As bees’ main source of protein and lipids, pollen is critical for their development, reproduction, and health. Plant species vary considerably in the macronutrient content of their pollen, and research in bee model systems has established that this variation both modulates performance and guides floral choice. Yet, how variation in pollen chemistry shapes interactions between plants and bees in natural communities is an open question, essential for both understanding the nutritional dynamics of plant–pollinator mutualisms and informing their conservation. To fill this gap, we asked how pollen nutrition (relative protein and lipid content) sampled from 109 co-flowering plant species structured visitation patterns observed among 75 subgenera of pollen-collecting bees in the Great Basin/Eastern Sierra region (USA). We found that the degree of similarity in co-flowering plant species’ pollen nutrition predicted similarity among their visitor communities, even after accounting for floral morphology and phylogeny. Consideration of pollen nutrition also shed light on the structure of this interaction network: Bee subgenera and plant genera were arranged into distinct, interconnected groups, delineated by differences in pollen macronutrient values, revealing potential nutritional niches. Importantly, variation in pollen nutrition alone (high in protein, high in lipid, or balanced) did not predict the diversity of bee visitors, indicating that plant species offering complementary pollen nutrition may be equally valuable in supporting bee diversity. Nutritional diversity should thus be a key consideration when selecting plants for habitat restoration, and a nutritionally explicit perspective is needed when considering reward systems involved in the community ecology of pollination.

Pollen nutrition structures bee and plant community interactions

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Significance
Nutrition is widely recognized as a key factor in addressing pollinator declines, and across the tree of life, plants vary in the protein and lipid content of their pollens offered to bees. However, within natural communities, we know surprisingly little about the nutritional value of plants to wild bees, and pollen nutrition has not previously been a priority when selecting plants for restoration efforts. We show that consideration of pollen macronutrient content can help explain patterns of interactions among wild bees visiting wildflowers to collect pollen, which sheds light on the nutritional basis of plant–bee mutualisms, and can help inform plans to restore bee habitat, conserve plant species, and design supplemental plantings for bees in agricultural and urban areas.

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The authors declare no competing interest.

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(14, 15, 35), offers a starting point to frame questions about how pollen macronutrients might structure bee–plant interactions (Fig. 1). We first assume that pollen-collecting bees have taxon-specific foraging targets (i.e., a particular ratio of nutrients that maximizes fitness; see the targets in Fig. 1) (4, 36). We can then visualize pollen macronutrient content (i.e., P:L ratios) of co-flowering plant species, the “nutritional landscape,” as “rails” (lines in Fig. 1), which pollen-foraging bees can “travel along” as they visit plants to collect pollen in pursuit of a target P:L ratio (arrows in Fig. 1). Using a GF approach to visualize the relationship between bee nutritional needs and the composition of available resources suggests that while some bees might exclusively visit plants whose pollen composition aligns with their target (Fig. 1, bee #1), others might combine visits to unbalanced plants to reach their intake target.

Fig. 1. Conceptual framework for considering bee nutritional niches and pollen foraging behavior. Plants’ positions in space represent their pollen protein and lipid concentrations and lines connecting them to the origin represent their protein:lipid (P:L) ratios. Target symbols represent hypothetical nutritional intake goals for different bee species. If groups of bees occupy similar targets, these may be considered nutritional niches. Direction and color of arrows illustrate how bees might use different foraging strategies while sharing similar resources to balance their diet to reach their target.

Question 1: How likely are plant species with nutritionally similar pollen to be visited by similarly composed communities of pollen-foraging bees? If most bees tend to forage for pollen relatively narrowly in line with their intake target (Fig. 1, bees #1 and #2), then we expect that plant species offering pollens of similar composition will share visitor communities, and plant species with dissimilar pollen nutrition will be visited by dissimilar bee communities. This signal would be lost however if bees combine visits to plants dispersed widely across nutritional space, if other floral traits more strongly influence pollen foraging behavior, or if bees simply visit the most abundant plants regardless of pollen nutrition.

Question 2: What role does pollen nutrition play in structuring broad patterns of pollen visitation networks? Here, we expected that bee and plant genera that occupy similar nutritional space and more frequently interact may represent nutritional niches (i.e., multiple bee subgenera might share similar intake targets in Fig. 1, and therefore share nutritionally similar host plants across space and time) (36, 41–44). Characterizing our interaction network into aggregated groups of frequently interacting pollen-collecting bees and plants [i.e., modules (44, 45)], we asked whether these modules were also composed of plant species that share similar nutritional values. If nutritional niches did not exist, we expected to find either no modules in the network, or variation in pollen nutrition equally distributed among modules.

