A Systems Approach to the Management of Arbuscular Mycorrhiza

Bioassay and Study of the Impact of Phosphorus Fertilization

Helena Kahiluoto

Department of Applied Biology
University of Helsinki, Finland

Academic dissertation in Plant Production Sciences, especially in Agroecology

To be presented, with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public criticism in Auditorium XIV, Unioninkatu 34, Helsinki, on January 19th, 2001, at 12 o´clock noon.

Helsinki 2000

Yliopistopaino
Helsinki 2000
ISBN 951-45-9630-7
ISSN 1457-8085

To Jouni, Jouki and Pyry

PREFACE

My professional identity is that of a researcher developing organic agriculture and, more generally, sustainable food systems. Sustainability implies a multidimensional point of view, and the practical strategy of organic farming is a strategy of systems management. Thus, it is not surprising that the systems approach enjoys lively discussion among researchers concerned with organic agriculture. The challenge of the systems approach for research on sustainable agriculture is often interpreted as a need to focus on the farm level or on higher organizational levels of the food system while the potential of a systems approach at lower organizational levels of agroecosystems have largely been disregarded. In this context, the present work not only involves research on the particular topic of arbuscular mycorrhiza (AM) in phosphorus (P) management but it demonstrates choices in the degree of systemism adopted in research on low system levels, and the impact of these choices on the research questions addressed, the methods applied and the conclusions drawn.

The attempt towards a systems approach determined the structure both of the work and of the thesis. The starting point is the features desired in a sustainable food system, and one of the major obstacles to sustainability, the present P management, is elucidated. The problematic situation in the P management is described, and the management of a phenomenon with good potential to improve the situation, AM, is spotlighted. The research questions are formulated and answers are sought with care for relevance to the sustainable food system. An effort is made to understand the functioning of the spotlighted subsystem of AM management by P fertilization, and reduction of any essential interactions is avoided. Finally, the results and conclusions are linked back to the context of the sustainable food system, and options to improve the P management are discussed.

This work concerns the possibilities to rely on ecosystem services in agriculture, in this case on AM. Within AM research, it represents a strategy to rely on AM fungal (AMF) field communities through management of the production system. This systems-oriented strategy is only now drawing attention in European research on AM, while the strategy to select, propagate and regularly inoculate exogenous AMF strains as "biological fertilizers" continues to dominate. The work reported in this thesis comprises the development of methods for AM studies of relevance for the SYSTEMS ORIENTED MANAGEMENT utilization strategy selected, and the application of these methods in bioassays and in the field. Except for minor parts of the method development, the work is published in the appended papers. Additionally, the impact of the systems approach on the work is studied.

In Juva, 2 October, 2000

Helena Kahiluoto

Summarized publications and the author's contribution

This thesis is based on the following articles, which were referred to in the text by their Roman numerals:

I Kahiluoto H, Ketoja E and Vestberg M 2000 Creation of a non-mycorrhizal control for a bioassay of AM effectiveness 1. Comparison of methods. Mycorrhiza 5, 241-258.

II Kahiluoto H and Vestberg M 2000 Creation of a non-mycorrhizal control for a bioassay of AM effectiveness 2. Benomyl application and soil sampling time. Mycorrhiza 5, 259-270.

III Thingstrup I, Kahiluoto H and Jakobsen I 2000 Phosphate transport by hyphae of field communities of arbuscular mycorrhizal fungi at two levels of P fertilization. Plant Soil 221,181-187.

IV Kahiluoto H, Ketoja E and Vestberg M 2000 Promotion of utilization of arbuscular mycorrhiza through reduced P fertilization 1. Bioassays in a growth chamber. Plant Soil. In press.

V Kahiluoto H, Ketoja E, Vestberg M and Saarela I 2000 Promotion of utilization of arbuscular mycorrhiza through reduced P fertilization 2. Field studies. Plant Soil. Accepted.

Helena Kahiluoto was responsible for the planning and performance of the experiments, interpretation of the results and reporting of the work for papers I, II and IV. The same concerns paper V, except for the determination of spore densities and identification of fungal species, which were done by Mauritz Vestberg. For paper III, Helena Kahiluoto collected, prepared and delivered the samples from a field experiment conducted by herself. She participated in the planning of the experiment which was performed by Ida Thingstrup in co-operation with Iver Jakobsen. Helena Kahiluoto wrote the manuscript based on a draft by Ida Thingstrup and comments by Iver Jakobsen. She was also responsible for the correspondence with the journal. Elise Ketoja analysed the data for papers I, IV and V, reported the statistical methods employed, drew the figures and commented on the manuscripts. She also assisted in the planning of the experiments and in the statistical analyses of paper II. Mauritz Vestberg was responsible for the Nordic project within which the present study was carried out. He made valuable comments on plans, experimental performance and reports, and drew the figures for paper II. Into Saarela was responsible for the long-term experiments utilized in this work; he read the manuscripts of papers IV and V and delivered useful comments.

CONTENTS

1 Introduction

1.1 The systems approach

This thesis comprises research work on a specific ecological topic and a methodologically oriented study on the challenges of applying a systems approach in research focusing on a relatively low organizational level of the agroecosystem.

The mechanistic - positivistic world-view and analytical scientific approach had clearly emerged by the 17th century in the work of Galileo, Descartes and Newton, in contrast to the former Aristotelian holistic-teleologic world-view. One-way cause - effect relations were sought by isolation of the phenomenon studied from the researcher, dividing it into discrete and manageable entities and studying these under controlled conditions (Bertalanffy, 1975). This paradigm (Kuhn, 1970) is often referred to as reductionist. According to the extreme reductionist view, the phenomena observed at a certain organizational level are explained through vertical reduction, i.e. solely by knowledge at a lower, even lowest possible, organization level.

Beginning with Einstein's theory of relativity (1905), a more systemic approach has evolved within science (Jantsch, 1975; Ackoff, 1983), and been formulated into a general theory of systems, for example by Bertalanffy (1973). According to the systems view, useful information about a phenomenon is not obtained by studying its components in isolation, because their interrelations determine the function of both the part and the whole (Capra, 1975; Bunge, 1985). The soft systems approach (e.g., Checkland, 1981), with its roots in phenomenology and hermeneutics, further assumes that every system can be described in several ways depending on the underlying world-view. This shift from an ontological systems orientation (hard systems methodology) to an epistemological one (soft systems methodology) implies that not only is the phenomenon studied interpreted as a system but also the inquiry into it (Checkland, 1988; Bawden, 1991). In this view, the interrelatedness between the different system levels is emphasized. Thus, the solutions emerge from a vision of the entity taken as a whole (Laszlo and Laszlo, 1997), reflecting a world-view that needs to be made explicit through a process of systemic inquiry (Bawden, 1995). This approach, participatory in its very nature (Laszlo and Laszlo, 1997), introduces the researcher as a responsible actor in the human activity system (also Alrøe and Kristensen, 1998).

Within agriculture, a plea for holism was made by movements questioning the sustainability of the dominant agricultural development, and by a number of individual scientists (e.g., Carson, 1962; White, 1967). Environmental and social problems were seen as deriving, in part, from the fragmented approach. Attempts to construct

research methodologies especially for agriculture, using a hard or soft systems approach, were made in third-world countries (farming systems research) and by Spedding (1979), Bawden et al. (1985), Conway (1985), Odum (1983, 1988) and others. According to Lockeretz and Boehncke (2000), in research on sustainable or, more specifically, organic agriculture, the challenge to do systems research has been offered and accepted, but still not met. In this discourse (Lieblein, 1995, 1997; Kristensen and Halberg, 1997; Alrøe and Kristensen, 1998; Lockeretz, 2000 etc.), the challenge is often interpreted as a need to focus on specific organizational levels ("*the* system-level"), usually on the farm level but sometimes higher on the food system. Taken to extremes this holistic (see Bunge, 1985) interpretation could, however, lead to "upward reductionism" (Bunge, 1985), i.e. to black box models with no consideration of the lower-level processes, or to an unrealistic attempt to include everything in every study (see Alrøe and Kristensen, 1998). Alternatively, this interpretation could result in disregarding the targets and context of the higher levels (suprasystems) and the constraints set by them for the focused level (Burkhardt, 1989; Wilson and Morren, 1990). Also, research on hierarchical levels lower than the farm have been taken as reductionist of necessity (e.g., Hoegh-Jensen, 1998).

The benefits of considering the system and subsystem character of the object and of the study have been largely disregarded in research focusing on low organization levels of the agroecosystem. Therefore, the present study attempts, as phrased by Lockeretz and Boehncke (2000), to "do the specific research with an eye toward also contributing to the longer-range and vaguer goal of development of systems research methods". It attempts to answer, besides the specific research questions, the methodological question posed by them, too: "How can we put the concept of systemism to work in this particular research?".

Reduction is relative. A system is always embedded in some larger system (Churchman, 1979; Bawden, 1991). This aspect of hierarchy in systems theory has also been renounced (e.g., Bookchin, 1982; Capra, 1995), but as Bunge (1985) reminds us, this plays on the ambiguity of the term "hierarchy". In systems theory, hierarchy only denotes a system composed of units subdivided into smaller units, with no dimension of power or value. Agriculture can be considered as involving numerous organizational levels, the understanding and management of any of which requires an understanding of processes on other organizational levels, especially the nearest ones (Bunge, 1985). This sets a requirement for continuous feedback between the different organizational levels in research, or a repeating process of analysis and synthesis, reduction and integration.

Interpreting research as a human activity system and recognizing researcher as an actor in it, viewing the natural scientific basis of agriculture as an agroecosys-

tem, and recognizing the role of the interrelationships of the system levels and subsystems for the character of the system, all present special challenges for research irrespective of the system level. Emphasis is placed on awareness of the developmental goals and function of the suprasystems (Wilson, 1984), the choice of systemic management strategy, and the prioritization and formulation of the research questions in this context. This is the value-bound, human, "soft" dimension of the systems approach which pertains as much to research focusing on lower organizational levels as to research on higher levels. Additionally, the complex and contextual nature of the systems investigated sets requirements on the methodology (Spedding, 1979).

In this study, these challenges were reflected in the choice of the sustainability discourse and thus of the target system for the phosphorus (P) management, of AM utilization in P management as the research object, of conceptualization of the object, of the utilization strategy, of the research methods and of the structure of this presentation (Figures 1 and 3). These choices and their influence are discussed in 3.1 Methodological considerations. The presentation proceeds from the target suprasystem towards the subsystem focused on and finally links the conclusions to the suprasystem of interest.

1.2 Sustainability and phosphorus

Sustainability discourses

The concept of sustainable development, introduced to common awareness by the Brundtland Committee (WCED, 1987), has been defined in numerous ways in various discourses. Burkhardt (1989), in agreement with the definition by WCED, showed that the legitimation for the goal of sustainability cannot be in a utilitarian orientation but only in the moral obligation to respect rights. Sustainability as a concept includes both a normative vision of desirable characteristics of a target system to be sustained, and the requirement that it can be sustained. The former aspect is the primary one (Thompson, 1992).

According to Douglass (1984), the different perspectives on sustainable agriculture are (1) food sufficiency, (2) conservation of resources (stewardship) and (3) encouragement of certain virtues and vitality of local communities (community orientation). Between the first two perspectives, the difference is over means, not ends, while the third one extends the concept to other goals (democracy, community and care) and thus includes social and cultural aspects (Burkhardt, 1989). The

last perspective sets constraints on the means employed for the goals of the second perspective, i.e. for sustaining the resource base for food production such as sparing nonrenewable resources and biodiversity and avoiding environmental hazards. In accordance with the last perspective, Thompson (1997) emphasizes functional integrity, i.e. consideration of the interactions of production practices with processes of renewal, avoidance of vulnerability and conservation of the capacity for resilience, all of them including both ecological and social dimensions. This systemic, community-oriented discourse on sustainability prefers means that are governed by the local community and thus empower it. This is the discourse on sustainability of organic agriculture (Burkhardt, 1989; Thompson, 1997; Alrøe and Kristensen, 1998; IFOAM, 1998).

P management

P management is one of the main keys to the development of ecologically sustainable food systems. Economically exploitable P deposits are a finite resource. Their utilization causes local environmental hazards and contributes to the depletion of fossil energy and thus to air pollution and climate change. Reliance on these resources also contributes to dispowerment of local rural communities. Furthermore, the P flow as fertilizers and feed to agriculture, and further either directly or through food consumption to waters, contributes to the devastation of aquatic ecosystems. P is the limiting factor for eutrophication in many aquatic systems (Fischer et al., 1995), including most temperate fresh waters such as the Finnish lakes. Together with nitrogen (N) it also is a minimum factor for eutrophication in the seas, including the Baltic Sea (Rekolainen et al., 1995). The current P input into seas is up to three-fold the input during the pre-agricultural period (Howarth et al., 1995). In public discussion this is considered to be a problematic situation.

In Finnish agriculture about 20 kg P ha a^{-1} is applied in fertilizers, but only 5 kg of this passes into the food produced (Granstedt, 1995). In the Nordic countries, 10-20% of the P uptake by crops ends up in sewage sludge, while a similar proportion finds its way to slaughter offal (Granstedt and Westberg, 1993; Peltola et al., 1995). Most of the P loss from the food system, or 60% of P in fertilizers and feeds, consists, however, of the P fixed to cultivated soils and leached from fields to surface waters. The P emissions from point sources have been notably reduced in Europe through control measures, but there has been no similar reduction in emissions from diffuse sources such as agriculture (Kauppi,1984; Rekolainen, 1989; Rekolainen et al., 1995). Thus, municipalities account for only 5% of the anthropogenic P loading. Looked at in another way, although only 7% of the land area of Finland is

cultivated, approximately 60% of the anthropogenic P loading (3300 t a^{-1}) or 50% of the corresponding algae-available loading originates from agriculture. Further, more than 90% of it (3000 t a^{-1}) originates from the field soils (Rekolainen, 1993; Ministry of the Environment, 1998). Erosion, surface runoff and transient flooding are the major mechanisms for P losses from field soils. The labile P and secondary P bound to Fe and Al oxides contribute to the loss of dissolved reactive P, which is immediately available for algal growth (Ekholm, 1994). Soil content of extractable P is the main factor affecting P losses from field soils, both in eroded particles and in dissolved form (Ekholm et al., 1999).

P plays a fundamental role in enzymatic reactions that depend on phosphorylation and thus it is essential for cell division and plant growth and development. Several to several tens of kilograms P ha^{-1} a^{-1} are taken up by crops in Northern Europe. P is present in soil solution only in trace amounts as inorganic ions that are immediately available for crops. Organic P only becomes plant-available through mineralization, and P in calcium phosphate minerals (most of soil P) only through slow weathering. Finnish cultivated soils tend to be naturally poor in plant-available P (Hartikainen, 1979), and use of P fertilizers was widely embraced to increase the productivity, in the 1950s. In Finland's fairly acid soils, a substantial amount of the added P accumulated in the soil in the form of Al and Fe phosphates (Hartikainen, 1991). The average field soil content of plant-available P extracted by acid ammonium acetate increased 2.2 times during 40 years, from 5.4 mg l^{-1} in 1955-1960 to 12.4 mg l^{-1} in 1991-1995. This change was accelerated by a simultaneous increase in soil pH (Mäntylahti, 1996). No further increase of P content has been observed since.

Sustainable P management requires changes at several system levels. A decrease in the P flow to agriculture in fertilizers and feeds would reduce losses and spare resources for most effective use. Redirecting the flow to regions where soil P is lowest and P effectiveness is highest in terms of low losses, high yield effect and high need for additional food supply would improve the situation. Organizational changes would be required for the necessary internal integration within the agriculture and food systems. Enhanced recycling of P from animal husbandry and human consumption to plant production would close P cycles reducing inputs and losses. By way of example, the proportion of easily recycled slaughter offal alone is equivalent to nearly half the P in the food we consume (Granstedt and Westberg, 1993). Nevertheless, the greatest potential for reducing P inputs to the food system, P losses from the system and P pollution of aquatic systems lies in a more efficient utilization by crops of the P in soil and in recycled organic matter. There are opportunities for this efficient utilization through

the biologically-mediated soil processes managed by every farmer. The key subsystem is arbuscular mycorrhiza.

1.3 Utilization of P by arbuscular mycorrhiza

Arbuscular mycorrhizal symbiosis

Arbuscular mycorrhiza (AM) is a system with a delicate balance between plant, fungus and soil (Mosse, 1986). It involves a symbiosis between plant and fungus, the mutualism of which is determined by the soil and other environmental factors. The fungal partner is an obligatory symbiont. It receives the carbon (C) for its metabolism and structures from the host plant, distributes it to the soil by biomass production and transports nutrients from the soil to the plant, benefitting the plant and the soil in several ways (see below). According to fossil evidence, the fungi and their host plants have co-evolved for 350 to 450 million years (Pirozynski and Malloch, 1975). AM is now formed by 80% of all plant species, including most agricultural crops (Trappe, 1987), and by approximately 150 species of soil-borne fungi belonging to six fungal genera (*Glomus, Sclerocystis, Acaulospora, Entrophospora, Gigaspora* and *Scutellospora*) of the single order Glomales within the Zygomycetes (Morton and Benny, 1990). These fungi are ubiquitous in terrestrial soils. Among the herbaceous plants only Brassicaceae, Chenopodiaceae, Urticaceae, Caryophyllaceae, Juncaceae, Cyperaceae and *Lupinus* (belonging to otherwise highly mycotrophic Leguminosae) almost consistently do not form mycorrhiza (Harley and Smith, 1983). The ability to utilize AM, i.e. mycorrhizal dependence (MD), varies greatly between the plant genotypes and environments.

Arbuscular mycorrhizal fungi (AMF) are a major component of soil microbial biomass (Hamel et al., 1991). AMF hyphae form a network within the soil and between plants. This network distributes C (Francis and Read, 1984) and nutrients (Heap and Newman, 1980). AM may equalize the small-scale variation in growth factors between plants, thus raising the average level of limiting factors. It supplies energy for soil processes, extending the rhizosphere to the much larger mycorrhizosphere. AM affects the evolution of the plant and microbial communities (on evolution of communities of co-evolving organisms see Bunge, 1985; Linderman, 1988; Francis and Read, 1994) and soil nutrient status and structure (Tisdall and Oades, 1982; Tisdall, 1991) at long term (Figure 2). Direct, short-term AM influences such as pathogen antagonism (Perrin, 1990), alleviation of drought (Nelsen, 1987; Ruiz-Lozano and Azcon, 1995) and heavy metal stresses (Heggo et al., 1990),

competition against ruderals (Francis and Read, 1994) and enhancement of photosynthetic rates (Kucey and Paul, 1982) and phytohormone levels (Allen et al., 1980) are well-established. Frequently observed is an increased uptake of less mobile nutrients, especially P, but also ammonium (NH_{4+}) (Johansen et al., 1993), Cu and zinc (Zn) (e.g., Singh et al., 1986; Kothari et al., 1990), potassium (K) (Bethlenfalvay et al., 1989), calsium (Ca) (Rhodes and Gerdemann, 1978a) and sulfur (S) (Rhodes and Gerdemann, 1978b). AM also promotes symbiotic N fixation (Barea et al., 1987) even if this may be related to improved P nutrition. Enhanced uptake of P is most often responsible for the growth increase of plants due to mycorrhization (Abbott and Robson, 1984). Up to 80% of the plant P, 60% of Cu and 25% of Zn can be delivered by external AMF hyphae extending as much as 12 cm from the root surface (Li et al., 1991; Marschner and Dell, 1994).

Utilization of P by AM

Increase in plant P uptake due to mycorrhization results mainly from the increased soil volume exploited by the mycorrhizal root system. P diffusion in soil is rate limiting for P uptake (Nye, 1977). The external AMF hyphae extend to soil volumes beyond the depletion zone around the roots (Sanders and Tinker, 1971), and to smaller soil pores and closer to the surfaces of soil particles than do the roots and root hairs (O'Keefe and Sylvia, 1992). Besides that, effective P acquisition by external hyphae is related to rapid formation of polyphosphates in the hyphae which maintain a low internal concentration of inorganic phosphates (Callow et al., 1978). A greater sink effect caused by an increased conversion of inorganic to organic phosphate in the leaves of mycorrhizal plants (Allen et al., 1981) and a greater affinity of the absorbing sites for $H_2PO_4^-$ in mycorrhizal roots have also been suggested (Cress et al., 1979). A lower pH than in the bulk soil has been observed in the close vicinity of AMF hyphae (Li et al., 1991), but the evidence for AM hyphal excretion of extracellular phosphatases contributing to mineralization is contradictory (Dodd et al., 1987; Joner et al., 1995; Joner and Jakobsen, 1995; Tarafdar and Marschner, 1994a,b). Thus, there is no conclusive evidence of a specific mechanism for P uptake from sparingly available inorganic or organic P sources by AM (Bolan, 1991; Joner and Jakobsen, 1994b). In addition to the direct effects, AM affects P uptake by plants via modified transpiration rates, enhanced microbial activity (Andrade et al., 1998) by, for example P solubilizing bacteria (Linderman, 1988), and improved soil structure. AMF hyphae mechanically bind soil aggregates (Graham et al., 1982) and produce fixing mucilage (Jastrow et al., 1998).

Irrespective of the specific mechanisms, the relative contribution of AM to plant P uptake is highest where concentrations of dissolved nutrients are low (Mosse,

1973) and where only sparingly soluble inorganic or organic sources are available (Bolan et al., 1987; Joner and Jakobsen, 1994b). Bolan et al. (1987) found mycorrhization to increase the uptake of P bound to strengite, an insoluble Fe oxide, six-fold for subterranean clover and 3.5-fold for ryegrass, whereas the corresponding increase in uptake of P from the soluble potassium dihydrogen phosphate was only 1.2-fold and from colloidal Fe 1.9-fold for subterranean clover. In a moderately P-deficient soil AM increased P uptake from organic sources such as plant material by two- to six-fold depending on the added amount (Joner and Jakobsen, 1994b) and 1.6-fold from bone meal (Kahiluoto and Vestberg, 1998), which is of great importance for P recycling to agriculture. These effects are evidently due to the close proximity of AMF hyphae to sites of mineralization (Dighton, 1991) and dissolution, and instant uptake of the released P, so that its fixation is intercepted (Bolan et al., 1987; Joner and Jakobsen, 1994a).

Fungal growth and respiration consumes C from the host plant. The amount transferred to hyphae is estimated to constitute 4-20% of the photoassimilated C (Kucey and Paul, 1982; Harris et al., 1985; Jakobsen and Rosendahl, 1990). At least part of this is compensated by the increased photosynthesis due to AM formation, through the sink effect (Herold, 1980; Snellgrove et al., 1986). The actual relationship between costs and benefits depends on the properties of the AMF and plant as well as on the properties, especially growth limiting factors, of the soil (e.g., P) and environment (e.g., light). The alternative strategy for a plant to acquire P is to invest in its roots, but the C cost per unit P is lower when AMF hyphae are employed (Harley, 1975). Thus, if P is the primary growth limiting factor, it is generally more profitable for the plant to invest in AM than in root growth. Effective AM can sustain acceptable crop growth at clearly lower soil concentration of extractable P than is possible without properly functioning AM. AM exploits the released P more effectively and reduces P losses to the water system also through hindering erosion.

1.4 Systems management of AM

Management strategies

AMF are widespread in field soils also in the Nordic countries such as in Finland (Vestberg, 1995). AM effectiveness varies considerably, however. The AM effectiveness in terms of plant growth and nutrient uptake, i.e. the contribution of AM to crop growth and nutrient uptake, is the combined result of plant dependence on AM, AMF community size and structure, soil and climatic conditions, and the compatibili-

ty among these factors. The AM effectiveness in a particular plant - fungus - soil combination in certain climatic conditions varies with time, and the combination varies with both time and space in the field. The variation in AM effectiveness between field soils is due to variation in natural conditions, including the initial situation when taken into cultivation, and to variation in management history.

The predominant AM utilization strategy in AM research has been to isolate and screen AMF for mass production, in order to inoculate crop plant material regularly with effective, exogenous fungi. Controlled inoculum production of an obligatory symbiont is expensive and resource consuming, however, as it requires crop cultivation in controlled conditions. As a farming strategy, regular inoculations represent a linear approach where AMF are seen as "biological fertilizers". The strategy ignores, and to be successful needs to suppress, soil ecological interactions. The effect of inoculation in the field is difficult to predict as it depends on factors related to host, soil and climate and the cultivation practices employed. The effect of inoculation also depends crucially on the effectiveness and competitiveness of the indigenous AMF communities, and on the competitive or synergistic reactions of the whole microbial community (Sen, 1992; Allen, 1993). Menge (1983) suggested that commercial use of mycorrhizal fungi is probably economically feasible only on disturbed sites, on fumigated soils and in greenhouses. Indeed, commercial mycorrhizal inoculum production and use has not been a success story.

