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Linking agricultural practices, mycorrhizal fungi, and traits mediating plant–insect interactions

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Abstract. Agricultural management has profound effects on soil communities. Activities such as fertilizer inputs can modify the composition of arbuscular mycorrhizal fungi (AMF) communities, which form important symbioses with the roots of most crop plants. Intensive conventional agricultural management may select for less mutualistic AMF with reduced benefits to host plants compared to organic management, but these differences are poorly understood. AMF are generally evaluated based on their direct growth effects on plants. However, mycorrhizal colonization also may alter plant traits such as tissue nutrients, defensive chemistry, or floral traits, which mediate important plant–insect interactions like herbivory and pollination. To determine the effect of AMF from different farming practices on plant performance and traits that putatively mediate species interactions, we performed a greenhouse study by inoculating *Cucumis sativus* (cucumber, Cucurbitaceae) with AMF from conventional farms, organic farms, and a commercial AMF inoculum. We measured growth and a suite of plant traits hypothesized to be important predictors of herbivore resistance and pollinator attraction. Several leaf and root traits and flower production were significantly affected by AMF inoculum. Both conventional and organic AMF reduced leaf P content but increased Na content compared to control and commercial AMF. Leaf defenses were unaffected by AMF treatments, but conventional AMF increased root cucurbitacin C, the primary defensive chemical of *C. sativus*, compared to organic AMF. These effects may have important consequences for herbivore preference and population dynamics. AMF from both organic and conventional farms decreased flower production relative to commercial and control treatments, which may reduce pollinator attraction and plant reproduction. AMF from both farm types also reduced seed germination, but effects on plant growth were limited. Our results suggest that studies only considering AMF effects on growth may overlook changes in plant traits that have the potential to influence interactions, and hence yield, on farms. Given the effects of AMF on plant traits documented here, and the great importance of both herbivores and pollinators to wild and cultivated plants, we advocate for comprehensive assessments of mycorrhizal effects in complex community contexts, with the aim of incorporating multispecies interactions both above and below the soil surface.

Key words: *aboveground–belowground effects; arbuscular mycorrhizal fungi; conventional farming;* Cucumis sativus; *cucurbitacin C; herbivore; organic farming; plant–insect interactions; pollinator*.

Introduction

Arbuscular mycorrhizal fungi (AMF) are abundant and widespread soil microbes that generally confer positive effects on growth and reproduction of wild and cultivated plants (Smith and Read 2008). In agriculture, these benefits may translate to yield increases in mycorrhizal crops (Gosling et al. 2006), and even help shift competitive relationships between crops and weeds (Daisog et al. 2012). However, agricultural practices, such as tillage regimes, nutrient inputs, and pesticide applications may affect AMF community composition, and even shape the evolution of particular species or strains (Oehl et al. 2003, 2004, 2005, Johansson et al. 2004, Gosling et al. 2006, Verbruggen and Kiers 2010, Schnoor et al. 2011, Yang et al. 2012). In particular, high inputs of N- and P-containing fertilizers may select for AMF that are poor mutualists, strains that provide few nutrients to plants while continuing to consume host carbohydrates, shifting mycorrhizal function toward the parasitism end of the "mutualism–parasitism continuum" (Johnson 1993, Johnson et al. 1997, Verbruggen and Kiers 2010, Johnson and Graham 2013).

A growing body of research has emerged which compares conventional and organic farming systems, with the aim of identifying specific management

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practices that affect mycorrhizal functioning (e.g., Verbruggen et al. 2012). For example, organic farming practices can lead to higher AMF colonization compared to conventional systems (Mäder et al. 2002), which might be attributed to differences in fertilizer inputs (synthetic vs. organically derived). But Gosling et al. (2006) emphasize that any high-P fertilizer, whether synthetic or organically derived, may reduce AMF function. Identifying soil characteristics that correlate with AMF function, including colonization or effects on plant traits, in a common environment may provide a predictive framework for determining how management practices influence the potential benefits of AMF in crop systems.

In general, past research has focused only on the direct effects of AMF on plants, such as changes in plant biomass and nutrient uptake, and how organic vs. conventional, or high- vs. low-input, agricultural systems influence these direct effects (Ryan et al. 1994, Scullion et al. 1998, Verbruggen et al. 2012). However, AMF also have important indirect effects on plants by altering interactions between plants and other community members. By influencing plant traits that mediate these interactions, AMF can modify interactions with both antagonists, such as herbivores, and mutualists, such as pollinators, that play important roles in agriculture. Insect herbivores can have profound impacts on crop yield by consuming both above- and belowground plant tissues (Peterson and Higley 2010), and insect pollination is necessary to produce many fruit and vegetable crops (Klein et al. 2007). If AMF communities affect the plant traits that mediate these interactions, they may affect crop yield as well.

By providing nutrients such as P and N to plants (Govindarajulu et al. 2005, Smith and Read 2008) or improving plant uptake of micronutrients and metal ions (Lee and George 2005), AMF affect plant nutrition, and thus host quality for above- and belowground insect herbivores. Likewise, AMF can modify plant traits for tolerance (Bennett and Bever 2007) and resistance (Bennett et al. 2009, Kempel et al. 2010). Although studies of AMF–plant–herbivore interactions have primarily been performed using nonagricultural plants, increased understanding of these interactions in managed systems may benefit agricultural sustainability and crop yield.

Compared to indirect effects of AMF on insect herbivores, AMF effects on plant–pollinator interactions are largely unknown. Colonization by AMF has the potential to influence pollinator behavior by increasing flower number (Schenck and Smith 1982, Lu and Koide 1994, Gange et al. 2005) and altering flower size (Gange and Smith 2005, Kiers et al. 2010, Varga and Kytöviita 2010), floral nectar production (Gange et al. 2005, Kiers et al. 2010), and floral volatiles (Becklin et al. 2011). Few studies have measured AMF effects on pollinator attraction in a field setting, but both Wolfe et al. (2005) and Gange and Smith (2005) showed that mycorrhizal plants attracted significantly more pollinators than nonmycorrhizal plants. Although the majority of crops throughout the world are dependent on pollination by animals (Aizen et al. 2008), the role of AMF in this vital ecosystem service is understudied.

