Mycorrhiza (2000) 9 : 241–258

ORIGINAL PAPER

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Creation of a non-mycorrhizal control for a bioassay of AM effectiveness

1. Comparison of methods

Received: 9 February 1999 / Accepted: 27 September 1999

Abstract The study aimed to create a control with suppressed mycorrhiza for assessing the effectiveness of field arbuscular mycorrhizal (AM) communities in a bioassay, in terms of plant growth and P uptake. The methods compared were benomyl incorporation into soil, γ-irradiation of soil by 10 and 3 kGy, and the use of a myc$^-$ mutant. The methods were examined on clay and loam. Two management histories were included with both soils to study the ability of the methods to differentiate AM effectiveness. For each soil type, two pot experiments were conducted in field soil, one to investigate the effects of the methods on soil nutrient status, and the other to study the effects on mycorrhization and plant response. The test plants, flax (*Linum usitatissimum*) and pea (*Pisum sativum*) myc$^+$ and myc$^-$ mutants, were grown in 1-l pots for 4 weeks in a growth chamber. To test the ability of the bioassay to reflect differences in AM effectiveness in the field, the mutants and benomyl were also studied in the field from which the loam for the pot experiments was obtained. The bioassay accurately represented the situation in the field and the use of benomyl appeared to be the most appropriate method currently available. The advantages were the ability to use a test plant responsive to AM, the use of less elevated nutrient concentrations than with irradiation, and thus the possibility to use untreated soil as the mycorrhizal treatment. The pea mutants proved unresponsive to AM, and reinoculation to irradiated soil resulted in only half the colonization rate in untreated soil. Benomyl may, however, lead to an underestimation of AM effectiveness because the control is not totally non-mycorrhizal. Its use also carries with it health and environmental risks.

Key words AM effectiveness · Bioassay · Indigenous AMF communities · Field soil · Management history

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Introduction

Most agricultural crops are hosts of the arbuscular mycorrhizal fungi (AMF) commonly present in field soils. Arbuscular mycorrhizal (AM) effects on crop growth, nutrient uptake and soil structure are of crucial agricultural interest. Those effects vary markedly with host, fungus and soil-related factors and they can be managed by cultivation practices. There is an increasing interest in utilizing AM by management of indigenous or introduced AMF instead of regular inoculation. As yet, there is little knowledge of the functioning and effects of field AMF communities, mainly because of methodological problems.

Plant response to mycorrhiza is not only related to propagule density, root colonization (Mosse 1972; Graham et al. 1982; Hetrick et al. 1992) and hyphal length (Abbott and Robson 1985; Jakobsen et al. 1992a; Buerkert and Robson 1994; Ravnskov and Jakobsen 1995) but also to functional aspects related to nutrient uptake. These have been studied by spatial separation of root and hyphal compartments (Schuepp et al. 1987a), often combined with the use of isotopes (Jakobsen et al. 1992b). This approach has been suggested for measuring AM function even in the field (Jakobsen 1994). These methods measure the potential of fungal hyphae to take up, translocate and transfer nutrients. They do not describe the total growth

effect of the multifunctional AM, nor the total AM effect on nutrient uptake because they do not take into account variation of the costs of symbiosis in terms of carbon expenditure, i.e. AM effects on the growth and nutrient uptake of the whole root system. Comparison with a control having blocked or reduced AM formation or functioning is thus the only way to measure the total growth or nutrient uptake effect of AM.

The AM effect on plants is the resultant of plant dependence on AM, AMF community size and structure, soil and climatic conditions, and the compatibility between these factors. The AM contribution to crop growth and nutrient uptake in a particular plant-fungus-soil combination within certain climatic conditions varies with time, and the combination varies both with time and space in the field. The mycorrhizal contribution to plant growth or nutrient uptake in this study is called mycorrhizal effectiveness. Relative mycorrhizal effectiveness (RME) describes the mycorrhizal contribution as a percentage of growth or nutrient uptake of the mycorrhizal plant using standard test plants and soil sampling times. RME is analogous to the concept of relative field mycorrhizal dependence (RFMD) introduced by Plenchette et al. (1983) to compare the mycorrhizal dependence of host plants, and to the mycorrhizal fungi contribution used by Kothari et al. (1991). RME estimated in a bioassay or within one or several growing seasons in the field indicates the crop-related AM effectiveness of the studied soil. The effect of AM on crop growth and nutrient uptake through changes in soil factors caused by AM in the long run is excluded in this kind of assessment.

Creating a non-mycorrhizal control for the field AMF community by partial sterilization of soil changes not only the soil microbial status but also e.g. the nitrogen (N), phosphorus (P) and soil toxin availability (Eno and Popenoe 1963; Messing 1965a,b), thus confounding measurement of AM effectiveness. These effects vary according to soil conditions (Eno and Popenoe 1964) and factors affecting them, such as former management (Powlson and Jenkinson 1976) and stage of the growing season. Among methods of partial sterilization, γ-irradiation has the least undesirable effects (Bowen and Rovira 1961; McLaren 1969; Thompson 1990). However, toxic elements such as Mn or Cu may increase (McLaren 1969). Irradiation doses (2.5 kGy or even 1 kGy) lower than usually recommended (10 kGy) may be enough to eliminate AMF infectivity with fewer changes in soil conditions (Jakobsen and Andersen 1982; Jakobsen 1984; Thompson 1990).

Fungicide treatment has also been used successfully to reduce AM activity. Fungicides seldom prevent mycorrhization completely but have fewer adverse soil effects than 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). Fungicide residues may also be toxic to reinoculated microbes. Benomyl, or the effective compound carbendazim, appears most effective in suppressing AM (e.g., Dodd and Jeffries 1989; West et al. 1993) but the fungicidal effects may be modulated by the AMF community structure (Schreiner and Bethlenfalvay 1996). Fungicides may also have phytotoxic effects.

Another possibility is to use isogenic myc$^-$ mutants of AM hosts (e.g., Bradbury et al. 1991; Balaji et al. 1994) as a non-mycorrhizal control. Adverse effects on soil can thus be avoided, but there are problems with their AM dependence, compatibility with indigenous AMF communities and agricultural relevance due to the limited selection of myc$^-$ mutants available. The phenotypic similarity in parameters other than mycorrhization should be tested thoroughly because the mutated genes may have functions other than regulating nodulation and mycorrhization (LaRue and Weeden 1994).

Although the limitations and advantages of individual methods to create non-mycorrhizal controls 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 risks of benomyl are greatest in field use (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 also have practical value if standardized for routine soil analyses of P availability with relevance to sustainable agriculture.

The objective of the present study was to determine the most appropriate method for creating a non-mycorrhizal control to assess AM effectiveness of field soil in a bioassay in growth chamber, in terms of crop growth and P uptake. The methods compared were incorporation of benomyl, γ-irradiation at the recommended or a lower dose, and use of a non-mycorrhizal mutant. The following criteria for an appropriate methodwere defined: 1) no or only small change in soil conditions, 2) satisfactory suppression of AM in the non-mycorrhizal control and little change in AMF colonization and AM functioning of the mycorrhizal treatment, and 3) difference only in mycorrhization between the mycorrhizal and non-mycorrhizal treatments. Furthermore, the differences in mycorrhizal effectiveness should be clearly indicated and should describe well the differences of effectiveness in the field. Therefore two management histories with presumably different effects on AM were included, and the use of mutants and benomyl were also studied directly in the field from which soil was taken. To increase the generality of the study, two different soil types with their indigenous AMF communities were employed.

Materials and methods

Soil treatments with clay

The abiotic properties of the untreated experimental soils are presented in Table 1. The objective of experiment 1 was to find out which soil treatment least changes the nutrient and toxic element status of clay, and whether the result depends on the farming system. The treatments are presented in Table 2. The clay soil was taken from adjacent fields of two farms with organic and conventional management practices at Loimaa in Southern Finland (60°49′N 23°9′E) on 6 November 1996. The sampling areas were 50 m long and 4 m wide 3–7 m from the field boundary and divided into five blocks. In each block, five subsamples were taken from the plow layer (0–20 cm) and combined into one sample.

The soil was sterilized in 5-cm layers in a moist state by Kolmi-Set Oy (Ilomantsi, Finland). The minimum point radiation doses were 3.1 and 9.6 kGy with average doses per sample of 3.3–3.9 kGy and 10.3–11.7 kGy. After sterilization, the soil layers were left open to detoxify. The water-holding capacity and water content of untreated soil samples were measured. Benomyl in benlate (du Pont de Nemours) 10 mg a.i. per kg soil in target moisture was suspended in water (500 mg l^{-1}) and carefully incorporated into the soil at the same time as the other soil samples were sterilized. The benomyl molecule contains 19% N but, according to the manufacturer, benlate contains no other plant nutrients or metals. Water was added to 60% water-holding capacity. The soil with benomyl was set in 3.5-l black PVC pots (height 165 mm, diameter 160 mm) uncovered and kept at a target moisture by watering to enable decomposition of the fungicide.

The soil samples were incubated in a laboratory at 22 °C. Soils with the same management history were kept together in each block. The order of the treatments within the management histories was random. The blocks corresponded to the blocks in the field. Soil was sampled 5 weeks after the soil treatments. Soil pH was determined by 0.01 M $CaCl_2$ extraction (Ryti 1965), plant-available soil P by water extraction (van der Paauw 1971) and sodium bicarbonate extraction (Olsen et al.1954), and the exchangeable cations potassium (K), calcium (Ca) and magnesium (Mg) by 1 M ammonium acetate extraction (Thomas 1982) of dried soil passed through a 2-mm sieve and analyzed by ICP. The soil mineral N content was determined from frozen (−18 °C) samples by 2 M KCl extraction and by measuring N_{NH4+} and N_{NO3-} concentrations colorimetrically with a Skalar autoanalyzer (Linden 1981; Keeney and Nelson 1982). The possibly toxic elements Al, Cu, Fe and Mn were extracted by acid ammonium acetate-ethylenediaminetetracetic acid solution (Lakanen and Erviö 1971) and analyzed by ICP.

