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Clonal growth traits of two *Prunella* species are determined by co-occurring arbuscular mycorrhizal fungi from a calcareous grassland

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Summary

1 The effects of three different isolates of co-occurring arbuscular mycorrhizal fungi (AMF) from the same calcareous grassland on clonal growth traits of *Prunella vulgaris* and *P. grandiflora* were investigated.

2 *P. vulgaris* and *P. grandiflora* were found to be highly dependent on AMF for growth in soil from the same calcareous grassland. The addition of phosphorus did not significantly increase the growth of either nonmycorrhizal or mycorrhizal *P. vulgaris*.

3 The three AMF isolates differed significantly in their effects on size of the two plant species. The AMF isolates also differed in their effects on stolon branching and stolon length and here the effects were not the same in the two plant species. The differential effects of AMF isolates on clonal growth traits were consistently independent of the level of AMF colonization throughout the experiment, indicating that the effects were not due to different rates of colonization. Some of the AMF isolate effects on stolon branching in *P. vulgaris* were also independent of the effects of AMF isolates on plant size. These results suggest that different AMF in a natural community have the potential to influence the growth, number of ramets and distribution of ramets in *Prunella* populations.

4 The strong differential effects of AMF isolates on clonal growth traits of *P. vulgaris* occurred in a homogeneous environment. Plasticity in such traits has previously been considered important for efficient resource foraging in a heterogeneous environment. Our results, however, indicate that different AMF in a community could strongly influence resource foraging strategies in clonal plants irrespective of environmental heterogeneity. The results also suggest that increasing the availability of phosphorus would be unlikely to alter these effects.

5 Our results point strongly to the potential importance of AMF diversity as a determinant of plant population structure in ecosystems.

Keywords: clonal plants, community structure, Glomales, morphological plasticity, mycorrhizal symbiosis, population structure, resource acquisition

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Introduction

Many plant species have the ability to propagate asexually, by clonal growth (Harper 1977; Eckert & Barrett 1993). Plasticity in plant growth traits, also called morphological plasticity, is considered important for the efficiency of resource acquisition for plants (Schlichting 1989; Turkington *et al.* 1991; Cheplick 1991, 1995). Experimental investigations have focused on the ability of plants to forage for resources through fine-scale leaf and root proliferation (Campbell *et al.* 1991; Jackson & Caldwell 1993a,b). If plants can respond morphologically to high resource patches this may allow them to maximize their resource acquisition and consequently their vegetative fitness. More recently, plasticity in spacer length, branching intensity and branching angle of stolons or rhizomes of

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clonal plants has been considered important for the production and placement of new ramets in new environments (de Kroon & Hutchings 1995). This plasticity could allow clonal plants to forage in a resource heterogeneous environment and thus maximize the placement of new ramets into high resource areas with high light intensity or high nutrient availability (Hutchings & de Kroon 1994; de Kroon & Hutchings 1995). Because environmental heterogeneity alters such growth traits the structure of clonal plant populations could also be strongly affected.

Plants, however, have other possibilities for acquiring resources below-ground, namely through a mutualistic association with soil fungi, known as arbuscular mycorrhizal fungi (AMF). AMF hyphae forage for nutrients in the soil and subsequently transfer them to the plant (Harley & Smith 1983). The benefit of the symbiosis for the plant has mainly been explained in terms of an improved nutrient supply, in particular by a better uptake of phosphate by mycorrhizal roots than by nonmycorrhizal roots, especially in nutrient poor soils (Koide 1991; Li *et al.* 1991). Thus, the following aspects of the interaction of plants with AMF should be taken into account when considering clonal growth traits and resource acquisition strategies. First, AMF form mycorrhizal symbioses with 80% of vascular plants (Harley & Harley 1987) and in almost all natural communities the roots of herbaceous plants are colonized by AMF. Secondly, plant species differ in their dependency on mycorrhizal fungi (Hetrick *et al.* 1988; Habte & Manjunath 1991; Koide & Li 1991) indicating that AMF could be more important for some plant species than for others. This difference in mycorrhizal dependency may be responsible for the strong effects of AMF on plant community structure in calcareous grasslands and annual plant communities (Grime *et al.* 1987; Gange *et al.* 1990; Sanders & Koide 1994). Thirdly, natural communities comprise many species or genetically different forms of AMF (Walker *et al.* 1982; Johnson 1993; Sanders *et al.* 1995; Bever *et al.* 1996). If these AMF species or genotypes were to alter plant growth or morphology in different ways, e.g. through their effectiveness in acquiring nutrients for the plant or through other unknown functions, then we hypothesize that interactions of a given plant with the different AMF present in a community could influence its clonal growth traits and increase its apparent plasticity.

Little attention has been paid to the role of different AMF species or genotypes in natural communities. This is mainly because of the apparent lack of specificity in the formation of the symbiosis under experimental conditions (Harley & Smith 1983). Experimental investigations, however, suggest that specific plant–AMF combinations can be more or less beneficial for both partners (Sanders 1993), meaning that selection pressures exist which could lead to the evolution of specificity. This is further supported by evidence showing that AMF differ in their potential to stimulate the growth of agricultural plants, through their effects on plant nutrition (Jensen 1982, 1984; Raju *et al.* 1990; Jakobsen *et al.* 1992) and different plant species have been shown to alter life history traits, such as sporulation and infectivity, of different native AMF (Sanders & Fitter 1992; Sanders 1993). Furthermore, previous studies have largely assumed that the benefit of AMF colonization is the result of improved phosphorus nutrition, although recent studies suggest that in natural ecosystems they could have other effects on plants, such as protection against pathogens, which are not a straightforward effect of improved plant phosphorus nutrition (Newsham *et al.* 1995).

