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The presence of aggressive ants is associated with fewer insect visits to and altered microbe communities in coffee flowers

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Abstract

The process of dispersal can shape ecological communities, but its influence is thought to be small compared to the effects of environmental variation or direct species interactions, particularly for microbial communities. Ants can influence movement patterns of insects and the microbes they vector, potentially affecting microbial establishment on plants, including in agroecosystems. Here, we examine how the presence of aggressive ants, which can influence floral visitation by bees and other pollinators, shapes the community composition of bacteria and fungi on coffee flowers in farms that differ in shade management intensity. We hypothesized that the presence of aggressive ants should reduce the frequency and diversity of floral visitors. Finally, we predicted that the effects of ants should be stronger in the low-shade farm, which has a less diverse community of floral visitors. We sampled microbial communities from nectar and pistils of coffee flowers near and far from nests of the aggressive ant *Azteca sericeasur* across two farms that vary in shade management and diversity of floral visitors. Bacterial and fungal community composition was characterized using Illumina sequencing of the 16S and ITS regions of the rRNA gene. Consistent with our expectation, *Azteca* presence was associated with a decrease in the number and diversity of visitors, visit duration and number of flowers visited. *Azteca* presence influenced microbial communities, but effects differed between farms. *Azteca* nests were associated with higher bacterial diversity in both farms, but the difference between flowers on trees with and without *Azteca* was greater in the high-shade farm. *Azteca* nests were associated with higher fungal diversity in the high-shade farm, but not the low-shade farm. In addition, the presence of ants was strongly associated with species composition of fungi and bacteria in flowers, but differentiation between ant and no-ant communities was greater in the low-shade farm. Specific operational taxonomic units (OTUs) were differentially associated with the presence of ants. We conclude that indirect interactions that influence dispersal may have large effects on microbial community composition, particularly in ephemeral microbial communities.

Keywords: *Azteca*; Community assembly; *Coffea arabica*; Chiapas; Mexico; Dispersal; Microbial ecology; Microbiome; Nectar chemistry

Introduction

Environmental constraints and direct species interactions are traditionally considered the main determinants of species membership in ecological communities. However, dispersal-driven processes are increasingly recognized for their role in

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community patterns (Ricklefs 1987; Hubbell 2001; Leibold et al. 2004). Theory and a growing body of evidence suggest that dispersal should play an important role in determining community structure and function through its effects on species presence, arrival order, and mass effects (Fukami 2015; Mittelbach & Schemske 2015). In microbial communities, environmental constraints and species interactions (e.g. competition and trophic interactions) are typically thought to structure communities (Lindström & Langenheder 2012; McFall-Ngai et al. 2013), while dispersal has been thought to play a smaller role, because microorganisms often have large effective dispersal distances and are able to survive in a dormant state for long time periods (Becking 1934; Finlay 2002). However, species-area and distance-decay relationships for microorganisms suggest that dispersal limitation may influence community composition (Green & Bohannan 2006; Zhou, Kang, Schadt, & Garten 2008; Vannette, Leopold, & Fukami 2016). In addition, many microorganisms rely on larger animals for transportation (known as phoresis) to specialized habitats. For example, fungal ascospores are carried by bark beetles among tree habitats (Moser, Perry, Bridges, & Yin 1995), and yeasts that grow in ephemeral habitats like floral nectar or rotting fruits often rely on phoresis by pollinators or flies to disperse among these habitats (Starmer, Barker, Phaff, & Fogleman 1986; Starmer & Fogleman 1986; Belisle, Peay, & Fukami 2012). The consequences of variation in dispersal for microbial community composition are not well-understood, particularly when microorganisms rely on phoretic dispersal (but see Ushio et al. 2014; Mittelbach et al. 2015).

