imply that the $^{14}CO_2$ was not evenly distributed around the chamber when the gas was sampled.

5.3.2 ^{14}C content of shoots

It is clear that the non-mycorrhizal plant shoots contained consistently greater concentrations of ^{14}C than mycorrhizal plants (ANOVA on $\log_e$ transformed data: $F = 35.9$, d.f. = 1, 50, $p < 0.001$; Figure 5.7). This may relate to the respiration rates of the different plants, or to the drain on shoot carbon by the AM fungi. Among the shoots of mycorrhizal plant species, the ^{14}C concentration was significantly higher at 8 hours from labelling than all the other times (ANOVA on $\log_e$ transformed data: $F = 8.6$, d.f. = 4, 25, $p < 0.001$, Tukey multiple comparison test $p < 0.05$).

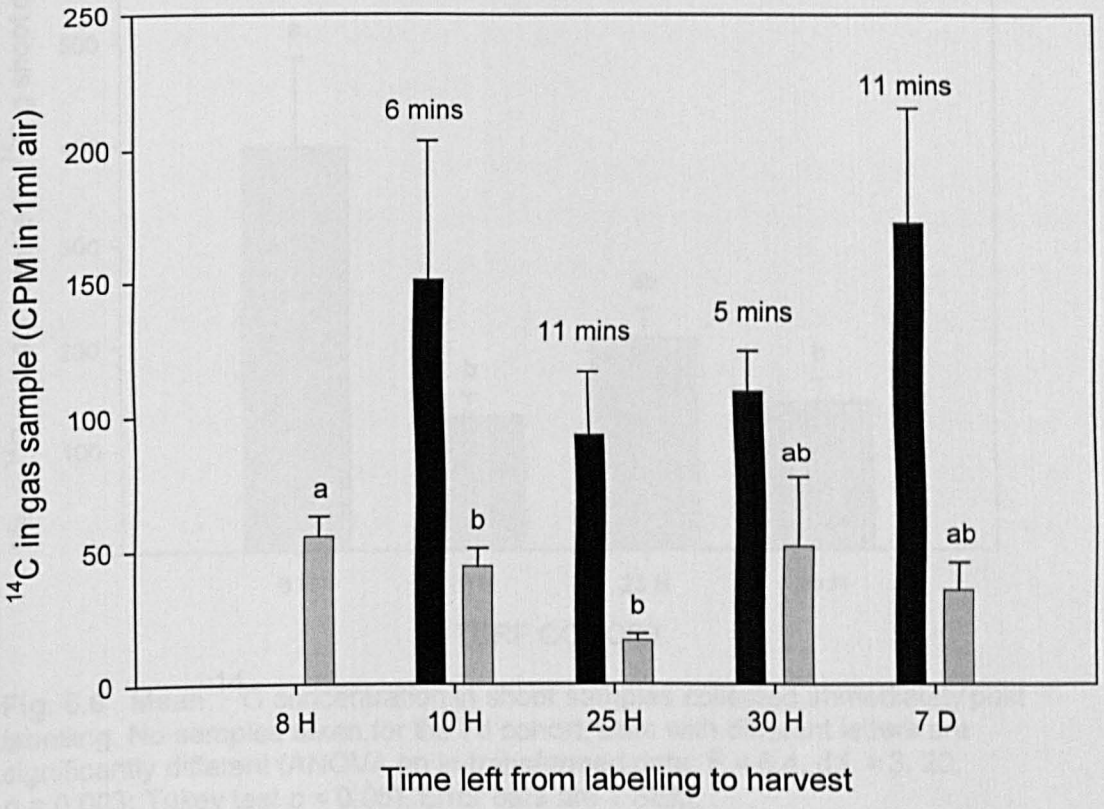

Fig. 5.5 Gas samples taken from chamber early in labelling (black bars, exact time shown above bar) and at the end of the 60 minute labelling period (grey bars), from cohorts of turfs left for different times until harvest. No early gas sample was taken for the 8 h turf cohort. Early gas samples are not significantly different (ANOVA on ln-transformed data: $F = 0.93$, d.f.= 3,20, $p = 0.443$); 60 minute gas samples with different letters are significantly different (ANOVA on ln-transformed data: $F = 3.89$, d.f. = 4,25, $p = 0.014$). Error bars are 1 SEM.

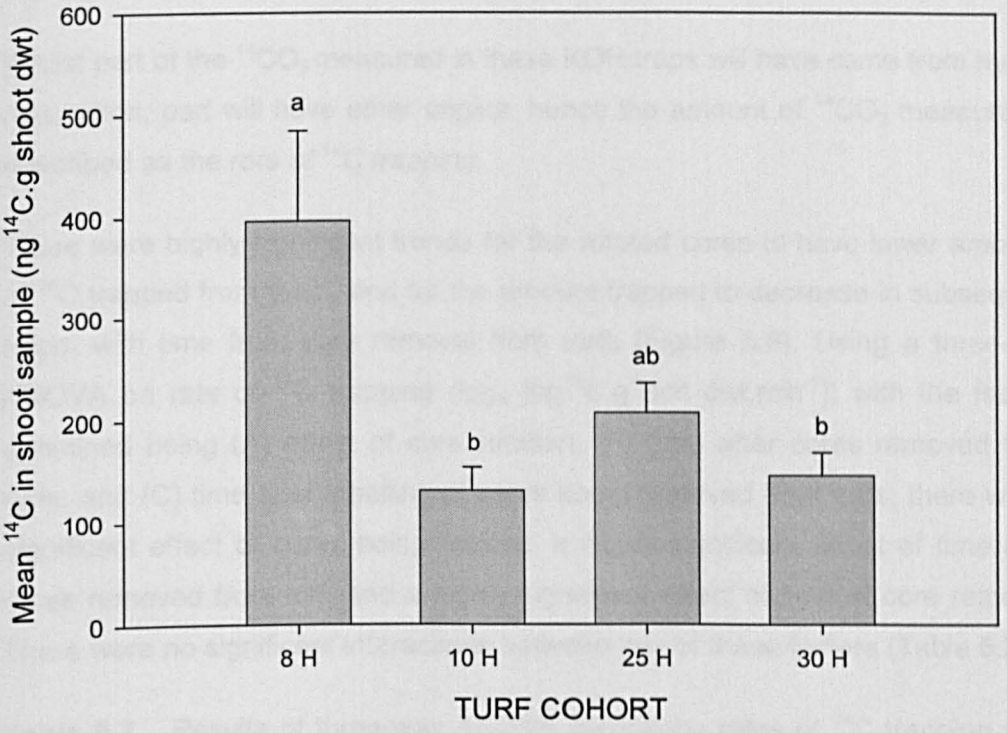

Fig. 5.6 Mean ^{14}C concentration in shoot samples collected immediately post labelling. No samples taken for the 7d cohort. Bars with different letters are significantly different (ANOVA on ln-transformed data: $F = 6.4$, d.f. = 3, 20, $p = 0.003$; Tukey test $p < 0.05$). Error bars are 1 SEM.

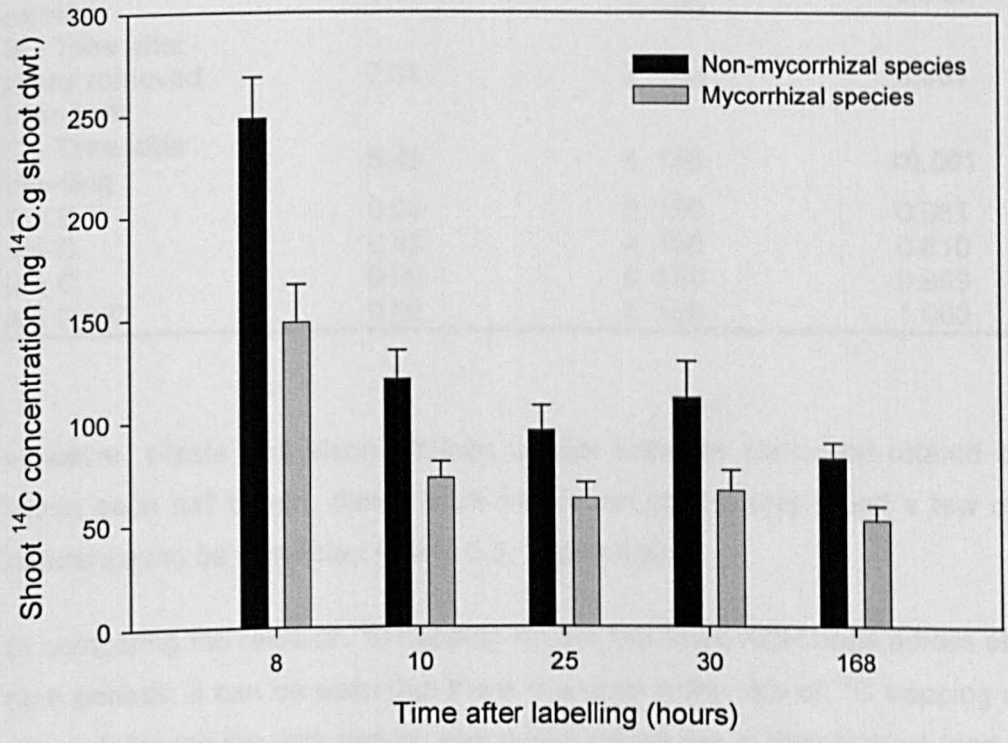

Fig. 5.7 Mean shoot ^{14}C concentrations in mycorrhizal and non-mycorrhizal plants, harvested at different intervals from labelling. Error bars are 1 SEM.

5.3.3 $^{14}CO_2$ trapping from whole cores

Whilst part of the $^{14}CO_2$ measured in these KOH traps will have come from hyphal respiration, part will have other origins, hence the amount of $^{14}CO_2$ measured is described as the *rate of ^{14}C trapping.*

There were highly significant trends for the rotated cores to have lower amounts of ^{14}C trapped from them, and for the amount trapped to decrease in subsequent traps, with time from core removal from turfs (Figure 5.8). Using a three-way ANOVA on rate of ^{14}C trapping ($\log_e$ [pg^{14}C.g^{-1}soil dwt.min^{-1}]) with the factors examined being (A) effect of core rotation, (B) time after cores removed from turfs, and (C) time after labelling of cores being removed from turfs, there was a significant effect of cores being rotated, a highly significant effect of time after cores removed from turf, and a highly significant effect of time of core removal. There were no significant interactions between any of these factors (Table 5.2).

Table 5.2 Results of three-way ANOVA comparing rates of ^{14}C trapping from static and rotated hyphal in-growth cores, which were removed from turfs at different times from labelling, during three subsequent traps.

Factors tested	F value	Degrees of freedom	*p* value
A - Effect of core rotation	8.43	1, 150	**0.004**
B - Time after cores removed from turfs	7.31	2, 150	**0.001**
C - Time after labelling	5.49	4, 150	**<0.001**
A x B	0.05	2, 150	0.951
A x C	0.40	4, 150	0.810
B x C	0.09	8, 150	0.999
A x B x C	0.06	8, 150	1.000

However, *t*-tests and Mann-Whitney U-tests between static and rotated cores within each turf cohort, during each incubation period, only found a few of the differences to be significant (Table 5.3, Figure 5.8).

In comparing the rates of ^{14}C trapping for the first three KOH traps across all five time periods, it can be seen that there is a drop in the rate of ^{14}C trapping at 25 hours, following the dark period, after which values rise to their highest (and most variable) at 7 days (Figures 5.8 and 5.9).

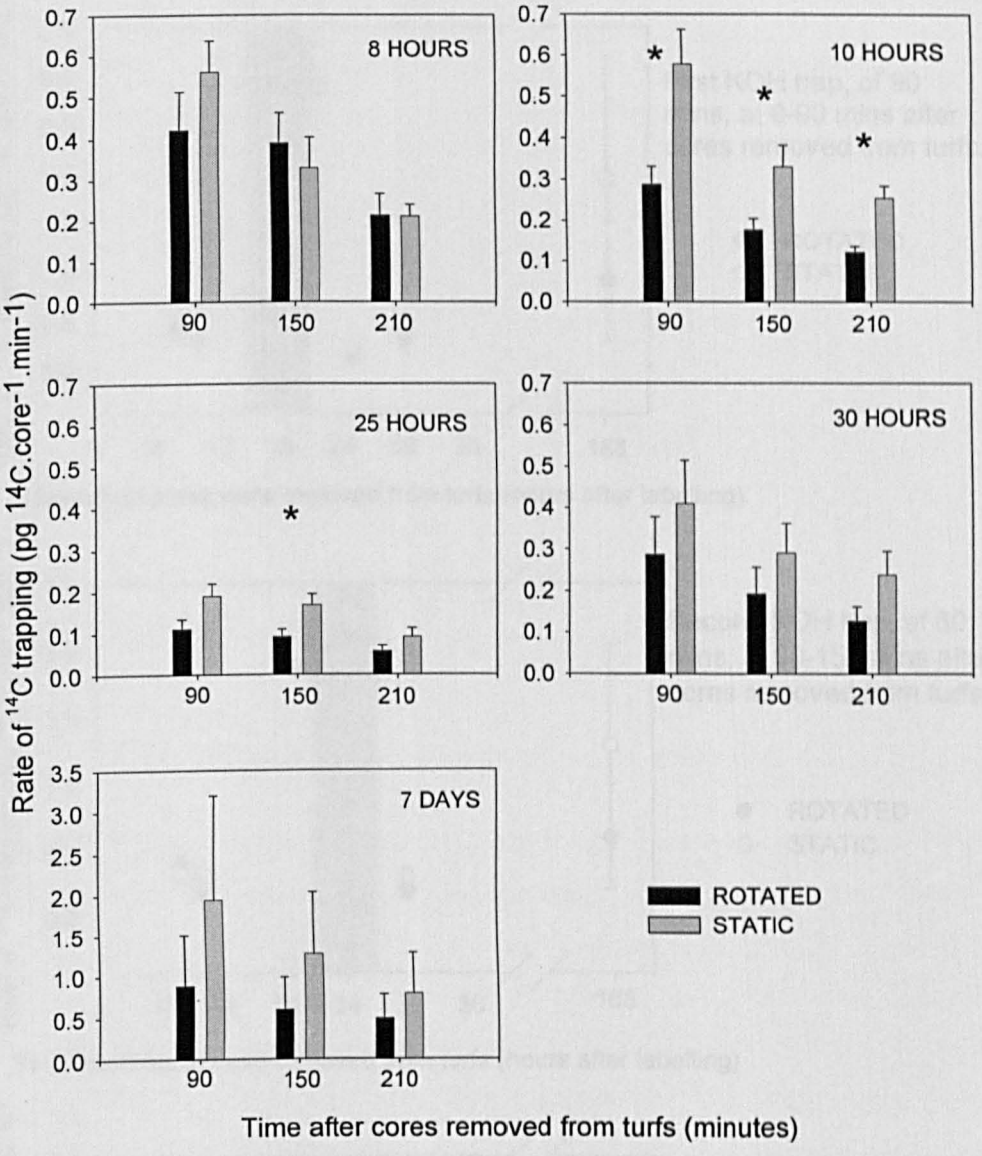

Fig. 5.8 Mean rates of ^{14}C trapping from rotated (black bars) and static (grey bars) cores, during three subsequent KOH traps. The cores were removed from turfs at various times from labelling, as shown on graphs. Pairs of bars shown with * are significantly different at $p<0.05$ (T-test or Mann-Whitney U, see table 5.1) Note the different scale of the 7 day graph.