Question 3: Do plants with different pollen nutritional profiles differ in their levels of specialization or generalization within interaction networks? Generalist species can provide resilience in ecological interactions, making them key to consider for pollinator habitat restoration efforts (46–48). We evaluated individual plant species’ visitation network–derived metrics of strength and specialization/generalization (e.g., strength, D’, effective partners, centrality), in relation to the nutritional value of their pollen. If generally, bees prioritize proteinaceous diets [as in bumble bees (15, 19, 49)], then we expected plants with higher P:L ratios would be visited more frequently by a higher taxonomic diversity of bees; or if bees prioritize lipids given their dietary importance (50–53), then plants with low P:L values may be more generalist. Alternatively, plants with intermediate “balanced” P:L values may attract the highest bee diversity and abundance. A lack of any relationship might suggest that plant species across the spectrum of pollen nutrition are equally important for supporting bees occupying different nutritional niches.

Results

The Pollen Nutritional Landscape. Great Basin/Eastern Sierra Sagebrush Steppe habitat. Considering that multiple floral traits other floral traits more strongly influence pollen foraging behavior, might structure their foraging for pollen protein and lipids among previously been characterized, how different wild bee species nutrition across multiple sites in the Great Basin/Eastern Sierra Sagebrush Steppe habitat. Considering that multiple floral traits might covary, we analyzed the role of pollen nutrition in explaining visitation patterns alongside floral morphology and plant phylogeny (12, 13, 37–40). We used this dataset to explore three related questions about the role of pollen macronutrient composition in community interactions: 1) How likely are plants with nutritionally similar pollens to be visited by similar bee communities? 2) What role does pollen nutrition play in structuring broad patterns of bee–flower interaction networks? 3) Do plants with different pollen nutritional profiles differ in their levels of specialization or generalization within interaction networks?

Due to an updated lipid analysis, we obtained P:L values higher than previously reported (11). The distribution of pollen protein and lipid concentrations, and P:L values, did not differ between sites (repeated measures mixed model with plant genus as a random effect; protein: \( F_{1,166} = 0.38, P = 0.91 \); lipid \( F_{1,166} = 6.45, P = 0.087 \); P:L: \( F_{1,173} = 0.16, P = 0.69 \); SI Appendix, Fig. S2 and Table S1) indicating that bee communities had similar foraging options across habitats even as plant species turned over. We note that our method of lipid analysis, the sulfo-phospho-vanillin assay (SPV), may be biased toward unsaturated fatty acids and thus
considered to be latent variables and were estimated by factor analysis (PCA). Pollen nutrition and floral morphology were traits (SI Appendix for floral morphology and phylogenetic covariance of these traits). We observed visiting plants to collect pollen, while accounting for floral morphology, we found that pollen P:L ratio and protein were the strongest metrics associated with floral morphology (facilitating that P:L ratios are a strong predictor variable for evaluating bee community composition directly with the P:L ratio as a fixed effect stratified by floral symmetry.

PCA plant scores closer in space along PC1 (22% variation explained) or PC2 (14%) axes shared similar bee communities in identity and visitation frequency. The most abundant bee subgenera, *Dialictus* and *Melanosmia*, were on the negative and positive ends of the PC1 axis respectively, while they both occupied the most negative values of PC2. Pollen nutrition was linearly related to plant scores along PC1 in both total effect (posterior distribution of slope mean (upper, lower 90% CI): P:L = 0.52 (0.39, 0.62), Nutrition factor = 0.54 (0.41, 0.66)) and while analyzing by morphology and phylogenetic covariance [P:L(radial) = 0.59 (0.08, 1.09), P:L(bilateral) = 0.63 (0.48, 0.78); Nutrition factor = 0.65 (0.50, 0.80)] indicating that the majority of variance in ordination was predicted by pollen nutrition (Fig. 4A and SI Appendix, Table S4). Likewise, PC2 scores were linearly related to pollen nutrition in total effect [P:L = −0.22 (−0.37, −0.07), Nutrition factor = −0.23 (−0.38, −0.07)] and especially after accounting for morphology and phylogenetic covariance [P:L(radial) = 0.10 (−0.56, 0.77), P:L(bilateral) = −0.58 (−0.73, −0.43); Nutrition factor = −0.58 (−0.74, −0.43)], yet for PC2 the relationship between variation in pollen nutrition and bee community composition was mainly within bilaterally symmetric flowers (SI Appendix, Table S4). That is, as pollen nutritional content differed among plant species, so did the visiting community of bees. Floral morphology also contributed to differences in bee communities as radial and bilateral flowers had different intercepts, yet this difference was dampened by including phylogenetic distances and the counterfactual effect of nutrition on morphology (nutrition-morphology = 0.70 (0.58, 0.82); Fig. 4B and SI Appendix, Table S4). Clearly, there was high phylogenetic covariance in plant nutritional values and morphologies (Ornstein–Uhlenbeck posterior $\eta^2 = 2.6$ vs. prior $\eta^2 = 1$, Fig. 4C and SI Appendix, Table S4); but including this variance in our models strengthened the association between floral nutrition and bee community likely because bee communities were more associated with plants whose pollen had a specific nutritional value regardless of plants’ (familial) relatedness.

