Plants other than animal-pollinated herbs provide wild bees with vital nutrients

Michał Filipiak *

Life History Evolution Team, Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, ul. Gronostajowa 7, Kraków 30-387, Poland

ARTICLE INFO

Keywords:
Agri-environment scheme
Diet
Weed
Nutrition
Wildflowers
Resource

ABSTRACT

Adult pollinators that forage primarily for energy have traditionally guided our understanding of plant–pollinator interactions, leading to assumptions about the importance of different plants to pollinators. Consequently, pollinator conservation strategies potentially overlook plants that balance juvenile diets. I studied a representative bee, Osmia bicornis, to explore the contributions of various plants to the nutritional quality of a pollinator diet. Using ecological stoichiometry and micronutrient ecology, I investigated the proportions of vital body-building chemical elements (C, N, P, S, K, Na, Ca, Mg, Fe, Zn, Mn and Cu; henceforth “nutrients”) in larval pollen loads. I considered botanical origin, location and sexual dimorphism in the nutritional niche as factors potentially influencing nutrient proportions in larval diets. Redundancy analysis indicated that the main factor determining nutrient proportion was pollen botanical origin, which accounted for 70.17% of the variation; location contributed 20.21%, and larval sex contributed 8.43%. Among the 30 taxa composing pollen loads, the main determinants of nutrient content were oak, maple, chestnut, the cabbage family, buttercup, and grasses. Trees and wind-pollinated plants provided important nutrients. Oak and maple were the main contributors of nutrients essential for growth and body development (N, P, S, Cu, and Zn). Grasses and buttercups were the main contributors of Na, which is vital for bees. Complex habitat management schemes extending beyond traditional seed mixes are essential for pollinator conservation, as non-obvious plant species provide important nutrients. Trees and wind-pollinated plants should be included in pollinator conservation efforts to create a complex nutritional landscape enabling balanced diets.

1. Introduction

The provision of nutrients lays the foundation for animal pollination (Van der Kooi et al., 2021; Willmer, 2011). Pollinators visit flowers to forage, enabling individuals to survive and produce offspring and allowing the entire pollinator population to function at optimum levels (Barraud et al., 2022; Parreño et al., 2022; Vaudo et al., 2024). Examined in this context, pollinator decline is linked to the inability to balance diets with optimal proportions of nutrients (Parreño et al., 2022). This inability to balance diets is due to changes in nutritional landscapes, i.e., the spatial distribution of the available nutrient resources (Lau et al., 2023). Consequently, alterations in the nutritional landscape are the result of anthropogenic modifications to the landscape and habitats, accompanied by
**Box 1**

The atoms forming the chemical elements are the most fundamental level of biological organisation, as organisms build growing bodies from atoms (based on [Filipiak and Filipiak, 2022](#)).

Organisms are composed of organic compounds, which are in essence, mutable and composed of chemical elements. Consequently, there exists a stoichiometric formula that precisely delineates the chemical composition of an organism’s body ([Sterner and Elser, 2002](#)). Within this context, life is shaped by the flow of nutrients through the food web. All organisms are components of Earth’s biogeochemical system and can be considered a small cog in a large wheel in which nutrients, i.e., immutable atoms forming chemical elements, flow from one sink to another in a never-ending cycle. The bodies of organisms are such sinks of nutrients, the most basic and immutable building blocks of which are the atoms forming the chemical elements. The biogeochemical origin of life and the fundamental natural laws that drive the functioning of all living organisms give rise to one of the most striking features of life on Earth: all living organisms, from bacteria to whales, are composed of atoms comprising the same approximately 25 chemical elements but in differing proportions ([Kasperski and Powers, 2016; Williams and Rickaby, 2012](#)). Consequently, all of the diversity of life and all interactions between lifeforms that can be comprehended have their mechanical origin in 25 chemical elements endlessly cycling within food webs. It is therefore accepted that the body of any organism must be constructed in accordance with specific proportions of chemical elements if it is to function fully and effectively. Ultimately, the fitness of an organism and its ability to function properly are shaped by the ability to build an optimal body (nutritional demand) based on the proportion of chemical elements supplied in the food (nutritional supply; Fig. B1) ([Filipiak and Filipiak, 2022](#)).

The growth and development of every cell, tissue, organism and population is subject to the law of conservation of mass, which is based on the immutable nature of atoms. Thus, organisms build their bodies by relying on thousands of chemical reactions, all of which must be stoichiometrically balanced (Fig. B1). Although organisms are composed of organic molecules, the production of every molecule, and consequently, of the whole organism, is limited by the stoichiometry of each molecule. Therefore, any molecule vital for the functioning of a given organism is available to this organism only if the proportion of elements currently available in the environment allows the construction of that molecule. The abovementioned rule can be applied at various scales, from cells to organisms to populations and communities to ecosystems. Therefore, this principle is a key feature of life, unifying ecology across scales and linking organisms with their environments ([Filipiak and Filipiak, 2022](#)). Organisms faced with stoichiometric mismatches exploit nutritional niches within the available nutritional landscapes.