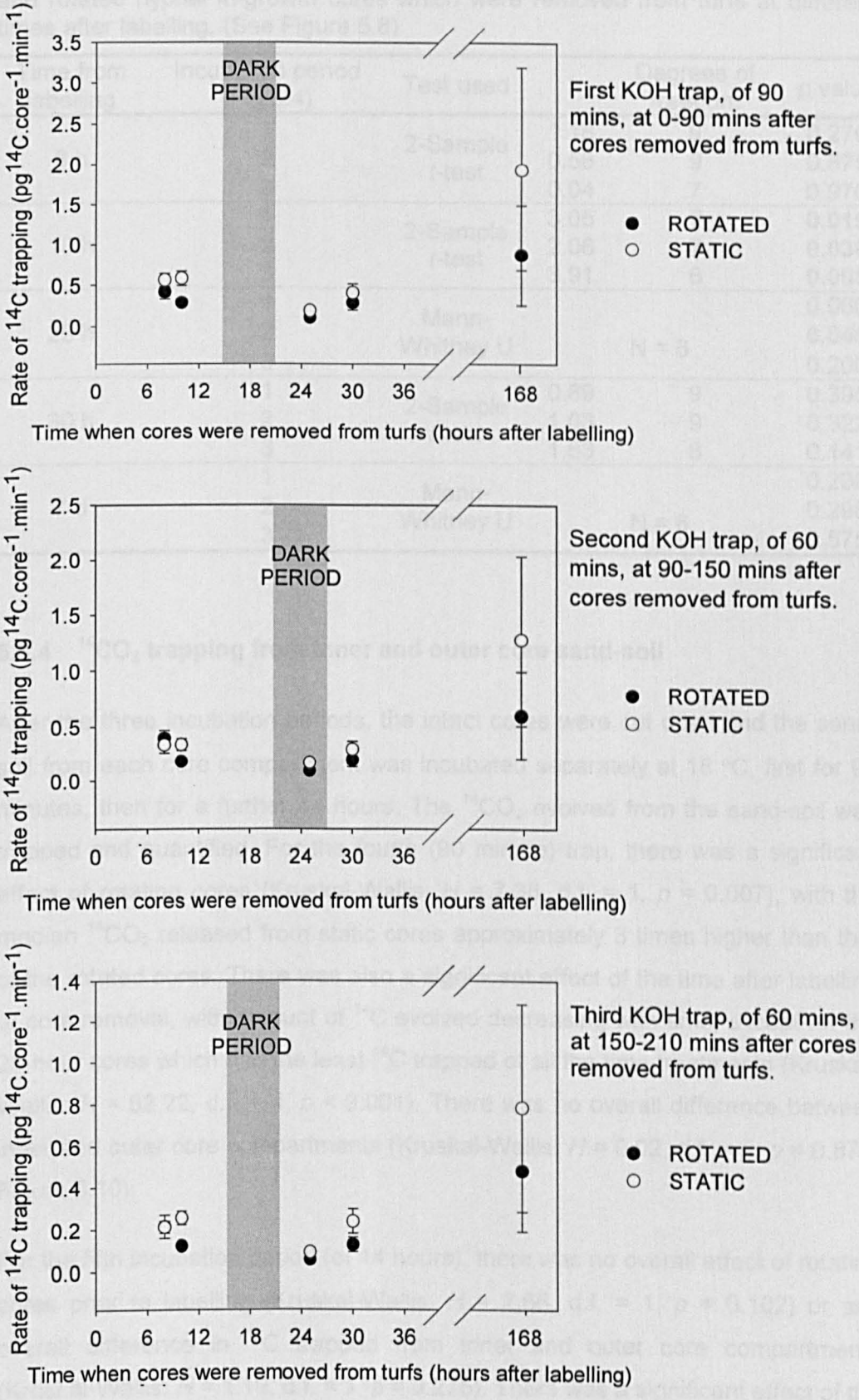

Fig. 5.9 Rate of ^{14}C trapping by the first, second, and third KOH traps, from static and rotated cores, which were removed from turfs at different times post-labelling. Note different scales. Error bars are 1 SEM.

Table 5.3 Results of statistical tests comparing rates of ^{14}C trapping from static and rotated hyphal in-growth cores which were removed from turfs at different times after labelling. (See Figure 5.8)

Time from labelling	Incubation period (Fig 5.4)	Test used	*t*	Degrees of freedom	*p* value
8 h	1	2-Sample *t*-test	1.18	9	0.270
	2		0.58	9	0.575
	3		0.04	7	0.970
10 h	1	2-Sample *t*-test	3.05	7	**0.019**
	2		2.06	7	**0.036**
	3		3.91	6	**0.008**
25 h	1	Mann-Whitney U		N = 6	0.066
	2				**0.045**
	3				0.200
30 h	1	2-Sample *t*-test	0.89	9	0.395
	2		1.03	9	0.322
	3		1.63	8	0.141
7 d	1	Mann-Whitney U		N = 6	0.298
	2				0.298
	3				0.575

5.3.4 $^{14}CO_2$ trapping from inner and outer core sand-soil

After the three incubation periods, the intact cores were cut open and the sand-soil from each core compartment was incubated separately at 18 °C, first for 90 minutes, then for a further 14 hours. The $^{14}CO_2$ evolved from the sand-soil was trapped and quantified. For the fourth (90 minute) trap, there was a significant effect of rotating cores (Kruskal-Wallis: H = 7.35, d.f. = 1, p = 0.007), with the median $^{14}CO_2$ released from static cores approximately 3 times higher than that of the rotated cores. There was also a significant effect of the time after labelling of core removal, with amount of ^{14}C evolved decreasing with time, except for the 25-hour cores which had the least ^{14}C trapped of all the time treatments (Kruskal-Wallis: H = 62.22, d.f. = 4, p < 0.001). There was no overall difference between inner and outer core compartments (Kruskal-Wallis: H = 0.02, d.f. = 1, p = 0.878; Figure 5.10).

For the fifth incubation period (of 14 hours), there was no overall effect of rotating cores prior to labelling (Kruskal-Wallis: H = 2.68, d.f. = 1, p = 0.102) or any overall difference in ^{14}C trapped from inner and outer core compartments (Kruskal-Wallis: H = 1.19, d.f. = 1, p = 0.275). There was a significant effect of the time when cores were removed after labelling, with the median values of ^{14}C trapped falling in the order 10 h > 8 h > 7 d > 30 h > 25 h (Kruskal-Wallis: H = 84.92, d.f. = 4, p < 0.001; Figure 5.11).

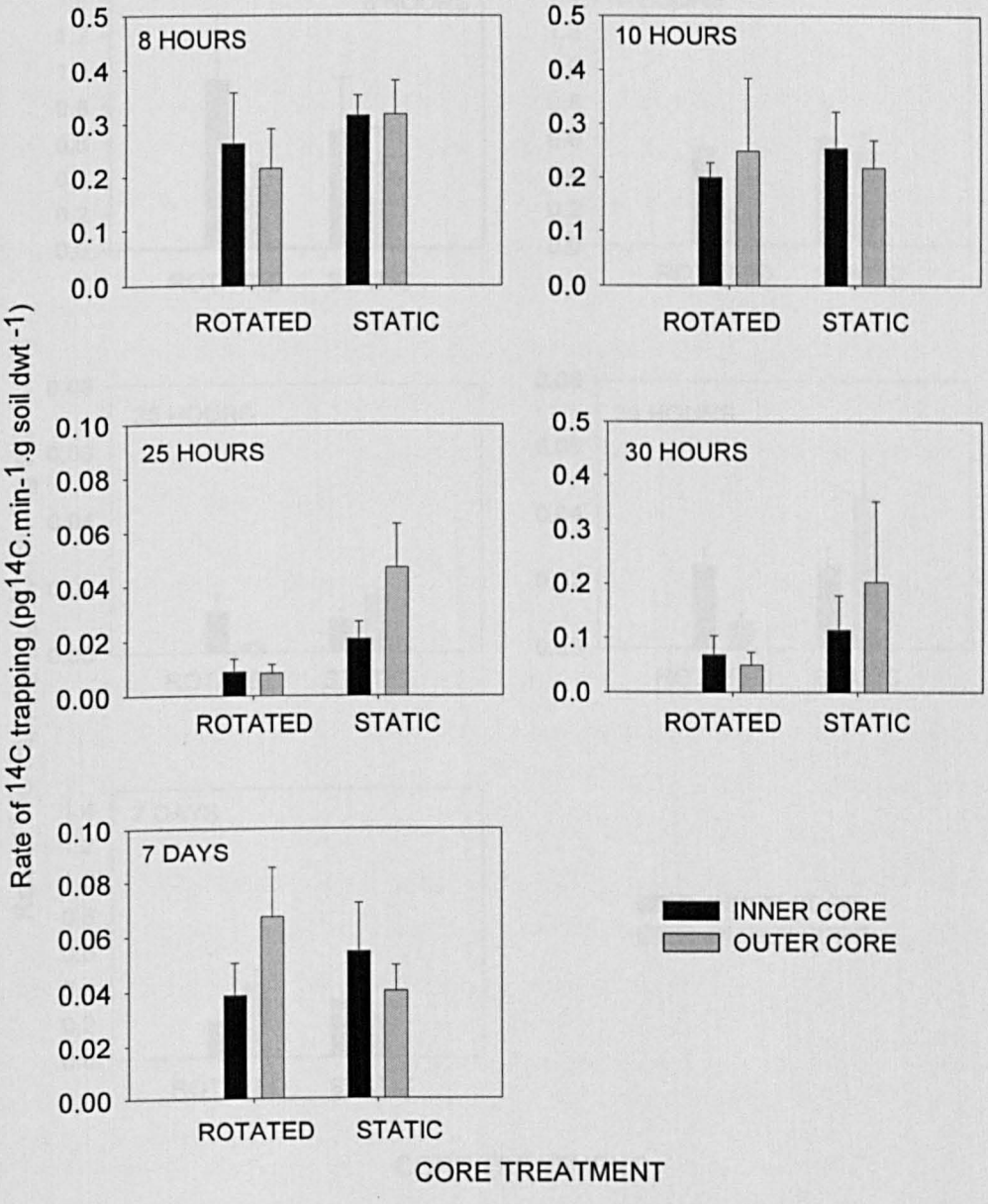

Fig. 5.10 Mean rate of ^{14}C trapping from soil from inner (black bars) and outer (grey bars) compartments of rotated and static cores, during the fourth (90 min) KOH trapping period. The cores were removed from turfs at various times after labelling, as shown on graphs. Note different scales on 25 hour and 7 day graphs. Error bars are 1 SEM.

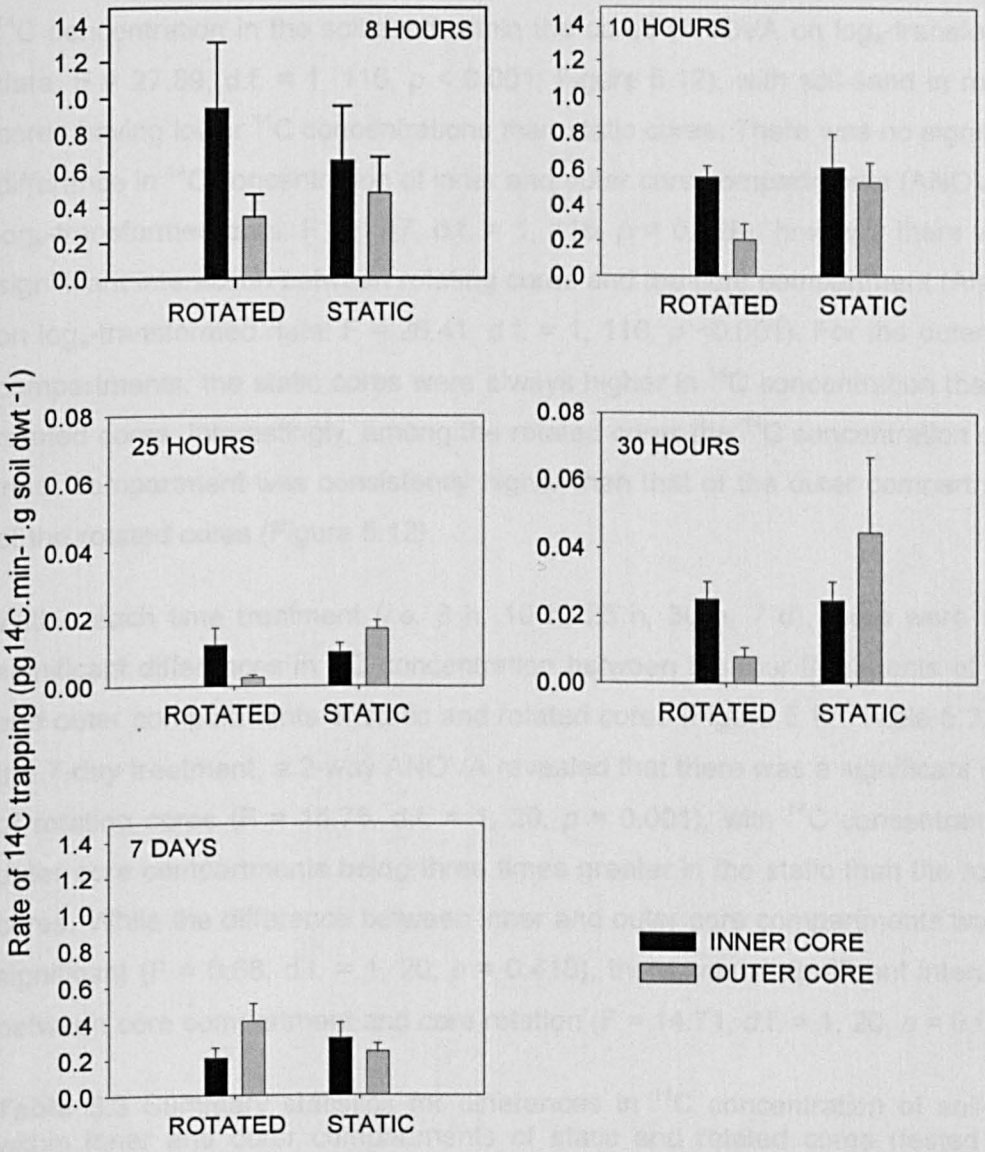

Fig. 5.11 Mean rate of ^{14}C trapping from soil from inner (black bars) and outer (grey bars) compartments of rotated and static cores, during the fifth (14 h) KOH trapping period. The cores were removed from turfs at various times after labelling, as shown on graphs. Note different scales on 25 hour and 30 hour graphs. Error bars are 1 SEM.

5.3.5 ^{14}C within hyphal in-growth cores

The rotation of core to sever hyphal connections had a highly significant effect on ^{14}C concentration in the soil-sand within the cores (ANOVA on $\log_e$-transformed data: $F = 27.89$, d.f. = 1, 116, $p < 0.001$; Figure 5.12), with soil-sand in rotated cores having lower ^{14}C concentrations than static cores. There was no significant difference in ^{14}C concentration of inner and outer core compartments (ANOVA on $\log_e$-transformed data: $F = 1.47$, d.f. = 1, 116, $p = 0.228$), however there was a significant interaction between rotating cores and the core compartment (ANOVA on $\log_e$-transformed data: $F = 26.41$, d.f. = 1, 116, $p < 0.001$). For the outer core compartments, the static cores were always higher in ^{14}C concentration than the rotated cores. Interestingly, among the rotated cores the ^{14}C concentration of the inner compartment was consistently higher than that of the outer compartments of the rotated cores (Figure 5.12).

Within each time treatment (*i.e.* 8 h, 10 h, 25 h, 30 h, 7 d), there were some significant differences in ^{14}C concentration between the four treatments of inner and outer compartments of static and rotated cores (Figure 5.12, Table 5.3). For the 7-day treatment, a 2-way ANOVA revealed that there was a significant effect of rotating cores ($F = 15.75$, d.f. = 1, 20, $p = 0.001$), with ^{14}C concentration of outer core compartments being three times greater in the static than the rotated cores. While the difference between inner and outer core compartments was not significant ($F = 0.68$, d.f. = 1, 20, $p = 0.418$), there was a significant interaction between core compartment and core rotation ($F = 14.71$, d.f. = 1, 20, $p = 0.001$).

Table 5.3 Summary statistics for differences in ^{14}C concentration of soil-sand within inner and outer compartments of static and rotated cores (tested as 4 treatment factors).

