

Mycorrhizal fungi suppress aggressive agricultural weeds

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Abstract Plant growth responses to arbuscular mycorrhizal fungi (AMF) are highly variable, ranging from mutualism in a wide range of plants, to antagonism in some non-mycorrhizal plant species and plants characteristic of disturbed environments. Many agricultural weeds are non mycorrhizal or originate from ruderal environments where AMF are rare or absent. This led us to hypothesize that AMF may suppress weed growth, a mycorrhizal attribute which has hardly been considered. We investigated the impact of AMF and AMF diversity (three versus one AMF taxon) on weed growth in experimental microcosms where a crop (sunflower) was grown together with six widespread weed species. The presence of

AMF reduced total weed biomass with 47% in microcosms where weeds were grown together with sunflower and with 25% in microcosms where weeds were grown alone. The biomass of two out of six weed species was significantly reduced by AMF (−66% & −59%) while the biomass of the four remaining weed species was only slightly reduced (−20% to −37%). Sunflower productivity was not influenced by AMF or AMF diversity. However, sunflower benefitted from AMF via enhanced phosphorus nutrition. The results indicate that the stimulation of arbuscular mycorrhizal fungi in agro-ecosystems may suppress some aggressive weeds.

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Abbreviations

AMF Arbuscular mycorrhizal Fungi

Introduction

Excessive weed growth is one of the biggest problems in agriculture causing between 10% and 30% of crop yield loss every year (Oerke and Dehne 1997). Hence, for the maintenance of crop production, it is essential to develop mechanisms by which weeds can effec-

tively be controlled. Herbicides are often used for weed control, but they can be expensive, can cause environmental problems and are not allowed in organic production systems. An increasing number of studies, therefore, investigate whether natural enemies of weeds can be used for their control (e.g. Scheepens et al. 2001; Hatcher and Melander 2003). In this study we focus on arbuscular mycorrhizal fungi (AMF), a widespread group of soil fungi that can enhance yield of several agricultural crops (Plenchette et al. 1983; Smith and Read 2008), especially when soil fertility is low. However, AMF may also suppress growth of agricultural weeds as was recently proposed by Jordan et al. (2000).

AMF form symbiotic associations with over two thirds of all terrestrial plant species (Trappe 1987) and form extensive mycelia networks in the soil (Giovannetti et al. 2004). AMF forage effectively for minerals such as phosphorus, zinc and copper that are delivered to the plant roots (Smith and Read 2008). Plants also benefit from AMF through enhanced water supply and disease protection (Auge 2001; Gosling et al. 2006; Sikes et al. 2009). As a consequence, AMF can promote plant productivity in natural and agricultural ecosystems (van der Heijden et al. 1998; Lekberg et al. 2007). However, AMF are not only beneficial, and interactions between plants and AMF can range from highly mutualistic to antagonistic where AMF reduce plant growth (Francis and Read 1994, 1995; Johnson et al. 1997; van der Heijden 2002; Klironomos 2003). For instance, studies performed with plants from natural communities show that AMF often have detrimental effects on non-hosts (Grime et al. 1987; Allen et al. 1989; van der Heijden et al. 1998), on plants grown at high nutrient availability or on plant species characteristic of ruderal environments where there is considerable disturbance (Francis and Read 1995).

Many agricultural weeds have a ruderal lifestyle and belong to families that comprise many non-hosts (e.g. Chenopodiaceae and Cruciferae—Harley and Harley 1987; Brundrett 2002; Wang and Qiu 2006). These observations suggest that AMF have the potential to suppress weed growth. Surprisingly however, little attention has been given to the effects of AMF on growth of major agricultural weeds. Moreover, even though some studies have indicated that AMF can reduce plant growth (see above), the large majority of mycorrhizal studies focussed on the positive effects of

AMF, ignoring the fact that an estimated 10–15% of all vascular plant species (that is approximately 17,000–39,000 species, including the model plant *Arabidopsis thaliana*) are non-mycorrhizal (Wang and Qiu 2006; Brundrett 2009).

Recent work has shown that the composition and diversity of AM fungal communities influence plant productivity and ecosystem functioning (van der Heijden et al. 1998; Vogelsang et al. 2006; Maherali and Klironomos 2007). Different plant species also respond differently to different AMF and some plant-fungal combinations are more compatible than others (e.g. Ravnskov and Jakobsen 1995; Avio et al. 2006; Scheublin et al. 2007). In some cases, some AMF taxa even reduce plant growth in one plant species, while promoting growth of other plant species (Klironomos 2003). It is still unclear whether weeds respond differently to different AMF and whether AM fungal diversity can suppress weed growth; in contrast to the positive effects of AM fungal diversity on some plant species (e.g. van der Heijden et al. 2006; Maherali and Klironomos 2007). Several studies have shown that the composition and diversity of AM fungal communities in agricultural ecosystems depend on land use intensity, crop rotation, fertility level and tillage intensity (Alguacil et al. 2008; Oehl et al. 2003, 2004; Hijri et al. 2006). Hence, such differences in AMF community composition may also affect weed growth, if weeds respond differently to different AMF communities.

In this study we established microcosms with one crop (sunflower) that co-occurred with six weed species typical of temperate environments. We tested the impact of AMF and different AMF taxa on crop productivity, crop nutrition and weed biomass. We hypothesized that (I) AMF suppress weed growth, (II) that the crop benefits from AMF and (III) that weed growth is more suppressed when several AMF (three versus one AMF taxon) are simultaneously present.

