COMMUNITY ECOLOGY – ORIGINAL RESEARCH

Bee community preference for an invasive thistle associated with higher pollen protein content

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Abstract

Non-native plant species reliant on insect pollination must attract novel pollinators in their introduced habitat to reproduce. Indeed, pollination services provided by resident floral visitors may contribute to the spread of non-native species, which may then affect the pollination services received by native plants. To determine the mechanisms by which an invasive thistle attracts pollinators in its introduced range, and whether its presence changes the pollinator visitation to native plant species, we compared bee visitation to native plants in the presence or absence of the invader. We experimentally tested the effect of a thistle invasion into a native plant community. We found that the non-native thistle was the most attractive of the plant species to visiting bee species. However, there was no effect of experimental treatment (presence of thistle) on bee abundance or visitation rate (bees per unit floral area per sample) to native plant species. Across 68 bee and 6 plant species, we found a significant correlation between pollen protein content and bee abundance and visitation rate. Thistle pollen also had a similar protein:lipid ratio to legumes, which correlated with bumble bee visitation. The high protein content of the thistle pollen, as compared to four native asters, may allow it to attract pollinators in novel ecosystems, and potentially contribute to its success as an invader. At the same time, this high protein pollen may act as a novel resource to pollinators in the thistle's invaded range.

Keywords Bees · *Carduus acanthoides* · Invasive species · Pollen nutrition · Pollination

Introduction

Non-native plant species have the potential to integrate into communities by providing attractive and nutritious floral resources to pollinators (Vilà et al. 2009), with implications

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for the structure of plant–pollinator interactions (Aizen et al. 2008; Stout and Morales 2009; Valdovinos et al. 2009; Russo et al. 2014). Invasive plants are often generalists in their interactions with pollinators, attracting a large number of species (Bartomeus et al. 2008; Russo et al. 2014, 2016). Moreover, some non-native plants are extremely attractive to resident pollinators in their invaded range (e.g., Lopezaraiza-Mikel et al. 2007), in some cases increasing the abundance of bee visitors to mixed communities of plants by over 300% (Russo et al. 2016). The ability to generally attract large numbers of pollinators is thought to be one mechanism facilitating the invasion of entomophilous non-native species into novel ecosystems (Richardson et al. 2007; Traveset and Richardson 2011) and, in some cases, there is a higher proportion of introduced plant species which depend on pollinators, relative to the proportion of pollinator-dependent native plants (Pyšek et al. 2011). Non-native plant species can also provide resources to resident pollinator species (Gleditsch and Carlo 2011), especially in highly modified agroecosystems (Pywell et al. 2005; Russo et al. 2016) and may even play central, stabilizing roles in plant–pollinator

networks (Stouffer et al. 2014; Russo et al. 2014 but see Kaiser-Bunbury et al. 2017). Non-native plants readily integrate into native plant–pollinator networks (Chittka and Schurkens 2001), suggesting that biotic pollination does not generally provide an effective barrier to invasion (Stout and Tiedeken 2017). Non-native plants may integrate into new communities if they have similar floral traits to native plant species and therefore exploit similar pollinator species (Morales and Traveset 2009; Gibson et al. 2012, but see Vilà et al. 2009; Maruyama et al. 2016). Moreover, in some cases, non-native species have higher flowering densities that can compete with less abundant native species (Seifan et al. 2014), while in others, there does not appear to be a density effect of the non-native species (Sun et al. 2013; Stanley and Stout 2014).

It is possible that invasive plants attract resident pollinators by providing high-quality nutritional resources. Bees are common pollinators, and pollen is the sole protein source for developing offspring in most bee species (Michener 2000) and mounting evidence suggests bees selectively forage to optimize pollen quality. Two bumble bee species (*Bombus impatiens* and *B. terrestris*) regulate their dietary intake to high protein to lipid ratios (Vaudo et al. 2016a) and *B. impatiens* preferentially visits plants with a pollen protein to lipid ratio of 5:1 (Vaudo et al. 2016b). Other studies suggest honey bees (*Apis mellifera*) and other bumble bees (*Bombus* spp.) forage for pollen based on its nutritional content (Cook et al. 2003; Hanley et al. 2008; Ruedenauer et al. 2016), though *A. mellifera* and *Bombus* spp. may base their foraging preferences on different pollen qualities (Leonhardt and Blüthgen 2012). In addition, some specialist bee species (e.g., *Colletes succinctus* group) switch to novel plant hosts with similar pollen nutritional content (Vanderplanck et al. 2017 but see Roulston and Cane, 2000). The quality of foraged pollen can be essential for the development and survival of offspring (Roulston and Cane 2002; Sedivy et al. 2011; Vanderplanck et al. 2014).

While individual bee species can forage selectively for pollen nutritional quality, the factors driving the preference of multispecies bee communities in mixed plant communities remain unknown. Complex floral traits, including the abundance or availability of nectar and pollen resources, may act synergistically with pollen nutritional quality to shape foraging behavior (Rasheed and Harder 1997). Pollinators make foraging decisions in a plant community based on the traits they are able to perceive. Some plants can restrict pollinator visitation with floral morphology (Harder and Thomson 1989), while both chemical and visual cues (e.g., floral display) are used to attract pollinators (Junker and Parachnowitsch 2015). Plants can use chemical tools to advertise nutritional content (e.g., pollen protein, lipid, and carbohydrate content) with volatile compounds (Dobson and Bergström 2000). In addition, the relative attractiveness of species may vary depending on the community context; in

other words, pollinators may actively compare the traits of one flower species with other available flower species (Junker and Parachnowitsch 2015) or visit more abundant and available flower species (Bartomeus 2013; Carvalheiro et al. 2014). Non-native species may therefore attract pollinators by producing more apparent or stronger signals or simply by being more abundant (Bartomeus et al. 2016).