Time treatment	Test	Test statistic	Degrees of Freedom	*p* value
8 h	1-way ANOVA	$F = 0.98$	3, 20	0.420
10 h	1-way ANOVA	$F = 2.36$	3, 20	0.102
25 h	Kruskal-Wallis	$H = 12.37$	3	**0.006**
30 h	Kruskal-Wallis	$H = 8.06$	3	**0.045**
7 d	1-way ANOVA	$F = 10.38$	3, 20	**< 0.001**

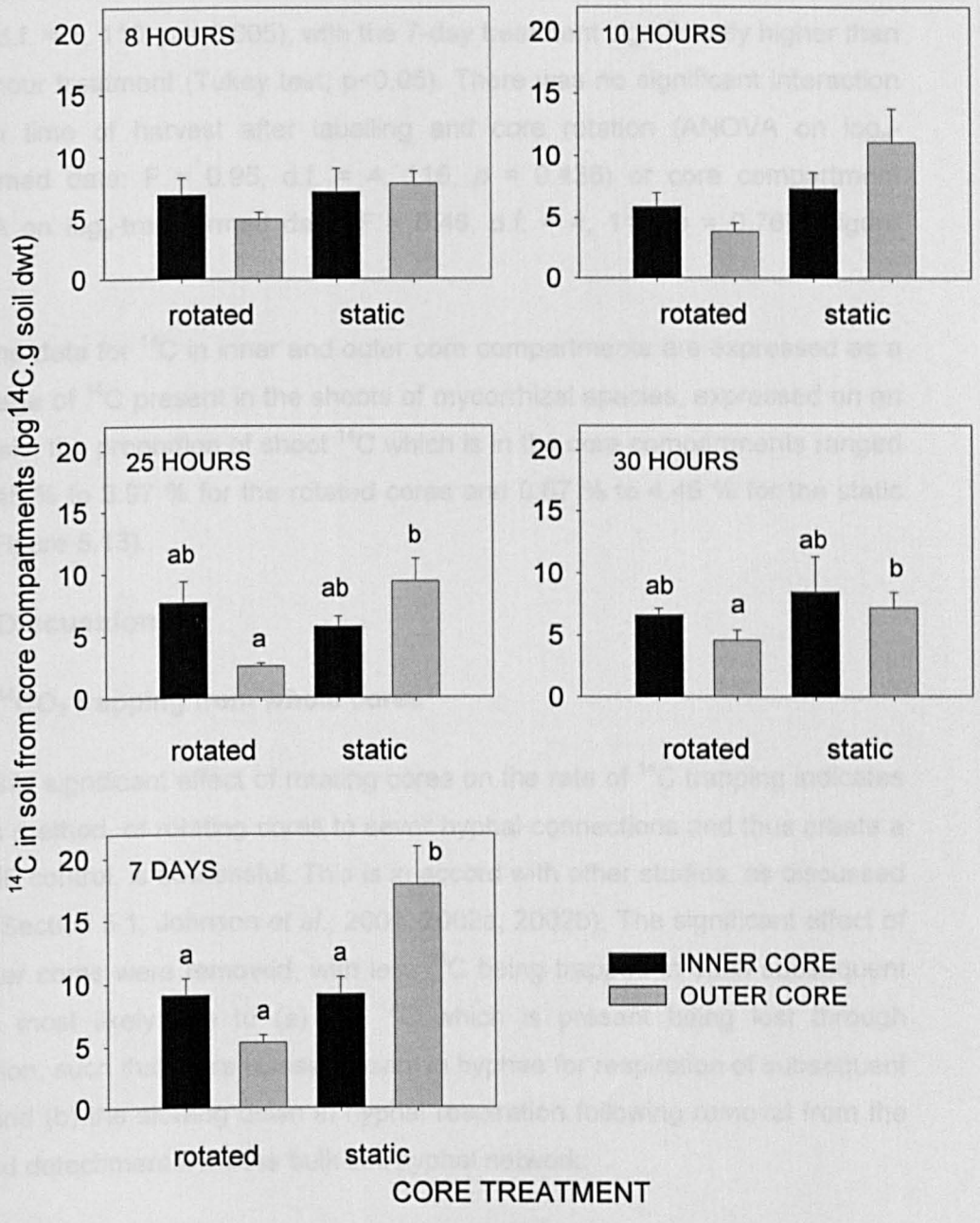

Fig. 5.12 Mean amount of ^{14}C in soil from inner (black bars) and outer (grey bars) compartments of rotated and static cores, removed from turfs at various times after labelling, as shown on graphs. Bars with different letters are significant different at $p < 0.05$ (Tukey test, or non-parametric multiple comparison test). Error bars are 1 SEM.

The length of time between labelling the system and harvesting the cores had a significant effect on core ^{14}C concentration (ANOVA on log_e-transformed data: $F = 4.00$, d.f. = 1, 110, $p = 0.005$), with the 7-day treatment significantly higher than the 30-hour treatment (Tukey test, $p<0.05$). There was no significant interaction between time of harvest after labelling and core rotation (ANOVA on log_e-transformed data: $F = 0.95$, d.f. = 4, 116, $p = 0.436$) or core compartment (ANOVA on log_e-transformed data: $F = 0.46$, d.f. = 4, 110, $p = 0.767$; Figure 5.12).

When the data for ^{14}C in inner and outer core compartments are expressed as a percentage of ^{14}C present in the shoots of mycorrhizal species, expressed on an area basis, the proportion of shoot ^{14}C which is in the core compartments ranged from 0.45 % to 3.97 % for the rotated cores and 0.67 % to 4.45 % for the static cores (Figure 5.13).

5.4 Discussion

5.4.1 $^{14}CO_2$ trapping from whole cores

The highly significant effect of rotating cores on the rate of ^{14}C trapping indicates that this method, of rotating cores to sever hyphal connections and thus create a non-AMF control, is successful. This is in accord with other studies, as discussed above (Section 5.1; Johnson *et al.*, 2001, 2002a, 2002b). The significant effect of time after cores were removed, with less ^{14}C being trapped in each subsequent trap, is most likely due to (a) the ^{14}C which is present being lost through respiration, such that there is less present in hyphae for respiration of subsequent traps, and (b) the slowing down in hyphal respiration following removal from the turfs and detachment from the bulk soil hyphal network.

The longer the time left between labelling and removing the cores, the lower the rate of ^{14}C trapping from the cores, with the exception of those cores left for 25 hours. This set of cores had lower rates of ^{14}C trapping, in each of the three subsequent traps. Lower ^{14}C trapping rates from the cores could indicate that there is a lower hyphal respiration rate, or that the pool of carbon being respired is more dilute in ^{14}C. The 25-hour harvest occurred 3 hours after the end of the dark period: thus, either the hyphae have a lower total respiration rate at this time, perhaps because the plant has only recently resumed photosynthesis and hence there is less photosynthate being translocated to the AMF, or, more likely, the hyphal respiration rate is relatively constant but the carbon supply which it is receiving originates in plant or fungal stores, formed prior to the pulse labelling

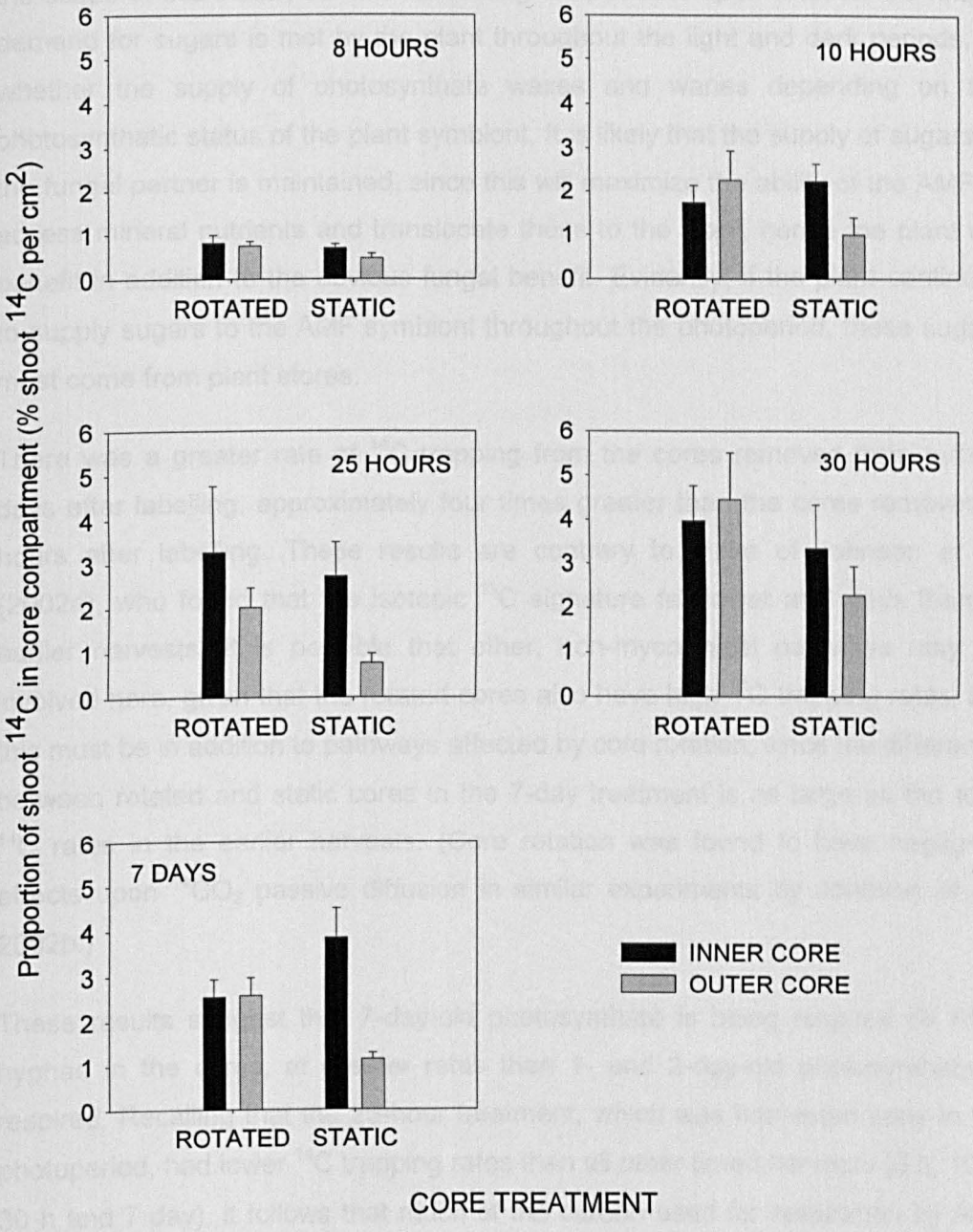

Fig. 5.13 ^{14}C content of inner and outer core compartments of rotated and static cores, as a percentage of ^{14}C content of shoots (of mycorrhizal plant species), harvested at different times after labelling. Error bars are 1 SEM.

event. If the latter is the case, the origin of the carbon being utilized by external hyphae early in the photoperiod as distinct from late in the photoperiod is outside the scope of this thesis, but it is interesting to speculate upon whether the fungal demand for sugars is met by the plant throughout the light and dark periods, or whether the supply of photosynthate waxes and wanes depending on the photosynthetic status of the plant symbiont. It is likely that the supply of sugars to the fungal partner is maintained, since this will maximize the ability of the AMF to access mineral nutrients and translocate these to the plant, hence the plant will benefit in addition to the obvious fungal benefit. Evidently, if the plant continues to supply sugars to the AMF symbiont throughout the photoperiod, these sugars must come from plant stores.

There was a greater rate of ^{14}C trapping from the cores removed from turfs 7 days after labelling, approximately four times greater than the cores removed 8 hours after labelling. These results are contrary to those of Johnson *et al.* (2002a), who found that the isotopic ^{13}C signature fell lower at 7 days than at earlier harvests. It is possible that other, non-mycorrhizal pathways may be involved here, given that the rotated cores also have high ^{14}C trapping rates, but this must be in addition to pathways affected by core rotation, since the difference between rotated and static cores in the 7-day treatment is as large as the total ^{14}C rates in the earlier harvests. (Core rotation was found to have negligible effects upon $^{14}CO_2$ passive diffusion in similar experiments by Johnson *et al.*, 2002b.)

These results suggest that 7-day-old photosynthate is being respired by AMF hyphae in the cores, at greater rates than 1- and 2-day-old photosynthate is respired. Recalling that the 25-hour treatment, which was harvested early in the photoperiod, had lower ^{14}C trapping rates than all other timed harvests (8 h, 10 h, 30 h and 7 day), it follows that much of the carbon used for respiration by AMF hyphae early in the photoperiod was fixed 7 days previously, whilst the carbon respired later in the photoperiod originates mainly in recent photosynthate. This hypothesis could be tested by measuring the total CO_2 evolution from cores in addition to the $^{14}CO_2$ evolution, removing the cores at different time intervals from ^{14}C labelling. However, the results related above are only from one experiment, and need repeating to verify whether the results reflect a true biological phenomenon or an anomalous experimental artefact.

5.4.2 $^{14}CO_2$ trapping from inner and outer core sand-soil

The trend observed in $^{14}CO_2$ trapping from whole cores, for $^{14}CO_2$ trapping to decrease with time from labelling, with the exception of the 25-hour treatment, was continued for the fourth and fifth KOH traps of soil from the inner and outer core compartments. This supports the hypothesis outlined above (Section 5.4.1). Whilst the overall effect of rotation was significant, the pattern of higher values for static cores, particularly static outer compartments, is only clear at the 25-hour and 30-hour treatments: the 8-hour, 10-hour and 7-day data have less apparent effects of either rotation or core compartment. The standard errors of these data are relatively large, which could mask some treatment effects. For both the fourth and fifth trapping periods the lack of a significant difference between inner and outer core compartments suggests that the distance from the bulk soil, of 0 – 5 mm or 5 – 16 mm, is not of great importance in terms of hyphal respiration.

It should be recalled that in order to measure the $^{14}CO_2$ evolution from the soil-sand within each compartment, the cores were split open and the soil within each compartment mixed, such that the hyphal network would be severely disturbed. This disturbance could prevent any trends in $^{14}CO_2$ evolution which occur in undisturbed systems being observed. Hence, in attempting to discern any differences between inner and outer core compartments, the data for the ^{14}C within the soil-sand is likely to hold greater meaning than the data for $^{14}CO_2$ evolution.

5.4.3 ^{14}C within hyphal in-growth core compartments

As with the evolution of $^{14}CO_2$ from cores, the ^{14}C concentration of the soil-sand within the cores was highest at 7 days after the pulse labelling event. This suggests that the ^{14}C has accumulated within the hyphae between the earlier harvests and that of 7 days.

Work published by Staddon *et al.* (2003) showed turnover of most pulse-derived hyphal carbon within 5 to 6 days, leading the authors to suggest that most AMF hyphae only live for 5 to 6 days. If this were the case, then most of the hyphae within the cores 7 days after ^{14}C pulse labelling would have grown subsequent to the labelling event. It would then follow that the carbon used for growth of new hyphae comes from plant (or fungal) stores, rather than from recent photosynthate. However, it is not proven that hyphae are so short-lived.

The outer core compartments showed a clear effect of core rotation, with static cores containing higher concentrations of ^{14}C than rotated cores. This is as expected, that the method of rotating the control cores severs hyphae and thus there is less ^{14}C, and it is concluded that the intact hyphae are transporting and containing ^{14}C compounds. However, for the inner core compartments, the pattern is rather different, with a lesser effect of rotation than on the outer compartments. Whilst this difference is not significant, it may be suggestive of non-AMF pathways for ^{14}C movement being of importance in the inner core compartments. An obvious pathway is that of plant roots breaking through the mesh barriers, but careful observation of each core suggested that roots had not been able to penetrate into the core compartments. It is possible that there may have been some algal or bryophyte growth on the surface of the soil-sand, which could directly fix $^{14}CO_2$ and confound interpretation of results. The outer compartments were more shaded by the tops of the frames of the dividing mesh barriers, and would thus be less likely to support autotrophic growth than the inner compartments, which received greater light. In the work of Johnson *et al.* (2001, 2002a, 2002b) there did not seem to be any such problem of autotrophic organisms colonising the surface of the cores, but the cores used in those experiments (*ibid.*) had a greater length of solid plastic at the top of the core, which would have shaded the surface of the sand within to a greater degree than the cores employed here. In any future work employing similar methods to those described in this chapter, it would be advisable to provide a gas-permeable covering to the cores, to prevent light reaching the substrate surface and reduce the likelihood of autotrophic growth within the cores.

Another possible pathway for $^{14}CO_2$ into the core system would be chemical (rather than biological). $^{14}CO_2$, which is concentrated in the soil air by one or two orders of magnitude, due to CO_2 production by roots and micro-organisms, and organic matter decomposition (Brook *et al.*, 1983), could combine with water to form HCO_3^-, a process which may be increased at lower pH. Whilst the pH of the soil-sand matrix in the cores was originally near neutral, it may have been lowered due to root exudation of protons. Any similar work in the future should measure pH of soil-sand at the end of the experiment, not solely at the outset, and could include control systems of moist cores which are in a sterile system, to elucidate any non-biological pathways for $^{14}CO_2$ to enter the cores.

5.4.4 Gas samples

The labelling of the turfs with $^{14}CO_2$ can be considered successful, since in all turfs the shoots became labelled with ^{14}C, and ^{14}C was detectable in the hyphal in-growth cores. The variability in gas samples taken early and late in the labelling period could be explained by insufficient homogenisation of the isotope through the air within the chamber, which is supported by the lack of any apparent relationship between ^{14}C concentration of gas samples and vegetation samples. This issue is addressed in the following chapter, where the labelling chamber was fitted with a fan to facilitate air circulation during labelling. It is also possible that when the isotope was injected into the chamber, some was lost from the syringe needle on the labelling hole, since variable contamination of these holes was noted. If this were the case, variable amounts of isotope for different turf systems could result in variable plant and core labelling between samples, which would confound the results. However, it is assumed here that any such variation is negligible, since the differences in ^{14}C uptake were not significant.