Materials and methods

Fungal material

The AMF used, all belonging to the genus *Glomus* (Phylum: Glomeromycota) are: *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, isolate IMA1 from UK (AMF A), *Glomus coronatum* Giovannetti, isolate

IMA3 (AMF B) and *Glomus intraradices* Schenck & Smith, isolate BEG 21 (AMF C). AMF A and B were obtained from pot-cultures maintained in the collection of the Department of Crop Plant Biology, University of Pisa, Italy and AMF C originated from Switzerland (see van der Heijden et al. 2006 for a description). Inoculum of each isolate was propagated for approximately three months on *Helianthus annuus* in pots filled with a sterilized mixture of loamy soil and terra green (1:1). The soil was collected near San Piero a Grado (Pisa, Italy).

Plant material

We established a model system with one crop species (*Helianthus annuus*—sunflower) and six weed species (*Amaranthus retroflexus* (Amaranthaceae), *Chenopodium album* (Chenopodiaceae), *Digitaria sanguinalis* (Poaceae), *Echinochloa crus-galli* (Poaceae), *Setaria viridis* (Poaceae), *Sinapis arvensis* (Brassicaceae)). These weed species are problematic to agriculture in temperate environments, where they often co-occur with sunflower and other crops (Bàrberi et al. 1996; Bàrberi and Bonari 2005). Moreover, *Chenopodium album* and *Echinochloa crus-galli* belong to the top ten of the World's most aggressive weeds (Holm et al. 1977). Three of the investigated weed species (*Amaranthus retroflexus*; *Chenopodium album* and *Sinapis arvensis*) are recognized as being non-mycorrhizal or poorly colonized by AMF (Harley and Harley 1987; Francis and Read 1995). Seeds of the weed species were obtained from the company Herbi-seed (www.herbi-seed.com). For sunflower we used the variety Ketil, which is often used by farmers in Italy.

Experimental model system

We established 63 microcosms in pots measuring 26.5×17×18 cm. These containers were filled with 12 kg (dry weight) of autoclaved sand collected from Dutch dunes at Castricum, on the North west coast of the Netherlands. Soil was collected from a former grassland/arable field in the dunes, of which about 1 m of the top soil was removed, resulting in a very nutrient poor sandy soil containing 0.64 mg N-NO₃ kg⁻¹, 0.85 mg N-NH₄ kg⁻¹ (both KCl-extractable), 0.30 mg P-PO₄ kg⁻¹ (NaHCO₃-extractable) and largely free of organic matter. The microcosms were inoculated with 550 g soil inoculum containing one of the three AMF species (three single AMF-species treatment: AMF A; AMF B;

AMF C); or a mixture of the three AMF species (AMF A+B+C); or with an autoclaved (121°C; 60 min.) soil mixture of these three AMF species (the non-mycorrhizal control treatment, NM). Eight sunflower seedlings and 30 weed seedlings (five seedlings per weed species) were planted together (sunflower + weed mixtures). This was done for five treatments, (AMF A; AMF B; AMF C; AMF A+B+C; NM), with 7 replicates per treatment. Fourteen other microcosms received only eight sunflower seedlings (sunflower monocultures). Seven of these microcosms were inoculated with AMF A+B+C and 7 microcosms remained non mycorrhizal, NM). Lastly, another 14 microcosms received only weed seedlings (weed monocultures). Seven of these microcosms were inoculated with AMF A+B+C and 7 microcosms remained non mycorrhizal, NM). Before planting, weed and sunflower seeds were cleaned with 1% commercial bleach for 10 min, washed with distilled water and germinated in moist sterile sand. Once germinated, seedlings were transplanted into the microcosms. Germination rates and germination time of all the plant species was tested before the experiment to ensure that equally aged seedlings were planted. The seedlings were 10 days old when transplanted into the microcosms.

Seedlings were planted at fixed distances from each other according to a predefined design where sunflower plants always occupied the same position along two central rows (simulating field conditions) while weeds were randomly planted. Planting design was randomized 63 times and assigned to each microcosm. This approach was chosen to avoid potential differences among treatments being confounded by neighbourhood interactions and initial plant species composition (van der Heijden et al. 2006). Seedlings that died within four weeks after planting were replaced so that each microcosm with sunflower and weeds contained 38 seedlings after 4 weeks. Microcosms with only sunflower or only weeds contained 8 sunflower seedlings or 30 weed seedlings, respectively. In order to avoid any risk of contamination during transplanting, pots were prepared as follows: about 8 kg of sterile soil and the inoculum were added to each pot and mixed carefully, subsequently this soil was covered with 2 kg of sterile sand.

Each microcosm received 65 ml of filtered washing of soil inoculum from the mixed AMF treatment (without AMF propagules) and of field soil, to correct for possible differences in microbial communities

between the different inocula, and to include microbial communities from the field. A total of 46 g soil of the mixed inoculum and 2.7 kg field soil was wet-sieved through a series of sieves to prepare the microbial wash. The finest sieve was 10 μm . Microcosms were watered three times a week with distilled water, and each microcosm was adjusted to equal soil water content every 2 weeks by weighing. Microcosms received a weekly fertilization comparable to 80 $\text{kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ for nitrogen, and a quarter-strength Hoagland's solution for phosphorus and other nutrients according to Hoagland and Arnon (1950). Microcosms were kept in a greenhouse with natural light conditions throughout the duration of the experiment (in Summer from April to August) and a minimum day and night temperature of 25 and 15°C, respectively.

Measurements and harvesting

Plant variables Microcosms were harvested after 14 weeks. Each individual plant was removed from the soil separately, and cleaned in water to remove parts of roots from other plants. Plant material of each plant species was subsequently pooled. Total biomass of sunflower was determined by adding sunflower root and shoot biomass. Total weed biomass was determined by adding weed root and shoot biomass of each weed species. The roots and shoots of each plant species were separated and root and shoot dry weight was determined for each plant species. The root–shoot ratio of sunflower and of each weed species was determined by dividing root dry mass with the total above ground shoot dry mass. Total dry weight of sunflower and weeds was used to calculate the Competitive Balance Index (C_b) according to Wilson (1988) (1).

$$C_b = \log_e[(W_{sw}/W_{ws})/(W_{ss}/W_{ww})] \quad (1)$$

where

W_{sw} dry weight of sunflower (s) grown together with weeds (w)

W_{ws} dry weight of weeds grown together with sunflower

W_{ss} dry weight of sunflower grown in monoculture

W_{ww} dry weight of weeds grown in monoculture

C_b values > 1 indicate a higher competitive ability for the crop relative to the weeds (Wilson 1988).