### Question 1

We asked how interspecific variation in pollen nutrition among plant species (protein and lipid content and P:L) contributed to the subgenera composition of bee communities we observed visiting plants to collect pollen, while accounting for floral morphology and phylogenetic covariance of these traits (SI Appendix, Fig. S6). We summarized bee communities associated with each plant species using principal components analysis (PCA). Pollen nutrition and floral morphology were considered to be latent variables and were estimated by factor analysis (SI Appendix, Fig. S6). We also conducted the analysis of bee community composition directly with the P:L ratio as a fixed effect stratified by floral symmetry.

### Question 2

The visitation network that we estimated from observations of pollen-collecting bees was partitioned into a handful of major modules (Fig. 5A), which represent bee subgenera and plant genera more likely to interact with each other than with undershoot the total lipid content of pollen (54). Nonetheless, digestible unsaturated fatty acids are important for bee health and perception (51–53) and thus comparative values across plant species have direct relevance to analyses of behavior and physiology.

We used factor analysis to provide estimates of pollen nutrition and morphology as latent variables based on measured pollen nutritional traits (protein $\mu g/mg$, lipid $\mu g/mg$, and P:L ratios) and morphological traits (floral symmetry, morphology, depth, and width) (SI Appendix, Table S2). Among our pollen nutrition metrics, P:L was the most strongly correlated with the pollen nutrition latent variable (factor loading: 1.0, SI Appendix, Table S2) indicating that P:L ratios are a strong predictor variable for evaluating pollen nutrition from both protein and lipid concentrations. Likewise, floral symmetry and open vs. tubular/complex flowers were the strongest metrics associated with floral morphology (factor loadings: 0.94 and 0.88, respectively) while depth or width were not as correlated with these other morphological factors (SI Appendix, Table S2).

Further testing the relationship between pollen nutrition and floral morphology, we found that pollen P:L ratio and protein concentration increased, and lipid concentration decreased, from radially to bilaterally symmetrical flowers (repeated measures mixed model with plant genus as a random effect; protein: $F_{3,75} = 25.82$, $P < 0.01$; lipid: $F_{3,75} = 9.87$, $P < 0.01$; P:L: $F_{3,75} = 36.07$, $P < 0.01$), and from open, to tubular, to complex morphologies (protein: $F_{3,75} = 12.65$, $P < 0.01$; lipid: $F_{3,75} = 5.98$, $P < 0.01$; P:L: $F_{3,75} = 22.63$, $P < 0.01$), meaning that complex flower morphology was generally associated with higher pollen P:L values (SI Appendix, Fig. S4 and Table S3).

Pollen nutritional profiles exhibited a phylogenetic signal among closely related plant species, yet these profiles (high or low P:L for instance) arose multiple times throughout the plant phylogeny (Fig. 3 and SI Appendix, Fig. S5): protein: $K = 0.59$, $\lambda = 0.98$; lipid: $K = 0.34$, $\lambda = 0.98$; P:L: $K = 0.08$, $\lambda = 0.83$.

### Question 1

We asked how interspecific variation in pollen nutrition among plant species (protein and lipid content and P:L) contributed to the subgenera composition of bee communities we observed visiting plants to collect pollen, while accounting for floral morphology and phylogenetic covariance of these traits (SI Appendix, Fig. S6). We summarized bee communities associated with each plant species using principal components analysis (PCA). Pollen nutrition and floral morphology were considered to be latent variables and were estimated by factor analysis (SI Appendix, Fig. S6). We also conducted the analysis of bee community composition directly with the P:L ratio as a fixed effect stratified by floral symmetry.

Fig. 2. Pollen nutritional landscape of the Great Basin/Eastern Sierra flora. Pollen protein and lipid concentrations ($\mu g/mg$) are arranged in two-dimensional nutritional space. Each marker represents an individual plant species, colored by plant family along a gradient from low to high pollen P:L (see Dataset S1 for individual species protein, lipid, and P:L values). Gray lines represent different P:L values.

**Family by Increasing P:L**

- **Malvaceae**
- **Lamiaceae**
- **Asteraceae**
- **Rhamnaceae**
- **Polygonaceae**
- **Amarillidaceae**
- **Brassicaceae**
- **Liliaceae**
- **Salicaceae**
- **Primulaceae**
- **Aatidaceae**
- **Ranunculaceae**
- **Onagraceae**
- **Montiaceae**
- **Phrymaceae**
- **Plantaginaceae**
- **Rosaceae**
- **Boraginaceae**
- **Apoaceae**
- **Loasaceae**
- **Caprifoliaceae**
- **Papaveraceae**
- **Malanthiaceae**
- **Grossulariaceae**
- **Valerianaceae**
- **Fabaceae**
- **Convulvulaceae**
- **Orobanchaceae**

