Plants interact with a variety of other community members that have the potential to indirectly influence each other through a shared host plant. Arbuscular mycorrhizal fungi (AMF) are generally considered plant mutualists because of their generally positive effects on plant nutrient status and growth. AMF may also have important indirect effects on plants by altering interactions with other community members. By influencing plant traits, AMF can modify aboveground interactions with both mutualists, such as pollinators, and antagonists, such as herbivores. Because herbivory and pollination can dramatically influence plant fitness, comprehensive assessment of plant–AMF interactions should include these indirect effects. To determine how AMF affect plant–insect interactions, we grew Cucumis sativus (Cucurbitaceae) under five AMF inoculum treatments and control. We measured plant growth, floral production, flower size, and foliar nutrient content of half the plants, and transferred the other half to a field setting to measure pollinator and herbivore preference of wild insects. Mycorrhizal treatment had no effect on plant biomass or floral traits but significantly affected leaf nutrients, pollinator behavior, and herbivore attack. Although total pollinator visitation did not vary with AMF treatment, pollinators exhibited taxon-specific responses, with honey bees, bumble bees, and Lepidoptera all responding differently to AMF treatments. Flower number and size were unaffected by treatments, suggesting that differences in pollinator preference were driven by other floral traits. Mycorrhiza influenced leaf K and Na, but these differences in leaf nutrients did not correspond to variation in herbivore attack. Overall, we found that AMF indirectly influence both antagonistic and mutualistic insects, but impacts depend on the identity of both the fungal partner and the interacting insect, underscoring the context-dependency of plant–AMF interactions.
direction and magnitude of AMF impacts on plant–pollinator interactions likely depend on both the pollinator and the AMF species colonizing the plant (Gehring and Bennett, 2009).

Insect herbivory is also frequently influenced by AMF colonization (Koricheva et al., 2009), and these effects may be due to mycorrhizal effects on plant biomass, nutrient content, or defenses (Bennett et al., 2006). For example, increased nutrient content of mycorrhizal plants may increase their quality as a food source for herbivores, but the resources made available by this interaction may also be allocated toward defense against herbivores (Vannette and Hunter, 2011). Additionally, AMF may also play an important role in induced resistance of plants against insects by priming the jasmonic acid-dependent responses of plants to herbivory (Duso and Arçón-Aguilar, 2007; Koricheva et al., 2009; Jung et al., 2012). Regardless of the underlying mechanisms, AMF can indirectly affect plant fitness through changes in herbivory.

The effects of AMF on pollination or herbivory are likely to differ among AMF species or strains. For example, both constitutive and induced levels of defensive chemicals in Plantago lanceolata varied among plants inoculated with three different AMF species (Bennett et al., 2009). In a recent study, performance of herbivores feeding on Fragaria virca varied when plants were inoculated with different strains of the AMF Rhizophagus irregularis (Roge et al., 2013). These results underscore the importance of examining multiple species in AMF–plant–insect interactions to understand the variation in indirect mycorrhizal effects (Gehring and Bennett, 2009). There are additional challenges to studying insect responses to mycorrhizal variation in a realistic field setting. For example, most field studies of these interactions have manipulated AMF by applying fungicide to plots and observing insect responses (Koricheva et al., 2009). Although this is an effective method of eliminating AMF from experimental plots, there may be unintended effects by altering non-mycorrhizal fungi and other soil organisms.

In this study we tested the hypothesis that plant–AMF interactions below-ground influence above-ground interactions between plants, herbivores and pollinators. We used an inoculation experiment to manipulate multiple species/strains of AMF in the roots of Cucumis sativus (cucumber, Cucurbitaceae). We transferred plants to an agricultural field setting, measured traits that may affect plant reproduction directly and indirectly, and determined pollinator and herbivore preferences. Although we made no specific predictions about the impacts of each inoculum on plant–insect interactions, we hypothesized that AMF-free plants would have reduced pollinator visitation and based on previous similar studies (Gange and Smith, 2005; Wolfe et al., 2005). Given the role of AMF in induced defenses of C. sativus (Barber, 2013), we also expected greater herbivory on these non-mycorrhizal plants.