Dried shoot material was ground in a ball mill, mixed thoroughly, and P and N concentrations of the shoot biomass of sunflower and of each weed species were determined. P concentration was determined by the molybdate blue ascorbic acid method (Watanabe and Olsen 1965). N concentration was determined by dry combustion on elemental analyzer Carlo Erba NA1500 series 2, Rodana, Italy. Only shoot material from the non-mycorrhizal control treatments and from microcosms inoculated with AMF A+B+C was used to determine the P and N concentration.

The shoot P and N content of sunflower per microcosm was determined by multiplying sunflower shoot P and N concentration of a microcosms with shoot dry weight of that microcosm. The average of the seven replicates per treatment was subsequently calculated and is presented. The shoot P and N content of each weed species was determined by multiplying P and N concentrations with shoot dry weight for each species per microcosm. The total shoot P and N content of the weeds (all weed species added) was subsequently determined by adding the shoot P and N content of each weeds species per microcosm. The average of all seven replicates per treatment was determined and is presented. Insufficient plant material was available to determine P and N concentrations for *A. retroflexus*. Moreover, for *S. arvensis*, plant material of some replicates of one treatment was pooled because the amount of available plant material was insufficient for N and P analysis. Due to its small size and absence of P and N concentrations, data for *A. retroflexus* were not used to calculate total shoot N and P content.

The N/P ratio of shoot from every plant species in this study (sunflower and weeds) was calculated to estimate which nutrient limits plant growth (Koerselman and Meuleman 1996). An N/P ratio below 14 indicates that N is limiting growth for wetland plants, while an N/P ratio above 16 indicates that P limits plant growth (Koerselman and Meuleman 1996). Gusewell (2004) estimated that N limitation for terrestrial plants occurs below an N/P ratio of 10, while P limitation usually occurs above an N/P ratio of 20.

Fungal variables After determining root dry weight, the same roots were softened in water for 1 day and stained with Trypan blue using lactic acid instead of phenol (Phillips and Hayman 1970). The percentage of root length colonized by AMF was estimated for

each species by grid-line intersect method using 100 intersections per sample under the microscope (Giovannetti and Mosse 1980). The percentage of arbuscules and vesicles, fungal structures important for AMF functioning, were also assessed using the grid line intersection method.

Statistical analysis

The experiment was set up as a randomized block design where each AMF treatment was replicated seven times. There were two blocks, reflecting microcosms that were established or harvested at the same moment. The microcosms and the blocks were randomized every second week. For each variable, a two way analysis of variance (ANOVA) (Proc GLM; SPSS version 10.1) was performed. The ANOVA consisted of two factors: AMF (with five levels or two levels); and block (with two levels). Both factors were treated as fixed effects. A significant block \times AMF effect was not expected, and was not included in the ANOVA model (Newman et al. 1997). The ANOVA was performed separately for the two treatments with sunflower monocultures, the two treatments with weed monocultures and the five treatments with microcosms where weeds and sunflower were grown in mixture (hence the AMF factor in the ANOVA consisted of two or five levels). If necessary, variables were transformed to meet the requirement of homoscedasticity. For dry weight data, a logarithmic transformation and for percentage root length colonized by AMF data an arcsine-transformation was performed. A non parametric Kruskal–Wallis test was performed for variables without homoscedasticity after transformation. In these cases a χ^2 test was performed as representative statistical test measure. Tukey's multiple comparisons test was performed to test which treatments differed from each other. The ANOVA (or the non-parametric equivalent) was performed for weed shoot biomass, AMF colonization levels, weed and sunflower shoot N concentration and the weed and sunflower shoot P concentration for each plant species. Such multiple testing of many plant species increases the chance of finding a significant result (Holm 1979). A sequential Bonferroni analysis was therefore performed as post hoc test to reduce the likelihood of increasing Type I errors (Holm 1979).

Results

Mycorrhizal colonization levels

The roots of sunflower were heavily colonized by AMF and colonization levels ranged from 56.5% in microcosms inoculated with *Glomus coronatum*, up to 88% in microcosms inoculated with *Glomus intraradices* (Table 1). Colonization levels differed significantly among microcosms inoculated with different single AMF taxa ($\chi^2=14.3$; $P=0.001$). The presence or absence of weeds did not influence AMF colonization levels of sunflower in the treatment with AMF A+B+C (Table 1).

Root colonization levels of the weeds ranged from 0.7% in *Sinapis arvensis* to 55% in *Setaria viridis*, when both were grown in microcosms without sunflower (Table 1). The AMF colonization levels of the weed species were always lower compared to those of sunflower grown in the same treatment. Roots of *Chenopodium album*, *Sinapis arvensis* and *Amaranthus retroflexus*, plant species thought to be non-mycorrhizal, were all colonized by AMF, but colonization levels were very low (15.1%, 2.6% and 4.2% respectively, averaged across all microcosms inoculated with AMF). Vesicles were observed in all treatments with AMF for *Amaranthus retroflexus*, *Chenopodium album*, *Digitaria sanguinalis*, *Echinochloa crus-galli*, *Setaria viridis* and in two out of five treatments for *Sinapis arvensis* (data not shown), showing that these typical mycorrhizal structures were present in all weed species investigated. Arbuscules were present in every treatment for sunflower (on average 43.3%), *Digitaria sanguinalis* (6.0%), *Echinochloa crus-galli* (18.3%) and *Setaria viridis* (17%). *Sinapis arvensis* and *Amaranthus retroflexus* had no arbuscules, while very few arbuscules were observed in *Chenopodium album* in two treatments (data not shown). Moreover, the average percentage of arbuscules in sunflower (43.3%) was significantly higher compared to any of the weed species. AMF were absent in control microcosms indicating that we successfully manipulated the presence of AMF.

