

# How to really help bees: nutrient demand and supply in a changing environment

Zuzanna Filipiak

Jagiellonian University in Cracow

Bozena Denisow

University of Life Sciences in Lublin

Ernest Stawiarz

University of Life Sciences in Lublin

Michał Filipiak (✉ [michal0filipiak@gmail.com](mailto:michal0filipiak@gmail.com))

Jagiellonian University <https://orcid.org/0000-0001-7438-4885>

---

## Article

**Keywords:** ecology, biogeochemistry, biodiversity, bees, conservation

**Posted Date:** April 20th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-443806/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

Local and global changes affect which pollen varieties are available to bees in the environment. Therefore, wild bees cannot always access the optimally balanced diet required for their survival. Our feeding experiment showed that the nutritional quality of the pollen diet eaten by bee larvae is shaped not by pollen diversity but by a specific pollen species composition that results in specific nutrients being scarce or sufficient; this species composition influences bee survivability, development and mass. We proposed that the functioning of bee populations and communities may depend on the floral diversity of the local habitat, which determines whether a nutritionally balanced pollen diet obtained from specific species can be provided to bee larvae. Holistically considering wild bee fitness and health and the different characteristics of the food base at both the ecosystem and bee biology levels can provide new, important knowledge for conserving bees and their critical ecological roles.

## Introduction

Bees are deeply entrenched in ecological systems and processes, and bee nutritional ecology, health and fitness are shaped by ecological interactions<sup>1–3</sup>. Although much is understood about the details of the functioning of bees and pollinators in general, connections among the various elements of knowledge about bee nutrition and well-being that would allow for a broad, ecosystem-oriented understanding of wild bee ecology and conservation are still lacking<sup>1–3</sup>. The functioning and prosperity of bee populations are influenced by human activities that cause changes in landscape structure, leading to habitat loss, fragmentation and degradation. As a result, bees may be subjected to deficiencies of food resources, which have been indicated as one of the most important drivers of bee decline<sup>4–6</sup>. The term “deficiency of food resources” should be emphasized here; it does not simply mean less food but rather refers to changes in floral species diversity and composition caused by landscape changes due to any agricultural, industrial, construction, or other anthropogenic activity.

The quantity and quality of food available in the environment both strongly impact consumer fitness and therefore determine the success of the consumer population<sup>7,8</sup>. Crop monocultures may increase the amount of food available for bees while at the same time decreasing its quality, i.e., the balance of its nutritional composition<sup>9–11</sup>. Monocultures can serve as nectar sources for adult bees<sup>12</sup>, but pollen from monocultures may be insufficient to meet the needs of developing bee larvae<sup>10</sup>. This is because nectar, which is rich in sugars, is extremely lacking in other nutrients<sup>13–15</sup>; thus, it is an important food for strongly energetically limited adults. In turn, for growing and developing larvae, pollen provides a source of body-building matter. Nevertheless, monofloral pollen from certain species is not nutritionally balanced enough to allow normal juvenile bee development<sup>10,16</sup>. Moreover, bee sexes differ in their nutritional needs and prefer different plant species as food sources<sup>17–19</sup>. However, bee conservation efforts are often based on simplistic assumptions regarding the nutritional ecology of one life stage (usually the adult stage) or sex (usually females). In reality, bee populations consist of individuals in various life stages and of different sexes. We believe that a holistic approach to bee conservation that is based on

details of bee biology but also considers bees as part of ecosystems and as being involved in ecological processes and interactions may be important for developing more effective management strategies for maintaining wild bee populations. Therefore, in this study, we connect the basic physiological mechanisms underlying bee nutritional needs with the functioning of bee populations within the context of ecosystems in which the flora offers either a nutritionally balanced or unbalanced food base.

In general, floral diversity has a positive impact on bee populations, providing large amounts of nectar and pollen with diverse botanical origins<sup>6,20-22</sup>. However, the mechanism underlying this positive impact is not understood. Here, we hypothesize that this mechanism is based on the differences between bee nutritional demand and environmental nutritional supply: bees are often unable to achieve a nutritional balance in their diets because only a strictly limited number of plant species provide the required resources in the needed proportion; these species are not available in certain landscapes that have specific floral compositions<sup>1,10,22,23</sup>. In recent years, a debate has developed over the importance of biological diversity to ecosystem functioning and ecosystem services. The economic value associated with biodiversity is a strong argument for conservation efforts; however, some studies indicate that this value has certain limitations<sup>24</sup>. For instance, only a small minority of bee species provide most of the crop pollination services worldwide<sup>24</sup>. This fact is most likely linked to bee nutritional needs; a more in-depth understanding of the nutritional requirements of wild bees is needed, and the relationship between floral species diversity and species composition could become a focal point for future conservation efforts.

Among the various measures of the nutritional balance of bee diets, the concept of stoichiometric mismatch and the ratios of atoms of vital chemical elements that form organic molecules seems to be one of the most ecologically relevant; this measure allows bee nutritional physiology and fitness to be connected directly with nutrient cycling throughout the whole food web, which is shaped by complicated synergy of ecological interactions<sup>8,25,26</sup>. The atoms of chemical elements in various proportions build the tissues and bodies of organisms. The most fundamental feature of these elements, which allows them to be used in ecological stoichiometry, is that these specific atoms cannot be transformed into different atoms by the organism. This feature distinguishes atoms from organic compounds. Within this context, ecological stoichiometry provides a common currency that links the ecology of organisms to life history trade-offs and evolutionary processes that are entrenched in the biogeochemical economy of life<sup>27</sup>. This currency is the ratio of atoms that compose the bodies of organisms and their food<sup>8</sup>. Consumers, especially herbivores, are faced with a high threshold of stoichiometric incompatibility (i.e., stoichiometric mismatch) between the chemical composition of their tissues and their food<sup>8,25,28</sup>. Within this context, the concepts of the biogeochemical niche and the stoichiometric niche were recently proposed<sup>29,30</sup>. Both concepts share similar ideas and focus on changes in the availability of particular atoms that are needed in specific proportions to build and maintain organisms as a factor driving the evolution of life and structuring the environment (hereafter, the term “stoichiometric niche” will be used). The stoichiometric niche is defined as a multivariate niche space occupied by a group of individuals with similar stoichiometries; specific species occupy specific niches, and each niche is stoichiometrically explicit<sup>29-31</sup>.