**STUDY SYSTEM**

Cucumis sativus is a widely cultivated, monococious annual vining plant that associates with multiple species of AMF. Mycorrhizae can influence flowering, fruit production, photosynthesis rates, and disease resistance in C. sativus (Zvimba and Knowles, 1999; Valentin et al., 2001; Hao et al., 2005; Kiers et al., 2010). Flowers of C. sativus open for a single day and are pollinated by a variety of generalist pollinators, including honey bees (Apis mellifera, Apidae), bumble bees (Bombus spp., Apidae), solitary bees (e.g., Halictidae), butterflies, and hoverflies (Syrphidae; Barber et al., 2012). All of these pollinators are common in western Massachusetts, USA, where this study took place. Throughout much of eastern North America, the most important herbivore of C. sativus is the specialist Aclydyma vittatum (Chrysomelidae), which accounts for virtually all leaf damage at the study site (Barber et al., 2012). Acalymma vittatum larvae feed on root tissue of host plants prior to pupation.

**EXPERIMENTAL DESIGN**

We surface sterilized C. sativus seeds (Marketmore 76, Johnny’s Selected Seeds, Winslow, ME, USA) using 5% bleach solution and germinated them in steam-sterilized potting mix (Fafard organic mix, Agawam, MA, USA). At transplanting, we inoculated 192 seedlings with one of six AMF treatments. For our fungal treatments, we chose three closely related fungal species: Glomus claro, G. custos (strain 010 Mycorvitro), and R. irregularis (strain 09 Mycorvitro, see Stockinger et al. (2009) for discussion of G. intraradices reclassification) in the Glomeraceae. These three species were chosen because they have been shown to differ in the growth benefits (i.e., P and N benefits) they confer to various host plants (Kiers et al., 2011; Ottus and Alginan, 2011; Verbruggen et al., 2012; Hart et al., 2013). The use of closely related AMF allowed us to focus on fungal cooperative strategy while excluding differences associated with radically contrasting life-history traits (Denison and Kiers, 2011). These fungal species were applied in liquid form (1 mL inoculum applied directly to seedling roots) from solubilized in vitro root cultures obtained from Estacion Experimental del Zaidin, Consejo Superior de Investigaciones Científicas, Granada, Spain. Fungal species were applied singly, but we also included a mixed treatment, which was composed of equal volumes (333 μL) of these three species together. The inoculum of these three species did not contain significantly different densities of spores (100 μL samples, n = 5 each, mean ± SE, G. claro 11.8 ± 3.1; G. custos 10.6 ± 1.6; R. irregularis 8.2 ± 2.4).

While these strains are well-characterized laboratory strains, we were also interested in studying the effects of commercial inoculum that farmers would apply in the field. Therefore, we included a commercial inoculum also composed of a R. irregularis strain (isolate DAOM 197198; hereafter we refer to this inoculum as “commercial” to distinguish it from the R. irregularis strain 09) produced by Myke Premier Tech Biotechnologies (Rivière-du-Loup, QC, Canada). The commercial inoculum was on a peroxide mixture that was added into the transplant pot (60 mL, ca. 120 spores). We also included a non-mycorrhizal control, in which plants received 1 mL water. The result was six AMF treatments (three single species, one mixture, one commercial inoculum, and one control). Both liquid and peroxide inocula also contained colonized root fragments and mycorrhizal hyphae as well as spores. Although peroxide from the commercial treatment represented only about 2.4% of the total volume of soil in the pot, it could potentially influence plants through changes in soil structure and increased drainage. However, peroxide does not influence soil cation exchange capacity and has little effect on soil nutrients.
To create a common soil growing medium for all plants, we mixed soil from an agricultural field at the study site with an equal volume of sand and autoclaved the mixture to sterilize it; characteristics of this soil mixture are presented in Table 1. We filled 2.5 L bleach-sterilized pots, lined with bleach-sterilized plastic mesh, with the soil mixture and transplanted inoculated seedlings on 1–2 June 2011. We transferred 16 plants from each group to the UMass Agricultural Research Center (South Deerfield, MA, USA) on 6 June to determine how AMF treatments affected leaf nutrient content in a field setting. We arranged plants in 16 blocks (rows) of six plants each (one plant/treatment/block, all plants spaced 2.5 m apart). We placed each pot into a black plastic tray filled with sand to create a barrier between the pot and AMF in the field soil. We allowed plants to grow under field conditions for 22 days, after which leaf tissues were collected and dried for nutrient analysis. We watered plants daily unless there was rainfall in the past 24 h.

