Date of preparation: 27 May 2009

Interactions between arbuscular mycorrhizal fungi and intraspecific competition affecting size and size inequality of *Plantago lanceolata* L.

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Running title: AM fungi and intraspecific competition
Word count of abstract: 324
Word count of full document: 7,584

Abstract

1 Intraspecific competition causes decreases in plant size and increases in size inequality. Arbuscular mycorrhizas usually increase the size and inequality of non-competing plants, but mycorrhizal effects often disappear when plants begin competing. Previous studies involving mycorrhizas and competition took place in either laboratory or field conditions and produced contrasting results. We hypothesised that mycorrhizal effects on size inequality would be determined by the experimental conditions, and conducted two simultaneous experiments to investigate how AM fungi and intraspecific competition determine size inequality in *Plantago lanceolata*.

2 In both field and controlled conditions, plant size was reduced when plants were competing, as expected. Most unexpectedly, size inequality was also reduced by competition. We conclude that the most likely reason is that plants were competing in a symmetric fashion, probably for nutrients. This is unlike most competitive situations, in which plant competition is strongly asymmetric.

3 Mycorrhizas had no effect on plant size or size inequality when plants were competing in either field or controlled conditions. We suggest that competition for nutrients was intense and negated any benefit the fungi could provide.

4 In non-competing plants, mycorrhizas also produced unexpected results. In field-grown plants, AM fungi increased plant size, but decreased size inequality. Mycorrhizal plants were more even in size, with very few very small individuals. In glasshouse conditions, mycorrhizal colonization was extremely high, and was generally antagonistic, causing a reduction in plant size. However, here mycorrhizas caused an increase in size inequality, supporting our original hypothesis. This was because most plants were heavily colonized and small, but a few had low levels of colonization and grew relatively large.

5 This study has important implications for understanding the forces that structure plant communities. AM fungi can have a variety of effects on size inequality and thus potentially important influences on long-term plant population dynamics, by affecting the genetic contribution of individuals to the next generation. However, these effects differ, depending on whether plants are competing or not, the degree of mycorrhizal colonization and the responsiveness of the plant to different colonization densities.

Key-words: Gini coefficient, Lorenz curves, intraspecific competition, mycorrhizas, size inequality, field grown plants, glasshouse plants, plant community, beneficial, antagonistic.

Introduction

Arbuscular mycorrhizal fungi have a wide variety of beneficial effects on their host plants, including enhanced growth through nutrient acquisition (Smith & Read 1997), fecundity (Koide 2000), competitive ability (e.g. West 1996), improved drought tolerance (e.g. Ruiz Lozano, Azcon & Gomez 1995) enhanced disease resistance (e.g. Borowicz 2001) and resistance to insect herbivores (Gehring & Whitham 2002). However, there are also many examples of AM colonization having a negative effect on plant growth and reproduction (e.g. Francis & Read 1995; Johnson, Graham & Smith 1997). Such negative effects may be explained by a degree of specificity in the symbiosis (Sanders 2002) or particular environmental conditions (such as high soil P) in which plants are grown (Gange & Ayres 1999).

The fact that plant species vary in their responses to AM colonization has led to studies of the role of these fungi in plant community structure. There are several experiments showing that AM fungi can increase the species richness of plant communities, either in microcosms or field situations (Grime *et al*. 1987; Gange, Brown & Sinclair 1993, van der Heijden *et al*. 1998), but the converse also occurs, as O'Connor, Smith & Smith (2002) and Hartnett & Wilson (1999) found that by reducing mycorrhizal occurrence with a fungicide, plant diversity or species richness subsequently increased.

Although no explicit test has been done, the mechanism by which these community effects occurs could well be a mycorrhizal effect on plant competition (van der Heijden 2002). Thus, if the competitive dominants in a community are strongly mycorrhizal, AM fungi will enhance their growth leading to suppression of weaker competitors and thus reduced species richness. Meanwhile, if the competitive dominants are weakly mycorrhizal or non-mycorrhizal, then AM fungi can enhance the growth of the mycorrhizal weaker competitors, promoting coexistence and an increased species richness. This simple description is, in reality, considerably more complicated, being affected by variations in mycorrhizal specificity and soil nutrient supply (Aerts 2002).

Implicit in the arguments regarding mycorrhizas and plant community structure is that the fungi can affect the balance of plant competition. A number of studies have shown that AM fungi can affect the outcome of interspecific competition (e.g. West 1996; Marler, Zabinski & Callaway 1999), particularly when there is a difference in responsiveness of the two plant species to fungal colonization (Watkinson & Freckleton 1997). However, in many plant communities, individuals of a given plant species are most likely to be growing in close proximity to members of their own species (Harper, 1977) and thus the role of AM fungi in affecting the outcome of intraspecific competition becomes critical.

Several studies have examined the effects of mycorrhizal presence on intraspecific competition in grasses (West 1996, but see also Watkinson & Freckleton 1997) and forbs (Shumway & Koide 1995; Moora & Zobel 1996; Facelli *et al*. 1999; Facelli & Facelli 2002). In all of these studies, mycorrhizas increased the intensity of competition. This could have consequences for the inequality in size seen within these populations.

High density plant populations are usually characterised by great inequality in size (Weiner & Thomas 1986), in which a few individuals usurp the majority of the available resource and the majority of individuals are small. These differences in size may be caused by any combination of environmental factors (such as nutrient availability or herbivores) and genetic differences between individuals, such as differential germination times or growth rates (Weiner 1990). Such inequality can have important consequences for the structure of plant populations, because an inequality in reproductive output will affect the genetic structure of subsequent generations (Shumway & Koide 1995). It can also affect the structure of the current generation, if self-thinning occurs, resulting in the death of smaller individuals (Weiner & Whigham 1988). An important question in plant community ecology is whether AM fungi can affect size inequality in competing plant populations. In theory, mycorrhizas could reduce size inequality, by increasing growth of weaker individuals, or increase it, by enhancing the growth of larger individuals at the expense of the weaker individuals. One aim of this paper is to address this question, using even-aged populations of *Plantago lanceolata* L., a strongly mycorrhizal forb (Gange & West 1994).