Agricultural practices may shape AMF communities, but a greater understanding of the effects of these changes on trait-mediated interactions with other community members, such as herbivores and pollinators, is needed (Fig. 1). If agricultural management selects for AMF communities that provide few nutrient benefits to plants (Verbruggen and Kiers 2010), then these fungi from agroecosystems may affect traits in a different way compared to studies using highly mutualistic fungi to inoculate plants. For example, the increases in flower production frequently seen in mycorrhizal plants are presumably due to increased nutrient access. If AMF in an agricultural setting provide fewer nutrients, then flower production and pollination services may decrease. In the case of herbivory, agricultural AMF could alter herbivore preference or performance via changing plant nutrient content or by reducing C available for allocation to C-based defenses (Vannette and Hunter 2011), resulting in plants that are more susceptible to herbivory.

Our aim was to understand how AMF exposed to different agricultural practices affect traits that mediate plant–herbivore and plant–pollinator interactions. We performed a greenhouse study in which we isolated the effects of organic and conventional farm-collected AMF from confounding environmental factors. We then compared effects of these farm AMF to commercial AMF and a non-AMF control. Using *Cucumis sativus* (cucumber, Cucurbitaceae), we inoculated plants with AMF from different management regimes and measured AMF colonization, plant growth, leaf and root quality traits known to mediate herbivory, and floral traits that influence pollination. We asked four questions: (1) Do soil characteristics differ between organic and conventional farms? (2) Do plant traits differ with AMF source (organic farms, conventional farms, commercial AMF, or nonmycorrhizal control)? (3) Do farm soil characteristics correlate with AMF colonization? (4) Are plant trait values correlated with the extent of AMF colonization?

By examining plant traits important to herbivory and pollination dynamics, our study extends AMF function beyond direct impacts on plant growth. Our aim is to place mycorrhizal effects in a comprehensive aboveground–belowground community context (Ohgushi 2005, Kaplan et al. 2008, van Dam and Heil 2011) and to determine how mycorrhizal interactions that vary from mutualistic to parasitic may alter other antagonistic (herbivory) or mutualistic (pollination) interactions. Exploring these indirect pathways in an agroecosystem allows us to assess how human impacts, particularly nutrient inputs, affect mycorrhizal function in a new light.

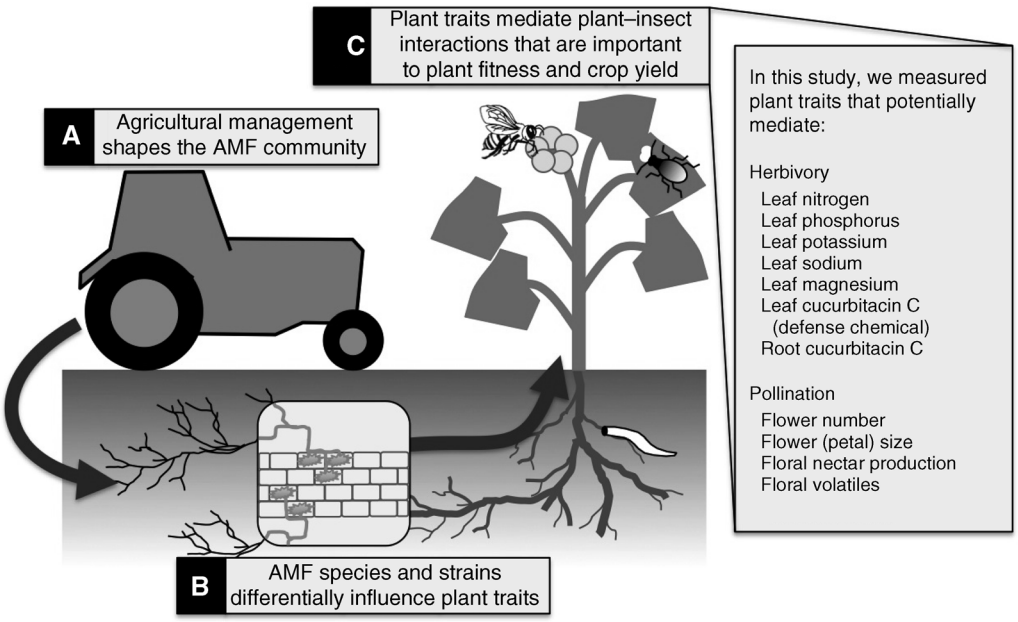

FIG. 1. Hypothetical indirect effects of agricultural management on plant–insect interactions. (A) Management techniques such as fertilization and tillage influence arbuscular mycorrhizal fungi (AMF) community composition by selecting for certain species or strains. (B) AMF species differ in their effects on host plant traits such as tissue nutrient content, defensive chemistry, and floral traits. (C) This variation in traits affects plant interactions with above- and belowground herbivores and with pollinators. We list the traits measured in this experiment that potentially mediate these interactions.

METHODS

Study system

Cucumis sativus is a widely cultivated annual plant. In 2011 alone, cucumbers were grown on 52 000 ha of land in the United States with a production value of US$362 million (National Agricultural Statistics Service 2012). Colonization of *C. sativus* by AMF affects flowering, fruit production, photosynthesis rates, and disease resistance (Trimble and Knowles 1995, Valentine et al. 2001, Hao et al. 2005, Kiers et al. 2010). The primary chemical defense of *C. sativus* is cucurbitacin C. Cucurbitacins are oxygenated tetracyclic triterpenes produced by Cucurbitaceae that act as feeding deterrents to most herbivores, but are phagostimulants for *Acalymma vittatum* (Chrysomelidae), a specialist beetle that is a widespread pest of cucurbit crops (Metcalf et al. 1980, Agrawal et al. 1999). *Cucumis sativus* is monoecious and relies on pollinators to vector pollen between male and female flowers. In the eastern United States, flowers are visited by a variety of generalist pollinators, including honey bees (*Apis mellifera*, Apidae), bumble bees (*Bombus* spp., Apidae), solitary bees (e.g., Halictidae, Andrenidae), butterflies, and hover-flies (Syrphidae) (Barber et al. 2012).