Soil treatments with loam

The soil properties are presented in Table 1. Experiment 2 was similar to experiment 1 on clay with the following exceptions. There were two sampling times and the double dose of benomyl was excluded from the soil treatments (Table 2). The loam soil originated from two P fertilization regimes, 0 and 45 kg P ha^{-1} a^{-1} since 1977, of an experiment in cereal rotation. The experiment took place at the North Savo Research Station of the Agricultural Research Centre of Finland at Maaninka in Central Finland (63°09′N 27°19′E). The samples were collected from each of the four blocks on 6 June 1995 before fertilization and again on 27 August 1995. The minimum point irradiation doses

Table 1 Abiotic properties of the experimental soils

	Soil texture			Organic matter		Exchangeable cations						
	<2 μm (%)	2–63 μm	>63 μm	pH_{CaCl_2} (%)		Ca (meq 100 g^{-1})	K	Mg	P_{NaHCO_3} (mg kg^{-1})	P_{H_2O}	N_{NH4+} (mg kg^{-1})	N_{NO_3}
Clay												
Autumn (Exp. 1, 3)												
Organic	47	37	16	5.6	5.39	7.80	0.29	1.54	24.7	2.5	2.0	2.3
Conventional	–	–	–	5.0	5.45	9.28	0.23	1.48	44.8	9.3	1.1	1.2
Loam												
June (Exp. 2)												
0 kg P ha^{-1} a^{-1}	8	50	42	2.7	5.49	4.26	0.09	0.37	27.2	6.1	1.6	5.7
45 kg P ha^{-1} a^{-1}	–	–	–	–	5.48	5.12	0.09	0.39	69.6	20.1	1.6	6.6
Autumn (Exp. 2, 4)												
0 kg P ha^{-1} a^{-1}	–	–	–	–	5.35	4.62	0.10	0.41	28.5	7.0	13.1	19.2
45 kg P ha^{-1} a^{-1}	–	–	–	–	5.37	4.72	0.06	0.29	74.1	21.7	11.8	18.7
Field experiment												
0 kg P ha^{-1} a^{-1}	8	50	42	2.7	5.53	4.78	0.06	0.40	29.5	6.6	2.0	0.5
45 kg P ha^{-1} a^{-1}	–	–	–	–	5.52	5.11	0.05	0.34	64.1	18.5	2.1	0.4

Table 2 Soil treatments and management histories for soils sampled in autumn for experiment 1 and in June and autumn for experiment 2 (*org* organic, *conv* conventional)

		Experiment 1 (Clay)		Experiment 2 (Loam)	
Soil treatment	Codes used in figures	Org	Conv	0 kg P ha^{-1} a^{-1} 0P	45 kg P ha^{-1} a^{-1} 45P
Untreated	Untreated	+	+	+	+
Benomyl 10 mg kg^{-1}	Ben10	+	+	+	+
Benomyl 20 mg kg^{-1}	Ben20	+	+	–	–
Irradiation 3 kGy	Irr3	+	+	+	+
Irradiation 10 kGy	Irr10	+	+	+	+

were 2.9 and 9.5 kGy with average doses per sample of 3.2–3.3 kGy and 11.5 kGy. Water was added to 50% water-holding capacity. Soil was sampled for analysis 6 weeks after the soil treatments.Bioassay on clay soil

In the bioassays (experiments 3 and 4), the methods to create a non-mycorrhizal control were evaluated in terms of mycorrhization and plant response (see above). Besides γ-irradiation and benomyl, the effect of equalizing the non-mycorrhizal soil microbiota by a sieved water extract of soil was also studied. A further mycorrhizal treatment was included by AMF reinoculation of the irradiated soil. The treatments are presented in Table 3.

The experiment was established on soil originating from the soil treatment experiment with clay (experiment 1). The soil mixtures were prepared and potted 4 weeks after irradiation or benomyl treatment. The water-holding capacity and water content of each soil sample was measured. Soil (650 g dry weight) was carefully mixed with inoculum and water separately for each 1-l (7×26 cm) black PVC pot without drainage. Water was added to 60% water-holding capacity. The irradiated soil was reinoculated by incorporating 5% w/w untreated soil. The non-mycorrhizal microbiota were inoculated by incorporating 21 g per kg dry weight sievings (37-μm sieve) of soil suspended in water. The suspension was prepared by mixing water with untreated soil originating from the same soil sample as the reinoculated soil (4 : 1 w/w) and incubating it for 3 h. The soil mixture was incubated for 10 days before sowing.

The test plant was oil-seed flax (*Linum usitatissimum* L.) cv. Linetta (Deutsche Saatveredelung, Lippstadt-Bremen). The seeds were pregerminated and three seeds were sown per pot. After emergence, they were thinned to one seedling per pot. In the growth chamber, the pots were organized in five blocks so that the soils of the blocks originated from separate blocks in the field. Within the blocks, the treatments, i.e. soil treatment by management history combinations, were in random order and the pots were circulated. The pots were watered to the individual target weight three times a week. Artificial lighting was given by 36 W Gro-Lux Fluorescent Tubes (Sylvania) with a 16-h day length and a temperature of $24/16\,^{\circ}C \pm 0.5\,^{\circ}C$. At emergence, the light intensity was 80–100 and at the top of harvested plants 135–170 μmol s^{-1} m^{-2}. The CO_2 concentration was 510–560 ppm at noon, and the relative humidity was 55–65%.

The experiments were harvested 28 days after sowing and the percentage root lengths colonized measured. A representative sample of the root system was cleared 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). The shoots and roots were cleaned and dried at 60 °C. Their P, K, Ca, Mg and Cu contents were analyzed by wet burning and ICP (Huang and Schulte 1985).

RME was 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. RME was determined for flax with benomyl and for 3 kGy and 10 kGy irradiation. The non-mycorrhizal treatments with reinoculation of non-mycorrhizal microbiota (Ben 10, Irr3 and Irr10, Table 3) were used.

Bioassay on loam

Experiment 4 on loam was similar to experiment 3 on clay with the following exceptions. In addition to the methods in experiment 3, the use of a non-mycorrhizal host plant mutant and the effect of benomyl incubation were studied. Reinoculation of AMF to irradiated soil by sieved water extract of soil was also compared to that by 5% w/w untreated soil, as used in experiment 3 (Table 3).

Soil from the soil treatment experiment with loam (experiment 2) sampled in the autumn was used. The soil mixtures were prepared and potted 3 weeks after the soil treatments. Fertilizers were used in an attempt to simulate the nutrient status of the sampled field soil at the start of the growing season. Soil of dry weight 725 g was carefully mixed with dissolved fertilizers, inoculum and water. The soil was mixed with 110 mg fertilizer (20% N, 15% K, 2% S, 1.5% Mg, 0.03% B, 0.0008% Se) (NK-lannos, Kemira Oy, Finland) and the soil from field plots with 45 kg P ha^{-1} a^{-1} additionally with 204 mg superphosphate (8.5% P, 20% Ca, 11% S) (Superfosfaatti, Kemira Oy, Finland), as used annually in the sampled field. After fertilization, the N_{NH4+} contents were 15.1 and 15.3 mg kg^{-1} and N_{NO3-} contents 25.5 and 26.6 mg kg^{-1} at the lower and higher P levels, respectively.

The sterilized soil was reinoculated alternatively by incorporating 5% w/w untreated soil or by adding 53 g per kg dry weight sievings (0.25-mm sieve) of a soil suspension prepared as for non-mycorrhizal reinoculation in experiment 3. The non-mycorrhizal microbiota were inoculated by incorporating 55 g per kg dry weight sievings (37-μm sieve) of the same suspension. Benomyl without incubation (10 mg kg^{-1}) was incorporated suspended in water (200 mg l^{-1}) immediately before sowing.

Besides the flax cv. Linetta used in experiment 3, two mutant lines of an early freezer pea (*Pisum sativum* L.) cv. Sparkle (Rogers Bros. Seed Co., Twin Falls, Idaho, USA) were also test-

Table 3 Host plants, treatments and management histories of soils used for bioassays in the growth chamber

Host plant	Benomyl (mg kg^{-1})	Irradiation (kGy)	Microbial reinoculatio n	Code used in figures and tables	Experiment 3 (Clay) Org	Conv	Experiment 4 (Loam) 0P	45P
Pea myc+	None	None	None		–	–	+	+
	10	None	None		–	–	+	+
Pea myc –	None	None	None		–	–	+	+
Flax	None	None	None	Untreated	+	+	+	+
	10	None	Non-AMF < 37 μm	Ben10	+	+	+	+
	None	None	None		+	+	+	+
	None[a]	None	None		–	–	+	+
	20	None	None		+	+	–	–
	None	3	Soil 5% w/w	Irr3 myc+	+	+	+	+
	None	3	Non-AMF < 37 μm	Irr3	+	+	+	+
	None	10	Soil 5% w/w	Irr10 myc+	+	+	+	+
	None	10	AMF < 250 μm		–	–	+	–
	None	10	Non-AMF < 37 μm	Irr10	+	+	+	+
	None	10	None		+	+	+	+

[a] no incubation

ed. Seeds of these near-isogenic, non-allelic, non-nodulating and non-nitrogen fixing (Nod^-, Fix^-) derivatives were supplied by T.A. LaRue, Boyce Thompson Institute for Plant Research, Ithaca, N.Y., USA. The mutant line N15 (*sym* 10) forms mycorrhiza but the other mutant line, R72 (*sym* 9), is not able to form AM (Balaji et al. 1994). In the studies by Kneen et al. (1994), the phenotypes of the mutants were similar in non-mycorrhizal conditions. The seeds were pregerminated and two pea and three flax seeds were sown per pot and thinned to one seedling per pot after emergence.