![Stoichiometric balancing of organismal growth and development](#)

**Fig. B1.** Stoichiometric balancing of organismal growth and development. Consumers ingest a prepackaged ratio of elements. If an organism forages for vital elements A and C, which are needed to maintain health and optimize fitness, it will never obtain only A and C from food. Instead, the whole composition of elements (A:B:C:D, etc.) will be provided in food-specific proportions that are either optimal or suboptimal for health and fitness of the organism. Stoichiometric mismatch, that is, incompatibility between the proportions of vital elements in food and those in the body of the consumer, has a negative effect on consumer fitness. (Figure source and detailed explanation: [Filipiak and Filipiak, 2022](#).)
shifts in species composition in the floras that provide floral resources to pollinators (Jones and Rader, 2022; Lau et al., 2023). Ultimately, human-driven modifications to landscapes and habitats lead to a change in the nutritional quality of the food available to pollinators, which has a detrimental impact on pollinator populations and communities, irrespective of alterations in the quantity of food available. This prompts the question of the contribution that individual plants make to the nutritional landscape for pollinators. Consequently, it is necessary to determine which plants should be given special attention in conservation measures oriented towards improving the quality of the pollinator food base.

The nutritional quality of food available to pollinators varies among plants in terms of chemical composition (Chau and Rehan, 2024; Lau et al., 2022; Vaudo et al., 2024). This means that different plants contribute differently to the nutritional balance of the pollinator’s diet, regardless of how often they are visited or how much food they provide. It is possible for a particular plant species to provide food that is rich in a key nutrient for a pollinator that uses this food as an essential but infrequent dietary supplement. Such sources of dietary balance are not obvious and are difficult to identify when we focus on the amount of food or the frequency of flower visits by pollinators. The roles of these species are even more challenging to recognize when pollinator research and conservation focus on a particular type of plant, such as perennial flowers. In contrast, pollinator diets are diverse and comprise more than only the obvious plant taxa that are typically included in pollinator conservation efforts. For example, pollinators readily use pollen from wind-pollinated plants, grasses, sedges and various non-obvious weeds (Joseph and Hardin, 2022; Rivermider et al., 2017; Saunders, 2018; Wilson et al., 2021). However, we do not know what role these food sources play in balancing pollinator diets and therefore to what extent they should be taken into account when planning and implementing conservation measures (Lau et al., 2023; Parreño et al., 2022). Within this context, understanding how different plants contribute to nutritional landscapes for pollinators is crucial for informing pollinator conservation efforts, related policies and management practices (Lau et al., 2023; Parreño et al., 2022; Pioletelli et al., 2024). In the following, I will refer to herbaceous animal-pollinated plants as ‘obvious’ pollinator-friendly plants, while ‘non-obvious’ plants will refer to trees and wind-pollinated plants.

Bee nutrition is primarily determined by the availability and nutritional quality of nectar and pollen sources (Danforth et al., 2019; Willmer, 2011). From the bee’s perspective, the chemical composition of pollen is crucial, as it is responsible for the correct development of the larva into a healthy adult of optimal body size with fully functioning organs (Filipiak et al., 2022; Leroy et al., 2023; Parreño et al., 2022; Rittschof and Denny, 2023). Pollen has a species-specific chemical profile in which proportions of vital nutrients may be nutritionally balanced or imbalanced (Parreño et al., 2022; Vaudo et al., 2024), and key plant species may provide bees with a balanced proportion of nutrients (Filipiak et al., 2022). Therefore, to consider the taxonomical diversity of pollen chemistry, I have studied the botanical origin of pollen gathered by wild bee adult females to be consumed by their larvae, the proportions of vital nutrients in the pollen and how these factors relate to each other.

To determine the mechanism underlying the contributions of different plant species to nutritional landscapes for pollinators, I used a representative model species with well-known biology, the wild solitary *Osmia* bee (Danforth et al., 2019; Michener, 2007). Among more than 20,000 living bee species, over 75% are solitary (Danforth et al., 2019), and depending on the geography, different *Osmia* species are used as model organisms in ecological and conservation biology studies (Eckerter et al., 2022; Nagamitsu et al., 2018; Słominski and Burkle, 2021; Yorstone et al., 2021). Furthermore, *Osmia* bees provide important ecosystem services for agriculture and forestry (Delaplane, 2021, 2023; Fliszkiewicz and Giedjasz, 2023; Kęy et al., 2023). *O. bicornis* is a highly efficient pollinator, and the pollination service provided by 500 specimens is equivalent to that provided by 2–4 colonies of honey bees (Splitl et al., 2022). The bee used in this study, *Osmia bicornis* L. (syn. *Osmia rufa* L., Hymenoptera: Megachilidae; red mason bee), is a generalist wild bee found in almost all of Europe, in the northern part of Africa and in western Asia (Splitl et al., 2022). As a generalist forager, *O. bicornis* is theoretically not dependent on any single plant species as a mandatory food source. For instance, Haider et al. (2014) reported that among 21 *Osmia* species, *O. bicornis* has the broadest host range, harvesting pollen from up to 19 plant families. This makes it an excellent model for investigating the roles of different plant species in providing a balanced proportion of nutrients in pollinator diets. The nutritional requirements of *O. bicornis* may differ between sexes (Filipiak, 2019; Filipiak and Filipiak, 2020); therefore, I have considered potential sexual dimorphism in the nutritional niche of *O. bicornis*.