Field selection and soil collection

We identified four conventional and four USDA-certified organic farms in western Massachusetts, USA. We focused on farms growing *Cucurbita*, which is confamilial to *Cucumis*, because few farms with *Cucumis* met our other criteria. Growers in the study region rotate a wide variety of annual crops, so growing *C. sativus* on soil that previously supported *Cucurbita* is ecologically realistic. Mycorrhizae from these sampled fields readily colonized *C. sativus* (see *Results*). Our approach was to grow cucumbers as a bioassay for AMF function generally. We asked growers to identify fields on their farms that met the following criteria: (1) currently growing *Cucurbita* sp. crops, (2) had at least three years of management records, and (3) never had commercial mycorrhizal inoculum added. We selected one to four fields at each farm, totaling nine conventional and nine organic fields. In August 2010, we collected 10 cm diameter, 15 cm deep soil cores from the base of nine *Cucurbita* plants evenly spaced throughout each field, including their small roots. These collections (total ~7.5 L of soil) were pooled and stored at 5°C. To sample variation in soil characteristics, we also collected 15 small (2.5 cm diameter, 15 cm deep) soil cores at evenly spaced points between rows (to avoid areas with direct fertilizer application) for analysis (University of Massachusetts Soil and Plant Tissue Testing Laboratory). Soil analysis determined soil pH, organic matter content, nitrate content, cation exchange capacity (CEC), P, K, Ca, Mg, Al, B, Mn, Zn, Cu, Fe, S, Ni, and Pb.

Inoculation experiment

Prior to inoculation, we sifted each pooled soil sample to separate root fragments. Roots were cut into 1 cm

lengths with sterilized scissors and remixed into the soil. We dried these samples (hereafter, farm inocula) in trays for two weeks. We sterilized 7.5-L pots with bleach solution, dried them, and filled each with 4 L of steam-sterilized bulk soil : sand mixture (50:50 volume/volume to maintain drainage). We collected soil from a field at the University of Massachusetts Center for Agriculture that had been under organic management for six years. Compared to farm-collected soils, the sterile bulk mixture had slightly higher pH, higher Ca, and lower organic matter content because of the added sand. However, CEC and most nutrients (nitrate, P, K, Mg, Al, B, Zn, Fe, Ni, and Pb; but not Mn, Cu, and S) were less than two standard deviations from the mean of farm-collected soils (Appendix B: Table B2). Growth of the same AMF strains can differ depending on soil conditions, and greater hyphal growth and plant benefit has been found in the fungi's local soil (Johnson et al. 2010). Because it was not feasible to factorially cross each AMF community with each farm's soil, we used a common soil source that was similar to sampled farm fields. Given its short history of organic cultivation, the source field was not more similar to one management type or the other.

We inoculated each pot by incorporating 200 mL of dried inocula into the top 5 cm of sterilized soil and adding 200 mL sterilized bulk soil mixture on top; this is equivalent to 4.5% inoculum by volume in each pot. For commercial AMF treatments, we added 200 mL *Rhizophagus irregularis* (syn. *Glomus intraradices*) on a perlite carrier (Myke, Premier Biotechnologies, Quebec, Canada), a strain selected for its beneficial effects on plants. Control plants received 200 mL sterilized soil mixture. Because commercial and control treatments lacked other soil microbes, we added 50 mL microbial filtrate created by pooling a small amount of each farm inocula and mixing with water to create a slurry that was filtered with 20-μm mesh, which excludes AMF. In total, there were 20 treatment levels (nine conventional fields, nine organic fields, commercial AMF, and control), each replicated 10 times. We arranged pots in a randomized block design on greenhouse benches, such that each bench was a block containing a single replicate of each of the 20 treatments, and re-randomized pot locations within each block every two weeks until plants were too large to move.

We sterilized *C. sativus* seeds (Marketmore 76, Johnny's Selected Seeds, Winslow, Maine, USA) in dilute bleach, rinsed them thoroughly, and planted three seeds per pot. We watered pots and recorded seed germination daily. After 14 d, pots lacking any successfully germinated seeds received transplanted extra seedlings from the same treatment or from control pots. Pots with organic inocula from one field lacked any successful germinants and were excluded from the experiment. Extra seedlings were removed, but ungerminated seeds remained in pots. We fertilized plants a single time after one month using a modified Hoagland's solution (half-strength N, quarter-strength P).

To measure direct effects on plant growth, we counted fully expanded leaves weekly and measured the length and width of the three most recently fully expanded leaves when plants were one month old. After eight or nine weeks, we collected, dried, and weighed aboveground biomass of each plant (all plants in a block collected on the same day). We carefully sifted roots from soil and rinsed and froze them at −80°C. We later dried and weighed roots from the first five blocks to determine belowground biomass. We collected a small sample of fine roots from each frozen root mass and stained them with trypan blue to determine AMF colonization using the magnified gridline intersect method (see Appendix A; McGonigle et al. 1990). The two-month duration of the experiment corresponds to the first two months of the growing season, when plants are most susceptible to herbivory effects and when pollinators are active (Barber et al. 2012).