Effects of AMF on weed and sunflower biomass

The total biomass of sunflower grown in mixture with weeds did not differ significantly among the different AMF treatments, ranging from 13.8 to 14.7 g (Fig. 1a).

Table 1 Percentage of root length colonized by AMF in *Helianthus annuus* (sunflower) and each of the following six weed species: *Setaria viridis*, *Digitaria sanguinalis*, *Echinochloa crus-galli*, *Chenopodium album*, *Sinapis arvensis* and *Amaranthus retroflexus*. Sunflower and weeds were grown in microcosms where the composition of the arbuscular mycorrhizal fungal (AMF) community was manipulated. Microcosms contained either no AMF (NM), one of three different AMF taxa (A, *Glomus mosseae*; B, *Glomus*

coronatum; C, *Glomus intraradices*) or all three AMF taxa (AMF A+B+C). Colonization levels are shown for microcosms where weeds are grown together with sunflower and for microcosms where sunflower and weeds are grown alone. The *P* value shows the significance level of the AMF factor in a two-way ANOVA (with AMF treatment and block as factors). This ANOVA was performed to test for differences among the four mycorrhizal treatments of microcosms with sunflower–weed mixtures

AMF treatment	Sunflower	Weed species					
		host weeds			non-host weeds		
		<i>Setaria</i>	<i>Digitaria</i>	<i>Echinochloa</i>	<i>Chenopodium</i>	<i>Sinapis</i>	<i>Amaranthus</i>
Weed–sunflower mixture							
NM ^a	0	0	0	0	0	0	0
AMF A	65.6bc	36.6bc	44.5a	45.1a	8.0b	3.4	4.3a
AMF B	56.5c	28.4c	37.8a	35.7a	8.2b	5.5	3.8a
AMF C	88.0a	44.7ab	32.8a	24.4b	16.8a	2.0	2.0a
AMF A+B+C	76.1ab	50.2a	41.5a	43.4a	14.8ab	1.2	7.0a
<i>P</i> -value ^a	<0.001	0.001	0.35	<0.001	<0.002	n.d. ^b	0.024
Weeds or sunflower grown alone							
NM	0	0	0	0	0	0	0
AMF A+B+C	81.8	54.8	43.9	39.3	27.8	0.7	3.9

P-values in bold show a significant difference among treatments after a correction for multiple testing. *P*-values of blocks are not shown. Different letters indicate a significant difference among treatments according to Tukey's test

^a The non-mycorrhizal treatment was excluded from statistical analysis; error degrees of freedom = 23

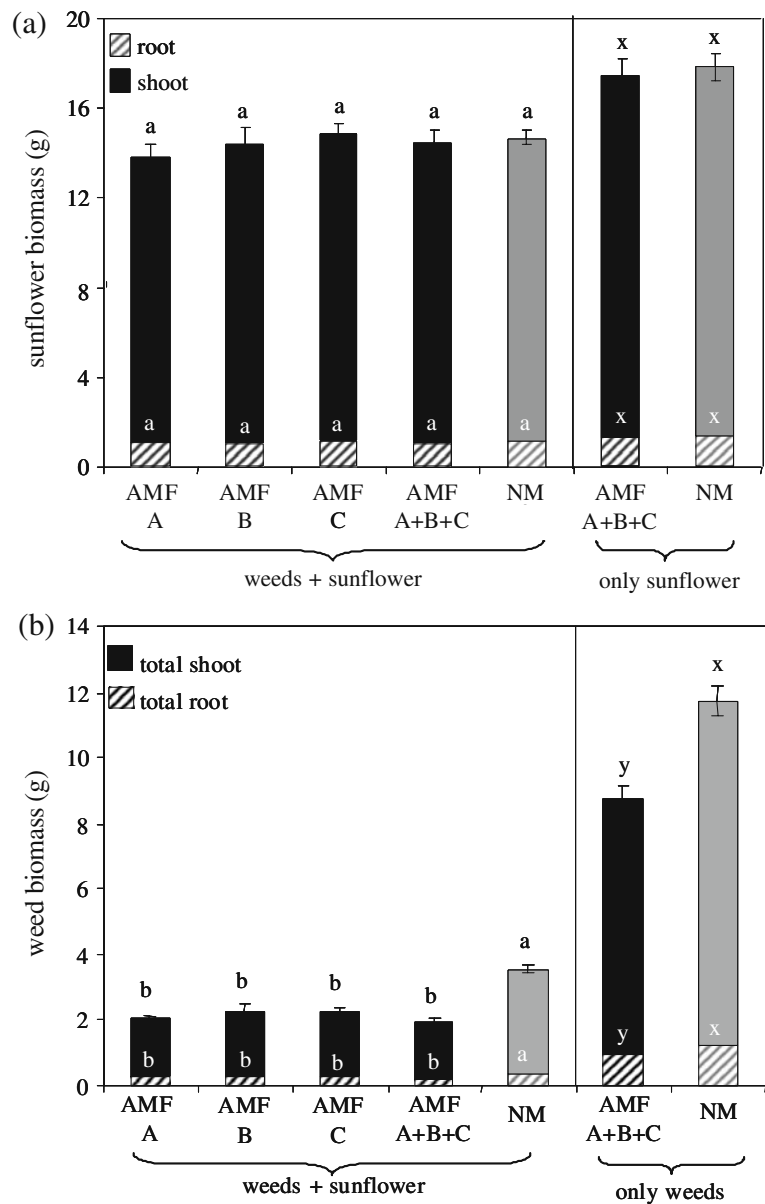
^b Only few samples were available and no statistical analysis was performed