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**Question 2.** The visitation network that we estimated from observations of pollen-collecting bees was partitioned into a handful of major modules (Fig. 5A), which represent bee subgenera and plant genera more likely to interact with each other than with...
members outside their group. The average pollen P:L of plant genera within modules differed between modules (Fig. 5 and SI Appendix, Table S5). These results held true when we manipulated the data in two ways: 1) Treating all plant genera in each module equally and taking the module average; or 2) weighing each plant genus by bee visitation frequency because some genera were more frequently visited for pollen than others, and thus more influential in the visitation network and likely to contribute more to bees' nutrient intake (Fig. 5C and SI Appendix, Table S5; see bee #3 in Fig. 1). The module with the highest average P:L had the widest distribution of P:L values, while the module with the lowest average P:L had a very narrow distribution of pollen P:L values collected by bees (Fig. 5B). Other modules exhibited intermediate yet decreasing distributions of P:L values from the high to low P:L modules. Bayesian predicted P:L distributions between modules were much narrower than observed values and clearly distinct between modules. Modules therefore may be used to delineate nutritional niches and predict potential intake targets for bees. These results were also corroborated by repeating the analysis such that the major modules remained consistent across iterations (SI Appendix, Table S5).

**Question 3.** We analyzed whether plant species' pollen nutrition was related to their network strength and degree of generalization/specialization in the pollen visitation interaction network, including the following species-level network metrics: strength, effective partners, nested rank, D', betweenness centrality, and closeness centrality. The data did not support the hypotheses that plants with differing nutritional values would be visited by a higher diversity and frequency of bee taxa (Fig. 6 and SI Appendix, Fig. S7 and Tables S6 and S7). Contrarily, there was a trend that increased P:L ratios values were associated with increased specialization mainly within bilaterally symmetric flowers (e.g., fewer effective partners, higher nested rank, and lower strength; Fig. 6 and SI Appendix, Fig. S7 and Tables S6 and S7). But posterior distributions of these slopes crossed 0, and the indices for D', betweenness centrality, weighted betweenness, and weighted
**Fig. 4.** The influence of pollen nutrition on bee community visitation, representing three processes analyzed in our model (see model 6 in SI Appendix, SI 3). (A) Standard scores of nutrition factors regressed against PC1. Each marker represents a different plant species, and associated visiting bee community, where distance between markers on the Y axis represents differences in bee visitors. Plant species markers are colored and shaped by their floral symmetry and morphology respectively. The trend line and shade represent the predicted slope and 90% credible interval of the effect of plant species’ pollen nutrition on bee community (SI Appendix, Table S4). (B) Counterfactual effect of nutrition on floral morphology representing the relationship between nutrition and morphology by manipulating pollen nutrition and predicting morphology. Markers represent individual plant species. As floral morphological complexity increases, so does its associated nutritional value. (C) Posterior distributions of phylogenetic signal against priors via Ornstein–Uhlenbeck covariance process. Highly related species share high covariance in pollen nutrition and morphology yet as distance increases, covariance in traits drops rapidly (Fig. 3). Legume image credit: Alexander Schmidt-Lebuhn, https://creativecommons.org/licenses/by-nc-sa/3.0/.

Closeness showed no trend (Fig. 6 and SI Appendix, Fig. S7 and Tables S6 and S7). Likewise, there was no indication that plants whose pollens had intermediate P:L nutritional values were more generalist, exhibiting very wide posterior distributions (Fig. 6 and SI Appendix, Fig. S7 and Tables S6 and S7). These results indicate that generally, plants with different pollen nutritional values were equally important and complementary in attracting diverse bee communities, and continuity within the nutritional landscape.

**Fig. 5.** Modules of Great Basin/Eastern Sierra bee–flower interactions representing potential nutritional niches. (A) Matrix of interactions where each blue square represents an observed interaction between a plant genus and bee subgenus, and the darkness of the square represents the frequency of the interaction. Data are standardized such that rows (i.e., plant genera) range from light to dark to compare relative visitation frequencies. Each module is outlined and labeled for plants and bees. (B) The raw distribution of P:L of each module weighted by visitation frequencies. (C) Bayesian posterior predictions of P:L associated with each module for analysis by both plant identity (each plant weighted equally) or weighed by visitation frequency. These data may be used as predictions of nutritional niches of bee–flower community interactions in the Great Basin/Eastern Sierra habitat.
may allow bees competing for resources to find alternative hosts with relatively comparable nutritional values.

**Discussion**

The nutritional threads that connect plants and pollinators within a community are impossible to see, challenging to study, and critically important for both understanding plant–pollinator mutualisms and informing their conservation. Although taxonomic patterns of pollen host plant specialization are a canonical theme in bee natural history (55), the role of pollen nutrition in explaining broad patterns of interactions has only recently received attention (10, 11, 32, 33, 49, 50), and has been often limited by the concept that a single dimension of pollen chemistry (largely, protein content) equates to quality. Yet, a parallel literature in nutritional ecology has long demonstrated that variation in multiple dimensions of macronutrient content is a primary driver of animal foraging behavior and trophic interactions more generally (3, 4). Understanding bee–host plant relationships may thus depend upon a nutritionally explicit consideration of reward composition, rather than plant taxonomic identity. A significant implication of our study is that generalist bees that visit unrelated plant species may indeed be cryptic nutritional specialists if those plants have chemically similar pollen. Likewise, we reveal that the natural distribution of nutritional diversity likely supports entire bee communities, and without this consideration of floral nutritional diversity, species-diverse restoration plantings could function as monocultures.