There are some consistent features of the studies that have examined the effects of AM fungi on size inequality. Firstly, they have produced quite similar results, in that mycorrhizas appear to increase size inequality when plants are grown at low density. At high densities, when resource competition is intense and nutrient depletion can occur, mycorrhizas have no effect on size inequality (Allsopp & Stock 1992; Facelli *et al*. 1999; Facelli & Facelli 2002). The one exception to this pattern is the work of Shumway & Koide (1995), in which AM fungi were found to increase the inequality in reproductive output of *Abutilon theophrasti* Medic. at both low and high density. It is interesting that the latter experiment was performed in the field, while other experiments have taken place in microcosms where nutrient limitation is likely to have occurred. Indeed, Facelli & Facelli (2002) suggest that at high density plantings, AM fungi deplete the available soil resources, with the subsequent limitation of plant growth negating the benefit derived from the symbiosis. Such a situation is much more likely to occur in controlled experiments and so we hypothesized that the effect of AM fungi on plant size inequality in crowded populations will depend on whether plants are grown in microcosms or in the field. In the former situation, plants experiencing intraspecific competition should exhibit no effects of mycorrhizas on size inequality, while in the latter a mycorrhizal effect may be apparent.

A second feature of previous studies is that the analysis of size inequality has been rather limited. Perhaps the most extensive was that of Shumway & Koide (1995), who examined inequality with Lorenz curves and the Gini coefficient. The Lorenz curve allows for graphical examination of the relative contribution of large or small individuals to a plant population, while the total amount of inequality (area under the curve between it and the line of equality) is summarised by the Gini coefficient. The concept of Lorenz curves and the Gini coefficient is summarised by Shumway & Koide (1995). Facelli & Facelli (2002) calculated just the Gini coefficient in their analysis of how mycorrhizas, intraspecific competition and nutrients affect size inequality in *Trifolium subterraneum* L. However, different Lorenz curves can possess identical Gini coefficients, thus the calculation of this statistic alone can produce misleading results if we are trying to understand how AM fungi affect the contribution of large or small plants to the total biomass of a population. Therefore, Damgaard & Weiner (2000) proposed an alternative statistic, the Lorenz Asymmetry Coefficient, and re-analysed the data of Shumway & Koide (1995). They were then able to show that the increase in reproductive inequality of *Abutilon theophrasti* when mycorrhizas were present was caused by the contribution of a small number of very large individuals. To date, no study has applied the methodology of Damgaard & Weiner (2000) to the analysis of mycorrhizal effects in competing plant populations. Here, we take this approach, enabling a more detailed analysis of how mycorrhizas affect plant size inequality.

Materials and Methods

STUDY SYSTEM

This investigation was carried out on *Plantago lanceolata* L. (Plantaginaceae), a common perennial forb that forms an arbuscular mycorrhiza and which shows a significant growth reduction when the mycorrhiza is reduced (Gange & West 1994). The investigation had two components: a field trial, in which plants were grown in natural soil and a controlled experiment, where plants were grown in pots of the same natural soil in a glasshouse. Both parts of this investigation were conducted simultaneously.

Seeds of *P. lanceolata* were sown in sterile potting compost (John Innes number 1, Roffey Ltd, Bournemouth, UK) and maintained at a temperature of 20°C. After 14 days, emerged seedlings were at the three leaf stage (two cotyledons plus one true leaf) and were selected for uniformity of size, based on the length of the true leaf. These were planted into the field and glasshouse trials.

FIELD TRIAL

An area of land at Silwood Park, Ascot, Berks measuring 500 m^2 was treated with the herbicide ‘Round Up’ (Monsanto plc, Leicester, UK) containing 360 g l^{-1} glyphosate in autumn, shallow ploughed in winter and hand raked in early spring, to remove any vegetation. A randomised block design was set out, consisting of four treatments, with 36 replicates of each. Two experimental conditions were created, consisting of presence or absence of intraspecific competition, with or without natural mycorrhizal colonization. The experiment was therefore a 2 x 2 factorial with four treatments in total. Non- competition plants consisted of one individual planted into the middle of a 0.5 m x 0.5 m plot, giving a density of 4 m^{-2} while competing plants consisted of 16 (in a 4 x 4 grid, i.e. 12.5 cm apart) (64 m^{-2}) in a 0.5 m x 0.5 m plot. These plant densities were chosen to represent the typical range of this species in early successional communities on this site (V.K. Brown, *pers. comm.*). Each plot was separated from its neighbour by 2 m and all other plants that appeared in the experimental plots through natural germination were hand-weeded out. Reduced mycorrhizal colonization was achieved by application of the fungicide ‘Rovral’ (Bayer Crop Science, Hauxton, UK) (containing 40% w/w iprodione) to the soil. This was applied at a rate of 2 g m^{-2} of formulated product at two week intervals from March to August. The soil was a sandy loam, with a pH of 5.4 and a bicarbonate extractable P content of 3.9 μg P g^{-1} and nitrogen content of 2.1 μg NO_3^- g^{-1}. Plants were watered immediately after transplanting, but once established, no supplementary water was given. A total of four plants did not survive transplanting and these were replaced within the first week of the trial. Thereafter, no plants died during the course of the experiment. The site was fenced to exclude rabbits and although molluscs were rare on the acidic sandy soil, a few pellets of the molluscicide MifaSlug (containing metaldehyde)

(Farmers Crop Chemicals Ltd, Worcester, UK) were placed around the perimeter of each plot once a month.

