How do belowground organisms influence plant–pollinator interactions?

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Abstract

Aims
The majority of angiosperms are pollinated by animals, and this interaction is of enormous importance in both agricultural and natural systems. Pollinator behavior is influenced by plants’ floral traits, and these traits may be modified by interactions with other community members. In recent years, knowledge of ecological linkages between above- and belowground organisms has grown tremendously. Soil communities are extremely diverse, and when their interactions with plants influence floral characteristics, they have the potential to alter pollinator attraction and visitation, but plant–pollinator interactions have been neglected in studies of the direct and indirect effects of soil organism–root interactions. Here, we review these belowground interactions, focusing on the effects of nitrogen-fixing bacteria, arbuscular mycorrhizal fungi and root-feeding herbivores, and their effects on floral traits and pollinators. Further, we identify gaps in our knowledge of these indirect effects and recommend promising directions and topics that should be addressed by future research.

Important Findings
Belowground organisms can influence a wide variety of floral traits that are important mediators of pollinator attraction, including the number and size of flowers and nectar and pollen production. Other traits that are known to influence pollinators in some plant species, such as floral volatiles, color and nectar composition, have rarely or never been examined in the context of belowground plant interactions. Despite clear effects on flowers, relatively few studies have measured pollinator responses to belowground interactions. When these indirect effects have been studied, both arbuscular mycorrhizal fungi and root herbivores were found to shift pollinator visitation patterns. Depending on the interaction, these changes may either increase or decrease pollinator attraction. Finally, we discuss future directions for ecological studies that will more fully integrate belowground ecology with pollination biology. We advocate a multilevel approach to these questions to not only document indirect effect pathways between soil interactions and pollination but also identify the mechanisms driving changes in pollinator impacts and the resultant effects on plant fitness. A more thorough understanding of these indirect interactions will advance ecological theory and may inform management strategies in agriculture and conservation biology.

Keywords: aboveground–belowground, pollinator, mycorrhizae, herbivore, floral traits, indirect effects

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INTRODUCTION

Pollinators represent a globally important component of biodiversity in both agricultural and natural terrestrial ecosystems. Recent studies estimate that 87.5% of angiosperms are pollinated by animals (Ollerton et al. 2011) and that over two-thirds of crop species rely on pollinators (Roubik 1995). Given their role in plant reproduction, pollinators can affect plant population dynamics (Ashman et al. 2004) and significantly influence the evolution of plant traits (Mitchell et al. 2009). However, both wild and domesticated pollinators are declining in many areas (Biesmeijer et al. 2006; Colla and Packer 2008; National Research Council of the National Academies 2007; Winfree et al. 2009), raising concerns about reduced pollination services (Aizen et al. 2008). These declines underscore the importance of understanding the ecological factors.
that influence plant–pollinator interactions, particularly if those factors may be affected by human land management or contemporary global change issues such as climate change and exotic species invasions.

Plant–pollinator interactions can be influenced by other community members. For example, leaf herbivores can affect a variety of floral traits that attract pollinators, including the number and size of flowers, nectar production or quality and floral volatiles (Adler 2007; Barber et al. 2012; Ivey and Carr 2005; Kessler and Halitschke 2009; Theis et al. 2009). Such changes in floral traits may reduce pollinator attraction and, consequently, plant fitness (Kessler and Halitschke 2009; Lehtila and Strauss 1997; Mothershead and Marquis 2000; Strauss et al. 1996; Strauss and Murch 2004). For example, wild radish plants that experienced herbivore damage had smaller and fewer flowers, leading to significantly less pollinator visitation (Lehtila and Strauss 1997). In some cases, floral herbivores can even directly prevent or deter pollinator visitation through damage to flowers (McCall and Irwin 2006).

