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Received: 8 December 2022 | Revised: 4 May 2023 | Accepted: 18 May 2023

DOI: 10.1002/ecy.4110

CONCEPTS & SYNTHESIS

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ECOLOGICAL SOCIETY OF AMERICA

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Funding information
BiodivERsA3 ERA-Net COFUND, Grant/Award Number: NutriB2; Narodowe Centrum Nauki, Grant/Award Number: 2019/31/Z/NZ8/04030; Uniwersytet Jagielloński w Krakowie, Grant/Award Number: Faculty of Biology (N18/DBS/000003)

Handling Editor: Jay A. Rosenheim

Abstract

Bees provide important ecological services, and many species are threatened globally, yet our knowledge of wild bee ecology and evolution is limited. While evolving from carnivorous ancestors, bees had to develop strategies for coping with limitations imposed on them by a plant-based diet, with nectar providing energy and essential amino acids and pollen as an extraordinary, protein- and lipid-rich food nutritionally similar to animal tissues. Both nectar and pollen display one characteristic common to plants, a high ratio of potassium to sodium (K:Na), potentially leading to bee underdevelopment, health problems, and death. We discuss why and how the ratio of K:Na contributes to bee ecology and evolution and how considering this factor in future studies will provide new knowledge, more accurately depicting the relationship of bees with their environments. Such knowledge is essential for understanding how plants and bees function and interact and is needed to effectively protect wild bees.

KEYWORDS
elementome, evolution, insect, interactions, ionomics, mutualism, nutrient cycle, plant, pollination, pollinator, potassium, sodium

INTRODUCTION: ROLES OF MICRONUTRIENT RATIOS IN ORGANISMAL ECOLOGY AND FITNESS

One of the major rules of life is the common evolutionary history of the biochemistry of all living organisms (Filipiak & Filipiak, 2022; Redfield, 1958; Williams & Rickaby, 2012). Matter composed of organic molecules makes up all living organisms on Earth. How these molecules formed and evolved is closely linked to the geological and chemical processes that have occurred on Earth since its formation, resulting in a co-evolving chemistry of the environment and life (Williams & da Silva, 2006; Williams & Rickaby, 2012). Within this context, one can speak of natural selection of chemical elements by living systems, as organisms concentrate certain elements while rejecting others (da Silva & Williams, 2001). This shared evolutionary history results in a fixed and rigorous set of elements that make up living organisms, from bacteria to whales (da Silva & Williams, 2001). Organisms are always composed of *atoms* of the same ~25 chemical *elements* (Kaspari & Powers, 2016; Williams & da Silva, 2006; Williams & Rickaby, 2012) (note: the text in italics is explained in Box 1 [glossary]). The proportion of all atoms that compose organismal bodies is homeostatic and, therefore, must be maintained at a relatively constant level

BOX 1 Glossary.

Atom—The most basic unit of matter building the environment and organisms. Atoms form chemical elements.
Ecological stoichiometry—Research framework that considers how organismal nutritional needs for basic body-building blocks shape their own and ecosystem ecology. It considers how the nutritional demands of organisms and the supply of nutrients in their environments affect organismal growth, organismal interactions with the biotic and abiotic world, and nutrient cycling in the whole ecosystem.
Element—The simplest substance consisting of atoms having a specific number of protons in the nucleus.
Ionomics—An approach to organismal biology linking physiological, ecological, and evolutionary phenomena with the nutritional needs of organisms, considering all of the ~25 vital chemical elements. It considers that the proportions of all atoms forming organismal bodies: (1) are important in driving phenotypic fitness-related traits; (2) may be adjusted within the limits of stoichiometric homeostasis; and (3) may be changed in response to environmental stressors.
Nutrient cycling—Describes how immutable atoms are transferred from the physical environment (atmosphere, hydrosphere, lithosphere) into organisms, being constantly incorporated into molecules that are being ceaselessly degraded. Matter composing the environment and organismal bodies is passed from one trophic level to the next, from rocks and soil to plants and other autotrophs, eaten by herbivores and microbivores, which are eaten in turn by predators. Dead bodies, shed body parts, secreta, and excreta are decomposed, and atoms constituting them are again incorporated into the atmosphere, rocks, and soil, and the cycle is repeated.
Nutritional mismatch—Incompatibility between the proportions of nutrients in food and those needed by the consumer that is eating that food.
Pollinivores—Organisms consuming pollen as a food source.
Puddling—Consuming nutrients from semi-liquid matter (e.g., mud, decaying plant and animal tissues, or dung) that is rich in elements or compounds that are scarce in other food sources.

regardless of the supply of these atoms in the environment (Sardans et al., 2021; Sterner & Elser, 2002). Two approaches are used in eco-evolutionary studies to learn why and how atoms of various chemical elements and their proportions shape organismal physiology, interactions, behaviors, life histories, and so forth (Filipiak & Filipiak, 2022): (1) *ecological stoichiometry* focuses on how the most basic law of nature, that is, the law of mass conservation, shapes the functioning of organisms, food webs, and ecosystems, resulting in a diversity of organismal forms and life strategies and influencing large-scale ecological phenomena such as *nutrient cycling* (Jeyasingh et al., 2014; Sardans et al., 2021; Sterner & Elser, 2002); and (2) *ionomics* looks at why various organisms have specific nutritional needs that shape their physiology and how this affects their interactions with the environment and with other species (Fernández-Martínez, 2021; Jeyasingh et al., 2014; Kaspari, 2021; Salt et al., 2008). Here, we discuss the potassium (K) to sodium (Na) ratio (K:Na), which is entrenched in both approaches, in relation to the evolution of a group of insects that are of critical importance in most terrestrial ecosystems: the bees (Hymenoptera: Anthophila).

The classical concept of ecological stoichiometry is built around causes and consequences of shifts in carbon (C), phosphorus (P), and nitrogen (N) ratios at different levels of biological organization (Sardans et al., 2021; Sterner & Elser, 2002). The dependence of organisms on certain ratios of atoms in their environment can be attributed to physiological trade-offs that impact the allocation of stoichiometrically limited molecules for critical organismal functions (Filipiak & Filipiak, 2022). For example, plant tissues and products have higher C:P and C:N ratios than tissues composing herbivores' bodies. Therefore, to fulfill their requirements for N and P, herbivores must ingest a large mass of plant tissue, which causes a high C surplus, which in turn requires special adaptations that are physiologically costly (Filipiak & Filipiak, 2022; Sterner & Elser, 2002). The cost is associated with developing mechanisms to dispose of excess C in a way that does not harm the organism. An excess of C in relation to the other elements may have a toxic effect on a non-adapted organism (Jones & Flynn, 2005) and negatively affects its fitness, resulting in associated costs such as body underdevelopment, prolonged development time or smaller body size (Filipiak & Filipiak, 2022). For example, the

larval development of xylophages, which feed on food with extremely high C to other element ratios, is prolonged by several years, the exact length depending on adult body size, which varies by species (Filipiak, 2018b). Importantly, all elements composing food are always provided in specific ratios. Therefore, 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, either optimal or suboptimal for the health and fitness of the organism (Box 2 and Figure 1). Studies are increasingly indicating that it is not exclusively C:N:P ratios that may help us understand the functioning of organisms and ecosystems: the relative proportions of all of the ~25 vital elements may be critical to this understanding (Filipiak & Filipiak, 2022; Kaspari, 2021; Sardans et al., 2021). This framework has recently been recognized as a fruitful approach for studying bee evolution and ecology (Filipiak & Filipiak, 2022; Lau et al., 2022).