To enable comparison with field results, the soils of the four blocks in the growth chamber originated from separate blocks in the field experiment. RME was determined for with benomyl, 3 kGy irradiation and 10 kGy irradiation.

Field experiment

In order to clarify how representative the results obtained in the bioassay are for field conditions, benomyl and the pea mutants were also studied in a field experiment. The potential of the mutants for assessing mycorrhizal effectiveness directly in the field was also investigated. The other test plant was barley (*Hordeum vulgare* L.) cv. Arra (Agricultural Research Centre of Finland). The treatments were myc^+ and myc^- mutants of pea, barley in untreated soil, and pea myc^+ and barley with benomyl 125 mg a.i. per kg soil divided into two applications. The field experiment had a split-plot design with four blocks. The main plots were the two P levels of the long-term experiment where the soil for experiments 2 and 4 was sampled. The methods of inhibiting AM functioning were randomized into subplots within each main plot.

The sizes of the subplots were 2.5 m^2 with barley and 1 m^2 with pea, 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 water corresponding to the water-holding capacity of the plow layer and incorporated by a rotary hoe to a depth of 16 cm. The field experiment was established after harrowing and fertilization on 6 June for barley and 28 June for pea. Benomyl was applied both just before sowing and 3 weeks before and after sowing of the pregerminated pea seeds and barley seeds, respectively. Every second pea plant and barley row was harvested at flowering, 40 and 42 days, and the rest at 75 and 84 days (11 September and 29 August) after sowing, respectively.

The soil sampled 42 days after fertilization and sowing was analyzed for the treatments with pea myc^+ in untreated soil, barley in untreated soil and barley with benomyl. The soil characteristics of the untreated soil (barley plots) 42 days after fertilization and sowing, on 18 July, are presented in Table 1. The percentage root length colonized was measured 40 days after sowing and at harvest. Plant P content was analyzed as in experiment 3. Shoot N content was measured at flowering by the Dumas method using a Leco FP-428 protein analyzer at 950 °C with a high flow profile. RME was determined for the pea mutants and pea myc^+ with benomyl. The temperature and precipitation at the location of the field experiment during the growing season are presented in Table 4.

Statistical methods

Preliminary examination of the effects of the treatments on soil nutrient and microbial status in loam (experiment 1) revealed that distribution of the response variables in June and in the autumn differed clearly with respect to location and/or spread. Therefore, the data for the two sampling times were modelled separately. The experimental design at both sampling times as well as in clay in the autumn (experiment 2) was a split-plot design where the whole-plot treatments (two management histories) were in a randomized complete-block design and the split-plot treatments (soil treatments) were randomized within each whole plot. Consequently, statistical analysis of the data was based on the common mixed model for a split-plot design including three fixed effects (management history, soil treatment and their interaction) and three random effects (block, whole-plot error and split-plot error). An exception was the P_{H2O} content in loam in June, for which the different P fertilization histories were analyzed separately as randomized complete-block designs. This was because benomyl treatment was applied only at the higher P level and the difference between the fertilization histories was obvious. In the field experiment, the analyses of the soil P_{H2O}, N_{NO3-}, N_{NH4+} and pH contents were based on the corresponding split-plot model where the two P levels were the whole-plot treatments, and the methods of inhibiting AM functioning were the split-plot treatments. All models were fitted using the residual maximum likelihood (REML) estimation method. Accordances of the data with the distribution assumptions of the models were checked by graphic plots. The equality of the spreads across groups was assessed by the spread-level plot (SAS 1991), and the residuals were checked for normality using the box plot (Tukey 1977). Furthermore, the residuals were plotted against the fitted values. Such a plot should have the appearance of a random scatter of points if the assumptions of the model are adequate. Planned comparisons between means were made by two-sided *t*-type tests or 95% confidence intervals (CI). If the 95% CI does not include zero, the difference between means is statistically significant at the 5% level. The analyses were performed by the MIXED procedure of the SAS/STAT software (Littell et al. 1996).

In the randomized complete-block experiments 3 and 4, AMF infection was evaluated by the gridline intersect method. The statistical analyses of the number of infected roots out of 100 intersections were based on the generalized linear mixed model (Littell et al. 1996), which is an extension of the mixed model to accommodate non-normally distributed errors. We assumed that y_{ijk}, the number of infected roots in the ijk^{th} block by management history by treatment combination, was binomially distributed with parameters n and π_{ijk}, where n is the total number of intersections (100) and π_{ijk} is the probability of occurrence of mycorrhizal colonization for a root segment. Further, the model included logit link function and was thus of the following form:

$$\log\left[\pi_{ijk}(1-\pi_{ijk})\right] = m + b_i + \alpha_j + \tau_k + (\alpha\tau)_{jk}$$

where m is the intercept, b_i is the random effect of block, α_j, τ_k, and $(\alpha\tau)_{jk}$ are the fixed effects of management history, treatment and their interaction, respectively. The block effects are assumed to be normally and independently distributed with zero means and constant variances. As there was more variation in the data than could be attributed to the usual binomial mean-variance

Table 4 Temperature and precipitation during the field experiment

Month	Temperature (°C) Mean	Minimum	Maximum	Effective temperature (sum °C)	Precipitation (mm)
June	16.6	5.2	28.8	489.9	78.2
July	15.0	3 .9	25.5	801.0	60.6
August	14.4	4.7	27.8	1093.7	79.4
September[a]	12.8	6.5	13.3	1178.6	7.3

[a] until 11 September

relationship, an extra-dispersion parameter was included in the model. Checking for systematic departures from the model was done by plotting the Pearson residuals against $2\sin^{-1}\sqrt{}$ fitted values. The adequacy of the link function was assessed by plotting the estimated linear predictor against the adjusted dependent variable (McCullagh and Nelder 1989). Comparisons between groups were made by two-sided *t*-type tests. In the field at harvest, the corresponding generalized linear mixed model for a split-plot design was applied. The analyses were performed by the GLIMMIX macro of the SAS/STAT software (Littell et al. 1996).

The statistical analyses of nutrient concentration, uptake rate and dry weight for shoots and roots as well as the analyses of mycorrhizal effectiveness were based on the common mixed model for a randomized complete-block design in loam (experiment 3) and in clay (experiment 4). In the field experiment, the split-plot model, which included P fertilization history, method and their interaction as fixed effects and block, whole-plot error and split-plot error as random effects, was used when analysing shoot P and N concentrations and uptake, shoot dry weight and RME in terms of N uptake measured at flowering. The data on RME in terms of shoot dry weight and P uptake had sampling time as an additional factor and so its main effect and interactions with the three fixed and random effects above were included in the model. Fitting and checking of the models and mean comparisons were performed as in the soil treatment experiments.

Results

Some individual observations are lacking due to occasional technical difficulties. On the basis of spread-level plots, a logarithmic transformation was applied to a few of the response variables to stabilize the variances between groups. The use of the transformation becomes apparent from the text below. Some data included discrepant observations whose discrepancy could not be explained. However, their influence on the results was examined by analyzing the data with and without them. In most cases, the influence was not considered critical and the results based on the whole data are presented; exceptions are mentioned in the text. The figures illustrating the distributions of certain response variables are based on the whole data. The presence of individual outlying values may have led to standard deviations being larger in some groups than in others.

Change in soil conditions

Clay (experiment 1)

One criterion for an appropriate method to create a non-mycorrhizal control was low change in soil nutrient conditions compared with untreated field soil. For soil P_{H2O}, however, the changes were negligible with all the methods. The effects of the treatments varied according to the management history ($F_{4,32} = 8.06$, $P < 0.001$), but this was due to practically insignificant differences in the mean P_{H2O} content in opposite directions within the two management histories (Fig. 1a).

For both farms, clearly the smallest change in soil $N_{soluble}$ content was produced by benomyl, which had no effect on N_{NH4+} and only slightly increased N_{NO3-} content (Fig. 1b, c). Doubling the benomyl dose increased the log-transformed N_{NO3-} content only negligibly in organically managed soil ($P = 0.03$). Generally, the effects of the treatments on the N_{NH4+} and N_{NO3-} contents varied slightly depending on the management history (for log-transformed N_{NO3-} $F_{4,32} = 3.63$, $P = 0.02$), being parallel but lower in soil with conventional management. Irradiation by 3 kGy increased the soil $N_{soluble}$ content considerably less than irradiation by 10 kGy. The reason was that 10 kGy destroyed a larger fraction of microbiota than 3 kGy and benomyl, thus causing an increase of N_{NH4+} content. For log N_{NO3-} content, however, no difference was found between irradiation doses ($P = 0.41$ and 0.12 for log N_{NO3-} in conventionally and organically managed soil, respectively).

Loam (experiment 2)

As for clay, the treatments had no marked effects on soil P_{H2O} in loam with either P fertilization history in June ($P > 0.32$) or in the autumn (Fig. 1a). Only in the autumn was there evidence of a treatment effect ($F_{3,18} = 4.07$, $P = 0.02$), but this was due to a practically insignificant difference between the two irradiation doses.

Regarding change of soil N status compared with untreated soil, the order of the methods did not depend on soil type or on sampling time (Fig. 1b, c). Nevertheless, the magnitudes of the changes tended to vary. With 3 kGy irradiation, the change was greater in loam than in clay, even if clearly smaller than with 10 kGy. Further, with benomyl the change in loam sampled in June was greater than in the autumn. In June, the loam N_{NO3-} content was increased by benomyl to a level between those of the two irradiation doses. However, taking into consideration the simultaneous decrease in the mean N_{NH4+} content, the increase in total $N_{soluble}$ content with benomyl even in June was clearly less than with either of the two irradiation doses. The increase with benomyl was 41% of that in the untreated soil, compared with 86% for irradiation. The differences or lack of differences in the distributions of N_{NH4+} and N_{NO3-} contents between the treatments were so obvious in loam that they made formal significance tests unnecessary.

Irradiation or benomyl treatment showed no ecologically significant effects on soil pH or K, Ca and Mg contents or phytotoxicity agents like Al, Fe, Cu and Mn in any experiment (data not shown).