However, studies of the indirect effects of plant interactions on pollination have focused almost exclusively on aboveground communities. In the last decade, there has been rapid growth in research on the ecological connections between above- and belowground communities, as well as wider recognition that interactions between species in the soil can both influence and be affected by aboveground organisms (Bardgett and Wardle 2003, 2010; van der Putten et al. 2009; Wardle et al. 2004). Soil communities can be incredibly diverse, and plant interactions with belowground organisms reflect this diversity (Scheu et al. 2005; Wardle et al. 2004). Soil community effects on plants have been described as occurring through both direct and indirect pathways (Wardle et al. 2004): an indirect pathway that results from activities of the detritus food web that influence nutrient availability to plants and a direct pathway which includes organisms that feed on or form associations with plant roots, such as root herbivores, mycorrhizal fungi and nitrogen-fixing bacteria. Both pathways may influence aboveground interactions between plants and other community members (Bardgett and Wardle 2010).

Although our understanding of the ways these belowground pathways influence aboveground communities has advanced considerably, research that crosses the soil boundary has generally focused on aboveground antagonisms. For example, studies have found that root herbivory may induce chemical defenses in leaves that change folivore preference, performance and effects on the host plant (Bezemer and van Dam 2005; Wäckers and Bezemer 2003). Reviews and meta-analyses have examined how herbivore performance or preference may be affected by soil organisms such as mycorrhizae (Koricheva et al. 2009) and root herbivores (Bezemer and van Dam 2005), and a recent series of articles on aboveground–belowground interactions included only studies on aboveground herbivores and pathogens (Heil 2011). Aboveground mutualisms largely have been ignored in these reviews, despite their potential importance for plant fitness.

Here, we argue that belowground interactions can have indirect effects on plant–pollinator interactions via changes in floral traits and should be incorporated into aboveground–belowground ecological theory. Studying the linkages between belowground interactions and pollination will advance theoretical knowledge of plant ecology and may have important applications in agriculture and conservation biology. For example, from an ecological networks perspective, the indirect interactions between belowground organisms and pollination may be an underappreciated avenue by which soil organisms influence plant community dynamics and succession if they influence the reproductive success of some plant community members over others (Bardgett and Wardle 2010). For the many crop plants that rely on animal pollinators (Roubik 1995), understanding the effects of both antagonistic and beneficial belowground interactions may be useful in designing management strategies that optimize pollination and thus maximize yield (Kremen et al. 2002). Similarly, management of belowground interactions may help conservation biologists to maximize pollination of rare plant species or to decrease reproduction of invasive plant species whose population dynamics are influenced strongly by seed production.

We review experimental studies that have manipulated interactions in the soil and measured floral traits or pollinator interactions. Focusing on the direct pathway, for which there is considerably more research, we demonstrate that belowground organisms commonly influence floral traits that mediate interactions between plants and pollinators. While few studies have yet addressed the actual impacts on pollinator behavior, a majority of those that have show effects on pollinator visitation. Finally, we propose future avenues of study that will extend the existing literature and allow ecologists to more fully integrate the currently separate field of pollination ecology and soil ecology.

**EFFECTS ON FLORAL TRAITS AND POLLINATOR ATTRACTION**

Although soil biodiversity spans all three taxonomic domains of life, research on belowground interactions with plants has concentrated on a limited subset of taxa. In this review, we focus on the three most-studied groups of soil organisms that may influence floral traits and pollinator attraction: nitrogen-fixing bacteria, mycorrhizal fungi and insect root herbivores. All are widespread in terrestrial ecosystems and can have strong impacts on plant growth (Smith and Read 2008; Sprent et al. 1987; van der Putten et al. 2009), but there is considerable variation in the number of studies investigating their effects on floral traits and pollination.

We sought studies that measured floral traits known to correlate with pollinator attraction and behavior (Fig. 1). For example, flower number and size, or inflorescence size, are often positively correlated with pollinator visitation (Buide 2006; Klinkhamer et al. 1989; Robertson and MacNair 1995;
Willmer 2011). Pollinator visitation and pollination quality may increase with nectar volume, sugar content and levels of other constituents such as amino acids (Adler 2000; Willmer 2011). Floral chemistry can also mediate plant–pollinator interactions. Plants produce an enormous variety of volatile organic compounds (VOCs) as attractants (Dudareva and Pichersky 2006), and floral tissues may contain defensive chemical that provide resistance against floral herbivores (McCall and Irwin 2006) while also influencing pollinators (Strauss et al. 2002).