To provide relevant information for pollinator conservation, it is important that studies be set in an ecologically relevant framework. To that end, I used the frameworks of ecological stoichiometry (Sterner and Elser, 2002) and micronutrient ecology (Kaspari, 2021); these frameworks can link bees with the chemical composition of their larval food, pollen, which is produced by plants from vital chemical elements mobilized from the environment (Filipiak and Filipiak, 2022). This approach allows the nutritional quality of food to be described in terms of the proportions of chemical elements (Box 1). Based on the law of conservation of mass and the stoichiometric balance of biogeochemical reactions, an organic nutrient will be available to a consumer only if the proportions of chemical elements currently available in the environment allow the nutrient to be synthesized (Filipiak and Filipiak, 2022; Box 1). Similarly, the chemical reactions leading to the production of fully functional cells, tissues, organs, and organisms must be stoichiometrically balanced (Sterner and Elser, 2002; Box 1). Within this context, organisms are limited by stoichiometric mismatches between the organismal demand for a species-specific proportion of body-building chemical elements and the environmental supply of these elements in consumed food (Filipiak and Filipiak, 2022; Box 1). As a result, organisms occupy unique multidimensional stoichiometric niches (Atkinson et al., 2020; González et al., 2017, 2018) defined by the proportion of multiple elements composing their bodies (Warnke et al., 2023; Xiao et al., 2024; Zhang et al., 2022). Thus, changes in resources may lead to stoichiometric mismatches experienced by individuals, where stoichiometric mismatches in specific organismal body-building elements between the requirements of the organism and the composition of consumed food negatively influence organismal populations and communities (Filipiak and Filipiak, 2022; Zhang et al., 2023; Zhang et al., 2024). Therefore, there exists species-specific variability in the tolerable proportion of elements in food (here, pollen) consumed by an organism (here, a wild bee), resulting in species-specific nutritional niches (Parreño et al., 2022).
My aim is to explain the role of different plants in creating nutritional landscapes for pollinators. I studied the proportions of 12 vital chemical elements (C, N, P, S, K, Na, Ca, Mg, Fe, Zn, Mn, and Cu; henceforth “nutrients”) in pollen collected by the wild bee O. bicornis for consumption by its larvae. My hypotheses are (1) that the proportion of nutrients forming the larval food of O. bicornis remains constant at various sites, (2) that this proportion is sex specific, (3) that this proportion depends predominantly on the taxa of pollen constituting the larval food, and (4) that pollen taxa other than animal-pollinated herbaceous plants contribute to the proportion of nutrients forming the larval food. I discuss the results considering their implications for pollinator conservation and related land management policies and practices that are currently focused on herbaceous animal-pollinated plants.

2. Materials and methods

2.1. Study sites and pollen sampling

The study was conducted in central and southern Poland (central Europe). The region is located in a moderate climate zone (Blazejczyk, 2006) (Fig. A1). Five sites with floristically diverse local conditions and land cover were selected. Local floral communities were inspected at each site, and each site was characterized based on the analysis of land cover maps (resolution of 1 m²) within a 500 m radius around the midpoint where the trap nest was located (see Supplementary materials for details). The 500 m radius around each nest reflects the usual O. bicornis foraging range (Gathmann and Tscharntke, 2002). Based on inspection and analysis, the following names were established to distinguish sites: urban green space (site 1), sub-urban wet meadow (site 2), rural dry meadow (site 3), sub-urban green space (site 4), and rural forest edge (site 5) (see Table A1).