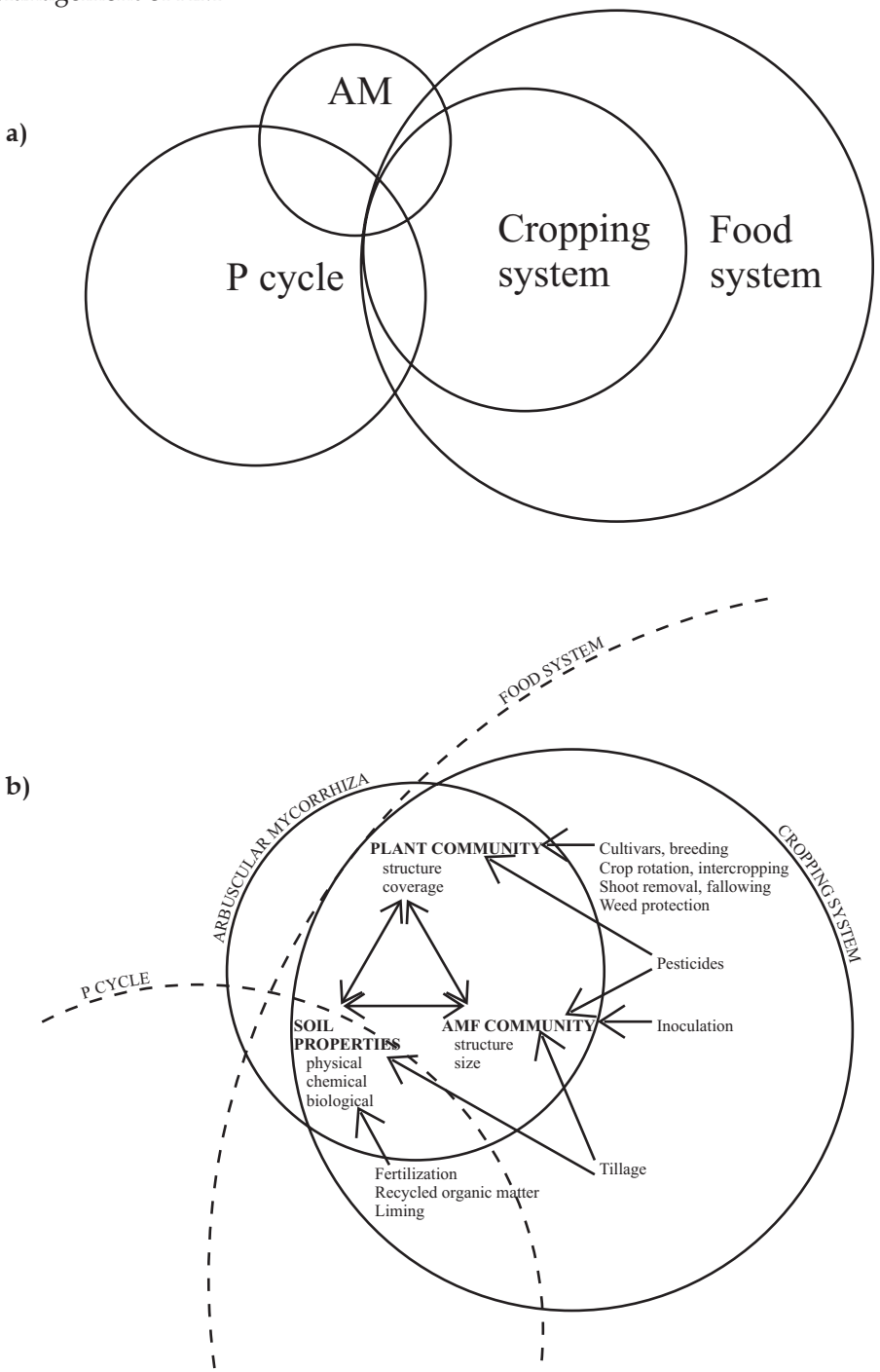
Our earlier study (Kahiluoto and Vestberg, 1998) indicated that the management history has an important influence on the variation in contribution of AM to plant growth and nutrient uptake in the field. The results suggested that regular inoculation of plant material can be of practical significance in field cropping only where the effectiveness of the indigenous AMF has been reduced through deleterious cultivation practices. Because it depends on of biologically mediated soil processes, sustainable farming strives to maintain high biological activity in the soil. This increases the probability that inoculation by exogenous organisms will be met by competitive reactions of soil that reduce the value of the inoculation strategy. Promoting instead the effectiveness of the indigenous AMF by modifying the farming system would be compatible with sustainable agriculture as a systems approach to understanding and utilizing the complex agroecological interactions (Reganold et al., 1990). This AM utilization strategy represents a paradigm only now taking form in European mycorrhizal research. It is in agreement with the goal of the community-oriented discourse of sustainability, which is to rely on local resources. Even if a more effective or more abundant AMF community were introduced, it would be necessary to manage field conditions to maintain it and favour its beneficial functions.

Systems management of AM

All partners in the tripartite system of plant, fungus and soil and their interactions are influenced by agricultural practices (Figure 1b). These influences can be utilized in order to promote reliance on AM in crop production. AMF species diversity as well as the species composition and size of the community vary considerably between field sites, as shown also in Finland (Vestberg, 1995). This is due to variation in climate, soil and vegetation, and to their history. The farming system as a whole (Douds et al., 1993, 1995), crop rotation (Dodd et al., 1990), fertilization (Gryndler and Lipavsky, 1995), liming (Wang et al., 1985) and tillage (Douds et al., 1995) have been shown to affect the species composition of AMF communities. Management practices may also affect the relative abundance of physiologically different clones, despite morphologically identical spores and thus no change in species composition (Morton, 1990). The structural features of a fungus, such as the proportions of spores, arbuscules, vesicles, internal hyphae and external hyphae, as well as its growth habit and physiology (Sanders, 1975), may be modified by imposed conditions (Johnson, 1993; Miller et al., 1994). Pesticides may have a drastic effects, not only on the size and composition of the AMF community but also on the structure of AMF individuals (Ocampo and Hayman, 1980). In addition, the tillage affects on the fungal individuals by breaking the hyphal networks or locating propagules deep in the soil by ploughing. Introduction of an exogenous fungus or an inoculum of indigenous fungi directly affects the AMF.

AM allows to be managed through modifying the plant partner. The C supply to the AMF is dependent on plant coverage and on shoot removal (Thompson, 1987; Bethlenfalvay et al., 1985) such as in fallowing, cutting and grazing. The AM dependence of host plants and their functional compatibility with the field AMF (Boyetchko and Tewari, 1994; Ravnskov and Jakobsen, 1995) are influenced by the diversity and structure of the plant community in both time and space (Dodd et al., 1990). As well as the interactions of more and less dependent and antagonistic (Fontenla et al., 1999) crops as well as the hyphal bridges transporting C and nutrients, especially from dead tissue, can all be exploited in the management of AM. There is also wide variation in the MD of temperate agricultural crop species, usually related to root morphology, root hair formation and seed P content (Baylis, 1975). Variation between cultivars has also been shown for example for wheat (Hetrick et al., 1992a,b) and barley (Boyetchko and Tewari, 1995). For wheat, there is a tendency for greater reliance on symbiosis in the older cultivated genotypes than in modern wheat cultivars (Manske, 1990; Hetrick et al., 1992a,b). Also, the response of MD to environmental gradients varies between plant species and cultivars (Hetrick et al., 1996). Breeding and choice of cultivar are thus important tools in AM management.

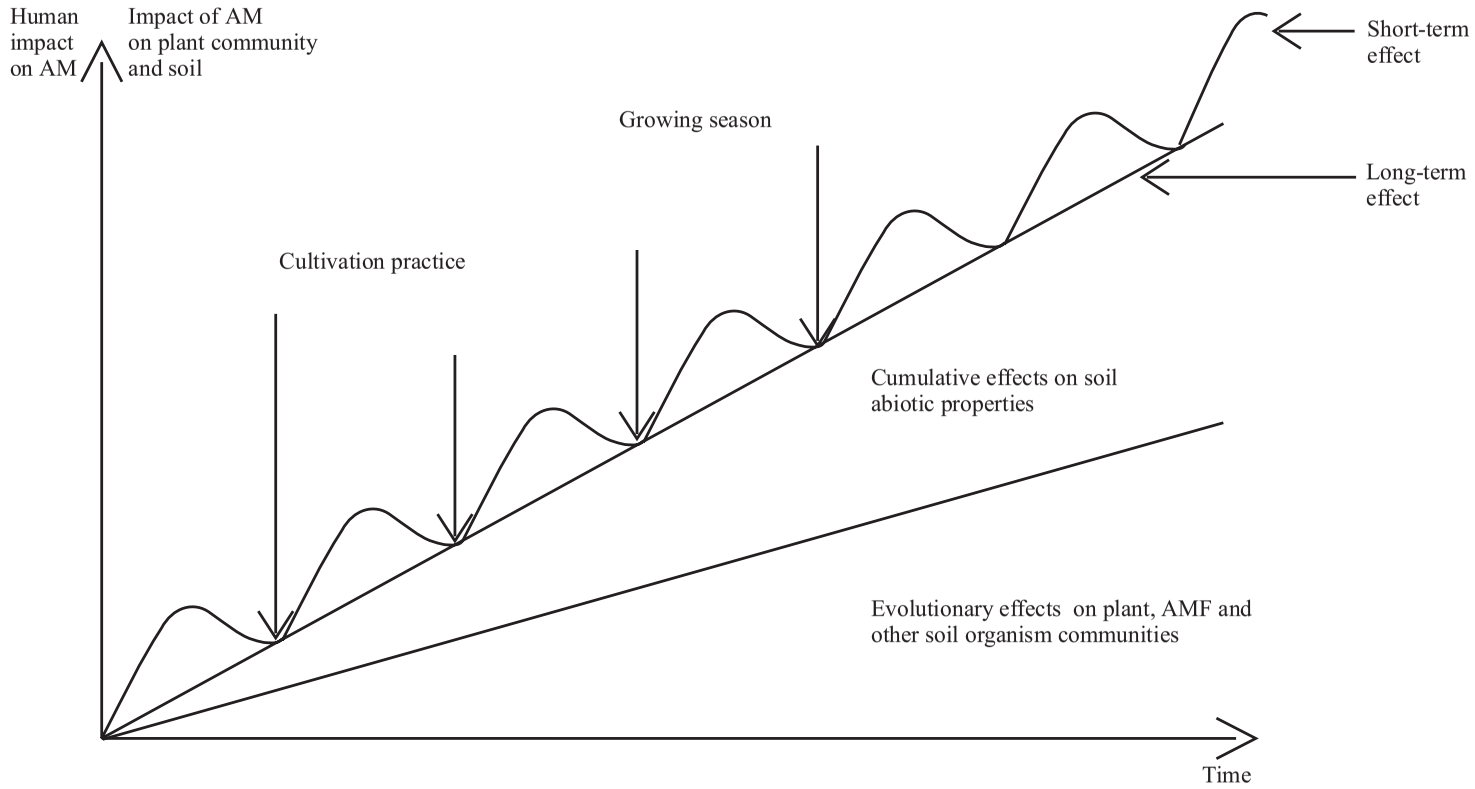
Figure 1. AM within the cropping system
a) Reference frame of the study
b) Management of AM



Soil biotic and abiotic conditions are crucial for AM formation and function. Favourable conditions can be created, for example, by modifying fertilization and organic matter content (St. John, 1983), liming (Graw, 1979; Raznikiewicz et al., 1994), irrigation (Nelsen, 1987) and aeration (Saif, 1983). The major factor affecting AM is soil P supply. Its influences on AM effectiveness are mediated through all three partners of the AM. A high soil P level not only decreases plant dependence on AM, i.e. the benefit from AM in relation to the cost to the plant. It also limits formation of AM mainly through elevated plant P concentration (Sanders, 1975; Menge et al., 1978) reducing the growth rate of infection units, the production of secondary external hyphae (Bruce et al., 1994) and spore germination (de Miranda and Harris, 1994). Higher soil P level could thus cumulatively decrease the size of the AMF community. Because of variation in sensitivity to P among AMF (Sylvia and Schenck, 1983; Thomson et al., 1986) it could also change the community structure and thus its functions and benefit to the crop. However, only a few studies have been reported on long-term effects of cumulative P fertilization on AM. Johnson (1993) and Gryndler and Lipavsky (1995) observed a negative impact of cumulative P fertilization on AMF infectivity and effectiveness together with a change of AMF species composition. Porter et al. (1978), using two replicates only, observed no response. Thingstrup et al. (1998) showed a decrease in AM infectivity and benefit on flax with increasing soil P levels in the field. The impact of P on mycorrhization also depends on the N supply (Sylvia and Neal, 1990).

Similarly to the AM effects on crop growth, also management effects on AM include both short-term and long-term effects (Figure 2). The short-term effect of a cultivation measure may be stronger (e.g., fresh P dressing) than or different (hyphal break by tillage) from the cumulative effect achieved through repetition of a cultivation measure. A notable effect on a microbial community may sometimes be achieved with a single dressing of a pesticide with a selective AMF effect (Schreiner and Bethlenfalvay, 1997), but usually repeated measures at long term are required.

Figure 2. Time perspective of the effect of cultivation practices on AM, and of the AM effect on crop production.



1.5 Research methods

Methodological choices

In research on systems management of AM for sustainability, choices are taken on the other hand in studying the impact of a management system on soil in general, and on the other hand in studying the impact on AM in particular. In regard to the impacts of the management system, the main aspects to consider are the practices studied in relation to the goal of sustainability, a relevant environment and a long perspective on the effects. The conditions prevailing before implementation of the treatments (AMF, weeds, soil), i.e. the starting point, are also of importance. The limited availability of relevant long-term experiments is the main constraint on this research approach. In regard to the assessment of the impact on AM, to assure relevance the observations should be made either in the field or in conditions as close to field conditions as possible. All the important interactions need to be considered. In drawing conclusions regarding further research needs and in guidelines for practical applications, the impact of management on other parts of the system should be taken into account as well.

The plant and soil responses to AM and the response of AM to management are difficult to predict on the basis of the existing mycorrhizal research. Most studies on AM functions have been carried out with use of sterilized soil and pure cultures of a small number of AMF taxa (Klironomos and Kendrick, 1993) with less relevant plant species and with single plants per pot. Particularly misleading is the exclusion of interactions with other soil microbes and between plants (Crush, 1976; Hetrick, 1989; Miller et al., 1994). Microbial interactions may drastically change AM function (Garbaye, 1991) as well as the competition for nutrients and the nutrient and C transport between plant individuals (Jackman and Mouat, 1972; Heap and Newman, 1980; Francis and Read, 1984). Furthermore, the AMF responsible for the effects should be identified, and more relevant indicators for AM effectiveness than colonization or hyphal length density are needed (Miller et al., 1994).

Our current poor understanding of the functioning and effects of field AMF communities is mainly attributable to methodological problems. Studies on indigenous AMF communities in field soils have concentrated on measuring the length of the colonized root and spore densities, and recently also hyphal length. However, plant and soil responses of importance for agriculture are not related solely to these variables. Hosts differ in their MD in ways not necessarily reflected in root colonization (Hetrick et al., 1992a,b) or hyphal growth, and functional host - fungus compatibility varies at similar levels of colonization and hyphal length (Ravn-

skov and Jakobsen, 1995). AMF differ in their ability to enhance plant growth and P uptake at comparable colonization levels (Mosse, 1972; Graham et al., 1982), often because of temporal differences in colonization or variation in the length of external hyphae among fungi (Graham et al., 1982; Kough and Linderman, 1986). Not even the length of external hyphae necessarily predicts the effectiveness (Abbott and Robson, 1985) because the spatial distribution and function of hyphae vary between AMF (Jakobsen et al., 1992a; Buerkert and Robson, 1994) and may respond in different ways to changed conditions (Miller et al., 1994). The species composition of an AMF community (taxonomic analysis) does not always explain the differences in effect at a similar level of hyphal length, because the clones within one morphological AMF species may vary drastically in function (Morton, 1990). Additionally, all hyphae may not be equally effective in P absorption since some hyphae seem to be specialized for absorption (Friese and Allen, 1991). The hyphal function, e.g. nutrient uptake, even of a certain AMF genotype, varies according to soil conditions, especially nutrient status. In addition, the functional efficiency of AM and of the structural parts of the fungal partner may vary in terms of nutrient inflow per carbon expenditure.

AM functioning has been investigated by comparing plant growth and nutrient uptake before and after a change of conditions or management (e.g., Evans and Miller, 1988), or following it in relation to colonization, as well as by studying the temporal coincidence of nutrient uptake and the development of colonization (Merryweather and Fitter, 1995). It is not possible, however, to determine the mycorrhizal contribution to crop response on the basis of changes in colonization levels only. Therefore, functional aspects related to nutrient uptake have been studied by spatial separation of root and hyphal compartments (Schuepp et al., 1987) by 30 to 60 μm screens, e.g. polytetrafluorethylene (PTFE) membranes which are impermeable for ions in aqueous solutions (Mäder et al., 1993). Compartmental methods are often combined with the use of isotopes (Jakobsen et al. 1992b). This approach has even been suggested for measuring AM functioning in the field (Jakobsen, 1994). Compartmental methods measure the potential of fungal hyphae to take up, translocate and transfer nutrients. However, they do not describe the total growth effect of the multifunctional AM. Neither do they describe the total AM effect on nutrient uptake, because they do not take into account the variation in the costs of symbiosis in terms of carbon expenditure, i.e. the AM effects on the growth and nutrient uptake of the whole root system. The mycorrhizal function, e.g. the hyphal P uptake, is not necessarily impaired even if the plant host does not display an increase in biomass or nutrient uptake in response to AM (Hetrick et al., 1996).

Non-mycorrhizal control

Comparison with a control with prevented or reduced AM formation or functioning is the only way to measure the total growth effect or the effect on the uptake of nutrients of the multifunctional, carbon consuming AM. The major challenge in the assessment of AM effectiveness then, is the creation of an appropriate non-mycorrhizal control, where AM is satisfactorily suppressed but otherwise no or only minor changes in soil conditions relative to the untreated field soil. With such a control, the differences in mycorrhizal effectiveness will be clearly indicated and they will describe well the relative differences in the field. The non-mycorrhizal control is most often produced by fumigation, steaming, autoclaving or irradiation. This partial sterilization of the soil destroys most of the microflora and fauna, causing a flush of decomposition of microbial cells and, to a lesser extent, decomposition of soil humus (McLaren, 1969; Powlson and Jenkinson, 1976). Thus it changing the soil organic matter and nutrient status, e.g. N and P availability (Eno and Popenoe, 1964; Jakobsen and Andersen, 1982). The changes are confounding because AM effectiveness varies greatly with soil nutrient status and soil biota (Linderman, 1988) and because the AM response to the changes depends on the AMF population, the plant and other conditions. Reinoculation of the microbes cannot be done satisfactorily either quantitatively or qualitatively.

Among methods of partial sterilization, gamma-irradiation has the least undesirable effects (Bowen and Rovira, 1961; McLaren, 1969; Thompson, 1990). It may, however, increase even soil toxins such as manganese (Mn) or copper (Cu) (McLaren, 1969; Fitter and Nichols, 1988). Irradiation doses (2.5 kGy, even 1 kGy) lower than usually recommended (10 kGy) may be sufficient to eliminate AMF infectivity while changing soil conditions considerably less (Jakobsen and Andersen, 1982; Jakobsen, 1984; Thompson, 1990). The dose requirements are dependent on the soil type, soil moisture and organism (McLaren, 1969). The side effects of sterilization vary according to soil conditions and previous management (Powlson and Jenkinson, 1976).

Fungicide treatment has been successfully used to reduce or eliminate AM activity. Fungicides seldom prevent mycorrhization completely, but their adverse effects on soil are less than those of sterilization. AM suppressing fungicides, however, may also affect other microflora, including plant pathogens (West et al., 1993). The effects may be rate-dependent (van Faassen, 1974). Further, fungicide residues may be toxic to reinoculated microbes. Fungicide comparisons commonly conclude that benomyl, or the effective compound carbendazim, is the most effective in suppressing AM (e.g., Dodd and Jeffries, 1989; West et al., 1993), but the fungicide effects may be modified by the AMF community structure (Schreiner and Bethlenfalvay, 1997). Creation of the non-mycorrhizal control with benomyl has

not always been successful, especially not in the field (Fitter, 1986; McGonigle and Fitter, 1988). Fungicides often have phytotoxic effects, as well. For a review of the usefulness of benomyl in the creation of a non-mycorrhizal control, see II.

A quite different approach to creating a control is to use isogenic myc$^-$ mutants of AM hosts (e.g., Bradbury et al., 1991; Balaji et al., 1994). Although the adverse effects on soil are avoided with these mutants, there are problems related to their AM dependence, compatibility with the indigenous AMF communities and agricultural relevance due to the limited selection of myc$^-$ mutants available. The phenotypic similarity in respects other than mycorrhization has to be tested thoroughly, also because the mutated genes may have several other functions in addition to regulating nodulation and mycorrhization (LaRue and Weeden, 1994).

Although the limitations and advantages of each method of creating the non-mycorrhizal control are known, the most promising methods have not been compared in the same study. Further, all these methods have limitations for use directly in the field. Irradiation is practically impossible in the field, large seed lots of myc$^-$ mutants are not available, and the environmental and safety risks of benomyl are greatest in field use. Benomyl is a potential human carcinogen according to EC and EPA classifications, with long persistence in soil (Torstensson and Wessen, 1984; Sinha et al., 1988). Thus there is a need for a bioassay of AM effectiveness with results representative of the situation in the field. A rapid bioassay in a growth chamber would have practical value if standardized for routine soil analyses of P availability with relevance to sustainable agriculture.

Soil sampling

Besides the creation of a non-mycorrhizal control, other important choices to be made in this kind of bioassay concern the representativeness of the soil sampling and the test plant species and cultivars. The main choices in ensuring the representativeness of soil sampling are sampling time, sampling method and size of the sampling area. Clearly, temporal variations in the amount, composition and vitality of AMF structures, in nutrient status and in other field soil characteristics will affect fungal infection, AM function and possibly also success in creation of the non-mycorrhizal control. Temporal variation in AMF infectivity has been observed (Sanders, 1993). However, little information is available on the dependence of mycorrhizal infectivity or effectiveness on season, especially in northern field soils. Use of composite samples is more practical than use of whole soil cores, and relevant for tilled soils. The maximum area for one composite sample is limited by the need to avoid mixing several, spatially separated field communities, so creating a new AMF community with new

interactions and thus new functional properties. Mixing of communities is unlikely on regularly cultivated fields (Douds and Millner, 1999).

Test plants

Issues to consider in the choice of test plant species and cultivars are the variation of MD between plant genotypes and the functional compatibility between plants and AMF communities (see above). An appropriate test plant gives a good response to AM. It is responsive over a wide range of fungal diversity and soil conditions, yet clearly indicates differences in AM effectiveness, for example between fungal communities or soil biotic or abiotic states. An appropriate test plant also predicts the relative responses of common crops of the agroecosystem concerned. The test plants should be relevant to that system and area therefore. In addition, the genetic variation between the plant individuals should be small so that an impractically large number of plants per pot or pots per treatment is not required to reveal the differences between the treatments.

1.6 Aims of the study

The effects of inoculation of exogenous AMF depend on several interacting abiotic and biotic factors. Since sustainable agriculture attempts to utilize and thus favour biologically-mediated soil processes, microbial interactions of inoculated AMF with the indigenous microbial communities are highly probable, and just as difficult to predict. Regular inoculation of plant material would thus appear to be an inappropriate strategy for utilizing AM. This was also indicated in a preliminary study (Kahiluoto and Vestberg, 1998). *The hypothesis formulated for further study, therefore, was that promoted reliance on indigenous AMF communities through management of the cropping system would have potential for the development of sustainable agriculture.* Understanding the mycorrhizal systems and their response to management is the prerequisite for their utilization and conservation. There was, however, little information about AM in non-sterile soils of managed ecosystems and few studies about the long-term effects of field management on functional aspects of AM. Mycorrhizal functioning in the Nordic climatic and edaphic conditions was particularly poorly documented. Therefore, long-term field experiments in Finland were utilized in this work.