A key first step toward understanding floral nutrition’s relevance to bee community ecology is to characterize its general patterns within plant communities. Rather than opportunistically sampling pollens across the tree of life, from plants used for pollinator habitat supplementation, or in agriculture (11–13, 56, 57), we sought to gain a nutritional snapshot of co-flowering plants within an ecological community. This allowed us to ask in real time whether pollen nutrition, on its own and in concert with other floral traits, explained broad patterns of wild bee pollen foraging. We found that, despite phenological and elevational turnover between sites, and independent of plant species richness at any given site, bee-visited plant communities shared similar distributions of pollen lipid and protein content, covering the aggregated distribution of observed values (Fig. 2 and SI Appendix, Fig. S2). This is significant in that natural areas had a “nutritionally complete” and diverse suite of pollen protein and lipid nutrients available to the bee community. After generating a visitation interaction network to specifically focus on pollen nutrition and pollen collection (and limiting the influence of nectar collection on visitation), we demonstrated that: 1) Pollen nutritional content and floral morphology are often correlated and both affect bee–flower associations, but nutritional content predicts bee communities when controlling for morphology (Fig. 4). 2) The most frequently interacting groups of bees and plants (i.e., modules of the interaction network) represent nutritional niches characterized by plants differing in average pollen P:L ratios (Fig. 5). 3) On its own, pollen macronutrient content does not predict floral generalization or strength in connecting the community (58, 59) (Fig. 6 and SI Appendix, Fig. S7). Thus, across its measured distribution, variation in pollen lipid and protein content is equally valuable in supporting a diverse bee community.

**The Nutritional Landscape, Floral Morphology, and Patterns in Wild Bee Visitation.** Reward nutrition does not function in isolation, and bee foraging patterns within a plant community are often associated with suites of covarying floral traits (39), many of which may be also predicted by relatedness. In line with previous studies (e.g., protein content: refs. 12 and 13), we found that more closely related plant species had pollens with
similar protein content, lipid content, and P:L ratios (Fig. 3 and SI Appendix, Fig. S5). Nutritional diversity at each site and across sites was thus maintained by abundant, species rich, and ubiquitous plant taxa that demonstrated consistent P:L values within their clade, yet differed between clades (e.g., Asteraceae with low P:L, Rosaceae and Apiaceae with intermediate P:L, and Penstemon (Plantaginaceae) or Lupinus (Fabaceae) with high P:L values). Our analysis also yielded an association between pollen nutrition and floral morphology, where higher complexity floral phenotypes tended to have higher pollen P:L ratios on average, as did bilaterally symmetric as compared to radially symmetric flowers (SI Appendix, Fig. S4). There were exceptions to this trend: Some complex flower morphologies such as Lotus and Vicia (Fabaceae) had lower P:L ratios than other legumes; and some simpler morphologies such as Cirsium (Asteraceae) or Purshia (Rosaceae) exhibited higher P:L ratios relative to their sister taxa (Fig. 3 and SI Appendix, Fig. S3).

Floral morphology and pollen chemistry have a complex functional interplay: complex morphology such as poricidal anthers and keel petals may restrict reward access to more behaviorally specialized bees (37, 40), and broad patterns of protein content correlate with these restrictive mechanisms (13, 60). The premise that complex flower morphology, here associated with higher P:L values, would lead to visitation by a less diverse community of bees (37, 38, 58), was only marginally supported by our data (Fig. 6 and SI Appendix, Fig. S7 and Table S6). For example, Pedicularis which has extremely high P:L values associated with poricidal anthers, was solely visited by bumble bees (Bombus spp.), yet other flowers with complex morphologies attracted a diversity of subgenera.

Even after accounting for the fact that closely related plant species often share similar pollen nutrition and floral morphology, variation in pollen macronutrient content explained variation in bee community composition, i.e., plants with similar pollen P:L ratios were frequently visited by bee communities comprised of similar subgenera (Figs. 4 and 5). For example, Rosaceae (with open radial flowers) and Brassicaceae (with more tubular radial flowers and fewer lines of symmetry) are not close relatives; yet these two groups nonetheless have similar P:L ratios and were visited by similar communities of pollen-collecting bees. Similarly, tubular radially symmetric flowers (e.g., Phacelia) and bilaterally symmetric keel flowers (e.g., Lupinus) with high P:L ratios shared similar communities of pollen-collecting bees. By the same token, plants with different pollen nutrition with similar morphologies were visited by disparate communities of bee subgenera. For example, among similarly shaped and highly related flowers, such as members of Asteraceae, Cirsium exhibited a higher P:L ratio than its relatives and was associated with a distinct community of bee visitors. Likewise, within Fabaceae, all of which have highly complex (keel petal) morphology, Lotus had very different P:L ratios than its relatives and was host to a different community of pollen-foraging visitors.