Moreover, the biomass of sunflower grown in microcosms inoculated with three AMF taxa did not differ from those in microcosms with only one AMF taxon or without AMF. In microcosms where sunflower was grown alone in monoculture, sunflower biomass ranged between 17.5 and 17.9 g (Fig. 1a). On average, the total biomass of sunflower grown alone in monocultures was 22% higher compared to microcosms where sunflower was grown in mixture together with weeds. Neither root nor shoot biomass of sunflower differed among treatments with or without AMF, either for sunflower monocultures or microcosms where sunflower was grown in mixture with weeds (Fig. 1a). The root–shoot ratio of sunflower grown respectively in monoculture or mixture with weeds did not significantly differ among the different AMF treatments ($F_{1,11}=0.395$, $P=0.55$ and $F_{4,29}=1.95$, $P=0.12$).

The total biomass of the weeds was, in contrast to that of sunflower, negatively affected by the presence of AMF (Fig. 1b). The total weed biomass in micro-

cosms with sunflower was on average 47% lower in microcosms with AMF, compared to microcosms without AMF (Fig. 1b). This effect was weaker (–25%), but still significantly different, when the weeds were grown alone (Fig. 1b). The biomass of *Amaranthus retroflexus*, *Digitaria sanguinalis*, *Setaria viridis* and *Sinapis arvensis* did not differ significantly between mycorrhizal and non-mycorrhizal microcosms, although the biomass was slightly reduced in mycorrhizal microcosms (Table 2). The biomass of *Echinochloa crus-galli* and *Chenopodium album* was significantly lower in microcosms where AMF were present (Table 2). Shoot dry weight of *Chenopodium album* and *Echinochloa crus-galli* grown in microcosms with sunflower was respectively 66% and 59% lower in the treatments with three AMF taxa compared to the non-mycorrhizal treatment, while a reduction of 37% and 32% was observed when grown without sunflower. The negative effects of AMF on weed biomass differed among the treatments with different AMF taxa for *Chenopodium album* ($F_{3,24}=6.9$; $P<0.002$; the

Fig. 1 Root and shoot dry mass of sunflower (a) and weeds (b) (mean \pm standard error) in microcosms simulating a sunflower cropping system. Sunflower and weeds were grown together in mixture or in monocultures. Microcosms contained either no AMF (NM), one of three different AMF taxa (A, *Glomus mosseae*; B, *Glomus coronatum*; C, *Glomus intraradices*) or all three AMF taxa (AMF A+B+C). Black bars represent shoot dry biomass in treatments with AMF, grey bars represent shoot dry biomass of non-mycorrhizal treatments and striped bars represent root dry biomass. Different letters above the bars indicate a significant difference ($P < 0.05$) among treatments for shoot biomass or root biomass (above striped bars) according to Tukey's test, performed separately for microcosms with or without weeds (a) or with or without sunflower (b). Standard error bars for shoot biomass are shown. Standard errors bars for root biomass were small and are not shown



non-mycorrhizal treatment was excluded from this analysis) with the highest suppression shown in the treatment with all three AMF and the treatment with AMF A. The biomass of the other weed species did not differ significantly between microcosms inoculated with different AMF (Table 2). Total root biomass of the weeds was lower in microcosms with AMF compared to non-mycorrhizal microcosms (Fig. 1b), with a slightly higher decrease when weeds were grown together with sunflower (–31%), compared to –24% when weeds were grown alone. The root–shoot ratio of

weeds grown in mixture with sunflower differed among the different AMF treatments ($F_{4,29}=2.80$; $P=0.05$). The root–shoot ratio of weeds were grown in monocultures was the same in the non-mycorrhizal and mycorrhizal treatment ($F_{1,11}=0.03$; $P=0.86$).

Effects of AMF on phosphorus and nitrogen acquisition

Phosphorous Sunflower shoot phosphorus uptake was significantly higher in microcosms inoculated

Table 2 Shoot biomass (g) of each of six weed species (*Setaria viridis*, *Digitaria sanguinalis*, *Echinochloa crus-galli*, *Chenopodium album*, *Sinapis arvensis* and *Amaranthus retroflexus*) in microcosms where the composition of the arbuscular mycorrhizal fungal (AMF) community was manipulated. Microcosms contained either no AMF

(NM), one of three different AMF taxa (A, *Glomus mosseae*; B, *Glomus coronatum*; C, *Glomus intraradices*) or all three AMF taxa (AMF A+B+C). The weeds were grown in microcosms with or without sunflower

AMF treatment	Weed species					
	host weeds			non-host weeds		
	<i>Setaria</i>	<i>Digitaria</i>	<i>Echinochloa</i>	<i>Chenopodium</i>	<i>Sinapis</i>	<i>Amaranthus</i>
weeds grown in microcosms with sunflower						
AMF A	0.48a	0.34a	0.52b	0.27c	0.19a	0.029a
AMF B	0.59a	0.27a	0.52b	0.38bc	0.23a	0.037a
AMF C	0.46a	0.27a	0.59b	0.44b	0.22a	0.032a
AMF A+B+C	0.48a	0.27a	0.43b	0.25c	0.24a	0.028a
NM	0.69a	0.38a	1.05a	0.75a	0.30a	0.045a
<i>P</i> -value	0.05	0.06	<0.001	<0.001	0.03	0.24
weeds grown in microcosms without sunflower						
AMF A+B+C	1.76a	1.12b	2.26a	1.20b	1.36a	0.12a
NM	1.89a	1.89a	3.30a	1.91a	1.33a	0.16a
<i>P</i> -value	0.59	0.002	0.06	0.003	0.89	0.087