We measured plant traits that influence herbivores by collecting leaves 3, 4, and 5 (counted from the base of the plant) from each plant when plants were one month old, when these leaves were fully expanded but not senescing. These leaves were dried at 60°C and ground. We used ground leaf tissue to determine cucurbitacin C content (see Appendix A) and content of N, P, Na, Mg, and K; these are elements that are known or suspected to be important in insect herbivore nutrition (Joern et al. 2012). We also determined cucurbitacin C content of roots after they were dried for belowground biomass measurement.

To measure plant traits that may mediate pollination, we measured flower number and size, nectar production, and floral volatiles because these are known to be important attraction cues for pollinators, especially honey bees, the most common pollinator of *C. sativus* in the study region (Duffield et al. 1993, Ashman et al. 2005). We counted male flowers five days per week (few female flowers were produced, possibly due to nutrient limitation). We measured the length and width of a single petal on three separate male flowers for each plant, and measured nectar production from a single flower per plant by extracting nectar with capillary tubes. Flowers are only open for a single day. All measurements were performed at midday to control for circadian changes in nectar production, and plants in a block were sampled on the same day when possible. We sampled floral scent from a single male flower on each plant using dynamic headspace sampling (see Appendix A). Floral volatiles were identified using gas chromatography–mass spectrometry. Because of an internal standard error, we were unable to calculate volatile concentrations. Volatiles are expressed instead as a proportion of the total volatile blend. We described floral volatiles in two ways: using principal components analysis (PCA, hereafter "scent PCA") and categorizing volatiles as monoterpenes, sesquiterpenes, and aromatics.

Analysis

To understand the relationships between agricultural management practices, AMF, and plant traits, our analysis had four steps: (1) examine how soil characteristics differ among organic and conventional farms; (2) compare plant traits and AMF colonization among AMF inoculation types (organic, conventional, commercial, or control); (3) determine if AMF colonization levels in experimental pots are related to soil characteristics from the source farm for each AMF inoculum; and (4) determine if plant traits are correlated with AMF colonization levels.

We described soil characteristics of farms using PCA of the soil laboratory analysis results (hereafter, "soil PCA") and compared principal component scores for organic and conventional farms with *t* tests. We used generalized linear mixed models (GLMMs), treating block (greenhouse bench) as a random factor, to compare plant traits among AMF inoculation types. For continuous response variables (including scent), models assumed Gaussian distribution and identity link. For count responses (flower number, AMF colonization), models assumed Poisson distribution and log link and were fit as individual-level random effects models to account for overdispersion. To analyze seed germination, we used binomial distribution with logit link, also incorporating individual-level random effects. In analyzing nectar production, we included date and time of collection as covariates. We used three a priori orthogonal contrasts to test for inoculum effects: control (no AMF inoculum) vs. AMF (commercial, conventional, and organic combined); commercial AMF vs. farm AMF (conventional and organic combined); and conventional vs. organic AMF.

To determine if AMF colonization varies with soil characteristics regardless of management type, we used generalized linear models to compare mean total AMF colonization and mean arbuscular colonization for each farm to first and second soil principal components. Note that farm is the unit of replication in this analysis. Finally, to determine if AMF colonization level influences plant traits, we used GLMMs to analyze the effect of total and arbuscular AMF colonization in each plant on the same traits examined in step one of the analysis. Rather than apply multiple comparisons adjustments such as sequential Bonferroni, which have been criticized as overly conservative in ecological experiments (Moran 2003), we present uncorrected *P* values and focus on effect sizes when appropriate to interpret results.

Results

Farm soil characteristics

Organic and conventional farm soil characteristics differed. In the soil PCA, the first two principal components explained 54% of the variation in soil characteristics (Appendix B: Table B1). Organic farm soils had significantly lower soil PC1 scores than conventional farms ($t_{15} = 4.00$, $P = 0.001$), indicating higher organic matter content, higher CEC, and lower P, K, and Zn than conventional farms. Soil PC2 scores did not differ ($t_{15} = 0.45$, $P = 0.658$). Soil characteristics for organic and conventional farms are summarized in Appendix B: Table B2.

Inoculum source effects on colonization and plant traits

Inocula from organic farms resulted in significantly higher total and arbuscular colonization than conventional farm inocula (Fig. 2A, Table 1). Colonization by commercial inoculum did not differ from combined farm AMF. Control treatments resulted in very low AMF colonization (<1%); this low level of colonization recorded was likely due to false positives when scoring root slides.

Neither above- or belowground biomass varied with inoculum treatment (Appendix C: Table C1), but commercial AMF plants had a significantly higher root : shoot ratio (Fig. 2B) and produced significantly larger leaves than farm AMF plants (Table 1). Seed germination rate was highest for control plants, and seeds in commercial AMF inoculum were significantly more likely to germinate than seeds in organic or conventional farm AMF (Table 1, Fig. 2C).

Inoculum treatments had no influence on leaf N content, but all AMF inocula reduced leaf P relative to the nonmycorrhizal control (Fig. 3A). Plants in farm AMF treatments had marginally lower leaf P than the commercial treatment, and leaf P was lower in conventional farms than organic farms (Table 2). Magnesium and K were unaffected by mycorrhizae treatments, but Na significantly increased with farm AMF, and particularly with organic AMF (Fig. 3B). Leaf cucurbitacin C content was not different among AMF treatments, but root cucurbitacins were marginally lower in commercial AMF than farm AMF plants (Fig. 3C). Plants in conventional AMF had higher root cucurbitacin content than organic AMF plants (Table 2).

Commercial AMF inoculum increased male flower production relative to farm AMF, but organic and conventional AMF did not differ (Fig. 2D, Table 3). Male flower petal size and nectar production did not differ with AMF treatments. Values of floral scent PC1 and PC2 (which together accounted for 36% of variation in scent composition; Appendix A: Table A1) were unrelated to inoculum type, and proportions of the three volatile classes (monoterpenes, sesquiterpenes, and benzyl compounds) also did not differ with treatment (Appendix A: Table A2).

