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**Wild bee is an important contributor to the nutrient cycle, as shown by biogeochemical niche analysis of *Osmia bicornis***

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## **Abstract**

I aimed to elucidate nutrient cycling through the flower visitation pathway. Thus, I investigated factors affecting the proportions of essential nutrients (C, N, P, S, K, Na, Ca, Mg, Fe, Zn, Mn, and Cu) that comprise the biogeochemical niche of the wild solitary bee, *Osmia bicornis*. The main factor was pollen taxonomy, with a central role of key species, including wind-pollinated trees and grasses. These plants contribute to nutrient cycling in a hitherto unexplored way. Calculating the nutrient amount translocated and partitioned in the food web involving *O. bicornis*, I proposed a conceptual representation of nutrient cycling through the flower visitation pathway. By linking wild bee with its environment through nutrient cycling, this study provides new insight into the ecosystem services provided by bees. It also demonstrates the role of wind-pollinated plants in pollinator nutrition and highlights the importance of complex landscape conservation beyond the traditionally used seed mixes for bee conservation.

## INTRODUCTION

The development of life on Earth is strongly influenced by the way organisms exploit, consume, assimilate and allocate nutrients (Slansky & Rodriguez 1987). This is seen as one of the main driving factors in evolution and biodiversity (Williams & da Silva 2006; Williams & Rickaby 2012). Within this context, organismal traits associated with foraging and nutrient processing are under selection pressure and significantly impact the elemental composition of an organism (Filipiak & Filipiak 2022; Jeyasingh & Weider 2007). Consequently, the ionome, which means a whole set of ~25 elements involving organismal bodies (Jeyasingh *et al.* 2014; Salt *et al.* 2008; Williams & da Silva 2006), is subject to selection (Jeyasingh *et al.* 2014, 2023; Kay & Vrede 2008). With reference to these fundamental laws of nature, the concept of the biogeochemical niche has been developed (Peñuelas *et al.* 2008, 2019).

The biogeochemical niche is a concept that addresses the specific requirements and exploitation of bioelements by different organisms (Peñuelas *et al.* 2008, 2019). Bioelements are a group of approximately 25 chemical elements, all present in varying proportions in every organism living on Earth (da Silva & Williams 2001; Kaspari 2021). This species-specific proportion of organismal ionomes is determined by the organism's structure and function in a particular environment where it has evolved and reflects the nutritional needs of the organism for a specific proportion of elements needed to build its fully functional body (Elser *et al.* 2000; Filipiak & Filipiak 2022; Sterner & Elser 2002). Therefore, the biogeochemical niche is defined by a unique combination of bioelements based on the species' individual needs and use for optimal functioning and structure.

By having specific requirements for the proportions of elements that make up their food, consumers play a role in regulating the flow of nutrients (Atkinson *et al.* 2017; Elser & Urabe

1999; Vanni *et al.* 2002). Consequently, the concept of the biogeochemical niche links consumers to ecosystem nutrient cycling. This means also that nutrient cycling is consumer-driven, with organisms acting as nutrient carriers within nutrient cycles (Filipiak & Filipiak 2022; Kitchell *et al.* 1979). The concept of the biogeochemical niche is also referred to as the stoichiometric niche (González *et al.* 2017; Zhang *et al.* 2022). In this study, I used the concept of the biogeochemical niche/stoichiometric niche to understand how pollinator-host interactions contribute to nutrient cycling through the flower visitation pathway.

Although significant progress has been made in comprehending the significance of pollinators, there is still a considerable amount of unknown information regarding their ecological importance (Ollerton 2021). In particular, the flow of nutrients through flower pollination remains largely unstudied and undervalued despite its crucial role in comprehending the preservation of terrestrial biodiversity (Ollerton 2021). This circumstance was most likely due to the lack of the necessary theoretical foundations, which have now become available (Filipiak & Filipiak 2022; Kaspari 2021; Zhang *et al.* 2022).

To study how pollinator-plant interactions shape the pollinator's biogeochemical niche and nutrient flow through the flower visitation pathway, I used a representative model species with well-known biology, a wild solitary *Osmia* bee (Danforth *et al.* 2019; Michener 2007; Raw 1972). From 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; Slominski & Burkle 2021; Yourstone *et al.* 2021). *Osmia bicornis* L. (syn. *Osmia rufa* L., Hymenoptera: Megachilidae; red mason bee) used in this study is a generalist wild bee found in almost all of Europe, in the northern part of Africa and in western Asia (Splitt *et al.* 2022).

Bee nutrition is primarily determined by the availability and nutritional quality of nectar and pollen sources (Danforth *et al.* 2019; Willmer 2011). The dietary requirements of bees can be seen as an interplay between the needs of both their immature (larval) and adult (imago) stages. While the food of the larvae (mainly pollen) is high in nutrients necessary for growth and development, it is chemically distinct from the energy-rich food of the adult bees (primarily nectar) (Willmer 2011). From a food web perspective, the most important bee food that plays a role in nutrient cycling is pollen because it is a concentrated, highly nutritious food that contains high proportions of essential nutrients. Pollen has a species-specific chemical profile, which includes proportions of vital elements that may be nutritionally balanced or imbalanced for specific bee species (Parreño *et al.* 2022). Key plant species may provide bees with a balanced proportion of specific nutrients (Filipiak *et al.* 2022). Therefore, to consider the taxonomical diversity of pollen chemistry, I have studied the botanical origin of pollen gathered by *O. bicornis* adult females to be consumed by their larvae, the proportions of the elements it contains and how these relate to each other. The nutritional requirements of *O. bicornis* may differ between sexes (Filipiak *et al.* 2021); therefore, I have considered potential sexual dimorphism in the biogeochemical niche of *O. bicornis*.

My aim is to elucidate the biological mechanism responsible for nutrient flow through the flower visitation pathway. My hypotheses are (1) that the proportion of studied nutrients (C, N, P, S, K, Na, Ca, Mg, Fe, Zn, Mn, and Cu) found in the larval food of *O. bicornis* remains constant in various landscapes within the range of variation in the *O. bicornis* biogeochemical niche, (2) that this proportion is maintained using key plant species that provide a balanced supply of specific nutrients, and (3) that the biogeochemical niche of *O. bicornis* is sex specific. I discuss the results in the context of nutrient cycling through the flower visitation pathway.

Therefore, to contextualize the study within nutrient cycling, I calculate the amount of nutrients translocated and partitioned in the food web involving *O. bicornis*. On this basis, I provide a conceptual representation of nutrient cycling through the flower visitation pathway.

## **METHODS**

### ***Study sites and pollen sampling***

The study took place in five different ecosystems that were suitable for *O. bicornis* and floristically distinct ((1) a ruderal ecosystem in the city, (2) wet meadow, (3) grassland, (4) allotments, and (5) forest edge) (see Appendix S1 for details). 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 in each of these ecosystems. 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 habitat for mother bees to form larval cells. A larval cell is a chamber inside a tube in which the female deposits the 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. Distinguishing between the sexes is easy because the daughter eggs are laid first and 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 (Splitt *et al.* 2022). Larval cells are provisioned by solitary bee mothers with pollen available from plants in the area. The species composition of available flowering plants depends on the available flora and changes throughout the season. The study was therefore designed to cover the widest possible range of pollen collected by this generalist bee.

Trap nests were inspected twice, approximately 3 weeks after the beginning of the study and approximately 6 weeks after the beginning. After 3 weeks, all tubes were removed from each trap nest, and 150 new tubes - completely empty - and 1000 new cocoons, were placed in the nests. From the removed tubes, three tubes were randomly selected from each trap nest. These tubes were filled with larval cells (the complete filling of a tube is easily observed because the filled tube is closed by the bee with a clay plug at the end). After another 3 weeks (6 weeks after the start of the study), all the tubes were again removed from each trap nest, and three filled tubes were selected at random for further analysis. The number of larval cells was checked in each tube and selected for further analysis. If the number was less than 10, the tube was discarded, and another tube was taken. 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. From these cells, all the deposited pollen was collected. Pollen from each of these four larval cells was then pooled (i.e., two pollen pools per tube, one female and one male) and homogenized to obtain a suitably sized sample for further analysis. In total, 60 analytical samples were used.