We designed an experiment to better understand how plant traits drive preferential visitation by bees to a non-native species. We focused this study on the invasive thistle, *Carduus acanthoides*, shown to be highly attractive to pollinators in its invaded range (Russo et al. 2016), despite the fact that members of the plant family Asteraceae are generally thought to have poor-quality pollen (Praz et al. 2008). We established communities of five native plant species and then experimentally introduced *C. acanthoides* into a subset of these. Four of the five native species used were asters, confamilial with the invader, to better compare nutritional traits that may vary within plant families. *Carduus acanthoides* was previously shown to be particularly attractive to bumble bees (Russo et al. 2016), thus, we also included a native legume in this study as a nutritional outgroup, because legume pollen has been shown to be preferred forage by bumble bees in some systems, and is sometimes found to have higher protein and lower lipid content than aster pollen (Pywell et al. 2005; Mata 2018). The legume we used here, *Chamaecrista fasciculata*, also has poricidal anthers, indicating it is targeting pollinators that can buzz pollinate, primarily bumble bees in the study region. We then measured the visitation rate of bees to different plant species, as well as plant traits including: number of inflorescences (proxy for the availability and abundance of floral resources), size of floral display, above-ground biomass, and three attributes of pollen nutritional quality (protein, lipid, and carbohydrate concentrations). Our objective was to answer three main questions: (1) is this invasive thistle more attractive than native confamilial species to resident pollinators?; (2) does the attractiveness of the invasive species affect the visitation rate to nearby native species?; (3) which traits of the invasive species allow it to preferentially attract bees?

Materials and methods

Experimental design

Six species of plants, including four native asters (*Helianthus annuus, Gaillardia pulchella, Rudbeckia hirta*, and *Coreopsis tinctoria*), and one native legume (*Chamaecrista fasciculata*) were grown from seed in 30 2 × 2 m plots at the Russell Larson Agricultural Research Center, Pennsylvania State University, PA, USA. These native plant species

were recommended for pollinator attractiveness, but were also chosen because plants in the family Asteraceae have sometimes been found to have low protein, high lipid, pollen [(Genissel et al. 2002), *H. annuus* (Human et al. 2007; Nicolson and Human 2013), *G. pulchella* (Mata 2018)], while some legumes have been shown to have relatively high protein, low lipid, pollen (Vaudo et al. 2016a; Mata 2018). Thus, we wanted to contrast the pollen quality within the aster family to an outgroup with potentially very different pollen quality. All plots were located on the edges of crop fields, and all were separated from one another by more than 100 m. This research farm includes many different crops managed in a variety of ways; for this reason, we used a blocked design to ensure plots within a block experienced a relatively similar background environment (Fig. S1). The research farm was located in the Ridge-and-Valley system of the Appalachian Mountains, and was bordered by conventional farmland (mostly corn and soy), fragmented forest habitat, and an approximately 39,000 ha state forest to the south. To establish the plots, 2 x 2 m patches of grass on the edges of agricultural fields were sprayed with glyphosate following manufacturer's instructions early in April. Three weeks later, each plot was seeded with 7.5 g of each of the native plant species and the plots were then covered in mulch. Because of low germination early in the spring, we reseeded the plots with an additional 7.5 g of each native plant species in late May. Contaminant plants were removed weekly and plots were watered during periods of drought (> 5 consecutive days without rain) throughout the season.

The 30 plots were placed into 6 blocks according to their location and surroundings, generally classified as adjacent to forest fragments, the state forest, or surrounded on all sides by farm fields (Fig. S1), and each of the five plots in each block was randomly assigned an experimental treatment. All experimental treatments concerned the presence of the invasive thistle (*C. acanthoides*): a control with no thistle, two early treatments (high and low intensity), and two late treatments (high and low intensity). We planted the thistles as seeds the previous autumn in a greenhouse and transplanted them to an open field to overwinter outdoors. We transferred rosettes to 5 gallon pots the following spring and watered them daily until their introduction to the experimental plots. We planted four individual thistles in the low-intensity treatment and eight individual thistles in the high-intensity treatment on the plot edges. Though *C. acanthoides* can sometimes reach high population densities, it does not compete well with other plant species in undisturbed habitat (Jongejans et al. 2007). Thus, flowering at lower densities is often found, such as the ones explored here, along with other species of flowering plants. Moreover, our objective was to provide a relatively similar abundance of each plant species, in which visiting insects could exhibit a preference. Relative to the native species in this study, the thistle produced both an intermediate floral display (Fig. S2) and above-ground biomass (Table S2).

For the early treatment, we planted the thistles around the plot before sampling began in July. At this stage, the thistles were bolting but not yet flowering and the remaining species in the plots were also not yet in flower. The thistle was the first species to bloom in July, followed by *C. tinctoria, C. fasciculata, H. annuus,* and *R. hirta,* in order. *Gaillardia pulchella* began flowering last in August (Fig. S2). The thistle individuals for the late treatment were planted while already flowering in the plots on 1 August for the remainder of sampling. This species generally flowers from early July until the first frost in Pennsylvania. We tested the intensity (Russo et al. 2014) and timing (Russo et al. 2013) of invasion, because previous research indicated they might impact the integration of invasive species with plant–pollinator communities (Russo et al. 2019).