Of all the manageable factors of the cropping system the soil P status has, besides the crop, the clearest effect on AM. At the same time the soil P status is the primary determining factor for the environmental load of Nordic agriculture, and the economically exploitable P deposits are very limited. P is also often mentioned

as the most problematic nutrient to replenish in sustainable agriculture. Therefore, and because there were long-term experiments in progress on *P fertilization* in Finland, this *was chosen as the pilot management practice to study, for testing the hypothesis* formulated above. The most prominent AM effect is enhancement of crop P nutrition which is also of most relevance to sustainable P management. This was taken, along with the total contribution to crop growth, as a focus in the description of the AM effects.

Our poor knowledge of the effects of field AMF communities and their response to management is mainly due to problems in the creation of a valid control with suppressed AM to assess the AM effectiveness in relevant conditions. The limitations and advantages of various methods of creating the control were known, but the most appropriate approach could not be chosen, because the methods had never been compared in the same study. Neither had any of them been further developed for assessing AM effectiveness. These methods are irradiation, especially by a low dose, use of fungicides such as benomyl, and use of non-mycorrhizal mutants. Because all these methods have limitations for use in the field, *there was a need for a bioassay that would be representative of the field.* In addition to the *control*, other choices requiring examination concerned the *soil sampling time* and the *test plant*.

The contribution that the systems approach, including the human, soft dimension, might make to research on low organizational levels of agroecosystems has largely been disregarded in discussions on improvement of research on sustainable agriculture. Accordingly, the choices of systemism and their influence on the research questions addressed, methods applied and conclusions drawn in this kind of research are demonstrated and discussed (3,1 Methodologial considerations). Use of methods focusing on different system levels of mycorrhiza to illustrate the impact of management on the role of mycorrhiza for plant P nutrition (Figure 3) also offered an opportunity to empirically *study the significance of the systemism* of methods *for the relevance of the results.*

The major research questions were

- Is it possible, in Nordic conditions, to promote utilization and conser vation of field AMF communities by management of the cropping sytem?
- Does P fertilization decrease the contribution of AM to plant growth and nutrient uptake, i.e. the AM effectiveness?
- How can the AM effectiveness of field soil be assessed?
- Do the conclusions in research on low organizational levels of agroecosystems depend on the systemism of the approach?

To be able to answer the first two questions it was necessary first to answer the last two. Therefore, the results are presented and discussed in opposite order to the deductive order of the questions above.

2 Materials and methods

Table 1 summarizes the experiments performed.

2.1 Long-term experiments

The impact of cumulative P fertilization on AM was studied in two long-term experiments begun in 1977. In both experiments, two P fertilization levels were utilized, 0 and 45 kg P $ha^{-1}a^{-1}$ (0P and 45P, respectively), applied in a randomized complete-block design with four blocks. The experiment with intermediate P availability was situated on loam at the North Savo Research Station of the Agricultural Research Centre of Finland at Maaninka (63°09'N 27°19´E) and the experiment with low P availability on clay at the Southwest Finland Research Station at Mietoinen (60°35'N 21°53´E). Before the establishment of the experiments, cereal had been cultivated, with fertilization including P. Both experiments involved a cereal rotation with autumn ploughing including barley and oats at Maaninka and barley, oats and spring wheat (rape in 1985 and 1992) at Mietoinen. Barley predominated on both locations, but especially at Maaninka. At the start of the experiments, the concentrations of soil dry weight for P_{total}, P_{NaHCO3} and P_{H2O} were 1.88 g kg^{-1}, 43.6 mg kg^{-1} and 11.4 mg kg^{-1} at Maaninka and 1.04 g kg^{-1}, 33.4 mg kg^{-1} and 4.6 mg kg^{-1} at Mietoinen. Between 1977 and 1988, P was given as superphosphate (9% P) and thereafter as double superphosphate (20% P). The annual rate of N application varied from 50 to 80 at Maaninka and 99 to 115 kg N ha^{-1} a^{-1} at Mietoinen. K was given at the rate of 75 kg^{-1} ha^{-1} a^{-1} at Maaninka while no K was applied at Mietoinen. The soil properties during this study are presented in paper IV: Table 1.

2.2 Method development

A method of creating a non-mycorrhizal control for assessing the AM effectiveness of field soil by a bioassay in a growth chamber was developed. The relative AM effectiveness (RME) was assessed as the relative contribution to the growth or nutrient uptake of the mycorrhizal plant. Two soil treatment experiments, six bioassays and one field experiment were performed. The development of the bioassay was based on the following criteria for an appropriate method: 1) no or only minor change in soil conditions compared to the untreated field soil, 2) satisfactory suppression of AM in the non-mycorrhizal control, and little change in AMF colonization and AM functioning of the mycorrhizal treatment as compared with untreated soil and 3) difference in mycorrhization only between the mycorrhizal and

Table 1. The major research questions and corresponding experiments

RESEARCH QUESTIONS	EXPERIMENT	SOIL	ARTICLE: Experiment	TREATMENTS
How can the AM effectiveness of field soil be assessed ?				
How to create the non-mycorrhizal control?	Method development			
- No confounding effects on soil?	- Soil treatment experiments	Mka (loam), clay	I: Exps. 1, 2	I: Table 2
- Effective AM suppression? No other effects?	- Bioassays	Mka (loam), clay	I: Exps. 3, 4	I: Table 3
- Most effective benomyl dose and application time?		Mka, Juva	II: Exp. 2	II: Table 3
- Benomyl doses and application times with no phytotoxicity?		Mka 0P	II: Exps. 3, 4	II: Table 3
Most appropriate soil sampling time?		Mka (loam), clay	II: Exp. 1	II: Table 1
Results representative of the field?	- Field experiment	Mka		
Is it possible to promote utilization and conservation of field AMF communities through management of the cropping system? Does P fertilization decrease the AM effectiveness?				
The impact of cumulative P?	Bioassays in the growth chamber			
- On AM infectivity and effectiveness in the original soil?	- Bioassay 1	Mka, Mie	IV: Bioassay 1	IV: Table 2
- On infectivity and effectiveness of AMF in standard conditions?	- Bioassay 2	Mka, Mie	IV: Bioassay 2	IV: p. 4
- On hyphal P transport capacity from a standard soil?	- ^{32}P transport from the hyphal compartment	Mka	III	III: p. 182
- On AM infectivity, effectiveness, spore density and species composition assessed in the field?	Field assays		Mka, Mie	V: Mka, Mie V: Table 1

non-mycorrhizal treatment. Furthermore, 4) the differences in mycorrhizal effectiveness should be clearly indicated and 5) describe well the differences of effectiveness in the field. Therefore two management histories with presumably different effect on AM were included, and the use of mutants and benomyl was studied also directly in the field where soil for bioassays was sampled. To increase the generality of the study, two different soil types with their indigenous AMF communities were employed.

Soil treatment experiments

To find out which soil treatment method of creating a non-mycorrhizal control least changes the nutrient and toxic element status of soil, and whether the result depends on the management history or soil type, two pot experiments were carried out in a growth chamber (I). The methods compared were the incorporation of higher and lower benomyl doses in the soil and gamma-irradiation of the soil with the recommended or a lower dose (I: Table 2). The clay soil samples were taken from adjacent fields of two farms with organic and conventional management practices, and the loam samples from the long-term experiment on P fertilization at Maaninka (above) (soil properties in I: Table 1). In each block, five subsamples were taken from the ploughed layer (0-20 cm) and combined into one sample per block. The soil was gamma-irradiated by 10 or 3 kGy in 5 cm layers, or treated with benomyl. Benomyl (Benlate®, E.I. du Pont de Nemours & Co. Inc., USA), 10 or 20 mg a.i. (kg soil at target moisture)$^{-1}$, was suspended in water and incorporated into the soil. The soil was kept open at the target moisture at 22 °C to detoxify after irradiation and to allow the benomyl to decompose. The blocks in the laboratory corresponded to the blocks in the field. The soil was sampled and analysed one month after the soil treatments.

Bioassays in the growth chamber

In two bioassays the methods of creating a non-mycorrhizal control were evaluated by criteria relevant to mycorrhization and plant response. Besides the gamma-irradiation and benomyl doses studied in the soil treatment experiments, the use of a non-mycorrhizal host plant mutant was also studied. In addition, the effect of benomyl incubation and equalizing of the non-mycorrhizal soil microbiota by sievings of untreated soil was investigated. Besides untreated soil, other mycorrhizal treatments were included, created by AMF reinoculation of the irradiated soil with untreated soil or soil sievings (I: Table 3). The bioassays were established on soil originating from the soil treatment experiments.

On the basis of the experiments, benomyl was chosen as the most appropriate

method of creating the non-mycorrhizal control. The use of benomyl was refined in five subsequent bioassays on method development. In one bioassay, the effectiveness of five benomyl doses and three benomyl application times in suppressing AM was compared. As well, the dependence of rate effect on different humus contents and indigenous AMF communities of the soil and on soil sampling time was studied (II: Table 3). Soil with no P applications from the long-term experiment on P fertilization at Maaninka, and soil from an organically managed field with a three-year-old clover grass in another location were used (soil properties at sowing in II: Table 2). The AMF communities of the two soils differed essentially.

Two additional bioassays were performed to confirm that the difference in plant response to the benomyl application (see bioassays above) between the mycorrhizal and non-mycorrhizal treatment was due only to mycorrhization. The phytotoxicity of the same five benomyl doses and same three application times was studied with four plant species in the Maaninka soil, irradiated by 10 kGy (II: Table 2).

A further bioassay examined the temporal variation in mycorrhizal infectivity (evaluated as percentage root length colonized) and effectiveness (evaluated as relative contribution of AM to plant growth) and in the effect of benomyl on AM (infectivity and effectiveness), as well as the dependence of the variation on the host plant and soil. Infectivity and effectiveness at seven sampling dates were compared with two host plants in three soils representing two soil types and climatic conditions and three different management histories (II: Table 1). Benomyl 10 mg (kg soil at target moisture)$^{-1}$ was applied immediately before sowing to create a control with suppressed AM. The loam soil originated from the long-term experiment on P fertilization at Maaninka and the clay soil from a farm with organic management. The same 2.5 m x 4 m plot of one block of the field experiment and one of the farm were sampled repeatedly (soil properties in II: Table 2).

Soil for the bioassays was sampled in the same way as in the soil treatment experiments. Fertilizers were used in an attempt to simulate the nutrient status of the sampled field soil at the start of the growing season. They were diluted in water and incorporated. The same dry weight of soil in each experiment was carefully mixed with fertilizers, inoculum and water, and with benomyl when this was used, separately for each 1 l (7x26 cm) black PVC pot without drainage. In the comparison of benomyl application times (II), one week after sowing the benomyl suspension was dressed from the top and injected through thin tubes. Water was added to 50 to 60% of water-holding capacity.

The test plant was oil-seed flax (*Linum usitatissimum* L.) cv. Linetta (Deutsche Saatveredelung, Lippstadt-Bremen GmbH). The pea mutants tested in method comparison (I) represented two phenotypically similar mutant lines, one mycor-

rhizal and one non-mycorrhizal, of an early freezer pea (*Pisum sativum* L.) cv. Sparkle (Rogers Bros. Seed Co., Twin falls, Idaho, USA). In addition to flax, barley (*Hordeum vulgare* L.) cv. Arra (Agricultural Research Centre of Finland) was used in the comparison of sampling times and barley, pea mutants and red clover (*Trifolium pratense*) cv. Bjursele were additional test plants in the phytotoxicity experiments (II). In unpublished test plant experiments, 10 northern crop plant species and several cultivars (e.g. for *Pisum sativum* mutant lines) were compared in bioassays carried out in untreated soil with benomyl-treated control and with different pot sizes, and in irradiated soil (see above) with different, inoculated AMF communities and different P levels. The seeds were pregerminated and after emergence were thinned to one (I) or three (II) seedlings per pot.

The pots were organized in the growth chamber in blocks so that the soils of the blocks originated from separate blocks in the field. Within the blocks the treatments were in random order, but in experiments with pairs of the same soil sample with and without benomyl the pots were located next to each other. The pots were watered three times a week to the target weight separately determined for each pot, and simultaneously the pots or pairs of pots were circulated. Artificial lighting was supplied by 36 W Gro-Lux Fluorescent Tubes (Sylvania, Germany) with a 16h/8h light/dark cycle. At emergence the light intensity was 80 to 100 and at the top of the harvested plants 135 to 170 $\mu mol\ s^{-1}m^{-2}$. The temperature was 24/16 or 17°C $\pm$ 0.5°C or 1.5°C depending on the experiment. The air CO_2 concentration varied from 510 to 560 ppm at noon, and the relative humidity was 55 to 65%. The experiments were harvested 28 days after sowing.

Field experiment

To clarify how representative the results obtained in the bioassay by the various methods are for the field conditions, benomyl and the pea mutants were also studied in a field experiment (I). Additionally, the potential of the mutants and benomyl for assessing mycorrhizal effectiveness directly in the field was investigated. The treatments were myc^+ and myc^- mutants of pea, barley in untreated soil, and pea myc^+ and barley with benomyl 125 mg a.i. $(kg\ soil)^{-1}$ divided into two applications. The pea mutant lines and the barley cultivar were the same as in the bioassays. The field experiment was of split-plot design with four blocks. The main plots were the two P levels of the long-term experiment at Maaninka. The methods of inhibiting AM functioning were randomized into subplots within each main plot.

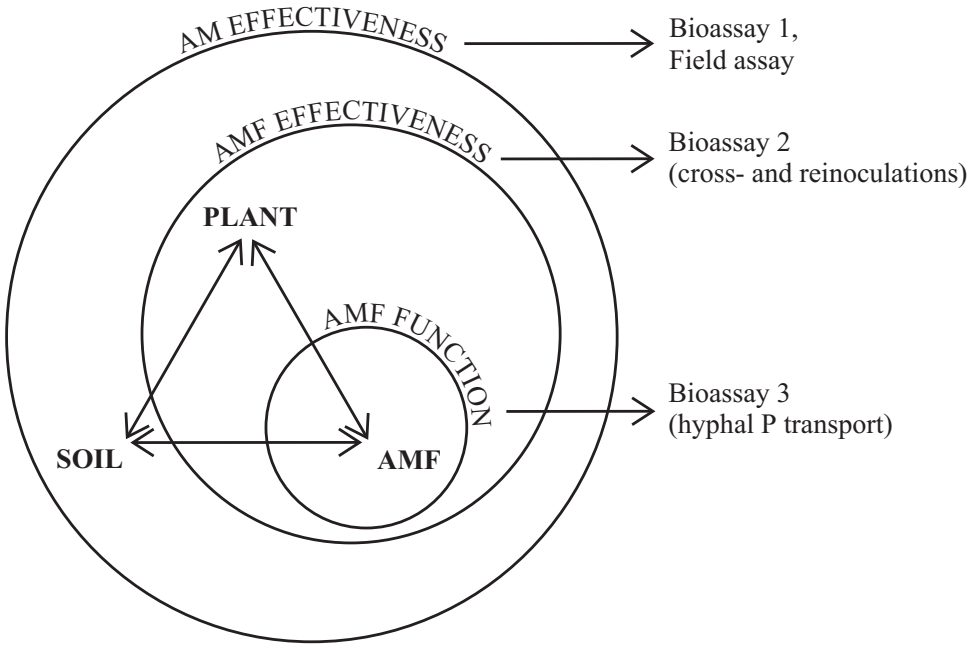
The size of the subplots with barley was 2.5 m^2 and the size of those with pea 1 m^2 consisting of one 2.5 m long pea mutant row with 8 pea plants and border rows around. The soil was moistened and benomyl was suspended in an amount of wa-

ter corresponding to the water-holding capacity of the ploughed layer and incorporated, in two applications, with a rotary hoe to a depth of 16 cm. The plots were fertilized according to the practice in the long-term experiment. Pea and barley were sampled at flowering and harvested at maturity.

2.3 Bioassays in the growth chamber

Three different bioassays were performed to test, at three different system levels of AM, the hypothesis that cumulative P fertilization reduces the AM effectiveness (Figure 3). Bioassay 1 studied the impact of P fertilization on total AM effectiveness and infectivity in field soil, and its dependence on N supply and crop. The bioassay was used to achieve valid non-mycorrhizal controls for assessing AM effectiveness, which is not always successful in the field. Bioassay 2 investigated the mode of action of cumulative P fertilization through changes in the properties of the AMF communities. The contributions to effects of the AMF communities were compared in standard conditions. Bioassay 3 clarified the impact of the cumulative P fertilization on the AMF hyphal P transport capacity. Bioassays 1 and 2 (IV) were carried out for both long-term field experiments, Maaninka and Mietoinen, while Bioassay 3 (III) was performed for the Maaninka field only.

Figure 3. System level of AM focused on in the assay



Soil samples from long-term experiments

The soil samples for bioassays in the growth chamber were taken from the long-term P fertilization experiments. For the field assay (see below), five fertilization treatments were randomized into subplots within each 0P and 45P whole plot. Because Bioassay 1 included the same treatments as the field assay, soil for the pots of the treatments was sampled from the subplots of the corresponding treatments in each block in the field. The results of the bioassays could thus be compared with the results of the field assays. For Bioassays 2 and 3, one sample per management history and block was taken from the whole plots of the 0P and 45P field treatments. For all the bioassays, five subsamples from each plot were collected from the ploughed layer (0-20 cm), and combined into one composite sample. The samples for Bioassays 1 and 2 were collected on 12 to 13 May 1997 before application of the fertilizer treatments of the field assay, and for Bioassay 3 on 11 September 1995 before ploughing. In addition, soil samples were collected from 0P and 45P plots to determine the effect of the treatments on the spore densities and species composition of the AMF community. Sampling was carried out on 14 June, 14 August and 30 October in 1995 at Maaninka, and 15 June, 18 August, and 21 October in 1998 at Mietoinen. Six subsamples per plot from each block were collected to a depth of 15 cm and pooled together to form one composite sample per block for both long-term fertilization treatments.

Bioassay 1

The impact of the two P fertilization histories of the Maaninka long-term field experiment (0P and 45P) on mycorrhizal infectivity, effectiveness and P response (Abbott and Robson, 1984) was studied with the developed bioassay (above, I, II) on flax (IV). The impact of omitting the last P application in soil with annual P dressings and the dependence of the P effect on N supply and the crop were also studied. For the Mietoinen field, only the effects of the P fertilization history and of omitting the last P dressing were investigated.

The treatments for the bioassay of the Maaninka field were as follows: 0P with three P application treatments, 0, 45 and 90 kg $ha^{-1}a^{-1}$ (0P+0P, 0P+45P, 0P+90P, respectively); a treatment with half of the moderate N dose with the annual treatment 0P+0P; and 45P with the same treatments (45P+0P, 45P+45P, 45P+90P and 45P+45P with half N dose). For the bioassay of the Mietoinen field the treatments were only 0P+0P, 45P+45P, 45P+0P. In addition to flax, red clover was included in the bioassays for both the Maaninka and Mietoinen fields, and barley was included in the bioassay for the Mietoinen field because the soil P

availability there was lower than at Maaninka. For every treatment there was a control with AM suppressed by benomyl.

Bioassay 1 was performed similarly to the bioassays with pairs of pots with and without benomyl described in Method development (II), with the following exceptions. N and K were diluted in water and applied as a drench. For clover no N was used. Superphosphate was mixed dry with a small amount of dry field soil and incorporated. The mean soil contents of P_{H2O} for all the treatments are presented in IV. Red clover (*Trifolium pratense* L.) cv. Jokioinen (Agricultural Research Centre of Finland) and barley (*Hordeum vulgare* L.) cv. Artturi (Boreal) were used. The temperature in the growth chamber was 24/16°C ± 3°C.

Bioassay 2

The difference in the infectivity and effectiveness of the AMF communities with the two P fertilization histories at Maaninka and Mietoinen and the dependence of this difference on the soil conditions were investigated in two cross-inoculation experiments, one for each field (IV). The method had been tested previously (I).

10% of the sample of untreated soil was stored at 5°C to be used as the microbial inoculum, and the rest of the sample was irradiated by 10 kGy. Besides irradiated soil (0P and 45P) there were three other factors in the experiment: inoculum soil (0P and 45P), inoculum amount (5 and 10%) and plant (flax and clover, see Bioassay 1). In the bioassay for Mietoinen, inoculum amount was not a factor and 5% was used. The difference in the effect of the inocula from the 0P and 45P soils on growth and nutrient uptake was interpreted as a difference in the effect of AMF. Untreated 0P and 45P soils with their AMF communities were reinoculated to both of the irradiated 0P and 45P soils in the amount of 5 or 10% w/w (dry weight) soil. Thus there were 16 irradiated soil by inoculum soil by inoculum amount by test plant combinations. The experiment was performed like the other bioassays but, because of the nutrient flush by partial sterilization, with no fertilization. The soil nutrient contents are given in IV.

Bioassay 3

The impact of the cumulative P fertilization on the P transport capacity of AMF hyphal length unit was assessed in Bioassay 3 (III). The soil samples were air-dried, sieved and stored until use in 1997. Pots were 1.5 l in volume with two compartments. The main compartment was for the root system including AMF. The other compartment was separated by a mesh and only the hyphae had access there (III: Figure 1). The main compartment contained untreated field soil from one of the P re-

gimes, while the hyphal compartment was filled with low-P soil irradiated by 10 kGy and mixed with carrier-free * HEX ^{32}P. Aqueous nutrient solution not containing P was mixed with all soils. The pots were used to measure the growth and P uptake of mycorrhizal plants and hyphal ^{32}P transport by the field communities of AMF. The contribution of AMF to plant growth and P uptake was measured, creating the control with benomyl as in Bioassay 1. The bioassay was performed similarly to Bioassays 1 and 2 with the following exceptions. The pots were placed in a completely randomized order in a growth chamber at 22°C/18°C and at a light intensity of 500-550 μmol m^{-2} s^{-1} PAR. N in aqueous solution was supplied to each pot during the growth period as required. The pots with no benomyl were harvested 23, 27 and 32 days after sowing, and the pots with benomyl 27 days after sowing.

2.4 Field assays

Even though the bioassays evidently show the relative impact of contrasting cropping histories on AM effectiveness (I), the conditions in a growth chamber assay are not exactly the same as in the field. Therefore, one field assay within each long-term field experiment (Maaninka and Mietoinen) was performed to clarify the practical significance of the impact of cumulative P fertilization on AM in northern field conditions (V).

The field assays were established in 1997 within the long-term field experiments, one for the Maaninka and one for the Mietoinen field. The impact on the same response variables and their dependence on the same interacting factors as in Bioassay 1 was studied on flax. Five fertilization treatments were randomized into subplots within each 0P and 45P whole plot. The treatments for both fields were the same as in Bioassay 1 for Maaninka, except that the additional test plant besides flax (red clover at Maaninka and barley at Mietoinen) was used only for the annual treatments 0P+0P and 45P+45P. The two subsubplot treatments with and without benomyl 125 mg a.i. (kg soil in the 20 cm deep plow layer)$^{-1}$ were randomized within the subplots. The full dose of N was at Maaninka 80 and at Mietoinen 109 kg ha^{-1}. For clover, no N was added.

The sizes of the subsubplots were 2.50 m^2 (1.23 m^2 harvested) for flax and barley and 3.50 m^2 (1.93 m^2 harvested) for red clover. Benomyl was incorporated as in the field experiment described in Method development above, but less water was used, and at Maaninka benomyl was added in one application (V). The soil nutrient contents of the treatments are presented in paper V. The crops were cultivated according to normal agricultural practices. The roots were sampled twice for assessment of AMF colonization and the shoots once before the har-

vest. In 1999, the experiment for barley at Mietoinen was repeated in the same plots because the harvested seed lots of 1997 were lost.

2.5 Analyses

Soil pH was determined by 0.01 M $CaCl_2$ extraction (Ryti, 1965). Plant-available soil P was determined by water extraction (van der Paauw, 1971) because water-extractable P well illustrates the P status of Finnish mineral soils (Hartikainen, 1982) and by sodium bicarbonate extraction (Olsen et al., 1954) to allow comparison with other European results. Total P content of soil was determined by wet digestion with concentrated H_2SO_4, H_2O_2 and HF, and the exchangeable cations by 1 M ammonium acetate extraction (Thomas, 1982), and analysed by inductively coupled plasma spectrometry (ICP). The soil mineral N content was determined from frozen (-18°C) samples by 2M KCl extraction and by measuring N_{NH4+} and N_{NO3-} concentrations colorimetrically with a Skalar autoanalyser (Linden, 1981, Keeney and Nelson, 1982). Aluminium (Al), Cu, iron (Fe) and Mn were extracted with acid ammonium acetate - ethylenediaminetetracetic acid solution (AAAc - EDTA) (Lakanen and Erviö, 1971) and determined by ICP.