### ***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, digested in 4:1 solution of nitric acid (70%) and perchloric acid (65%) using a hotplate, suitable for analysis of the concentrations of K, Ca, Mg, Fe, Zn, Mn, Cu, Na and P. Once the digestion was complete, all the acid was evaporated and the samples were filled with 30 ml of deionized water – this particular step is important to obtain accurate results when



measuring elemental concentrations, especially for Na and K, (2) approximately 6-8 mg dm was used for direct analysis of the 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 automatic CHNS analyzer; 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 that was observed under the microscope as described by Splitt *et al.* (2021). This method has been confirmed to be accurate in previous research (Filipiak *et al.* 2022).

### ***Statistical analyses***

The data were analyzed first using a permutation analysis of variance (PERMANOVA) in Past 4.12 (Hammer *et al.* 2001) to test whether pollen (1) botanical origin and (2) elemental composition differed between ecosystems and sexes. This analysis was complemented with a redundancy analysis (RDA) performed in Canoco 5 (Šmilauer & Lepš 2014). This allowed to investigate the relationship between three factors, i.e., (1) pollen botanical origin, (2) specific habitat where pollen was collected, (3) the sex of the larvae for which the pollen was collected, and pollen elemental composition.

## **RESULTS**

The botanical composition of the pollen loads varied with both habitat and bee sex, and the sex difference varied with habitat (indicated by a significant interaction between sex and habitat; Table 1). The proportion of elements composing pollen loads varied with both habitat and bee sex (Table 1). The RDA showed that these differences were driven mainly by *Quercus* preferred for females and associated with P, Cu and S concentrations, and *Ranunculus* and *Poaceae* (associated with Na), which together with *Acer* (associated with Zn) were preferred for males. *Brassicaceae*, associated with C and found in high proportions in habitat 3 (grassland), especially in female pollen loads, and with high N concentrations, were found in pollen loads collected in habitat 5 (forest edge). Importantly, the position of N on the plot between vectors associated with *Quercus* and *Acer* reflects the high N concentrations observed in both *Quercus*-rich and *Acer*-rich pollen loads. The relationships between the botanical composition of pollen loads, habitat and progeny sex on the elemental proportions together explained 34.80% of the variation (Fig. 1). Regarding the botanical composition of pollen loads, the interaction between sex and habitat (Table 1) was related mainly to the uniqueness of habitat 3 (grassland), for which much *Brassicaceae* pollen in the female larvae provisions occurred, and habitat 2 (wet meadow), for which the largest proportion of *Ranunculus* pollen in the male larvae provisions occurred. In all the habitats studied, there was a consistent effect of *Quercus* preference for female larvae associated with Cu, P, S and N concentrations and *Ranunculus* and *Poaceae* preference for male larvae associated with Na concentration (Fig. 1).

In the next step, I explored to what degree individual biological factors, i.e., (1) pollen botanical origin connected with taxonomy-specific nutritional composition, (2) habitat connected with environmental factors influencing plant chemistry, and (3) larval sex, which may be an indirect factor connected with sexual dimorphism in nutritional needs, explain the observed

variation in proportions of elements composing larval pollen loads (Fig. 1). Most of this variance (32.84%) was explained by the botanical origin of the pollen, followed by the habitat (16.34%), and sex contributed the least (8.43%) to the observed variance (Fig. 1). Magnesium, Ca and K concentrations showed the strongest relationship with the environment from all the elements studied, linked to their high concentrations in pollen loads collected in habitat 1 (ruderal) (Fig. 1). Sexual differences were mainly associated with relatively high concentrations of C, P, S and N in female pollen loads and relatively high concentrations of Na and K in male pollen loads (Fig. 1).

Since the elemental composition of pollen loads was mostly related to their botanical origin, I further explored this phenomenon to reveal central pollen taxa behind this effect. To that end, in the next analysis, I considered only pollen taxa whose number of grains exceeded 10% of the total pollen load. By employing this approach, the impact of rare species on the overall outcome was eliminated. In this case, the RDA of the influence of the botanical origin of pollen on its elemental composition explained 30.76% of the variance (Fig. 2). This analysis revealed that 17 pollen taxa, including wind-pollinated trees and grasses, as shown in Fig. 2, play a central role in shaping the biogeochemical niche of *O. bicornis*.

Overall, the proportion of 17 pollen taxa (*Acer*, *Aesculus*, *Brassicaceae*, *Carpinus*, *Frangula*, *Iris*, *Juglans*, *Lonicera*, *Malus*, *Papaver*, *Poaceae*, *Prunus*, *Quercus*, *Ranunculus*, *Rubus*, *Salix*, and *Viola*) and the habitat were the main determinants of the multielement stoichiometry of pollen loads consumed by *O. bicornis* in this study. An additional factor that shaped the pollen load stoichiometry was larval sex; however, this effect was indirect and related to high concentrations of *Quercus* and *Brassicaceae* in female pollen loads and high concentrations of *Ranunculus* and *Poaceae* in male pollen loads.

The concentrations of different elements in the pollen load showed specific 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 Mg and Fe concentrations were the most variable, with coefficients of variation above 0.5 for both females and males (Table 2).

## DISCUSSION

Vital nutrients are concentrated in pollen, making it an exceptionally rich source of sustenance for herbivores (Roulston & Cane 2000; Willmer 2011). For example, comparing concentrations of elements composing pollen with another important pathway of nutrient cycling, i.e., forest litter, it was shown that vital nutrients are concentrated in pollen approximately from 2 (for Cu) to 12 (for P) times more than in litter (with N and S 5-fold more concentrated in pollen) (Filipiak 2016). Therefore, to fully understand the results of this study, the context of the amounts of nutrients transported through the flower visitation pathway is needed. Accordingly, in Box 1 and Fig. 3, I present a conceptual representation of a bee's role in nutrient cycling, using *O. bicornis* as an example. The presented calculations (Box 1 and Fig. 3) suggest that the plant-pollen-consumer pathway plays a significant role in ecosystem nutrient cycling and demonstrate that animal-collected pollen can be an essential factor for nutrient inputs into the trophic network. Its significance is evident when compared to other pathways of nutrient cycling. For example, for just one bee species, i.e., *O. bicornis* studied here, masses of nutrients translocated from flowers to bee nests and ramifying further in the food web are similar to the nutrient consumptions by forest and grassland birds and surpass the annual production of ungulates (Box 1). These nutrients, mobilized by plants from the environment, allocated to pollen and consumed by bee

larvae, continue to propagate in the food web, shaping ecological processes and interactions (Filipiak & Filipiak 2022; Kaspari 2021). Therefore, the example of *O. bicornis* shows that even a single species of pollen-eating insect can, through pollen translocation and consumption, be a vector moving much nutrients in a food web. The discussion of the biogeochemical niche of *O. bicornis*, presented below, provides a first hint into understanding the flower visitation pathway of nutrient cycling.

The biogeochemical niche of *O. bicornis* in this study was shaped by key plant species ensuring a stable proportion of vital nutrients composing pollen loads consumed by bee larvae across different habitats. Wind-pollinated (anemophilous) trees and grasses contributed highly to the biogeochemical niche. This contradicts common beliefs about how bees function, according to which bees mainly feed on insect-pollinated (zoophilous) perennial flowers, but is confirmed by previous research showing *O. bicornis* preferences toward tree and grass pollen (Casanelles-Abella *et al.* 2022b; Eckerter *et al.* 2022; Splitt *et al.* 2022). 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 confirm this value regarding trees (Allen & 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). Thus, wind-pollinated plants may contribute to nutrient cycling in green food webs in a hitherto unrecognized way. Importantly, this finding shows that pollinators, such as bees, are not only involved in the plant-pollen-pollinator pathway of nutrient cycling but are also part of the more general plant-pollen-consumer pathway, involving pollen produced by both zoophilous and anemophilous plants.

My first hypothesis regarding the constant proportion of elements in the larval diet was partially confirmed. The proportion of most elements was constant, except for Mg, Ca and K, which showed higher concentrations in habitat 1, i.e., ruderal habitat in a city than in other studied habitats. As predicted in my second hypothesis, key plant species maintained this ratio, with oak (*Quercus*), maple (*Acer*), cabbage family (*Brassicaceae*), buttercup (*Ranunculus*), and grasses (*Poaceae*) contributing most, and chestnut (*Aesculus*), fruit trees (*Prunus* and *Malus*), black locust (*Robinia*), walnut (*Juglans*), poppy (*Papaver*), and *Viola* being additional important contributors. Pollen from all these plants not only strongly contributed to the observed variation in element proportions but was also found in pollen loads in proportions exceeding 10%. Importantly, the botanical origin of pollen loads was the factor contributing the most to the observed variance in elemental proportions in pollen loads, explaining 32.84% of the variance. The third hypothesis of sexual dimorphism in the biogeochemical niche of *O. bicornis* was confirmed. Female and male pollen loads were composed of similar pollen taxa but mixed in different proportions, resulting in sex-specific proportions of elements in pollen loads. However, sexual dimorphism contributed little to the biogeochemical niche realized by *O. bicornis*, explaining 8.43% of the observed variance in elemental proportions in pollen loads.