For bees, K:Na seems to be particularly important, as during their evolution, bees had to solve an ecophysiological problem when switching from the low-K:Na carnivorous diet of their ancestors to the high-K:Na herbivorous diet that most species now consume. The switch from a carnivorous to an herbivorous diet clearly shaped the diverse physiologies, ecologies, nesting strategies and behaviors of the more than 20,000 living species of bees. But to date, there has been little discussion of this in relation to K:Na. We discuss the expectations of this dietary switch first by exploring how the fitness of all consumers is influenced by K:Na in food. Second, we focus on bees and their preferred K:Na and trade-offs resulting from changes in the switch from a carnivorous to an herbivorous diet. Finally, we discuss the implications of this for interactions between plants and bees and nutrient cycling in ecosystems.

CONSUMER FITNESS IS INFLUENCED BY FOOD K:Na, WHICH LIMITS THE FUNCTIONING OF HERBIVORES

For plants, Na metabolic function is minor, and the concentration of Na is low in most plant tissues and products. However, for herbivores, Na is a key nutrient

BOX 2 Organisms are made of matter: the role of food stoichiometry in organismal growth, development, and fitness.

All living organisms are composed of atoms of ~25 chemical elements (H, Na, K, Mg, Ca, C, N, O, P, S, Cl, V, Cr, Mo, Mn, Fe, Co, Ni, Cu, Zn, B, Si, Se, F, and I) that serve as their basic building blocks, which combine to form diverse molecules with different functions (da Silva & Williams, 2001; Williams & Rickaby, 2012). Throughout their growth and development, organisms acquire all the necessary building blocks to form their adult bodies (Filipiak & Filipiak, 2022). The composition of an adult body in terms of its constituent atoms is known as organismal stoichiometry and varies among different taxonomic groups. The stoichiometry of the adult body reflects the resources gathered during organismal growth. To build a fully functioning, healthy adult body, organisms undergo a vast number of chemical reactions, each of which must be stoichiometrically balanced (Sterner & Elser, 2008). This means that the availability and balance of vital elements determine an organism's ability to grow and develop optimally. However, mismatches can occur between the atomic composition of the adult body and the juvenile food source (Figure 1; Filipiak & Filipiak, 2022).

Discrepancies between the stoichiometry of food and that of the consumer's body result in high physiological effort of the consumer to absorb nutrients in the necessary proportions, leading to costs such as prolonged development time or decreased body size (Figure 1; Filipiak & Filipiak, 2022). In this way, stoichiometric balance is a key feature of life that links organisms with their environments and affects ecological interactions between organisms, individual life histories, and species evolution.

Within this context, juvenile bees (larvae) are significantly more limited by the nutritional balance of the diet than adults (imagines) due to the different energy and body-building requirements of these stages. While adult bees have access to energy-rich food that can meet their functional requirements (e.g., locomotion, respiration, and healing), an inadequate nutritional balance within the diet of juvenile bees can limit the growth and development of individuals, negatively affecting populations (Filipiak, Denisow, et al., 2022; Parreño et al., 2022).

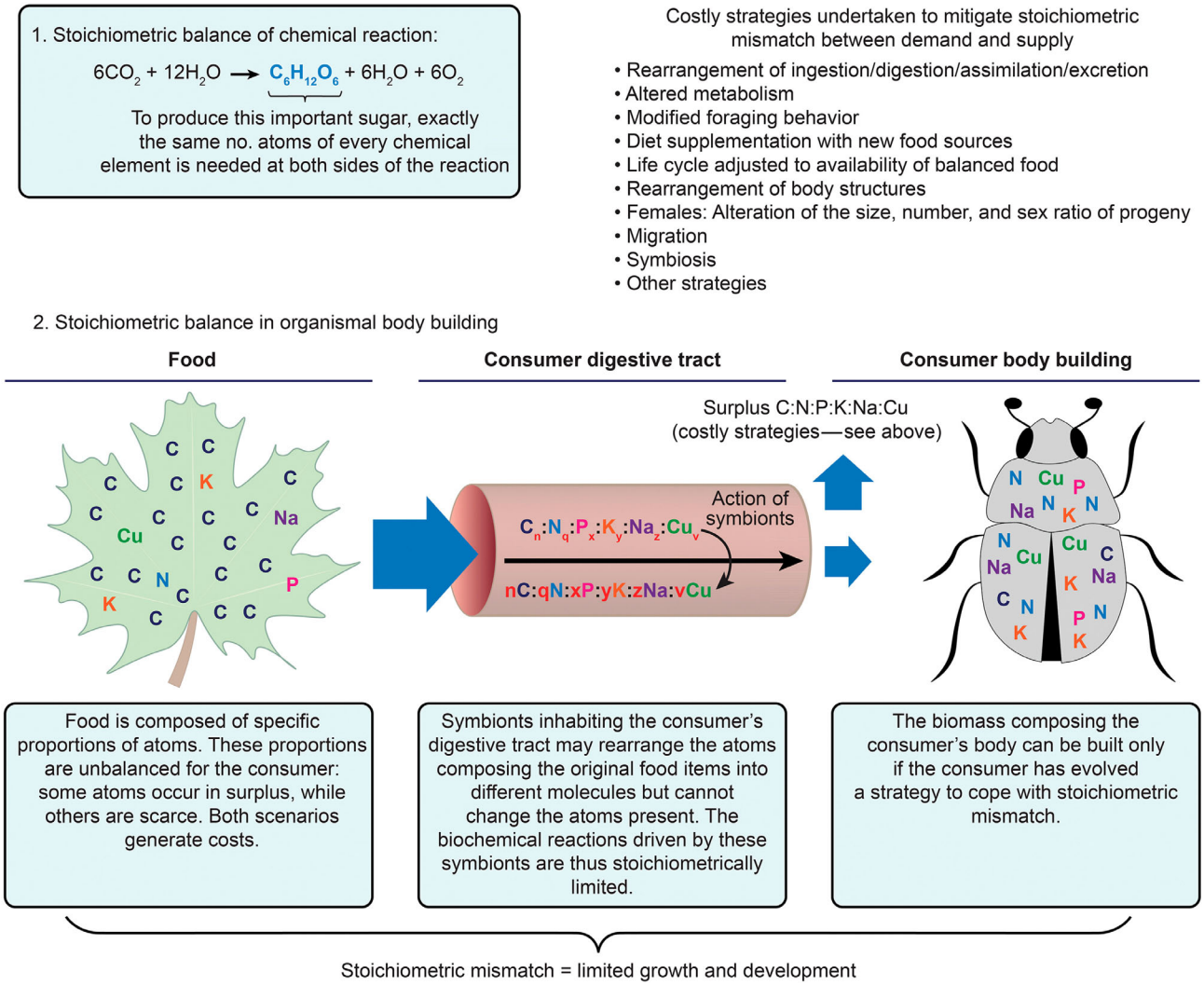

FIGURE 1 Stoichiometric balancing of organismal growth and development (first published in Filipiak & Filipiak, 2022).