5.4.5 Shoot ^{14}C uptake

The shoot ^{14}C concentration fell between 8 and 10 hours from labelling: this is most likely due to plant respiration, and allocation below ground, of the recent photosynthate. The consistent difference in shoot ^{14}C concentration of mycorrhizal and non-mycorrhizal (NM) plant species may partly represent that proportion of carbon which is allocated to AMF symbionts, however the mean difference between mycorrhizal and NM is 39 %. This is a far greater proportion of carbon than the literature suggests is ever allocated to AMF, and therefore it is likely that the NM plants may also have higher C fixation rates or lower respiration rates than the mycorrhizal plant species.

5.4.6 Overview

This novel use of double-walled hyphal in-growth cores provides a useful method for excluding both plant roots and root hairs, creating hyphal compartments. The quantities of ^{14}C measured in the inner and outer core compartments were comparable to each other, suggesting that transport via root hairs is not a significant problem when using mesh barriers such as these. However, it is likely that non-hyphal pathways were also responsible for transporting ^{14}C into the cores, since the rotated cores had relatively high values of ^{14}C both within the cores and of $^{14}CO_2$ evolved from them. Core rotation had a consistent

depressive effect on the amount of $^{14}CO_2$ which evolved from the cores, and on the amount of ^{14}C in the soil-sand of the outer cores, but had negligible effect on the ^{14}C within the inner core compartments. This suggests that there may be a non-hyphal pathway for ^{14}C into the inner core compartments, an issue which should be resolved in future work using such systems.

The changes in ^{14}C concentration with time showed higher amounts of ^{14}C in the cores and evolving from the cores at 7 days than at 8, 10, 25 and 30 hours after labelling. This is in direct contrast to other work (Johnson *et al.*, 2002a) where the 7-day measurements were below earlier measurements, suggesting that these 7-day data may not be representative of typical AMF functioning.

Chapter 6:

Carbon flux from plant shoots through soil to double-walled hyphal in-growth cores in soil monoliths from different management intensities

Chapter 6: Carbon flux from plant shoots through soil to double-walled hyphal in-growth cores in soil monoliths from different management intensities

6.1 Introduction

Intensive agriculture creates an environment which is sub-optimal for many AMF species, through ploughing and thus breaking up hyphal networks within the soil, through fertilisation which reduces the plant's need for AMF, and through application of agrochemicals and growth of non-mycorrhizal crop species, which directly decrease the frequency of AMF (Chapter 1 and references therein). In attempting to create an environment in which the agricultural plant can flourish, and hence provide high yields, the impacts upon the AMF have historically been ignored. Johnson *et al.* (1997) suggest that when land is fertilised, such that the plant can access mineral nutrients without the aid of AMF, the benefits of the mycorrhizal symbiosis may be reduced to the extent that there may be a switch from mutualism to parasitism, where the carbon cost of AMF is not offset by any increase in mineral nutrition. Evidence for this was reported by Graham and Eissenstat (1998), who found that fungicide treatment of root zones of mycorrhizal citrus trees increased the growth rate of Valencia orange by 5 - 17 % after three growing seasons, and these trees lacking active mycorrhiza did not appear to be phosphorus limited. Their results suggest that the mycorrhizal fungi are requisitioning carbon which in the absence of active AMF is used for plant growth.

Johnson (1993) found evidence that soil fertilisation altered AMF species composition, decreasing relative spore abundance of *Gigaspora gigantea, Gigaspora margarita, Scutellospora calospora,* and *Glomus occultum,* whilst the relative spore abundance of *Glomus intraradix* increased. When the communities of AMF from either fertilised or unfertilised plots were inoculated onto *Andropogon gerardii* grass grown in a glasshouse, the plants with AMF from fertilised plots were smaller after 1 month and produced fewer inflorescences after 3 months, compared to unfertilised plots. Johnson (1993) suggested that the AMF from fertilised plots were exerting a greater *net* carbon cost, an assertion which she supported by the existence of a lower frequency of hyphae and arbuscules in mycorrhizal roots from fertilised plots (which presumably decreased their ability to forage for mineral nutrients and transfer them to the plant), but no difference in quantity of vesicles between fertilised and unfertilised treatments,

implying similar carbon demand by the AMF. It should be recalled that carbon transfer from plant to fungus is not in itself a cost, and is beneficial to the plant where the carbon directly contributes to increased nutrient uptake. However, where two different AMF species receive the same amounts of carbon, but with one species this results in greater plant nutrient uptake than the other AMF species, there is a greater net carbon cost of the latter AMF species. Thus there is a continuum between mutualism and parasitism, such that particular AMF species may be described as *less* mutualistic than other AMF species, yet they may still be mutualists.

A study in which mycorrhizal status of barley and wheat was examined 10 years after fertilisation had ceased, in plots which had previously received 0, 17.5, or 52.5 kg P ha^{-1} y^{-1} (for 23 years), suggested that even after a decade without P fertilisation, the AMF in the highest P treatment plots were less mutualistic than those in the lower P treatment plots (Dekkers and van der Werff, 2001). The authors inferred the degree of mutualism from the ratio of arbuscular colonisation to total colonisation, since they proposed that the ratio of arbuscular to total colonisation is an indication of benefit of the mycorrhizal symbiosis to the plant, arbuscules being the site of nutrient exchange, and total colonisation indicating the cost to the plant, since the fungal carbon is plant-derived. Thus a more mutualistic AMF confers greater benefits and exerts lesser costs (has greater arbuscular colonisation relative to total colonisation). Total and hyphal AMF root length colonisation increased with amount of P formerly applied, in both barley and wheat, whilst arbuscular colonisation showed the reverse trend. The ratio of arbuscular to total colonisation was significantly higher, in both barley and wheat, in the plots which had not been fertilised for 33 years. Data for production of external hyphae was not given: this would have enabled a more exact measure of the AMF biomass, and may also have indicated the foraging ability of the AMF.

AMF differ in their production and patterns of growth of external mycelia through soil (Abbott *et al.*, 1992), and in the biomass of spores which they produce (Sieverding *et al.*, 1989). It is therefore likely that demand on the host plant for C will vary with AMF species, although this relationship is not straightforward, since in a comparison of three AMF species grown with cucumber plants, *Scutellospora calospora* received the most C from the plant but was found to produce least fungal biomass and to give the plant the least amount of P (Pearson and Jakobsen, 1993). Such a fungal species is presumably less mutualistic, although cost-benefit analyses based solely upon C and P transfer may not give an accurate representation of the symbiosis if other benefits are conferred, such as

protection from root pathogens or access to micronutrients, or if other costs are entailed, such as improved shoot nutrient status leading to increased herbivory. Analyses of costs and benefits are limited by the experimental design, such as the distance of hyphal compartments from the plant roots, which will affect results. Certain fungi may be more affected by experimental conditions than others, for example a species which is effective at P uptake over short distances to the root may be identified as a less mutualistic fungus in an experiment which examines P uptake over greater distances.

The timing and amount of nutrient supply can affect AMF carbon dynamics. Gavito and Olsson (2003) found that in treatments where soil had a single large, early organic nutrient amendment (1 mg bakers yeast per gram sand, mixed in at the start of the experiment) there was a cumulatively higher allocation of carbon to fungal storage lipids than in systems which had continuous but low nutrient supply (nutrient solutions added twice each week). They also showed that allocation of carbon to hyphal proliferation or storage lipids in AMF was related to plant carbon availability.

Chapters 3 and 4 of this thesis showed that an increase in management intensity can result in a decrease in phosphorus translocated from AMF to plant hosts. Assuming that differing management intensities exert differing selection pressures, and result in functionally distinct communities of AMF, the quantity of carbon translocated from plant to AMF may also vary with management intensity. Carbon may be allocated by the AMF in a number of ways: to production of internal and external mycelium, to arbuscules, vesicles, or spores. A highly mutualistic mycorrhizal fungus would likely allocate a larger proportion of carbon to produce more arbuscules, increasing potential rates of nutrient transfer (Johnson, 1993; Dekkers and van der Werff, 2001), and to producing greater amounts of external mycelium, increasing its foraging ability, since total lengths of external AMF hyphae can be related to hyphal uptake of P (Jakobsen *et al.*, 2001). Ploughing will break up external hyphal networks and hence could discourage such highly mutualistic fungi. A less mutualistic mycorrhizal fungus might prioritise carbon allocation to spore production, over absorptive fungal biomass which is of benefit to the plant symbiont, and such an AMF species would be more successful in an environment subjected to ploughing, the removal of host plants and the growth of non-mycorrhizal crops. Thus it is likely that carbon allocation between fungal compartments will differ in unmanaged and intensively farmed land; however, whether the net amount of carbon received by AMF will vary with management intensity is unclear. It has been suggested that in

nutrient limited soils, plants release more carbohydrates through the roots and hence facilitate extensive formation of mycorrhiza, thus enhancing nutrient uptake (Schwab *et al.*, 1991). However, Johnson (1993) suggested that where the plant allocates less carbohydrates to root exudates, aggressive strains of AMF which are still able to extract carbon from the roots are selected for.

The question of how carbon transfer is controlled, by the plant or fungal symbiont, is pertinent here, since a less mutualistic fungus will exert a greater drain on plant carbon whilst giving minimal nutritional benefit to the plant. If the plant was fully in control of carbon transfer, it should then decrease carbon allocation to AMF which are of little net benefit. However, studies which have shown that AMF are limiting to plant growth or fitness indicate that the plant may not be able to control the transfer of its resources to AMF (Graham and Eissenstat, 1998).

6.1.1 Aims and hypotheses

This study aims to determine whether management intensity affects carbon flux from plants into AMF external to the root. It is hypothesised that intensive management selects for less mutualistic AMF, and hence movement of carbon from plant to AMF external biomass will be greater (a) in ploughed systems than in undisturbed field margins, and (b) in conventionally managed fields than in fields under organic management. The respiration rates of external AMF hyphae, as measured by trapping $^{14}CO_2$, are hypothesized to follow the same pattern as the biomass-carbon.

If the results of this experiment mirror those of Chapter 5 in which this double-walled core design was piloted, it is expected that core rotation will decrease carbon flux to the outer compartments, but will have little impact upon the inner compartments of the cores. (Possible reasons for this were discussed in Section 5.4.3.)

This experiment complements that of Chapter 4, which quantified hyphal uptake of phosphorus to plant shoots, using the same study sites but quantifying carbon flux from plants to AMF. The experimental system is similar to that of Chapter 5, using double-walled hyphal in-growth cores to create hyphal compartments, and rotating some cores to sever hyphal connections and thus create non-mycorrhizal controls.

6.2 Materials and Methods

6.2.1 Experimental design

Turfs were removed from fields under Winter wheat, from 4 organic and 4 conventional fields, plus from the margin of each field (giving 4 treatments from 16 sites, detailed in Table 2.1 and Section 2.2). The turfs were fitted into plastic boxes 20 cm by 17 cm by 13 cm depth, with drainage holes. One such turf was prepared from each field or field margin, and 6 double-walled cores were inserted into each box (Figure 6.1). These cores allow free in-growth of hyphae whilst excluding plant roots, and were filled with a standard 1:1 soil-sand mix. The double-walled cores were constructed as described previously (Section 5.2.2). The soil for this standard mix came from a permanent pasture at Quenby, Leicestershire, with low phosphate status (Section 2.2), which had been sieved to 2 mm, mixed with washed and autoclaved (121 °C for 20 minutes) silica sand, and stored in the dark for over 10 months (Sections 3.2.2 and 5.2.2).

The turfs were removed from the field sites in March 2002 and fitted into the boxes; the hyphal in-growth cores were inserted into holes made using a 32 mm diameter soil corer. They were left for 6 weeks for the system to recover from transplantation shock, then the herbicide was added (Section 4.2.2) and the turf-core system was left barren for 4 weeks. The turfs were re-sown with a mixture of meadow grasses and wild flowers (Section 4.2.2), and maintained in a glasshouse (Section 4.2.1) until November 2002, providing ample time for the plants to grow and the hyphal networks to become well established. Figure 6.3 shows that the re-sown plants grew well, with comparable above-ground biomasses.

After allowing time for hyphae to grow throughout the cores, the turfs were pulse-labelled with ^{14}C and analysed for ^{14}C uptake by hyphae, shoots, and soil. The double wall of the hyphal in-growth core enabled movement of ^{14}C to be measured at different distances from the plant root compartment, and also created a central compartment free from any root hairs which might protrude through the outer mesh. Half of the cores in each box were rotated 90° immediately prior to labelling, to sever hyphal connections and create non-mycorrhizal controls to enable measurement of non-hyphal movement of ^{14}C.

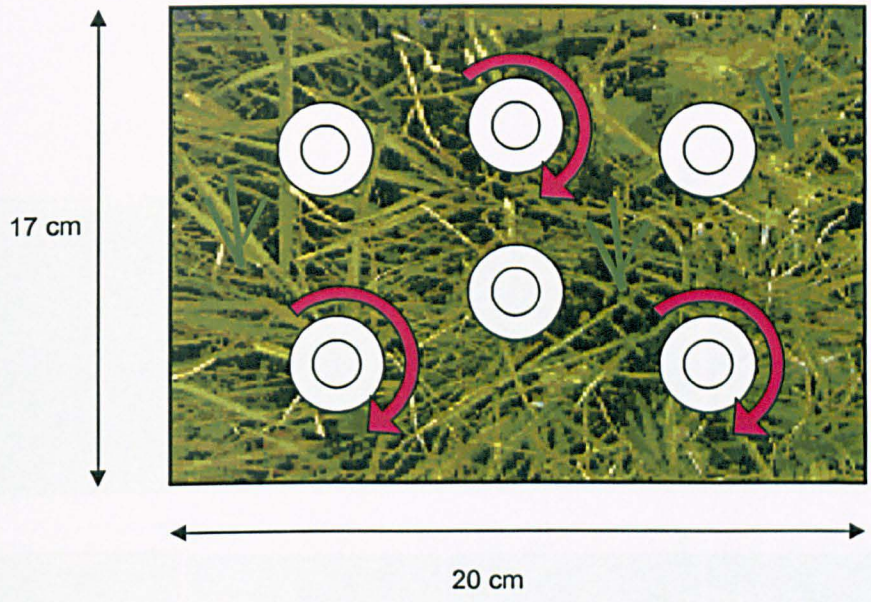

Fig. 6.1 Plan-view diagram of turf-core system, with 6 double-walled hyphal in-growth cores, 3 of which were rotated prior to pulse labelling.

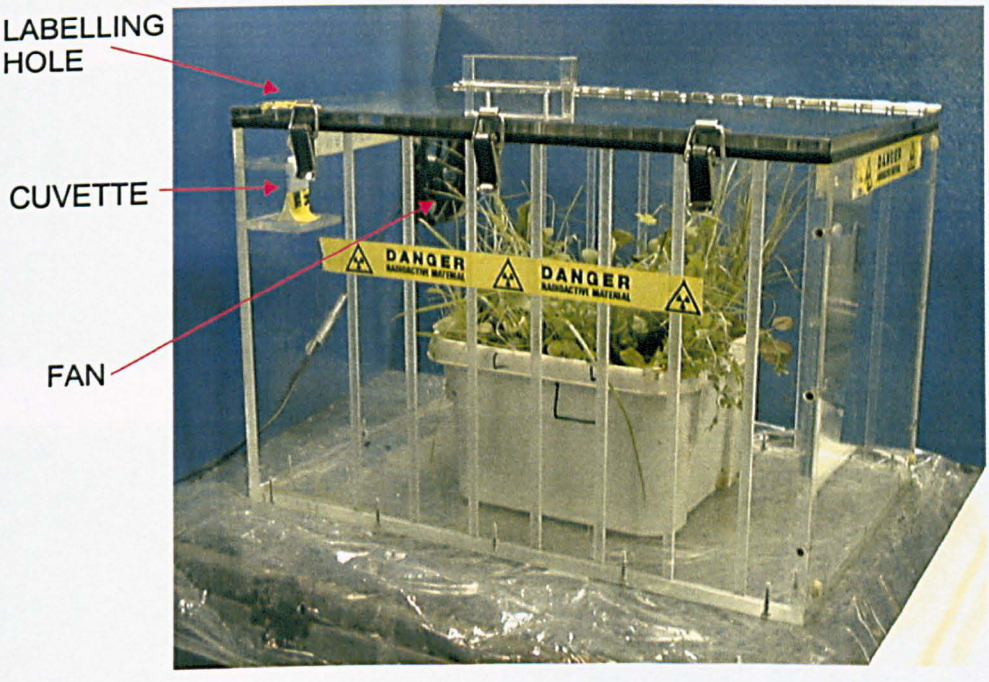

Fig. 6.2 Labelling chamber containing turf-core system.