Most previous studies on the differential effects of AMF have assessed their potential to improve crop growth in agricultural soils rather than being carried out in an ecological context. Consequently, investigations comparing the effects of AMF were often carried out in soils which were different to the origin of the AMF isolate and/or plants were inoculated with isolates with which they would have been unlikely to form a symbiosis in the natural environment. In addition, it is possible that because many of the AMF used in these experiments have been isolated from agricultural soils, where P fertilizers have been applied for many years, selection may have already occurred for less mutualistic AMF (Johnson 1993). It is unknown therefore to what extent co-occurring AMF from one community differ in their effects on plant growth from the same ecosystem and in the same soil.

The aims of our study were to investigate whether (1) three different co-occurring AMF from a calcareous grassland could potentially alter the growth of two co-occurring plant species from the same grassland; (2) whether different AMF could differentially affect clonal growth traits of plants and (3) whether any differential effects might influence the morphological plasticity of clonal plants, irrespective of resource heterogeneity. For this investigation, we have used the plant species *Prunella vulgaris* L. and *Prunella grandiflora* (L.) Scholler, both of which co-occur in species-rich calcareous grasslands in Switzerland. Both species have a prostrate growth habit and can produce branching stolons, from which new ramets can become established. These species were chosen because preliminary investigations indicated that these two species are almost entirely dependent on AMF for growth in calcareous soil (R. Streitwolf-Engel, unpublished), and because morphological plasticity of growth traits in varying environments are also being studied in natural populations of these plants in the same calcareous grassland (Schmid *et al.* 1996).

In an initial experiment, *P. vulgaris* was grown with and without a mixed AMF community from a calcareous grassland at different levels of phosphorus fertilization. Different AMF isolates were obtained

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from the field site and two further experiments were designed to compare the differential effects of these isolates on clonal growth traits of *P. vulgaris* and *P. grandiflora*.

Materials and methods

SITE DESCRIPTION, PLANT AND FUNGAL MATERIAL

The study site, Nenzlinger Weide, is a calcareous grassland in the Jura Mountains, Switzerland (grid reference 255 609 of the Landeskarte der Schweiz, sheet 1067). The site is 450 m above sea level and supports a diverse flora, characteristic of calcareous grassland and which fits to the phytosociological description of *mesobrometum*. The soil is a rendzina, with a clayey to silty clayey texture (pH 6.8–7.6). Seeds of *P. vulgaris* and *P. grandiflora* used in expts 1 and 2 were collected in August 1993 from plants randomly selected at Nenzinger Weide. Seeds from many individuals were mixed and therefore the seeds used for the experiments are likely to comprise a mixture of genotypes. Three cultures of AMF from Nenzlinger Weide were established. Cultures of *Glomus geosporum* (Nicol. & Gerd.) Walker (BEG 18) and *Glomus* sp. (BEG 19) originated from single spores of these fungi which were extracted from the field soil by a sucrose centrifugation technique (Walker *et al.* 1982). *Glomus* sp. (BEG 19) exhibits morphological features which are similar to both *Glomus constrictum* Trappe and to *Glomus botryoides* Rothwell & Victor. *Glomus geosporum* (BEG 18) and *Glomus* sp. (BEG 19) were maintained in pot culture with *Trifolium pratense* L. The third culture, *Glomus* sp. (Basle Pi), was obtained from the roots of *P. vulgaris* growing at Nenzlinger Weide. The roots of intact *P. vulgaris* were carefully washed to remove soil and planted into a sterile mixture of sand and expanded clay (1:1 v:v). After several months sporocarps produced in the substrate were used to inoculate plants. The spores were similar in morphology to *Glomus microcarpum* Tulasne & Tulasne. All cultures are maintained in the Botanical Institute of the University of Basle.

PLANT GROWTH CONDITIONS

Experiment 1

Seeds of *P. vulgaris* were surface-sterilized for 3 min in 0.75% sodium hypochlorite and sown into trays containing an autoclaved mixture of sand, loam and organic matter in the form of leaf compost (3:2:1, v:v) on 28 March 1994. Fourteen days after sowing, 48 seedlings were transplanted to pots (10 cm in diameter, 8.5 cm high) containing a mixture of autoclaved (40 min/121 °C) field soil and quartz sand (1:1 v:v). Seedlings were selected for uniformity in size and development.

Half of the plants were inoculated by placing 8 g of unsterilized field soil containing indigenous AMF next to the seedling. In the nonmycorrhizal treatment plants were mock-inoculated with autoclaved field soil and were given soil washings (32-μm sieve) of field soil to ensure the presence of non-AMF soil microflora which would have been killed during sterilization (Koide & Li 1989).

Plants were grown in the glasshouse (day 14 h, 27 °C/night 10 h, 20 °C) and were watered every three days with 50 mL of tap water. Following the third week after transplanting, mycorrhizal and nonmycorrhizal plants were fertilized twice a week with 10 mL of full strength Hoagland solution containing either 0%, 20%, 40%, 60%, 80% or 100% of the full P content. The concentration of K in the solutions with reduced P concentrations was adjusted accordingly with KCl.

The plants were arranged in the glasshouse in a complete randomized block design, with four blocks and one of the 12 treatment combinations in each block. Plants were harvested 61 days after inoculation.

Experiment 2

Seeds of *P. vulgaris* and *P. grandiflora* were surface-sterilized and sown into trays on 15 April 1994, as described above. Twenty-one days after sowing, 24 seedlings of *P. vulgaris* and 24 seedlings of *P. grandiflora* were transplanted singly into pots. The substrate mixture was the same as that used in expt 1. Plants were inoculated with 8 g of soil inoculum of one of the three AMF isolates. Plants in the nonmycorrhizal treatment all received the same amount of an autoclaved mixture of soil inoculum from the three cultures. Sieved washings (32 μm sieve) of a mixture of the three inocula were given to all plants. The growth conditions in the glasshouse (temperature, day-length, watering and addition of nutrients) were the same as those described in expt 1.