Plants were maintained for 20 weeks after which time each was carefully dug up and the roots washed free of soil. The extreme sandy nature of the soil meant that we were able to recover virtually all of each root system intact. Total vegetative biomass (separately for roots and shoots) was recorded as dry weight and the number of inflorescences counted on every plant. To minimise edge effects, we conducted our analyses (below) using the means of the four plants in the middle of the plot, in a similar manner to the designs of Shumway & Koide (1995) and Facelli & Facelli (2002). Before drying, a 2 g portion of fresh root was removed from each plant and subjected to autofluorescence microscopy for the quantification of mycorrhizal colonization. Roots were washed, placed on microscope slides and examined at x 200 using a Zeiss Axiophott epifluorescence microscope equipped with a UV lamp and filters giving a transmission of 450-490 nm blue. Under these conditions, the arbuscules fluoresce (Ames, Ingham & Reid 1982) and arbuscular colonization was recorded as percent root length colonized (% RLC) by the cross hair eye piece method of McGonigle *et al.* (1990). Values for dry root biomass were corrected for the loss of the 2g sample in each case. This method was chosen because it produces more consistent and reliable results in *P. lanceolata* than any of the conventional stains (Gange *et al.* 1999), however its disadvantage is that any non-mycorrhizal fungal material will not be seen. Therefore, we also subjected roots to a conventional staining procedure (Vierheilig *et al.* 1998), to check for infection by non-mycorrhizal fungi.

GLASSHOUSE EXPERIMENT

The experiment was conducted under controlled conditions in a glasshouse at the University of East London, Stratford, UK. Seedlings at the three leaf stage (see above) were transplanted into 250 mm diameter pots containing 24 l of soil taken from an area adjacent to that of the field study area at Silwood Park. The soil was placed into the pots and allowed to equilibrate for a two month period prior to transplanting. After this time, N and P contents were measured and found to be 2.9 μg NO_3^- g^{-1} and 4.4 μg P g^{-1} respectively. Neither of these two values were significantly different from those obtained in the field site ($P > 0.05$).

The no competition treatment consisted of one plant in the middle of each pot (equivalent to 20 m^{-2}), while the competition treatment consisted of 3 plants, each 12.5 cm apart (equivalent to a density of 61 m^{-2}). Therefore, plant densities in this experiment were as similar as possible to those in the field trial. Within blocks, competition pots were arranged adjacent to each other on the glasshouse bench, with extra ‘dummy’ pots around the edge. Only pots inside this arrangement (i.e. not edge pots) were sampled, to minimise edge effects and to be as close a mimic as possible of the field plot design and those of Shumway & Koide (1995) and Facelli & Facelli (2002). Mycorrhizas were reduced by addition of iprodione at the same application rate as in the field (i.e. 2 g m^{-2}, 0.1 g per pot) applied at two week intervals. There were 25 replicate pots of each of the four treatments and these were arranged in a randomised block design on the glasshouse bench.

Plants were maintained for 20 weeks, during which time no supplementary fertiliser was given, but each pot received variable amounts of water per week, to maintain a soil moisture level equal to that occurring in the field. At the end of the growth period, all plants were carefully removed from the pots and their roots washed free of soil. Foliar and root biomass was obtained for all individual plants, but for those in the competition treatment, roots could not be separated and so mean biomass per pot was calculated by dividing the total by three. Dry biomass was recorded, together with the total number of inflorescences produced per plant. Mycorrhizal colonization of each plant was obtained in an identical manner to that in the field trial.

STATISTICAL ANALYSIS

Plant growth data (foliar and root biomass and flower number) were tested for normality and homogeneity of variances prior to analysis, and underwent log transformation, where appropriate. Mycorrhizal percentage colonization data were subjected to the angular transformation prior to analysis (Zar, 1996). For non-competing plants, we examined the relation between mycorrhizal colonization and the degree of ‘benefit’ received by the plant, (defined as the percentage change in a parameter of a mycorrhizal plant relative to a mean value for plants without AM colonization (Gange & Ayres 1999)). Foliar biomass was used as the response variable in this analysis.

Data were analysed by Randomised block analysis of variance, including mycorrhizas and competition as main effects, using the UNISTAT® statistical package. To examine size inequality, we calculated the Gini coefficient (Damgaard & Weiner 2000) and constructed Lorenz curves for each treatment, as described by Shumway & Koide (1995), to examine the relative contribution of large or small individuals to the inequality of the populations. If all individuals in a population are the same size, then the Lorenz curve is a straight diagonal line, called the line of equality (Damgaard & Weiner 2000). Otherwise, it is a curve below the line and the area between it and the line is measured by the Gini coefficient or ratio, defined as the ratio of the area bounded by the line and the curve to the total area beneath the line. In competition treatments, the coefficient was calculated using the four middle plants (field plots) or all three plants (glasshouse pots), with each plot or pot as a replicate. As it is possible for different Lorenz curves to have the same Gini coefficient, the Lorenz Asymmetry Coefficient (S) was calculated in each case, following Damgaard & Weiner (2000). This is done by measuring the asymmetry of the Lorenz curve around the axis of symmetry (the other diagonal). Specifically, the Asymmetry Coefficient is the point at which the slope of the Lorenz curve is equal to 1 (i.e. equal to that of the line of equality) and can be used to examine whether the total biomass of a population is being made up by a few very large individuals (curve ‘a’ in Damgaard & Weiner 2000) or many small individuals (curve ‘b’ in the same paper). When the Lorenz curve is parallel with the line of equality at the axis of symmetry, S will equal 1, since all individuals are the same size. If the point at which the Lorenz curve is parallel with the line of equality occurs below the axis of symmetry, $S < 1$, which is indicative of a population with many small individuals that contribute little to the population’s total biomass. If the point at which the Lorenz curve is parallel with the line of equality occurs above the axis of symmetry, $S > 1$, indicative of a population with a few very large individuals which contribute the majority of the population’s biomass. Confidence intervals for S were obtained with a bootstrap procedure (Dixon *et al.* 1987).

Results

MYCORRHIZAL COLONIZATION

In both field and glasshouse grown plants, application of fungicide was successful in reducing the abundance of AM fungi (Fig. 1). Infection by non-mycorrhizal fungi was extremely low and the highest level recorded in any sample was that for glasshouse grown plants in the non-fungicide treatment at 3.1% RLC (Root Length Colonized). It is therefore most unlikely that any confounding effects of non-mycorrhizal fungi existed. In contrast, levels of arbuscular colonization were exceptionally high in glasshouse plants, with a mean of 50% in non-competing, untreated plants (Fig. 1b). Some individual plants in this treatment had levels of arbuscular colonization alone over 70%.