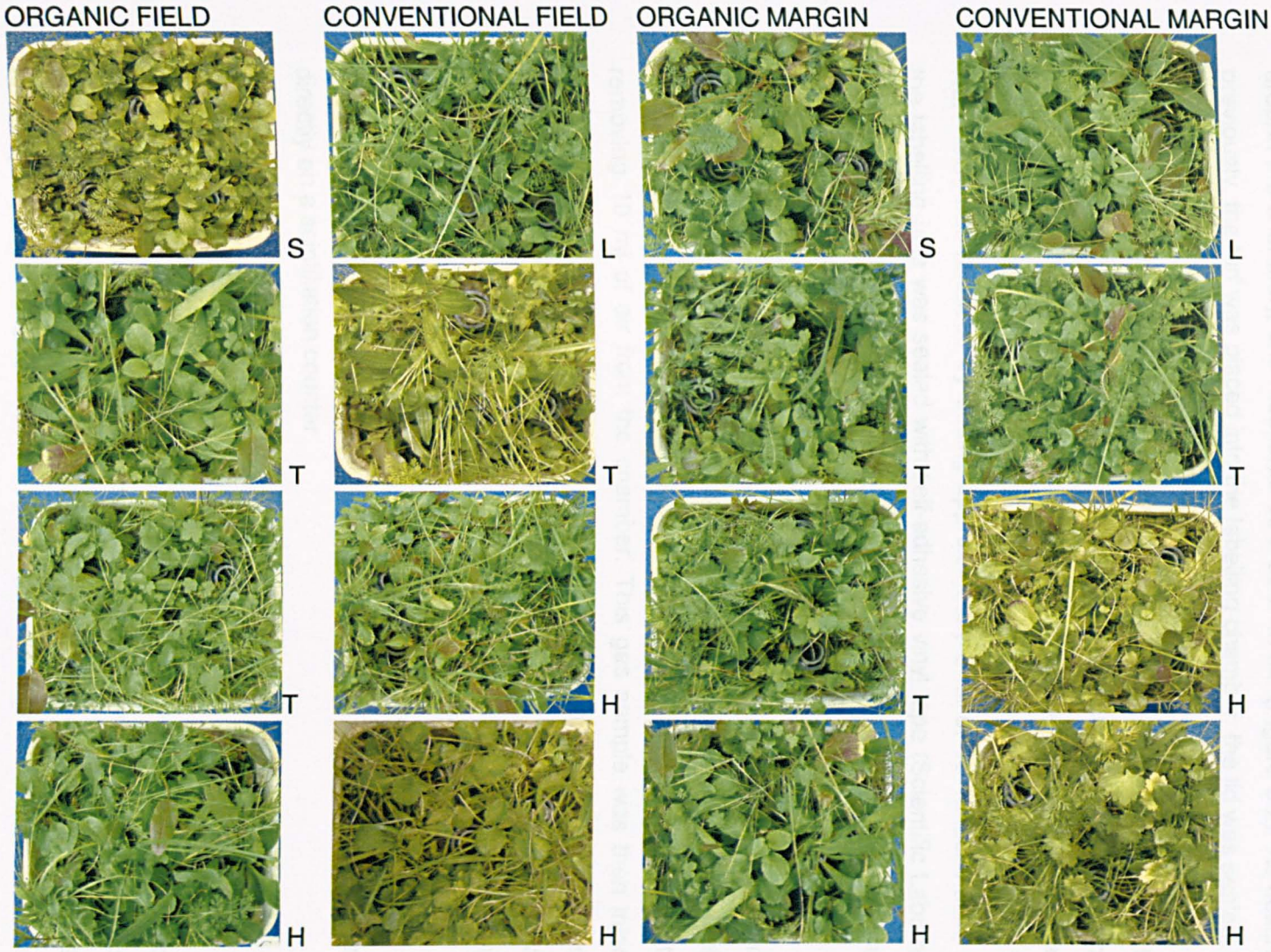

Fig. 6.3 Comparison of turfs from organic and conventional fields and their margins, which were re-sown with standardised mixed seed. Turfs originated from Stoughton (S), Loddington (L), Terrington (T) or High Mowthorpe (H). Double walled hyphal in-growth cores are partially visible.

6.2.2 Pulse Labelling turfs with ^{14}C

A similar labelling system was used as described in Chapter 5, however this labelling chamber possessed a rubber seal around the door, contained a small electric fan (useful both in cooling the turf and in distributing the $^{14}CO_2$ evenly around the chamber), and measured 30 x 30 x 45 cm (Figure 6.2). As described previously, the turf was placed into the labelling chamber, the lid was sealed, and the radioisotope was injected into the cuvette as $NaH^{14}CO_3$ (specific activity 2.0 GBq/mmol, Lot no.305517, obtained from ICN Pharmaceuticals Inc., Basingstoke, U.K.). The amount of isotope used per turf was calculated to be directly comparable with the amount used in the earlier ^{14}C experiment, based upon soil volume. Hence 1.4 MBq (18.9 µl) was added to each turf system. The $NaH^{14}CO_3$ was released by adding approximately 3 ml of 10 % lactic acid, and the labelling hole was sealed with self-adhesive vinyl tape (Scientific Laboratory Supplies, Nottingham, U.K.). The fan was then switched on, and the system left for 2 hours under lights (340 µmol m^{-2} s^{-2}).

At 10, 60 and 120 minutes after the $^{14}CO_2$ was released, the gas in the chamber was sampled. This was done by inserting a syringe needle through the labelling hole, pumping the syringe 3 times to mix the air within the chamber, then removing 10 ml of air from the chamber. This gas sample was then injected through a Suba seal into a plastic scintillation vial containing 7 ml Carbosorb (Packard) and 7 ml Permafluor (Packard), which had had a vacuum created within the vial by syringing out 20 ml of air from the vial. The syringe was pumped several times with the needle extending through the Suba seal into the vial headspace, over at least 15 minutes. The syringe was then finally evacuated and removed, and the vials were quickly capped. These samples were counted directly on a scintillation counter.

6.2.3 Harvest and incubation of cores

After the 2-hour labelling period the chamber was placed into a fume cupboard and opened. A small sample of representative vegetation was removed and oven dried at 80 °C. Following 15 minutes in the fume cupboard, the turf was placed under lights (8-hour night) in a controlled environment growth room. At 24 hours subsequent to labelling, the turf was destructively harvested: all shoots were removed and oven dried, a core of bulk soil was taken from the centre of the turf and oven dried, the hyphal in-growth cores were removed and placed in a conductrimetric respirometer (Nodgren Innovations AB, Nordgren, 1998) at

22 °C. The harvest time of 24 hours after pulse labelling was chosen since it was convenient, not requiring nocturnal work as for the previous chapter. The experiment described in Chapter 5 had ascertained that at 25 hours after labelling there was measurable ^{14}C in the hyphal in-growth cores, so this was deemed to be a suitable time.

The respiration of each intact core was calculated from measuring the changing conductance of a 10 ml KOH (0.06 M) trap in a sealed container incubated at 22 °C. Samples (1 ml) of this KOH trap were taken at 1.5, 2.5 and 3.5 hours from the time of the core being placed in the respirometer, and their ^{14}C content determined by scintillation counting. The intact cores were then removed, weighed, and split open. The soil-sand mixture contained by each core compartment was scraped out, homogenised, a subsample taken and dried, and the remainder placed in a sealed container in the respirometer for 18 hours.

6.2.4 Mycorrhizal status of plants

Following removal of the hyphal in-growth cores, 24 hours following ^{14}C labelling, and prior to removal of all shoots, a single *Plantago lanceolata* plant was removed from each turf for verification of mycorrhizal status in each turf. The roots were washed in distilled water, cleared and stained (Section 2.3.10), and examined at x 100 and x 400 for presence or absence of mycorrhizal colonisation (Section 2.3.11). All samples were verified as mycorrhizal; more detailed information on AMF colonisation in these turfs was given in Section 4.3.4 (Figure 4.7) where it was found that there were no significant differences in total AMF root length colonisation between management treatments.

6.2.5 Investigation of soil sample drying method

To test whether any loss of ^{14}C from bulk soil samples occurred when oven-drying the samples at 80°C, parallel samples were taken and frozen (-20 °C), then freeze dried. Three soil cores for each drying method were taken from each of three turfs, dried, the cores homogenised using a pestle and mortar, and nine subsamples taken from each soil core. These subsamples were oxidized and their ^{14}C concentration determined.

The mean and standard error for these 9 subsamples were calculated, and comparisons made between the 6 cores. No significant difference was found between freeze dried and oven dried samples (ANOVA: d.f. = 1, 159, $F = 0.08$, $p = 0.773$).

Table 6.1 ^{14}C concentration of bulk soil cores, which were either freeze-dried or oven-dried.

Original management	Freeze dried		Oven dried	
	Mean ^{14}C per core of bulk soil ($fg^{14}C.g^{-1}$soil dwt.)	SEM	Mean ^{14}C per core of bulk soil ($fg^{14}C.g^{-1}$soil dwt.)	SEM
Organic wheat field	380.33	10.30	326.92	9.38
	410.95	21.32	412.72	11.85
	335.27	22.78	301.29	4.80
Conventional wheat field	472.17	16.59	469.57	17.49
	375.16	9.48	421.30	23.45
	401.22	17.88	476.84	17.71
Conventional field margin	413.23	17.28	298.52	5.51
	364.83	14.42	414.57	12.37
	248.00	9.59	254.84	6.54

6.3 Results

6.3.1 ^{14}C content of gas samples and initial shoot samples

The samples of vegetation taken immediately post-labelling had an overall mean of 1148 fg ^{14}C g^{-1} shoot dwt, and a standard error of 165 fg ^{14}C g^{-1}shoot dwt. There were no significant differences in ^{14}C concentration of shoot samples between organic and conventional treatments (ANOVA of $\log_e$-transformed data: $F = 0.42$, d.f. = 1, 10, $p = 0.530$), margin and field treatments (ANOVA of $\log_e$-transformed data: $F = 0.52$, d.f. = 1, 10, $p = 0.486$), and no interaction between these treatments (ANOVA of $\log_e$-transformed data: $F = 1.42$, d.f. = 1, 10, $p = 0.255$).

The gas samples taken from the labelling chamber during labelling tended to show a decrease in ^{14}C concentration through the 2 hours of labelling, although the mean concentrations for the conventional margins appear constant (Table 6.2). There were no significant differences in chamber ^{14}C concentration between the management treatments for either the 10-minute gas samples (Kruskal-Wallis : $H = 4.67$, d.f. = 3, $p = 0.197$), the 60-minute gas samples (Kruskal-Wallis : $H = 2.60$, d.f. = 3, $p = 0.458$), or those taken at 2 hours from releasing the $^{14}CO_2$ (Kruskal-Wallis : $H = 5.37$, d.f. = 3, $p = 0.147$). This implies that the shoot uptake of ^{14}C was comparable across all management treatments.

Table 6.2 ^{14}C concentration (pg ^{14}C ml^{-1} air) within labelling chamber at 10, 60 and 120 minutes after releasing the $^{14}CO_2$.

Management	10 mins		60 mins		120 mins	
	Mean	SEM	Mean	SEM	Mean	SEM
Conventional	5.124	1.251	2.886	0.001	2.558	0.522
Conventional margin	3.667	0.387	3.864	0.589	3.847	0.664
Organic	3.058	0.323	2.936	0.238	2.244	0.258
Organic margin	3.501	0.058	3.408	0.438	2.572	0.256

6.3.2 ^{14}C in soil-sand within core compartments

When comparing the ^{14}C concentration of the soil-sand of inner and outer core compartments, under organic and conventional fields and their margins, it is clear that the core rotation impacts strongly on the outer core compartments, but has no apparent impact upon the inner compartments (Figure 6.4).

Examining the soil-sand ^{14}C concentration data of only the outer core compartments shows an effect of management: when the values for rotated cores are subtracted from those of intact cores (to give quantity of ^{14}C transported by AMF), there was a significant effect of management (Figure 6.5; 1-way ANOVA on $\log_e$-transformed data: $F = 2.98$, d.f. = 3, 44, $p = 0.042$). The conventional field treatment had significantly higher ^{14}C concentration than the organic field treatment (Tukey test, $p < 0.05$), whilst the margin treatments had intermediate ^{14}C concentrations and were not significantly different from one another. This implies that the AMF external mycelium exerts a greater drain on plant photosynthate in conventional fields than in organic fields.

6.3.3 ^{14}C trapped from whole cores

For each of the three trapping periods, the rotated cores had less ^{14}C trapped than the intact cores, except for the organic margin samples during the second trapping period (Figures 6.6 – 6.8, Table 6.3).

To examine whether there were any effects of management treatment on hyphal $^{14}CO_2$ respiration, Kruskal-Wallis tests were performed on the difference between static and rotated cores (Table 6.4). Although all the p values were quite low, the tests showed that management was only a significant factor during the third

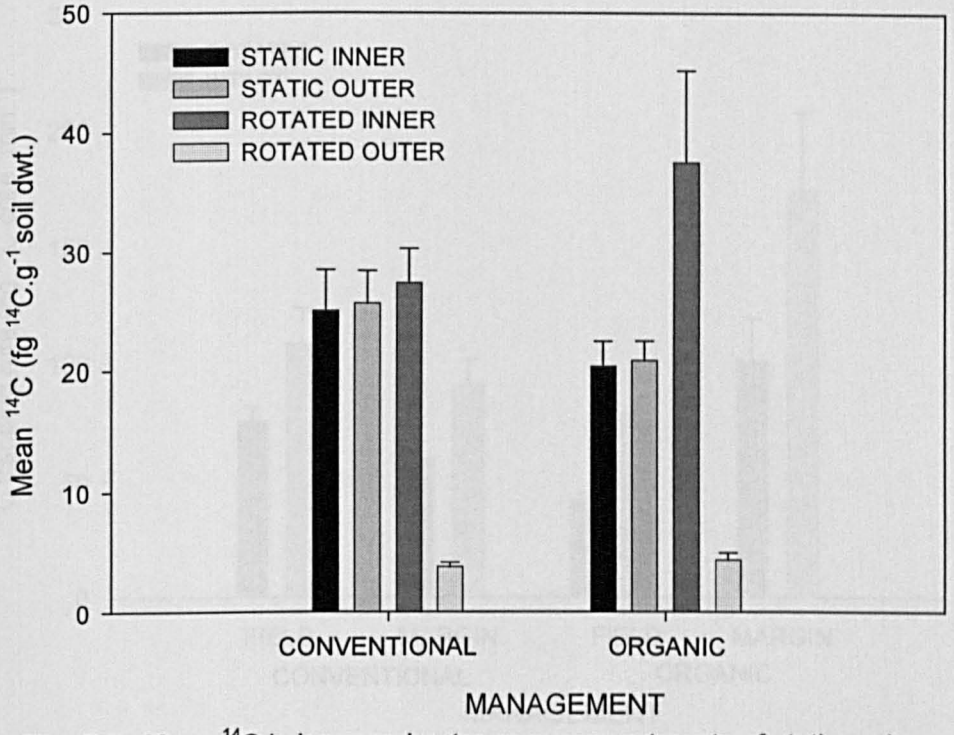

Fig. 6.4 Mean ^{14}C in inner and outer core compartments of static and rotated cores, from turfs originating in conventional and organic land. Error bars are 1 SEM.