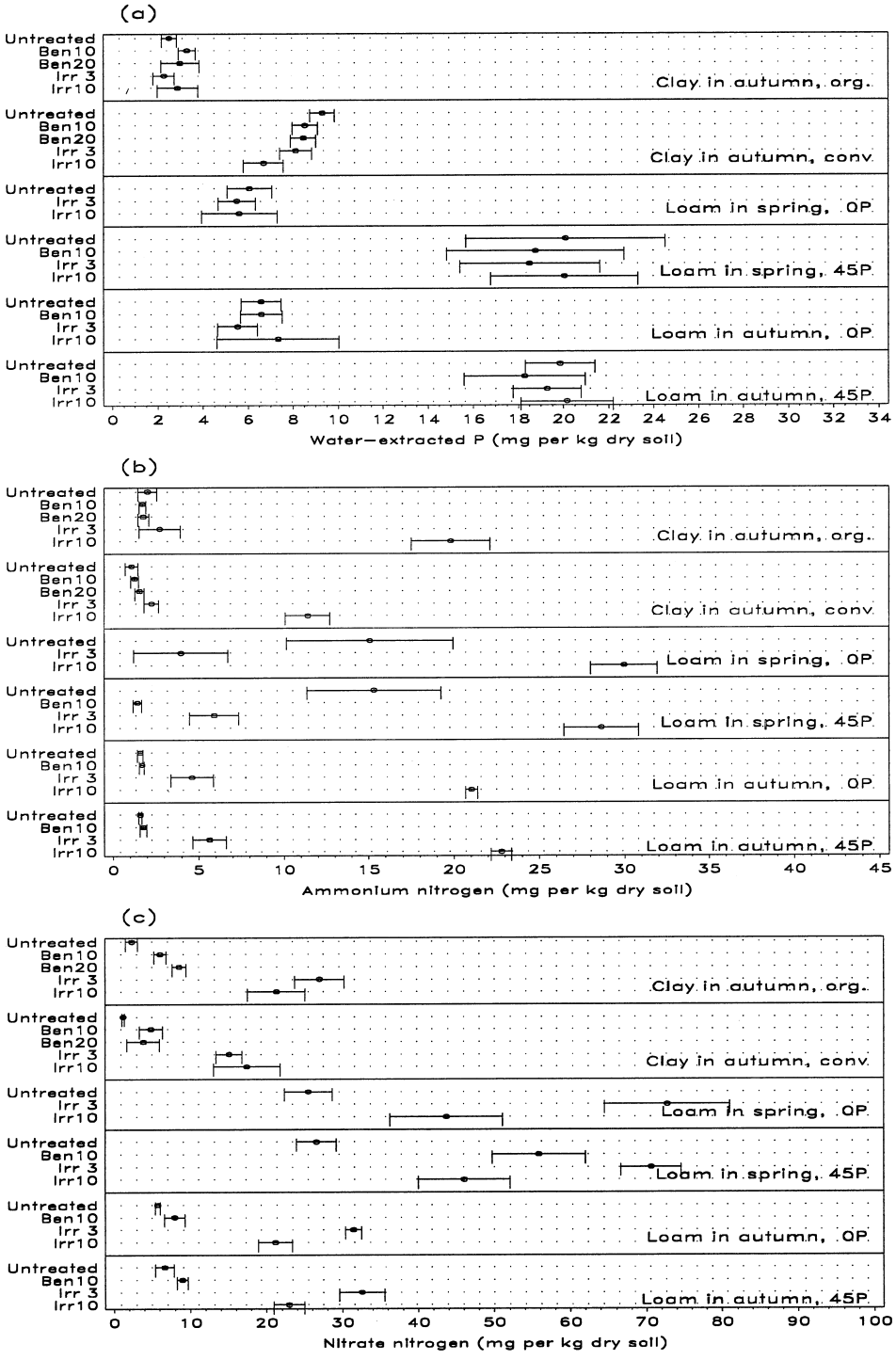


Fig. 1 Effect of soil treatments on **a** water-extracted P (P_{H2O}), **b** ammonium nitrogen (N_{NH4+}) and **c** nitrate nitrogen (N_{NO3-}) concentrations. Values are means of five (experiment 1) or four (experiment 2) replicates. For treatment codes, see Table 2; *bars* standard deviations

Change in AM colonization

Clay (experiment 3)

One criterion for an appropriate non-mycorrhizal control was satisfactory suppression of AMF colonization. With all the methods, the suppression was satisfactory in the non-mycorrhizal control. Irradiation by 10 kGy prevented infection completely and it was, therefore, excluded from modelling of the data. The effects of the methods were slightly dependent on the management history ($F_{6,52} = 1.99$, $P = 0.08$) with greater differences in organically managed soil where colonization was higher. Evidence of difference between the non-mycorrhizal treatments was observed only there, and solely between benomyl and 3 kGy irradiation ($P = 0.02$, $P > 0.12$ for other differences), irradiation being slightly more suppressive. No effect of doubling

the benomyl dose or of reinoculation of the non-mycorrhizal microflora to the controls with suppressed mycorrhization was found on any response variable related to mycorrhization or plant response.

The mycorrhizal treatment should result in colonization levels comparable to those obtained with untreated soil. The negligible changes in soil nutrients with benomyl treatment allowed use of untreated soil as the mycorrhizal treatment for benomyl. In addition, creation of the mycorrhizal treatment by reinoculation of benomyl-treated soil was impossible due to the long persistence of benomyl in soil. Conversely, the drastic changes in soil nutrient status by irradiation made it necessary to use irradiated, reinoculated soil as the mycorrhizal treatment. However, reinoculation did not result in satisfactory colonization. There was a notable difference in colonization between untreated and irradiated, reinoculated soil (PP\s) 0.32) (Fig. 2).

Loam (experiment 4)

The effect of the methods on colonization depended slightly on the soil type, obviously due to differences in the AM potential of the soils. In loam, colonization was higher than in clay, and the effects of the methods were dependent also on the management history ($F_{11,68} = 4.00$, $P < 0.001$). Compared with clay, benomyl at the lower P level suppressed mycorrhization less (Fig. 2) and differed clearly from irradiation ($P = 0.001$ and 0.12 at the lower and higher P levels, respectively, when non-mycorrhizal microflora were reinoculated). Incubation of soil with benomyl for 1 month before sowing had no statistically significant effect on any response variable compared with incorporation of benomyl immediately before sowing. Irradiation by 3 kGy was also as effective as 10 kGy ($P > 0.11$ for difference). Pea myc$^-$ was not infected and, therefore, not included in the statistical modelling of data.

Similarly to clay, reinoculation of irradiated soil did not result in colonization rates at all comparable to untreated soil. As in clay, colonization due to reinoculation depended on management history being closer to untreated soil at the lower P level (Fig. 2, $P < 0.001$ for the difference at both P levels). The colonization rate if reinoculated by a water extract of soil differed from that achieved by untreated soil, 5% w/w ($P < 0.001$) being quite unsatisfactory.

Change in AM functioning

Clay (experiment 3)

Differences in plant P content and growth reflected differences in AM functioning, taking into consideration also the changes in soil nutrient status. The effects contribute to estimates of mycorrhizal effectiveness and explain the differences between the methods. The non-mycorrhizal control must show a sufficient suppression of mycorrhizal functioning. In clay, no clear evidence of difference was found in suppression of plant P content by the different methods (Fig. 3, Table 5). However, higher shoot growth in

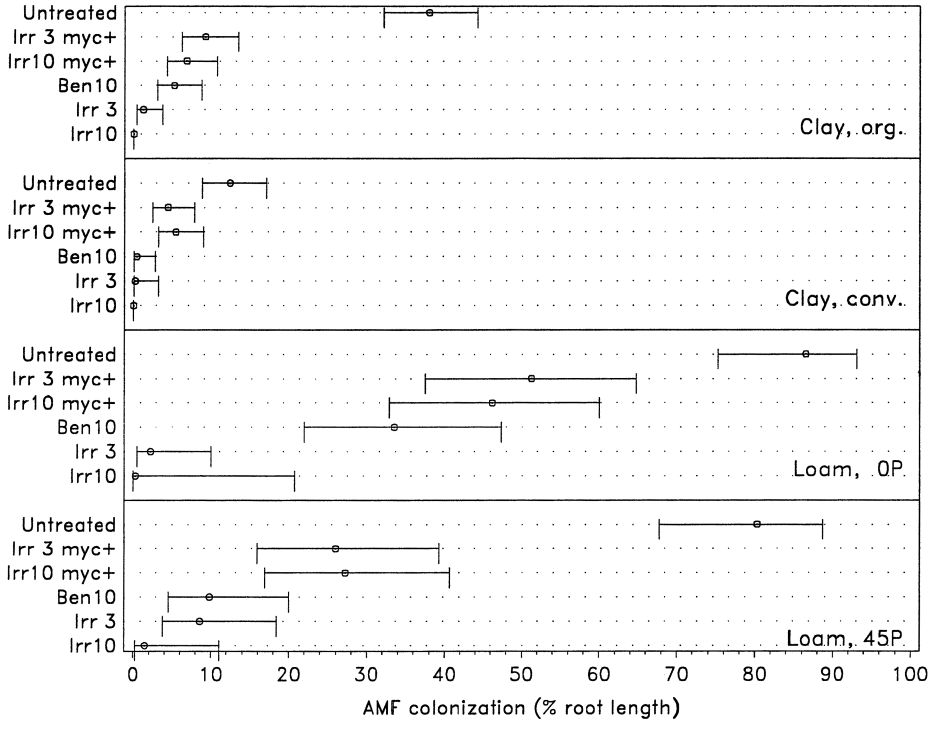


Fig. 2 Effect of methods on AMF colonization of flax in the bioassays. Values are means of five (experiment 3) or four (experiment 4) replicates. For treatment codes, see Table 3; *bars* 95% confidence intervals

conventionally managed soil irradiated by 3 kGy than in benomyl-treated soil was found due to nutrient release after irradiation (Fig. 4, Table 6).