Pollen can help maintain food webs by providing nutrients to a wide range of organisms (Filipiak 2016). However, to date, there is no data on the proportions and amounts of nutrients moved through the flower visitation pathway of nutrient cycling (Ollerton 2021). The current study shows a high contribution of wind-pollinated plants to the diet of an obligate pollen-feeder, with oak and maple contributing most to dietary proportions of major elements involved in growth and body development (N, P, and S). Oak and maple additionally contributed the most to the dietary proportions of two essential trace elements, i.e., Cu, and Zn. Wind-pollinated grasses

contributed highly to the Na proportion, which is crucial for bees because of the generally high  $K:Na$  in plant tissues, which is harmful for herbivores (Filipiak *et al.* 2023; Kaspari 2020).

All living things depend heavily on N and P for their proper functioning, and the way these elements move and are recycled in the environment is largely controlled by biological mechanisms (Sterner & Elser 2002). These elements have therefore played (Williams & Rickaby 2012), and continue to play (Jeyasingh *et al.* 2023), an important role in biochemical evolution, shaping the life histories of organisms and their ecological interactions (Isanta-Navarro *et al.* 2022; Sterner & Elser 2002). Assuming that N and P have played an essential role in the evolution of plant-pollinator interactions due to their biological significance, it is reasonable to anticipate that animal-pollinated plants would exhibit higher concentrations of these elements in their pollen than wind-pollinated plants. The present study suggests that this may not always be the case. Wind-pollinated oak provided bees, especially females, with a high proportion of N and P and was often the dominant species in pollen loads collected by *O. bicornis*. Similarly, wind-pollinated maple provided bees with a high proportion of N. This highlights the previously unrecognized role of oak and maple in mobilizing N and P from the environment and introducing them into the terrestrial green food web.

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. *Brassica napus* monocultures flower in the nest sites at the time of pollen load collection, and thus, *B. napus* is most likely the main contributor to the pollen loads with *Brassicaceae*. 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.

Another particularly interesting element is Na, since herbivores cope with a generally high *K:Na* ratio in their food, potentially causing health problems, underdevelopment and reduced fitness (Filipiak *et al.* 2023; Kaspari 2020, 2021). Given current knowledge, it is not clear why grasses contribute to the Na concentration in the bee's diet: grasses are anemophilous, it is therefore not expected that grasses interact with pollinators regulating the nutritional composition of pollen. However, the contribution of *Ranunculus* to dietary Na may be interpreted considering plant-pollinator interaction. Plants in the genus *Ranunculus* are insect-pollinated, and perhaps the high Na content of their pollen is the result of a strategy to increase the likelihood of pollination, as suggested by (Finkelstein *et al.* 2022) and described by Filipiak *et al.* (2023). This hypothesis requires further research.

In all studied habitats, *O. bicornis* realized a consistent biogeochemical niche regarding the proportion of N, P, S, Na, Fe, Zn, Mn, and Cu. Of these elements, N, P, S, Na, and Cu concentrations had the lowest variance, suggesting their conservative selection. This is particularly noteworthy given the great variability in concentrations of these elements in the environment, from the soil to the pollen collected by bees (Filipiak *et al.* 2022; Kaspari 2021; Peñuelas *et al.* 2019). It is therefore possible that *O. bicornis* optimizes its pollen collection to acquire a balanced proportion of key nutrients, which are necessary for optimal larval growth and development and ultimately for maximizing fitness. The biological mechanism underlying this optimization has already been proposed by Filipiak *et al.* (2022), who showed that the proportion of different vital elements composing pollen loads consumed by *O. bicornis* larvae is determined by the species composition of the pollen loads and affects bee fitness. In fact, in the



present study, the proportion of elements that make up the biogeochemical niche of *O. bicornis* was primarily shaped by the botanical origin of the collected pollen loads. This suggests that nutrient cycling through the flower visitation pathways is driven by the nutritional needs of the pollen consumer, in this case, *O. bicornis*, which preferentially collects pollen species to obtain nutritionally balanced larval food.

Animals can have a major impact on nutrient availability in ecosystems (Herzog *et al.* 2023; Atkinson & Forshay 2022; Clay *et al.* 2013; McInturf *et al.* 2019). All living things are part of the biogeochemical cycle of the Earth and can be thought of as small cogs in the great wheel of atoms flowing from one sink to another in an endless circle (Kaspari 2021; Schlesinger & Bernhardt 2020; Vernadsky 1924). However, we know little about the function of different organisms as vectors composed of a set of energy-driven chemical elements that are alive, need to optimize their own fitness, and additionally move and shape the environment, contributing to nutrient cycling through realized ecological interactions (Filipiak & Filipiak 2022). More recently, Ollerton (2021) pointed out that the role of bees and other pollinators in nutrient cycling in food webs and ecosystems has not yet been studied, and we have no knowledge of it. The current study suggests that the role of bees in nutrient cycling may be high and undervalued, making it one of the most important problems to be solved in ecology.

In recent years, the decline in pollinators has been attributed to poor nutrition resulting from landscape changes caused by human activities (LeBuhn & Vargas Luna 2021; Leroy *et al.*, 2023; López-Uribe *et al.* 2020; Parreño *et al.* 2022). Although this is a widely accepted view, the underlying mechanisms by which pollinators depend on their nutritional landscapes are not yet fully understood (Lau *et al.* 2023; Pioltelli *et al.* 2023; Stevenson *et al.* 2022). Thus, it is crucial to explore the dependence of pollinators on their nutritional landscapes in greater depth

(Gillespie *et al.* 2022; Leroy *et al.*, 2023; Stevenson *et al.* 2022), including the biology of the chemical elements (Lau *et al.* 2022; Ausma *et al.* 2021; van der Kooi *et al.* 2021). Pollinator studies have frequently emphasized the importance of floral diversity as a critical factor impacting pollinator diversity. 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 the observed patterns. Instead, basic chemical, physical, biological, and biogeochemical processes and phenomena often better explain these patterns (Bianchi 2021; Huang & Salt 2016; Kaspari 2021). In this regard, the current study has demonstrated that plant taxa such as oak, maple, willow, blackberry, fruit tree, and grass, which are not typically considered in conservation efforts aimed at enhancing the nutritional base of wild bees, play a critical role in balancing the diet of the generalist 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 generalist bees. These considerations should be accounted for when designing landscapes and planning urban areas.

## **CONCLUSIONS AND FUTURE DIRECTIONS**

The biology and ecology of *Osmia bicornis*, a model organism in bee and pollinator research, is well studied. Therefore, the present study could build on a solid foundation when discussing the results, leading to uniquely interesting conclusions regarding the potential role of a wild bee in nutrient cycling. However, it is difficult to generalize at this stage. Even closely related generalist solitary bee species differ in their preference for pollen species and their proportions (Casanelles-Abella *et al.* 2022a; Eckerter *et al.* 2022), which can translate into the proportion of

elements that make up their biogeochemical niches. As a result, varying amounts of different elements can be introduced into the food web with the participation of different bee species. It is not possible to apply the data and calculations presented here to all (more than 20,000 species) bees. They are mono-, oligo-, and poly-lectic, have different biology, and interact specifically with the environment and other organisms. As such, the following questions arise: How many different nutrients do various bee species in their various ecosystems translocate between flowers and other environmental compartments? How much of the acquired nutrients from the environment do plants allocate to pollen, and what are the life history trade-offs involved in doing so? How does the flower visitation pathway of nutrient cycling contribute to the functioning of the entire food web? What fraction of all matter in the ecosystem is cycling with the contribution of this pathway? Answering these questions will greatly enhance our understanding of bees' roles in ecosystem functions. The current focus is on bees' roles as pollinators, but this study showed that bees' roles go beyond pollination, and even a single species can importantly contribute to local-scale nutrient cycling.