The effects of soil treatments on microbial activity were not only studied through changes in soil nutrient status (I). In the field experiment, the effect of benomyl and AM formation of pea mutants on decomposition was studied by the mesh bag method (Berg et al., 1987, Müller, 1987). 1 to 1.1 g air-dry clover leaves (without petioles) and barley straw of a known moisture content were weighed into polyester bags (mesh size 0.2x0.2 mm, size of bags 5x5cm), which were placed in the soil in an upright position, the top at 5 cm below the soil surface and with 2 to 3 cm between bags. Because of the scarcity of bags, the clover leaf bags were set only in the soil of the lower P level. One clover leaf bag per plot was dug up one and two months later, and one clover leaf bag and one barley straw bag were dug up at harvest. The bags were gently washed and dried at 60 °C overnight. The decrease in dry weight was measured.

The spore densities were determined by centrifugation and sugar flotation (Väre et al., 1992) and spores were identified. Diversity indexes were not calculated owing to the relatively high proportion of unidentifiable spores (V). A representative sample of the root system was cleared with KOH (Kormanik and McGraw, 1982) and stained with methyl blue (Grace and Stribley, 1991) and the percentage of colonized root length was determined by the gridline intersect method (Giovannetti and Mosse, 1980). Hyphal length was determined from samples taken 5 to 6 cm below the soil surface in the pots (Jakobsen et al., 1992a) (II, III).

The shoots and roots were cleaned and dried at 60 ºC. Root dry weight was not

determined in the bioassays of studies II and IV because of the lack of consistent differences in root growth or P uptake response to AM in method comparisons (I, II). Plant P, K, Ca, Mg, Cu, Zn and S contents were determined by wet burning and ICP by ARL 3580 OES (Huang and Schulte, 1985). For study III, root and shoot P concentrations were determined in the diluted digests by the molybdate blue method (Murphy and Riley, 1962). Shoot N content was measured by the Dumas method using a Leco® FP-428 protein analyser at 950 °C with a high flow profile. Dried plant materials were digested in a solution of nitric/perchloric acid (4:1, v/v). The diluted digest was analysed for ^{32}P content with a Packard TR1900 liquid scintillation counter using the Cerenkov analysis. The inflow of ^{32}P through the hyphae to the plants was estimated as an overall mean of both P levels by using the equation of Nye and Tinker (1977): $Inflow_{1\text{-}2} = (^{32}P_2\text{-}^{32}P_1) \times (\ln H_2\text{-}\ln H_1) \times (t_2\text{-}t_1)^{-1} \times (H_2\text{-}H_1)^{-1}$ where ^{32}P is content of ^{32}P in plants, H is hyphal length in labelled soil, t is time of harvest, and subscript numbers refer to the two different times of harvest.

The relative mycorrhizal effectiveness (RME) is presented as the mycorrhizal contribution to the growth (or nutrient uptake) of the mycorrhizal plant and defined by the following formula: RME (%) = $[(Y^{myc+} - Y^{myc-}) / (Y^{myc+})] \times 100$ where Y^{myc+} and Y^{myc-} are the dry weights (or nutrient uptake rates) of the mycorrhizal treatment and the control with inhibited AM functioning, respectively. The absolute contribution of AM is, correspondingly, the difference in growth or nutrient uptake between the mycorrhizal treatment and the control with inhibited mycorrhiza.

2.6 Statistical methods

The statistical analyses of nutrient concentrations, uptake rates and dry weights, as well as the analyses of mycorrhizal effectiveness and decomposition, were based on common linear mixed models (Littell et al., 1996). All models were fitted using the residual maximum likelihood (REML) estimation method. The distributional assumptions of the models, normality of the response variable and variance homogeneity across groups were checked by graphic plots. Planned comparisons between means were made by two-sided t-type tests or 95% confidence intervals, except in the field assays, where 90% confidence intervals were used.

Generalized linear mixed models (Littell et al., 1996) were used in the statistical analyses of the number of infected roots and spore densities; in accordance with their names, these models are based on more general assumptions than linear mixed models. The response variables, for instance, do not need to be normally distributed but other probability distributions can be used. Furthermore, in linear mixed models M_i, the expected value of the observations y_i is assumed to be a line-

ar function of the explanatory variables but in generalized linear mixed models some function of M_i, $g(M_i)$, is assumed to be linearly related to the explanatory variables. The function g is called a link function. In analysing the number of infected roots, we assumed that the response was binomially distributed and that the logit link function was adequate. In the analyses of the spore densities, a Poisson distribution and identity link function were used. Accordances of the data with the models were checked by graphic plots. Comparisons between groups were made by two-sided t-type tests. Some data included discrepant observations whose discrepancy could not be explained and their influence on the results was examined by analysing the data with and without them. The analyses were performed using SAS software (Littell et al., 1996). The statistical methods used in III are specified in III: Tables 2 and 3.

3 Results and discussion

3.1 Methodological considerations

The effort to relevance for sustainability with a community orientation (Douglass, 1984; Thompson, 1992, 1997; Alrøe and Kristensen, 1998; IFOAM, 1998, see 1.2) is reflected in this study by an attempt to view the food, agricultural and natural resource situations as if they were systems, and finally focus on subsystems, not the other way around (Wilson and Morren, 1990). Characteristics of the soft systems approach (see 1.1) thus appear in the explicit choice of the community-oriented, systemic perspective of sustainability as the starting point, in the description of the problematic situation and the improved human activity system within this reference frame (in 1.2), in the prioritization (in 1.2) and conceptualization (in 1.3, discussed below) of the research object, and in the choice of a systems management strategy (in 1.4, discussed below). These choices were further reflected in the choice of research methods (in 1.5, discussed below). No particular systems methodology (e.g., Spedding, 1979; Checkland, 1981; Bawden et al., 1985; Conway, 1985) was implemented, however. A more participatory and interdisciplinary approach would be both possible and useful for embracing multiple perspectives in perceiving the problem situation as well as in research for management of AM. In this section, methodological choices of this work, and their impact, are described and discussed. The focus is in the choices related to systemism.

The discourse of sustainability relied on determines the strategy to be employed for management of AM (see 1.2): an inoculation strategy resting on non-renewable resources, exogenous biological material and remote expertise for inoculum production in growth chambers and greenhouses is applied, or a systems management strategy which relies on local ecosystem services such as field AMF communities which was the strategy of this work (see 1.4). Choices of ultimate goals of management of AM concern the maximizing of yield, maximizing the efficiency of P in fertilizers while compromising or not compromising the yield per unit field area (Gianinazzi-Pearson, 1986; Mosse, 1986; Abbott and Robson, 1991b; Miller et al., 1994), or maximizing the reliance on AM such as here, and on P in soil and recycled organic matter. Minimum P loss to waters could be sought for within the constraints of each of these choices. Yet an other goal could be to maximize profit within given economic constraints (Miller et al., 1994). The choice of major importance is, whether to accept substitution of finite and imported resources such as P fertilizers, the use of which loads the environment, for a local resource, field area. These choices determine, whether it is preferable to maximize RME, i.e. relative contribution of AM needed to maximize the reliance on AM, or to maximize the absolute contribution of AM to plant growth (compare III, IV, V). The impacts of management on the relative and absolute contribution of AM to nutrient uptake and growth were similar in this study, if the field conditions were simulated (IV, V), but not in novel conditions (III, see below). All the above choices affect the conclusions on the management options.

A conceptual framework for management of AM in the field by inoculation with selected AMF or by choice of agricultural practice was discussed by Abbott and Robson (1991b), who set a goal of maximizing mycorrhization (see also Smith et al., 1992). Miller et al. (1994) further discussed use of soil and crop management practices as an alternative strategy to inoculations, to promote AM effectiveness in terms of plant growth. Their approach represents a notable step towards a more systemic approach for they focus on and utilize interrelations between plant, soil and fungus. Nevertheless, AMF and not the interactions of the three partners (plant, fungus, soil) was taken as the object of management. The same applies to the work of Johnson (1993) and Johnson et al. (1992a,b). Conceptual extension was needed. Besides the changes obtainable in AMF individuals and the AMF community, the changes in the effects of the unchanged fungal structures deserve attention. Changes in these effects can be imposed by modifying soil conditions (e.g. soil P status and P sources), or by modifying plant-genotypic AM dependence and characteristics of the plant community. This expanded focus embraces the interrelations both between the three partners and between the hierarchical levels and

scales of the plant and soil communities, farm (e.g. grazing animals) and food system (e.g. recycling of organic matter) as the management object. This allows a wider selection of modes of action for implementation (see 1.4 and Figure 1). Attempts towards a systems approach was presented also by Johnson et al. (1997).

The results of our study emphasize the need for this kind of conceptual enhancement. They indicate that the impact of management on the contribution of AM to crop growth or nutrient uptake is often more due to a change in the conditions in which the AMF individual or AMF community functions than to a change in the size or structure of AMF individuals or the AMF community (III, IV, V, Kahiluoto and Vestberg, 1999). Thus, approaching mycorrhiza as a system with a delicate balance between plant, fungus and soil instead of focusing on AMF as the management object, crucially affected the results.

In the context of sustainability, in a study on the management effects on AM it is necessary to include the long-term impact of a repeated cultivation practice. Moreover, the long-term and short-term impacts need to be distinguished (see 1.3, Figure 2). This was emphasized by the qualitative difference in the effect of a single P application and the cumulative effect of the annual applications (IV: Figures 1b,d and 2b,d, Table 5; V: Figures 1 to 4, b,d, Table 5). The difference was even more dramatic in a cropping system comparison, where the preceding-crop effect could overwhelm the long-term effect of a system (Kahiluoto and Vestberg, 1999). Both cumulative effects through abiotic soil properties on AM (IV: Figures 1b,d and 2b,d compared with Table 7; V: Figures 1 to 4, b,d, compared with Table 7), and micro-evolutionary effects on AMF (IV: Table 7) by P fertilization were shown. However, the same species composition of the AMF community seemed to dominate over the whole long-term experimental field at both sites, with no clear response to the P histories (V: Table 7). This was also found elsewhere (Kahiluoto and Vestberg, 1999). There remains some uncertainty, however, as to the validity of the latter finding due to the practical problems of preventing the distribution of the AMF propagules to other experimental plots by hyphal growth, surface waters, soil (Sibbesen et al., 1995) and machines and on the feet of people and animals. Use of large plots spatially separated from each other, the construction of permanent barriers between plots, and cleaning of machines between the treatments would reduce the problem. The problem could be avoided in a comparison of a farm pair with adjacent fields with contrasting management systems and separated by a ditch. The impact of long-term management on the AMF community needs to be confirmed using such an approach (Reganold et al., 1987; Kahiluoto and Vestberg, 2000).

Because of the difficulties of creating a valid and relevant non-mycorrhizal control directly in the field (Fitter, 1986; McGonigle and Fitter, 1988; Thingstrup et al.,

1998), relative AM effectiveness (RME) was assessed with of a bioassay in a growth chamber. Both the bioassay (I, II, IV) and the field assay (I, V) have one notable limitation, however. RME estimated in a bioassay or within one or a few growing seasons in the field indicates an acute, crop-related AM effectiveness of the soil studied. Thus, the effect of AM on crop growth and nutrient uptake through changes in soil factors caused by AM at long term is excluded in this kind of assessment (see 1.3, Figure 2). The indirect AM effects through soil could be included only with a long-term control in the field, the creation of which is impracticable. Neither are the environmental influences of AM directly covered. The bioassay that was developed simulates arable cropping more successfully than long-term grasslands because the conditions of infection with no established, overwintering hyphal networks (Addy et al., 1994) are more relevant to this. This bioassay represents a higher system level (soil-plant system) and yields results more relevant to plant nutrient availability than chemical extractions, which exclude the biologically mediated processes. The difference in system level focused on, between the two approaches, is analogous to the difference between the process-focused assessment of decomposition of plant remains in this study as a relevant indicator for soil biological activity (see 3.3 Conceptual model of the impact of P on AM, *Time perspective*) and, for example, assessment of enzyme activities or soil respiration. Because of its holistic tendency (Bunge, 1985), the bioassay approach still requires a well-planned strategy for problem determination and operationalization.

Choices related to the degree of systemism also affect the relevance of the bioassay results. As an example, in AM research nutrients other than P are commonly supplied in excess, in an attempt to determine the cause - effect relation of the P supply only. This reductionist approach disregards essential interactions. In our work, results were different when N was and was not applied in excess dose (III: Table 1 compared with I: Figures 3c, 4a and 5a; II: Figure 1b; IV: Figures 1a,b and 2 a,b). When the nutrient status in the Maaninka field was simulated in the bioassay (I, II, IV), both the relative (RME) and the absolute contribution of AM to growth and nutrient uptake was noticeably lower in soil with cumulative P than in soil with no P applications. In contrast, when N was in excess, no difference in absolute contribution of AM to P uptake between the histories was observed. The reason is that in P-fertilized soil with no limiting P notably faster growth was achieved when N was in excess than when N was limiting such as it was in the field. This means that P uptake was increased when N was in excess and so too the absolute contribution of AM to P uptake. In addition, N supply interacts with the impact of P on mycorrhization (V, Sylvia and Neal, 1990) and therefore may interact with the impact of P on AM effectiveness, too. The results obtained with excess of other nu-

trients do not correspond to the field situation, especially not in sustainable agriculture. As an alternative to field simulation, an abundance of other nutrients could be included as an additional factor to bioassays such as for N in experiments reported in papers IV and V. Another example of choices related to systemism in the bioassays is, whether the soil is used fresh or as air-dried, sieved and stored according to a commonly applied practice (compare III to I, II, IV). This may affect the AMF community and its functions as well as the interacting soil conditions, and thus the conclusions. Johnson et al. (1999), too, emphasize the importance of mimicking the field conditions in bioassays assessing AM.

The dominating approach to elucidating underlying mechanisms is to break down a complex system and focus on the key part in standardized conditions. This approach is demonstrated in the current practice of assaying the hyphal P or N transport from the ^{32}P- or ^{15}N-labelled hyphal compartment (see 1.5). The assay is valuable in describing one possible mode of action of a management on AM: the impact on the potential of a hyphal length unit of the AMF community to take up and transport nutrients from soil with a standard nutrient status (Figure 4). If the plants grow in the untreated field soil with the management studied (III), then the impact of the management on total nutrient transport potential of the AMF communities from standard soil can be determined. However, conclusions drawn on the basis of this assay about the contribution of AM to the P or N uptake by crops, even for the experimental conditions, are misleading (compare e.g. Schweiger and Jakobsen, 1999; Mäder et al., 2000). Essential interactions, such as the C costs of AM which decrease nutrient uptake by roots, are here ignored. High share of hyphal P uptake does not necessarily mean that AM increases plant P uptake, because AM may be a more costly strategy than uptake by roots for a plant with an abundant nutrient supply (V). The relation of costs and beneficial functions may also vary among AMF communities (e.g., Johnson, 1993) and with other conditions. Variation in C costs often determines the difference in AM effect more than does variation in the function (Raju et al., 1990).

Hyphal assay did not reveal any clear difference between the P histories in hyphal P transport capacity, but there were notable differences in the contribution of AM to P uptake (higher organization level) in field soil (IV: Figures 1a,b and 2a,b, Table 4; V: Figures 2, 4, Table 4). This result shows how important it is to relate the conclusions to the system level considered (Figure 3), and to include all the essential interactions on the level where the results are applied. Further, the above examples emphasize that the suprasystem of interest determines the approach appropriate to obtaining relevant results in research on the subsystems.

3.2 Bioassay of AM effectiveness

Treatment with benomyl was the most appropriate method for creating the non-mycorrhizal control for assessing AM effectiveness of field soils (I). Representative results were obtained with use of a standard test plant and standard soil sampling time. In contrast to the minor changes in N supply caused by benomyl, irrespective of the dose, irradiation increased the N supply manifold due to decomposition flush (I: Figure 1b,c). In clay with high organic matter content, the increase caused by the common dose of 10 kGy was considerably higher than the increase with the low dose of 3 kGy. The 10 kGy dose also reduced nitrification, as shown by the high proportion of N_{NH4}. Irradiation improved P availability, too, despite rapid fixation by soil and no increase in extractable P after the flush (I: Figure 1a). The change was shown by a higher P concentration and doubled P uptake by the mycorrhizal flax at the lower P level in soil irradiated by 10 kGy than in soil irradiated by 3 kGy (I: Figure 3a,c, Table 5). The same phenomenon was observed earlier by Thompson (1990). In contrast to the small increases in soil extractable P sometimes observed with irradiation (Eno and Popenoe, 1964; Jakobsen and Andersen, 1982; Jakobsen, 1984), no increase was or has been shown with the benomyl treatment (I: Figure 1a; Fitter and Nichols, 1988; Bentivenga and Hetrick, 1991). Where P was not limiting, the elevated N supply increased growth in irradiated soil relative to benomyl-treated soil.

The drastic change in nutrient and microbial conditions caused by irradiation required the use of irradiated and reinoculated soil as the mycorrhizal treatment for comparison with the irradiated control, while untreated soil could be used with benomyl. The considerably lower percentage of root length colonized in reinoculated than in untreated soil (I: Figure 2), also observed by Trouvelot et al. (1996), notably reduced growth. The effect of inoculation with soil extract was even more unsatisfactory than inoculation with 5% (w/w) untreated soil. In the control created by irradiation, suppression of AMF colonization was almost complete even with the lower irradiation dose. The incomplete suppression by benomyl was not reflected as a difference in growth or P uptake relative to the irradiated soil (I: Figures 3c, 4a). In fact, hyphal length correlated better than colonization level with the effect of benomyl on growth and P uptake (II, III). Hyphal length (II, III) or hyphal P transport (III) in the benomyl-treated soil did not differ from those values in the irradiated soil. When incorporated in soil, benomyl probably mainly affects the extramatrical hyphae and secondary hyphal growth, while the internal colonization which is protected by the plant tissue is not reduced to the same extent. Accordingly, notable reductions in plant growth and P uptake were observed with benomyl

despite a relatively high percentage of root colonized or no effect on root colonization (I: Figures 2, 3c, 4a; Tables 5, 6; II: Figure 1; III: Table 1). The same was observed in earlier studies (e.g. Bailey and Safir, 1978). The total root length colonized responds to benomyl more conclusively than does percentage colonization (III: Table 1) because benomyl suppresses AM functioning and may therefore decrease plant and also root growth.

No problems were observed in the validity of the measurement since, other than mycorrhization, no evidence of differences was found between the chosen mycorrhizal treatments and the controls (I). This was also true for the myc^+ and myc^- mutant lines, in agreement with the earlier observations on phenotypic similarity in non-mycorrhizal conditions (Kneen et al., 1994). Unfortunately, the lines did not respond to AM. Reinoculation of the non-mycorrhizal microbiota to the controls with suppressed mycorrhization seemed to be unnecessary because it did not affect any of the results in the bioassay (I). Nor did benomyl have any measurable effect on the decomposition of plant remains in the field (unpublished results, 2.2 Method development, *Field experiment*). The effects of benomyl on soil nutrients and microflora have generally been weak (Helweg, 1973; van Faassen, 1974; de Bertoldi et al., 1977; Habte, 1997). However, in some cases AM effectiveness might be underestimated with benomyl treatment, because traces of mycorrhization and the interference of root pathogens cannot be excluded.

The assay with the control created using benomyl, exhibited the highest RME or contribution to growth and P uptake (I: Figure 5, Tables 5, 6), mainly due to higher mycorrhization in the mycorrhizal treatment. No clear difference in the methods was evident in the ability to differentiate AM effectiveness between the management histories, but the tendency observed in clay was in favour of the benomyl method. The effects on roots were inconsistent and should be excluded when calculating RME (I, II). Root growth response is not of interest either, because the hyphae compensate root functions.

Thus, benomyl proved to be the most appropriate method of creating the control because it allowed the use of a responsive host with no limitation on the selection of non-mycorrhizal mutants, and the distortion of assessment was less than with the various irradiation doses. The most effective benomyl dose and application time with no phytotoxicity was determined (II): incorporation of benomyl at 20 mg kg^{-1} (kg soil at target moisture)$^{-1}$, i.e. 25 mg (kg dry soil)$^{-1}$, immediately before sowing provided the most effective suppression of AM, irrespective of the soil and sampling time (II: Figures 2, 3a,c, 4a, 5, 6, Table 4). The improvement over the dose of 10 mg kg^{-1} was not great, however (II: Figures 3c, 4a, Table 4). This is in agreement with the results of Schreiner and Bethlenfalvay (1997) who showed that benomyl applied at 20 mg (kg

air-dry soil)$^{-1}$ inhibited spore germination in all three AMF isolates tested, in contrast to 10 mg kg^{-1} which had variable effects on the isolates. Injection one week after sowing was less effective, possibly because of uneven penetration. This was also the only benomyl treatment with a clear phytotoxic effect.

Although the success of benomyl in creating a non-mycorrhizal control was not dependent on soil sampling time (II: Figure 1), the most appropriate sampling time for assessing AM effectiveness in terms of growth or nutrient uptake was in spring, before the start of the growing season. Infectivity was highest after the spring thaw, and after a decline in summer it increased towards the autumn, irrespective of soil or management history. This pattern was evidently due to variation in the density of vital propagules and P availability. Temporal variation in AM infectivity has also been observed by Sanders (1993). In soils with high AM potential, RME followed infectivity: it was highest in the spring and high again in the autumn, irrespective of the plant species. In soil with a high P level and thus low AM potential, however, the temporal variation in RME was opposite to that in soils with high AM potential. Evidently, the heavy mycorrhization in soil sampled in spring and autumn increased the C cost while the benefit from AM at the high P level was small. Therefore, in summer the opposite patterns in RME in soils with high and low AM potential reduced the difference in RME between the soils. The other possible sampling time besides spring is autumn, when infectivity and effectiveness are close to that in spring. Sampling in spring is more relevant to the conditions of infection in the field, however, and the difficulties of autumn sampling in simulating the nutrient status at infection are avoided.

Linseed flax (*Linum usitatissimum*) cv. Linetta proved to be the most appropriate standard test plant. It was responsive to AM over a wide range of fungal diversity and soil conditions, while clearly indicating differences in mycorrhizal effectiveness between fungal communities and soil states (unpublished results of test plant comparisons, data not shown; I, II: Figures 1, 5, 6, Table 4; IV: Figures 1, 2, Tables 3, 4, 5, 7). Flax is also a crop of increasing economic importance. Because of the functional compatibility of the three partners of AM (plant-soil-fungus) (unpublished results, IV: Tables 2, 7) an other plant species relevant to the cropping system in question should be included as well. Appropriate choices for northern sustainable agriculture with soils of relatively low P are red clover and white clover (*Trifolium repens*). They, however, require a greater number of plant individuals per pot or more pots per treatment than flax, owing to high genotypic variation between the individual plants (unpublished results, IV). Use of myc^{-} mutants would avoid the disruptive soil treatments as well as the safety and environmental problems of benomyl. However, their use would require development of a selection of isogenic,

phenotypically comparable pairs of mutants of genotypes responsive to AM in a variety of conditions, and relevant to various agroecosystems. For infectivity studies, Johnson et al. (1999) have recommended the use of a standard test plant (e.g. *Trifolium repens*), or as a possible alternative, a plant species relevant to the system.

The colonization levels in the bioassay were close to those in the field in July, confirming the appropriateness of the bioassay in assessing infectivity (IV: Tables 3, 7; V: Table 3). Parallel differences in RME were generally found between the management histories in the field and the bioassay in the growth chamber (I: Table 7, IV: Figures 1b,d and 2b,d, Table 4, in comparison with V: Figures 1 to 4, b,d, Table 4). It turned out, however, that the transient negative response due to a very low P supply might confound the conclusions of a short bioassay. Thus, in assessing the AM effectiveness of soils with P_{H2O} of about 5 mg kg^{-1} or below, the inclusion of a test plant with low P demand such as clover is useful. Still, this study indicates that the C consumption by continuing hyphal growth may be crucial for the total plant response to AM at harvest. To better predict that response, a longer growth period than four weeks, possibly combined with increased soil volume depending on the test plant, would be useful. Johnson et al. (1999) have pointed out that the optimum duration of bioassays on mycorrhiza should be determined in a pilot study for each system. They suggested a duration of 2-6 weeks for AM, yet discussed the assessment of infectivity only.