To obtain pollen, a trap nest containing 150 empty Phragmites reed tubes (20–30 cm long, 0.5–1 cm in diameter, open on one side) was placed at each site. One reed tube was considered one replicate, representing pollen collected by a single bee. At each trap nest, 1000 cocoons containing O. bicornis specimens were placed in late April 2022 (supplier: BioDar; https://www.shop.biodar.com.pl/). The space inside the tubes offered a desirable nesting space for mother bees to form larval cells. A larval cell is a chamber inside a tube in which the female deposits collected pollen, lays an egg and then closes the chamber with a clay septum (Splitt et al., 2022). The female determines the sex of the egg by fertilizing it or not (Filipiak, 2018). Distinguishing between the sexes is easy because the daughter eggs are laid first and the son eggs are laid closer to the opening of the tube; additionally, the eggs of the daughters are supplied with more pollen than those of the sons (Filipiak, 2018; Splitt et al., 2022). Larval cells are provisioned by solitary bee mothers with pollen from plants in the area. The species composition of available flowering plants depends on the local flora and changes throughout the season. The study was therefore designed to cover the widest possible range of pollen collected by this generalist bee.

To ensure the collection of as wide a range of pollen as possible by the bee, the trap nests were inspected twice. The inspection period covered six weeks of pollen collection, corresponding to the natural activity period of O. bicornis, and covered the flowering periods of a variety of plants flowering from late April to early June. Trap nests were inspected for the first time approximately 3 weeks

Table 1
Summary of the RDA results. Canonical eigenvalues correspond to the percentage of variation explained by the explanatory variables. The response variables are the concentrations of nutrients (C, N, P, S, K, Na, Ca, Mg, Fe, Zn, Mn and Cu) in pollen loads. The explanatory variables are botanical origin (percentages of pollen grains belonging to 30 different taxa found in pollen loads), sampling site (five different sites) and larval sex (sex of the bee larva for which the pollen load was prepared by its mother). N = 60 (number of all investigated pollen loads). Full model: the effects of the botanical composition of pollen loads, sampling site, and larval sex on the variation in nutrient proportions in pollen loads. Partial models: the separate effects of (A) botanical origin, (B) sampling site, and (C) larval sex on the variation in nutrient proportions in pollen loads. Figure numbers correspond to the graphical representations of the RDA results shown below.

<table>
<thead>
<tr>
<th>RDA model</th>
<th>F</th>
<th>p</th>
<th>Canonical eigenvalues</th>
<th>Statistics summary for axes 1–4</th>
<th>Axis 1</th>
<th>Axis 2</th>
<th>Axis 3</th>
<th>Axis 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full model</td>
<td>2.7</td>
<td>0.002</td>
<td>78.41%</td>
<td>Eigenvalues</td>
<td>0.2061</td>
<td>0.1418</td>
<td>0.1072</td>
<td>0.0984</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cumulative explained variation</td>
<td>20.61</td>
<td>34.80</td>
<td>45.51</td>
<td>55.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pseudo-canonical correlation</td>
<td>0.9645</td>
<td>0.9595</td>
<td>0.945</td>
<td>0.9171</td>
</tr>
<tr>
<td>Botanical origin</td>
<td>2.4</td>
<td>0.002</td>
<td>70.17%</td>
<td>Eigenvalues</td>
<td>0.1988</td>
<td>0.1297</td>
<td>0.1028</td>
<td>0.0896</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cumulative explained variation</td>
<td>19.88</td>
<td>32.84</td>
<td>43.12</td>
<td>52.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pseudo-canonical correlation</td>
<td>0.9494</td>
<td>0.9292</td>
<td>0.9248</td>
<td>0.8748</td>
</tr>
<tr>
<td>Sampling site</td>
<td>3.5</td>
<td>0.002</td>
<td>20.21%</td>
<td>Eigenvalues</td>
<td>0.0953</td>
<td>0.0681</td>
<td>0.025</td>
<td>0.0136</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cumulative explained variation</td>
<td>9.53</td>
<td>16.34</td>
<td>18.85</td>
<td>20.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pseudo-canonical correlation</td>
<td>0.7834</td>
<td>0.6919</td>
<td>0.4881</td>
<td>0.4501</td>
</tr>
<tr>
<td>Larval sex</td>
<td>5.3</td>
<td>0.002</td>
<td>8.43%</td>
<td>Eigenvalues</td>
<td>0.0843</td>
<td>0.1964</td>
<td>0.1505</td>
<td>0.1236</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cumulative explained variation</td>
<td>8.43</td>
<td>28.07</td>
<td>43.12</td>
<td>55.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pseudo-canonical correlation</td>
<td>0.705</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
after the beginning of the study and for the second time approximately 6 weeks after the beginning of the study. After 3 weeks, all tubes were removed from each trap nest, and 150 new (completely empty) tubes, as well as 1000 new cocoons, were placed in the trap nests. Three tubes for each trap nest and sampling point were randomly selected from the removed tubes (i.e., after 3 and 6 weeks). Tubes filled with larval cells were selected for pollen collection; complete filling of a tube was easily observed because filled tubes were closed by the bee with a clay plug at the end. The larval cells in each tube were counted, and certain tubes were selected for further analysis. If the number of cells was less than 10, the tube was discarded, and another tube was selected. There were between 12 and 18 larval cells in the tubes selected for further analysis. From each tube, 4 posterior (female) and 4 anterior (male) larval cells were selected. All the deposited pollen in these cells was collected. Subsequently, the pollen from each set of four larval cells (i.e., two pollen pools per tube, one female and one male) was pooled and homogenized to obtain a suitably sized sample for further analysis. Note that solitary bees do not form colonies. For each sampling site, six reed tubes, each of which had been used by a different female bee, were sampled. Thus, for each site and sex, \( n = 6 \) true replicates (i.e., tubes) were obtained. The remaining tubes were used for another study. In total, 60 samples were analysed, \( (6 \text{ samples} \times 5 \text{ sites} \times 2 \text{ sexes}) \).