Beginning in July, we checked each plot in the morning and afternoon every week. Sampling only occurred on plants in flower; as species and plots began flowering at different times, this resulted in variable sampling intensity among the different species (Table S1, Fig. S3). On each sampling date throughout the flowering season (July and August), each plant species flowering in the plot was sampled for 5 min by insect vacuum and all insect visitors were collected. The order in which we sampled the different flowering plant species was randomized on each visit. The insect vacuum was a modified handheld vacuum with low power, which was essential to prevent damage to the flowering plants in the experimental study; a hand net would have damaged the plants and made it impossible to track plant health over the course of the season. The insect vacuum was only turned on when an insect contacted the reproductive parts of the flower for more than one second, and the sound of the vacuum did not appear to alter pollinator behavior (*pers. obs,* L. Russo). These collections provided data for the number of insects visiting the inflorescences of a given plant species over a 5-min sample, for the duration of its flowering period. At each sampling date, we also recorded the number of inflorescences of each species. This allowed us to measure the abundance of the floral resources provided by each species at each sampling event. We also obtained an average diameter of the inflorescence of each species and used the number of individual inflorescences multiplied by the average size of the floral unit to calculate the total size of the floral display for each sample. At the end of the season, we conducted an above-ground destructive census. We dried all above-ground biomass in a drying oven for a minimum of 48 h and weighed the dry biomass of each species (Table S2).

We spent a total of 58.3 h (700 5-min samples) sampling insects during the course of the field season, divided unevenly between the plant species due to differences in the timing of flowering and unequal flowering across the plots

(Table S1, Fig. S3). Sampling was evenly divided between morning (9 am–12 pm) and afternoon (12 pm–4 pm) to ensure we accounted for variation in visitation rates during different times of the day (Vaudo et al. 2014). Some additional sampling (not included here) demonstrated little bee activity outside of sampling hours (L. Russo, unpub. data). Cool, wet mornings were common in the field during this field season and precluded most early bee activity. For the purposes of this study, we focus on the bee visitors to the different plant species, as they are completely dependent on pollen as a protein source for their offspring. We identified the majority (98%) of the bee specimens to species with help from Samuel Droege, a bee taxonomist with the United States Geological Survey (USGS).

Abundance vs. visitation rate

We calculated bee visitation in two different ways. First, we totaled the number of individuals collected in a 5-min sample on each species: the bee abundance. Averaged across the samples for a given species, abundance described the average number of bees per 5 min independent of the number of inflorescences. We used this abundance value to compare the relative importance of different plant traits across the six species, but not to compare bee preference.

Because the size of the floral display may affect the total number of visitors to a given species during a sample, we also calculated visitation rate. The visitation rate was the number of bee individuals per 5-min sample divided by the product of inflorescence number and average floral area of a given plant species. Visitation rate considered bees per unit floral area per 5-min sample. This allowed us to better understand the relative attractiveness of plants with different floral displays to bees, controlling for the number of inflorescences and size of the display. It was important to consider floral area because it related to the abundance of the floral resource provided, in addition to the quality of those resources.

Pollen collection and analysis

We did not collect pollen from the plants in the experiment to avoid interfering with pollinator visitation. Instead, pollen was collected from separately grown potted individuals of each plant species in the same potting soil and greenhouse conditions (22–27 °C, 50–60% humidity). To acquire sufficient pollen for the nutritional analyses, pollen was pooled from many individuals of each species. To collect pollen from the asters, we removed inflorescences and placed them in a water tube, and then into an isolation chamber (see Costa and Yang 2009). We let pollen fall passively and used a razor to remove any additional pollen sticking to anthers. The legume (*C. fasciculata*) required buzz pollination, so we used an electric toothbrush to buzz the anthers. Pollen

was stored in a − 80 °C freezer until it could be analyzed for nutritional content. We used a Bradford assay to measure protein content, and a modified Van Handel and Day (1988) protocol for carbohydrate and lipid content. We analyzed protein, lipid, and carbohydrate content in three subsamples of pooled pollen from each species. A full description of the methodology used to determine pollen protein, lipid, and carbohydrate concentrations is presented in Vaudo et al. (2016a). We did not evaluate nectar quality in this study as we were focused on pollen as an essential resource that bees use to provision their offspring (Roulston and Cane 2000); however, nectar may also contribute to the relative attractiveness of different flower species and therefore guide foraging preferences (Heil 2011).

Data analysis

First, we tested for the presence of spatial autocorrelation in the communities of bees visiting the research plots. To determine whether plots closer in proximity had a more similar bee community, we used a Mantel test to calculate the correlation between two matrices: (1) a matrix of the pairwise physical distances, in meters, between plots and (2) a matrix of average pairwise Euclidean distances between the interaction matrices of the different plots.

Next, we tested whether the experimental design had the desired effect using generalized linear mixed effects models (glmms) to build four different models: (1) whether the experimental treatment affected the number of thistle inflorescences produced in different plots (a proxy for the intensity of the invasion of the thistle), (2) whether the treatment affected the total number of bees collected at each plot, (3) whether there was a significant relationship between the number of thistle inflorescences and the abundance of bees, and (4) whether there was a relationship between the number of thistle inflorescences and the visitation rate of bees (R Core Team 2013). For all four of these models, time, date, and block were used as random effects and the experimental treatment was the fixed effect.

Because there was no significant difference between the number of thistle inflorescences produced in the high- and low-intensity treatments (see below), we instead tested whether the number of thistle inflorescences affected the visitation rate (number of bees collected per unit floral area over each 5 min sample) of the other plant species. We separately tested whether the timing of the treatment, or the presence or absence of the thistle, affected the visitation rate of bees to the other plant species. Because *G. pulchella* received very few visits across the experiment, we were not able to conduct this analysis with this species.