The plants were arranged in the glasshouse in a complete randomized block design, with six blocks and one of each of the eight treatment combinations represented in each block. Plants were harvested 94 days after inoculation.

Experiment 3

P. vulgaris seeds were planted into a tray containing a sterile mixture of quartz sand and a loamy soil (1:1 v:v) on 29 June 1994. After four weeks, 40 seedlings were transplanted singly into pots in a volume of 750 cm^3 of the same autoclaved calcareous soil and quartz sand mixture (1:1 v:v) as used in expt 1. Seedlings of the same size were selected for transplantation.

Thirty plants were inoculated with 8 g of one of the AMF cultures, or for the nonmycorrhizal treatment,

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given 8 g of an autoclaved mixture of the three inocula. Each pot received a sieved (32-μm sieve) washing of a mixture of field soil and all three inocula (Koide & Li 1989) containing natural bacteria and fungi but no mycorrhizal propagules.

Plants were kept in climate chambers with a simulated summer climate of 25 °C day and 16 °C night, with natural lighting. Additional lighting was provided by two 1000 W daylight halogen lights which were switched on if the natural light level fell below 180 μm m^{-2} s^{-1}. Day-length for additional lighting and temperature was 16 h day^{-1}. Plants were watered every two days with 50 mL of water and no additional nutrient solution was given.

Pots were arranged in the growth chambers in a block design where each growth chamber contained an equal number of replicates of each of four mycorrhizal treatments and each block contained one replicate of each mycorrhizal treatment. Position in the block was randomized and each week when the pots from one chamber were moved to another chamber the positions of the pots were randomized again and the positions of the blocks within the chamber were also randomized.

Plants were harvested on 30 November 1994, 18 weeks after AMF treatments were started.

MEASUREMENT OF LEAF AREA, PHOSPHORUS CONTENT AND AMF COLONIZATION

During expts 1, 2 and 3 plant growth was recorded by measuring total leaf area, leaf number (all living leaves with an area larger than 0.06 cm^2) and number of stolons at intervals of 10 or 20 days (except expt 3, every 14 days). Total leaf area of the plants was estimated by multiplying leaf length × leaf width of single leaves × 0.714. The factor of 0.714 was determined in a preliminary study where leaf area was measured with a LI-COR LI3000A leaf area meter (Li-Cor Inc., Lincoln, NE, USA). In all preliminary investigations estimated leaf area calculated using the factor 0.714 was an accurate measure of actual leaf area with no significant deviation (data not shown). At the final harvest of each experiment, actual leaf area was measured with the leaf area meter. Relative growth rates (RGR) were calculated for every plant from the leaf area data from two subsequent time-points. In expt 2, at 56, 69 and 84 days following inoculation with AMF, a soil core was removed from the pot with a 2-cm-diameter × 10-cm-long cork borer. The roots were carefully washed out of the soil core and were cleared in KOH and stained with Trypan blue (Phillips & Hayman 1970). Percentage of AMF colonization of roots was estimated using the grid-line intersection method (Giovannetti & Mosse 1980). The hole left by the removal of the soil core was filled in with a sterilized mixture of the soil–sand substrate.

At the final harvest, leaf area, leaf number, stolon number and length of stolons were recorded. The fresh weight of the roots was determined and a 25% fresh weight subsample was used to measure root length and was subsequently stained with Trypan blue for estimation of AMF colonization (as described above). Leaves, stolons, flower heads and the remaining 75% of the roots were dried separately and weighed. These parts were ground, mixed and aliquots were analysed for phosphorus content (Watanabe & Olsen 1965).

STATISTICAL ANALYSIS

Variables measured in expts 1, 2 and 3 were analysed as complete randomized block designs using the SUPERANOVA statistical package (Abacus Concepts Inc., California). All proportional values, such as percentage root length colonized by AMF, were arcsine square root transformed before ANOVA and data which did not exhibit a normal distribution were transformed accordingly (Zar 1984). For each variable Bartlett's test was used to determine whether variances were equal between treatments and ANOVA was only performed on variables exhibiting equal variance. Mean contrasts were carried out using the least significant difference (LSD) and were only performed where a significant mycorrhizal treatment or plant species effect was shown by ANOVA. Because there were large differences in the growth of nonmycorrhizal and mycorrhizal plants in expts 2 and 3, ANOVA was performed with and without the nonmycorrhizal treatment. The inclusion of the nonmycorrhizal treatment as a level could result in a significant mycorrhizal effect, which does not necessarily reflect a significant effect of the different AMF isolates.

Results

EXPT 1: RESPONSE OF *P. VULGARIS* PLANTS TO AMF INOCULATION AND PHOSPHORUS FERTILIZATION

Nonmycorrhizal plants never developed more than four small leaves at any time, independent of P fertilization. At the final harvest nonmycorrhizal plants had a mean leaf area of 0.59 cm^2 compared to 24.54 cm^2 in mycorrhizal plants. The growth rate (calculated from leaf area) of nonmycorrhizal plants was negative in 11 cases and lead to the death of nine nonmycorrhizal plants during the experiment. One plant inoculated with AMF also died.

The dry weight of *P. vulgaris* inoculated with indigenous AMF was 23.8 times higher than the dry weight of nonmycorrhizal *P. vulgaris*. Fertilization with different concentrations of P did not have a significant effect on dry weight in either mycorrhizal or

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nonmycorrhizal plants (Fig. 1a). The mean total dry weight of mycorrhizal plants fertilized with the 60% P treatment was higher than any of the other P treatments although this was not a significant effect.

A significantly greater proportion of the roots of plants treated with 80% P was colonized by AMF compared to plants with 20% P. There was, however, no obvious trend indicating that increasing P concentrations lead to any change in AMF colonization (Fig. 1b).