For each focal soil group (nitrogen-fixing bacteria, mycorrhizal fungi and insect root herbivores), we review what effects on floral traits have been found and, when it has been studied, how these affect pollination. We also identify or speculate on the mechanisms underlying these effects.

### Nitrogen-fixing bacteria

Despite the importance of the nitrogen-fixing symbiosis of legumes and other plants in ecosystems worldwide, there has been surprisingly no investigation of the impacts of nitrogen-fixing bacteria on plant–pollinator interactions, although some studies have addressed effects on flower production. For example, in some cultivars of Glycine max (Fabaceae), inoculation with Rhizobia increased inflorescences (Megueni et al. 2006). In one manipulative study, nodules were clipped off to mimic a belowground herbivore of Lupinus nanus (Fabaceae), and this tended to reduce flower number (Rudgers and Hoeksema 2003), although whether this was due entirely to the loss of the bacteria is unclear, as the plants were damaged as well. These positive effects of bacteria on flower production are likely due to their nitrogen-provisioning function. Supplemental nitrogen can increase floral displays and pollinator visitation in plants that do not harbor rhizobial symbionts (Burkle and Irwin 2008; Hoover et al. 2012; Muñoz et al. 2005; Soper Gorden and Adler 2013), and increased nitrogen supply by rhizobial bacteria are expected to have a similar effect. Whether increased floral production increases plant
fitness through pollination services remains to be tested, as no studies have yet addressed this question. However, a study that took advantage of a non-nodulating G. max mutant found that these plants produced pollen with a lower germination rate than the nodulating cultivar (Gwata et al. 2003), suggesting that root symbiont bacteria may increase the success of pollination services when they do occur. Clearly, the implications of these symbiotic relationships for plant–pollinator interactions are poorly understood and await further study.

An additional question concerns the impacts of plant growth-promoting rhizobacteria on non-legumes. A recent study of two cultivated Solanaceae inoculated with *Rhizobium* found that the bacteria colonized their roots and increased both growth and flower production (García-Fraile et al. 2012). The authors attribute these effects to bacterial production of the plant hormone indole acetic acid and compounds that benefit iron nutrition in the plants. If these effects of rhizobacteria on flowers in non-legumes are widespread, it may provide an additional mechanism by which soil bacteria could influence aboveground interactions.

### Mycorrhizal fungi

Most plants form symbiotic associations with soil fungi, and the growth benefits that plants receive from these mycorrhizal associations are well documented, although these effects vary with different fungal species and under differing environmental conditions (Smith and Read 2008). In general, mycorrhizae transfer mineral nutrients or water to plants in exchange for photosynthetically derived carbohydrates, leading to increased plant growth and reproduction (Smith and Read 2008). Mycorrhizal fungi also affect floral traits with potentially important consequences for pollination. Research on floral traits and pollinators has focused on arbuscular mycorrhizae (AMF, members of Glomeromycota) rather than ectomycorrhizae (ECM, within Basidiomycota, Ascomycota and Zygomycota). Some plants that depend on pollinators for reproduction, such as some Rosaceae, associate with both AMF and ECM (Wang and Qiu 2006), indicating that future studies could examine the role of ECM in plant–pollinator interactions. We examine the impacts of AMF on flowers and their pollinators.

Experiments comparing plants with AMF to nonmycorrhizal controls have frequently demonstrated an increase in flower number (Bryla and Koide 1990; Dodd et al. 1983; Gange et al. 2005; Gange and Smith 2005; Koide 2000; Lu and Koide 1994, Poulton et al. 2001a, 2001b, 2002; Perner et al. 2007; Scagel 2004; Schenck and Smith 1982, Varga and Kytöviita 2010; Wolfe et al. 2005), and one study found that flower size was increased by AMF on *Datura stramonium* (Solanaceae) plants that were not light-limited (Aguilar-Chama and Guevara 2012). These increases in floral display are presumably due to greater nutrient supply, particularly phosphorus, to plants. For example, Poulton et al. (2002) manipulated AMF status and soil phosphorus content for *Lycopersicon esculentum* (Solanaceae). Plants grown with AMF in a low-phosphorus environment produced a similar number of flowers compared with nonmycorrhizal plants in high-phosphorus soil, and both of these treatments resulted in more flowers than control (nonmycorrhizal, low-phosphorus) plants. These results support a hypothesized mechanism that AMF colonization can promote flower production and display by increasing plant phosphorus nutrition.