A small decrease in AM functioning in the mycorrhizal treatment compared with untreated soil was aimed at. In irradiated and reinoculated clay, however, flax shoot and root P concentrations and uptake were greatly reduced (Fig. 3, Table 5) despite the absence of clear differences in soil P availability (see above). In general, plant Cu concentration and uptake varied as for P concentration and uptake.

Loam (experiment 4)

The ability of the methods to suppress P uptake and growth in the non-mycorrhizal control depended both on the soil type and P level. The only slight difference in effect in clay and loam reflected a difference in AM potential between the soils. Dependence on P level reflected the different effects of the methods on soil N availability. In contrast to clay, with no difference apparent between the methods, irradiation in loam, with higher colonization, was slightly more suppressive than benomyl at the lower P level. Here only

Table 5 Results of analyses of variance and method comparisons for flax P concentration and uptake. See Table 3 for treatment codes

	P concentration mg kg^{-1}				P uptake μg $plant^{-1}$			
	Clay (Exp. 3)		Loam (Exp. 4)		Clay (Exp. 3)		Loam (Exp. 4)	
			OP	45P			OP	45P
Shoot								
Non-mycorrhizal treatments								
Ben10 – Irr [b]								
Difference	+0.13		+0.60	−0.80	−5		+40	−172
95% CI	−0.01, +0.28		+0.03, +1.16	−1.36, −0.23	−26,+17		−31, +111	−243, −101
Irr3 – Irr10								
Difference	+0.06		+0.02	−0.48	−22		+21	−12
95% CI	−0.10, +0.22		−0.64, +0.68	−1.14, +0.18	−104, +60		−3, +45	−94, +70
Mycorrhizal treatments								
Untreated – Irr myc+[a]								
Difference	Distributions[d]		+1.69	+1.31	Distributions[d]		+346	+20
95% CI	clearly different		+1.12, +2.25	+0.74, +1.88	clearly different		+275, +417	−51, +91
Irr3 myc+ – Irr10 myc+								
Difference	−0.02		−1.36	−0.22	−12		−125	−18
95% CI	−0.18, +0.14		−2.01, −0.70	−0.87, +0.44	−36, +12		−207, −44	−98, +64
Method	$F_{7,59}=5.35$, $P<0.001$		$F_{9,56}=30.95$, $P<0.001$		$F_{7,58}=3.51$, $P=0.003$		$F_{9,56}=33.12$, $P<0.001$	
Management history × method	$F_{7,59}=0.67$, $P=0.70$		$F_{9,56}=6.16$, $P<0.001$		$F_{7,58}=0,26$, $P=0.97$		$F_{9,56}=9.39$, $P<0.001$	
	Org	Conv	OP	45P	Org	Conv	OP	45P
Root[c]								
Non-mycorrhizal treatments								
Ben10 – Irr[b]								
Difference	+0.10	−0.76	+0.62	−0.59	−8	−58	+15	−38
95% CI	−0.14, +0.33	−1.46, −0.06	+0.14, +1.09	−1.07, −0.11	−32, +15	−109, −6	−19, +49	−72, −4
Irr3 – Irr10								
Difference	−0.13	−0.29	−0.11	−0.02	+0.3	+3	−29	+8
95% CI	−0.40, +0.14	−1.13, +0.55	−0.66, +0.44	−0.57, +0.54	−27, +27	−59, +65	−68, +11	−31, +48
Mycorrhizal treatments								
Untreated – Irr myc+[a]								
Difference	Distributions[d]		+0.84	+0.07	Distributions [d]		+29	−61
95% CI	clearly different		+0.36, +1.32	−0.41, +0.55	clearly different		−5, +63	−95, −27
Irr3 myc+ – Irr10 myc+								
Difference	−0.09	−1.38	−0.70	+0.39	−16	−89	−10	+7
95% CI	−0.36, +0.18	−2.17, −0.59	−1.25, −0.15	−0.17, +0.94	−43, +11	−147, −30	−50, +29	−33, +46
Method	Org. $F_{7,28}=4.63$, $P<0.005$ Conv. $F_{7,27}=5.60$, $P<0.001$		$F_{9,57}=8.40$, $P<0.001$		Org.. $F_{7,28}=3.03$, $P=0.02$ Conv. $F_{7,27}=5.50$, $P<0.001$		$F_{9,57}=3.50$, $P<0.005$	
Management history × method			$F_{9,57}=6.47$, $P<0.001$				$F_{9,57}=5.18$, $P<0.001$	

[a] Average of means for irradiation doses, reinoculated by soil 5% w/w
[b] Average of means for irradiation doses, reinoculated by non-AMF < 37 μm
[c] Data in experiments for soil of each farm were analyzed separately due to higher spread of distribution in soil with conventional management
[d] For untreated soil, the location and spread of distribution differed so clearly from those of the other treatments that it was excluded when modelling data

plant P concentration was lower in irradiated soil than in the more mycorrhizal flax in benomyl-treated soil, showing that the incomplete AM suppression by benomyl had hardly any functional effect in comparison with irradiated soil. On the contrary, at the higher P level where growth was limited by N rather than by P, P concentration, P uptake and dry weight were higher in irradiated than in benomyl-treated soil (Figs. 3, 4, Tables 5, 6).

Greater differences in growth and P content were observed between the mycorrhizal treatments than the non-mycorrhizal treatments. As in clay from farms, at the lower P level of loam, flax shoot P uptake (Fig. 3, Table 5) and growth (Fig. 4, Table 6) were considerably higher in untreated soil than in irradiated, reinoculated soil because of lower mycorrhization by reinoculation. At the higher P level, P was not the limiting factor and so the benefit from the higher

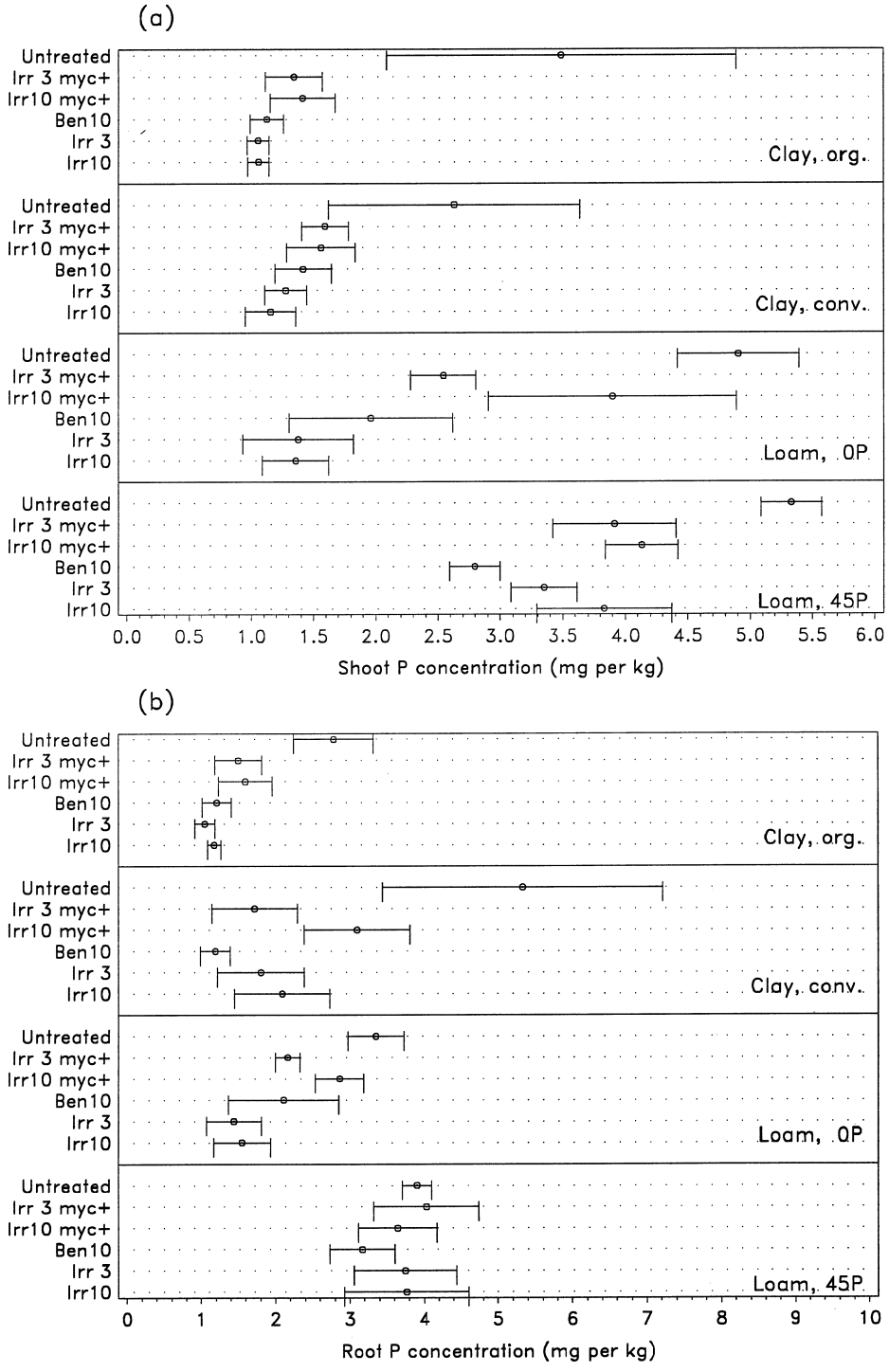
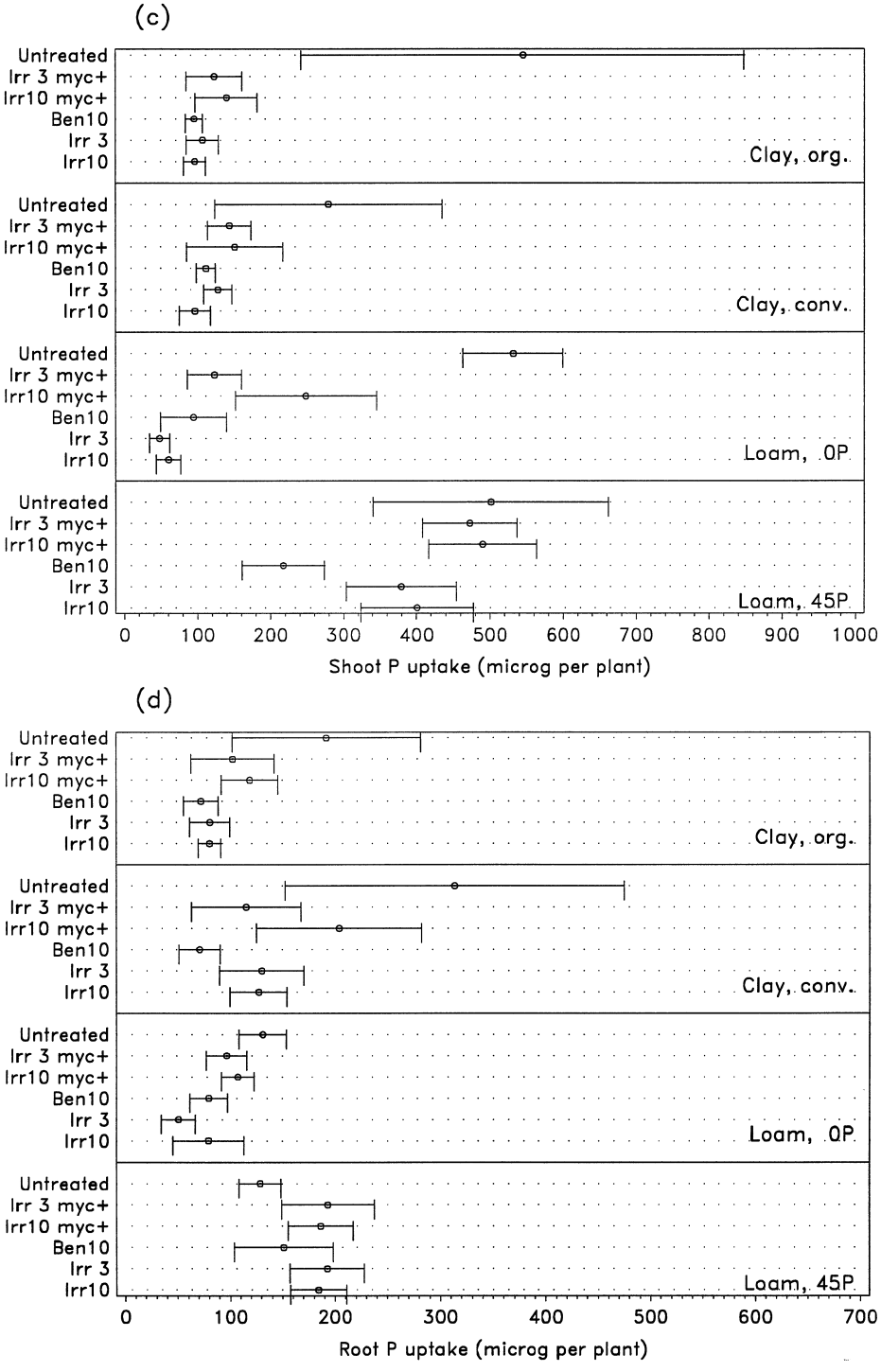