EXPT 2: PLANT GROWTH RESPONSE TO DIFFERENT AMF ISOLATES

Nonmycorrhizal *P. vulgaris* and *P. grandiflora* grew very little throughout the experiment. The mean total leaf area of nonmycorrhizal *P. vulgaris* and *P. grandiflora* was 0.48 cm^2 and 0.54 cm^2, respectively, compared to 160.16 cm^2 and 112.88 cm^2 for mycorrhizal plants. Four mycorrhizal plants and seven nonmycorrhizal plants died by the final harvest. Because of the large differences in size between mycorrhizal and nonmycorrhizal *Prunella*, data for the nonmycorrhizal treatments were excluded from further statistical analyses where tests on the differential effects of AMF isolates were performed.

At the final harvest, the three different AMF isolates had occupied significantly different proportions of the *Prunella* roots (Fig. 2a), irrespective of plant species. *Glomus* sp. (Basle Pi) colonized a significantly greater proportion of the root length than either *G. geosporum* and *Glomus* sp. (BEG 19). Mycorrhizal colonization of roots removed at 56, 69 and 84 days following inoculation all showed exactly the same trend (data not shown). At each of these dates, the percentage of AMF colonization in the roots sig-

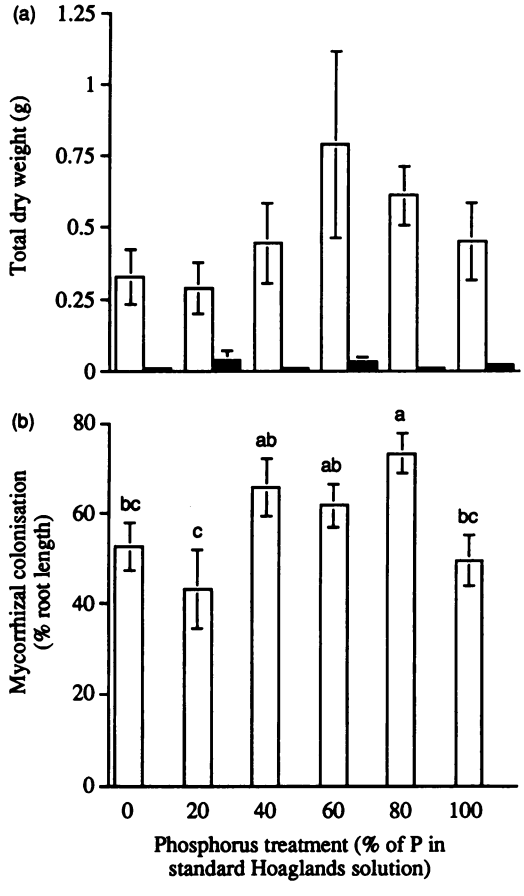

Fig. 1 Mean (a) total dry weight and (b) percentage of root length colonized by AMF in *Prunella vulgaris* plants fertilized with Hoaglands solution containing 0%, 20%, 40%, 60%, 80% or 100% of the total P concentration in standard Hoaglands solution: (□) plants inoculated with AMF; (■) uninoculated plants. Bars show (±) one standard error. Different letters above bars indicate a significant difference ($P \leqslant 0.05$) according to the LSD test. *F*-ratios for the P treatment effect using the data presented in (a) and (b) as dependent variables, were $F_{5,23} = 0.605$, $P \leqslant 0.69$ and $F_{5,14} = 4.185$, $P \leqslant 0.016$, respectively.

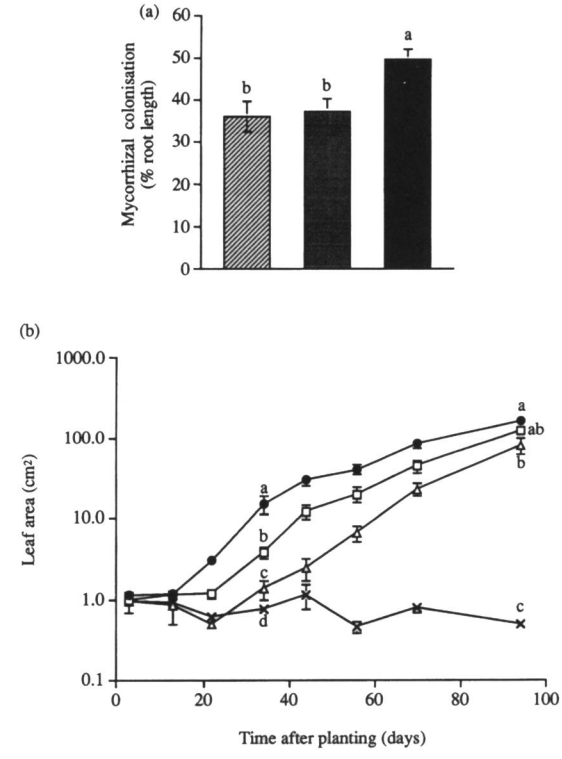

Fig. 2 (a) Percentage root length colonized by AMF in *P. vulgaris* and *P. grandiflora*, 94 days following inoculation with one of three AMF isolates. (▨) *Glomus geosporum* (BEG 18), (▤) *Glomus* sp. (BEG 19), (■) *Glomus* sp. (Basle Pi). Values are means of the two plant species. Different letters above bars indicate a significant difference ($P \leqslant 0.05$) according to the LSD test. (b) Time course of mean total plant leaf area in *Prunella* spp. inoculated with either one of three different AMF isolates or uninoculated. (□) *Glomus geosporum* (BEG 18), (△) *Glomus* sp. (BEG 19), (●) *Glomus* sp. (Basle Pi), (×) uninoculated. Values are means of the two plant species. Bars show (±) one standard error. Different letters next to points at 34 days following planting indicate the first time point at which all mycorrhizal treatments differ from each other. Different letters next to points at day 94 show the difference among AMF isolate treatments at the final harvest. Bars show (±) one standard error. *F*-ratios for the AMF isolate effect using the data presented in (a) and (b) at 94 days as dependent variables and excluding the nonmycorrhizal plants, were $F_{2,20} = 6.14$, $P \leqslant 0.008$ and $F_{2,21} = 4.47$, $P \leqslant 0.024$, respectively.