However, increased flowering in response to AMF are not universal, as a few investigators have found that AMF can reduce flower number (Cahill et al. 2008; Ganade and Brown 1997; Nuortila et al. 2004) or have no effect (Barber et al. 2013; Cahill et al. 2008; Gange and Smith 2005; Philip et al. 2001; Schenck and Smith 1982). In two studies where AMF reduced flower number or size, reductions were found only in plant species with limited reliance on AMF (Cahill et al. 2008; Ganade and Brown 1997). In both studies, AMF manipulations were performed on plant communities in a field setting, and in both cases, the authors attributed the results to increased growth and competition from other plants in the community that were more heavily dependent on AMF. When investigators reduced AMF by applying fungicide to field plots, nonmycorrhizal plants benefited at the expense of AMF-dependent species. Thus, for plant species that rely on AMF associations, the presence of mycorrhizae tends to increase floral production, but plant community context may be an important mediator. Additionally, plant flowering responses to nutrient supplementation by AMF may depend on plant investment strategies since plants may allocate phosphorus to vegetative or root growth rather than reproductive structures (Scagel and Schreiner 2006).

The effects of AMF colonization on flower number or size may also depend on plant breeding system. In a gynodioecious plant, AMF increased flower size of hermaphrodite individuals, but not females (Varga and Kytöviita 2010). Similarly, male flower number increased with AMF in monoecious *Cucurbita foetidissima* (Cucurbitaceae) (Pendleton 2000). Colonization by AMF also increased male flower size in monoecious *Cucumis sativus* (Cucurbitaceae), but female size was not measured (Kiers et al. 2010). Such changes may be particularly important since pollinators frequently prefer male or hermaphrodite flowers over female flowers in plants with gender dimorphism (Ashman et al. 2000; Askkainen and Mutikainen 2005; Huang et al. 2006). If AMF consistently boosts male function in plants, we may expect stronger indirect effects on pollinator attraction and services. Researchers measuring pollen have also consistently found increased pollen production and size in plants colonized by AMF (Lau et al. 1995; Philip et al. 2001; Poulton et al. 2001a, 2001b; Varga and Kytöviita 2010), which could further increase attraction of pollen-feeding insects or contribute to male fitness success.

Nectar is another valuable reward that plays an important role in pollinator preference. AMF might be predicted to increase nectar production because mycorrhization improves plant water use efficiency (Kaya et al. 2003; Ruiz-Lozano and Aroca 2010), but their use of plant carbohydrates could reduce...
nectar sugar concentrations (Varga and Kytöviita 2010). In the two studies that measured nectar volume in response to mycorrhizae, AMF increased nectar production (Gange and Smith 2005; Kiers et al. 2010). Gange and Smith (2005) found an increase in sugar content with the presence of AMF in one species examined, but there was no change in two other species. As with many indirect effects, the influence of AMF on nectar volume and constituents is likely context-dependent, varying among locations and with abiotic conditions such as light or carbon availability (Hoover et al. 2012). Low-nutrient (especially low phosphorus) and other stressful conditions may enhance AMF effects on nectar traits. In a comparison of two alpine habitats, fungicide application caused higher nectar sugar content in the site with lower soil nutrients (Becklin et al. 2011). Although this suggests that AMF may reduce nectar sugar, the authors point out that this could be due to pathogenic fungi as well.

Floral VOCs can be important pollinator attractants and are used by pollinators to make choices about flower visitation (Dudareva and Pichersky 2006). Only Becklin et al. (2011) have measured VOCs in response to AMF, finding reduced total VOC production and fewer individual compounds as AMF colonization increased. However, this effect was only observed in one of two study sites, further emphasizing the context dependency of AMF effects on floral traits.