This study also showed that wind-pollinated plants contribute to nutrient cycling in a previously unexplored way. Wind-pollinated plants introduce large amounts of vital elements directly into the terrestrial green food web through their pollen, which is consumed by bees. From a conservation perspective, by highlighting the important role of wind-pollinated plants, including trees and grasses, in the diet of a generalist wild bee, this study contributes to our understanding of how landscape change can affect pollinator decline.

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The funding sources had no involvement in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

## **AUTHOR CONTRIBUTIONS**

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

## BOXES

### **Box 1. A conceptual representation of the role of wild solitary bee in nutrient cycling in terrestrial ecosystem.**

Splitt *et al.* (2022) reviewed current knowledge on *O. bicornis* biology, providing the following information. In nature, *O. bicornis* live in groups of 50-250 individuals per site. An average population density of 15 females per 1 km<sup>2</sup> was estimated. On average, a single female will produce 30 larval cells, i.e., 30 offspring. The average dry mass of the collected pollen load is 155 mg for male larvae and 280 mg for females (Filipiak *et al.* 2021). Although *O. bicornis* invests available resources conditionally into its offspring sex, depending on environmental factors, level of parasitism, nutritional resources, and mother size and age (Seidelmann *et al.* 2010; Splitt *et al.* 2022), at the population level, this investment is balanced (Seidelmann *et al.* 2010); therefore, one can assume a 0.5-0.5 sex ratio of the model population as well as 0.5-0.5 production of female and male progeny. Consequently, combining the above information with the average concentrations of elements in the pollen loads examined in this study, it can be calculated that, under fully natural conditions, an average population of *O. bicornis* from a single site would translocate the following amounts of elements from plants to bee nests annually ((detailed explanation of how these values were calculated is given in Appendix S1; from 50 to 250 individuals = from 25 to 125 females \* (15 female larval cells and 15 male cells)): C 79.1–395.5 g; N 7.2–35.8 g; K 2.3–11.3 g; P 0.8–3.9 g; Ca 0.5–2.5 g; S 0.5–2.3 g; Mg 0.4–2.0 g; Na 42.1–210.5 mg; Fe 37.4–187.1 mg; Mn 6.9–34.3 mg; Zn 8.2–41.1 mg, and Cu 2.1–10.7 mg. Similarly, the amounts of elements translocated by the bees per square kilometer annually are C 47.5 g/km<sup>2</sup>; N 4.3 g/km<sup>2</sup>; K 1.4 g/km<sup>2</sup>; P 0.5 g/km<sup>2</sup>; Ca 0.3 g/km<sup>2</sup>; S 0.3 g/km<sup>2</sup>; Mg 0.2 g/km<sup>2</sup>;

Na 25.3 mg/km<sup>2</sup>; Fe 22.4 mg/km<sup>2</sup>; Mn 4.1 mg/km<sup>2</sup>; Zn 4.9 mg/km<sup>2</sup>; Cu 1.3 mg/km<sup>2</sup>. These amounts are considerable and significant for ecosystem functions. They are, e.g., in a similar range of values as the yearly consumption of elements by forest and meadow birds, 100-1000 times higher than the amounts of elements in the annual production of forest rodents, small birds, carnivores, and rhizophagous insects, and 10-100 times higher than the annual production of ungulate and saproxylophage communities measured in the European forest (Grodzinski *et al.* 1984). Finally, these amounts, involving a single bee species, are 10-100 times lower than the annual input of elements through litterfall in forests, involving all litterfall-producing species (Grodzinski *et al.* 1984; Ukonmaanaho *et al.* 2008). Therefore, the flower visitation pathway of nutrient cycling is an important pathway for element cycling in ecosystems and is responsible for distributing much of the biomass.

Elements translocated by bees into their nests are further assimilated by larvae, ramifying through the green food web in newly built bee bodies or entering the brown food web as depleted cocoons and excreta. Filipiak *et al.* (2021) provided data on the assimilation rates of elements from consumed pollen loads to *O. bicornis* bodies, as well as on the dry masses and elemental concentrations of bee bodies, cocoons, and excreta. Using these data, it is possible to estimate the amounts of elements that, after ingestion by bee larvae from pollen loads, enter different compartments of the nutrient cycle. Based on these data and the elemental quantities presented above and assuming 33% mortality during development, as given by (Ostap-Chec *et al.* 2021), I provide a conceptual representation of the plant-pollen-pollinator pathway's contribution to nutrient cycling in an ecosystem in Fig. 3. A detailed explanation of the calculations is given in Appendix S1.

*O. bicornis* is used commercially as a crop pollinator (Bilinski & Teper 2004; Splitt *et al.* 2022; Teper & Bilinski 2009; Wilkaniec & Radajewska 1997), in hobby gardening or bred by private individuals (Gaston *et al.* 2005; Goulson 2021; Lemelin 2012). These activities can increase the natural population size by a factor of 2.8 (Gruber *et al.* 2011; Steffan-Dewenter & Schiele 2008), thus increasing the bee's contribution to nutrient cycling.

## TABLES

**Table 1.** Both the botanical and elemental composition of pollen loads collected by *O. bicornis* mothers differed between habitats and depended on the progeny sex. Regarding botanical composition, the sex difference varied with habitat (significant interaction between habitat and sex). The results of two-way PERMANOVA

Variables	df	F	p
Botanical composition			
Habitat	4	2.90	<b>0.0001</b>
Sex	1	6.55	<b>0.0001</b>
Habitat × sex interaction	4	1.59	<b>0.0488</b>
Residual	50		
Elemental composition			
Habitat	4	3.19	<b>0.0055</b>
Sex	1	7.73	<b>0.0015</b>
Habitat × sex interaction	4	0.83	0.5607
Residual	50		

Statistical significance is indicated in bold.