2.2. Chemical analyses

Each homogenized analytical sample was vacuum dried to a dry mass (dm), and three subsamples were obtained: (1) approximately 250–300 mg dm was used to prepare a liquid solution subsample and digested in a 4:1 solution of nitric acid (70%) and perchloric acid (65%) using a hotplate for analysis of the concentrations of K, Ca, Mg, Fe, Zn, Mn, Cu, Na and P. Once the digestion was complete, the acid was evaporated, and the samples were reconstituted in 30 ml of deionized water; (2) approximately 6–8 mg dm was used for direct analysis of C, N and S concentrations; and (3) the remainder was used for analysis of the botanical origin of the pollen. Carbon, N and S concentrations were determined using a Vario EL III Elementar automatic CHNS analyser; K, Ca, Mg, Fe, Zn, Mn, Cu and Na were determined by atomic absorption spectrometry (Perkin-Elmer AAnalyst 200 and Perkin-Elmer AAnalyst 800); and P was determined by colorimetry (MLE FIA). The following reference materials were used: NIST SRM 1575a, NIST SRM 1577c, NRC DOLT-5, and NRC BOVM-1. The botanical analysis involved identifying the species present in each pollen pool by counting the percentage of pollen grains of each taxon observed under a microscope as described by Splitt et al. (2021).

2.3. Statistical analyses

I analysed the data via redundancy analysis (RDA) in Canoco 5 (Smilauer and Leps, 2014). RDA is often described as a direct extension of multiple regression because RDA models the effect of an explanatory matrix X on a response matrix Y (Capblancq and Forester, 2021; Zuur et al., 2007). This approach allowed me to investigate the relationship between the response variables (i.e., nutrient concentrations) and explanatory variables (i.e., pollen botanical origin, site where pollen was collected, and sex of the larvae for which the pollen was collected). Because the p value for the whole model was significant \( (p < 0.05) \), I performed separate RDAs to test each hypothesis. To test the first hypothesis, I investigated the relationship between nutrient concentrations and site. To test the second hypothesis, I explored the relationship between nutrient concentrations and larval sex. Then, I tested the third hypothesis by investigating the relationship between nutrient concentrations and the botanical origin of pollen loads (percentages of pollen grains belonging to each of 30 taxa composing the pollen loads). Finally, to explore the contributions of animal-pollinated herbaceous plants

![Fig. 1. Effects of the botanical composition of pollen loads, sampling site, and larval sex on the variation in nutrient proportions. Redundancy analysis (RDA) plot (explanatory variables: concentrations of pollen species in pollen loads, sampling site, and bee sex; response variables: nutrient concentrations; for details see Table 1). The first two axes are presented. The percentage of variation explained is given for both axes. The vector lengths represent the contribution to the pattern shown (longer vector = greater contribution). The direction of the vector shows the axis to which it contributes.](image-url)
vs. other, non-obvious plants to nutrient balance in the larval diet, I investigated the relationship between nutrient concentrations and the proportion of pollen grains belonging to 4 plant groups (animal-pollinated herbaceous plants, wind-pollinated herbaceous plants, animal-pollinated trees and shrubs, and wind-pollinated trees and shrubs) in pollen loads.

3. Results

I analysed how the nutrient proportions in pollen loads are influenced by botanical composition, sampling site, and larval sex to assess the role of different plants in creating nutritional landscapes for pollinators. The proportion of nutrients in pollen loads varied with botanical composition, sampling site and larval sex (Table 1 and Fig. 1). The relationships of the botanical composition of pollen loads, sampling site and larval sex with nutrient proportion together explained 78.41% of the variation (Table 1). The factor most strongly corresponding to pollen load nutrient proportion was botanical composition, which accounted for 70.17% of the total variation (Table 1). Sampling site accounted for 20.21%, and larval sex accounted for 8.43% of the variation (Table 1).