Intraspecific competition significantly reduced AM colonization in both field ($F_{1,140} = 38.2$, $P < 0.001$) and glasshouse plants ($F_{1,96} = 12.5$, $P < 0.001$). In field plants, there was a significant interaction term between mycorrhizas and competition ($F_{1,140} = 6.9$, $P < 0.01$), because the fungicide effect was only clearly seen when plants were not competing (Fig. 1a).

PLANT GROWTH

Not surprisingly, plants undergoing competition produced significantly smaller amounts of both foliar and root biomass than those not competing, in both experiments. Of more interest was the fact that AM fungi also affected biomass, but this was not consistent between the experiments. In field-grown plants, mycorrhizas resulted in plants with greater foliar biomass. However, because this effect was only seen in non-competing plants, there was a significant interaction term between mycorrhizas and competition. No interaction was seen with root biomass, as mycorrhizas increased the amount of root, irrespective of the density at which plants were grown (Table 1). In glasshouse plants, however, mycorrhizas decreased both foliar and root biomass significantly. In both parameters, there was a significant interaction between the treatments, as the mycorrhizal-induced reduction in growth was only seen in non-competing plants, where the response was quite dramatic, with mycorrhizas causing a reduction of over 25% in each case.

Mycorrhizas had no effect on the root/shoot ratio in either experiment, but this parameter was consistently increased by competition. In the field trial, non-competing plants produced more shoot than root biomass, giving a ratio less than unity, whilst the reverse was true for competing plants where ratios were greater than one (Table 1). This resulted in a significant interaction term for root/shoot ratio in field grown plants. In glasshouse plants, however, all treatments produced ratios over one, (indicating a greater amount of root), but the effect of competition was still significant, albeit weak.

The number of flowering stems was greatly reduced by competition in both experiments, a likely result of the overall effects on plant size. The mycorrhizal effect was not consistent because inflorescence number was significantly increased by mycorrhizas in non-competing, field grown plants, but unaffected by AM fungi when plants experienced competition. This resulted in a significant interaction term for field grown plants (Table 1). In contrast, the number of flowering stems produced by glasshouse plants was unaffected by mycorrhizas, even though overall foliar biomass was altered (Table 1).

For plants grown in the field, the range in colonization across fungicide-treated and untreated plants was 2 – 35%. A significant positive relationship was found, which was fitted best by a second order polynomial ($F_{2,70} = 155.5$, $P < 0.001$, $R^2 = 81.6\%$) (Fig. 2a). This indicates that the association with AM fungi was generally beneficial to the plants. Meanwhile for glasshouse plants, the range in colonization was 9 – 71% and a significant negative relationship was obtained, also fitted by a second order polynomial ($F_{2,48} = 37.4$, $R^2 = 60.9\%$) (Fig. 2b). This indicates that the association with AM fungi was mostly antagonistic to the plants. In the latter experiment, plants with very high levels of colonization were smaller than mycorrhizal free plants grown in the same conditions.

SIZE INEQUALITY

It should be noted that comparisons of Gini coefficients are only unambiguous if populations share the same type of Lorenz curve. As this was not so in this study, we report qualitative differences between the coefficients only.

In field grown plants, size inequality was reduced by competition, as indicated by the reductions in Gini coefficients (Fig. 3a). Mycorrhizas also had an effect on size inequality, which varied according to the level of competition. In non-competing plants, AM fungi reduced inequality by about 25%. However, in the competition treatments, no effect of mycorrhizas was found (Fig. 3a). These results form an interesting comparison to those of total foliar biomass (Table 1), because when mycorrhizas increased plant size, inequality was reduced.

The reduction in total inequality in competition treatments can be seen clearly in the two Lorenz curves being closer to the line of equality than either of the two non-competition curves (Fig. 3b). When plants were grown singly, the Asymmetry Coefficient, S, was 0.872 for mycorrhizal plants and 0.713 for plants where mycorrhizas were reduced. The interpretation of this is that as the mycorrhizal coefficient is closer to one, this population contained fewer very small individuals and plants were more even in size. However, when plants experienced competition, S for mycorrhizal plants was 1.105, while that for reduced-mycorrhizal plants was 1.045. These coefficients are significantly ($P < 0.05$) greater than those for non-competing plants, but much closer to unity, and indicate that in competing populations, a smaller degree of asymmetry existed. However, in these competing populations, mycorrhizas were found to have no effect on total size (Table 1), no effect on inequality and no effect on the relative proportions of large and small plants. In summary, when plants were grown without competition, mycorrhizas increased plant size and made the population to be more even in size, by causing there to be fewer very small plants. However, the mycorrhizal effects did not occur when plants were competing.

In glasshouse plants, competition again reduced total inequality(Fig. 4a). In non-competing plants, AM fungi increased inequality by about 20%, the opposite to the situation observed in field-grown plants. However, when glasshouse plants were competing, mycorrhizas had no effect on inequality (Fig. 4a), the same as was observed with field grown plants.

When plants were grown singly, the Asymmetry coefficient *S* was 1.164 for mycorrhizal plants, but only 0.92 for plants with reduced mycorrhizas. This shows that the mycorrhizal plant population exhibited a greater degree of asymmetry, with a greater proportion of large plants than the non-mycorrhizal population. When plants experienced competition, *S* was 1.102 for mycorrhizal plants and 1.158 for those where mycorrhizas were reduced. Therefore, as with field plants, mycorrhizas had no effect on foliar biomass or size inequality in competing populations. In summary, when plants were grown without competition, mycorrhizas reduced plant size and made the population to be less even in size, because of a few very large plants. However, this mycorrhizal benefit on a few individuals disappeared when plants were competing.