To obtain stoichiometrically balanced food, various organisms with specific optimal elemental phenotypes (also called ionomes) may prefer various food sources that provide nutrients in proportions that reflect their nutritional demand<sup>31</sup>. Feeding on stoichiometrically unbalanced food, which would result in decreased concentrations of some elements and therefore an unbalanced stoichiometric ratio, may negatively affect bee fitness<sup>18</sup>. Nutritional niches (irrespective of the specific definition applied) may differ between sexes, imposing sex-specific nutritional constraints on organisms<sup>31,32</sup>. It has been suggested that in the case of wild bees, sexual dimorphism in nutritional demand may be an important factor for and should be considered in bee conservation efforts that focus on providing balanced diets to bees<sup>17–19</sup>.

Here, we propose and investigate the mechanisms underlying the link between floral diversity and bee performance. We hypothesize that (1) the nutritional quality of bee larval food is connected to both the species diversity and species composition of the pollen constituting this food but that (2) the factor directly driving the nutritional quality of the food is the species composition and not the species diversity, because (3) the species composition of the pollen provisions, i.e., the presence of pollen from specific plant species, is associated with the stoichiometric phenotype of the pollen, which reflects its ability to provide atoms of chemical elements in the specific proportions that meet or do not meet bee nutritional demands. We investigated the abovementioned ideas and hypotheses using a solitary bee model system. Utilizing this model organism, we were able to perform feeding experiment controlling and measuring independently (1) the pollen diversity and species composition of larval food, (2) the stoichiometric phenotype of this food and (3) the different nutritional needs of bee females and males (Figs. 1 and 2). Additionally, this study system provided us with a unique opportunity to determine the nutritional needs of female and male bees and to address a general ecosystem ecology problem, that is, the life history of an organism embedded within an ecosystem. We believe that this approach makes the evaluation of floral resource limitations in bees ecologically relevant and may provide precise tools for use in conservation actions. Such precise tools are needed because, as the nutritional ecology of wild bees is still not well understood, management actions to support bee habitats are not effective and often fail<sup>33–35</sup>.

## Results

### Pollen pools – nutritional quality, species composition and species diversity

The pollen pools used in the feeding experiment differed in their nutritional quality, as reflected by the concentrations and proportions of the studied elements (Fig. 3). The control-Osmia, Apis1 and Apis2 pollens had similar nutritional quality, -CuZn1 and -CuZn2 were scarce in Cu and Zn, -KP was scarce in K and P, and -NaP was scarce in Na and P (Fig. 3). The exact concentrations of the studied elements are shown in Supplementary Table S2. There was a strong correlation between the elemental composition of the pollen mixtures and the presence of pollen from specific plant species: scarcity of Cu and Zn was associated with a predominance of *Brassica napus* pollen, scarcity of K and P was associated with a

predominance of *Anthriscus* sp. pollen, and scarcity of Na and P was associated with a predominance of *Aesculus* sp. pollen.

The pollen pools differed in both species composition and species diversity. -CuZn1 was the least diverse, and -KP was the most diverse (Tables 1 and 2). The most diverse pollen pool (-KP) also had the lowest dominance value, whereas the -CuZn1 and -CuZn2 pollens, composed of approx. 80–90% of *B. napus* pollen grains, were the most strongly dominated by a single species (Tables 1 and 2). All the nutritionally balanced pollen pools, i.e., control-Osmia, Apis1, and Apis2, had average species diversity as expressed by the Fisher's alpha and D indices among all studied pools (Table 1) but differed in their species composition (Table 2).

Table 1

Diversity indices characterizing the pollen pools used in the feeding experiment based on the number of bee specimens and the number of taxa of pollen grains composing the pools.

	Control-Osmia	Apis1	Apis2	-CuZn1	-CuZn2	-KP	-NaP
Number of taxa	16	21	21	11	27	31	27
Number of specimens	946	1008	1174	997	1016	1471	936
Dominance (D)	0.26	0.25	0.33	0.84	0.80	0.15	0.44
Fisher's alpha	2.74	3.75	3.63	1.73	5.09	5.55	5.19
The dominance (D) value ranges from 0 (all taxa are equally present) to 1 (one taxon dominates the community completely). For Fisher's alpha, a higher value indicates more diversity. See Supplementary Table S1 for details.							

Table 2  
Most numerous pollen species composing the pollen pools used in the feeding experiment.