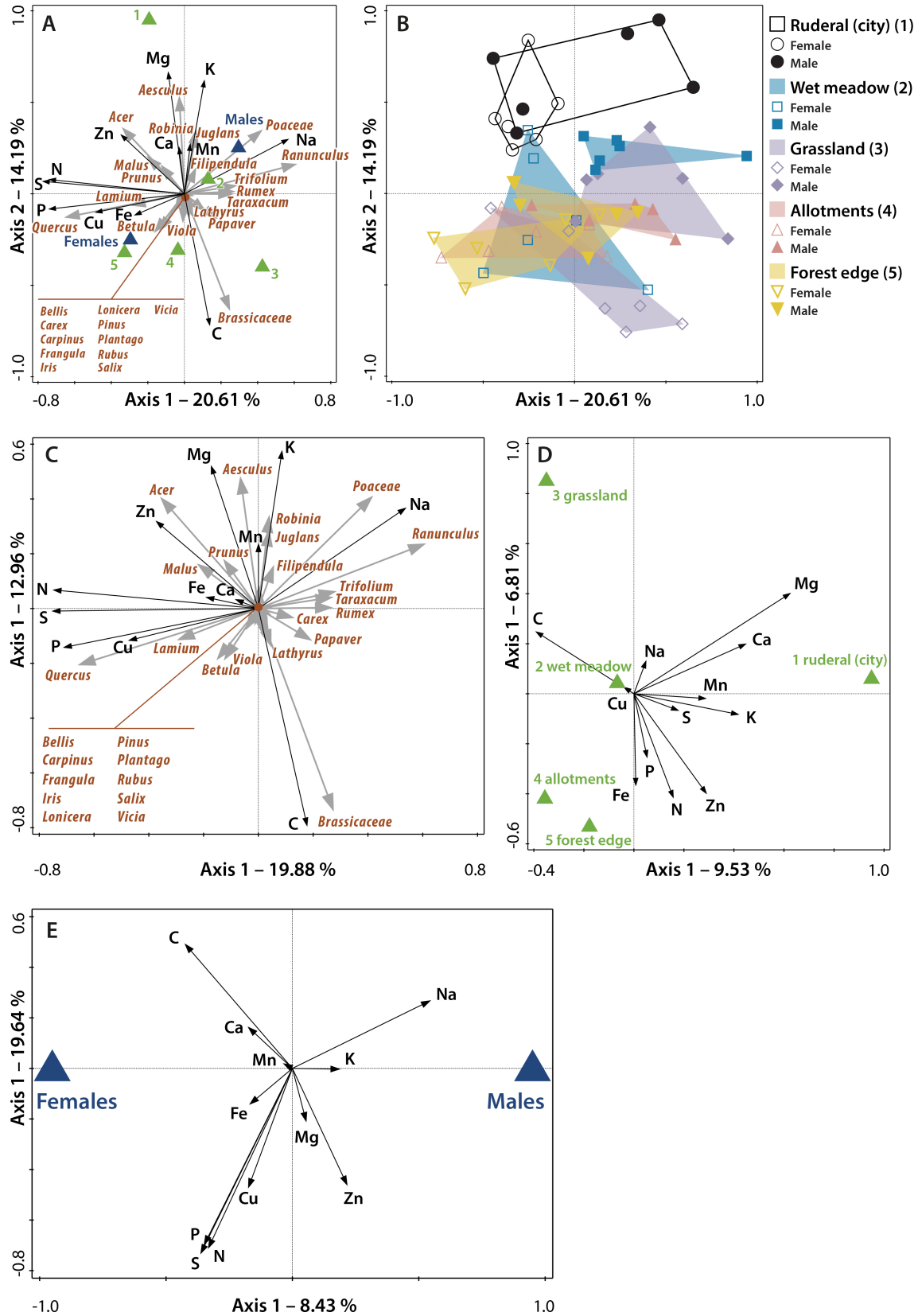


**Table 2.** Elemental composition and its variation in pollen loads collected by *O. bicornis*

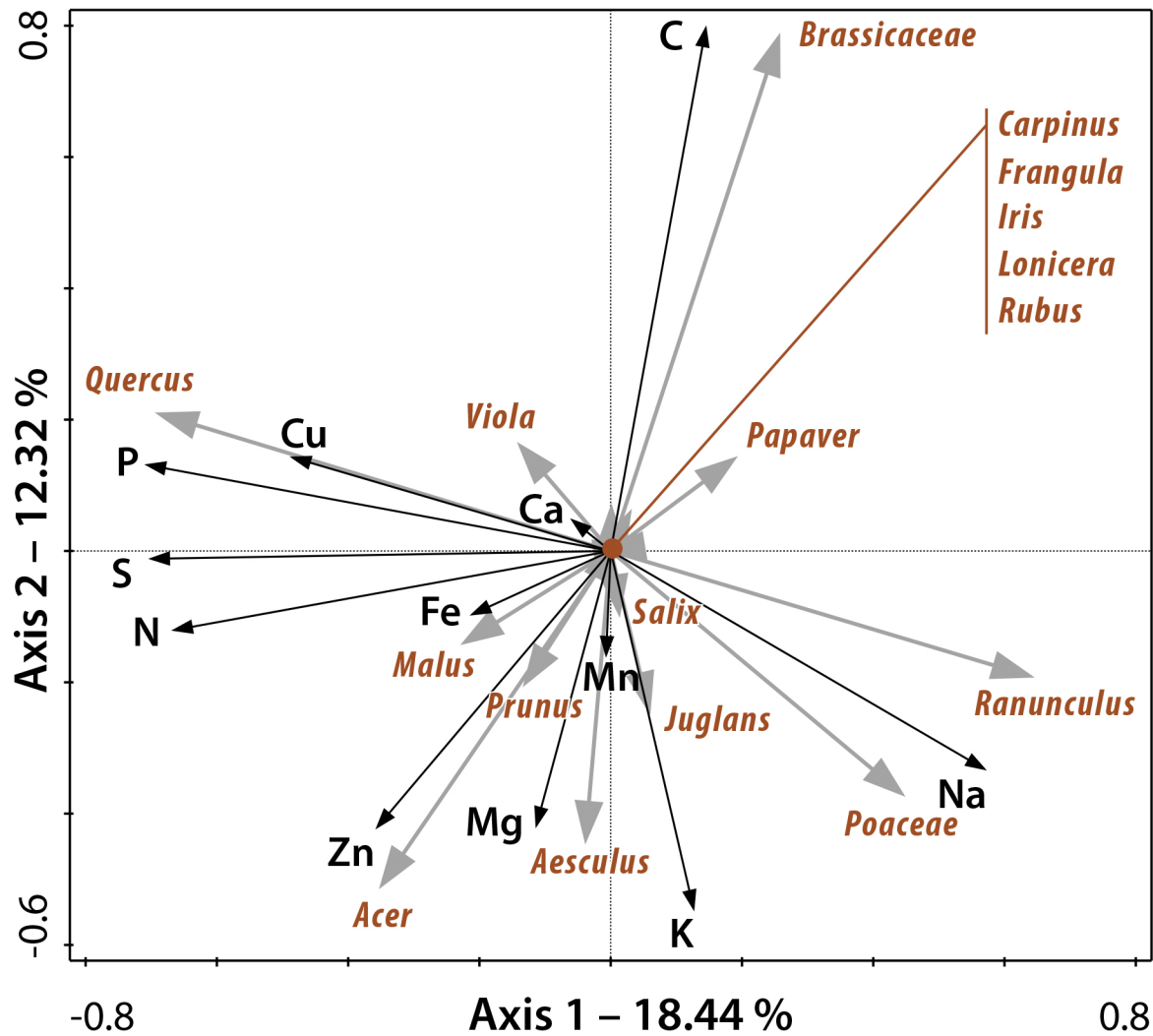
Variables	%					ppm						
	C	N	P	S	K	Ca	Mg	Na	Fe	Zn	Cu	Mn
Females												
Mean	49.57	4.54	0.50	0.30	1.33	0.32	0.24	226.3	244.38	48.22	13.5	42.63
Median	48.26	4.78	0.47	0.30	1.34	0.35	0.19	219.02	208.22	46.31	14.62	36.26
Min	44.85	2.98	0.31	0.18	0.49	0.03	0.07	64.91	42.19	29.92	5.04	13.38
Max	58.27	5.32	0.69	0.41	2.98	0.54	0.56	371.38	524.94	71.55	18.63	155.7
max/min	1.3	1.79	2.23	2.28	6.08	18.00	8.00	5.72	12.44	2.39	3.70	11.64
CV	0.08	0.14	0.17	0.18	0.33	0.42	0.56	0.26	0.56	0.22	0.26	0.63
Males												
Mean	46.53	4.11	0.44	0.25	1.5	0.27	0.25	315.44	202.16	54.26	12.33	41.13
Median	46.45	4.00	0.42	0.27	1.49	0.29	0.15	310.97	181.5	50.4	12.57	34.21
Min	40.81	2.74	0.35	0.11	0.55	0.04	0.07	179.63	64.32	25.37	5.3	16.27
Max	51.63	5.21	0.59	0.38	2.44	0.58	0.57	501.93	539.35	89.66	18.37	82.92
max/min	1.27	1.90	1.69	3.45	4.44	14.5	8.14	2.79	8.39	3.53	3.47	5.10
CV	0.05	0.15	0.13	0.25	0.29	0.52	0.69	0.25	0.55	0.30	0.25	0.40