The *P* value shows the significance level of the AMF factor according to a two-way ANOVA (with AMF treatment and block as factors), or a non parametric Kruskal–Wallis test (for *Sinapis arvensis* and *Amaranthus retroflexus*), to test for differences among AMF treatments. *P*-values in bold show a significant difference among treatments, after a correction for multiple testing. *P*-values of blocks are not shown. The ANOVA was performed separately for microcosms with or without sunflower. Different letters indicate a significant difference ($P < 0.05$) among treatments according to Tukey's test performed for microcosms with sunflower and a regular *t*-test for microcosms without sunflower

with AMF compared to control microcosms without AMF (Fig. 2a). The presence of AMF increased sunflower shoot P content on average with 48% when it was grown together with weeds, and with 79% when sunflower was grown in monoculture, compared to the non-mycorrhizal treatment. The shoot P content of sunflower grown in mycorrhizal microcosms without weeds was significantly higher (+43%) compared to the shoot P content of sunflower grown in mycorrhizal microcosms with weeds (Fig. 2a; $t=4.89$; $P < 0.001$). In contrast to this, the shoot P content of sunflower did not differ between sunflower monocultures without AMF and sunflower–weed mixtures without AMF (Fig. 2a; $t=1.76$; $P=0.10$). This indicates that sunflower was more effective in obtaining phosphorus in the absence of weeds and with AMF. The shoot P content of sunflower did not differ among microcosms inoculated with different AMF (data not shown).

The P concentration of sunflower was also significantly higher in microcosms inoculated with AMF

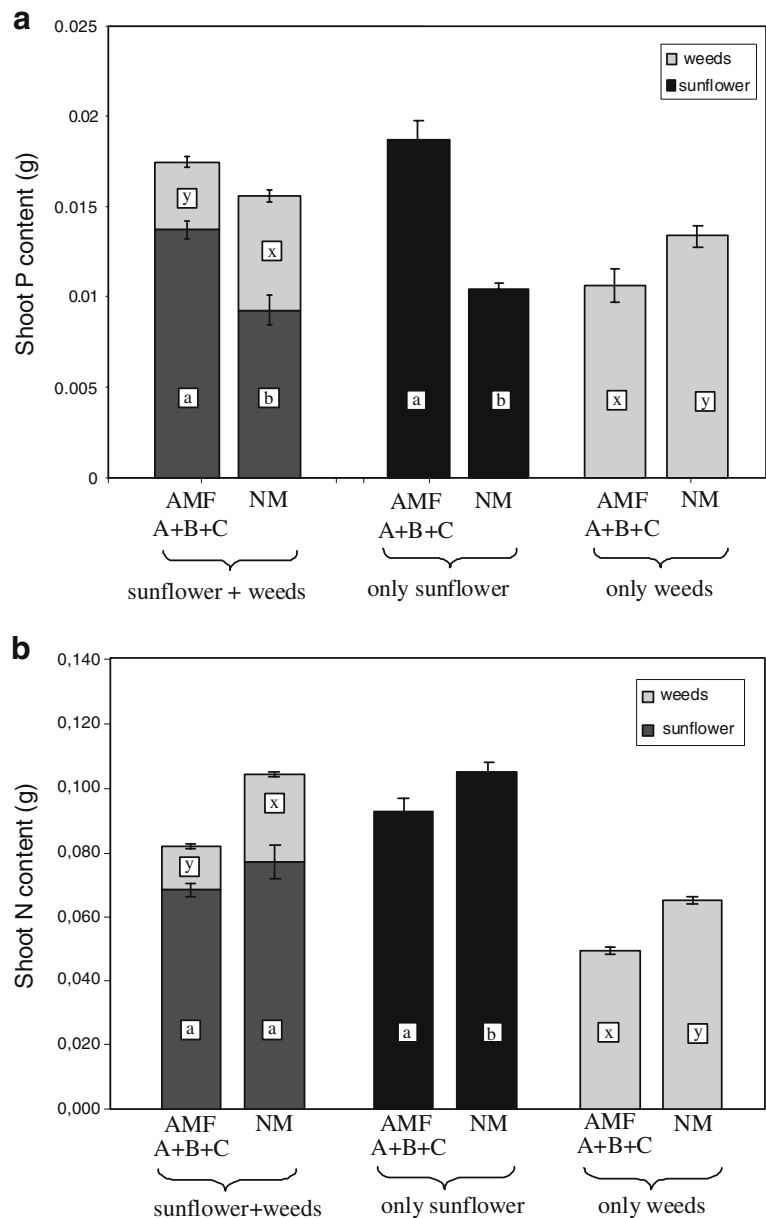
compared to non-inoculated microcosms, both when sunflower was grown alone or in combination with weeds (Table 3).

In contrast to sunflower, the shoot P content of the weeds (all weed species added) was significantly reduced when AMF were present, both when weeds were grown alone (−9%) or when grown together with sunflower (−41%) (Fig. 2a). Although total shoot weed P uptake was reduced in microcosms with AMF, one weed species (*Digitaria sanguinalis*) had a significantly higher P content in microcosms inoculated with AMF (data not shown).

Moreover, each of the investigated weed species, except *Sinapis arvensis*, had a significant higher P concentration in microcosms inoculated with AMF (Table 3).

Nitrogen The shoot nitrogen content of sunflower did not differ between non-mycorrhizal microcosms and microcosm inoculated with AMF A+B+C, when sunflower was grown in mixtures with weeds (Fig. 2b).