Fig. 3 Effect of methods on flax **a, b** P concentration and **c, d** P uptake in the bioassays. Values are means of five (experiment 3) or four (experiment 4) replicates. For treatment codes, see Table 3; *bars* standard deviations

mycorrhization in untreated soil was small. Thus, the situation was the opposite to the lower P level and dry weight due to N flush by irradiation, and there were no differences in P uptake. A further indication of reduced AM effectiveness in irradiated, reinoculated soil was that shoot growth at the higher P level was double that at the lower P level, while in untreated soil it was similar.

Flax P contents achieved by the different ways to reinoculate AMF were compared in order to find that giving higher P content and thus higher mycorrhizal functioning. At the lower P level, there was a difference in shoot P concentration between irradiated soil reinoculated by soil extract and by 5% w/w untreated soil ($P < 0.001$). Soil extract resulted in 2.44 mg kg^{-1} (95% CI: +1.78, +3.10) lower shoot P concentration than reinoculation by 5% w/w untreated soil (1.46 and



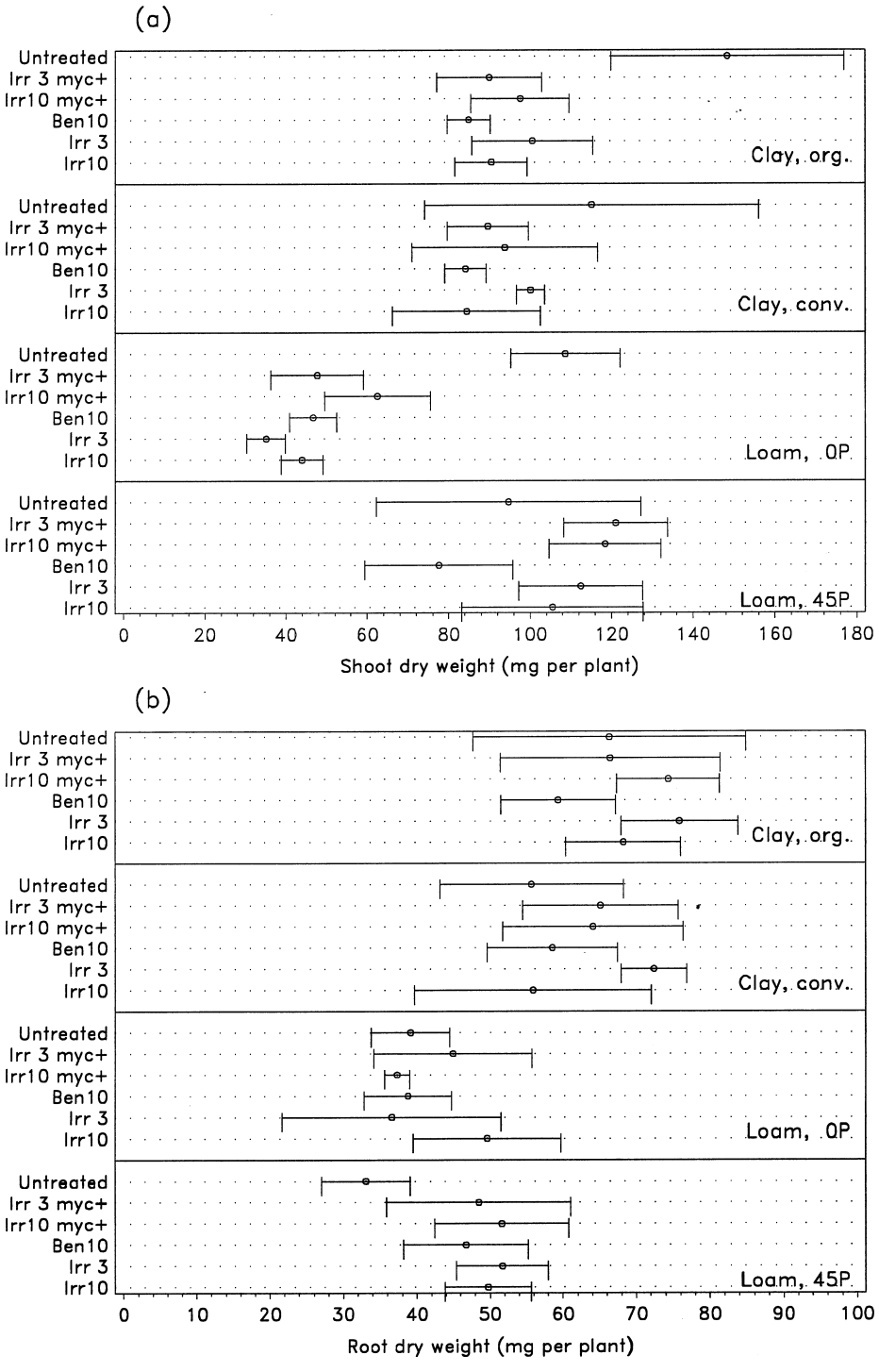


Fig. 4 Effect of methods on flax **a** shoot and **b** root growth in the bioassays. Values are means of five (experiment 3) or four (experiment 4) replicates. For treatment codes, see Table 3; *bars* standard deviations

3.90 mg kg^{-1} for extract and 5% w/w, respectively). The effects on shoot P uptake and root P concentration were similar. The difference between the means of shoot P uptake for inoculation by extract and 5% w/w (60 v. 250 µg $plant^{-1}$) was −190 µg $plant^{-1}$ (95% CI: −269, −105). Correspondingly, the difference between the means of root P concentration (1.79 v. 2.87 mg kg^{-1}) was −1.08 mg kg^{-1} (95% CI: −1.63, −0.53). There was no evidence of an effect on root P uptake ($P = 0.07$).