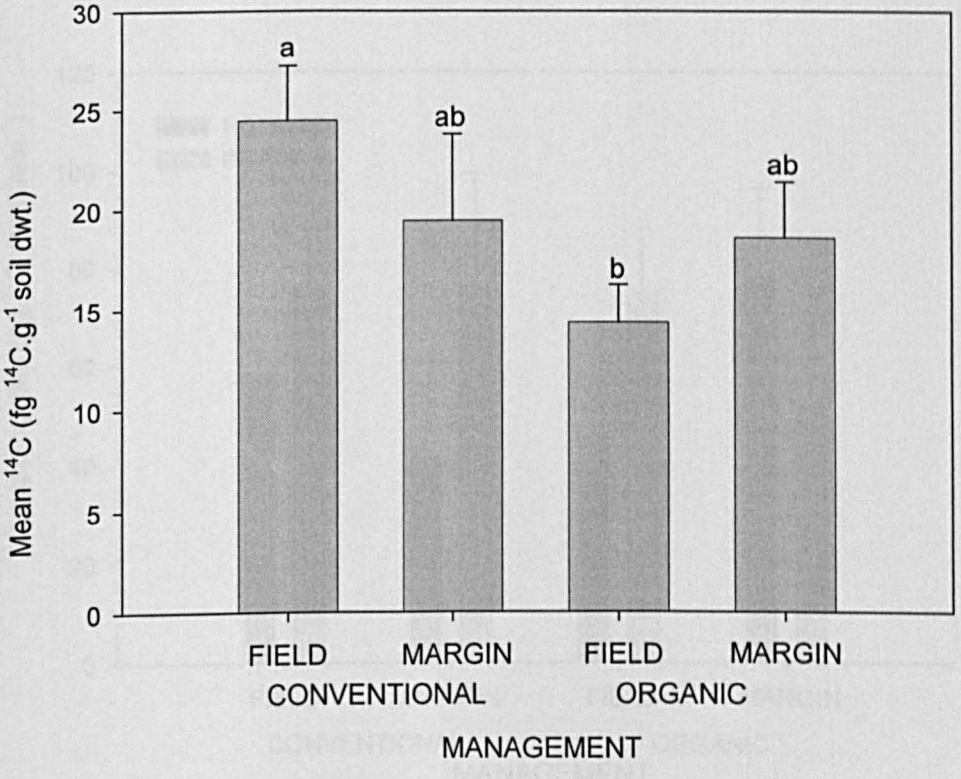

Fig. 6.5 Mean differences between static and rotated cores, in ^{14}C concentration of soil-sand in outer core compartments. Bars with the same letter are not significantly different (Tukey test, $p > 0.05$). Error bars are 1 SEM.

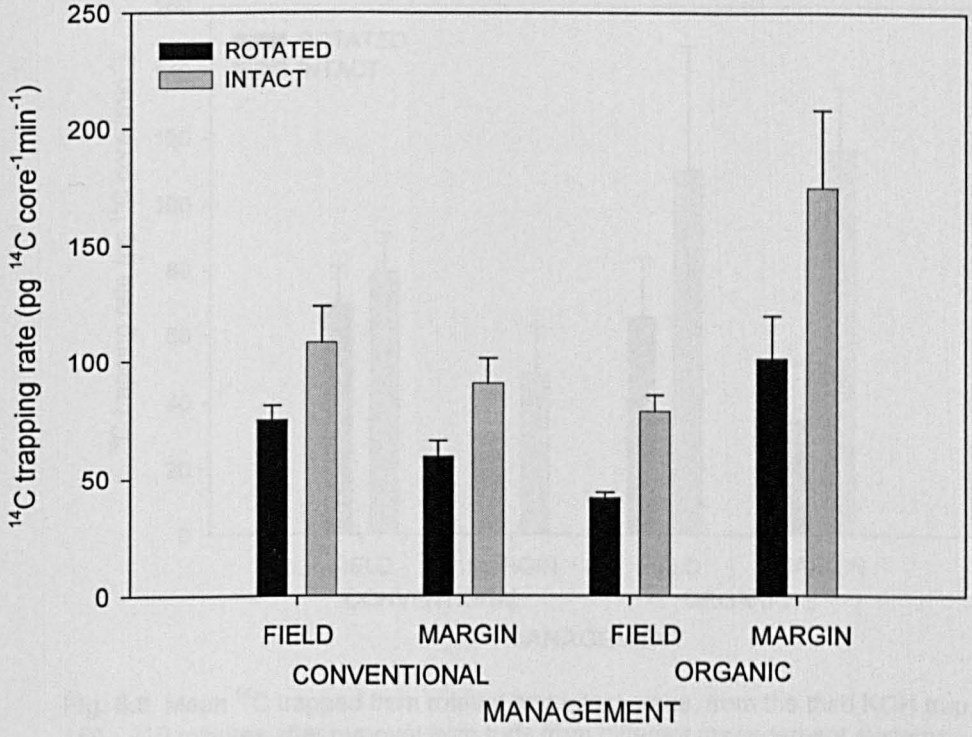

Fig. 6.6 Mean ^{14}C trapping rate from rotated and intact cores, from the first KOH trap (of 90 mins) after removal from turfs from different management systems. Error bars are 1 SEM.

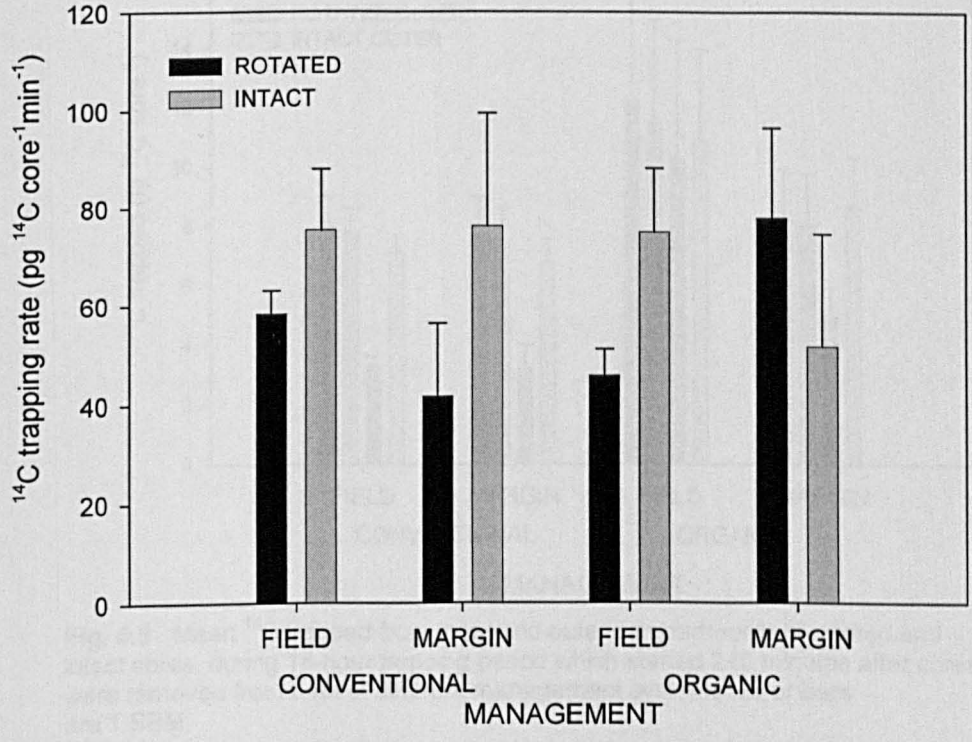

Fig. 6.7 Mean ^{14}C trapping rate from rotated and intact cores, from the second KOH trap, 90 - 150 minutes after removal from turfs from different management systems. Error bars are 1 SEM.

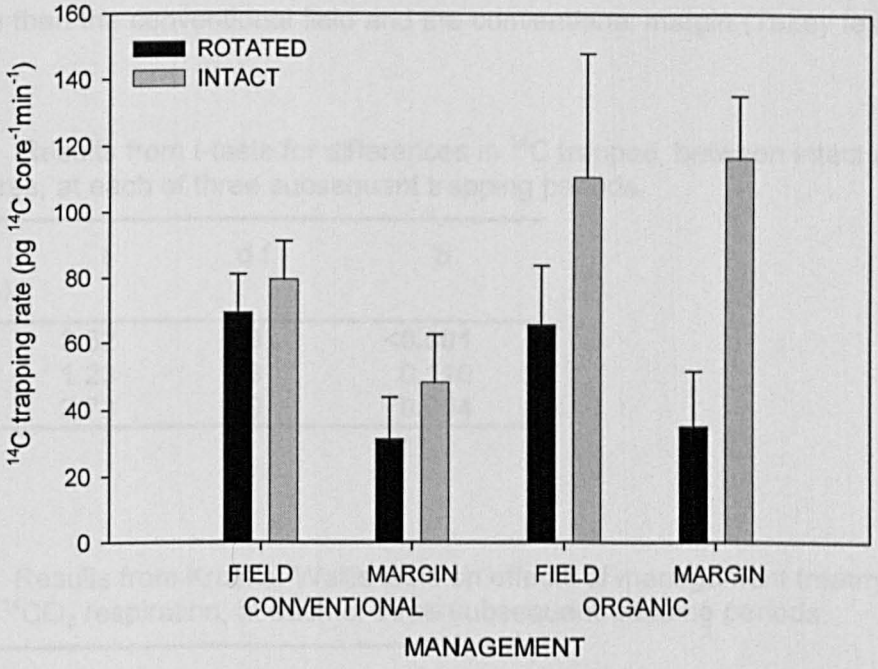

Fig. 6.8 Mean ^{14}C trapped from rotated and intact cores, from the third KOH trap, 150 - 210 minutes after removal from turfs from different management systems. Error bars are 1 SEM.

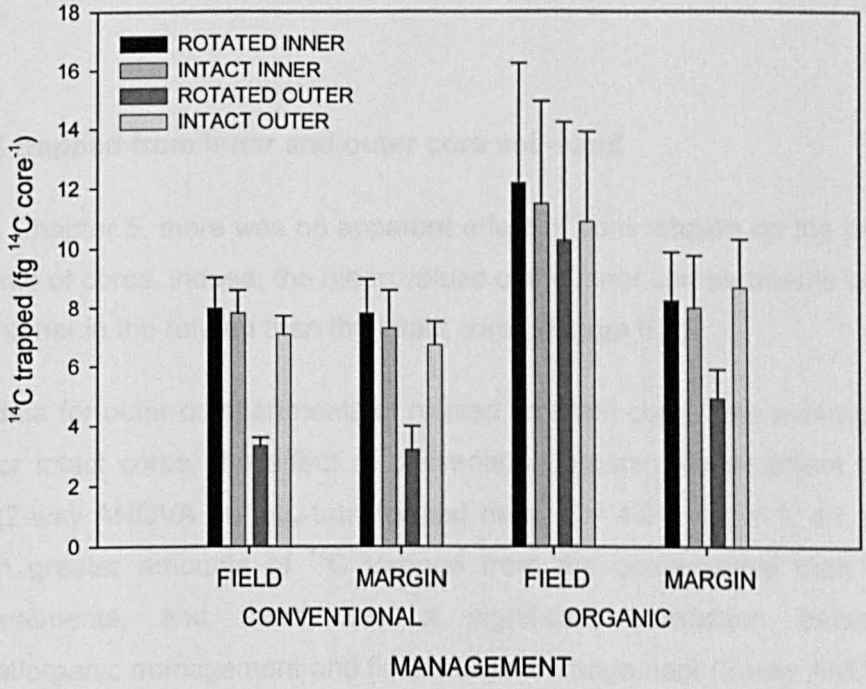

Fig. 6.9 Mean ^{14}C trapped from inner and outer compartments of rotated and intact cores, during 18-hour trapping period which started 240 minutes after cores were removed from turfs of different management systems. Error bars are 1 SEM.

trapping period, where the organic margin had significantly greater hyphal $^{14}CO_2$ respiration than the conventional field and the conventional margin (Tukey test, p <0.05).

Table 6.3 Results from *t*-tests for differences in ^{14}C trapped, between intact and rotated cores, at each of three subsequent trapping periods.

Trapping time (mins)	*t*	d.f.	*p*
0 - 90	3.52	73	**<0.001**
90 - 150	1.23	83	0.110
150 - 210	2.73	80	**0.004**

Table 6.4 Results from Kruskal-Wallis tests on effects of management treatment on hyphal $^{14}CO_2$ respiration, at each of three subsequent trapping periods.

Trapping time (mins)	H	d.f.	*p*
0 - 90	4.77	3	0.190
90 - 150	5.97	3	0.113
150 - 210	9.57	3	**0.023**

6.3.4 ^{14}C trapped from inner and outer core soil-sand

As found in Chapter 5, there was no apparent effect of core rotation on the inner compartments of cores, indeed, the mean values of the inner compartments were marginally higher in the rotated than the intact cores (Figure 6.9).

When the data for outer compartments of rotated (control) cores was subtracted from that for intact cores, the effect of conventional/organic management was significant (2-way ANOVA on $\log_e$-transformed data: F = 4.01, d.f. = 1, 44, p = 0.051), with greater amounts of ^{14}C trapped from the conventional than the organic treatments, and there was a significant interaction between conventional/organic management and field/margin management (2-way ANOVA on $\log_e$-transformed data : F = 4.56, d.f. = 1, 44, p = 0.038). There was no significant difference between turfs from fields or their margins (2-way ANOVA on $\log_e$-transformed data: F = 0.37, d.f. = 1, 44, p = 0.545; Figure 6.10).

Examining the data for fields (excluding that for the margins), the difference in ^{14}C trapped from soil-sand between rotated and intact outer core compartments showed that conventional fields evolved significantly more $^{14}CO_2$ than organic

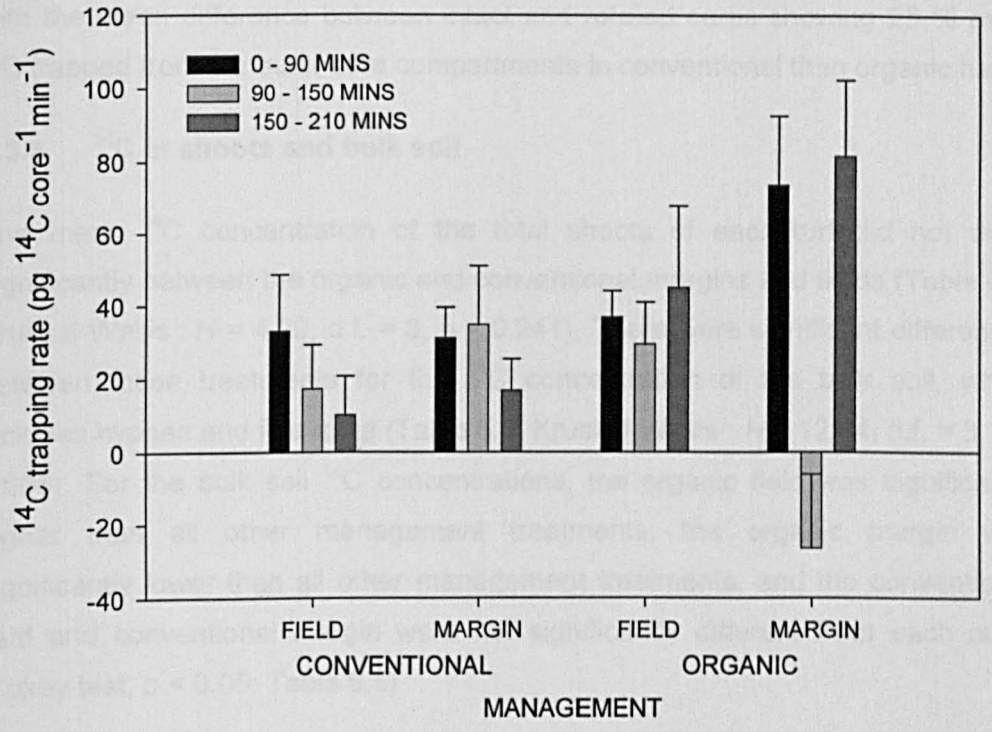

Fig. 6.10 Mean differences in ^{14}C trapping rate between intact and rotated cores during three subsequent KOH trapping periods, following removal of cores from turfs from different management systems, 24 hours after ^{14}C labelling. Error bars are 1 SEM.