Pollen species	Percentage of grains in the pollen pool (%)							
	Control-Osmia	Apis1	Apis2	-CuZn1	-CuZn2	-KP	-NaP	
<i>Quercus</i>	41.0	8.2	0.0	0.7	1.0	0.5	0.1	
<i>Salix</i>	29.0	0.1	0.0	0.2	0.6	3.8	3.8	
<i>Brassica napus</i>	0.0	36.3	0.0	91.5	89.3	0.0	0.0	
Other <i>Brassicaceae</i>	1.2	0.0	1.6	0.0	0.0	23.2	3.0	
<i>Acer</i>	4.1	26.5	0.0	2.8	1.3	0.1	0.2	
<i>Parthenocissus</i>	0.0	0.0	44.0	0.0	0.1	0.1	0.1	
<i>Filipendula</i>	0.0	0.0	36.5	0.0	0.0	16.2	1.5	
<i>Anthriscus</i>	0.0	0.4	0.0	1.6	0.1	24.8	0.7	
<i>Aesculus</i>	0.6	0.1	0.1	0.0	0.6	0.2	65.0	

For each pollen pool, the values for the pollen species composing more than 20% of the pool are shown in bold. All species found in the pollen pools and their percentages in each pollen pool are presented in Supplementary Table S1.

## Mortality

Bee mortality differed among treatments, and the differences in mortality were sex dependent (Fig. 4). The -CuZn1 and -CuZn2 treatments without supplemental nutrients resulted in increased mortality compared with the control in both sexes, and this effect was stronger for males than for females. This effect was mitigated in females when both Cu and Zn were supplied. In males, the effect was mitigated when Zn was added, but adding both Cu and Zn had a stronger mitigating effect. The mitigating effect caused by the addition of both Cu and Zn was stronger for males than for females. The -KP treatment without supplemental nutrients resulted in increased mortality, and this effect was stronger for males than for females. The addition of either K or K and P caused an even stronger increase in female mortality but did not have any effect on males. The -NaP treatment without supplemental nutrients resulted in increased mortality in both sexes, but this effect was stronger for females (100% mortality) than for males. The addition of either Na or Na and P mitigated this effect only for females, and the addition of both Na and P had a stronger mitigating effect than adding only Na.

## Cocoon development

Cocoon mass was not correlated with cocoon development, and adult mass was moderately correlated with cocoon mass (Table 3).

Table 3  
Correlations between adult mass, cocoon mass and cocoon development.

	Females		Males	
	R	p	R	p
Adult mass vs cocoon mass	0.48	$9 \times 10^{-17}$	0.49	$2 \times 10^{-17}$
Adult mass vs cocoon development	0.22	0.00025	0.29	$2 \times 10^{-6}$
Cocoon mass vs cocoon development	0.19	0.00213	0.19	0.00196
Spearman's R coefficient, p < 0.05. For both sexes, adult mass was moderately correlated with cocoon mass and slightly correlated with cocoon development, while cocoon mass was not correlated with cocoon development.				

The effect of diet on cocoon development differed among treatments, and these differences were sex dependent (Fig. 5). The -CuZn1 and -CuZn2 treatments without supplemental nutrients resulted in underdeveloped cocoons in both sexes. This effect was mitigated in females when both Cu and Zn were added. In the case of males, mitigation was observed when Zn was added, but adding both Cu and Zn had a stronger mitigating effect. The -KP treatment without supplemental nutrients resulted in underdeveloped cocoons in both sexes. Supplementation with either K or K and P mitigated this effect in males. -NaP treatment without supplemental nutrients resulted in underdeveloped cocoons in males, and supplementation, whether with Na or Na and P, had no effect.

### Adult mass and cocoon mass

The effect of diet on the masses of cocoons and adults differed between treatments, and these differences were sex dependent (Fig. 6). The -CuZn1 and -CuZn2 treatments without supplemental nutrients resulted in reduced body mass in both sexes, and the effect was stronger for males than for females. At the same time only the female cocoon mass was reduced in these treatments. Supplementation with both Cu and Zn mitigated the negative effect on female body mass but not that on cocoon mass. For males, the negative effect on their body mass was mitigated by supplementation with solely Cu, solely Zn, and both Cu and Zn. The -KP treatment without supplemental nutrients reduced both body mass and cocoon mass in both sexes. Supplementation with both K and P mitigated the negative effect on male cocoon mass but not that on male body mass. The -NaP treatment reduced both body mass and cocoon mass in males, and supplementation, whether with Na or Na and P, had no mitigating effect.

## Discussion

The links between floral diversity and bee functioning and between floral species composition and bee functioning are unclear, and a better integration of the approaches and frameworks scattered among various studies is needed<sup>2,22,23,36,37</sup>. Here, we provide a point of view rooted in organismal bee physiology

and further extend it over the whole ecosystem, of which the bee population and the floral composition form a part. We suggest that bee fitness may be shaped by the availability of vital nutrients at specific concentrations associated with specific key plant species. We conclude that more diverse floras provide bees with the opportunity to use their preferred resources; however, the direct mechanism driving the increase in bee fitness in diverse environments is the diet balance achieved with the pollen of key plant species.