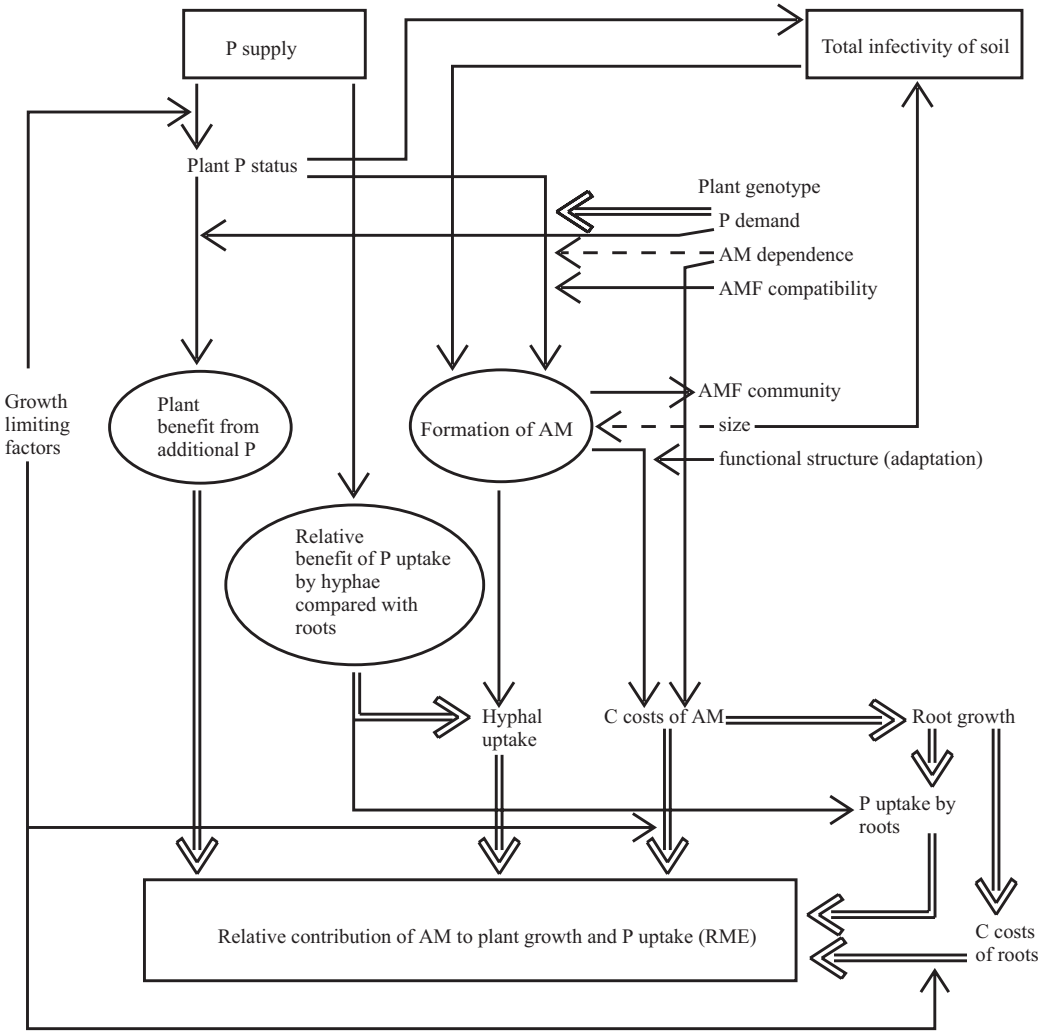
The superiority of benomyl and the most appropriate soil sampling time, benomyl dose and application time did not vary for the various soils, with their different AMF communities and origins from various latitudes and coastal to inland climatic conditions within Finland (I: Figures 2, 3, 4, 5, Tables 5, 6; II: Figures 1, 2, 3, 4, Table 4). The results might thus be applicable to most North European field soils and even other soils subject to thaw. This bioassay serves research on management of field AMF and allows standardization for practical purposes. The chemical extraction of soil P could be complemented by the determination of RME, and possibly infectivity, in this kind of bioassay as a means of better describing field soil P availability and its problems.

In conclusion, for mycorrhizal infectivity and relative AM effectiveness of field soils, representative results are achieved in a bioassay of soil sampled in the spring after the thaw. The most appropriate control is created with suppression of mycorrhization by 20 mg (kg soil in target moisture)$^{-1}$ benomyl incorporated in the soil immediately before sowing. Flax is an appropriate test plant together with a crop relevant to the particular agroecosystem.

3.3 Conceptual model of the impact of P on AM

A conceptual model of the impact of P on AM effectiveness is presented on the basis of the suggestions of this study, supplemented by earlier findings. The model is an attempt to understand the dynamics of the interaction of the P supply and the contribution of AM to plant growth and nutrient uptake, as well as the relative importance of the interacting phenomena in the conditions of northern agriculture. The interactions described are illustrated in Figure 4. The end purpose is to offer a conceptual framework for AM management by manipulating the P supply and a starting point for applied extensions to include AM management in the design of farming systems.

Figure 4. A conceptual model of the impact of P on AM



As a total effect, within the range of soil-extractable P with positive AM response, cumulative P fertilization decreases the contribution of AM to plant nutrient uptake and growth (IV: Figures 1b,d and 2b,d, Table 4; V: Figures 2b,d and 4b,d, Table 4; Thingstrup et al., 1998). At intermediate and higher soil P levels with no or a negative growth response to AM, the effect may be reverse at later growth stages due to the suppression of AM by increased P supply (V: Figures 1d and 3d). A suboptimal P supply decreases the benefit from AM due to insufficient P uptake to compensate the AM costs (IV: Figures 1d and 2d). Thus, extremely low soil P may at least transiently result in growth depressions for crops with high P demand or low ability to utilize AM.

Benefits

P supply affects the tripartite AM via the soil and further through the host plant. An effect of major importance for *AM effectiveness in terms of plant growth and nutrient uptake* appears to be that increased soil concentration of extractable P elevates the P concentration in the plant thus decreasing *the benefit of additional P* and thus the benefit of AM for the plant (IV, V). It evidently suppresses *AM formation* mainly via elevated plant P concentration (Sanders, 1975; Menge et al., 1978); the evidence for a direct effect through the soil on the extramatrical fungal growth is contradictory (Abbott et al., 1984; de Miranda et al., 1989; de Miranda and Harris, 1994; Olsson et al., 1997). A reduction in spore germination under conditions of high soil P has, however, been shown (de Miranda and Harris, 1994). The P supply that decreases AM formation depends on the *plant genotype*, evidently being related to the *plant P demand* (IV). In the long term, therefore, increased soil P content cumulatively decreases *the size of the AMF community* (V). With the increase in soil and plant P and the cumulative decrease in size of the AMF community, *the total infectivity of the soil* is decreased, as observed throughout the present study (III: Tables 1, 2; IV: Table 3; V: Table 3). Because of the variation among AMF in sensitivity to P (Sylvia and Schenck, 1983; Thomson et al., 1986), cumulative P also appears to cause a change in *the community structure* and thus in its functions (IV: Table 7; Johnson, 1993). The functional change was not related to nutrient uptake (IV), neither was any change observed in the P transport capacity per hyphal length unit due to P fertilization in the long term (III: Figure 2, Table 2). This is in accordance with the results of Jakobsen and Vestberg (1998), who showed interspecific variation in the P uptake potential of *Glomus fistulosum* and *G. mosseae*, but no intraspecific variation among the isolates. At Maaninka, *G. mosseae* was dominant according to spore densities (V: Table 7). During the 20 years of study, no P fertilization effect on the AMF species

composition was observed in the two fields investigated (III, V: Table 7). This is in contrast to some other studies (Johnson, 1993; Gryndler and Lipavsky, 1995).

The decrease in benefits from AM caused by an increase in soil P supply seems to be *partly compensated*. First, an increase in the soil content of extractable P increases the P supply available for *hyphal uptake*. The role of this appears clearly at the start of plant growth when the soil P is extremely low (IV: Figures 1d, 2d), and seems to appear occasionally when it is supraoptimal for mycorrhizal benefit (V: Figures 1d, 3d) (see below). Secondly, in the long term the AMF communities seem to show *a feedback tendency* by *adaptation* to cumulative changes in soil P in functional terms, as shown here as a result of 20 years of influence (IV: Table 7). This was not observed by Johnson (1993) and Gryndler and Lipavsky (1995) within eight- and ten-year periods, respectively. This adaptation seems, however, to be of no practical benefit. Either it is not able to compensate the other, contrary effects of high P contributing to AM effectiveness, or such an evolution would require an extremely long time (IV).

Costs

By decreasing mycorrhization the enhanced P supply also reduces *AM costs* (V: Figure 1d). The C expenditure for the build-up and metabolism of fungal tissue and more generally greater below-ground C expenditure (Eissenstat et al., 1993) decreases along with the suppression of root colonization and the combined suppression of growth of extramatrical fungal structures such as hyphae. The *functional differences* observed in the AMF communities from different P histories were not related to P uptake (IV), but could be related to C expenditure. Johnson (1993), too, found less mutualistic AMF with more C consuming structures in soil with cumulated P fertilization than in unfertilized soil. Raju et al. (1990) observed more genotypic differences between AMF and plants in costs than in benefits of AM. The difference in benefits and costs from AM calculated as C determines the contribution of AM to plant growth. Simultaneously with the effect on AM, soil P supply also affects the alternative P acquisition mode, or growth and P uptake of roots. When increased P supply suppresses C expenditure by AM, a greater share of C is available for *root growth*, thus enhancing *nutrient uptake by roots*. The simultaneous increase in P supply for roots results in a *decreased relative benefit of uptake by hyphae*, which encompass larger soil volumes and smaller soil pores. Thus, the observed hyphal P uptake does not mean that AM would necessarily benefit the plant (compare Schweiger and Jakobsen, 1999). The C costs of AM in comparison with the costs of an alternative P acquisition mode, i.e. uptake by roots, are crucial (V: Figure 1d). The relative P:C efficiency of the uptake by hyphae and roots evidently depends most on the interrelations of the plant and soil P status (V).

There are, however, *compensating mechanisms* for the AM costs as well. AMF may utilize C that in any event would be lost from the roots by exudation (Schwab et al., 1991), but this has only a minor role (Tinker et al., 1994). Also, AM may slightly increase the plant photosynthetic rate per unit leaf area (Snellgrove et al., 1986). The plant could react to AMF demand by increased photosynthesis through the sink-effect, or the plant may have a surplus of carbohydrate (Fitter, 1991). The last alternative is evidently of major importance, being related to the prevailing conditions of *growth limitation*. Accordingly, when the soil P supply is not limiting, AM often depresses growth (Crush, 1976; Buwalda and Goh, 1982; I: Figure 5a; V: Figures 1b,d and 2b,d, Table 4) and conversely.

Factors interacting with the impact of P on AM

The plant genotype determines the *plant P demand* in relation to other growth factors, and thus the potential benefit of additional P. The results demonstrated notable variation in the crops in both the location and width of the range of the soil P content with positive response to AM (IV: Figures 1b,d and 2b,d). The location is evidently mainly determined by the plant P demand, while the width of the range and the maximum *AM dependence* of the crop depend on several factors such as the alternative P acquiring strategies (e.g., Baylis, 1975) and the ability to cover the C costs by AM (IV). For example, the low MD of barley lifted the lower threshold P level in relation to its P demand and increased the growth depression by AM, in comparison with flax with its higher P demand and MD. The functional compatibility of some crops with the AMF communities in Nordic field soils (Boyetchko and Tewari, 1995; Ravnskov and Jakobsen, 1995) seems to vary notably. Thus, the AMF infectivity and contribution of AM to flax growth was clearly higher in Maaninka than in Mietoinen soil at the same soil content of extractable P in the bioassay simulating the start of the growing season (IV); it was similar in July in the field, and the opposite at harvest (IV: Figures 1b,d, 2b,d, V: Figures 1 to 4, b,d). In contrast, the contribution to clover growth was fairly similar in the two soils at the same P level in the bioassay. The difference in compatibility for the two plants seems not to be explained by the cropping history of the fields since neither clover nor flax had been grown at either location for at least 20 years. The difference in P demand of clover and flax did, however, coincide with the difference in P availability of the soils, the AMF of which caused the higher root colonization for clover and flax (Mietoinen and Maaninka, respectively) (compare Johnson et al., 1992b) (IV).

All *growth factors* of importance, especially those that limit growth most, affect the C supply for the AMF. In addition, the degree of limitation by P in relation to

the limitation by other growth factors is crucial for its effect. Among the most important of these other factors are light, temperature and water conditions, the supply of nutrients other than P, and interacting soil organisms. The relationship between these factors determines the P thresholds for benefit from AM and optimal mycorrhization (below). Their manageability in field conditions determines their relative importance in agriculture.

The N supply affects the P response of AM (IV: Table 6; V: Table 6). In the field, halving the moderate N supply prevented suppression of colonization by high P (V), as also shown previously (Sylvia and Neal, 1990). The effect on colonization may involve specific physiological mechanisms (Sylvia and Neal, 1990; Abbott and Robson, 1991a). The interaction of N with the P impact on the contribution of AM seems to be more complex, however, depending on the roles of colonization rate (see below) and growth limitation. If increase in N supply causes P to become more growth limiting than N, this increases the contribution of AM (V: Table 6, see below).

Thresholds for positive AM response

When the soil P supply increases, the benefit of the additional P transported by AMF to the plant decreases. Most often, the benefit seems to decrease clearly before the P concentration of the plant noticeably suppresses the AM and thus reduces the C cost (IV: Figures 1b,d, Table 4; V: Figures 1d and 2d, Table 4). This leads to *growth depressions.* Crop growth suppressions by AM seem to be of importance in the common conditions of northern field soils, as e.g. in the Maaninka field which represents an intermediate content of extractable P for Finnish field soils at present, and common conventional cropping practices. The generality of growth suppressions by AM has been undervalued, but received more attention in recent years (Johnson et al., 1997; Graham and Eissenstat, 1998). The *AMF community*, its size, adaptation to soil P status and functional compatibility with the crop (IV: Figure 1b,d, Table 7; Boyetchko and Tewari, 1995; Ravnskov and Jakobsen, 1995) also affect the threshold P levels and the relative contribution of AM. In the bioassay, the relative contribution of AM to growth was roughly three times as high and its contribution to P uptake double for flax in the Maaninka soil compared with the Mietoinen soil at corresponding P availability (IV: Figures 1b,d and 2b,d). When N was in surplus in the Maaninka soil, P still limited growth even in soil with cumulated P applications (III: Tables 1,3). In this soil the absolute contribution of AM was lower with lower N supply (V: Table 6). The actual threshold for positive plant response to AM is *related to factors limiting growth* such as N supply. Thus, the threshold also depends on the host plant and its P demand and AM dependency (IV: Figures 1b,d and 2b,d). In the Maaninka field (P_{H2O} 5 to 18 mg kg^{-1}), AM caused

growth depression for flax, barley and pea, while for red clover with low P demand the soil P supply was high enough to almost totally suppress the early mycorrhization, leading to *no AM response* (I: Table 7, IV: Table 3; V: Figure 1d, Table 3).

In addition to the upper threshold P content, *a lower threshold* for positive P response is sometimes observed. Plant AM response may be transiently negative due to too low soil P supply during the first weeks of growth, up to 9 weeks after sowing (Bethlenfalvay et al., 1982; Koide 1985). Presumably the build-up of the AMF structures then consumes more C than can be compensated by the growth effect of the fungal P uptake from extremely P-deficient soil. Additionally, the fungal partner may directly compete with the host plant for the P resources if the P supply is very low (Crush, 1973). This situation was demonstrated in the unfertilized Mietoinen soil with P_{H2O} 2.7 mg kg^{-1} P in the bioassay for flax with its high P demand and for barley with its poor ability to utilize AM, but not for red clover (IV: Figure 1b,d). The same situation was not observed directly in the field in the same soil when this was sampled 50 days after sowing. This was because of its *transient character*, or not sufficiently low P supply to cause continuous growth depression (V: Figure 2b).

However, the range of soil content of extractable P with positive plant response to AM seems not always to be a continuum. Particularly at soil P concentrations above the upper threshold for positive AM response, situations sometimes seem to occur where the ratio of the degree of mycorrhization to the P supply for hyphal uptake occasionally becomes beneficial for the plant. The beneficial ratio then causes a positive response by AM and possibly also by plant growth to an increase in the P supply (V: Figure 1d). This phenomenon is evidently of no practical importance, however, because it is difficult to utilize. One example of this phenomenon is the following:

At Mietoinen in July, fresh additions of 90 kg P ha^{-1} to the annually fertilized soil resulted in the highest flax growth and contribution of AM to growth and P uptake (V: Figures 3a,b and 4a,b). Yet, the flax P concentration was lower with 90 kg P ha^{-1} than with 45 kg P ha^{-1}. Nor did the higher P addition increase the growth of flax with suppressed AM relative to the lower P levels. This indicates that the lower mycorrhization but higher P supply for uptake by the scarcer hyphae with 90 kg P ha^{-1} compared with 45 kg P ha^{-1} allowed a higher share of C to be used for growth and thus more *efficient P utilization*, in terms of P uptake in relation to the C cost, than the 45 kg P ha^{-1} application. The alternative explanation of benefits not related to P nutrition is improbable because the effect appeared upon the addition of P. This example demonstrates the complexity of the impact of P on AM, the complexity of the growth limitation, and the important role of the P impact on the costs and not only on the beneficial functions of AM (Raju et al., 1990).

Optimum mycorrhization

There seems to be an optimum range of mycorrhization, which varies considerably between host *plant genotypes* (Pande and Tarafdar, 1999) but also depends on soil P and N status, and evidently more generally on *the relations between growth limiting factors* (IV, V). The relation between mycorrhization and growth response may also depend on the *AMF community* (Mosse, 1972; Graham et al., 1982; Ravnskov and Jakobsen, 1995). Not only the hyphal length, growth habit and functioning (Kough and Linderman, 1986; Jakobsen et al., 1992a,b; Buerkert and Robson, 1994; Jakobsen and Vestberg, 1998), but presumably also the C cost per functional hyphal length unit (e.g. hyphal robustness) vary considerably among AMF and so affect their efficiency of P uptake and other benefits in relation to C expenditure.

Additionally, the results of this study show that the critical level of mycorrhization is not a minimum, but an optimum range, that *can also be exceeded* (see Fitter, 1991), and they suggest that the exceeding might often be the case in agricultural fields (V: Figures 1d and 2d). This finding contradicts the common assumption that the extent of AM formation is the limiting factor for enhancement of the P uptake of crops by AM (e.g. Abbott and Robson, 1984; Mosse, 1986; Abbott and Robson, 1991a; Smith et al., 1992), and that if mycorrhizas form rapidly and extensively, it is likely that maximum benefits from AM are being gained (Abbott and Robson, 1991b). Both growth limitation by different factors and the proportions of fungal structures *vary with the stage of growth,* thus affecting the optimal rate of mycorrhization. Later in the growing season the plant's P demand is often lower while the C expenditure of the large, continuously growing mycelium and possible formation of carbohydrate storage vesicles is higher.

Time perspective

We found that the short-term effect of P fertilization may differ notably from the long-term effect, not only quantitatively but qualitatively (see also Figure 2). In cases where the P fertilization had a clear impact, *omitting the P application* before sowing resulted in higher colonization than did including it (IV: Tables 4 and 5; V), and also in higher contribution of AM to plant growth and P uptake at the start of the growing season (IV: Figures 1b,d and 2b,d). Thus, freshly added P at growth initiation caused a peak in plant P concentration and suppressed AMF colonization and function more than would be expected from its effect on soil P_{H2O}. It is the concentration of P within the plant and not the soil that leads to a reduction in mycorrhization (see above). However, at harvest the added P tended to have an opposite effect on AM effectiveness (V: Figures 1 to 4, d). Clearly the *rate of mycorrhization*

was no longer the limiting factor for AM effect on the plant at the latter half of the growing season. Hence, at the same level of soil extractable P the more abundant mycorrhization in the soil with the omitted P dressing caused a higher net cost than the mycorrhization in the fertilized soil.

The long-term effects of P fertilization on AM include *evolutionary changes* in AMF (Morton, 1990) and the AMF community and in other microbial communities (Bunge, 1985) interacting with it (Linderman, 1988; Garbaye, 1991). Within 20 years, neither the effects observed on the structure of the AMF communities (see *Benefits*, IV: Table 7; compare with V: Table 7) nor possible influences on the other microbial communities reinoculated to irradiated soil (I) seemed to be of ecological significance for AM effectiveness. No long-term P fertilization effect on the ability of the soil to decompose plant remains was found either ($p>0.81$, unpublished results of the mesh bag assay in the field experiment, see 2.2 Method development). The *cumulative effects* on the soil P content and thus on the environment of the microbes were far more prominent, overwhelming the effects on the microbial community including the effects on the size of the AMF community.

For the long-term perspective of sustainability, this indication of the adaptation of the functional structure of the AMF communities (IV: Table 7) is highly important. Since no changes in species composition were observed (V, yet with a fairly high proportion of unidentifiable spores), the functional change may have proceeded through changes in the relative abundance of physiologically different clones despite morphologically identical spores (Morton, 1990). Measures of taxonomic and functional diversity are often poorly correlated. Species definitions rely on phenotypic or genetic characteristics that may have no direct relationship to soil processes (Lee and Pankhurst, 1992). The long time the adaptional changes seem to require, decreases the *resilience* of the system towards low-input conditions. The same concerns the cumulative P effects, as the changes in soil P status are slow. Therefore, the improvement in AM effectiveness is slow even after P fertilization is omitted or reduced (Porter et al., 1978; V: Figure 1b).

In addition to the long-term effects of P on AM there is a *cumulative impact by AM on the soil* (Figure 2). The soil effects include effects on soil structure (Tisdall and Oades, 1979; Tisdall, 1991) which may lead to reduced erosion (Reganold et al., 1987) and effects through plant and AMF growth on soil nutrient and carbon content. They also include *evolutionary* effects, as *feed-back* to the AMF community and the interacting microbial and plant communities. Quantification of these effects is difficult and was attempted only for AM effects on soil structure (to be published elsewhere).

Characteristics of the P supply

As regards P supply, not only the quantitative aspect is important for AM effectiveness, but also the relative availability of the soil inorganic and organic sources and the added organic matter and inorganic fertilizers to the plant and to AM is decisive (Mosse, 1973; Bolan et al., 1987; Joner and Jakobsen, 1994b, see also 1.3 P utilization by arbuscular mycorrhiza). The evidence for the superiority of AM in utilizing P in organic matter is of notable practical importance for sustainable agriculture (Kahiluoto and Vestberg, 1998; Kahiluoto and Vestberg, 2000). It has also been suggested that band application of P would allow AM functioning in roots outside the band (Lu et al., 1994), but the evidence is in disagreement with the findings of several authors (e.g., Menge et al., 1978; de Miranda et al., 1989). These aspects were not covered in the present study.

The evidence from the present study suggests that the temporal variation in P availability is a major determinant for AM development, functioning and total effect in field soils. The variation is closely linked to the form of P, but interacts with climatic and other factors and microbial activity. Early infection is considered important for AM effectiveness (e.g., Smith et al., 1992), but combined with extremely low P supply it may in fact considerably retard plant and AM development (IV: Figures 1d and 2d). Above the threshold P supply required for the early infection to be effective, abundant plant-available P at the start of the growing season seems to decrease AM utilization (IV: Figures 1, 2; V). Thus, slower and more continuous P release during the growing season might promote it. As indicated by the results, if the soil P supply is not low, the plant response may be strongly negative later in the season (V). In that case, an increase in plant-available P in the middle of the growing season might prevent excess mycorrhization and favour plant fitness later in the season. However, this benefit would require a rapidly growing plant community with an extensive root system to prevent environmental hazards.

3.4 Management of AM by P fertilization

Practical significance of the impact of P on AM

At the low soil P supply, the cumulative P fertilization reduced the contribution of AM to crop growth and nutrient uptake during the first half of the season in the field. For flax, this was the case through the whole season, while for barley the effect of P fertilization remained unclear at harvest in both soils (V: Figures 2b,d and 4b,d, Table 4). At an intermediate initial soil P status the contribution of AM to

crop growth was similarly reduced by the cumulative P fertilization during the first half of the growing season (V: Figure 1b), but thereafter AM was not beneficial, irrespective of P fertilization (V: Figure 1d). For clover, the intermediate soil P supply was high enough to retard mycorrhization and prevent AM response irrespective of fertilization.