The remaining 96 plants were maintained in a greenhouse with natural light and locations rotated on greenhouse benches weekly. On 1 July we transported these plants to the same field to determine how AMF treatments affected plant characteristics and interactions with AMF, herbivores, and pollinators (Barber et al., 2011, 2012). This ensured that responses were due to transferring them to the field prevented early season herbivory, which affects interactions with AMF, herbivores, and pollinators. We filled 2.5 L bleach-sterilized pots, lined with bleach-sterilized plastic mesh, with the sterilized soil mixture. We placed each pot into a black plastic tray filled with sand to create a barrier between the pot and AMF in the field soil. We allowed plants to grow under field conditions for 22 days, after which leaf tissues were collected and dried for nutrient analysis. We watered plants daily unless there was rainfall in the past 24 h.

We placed each pot into a black plastic tray filled with sand to create a barrier between the pot and AMF in the field soil. We allowed plants to grow under field conditions for 22 days, after which leaf tissues were collected and dried for nutrient analysis. We watered plants daily unless there was rainfall in the past 24 h.

Table 1 | Characteristics of sterilized soil mixture (mean ± 1 SE, n = 2).

<table>
<thead>
<tr>
<th>Soil characteristic</th>
<th>µM</th>
<th>ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.15 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>% organic matter</td>
<td>3.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>CEC</td>
<td>3.65 ± 0.55</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>22.5 ± 6.5</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>69.0 ± 6.0</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>918 ± 134</td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>38.5 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Al</td>
<td>19.0 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.1 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>21.8 ± 5.8</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>12 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.9 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>2.0 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>340.0 ± 10.8</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>2.95 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

CEC = cation exchange capacity; all measurements (other than pH, % organic matter, and CEC) are ppm.
Arbuscular mycorrhizal fungi treatments also did not affect the proportion of flowers probed by plant pollinators in total but did affect the proportion probed by honey bees and by Lepidoptera (Table 3). Honey bees probed a significantly lower proportion of flowers on plants inoculated with each of the single species inocula compared to AMF-free control plants, and honey bee probes were no more likely on mixture plants than single species plants (Figure 3C). Lepidoptera tended to probe a higher proportion of

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Results of GLMM analyses of AMF treatment effects on leaf nutrient content.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$z^2$/Wald z</td>
</tr>
<tr>
<td>Leaf N</td>
<td>3.49</td>
</tr>
<tr>
<td>Leaf P</td>
<td>9.56</td>
</tr>
<tr>
<td>Leaf K</td>
<td>12.97</td>
</tr>
<tr>
<td>Control vs. G. clarum</td>
<td>1.32</td>
</tr>
<tr>
<td>Control vs. G. custos</td>
<td>1.55</td>
</tr>
<tr>
<td>Control vs. R. irregularis</td>
<td>1.08</td>
</tr>
<tr>
<td>Control vs. commercial</td>
<td>2.52</td>
</tr>
<tr>
<td>Mixture vs. single</td>
<td>2.30</td>
</tr>
<tr>
<td>Leaf Na</td>
<td>19.35</td>
</tr>
<tr>
<td>Control vs. G. clarum</td>
<td>1.16</td>
</tr>
<tr>
<td>Control vs. G. custos</td>
<td>0.79</td>
</tr>
<tr>
<td>Control vs. R. irregularis</td>
<td>0.02</td>
</tr>
<tr>
<td>Control vs. commercial</td>
<td>3.51</td>
</tr>
<tr>
<td>Mixture vs. single</td>
<td>2.08</td>
</tr>
</tbody>
</table>