**Nutritional Niches and Network Structure.** Bees must navigate a variable environment in search of an appropriate diet that has direct fitness consequences (16, 50, 61). We know from manipulative experiments that pollen macronutrient content plays a key role in multiple dimensions of bee performance: For example, pollen protein and lipid content shape ovary activation and egg weight, larval weight and survival, learning and memory, adult immune function and survival, and colony reproduction (reviewed in ref. 11). Given this, we asked what role the pollen nutritional landscape found in natural communities played in structuring interactions with wild bees and found that explicit consideration of pollen macronutrient content shed light on bee–flower interaction networks in three ways.

First (Question 1), we asked how likely nutritionally similar plants are to be visited by similar bee communities. Our analysis revealed a diversity of strategies in how bees may achieve their macronutrient targets, via differing mechanisms of nutrient balancing. Namely, some similar bee communities (i.e., points near each other on PC1) are associated with plants that offer a range of pollen nutritional values (Figs. 4 and 5), indicating that some bees forage widely around their nutritional targets (if bees only foraged close to their nutritional target, we would expect to see the plant species’ PC scores to lie close to the line of regression), perhaps even exhibiting a particular level of nutritional tolerance (33) (e.g., Halictidae genera Lasioglossum and Halictus, Fig. 5). In these cases, it may be that no single plant species’ pollen aligns with bees’ nutritional needs. Consistent with this pattern, we show that even some bee species classically considered to specialize on pollen from a restricted number of host plants (i.e., those considered oligolectic) do not forage from taxa strictly close to a single macronutrient ratio (e.g., O. cornifrons exhibits preferences for Fabaceae and Rosaceae which differ in P:L values (62), or Megachile here visiting Fabaceae and Asteraceae at opposite ends of the P:L spectrum).

Second (Question 2), we asked what role pollen nutrition plays in structuring broad patterns of bee–flower interaction networks. By assigning the most frequently interacting groups of bees and plants to modules and determining that these modules differ in pollen P:L ratios, we demonstrate that, even with environmental and community variation across our sites, the broad structure of the interaction network is comprised of nutritional niches (44, 63) (Fig. 5), providing an estimate of bees’ nutritional intake targets and foraging strategies to obtain them. At a community level, bee nutritional niches may be established by bees sharing resources in a landscape yet occupying distinct nutritional space by collecting different macronutrient ratios based on collection frequencies or quantities from pollen hosts (36): e.g., compare Bombus and Melanomia to Dialictus and Lasioglossum in Fig. 5. For example, bees and plants associated with the module characterized by the largest mean P:L value had the widest distribution of plants’ P:L values, yet this module housed many of the most morphologically complex flowers (Fig. 5). Perhaps bees in this module, while requiring high protein pollen, still need to obtain lipids from plants to balance their high protein diet, resulting in visitation to a wider distribution of nutritional values. On the opposite end, we see a high level of nutritional specialization associated with the lowest P:L value module. This module was dominated by the family Asteraceae, and the bees foraged very narrowly within this group (64, 65). Finally, the modules with the highest numbers of plant and bee subgenera occupied intermediate P:L values and housed a spectrum of bees with both locally observed generalist and specialist foraging patterns. Supporting previous research, bumble bees (Bombus spp.) occupied the highest P:L module confirming they were assigned the niche shown in laboratory-based experiments to support their developmental needs (14–16, 18, 19). Interestingly, we found that related bee subgenera frequently differed in their assigned modules and potential intake targets (e.g., Andrena, Perdita, Osmia, Anthophora, etc. Fig. 5) indicating a potential mechanism of dietary adaptive radiation.

Finally (Question 3), we found that differences in pollen nutrition did not predict the strength or generalization of plants’ roles in the network (Fig. 6 and SI Appendix, Fig. S7): Plants from any nutritional niche can be specialist or generalist, be of equal strength within an interaction network, and support diverse bee
communities. Further, the hypothesis that the most intermediate P:L values could support most bees by overlapping with most intake targets, and therefore create resilience in the network, was not supported here, nor was the hypothesis that high protein or high lipid content would drive preferences of most bee species (based on the presumption that a single macronutrient’s concentration equates to quality).