(Kaspari, 2020), for example, it is essential for the operation of expensive Na–K ATPases, which account for 25%–75% of the total ATP budget of cells. Na is also responsible for electrolytic balances and fluxes, such as neural activity, and for the stability of polyelectrolytes, such as DNA, and it is involved in the binding of pyrophosphate of nucleoside triphosphates (da Silva & Williams, 2001). In contrast, for plants, K is an important nutrient that is highly concentrated in their tissues and products (Amtmann & Rubio, 2012; Danchin & Nikel, 2019; Sardans & Peñuelas, 2015; White & Karley, 2010), while for plant consumers, too high a K concentration in the diet is limiting and may lead to underdevelopment, health problems, and death of the animal (Kaspari, 2020). Negative impacts of Na scarcity on living and dead plant matter consumers have been reported in various groups of animals, both invertebrates and vertebrates, from beetles to elephants (Demi et al., 2021; Kaspari, 2020; Welti & Kaspari, 2021).

Sodium and potassium are physiologically bound, as they are jointly involved in fundamental physiological processes (da Silva & Williams, 2001; Kaspari, 2020). For example, for consumers, a balanced K:Na ratio is required to maintain their microbiome, Na–K ATPases, osmoregulation, phosphate homeostasis and sensing, and adaptation to cold and heat, among other functions, whereas an unbalanced K:Na in food may increase consumer mortality and cause underdevelopment (Dow, 2017; Kaspari, 2020; Pedersen & Zachariassen, 2002). The dynamic homeostasis of these elements remains so precise that slight variations of less than 1% may be a sign of weakness in the organism (da Silva & Williams, 2001). It is beyond the scope of this manuscript to go into the particulars of the biological significance of the elements, and more details can be found in Kaspari (2020) and da Silva and Williams (2001). Nevertheless, we emphasize that both K and Na have an effect on consumer behavior and performance, and thus, as described in the previous

section, consideration of K:Na, and not each element individually, might be critical to understanding the nutritional physiology and ecology of the consumers being studied.

A unique feature of plant tissues and products is a high K:Na (Figure 2), which must be overcome by consumers of plant matter, imposing physiological constraints (Box 2 and Figure 1). Hence, it could be hypothesized that an unbalanced food K:Na might reduce fitness in plant consumers. As a result, plant consumers must employ strategies to cope with constraints imposed by a high K:Na in plant tissues and products. Balance of the whole-body K:Na can be regulated physiologically and behaviorally. In herbivorous insects, a few studies have depicted internal salt regulation, fluctuations in K and Na concentrations, and K:Na in hemolymph in relation to food intake (e.g., Boné, 1947; Wyatt, 1961). Other studies have shown that insects can regulate Na intake behaviorally, for example, by *puddling*, drinking animal secreta, or performing cannibalistic behavior (Bänziger & Bänziger, 2010; Dangles & Casas, 2012; de la Rosa, 2014; Kaspari, 2020; Simpson et al., 2006). These mechanisms may be factors driving the ecology and evolution of insects, in turn shaping plant-insect interactions and nutrient cycling in food webs.

BEE PREFERENCES FOR K:Na

Pollen and nectar are the principal food sources for bees. Adults mainly consume nectar (or honeydew), whereas juvenile bees rely on protein-rich pollen to fulfill their body-building requirements (Danforth et al., 2019). In diverse bee species, pollen may be augmented with varying proportions of nectar and/or adult saliva to form bee bread or brood provisions (Danforth et al., 2019). Adult bees, on the other hand, primarily consume carbohydrate-rich nectar to meet their energetic needs (but see Cane, 2016; Urban-Mead et al., 2022). While there is limited knowledge on bees' dietary regulation in regard to the K:Na ratio, a few studies have indicated that bees exhibit a preference toward the intake of K and Na in a particular proportion, which could be linked to their respective quantities in the bees' primary food sources (i.e., pollen and nectar). For

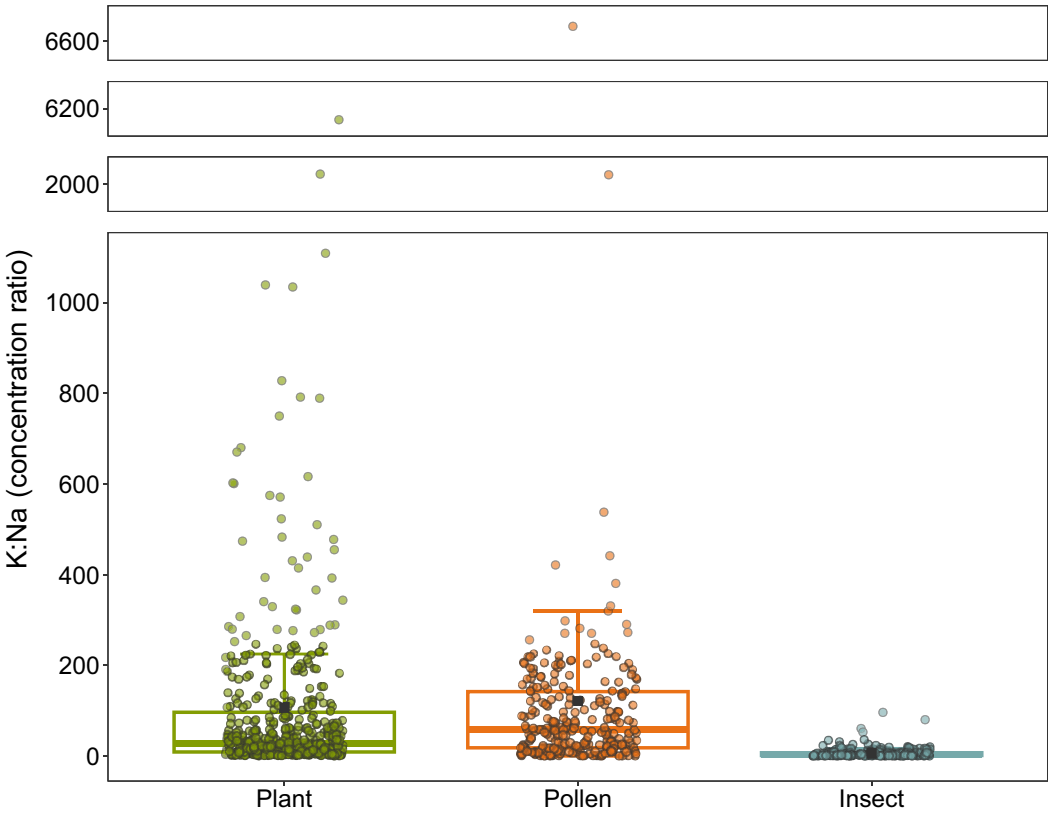

FIGURE 2 Pollen K:Na compared to the K:Na in insect bodies and in plants. The insect K:Na is ~15 times lower than the K:Na in all plant tissues and products, including pollen, imposing limitations on herbivorous and detritivorous insects. Results are based on data from the literature, please see https://doi.org/10.5281/zenodo.7951112 for details. Box and whisker plot with individual data points overlaid (pollen, $n = 253$; plant, $n = 462$; insect, $n = 227$). Boxes, first and third quartiles; whiskers, extend to values ≤1.5 times the interquartile range of the box; square sign, mean value; center line, median. Note that the *y*-axis is broken to accommodate the range.