To distinguish between factors associated with bee preference among the six species, we obtained an average bee abundance per five minute sample for each plant species by

averaging across all samples collected in the entire season, and across all the plots sampled, for each plant species. We then calculated the Pearson correlation coefficient of bee abundance with each of the following plant traits: average number of inflorescences, inflorescence diameter, biomass, and protein, lipid, and carbohydrate content of the pollen. Because the protein to lipid ratio of pollen has previously been shown to be an important factor determining bumble bee preference (Vaudo et al. 2016a, b), we also tested the correlation between the average protein:lipid ratio of each species and its visiting bee abundance.

We then separated the bees visiting native plants in control plots from the bees visiting native plants in treatment plots and tested whether the presence of the thistle changed (1) the abundance of bees visiting native plants (2) whether the relationship between the bee abundance and plant traits differed. To test the effect of the thistle on bee abundance per sample on the native plant species, we used a GLMM with a fixed effect of the presence/absence of the thistle, random effects of date, time, and block, and a Poisson distribution. We repeated the above test for the relationship between bee abundance and plant traits with a Pearson correlation coefficient for the native plants in control plots and treatment plots separately.

We used visitation rate to compare preference of bees between different plant species. Because inflorescence count and inflorescence size in part determine visitation rate, we tested its correlation only with biomass and four aspects of pollen quality (protein, lipid, and carbohydrate content, and protein:lipid ratio). We repeated these analyses with subsets of special interest, including the honey bee (*A. mellifera*), bumble bee genus (*Bombus*), most abundant bee species in our samples (*Halictus ligatus*, a social halictid), male and cleptoparasitic bees, and females of pollen-collecting species.

To further determine the model that best predicted bee visitation to the different plant species, we used the dredge function in the MuMIn package in R (Barton 2018). This function compared all possible models, and then ranked them according to their AICc value. For these models, we used the means of all predictors (plant traits) that were not correlated, log normalizing biomass as it was on a very different scale from the rest of the predictors, with the abundance of the bees at each sample as the response. We then reported all models with a delta of < 2, and their r^2 values. We repeated this model selection process with and without the thistle.

Results

Overall, we collected 3,749 bee specimens, representing 68 species (Table S3). A Mantel test found no significant correlation between the physical proximity of different plots

and the bee communities visiting those plots (Mantel r stat − 0.04, $P = 0.63$), suggesting there was no spatial autocorrelation (Fig. S4). When testing the number of inflorescences produced by the thistles in the different experimental treatments, we found that there was no significant difference between the high-intensity and low-intensity early treatments (est − 4.55, t value − 1.45, P value = 0.15). There was a significant relationship between the number of thistle inflorescences and the number of bees collected at each plot (est 0.23, t value 9.49, $P \ll 0.001$), so we used the number of thistle inflorescences as a proxy for the intensity of the invasion instead of the categorical (high vs. low) treatments.

When the visitation rate was averaged across all plots (including control and treatment plots), the visitation rates to different plant species differed significantly (Fig. 1). The invasive thistle, *Carduus acanthoides*, had the highest visitation rate (number of bees per unit floral area in a 5-min sample), while the native legume, *C. fasciculata*, had the lowest visitation rate (Fig. 1), probably because its floral morphology (poricidal anthers) restricts pollinator access to
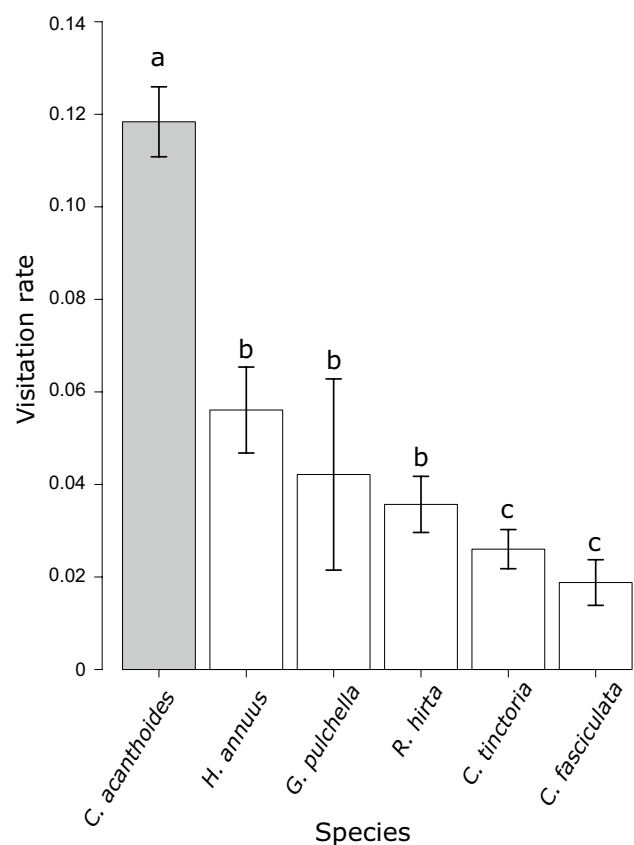

Fig. 1 The visitation rate (number of bees per floral area per 5-min sample) averaged across plots and sampling events for the six different plant species in this study, with standard error bars. Using a GLMM, we show the invasive thistle (*C. acanthoides*, in gray) had a significantly higher rate of visitation than the native plant species (in white)

pollen. There was no effect of the number of thistle inflorescences, the presence/absence of the thistle, or the timing of the thistle invasion on the visitation rates to *C. fasciculata, C. tinctoria, H. annuus,* or *R. hirta* (Table S4). In addition, there was no difference in the abundance of bees visiting native plants in control plots vs treatment plots (est 0.12, *z* value 1.77, $P = 0.08$).