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

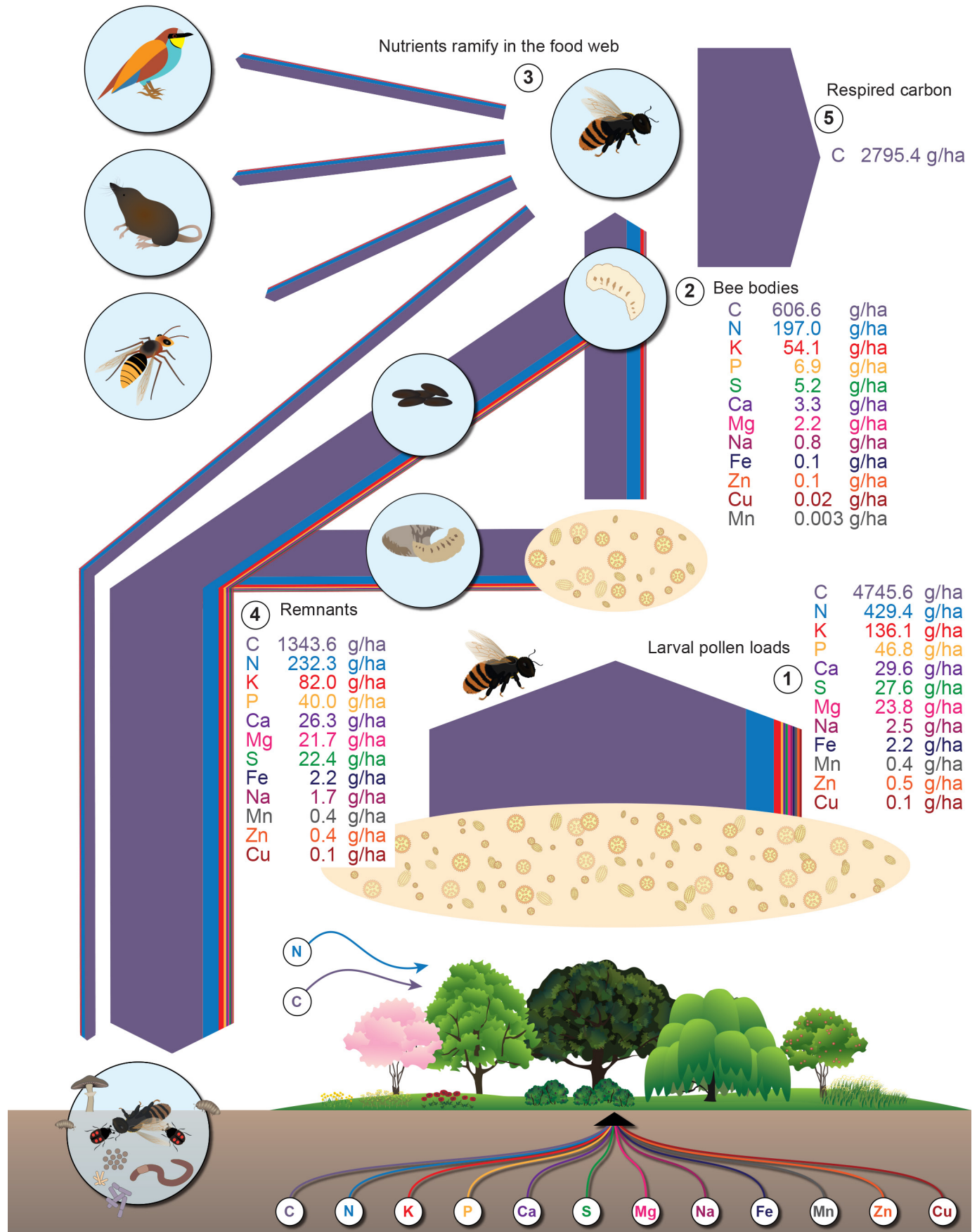
# FIGURES



**Fig. 1.** The effects of the botanical composition of pollen loads, habitat, and progeny sex on the variation in elemental proportions. RDA plot. 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. A – RDA plot summarizing the effects of habitat, larval sex, and botanical origin of pollen loads on the elemental composition of pollen loads; B – RDA plot showing the locations of all measured pollen loads with indication of habitat and larval sex; C, D, and E – RDA plot showing the separate effects of (C) botanical origin, (D) habitat, and (E) larval sex on the elemental proportion variation in pollen loads



**Fig. 2.** The effect of botanical origin, considering only pollen major species (more than 10% of the total pollen load), on the elemental proportion variation in pollen loads. RDA plot. 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.



**Fig. 3.** A conceptual representation of nutrient cycling through the flower visitation pathway and further ramification of nutrients in the food web. Plants acquire the necessary amount of nutrients from the environment. They allocate some of them in pollen in a certain proportion. At present, the trade-offs involved are unknown and, therefore, the mechanisms that determine the proportion of nutrients allocated in pollen are also unknown. However, to obtain an idea of bees' roles in nutrient cycling, it is possible to roughly calculate the amount of nutrient cycling in the ecosystem with the help of the model wild bee *Osmia bicornis*. A population of these bees collects pollen containing the amounts of nutrients given in point (1). Some of these nutrients, amounts given in point (2), are assimilated into the bodies of the newly formed generation and ramify in the food web (point 3; values cannot be calculated). The remaining nutrients are left in the bees' nests in the form of the offspring that did not survive, excreta, cocoons, and the remains of uneaten pollen (point 4). These nutrients are used by detritivores. In addition, some carbon is released into the atmosphere as a result of respiration (point 5). The values calculated here, although approximate, provide insight into the contribution of a wild bee species, *O. bicornis*, to local nutrient cycling. These values are significant in the context of the flow of elements through other pathways in the ecosystem, as discussed in Box 1. A detailed explanation of how these values were calculated is given in Box 1 and Appendix S1.

## REFERENCES

- Allen, G. & Davies, R.G. (2023). Canopy sampling reveals hidden potential value of woodland trees for wild bee assemblages. *Insect Conserv Divers*, 16, 33-46. doi: 10.1111/icad.12606
- Atkinson, C.L., Capps, K.A., Rugenski, A.T. & Vanni, M.J. (2017). Consumer-driven nutrient dynamics in freshwater ecosystems: From individuals to ecosystems. *Biol Rev*, 92, 2003-2023. doi: 10.1111/brv.12318
- Atkinson, C.L. & Forshay, K.J. (2022). Community patch dynamics governs direct and indirect nutrient recycling by aggregated animals across spatial scales. *Funct Ecol*, 36, 595-606. doi: 10.1111/1365-2435.13982
- Ausma, T., Bansal, V., Kraaij, M., Verloop, A.C.M., Gasperl, A., Müller, M. *et al.* (2021). Floral displays suffer from sulphur deprivation. *Environ Exp Bot*, 192, 104656. doi: 10.1016/j.envexpbot.2021.104656
- Bianchi, T.S. (2021). The evolution of biogeochemistry: *Revisited*. *Biogeochemistry*, 154, 141-181. doi: 10.1007/s10533-020-00708-0
- Bilinski, M. & Teper, D. (2004). Rearing and utilization of the red mason bee - *Osmia rufa* L. (Hymenoptera, Megachilidae) for orchard pollination. *J Apic Sci*, 48, 69-74.
- Casanelles-Abella, J., Keller, A., Müller, S., Aleixo, C., Alós-Orti, M., Chiron, F. *et al.* (2022a). Wild bee larval food composition in five European cities. *Ecology*, 103, e3740. doi: 10.1002/ecy.3740
- Casanelles-Abella, J., Müller, S., Keller, A., Aleixo, C., Alós Orti, M., Chiron, F. *et al.* (2022b). How wild bees find a way in European cities: Pollen metabarcoding unravels multiple

- feeding strategies and their effects on distribution patterns in four wild bee species. *J Appl Ecol*, 59, 457-470. doi: 10.1111/1365-2664.14063
- Clay, N.A., Lucas, J., Kaspari, M. & Kay, A.D. (2013). Manna from heaven: Refuse from an arboreal ant links aboveground and belowground processes in a lowland tropical forest. *Ecosphere*, 4, art141. doi: 10.1890/ES13-00220.1
- Cunningham-Minnick, M.J., Milam, J., Kane, B., Roberts, H.P. & King, D.I. (2023). Abundant, distinct, and seasonally dynamic bee community in the canopy-aerosphere interface above a temperate forest. *Ecol Evol*, 13, e9739. doi: 10.1002/ece3.9739
- da Silva, J.J.R.F. & Williams, R.J.P. (2001). *The biological chemistry of the elements: The inorganic chemistry of life*. OUP Oxford, New York, p. 600
- Danforth, B.N., Minckley, R.L. & Neff, J.L. (2019). *The solitary bees: Biology, evolution, conservation*. Princeton University Press, Princeton, New Jersey, p. 488
- Eckerter, P.W., Albrecht, M., Herzog, F. & Entling, M.H. (2022). Floral resource distribution and fitness consequences for two solitary bee species in agricultural landscapes. *Basic Appl Ecol*, 65, 1-15. doi: 10.1016/j.baae.2022.09.005
- Elser, J.J., Fagan, W.F., Denno, R.F., Dobberfuhl, D.R., Folarin, A., Huberty, A. *et al.* (2000). Nutritional constraints in terrestrial and freshwater food webs. *Nature*, 408, 578-580. doi: 10.1038/35046058
- Elser, J.J. & Urabe, J. (1999). The stoichiometry of consumer-driven nutrient recycling: Theory, observations, and consequences. *Ecology*, 80, 735-751. Filipiak, M. (2016). Pollen stoichiometry may influence detrital terrestrial and aquatic food webs. *Front Ecol Evol*, 4, 138. [https://doi.org/10.1890/0012-9658\(1999\)080\[0735:TSOCDN\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1999)080[0735:TSOCDN]2.0.CO;2)