Tests of overall effect of AMF treatment are $z^2$ tests, when significant, contrasts used Wald z tests with P-values adjusted for multiple comparisons using the Westfall method (see Analysis).

Survey date, there were four possible responses (0, 1, 2, or 3 leaves damaged). This is equivalent to a repeated measures analysis, but in a binomial linear model framework. For continuous response variables (flower size, aboveground and belowground plant growth, proportion flowers probed), we used Gaussian errors and identity link.

The goal of this experiment was to determine whether AMF inoculum influenced plant traits and plant–insect interactions; we did not have specific predictions about how individual inocula might differ compared to each other. We used likelihood ratio tests to compare models with and without AMF treatment, which compare likelihood ratios to a $\chi^2$ distribution. When this test was significant at $P < 0.05$, we compared each single species inoculum (G. clarum, G. custos, R. irregularis, and commercial) to the AMF-free control. We also tested one additional a priori hypothesis contrasting the mixture treatment of G. clarum, G. custos, and R. irregularis with the three independent treatments of these species combined. This tests if these AMF species have additive or interactive effects when combined; a significant contrast indicates that the species in combination interact to produce a response different from average responses to single species colonizations. We used the multcomp package (Hothorn et al., 2008) to perform contrasts, adjusting P-values for multiple comparisons using the Westfall method, as implemented in the glht() function of multcomp.

RESULTS

PLANT MEASUREMENTS

Colonization varied significantly among the inoculation treatments ($\chi^2 = 46.51, df = 5, P = 0.001$), with the mix inoculum treatment showing the highest colonization levels. No AMF structures were observed in control plants (Figure 1). AMF inoculation treatment did not affect total plant biomass ($\chi^2 = 7.59, df = 5, P = 0.181$) or root:shoot ratio ($\chi^2 = 4.46, df = 5, P = 0.486$). There were also no differences in total flower number ($\chi^2 = 4.40, df = 5, P = 0.492$) or flower petal size ($\chi^2 = 3.34, df = 5, P = 0.634$). AMF treatments did affect leaf nutrient content, with a significant effect on leaf K and Na and a marginally significant effect on leaf P (Table 2). Leaf K and Na were significantly increased by commercial AMF compared to control ($\chi^2 = 5.62, df = 1, P = 0.017$). AMF inoculation treatment did affect leaf nutrient content, with a significant effect on leaf K and Na and a marginally significant effect on leaf P ($\chi^2 = 2.30, df = 1, P = 0.127$).

POLLINATION

We observed 2,498 plant visits by pollinators and 4,254 individual flower probes. Although AMF treatments did not influence total pollinator visitation, visitation of several pollinator taxa varied with AMF inoculum (Table 3). There was a significant effect of AMF treatment on visitation by bumble bees (Figure 3A) and Lepidoptera (Figure 3B) and a marginally significant effect on honey bee visitation. Bumble bee visitation was greatest to plants inoculated with R. irregularis; this difference was marginally significant after controlling for multiple comparisons. Similarly, although AMF affected Lepidoptera visitation overall, visits to particular treatments did not differ in any individual contrasts (Table 3).
FIGURE 2 | Effects of AMF inoculation treatments on (A) leaf Na content and (B) leaf K content. Values are fitted means expressed in parts per million ± 1 SE for Na and parts per thousand ± 1 SE for K. Asterisk indicates significant difference from non-mycorrhizal control.