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nificantly differed according to AMF isolate treatment (*F* ratios for main AMF isolate effects, excluding the nonmycorrhizal treatment, were $F_{2,29} = 3.63$ ($P \leqslant 0.05$), 5.51 ($P \leqslant 0.009$), 4.03 ($P \leqslant 0.02$), at 56, 69 and 84 days, respectively). There were no significant plant-species–AMF-isolate interactions on the percentage of AMF colonization at any time, meaning that AMF colonization by an isolate did not differ between *P. vulgaris* and *P. grandiflora*.

There was also a significant AMF isolate effect on total leaf area at 34, 44, 56, 69, 84 and 96 days following inoculation. At each date, plants inoculated with *Glomus* sp. (Basle Pi) had a larger mean total leaf area compared to those inoculated with *Glomus* sp. (BEG 19) (Fig. 2b), irrespective of plant species. There were no significant plant-species–AMF-isolate interactions on leaf area at any date meaning that the effect of AMF isolates on leaf area was the same in both plant species. The differential effect of the AMF isolates on plant growth, as measured by leaf area, could be seen before the final harvest (Fig. 2b). All the AMF isolates gave a significant growth stimulation to the mycorrhizal plants compared to nonmycorrhizal plants, 34 days following inoculation ($F_{3,35} = 26.49$, $P \leqslant 0.0001$) and by this time leaf areas among plants inoculated with different AMF isolates were also significantly different ($F_{2,25} = 41.22$, $P \leqslant 0.0001$). There were significant AMF isolate effects on the RGR of the plants both for the interval between day 56 and day 69 ($F_{2,22} = 4.26$, $P \leqslant 0.028$) and for the interval between day 69 and day 94 ($F_{2,21} = 11.34$, $P \leqslant 0.0005$).

There was, however, a significant plant-species–AMF-isolate interaction on total plant dry weight (Fig. 3a). *P. vulgaris* inoculated with *Glomus* sp. (Basle Pi) had a significantly higher dry weight than *P. grandiflora* inoculated with the same AMF or either plant species inoculated with any other AMF. *P. grandiflora* inoculated with *Glomus* sp. (BEG 19) were significantly smaller than *P. grandiflora* inoculated with *G. geosporum* or *P. vulgaris* inoculated with either *G. geosporum* or *Glomus* sp. (Basle Pi). There was also a significant plant-species–AMF-isolate interaction on above-ground dry weight ($F_{2,21} = 3.87$, $P \leqslant 0.037$; data not shown) and the same patterns occurred as for total plant dry weight. No significant plant-species–AMF-isolate interactions occurred for root dry weight ($F_{2,21} = 1.69$, $P \leqslant 0.21$) or total root length ($F_{2,21} = 4.18$, $P \leqslant 0.91$).

Significant plant-species–AMF-isolate interactions also occurred for the total number of leaves (Fig. 3b). *P. vulgaris* plants colonized by *Glomus* sp. (Basle Pi) had a significantly higher leaf number compared to plants colonized by any of the other AMF isolates. *P. vulgaris* colonized with *Glomus* sp. (BEG 19) had a significantly higher number of leaves than *P. grandiflora* colonized by the same AMF isolate. The significant plant-species–AMF-isolate effect started at 34 days following inoculation (*F*-ratio for the interaction term, excluding the nonmycorrhizal treatment, was $F_{2,30} = 6.11$, $P \leqslant 0.006$) and occurred at every subsequent measurement until the final harvest.

There were no significant AMF isolate effects or plant-species–AMF-isolate interactions on phosphorus concentrations of the whole plants (Fig. 3c) or in the roots, stems or leaves. There was, however, a significant plant-species–AMF-isolate interaction for the total phosphorus content of the plants ($F_{2,21} = 6.36$, $P \leqslant 0007$; data not shown), and this reflected the differences in total dry weight of the two plant species inoculated with the different AMF isolates (see Fig. 1).

There were significant plant-species–AMF-isolate interactions for the number of branches of the stolons (Fig. 3d). This significant interaction first occurred at day 44 following inoculation (*F*-ratio for the interaction term, excluding the nonmycorrhizal plants, was $F_{2,30} = 13.10$, $P \leqslant 0.0001$) and continued until the final harvest. There was also a significant plant-species–AMF-isolate interaction on total length of stolons (Fig. 3e). The stolons of *P. vulgaris* inoculated with *Glomus* sp. (Basle Pi) branched significantly more (Fig. 3d) and were significantly longer (Fig. 3e) than in any other treatment combination. Stolons of *P. grandiflora* inoculated with either *Glomus* sp. (BEG 19) or *Glomus* sp. (Basle Pi) were significantly shorter than the stolons of the *P. vulgaris* plants inoculated with any one of the three AMF isolates (Fig. 3e).

EXPT 3: GROWTH RESPONSE OF *P. VULGARIS* TO DIFFERENT AMF ISOLATES

The majority of plant and fungal variables were significantly and strongly affected by mycorrhizal treatment (Figs 4 and 5). Each of the AMF isolates differed significantly from the others in the percentage of root length they colonized and the percentage occupied by arbuscules and vesicles (Fig. 4). *G. geosporum* and *Glomus* sp. (Basle Pi) colonized a greater proportion of the roots than *Glomus* sp. (BEG 19), although *Glomus* sp. (Basle Pi) produced significantly more arbuscules and vesicles than either of the other two isolates.