The aspects of the pollen quality that we measured here (protein, lipid, and carbohydrate content) varied among the plant species (Fig. 2). The invasive thistle (*C. acanthoides*) had by far the highest protein content (Fig. 2c). It had a lower lipid content than the native asters, except *H. annuus*, but higher lipid content than the native legume, *C. fasciculata* (Fig. 2a). The balance between these two factors led to the

thistle having a similar protein:lipid ratio but higher protein and lipid concentrations than the legume, and a much higher protein:lipid ratio than the native asters (Fig. 2b). There was no clear difference between the non-native and native asters in terms of sugar content, but the legume had a much lower sugar content than the asters (Fig. 2d). The six different plant species also varied in other factors we measured (Table S2).

Within a given plant species, the number of inflorescences was significantly correlated with bee abundance ($P < 0.05$, Fig. S5), though this relationship was not significant across species (Fig. 3a). In other words, the abundance of the floral resource alone was not associated with foraging preferences among these plant species. The average inflorescence size and biomass of each species also did not correlate
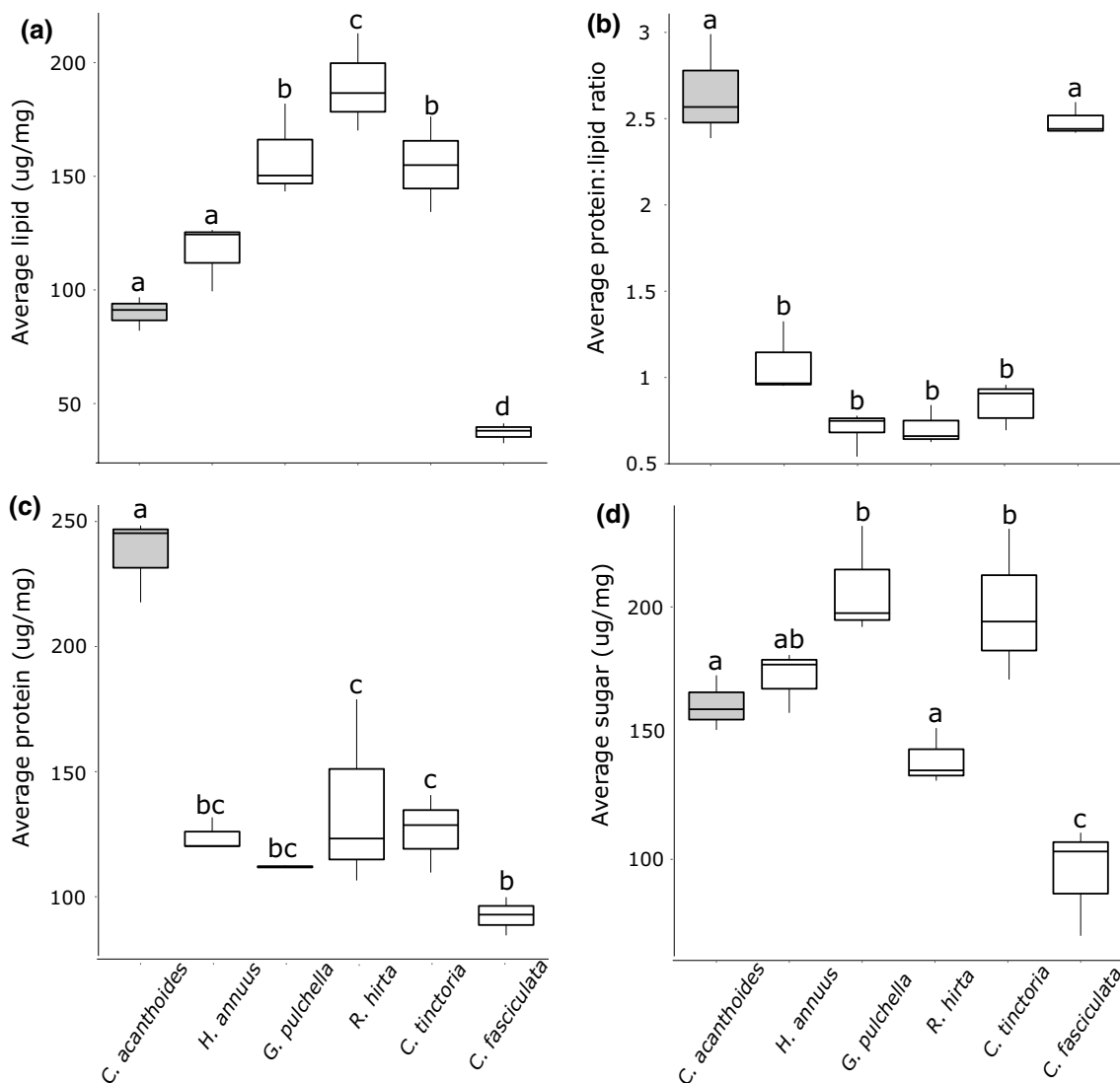

Fig. 2 Measured attributes of pollen quality in the six different plant species, including average lipid (**a**), protein:lipid ratio (**b**), protein (**c**), and carbohydrate (**d**) concentrations. The invasive thistle (*C. acanthoides*) is in gray and the native species are in white. For these box

and whisker plots, the whiskers show the extent of the largest and smallest observations, the box is bounded by the lower and upper quartile, and the median value is marked with a horizontal line

with average bee abundance across plant species. Indeed, the only significant correlation between plant traits and bee abundance across species was with the protein content of the pollen (Fig. 3e). This correlation also had a high r value (0.9) across all plant species. Though other studies found protein:lipid ratio drove the visitation of some bumble bee species (Vaudo et al. 2016a, b), the relationship between this trait and the abundance of the overall bee community was not significant ($P > 0.05$, Fig. 3d). Because bee species richness was tightly correlated with bee abundance ($r = 0.99$), we did not repeat these tests with species richness. Using the model selection process, we identified the best model for bee abundance (i.e., the model with the lowest AICc score) as a model which includes inflorescence size, biomass, and protein content as predictors ($r^2 = 0.26$, Table 1, Fig. 4). Though inflorescence size and biomass were not individually correlated with bee abundance, they did improve the fit of the model overall. When we used visitation rate (number of bee visitors per floral area per 5-min sample) instead of abundance (number of bees per sample, not controlled for

floral display) as a response variable, visitation rate was significantly and strongly correlated with protein concentration, but not biomass, protein:lipid ratio, or lipid or carbohydrate concentration (Fig. S6).