Even if no difference between the treatments was apparent in soil-extractable P, irradiation obviously also interfered with mycorrhizal effectiveness by improving soil P availability to mycorrhizal but not to non-mycorrhizal plants. This was shown by higher plant P concentration and double shoot P uptake in

Table 6 Results of analyses of variance and method comparisons for flax growth. See Table 3 for treatment codes

	Shoot dry wt. mg plant^{-1}			Root dry wt. mg plant^{-1}	
	Clay (Exp. 3)	Loam (Exp. 4) OP	45P	Clay (Exp. 3)	Loam (Exp. 4)
Non-mycorrhizal treatments					
Ben10 – Irr[b]					
Difference	−10	+7	−31	−10	−4
95% CI	−19, −1	−9, +23	−48, −15	−17, −1	−12, +3
Irr3 -Irr10					
Difference	+13	−9	+7	+12	−6
95% CI	+3, +23	−28, +10	−12, +26	+3, +21	−14, +3
Mycorrhizal treatments					
Untreated – Irr myc[a]					
Difference	Distributions[c]	+54	−25	−7	−10
95% CI	clearly different	+37, +70	−41, −9	−15, +2	−17, −2
Irr3 myc+ - Irr10 myc+					
Difference	−6	−15	+3	−4	+2
95% CI	−16, +4	−34, +4	−16, +21	−13, +6	−6, +11
Method	$F_{7,98}=2.32$, $P=0.04$	$F_{9,57}=10.93$	$P<0.001$	$F_{8,68}=2.38$, $P=0.03$	$F_{9,57}=1.99$, $P=0.06$
Management history × method	$F_{7,59}=0.22$, $P=0.98$	$F_{9,57}=9.17$	$P<0.001$	$F_{8,68}=1.01$, $P=0.44$	$F_{9,57}=1.20$, $P=0.31$

[a] Average of means for irradiation doses, reinoculated by soil 5% w/w
[b] Average of means for irradiation doses, reinoculated by non-AMF < 37 μm
[c] For untreated soil, location and spread of distribution differed so clearly from those of the other treatments that it was excluded when modelling data

soil irradiated by 10 kGy than 3 kGy at the lower P level when reinoculated by AMF, and no such difference when not reinoculated (Fig. 3, Table 5).

Difference other than mycorrhization between pea myc$^+$ and myc$^-$

Mycorrhization should be the only difference between the mycorrhizal and non-mycorrhizal treatments. When P content and growth of pea myc$^-$ were compared to those of pea myc$^+$ with benomyl in loam (experiment 4), there was no problem with the validity of the myc$^-$ mutant R72 as the control for the corresponding mycorrhizal pea mutant N15. There was some evidence of a difference between pea myc– in untreated soil and pea myc+ in benomyl-treated soil in terms of shoot concentration and root P concentration and uptake. The differences in shoot P concentration could, however, be explained by pea myc$^+$ with benomyl being slightly mycorrhizal, unlike pea myc$^-$. The higher root P concentration and P uptake in pea myc$^-$ than in pea myc$^+$ with benomyl remain unexplained, but the root effects were generally less consistent than the shoot effects (data not presented).

Phytotoxicity to the host plants of the benomyl doses used could be excluded. The results of the phytotoxicity studies will be published separately.

Differences in mycorrhizal effectiveness

Clay (experiment 3)

An appropriate method should clearly indicate differences in mycorrhizal effectiveness. This criterion was examined through two management histories with presumedly different effects on AM. There was, however, no clear evidence of a difference between the methods. This was shown by the lack of interactions between method and management history ($P>0.13$ for shoots and roots). The greatest differences in means of RME between organic and conventional farms were obtained by benomyl, being 19% for growth and 36% for P uptake. However, these mean differences dropped to 4 and 13%, respectively, when one outlier was exluded from the data of the conventional farm. Figure 5 illustrates the distributions of RME in terms of flax shoot growth and P uptake for the whole data. The differences between the management histories in RME for root growth or nutrient content were generally smaller than the corresponding differences for shoots.

The clearly highest values of RME were achieved by use of benomyl with flax as a test plant. The method's main effects were statistically significant in terms of shoot dry weight ($F_{2,19}=14.60$, $P<0.001$), shoot P uptake ($F_{3,21}=12.31$, $P<0.001$) and root P uptake ($F_{2,19}=13.86$, $P<0.001$), but not in terms of root dry weight ($F_{2,20}=2.48$, $P=0.11$). Furthermore, differences between methods were found in terms of K and Cu uptake ($P<0.001$ for shoots and $P<0.03$ for roots). There was a difference between benomyl and irradia-

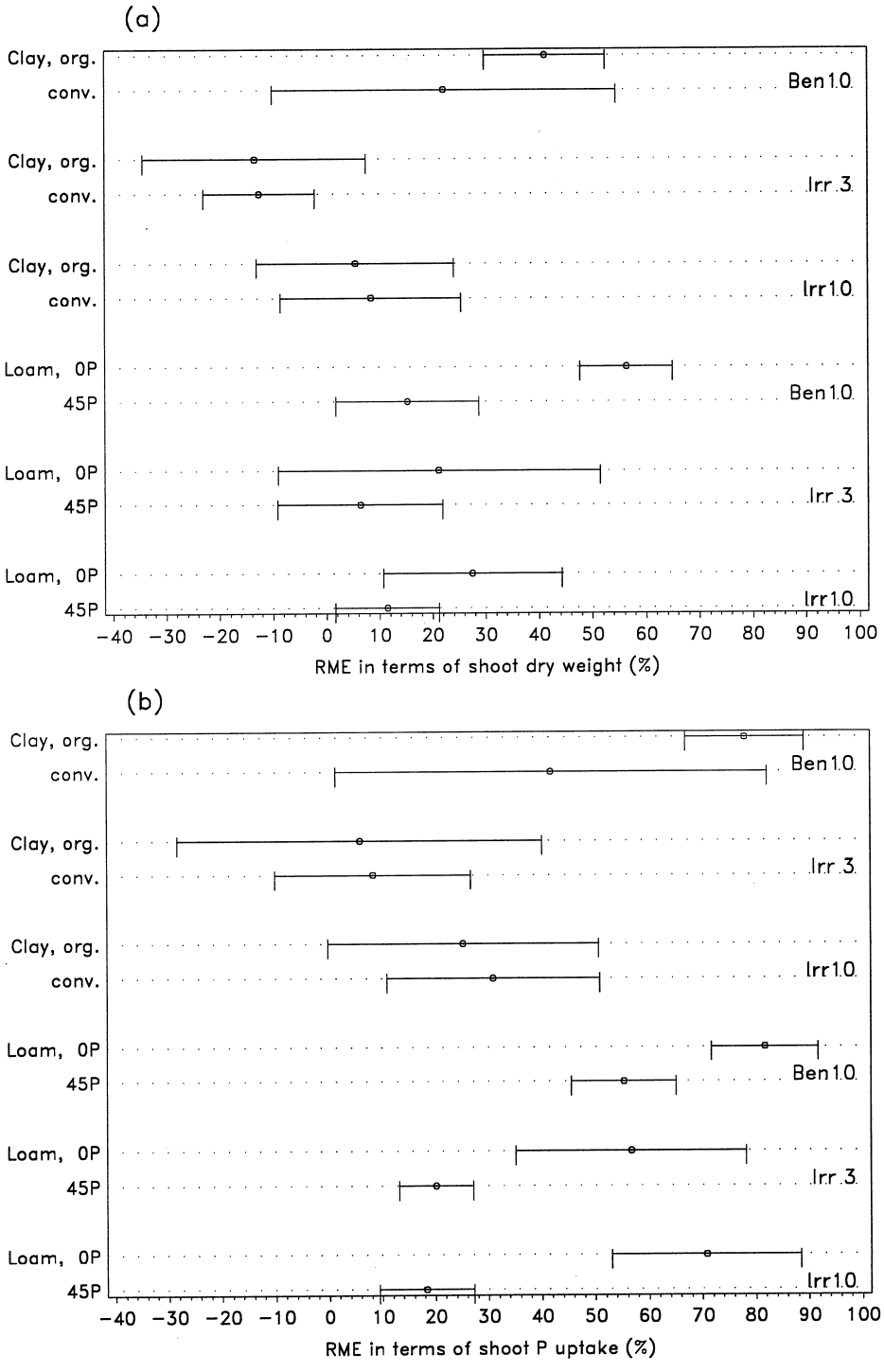


Fig. 5 Effect of methods on RME in terms of flax **a** shoot growth and **b** P uptake in the bioassays. Values are means of five (experiment 3) or four (experiment 4) replicates. For treatment codes, see Table 3; *bars* standard deviations

tion ($P<0.001$ for dry weight and P and K uptake, $P=0.004$ for Cu uptake) and between the two irradiation doses ($P=0.02$ and 0.05, 0.005 and 0.05 for dry weight and uptake of P, K and Cu, respectively), the higher dose resulting in higher RME. The differences between the doses are not explained by the response variables of the study. In general, the estimates of RME were parallel but considerably smaller for root growth and nutrient content than for shoots.

Loam (experiment 4)

The ability of the methods to indicate differences in mycorrhizal effectiveness did not depend on the soil type. There was no clear evidence of interactions between method and management history in loam ($p>0.06$ for RME in terms of growth and nutrient uptake of shoots and roots). As regards RME in terms of shoot growth, the greatest difference between the P

levels (41%) was obtained with benomyl, as in clay, (Fig. 5). With the irradiation doses 3 and 10 kGy, the differences were 15% and 16%, respectively. However, when one outlier from the data of 3 kGy was exluded, the difference for this method increased to 29%. For shoot P uptake, the irradiation doses resulted in a greater difference between the P levels than use of benomyl. RME for all the response variables with all the methods was higher at the lower P level than at the higher level (Fig. 5), except for RME in terms of root growth after 10 kGy irradiation.

The differences in RME between the methods per se in loam were otherwise consistent with those in clay, except that there was no difference between the irradiation doses ($P = 0.54$ and 0.35 for shoot dry weight and P uptake, respectively) (Fig. 5). The main effects of the methods were statistically significant in terms of shoot dry weight ($F_{3,21} = 12.00$, $PF_{3,21} = 39.71$, $PF_{3,21} = 4.84$, $P = 0.01$), but not in terms of root dry weight ($F_{3,21} = 2.28$, $P = 0.11$). In contrast to flax, no shoot or root growth or nutrient uptake response to mycorrhiza was shown by the pea mutants, irrespective of whether a non-mycorrhizal mutant or benomyl treatment was used as the non-mycorrhizal control. This was reflected in the low, often negative values of RME with the pea mutants (Table 7).