Table 3 | Results of GLMM analyses of AMF treatment effects on total number of pollinator visits per plant and proportion of flowers probed, by pollinator taxa.

<table>
<thead>
<tr>
<th>Pollinator Taxa</th>
<th>Total pollinator visits</th>
<th>Proportion flowers probed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>χ²/Wald z</td>
<td>P</td>
</tr>
<tr>
<td>All pollinators</td>
<td>2.00</td>
<td>0.850</td>
</tr>
<tr>
<td>Honey bees (Apis mellifera)</td>
<td>3.70</td>
<td>0.084</td>
</tr>
<tr>
<td>Control vs. G. clarum</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Control vs. G. custos</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Control vs. R. irregularis</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Control vs. commercial</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mixture vs. single</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Bumble bees (Bombus spp.)</td>
<td>11.20</td>
<td>0.048</td>
</tr>
<tr>
<td>Control vs. G. clarum</td>
<td>0.03</td>
<td>0.720</td>
</tr>
<tr>
<td>Control vs. G. custos</td>
<td>0.94</td>
<td>0.720</td>
</tr>
<tr>
<td>Control vs. R. irregularis</td>
<td>2.36</td>
<td>0.075</td>
</tr>
<tr>
<td>Control vs. commercial</td>
<td>0.94</td>
<td>0.720</td>
</tr>
<tr>
<td>Mixture vs. single</td>
<td>1.01</td>
<td>0.720</td>
</tr>
<tr>
<td>Lepidoptera</td>
<td>13.52</td>
<td>0.001</td>
</tr>
<tr>
<td>Control vs. G. clarum</td>
<td>1.98</td>
<td>0.144</td>
</tr>
<tr>
<td>Control vs. G. custos</td>
<td>0.66</td>
<td>0.100</td>
</tr>
<tr>
<td>Control vs. R. irregularis</td>
<td>0.72</td>
<td>0.701</td>
</tr>
<tr>
<td>Control vs. commerial</td>
<td>1.15</td>
<td>0.515</td>
</tr>
<tr>
<td>Mixture vs. single</td>
<td>2.12</td>
<td>0.135</td>
</tr>
<tr>
<td>Hymenoptera</td>
<td>5.79</td>
<td>0.327</td>
</tr>
<tr>
<td>Apidae</td>
<td>2.45</td>
<td>0.784</td>
</tr>
</tbody>
</table>

Tests of overall effect of AMF treatment are χ² tests; when significant, contrasts used Wald z tests with P-values adjusted for multiple comparisons using the Westfall method (see Analysis). Notes: d.f. = 5 for all χ² tests, d.f. = 1 for all Wald z tests. Bold indicates results with P < 0.05.
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**FIGURE 3** | Effects of AMF inoculation treatments on (A) bumble bee visits per plant, (B) Lepidoptera visits per plant, (C) honey bee probes per flower, and (D) Lepidoptera probes per flower. Values are fitted means ± 1 SE. Asterisks indicates significant differences based on a priori contrasts between non-mycorrhizal control and each single species inoculum (G. clarum, G. custos, R. irregularis, and commercial). Double dagger indicates significant difference based on a priori contrast between the mixed inoculum and its component single-species inoculum.

flowers on plants inoculated with G. clarum or the three species mixture, although only the mixture vs. single species contrast was significant (Figure 3D).

**HERBIVORY**

Arbuscular mycorrhizal fungi significantly affected herbivore damage (Table 4, Figure 4), a result that was likely driven by higher probability of damage to mixed inoculum and commercial inoculum plants. Date was also significant ($\chi^2 = 96.00, P < 0.001$), because herbivore damage was much more prevalent on the earlier survey date. However, after adjusting for multiple comparisons, no AMF treatment contrasts were significant.