The abundance and visitation rate of bees just to the native species (excluding thistle data) did not correlate with any of the pollen nutritional qualities (protein, lipid, sugar, or protein:lipid ratio). Instead, bee abundance correlated strongly with the average number of inflorescences ($r = 0.89$, $P = 0.04$), biomass ($r = 0.97$, $P < 0.01$), and total floral area ($r = 0.96$, $P < 0.01$) of each species (Fig. S7). We found only minor differences in the correlations between bee abundance on native plants in control vs treatment plots, suggesting that the presence of the thistle did not affect the relationship between the plant traits and bee abundance on native plants. Bee abundance on native plants in both control and treatment plots correlated significantly only with the number of inflorescences (control: $r = 0.94$, $P = 0.02$; thistle present: $r = 0.88$, $P = 0.0497$) and biomass (control: $r = 0.98$, $P = 0.003$; thistle present: $r = 0.92$, $P = 0.03$). This suggested
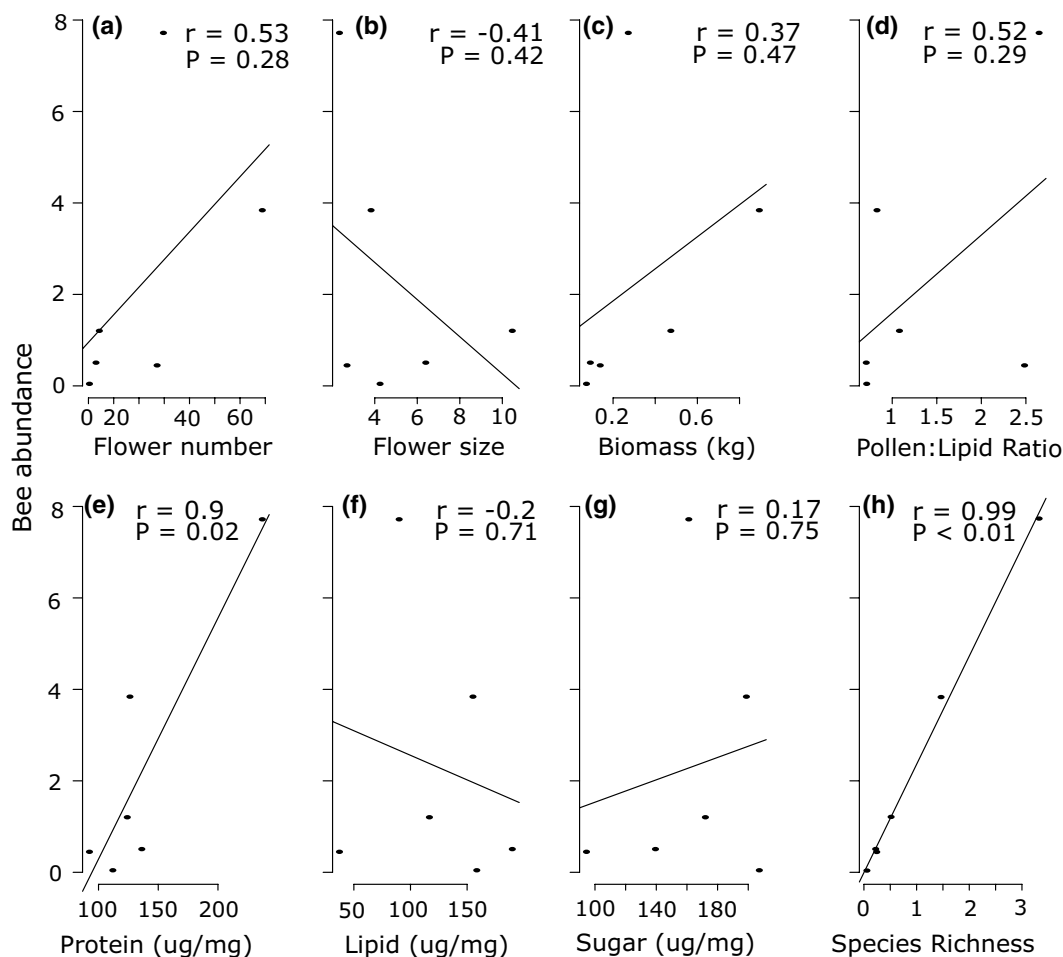

Fig. 3 Correlations between means of measured plant traits and bee abundance (number of bee visits per sample). The *r* value is reported as a measure of the strength of the correlation, and *P* values indicate the significance of the relationship

Table 1 Model selection process for identifying the best predictors of bee abundance (including all plant species) based on AICc value

| (Int)	Inflorescence size	Lipid	Log (biomass)	Protein	Sugar	Degrees of freedom	LogLik	AICc	Delta	Weight	r^2
− 0.68	− 0.17		1.84	0.06		5	− 2214.99	4440.1	0	0.209	0.26
− 2			1.72	0.06		4	− 2216.45	4441	0.89	0.134	0.25
− 0.83	− 0.19	0.002	1.79	0.06		6	− 2214.96	4442	1.97	0.078	0.26
− 38.62	− 0.46	− 0.09	− 7.97		0.3	6	− 2214.96	4442	1.97	0.078	0.26
− 7.77	− 0.24	− 0.01		0.05	0.05	6	− 2214.96	4442	1.97	0.078	0.26
− 1.53	− 0.19		1.61	0.06	0.006	6	− 2214.96	4442	1.97	0.078	0.26
24.76		0.06	8.4	0.1	− 0.2	6	− 2214.96	4442	1.97	0.078	0.26
− 0.83	− 0.19	0.002	1.79	0.06		6	− 2214.96	4442	1.97	0.078	0.26