The ability to describe the differences of effectiveness in the field

The bioassay was developed to describe differences in AM effectiveness in the field. Therefore, the differences in RME between the P fertilization histories obtained in the bioassay in loam (experiment 4) were compared with those obtained directly in the field from which soil for the bioassay was taken. The results in the field and in the bioassay were fairly similar, irrespective of the method (Table 7). There was also no evidence in the field that the differences between the management histories in RME in terms of growth and P and N uptake varied with the method. There were only tendencies of the interaction between management history and sampling time in terms of shoot P uptake ($F_{1,3} = 7.93$, $P = 0.07$) and shoot dry weight ($F_{1,3} = 5.50$, $P = 0.10$, with one suspect outlier deleted). The differences in RME between the P fertilization histories were notable at flowering of pea, with the RME for the lower P level always being higher in accordance with the bioassay in the growth chamber. The results at harvest were more inconsistent. The potential for using the pea mutants to assess mycorrhizal effectiveness directly in the field turned out to be small due to low mycorrhizal dependence of the mutants, even in the field.

There was no evidence of a benomyl effect on log-transformed soil P_{H2O} content, pH or N_{NO3-} or N_{NH4+} contents, in accordance with the bioassay in pots. The higher benomyl rate in the field reduced colonization close to zero, even at harvest. Pea myc$^-$ was also not infected in the field. Similar to the bioassay, pea myc$^-$ in the field in untreated soil differed somewhat from pea myc$^+$ in benomyl-treated soil with respect to shoot P and N concentrations. This was possibly due to release of small amounts of N mineralized from fungal cells killed by benomyl, although there were no measurable differences in soil $N_{soluble}$ concentration. The results for barley will be published separately.

Discussion

The criteria for creating a non-mycorrhizal control were tested successfully in the experimental set-up. The bioassay represented well the field situation, irrespective of the method used to create the non-mycorrhizal control. Parallel differences in RME between management histories in the field and the growth chamber bioassay for pea were found. The differences in the bioassays were smaller rather than greater than in the field at flowering, especially in terms of P uptake. Obviously the limited growth space in pots decreased the mycorrhizal benefit for pea, but not for flax with its smaller root system.

No clear difference was found between the methods in their ability to differentiate AM effectiveness between the management histories. This was due to high variation in shoot growth and P uptake in untreated soil, which was the mycorrhizal treatment for benomyl use, when the AM potential was fairly

Table 7 Correspondence between mean RME values obtained in a pot experiment in the growth chamber (experiment 4) and in the field at flowering. Standard deviations are in parentheses (*n* number of observations)

		RME (shoot dry wt., %)			RME (shoot P uptake , %)		
		0P	45P	difference 0P – 45P	0P	45P	difference 0P – 45P
Pea mutants	n						
Growth chamber (Exp. 4)	4	–3 (3)	–32 (31)	29	+17 (8)	–16 (16)	33
Field	4	–20 (58)	–50 (52)	30	+22 (45)	–75 (111)	97
Pea myc+ with benomyl							
Growth chamber (Exp. 4)	4	–2 (33)	–15 (64)	13	+25 (18)	–7 (43)	32
Field	4	+18 (49)	–15 (25)	33	+21 (53)	–27 (50)	48

low, as in clay, and at the higher P level of loam. This reflected high variation of AM effectiveness in these fields. The same management history had a higher RME in loam irrespective of the method, both in the bioassay and in the field. In clay, with a lower AM potential, the management histories differed slightly only when benomyl was used to create the control.

Use of benomyl produced the highest RME or contribution to growth and P uptake. The differences in RME obtained by the various methods were due rather to differences in growth and nutrient uptake of the mycorrhizal compared with the non-mycorrhizal treatments. This was mainly caused by the considerably lower percentage root length colonized in irradiated, reinoculated soil than in untreated soil, as also observed by Trouvelot et al. (1996). The effect of inoculation with soil extract was even less satisfactory than with 5% (w/w) untreated soil. Differences between the mycorrhizal treatments also arose from changes in soil nutrients. The variation in P uptake was generally higher notable than that in dry weight due to parallel differences in plant P concentration.

There were, also differences in growth and P uptake between the non-mycorrhizal treatments. In soils with moderately high P, where N was the growth limiting factor, irradiation increased growth and P uptake compared with benomyl treatment. This was the result of N flush and improved P availability. In contrast, the incomplete AM suppression by benomyl was not reflected in growth or nutrient uptake, except for only a slight increase in growth in loam with a lower P and higher AM effectiveness than 3 kGy irradiation. Colonization rates were reduced by benomyl incorporation somewhat more than in former studies either in pots applied as a soil drench at sowing (Bailey and Safir 1978; Kough et al. 1987), in the field applied as a drench of soil cores (Merryweather and Fitter 1996) or incorporated into soil (Lu and Miller 1989). The effect of benomyl was not changed by incubation for 1 month before sowing or by doubling the dose. The slightly higher RME obtained by 10 than by 3 kGy in clay soil was exceptionally due to difference in the growth of the non-mycorrhizal controls. A possible explanation is increased availability of soil toxic agents, which would be alleviated by mycorrhiza (e.g., Schuepp et al. 1987b). However, phytotoxic agents formerly reported to be increased by γ-irradiation (Mn and Cu) or by steam sterilization (Al) were excluded. Reinoculation of the non-mycorrhizal microbiota to the controls with suppressed mycorrhization seemed not to be necessary as it did not affect the results.

P, even if mineralized by partial sterilization, was quickly fixed by soils. However, the altered P conditions were manifested at the lower P level by higher P uptake and P concentration in mycorrhizal (but not non-mycorrhizal) flax in soil irradiated with 10 than with 3 kGy, a phenomenon observed earlier by Thompson (1990). This was caused by the sterilization of a larger fraction of the soil microbiota by the higher dose, as shown by a higher share of N_{NH4+} in $N_{soluble}$ due to decreased nitrification (Popenoe and Eno 1962). The mineralized P, even though no longer detectable as P_{H2O}, is obviously fixed to iron and aluminium phosphates, adding to the pool of labile phosphate which is more effectively utilized by mycorrhizal than by non-mycorrhizal plants (Bolan et al. 1987). Small increases in soil concentration of plant-available P due to partial sterilization have been reported occasionally (Eno and Popenoe 1964; Jakobsen and Andersen 1982; Jakobsen 1984) but mostly not detected (e.g., Thompson 1990; Jawson et al. 1993; Ellis et al. 1995). Nor has such an increase been observed with benomyl treatment (Fitter and Nichols 1988; Bentivenga and Hetrick 1991).

The change in soil N status confounds assessment of mycorrhizal effectiveness because it affects plant growth and interacts with the P effect on mycorrhizal formation (Sylvia and Neal 1990). The manyfold increases in soluble N content due to the decomposition flush depended on the sampling time, obviously due to variation in the microbial biomass from which most of the N flush originates (McLaren 1969). Lowering the irradiation dose below one-third of usual rate produced a considerably smaller increase in the $N_{soluble}$ content of clay soil, especially that with an organic management history. The effects of benomyl on plant-available N in soil were small, except in one of eight combinations of soil, management history and sampling time, i.e. in pots with loam sampled in June, and even then the effect was only half that of irradiation. There was also no effect by doubling the dose. Increases in foliar N content have been observed by considerably higher, regularly repeated benomyl applications (Cade-Menun and Berch 1997). Earlier comparisons of the effects of benomyl and partial sterilization on soil N status are not known to the authors.

No problem was found with the pea myc^- mutant R72, cv. Sparkle as a control for the corresponding myc^+ mutant N15 in the present study, in accordance with former results on their phenotypic similarity in the non-mycorrhizal state (Kneen et al. 1994). In contrast to these mutants with low mycorrhizal dependence, Trouvelot et al. (1996) observed a notable difference in growth and P uptake between the wild-type Frisson cultivar (nod^+ myc^+) and its nod^- myc^- mutant fertilized with N in a field study at P levels comparable to the present study. The availability of isogenic myc^+ and myc^- mutants is, unfortunately, very limited. Their use would avoid disruptive soil treatments and the safety and environmental problems of benomyl use. The development of a selection of isogenic, phenotypically comparable mutants of genotypes responsive to AM in a variety of conditions relevant to various agroecosystems would be beneficial.

In conclusion, use of benomyl is the most appropriate method currently available to create a non-mycorrhizal control for field AMF communities, irrespec-

tive of soil type and management history. This allowed the use of a responsive host and produced less distortion of assessment than irradiation. Thus it was also possible to use untreated soil as the mycorrhizal treatment, reflecting the potential of the whole field AMF population. No phytotoxic effect of benomyl was observed. Use of this fungicide may, however, lead to underestimation of AM effectiveness because the control is not totally non-mycorrhizal and because effects on root pathogens cannot be excluded. Irradiation with a low dose of 3 kGy suppressed mycorrhization sufficiently and changed the soil nutrient conditions less than the commonly used 10-kGy dose.

This bioassay serves research on management of field AMF and allows standardization even for practical purposes. Conventional soil P extraction could be complemented by the determination of RME and possibly infectivity in this kind of bioassay, to better describe field soil P availability and its problems. The benomyl dose and application time still require optimization, as does the soil sampling time and test plants.

Acknowledgements Financial support from The Finnish Academy via the Nordic Joint Committee for Agricultural Research (grant no. 93) is gratefully acknowledged. The authors wish to thank Into Saarela for permitting utilization of the long-term experiment on cumulative P dressings, Seija Pajari and Teemu Perho for cooperation on studying their field soil, Asko Hannukkala for useful discussions on the use of benomyl, Merja Eurola and Leila Lindstedt for plant and soil analyses and Riitta Bagge, Päivi Hämäläinen, Leena Juuti, Pauliina Lehtinen, Pekka Leinonen, Anu Marjo, Juhani Mäkelä, Tuure Peltonen and Kaija Rimppi for technical assistance.

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