As regards flax, in the field with soil P_{H2O} 2.5 to 5.0 mg kg^{-1} (Mietoinen), a clear growth benefit was obtained from AM (V: Figure 2d). This is approximately in agreement with the results for Danish field soils reported by Thingstrup et al. (1998). The threshold for positive AM contribution to the P uptake of flax was approximately P_{H2O} 10 mg kg^{-1} (V: Figure 4d). The soil P level of P_{H2O} 5.4 to 21.3 mg kg^{-1} (Maaninka) seemed to be too high for mycorrhizal yield benefit for any of the three plant species (V: Figure 1d). At harvest the well-developed AM depressed flax growth there in unfertilized soil (P_{H2O} 5.4 mg kg^{-1}) because in the later stage of the growth the benefit obtainable from additional P was not sufficient to compensate the costs (Graham and Eissenstat, 1998. However, in the regularly fertilized soil of the intermediate initial P level (Maaninka, P_{H2O} 17.9 to 21.3 mg kg^{-1}) mycorrhization was limited by the higher P content. Despite this, the lower C costs than in unfertilized soil were covered by the higher P supply for hyphal uptake only in the soil with the annual P dressing (P_{H2O} 18.8 mg kg^{-1}). This situation resulted in increased nutrient uptake but no growth benefit (V: Figures 1d, 3d). Compensation of the AM of unfertilized soil for flax growth and P uptake until July required 90 kg P ha^{-1} in superphosphate at the low initial P level (Mietoinen) and correspondingly 45 kg at the intermediate P level (Maaninka) in soil with hindered AM functioning (V: Figures 1 to 4, a). At the low P status (Mietoinen) the situation remained the same until harvest contrary to the intermediate soil P status (Maaninka) where the benefit from AM disappeared (V: Figures 1 to 4, c).

For flax at harvest, the highest relative AM contributions were found in soil with lowest extractable P content; these contributions were 35% to P uptake, 17% to growth and 18% to seed dry weight (V: Figures 2d, 4d, Table 4, data in the text). The maximum reductions in P uptake, growth and seed dry weight of flax due to AM were found in the soil with intermediate initial soil P status with no cumulative P fertilization and were 34, 42 and 56%, respectively (V: Figures 1d, 3d, data in the text). The management benefit, or increase in benefit from AM to flax due to omitting the cumulative P fertilization, was at the low soil P supply 30% of seed dry weight and 35% of seed and total P uptake at harvest (V: Figure 4d, Table 4, data in the text). Here AM largely compensated the yield effects of P fertilization of 45 kg P ha^{-1} a^{-1} for 20 years (V: Figure 2c). The more effective AM in unfertilized soil resulted in even higher growth, seed yield and seed nutrient concentrations and equal P uptake rela-

tive to soil with cumulative P (V: Figure 2c, 4c, data in the text). At the intermediate initial soil P status, the P fertilization was required to suppress mycorrhization and thus reduce the flax growth depression by AM. Even there, only growth and P uptake of flax benefitted from cumulated P (V: Figures 1c, 3c) while the seed yields and nutrient concentrations responded to the annual fertilizations only if the P application at the start of the growing season was omitted (data not shown).

Red clover requires less P than flax and its AM dependency is lower (IV: Figure 1a, unpublished comparison of test plants, see 3.2). In field soil with low P status (Mietoinen, P_{H2O} 3 to 7 mg kg^{-1}), clover tended to receive more benefit from AM with no P fertilization. This was indicated in the bioassays, which well represented the situation in the field until July when clover is harvested (IV: Figure 1d). At the intermediate soil P status (Maaninka), P was not limiting for clover growth even in the unfertilized soil. The high soil P status in relation to the P demand of clover and the incompatibility with its AMF community (IV: Table 7, Figure 1b,d) retarded mycorrhization, leading to no clear AM response (V: Figure 1d, 3d).

Barley benefitted from AM in unfertilized soil until July at both locations but more at the lower P level. In the annually fertilized soil there was no net response to AM at the location with low soil P supply (Mietoinen), and a growth depression of nearly 40% due to AM at the location with intermediate soil P supply (Maaninka) (V: Figures 1b, 2b). At harvest the difference between the P histories remained unclear for barley and there was no net benefit from AM (V: Figures 1d, 2d, Table 4). Yet, the barley seed yield benefitted only slightly from the cumulated P at the low soil P level (Mietoinen) and not at all at the intermediate P level (Maaninka) (V: data not presented). The seed P concentration tended, however, to be higher in the annually fertilized soil than in soil with no added P, as N rather than P limited growth in the fertilized soil (V: data not presented). In a study by Saarela et al. (1995) carried out during 15 to 18 years at 24 locations, the difference in cereal yields between the unfertilized plots and plots fertilized with 15 to 60 kg P ha^{-1} a^{-1} was on an average 11% from the yield with fertilization. At Mietoinen the difference was 13% and at Maaninka 8%. Evidently, the net cost of AM in the fertilized soil contributes to keeping the differences small.

The enhancement of nutrient uptake by AM was notable for Zn, P, Cu and even K in loam with intermediate P supply (Maaninka), while only the P effect was important in clay with low P supply (Mietoinen) (V: data in the text). AM elevated seed concentrations of P, Cu and Zn as previously reported (Pacovsky, 1986), also when no yield benefit was achieved (Gildon and Tinker, 1983). AM considerably enhanced N uptake as well but the effect was clear only until July (IV, V: Table 6). In addition, no growth or yield reductions at halved N supply were observed at

Mietoinen, where P evidently limited growth more than N, irrespective of the P history (V: data not presented). This finding demonstrates the importance of adjusting the supplies of various nutrients to each other for improving their efficiency. In principle, mycorrhizal growth depression by increased P could be the result not only of C cost with no compensating benefit, but also of the suppression by high P of benefits from AM not related to P (e.g., Singh et al., 1986; Thompson, 1996). Evidence of lower reductions in benefit from AM for uptake of other nutrients than P in fertilized soil was not obtained, however (IV, V).

The main reason for the difference in P impact on AM between the two locations is evidently the difference in P status and thus in the benefit of additional P to the plant. A contribution by a less efficient AMF community in terms of acquired P per unit expended C at Maaninka is also possible. This interpretation is suggested by the higher benefit from AM for flax at Mietoinen than at Maaninka at equivalent soil contents of extractable P at harvest in the field (V: Figures 1 to 4, d). Caution is required, however, because the P availability of different soils is difficult to compare by chemical measurements. The soil P supply and P:C efficiency of the evolved AMF community may also interact. Johnson (1993) found evidence for the hypothesis that plants with no nutrient stress release less carbohydrates and therefore create a selective pressure favouring AMF strains that most aggressively acquire host carbohydrate. Thus, less beneficial or even detrimental AMF were selected in fertile or fertilized soil. The AMF species compositions of the two fields were not very different, as the species with clearly dominating spore densities on the two locations are closely related (V: Table 7). However, as stated above, the differences in the functional properties of AMF may even occur intraspecifically.

Potential to promote reliance on AM in crop P uptake and growth

The considerable yield losses found at the intermediate field soil P levels occur because crops have no use for the P uptake potential of AM, while the fungal C costs still have to be paid. This reduces the effectiveness of P fertilization for growth. The yield losses can be avoided and the potential of AM can be utilized only by reducing the soil P supply through omitting P fertilization. Alternatively, P has to be used in surplus, as in fertilizing at the intermediate soil P level (Maaninka) just to suppress the detrimental AM. However, immediate improvements in AM utilization through reduced P fertilization seem not to be obtainable. Omitting solely the last P application improved the contribution of AM in the Mietoinen field only until July (IV: Figures 1d, 2d, Table 5; V: Figures 2b, 4b, Table 5), while the effect was disappeared until the harvest (V: Figures 2d, 4d, Table 5). Approximately one fourth of the area under cultivation in Finland at present represents the P status with positive AM response

for flax and clover in this study. Yet, the upper threshold for positive AM response obtained in this work roughly corresponds to the average concentration of soil extractable P in Finnish field soils until the late sixties (Mäntylahti, 1996), based on the relation between soil water-extractable and ammonium-acetate-extractable P proposed by Puustinen et al. (1994). At a relatively low extractable P content of field soil, P_{H2O} 2.5 mg kg^{-1}, AM compensated the 20 years' annual P fertilization of 45 kg ha^{-1} for flax, with its high AM dependence. Even in this range of P status the yield compensation by AM was incomplete for clover, and for barley no benefit from AM was shown at harvest. There are, however, possibilities to improve the benefit from the symbiosis. By exploiting these possibilities even the thresholds of soil P with a positive response to AM could be shifted.

The genotypic variation in AM dependence of plants offers a way to increased reliance on AM in crop P uptake through crop selection and breeding. Manske (1990) and Hetrick et al. (1992a,b) showed a strong genetic basis for the differences in MD among wheat cultivars. The consistent mycorrhizal dependence of cultivars released before 1950, in contrast to modern cultivars, suggests that modern breeding practices have reduced the dependence on mycorrhizal symbiosis. This may well be true for cereals in general. Behind this development lie both breeding goals and the selection conditions serving these goals. High yields of good processing quality in intensive cultivation have been the major goal, while the ability to utilize soil biologically mediated processes has been disregarded. In soil with intermediate or high content of extractable P where the selection has been performed, AM is either of no importance or a stress factor for the crops. The differences in AM dependence between barley cultivars (Boyetchko and Tewari, 1995) also provide potential for breeding.

For barley, the reason for the poor ability to utilize AM seems not to be poor colonization (V: Table 3) or sporulation (unpublished results by Mauritz Vestberg; V: data in the text), nor a deficient ability to divert photosynthates to the root system (IV: Figures 1d, 2d; V: Figures 1b, 2b,d, 3b, 4b,d), contrary to the suggestion by Sanders (1997). Rather, the problem seems to be the small net benefit drained from the formed symbiosis, at least for the three Finnish barley cultivars used in this study (IV, V, unpublished results of the test plant comparison). The observation is in accordance with that of Raju et al. (1990), who showed that the genotypic differences between both host plants and AMF were pronounced for calculated costs, but not for benefits. It is also possible that the AMF communities of the experimental fields were exceptionally poorly compatible with barley. The AMF species compositions of the two fields were common for temperate field soils (V: Table 7). However, both fields had been in cereal cultivation for at least the past 20 years.

The Maaninka field was almost monocropped with barley, while at Mietoinen barley was cultivated every third year on an average. Johnson et al. (1992a) have concluded that the dominant crop of the system may show even least mutualism with the evolved AMF community. The explanation could be that, in a managed ecosystem, the plant community, unlike the soil microbial community, is not under selection pressure. Therefore, in a monoculture an AMF community might evolve which draws maximum benefit from the host plant as a C source but disregards the mutualistic potential. Thus, the cereal monoculture could have caused a selective pressure favouring AMF species or strains with less benefit to barley, at Maaninka together with a relatively high P level (see above). This situation could be prevented by a proper crop rotation. Even the introduction of more compatible AMF from regions where the cereals originate has been suggested. Introduction of a new, more effective AMF community could also be relevant in cases where the indigenous community or its functioning have been deteriorated through mismanagement, but the unpredictable interactions in the soil community would first need to be carefully studied.

In addition to crop characteristics (IV, V), the temporal dynamics of P supply (V) appear to be important in redesigning of cropping systems for maximum reliance on AM in P management (Miller et al., 1994; Thompson, 1994). The timing of the P supply in relation to the plant and AM development, and the relative availability of P to AM and the plant roots (see 3.3 Conceptual model of the impact of P on AM, *Time perspective*) appear to be key aspects. Both are closely linked to the form of the P addition and to the whole P management of the agriculture and the food system. Further research on the interrelations of AM and the cropping system on several organizational levels is needed, however, to utilize the potential of AM. The present study showed that more emphasis should be devoted to the soil and plant partners of AM as the objects of management in order to utilize the existing AMF structures. Further, maximization of the P:C efficiency of AM should be adopted as the management goal, not maximum mycorrhization or hyphal P uptake. Attention should be focused on efficiency relative to alternative P acquisition modes such as roots, and on the temporal dynamics.

Potential of AM to promote sustainability of P managements

Even though the differences in AM utilization were notable, only some slight yield benefits by the cumulated P were achieved (V). On the other hand, the P emissions from the unfertilized soil of the field with low P status (Mietoinen) were only 11 to 29% of those from the fertilized soil of the same experimental field (V). These figures were obtained with the models of Jansson (1998) and Ekholm et al. (1999) for

the content of dissolved reactive P (DRP) in waters as a function of soil extractable P (V). The models are based on measurements in soils representative of the Mietoinen field, and the observed relations between the water-extractable and ammonium-acetate-extractable P in Finnish clay soils (Puustinen et al., 1994) were applied in the above estimation. The losses are less in soil not fertilized with P than in fertilized soil, not only because of lower soil P status as such but also because of more effective AM functions. These functions include more complete soil depletion and immediate uptake close to point sources by AMF hyphae which intercept leaching and fixation (Bolan et al., 1987; Joner and Jakobsen, 1994a) and, as well, reduce erosion (see above; Ekholm, 1998). These AM effects should be quantified and their management clarified.

If P fertilizers are not applied, the recycling of P within the food system needs to be as complete as possible. The unavoidable P losses from the system should be replaced, by returning P which finds its way to watercourses, back to the field soil in organic matter and for example through utilization of coarsefish. Also, the potential quantity of P supply through weathering needs to be elucidated. It might be enhanced in low-input conditions with high biological activity and with a lowered P concentration on particle surfaces due to uptake by AMF hyphae (Dighton, 1991). AM also has the potential to enhance utilization of the fertilizer P accumulated in soil mainly as Al- and Fe-bound P (Bolan et al., 1987; Hartikainen, 1991). The content of extractable P in previously fertilized soil is lowered for some time, but might eventually approach to the native content and reach a balance. The rate of decrease in soil P due to omitting fertilization seems to greatly depend on the initial soil P content, being higher, if the starting point is high (Saarela et al., 1995). In the Finnish long-term experiments on P fertilization, the decrease seemed to retard or cease at some locations even within ten years. The balance could possibly be obtained and sustained at a desired, lower level, by management of the intensity and processes of the cropping system.

More effective AM delivers satisfactory yields at lower P supply than does less effective AM, because the input-output ratio of P is considerably affected by AM effectiveness (IV: Figure 2a,c; V: Figures 3a,c, 4a,c). This is an example of the change in nutrient balance by management of the process without a change in the amount of inputs, i.e. without a change in the intensity of the system. In principle, accelerating the rate of nutrient cycling between the plant community and soil, or encompassing a larger part of soil P in the cycle, should increase the productivity per time unit even without a change in the amount of either inputs or losses. On the other hand, as we showed (IV, V), the intensity of cultivation also drastically changes the process. Extensive utilization of biologically contributed soil processes

such as AM requires less intensive cultivation practices. The ultimate limits for increasing plant productivity through optimization of a low-input system are not known. Nevertheless, at the present stage of technical development, AM cannot totally compensate the yield loss at the lower P supply for most crops. Instead of maximizing the output per unit field area, an alternative would be to maximize the output per unit of non-renewable resources or the reliance on local resources. The yield addition per unit added P decreases as the P supply increases. Further, the major factor determining the P losses to waters is the soil P supply. The increase of loss in relation to soil extractable P may be linear (Ekholm et al., 1999) within the same field but above a certain threshold even exponential (Heckrath et al., 1995). Thus, a reduction in soil P supply and a corresponding extension of the field area should lead to a decreased P load to waters with no reduction in the total production. The simultaneously increased effectiveness of P uptake by AM would both decrease the required field area as a feed-back phenomenon and intensify the depletion of soil algae-available P, further reducing P emissions to waters.

AM is an example of ecosystem services which are impaired or even irreversibly lost with a high intensity cultivation (IV Björklund et al., 1999). The use of external inputs to intensify production compensates part of these ecosystem services. Simultaneously the intensification often suppresses other contributions which are not compensated, or are even impossible to compensate. This is the case with AM functions other than P uptake, e.g. uptake of Zn and Cu (IV, V; Thompson, 1996), formation and stabilization of soil structure, distribution of C to soil microbiota and enhancement of bacterial solubilization of P (Linderman, 1988). In addition, even a considerable proportion of the inputs may in fact be expended to suppress these ecosystem processes. Growth suppression by AM in the field with the intermediate soil P availability but not in that with high availability (Maaninka, V: Figure 1d) demonstrates this phenomenon. External inputs are needed not only to compensate the benefit from AM that is obtainable at low P but also to compensate the net cost that occurs at the intermediate P supply, or alternatively to elevate the supply high enough to totally suppress mycorrhization. The irreversible or extremely slowly reversible adaptation of the AMF communities to extreme P conditions (IV: Table 7) is a loss in ecosystem services and decreases resilience. This decreases the flexibility and would be hazardous if the access to external resources were lowered.

The ultimate goal of sustainable soil management depends on the discourse of sustainability relied on. The goal could be maximum productivity per unit field area, in accordance with ”food sufficiency”, a concept of sustainability with a reduced perspective, possibly attempting to find an optimum concentration of extractable nutrients while taking into account the loss to waters. Alternatively, the

goal could be maximum reliance on the ecosystem services in coincidence with a community-oriented discourse that builds on utilization of local resources and emphasises the conservation of systems integrity, resilience and flexibility. The goal of reliance on ecosystem services such as mycorrhiza questions the reductionist approach to soil fertility that focuses on concentrations of extractable nutrients. In addition, it questions the holistic concept (see 1.1 Systems approach) of soil quality index (Karlen et al., 1997; refined e.g. by Glover et al., 2000) and indicators that disregard understanding the underlying processes and interactions, as well as the interrelatedness of the system levels (compare Sojka and Upchurch, 1999). The same value of an indicator may provide quite different information, depending on its interactions with other parts of the system, as shown for mycorrhization and mycorrhizal functions in this work (V). Also, the criteria for soil quality depend on the goals and strategy of soil management. Reliance on ecosystem services demands a systems-oriented concept of soil quality. Understanding the plant-soil system and its interrelations with food systems and the ecosystem forms the basis for the assessment and management of soil processes to sustain the capacity of soil to function as a vital system serving multiple and varied goals.

3.5 Conclusions

• *Do the conclusions depend on the systemism of the approach in research on low organizational leves of agroecosystems?*

This study demonstrates that, even in research at lower system levels, choices are made regarding the degree of systemism, including its soft dimension (see Methodological considerations, 3.1). Here, these choices encompassed the desirable characteristics of the target system, the AM management strategy, the definition of the research object, the research methods and even the response variables. The choices affected the results and conclusions. This was also shown in a comparison of results obtained by methods which represent different degrees of systemism. It was shown to be important to relate the conclusions to the system level considered. Moreover, the higher system level of interest affects the choice of appropriate approach even for research on the subsystems.

• *How can the contribution to plant growth and nutrient uptake, i.e. the mycorrhizal effectiveness, be assessed?*

Representative results for mycorrhizal infectivity and relative mycorrhizal effectiveness in field soils were obtained using a bioassay that involved sampling of the soil after the spring thaw and creation of a control with suppressed mycorrhization through incorporation of 20 mg (kg soil in target moisture)$^{-1}$ benomyl to the soil immediately before sowing. Flax proved to be an appropriate test plant. The use of an additional crop plant relevant to the agroecosystem concerned improves the relevance of the results. Prolonging the bioassay for more than the four weeks used would improve its representativeness beyond the range of soil P level with a positive response to AM. The bioassay allows standardization for practical purposes, to improve prediction of field soil P availability and its problems, especially in sustainable agriculture.

• *Does P fertilization decrease the contribution of AM to plant growth and nutrient uptake ?*

P fertilization always decreased the AM infectivity, and usually the AM effectiveness as well. Cumulative P fertilization decreased the size of the AMF communities, but did not affect the species composition or hyphal P transport capacity. Extreme P conditions caused functional intraspecific adaptation of the AMF communities. The adaptation was not related to P uptake but could be related to C costs, as earlier observed. The major practical impact of P fertilization in a 20 year's period was the change in the conditions in which the AMF functioned, not in the AMF themselves. For red clover with its low P demand the lack of AM response at the intermediate P level of Finnish field soils was due to suppressed mycorrhization. For flax and barley the major factor limiting the response to AM in field soils seemed to be too high and costly mycorrhization in relation to the benefit obtained. Thus, at the intermediate soil P level, cumulative P fertilization prevented yield loss due to AM because it reduced mycorrhization. The net benefit did not depend on variation in hyphal P transport potential but on the rate of plant growth limitation by P in relation to other growth limiting factors such as N, and on the relative P:C efficiency compared with the alternative P acquiring modes. Therefore, the main focus of AM utilization in crop production should be on the management of soil conditions and plant communities to promote net benefits of AM to the plant, rather than on favouring AMF. Management of costs and net benefits of AM requires more attention than the management of AM benefits. The temporal dynamics of P supply appear to be of major importance in AM management. The role of AM effects on soil should also be elucidated.

• *Is it possible to promote utilization and conservation of field AMF communities in Nordic conditions by management of the cropping system?*

Promotion of reliance on AM in northern agricultural fields, through modification of cropping systems and cultivation practices, is of practical significance. In soil with an initial content of P_{H2O} of 4.6 mg kg^{-1}, the management benefit, or increase in contribution of AM to growth, obtained by omitting the annual P fertilization of 45 kg ha^{-1} for 20 years was, for flax, 30% of seed dry weight, 35% of seed P content and 35% of total P uptake at harvest. AM more than compensated the annual P fertilization for flax, the compensation was not complete for red clover, and the benefit for barley disappeared until the harvest. The highest relative contribution of AM to flax P uptake was 35% and to seed dry weight 18%. In fields with intermediate and higher P levels (initial P_{H2O} 11.4 mg kg^{-1}), no yield benefits were obtained from AM, and reductions in seed dry weight by AM were as much as 56% from the weight with suppressed AM. Enhanced reliance on AM in crop P management reduced the environmental load, such as losses to water. Reliance on AM also conserved the ability of the AMF communities to contribute to crop growth and P nutrition in low-input conditions, and thus conserved the resilience of the soil ecosystem. AM deserves to be involved in the development of sustainable production systems as well as in breeding and soil quality assessment programmes serving sustainable agriculture. The environmental effects of AM should be elucidated and quantified.

4 Summary

The aim of this study was to find out whether utilization of arbuscular mycorrhiza (AM), in crop production in Nordic conditions, can be promoted through management of the cropping system. P fertilization was chosen as the pilot system to manage because it has a major effect on AM and is because it problematic from the viewpoint of sustainability. Our scant knowledge of AM functioning and its effects in the field is mainly due to the methodological problems of research. Therefore, a bioassay of AM effectiveness was developed. In addition, the work demonstrated the challenge of a systems approach to research on low organizational levels of the agroecosystem. The starting point was the goal of sustainable phosphorus management in the food system, focused down to the AM level. The conclusions were then linked back to the context of the target suprasystem.

The study demonstrated that, even in research at lower system levels, choices

are made regarding the degree of systemism, and the soft dimension of systems approach is also relevant. The higher system level of interest affects the approach to be taken in research on the subsystems, and it is important that the conclusions are related to the system level considered.

Representative results for AM infectivity and effectiveness in field soils were obtained in a bioassay involving sampling of the field soil in the spring, after the thaw, and creating a control with suppressed AM through the incorporation of 20 mg (kg soil in target moisture)$^{-1}$ benomyl in the soil immediately before sowing. Flax proved to be an appropriate test plant together with a relevant host. This bioassay also allows standardization for the practical purpose improving the prediction of field soil P availability and its problems, especially in sustainable agriculture.

Understanding the interrelations of the tripartite system of mycorrhiza encompassing plant, soil and AMF is a prerequisite for management of AM. The influence of P on AM formation and effectiveness in terms of growth and nutrient uptake of barley, red clover and flax was investigated. As well, the effect of P on the size, composition and functioning of the field AMF communities was clarified. The impact of the P history on P response and the immediate effect of omitting P application and halving N fertilization were elucidated with flax. Two long-term field experiments representing contrasting soil types with low and intermediate contents of extractable P were utilized. Besides the bioassay for AM effectiveness developed, two other bioassays and a field assay were employed. A conceptual model of the impact of P on AM effectiveness was presented.