Models with a delta < 2 are included
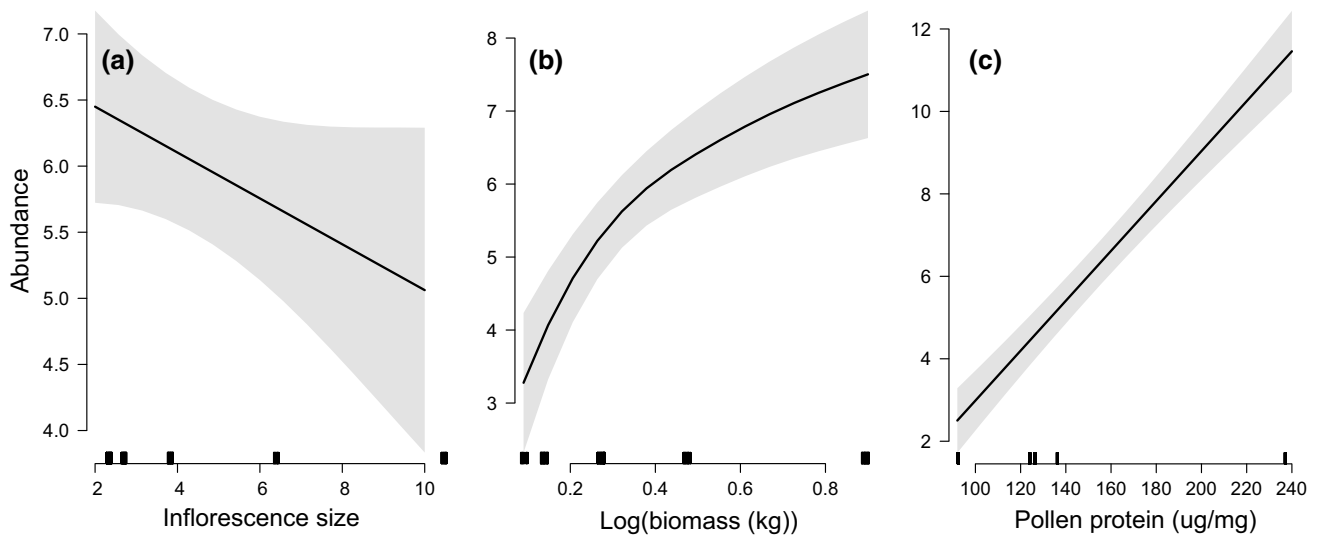
Fig. 4 Effects of the main predictors in the best model chosen by the model selection process (lowest AICc) for bee abundance. The shaded areas around the lines represent the confidence intervals around the predicted relationship between the predictor and response variable

abundance, rather than pollen protein, of the floral resource was more important in guiding bee foraging preferences among the native plant species. The visitation rate of the bees (excluding the thistle) was not significantly correlated with the plant biomass, or any of the nutritional qualities of the pollen. Similarly, there were no significant correlations between the visitation rate to native plants and any measured plant trait in control or treatment plots. In light of these similarities, and to increase replication, we used visitation to native plants in all plots to run the model selection process. The best model identified included inflorescence size and sugar content as predictors ($r^2 = 0.15$, Table S5, Fig. S8). Once again, though sugar content was not individually correlated with bee visitation, it remained as a predictor in the model of best fit.

Of the subsets analyzed, *Bombus* species, pollen-collecting, and male and cleptoparasitic bees all had a highest

visitation rate on *C. acanthoides*. *H. ligatus* and *A. mellifera* had similar visitation rates across all plant species (Fig. S9). The visitation of these subsets was also associated with pollen protein content, except for *H. ligatus,* whose visitation did not correlate with any measured plant traits (Fig. S10). Notably, the visitation of bees of the genus *Bombus* also correlated with the protein:lipid ratio of the pollen (Fig. S10A).

Discussion

Plant–pollinator interactions are considered by many to be structured by coevolution (Jordano et al. 2002). However, despite the absence of a coevolutionary past, non-native species are often highly attractive to novel pollinators (Lopezaraiza-Mikel et al. 2007; Russo et al. 2016). That they are able to integrate into novel plant–pollinator communities

indicates non-native plants are able to co-opt the same visual and chemical mechanisms native species use to attract floral visitors. The ease with which non-native species integrate into resident pollination networks may also facilitate their invasion into novel ecosystems (Richardson et al. 2007; Traveset and Richardson 2011). We examined the preferences of a bee community for visual and nutritional traits of one plant invader (*C. acanthoides*) attractive to resident pollinators in its invaded range (Russo et al. 2016), relative to five native plant species. The five native species comprised four species confamilial with the invader to determine whether the invader had similar visual and nutritional qualities as native members of the same family. To contrast, we also included one native legume, as legumes are preferred by some bee species (Pywell et al. 2005), and are thought to have a different nutritional profile (e.g., Mata 2018).