- Filipiak, M. & Filipiak, Z.M. (2022). Application of ionomics and ecological stoichiometry in conservation biology: Nutrient demand and supply in a changing environment. *Biol Conserv*, 272, 109622. doi: 10.1016/j.biocon.2022.109622
- Filipiak, M., Woyciechowski, M. & Czarnoleski, M. (2021). Stoichiometric niche, nutrient partitioning and resource allocation in a solitary bee are sex-specific and phosphorous is allocated mainly to the cocoon. *Sci Rep*, 11, 652. doi: 10.1038/s41598-020-79647-7
- Filipiak, Z.M., Denisow, B., Stawiarz, E. & Filipiak, M. (2022). Unravelling the dependence of a wild bee on floral diversity and composition using a feeding experiment. *Sci Total Environ*, 820, 153326. doi: 10.1016/j.scitotenv.2022.153326
- Filipiak, Z.M., Ollerton, J. & Filipiak, M. (2023). Uncovering the significance of the ratio of food K:Na in bee ecology and evolution. *Ecology*, e4110. doi: 10.1002/ecy.4110
- Finkelstein, C.J., CaraDonna, P.J., Gruver, A., Welti, E.A.R., Kaspari, M. & Sanders, N.J. (2022). Sodium-enriched floral nectar increases pollinator visitation rate and diversity. *Biol Lett*, 18, 20220016. doi: 10.1098/rsbl.2022.0016
- Gaston, K.J., Smith, R.M., Thompson, K. & Warren, P.H. (2005). Urban domestic gardens (II): Experimental tests of methods for increasing biodiversity. *Biodivers Conserv*, 14, 395-413. doi: 10.1007/s10531-004-6066-x
- Gillespie, M.A.K., Baude, M., Biesmeijer, J., Boatman, N., Budge, G.E., Crowe, A. *et al.* (2022). Landscape-scale drivers of pollinator communities may depend on land-use configuration. *Philos Trans R Soc Lond B Biol Sci*, 377, 20210172. doi: 10.1098/rstb.2021.0172

- González, A.L., Dézerald, O., Marquet, P.A., Romero, G.Q. & Srivastava, D.S. (2017). The multidimensional stoichiometric niche. *Front Ecol Evol*, 5, 110. doi: 10.3389/fevo.2017.00110
- Goulson, D. (2021). *Gardening for bumblebees: A practical guide to creating a paradise for pollinators*. Random House, New York, p. 304
- Grodzinski, W., Weiner, J. & Maycock, P. (1984). Forest ecosystems in industrial regions: Studies on the cycling of energy nutrients and pollutants in the Niepołomice forest Southern Poland. In: *Ecological studies*, eds. Grodzinski, W., Weiner, J. & Maycock, P. Springer Berlin Heidelberg, Berlin, Heidelberg, p. 277. <https://doi.org/10.1007/978-3-642-69802-6>
- Gruber, B., Eckel, K., Everaars, J. & Dormann, C.F. (2011). On managing the red mason bee (*Osmia bicornis*) in apple orchards. *Apidologie*, 42, 564-576. doi: 10.1007/s13592-011-0059-z
- Hammer, O., Harper, D. & Ryan, P. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontol Electron*, 4, 1-9.
- Herzog, C., J. T. Reeves, Y. Ipek, A. Jilling, D. Hawlena, & Wilder, S.M. (2023). Multi-elemental consumer-driven nutrient cycling when predators feed on different prey. *Oecologia*, 202, 729–742. doi: 10.1007/s00442-023-05431-9
- Huang, X.-Y. & Salt, David E. (2016). Plant ionomics: From elemental profiling to environmental adaptation. *Mol Plant*, 9, 787-797. doi: 10.1016/j.molp.2016.05.003
- Isanta-Navarro, J., Prater, C., Peoples, L.M., Loladze, I., Phan, T., Jeyasingh, P.D. *et al.* (2022). Revisiting the growth rate hypothesis: Towards a holistic stoichiometric understanding of growth. *Ecol Lett*, 25, 2324-2339. doi: 10.1111/ele.14096

- Jauker, F., Peter, F., Wolters, V. & Diekötter, T. (2012). Early reproductive benefits of mass-flowering crops to the solitary bee *Osmia rufa* outbalance post-flowering disadvantages. *Basic Appl Ecol*, 13, 268-276. doi: 10.1016/j.baae.2012.03.010
- Jeyasingh, P.D., Cothran, R.D. & Tobler, M. (2014). Testing the ecological consequences of evolutionary change using elements. *Ecol Evol*, 4, 528-538. doi: 10.1002/ece3.950
- Jeyasingh, P.D., Sherman, R.E., Prater, C., Pulkkinen, K. & Ketola, T. (2023). Adaptation to a limiting element involves mitigation of multiple elemental imbalances. *J R Soc Interface*, 20, 20220472. doi: 10.1098/rsif.2022.0472
- Jeyasingh, P.D. & Weider, L.J. (2007). Fundamental links between genes and elements: Evolutionary implications of ecological stoichiometry. *Mol Ecol*, 16, 4649-4661. doi: 10.1111/j.1365-294X.2007.03558.x
- Kaspari, M. (2020). The seventh macronutrient: How sodium shortfall ramifies through populations, food webs and ecosystems. *Ecol Lett*, 23, 1153-1168. doi: 10.1111/ele.13517
- Kaspari, M. (2021). The invisible hand of the periodic table: How micronutrients shape ecology. *Annu Rev Ecol Evol Syst*, 52, 199-219. doi: 10.1146/annurev-ecolsys-012021-090118
- Kay, A.D. & Vrede, T. (2008). Evolutionary and biochemical aspects. In: *Encyclopedia of ecology*, eds. Jørgensen, S.E. & Fath, B.D. Academic Press, Oxford, pp. 1472-1481. doi: 10.1016/B978-008045405-4.00305-0
- Kitchell, J.F., O'Neill, R.V., Webb, D., Gallepp, G.W., Bartell, S.M., Koonce, J.F. *et al.* (1979). Consumer regulation of nutrient cycling. *BioScience*, 29, 28-34. doi: 10.2307/1307570
- Lau, P. W., I. L. Esquivel, K. A. Parys, K. J. Hung, & Chakrabarti, P. (2023). The nutritional landscape in agroecosystems: a review on how resources and management practices can

- shape pollinator health in agricultural environments. *Annals of the Entomological Society of America*, 116, 261–275. doi: 10.1093/aesa/saad023
- Lau, P., P. Lesne, R. J. Grebenok, J. Rangel, & Behmer, S. T. (2022). Assessing pollen nutrient content: a unifying approach for the study of bee nutritional ecology. *Philosophical Transactions of the Royal Society B: Biological Sciences* 377:20210510. doi: 10.1098/rstb.2021.0510
- LeBuhn, G. & Vargas Luna, J. (2021). Pollinator decline: What do we know about the drivers of solitary bee declines?. *Curr Opin Insect Sci*, 46, 106-111. doi: 10.1016/j.cois.2021.05.004
- Leroy, C., Brunet, J., Henry, M., & Alaux, C. (2023). Using physiology to better support wild bee conservation. *Conservation Physiology*, 11(1), 1–15. doi: 10.1093/conphys/coac076
- Lemelin, R.H. (2012). *The management of insects in recreation and tourism*. Cambridge University Press, New York, Melbourne, p. 370
- López-Urbe, M.M., Ricigliano, V.A. & Simone-Finstrom, M. (2020). Defining pollinator health: A holistic approach based on ecological, genetic, and physiological factors. *Annu Rev Anim Biosci*, 8, 269-294. doi: 10.1146/annurev-animal-020518-115045
- McInturf, A.G., Pollack, L., Yang, L.H. & Spiegel, O. (2019). Vectors with autonomy: What distinguishes animal-mediated nutrient transport from abiotic vectors?. *Biol Rev Camb Philos Soc*, 94, 1761-1773. doi: 10.1111/brv.12525
- Michener, C.D. (2007). *The bees of the world*. Johns Hopkins University Press, Baltimore, US, p. 953
- Nagamitsu, T., Suzuki, M.F., Mine, S., Taki, H., Shuri, K., Kikuchi, S. *et al.* (2018). Effects of forest loss and fragmentation on pollen diets and provision mass of the mason bee, *Osmia cornifrons*, in central Japan. *Ecol Entomol*, 43, 245-254. doi: 10.1111/een.12494