Here, we examine the effect of modified dispersal on the diversity and composition of microbial communities in the flowers of *Coffea arabica* (Rubiaceae). We focus on flowers, because these habitats are ephemeral and initially host little to no culturable microorganisms (e.g. Herrera, Canto, Pozo, & Bazaga 2010; Belisle et al. 2012), and as a result, represent the initial stages of community assembly, where effects of dispersal can be disentangled from other processes. We sampled microbial communities on floral stigmas and in nectar of coffee flowers, which are open between 2–5 days (Jiménez-Castano & Castillo-Zapata 1976; Free 1993). We sampled floral nectar and stigmas because they likely differ in suitability for microbial growth and may host different microbial communities (Junker & Keller 2015). Nectar acts as a strong biological filter, and only a phylogenetically restricted subset of microorganisms with particular adaptations has been found to attain high abundance in this environment (e.g. Herrera et al. 2010; Alvarez-Perez, Herrera, & de Vega 2012). Microbial growth can depend on nectar sugar concentration and composition–which determines water activity–and the concentration of secondary compounds (Vannette & Fukami 2016), including caffeine, which is found in coffee nectar. In addition to the utility of microbial communities for testing ecological theory (Srivastava et al. 2004), nectar-inhabiting microbial communities can also differentially influence plant-pollinator interactions (Herrera, Pozo, & Medrano 2013; Vannette, Gauthier, Fukami 2013; Schaeffer & Irwin 2014), depending on their composition. As a result, characterizing taxonomic contribution and species interactions that contribute to variation in composition may be ecologically important in some cases. Here, we characterize microbial composition in coffee nectar and stigmas, its association with sugar composition of nectar and association with the presence of ants, but do not examine functional consequences for plants or pollinators.

Within coffee agroecosystems, aggressive arboreal ants are dominant ecological players with the potential to influence microbial dispersal both directly and indirectly. Ants can facilitate the dispersal of specific microbes (de Vega & Herrera 2013), including entomopathogenic fungi and fungi that parasitize rust in coffee systems (Philpott 2010; Vandermeer, Perfecto, & Philpott 2010; Jackson, Zemenick, & Huerta 2012) and yeast to floral nectar (de Vega & Herrera 2013). Aggressive ants also influence the abundance, diversity and composition of other insects that may vector microorganisms. Specifically, aggressive ants (e.g., Argentine ants, red imported fire ants, and pavement ants) are renowned for negatively affecting pollination services in many plant species, both by chasing pollinators (bees) away from plants, stealing nectar without pollinating (Inouye 1980), causing mechanical damage to floral tissue, or leaving deterrent scent marks on flowers (Lach 2007; LeVan, Hung, McCann, Ludka, & Holway 2014; Sidhu & Rankin 2016). In coffee agroecosystems, the dominant arboreal ant *Azteca sericeasur* tends scale insects on coffee plants, defends scales and coffee plants from both herbivores and predators, and is aggressive towards a number of other ant species (Vandermeer et al. 2010). Because this ant is generally aggressive towards all insects it encounters, and because other aggressive ant species negatively affect pollinators, we hypothesize that *A. sericeasur* alters pollinator visits to coffee flowers, ultimately influencing diversity and structure of the microbial communities in coffee flowers.

Coffee is grown under varying management intensity, and cultivation conditions may also influence microbial dispersal. At one extreme, coffee is cultivated in the understory of a diverse and dense canopy of shade trees and at the other extreme, coffee is produced under intensive management with minimal shade tree cover (Perfecto, Rice, Greenberg, & Van der Voort 1996; Moguel & Toledo 1999). Shade coffee farms with high vegetation complexity support a higher abundance, richness, and a distinct community composition of many insects, including pollinators and other floral visitors, than do farms with less complex vegetation or without shade (Klein, Steffan-Dewenter, Buchori, & Tscharntke 2002; Armbrecht, Rivera, & Perfecto 2005; Philpott et al. 2008a; Jha & Vandermeer 2010). Changes in the insect species present, and their interactions with one another may also influence the floral microbial community.

In this study, we tested the hypothesis that the presence of aggressive ants influences microbial communities in flowers. We hypothesized that ant presence will (1) reduce floral