In our study, the pollen mixtures that were both the most diverse and the least diverse in terms of floral species had limiting effects on bee growth, development and fitness. This phenomenon was associated with the presence of specific plant species that are responsible for dietary imbalances in terms of certain nutrient elements. Diminished concentrations of Cu and Zn were associated with the dominance of *Brassica napus*; a lack of K and P was associated with a predominance of *Anthriscus sp.*, and other *Brassicaceae*; and a lack of Na and P was associated with a predominance of *Aesculus sp.* Interestingly, the two diets (-CuZn1 and -CuZn2) with a high percentage of *B. napus* pollen were characterized by similar dominance D indices but relatively distinct Fisher's alpha indices, and both diets had similar negative effects on bee survivability and development. However, the addition of Cu and Zn to these diets mitigated their negative effects. Therefore, *B. napus* pollen, if highly concentrated in the larval diet, increases bee mortality, decreases body mass, and inhibits cocoon development, and this effect is driven by the scarcity of Cu and Zn. Our results are in accordance with those of previous studies<sup>37,38</sup>. Klaus et al.<sup>37</sup> observed diminished reproduction of *O. bicornis* in monofloral habitats (100% oilseed rape) compared with that in habitats with more complex floral resources (50% wildflowers: 50% oilseed rape) in a semifield experiment with pesticides. Holzschuh et al.<sup>38</sup> in turn found that almost no *O. bicornis* reproduction occurred in isolated oilseed rape fields that were not adjacent to grasslands, whereas reproduction was prominent in oilseed rape fields that were adjacent to grasslands as well as in grasslands that were adjacent to oilseed rape fields. Our study is the first to reveal the mechanism behind the widely known phenomenon of the need for diverse floral resources in the vicinity of *B. napus* monocultures, namely, the deficiency of specific nutritional elements (Cu and Zn), which in turn can be mitigated by the presence of specific plant species whose pollen is rich in those elements, e.g., *Filipendula sp.*

A negative effect was also imposed on bee growth, development and fitness by the two most diverse diets, which had either no dominant pollen species (treatment -KP) or moderately dominant pollen species (treatment -NaP). This negative effect was most likely caused by the specific species composition of these diets, which resulted in a scarcity of vital nutrients (either K and P or Na and P); however, additional causal factors cannot be excluded. The supplementation of -KP with K or both K and P had no effect on females. In males, the supplementation of the -KP pollen pool had a positive effect on cocoon development and mass, indicating that to some extent, the negative effects were associated with K and P deficiency. Regarding -NaP, the positive effect on the studied parameters after supplementation was more pronounced than that in the supplemented -KP diets, but the survival, cocoon development and masses still did not reach the levels of the control individuals. In general, the relatively high diversity

indices calculated for -KP and -NaP do not imply an adequate diet, and even supplementation did not mitigate the negative effects of these diets; certain other factors may have been related to the observed results, e.g., colimiting nutrients (apart from K and P) or the presence of poisonous substances<sup>39</sup>. These additional effects might also be related to the specific species composition of the pollen, with -KP having a 25% concentration of *Anthriscus* pollen and -NaP having a 65% concentration of *Aesculus* pollen.

In contrast, the pollen diet collected in nature by *O. bicornis* (Control-Osmia) and the two *Apis* diets (Apis1 and Apis2), which did not have any negative effects on the bees, had moderate pollen species diversity and dominance. What differentiated these diets from the limiting diets was the specific pollen species composition, which resulted in a stoichiometric phenotype that was nutritionally balanced for *O. bicornis*. Therefore, we infer that, considering the pollen pool available directly to bee larvae, the species composition of the larval pollen diet is more important to an adequate nutritional balance than the diversity of the larval pollen diet. However, we emphasize that the pollen pool that is directly available to bee larvae is not identical to the pollen pool provided by particular flora in the environment to adult bees that collect pollen for their larvae. Therefore, below, we extend our point of view to the whole ecosystem.

The ontogenetic development of individuals and the transfers of energy and matter that occur during this development have implications for the functioning of populations and communities<sup>40</sup> as well as for the whole food web and the functioning of ecosystems<sup>41</sup>. In this study, we have shown how the capability of wild bees to compose a nutritionally balanced diet influences the mortality and development of individuals. The results of our study may be further translated to the population level, as described later in this paragraph and in Fig. 7. Let us consider two populations of *O. bicornis* with the same number of individuals and with a sex ratio of 0.5 females:0.5 males (for clarity, regardless of the sex ratios that are most common in nature). Both populations are established in seminatural environments consisting of a rapeseed monoculture surrounded by many flowering plants and some trees that produce large amounts of pollen from early to late spring, i.e. during the time window utilized by *O. bicornis* females to collect pollen for their progeny. Population (a) is established in a diverse flora offering eight pollen species (Fig. 7a), and population (b) is established in a poor flora offering only four pollen species (Fig. 7b). Population (a) thrives and prospers because the bees are able to collect pollen from their preferred species and thereby compose a nutritionally balanced diet that allows for proper larval development. Therefore, in the next generation, the number of individuals increases, and the bees are appropriately sized and healthy. In the case of population (b), the poor flora offers mainly stoichiometrically unbalanced pollen, resulting in stoichiometric mismatches<sup>25,26</sup> for the bees. The bees experience high mortality, generate underdeveloped cocoons that further increase their mortality and have small body sizes. Since the negative effects of dietary imbalances affect females to a greater degree than males, as shown in the current study, the next generation is dominated by males. Moreover, smaller bees can fly only shorter distances and can carry less pollen to their progeny than larger bees, which further negatively affects future generations. Overall, population (b) is in decline in this scenario. Therefore, even though all the pollen species occurring in flora (b) also occurred in flora (a), the additional species that occurred only in flora (a) allowed bee population (a) to thrive and prosper. This positive effect on bee populations

happens more often in diverse floras than in poor floras simply by chance – the more plant species are available, the greater the chance of finding pollen that allows a nutritionally balanced diet. Therefore, the floral composition may shape bee populations by controlling the nutritional supply available to bees. The occurrence of key plant species that provide the correct dietary stoichiometric balance for bees may be a factor in shaping bee populations. Access to these key plant species is essential for bee growth and development regardless of whether the pollen from these plants is gathered intentionally or not. Bee populations are influenced by the nutritional balance of the bee larval diet, and this balance depends on the floral composition of the bee habitat (the stoichiometric niche). High floral diversity may be necessary to maintain populations of pollen eaters by providing key plant species that allow for dietary nutrient balancing; single-species crop plantations, even if they are rich in nectar and pollen, might limit bee development. Consequently, changes in local floral communities may shape bee colonies, populations and communities. Therefore, not only the quantity but also the quality of food sources for bees should be considered in intervention strategies aimed at improving the nutritional base for bees.