Mycorrhizal treatment also affected plant growth and allocation of biomass (Fig. 5). As in expts 1 and 2, nonmycorrhizal *P. vulgaris* grew very little during the experiment and in all cases the nonmycorrhizal plants were significantly different in size, stolon branching, leaf number and leaf area from any of the mycorrhizal treatments. Differences in plant growth and biomass allocation can also be attributed to effects of the three AMF isolates. Total dry weight was significantly lower in plants inoculated with *Glomus* sp. (BEG 19) than in plants inoculated with either of the other two isolates (Fig. 5a). This effect is largely due to differences in stolon dry weight (Fig. 5b) and leaf dry weight (Fig. 5c). Root dry weight followed the same trend but was not significantly different among

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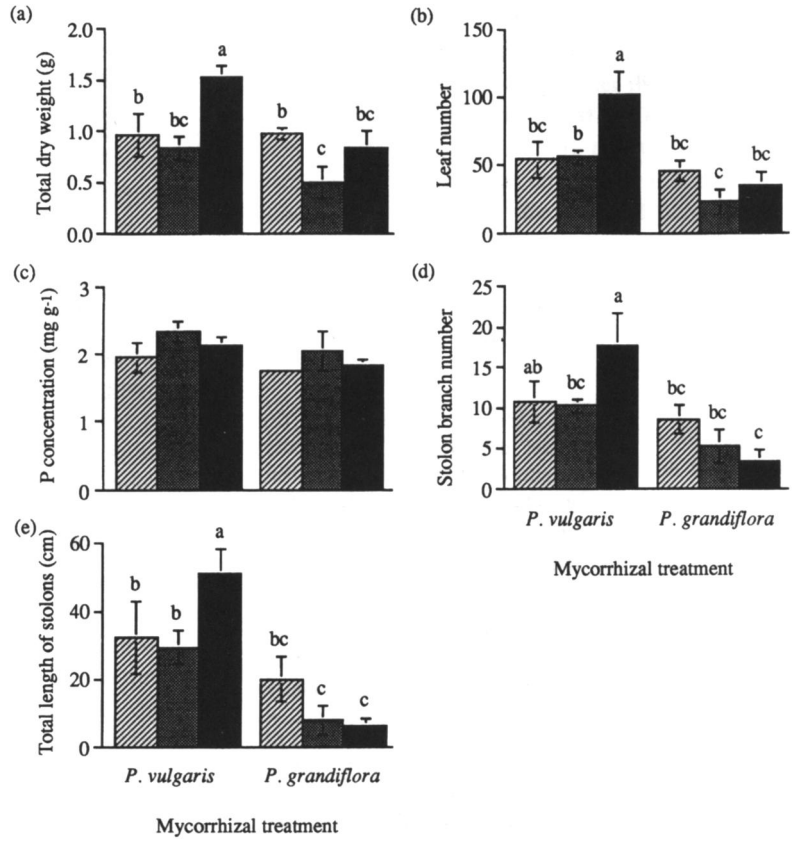

Fig. 3 Mean (a) total dry weight; (b) leaf number; (c) P concentration; (d) number of branches in stolons and (e) total length of stolons in *P. vulgaris* and *P. grandiflora* inoculated with one of the three AMF isolates: (▨) *Glomus geosporum* (BEG 18), (▩) *Glomus* sp. (BEG 19), (■) *Glomus* sp. (Basle Pi). Different letters above bars indicate significant difference according to the LSD test ($P \leqslant 0.05$). Letters are not shown above bars in (c) because the interaction term is not significant and means comparisons are therefore not appropriate. *F*-ratios of the interaction term, for the data presented in (a–e) as dependent variables, were $F_{2,21} = 3.93$, $P \leqslant 0.033$; $F_{2,21} = 3.92$, $P \leqslant 0.036$; $F_{2,21} = 2.49$, $P \leqslant 0.11$; $F_{2,21} = 3.49$, $P \leqslant 0.049$ and $F_{2,21} = 3.51$, $P \leqslant 0.046$, respectively.

plants inoculated with different AMF isolates (data not shown). Although plants inoculated with *Glomus geosporum* and *Glomus* sp. (Basle Pi) exhibited similar stolon dry weights, plants inoculated with *Glomus* sp. (Basle Pi) branched significantly more than plants inoculated with either of the other two isolates (Fig. 5d). There was no significant effect of AMF isolates on the leaf area of the plants (Fig. 5e).

Discussion

MYCORRHIZAL DEPENDENCY OF *PRUNELLA* SPP.

The results from the three experiments show that *P. vulgaris* and *P. grandiflora* are highly dependent on AMF in the calcareous soil used in this investigation. Although the effects of AMF on the growth of *Pru-*

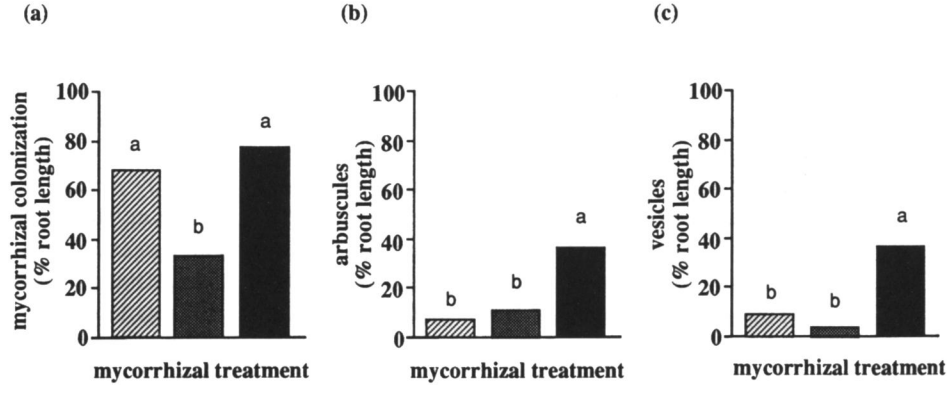

Fig. 4 Mean values of mycorrhizal colonization in the roots of *P. vulgaris* inoculated with three different isolates of AMF: (▨) *Glomus geosporum* (BEG 18), (▩) *Glomus* sp. (BEG 19), (■) *Glomus* sp. (Basle Pi).(a) percentage root length occupied by all AMF structures (hyphae, arbuscules and vesicles);. (b) percentage root length occupied by arbuscules;. (c) percentage root length occupied by vesicles. *F*-ratios of ANOVA, with mycorrhizal colonization, arbuscules or vesicles as dependent variables, were $F_{2,24} = 31.84$ ($P \leqslant 0.0001$), $F_{2,24} = 31.21$ ($P \leqslant 0.0001$), $F_{2,24} = 12.80$ ($P \leqslant 0.0002$), respectively. Different letters above bars indicate a significant difference ($P \leqslant 0.05$) according to the LSD test.