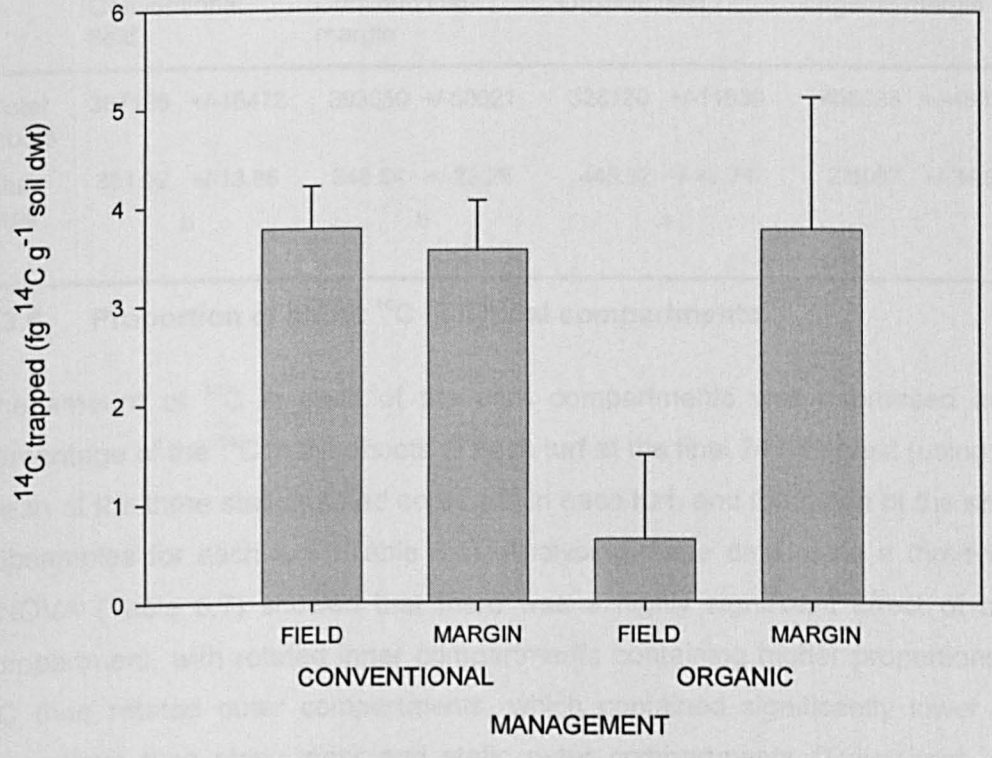

Fig. 6.11 Mean differences between intact and rotated cores for ^{14}C trapped from soil-sand from outer core compartments of organic and conventional fields and their margins. Error bars are 1 SEM.

fields (2-sample *t*-test on log_e-transformed data: t = 2.60, d.f. = 22, p = 0.016), with the mean difference between intact and rotated cores showing 25 % more ^{14}C trapped from the outer core compartments in conventional than organic fields.

6.3.5 ^{14}C in shoots and bulk soil

The mean ^{14}C concentration of the total shoots of each turf did not differ significantly between the organic and conventional margins and fields (Table 6.5, Kruskal-Wallis : H = 4.20, d.f. = 3, p = 0.241). There were significant differences between these treatments for the ^{14}C concentration of the bulk soil, which includes hyphae and fine roots (Table 6.5, Kruskal-Wallis : H = 12.94, d.f. = 3, p = 0.005). For the bulk soil ^{14}C concentrations, the organic field was significantly higher than all other management treatments, the organic margin was significantly lower than all other management treatments, and the conventional field and conventional margin were not significantly different from each other (Tukey test, $p < 0.05$; Table 6.5)

Table 6.5 Mean ^{14}C concentrations (fg ^{14}C g^{-1} dwt, +/- standard error) of total shoots and bulk soil, 24 h after pulse labelling. Values with different letters are significantly different (Kruskal-Wallis : H = 12.94, d.f. = 3, p = 0.005, Tukey multiple comparison test).

	Conventional field	Conventional margin	Organic field	Organic margin
Total shoots	387106 +/-16472	393050 +/-50021	328120 +/-11830	455038 +/-48896
Bulk soil	331.92 +/-13.85 b	348.54 +/-23.26 b	445.82 +/-41.74 a	229.67 +/-34.65 c

6.3.6 Proportion of shoot ^{14}C in hyphal compartments

The amount of ^{14}C in each of the core compartments was expressed as a percentage of the ^{14}C in the shoots of each turf at the final 24 h harvest (using the mean of the three static/rotated cores within each turf, and the mean of the shoot subsamples for each turf ; Table 6.6). Analysing these data using a three-way ANOVA (Table 6.7) showed that there was a highly significant effect of core compartment, with rotated inner compartments containing higher proportions of ^{14}C than rotated outer compartments, which contained significantly lower ^{14}C proportions than static inner and static outer compartments (Tukey test, $p <$ 0.05). The three-way ANOVA also showed a significant interaction between conventional/organic and field/margin, where proportion of ^{14}C in cores was much higher in conventional fields than in organic fields, whereas the margins had

Management	Rotated inner compartment		Rotated outer compartment		Static inner compartment		Static outer compartment	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Conv. field	0.0083495	0.0032323	0.0009805	0.0001784	0.0076918	0.0022500	0.0074645	0.0008155
Conv. margin	0.0072716	0.0007677	0.0009372	0.0002019	0.0062487	0.0016303	0.0059453	0.0009251
Organic field	0.0073894	0.0021859	0.0010443	0.0001135	0.0039853	0.0008275	0.0046793	0.0015747
Organic margin	0.0131202	0.0036248	0.0012026	0.0002821	0.0074674	0.0006291	0.0067674	0.0006791

Table 6.6 Mean proportions of ^{14}C in soil-sand of different core compartments, as a percentage of ^{14}C in shoots, 24 hours after pulse-labelling.

Factor	F	d.f.	*p*
A - Core compartment	16.86	3, 63	**<0.001**
B - Organic/conventional	0.01	1, 63	0.907
C - Field/margin	1.28	1, 63	0.263
A x B	1.07	3, 63	0.372
A x C	0.39	3, 63	0.757
B x C	5.70	1, 63	**0.021**
A x B x C	0.73	3, 63	0.538

Table 6.7 Results of a 3-way ANOVA on proportions of ^{14}C in soil-sand (as a percentage of ^{14}C in shoots), of different core compartments in turfs from organic and conventional fields and their margins.

similar values, the organic margins having only slightly higher values than conventional margins (Tables 6.6 and 6.7).

6.4 Discussion

6.4.1 Core compartment ^{14}C concentration

As found in Chapter 5, core rotation had a differential effect on the inner and outer core compartments, decreasing the ^{14}C concentration found in the outer compartments but having no effect on the inner compartments. This phenomenon was discussed earlier (Section 5.4.3) and hence is not repeated here.

The significant management effect found on the AMF ^{14}C (*i.e.* difference between static and rotated samples) of outer core compartments, with the conventional field giving higher values than the organic field, is interesting. This shows that the type of agricultural management impacts upon the flux of carbon from plant to AMF in the soil. What this experiment cannot show is whether the higher values of ^{14}C measured in the cores from conventional field turfs was due to a greater production of AMF hyphae, or of AMF spores. If the allocation was to hyphae, this would suggest that the AMF in conventional fields would have greater foraging ability, assuming that the main function of the external mycelium is to forage for nutrients (rather than colonise other plant roots). However, the results from Chapter 4 suggested that the reverse was true, that AMF in conventional fields have a lesser ability to acquire phosphorus than AMF in organic fields. The alternate conclusion must be that the additional ^{14}C found in the conventional field cores is allocated to greater spore production. As suggested above (Section 6.1), AMF which are less mutualistic, and those in disturbed environments or where root-to-root colonisation is rarer than spore-to-root colonisation, are likely to prioritise resources to spore production over nutrient foraging which will benefit the plant. Further research is needed to test such a hypothesis, examining net quantities, and relative allocation, of carbon to AMF spores and external mycelium in land under different management intensities.

Conventional fields tend to have fewer weeds than organic fields, which rely on mechanical rather than chemical means to control weeds. This could provide some explanation as to why such a difference in C partitioning to AMF compartments may exist between the conventional and organic fields, since

weeds which remain in organic fields that are mycorrhizal may act as important havens for AMF when the main crop is harvested.

The fact that the margin turfs had intermediate values of ^{14}C in the AMF compartments is not easily explained. If the hypothesis were true that there is greater ^{14}C allocated to spore production in disturbed environments or cropped environments, it would follow that there will be even less carbon allocation to spores in the field margins where there is constant presence of mycorrhizal species and the land is not subject to ploughing. However, in such environments there may be greater production of AMF external mycelium, which could explain the results seen here. Again, such a conclusion must remain supposition until such time as further studies validate it.

6.4.2 Trapping of $^{14}CO_2$ from cores and from soil-sand from core compartments

Core rotation decreased the amount of $^{14}CO_2$ trapped from the cores (in all but one instance) and this again upholds the method of core rotation to sever hyphae as an effective treatment. The finding that management had a significant effect upon hyphal respiration (the difference between $^{14}CO_2$ trapped from static and rotated cores), with greater hyphal respiration in organic margins than conventional fields and margins, contrasts with the findings pertaining to the amounts of ^{14}C within the cores. That the patterns of hyphal respiration rates differ from the patterns of ^{14}C allocated to the cores is evidence of functional differences between these agricultural management systems. This data could be seen to support the suggestion above that in the conventional systems the carbon is allocated to spores rather than to active foraging hyphae which are likely to respire at greater rates. Again, this novel research has shown that this is an area which will be fruitful to research further, and that the current lack of knowledge of mycorrhizal functional differences between different environments needs to be redressed.

As for the ^{14}C within the cores, the ^{14}C trapping from inner core compartments was unaffected by the core rotation treatment, a methodological issue which requires resolving (*c.f.* Section 5.4.3). Examining the data for the differences in $^{14}CO_2$ trapping between static and intact outer cores only, again shows a pattern different to that for core ^{14}C concentration and different again from that for whole-core $^{14}CO_2$ trapping. Since these differences are all significant and marked, they can not simply be discounted as experimental error, but rather as indications of

how complex the mycorrhizal functional differences seem to be among these management systems.

6.4.3 Concentration of ^{14}C in shoots and bulk soil

The lack of any significant difference in ^{14}C concentration between the initial shoot samples from the different turfs meant that the below-ground comparisons of ^{14}C allocation were valid, since comparable amounts of ^{14}C were likely to have entered the system. The similar lack in significant difference among shoot collected at the final harvest showed that, presumably, comparable amounts of ^{14}C had moved from above-ground to below-ground. This would suggest that in comparing the different management systems, it may be carbon partitioning that varies more than the total carbon drain on the plant. However, such a proposal needs to be examined in detail through further study.

The variation in ^{14}C in the bulk soil (soil, fine roots, hyphae, other micro-organisms etc.) may be partly due to the heterogeneity of the soil, which may have had very variable proportions of fine roots, for instance. However, the differences were marked, with the greatest difference being between organic field (low ^{14}C concentration) and organic margin (high ^{14}C concentration). Comparing this to the findings for ^{14}C concentration in the soil-sand of the cores, in both cores and in bulk soil the organic field was lower in ^{14}C concentration than the organic margin, however within the cores the conventional field had the highest value of all the management systems. Again, these apparent differences in carbon partitioning between management systems offer interesting avenues for future research to follow up.

6.4.4 Proportion of shoot ^{14}C in hyphal compartments

That the proportion of shoot ^{14}C that was in hyphal compartments followed the pattern for the ^{14}C concentrations in hyphal compartments is unsurprising given that the amounts of ^{14}C in shoots did not vary with treatment. The proportions reported here are far lower than those reported for the experiment in Chapter 5. This could be a reflection of the trauma which these systems endured in having all their indigenous vegetation killed by herbicide and remaining barren of flora for 4 weeks, or of the plant species composition being artificially altered. The latter could be an important factor if it is the case that AMF, whilst perhaps not being host-specific in forming mycorrhizal associations, are specific in their functional relationships with their host plants. If the lower proportions of ^{14}C being transported to hyphal compartments are due to trauma caused by the

experimental design, then one would expect that actual values would be greater, and thus the results found in this chapter may be magnified in the field. The lower proportions in this chapter, compared to Chapter 5, could also be a reflection of a differential in function between agricultural systems and the semi-natural calcareous grassland studied in Chapter 5.

6.4.5 Appraisal of methodology

The treatment of the turfs to standardize the vegetation enabled direct comparisons of the different management systems, including field margins which naturally have very different flora from agricultural fields. By standardizing the vegetation, it was possible to attribute results to the effects of management, rather than to the presence of different plant species and above-ground biomass. However, this treatment was severe, and may potentially have affected the systems. In designing this experiment it was presumed that any such effects of the method would affect all turfs similarly, yet this supposition is not proven and it is plausible that differential effects may result from the fact that the conventional fields have a history of agrochemical applications. Such difficulties are inherent to studying complex systems such as these, and it seems that any experimental method will have drawbacks since it is not possible to control variables (such as plant species) independently of all other variables in the system.

6.4.6 Overview

This chapter has shown that there are differences between organic and conventional fields and their margins in terms of carbon flux into, and out from, external AMF hyphae. These changes in allocation are not straightforward to interpret, and further research is necessary before conclusions can be made as to exactly how the carbon partitioning and flux is different in different management systems. These results suggest that in conventional fields, more carbon is allocated to AMF external to the root, *i.e.* to hyphae and/or spores. Given the findings of Chapter 4, that conventional fields had the lowest ^{33}P uptake to plant shoots, it seems that this extra carbon allocated to AMF external to the root does not increase the AMF foraging ability, and hence may be of little benefit to the plant. This chapter also found that hyphal respiration of pulse-derived carbon seemed higher in organic margins, perhaps indicative of functional differences whereupon AMF in organic margins are more metabolically active (and transport more phosphorus to the plant shoots: Chapter 4), whilst incorporating less pulse-derived carbon into biomass than the AMF in conventional fields. This experiment was intended to determine whether

management intensity affects carbon flux from plants into AMF external to the root, and has shown that this is indeed the case.

Very little research has been carried out in this area of mycorrhizal carbon flux in different management systems. The most relevant work was a ^{13}C labelling and nutrient amendment experiment, in which Gavito and Olsson (2003) found that where plant hosts were less nutrient limited and produced more inflorescences, there was less carbon allocated below ground. They also found that, 7 days following ^{13}C pulse labelling, allocation of C to storage lipids (NLFA 16:1ω5) relative to AMF biomass indicator lipids (PLFA 16:1ω5) was greater in hyphal compartments which were unamended with nutrients than in hyphal compartments amended with nutrient solutions or dry yeast (*ibid.*). Their results also showed that hyphal length density, hyphal biomass (PLFA 16:1ω5), and AMF storage lipids (NLFA 16:1ω5) were significantly greater in nutrient amended compartments than unamended compartments; the amount of storage lipids (NLFA 16:1ω5) was significantly affected by the type of nutrient amendment, being greatest where all nutrients except P were added in solution, intermediate where only a P solution was added, and least where there was the organic amendment of dry yeast. The relative allocation of C to storage lipids (NLFA 16:1ω5/PLFA 16:1ω5) was least in the organic amendment, and was greater in the unamended compartments. This type of work is able to elucidate differences in carbon allocation below-ground, and it is this type of approach which will be useful in deepening understanding of the findings of this chapter.

Chapter 7 :
General discussion

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7.1 Synthesis

Modern intensive agriculture has created environments which are likely to impact upon the functioning of arbuscular mycorrhiza, through practices such as fertilisation, ploughing, use of pesticides, growth of non-host crops and monocultures (Chapter 1). This thesis has examined differences in arbuscular mycorrhizal functioning in land under different management intensities, such as organic and conventional agriculture. Chapter 3 showed that marked differences in AMF P uptake and translocation to plant roots can occur, with turfs from organic pasture land having far greater ^{33}P translocated to shoots via hyphae than turfs from conventional pasture, conventional cropped land or integrated cropped land. These findings were further explored in Chapter 4, where more comparable systems (with standardised plant species) were used from a wider range of sites across England. Whilst there was a trend for increased management intensity to equate to decreased hyphal ^{33}P translocation to plant shoots, the differences were not so marked and not statistically significant. This was perhaps not surprising, given the many variables and confounding factors involved in using different soil types from a range of sites, with relatively low sample replication. It was concluded that those results were biologically meaningful, that hyphal P translocation can be affected by agricultural management.

Mycorrhizal plant phosphorus uptake has often been seen as a main benefit to plants of arbuscular mycorrhizal status, whilst a factor which can sometimes be viewed as a cost to the plant is the quantity of carbon which the plant provides to the fungus, and this element of the symbiosis was studied in Chapters 5 and 6. Although Chapter 5 found some complications in using the doubly-compartmented hyphal in-growth cores, namely that the inner compartments were little affected by core rotation, Chapter 6 showed that using this approach could elucidate some differences in ^{14}C translocation from plants to hyphal compartments in turfs from different management systems. It was shown that even with an experimental design with many potentially confounding factors and low replication, significant differences could be found in the ^{14}C translocated to hyphae, with more ^{14}C translocated to those in conventional fields than those in organic fields.