instance, Petanidou (2007) hypothesized that nectars highly attractive to pollinators are selected on the basis of a low K:Na, since high concentrations of K are a deterrent to pollinators and high concentrations of Na are more favored by them. It was indicated that bees actively seek alternatives to pollen as a source of Na, including so-called "dirty water," animal secretions or carrion (Bänziger & Bänziger, 2010; Dangles & Casas, 2012; de la Rosa, 2014; de Sousa et al., 2022; Dorian & Bonoan, 2021; Khan et al., 2021), and this behavior may be utilized to balance the K:Na ratio of the overall diet (Cairns et al., 2021; Filipiak et al., 2017). Importantly, almost all studies examining bee elemental preferences considered concentrations of single elements (K % and Na %) without referring to K:Na. For example, a deterrent effect of a high K concentration in nectar-like food was shown for the stingless bees *Trigona nigra* and *Nannotrigona perilampoides* and the social wasp *Polybia* sp. (Afik et al., 2014). Threshold K concentrations inducing this effect differ between taxa and are probably related to the K concentration in their natural food (Afik et al., 2014). The deterrent effect of carrion food baits soaked with water, K, Ca, and Mg, but not Na, was shown for five species of stingless bees, suggesting bee preferences for Na (Dorian & Bonoan, 2021). Preferences for bait solutions rich in Na were also shown for various bee species in Brunei Darussalam, and the effect was more pronounced for social bees (Roubik, 1996). Similar preferences were reported for the Western honey bee *A. mellifera* offered different mineral solutions to reflect the dirty water that bees seek (Bonoan et al., 2017). The only study to date considering bee preferences for both concentrations and ratios of elements in water showed that *A. mellifera* preferences are driven by the K:Na ratio and not by concentrations of Na, K, or other nutrients (Cairns et al., 2021). While some studies focus solely on Na concentration as a supplement and others on K concentration as a repellent, we emphasize that the most accurate approach is to also consider the K:Na ratio in the bee diet, as K and Na concentrations alone do not encompass the complexity of the ecophysiology of organisms. Overall, these few examples indicate that the bees' primary food source might not be sufficient to balance K:Na in the diet. Therefore, they seek sodium from other sources, such as mud, dirty water, or carrion. Could this behavior be related to their evolution?

TRADE-OFFS EXPERIENCED WHEN EVOLVING FROM CARNIVORY TO HERBIVORY

For a long time, bees were considered to be the sister group to Spheciform hunting wasps; however, according to current knowledge, bees actually belong within this clade of wasps (Danforth et al., 2019; Murray et al., 2018). Therefore, bees are vegetarian wasps that somehow were able to overcome the nutritional disparities between animal and plant diets. This transition from carnivore to herbivore has also occurred in other Hymenoptera, for example, the "pollen wasps" (Vespidae: Masarinae) (Gess, 1996). The trade-offs that these insects faced when evolving herbivory from carnivory represent an interesting eco-evolutionary problem. On the one hand, herbivory is a desirable strategy since plant tissues and products are available in large quantities and are easily accessible (they do not run away; Figure 3). On the other hand, herbivory is tricky because plant-derived food is nutritionally imbalanced and hard to digest due to the presence of cellulose, hemicellulose, and lignin. In addition, all plants produce toxic compounds to deter various groups of herbivores; therefore, plant consumers must acquire costly adaptations to profit from plant consumption (Figure 3).

However, pollen is a different type of plant food (Figure 3). Similar to other plant tissues, it is usually easily available, although some plants have evolved pollen-defensive strategies involving limiting access only to groups of pollinators with appropriate behaviors or physical strengths, for example, "buzz pollinated" flowers or groups such as the asclepiads (Apocynaceae) and orchids (Orchidaceae) that possess pollinia (De Luca & Vallejo-Marín, 2013; Johnson & Edwards, 2000; Ollerton et al., 2019). Importantly, pollen is more similar to animal tissues than to plant tissues in terms of nutritional value (Box 3) (Willmer, 2011). Additionally, special adaptations are not needed to digest pollen, since pollen grain walls can easily be destroyed mechanically or through pseudo-germination and osmotic shock (Greenfield, 1999; Roulston & Cane, 2000; Willmer, 2011; see Box 3 for an explanation). Consequently, it may not be so surprising that some insect groups have abandoned carnivory in favor of feeding on pollen (Figure 3). However, although similar to animal tissues in terms of digestibility and nutrition, pollen shares a common trait of all plant tissues and products, that is, a low concentration of Na and high concentration of K (reviews of pollen elemental composition are available in (Filipiak et al., 2017; Filipiak & Weiner, 2017)). Therefore, the largest constraint to be overcome when evolving from carnivory to *pollinivory* is most likely a high pollen K:Na (Figures 2 and 3).

During growth and development, all organisms need food composed of atoms in specific, balanced proportions to build optimal adult bodies equipped with all the necessary organs and morphological structures needed to be healthy and optimize fitness (Sterner & Elser, 2002). A balanced K:Na in bee larval food is an important part of building those optimal bodies and is needed to improve