Although these analyses suggest pollen macronutrients help structure bee–plant interactions, it is likely that additional ecological factors and reward traits operate alongside pollen to shape visitation patterns. For instance, competition among communities of floral visitors can influence which plants are accessible at any given time (66). Our network thus represents the realized, rather than fundamental, diet choices of bees; from a metanetwork perspective, differing levels and patterns of competition across sites could explain some of the intraspecific variation in floral visitation we observed. Like other foragers who must cope with suboptimal dietary prospects (for many reasons, including competition), bees might choose alternative yet still complementary hosts in pursuit of their nutritional target, as predicted by nutritional geometry theory (3, 36). A second important consideration relates to the chemical complexity of floral resources. While we focused here on pollen protein and lipids due to the evidence of their functional relevance to bee reproduction and floral choice (11), pollen micronutrients, such as essential fatty acids (51–53), amino acids (17, 35, 56), and sterols (50), as well as microbial symbionts (67), and specialized metabolites (21, 22), may also guide foraging preferences, but were not analyzed here. The degree to which chemical or physical pollen defenses covary with macronutrient content within an ecological community is an obvious but, to our knowledge, completely open question worthy of future consideration. More broadly, while we limited our observations to pollen-collecting bees, nectar collection often occurs alongside that of pollen, potentially adding some degree of noise to the visitation dataset as nectar traits can influence floral choice and response to pollen chemistry (68). Analysis of individual bee pollen loads to determine the mix of collected pollen would help refine our estimates of bee species-specific nutritional values (32) (SI Appendix, SI 4).

Potential Applications. The nutritional insights our study reveals may improve our ability to predict plant–pollinator interactions in altered landscapes, design habitat restoration protocols, and inform conservation efforts. A major concern in addressing wild bee population declines is the simple question, what plants to plant for bees? There are many factors to consider (10, 31, 69–72), but pollen is a critical nutritional resource for bees that our work shows deserves urgent attention. Beyond its relevance to larval and adult performance, floral nutrition can buffer bees against pathogens, environmental stress, and pesticides (20, 33, 34, 73–78). Here, we found that pollen nutrition across its community distribution attracted a diversity of bee visitors, and thus, pollen nutritional diversity may be critical for supporting bees that occupy different nutritional niches. In short, when it comes to pollen, a single plant species is unlikely to satisfy the nutritional needs of bee communities, even if it attracts the highest species richness, as a single pollen nutritional value may not represent the same quality for all bee species. As nutritional diversity was widely distributed in the natural systems we surveyed in this study, supporting bee functional and phylogenetic diversity in working lands may be in part accomplished through plant lists that represent the span of macronutrient values observed here or measured at baseline conditions specific to a region. The potential for plasticity in these critical dimensions of pollen chemistry and their effects on pollinators is a critical next step of research in the face of climate change.
architecture using categories modified from ref. 88: “open” (open/bowl flow-
ers with anthers fully exposed), “tubular” (tubular flowers with more recessed
anthers), and “complex” (keel and poricidal flowers where pollen is not exposed). Finally, we categorized floral symmetry as radial (actinomorphic, ≥2 planes of
symmetry) or bilateral (zygomorphic, 1 plane of symmetry). For each species, we
measured 5 to 10 individual flowers to estimate floral size, including average
width (corolla diameter) and depth (from flower base to anther tip; Dataset S1C).

**Pollen collection and nutritional analysis.** We analyzed a total of 109 species
for pollen nutritional content. We harvested at least 10 mg pollen by hand from
each plant species using techniques described in ref. 11 that include collecting
pollen from flowers directly or extracting pollen from anthers (SI Appendix, SI 2
and Dataset S1C). We determined fresh pollen protein and lipid concentration
(μg/mg) and P:L ratios via established protocols slightly modified from ref. 11
(see the step–by-step protocol in SI Appendix, SI 2). Briefly, three 1 mg replicates
from pooled pollen samples were used for both pollen and lipid analysis. Protein
concentration was analyzed with the Bradford assay using a NaOH extraction
buffer for 24 h (disrupting ~78% of pollen grains; see table in SI Appendix, SI 2),
and lipid content via SPV using chloroform:methanol:water lipid extraction. SPV
lipid analysis is commonly used to estimate total lipid content but may be biased
in the direction of unsaturated fatty acids and exclude undigestible pollen lipid components
e.g., sporopollenin of the pollen exine). It may thus underestimate the total
lipid content of pollen (54). However, these digestible unsaturated fatty acids
e.g., omega-3, omega-6, omega-9) are deemed important for bee health and
perception (51–53), and thus comparative values across plant species have direct
relevance to behavior and physiology.

**Data analysis.**

**Question 1.**

**Principal components of bee–wildflower visitation.** The pollen-collecting
bee–flower interaction matrix contained 2,261 interactions between 101 plant
species and 73 bee subgenera (Dataset S1D). We standardized the interaction
matrix by dividing the number of bees in each subgenus by the total number of
bees per minute observing each plant species to create a visitation frequency matrix
(plant species by number of bees observed per minute observation). We conducted
PCA on this interaction frequency matrix using the R package “vegan” (function
rda and with row total standardization using the function decorand (89)). Site
scores represent individual plant species and their associated community of bee
visitors where distance along either PC axis represents similarity in the composi-
tion of these communities. We used these scores as response variables in further
analyses (Dataset S1C).