The demand for resources for growth and development, as reflected in organismal stoichiometry, is usually studied by comparing different species, but research has also started to focus on individual variations in the chemical compositions of bodies; much of this variation is expected to be attributable to sex differences<sup>19,31,32</sup>. This is expected because processes involved in life history evolution and population dynamics are likely to differentially affect females and males, thus imposing sex-specific nutritional limitations<sup>27,32</sup>. By considering such within-species variance, evaluations of resource limitations in a given species can increase their ecological relevance. In our previous study, we presented the idea that both sexes of *O. bicornis* have different stoichiometric niches<sup>19</sup>. It has also been hypothesized that *O. bicornis* females collect pollen species in proportions that reflect the sex-specific nutritional needs of their daughters and sons<sup>10,42</sup>. Sex-specific differences in stoichiometric phenotypes that can be reflected in stoichiometric niches have also been detected in other invertebrates, including amphipods and spiders<sup>31,43</sup>. Our preliminary experiment showed that the scarcity of specific nutrients in a larval diet indeed impacted bee development in a sex-dependent manner<sup>18</sup>, as predicted by theoretical calculations based on stoichiometric phenotypes and stoichiometric mismatches between consumers and their food<sup>10</sup>. The current study provides the first detailed insight into this phenomenon that is based on a large pool of specimens utilizing different nutritional treatments. We have shown that the scarcity of specific nutrients (atoms of vital chemical elements) in bee larval food shapes the fitness of bee individuals in a sex-dependent manner. Females were strongly negatively affected by the scarcity of Na and P, which resulted in higher mortality rates for females than for males. In fact, the Na- and P-scarce treatment was the only treatment resulting in 100% mortality (only for females); this effect was mitigated when the diet was supplemented with Na and mitigated even more when the diet was supplemented with Na and P. Interestingly, apart from the obvious functions related to maintaining transmembrane electrochemical potential differences in living cells<sup>44</sup>, Na plays important roles in regulating the assimilation of N and especially P from food, in phosphate homeostasis and in phosphate sensing at the cellular and organismal levels<sup>45–47</sup>. This function may be more important for P-limited females that need

to develop their ovaries and to produce eggs than for males that do not develop N- and P-demanding reproductive apparatuses<sup>19</sup>. In contrast, males had higher mortality than females when fed on pollen scarce in K; while K scarcity resulted in decreased masses of bodies and cocoons of both sexes, supplementation with K had a positive effect only on male cocoon masses. These results are in line with those of a previous study showing that Na and K are assimilated and allocated to specific functions differently in both sexes of *O. bicornis*<sup>19</sup>. Males experienced higher mortality than females when feeding on pollen that was scarce in Cu and Zn. The scarcity of Cu and Zn also resulted in lower female cocoon mass but had no effect on male cocoon mass. Both sexes also developed lower body masses when fed on pollen that was low in both Cu and Zn, but supplementation with Zn mitigated this effect for males (supplementation with both Cu and Zn had a positive effect on both sexes). These differences are again in line with our study considering the elemental budget, assimilation and allocation of elements by *O. bicornis* and are discussed therein<sup>19</sup>. Together, these detailed results show that in terms of the dietary nutrient balance, individual fitness is regulated differently and is linked to a sex-specific optimal proportion of nutrients for the larval food of bees. This is important information that should be considered when designing regulations, laws and actions aimed at wild bee conservation. Existing regulations, laws and actions are based mostly on data obtained for females, especially adult females, and related to their energetic needs rather than to detailed nutritional biology data.

Vaudo et al.<sup>9</sup> stated that bee population declines are linked to nutritional shortages and that possible host plant species vary in their nutritional quality; thus, knowledge of bee nutrition should be applied to the selection of floral resources during habitat restoration. The authors also observed that little is known about the nutritional requirements of bees, which remains true. A better understanding of the nutritional ecology of wild bees may be one of the most critical focus areas in bee ecology<sup>25,48</sup>. The attractiveness of wildflower mixtures for wild bees has been shown to depend on several key plant species<sup>49</sup>, but future studies that link bee nutrition to bee life history traits and fitness in the context of floral preferences and floral habitat composition are needed to elucidate the dependency of bees on these factors. Nutrient collapse in plant tissues has been reported in recent studies; specifically, elevated concentrations of atmospheric CO<sub>2</sub> reduce the concentrations of important nutrients in plant tissues<sup>50</sup>, including the nutrients in the pollen utilized by bees<sup>51</sup>. In this context, understanding the demands of growing bees for a nutritionally balanced diet is even more important; apart from contributing to the development of ecology and evolution studies, the current project will also impact conservation biology.

## Methods

We investigated the influence of various larval diets on bee fitness. The diets were composed of pollen mixtures differing in species diversity, species composition, and nutritional quality, as reflected in the concentrations and proportions of studied elements (the P, Na, K, Zn and Cu; the diets were either nutritionally balanced or low in specific elements). To allow for the clear interpretation of the obtained results, every diet that was low in specific elements was provided followed by a diet composed of the same pollen mixture that was artificially supplemented with a salt of the lacking element(s). Our

experimental setup, described below, allowed us to (1) study the direct effects of the nutritional quality of the diet on bees, (2) study the indirect effect of plant diversity on bees, and, most importantly, (3) determine whether these effects are related to each other. Such an approach allowed us to clearly indicate which factor was responsible for the obtained results. Moreover, because we studied different features of the food base, the setup allowed us to interpret the results in the context of natural conditions; this increased the ecological relevance of our evaluation of the effects of resource limitation on bees. We used a model organism and experimental design based on our preliminary studies:<sup>18,19</sup>. Bee fitness was represented by several life history traits, namely, (1) mortality during larval development, (2) adult body mass, (3) cocoon mass, and (4) cocoon development state (cf.<sup>18</sup>).