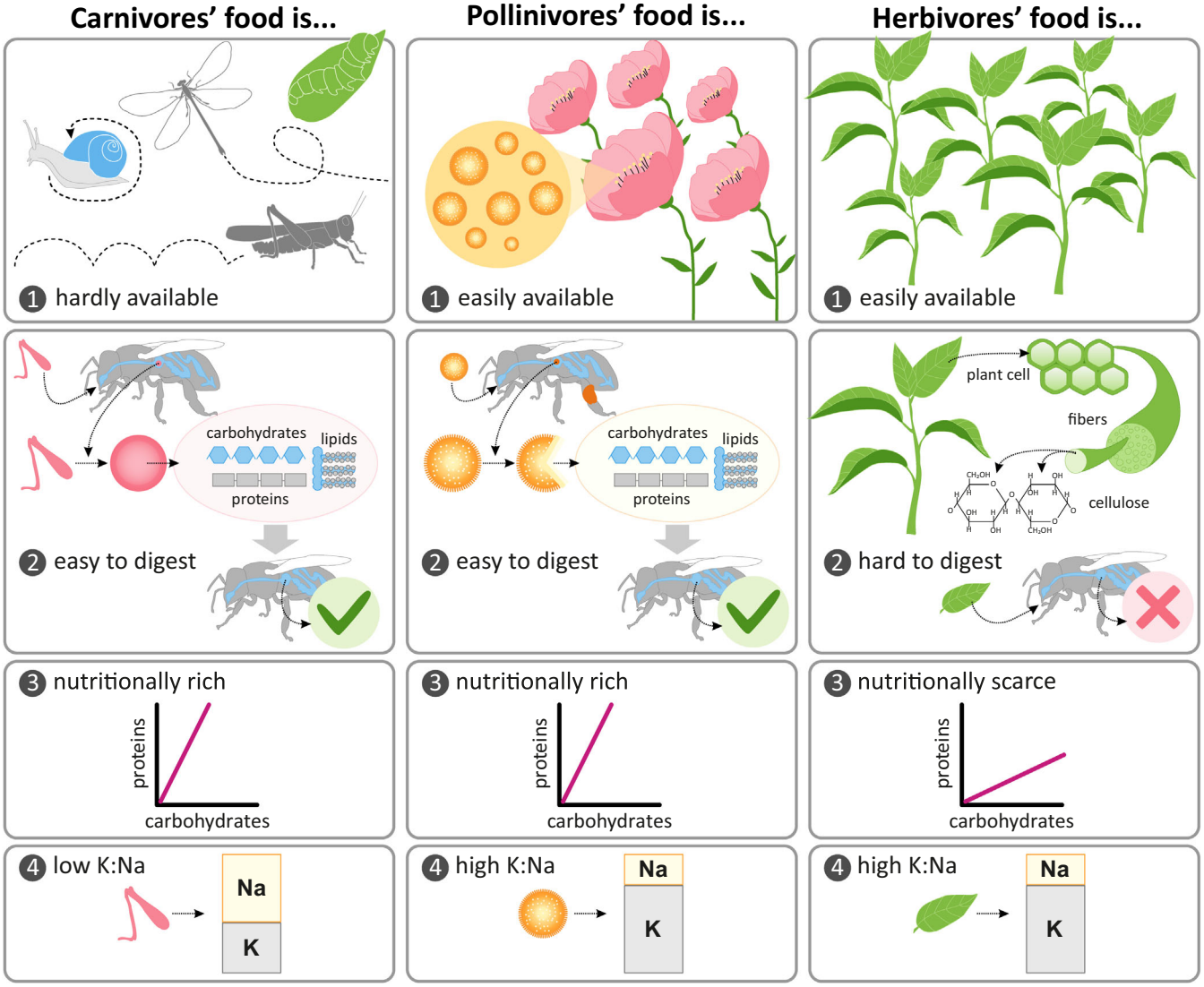

FIGURE 3 Main benefits and challenges shaping the physiology, behavior, life history and evolution of insects employing feeding strategies of carnivory (left panel), pollinivory (center panel), and herbivory (right panel). Benefits and challenges were distinguished based on food source (1) availability, (2) digestibility, (3) nutritional quality expressed as protein:carbohydrate ratio, and (4) K:Na ratio. Illustrations by Zuzanna M. Filipiak.

development and ensure larval survival (Filipiak, Denisow, et al., 2022). A balanced K:Na is also involved in the further functioning of adults, since specific concentrations of K and Na ions in their hemolymph must be physiologically maintained (Wyatt, 1961). Carnivorous insects have a lower K:Na in the hemolymph than herbivores, and the transition from Na-rich (which is a feature of the more basal groups of insects) to K-rich (characteristic of more derived insects) hemolymph occurred over the course of bee evolution (Wyatt, 1961). Progressive adaptation to a plant diet evolved in hymenopterans (i.e., bees and related insects) to reduce the level of maintenance needed to retain a low hemolymph K:Na (Boné, 1947; Wyatt, 1961). Such adaptation, involving an increase in the basal K:Na in hemolymph, was most likely costly due to the high energy expenditure associated with Na-K pumps, which consume 25%–75% of a cell's ATP budget (da Silva & Williams, 2001).

K:Na regulation by bees can be caused by physiological and behavioral mechanisms. For instance, the evolution of some bees (e.g., *Xylocopa capitata*, foraging on plants with very low Na concentrations) resulted in an exceptionally low Na content in their body tissues and fluids (females 30 μmol Na/g dry mass, males 47 μmol Na/g dry mass) in comparison with other insects (e.g., *Periplaneta americana*, ca. 125 μmol Na/g dry mass; *Thymelicus lineola*, ca. 407 μmol Na/g dry mass), as reported by Nicolson (1990). It was suggested that *X. capitata* bees depend on recycling almost all of the Na entering their rectum and on more complicated

BOX 3 Digestibility and nutritional quality of pollen.

Pollen has a protective wall that safeguards it against chemical degradation and protects it from microbial degradation, making it a useful tool in paleopalynology (Brooks et al., 1971). However, the same wall does not provide sufficient protection against mechanical damage. Pollen digestion in pollen consumers is mediated by mechanical damage to the pollen grains, allowing access to the nutritious cytoplasm (e.g., Gilbert, 1972; Haslett, 1983; Howell, 1974; Kirk, 1984; Nicolson, 1994; Roulston & Cane, 2000; Simpson & Neff, 1983; Turner, 1984; Willmer, 2011). These mechanisms include (1) direct mechanical damage caused by crushing, cutting, or chewing before swallowing; (2) opening of the germination pore during pseudo-germination in the gut; and (3) damage caused by osmotic pressure (Roulston & Cane, 2000; Willmer, 2011). These are simple mechanisms that do not require specific adaptations. Pollen germination can occur in vitro in the presence of solutions of sugar and water (Stanley & Linskens, 1974) and occurs in the consumer's gut (Roulston & Cane, 2000; Willmer, 2011), including in that of bees (Dobson & Peng, 1997; Kroon et al., 1974; Peng et al., 1985). Many invertebrates not specialized in pollen consumption, such as various spiders (Pfannenstiel, 2012; Smith & Mommsen, 1984; Wilder, 2011) and mantids (Beckman & Hurd, 2003), other predators, ants, various beetles (Lundgren, 2009), and many others (Filipiak, 2016), as well as vertebrates such as frogs, lizards, birds, bats, rodents, marsupials, and monkeys, supplement their diet with pollen due to its exceptional nutritional quality (Grant, 1996; Kloh et al., 2018; Mellink & Riojas-López, 2002; Olesen & Valido, 2003; Tattersall & Sussman, 1975; Willmer, 2011). Pollen is also commercially available as a healthy food supplement for humans, who also have access to the nutrient-rich interior of pollen grains through the above mechanisms.