Given the generally positive impacts of AMF colonization on traits associated with pollinator attraction (increased flower number and size, increased pollen and nectar production), AMF may be expected to increase pollinator visitation. In fact, of five studies that have quantified pollinator behavior in response to AMF, three have demonstrated increased pollinator visitation. Wolfe et al. (2005) found increased visits to Chamerion angustifolium (Onagraceae) on plants with AMF, and Gange and Smith (2005) documented similar patterns for all three species of Asteraceae they studied. Of six focal species studied by Cahill et al. (2008), two (both Asteraceae) experienced greater visitation rates with AMF present; one species (Cerastium arvense, Caryophyllaceae) received more visits in the absence of AMF, although again the authors attribute this to reduced competition from other community members that associate more strongly with AMF, as discussed above.

However, experimental designs that manipulate multiple AMF species and measure responses of multiple pollinator species reveal more complex interactions. Varga and Kytöviita (2010) inoculated plants with two AMF species and found that one species decreased visits by non-bumble bee hymenopterans; with the other species of AMF and other insects, there were no effects. Similarly, an experiment using four different AMF species/strains found variation in attraction of bumble bees, honey bees and Lepidoptera (Barber et al. 2013). Bumble bees and Lepidoptera tended to prefer plants inoculated with different AMF species, while honey bees probed more flowers on AMF-free control plants. The mechanisms for these changes in pollinator behavior are unknown, as visitation patterns were not associated with changes in flower number or size in either experiment.

It is important to note that only one of the studies discussed here (Becklin et al. 2011) has examined if changes in pollinator visitation due to the presence of AMF affect plant fitness. Although increases in fruit or seed production on AMF-colonized plants are documented (Gange and Smith 2005; Poulton et al. 2002), this increase may be due to either the direct effects of mycorrhizal nutrient uptake or changes in pollination (or by the combined effects of both). Experiments such as pollen receipt manipulations are necessary to understand the mechanism behind these patterns. Becklin et al. (2011) combined a hand pollination treatment with a fungicide treatment and showed that, while both mycorrhizal and nonmycorrhizal plants were pollen-limited, AMF did not affect pollen receipt, and at one site fungicide treatment increased seed production, although this may have been due to suppression of pathogenic fungi rather than AMF. Overall, results suggest that, while the presence of AMF tends to increase floral traits that may attract pollinators and increase pollination visitation in some cases, these effects and the impact on plant fitness are still poorly understood and likely depend in part on the fungal community colonizing the plant and the species of pollinator involved.

### Root herbivores

Although the vast majority of research on plant–herbivore interactions has examined herbivores that feed on aboveground plant tissues (van der Putten et al. 2001), root herbivores have been shown to have significant effects on plant growth and fitness (Barber et al. 2011; Strong et al. 1995; Zvereva and Kozlov 2012). As with plant–AMF interactions, if belowground herbivores influence floral traits, they have the potential to alter plant fitness through changes in plant–pollinator interactions.

Herbivore damage to aboveground tissues often reduces flower production (e.g. Lehtila and Strauss 1997; Quesada et al. 1995), but root herbivores have less consistent effects on flowers. Of six studies that manipulated root herbivory, four found no impact on flower number (Hladun and Adler 2009; Poveda et al. 2003, 2005; Rudgers and Hoeksema 2003). Two experiments documented reductions in flower production following root damage: the first used artificial root damage and found a decrease in total flower number (Reichman and Smith 1991), and the second showed that root herbivory by insects in a monococious plant reduced female, but not male, flowers (Barber et al. 2011). In both of these studies, root herbivory also had strong overall effects on plant biomass, whereas in studies where root herbivory did not influence flower production, plant biomass was unaffected (Poveda et al. 2003, 2005). This suggests that, at least in some cases, root herbivory by insects may impact flower number through removing or preventing uptake of resources needed for growth and flower production, leading to fewer flowers or fewer of the more expensive female flowers.
Root herbivores may affect floral characteristics, but few studies have examined these effects. Flower size was unchanged by root herbivory in two Cucurbitaceae species (Barber et al. 2011; Hladun and Adler 2009) and in one Brassicaceae species (Poveda et al. 2005). However, Masters et al. (2001) used soil insecticides to show that root herbivores reduced flower head size in a thistle (Asteraceae). Given that Cirsium flower heads are composed of multiple florets, this result may be more akin to the reduction in flower number discussed above. Only two studies have examined nectar responses, finding that root herbivory did not affect nectar production (Poveda et al. 2005) or sugar content (Hladun and Adler 2009). It is evident that there is still a large gap in our knowledge about how root herbivores affect flowers. Not only is the current body of literature inconclusive, but many traits (e.g., floral defenses, VOCs, nectar constituents and flower color or shape) have not been studied in response to root herbivores at all.