Discussion

In order to understand how AM fungi affect plant coexistence and the structure of communities, experiments need to be performed that address the responses of plants at the population level, using realistic mycorrhizal communities (Hart, Reader & Klironomos 2003). A fundamental aspect of any plant population is the degree of variability or inequality in size. As plant size and reproduction are often correlated, inequality in size will mean inequality in reproductive output, which will influence the range of genetic variation in subsequent generations (Weiner 1988). Intraspecific competition has been shown to increase the inequality in size of a range of plant species (e.g. Weiner & Thomas 1986; Weiner, Mallory & Kennedy 1990; Weiner *et al*. 2001), due to asymmetric competition between plants. In asymmetric competition, a few plants usurp the majority of the resources and grow very large, while the vast majority are small (Weiner 1990). However, some previous studies have found that intraspecific competition has no effect on size inequality. Facelli & Facelli (2002) found that in the absence of mycorrhizas, the Gini coefficient was identical in plants of *T. subterraneum* grown at low and high density and Shumway & Koide (1995) found a very similar result in low and high density non-mycorrhizal populations of *A. theophrasti*. However, in our study we found consistently that competition reduced the amount of inequality in populations, although the extent of this reduction depended on the presence of mycorrhizas. Two factors might account for competition leading to a reduction in size inequality. Firstly, if self-thinning occurs, in which the smallest plants die, this will lead to a reduction in inequality (Weiner & Thomas 1986). However, this cannot be the reason for our observations, as none of the plants died in our experiment. The second possibility is that competition between plants was more symmetric, with a relatively even distribution of resources between each individual. If interactions are symmetric, competition will act to slow the growth of all plants and thus reduce the divergence in size, leading to a reduction in size inequality (Weiner & Thomas 1986). Symmetric competition is unusual in plant populations, and may occur when plants are at the seedling stage and competition is only for nutrients. When plants grow larger, competition for nutrients may be size symmetric (Schwinning & Weiner 1998), although this depends on the distribution of resources (Rajaniemi 2003). If plants are grown at low density, then competition for light may also be symmetric, but at high density, dominance and suppression (asymmetric competition) is to be expected (Schwinning 1996). It is interesting that symmetric competition was reported by Turner & Rabinowitz (1983), working with the grass *Festuca paradoxa* Desv. These authors suggested that the graminoid growth form was less likely to produce competition for light and it is possible that a similar event occurred in our populations. *P. lanceolata* is a rosette hemicryptophyte, with the majority of biomass invested in leaf material. Although our plants were grown close enough together so that mutual shading occurred, it is possible that competition for light was of much less relevance than for nutrients. The field site was fully exposed to the sun and the glasshouse provided ample light, but the soil was nutrient-poor (particularly in P) and so competition in our populations may have been primarily for nutrients, meaning that it was relatively symmetric. This situation would have been exacerbated by the fact that our plants were even aged and even sized when the experiment began. It is known that differences in germination rate and subsequent growth rate can contribute to the size hierarchies seen in plant populations (Schwinning & Weiner 1998), but as our plants were all the same age and size at the beginning of the experiments, no individual would have possessed an initial advantage.

To date, there have been few studies of how AM fungi can affect inequality in size in plant populations. In general, experiments have involved plants grown at low and high densities, with and without the addition of mycorrhizal inoculum. When grown at low density (N.B. the definition of ‘low’ varies greatly between studies and generally has not used plants grown without competition, as in this study), mycorrhizas have increased competitive asymmetry, leading to an increase in size inequality (Allsopp & Stock 1992; Shumway & Koide 1995; Facelli & Facelli 2002). However, when plants experience intense competition, mycorrhizas usually have no effect on inequality. In the current study, mycorrhizas had no effect on plant size or inequality in size when intraspecific competition was occurring, similar to the findings of Allsopp & Stock (1992) and Facelli & Facelli (2002). When plant density is high, the density of roots means that the mycorrhizal mycelium becomes less important for nutrient absorption, as nutrients become depleted locally (Koide 1991). Therefore, our original hypothesis, that mycorrhizal effects on inequality in crowded populations should differ in field and glasshouse was rejected. It would seem that in both situations, nutrient limitation occurred, negating any benefit that the mycorrhizas could provide.

However, when plants were grown without competition, our experiments produced results that were in contrast to previous studies. *P. lanceolata* is a strongly mycotrophic forb that has shown enhanced growth from mycorrhizal colonization in previous field trials (Gange & West 1994; Gange, Bower & Brown 2002). In this respect, our field data was not unusual, as plants with mycorrhizas were considerably larger than those where the association was reduced. However, the size inequality of the mycorrhizal plants was much smaller. Analysis of the Lorenz curves showed that this was because the mycorrhizal plant population contained fewer plants in the smallest size classes. This may again be a result of the fact that plants in the current experiment were of the same age. If seeds germinate naturally and there is a difference in germination times, then the growth rate of early-germinating individuals that become mycorrhizal will be enhanced, leading to a fungal-induced increase in size inequality (Weiner 1990). Our data show that if plants have synchronous germination, then competition is likely to be more symmetric, as all individuals probably became colonized at the same time. It would be instructive to examine the effects of mycorrhizas on size inequality of populations naturally establishing from seed, rather than planted seedlings. These data alone show how the conditions of an experiment may affect the development of plant size hierarchies.

An even better example of experimental variation is provided by the results from non-competing plants grown in the glasshouse. Mycorrhizal colonization levels in these were extremely high and even when fungicide was applied, the abundance of arbuscules was reduced to a level approximately equal to that of the untreated plants in the field. At these extraordinary high levels of arbuscular colonization, the mycorrhizas appeared to be antagonistic to *P. lanceolata*. It is possible that application of fungicide killed pathogens, but as levels of non-mycorrhizal fungi were so low in the roots, we do not consider this as a viable explanation. The relationships between colonization levels and plant performance clearly showed a curvilinear relation, as predicted by Gange & Ayres (1999). To our knowledge, this is the first report of mycorrhizal antagonism in this plant, almost certainly caused by the fungi being carbon parasites (Gange & Ayres 1999). As the plants were grown in pots, nutrient depletion may well have occurred and thus the benefit to the plant was outweighed by loss of carbon to the mycorrhizas. In this case, the mycorrhizal plants showed an increase in inequality because most plants were very heavily colonized and therefore small, but a few had much lower levels of colonization and appeared to benefit from the association and grew very large. When fungicide was applied, colonization was reduced, the antagonistic effect of the mycorrhizas was lessened and mean plant size increased. This population was more even in size, and no individual was very large relative to the others. As Gange & Ayres (1999) state, few studies consider the responses of individual plants to mycorrhizal colonization and our data show that the degree of colonization that plants experience is likely to be a hitherto unconsidered factor in affecting the development of size inequality in plant populations.