The nutritional quality of pollen is high and, although taxonomically variable, comparable to that of various animal tissues (Willmer, 2011). Pollen has a total protein content of ~1.5%–61%, a total lipid content of 0.5%–24.6%, and an energy value of 16–28 J/g. (Ruedenauer et al., 2019; Vaudo et al., 2020; Willmer, 2011). The amino acid composition of most pollen species is generally balanced in relation to animal requirements even though the proportions of individual amino acids vary taxonomically (Roulston & Cane, 2000; Weiner et al., 2010). Pollen is composed of all amino acids and fatty acids essential for animals, as well as important sugars, polyphenols, and various antioxidants (El Ghouizi et al., 2023; Thakur & Nanda, 2020). It is also rich in vitamins (El Ghouizi et al., 2023; Thakur & Nanda, 2020). Pollen has been shown to be an excellent source of vital nutrients due to its chemical composition translating into its nutritional and physico-chemical properties (Aylanc et al., 2021; Khalifa et al., 2021; Thakur & Nanda, 2020).

regulation of K, probably involving K resorption in the ileum (Nicolson, 1990). *Apis mellifera*, on the other hand, has evolved adaptive behavior such as actively seeking Na-rich fluids to forage on (Cairns et al., 2021; Filipiak et al., 2017; Lau & Nieh, 2016) and increases the K:Na ratio in its urine in response to the increased K:Na in nectar, leaving the ratio of these two elements unchanged in its hemolymph (Nicolson & Worswick, 1990). Accordingly, *A. mellifera* has a relatively high whole-body Na concentration compared with that of other herbivorous insects (Filipiak et al., 2017). Cannibalism occurs in *A. mellifera* colonies (Schmickl & Crailsheim, 2001; Siefert et al., 2021) and is worth considering since it might contribute to the production of K:Na balanced "jelly" that is fed to larvae. Moreover, some bee species are obligatory necrophages (Figueroa et al., 2021; Roubik, 1982). Cannibalism and necrophagy in bees may provide adaptive benefits analogous to those in other organisms (such as saproxylophages) that feed on nutritionally unbalanced sources (Filipiak, 2018b; Mattson, 1980). Consumption of young or deceased conspecifics with a chemically balanced body composition (i.e., with the same stoichiometry as the consumer) is a feeding strategy used to mitigate the effects of stoichiometric mismatches (Filipiak & Filipiak, 2022) experienced by the consumer (Filipiak, 2018b; Mattson, 1980). Obtaining a dietary K:Na balance might be a factor contributing to these foraging behaviors.

Importantly, microorganisms that colonize pollen provisions or live in the digestive tract of bees cannot alter the overall K:Na in the food consumed by bees. This is because microbes cannot destroy or transform atoms. Although microorganisms can rearrange atoms into different chemical compounds, which may affect the digestibility of food, provide needed organic molecules, and facilitate the excretion of some of the excess carbon, they cannot affect the stoichiometric imbalances (Filipiak,

2018b), as depicted in Figure 1. Overall, we hypothesize that an unbalanced K:Na in the diet might be among the major problems to overcome for carnivorous wasps evolving into pollen-eating bees.

BEE–PLANT INTERACTIONS

Plants are limited by the availability of mineral nutrients in soil and thus have frequently evolved mutualistic relationships with fungi and other microbes. This limitation may impose trade-offs, and thus costs (including maintenance of mutualisms), in developing fitness-related traits, as suggested by Filipiak, Shields, et al. (2022). For example, some proportion of minerals acquired from soil is invested to create pollen and nectar, which are then gathered and eaten by bees, and another proportion is allocated in other parts of the plant, such as leaves and stems, which could be eaten by leaf-eaters, for example, by butterflies, moths, or beetles. It seems that the allocation of specific elements within a plant's organs or tissues could be contingent upon that plant's interactions with other organisms. Recently, it was suggested that plants may allocate their stored Na to specific organs, tissues, or products to attract or repel herbivores (Kaspari, 2020, 2021). This suggestion is compelling when considering the role of K:Na in the evolution of plants and their interactions with bees.

When considering plant–pollinator interactions, it is important to realize that the primary function of pollen is plant reproduction; however, for the majority of flowering plants, the involvement of pollinators is a prerequisite for this process to occur (Ollerton et al., 2011). In turn, many pollinators, including bees, rely largely on floral resources as food sources to maintain their growth, development, and well-being. As a result, numerous plants and pollinators interact with each other, although their interests may conflict (Nicholls & de Ibarra, 2017; van der Kooi et al., 2021; Willmer, 2011). Moreover, when discussing plant-pollinator interactions, it should be remembered that the demand for pollen and nectar may fluctuate depending on the pollinator's taxonomic identity or life stage. Nectar is the main food of most adult pollinators, whereas their larvae may belong to various feeding guilds—bee larvae feed on pollen, but larvae of other pollinators feed on a vast range of resources, including leaves, wood, fungi, animal flesh, and decomposing organic matter. For juvenile bees, pollen is the primary food source, although it could be mixed to some extent with nectar or adult saliva. Considering this, one may assume that various mechanisms drive the chemical composition of nectar and pollen to different degrees. Since different trade-offs occur for plants nutritionally altering their pollen and nectar during evolution with bees, the plant products should be discussed separately with regard to bee-plant interactions.

Nectar is relatively inexpensive to produce because it consists mostly of water and carbohydrates. Since only 10% of nectar dry weight consists of non-sugar compounds (Roy et al., 2017), it may be roughly estimated that nectar consists of almost 50% C, 44% O, and 6% H, with only trace amounts of other elements (nectar elemental concentrations are given in Filipiak et al., 2017). The availability of water may limit the production of nectar; however, access to atoms of C and O used to build carbohydrates is almost unlimited (these atoms are acquired from the air). Nectar traits are heritable (Parachnowitsch et al., 2019); however, the hypothesis that nectar composition is under pollinator-mediated selection has not been supported by evidence. This has been identified as one of the most conspicuous gaps in our knowledge of the evolutionary ecology of nectar (Parachnowitsch et al., 2019; Pyke, 2016). This gap has recently gained attention in the form of the "salty nectar hypothesis," which predicts that plants concentrate Na in nectar to compete for pollinators (Kaspari, 2020). The first study testing this hypothesis did not find strong evidence to support it (Welti & Kaspari, 2021). However, the authors measured whole floral heads, not pollen or nectar directly; therefore, the feasibility of detecting Na-enriched pollen/nectar, even if it was present, was likely low because of the overwhelming mass of plant structural tissues, which are scarce in Na (Welti & Kaspari, 2021). The validity of this hypothesis was confirmed by the experimental enrichment of flower nectar with Na (Finkelstein et al., 2022a; related correspondence: Pyke & Ren, 2022, and rebuttal: Finkelstein et al., 2022b). Both the number of visits by pollinators and the number of visiting pollinator species were increased in plants with Na-enriched nectar (Finkelstein et al., 2022a).

In contrast, considering the expense associated with pollen production, it could be regarded as costly. This is because cells constituting pollen consist of a suite of all of the ~25 elements that build all living things (da Silva & Williams, 2001; Kaspari & Powers, 2016). Many of these elements are available for plants only via soil in scarce and limiting amounts; therefore, trade-offs may occur between allocating available atoms to pollen and to other plant tissues. As a consequence, plants experience life history trade-offs caused by these limitations due to the nutritional needs of pollinators. Such trade-offs would result in different amounts of acquired chemical elements allocated to specific functions and ultimately in different concentrations of vital organic compounds in plant organs and products (leaves, stems, nectar, pollen, etc.), shaping plant interactions with consumers (pollinators, leaf eaters, etc.) (Kaspari et al., 2021).