Fig. 2 Shoot phosphorus content (a) and shoot nitrogen content (b) of sunflower and weeds (mean \pm standard error) in control microcosms without AMF (NM) or in microcosms inoculated with three AMF taxa (AMF A+B+C). Sunflower and weeds were grown alone in monoculture or together in mixture. Sunflower and weed data are shown in *black* or *gray* respectively. Different letters within the columns indicate a significant difference ($P < 0.05$) between the non-mycorrhizal treatment and the treatment with three AMF taxa according to a *t*-test performed for sunflower (letters *a* and *b*) or weeds letters (*x* and *y*). This *t*-test was performed separately for microcosms with weeds and sunflower, or microcosms with only sunflower or only weeds



However, when sunflower was grown alone, it had a significant higher shoot N content in microcosms without AMF compared to microcosms with AMF A+B+C (Fig. 2b).

The shoot nitrogen content of weeds (all weed species added) was significantly reduced in microcosms with AMF where weeds were grown together with sunflower (Fig. 2b).

The shoot nitrogen concentration of the single weed species was either enhanced (*Digitaria sanguinalis*), not affected (*Setaria viridis*), or reduced (*Chenopodium*

album and *Echinochloa crus-galli*) when microcosms with AMF were compared with non-mycorrhizal ones (Table 3).

The N/P ratios of plant material in this study ranged from 0.37 for *Digitaria sanguinalis* grown in microcosms with sunflower that were inoculated with AMF A+B+C to 11.05 for *Setaria viridis* grown in non-mycorrhizal microcosms without sunflower. The average N/P ratio of sunflower in microcosms with weeds and with AMF was 5.0 while it was significantly higher (8.3) in non-mycorrhizal microcosms with weeds.

Table 3 Shoot N concentration ($\text{mg}\cdot\text{g}^{-1}$) and shoot P concentration ($\text{mg}\cdot\text{g}^{-1}$) of *Helianthus annuus* (sunflower) and each of six weed species (*Setaria viridis*, *Digitaria sanguinalis*, *Echinochloa crus-galli*, *Chenopodium album*, *Sinapis arvensis* and *Amaranthus retroflexus*) which were grown in microcosms where the composition of the arbuscular mycorrhizal fungal (AMF) community was

manipulated. Microcosms contained either no AMF (NM), one of three different AMF taxa (A, *Glomus mosseae*; B, *Glomus coronatum*; C, *Glomus intraradices*) or all three AMF taxa (AMF A+B+C). Weeds and sunflower were grown together in mixture (WS) or sunflower (S) and weeds (W) were grown alone

AMF treatment		Sunflower	Weed species						
			host weeds			non-host weeds			
			<i>Setaria</i>	<i>Digitaria</i>	<i>Echinochloa</i>	<i>Chenopodium</i>	<i>Sinapis</i>	<i>Amaranthus</i>	
<i>N concentration ($\text{mg}\cdot\text{g}^{-1}$)</i>									
WS	NM	5.71a	11.22a	6.31b	6.56a	9.93a	9.44	n.d.	
WS	AMF A+B+C	5.11a	11.68a	7.51a	5.79b	7.40b	6.77 δ	n.d.	
S	NM	6.43a							
S	AMF A+B+C	5.77a							
W	NM		8.26a	4.05b	4.82a	8.62a	7.00a	n.d.	
W	AMF A+B+C		8.24a	5.40a	5.13a	6.78b	5.87b	n.d.	
<i>P concentration ($\text{mg}\cdot\text{g}^{-1}$)</i>									
WS	NM	0.68b	1.16b	0.64b	1.49b	3.97b	2.96	n.d.	
WS	AMF A+B+C	1.03a	1.63a	1.99a	1.67a	4.36a	n.d.	n.d.	
S	NM	0.66b							
S	AMF A+B+C	1.23a							
W	NM		0.76b	0.47b	1.12b	2.49a	2.50a	n.d.	
W	AMF A+B+C		1.18a	1.08a	1.26a	2.89a	2.40a	n.d.	

Different letters in bold indicate a significant difference ($P < 0.05$) between the non-mycorrhizal treatment and the treatment with AMF A+B+C, per plant species and for each variable according to a *t*-test followed by a correction for multiple testing. δ = only 1 sample was used for analysis

Effects of AMF on sunflower–weed interactions

The sunflower shoot biomass and the total weed shoot biomass from mixtures and monocultures were compared to investigate whether competitive interactions between sunflower and weeds were mediated by AMF. The competitive ability of sunflower was significantly higher when AMF were present ($C_b = 1.36 \pm 0.10$) compared to when AMF were absent ($C_b = 0.99 \pm 0.074$) ($P = 0.016$).

Discussion

This study shows that AMF have the ability to suppress growth of some aggressive agricultural weeds, including *Chenopodium album* and *Echinochloa crus-galli*, which belong to the top ten of the world's most aggressive weeds. The investigated weed species were grown together with a crop, sunflower, which benefited from

AMF through improved phosphorus uptake. This result points to a novel characteristic of the mycorrhizal symbiosis, namely that AMF have the ability to suppress unwanted weed species, while at the same time promoting nutrition of the target crop species. This work supports two earlier reports by Vatovec et al. (2005) and Jordan and Huerd (2008) which indicate that soil fungi can suppress a range of agricultural weeds.

Previous studies have shown that plants respond differently to different AMF (Ravnskov and Jakobsen 1995; Avio et al. 2006) and that AMF diversity can promote plant productivity and plant nutrition (van der Heijden et al. 1998; Vogelsang et al. 2006; Maherali and Klironomos 2007). The effects of different AMF taxa and AMF diversity on weed growth had not been tested before. In this experiment, differences in weed biomass between the various treatments with different AMF were small (only *Chenopodium album* responded differently to different AMF) and AMF diversity (three

AMF taxa versus one) did not lead to reduced weed biomass. Moreover, sunflower biomass and P uptake was not enhanced in microcosms with three AMF compared to one AMF showing that AMF diversity effects were small. This suggests that the investigated fungi had similar physiological effects on the plants, even though root colonization levels of sunflower and some of the investigated weed species differed significantly among treatments with different AMF taxa.