However, despite the weakly negative or neutral effects on flowers and floral traits, three of the four published studies that have measured pollinator behavior in the presence and absence of root herbivory found that root damage paradoxically increased pollinator attraction. In two separate experiments, Poveda et al. (2003, 2005) showed that honey bee (Apis mellifera) visitation increased on wild mustard (Sinapis arvensis, Brassicaceae) when they were attacked by root herbivores. In cucumber (Cucumis sativus, Cucurbitaceae), honey bees also preferred plants with enhanced root herbivory, spending longer times probing individual flowers than on plants where root herbivory was not increased (Barber et al. 2011). However, this effect was absent in a similar study of the confamilial Cucurbita moschata (Hladun and Adler 2009). The mechanism of increased attraction in these studies is unknown, as none of these studies found changes in flower number, size or nectar production as a result of root herbivory. More subtle floral characteristics involved in pollinator choice, such as scent (Raguso 2008) or nectar constituents including sugar composition (Herrera et al. 2006), amino acids (Gardener and Gillman 2002) or toxins (Adler 2000), should be carefully examined following root damage to determine how these traits influence pollinator behavior.

**FUTURE DIRECTIONS**

The examples discussed above demonstrate that interactions between plants and soil organisms have the potential to modify plant–pollinator interactions and, in some cases, have been shown to affect pollinator visitation. However, significant gaps remain in our understanding of these multispecies interactions, inhibiting our ability to fully integrate belowground ecology with pollination ecology. It is clear that ecologists need to carry out more experiments actually measuring pollinator responses to belowground interactions (Fig. 1), but these studies should be designed to elucidate the trait-based mechanisms and consequences of changes in pollinator behaviors for plant fitness. In this section, we identify pressing needs in this field and suggest approaches that will fill these knowledge gaps. We structure this discussion around a four-level theoretical framework (Fig. 1) of the interacting organisms: belowground organisms, plant floral traits, pollinators and plant fitness. There are opportunities at all of these levels to incorporate manipulations or measurements that will result in a more comprehensive understanding of the importance of these interactions.

**Belowground organisms**

As highlighted earlier, the taxonomic range of belowground organisms investigated for effects on floral traits and pollinators is limited. Nitrogen-fixing bacteria are known to affect flower production, but they have not been incorporated into pollination studies. Other soil organisms, including root pathogens, nematodes and ECM, have never been studied in the context of their effects on pollinators. These unexplored soil organisms may alter floral characteristics similarly to AMF and root herbivores discussed above. For instance, root-knot nematodes (Meloidogyne spp.) have been studied in a wide variety of agricultural and horticultural crops, where they can delay flowering and reduce flower production (Hesamedin 2006; Wang and McSorley 2005). Fungal and bacterial soil pathogens that infect plant roots may have similar effects: pathogenic fungi reduced flower size in Hydrophyllum appendiculatum (Hydrophyllaceae), but the impact on pollination was not assessed (Marr and Marshall 2006). There is great potential for research on how the systemic effects of plant pathogens, nematodes, nitrogen-fixing bacteria and ECM may influence floral traits and pollination services.