AMF colonization levels, soil characteristics, and plant traits

Mycorrhizal colonization was lower when AMF inoculum was obtained from soil with high organic matter content and CEC and low P, K, and Zn. Mean

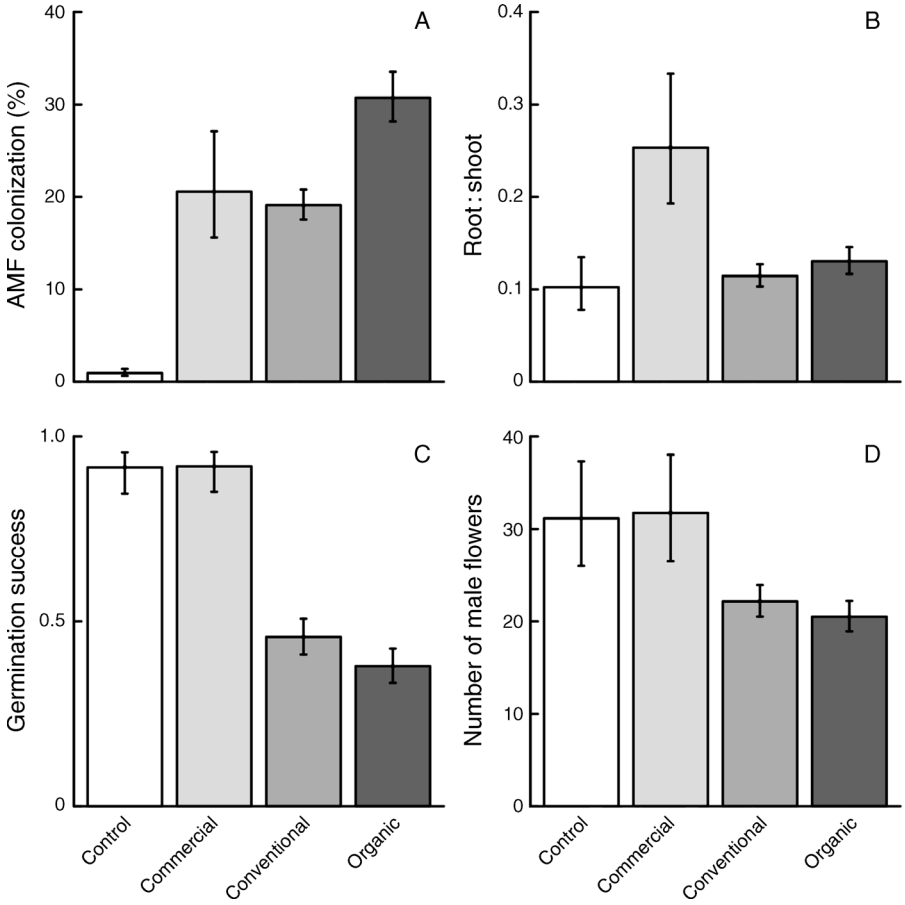

FIG. 2. Effects of AMF inoculation treatments on (A) total AMF colonization, (B) root : shoot biomass ratio, (C) proportion of seeds germinating, and (D) male flower production. Values are fitted means ± SE. Cucurbitacin C content is expressed as unitless relative values because a standard was not available for quantitation (see Appendix A).

total and arbuscular colonization of greenhouse plants significantly declined with soil PC1 of the 17 farm fields studied (β [estimate ± SE]; total, −2.49 ± 0.88, $t = 2.81$, $P = 0.013$; arbuscular, −1.61 ± 0.59, $t = 2.74$, $P = 0.015$), but colonization was unrelated to PC2 (all $P > 0.3$). All plant traits were unrelated to total or arbuscular AMF colonization, except root cucurbitacin, which significantly increased with both colonization measures (Appendix C: Table C2).

DISCUSSION

Our goal was to understand how AMF from different agricultural management regimes influence crop host traits. We examined both direct effects, such as seed germination and plant growth, and changes in traits that could indirectly influence growth and yield by mediating interactions with herbivores and pollinators. Using AMF communities from organic and conventional

TABLE 1. Results of generalized linear mixed models (GLMM) analyses of arbuscular mycorrhizal fungi (AMF) inocula on total AMF colonization, arbuscular colonization, seed germination, root : shoot ratio, and mean leaf size.

Contrast	Control vs. AMF			Commercial vs. farm			Organic vs. conventional		
	β ± SE	Wald	P	β ± SE	Wald	P	β ± SE	Wald	P
Total AMF	−0.80 ± 0.10	7.902	**<0.001**	−0.11 ± 0.19	0.579	0.562	0.24 ± 0.06	3.912	**<0.001**
Arbuscules	−1.30 ± 0.30	4.351	**<0.001**	−0.13 ± 0.22	0.598	0.550	0.29 ± 0.07	3.975	**<0.001**
Seed germination	0.45 ± 0.18	2.498	**0.013**	1.85 ± 0.46	4.002	**<0.001**	−0.16 ± 0.10	1.604	0.109
Root : shoot ratio	0.10 ± 0.07	1.465	0.147	−0.49 ± 0.18	2.642	**0.010**	−0.06 ± 0.07	0.979	0.331
Leaf size	6.73 ± 7.61	0.884	0.378	66.63 ± 19.58	3.403	**<0.001**	−3.40 ± 6.95	0.489	0.625

Notes: Values of $\beta > 0$ indicate control > AMF, commercial > farm, or organic > conventional. Total AMF, arbuscules, and root : shoot ratio coefficients are on a log scale; seed germination coefficients are expressed in logit form. The Wald statistic is z for total AMF, arbuscles, and seed germination, and t for root : shoot ratio and leaf size. The Wald z test statistic was used in Poisson models, and the Wald t was used in Gaussian models.