## Model organism

We obtained specimens of the wild solitary bee *Osmia bicornis* (syn. *O. rufa*, Hymenoptera: Megachilidae) from a trap nest containing 500 empty cane stems (25–30 cm in length, 0.6–1 cm in diameter) mounted in one case (Kraków, Poland; 50° 01' 35" N; 19° 54' 05" E; elevation: 213 m.a.s.l., MAAT: 8.7°C, MAP: 679 mm). Female bees formed larval cells inside every stem (Fig. 1). This system allowed for easy sex determination, since female progeny are usually located in the rear part of the stem, while males can be found near the entrance (Fig. 1;<sup>18</sup>). The stems were checked daily for the presence of larval brood cells, and 500 stems containing 1–3 larval cells with female eggs were first collected to obtain female specimens to be used in the feeding experiment. New empty cane stems were then mounted to allow the adult bees to continue laying eggs, and the stems were again checked on a daily basis. Every completed stem, i.e., those that were filled with eggs and closed with mud, was collected to obtain male specimens to be used in the feeding experiment. All stems were kept at 21°C, 60% relative humidity, and a 12:12 light:dark photoperiod to allow the eggs to hatch. Three-day-old larvae were used for the experiment due to the fragility and sensitivity of eggs and the possibility of mechanical damage to the eggs during the transfer to experimental containers<sup>18</sup>. To eliminate possible genetic biases, only one female and one male specimen from each stem was collected to be used in the feeding experiment.

## Experimental design

With a feeding experiment, we studied whether and how fitness-related life history traits (the mortality, adult body mass, cocoon mass and cocoon development) of wild solitary bees (*O. bicornis*) depended on the species diversity and nutritional composition of the pollen provided as larval food. We studied the abovementioned fitness-related life history traits since (1) mortality is the most relevant trait to study; (2) cocoons are secretions made for a specific purpose to enhance bee fitness (providing protection during prewintering and overwintering, i.e., during the first ten months of the adult part of the life cycle)<sup>52,53</sup>; and (3) the mass of adults is correlated with the fitness of *O. bicornis* solitary bee females (this effect is not observed in males<sup>54,55</sup>).

Twenty-five replicates (Eppendorf tubes, 2 ml) were prepared per treatment and sex. The tubes were filled with specific pollen diets (see below). The amount of pollen corresponded to the dry mass of pollen provisions typically found in nature, i.e.,  $195 \pm 5$  mg dm for females and  $140 \pm 5$  mg dm for males. The

dry pollen loads were complemented with either demineralized water (in the case of experimental controls and the pollen mixtures that were low in specific nutrients) or salt solutions (in the case of the pollen mixtures that were low in specific nutrients to obtain treatments with the same pollen species composition but different nutritional quality) in an amount that was ca. 25% of the dry pollen mass. Pollen collected by *Osmia* in nature is considered to be nutritionally balanced for larvae; thus, the treatments supplemented with salt solutions had concentrations of elements that were similar to those of pollen mixtures collected by *Osmia* females and provided to their larval progeny. Before starting the experiment, the Eppendorf tubes were left for 24 h to allow the water and salt solutions to penetrate the pollen loads. Three-day-old larvae were assigned to treatments, with one individual per Eppendorf tube. All experimental tubes were kept at 21°C and 60% RH under a 12:12 (L:D)-h photoperiod for 3 months. The exposure period was long enough to ensure that all larvae had gone through the life cycle to reach adulthood, i.e., to the stage where fully developed individuals hibernated in their cocoons<sup>53,56</sup>. At the end of exposure, cocoons and undeveloped individuals were collected to determine the degree of cocoon development. Then, live bees were extracted from cocoons, and the mortality rate was assessed. Afterwards, the individuals (i.e., the adult bodies and their cocoons) were frozen and dried using a vacuum drier (80°C, 48 h) to obtain their dry mass. This procedure was previously used in preliminary experiment<sup>18</sup>.

### Pollen diets

We established seventeen larval pollen diets differing in their elemental composition and species composition. For every diet that was low in specific nutrients, we also prepared the same diet supplemented with the lacking nutrients. The nutritional quality of the diets was measured in terms of the balanced/unbalanced proportions of vital nutritional elements (P, Na, K, Zn and Cu), as in previous studies<sup>10,18</sup>. Balanced nutritional quality was defined as the proportion of the measured elements found in pollen collected by *O. bicornis* in nature (Control-Osmia pollen diet). Unbalanced nutritional quality was defined as a scarcity (decreased concentration) of one or more elements in relation to the level of those elements in the Control-Osmia pollen diet.