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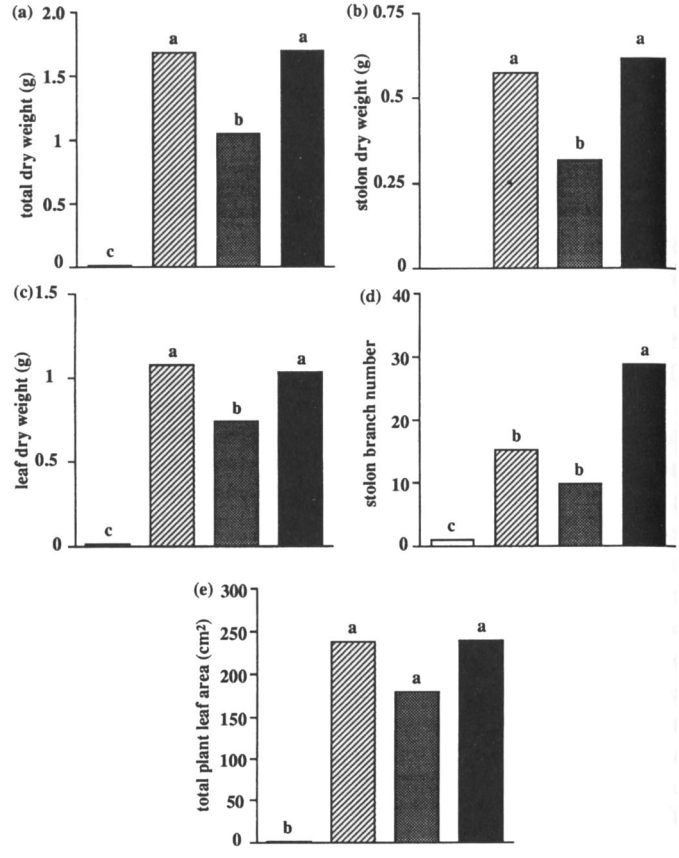

Fig. 5 Mean (a) total dry weight; (b) stolon dry weight; (c) leaf dry weight; (d) stolon branch number; (e) total plant leaf area for *P. vulgaris* inoculated with one of three AMF isolates (see Fig. 4 for legend) or not inoculated with AMF. *F*-ratios of ANOVA, for data presented in (a–e) as dependent variables, were $F_{3,31} = 32.01$ ($P \leqslant 0.0001$), $F_{2,24} = 10.48$ ($P \leqslant 0.0005$), $F_{3,31} = 27.81$ ($P \leqslant 0.0001$), $F_{2,24} = 21.12$ ($P \leqslant 0.0001$), $F_{2,29} = 18.61$ ($P \leqslant 0.0001$), respectively. Different letters above bars indicate a significant difference ($P \leqslant 0.05$) according to the LSD test.

nella spp. have not previously been investigated, another study indicated that several other low growing subordinate species of calcareous grasslands were strongly mycorrhizal dependent (Grime *et al.* 1987). There are numerous reports in the literature showing that mycorrhizal dependency can be overcome or partly overcome by the addition of inorganic phosphate. Our results from expt 1 show that this is not the case for *Prunella vulgaris* and they indicate that the effect of AMF is not related to phosphate acquisition. Because all plants in expt 1 received a nutrient solution with a full complement of other essential nutrients, we suspect that the main mycorrhizal effect on the growth of *P. vulgaris* is not due to improved acquisition of other mineral nutrients by AMF either. Other reports have suggested that interactions occur between AMF and root pathogens (Newsham *et al.* 1995), although the nonmycorrhizal *Prunella* in expts 1, 2 and 3 did not show any obvious symptoms on the leaves or the roots which would have suggested that they had become infected by pathogenic organisms. Thus, the reason for the strong dependency of *Prunella* on AMF remains to be elucidated.

EFFECTS OF DIFFERENT CO-OCCURRING AMF ON CLONAL GROWTH TRAITS OF *PRUNELLA* SPP.

Our results indicate that different AMF species can differentially influence clonal growth traits of two *Prunella* spp. Plant-species–AMF-isolate interactions occurred for clonal growth traits (stolon branching & stolon length) in the two *Prunella* spp. (Fig. 3). This means not only that different AMF fungi have different effects on clonal growth traits but also that the effect is not the same in each plant species. If this effect also occurs in other co-occurring plant species then the presence or absence of different AMF species would have the potential to affect not only the population structure of plant species but also the structure of a plant community.

The differential effects of AMF isolates on the length of stolons and the branching of the stolons in *P. vulgaris* in expt 2 appeared to be consistent with the effects of the AMF isolates on the size of the plants (Fig. 3). Although it was not possible to separate effects of growth and branching in expt 2, the growth rate of plants inoculated with *G. geosporum* significantly increased between 69 days and the final harvest at 94 days, suggesting that if the experiment had been extended, the size of plants inoculated with this AMF isolate could potentially have reached that of plants inoculated with *Glomus* sp. (Basle Pi). In expt 3, indeed we showed that 126 days after inoculation plants inoculated with *G. geosporum* were as large as those inoculated with *Glomus* sp. (Basle Pi), even though stolon branching was significantly lower (Fig. 5). This suggests that the effect of AMF on stolon branching is independent of AMF effects on size.