We found the invasive thistle not only had the highest rate of visitation, but also the highest protein content of any of the plant species analyzed. Furthermore, there was a strong significant correlation between the pollen protein content and bee visitation rate over the course of the experiment. Pollen protein was included in the best model based on the model selection process and was consistently associated with bee visitation in subsets of the bees captured, including pollen-collecting bees, bumble bees, honey bees, and male/cleptoparasitic bees. Interestingly, the visitation of the most abundant bee in this study, *H. ligatus*, was not correlated with plant pollen protein, or any other measured trait. Another species of halictid bee was previously shown to not detect pollen protein content, despite the fact that protein content correlated with the ultimate body size of its offspring (Roulston and Cane 2002). The importance of pollen protein to bumble bee foraging behavior is well established (e.g., Rasheed and Harder, 1997; Vaudo et al. 2016a, b). On the other hand, results are mixed on the ability of honey bees to detect protein content (e.g., Pernal and Currie 2002; Cook et al. 2003). Future research may investigate whether other species of halictid bees, or bees of other families, are unable to detect pollen protein content.

Using just bee abundance (not visitation rate), traits, such as the number or size of the inflorescences, or above-ground biomass, did not correlate with bee abundance across all species, but the inflorescence size and above-ground biomass were included as predictors in the best model selected (Table 1; Fig. 4). This indicates that these two factors help to explain some of the variation in bee abundance. Using a visitation rate model, pollen lipid and carbohydrate content did not correlate with bee visitation overall or in any subset we analyzed. The pollen protein:lipid ratio of the plant species in this study did significantly correlate with bumble bee visitation rate, consistent with previous studies on factors driving bumble bee visitation (Vaudo et al. 2016a, b). Furthermore, the protein:lipid ratio of the thistle

significantly differed from the other asters, but not from the legume (Fig. 2b).

When we excluded the thistle from the analysis, we found the plant traits associated with bee visitation changed. Without the thistle, pollen nutrition did not affect bee abundance or visitation rates to the other plant species. Instead, the number and size of inflorescences, as well as biomass, determined bee visitation among the native plant species. Moreover, there was no difference between the correlation between these plant traits and bee abundance on the native plants in control plots vs in treatment plots, suggesting the presence of the thistle did not change the bee foraging preferences when selecting among the native plant species. In the best model selected when the thistle was excluded, the inflorescence size and sugar content were included (Table S5, Fig. S8). This is likely due to relatively little variation in the pollen nutrition of the four native asters and a low bee visitation to the legume in general, likely due to its need for buzz pollination. When pollen nutrition varies little, bees may use other cues, such as the size of the floral display, to make their foraging selections.

It is also possible that plant attributes other than the ones we measured may have a role in driving pollinator foraging preference. For example, we measured the abundance of the floral resources through the proxy of total floral display (floral area × number of inflorescences), but the volume of pollen or nectar produced by individual inflorescences may vary between plant species and thus contribute to foraging behavior. However, it seems likely that the volume of the pollen produced and its quality are not correlated. Bumble bees have been shown to forage based on the highest availability of pollen protein in a community context, including both pollen protein content and pollen volume (Rasheed and Harder 1997). The quality of the pollen as a resource may also vary based on attributes we were not able to measure, such as the presence of particular amino acids (Leonhardt and Blüthgen 2012), the availability of micronutrients (Harmanescu et al. 2012), or secondary plant compounds (Irwin et al. 2014). Furthermore, we did not evaluate nectar quality in this study, though it is an attractant and may also contribute to foraging preferences among bees (Heil 2011). Other factors that have been shown to affect the foraging behavior of bees include floral morphology (Harder 1983), competition (Goulson et al. 1998), and even caffeine (Thomson et al. 2015).

Another possible explanation is the thistle has a significantly different volatile chemical profile that attracts bee visitors or indicates the protein content of its pollen, as pollen odors can be important determinants of pollinator visitation (Dobson and Bergström 2000; Raguso 2008). Future research may determine whether pollen with higher protein content has a different volatile signature, and if so, whether these volatiles significantly predict floral visitation rates of

bee communities. Volatile compounds advertising nectar availability and quality may also play an important role in attraction. It is possible differences in nectar quality mirror differences in pollen protein in the species we studied: another valuable avenue for future research. On the other hand, bees may be using the taste of the pollen to guide their foraging choices (Ruedenauer et al. 2015). It would be interesting to determine whether this invasive thistle has a similar protein content in its native range, or whether the increase in pollen protein content is an adaptation to attract novel pollinators in its invaded range.

Interestingly, the visitation rate to different plant species in this study was not significantly affected by the presence of this highly attractive invasive plant. This is consistent with a previous study on *C. acanthoides*, suggesting though the thistle was highly attractive itself, it did not affect visitation to nearby plant species (Russo et al. 2016). This may suggest there is little overall competition for pollinators among the inflorescences in this system. Other research on the impact of non-native plants on the pollination of native plants has been mixed, with some studies showing an overall competitive effect (Morales and Traveset 2009), and others showing no strong effects (Charlebois and Sargent 2017). However, it is also possible competition was occurring in ways we did not measure, for example, by increasing the proportion of non-target pollen deposition (Lopezaraiza-Mikel et al. 2007, but see Moragues and Traveset 2005). Although high-intensity agroecosystems can have a negative effect on bee abundance, the agricultural area where this study was conducted has many patches of forest and habitat to support larger wild bee populations (Fig. S1) (Park et al. 2015).

A large number of bees, representing 68 species, visited these 6 plant species. These bees exhibited a strong preference for the invasive thistle, visiting it at a much higher rate than the other plant species. Though other plant traits (e.g., volatile chemicals) may play a role in attracting these bees, this study provides support for the idea that pollinators are optimizing their foraging effort by preferentially visiting species providing high-quality floral resources. Invasive species that provide high-quality resources may in turn gain enhanced pollination services, raising the hypothesis that the nutritional content of floral resources may be an important factor facilitating the invasion of non-native species to novel floral markets.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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