### Pollen collection and analyses (Fig. 2 – stages 1 and 2)

To prepare the diets for the different experimental treatments, we first purchased five packs of honey bee polyfloral pollen pellets from different manufacturers (Fig. 2 – stage 1). We divided the pollen from each pack according to color by the naked eye to obtain pollen pellet pools that had specific species compositions and nutritional qualities. The control-Osmia pollen was collected from *Osmia* nests. Every pollen pool was mixed manually to obtain a homogenous powder and then freeze-dried to obtain its dry mass (dm) without changing the pollen nutritional properties, as freeze-drying allows vital molecules to be preserved. Then, the pools were analyzed stoichiometrically, i.e., The proportions of various elements were measured and compared in order to select the pollen pools to be used in the feeding experiments (Fig. 2 – stage 2a). The chemical analyses considered five elements and their ratios: Zn, Cu, Na, K and P. We compared the nutritional quality of the pollen types as reflected in their stoichiometric phenotypes (i.e., the proportions of elements; see<sup>10,25</sup>). Control-Osmia pollen, *Apis*-collected pollen pellets that were

nutritionally similar to control-Osmia pollen (two pollen pools, namely: Apis1 and Apis2) and Apis-collected pollen pellets lacking vital elements (four pollen pools that were scarce in Zn, Cu, Na, K and P, namely: -CuZn1, -CuZn2, -KP, and -NaP) were chosen to be used in the feeding experiment. These pollen pools were analyzed botanically, i.e., their species composition and diversity were investigated (Fig. 2 – stage 2). The exact species composition (the percentage of every noted taxon) of each distinct pollen pool was estimated by counting the pollen grains under a microscope using two samples, each of 3.5 g dry mass (d.m.), from every homogenized pollen pool.

#### Pollen pool selection and treatments used in feeding experiment (Fig. 2 – stages 3 and 4)

The following treatments were established (Fig. 2 – stage 3): (i) Control-Osmia, the natural larval food collected from *O. bicornis* nests; (ii) Apis1 and Apis2, two unsorted honeybee pollen pellets that were similar to control-Osmia in terms of their nutritional quality but differed in their species composition and diversity; and (iii) four diets that were low in specific nutrient(s), namely: -CuZn1, -CuZn2, -KP, and -NaP. Additionally, each diet described in (iii) was accompanied by a diet containing the same pollen pool but supplemented with salt of the scarce element; the concentration of the salt was adjusted to reflect the concentration of the corresponding element in Control-Osmia. The pollen pools obtained allowed us to investigate the effects of a scarcity of five nutrient elements, Cu, Zn, Na, K and P, in pollen mixtures of various botanical origins. Following the results of a preliminary experiment<sup>18</sup>, we used ZnCl<sub>2</sub> and CuCl<sub>2</sub> to supplement Zn and Cu in the bee diets, respectively, and used KCl, KH<sub>2</sub>PO<sub>4</sub>, NaCl, and NaH<sub>2</sub>PO<sub>4</sub> to supplement Na, K and P. All the treatments used in the feeding experiment are shown in Fig. 2 – stage 4.

#### Chemical analyses

The Zn, Cu, Na, and K concentrations were determined using atomic absorption spectrometry (Perkin-Elmer AAnalyst 200 and Perkin-Elmer AAnalyst 800), and the P content was determined by colorimetry (MLE FIA). Homogenized and dried samples were used to prepare a liquid solution (digested on a hotplate in a 4:1 mixture of nitric acid (70%) and hydrogen peroxide (30%)) that allowed us to perform the analyses. To determine the analytical precision, certified reference materials (bush, NCS DC 73349; chicken, NCS ZC 73016; and bovine muscle powder, RM8415) were tested with the samples.

#### Statistical analyses (Fig. 2 – stage 5)

We used a series of analyses to explore the relationships between the species composition, diversity and nutritional quality of pollen and its influences on female and male bee fitness.

For the pollen pools used in the feeding experiment, we calculated Fisher's alpha as the index of diversity and Dominance D (i.e. 1 minus the Simpson index; ranging from 0 (all taxa are equally present) to 1 (one taxon dominates the community completely))<sup>57–59</sup> as the index of dominance, calculated using PAST 4.05<sup>60,61</sup>. Fisher's alpha was used because it is suitable for a data set characterized by a high number of rare species, which was the case in the present study (see Supplementary Table S1).

We investigated differences in nutritional quality (i.e., the stoichiometric phenotype) between pollen pools and correlated the nutritional quality (i.e., the stoichiometric phenotype) of the pollen pools with their species composition using redundancy analysis (RDA). RDA was performed only on the pollen species that contributed the most to each pollen pool (i.e., the concentration of pollen grains in a specific pollen pool had to be higher than 20%) (CANOCO 5<sup>62</sup>).

To consider bee fitness, we first analyzed the entire dataset for mortality (Fig. 2 – stage 5). To maintain ecological relevance, we assessed the differences in mortality using the following procedure (chi-square test,  $p < 0.05$ , Statistica 13): to study the effects of pollen species composition/diversity on bees via nutritional scarcity we first compared, for each sex separately, the results for control-Osmia (1) with those for the other pollen pools that were not supplemented with nutrients, i.e., control-Osmia vs. (Apis1, Apis2, -ZnCu1 + H<sub>2</sub>O, -ZnCu + H<sub>2</sub>O, -KP + H<sub>2</sub>O, and -Na + H<sub>2</sub>O) and (2) with those for the other pollen pools that were fully supplemented with nutrients, i.e., control-Osmia vs. (-CuZn1 + CuZn, -CuZn2 + CuZn, -KP + KP, and -NaP + NaP). Second, to investigate the power of the effect of nutritional scarcity, we compared, for each sex separately, every nutrient(s)-scarce treatment with the corresponding nutrient(s)-supplemented treatments. Third, to assess the sexual differences in the impact of the scarcity of specific nutrients on fitness, we compared the results for females from each treatment to the results for males from the same treatment.