The allocation of Na, however, might be of low cost for plants, as Na is a relatively common element and of

minor function for plants. Whether plants regulate K:Na in pollen to attract bees remains an open question. According to a literature review of 11 elements studied to date in pollen, Na concentrations are the most variable, while K concentrations are among the most stable (Filipiak & Weiner, 2017). This finding suggests that plants might regulate K:Na in pollen by increasing/decreasing Na concentrations. However, the only study to date that has investigated this suggestion has shown that in the case of sunflower (*Helianthus annuus*) pollen, such regulation probably does not occur, at least in the short term of a single growth period (Filipiak, Shields, et al., 2022). However, some plants produce two types of pollen, one used to reward the bees and the other for reproduction (Luo et al., 2008). The question arises: why?

Perhaps the two types of pollen differ in their chemical composition, optimized to perform a specific function (food for the pollinator vs. reproduction of the plant). A recent study suggests that this may indeed be the case regarding certain organic molecules (i.e., different proteins and lipids) (Huang & Gong, 2022). However, it is not known whether this also applies to K:Na (Filipiak, Shields, et al., 2022). There are two possible eco-evolutionary mechanisms related to the exceptional Na variability observed in pollen: (1) some plant species might have high Na concentrations in pollen, since they produce pollen not solely to reproduce but also as a reward for pollinators (Nicholls & de Ibarra, 2017), while (2) other plants might reduce the concentration of Na in pollen to discourage pollen eating.

AVENUES FOR FUTURE RESEARCH: FROM THE HEALTH AND FITNESS OF INDIVIDUALS TO THE FUNCTIONING OF FOOD WEBS AND NUTRIENT CYCLING IN ECOSYSTEMS

Nutrient cycling in food webs and ecosystems is driven by the nutritional needs of consumers and is shaped by *nutritional mismatches* between adjacent links in the food chain (Filipiak & Filipiak, 2022; Sardans et al., 2021; Sterner & Elser, 2002). Regarding nutrient cycling via the soil–plant–pollen/nectar–consumer pathway, our knowledge is extremely limited. We hypothesize that since K:Na is an important factor generating eco-evolutionary trade-offs, it influences nutrient cycling in food webs and ecosystems, shaping ecological interactions via nutritional mismatch (Figure 4). To date, bees have not been considered in studies on nutrient cycling. We know that a high K:Na in food causes acute paralysis and death in bees (Kaspari, 2020). We also know that bees prefer a low K:Na in water (Cairns et al., 2021). We have some knowledge of a similar phenomenon in herbivorous mammals, as too high a K:Na in plant tissues causes tetany, diarrhea, and death in large mammals, therefore shaping their feeding behavior (Kaspari, 2020). As a consequence, nutrient cycling in ecosystems may be altered via changes in the amounts and proportions of nutrients composing animal excreta as well as via changes in the spatial distribution of these nutrients (Sitters et al., 2015, 2017). The way in which this might work is illustrated by the example of *Azteca trigona*, an ant inhabiting Neotropical forests (Clay et al., 2013), which highlights the crucial role of insects in nutrient cycling. According to the authors, *A. trigona* creates concentrated and long-lasting nutrient pathways between the canopy and the forest floor. The ants produce a steady flow of nutrients in the form of refuse, which is more concentrated than leaf litter in decomposition-enhancing chemical elements. These nutrients are propagated up the food web, supporting the food chain, increasing the population sizes of decomposers and predators, and accelerating decomposition on the forest floor (Clay et al., 2013). Similarly, the experimental addition of K and Na to a tallgrass prairie ecosystem has affected the food web, altering plant ionomes and biomass and shaping invertebrate abundance (Kaspari & Welti, 2023).

How large the role of bees and other pollinators is in nutrient cycling in food webs and ecosystems has yet to be investigated (Ollerton, 2021a). Currently, one can only speculate that, considering the large amount of biomass relocated by bees in ecosystems, bees play an important role in the mobilization and flow of nutrients from soil through plant pollen and nectar to make them available for other links of the food chain. For instance, the importance of plant-pollinator interactions for carbon cycling and its deposition in soil systems and how this might contribute to climate change mitigation remain unknown (Ollerton, 2021b). Currently, all that can be said with certainty is not only that bee bodies are vehicles relocating nutrients within ecosystems but also that bee cocoons and excreta may play a role in local-scale nutrient cycling (Filipiak et al., 2021).

Kaspari and colleagues have suggested that in response to their interactions with herbivores and pollinators, plants may modulate Na concentrations in their tissues and products (Finkelstein et al., 2022a; Kaspari, 2020, 2021). In fact, a review of the literature on the chemical composition of pollen revealed that of the 11 elements studied, K levels are relatively constant, while Na levels vary widely (Filipiak et al., 2017). Furthermore, herbivores tend to prefer plant tissues and products with elevated Na (Borer et al., 2019; Finkelstein et al., 2022a; Nicolson & Worswick, 1990). Hence, it is conceivable that the soil–plant–bee pathway of the nutrient cycle is influenced by the plants' ability to use K:Na