In natural communities, mycorrhizal colonization of *P. lanceolata* varies greatly over the course of a growing season (Gange *et al*. 2002). It is also highly likely that the species composition of fungi in the root system changes seasonally, as molecular studies have shown that this happens in other plants (Helgason, Fitter & Young 1999). Furthermore, mycorrhizal species show spatial heterogeneity in their distributions (Hart & Klironomos 2002). Given that different AM species or combinations can have different effects on plant growth (Sanders 2002), it is likely that they will also have different effects on size inequality. It is remotely possible that the soil in our glasshouse pots contained different fungal species to that in our field plots. As the soil was taken from an area adjacent to the field site, we consider this very unlikely, but future experiments on size variability would benefit from a molecular investigation of the species composition in the roots. If we are to understand how AM fungi affect the development of inequality in plant populations then experiments need to be performed with different fungal combinations, as recommended by Hart *et al*. (2003).

It is known that perennial forbs exhibit a range in responses to natural mycorrhizal colonization, from negative to positive (Wilson *et al*. 2001). The differential effects of mycorrhizas on plants can lead to changes in plant community structure, mediated through interspecific competition (Smith, Hartnett & Wilson 1999). It would therefore be rewarding to examine the effects of mycorrhizas on size inequality of plant species that respond positively or negatively to mycorrhizal colonization. Hart *et al*. (2003) argue that future experiments of this type should take place in macrocosms, because of the difficulty in manipulating mycorrhizas in the field. However, the fact that our experiments have produced quite different conclusions suggests that a dual approach of laboratory and field does have merit. Controlled experiments will lose much of the natural variability in mycorrhizal spatial and temporal distributions, which could mask important effects on the inequality within populations. The fact that we have found differing effects of the fungi on size inequality suggests that mycorrhizas may have profound effects on long-term plant population dynamics, by altering the genetic contribution of individuals from one generation to the next.

Acknowledgements

We are grateful to the University of East London for funding the glasshouse experiment. D.M.A. was supported by the Natural Environment Research Council.

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Table 1 Means (with one SE in parentheses) and summary of statistical analysis of growth parameters of *Plantago lanceolata*, grown in conditions of low or high density, with mycorrhizas (+AM) or with reduced mycorrhizas (-AM). Statistical values tabulated are *F* ratios from ANOVA, testing for the main effect of mycorrhizas (M), intraspecific competition (C) or the interaction between them (M*C). Degrees of freedom for field plants: 1,140 and for glasshouse plants 1,96. Superscript notation is *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

	- Competition		+ Competition		ANOVA summary		
	+ AM	- AM	+AM	-AM	M	C	M*C
Field grown plants							
Foliar biomass, g	27.8 (2.2)	18.5 (1.9)	5.9 (0.3)	5.7 (0.3)	11.3***	231.3***	8.7**
Root biomass, g	23.5 (2.1)	18.1 (1.8)	13.3 (0.6)	11.7 (0.4)	7.2**	30.1***	0.9
Root/shoot ratio	0.65 (0.06)	0.87 (0.09)	1.57 (0.06)	1.45 (0.07)	0.5	104.3***	5.1*
Inflorescence number	39.6 (2.5)	31.6 (2.5)	13.9 (0.9)	13.3 (0.4)	6.1*	183.3***	4.8*
Glasshouse plants							
Foliar biomass, g	8.8 (0.9)	11.9 (0.9)	4.2 (0.3)	4.1 (0.2)	4.6*	136.7***	6.2*
Root biomass, g	15.6 (1.5)	20.9 (1.6)	8.3 (0.4)	8.1 (0.5)	4.1*	82.9***	5.6*
Root/shoot ratio	1.6 (0.1)	1.6 (0.1)	2.0 (0.2)	2.0 (0.1)	0.04	4.4*	0.0
Inflorescence number	30.8 (2.5)	30.4 (2.8)	10.1 (0.8)	10.4 (0.6)	0.01	70.4***	0.7

Figure legends

Fig. 1 Mycorrhizal colonization of *Plantago lanceolata*, measured by percent root length colonized (% RLC, arbuscules only) and grown with or without competition (see text for explanation). Open bars: natural mycorrhizal levels, shaded bars: application of fungicide to reduce colonization.

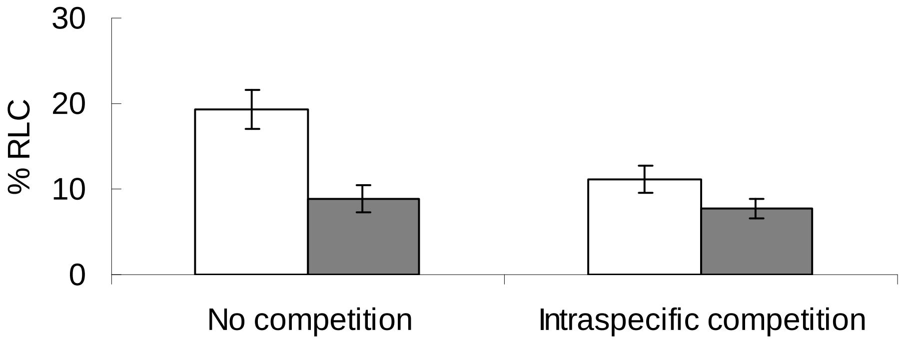
Fig 2 Relationships between mycorrhizal colonization and the degree of 'benefit' (sensu Gange & Ayres 1999) derived by the plant. Data portrayed is that for all low density plants, combined across fungicide treatments. The equation of the fitted line for field grown plants is $y = 6.6x - 0.1x^2$ while that for glasshouse plants is $y = 9.1x - 0.1x^2$.

Fig. 3 Graphical analysis of inequality in field grown plants. Total inequality is measured by the Gini coefficient in non-competing and competing plants. Open bars: natural mycorrhizal levels, shaded bars: application of fungicide to reduce colonization. Lower graph shows the Lorenz curve for each treatment. +C and -C: with and without competition respectively; +AM and –AM indicate natural mycorrhizal levels or reduced levels. The diagonal solid line is the line of equality.