This final chapter aims to draw together the results of the previous chapters, placing the work in the context of varying degrees of AMF mutualism, and also to identify areas for future research.

7.2 A continuum between mutualism and parasitism

The ubiquitous and intimately close symbiosis between mycorrhizal plants and fungi has evolved through mutual benefit. Each partner in the symbiosis is able to provide a commodity by which the other partner benefits. In such a case, there could be a tendency to conceive of this intimate partnership as co-operative or even altruistic, but as Herre *et al.* (1999) stated, "mutualisms are best viewed as reciprocal exploitations that nonetheless provide net benefits to each partner". Thus it becomes evident that there may be potential conflicts of interests between plants and fungi, such as could create selection for less mutualistic, or even parasitic relationships. As this thesis has already begun to examine (Chapters 1 and 6), the conditions created by farming, and by intensive agriculture in particular, can exert pressures which may result in the symbiosis becoming less mutualistic.

Various studies have noted plant growth can be greater where the AMF are inhibited or absent from the system (Graham and Eissenstat, 1998), whilst in the case of myco-heterotrophy (Leake, 1994) it seems that the plant receives significant benefit whilst the fungi receive nothing in return. In any symbiosis, mutualistic or not, each party has its own fitness as a priority, and the symbiosis is therefore at least potentially open to cheating.

7.2.1 Evidence for management intensity affecting degree of mutualism

It is possible to calculate a hyphal transfer ratio, which represents the amount of ^{14}C received by hyphae per unit of ^{33}P given to the plant shoots via hyphae. This ratio can be used as an indicator of the degree of mutualism within the mycorrhizal symbiosis. The results of such a comparison are given here, with ^{33}P and ^{14}C values paired on a field basis (using the mean values for replicates within each field, or within each margin), using the data for hyphal ^{14}C within outer core compartments (*i.e.* the difference between static and rotated cores), and for shoot ^{33}P transferred from hyphal compartments at 1 cm from the bulk soil (Fig 7.1). These results show that the mean hyphal transfer ratio is significantly greater under conventional than under organic management (mean hyphal transfer ratio 1.95 (SEM 0.43) for conventional, and 0.993 (SEM 0.20) for organic turfs; 1-tailed *t*-test: $t = 2.01$, d.f. = 14, $p = 0.032$). Whilst the hyphal transfer ratios were lower

for margins than fields in both management systems, this was not significant (mean hyphal transfer ratio 1.58 (SEM 0.35) for field, and 1.37 (SEM 0.41) for margin turfs; 1-tailed *t*-test: t = 0.39, d.f. = 14, p = 0.352). (Results from an ANOVA given in Table 7.1.)

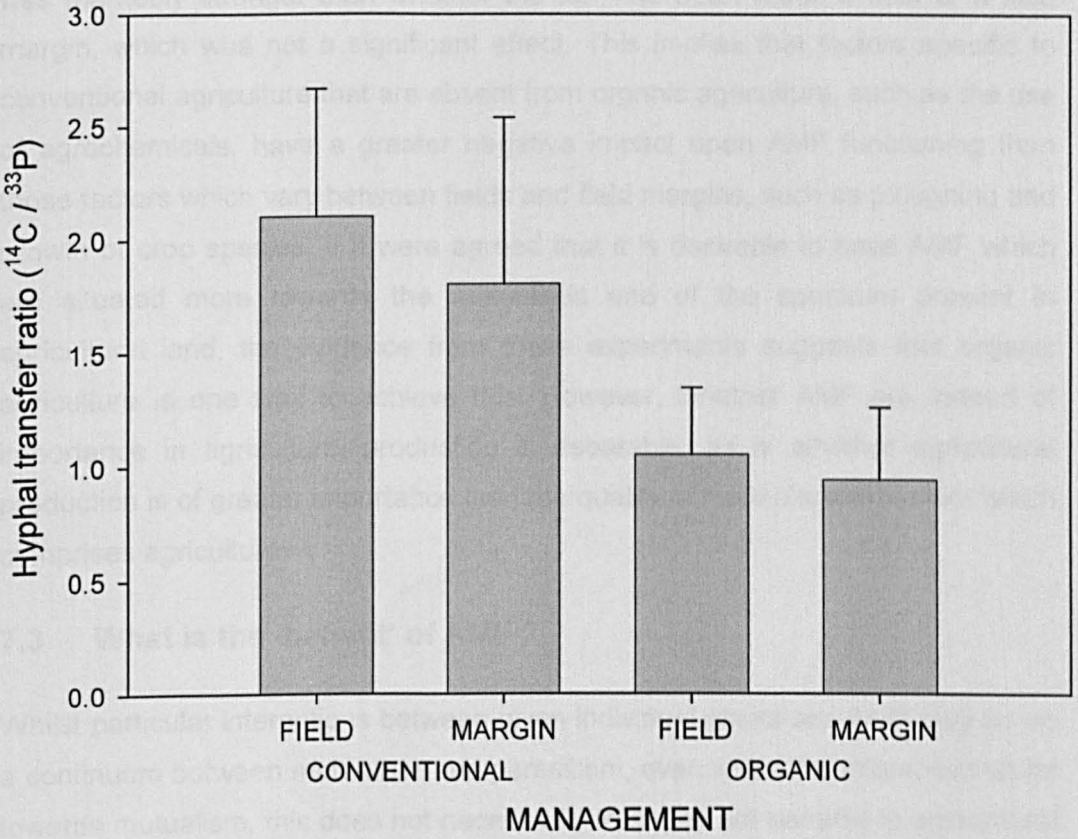

Fig. 7.1 Mean hyphal transfer ratio, the amount of ^{14}C transferred to outer core compartments by hyphae (difference between static and rotated cores, fg ^{14}C g^{-1} soil dwt), divided by shoot ^{33}P transferred from hyphal compartments at 1 cm from bulk soil (shoot ^{33}P ng g^{-1}).

Table 7.1 Results from ANOVA on effects of management on hyphal transfer ratio. (D.f.= 1, 15)

Factor	F	*p*
Conventional/organic	3.51	0.085
Field/margin	0.17	0.691
Interaction	0.03	0.858

The *t*-test upholds the hypothesis that the hyphal transfer ratio is greater in conventional than in organic agriculture, although the results from the ANOVA

are not so conclusive. Whilst the ANOVA results are not significant at the customary cut-off point of $p = 0.05$, they nonetheless show that with a sample size of 16 fields or margins sampled from across England, there is a 90 % probability that the hypothesis is true.

An interesting finding was that the effect of conventional or organic management was markedly stronger than whether the turf had been within a field or a field margin, which was not a significant effect. This implies that factors specific to conventional agriculture that are absent from organic agriculture, such as the use of agrochemicals, have a greater negative impact upon AMF functioning than those factors which vary between fields and field margins, such as ploughing and growth of crop species. If it were agreed that it is desirable to have AMF which are situated more towards the mutualistic end of the spectrum present in agricultural land, the evidence from these experiments suggests that organic agriculture is one way to achieve this. However, whether AMF are indeed of importance in agricultural production is debatable, as is whether agricultural production is of greater importance than the quality of the biological system which comprises agriculture.

7.3 What is the 'benefit' of AMF ?

Whilst particular interactions between given individual plants and AMF may be on a continuum between mutualism and parasitism, even when the interaction tends towards mutualism, this does not necessarily infer any net benefits to agricultural production. Indeed, whether the overall outcome is seen as "beneficial" depends on both what criteria are used to measure benefit, and upon available knowledge about the system. Ryan and Graham (2002) suggested in a review paper that AMF do not play a vital role in the nutrition and growth of plants in many production-orientated agricultural systems. Examining the evidence from Australia, they observed that AMF had been found to be beneficial to plant growth and nutrition in areas where farmers had not traditionally applied phosphate fertiliser and where soils were very low in phosphorus (*ibid.*). However, when P fertilisers were applied, the presence or absence of AMF had no effect upon plant growth or P uptake (Ryan and Angus, unpublished data cited in Ryan and Graham, 2002). Thus where farmers are concerned solely with yield, they may perceive greater benefits in applying P fertilisers than in their crops having AMF associations.

Chapter 1 outlined the 20th century trend towards increasing agricultural production, which led to overproduction and food mountains in Europe, and the

changes in government policy to move towards a more sustainable mode of agriculture. Thus today it is not simply yields that agriculture is concerned with, but a range of aspects of the farming system. Even when there is no benefit of AMF on crop growth or P content, there can be other benefits such as increased grain zinc content (Ryan and Graham, 2002). Consumers are concerned over the amounts of vitamins and minerals contained in their diets, such that in 2000 the U.K. retail market for these and other supplements was worth £335 million (Mintel, 2001). Of this market, mineral supplements made up 7.7 %, with zinc being the second most popular mineral (after iron; *ibid.*). There is also evidence that AMF greatly increase the aggregate stability of soils through their production of glomalin (Rillig *et al.*, 1999), and may therefore help limit soil erosion. Such a benefit will only accrue over long time periods, and hence is difficult to include in any cost-benefit analysis.

7.4 Difficulties inherent to this project

There were many problems faced by this project. The nature of the environment being studied is not readily conducive to scientific exploration, due to many confounding factors. These experiments used turfs from the field, since this is closer to the field situation than alternatives (for example, transplanting plants into sand); yet this brings a lack of control over experimental conditions. The soil itself, which exerts such a strong influence both on AMF and on plants, is heterogeneous and may vary widely in nutrient concentration within a single field, as well as between fields. It would therefore be desirable to have large numbers of replicate turfs from each field which was studied, however this was not logistically possible, and in any case unlikely to be acceptable to the farm managers. The history of each field which was studied is likely to vary widely, and limited information on management histories was available. As explained earlier in this thesis, many agricultural management practices can impact upon AMF, such as fertilisation, ploughing and crop species (Chapter 1). Thus the varying management histories (even within each management treatment) will have impacted upon the systems and may have affected the results of the experiments carried out. Ideally, work such as this would be carried out on land which had been under standardised and documented management for a long time, as were the Swiss trials by Mäder *et al.* (2000, 2002), but no such land was available in England for this project to study.

The mycorrhizal system itself, consisting of a community of AMF and various plant species, is likely to be species specific (although there is still a lack of

evidence on this subject; Sanders, 2002), such that changing either the plant species, or indirectly affecting AMF community composition, may impact upon the functioning of the system (Johnson 1993; Smith 2000; Mäder *et al.*, 2000, 2002). The question of which plant species are used for experiments such as these is complex, since the functioning of the system as it is in the field may only be operable when the indigenous plant species are present, as in Chapter 3. However, where different management systems are being compared which have different indigenous vegetation, any measured differences in AMF functioning may be due to either plant species or management, it is impossible to conclude which. This issue was addressed in Chapters 4 and 6, by standardizing the vegetation, however this artificial manipulation of the system may of itself have altered its functioning. Thus, as with any experiment, the results can only be applied with certainty to systems under identical conditions and treatments, and any extrapolation to wider circumstances must be made with a degree of caution.

The methods employed for this project, of labelling with radioisotope tracers, bring in their own variability to the results. The nature of radioactive decay is that it is a little stochastic over time, and this could introduce additional noise to the data, possibly masking trends which do exist. This additional variation within results should be countered by using a large number of replicate samples, but this was limited by resources available to the project.

An additional difficulty faced by this project was the major outbreak of Foot and Mouth Disease across England in 2001. This outbreak co-incided with planned sampling from fields which were situated on dairy farms, and quarantine regulations delayed field sampling for months.

7.5 Directions for future research

Since resources for this project were limited, it is inevitable that the findings must be tentative and in need of further exploration and validation. Various interesting results have been described in the previous chapters, all of which merit further investigation. The differences found in phosphorus and carbon transfer between mycorrhizal partners, and the changes in hyphal C:P transfer ratio, in turfs from different management systems, were found by studies which had very low replication given the many confounding factors (as mentioned above, Section 6.4). An obvious follow-on to this work would be to carry out similar experiments with much greater replication and screening an even broader range of soil types, preferably with standardised management histories.

Given that differences have been found in the function of AMF in these different environments, it is likely that the actual identity of the AMF varies. Molecular techniques have been used elsewhere to show that AMF diversity and community composition is different in different environments (Helgason *et al.*, 1998, 2002; Daniell *et al.*, 2001), and these techniques should be applied to study the identity of AMF in agricultural land under different management regimes. If there are seasonal changes related to crop sowing and harvesting which are considered negative, these could perhaps be ameliorated by maintaining plant cover through most of the year.

At present, rather little is known about how beneficial AMF are to the farmer overall, and research should be carried out into this question. However, the issues here are complex, and what may seem profitable in the present climate (*e.g.* crop productivity or crop health) may not concur with what will be beneficial in the long term (*e.g.* protection against soil erosion, micronutrient nutrition). One difficulty with this type of research is that an emphasis is often placed upon profitability, and a monetary value placed upon different components of the system, with components or phenomena which cannot be assigned a monetary value being designated as having no value, which may be far from realistic.

Molecular techniques and the more traditional methods of examining morphology have shown that the abundance of AMF species can change through the year (*e.g.* Merryweather and Fitter, 1998; Helgason *et al.*, 1999). It may be that this is also the case in land such as that studied in this project, and it is unknown whether any seasonal differences in AMF presence or function exist between systems under different management intensities. The possible seasonal changes in presence of particular AMF species in agricultural land have yet to be elucidated, and this needs to be investigated.

A factor which was outside the scope of this project is the amount of time which land has been under a given management system. If there are differences in mycorrhizal functioning between management systems, it follows that these changes take some time to arise. There are some suggestions in the literature (Miller and Jackson, 1998; Dekkers and van der Werff, 2001) that AMF may be affected by previous management for 10 years, and perhaps longer. What is presently unknown is how long changes in mycorrhizal functioning may take to stabilise following changes in management, and what are the factors driving such change. This would be a useful area to research, since the knowledge could potentially be applied when land is converted from conventional to organic

management, to increase this rate of change through conducive management practices, and thus gain the maximum benefit from AMF.

The carbon radioisotope work (Chapter 5) found intriguing patterns in amounts of carbon in hyphal compartments as time from labelling progressed. Whilst this could be experimental artefact, it may reflect changes in plant carbon pools which are used to source the AMF carbon demand, during the photoperiod versus the dark period. Published work examining carbon flux through the mycorrhizal system which had looked at the effects of time has tended to take measurements at the same time each day. However, if there are changes through the light-dark period, this would be worth researching to better understand the impact of the AMF upon their plant hosts.

Two novel methods were pioneered in this project: the use of anion-exchange resin membranes as a source for ^{33}P, and the use of double-walled hyphal in-growth cores. The anion-exchange resin membranes seemed highly successful, and should provide a useful tool in future work. The double-walled hyphal in-growth cores need to be further examined, under a range of conditions, to verify them as a useful addition to the limited armoury available to mycorrhizal researchers for creating non-mycorrhizal controls.

7.6 Conclusions

Whilst these experiments, (as most other experiments,) must be qualified with a note of caution due to their limited scope (particularly in terms of replication and the issues discussed above, in Section 7.4), several conclusions can be made from this work.

1. Anion-exchange resin membranes provide a successful method for providing a defined phosphorus source (Chapters 3 and 4).

2. Double-walled hyphal in-growth cores show treatment effects in the outer compartments, but there is a non-hyphal pathway for carbon transfer into the inner core compartments and further investigation of this design core is therefore needed (Chapters 5 and 6).

3. There were large differences in hyphal P uptake in turfs from different management systems; namely there was far greater hyphal P uptake to plants in organic pasture than in conventional pasture, conventional wheat fields or wheat fields under integrated management (Chapter 3).

4. There appeared to be a trend of increasing hyphal P uptake to plants with decreasing management intensity, with organic versus conventional management having a stronger effect than whether turfs originated in fields or in field margins (Chapter 4).

5. There were significant differences in carbon allocation between organic and conventional fields and their margins. Hyphal respiration of pulse-derived carbon was higher in organic margins than conventional fields and their margins in intact cores, whilst in outer core compartments 25 % more ^{14}C was trapped in conventional than organic fields. More carbon was allocated to AMF external to the root in conventional fields than organic fields (Chapter 6).

6. The effect of conventional or organic management was stronger than whether the turf had been within a field or a field margin, for most variables measured.

7. The hyphal transfer ratio of carbon to phosphorus is affected by management intensity, suggesting that AMF are less mutualistic in systems under higher management intensities (Chapter 7).

These results are important for understanding arbuscular mycorrhizal functioning in different agricultural systems, an area which is under-researched and yet is of great relevance in today's agricultural climate, where organic and other alternatives to conventional agriculture are increasing in prevalence.

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