Variations in growth effects by different AMF taxa appear to be largest at the genus level, not at the species or isolate level (Hart and Klironomos 2002). Hence, this indicates that it is more likely to find complementary effects of AMF diversity when different AMF genera (with different strategies) are present (de la Providencia et al. 2005). The fact that all AMF taxa used in this study belonged to the same genus (*Glomus*) may, thus, explain why there was no effect of AMF diversity on plant productivity. European arable soils subjected to regular ploughing are regularly dominated by members of the *Glomeraceae* (Daniell et al. 2001; Hijri et al. 2006; Alguacil et al. 2008; Verbruggen et al. unpublished results). Hence, the use of only *Glomus* species in our experiment is comparable to a situation regularly found in the field. However, in some agricultural fields other AMF genera are also present; sometimes even abundant (Oehl et al. 2004; Hijri et al. 2006; Jansa et al. 2002). Hence, in order to assess the potential impact of AMF diversity on crop-weed interactions in such fields, more diverse AMF assemblages should be used.

We identified several potential mechanisms that could explain the negative effects of AMF on weeds. We distinguish between direct and indirect effects. The induction of plant defence and production of toxic compounds by AMF (Francis and Read 1994, 1995) can be considered as direct effects. We are currently investigating whether AMF suppress weed growth by exuding allelopathic compounds and inducing defence responses (Allen et al. 1989; Giovannetti and Lioi 1990; Francis and Read 1994). However, we don't know whether this occurred in this experiment.

There are also several indirect mechanisms by which AMF can suppress weeds. These include: (I) the non-mycorrhizal weeds may have reduced access to nutrients already taken up by AMF and transported to sunflower or mycorrhizal weeds, (II) AMF preferentially allocate nutrients to the crop and, (III) weeds invest carbon in mycorrhizal networks but receive no benefit in return. The three weeds considered to be

non hosts (*Chenopodium album*, *Sinapis arvensis* & *Amaranthus retroflexus*—Harley and Harley 1987) contained significantly lower amounts of phosphorus than sunflower, providing potential evidence for the first mechanism (see below). Moreover, sunflower obtained 48% more phosphorus (P) when AMF were present, while AMF reduced weed P content of the three mycorrhizal weeds (*Digitaria sanguinalis*, *Echinochloa crus-galli*, *Setaria viridis*) by 21%. This provides evidence for mechanism two. It is, however, interesting to note that the P concentrations of the three mycorrhizal weeds was enhanced in microcosms with AMF, indicating that other mechanisms are important. Moreover, the N/P ratio of sunflower ranged from 4.7 to 9.7 and that of weeds ranged from 0.4 to 11, indicating that both sunflower and weeds were nitrogen limited (Koerselman and Meuleman 1996) and not phosphorus limited. Thus, although sunflower obtained more P than weeds, it is unlikely that this explained reduced weed growth.

The three mycorrhizal weeds and sunflower were colonized by each AMF taxon (in the single AMF treatments) indicating that these plants were interconnected by the same mycorrhizal network. We did not determine how much carbon each plant allocated to the mycorrhizal networks, but it is possible that cost-benefit relationships varied among these plant species and that sunflower obtained most benefit. All weed species had lower fungal colonization levels and contained less arbuscules, fungal structures important for nutrient exchange, than sunflower. Hence, this indicates that sunflower was functionally more compatible with AMF. It is important to note that weed responses to AMF are weed-species specific (see Table 2). Hence, other weed communities might respond differently to AMF compared to the weed community investigated in this study.

Our results also suggest that AMF changed competitive interactions between sunflower and weeds, confirming earlier studies for other plant species combinations (e.g. Hetrick et al. 1994; Schroeder-Moreno and Janos 2008; Scheublin et al. 2007). We had pooled all weed species together into one group (weeds) in order to calculate competitive interactions with sunflower (see Materials and methods). Future work should investigate sunflower-weed interactions with only one weed species and consider factors such as plant density, harvest time, size differences among plants, which are all known to influence the outcome of competition among plants (e.g. Gibson et al. 1999).

Although our results support the hypothesis that weeds respond negatively to AMF, additional work is needed including a test for the response of a much wider range of weed species, and testing the effects of different soil types, fertility levels and different crop species on weed growth and crop-weed interactions. Moreover, it is important to consider that some important crops (notably sugar beet and oilseed rape) are non-mycorrhizal. It is unlikely that these crops benefit from AMF and that AMF suppress weeds that grow with these crops.

Most agricultural systems, such as the one simulated in this study, can be compared with early successional plant communities, due regular disturbance and high nutrient availability. Plant species characteristic for these conditions are often non-mycorrhizal and/or have low responsiveness to AMF (Janos 1980; Read 1989). Several studies report that the arrival of AMF in early successional communities can drive succession and initiates the replacement of non-mycorrhizal plant species by mycorrhizal plant species (e.g. Allen and Allen 1988). Many weed species, including some of the weeds tested here, have their natural niche in early successional communities such as fore-dunes or drift lines near streams. Hence, our results, at least in part, confirm early observations that AMF suppress some of these ruderal plant species. This points to the role of AMF as ecosystem engineers and drivers of succession.

Previous work has emphasized that AMF are important for the sustainability of agricultural ecosystems by enhancing crop nutrition (Plenchette et al. 1983; Gosling et al. 2006), by reducing nutrient leaching losses after heavy rain (van der Heijden 2009), by providing protection against stress and disease (Auge 2001; Sikes et al. 2009) and by improving soil structure (Rillig and Mummey 2006). This study indicates that in addition to this, AMF can suppress aggressive agricultural weeds.

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