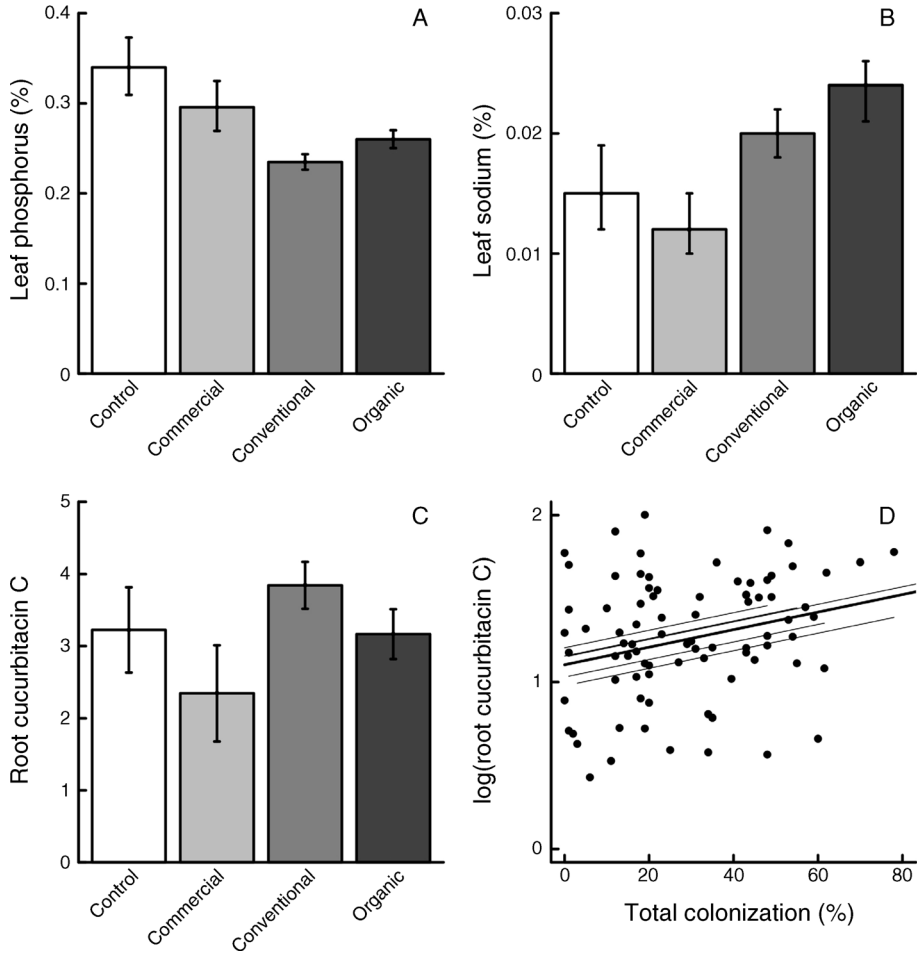

FIG. 3. Effects of AMF inoculation treatments on (A) leaf percentage phosphorus, (B) leaf percentage sodium, and (C) root cucurbitacin C content. Values are fitted means ± SE. (D) Relationship between total AMF colonization and log-transformed root cucurbitacin C content, where the bold line represents overall model fit and thin lines represent fitted relationships for each of five blocks. In panels (C) and (D), cucurbitacin C content is expressed as unitless relative values because a standard was not available for quantitation (see Appendix A).

farms, we found that inoculum type had a strong impact on crop traits, such as leaf nutrients, root defensive chemistry, and floral production, but we found no dramatic changes in plant biomass. We discuss results and the implications for AMF community function in the context of agricultural land management.

Soil conditions and AMF colonization

Farm management type strongly affected soil characteristics and presumably AMF communities as well. Soil from organic farms had significantly higher organic matter content than conventional farms and, as a result, higher CEC. Conventional farm soil was higher in P, K,

TABLE 2. Results of GLMM analyses of AMF inocula effects on leaf and root chemistry.

Contrast	Control vs. AMF			Commercial vs. farm			Organic vs. conventional		
	β ± SE	Wald *t*	*P*	β ± SE	Wald *t*	*P*	β ± SE	Wald *t*	*P*
Leaf nitrogen	−0.04 ± 0.04	0.931	0.354	−0.10 ± 0.10	0.995	0.323	−0.00 ± 0.04	0.022	0.982
Leaf phosphorus	0.06 ± 0.02	2.670	**0.009**	0.12 ± 0.06	1.925	0.058	0.05 ± 0.02	2.314	**0.023**
Leaf sodium	−0.04 ± 0.05	0.771	0.443	−0.36 ± 0.14	2.608	**0.011**	0.09 ± 0.05	1.908	0.060
Root cucurbitacin C	2.65 ± 15.77	0.168	0.867	−77.39 ± 39.43	−1.963	0.053	−33.94 ± 14.23	−2.385	**0.019**
Leaf cucurbitacin C	0.02 ± 0.02	0.997	0.321	0.05 ± 0.05	1.014	0.312	−0.00 ± 0.02	0.155	0.877
Leaf potassium	0.02 ± 0.02	0.748	0.456	0.09 ± 0.06	1.434	0.155	−0.01 ± 0.02	0.234	0.815

Notes: Values of $\beta > 0$ indicate control > AMF, commercial > farm, or organic > conventional. Coefficients for all response variables except root cucurbitacin C are on a log scale.

TABLE 3. Results of GLMM analyses of AMF inocula on floral traits.