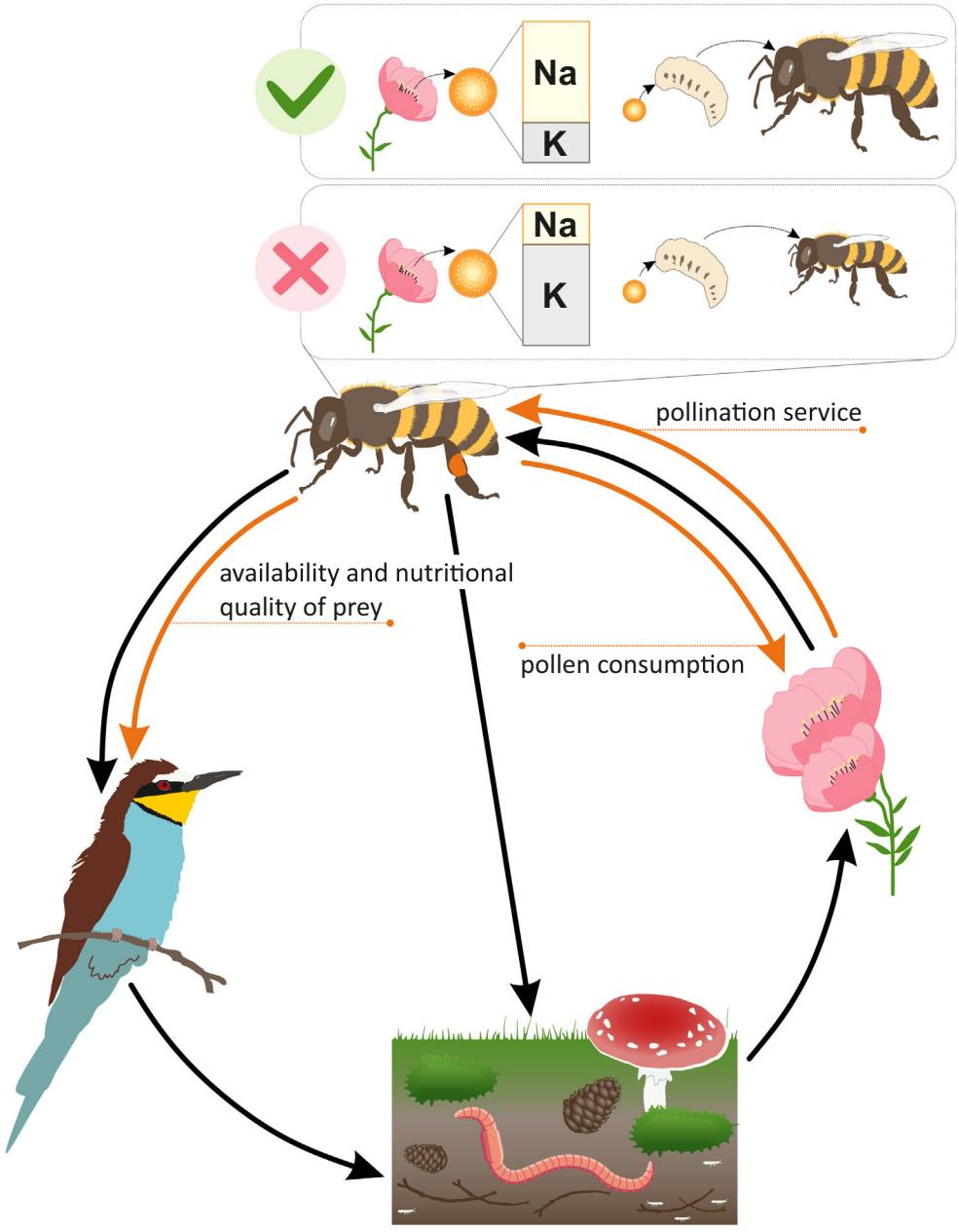

FIGURE 4 Nutritional mismatches originating from K:Na may shape the functioning of the food web and nutrient cycling in ecosystems. Black arrows, nutrient cycling; orange arrows, ecological interactions. Interactions between components of the food web are driven by stoichiometric mismatch in K:Na. Plants mobilize atoms of chemical elements from soil and allocate a specific proportion of these atoms to pollen (black arrow). Bee development, survivability, size at maturity, and health depend on the pollen K:Na (the ✓ and ✕ signs indicate whether limiting effects occur). This limitation ramifies further in the food web, affecting bee predators and decomposers that forage on bee excreta, cocoons, and dead bodies (orange arrows indicate ecological interactions, and black arrows indicate the flow of nutrients). The orange arrow from bee to plant indicates the potential impact of the bee's nutritional needs on the plant's allocation of a particular K:Na ratio to the pollen. Illustrations by Zuzanna M. Filipiak.

regulation in their pollinator rewards (but see Filipiak, Shields, et al., 2022). The impact of plant-pollinator nutritional interactions on nutrient cycling within ecosystems remains to be thoroughly explored, especially considering the significance of the soil–plant–pollinator pathway of nutrient cycling for the functioning of food webs, ecosystems, and the well-being of humans.

CONCLUDING REMARKS

Nutrients are often envisioned only as organic molecules available in the form of proteins, lipids, carbohydrates, phospholipids, vitamins, essential amino acids, and so forth. However, it is crucial to contemplate alternative perspectives that enable a comprehensive evaluation of

BOX 4 Outstanding questions.

1. To what degree does the functioning of individual bees, their populations and communities depend on the availability of a balanced K:Na in their food?
2. Do plants manipulate their nectar and pollen K:Na to attract or repel potential consumers?
3. If so, can plants produce two types of pollen with different K:Na ratios and regulate interactions with bees via the pollen K:Na?
4. How is nectar and pollen K:Na associated with particular plant species? Do plants employing different strategies (e.g., bee pollinated vs. butterfly pollinated vs. wind pollinated) differ in their pollen K:Na?
5. To what extent do nectar and pollen K:Na depend on the nutritional properties of soil?
6. Considering various bee species, all of which have specific feeding preferences but may be limited by their food K:Na, how do foraging behavior and feeding preferences related to K:Na in food affect nutrient cycling in ecosystems?

the food web continuum (e.g., soil–plant–pollinator). This may be achieved by examining the interactions between its constituents via stoichiometric mismatches between resources and consumers (Filipiak & Filipiak, 2022). We still do not fully understand the mechanisms driving nectar and pollen nutritional ecology and how this in turn shapes ecological interactions and ecosystem functioning. For example, regarding K:Na, we present the most important questions and problems to be solved in Box 4. Nutritional balancing of bee food is not as simple and straightforward as we often assume. First, food quantity cannot replace food quality, as too much nutritionally unbalanced food has toxic effects on consumers (Jones & Flynn, 2005). Second, balanced adult nutrition is not enough to maintain stable and healthy populations and communities, as immature stages have different nutritional needs than adults (Filipiak, 2018a). These phenomena remain largely unexplored and pose a challenge for future studies dealing with the nutritional, trophic, and evolutionary ecologies and conservation of bees worldwide. Therefore, we should gain knowledge on bees' demands for and ecosystems' supply of a balanced K:Na in bee food sources. Pollinators make their own choices as to which flowers to forage on (Nicholls & de Ibarra, 2017; Willmer, 2011), and there is a positive correlation between plant diversity and pollinator diversity within communities (Ollerton, 2017). At the same time, some key plant species provide vital nutrients in proportions needed by bees (Filipiak, Denisow, et al., 2022), and Na may be supplemented from non-living components of ecosystems (Cairns et al., 2021; Filipiak et al., 2017). However, it is not yet clear the extent to which plant different species contribute to the K:Na balance in bee diets. Furthermore, we do not know to what extent floral resources in general contribute to the K:Na balance and to what extent this balance depends on complementary resources such as manure, ponds, puddles, carrion, and animal secreta and excreta. In relation to landscape restoration and management interventions for pollinators, these "supplements" comprise one of the three vertices of the "Requirements of Pollinators Triangle" suggested by Ollerton (2021a). Therefore, improved knowledge on the nutritional mechanisms underlying bee-plant and bee-ecosystem relationships will allow us to apply appropriate bee conservation actions and improve our efforts aimed at mitigating bee declines.

AUTHOR CONTRIBUTIONS

Zuzanna M. Filipiak and Michał Filipiak conceived the study. Zuzanna M. Filipiak conducted a literature search, analyzed the data retrieved, and wrote the first draft of the manuscript. All authors (Zuzanna M. Filipiak, Michał Filipiak, and Jeff Ollerton) contributed substantially to writing and revisions.

ACKNOWLEDGMENTS

This work was funded/supported by Jagiellonian University, Faculty of Biology (N18/DBS/000003), and by the 2018–2019 BiodivERsA joint call for research proposals under the BiodivERsA3 ERA-Net COFUND program in association with the National Science Centre, Poland (grant no. 2019/31/Z/NZ8/04030). The open-access publication of this article was funded by the program "Excellence Initiative – Research University" at the Faculty of Biology of the Jagiellonian University in Kraków, Poland.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

This study did not generate new data. The data set and literature used to create Figure 1 are available in Filipiak et al. (2023) in Zenodo at https://doi.org/10.5281/zenodo.7951112.

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How to cite this article: Filipiak, Zuzanna M., Jeff Ollerton, and Michał Filipiak. 2023. "Uncovering the Significance of the Ratio of Food K:Na in Bee Ecology and Evolution." *Ecology* 104(8): e4110. https://doi.org/10.1002/ecy.4110