Fig. 4 Graphical analysis of inequality in glasshouse grown plants. Legend as in Fig. 3.

Fig. 1

(a) Field grown plants



(b) Glasshouse plants

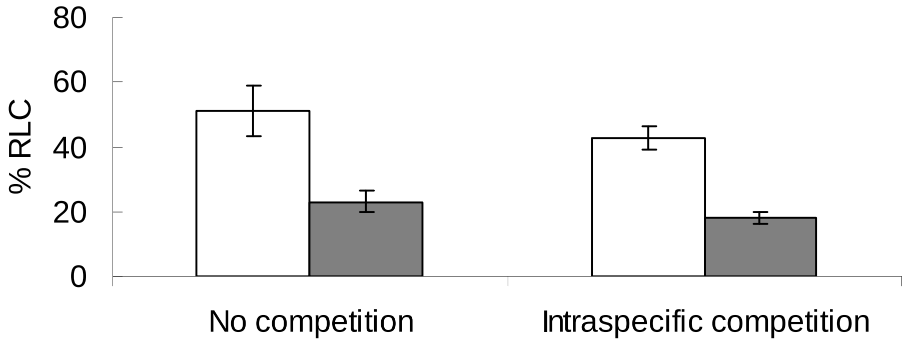
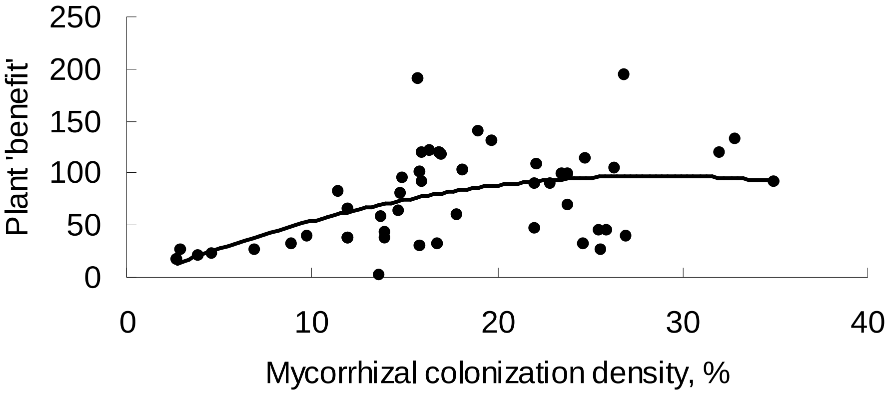