**Factor analysis.** The latent variables “nutrition” and “morphology” were esti-
mated via factor analysis in JMP Pro v16 (SAS Institute Inc., Cary, NC). For nutrition,
we used standard scores of plant species’ pollen protein μg/mg, lipid μg/mg, and
P:L ratio. For morphology, we used standard scores of floral width and depth;
floral symmetry was scored as 0 for radial or 1 for bilateral; floral shape of open/
tubular vs. complex was scored as 0 or 1, and floral shape as open vs. complex/
tubular as 0 or 1 (Dataset S1C). We first used exploratory factor analysis of two
factors including all variables to determine factor loadings and correlations. We
then assigned variables to two separate factors associated with nutrition (protein
concentration, lipid concentration, and P:L ratio) and morphology (symmetry, morphology, depth, and width), such that the effects of nutrition and morphology
could be analyzed independently (SI Appendix, Fig. S6). We exported plant
species factor scores for both nutrition and morphology to be used in further
analyses (Dataset S1Q).

**Plant phylogeny.** We tested for phylogenetic signal (Pagel’s λ and Blomberg’s
K) in pollen nutrition using the R packages “ape” (90), “pez” (91), and “phytools”
(92). We pruned the plant phylogenetic tree published by Smith and Groom (93)
for plant species included in our dataset (Dataset S1Q). For species not in the
tree, we chose closely related plant species in the same genus (only replacing
Eremalche where Sidalcea was unavailable). For plants that we did not identify
to species (e.g., Salix), we chose a species in the tree that occupied our study
region. We created a between-species phylogenetic distance matrix using the
cophenetic function in base R “stats” package and standardized it by dividing all
pairwise distances by the maximum distance.

**Phylogenetic multiple regression.** We conducted Bayesian analyses using the
R package “rethinking” (94) which uses “Stan” (95) and Hamilton MCMC to deter-
mine posterior distributions. We analyzed the effect of nutrition and morphology
on the bee communities associated with each plant species (represented by plant
species’ PC1 or PC2 scores) in two separate ways (SI Appendix, Fig. S6 and SI 3): 1
nutrition represented by pollen P:L ratios and morphology represented by
floral symmetry (models 1 to 3), and 2) nutrition and morphology represented
by their factor analysis latent variable scores (models 4 to 6). We formulated three
models for each: 1) total effect of floral nutrition on bee community associated
with each plant species (models 1 and 4), 2) the stratified or counterfactual effect
of floral nutrition and floral morphology on bee community (models 2 and 5),
and 3) the stratified or counterfactual effect of floral nutrition and morphology
including phylogenetic covariance ([Ornstein–Uhlenbeck process (94) of these
factors on bee community (models 3 and 6)]. We used standardized scores of all
input and response variables for analysis.

**Question 2.** We used network module analysis to test the hypothesis that groups
of frequently interacting bees and plants represent nutritional niches. Modules
represent aggregated sets of interacting bees and plants, where within-module
interactions are generally stronger than between modules (44, 45). To approximate
nutritional niches, we analyzed the plants at the genus level because we wanted to
represent groups of plants and bees over space and time that may have not
been associated at a single site due to high levels of species turnover, and
because we wanted to account for their similarity in pollen nutrition revealed
through phylogenetic analysis. Using the R package “bipartite” (96–98) we
determined modules using the function metaComputeModules, iterating the
analysis 1,000 times; this analysis assigns each plant genus and bee subspecies
as a module identity. We repeated the analysis to verify consistency in module
identification. We conducted Bayesian analyses using the R package “rethink-
ing” and used adaptive priors to compare the distribution of P:L ratios of plant
genera between modules by 1) plant genus identity where each plant genus
within their assigned module was given equal weight, and 2) plant genus
weighted by frequency of bee visitation within each module such that plants
in the module that are more preferred or more visited (and likely contribute
more to bees’ nutrient balancing) are more accurately represented (Model 7,
SI Appendix, SI 3).

**Question 3.** To evaluate whether plant species differ in their level of speciali-
zation in interaction networks based on pollen nutrition, we used the package
“bipartite” and function specieslevel (97) to output the following indices for
our focal plant species in the visitation frequency metric: “species strength” (a
quantitative measure of a plant’s importance in the community via interaction
frequency to a diversity of pollinators (99), “effective partners” (a standardized
metric of bee diversity visiting each plant), “nested rank” (a metric of plant
specialization/generalization based on its discrimination from random selection
of partners (100), and both weighted and unweighted “betweenness centrality”
(the importance of a species as a connector in the network) and “closeness
centrality” (the proximity of a species to all other species, or importance
to connectedness of the network) (101). These indices were chosen as a diverse
set of typical analyses used to measure species-level specialization/generaliza-
tion from plant–pollinator networks. We then ran Bayesian analysis on the
linear effect of pollen nutrition on these indices using either P:L and symme-
try, or nutrition and morphology factor scores (see models 2 and 4). To test
whether plant species with pollens of intermediate values were more generalist
than those with extreme high or low values, we ran Bayesian analysis on the
quadratic effect of pollen nutrition on their network indices using either P:L or
nutrition factor scores (model 8).

**Data, Materials, and Software Availability.** All study data are included in the
article and/or supporting information.

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