The examples discussed in this review have generally focused on simple indirect pathways in which a belowground organism alters floral traits with consequences (measured or potential) for pollinator attraction. However, natural communities tend to be much more complex, and other multispecies interaction pathways could affect belowground organism–plant–pollinator interactions. For example, establishment of AMF in roots can prime plants’ induced defense responses (Pozo and Azcón-Aguilar 2007), which may diminish the impacts of subsequent belowground herbivores. The direct measurement of interactions between belowground organisms is relatively rare. We do not know how frequently belowground interactions modify each other’s impacts on plants, including floral traits and interactions with other plant–insect interactions such as pollinators. Future work should include studies that integrate several belowground interactions and their effects on floral traits and pollination.

Lastly, in their review of mycorrhizae–plant–insect interactions, Gehring and Bennett (2009) drew attention to the widespread use of single-species inoculation experiments despite knowledge that different fungal species and strains may differ in the magnitude, or even the direction, of their effects on plants and insects. For example, a study that examined three fungi in different genera found significant differences in their...
effects on plant growth and plant responses to herbivory; one species increased plant tolerance to herbivory while another decreased tolerance (Bennett and Bever 2007). The two studies that manipulated multiple AMF (Barber et al. 2013; Varga and Kyttöviita 2010) demonstrated species- or strain-specific effects of fungi on pollinators. For this reason, future work on the role of AMF in plant–pollinator interactions will require testing across multiple AMF taxa or inoculating with entire AMF communities (Gehring and Bennett 2009). A similar approach to studying different nitrogen-fixing bacteria would also be informative. Such community-inoculation approaches may be most useful in understanding the influences of global change drivers such as climate change or land-cover alterations that may cause shifts in the composition of entire belowground microbial communities (Bellgard and Williams 2011; Fitter et al. 2000). Parallel issues arise in studies of root herbivory effects because plants can be attacked by multiple species of belowground herbivores, yet few studies look at the effects more than one belowground herbivore on plants (Erwin et al. 2013).

Floral traits
We highlighted several plant traits that are potentially important for pollinator attraction and pollination services. A thorough understanding of how belowground organisms influence plant–pollinator interactions will require more comprehensive studies of plant traits, beyond the most commonly studied characteristics, like flower number and size. These include nectar constituents such as amino acids, pathogens and carbohydrate ratios (Willmer 2011), as well as floral and nectar defenses (Adler and Irwin 2005; McCall and Karban 2006) and VOCs (Pichersky and Gershenzon 2002). Positive and negative belowground interactions may also influence plant morphology such as plant height (another important predictor of pollinator attraction; Gómez 2003) or floral morphology, including flower color and symmetry (Hoballah et al. 2007; Irwin and Strauss 2005; Møller and Eriksson 1995; Wignall et al. 2006).

Both AMF and root herbivores can also influence aboveground induced defense responses in leaves (Erb et al. 2008; Kaplan et al. 2008; Kempel et al. 2010), but whether these effects extend to floral tissues in the same way that leaf herbivory can (Adler et al. 2006; McCall and Karban 2006) is unknown. Levels of chemical defenses in petals, anthers and nectar are often directly proportional to those in leaves (Adler 2000), suggesting that the induced defense response initiated by belowground interactions may extend to floral tissues. Since defenses in flower tissue often deter or even harm pollinators (Adler and Irwin 2005; Crane 1977; Gegear et al. 2007), this may lead to reduced pollinator visitation or decreased pollination success (Adler and Irwin 2012). Thus, investigation into whether floral defense levels are altered by belowground interactions is warranted.