Botanical composition significantly determined the nutrient composition of pollen loads (Table 1), with different plant taxa contributing differently to the concentrations and ratios of all the studied nutrients (Fig. 2A). Among the 30 plant species found in various proportions in the pollen loads, 6 taxa (Acer, Aesculus, Brassicaceae, Poaceae, Quercus, and Ranunculus) contributed the most to the pollen load nutrient proportions. The proportions of Quercus and Acer corresponded strongly to the concentration of N in pollen.
loads. The proportion of Quercus was also related to the concentrations of P, Cu, and S, and the proportion of Acer was related to the concentration of Zn. Brassicaceae were related to C, Poaceae and Ranunculus were related to Na, and Aesculus was related to K and Mg.

The effect of sampling site was weaker than the effect of botanical composition (Table 1) and affected 5 out of the 12 nutrients studied: Mg, Ca, K, and Mn, which had greater concentrations in pollen from site 1 than in pollen from all other sites; and Fe, which had the highest concentration in pollen from sites 4 and 5 and the lowest concentration in pollen from site 3 (Fig. 2B).

Male pollen loads were positively correlated with the proportions of Poaceae and Ranunculus and had higher Na concentrations than female pollen loads. Female pollen loads correlated positively with the proportion of Quercus and had higher concentrations of P, S, N, and C than male pollen loads (Figs. 1 and 2C).

The next step was to investigate the degree of nutritional contribution to pollen loads of obvious pollinator-friendly plants vs. other plants not considered obvious pollinator food sources. To that end, I analysed the effect of 4 groups of plants on the nutrient composition of pollen loads (RDA; Fig. 3). Animal-pollinated herbaceous plants, representing obvious pollinator-friendly plants, contributed strongly to the concentrations of C and Na in pollen loads. Wind-pollinated herbaceous plants contributed the most to the concentration of Na. Trees and shrubs contributed strongly to the concentration of N. Animal-pollinated trees contributed the most to the concentrations of Zn and K, while wind-pollinated trees contributed the most to the concentrations of P, Cu, and S.

To explore the tightness of the different nutrients studied in creating a nutritional niche, I calculated the variance of nutrient concentration in pollen loads. The concentrations of different nutrients in pollen loads showed specific nutrient variability (Table 2). The most conservative were the concentrations of C, N, P, S, Na, and Cu, with coefficients of variation below 0.3 for both females and males, whereas the Mg and Fe concentrations were the most variable, with coefficients of variation above 0.5 for both females and males (Table 2).


**Table 2**
Nutritional composition and variation in pollen loads collected by *O. bicornis*.

<table>
<thead>
<tr>
<th>Variables</th>
<th>% in dry mass</th>
<th>ppm in dry mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>N</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>49.57</td>
<td>4.54</td>
</tr>
<tr>
<td>Median</td>
<td>48.26</td>
<td>4.78</td>
</tr>
<tr>
<td>Min</td>
<td>44.85</td>
<td>2.98</td>
</tr>
<tr>
<td>Max</td>
<td>58.27</td>
<td>5.32</td>
</tr>
<tr>
<td>max/min</td>
<td>1.30</td>
<td>1.79</td>
</tr>
<tr>
<td>CV</td>
<td>0.08</td>
<td>0.14</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>46.53</td>
<td>4.11</td>
</tr>
<tr>
<td>Median</td>
<td>46.45</td>
<td>4.00</td>
</tr>
<tr>
<td>Min</td>
<td>40.81</td>
<td>2.74</td>
</tr>
<tr>
<td>Max</td>
<td>51.63</td>
<td>5.21</td>
</tr>
<tr>
<td>max/min</td>
<td>1.27</td>
<td>1.90</td>
</tr>
<tr>
<td>CV</td>
<td>0.05</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Abbreviations: CV: coefficient of variation; max/min: maximal concentration divided by the minimal concentration.
4. Discussion

My aim was to explain the roles of different plants in creating nutritional landscapes for pollinators. I have considered ‘obvious’ pollinator-friendly plants (herbaceous animal-pollinated plants) and ‘non-obvious’ plants (trees and wind-pollinated plants) as sources of vital nutrients that make up the diet of wild bee larvae. Non-obvious plants, including wind-pollinated trees and grasses, strongly influenced the concentrations of vital nutrients in the larval diet of *O. bicornis* and thus the nutritional quality of this diet. Only recently have we begun to explore the hidden potential value of different wind-pollinated plants to wild assemblages of different bees, and recent works on the subject have confirmed this value in trees (Allen and Davies, 2023; Cunningham-Minnick et al., 2023; Urban-Mead et al., 2023), grasses, sedges and various weeds (Rivernider et al., 2017; Saunders, 2018; Wilson et al., 2021). Until now, however, it has not been clear whether and how these plants contribute to improving the nutritional quality of the bee diet. Here, I have shown that the pollen of wind-pollinated grasses is an important source of Na, which is one of the most limiting nutrients for bees (Filipiak et al., 2023). Moreover, trees, including wind-pollinated *Quercus*, provide *O. bicornis* with the protein-building nutrients N, P, and S (da Silva and Williams, 2001).