Fig. 2

(a) Field grown plants



(b) Glasshouse plants

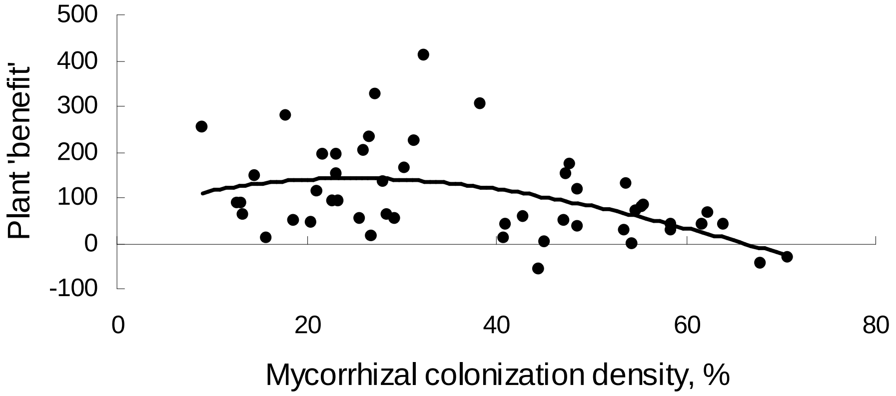


Fig. 3

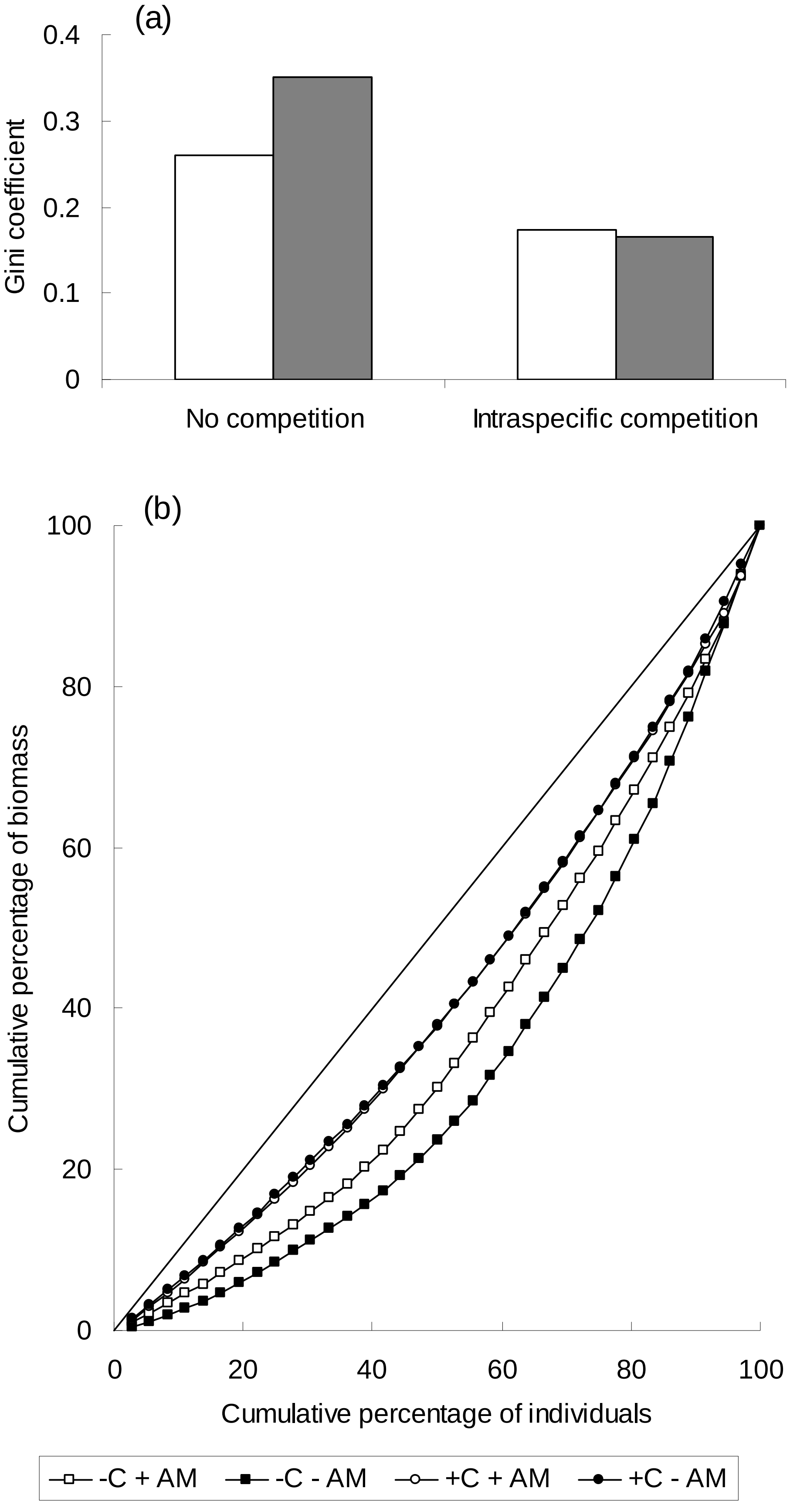
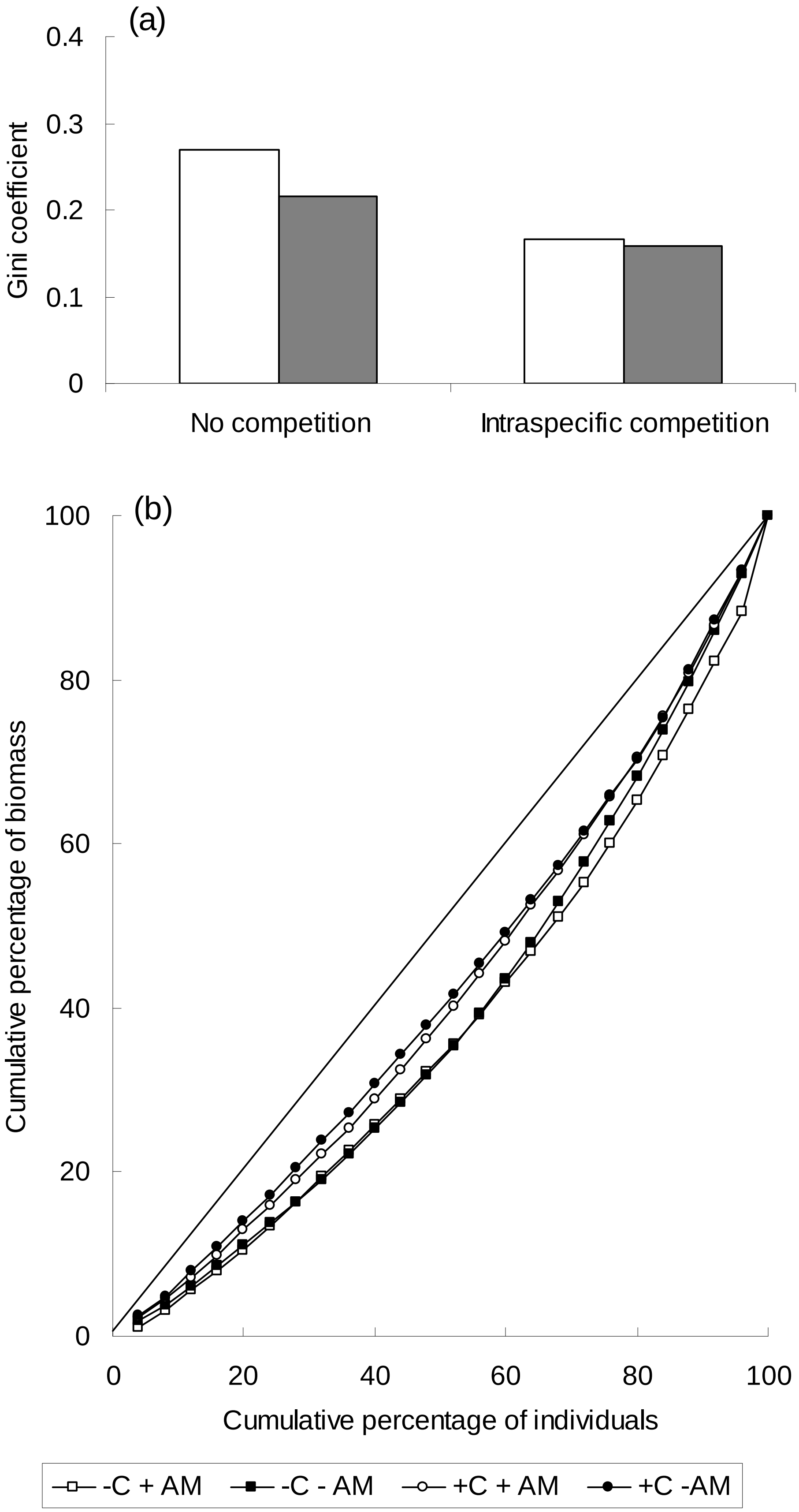

(a)
Gini coefficient
0.4
0.3
0.2
0.1
0
No competition
Intraspecific competition
(b)
Cumulative percentage of biomass
100
80
60
40
20
0
0
20
40
60
80
100
Cumulative percentage of individuals
-C + AM
-C - AM
+C + AM
+C - AM

Fig. 4


(a)
Gini coefficient
0.4
0.3
0.2
0.1
0
No competition
Intraspecific competition
(b)
Cumulative percentage of biomass
100
80
60
40
20
0
Cumulative percentage of individuals
0
20
40
60
80
100
-C + AM
-C - AM
+C + AM
+C -AM