Additionally, indirect effects of belowground organisms could extend to aboveground floral antagonists if plant traits that mediate the behavior of these enemies are affected, especially since floral antagonists often target the same traits as pollinators. Nectar larcenists (Becklin et al. 2011) and florivores cause damage that can decrease attractiveness of flowers to pollinators, and their activity can directly interfere with pollinator access to flowers (McCarrn and Irwin 2006). Wäckers and Bezemer (2003) showed that artificial and natural root herbivory dramatically increased extrafloral nectaries (EFN) production in cotton (Gossypium herbaceum, Malvaceae). If EFN alters the foraging behaviors of ants by reducing foraging on floral nectar sources (Wagner and Kay 2002), it might reduce nectar robbing by ants or ant aggressiveness towards pollinators, thereby increasing pollination. Alternatively, increased EFNs could recruit more ants to a plant, thus increasing ant-pollinator antagonisms. Variation in nectar composition may influence floral antagonists in addition to ‘honest’ pollinators (Adler and Irwin 2005; Kessler et al. 2008), further modifying effects on plant fitness through direct damage or indirectly by changing pollinator preference (Irwin et al. 2010; McCall and Irwin 2006). However, while there are few studies of how belowground organisms affect pollinators, there are none that investigate how belowground organisms affect floral antagonists, suggesting another important avenue for future research.

Finally, flowering phenology represents a plant trait of particular contemporary concern. Phenological shifts due to climate change may alter plant–pollinator interactions, but they are difficult to predict given the variability in responses of plants and pollinators (Hegland et al. 2009; Memmott et al. 2007). AMF can increase flowering duration (Bryla and Koide 1990; Lu and Koide 1994; Sun et al. 2008), while root herbivory may shorten it (Nötztold et al. 1997), with the potential for conflicting selection pressures. If these effects of AMF or belowground herbivores interact with earlier or warmer spring temperatures to influence flowering phenology, it could have important demographic consequences for plants by magnifying or attenuating phenological shifts. The outcomes of phenological changes in plants will often depend on the direction and magnitude of concomitant shifts in pollinators. For example, a longer flowering period only benefits plants if pollinators are present during this extended time. In fact, there is some evidence that plant species that are constrained by pollinator reliance may not be able to adapt as quickly to seasonality changes as a result of climate change (Rafferty and Ivie 2011). Nonetheless, increased flowering duration could help buffer against phenological mismatches that may occur under climate change (Memmott et al. 2007), although further research is still needed in this area.

Pollinators
Pollinators vary in their effectiveness and the breadth of plant species they visit (Gange and Smith 2005). Further, different pollinator species may show unique responses to variation in plant traits (Varga and Kyttöviita 2010). This underscores
the importance of an inclusive community-level view when examining pollinator responses and the necessity of measuring pollinator visitation at a higher resolution than just the total number of insect visits to a plant (Barber et al. 2013). Understanding interspecific differences among pollinators may become especially important if climate change shifts the ranges and phenologies of both plants and their pollinators (Gordo and Sanz 2005; Memmott et al. 2007), shuffling community composition.

**Plant fitness**

Despite extensive research on the direct effects of belowground organisms on plants, the consequences of these effects for plant fitness through changes in pollination services are almost entirely unknown. This is an important gap, considering the role of pollination in plant fitness, as well as how its variation in pollination by insects can drive selection for plant traits (Bronstein et al. 2006; Fenster et al. 2004). Experiments like those reviewed above should be extended to evaluate plant fitness outcomes such as seed production or germination success. Factorial experiments combining manipulations of belowground organisms with pollen receipt manipulations can reveal if soil interaction effects on plant fitness are due to changes in pollination services or direct effects of the interaction (Knight et al. 2006).

**CONCLUSION**

The studies we reviewed here clearly demonstrate that belowground interactions between plants’ roots and other soil organisms may have important consequences for aboveground plant–pollinator interactions. Through changes in plant traits that can mediate attraction and behavior of animal pollinators, belowground organisms such as nitrogen-fixing bacteria, mycorrhizal fungi and root-feeding herbivores can play an underappreciated role in an important component of plant fitness and population dynamics. Our suggestions for future avenues of research represent broad topics that can further integrate and advance our knowledge of aboveground–belowground linkages. A community-level view that considers multiple species and interactions and attempts to identify trait-based mechanisms provides the best approach to this topic. Given widespread concerns about pollinator losses and drastically changing climate, this understanding takes on particular importance, as it may inform management decisions necessary to maintain biodiversity and the important services that pollinators provide.

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**REFERENCES**