My first hypothesis regarding the constant proportion of nutrients in the larval diet was partially confirmed. The proportion of most nutrients, except for Mg, Ca, Mn, Fe and K, was constant across sites. The effect of site-related variation in nutrient concentrations was low, accounting for 20.21% of the observed variance in nutrient proportions in pollen loads. It is likely that Mg, Ca, Mn, Fe and K are less important than other nutrients to bees. Mg, Mn, Fe and Ca concentrations had the highest variance in pollen loads, suggesting that their collection was not optimized. High K concentrations are detrimental or even potentially lethal to bees (Filipiak et al., 2023). There is a lack of published research on this topic that could be the subject of discussion; I am unaware of any studies investigating elemental concentrations in *O. bicornis* pollen from different areas.

The second hypothesis of sexual dimorphism in the nutritional niche of *O. bicornis* was confirmed. Female and male pollen loads were composed of similar pollen taxa, but the taxa were mixed in different proportions, resulting in sex-specific proportions of nutrients in pollen loads. Although statistically significant, sexual dimorphism contributed little to the nutritional niche realized by *O. bicornis*, explaining 8.43% of the observed variance in nutrient proportions in pollen loads. The nutritional requirements of *O. bicornis* females and males are distinct, with different proportions of nutrients in food being optimal for each sex (Filipiak, 2019; Filipiak and Filipiak, 2020). The proportion of nutrients supplied to the larvae by pollen influences fitness-related life history traits such as survival, body size and cocoon formation (Filipiak et al., 2022). This influence is sex-specific (Filipiak and Filipiak, 2020). The bee population inhabits the local flora, which consists of certain proportions of specific plant species. These specific proportions of given plants create a nutritional niche, the quality of which is related to the chemical composition of the pollen of these plants. The mother bees collect the pollen and decide whether or not to fertilise the egg, which determines the sex of the offspring. Therefore, the sex of the offspring may be a result of the species composition of the flora inhabited and may be biased towards either females or males in the specific landscape. In this way, the size and function of the bee population depends indirectly on the species composition and diversity of the flora and directly on the ability of the developing larvae to exploit an optimal nutritional niche (Filipiak et al., 2022).

As predicted in my third hypothesis, pollen taxa maintained the proportion of nutrients in larval food, as the botanical origin of pollen loads was the factor contributing the most to the nutritional niche, explaining 70.17% of the variance. Among the 30 pollen taxa, oak (*Quercus*; wind-pollinated tree), maple (*Acer*; animal-pollinated tree), chestnut (*Aesculus*, animal-pollinated tree), the cabbage family (*Brassicaceae*; animal-pollinated herbs), buttercup (*Ranunculus*; animal-pollinated herb), and grasses (*Poaceae*; wind-pollinated herbs) contributed the most to the nutritional composition of pollen loads, confirming my fourth hypothesis that non-obvious pollen taxa other than animal-pollinated herbaceous plants contribute to the proportion of nutrients in larval food.

Oak and maple contributed the highest proportions of major elements involved in growth and body development, i.e., N, P, and S (da Silva and Williams, 2001; Kaspari, 2021; Sterner and Elser, 2002). Oak and maple also contributed the most to the dietary proportions of two essential trace elements, i.e., Cu and Zn. For example, Cu is involved in chitin production, protective mechanisms and the formation of critical enzymes and proteins, while Zn is the most important ion involved in regulating cell metabolism; Zn enzymes control DNA expression, and Zn fingers enhance protein folding (da Silva and Williams, 2001). Grasses and buttercup contributed greatly to the Na proportion, which is crucial for bees because the generally high K:Na ratio in pollen is harmful to bees (Filipiak et al., 2023; Kaspari, 2020).

Interestingly, a high concentration of C in the pollen loads rich in *Brassicaceae* pollen was observed; however, this phenomenon is unlikely to be directly related to the chemical composition of the pollen. At the time of pollen load collection, *Brassica napus* monocultures flowered in the nest sites, where high levels of *B. napus* pollen were found in pollen loads. Most likely, bees use *B. napus* as a source of energy-rich nectar, which they include in larval provisions (Jauker et al., 2012; Yourstone et al., 2021). This could lead to an increased amount of C in the pollen loads, as nectar consists mostly of water and C-rich carbohydrates. Although nectar-derived carbohydrates are a source of energy and an important contributor to the overall nutritional balance of the bee diet (Austin and Gilbert, 2021), *B. napus* pollen is nutritionally unbalanced in terms of imposing a high stoichiometric mismatch on the bee (Filipiak, 2019). Because of this stoichiometric mismatch, overrepresentation of *B. napus* pollen in the diet of *O. bicornis* larvae is detrimental to bee growth, development and fitness, resulting in increased larval mortality, cocoon underdevelopment, and reduced adult body mass (Filipiak et al., 2022). Therefore, *B. napus* should not be interpreted as a species that provides vital nutrients in pollen for bee larvae.