**DISCUSSION**

Species interactions can be very context-dependent, and outcomes will vary depending on various biotic and abiotic factors. This is evident in natural systems as well as in agroecosystems (Tscharntke et al., 2008). In agricultural fields, mycorrhizal and other symbioses may modify a range of plant traits that alter the nature or frequency of plant–insect interactions important for plant reproduction. This can happen via direct effects on plant growth, nutrition, and other traits. Here, we found that associations between plants and AMF influenced aboveground plant

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**Table 4** | Results of GLMM analyses of AMF treatment effects on herbivory.

<table>
<thead>
<tr>
<th></th>
<th>$\chi^2$</th>
<th>Wald z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbivory</td>
<td>12.49</td>
<td>0.029</td>
<td></td>
</tr>
<tr>
<td>Control vs. G. clarum</td>
<td>0.53</td>
<td>0.820</td>
<td></td>
</tr>
<tr>
<td>Control vs. G. custos</td>
<td>1.62</td>
<td>0.247</td>
<td></td>
</tr>
<tr>
<td>Control vs. R. irregularis</td>
<td>0.13</td>
<td>0.896</td>
<td></td>
</tr>
<tr>
<td>Control vs. commercial</td>
<td>1.95</td>
<td>0.158</td>
<td></td>
</tr>
<tr>
<td>Mixture vs. single</td>
<td>2.23</td>
<td>0.107</td>
<td></td>
</tr>
</tbody>
</table>

Notes: d.f. = 5 for $\chi^2$ test, d.f. = 1 for all Wald z tests. Bold indicates results with $P < 0.05$. 

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This page contains a figure and a table related to the study on arbuscular mycorrhizae and plant–insect interactions. The figure illustrates the effects of AMF inoculation treatments on bumble bee visits, Lepidoptera visits, honey bee probes, and Lepidoptera probes. The table provides results of GLMM analyses for AMF treatment effects on herbivory, indicating significant differences in herbivory damage across different inoculation treatments.
interactions with both pollinators and herbivores and that these effects differed among both AMF and insect species, highlighting the context-dependent nature of these interactions. Colonization varied significantly among AMF treatments, with the highest colonization (fungal structures present in >54% of root length on average) by the mix treatment containing three AMF species. This level is greater than the average colonization by any of the component species alone and greater than the sum of these single species, suggesting there may be synergistic interactions that benefit the fungi when multiple species are present. The lowest colonization, other than the AMF-free control, was by R. irregularis. This was much lower than the commercial inoculum, also a strain of R. irregularis, illustrating the wide variation in colonization potential possible among even taxa categorized as conspecific (Stockinger et al., 2009; Roger et al., 2013).

POLLINATION

Inoculation by different AMF species influenced the behavior of several taxonomic groups of insect pollinators. Honey bees, bumble bees, and Lepidoptera behavior varied with inoculation treatment, and the patterns of visitation and flower probing differed among these groups. Differences in visitation to plants by bumble bees was driven by apparent greater preference for plants inoculated with R. irregularis, although this contrast was marginally significant after adjustment. Similarly, there was a trend toward greater preference by Lepidoptera for plants inoculated with G. clarum. While the decision to begin foraging on a less-rewarding plant may be terminated before all flowers have been visited. We found that the proportion of flowers probed by both honey bees and Lepidoptera varied with AMF inoculation treatment. Honey bee flower probing rates were significantly reduced on plants that had been inoculated with single species of AMF compared to non-mycorrhizal controls; this is surprising given that AMF usually increase floral reward and pollinator preference (Gange and Smith, 2003; Gange et al., 2005; Aguilar-Chama and Garvera, 2012). Lepidoptera flower probes were significantly greater for mixture plants than single species inocula plants combined, suggesting that the three AMF had interactive effects on floral traits that increased Lepidoptera preference.