- Ollerton, J. (2021). *Pollinators and pollination: Nature and society*. Pelagic Publishing, Exeter, p. 300
- Ostap-Chec, M., Kierat, J., Kuszewska, K. & Woyciechowski, M. (2021). Red mason bee (*Osmia bicornis*) thermal preferences for nest sites and their effects on offspring survival. *Apidologie*, 52, 707-719. doi: 10.1007/s13592-021-00858-6
- Parreño, M.A., Alaux, C., Brunet, J.L., Buydens, L., Filipiak, M., Henry, M. *et al.* (2022). Critical links between biodiversity and health in wild bee conservation. *Trends Ecol Evol*, 37, 309-321. doi: 10.1016/j.tree.2021.11.013
- Peñuelas, J., Fernández-Martínez, M., Ciais, P., Jou, D., Piao, S., Obersteiner, M. *et al.* (2019). The bioelements, the elementome, and the biogeochemical niche. *Ecology*, 100, e02652. doi: 10.1002/ecy.2652
- Penuelas, J., Sardans, J., Ogaya, R. & Estiarte, M. (2008). Nutrient stoichiometric relations and biogeochemical niche in coexisting plant species: Effect of simulated climate change. *Pol J Ecol*, 56, 613-622.
- Pioltelli, E., Guzzetti, L., Ouled Larbi, M., Labra, M., Galimberti, A. & Biella, P. (2023) Landscape fragmentation constrains bumblebee nutritional ecology and foraging dynamics. *Authorea Preprints*. doi: 10.22541/au.168989255.56800434/v1
- Raw, A. (1972). The biology of the solitary bee *Osmia rufa* (L.) (Megachilidae). *Trans R Entomol Soc Lond*, 124, 213-229. doi: 10.1111/j.1365-2311.1972.tb00364.x
- Rivernider, R., Venturini, E. & Drummond, F.A. (2017). *Phleum pratense* (Poales: Poaceae), a Pollen Forage for native bumble bees *bombus latrielle* (Hymenoptera: Apidae). *J Kans Entomol Soc*, 90, 63-68. doi: 10.2317/0022-8567-90.1.63

- Roulston, T.H. & Cane, J.H. (2000). Pollen nutritional content and digestibility for animals. *Plant Syst Evol*, 222, 187-209. <http://link.springer.com/article/10.1007/BF00984102>
- Salt, D.E., Baxter, I. & Lahner, B. (2008). Ionomics and the study of the plant ionome. *Annu Rev Plant Biol*, 59, 709-733. doi: 10.1146/annurev.arplant.59.032607.092942
- Saunders, M.E. (2018). Insect pollinators collect pollen from wind-pollinated plants: Implications for pollination ecology and sustainable agriculture. *Insect Conserv Divers*, 11, 13-31. doi: 10.1111/icad.12243
- Schlesinger, W.H. & Bernhardt, E.S. (2020). *Biogeochemistry: An analysis of global change*. Elsevier Science, Massachusetts, US, p. 762
- Seidelmann, K., Ulbrich, K. & Mielenz, N. (2010). Conditional sex allocation in the red mason bee, *Osmia rufa*. *Behav Ecol Sociobiol*, 64, 337-347. doi: 10.1007/s00265-009-0850-2
- Slansky, F. & Rodriguez, J.G. (1987). *Nutritional ecology of insects, mites, spiders, and related invertebrates*. Wiley, New Jersey, US, p. 1,016
- Slominski, A.H. & Burkle, L.A. (2021). Asynchrony between solitary bee emergence and flower availability reduces flower visitation rate and may affect offspring size. *Basic Appl Ecol*, 56, 345-357. doi: 10.1016/j.baae.2021.08.003
- Šmilauer, P. & Lepš, J. (2014). *Multivariate analysis of ecological data using CANOCO 5*. Cambridge University Press, Cambridge, p. 362
- Splitt, A., Schulz, M. & Skórka, P. (2022). Current state of knowledge on the biology and breeding of the solitary bee – *Osmia bicornis*. *J Apic Res*, 61, 163-179. doi: 10.1080/00218839.2021.1957610

- Splitt, A., Skórka, P., Strachecka, A., Borański, M. & Teper, D. (2021). Keep trees for bees: Pollen collection by *Osmia bicornis* along the urbanization gradient. *Urban For Urban Green*, 64, 127250. doi: 10.1016/j.ufug.2021.127250
- Steffan-Dewenter, I. & Schiele, S. (2008). Do resources or natural enemies drive bee population dynamics in fragmented habitats?. *Ecology*, 89, 1375-1387. 10.1890/06-1323.1
- Sterner, R.W. & Elser, J.J. (2002). *Ecological stoichiometry: The biology of elements from molecules to the biosphere*. Princeton University Press, New Jersey, p. 439
- Stevenson, P.C., Koch, H., Nicolson, S.W. & Brown, M.J.F. (2022). Natural processes influencing pollinator health. *Philos Trans R Soc Lond B Biol Sci*, 377, 20210154. doi: 10.1098/rstb.2021.0154
- Teper, D. & Bilinski, M. (2009). Red mason bee (*Osmia rufa* L.) as a pollinator of rape plantations. *J Apic Sci*, 53, 115-120.
- Ukonmaanaho, L., Merilä, P., Nöjd, P. & Nieminen, T. (2008). Litterfall production and nutrient return to the forest floor in scots pine and Norway spruce stands in Finland. *Boreal Environ Res*, 13, 67-91.
- Urban-Mead, K.R., van Dyke, M., Muñiz, P., Young, A.D., Danforth, B.N. & McArt, S.H. (2023). Early spring orchard pollinators spill over from resource-rich adjacent forest patches. *J Appl Ecol*, 60, 553-564. doi: 10.1111/1365-2664.14350
- van der Kooij, C.J., Vallejo-Marín, M. & Leonhardt, S.D. (2021). Mutualisms and (A)symmetry in plant-pollinator interactions. *Curr Biol*, 31, R91-R99. doi: 10.1016/j.cub.2020.11.020
- Vanni, M.J., Flecker, A.S., Hood, J.M. & Headworth, J.L. (2002). Stoichiometry of nutrient recycling by vertebrates in a tropical stream: Linking species identity and ecosystem processes. *Ecol Lett*, 5, 285-293. doi: 10.1046/j.1461-0248.2002.00314.x

- Vernadsky, W. (1924). *La géochimie*. Alcan, Paryż, p. 404
- Wilkaniec, Z. & Radajewska, B. (1997). Solitary bee *Osmia rufa* L. (Apoidea, Megachilidae) as pollinator of strawberry cultivated in an unheated plastic tunnel. *Acta Hortic*, 439, 489-494. doi: 10.17660/ActaHortic.1997.439.83
- Williams, R.J.P. & da Silva, J.J.R.F. (2006). *The chemistry of evolution: The development of our ecosystem*. Elsevier Science, Netherlands, p. 481. doi: 10.1016/B978-0-444-52115-6.X5042-8
- Williams, R.J.P. & Rickaby, R. (2012). *Evolution's destiny: Co-evolving chemistry of the environment and life*. Royal Society of Chemistry, Cambridge, UK, p. 342. doi: 10.1039/9781849735599
- Willmer, P. (2011). *Pollination and floral ecology*. Princeton University Press, Princeton, New Jersey, p. 778
- Wilson, R.S., Keller, A., Shapcott, A., Leonhardt, S.D., Sickel, W., Hardwick, J.L. *et al.* (2021). Many small rather than few large sources identified in long-term bee pollen diets in agroecosystems. *Agric Ecosyst Environ*, 310, 107296. doi: 10.1016/j.agee.2020.107296
- Yourstone, J., Karlsson, M., Klatt, B.K., Olsson, O. & Smith, H.G. (2021). Effects of crop and non-crop resources and competition: High importance of trees and oilseed rape for solitary bee reproduction. *Biol Conserv*, 261, 109249. doi: 10.1016/j.biocon.2021.109249
- Zhang, B., Chen, H., Deng, M., Li, J., González, A.L. & Wang, S. (2022). High dimensionality of stoichiometric niches in soil fauna. *Ecology*, 103, e3741. doi: 10.1002/ecy.3741