Among the studied elements, the N, P, S, Na, and Cu concentrations had the lowest variance in pollen loads, suggesting their conservative selection. This is particularly noteworthy given the great variability in the concentrations of these elements in the environment, from the soil to pollen collected by bees (Filipiak et al., 2022; Kaspari, 2021; Penuelas et al., 2019). It is therefore possible that *O. bicornis* optimizes its pollen collection to acquire a balanced proportion of these key nutrients, which are necessary for optimal larval growth and development and ultimately for maximizing fitness. All five nutrients play crucial roles in organisal
growth and development (da Silva and Williams, 2001).

Pollinator studies have frequently emphasized the importance of floral diversity as a critical factor impacting pollinator diversity (Lane et al., 2020; Parreño et al., 2022). However, the functioning of organismal populations and communities is complex, and larger-scale phenomena such as floral diversity may not be the primary driver of observed patterns. Instead, basic chemical, physical, biological, and biogeochemical processes sometimes better explain these patterns (Bianchi, 2021; Huang and Salt, 2016; Kaspari, 2021). In this regard, the current study has demonstrated that wind-pollinated herbaceous plants and trees, which are not typically considered in conservation efforts aimed at enhancing the nutritional base of wild bees, play important roles in balancing the diet of the generality bee O. bicornis. Therefore, focusing solely on animal-pollinated or perennial flowering plants as sources of nutrients for pollinators may not be advantageous for all pollinators and may even contribute to nutritional imbalances in the diets of generality bees. These considerations should be accounted for when designing landscapes and planning urban areas.

Current strategies for the conservation of pollinators may be inadequate as practical land management measures are not being informed by advances in our understanding of the functional roles of plants for pollinators (Glenny et al., 2023). Consequently, there is still a lack of information on the best methods for pollinator conservation (Glenny et al., 2023). In this context, it is important to be aware that many of the recommended seed mixtures for pollinators may not be optimal for wild bees and therefore may not be fulfilling their function, as demonstrated, e.g., by (Gresty et al., 2018; Piko et al., 2021; Simanonok et al., 2021). This is why attempts have been made to create better seed mixtures (Kuppler et al., 2023; Nichols et al., 2022, 2023). These efforts should be applauded and taken into account in conservation policies and land management strategies while recognizing the complexity of pollinator nutritional ecology, which requires the inclusion of a complementary approach to seed mixtures that introduces additional, non-obvious sources of nutrients essential to pollinators. Consideration should therefore be given to wind-pollinated plants and plants that are not frequently visited by pollinator adults but are critical for providing important nutrients for pollinator juveniles. This calls for a comprehensive approach to creating nutritional landscapes for pollinators, which should include traditional seed mixes combined with non-obvious sources of nutrients, from grasses and herbaceous plants to shrubs and trees, both insect-pollinated and wind-pollinated, to provide both the nectar needed by adults and the pollen to balance the diet to ensure population sustainability. In this context, woody habitats (Edelkind-Vealey et al., 2024; Ulyshen et al., 2023), linear woodlots (Sobieraj-Betlińska et al., 2023), forest and grassland habitats (Edelkind-Vealey et al., 2024; Ulyshen et al., 2023; Vujanović et al., 2023) and patches of local habitats providing non-obvious forage resources (Dylewski et al., 2022; Joseph and Hardin, 2022; Twerd et al., 2019; Twerd and Sobieraj-Betlińska, 2020) are valuable tools for improving the food base of pollinators, complementing the widespread use of seed mixes with plants that contribute to the dietary balance of pollinators.

5. Conclusions

Even polylectic bees such as O. bicornis, which feeds on a diverse range of plant species, may depend on only a few specific plant taxa to balance their larval diet. Failure to consider such key plant species in conservation efforts aimed at improving the nutritional base of wild bees can result in nutritional imbalances in the diet of even generalist bees. Focusing conservation efforts on wind-pollinated plants as sources of nutrients for pollinators may be counterintuitive; however, a balanced nutritional landscape consisting of specific plant species that provide nutrients in optimal proportions is a crucial functional component of floral diversity. These considerations should be taken into account when designing landscapes and planning urban areas to create more nutritionally complex landscapes.

Role of the funding source

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contributions

MF conceived of the study, collected the field data, analysed the data, and wrote the manuscript.

Funding

Funding for this study was provided by the National Science Centre, Poland (2019/33/B/NZ8/01700) and Jagiellonian University, Faculty of Biology, Institute of Environmental Sciences (N18/DBS/00022).

CRedit authorship contribution statement

Michał Filipiak: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The author has no conflict of interest to report.
Data availability

Data are available in the article and in the supplementary material.

Acknowledgements

I thank my colleagues from the Institute of Environmental Sciences for their help with the spatial data analysis.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2024.e02984.

References