Contrast	Number of male flowers			Petal size			Nectar production		
	β ± SE	Wald *z*	*P*	β ± SE	Wald *t*	*P*	β ± SE	Wald *t*	*P*
Control vs. AMF	0.06 ± 0.05	1.33	0.184	5.69 ± 5.78	0.984	0.326	−0.10 ± 0.13	0.734	0.464
Commercial vs. farm	**0.27 ± 0.12**	**2.23**	**0.026**	−3.18 ± 14.88	0.214	0.831	−0.03 ± 0.34	0.078	0.938
Organic vs. conventional	−0.04 ± 0.04	−0.89	0.372	−3.89 ± 5.30	0.735	0.463	−0.08 ± 0.12	0.652	0.515

Notes: Values of $\beta > 0$ indicate control > AMF, commercial > farm, or organic > conventional. Male flower coefficients are on a log scale. The nectar production model included date ($F_{2,172} = 1.09$, $P = 0.337$) and time ($F_{1,172} = 2.37$, $P = 0.126$) as nonsignificant covariates (see *Methods: Analysis*).

and Zn, probably due to greater fertilizer addition. Although we did not attempt to describe the species composition of the AMF communities at our collection sites, conventional farming practices and agricultural intensification (greater soil pesticide and fertilizer inputs, less crop diversity) can decrease both abundance and diversity of AMF (Oehl et al. 2003, 2004). Recently, molecular methods that allow higher-resolution descriptions of community composition have verified these results, demonstrating that AMF communities in organic fields tend to be more similar to those in unmanaged grasslands than those of conventional farms (Verbruggen et al. 2010).

Although AMF colonization intensity in *C. sativus* roots differed between organic and conventional farms, this was generally unrelated to plant performance and other traits. Plants inoculated with AMF from organic farms had 61% greater root colonization than those inoculated with conventional farm AMF (Fig. 2A). However, root colonization was not correlated with any measured plant traits except root cucurbitacin C content. This confirms previous reports that percentage AMF colonization is often a less helpful metric in predicting mycorrhizal impacts on plants than fungal identity, given the species- or strain-specific effects observed in controlled inoculation experiments (Ruiz-Lozano et al. 1995, Bennett and Bever 2007, Gehring and Bennett 2009, Verbruggen and Kiers 2010).

Direct effects

There were no differences in root or shoot biomass among inoculum treatments, indicating that AMF did not affect total plant growth relative to non-AMF or commercial AMF controls. Although this could be an artifact of a greenhouse experiment, a lack of growth effect underscores the fact that AMF are not always beneficial mutualists when evaluated solely by their effects on plant biomass. In a similar study of AMF from organic and conventional maize fields, plant biomass was significantly reduced by farm AMF compared to sterile control (Verbruggen et al. 2012). In the current study, root : shoot ratio was significantly higher in plants with commercial AMF, which may indicate greater plant investment in belowground growth or that the commercial strain of *R. irregularis* is a strong carbon sink at the expense of aboveground plant growth. Because plants allocate growth to the sphere where resources are limited (Alpert 1991), the commercial AMF may not have provided access to sufficient nutrients, resulting in plants that responded by increasing belowground growth. However, under this mechanism we might expect a similar result for control plants that received no nutrient benefit from AMF, but we did not find increased belowground growth in control plants.

Both organic and conventional farm AMF reduced seed germination compared to commercial AMF and AMF-free control. This result is surprising given that AMF effects are generally thought to occur after a seedling begins to grow and the fungi colonize root cells (Smith and Read 2008). One potential explanation is that a soil pathogen from farm inocula reduced germination. However, we added an AMF-free microbial filtrate pooled from all farm sources to control plants, which presumably would have caused a similar result if germination-reducing pathogens were present. However, control plants exhibited >90% germination, while germination rates for organic and conventional AMF were both <50%. Inoculum from one organic farm completely prevented seed germination, demonstrating that this pattern could be important in some agricultural settings. We are unaware of a mechanism that could explain these results, although a recent report that AMF spore exudates suppress seed germination of a parasitic plant (Louarn et al. 2012) suggests further studies of this phenomenon are warranted.

Leaf and root traits

Mycorrhizal inoculum source affected several leaf and root traits that influence herbivores and herbivory, including nutrient content and defensive chemistry. Surprisingly, plants grown with AMF had lower leaf P content than non-AMF plants, with farm AMF in general (and conventional farm AMF in particular) reducing leaf P. Leaf P may have been low if plants preferentially allocated P to roots, which could benefit root-feeding herbivores, but we did not measure root nutrient content. Although ecologists often focus on N availability in host plants as a limiting factor for herbivores, P can be also be important. Limited P reduced the growth of both generalist (Janssen 1994) and specialist caterpillars (Perkins et al. 2004) and the growth and survival of a specialist planthopper (Huberty and Denno 2006), although P effects may depend

on the availability of N and other micronutrients, such as Mg and S (Clancy and King 1993, Busch and Phelan 1999).

By contrast, AMF derived from both organic and conventional farms resulted in plants with nearly double the Na content of commercial AMF plants. Sodium has recently received greater attention as an important nutrient for herbivores given its generally low concentration in plant relative to herbivore tissues (Kaspari et al. 2008, Behmer and Joern 2012, Chavarria Pizarro et al. 2012, Joern et al. 2012). Thus, farm AMF communities may have mixed effects on foliage nutrient quality for herbivores by decreasing availability of one potentially limiting element (P) but increasing another (Na). However, organic farm AMF might be expected to have a more positive effect on herbivores because it reduced P less and increased Na more than conventional farm AMF, although the P difference is relatively small (Fig. 2C, D). Behmer and Joern (2012) emphasize that polyphagous insect herbivores with access to multiple host species may be able to balance nutrient intake through selective foraging (Pulliam 1975, Bernays et al. 1994), but this may not be possible for insect herbivores on farms, where crops are usually planted as monocultures. Insect pests facing an entire field of similar plants may undergo population booms if a limiting nutrient is promoted; conversely, crops may be less susceptible to attack if another limiting nutrient is scarce.