P fertilization consistently decreased AMF infectivity and generally AM effectiveness, too. Cumulative P fertilization decreased the size of the AMF communities but did not affect the hyphal P transport capacity, or, in contrast to some other studies, the species composition. Instead, functional intraspecific adaptation of the AMF communities to extreme P conditions was observed. However, the mode of action of cumulative P fertilization of major practical importance was the change in the soil conditions in which the AMF functioned. In some cases the restriction on contribution of AM to crop growth and nutrient uptake in the field seemed to be a low rate of mycorrhization due to incompatibility of the plant with the soil P status or with the AMF community evolved. Most often, however, the benefit appeared to be limited by plant and soil factors which cause AM to be a net cost.

AM appears to be an ecosystem service that is impaired or lost by intensive cultivation. In soil representing the present lower end of the P supply of Finland's field soils, but the average status of the late sixties, the higher AM effectiveness in soil with no added P for 20 years compensated the annual P fertilization of 45 kg ha^{-1} (soil P_{H2O} 2.5 v. 9.5 mg kg^{-1}) for flax. The compensation was not complete for red clover. In

contrast to July, at harvest barley received no benefit from AM and only a slight benefit from cumulated P. The management benefit, or increase in relative contribution of AM to growth by omitting the annual P fertilizations of 45 kg ha^{-1}, was in the low P soil, for flax, 30% of seed dry weight, 35% of seed P content and 35% of the total P uptake at harvest in the field. The contribution of AM in low P soil was up to 35% of P uptake and 18% of dry weight. No benefit from AM was found in soil with an intermediate or high content of extractable P (P_{H2O} 5.4 to 21.3 mg kg^{-1}). Instead, yield reductions of as much as 56% were recorded up to a P level that was sufficient to hinder mycorrhization. The results suggest that AM deserves to be considered in the development of sustainable production systems as well as in breeding and soil quality assessment programmes serving sustainable agriculture.

Acknowledgements

The present study was part of a Nordic project conducted at the Agricultural Reseach Centre of Finland (MTT) during 1995 to 1998. It was financially supported by the Finnish Academy via the Nordic Joint Committee for Agricultural Research (NKJ). I acknowledge my supervising professors Juha Helenius and Eija Pehu for their invaluable support and advice. I am most grateful to my supervisor Mauritz Vestberg who introduced me to the world of mycorrhiza, and to him and Elise Ketoja for invaluable guidance, constructive criticism and patient support throughout the study. The preexaminers of my thesis, professors Kristina Lindström and Bengt Söderström, gave many helpful comments to the manuscript. I extend my gratitude to Juhani Mäkelä and Reijo Björkbacka for skillful assistance with the field experiments. Further, I wish to express my warmest thanks to my special team Riitta Bagge, Ilkka Haarala, Pirjo Ikonen, Leena Juuti, Hilkka Järveläinen, Anja Kammonen, Aila Koskinen, Ulla Lautiainen, Pauliina Lehtinen, Pekka Leinonen, Anu Marjo, Hannu Parkkinen, Tuure Peltonen, Kaija Rimppi and Esa Teittinen whose careful work made the study possible. With them I also spent countless enjoyable hours. I am indebted to Elvi and Aaro Rajala, Seija Pajari and Teemu Perho for kind hospitality and the opportunity to utilize their field soil. I further acknowledge Iver Jakobsen, Erik Joner and Ida Thingstrup as well as Kielo Haahtela, Helinä Hartikainen and Robin Sen for fruitful discussions and good advice, Merja Eurola, Leila Lindstedt and Taina Lilja for plant and soil analyses, Matti Latvala for drawing figures, all the other persons who contributed to the work, and my superiors and colleagues especially in Partala, for many kinds of support and help, and for the opportunity to perform this study. Laura Seppänen critically read the introduction, and Sevastiana Ruusamo and Kathleen Ahonen revised my English. Finally, I wish to extend my dearest thanks to my family for the loving support and patience, and for allowing me never to forget what is truly important.

References

Abbott LK and Robson AD 1984 The effect of mycorrhizae on plant growth. *In* VA mycorrhiza. Eds. CL Powell and DJ Bagyaraj. pp. 113-130. CRC Press, Boca Raton.

Abbott LK and Robson AD 1985 Formation of external hyphae in soil by four species of vesicular arbuscular mycorrhizal fungi. New Phytol. 99, 245-255.

Abbott LK and Robson AD 1991a Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. Agric. Ecosyst. Environ. 35, 121-150.

Abbott LK and Robson AD 1991b Field management of mycorrhizal fungi. *In* The rhizosphere and plant growth. Eds. DL Keister and PB Cregan. pp. 355-362. Kluwer Academic Publishers, Netherlands.

Abbott LK, Robson AD, De Boer G 1984 The effect of phosphorus on the formation of hyphae in soil by the vesicular-arbuscular mycorrhizal fungus, *Glomus fasciculatum*. New Phytol. 97, 437-446.

Ackoff RL 1983 The future of operational research is past. J. Operational Res. Soc. 30, 93-104.

Addy HD, Schaffer GF, Miller MH and Peterson RL 1994 Survival of the external mycelium of a VAM fungus in frozen soil over winter. Mycorrhiza 5, 1-5.

Allen MF 1993 The ecology of mycorrhizae. University Press, Cambridge. 184 p.

Allen MF, Moore TS and Christensen M 1980 Phytohormone changes in *Bouteloua gracilis* infected by vesicular-arbuscular mycorrhiza. I. Cytokinin increases in the host plant. Can. J. Bot. 58, 371-374.

Allen MF, Sexton JC, Moore TS and Christensen M 1981 Influence of phosphate source on vesicular arbuscular mycorrhizae of *Bouteloua gracilis*. New Phytol. 87, 687.

Alrøe HF and Kristensen ES 1998 Sustainability and organic farming. *Paper presented at the First SREN Workshop on Research Methodologies in Organic Farming, FAO, 30 Sept. -3 Oct, 1998, Frick, Switzerland*.

Andrade G, Mihara KL, Linderman RG and Bethlenfalvay GJ 1998 Soil aggregation status and rhizobacteria in the mycorrhizosphere. Plant Soil 202, 89-96.

Bailey JE and Safir GR 1978 Effect of benomyl on soybean endomycorrhizae. Phytopath. 8, 1810-1812.

Balaji B, Ba AM, LaRue T, Tepfer D and Piche´ Y 1994 *Pisum sativum* mutants insensitive to nodulation are also insensitive to invasion in vitro by the fungus, *Gigaspora margarita*. Plant Sci. 102, 195-203.

Barea JM, Azcon-Aguilar C and Azcon R 1987 Vesicular-arbuscular mycorrhiza improve both symbiotic N2 fixation and N uptake from soil as assessed with a 15N technique under field conditions. New Phytol. 106, 717-725.

Bawden RJ 1991 Systems thinking and practice in agriculture. J. Dairy Sci. 74, 2362-2373.

Bawden RJ 1995 On the systems dimension in FSR. J. Farming Systems Research-Extension 5, 1-18.

Bawden RJ, Ison RL, Macadam RD, Packham RC and Valentine I 1985 A research paradigm for systems agriculture. *In* Agricultural systems research for developing countries. Ed. JV Remenye. pp. 31-42. Canberra.

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

Bentivenga SP and Hetrick BAD 1991 Relationship between mycorrhizal activity, burning, and plant productivity in tallgrass prairie. Can. J. Bot. 69, 2597-2601.

Berg B, Müller MM and Wessen B 1987 Decomposition of red clover (*Trifolium pratense*) roots. Soil Biol. Biochem. 19, 589-593

Bertalanffy L von 1973 General system theory. Foundations, developments, applications. George Braziller, Inc., N.Y. 311 p.

Bertalanffy L von 1975 Perspectives on general system theory, scientific-philosophical studies. George Braziller, Inc., N.Y.

de Bertoldi M, Griselli M and Rambelli A 1977 Effects of soil applications of benomyl and captan on the growth of onions and the occurrence of endophytic mycorrhizas and rhizosphere microbes. Ann. Appl. Biol. 86, 114-115.

Bethlenfalvay GJ, Brown MS and Pacovsky RS 1982 Parasitic and mutualistic associations between a mycorrhizal fungus and soybean: development of the host plant. Phytopath. 72, 889-893.

Bethlenfalvay GJ, Evans RA and Lesperance AL 1985 Mycorrhizal colonization of crested wheatgrass as influenced by grazing. Agron. J. 77, 233-236.

Bethlenfalvay GJ, Franson RL, Brown MS and Mihara KL 1989 The *Glycine*-*Glomus*-*Bradyrhizobium* symbiosis. IX. Nutritional, morphological and physiological responses of nodulated soybean to geographic isolates of the mycorrhizal fungus *Glomus mosseae*. Physiol. Plant 76, 226-232.

Björklund J, Limburg KE and Rydberg T 1999 Impact of production intensity on the ability of the agricultural landscape to generate ecosystem services: an

example from Sweden. Ecol. Economics 29, 269-291.
Bolan NS, Robson AD and Barrow NJ 1987 Effects of vesicular-arbuscular mycorrhiza on the availability of iron phosphates to plants. Plant Soil 99, 401-410.
Bolan NS 1991 A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant Soil 134, 189-207.
Bookchin M 1982 The ecology of freedom: The emergence and dissolution of hierarchy. Cheshire Books, Palo Alto.
Bowen GD and Rovira AD 1961 Plant growth in irradiated soil. Nature 191, 936-937.
Boyetchko SM and Tewari JP 1995 Susceptibility of barley cultivars to vesicular arbuscular mycorrhizal fungi. Can. J. Plant Sci. 75, 269-275.
Bradbury SM, Peterson RL and Bowley SR 1991 Interactions between three alfalfa nodulation genotypes and two *Glomus* species. New Phytol. 119, 115-120.
Bruce A, Smith SE and Tester M 1994 The development of mycorrhizal infection in cucumber: effects of P supply on root growth, formation of entry points and growth of infection units. New Phytol. 127, 507-514.
Buerkert B and Robson A 1994 ^{65}Zn uptake in subterranean clover (*Trifolium subterraneum* L.) by three vesicular-arbuscular mycorrhizal fungi in a root-free sandy soil. Soil Biol. Biochem. 26,1117-1124.
Bunge M 1985 Treatise on basic philosophy. Vol. 7, Epistemology and methodology III: Philosophy of science and technology. Part II: Life science, social science and technology. Reidel, Dordrecht. 341 p.
Burkhardt J 1989 The morality behind sustainability. J. Agric. Ethics 2, 113-128.
Buwalda JG and Goh KM 1982 Host-fungus competition for carbon as a cause of growth depressions in vesicular arbuscular mycorrhizal ryegrass. Soil Biol. Biochem. 14, 103-106.
Callow JA, Capaccio LCM, Parish G and Tinker PB 1978 Detection and estimation of polyphosphate in vesicular-arbuscular mycorrhizas. New Phytol. 80, 125.
Capra F 1975 The tao of physics. Shambala Publications, Colorado. 330 p.
Capra F 1995 Deep ecology. A new paradigm. *In* Deep ecology for the 21st century. Ed. G. Session. pp. 19-25. Shambala, Boston.
Carson R 1962 Silent spring. Houghton Miflin, Boston.
Checkland PB 1981 Systems thinking, systems practice. John Wiley & Sons Ltd, N.Y. 330 p.
Checkland PB 1988 Images of systems and the systems image: presidential address to ISGSR. J. Appl. Syst. Anal. 15, 37.
Churchman CW 1979 The systems approach. Dell, N.Y.

Conway GR 1985 Agroecosystem analysis. Agric. Administr. 20, 31-55.
Cress WA, Throneberry GO and Lindsey DL 1979 Kinetics of phosphorus absorption by mycorrhizal and nonmycorrhizal tomato roots. Pl. Physiol. 64, 484-487.
Crush JR 1973 The effect of *Rhizophagus tenuis* mycorrhizas on ryegrass, cocksfoot and sweet vernal. New Phytol. 72, 965-973.
Crush JR 1976 Endomycorrhizas and legume growth in some soils of the Mackenzie Basin Canterbury, New Zealand. New Zealand J. Agric. Res. 19, 473-476.
Dighton J 1991 Acquisition of nutrients from organic resources by mycorrhizal autotrophic plants. Experientia 47, 362-369.
Dodd JC, Burton CC, Burns RG and Jeffries PJ 1987 Phosphatase activity associated with the roots and the rhizosphere of plants infected with vesicular-arbuscular mycorrhizal fungi. New Phytol. 107, 163-172.
Dodd J, Arias I, Koomen I and Hayman DS 1990 The management of populations of vesicular-arbuscular mycorrhizal fungi in acid-infertile soils of a savanna ecosystem II. The effects of pre-cropping on the spore populations of native and introduced VAM fungi. Plant Soil 122, 241-247.
Dodd JC and Jeffries P 1989 Effect of fungicides on three vesicular arbuscular mycorrhizal fungi associated with winter wheat (*Triticum aestivum* L.). Biol. Fertil. Soils 7, 120-128.
Douds DD, Janke RR and Peters SE 1993 VAM fungus spore populations and colonization of roots of maize and soybean under conventional and low-input sustainable agriculture. Agric. Ecosyst. Environ. 43, 325-335.
Douds DD Jr and Millner PD 1999 Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. Agric. Ecosyst. Environ. 74, 77-93.
Douds DD Jr, Galvez L, Janke RR and Wagoner P 1995 Effect of tillage and farming system upon populations and distribution of vesicular arbuscular mycorrhizal fungi. Agric. Ecosyst. Environ., 52, 111-118.
Douglass GK 1984 The meanings of agricultural sustainability. *In* Agricultural sustainability in a changing world order 1-29. Ed. GK Douglass, Westview press, Boulder, Colorado.
Eissenstat DM, Graham JH, Syvertsen JP and Drouillard DL 1993 Carbon economy of sour orange in relation to mycorrhizal colonization and phosphorus status. Ann. Bot. 71, 1-10.
Ekholm P 1994 Bioavailability of phosphorus in agriculturally loaded rivers in southern Finland. Hydrobiologia 287, 179-194.
Ekholm P 1998 Algae-available phosphorus originating from agriculture and municipalities. Monographs of the Boreal Environ. Res. 11. 60p. Helsinki.

Ekholm P, Kallio K, Turtola E, Rekolainen S and Puustinen M 1999 Simulation of dissolved phosphorus from cropped and grassed clayey soils in southern Finland. Agric. Ecosyst. Environ. 72, 271-283.

Eno C and Popenoe H 1964 Gamma radiation compared with steam and methyl bromide as a soil sterilizing agent. Soil Sci. Soc. Proc. 28, 533-535.

Evans D and Miller MH 1988 Vesicular-arbuscular mycorrhizas and the soil disturbance-induced reduction of nutrient absorption in maize. I Causal relations. New Phytol. 110, 67-74.

van Faassen HG 1974 Effect of fungicide benomyl on some metabolic processes, and on numbers of bacteria and actinomycetes in the soil. Soil Biol. Biochem. 6, 131-133.

Fischer TR, Melack JM, Grobbelaar JU and Howarth RW 1995 Nutrient limitation of phytoplankton and eutrophication of inland, estuarine and marine waters. *In* Phosphorus in the global environment. Ed. H Tiessen. pp. 301-322. John Wiley & Sons, Chichester.

Fitter AH 1986 Effect of benomyl on leaf phosphorus concentration in alpine grasslands: a test of mycorrhizal benefit. New Phytol. 103, 767-776.

Fitter AH 1991 Costs and benefits of mycorrhizas: implications for functioning under natural conditions. Experientia 47, 350-355.

Fitter AH and Nichols R 1988 The use of benomyl to control infection by vesicular-arbuscular mycorrhizal fungi. New Phytol. 110, 201-206.

Fontenla S, Garcia-Romera I and Ocampo JA 1999 Negative influence of non-host plants on the colonization of *Pisum sativum* by the arbuscular mycorrhizal fungus *Glomus mosseae*. Soil Biol. Biochem. 31, 1591-1597.

Francis R and Read DJ 1984 Direct transfer of carbon between plants connected by vesicular-arbuscular mycorrhizal mycelium. Nature 307, 53-56.

Francis R and Read DJ 1994 The contributions of mycorrhizal fungi to the determination of plant community structure. Plant Soil 159, 11-25.

Friese CF and Allen MF 1991 The spread of VA mycorrhizal fungal hyphae in the soil: Inoculum types and external hyphal architecture. Mycologia 83, 404-418.

Garbaye J 1991 Biological interactions in the mycorrhizosphere. Experientia 47, 370-375.

Gianinazzi-Pearson V 1986 A potential for better use of phosphate fertilizer. Fert. Agric. 92, 3-8.

Gildon A and Tinker PB 1983 Interactions of vesicular-arbuscular mycorrhizal infections and heavy metals in plants. II. The effects of infection on uptake of copper. New Phytol. 95, 263-268.

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

Glover JD, Reganold JP and Andrews PK 2000 Systematic method for rating soil quality of conventional, organic, and integrated apple orchards in Washington State. Agric. Ecosyst. Environ. 80, 29-45.

Grace C and Stribley DP 1991 A safer procedure for routine staining of vesicular mycorrhizal fungi. Mycol. Res. 95, 1160-1162.

Graham JH and Eissenstat DM 1998 Field evidence for the carbon cost of citrus mycorrhizas. New Phytol. 140, 103-110.

Graham JH, Linderman RG and Menge JA 1982 Development of external hyphae by different isolates of mycorrhizal *Glomus* spp. in relation to root colonization and growth of Troyer Citrange. New Phytol. 91, 183-189.

Granstedt A 1995 Ekologiskt lantbruk - ett resursekonomiskt brukande av jorden. Organic agriculture - resource-economical cultivation of soil. (In Swedish). pp. 39-51. *In* Luonnonmukaisen tuotannon tutkimusseminaari. Reseach seminar in organic agriculture 13.- 14.2.1995. Agricultural Research Centre of Finland. Jokioinen.

Granstedt A and Westberg L 1993 Flöden av växtnäring i jordbruk och samhälle. Plant nutrient flows in agriculture and society. (In Swedish). Aktuellt från lantbruksuniversitetet 416. Swedish Agricultural University. Uppsala. 32 p.

Graw D 1979 The influence of soil pH on the efficiency of vesicular arbuscular mycorrhiza. New Phytol. 82, 687-695.

Gryndler M and Lipavsky J 1995 Effect of phosphate fertilization on the populations of arbuscular mycorrhizal fungi. Rostlinna Vyroba 41, 533-540.

Habte M 1997 Use of Benlate to obtain soil free of arbuscular mycorrhizal fungal activity for greenhouse investigations. Arid Soil Res. Rehabil. 11, 151-161.

Hamel C, Neeser C, Bannates-Cartin U and Smith DL 1991 Endomycorrhizal fungal mediate 15N transfer from soybean to maize in non-fumigated soil. Plant Soil 138, 41-47.

Harley JL 1975 Problems in mycotrophy. *In* Endomycorrhizas. Eds. FE Sanders, B Mosse and PB Tinker. pp.1-24. Academic Press, London.

Harley JL and Smith SE 1983 Mycorrhizal symbiosis. Academic Press, London. 483 p.

Harris D, Pacovsky RS and Paul EA 1985 Carbon economy of soybean, *Rhizobium*, *Glomus* associations. New Phytol. 101, 427-440.

Hartikainen H 1979 Phosphorus and its reactions in terrestrial soils and lake sediments. J. Sci. Agric. Soc. Finl. 51, 537-624.

Hartikainen H 1982 Water soluble phosphorus in Finnish mineral soils and its dependence on soil properties. J. Sci. Agric. Soc. Finl. 54, 89-98.
Hartikainen H 1991 Potential mobility of accumulated phosphorus in soil as estimated by the indices of Q/I plots and by extractant. Soil Sci. 152, 204-209.
Heap AJ and Newman EI 1980 The influence of vesicular-arbuscular mycorrhizass on phosphorus transfer between plants. New Phytol 85, 173-179.
Heggo A, Angle JS and Chaney RL 1990 Effects of vesicular-arbuscular mycorrhizal fungi on heavy metal uptake by soybeans. Soil Biol. Biochem. 22, 865-869.
Heckrath G, Brookes PC, Poulton PR and Gouldin KWT 1995 Phosphorus leaching from soils containing different phosphorus concentrations in the Broadbalk experiment. J. Environ. Qual. 24, 904-910.
Helweg A 1973 Undersoegelser over fungicidet benomyl i jord. II. Benomyls indflydelse på jordbundens mikroflora. Investigations on the fungicide benomyl in soil. II. The effect of benomyl on soil microflora. (In Danish). Statens Forsogsvirksomhed i Plantekultur 1097, 375-384
Herold A 1980 Regulation of photosynthesis by sink activity - the missing link. New Phytol. 86, 131-144.
Hetrick BAD 1989 Acquisition of phosphorus by VA mycorrhizal fungi and the growth responses of their host plants. *In* Nitrogen, phosphorus and sulphur utilization by fungi. Eds. L Boddy, R Marchant and DJ Read British Mycol. Soc., Cambridge Univ. Press, Cambridge.
Hetrick BAD, Wilson GWT, Cox TS 1992a Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors. Can. J. Bot. 70, 2032-2040.
Hetrick BAD, Wilson GWT, Cox TS 1992b Mycorrhizal dependence of modern wheat cultivars and ancestors: a synthesis. Can. J. Bot. 71, 512-518.
Hetrick BAD, Wilson GWT and Todd TC 1996 Mycorrhizal response in wheat cultivars: relationship to phosphorus. Can. J. Bot. 74, 19-25.
Hoegh-Jensen H 1998 Systems theory as a scientific approach towards organic farming. Biol. Agric. Hortic. 16, 37-52.
Howarth RW, Jensen HS, Marino R and Postma H 1995 Transport to and processing of P in near-shore and oceanic waters. *In* Phosphorus in the global environment. Ed. H Tiessen. pp 323-345. John Wiley & Sons, Chichester.
Huang C-YL and Schulte EE 1985 Digestion of plant tissue for analysis by ICP emission spectroscopy. Commun. in Soil Sci. Plant Anal. 16, 943-958.
IFOAM International Federation of Organic Agriculture Movements 1998 Basic standards for organic production and processing. 60 p. Werbedruck, St. Wendel.

Jackman RH and Mouat MCH 1972 Competition between grass and clover for phosphate. I Effect of browntop (*Agrostis tenuis* Sibth.) on white clover (*Trifolium repens* L.) growth and nitrogen fixation. N.Z. J. Agric. Res. 15, 653-666.

Jakobsen I 1984 Mycorrhizal infectivity of soil eliminated by low doses of ionizing radiation. Soil Biol. Biochem. 16, 181-182.

Jakobsen I 1994 Research approaches to study the functioning of vesicular-arbuscular mycorrhizas in the field. Plant Soil 159,141-147.

Jakobsen I and Andersen AJ 1982 Vesicular-arbuscular mycorrhiza and growth in barley. Effects of irradiation and heating of soil. Soil Biol. Biochem. 14, 171-178.

Jakobsen I and Rosendahl L 1990 Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. New Phytol. 115, 77-83.

Jakobsen I and Vestberg M 1998 Limited intraspecific variation in phosphorus uptake potential among isolates of *Glomus fistulosum* and *G. mosseae*. In Programme and Abstracts. Second Int. Conf. on Mycorrhiza, Uppsala, Sweden. 89 p.

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

Jakobsen I, Abbott LK, Robson AD 1992b External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 2. Hyphal transport of ^{32}P over defined distances. New Phytol. 120, 509-516.

Jansson H 1998 Maan helppoliukoinen fosfori valumavesien fosforipitoisuuden säätelijänä. Soil soluble phosphorus as the determinant of the phosphorus concentration of runoff waters. (In Finnish). Paper presented at Agro-Food symposium, February 3-5, 1998, Tampere, Finland.

Jantsch E 1975 Design for evolution. George Braziller, N.Y.

Jastrow JD, Miller RM and Lussenhop J 1998 Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. Soil Biol. Biochem. 30, 905-916.

Johansen A, Jakobsen I and Jensen ES 1993 Hyphal transport by a vesicular arbuscular mycorrhizal fungus of N applied to the soil as ammonium or nitrate. Biol. Fertil. Soils 16, 66-70.

Johnson NC 1993 Can fertilization of soil select less mutualistic mycorrhizae. Ecol. Applic. 3, 749-757.

Johnson NC, Copeland PJ, Crookston RK and Pfleger FL 1992a Mycorrhizae: Possible explanation for yield decline with continuous corn and soybean. Agron. J. 84, 387-390.

Johnson NC, Tilman D and Wedin D 1992b Plant and soil controls on mycorrhizal fungal communities. Ecol. 73, 2034-2042.