Previous studies have found that the effects of AMF on pollinator behavior differ among plant species and pollinator taxa. Gange and Smith (2005) compared pollinator visitation to mycorrhizal and non-mycorrhizal individuals of three plant species. Although AMF generally increased visitation the effect differed with specific combinations of pollinator taxa and plant species, with increased Hymenoptera visitation to two species and Diptera visitation to the other species. Similarly, we show that visitation or flower probes varied by taxa (bumble bees, honey bees, and Lepidoptera) and inoculum type. This suggests that AMF species or strains alter plant traits in different ways, and that these pollinator taxa differ in their response to these traits. In a study that manipulated two AMF species, including R. irregularis, pollinator visitation increased with both AMF, although only a subset of the pollinator community was examined (Wolfe et al., 2005). Of the few prior experiments on AMF effects on pollinator behavior, only one (Varga and Kytöviita, 2010) both manipulated AMF species identity and examined multiple pollinator taxa, as we did here. Interestingly, they found reduced Syrphidae visitation to female Geranium sylvaticum (Geraniaceae) when plants were inoculated with one species of AMF, compared to control and the other AMF species. They also showed reduced visitation by small Hymenoptera to plants inoculated with the other species. Syrphidae and small Hymenoptera (here, Halictidae) were also common visitors in our experiment, but we found no effects of AMF inoculation on visitation or flowers probed. Taken together, these results indicate that different AMF species likely have distinct effects on floral traits and that pollinators have taxa-specific responses to these trait changes.

Pollinators responded to AMF treatments, despite the lack of treatment effects on the floral traits we measured. Gange and Smith (2005) attributed increased pollinator visitation on mycorrhizal plants to greater inflorescence number or size for two Asteraceae species. However, our AMF treatments did not affect flower number or size, which contrasts with many studies that find increased flower production in plants associating with AMF (Beyla and Kneze, 1999; Perrar et al., 2007; Varga and Kytöviita, 2010). In previous work R. irregularis increased male flower production and flower diameter in C. sativus, although the effect on flower size was eliminated by addition of methyl jasmonate (Kiers et al., 2010). Herbivore attack triggers jasmonic acid responses in plants (Farmer et al., 2003), so insect herbivory on our plants in the field may have erased any positive effects of AMF on floral traits. However, herbivore attack was not significantly correlated with total flower production or male flower size (data not shown). Nectar production and composition and floral volatiles can also
have profound effects on pollinator behavior (Schemske and Bradshaw, 1999; Dudareva and Pichersky, 2006; Adler, 2007), but were not measured in this study. Effects of AMF on nectar quantity and quality vary among plant species (Tilman and Smith, 2005; Becklin et al., 2011), and AMF use of plant photosynthates may reduce plant carbohydrate availability for nectar (Laird and Addicott, 2007). Nectar production in C. sativus, like flower size, was also reduced by methyl jasmonate application (Kiers et al., 2010), so herbivory may interact with mycorrhizal status to affect nectar. Floral scent from volatile production affects pollinator attraction, and experimental elimination of soil fungal communities altered volatile production in Polemonium viscosum (Becklin et al., 2011). However, inoculation with commercial AMF or farm AMF controls had no effect on C. sativus volatiles compared with non-mycorrhizal controls (Barber et al. unpublished data), suggesting that this trait may not explain the indirect effects of AMF on pollinators observed here.