Chemical defense traits also responded to AMF source, but interestingly only in root tissue. Concentrations of cucurbitacin C, the putative primary defensive chemical in *C. sativus*, tended to be lower with commercial compared to farm AMF, and were significantly higher in conventional compared to organic farm AMF. This result is of particular importance for *C. sativus* and other cucurbits, because the specialist herbivore *A. vittatum* feeds on roots as larvae and has strong direct and indirect effects on *C. sativus*, including changes in pollinator attraction (Barber et al. 2011). In studies of dandelion (*Taraxacum officinale*), mycorrhizal colonization reduced performance and survival of a root-feeding weevil larva (Gange et al. 1994). An experiment in strawberry (*Fragaria* x *ananassa*) produced similar results when either of two AMF species was inoculated independently, but the effect disappeared on plants colonized by both species together (Gange 2001). This suggests that, as in our study, effects on plant defenses vary among AMF species.

Leaf cucurbitacin levels were unaffected by our inoculation treatments. In a meta-analysis of mycorrhizal effects on herbivores and herbivory, Koricheva et al. (2009) showed that enhanced resistance to herbivores by AMF colonization occurred similarly in both above- and belowground tissues. They interpreted this result as evidence of systemic effects of AMF on plant defenses, but we show that these effects may be localized to different plant organs, consistent with Koricheva et al.'s (2009) original hypothesis that AMF effects would be most apparent on herbivores feeding at the site of colonization. Interestingly, root cucurbitacins increased with greater AMF colonization (Fig. 3D), although conventional farms, which had higher cucurbitacin C root content, had lower colonization levels than organic or commercial treatments. This further highlights our suggestion that AMF effects on plant defenses emerge as specific effects of the community composition present in the soil under different management practices.

Floral traits

Both organic and conventional farm AMF reduced flower production, which would likely reduce pollination services in the field. Compared to nonmycorrhizal *C. sativus*, plants inoculated with farm AMF produced 32% fewer male flowers. Reduced floral production significantly reduces pollinator attraction in this system (Barber et al. 2012). Based on results from this past study, where the average plant produced 280 flowers, a 32% reduction in flowers would translate to a 23% reduction in the number of pollinators visiting that plant (visits $= 0.041 \times$ flowers $+ 4.482$, $R^2 = 0.46$). Although some AMF may alter floral traits (Gange and Smith 2005, Becklin et al. 2011) and, in *C. sativus*, increase flower size under high-P conditions (Kiers et al. 2010), the size, nectar content, and scent of flowers were unaffected in this experiment. Under more limited nutrient conditions, floral production may be simply a function of nutrient availability, with plants producing fewer or more flowers that are similar in quality. This may also explain the small number of female flowers produced, which likely require greater resource investment than male flowers. Given that we found AMF effects on male flower number but not plant growth, *C. sativus* may allocate limited resources to growth before investing in flowers, and to male flowers before female flowers given the lower cost of male reproductive function (Silvertown 1987). If so, this suggests that the influence of different AMF communities on floral traits is a quantitative effect, rather than an influence on flower quality.

Conclusions

AMF source significantly affected several plant traits that are important mediators of plant–insect interactions. Leaves from plants inoculated with organic AMF had significantly higher P content, and there was a trend toward higher leaf Na. Although organic AMF may provide nutrient uptake benefits to plants, this did not translate into other benefits such as increased growth or flower production. Organic- and conventional-inoculated plants also differed in root chemical defenses, with AMF from conventional farms increasing root cucurbitacin C content. This could have important effects on plant growth and reproduction because root herbivory has strong direct and indirect effects on *C. sativus*, including reduced growth, flower production, and seed

production, as well as changes in pollinator preference (Barber et al. 2011).

In this series of experiments, other traits, including biomass, did not differ between plants inoculated with AMF communities from organic vs. conventional management farms. However, compared to commercial inoculum (containing a strain selected to be generally mutualistic) and nonmycorrhizal control, both types of farm AMF reduced leaf P, flower production, and leaf size. Given that both organically and conventionally managed farms are applying large quantities of nutrients to their fields, fertilization may have a negative impact on the quality of AMF, whether the nutrients are in a mineral or organic form (Gosling et al. 2006). In a recent experiment, inocula from agricultural settings (both organic and conventional) reduced maize growth in pots, with plant productivity inversely correlated with AMF abundance (Verbruggen et al. 2012). Despite this negative effect, inocula were shown to reduce P leaching in the system after simulated rain events, suggesting that positive effects on one ecosystem service (reduced P leaching) may be linked with negative effects on another ecosystem service (crop production) (Verbruggen et al. 2012).

A key area of future research is to examine how multispecies interactions (e.g., plant–pollinator or plant–herbivore) are modified in the presence of different AMF species or communities. There have been recent advancements in understanding how soil mutualists can change plant–plant competitive interactions (Bever et al. 2010, Daisog et al. 2012), but there are still many outstanding questions about how AMF influence herbivory and pollination, especially in a field setting (Gange and Smith 2005, Koricheva et al. 2009). For example, because herbivores can reduce plant photosynthetic area, AMF that enhance plant resistance to herbivores may have the same carbon benefits to AMF as increased plant growth. These benefits could help select for defense-enhancing AMF within soil communities. Indirect effects on pollinator attraction may be of particular importance in light of concerns about declines in pollination services (Biesmeijer et al. 2006, National Research Council of the National Academies 2007).

Soil microbial communities are an integral part of agroecosystems, with potential to provide both benefits and costs to farmers. The management decisions of farmers drive evolutionary selection in these diverse communities (Verbruggen and Kiers 2010). Continued work on these questions may allow us to provide specific management recommendations that can increase yield and feed an increasingly populous world.

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Supplemental Material

Appendix A

Methodological details of AMF staining, cucurbitacin analysis, and floral volatile analysis, with volatile results (*Ecological Archives* A023-078-A1).

Appendix B

Soil characteristics from sampled conventional and organic fields (*Ecological Archives* A023-078-A2).

Appendix C

Results for statistical analyses of AMF treatments on plant biomass and AMF colonization on plant traits (*Ecological Archives* A023-078-A3).