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ARE DIFFERENTIAL AMF ISOLATE EFFECTS INDEPENDENT OF THE COLONIZATION STAGE OF AMF?

Our results demonstrate that different AMF isolates (that co-occur in a natural community) affect clonal growth traits differently. An alternative hypothesis could be that isolates do not differ in their effects on growth in *Prunella* spp. but that the effects are dependent on the rate of colonization by the fungus and that in expts 2 and 3 the fungi differed in their rate of colonization. For the following reasons, the results of expt 2 allow us to reject the alternative hypothesis. Although the fungal isolates do indeed differ in their colonization of *Prunella* roots, the colonization of each AMF isolate was the same in the two species at the final harvest (Fig. 2), indicated by the main AMF isolate effect and a lack of a plant-species–AMF-isolate interaction. This effect was observed at each measurement made before the final harvest. However, effects of AMF isolates on several variables of plant growth (plant weight, leaf number, stolon branch number and stolon length) differed between the two plant species, indicated by the significant plant-species–AMF-isolate interactions (Fig. 3). These interactions also occurred at measurements prior to the final harvest. The differential effects of AMF isolates must therefore be independent of the developmental stage of the AMF. In addition, because the main AMF isolate effects on AMF colonization and the interactions for plant growth and clonal growth traits were consistent at measurements before the final harvest it is unlikely that the effects could be due to a delayed response to differences in AMF colonization at an earlier time-point in the experiment. Furthermore, the results of expt 3 show that colonization of *P. vulgaris* by *G. geosporum* (BEG 18) and *Glomus* sp. (Basle Pi) were not significantly different, although *Glomus* sp. (Basle Pi) induced significantly greater stolon branching than *G. geosporum* (BEG 18). We conclude therefore that that different AMF isolates differentially affect the clonal growth of plants.

POTENTIAL EFFECTS OF DIFFERENT AMF SPECIES ON FORAGING STRATEGIES OF CLONAL PLANT SPECIES

Plasticity in clonal growth traits such as branching intensity and stolon length in response to environmental heterogeneity are thought to determine a plant's ability to forage for resources (Hutchings & de Kroon 1994; de Kroon & Hutchings 1995). The AMF effects on branching observed here occurred in a resource homogeneous environment. This suggests that interactions between clonal plants and the different AMF fungal species in a community could also have the potential to strongly affect the foraging of clonal plants, and therefore their fitness, independently of resource heterogeneity. The results from expt 1 suggest that increasing a likely limiting resource, such as P, does not alter the way in which the plants will respond to AMF. The results from expt 2 would suggest therefore that the effect of the different AMF species on foraging strategies of *P. vulgaris* would not be affected by a P heterogeneous environment.

The significance of the effects of AMF on resource foraging strategies could be dependent on scale and growth habit of the clonal plant. In another investigation the same three AMF isolates which we used in expts 2 and 3 were able to grow at least 13–14 cm from the root system of *P. vulgaris*, although the maximum distance from the root which the hyphae will reach has not been tested (data not shown). If the effect of AMF on *P. vulgaris* is to increase resource acquisition below-ground then their effects could be more important to the plant than small scale changes in plant growth which will determine the placement of ramets in new resource patches. New ramets of *Prunella vulgaris* are usually formed a few centimetres from the parent and would be unlikely to be placed 13–14 centimetres away. The placement of ramets is likely, however, to affect the above-ground acquisition of resources, which is important for growth of AMF as well as the plant.

POTENTIAL OF DIFFERENT AMF TO INFLUENCE THE STRUCTURE OF *PRUNELLA* SPP. POPULATIONS

The strong mycorrhizal dependency of *Prunella* spp. may in itself be fairly irrelevant to the ecology of these species because very few soils in temperate ecosystems are completely devoid of AMF. Our results, however, indicate that different co-occurring AMF species can influence clonal growth traits which would influence the vegetative fitness of a given plant, the number of ramets and their spatial distribution in a population.

Whether and how the effects of different AMF species would affect the population structure or resource foraging strategies of *Prunella* in a natural community will be dependent on several factors which are currently unknown. First, the specificity of AMF in natural communities is unknown. In pot experiments the symbiosis seems to be relatively unspecific but whether this lack of specificity actually occurs in a natural community where some combinations of AMF are more beneficial to both partners than other combinations is unknown. Secondly, the spatial distribution of AMF species in natural communities is unknown and therefore whether new ramets of *Prunella* would be likely to become colonized by different AMF species by placement in a new position in the community is unclear. Thirdly, the effects of several AMF species colonizing one *Prunella* individual has not been compared to the effects of a single AMF isolate on *Prunella* growth. Finally, the effects of

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AMF on *Prunella* growth have been investigated with mixed genotypes of plants. Whether the effects of AMF diversity on plasticity of *Prunella* will override the genotypic differences in the clonal growth traits has not been investigated. Experiments are now being designed to answer these questions.

Conclusions

In this investigation we have shown that, through their differential effects on clonal growth traits of plants, different AMF (which co-occur in natural communities) can alter the foraging strategies of plant species, irrespective of resource heterogeneity and thus can potentially affect plant population structure. Furthermore, because the effects of an AMF isolate on clonal growth differs between plant species, co-occurring AMF also have the potential to affect plant community structure. To what extent AMF community structure could be a determinant of plant community structure will remain unknown until the effects of co-occurring AMF on other plant species from the community are investigated. Considering the results of this study, we suggest that future investigations on the determinants of clonal growth, resource acquisition, population structure and community structure should consider the presence of AMF which co-occur with plants in natural communities.

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