Johnson NC, Graham JH and Smith FA 1997 Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytol. 135, 575-585.

Johnson NC, O'Dell TE and Bledsoe CS 1999 Methods for ecological studies of mycorrhizae. Standard soil methods for long-term ecological research 18, 378-412.

Joner EJ and Jakobsen I 1994a Contribution by two arbuscular mycorrhizal fungi to P uptake by cucumber (*Cucumis sativus* L.) from ^{32}P-labelled organic matter during mineralization in soil. Plant Soil 163, 203-209.

Joner EJ and Jakobsen I 1994b Uptake of ^{32}P from labelled organic matter by mycorrhizal and non-mycorrhizal subterranean clover (*Trifolium subterraneum* L.). Plant Soil 172, 221-227.

Joner EJ, Magid J, Gahoonia, TS and Jakobsen I 1995 P depletion and activity of phosphatases in the rhizosphere of mycorrhizal and non-mycorrhizal cucumber (*Cucumis sativus* L.). Soil Biol. Biochem. 27, 1145-1151.

Joner EJ and Jakobsen I 1995 Growth and extracellular phosphatase activity of arbuscular mycorrhizal hyphae as influenced by soil organic matter. Soil Biol. Biochem. 27, 1153-1159.

Kahiluoto H and Vestberg M 1998 The effect of arbuscular mycorrhiza on biomass production and phosphorus uptake from sparingly soluble sources by leek (*Allium porrum* L.) in Finnish field soils. Biol. Agric. Hort. 16, 65-85.

Kahiluoto H and Vestberg M 1999 Impact of cropping system on mycorrhiza. *In* Designing and testing crop rotations for organic farming. Eds. JE Olesen, R Eltun, MJ Gooding, ES Jensen and U Köpke. DARCOF Report no. 1. pp. 305-310.

Kahiluoto H and Vestberg M 2000 Utilization of mycorrhiza by system management. *In* Proc. 13th Int. IFOAM Sci. Conf. Eds. Th. Alföldi, W. Lockeretz and U. Niggli. p. 12.

Karlen DL, Mausbach MJ, Doran JW, Cline RG, Harris RF and Schuman GE 1997 Soil quality: A concept, definition, and framework for evaluation. Soil Sci. Soc. Am. J. 61, 4-10.

Kauppi L 1984 The contribution of agricultural loading to eutrophication in Finnish lakes. Wat. Sci. Tech. 17, 1133-1140.

Keeney DR and Nelson DW 1982 Nitrogen - Inorganic forms. Methods of Soil Analysis. Part 2. Chemical and microbiological properties. Agron. 9. pp 643-698. Madison, Wisconsin.

Klironomos JN and Kendrick WB 1993 Research on Mycorrhizas - Trends in the Past 40 years as expressed in the Mycolit Database. New Phytol. 125, 595-600.

Kneen BE, Weeden NF and LaRue TA 1994 Non-nodulating mutants of *Pisum sativum* L. cv. Sparkle. J. Heredity 85, 129-133.

Koide R 1985 The nature of growth depressions in sunflower caused by vesicular-arbuscular mycorrhizal infection. New Phytol. 99, 449-462.

Kormanik P P and McGraw A C 1982 Quantification of vesicular arbuscular mycorrhizae in plant roots. *In* Methods and principles of mycorrhizal research. Ed. NC Schenk. pp. 37-45. Amer. Phytopathol. Soc., St. Paul, Minn.

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

Kough JL and Linderman RG 1986 Monitoring extra-matrical hyphae of a vesicular-arbuscular mycorrhizal fungus with an immunofluorescence assay and the soil aggregation technique. Soil Biol. Biochem. 18, 309-313.

Kucey RMN and Paul EA 1982 Carbon flow, photosynthesis and N_2 fixation in mycorrhizal and nodulated Faba beans (*Vicia faba* L.) Soil Biol. Biochem. 14, 407-412.

Kuhn TS 1970 The structure of scientific revolution. University of Chicago Press, Chicago. 210 p.

Kristensen ES and Halberg N 1997 A systems approach for assessing sustainability in livestock farms. *In* Livestock farming systems – More than food production. Proc. 4th int. symposium on livestock farming systems. EEAP Publ. No 89, 16-30.

Lakanen E and Erviö R 1971 A comparison of eight extractants for the determination of plant available micronutrients in soils. Acta Agr. Fenn. 123, 223-232.

LaRue TA and Weeden NF 1994 The symbiosis genes of the host. *In* Proc. 1st European nitrogen fixation conf. Eds. GB Kiss and G Endre. Szeged, Hungary.

Laszlo, E. And Laszlo, A. 1997 The contribution of the systems sciences to the humanities. Syst. Res. Behav. Sci. 14, 5-19.

Lee KE and Pankhurst CE 1992 Soil organisms and sustainable productivity. Aust. J. Soil Res. 30, 855-892.

Li X-L, George E and Marschner H 1991 Phosphorus depletion and pH decrease at the root-soil and hyphae-soil interfaces of VA mycorrhiza white clover fertilized with ammonium. New Phytol. 119, 397-404.

Lieblein G (ed.) 1995 Research methods in ecological agriculture. First Nordic postgraduate course in ecological agriculture. Dep. Hortic. Crop Sci., Agric. Univ. Norway (NLH).

Lieblein G (ed.) 1997 From farming systems to food systems. Third Nordic postgraduate course in ecological agriculture. Dep. Hortic. Crop Sci., Agric. Univ. Norway (NLH).

Linden B 1981 Ammonium- och nitratkvävets rörelser och fördelning i marken. II Metoder för mineralkväveprovtagning och analys. Movements and distribution of ammonium and nitrate nitrogen in soil. (In Swedish). Sveriges Lantbr.univ. Rapp. 137, 1-79.

Linderman RG 1988 Mycorrhizal interactions with the rhizosphere microflora: The mycorrhizosphere effect. Phytopath. 78, 366-371. Littell RC, Milliken GA, Stroup WW and Wolfinger RD 1996 SAS system for mixed models. SAS Insitute Inc. Cary, NC.

Lockeretz W 2000 Organic farming research, today and tomorrow. *In* Proc. 13th Int. IFOAM Sci. Conf. Eds. T. Alföldi, W. Lockeretz and U. Niggli. pp. 718-720. Hochschulverlag, Zurich.

Lockeretz W and Boehncke E 2000 Agricultural systems research. *In* Proc. 2nd NAHWOA Workshop, Gordoba, Spain. pp. 5-12.

Lu S, Braunberger PG and Miller MH 1994 Response of vesicular-arbuscular mycorrhizas of maize to various rates of P addition to different rooting zones. Plant Soil 158, 119-129.

Mäder P, Vierheilig M Alt and Wiemken A 1993 Boundaries between soil compartments formed by microporous hydrophobic membranes (GORE TEX®) can be crossed by vesicular-arbuscular mycorrhizal fungi but not by ions in the soil solution. Plant Soil 152, 201-206.

Mäder P, Vierheilig H, Streitwolf-Engel R, Boller T, Frey B, Christie P and Wiemken A 2000 Transport of ^{15}N from soil compartment separated by a polutetrafluoroethylene membrane to plant roots via the hyphae of arbuscular mycorrhizal fungi. New Phytol. 146, 155-161.

Manske GGB 1990 Genetical analysis of the efficiency of VA mycorrhiza with spring wheat. I. Genotypical differences and a reciprocal cross between an efficient and non-efficient variety. *In* Genetic aspects of plant mineral nutrition. Eds. N El Bassam et al. Kluwer Academic Publishers, Netherlands, pp. 397-405.

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

McGonigle TP and Fitter AH 1988 Growth and phosphorus inflows of *Trifolium repens* with a range of indigenous vesicular-arbuscular mycorrhizal infection levels under field conditions. New Phytol. 108, 59-65.

McLaren AD 1969 Radiation as a technique in soil biology and biochemistry. Soil Biol. Biochem. 1, 63-73.

Menge JA 1983 Utilization of vesicular-arbuscular mycorrhizal fungi in agriculture. Can. J. Bot. 61, 1015-1024.

Menge JA, Steirle D, Bagyaraj DJ, Johnson ELV and Leonard RT 1978 Phosphorus concentrations in plants responsible for inhibition of mycorrhizal infection. New Phytol. 80, 575-578.

Merryweather J and Fitter A 1995 Phosphorus and carbon budgets: mycorrhizal contribution in *Hyacinthoides non-scripta* (L.) Chouard ex Rothm. under natural conditions. New Phytol. 129, 619-627.

Miller M, McGonigle T and Addy H 1994 An economic approach to evaluate the role of mycorrhizas in managed ecosystems. Plant Soil 159, 27-35.

Ministry of the Environment 1998 Vesiensuojelun tavoitteet vuoteen 2005. The goals of water protection until the year 2005. (In Finnish). The Finnish Environment 226, Ministry of the Environment, Helsinki, Finland.

de Miranda JCC, Harris PJ and Wild A 1989 Effects of soil and plant phosphorus concentrations on vesicular-arbuscular mycorrhiza in sorghum plants. New Phytol. 123, 405-410.

de Miranda JCC and Harris PJ 1994 Effects of soil phosphorus on spore germination and hyphal growth of arbuscular mycorrhizal fungi. New Phytol. 128, 103-108.

Morton JB 1990 Species and clones of arbuscular mycorrhizal fungi (Glomales, Zygomycetes): their role in macro- and micro-evolutionary processes. Mycotaxon 37, 493-515.

Morton JB and Benny GL 1990 Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): A new order, Glomales, two new suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. Mycotaxon 37, 471-491.

Mosse B 1972 Effects of different Endogone strains on the growth of *Paspalum notatum*. Nature 239, 221-223.
Mosse B 1973 Advances in the study of vesicular-arbuscular mycorrhiza. Ann. Rev. Phytopath. 11, 171-196.
Mosse B 1986 Mycorrhiza in sustainable agriculture. Biol. Agric. Hortic. 3, 191-209.
Müller MM 1987 The release and fate of clover nitrogen in soil. Acad. Diss. Dep. General Microbiol. Univ. Helsinki. Helsinki. 53 p. 6 encl.
Murphy J and Riley JP 1962 A modified single solution method for the determination of phosphate in natural water. Anal. Chim. Acta 27, 31-36.
Mäntylahti V 1996 Viljelymaiden fosforipitoisuuden kehitys. The development of the P concentration in field soils. (In Finnish). *In* Viljelymaiden fosforitalous. pp. 6-7. Agricultural Research Centre of Finland, Jokioinen.
Nelsen CE 1987 The water relations of vesicular-arbuscular mycorrhizal systems. *In* Ecophysiology of mycorrhizal plants. Ed. GR Safir. pp. 71-91. Boca Raton.
Nye PB 1977 The rate-limiting step in plant nutrient absorption from soil. Soil Sci. 123, 292-297.
Nye PH and Tinker PB 1977 Solute movement in the soil-root system. Blackwell Scientific Publications, Oxford. 342 p.
Ocampo JA and Hayman DS 1980 Effects of pesticides on mycorrhiza in field-grown barley, maize and potatoes. Trans. Br. Mycol. Soc. 74, 413-416.
Odum HT 1983 Systems ecology. Wiley, N.Y. 644 p.
Odum HT 1988 Self organization, transformity, and information. Science 242, 1132-1139.
O'Keefe DM and Sylvia DM 1992 Chronology and mechanisms of P-uptake by mycorrhizal sweet potato plants. New Phytol. 122, 651-659.
Olsen SR, Cole CV, Watanabe FS and Dean LA 1954 Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Dept. Agric. Circ. 939, 1-19.
Olsson PA, Bååth E and Jakobsen I 1997 Phosphorus effects on mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by fatty acid signatures. Appl. Environ. Microb. 63, 3531-3538.
van der Paauw F 1971 An effective water extraction method for the determination of plant-available soil phosphorus. Plant Soil 34, 467-481.
Pacovsky RS 1986 Micronutrient uptake and distribution in mycorrhizal or phosphorus-fertilized soybeans. Plant Soil 95, 379-388.
Pande M and Tarafdar JC 1999 Critical level of VAM fungal propagules in different arid crops. Agrochimica 43, 187-192.
Peltola R, Seuri PM, Granstedt A, Parviainen T and Vehkasalo V 1995 Ympäristö-

taloudellisesti kestävän maatalouden mahdollisuudet Mikkelin läänissä. The opportunities of environmental-economically sustainable agriculture in the county of St Michel. (In Finnish). University of Helsinki, Rural Research and Training Centre, St. Michel. Publ. 42. 81 p.

Perrin R 1990 Interactions between mycorrhizae and diseases caused by soil-borne fungi. Soil Use Managem. 6, 189-195.

Pirozynski KA and Malloch DW 1975 The origin of land plants: a matter of mycotrophism. Biosystems 6, 153-164.

Porter WM, Abbott LK and Robson AD 1978 Effect of rate of application of superphosphate on populations of vesicular arbuscular endophytes. Austr. J. Experim. Agric. Animal Husb. 18, 573-578.

Powlson DS and Jenkinson DS 1976 The effects of biocidal treatments on metabolism in soil II. Gamma radiation, autoclaving, air-drying and fumigation. Soil Biol. Biochem. 8, 179-188.

Puustinen M, Merilä E, Palko J and Seuna P 1994 Kuivatustila, viljelykäytäntö ja vesistökuormitukseen vaikuttavat ominaisuudet Suomen pelloilla. The drainage, cultivation practice and the soil properties influencing to the loading on waters by the Finnish fields. (In Finnish.) Publ. National Board of Waters and the Environment A 198, 319 p.

Raju PS, Clark RB, Ellis JR, Duncan RR and Maranwille JW 1990 Benefit and cost analysis and phosphorus efficiency of VA mycorrhizal fungi colonizations with sorghum (*Sorghum bicolor*) genotypes grown at varied phosphorus levels. Plant Soil 124, 199-204.

Ravnskov S and Jakobsen I 1995 Functional compatibility in arbuscular mycorrhizas measured as hyphal P transport to the plant. New Phytol. 129, 611-618.

Raznikiewicz H, Carlgren K and Mårtensson A 1994 Impact of phosphorus fertilization and liming on the presence of arbuscular mycorrhizal spores in a Swedish long-term field experiment. Swedish J. Agric. Res. 24, 157-164.

Reganold JP, Elliott LF and Unger YL 1987 Long-term effects of organic and conventional farming on soil erosion. Nature 330, 370-372.

Reganold JP, Papendick RI and Parr JF 1990 Sustainable agriculture. Sci. Am. 262, 72-78.

Rekolainen S 1989 Phosphorus and nitrogen load from forest and agricultural areas in Finland. Aqua Fenn. 19, 95-107.

Rekolainen S 1993 Assesment and mitigation of agricultural water pollution. Publ. Wat. Env. Res. Inst. 12, National Board of Waters and the Environment, Helsinki, Finland.

Rekolainen S, Pitkänen H, Bleeker A and Felix S 1995 Nitrogen and phosphorus fluxes from Finnish Agricultural areas to the Baltic Sea. Nord. Hydrol. 26, 55-72.

Rhodes LH and Gerdemann JW 1978a Translocation of calsium and phosphate by external hyphae of vesicular arbuscular mycorrhizae. Soil Sci. 126, 125-126.

Rhodes LH and Gerdemann JW 1978b Influence of phosphorus nutrition on sulfur uptake by vesicular arbuscular mycorrhizae of onion. Soil Biol. Biochem. 10, 361-364.

Ruiz-Lozano JM and Azcon R 1995 Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. Physiologia plantarum 95, 472-478.

Ryti R 1965 On the determination of soil pH. J. Sci. Agric. Soc. Finl. 37, 51-60.

Saarela I, Järvi A, Hakkola H and Rinne K 1995 Phosphorus fertilizer rate trials, 1977-1994. Effects of the rate of annual phosphorus application on soil fertility and yields of field crops in long-term field experiments. In Finnish with a summary in English. Agricultural Research Centre of Finland, Tiedote 16/95. 94 p.

Saif SR 1983 The influence of soil aeration on the efficiency of vesicular-arbuscular mycorrhizas. II. Effect of soil oxygen on growth and mineral uptake in *Eupatorium odoratum* L., *Sorghum bicolor* (L.) Moench and *Guizotia abyssinica* (L.f.) Cass. inoculated with vesicular-arbuscular mycorrhizal fungi. New Phytol. 95, 405-417.

Sanders FE 1975 The effect of foliar-applied phosphate on the mycorrhizal infections of onion roots. *In* Endomycorrhizas. Eds. FE Sanders, B Mosse, PB Tinker. pp. 261-276. Academic Press, London.

Sanders FE 1997 Plant breeding as a tool for improving the acquisition and use of P by plants. *In* COST-Action 821. Arbuscular mycorrhizas in sustainable soil-plant systems. Report of 1996 activities. p. 162. European communities. Luxembourg.

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

Sanders IR 1993 Temporal infectivity and specificity of vesicular-arbuscular mycorrhizas in coexisting grassland species. Oecologia 93, 349-355.

Schreiner RP and Bethlenfalvay GJ 1997 Mycorrhizae, biocides, and biocontrol. 4. Response of a mixed culture of arbuscular mycorrhizal fungi and host plant to three fungicides. Biol. Fertil. Soils 23, 189-195.

Schuepp H, Miller DD and Bodmer M 1987 A new technique for monitoring hyphal growth of vesicular arbuscular mycorrhizal fungi through soil. Trans. Br. Myc. Soc. 89, 429-435.

Schwab SM, Menge JA and Tinker PB 1991 Regulation of nutrient transfer between host and fungus in VA mycorrhizae. New Phytol. 117, 387-398.

Schweiger PF and Jakobsen I 1999 Direct measurement of arbuscular mycorrhizal phosphorus uptake into field-grown winter wheat. Agron. J. 91, 998-1002.

Sen R 1992 Isozyme analyses of mycorrhizal fungi and their mycorrhiza in identification, population biology and competition studies. Acad. diss. in general microbiol., Helsinki University. NurmiPrint Oy, Nurmijärvi. 359 p.

Sibbesen E, Skjoeth F and Christensen BT 1995 Soil and substance movement between plots in long-term field experiments. In The Askov long-term experiments on animal manure and mineral fertilizers, 100th Anniversary Workshop: Askov Experimental Station, 1994. Eds. BT Christensen and Trentemoeller. Tjele Danish Institute of Plant and Soil Science.

Singh JP, Karamanos RE and Stewart JWB 1986 Phosphorus-induced zinc deficiency in wheat on residual phosphorus plots. Agron. J. 78, 668-675.

Sinha AP, Singh K and Mukhopadhyay AN 1988 Soil fungicides, Vol I. CRC Press, Boca Raton, Florida.

Smith SE, Robson D and Abbott LK 1992 The involvement of mycorrhizas in assessment of genetically dependent efficiency of nutrient uptake and use. Plant Soil 146, 169-179.

Snellgrove RC, Stribley DP, Tinker PB and Lawlor DW 1986 The effect of vesicular-arbuscular mycorrhizal infection on photosynthesis and carbon distribution in leek plants. In proc. 1st ESM, Dijon, France.

Sojka RE and Upchurch DR 1999 Reservations regarding the soil quality concept. Soil Sci. Soc. Am. J. 63, 1039-1054.

Spedding CRW 1979 An introduction to agricultural systems. Elsevier Appl. Sci. Publ., London. 69 p.

St. John TV, Coleman DC and Reid CPP 1983 Association of vesicular arbuscular mycorrhizal hyphae with organic particles. Ecol. 64, 957-959.

Sylvia DM 1990 Distribution, structure and function of external hyphae of vesicular-arbuscular mycorrhizal fungi. *In* Rhizosphere dynamics. Eds. JE Box and LH Hammond. pp. 144-167. Westview Press, Boulder.

Sylvia DM and Neal LH 1990 Nitrogen affects the phosphorus response of VA mycorrhiza. New Phytol. 115, 303-310.

Sylvia DM and Schenck NC 1983 Application of superphosphate to mycorrhizal plants stimulates sporulation of phosphorus-tolerant vesicular-arbuscular mycorrhizal fungi. New Phytol. 95, 655-661.

Tarafdar JC and Marschner H 1994a Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. Soil Biol. Biochem., 26, 387, 395.

Tarafdar JC and Marschner H 1994b Efficiency of VAM hyphae in utilisation of organic phosphorus by wheat plants. Soil Sci. Plant Nutr. 40, 593-600.

Thingstrup I, Rubaek G, Sibbesen E and Jakobsen I 1998 Flax (*Linum usitatissimum* L.) depends on arbuscular mycorrhizal fungi for growth and P uptake at intermediate but not high soil P levels in the field. Plant Soil 203, 37-46.

Thomas GW 1982 Exchangeable cations. *In* Eds. AL Page, RH Miller, DR Keeney. Methods of soil analysis, part 2. Amer. Soc. Agron. Inc., p. 159. Madison, Wisc.

Thomson BD, Robson AD and Abbott LK 1986 Effects of phosphorus on the formation of mycorrhizas by *Gigaspora calospora* and *Glomus fasciculatum* in relation to root carbohydrates. New Phytol. 103, 751-765.

Thompson JP 1987 Decline of vesicular-arbuscular mycorrhizae in long fallow disorder of field crops, and its expression in phosphorus deficiency of sunflower. Aust. J. Agric. Res. 38, 847-867.

Thompson JP 1990 Soil sterilization methods to show VA-mycorrhizae aid P and Zn nutrition of wheat in vertisols. Soil Biol. Biochem. 22, 229-240.

Thompson JP 1994 What is the potential for management of mycorrhizas in agriculture? *In* Management of mycorrhizas in agriculture, horticulture and forestry. Eds. LK Abbott and N Malajzuk. pp. 191-200. Kluwer Academic Publishers, Dordrecht/Boston/London.

Thompson JP 1996 Correction of dual phosphorus and zinc deficiencies of linseed (*Linum usitatissimum* L.) with cultures of vesicular-arbuscular mycorrhizal fungi. Soil Biol. Biochem. 28, 941-951.

Thompson PB 1992 The varieties of sustainability. Agric. Human Values 9, 11-19.

Thompson PB 1997 The varieties of sustainability in livestock farming. *In* Livestock farming systems – more than food production, proc. 4th int. symp. livestock farming systems. Ed. JT Sörensen. EEAP Publ. No. 89, 5-15.

Tinker PB, Durall DM and Jones, MD 1994 Carbon use efficiency in mycorrhizas: theory and sample calculations. New Phytol. 128, 115-122.

Tisdall JM and Oades JM 1982 Organic matter and water-stable aggregates in soils. J Soil Sci 33, 141-163

Tisdall JM 1991 Fungal hyphae and structural stability of soil. Aust. J. Soil Res. 29, 729-743.

Torstensson L and Wessen B 1984 Interactions between the fungicide benomyl and soil micro-organisms. Soil Biol. Biochem. 16, 445-452.

Trappe JM 1987 Phylogenetic and ecological aspects of mycotrophy in the angiosperms from an evolutionary standpoint. In Ecophysiology of VA mycorrhizal plants. Ed. GR Safir. pp. 5-25. CRC Press, Boca Raton.

Trouvelot A, Lovato P and Gianinazzi S 1996 Agronomical interest of arbuscular mycorrhizas: Comparative field growth of myc+ and myc- peas. *In* Mycorrhizas in integrated systems from genes to plant development. Eds. C Azcon-Aguilar and JM Barea. pp. 595-598. Granada.

Vestberg M 1995 Occurrence of some Glomales in Finland. Mycorrhiza 5, 329-336.

Väre H, Vestberg M and Eurola S 1992 Mycorrhiza and root-associated fungi in Spitsbergen. Mycorrhiza 1, 93-104.

Wang GM, Stribley DP, Tinker PB and Walker C 1985 Soil pH and vesicular-arbuscular mycorrhizas. *In* Ecological Interactions in Soil. Ed. AH Fitter. pp. 219-224. Blackwell Scientific.

WCED World Commission on Environment and Development 1987. Our common future. Oxford University press, Oxford and New York.

West HM, Fitter AH and Watkinson AR 1993 The influence of three biocides on the fungal associates of the roots of *Vulpia ciliata* ssp. *ambigua* under natural conditions. J. Ecol. 81, 345-350.

White L Jr 1967 Historical roots of our ecological crisis. Science 155, 1203-1207.

Wilson B 1984 Systems: Concepts, methodologies, and applications. John Wiley & Sons, Chichester. 339 p.

Wilson K and Morren GEB Jr. 1990 Systems approaches for improvement in agriculture and resource management. Macmillan Publishing Company, N.Y. 361 p.