HERBIVORY
Mycorrhizal treatment significantly affected the probability of herbivore damage to leaves, with probability of attack varying from 0.3 in plants inoculated with G. clarum to nearly 0.6 in plants with a mixture of AMF species. Control plant herbivory was intermediate, so individual treatments did not differ significantly from control (Figure 4). Inoculation affected leaf nutrient content, but surprisingly not P or N, the nutrients that are most frequently studied in plant–AMF research. Rather, commercial AMF inoculation significantly increased leaf K and Na content relative to non-mycorrhizal plants, although the increase in K was modest. Recent work has emphasized the potential importance of less-studied elements that exist in both organic molecules and ionic forms, but are essential to herbivores (Behmer and Joern, 2012; Joern et al., 2012). Sodium can be limiting for insect herbivores because it occurs in low concentration in plant tissues (Kaspari et al., 2008; Behmer and Joern, 2012; Chavarria Pizarro et al., 2012), and potassium was also identified as a predictor of insect herbivore abundance (Joern et al., 2012). If AMF alter plant concentrations of these elements (in organic or inorganic forms) that are important to insect nutrition, it may provide an additional mechanism of indirect mycorrhizal effects on insect herbivore preference and performance. Future work should address whether the magnitudes of these differences in elemental content (20–50 ppm Na, 1–2 ppt K) are sufficient to influence insect herbivore preference or performance.

Treatment effects on herbivory could also be caused by AMF influences on plant defenses. Colonization of plant roots by AMF is thought to induce both local and systemic responses that allow the plant to respond more rapidly or efficiently to attack by herbivores or pathogens (Jung et al., 2012). Mycorrhization increased induced defenses against a generalist herbivore (Spodoptera exigua) in C. sativus, with herbivores consuming more leaf tissue on induced mycorrhizal plants without increasing their biomass (Barber, 2013). Given this finding, we would expect reduced herbivory on inoculated plants compared to control plants in this experiment, but instead we found lower herbivory on control plants (Figure 4). This may in part be explained by the dominant wild herbivore in this agroecosystem, Acalymma vittatum (striped cucumber beetle), a specialist that responds positively to cucurbitacins, the chemical defensive chemicals in Cucumis and its relatives (Metcalfe et al., 1980). The role of AMF in inducing plant defenses may be more important for generalist herbivores than specialists. This hypothesis is supported by a meta-analysis of AMF–herbivore experiments that found more negative effects of mycorrhizae on generalist chewing herbivores than on specialists (Koricheva et al., 2009).

CONCLUSION
The outcomes of plant–AMF interactions have historically focused on the direct effects of the fungi on plants, such as plant growth or nutrient content. However, plant growth and fitness are also influenced by community members, whose interactions may be modified by AMF-driven changes in plant traits. Here we show that colonization by different AMF species has consequences for pollinator visitation and herbivory in an agroecosystem, but these effects vary with both AMF and insect identity. For AMF–plant–pollinator interactions, future work should focus on the multiple floral traits that can mediate pollination, including how different AMF species (or AMF communities from different ecological contexts) influence floral traits like visual cues, nectar production and composition, and floral scent. A more detailed understanding of AMF effects on these flower traits will allow better predictions of pollinator responses based on the floral signals used by different pollinator taxa. Similarly, understanding AMF effects on herbivory will require experiments that measure plant nutrients and chemical defenses in a field setting or controlled, but ecologically realistic, laboratory conditions. Our results demonstrate the potential for above- and belowground communities to interact in complex ways via species-specific responses of insects and their effects on plant fitness. Thus, even in relatively simple agroecosystems, diversity may provide an important buffer maintaining critical species interactions.

ACKNOWLEDGMENTS
We are grateful to the assistants who worked on this project, especially J. Kelly, Z. Dowling, C. Thompson, E. Fitzpatrick, J. Muschinski, K. Nelson, and E. Zyskowski. Thanks also to greenhouse support and the UMass Center for Agriculture staff. This work was supported by USDA NRI 2008-02346 to Lynn S. Adler, E. Toby Kiers, and Ruth V. Hazard, Dutch Science Foundation grants (NWO: meersrood 836.10.001 and vidi 864.10.005) to E. Toby Kiers, and Northern Illinois University to Nicholas A. Barber.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 26 April 2013; accepted: 11 August 2013; published online: 05 September 2013.


This article was submitted to Plant-Microbe Interaction, a section of the journal Frontiers in Plant Science.

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