In the second step of considering bee fitness, we analyzed the remaining life history traits (cocoon development, cocoon mass, and adult body mass) measured in the specimens that survived the feeding experiments. Before performing detailed analyses, we checked whether and to what degree the measured traits were correlated. To that end, we calculated the Spearman's R coefficient for the whole dataset for all traits for both sexes separately (adult mass vs cocoon mass, adult mass vs cocoon development and cocoon mass vs cocoon development; Statistica 13). Since the measured life history traits were not strongly correlated, in the following steps, we investigated how each trait was exclusively influenced by the different diets used in the feeding experiment. We assessed cocoon development with Fisher's exact probability test ( $p < 0.05$ , VassarStats<sup>63</sup>). The degree of cocoon development was assessed by qualitative analysis. Four stages of cocoon development were distinguished: (1) a fully developed cocoon that covered the whole bee body and consisted of a hard material; these cocoons were impossible to tear with bare hands but could be cut with a knife because they were sufficiently hard; (2) an almost-developed cocoon that covered the whole bee body but consisted of a soft material; these cocoons were impossible to cut with a knife because they were too soft, but they could be torn by hand; (3) an underdeveloped cocoon that covered only part of the bee body and was soft; and (4) a very underdeveloped cocoon that did not cover any part of the bee body and consisted only of "woolly" matter. See supplementary Fig. S1 for details.

We performed statistical comparisons between the same groups as in the case of mortality but considered only groups having at least five surviving specimens to avoid artificial statistical results. For the data on the masses of the adult bees and their cocoons, we performed ANOVA ( $p < 0.05$ ; Statistica 13).

# Declarations

## Data availability

Source data are provided with this paper. All relevant data in this study are available from the corresponding author upon request.

## Code availability

Not applicable.

## Acknowledgements

Funding for this study was provided by the National Science Centre, Poland (2019/33/B/NZ8/01700), and Jagiellonian University, Faculty of Biology (N18/DBS/000003). The open-access publication of this article was funded by the Priority Research Area BioS under the program “Excellence Initiative–Research University” at Jagiellonian University in Krakow.

## Author contributions

ZMF and MF designed the study, performed the experiments and analyzed the data, ZMF wrote the manuscript, BD and ES analyzed the data, provided helpful discussion and gave critical contributions to the manuscript preparation, MF supervised the study, provided helpful discussion and gave critical contributions to the manuscript preparation.

## Competing interests

The authors declare no competing interests.

# References

1. van der Kooi, C. J., Vallejo-Marín, M. & Leonhardt, S. D. Mutualisms and (A)symmetry in plant-pollinator interactions. *Curr. Biol.* **31**, R91-R99 (2021).
2. Leonhardt, S. D., Lihoreau, M. & Spaethe, J. Mechanisms of nutritional resource exploitation by insects. *Insects* **11**, 570 (2020).
3. Ollerton, J. *Pollinators and Pollination: Nature and Society* (Pelagic Publishing, Exeter, 2021).
4. Goulson, D., Nicholls, E., Botias, C. & Rotheray, E. L. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* **347**, 1255957 (2015).
5. Roulston, T. A. H. & Goodell, K. The role of resources and risks in regulating wild bee populations. *Annu. Rev. Entomol.* **56**, 293-312 (2011).
6. Belsky, J. & Joshi, N. K. Impact of biotic and abiotic stressors on managed and feral bees. *Insects* **10**, 233 (2019).

7. Simpson, S. J. & Raubenheimer, D. The nature of nutrition: a unifying framework. *Aust. J. Zool.* **59**, 350 (2011).
8. Sterner, R. W. & Elser, J. J. *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere* (Princeton University Press, Princeton, 2002).
9. Vaudo, A. D., Tooker, J. F., Grozinger, C. M. & Patch, H. M. Bee nutrition and floral resource restoration. *Curr. Opin. Insect Sci.* **10**, 133-141 (2015).
10. Filipiak, M. Key pollen host plants provide balanced diets for wild bee larvae: a lesson for planting flower strips and hedgerows. *J. Appl. Ecol.* **56**, 1410-1418 (2019).
11. Scheper, J. et al. Museum specimens reveal loss of pollen host plants as key factor driving wild bee decline in The Netherlands. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 17552-17557 (2014).
12. Jauker, F., Peter, F., Wolters, V. & Diekötter, T. Early reproductive benefits of mass-flowering crops to the solitary bee *Osmia rufa* outbalance post-flowering disadvantages. *Basic Appl. Ecol.* **13**, 268-276 (2012).
13. Filipiak, M. et al. Ecological stoichiometry of the honeybee: Pollen diversity and adequate species composition are needed to mitigate limitations imposed on the growth and development of bees by pollen quality. *PLoS One* **12**, e0183236 (2017).
14. Willmer, P. *Pollination and Floral Ecology* (Princeton University Press, Princeton, 2011).
15. Nicolson, S. W. & Thornburg, R. W. Nectar chemistry in *Nectaries and Nectar* (eds Nicolson, S. W., Nepi, M. & Pacini, E.) 215-264 (Springer Netherlands, Dordrecht, 2007).
16. Lawson, S. P., Kennedy, K. B. & Rehan, S. M. Pollen composition significantly impacts the development and survival of the native small carpenter bee, *Ceratina calcarata*. *Ecol. Entomol.* **46**, 232-239 (2020).
17. Roswell, M., Dushoff, J. & Winfree, R. Male and female bees show large differences in floral preference. *PLoS One* **14**, e0214909 (2019).
18. Filipiak, Z. M. & Filipiak, M. The scarcity of specific nutrients in wild bee larval food negatively influences certain life history traits. *Biology* **9**, 462 (2020).
19. Filipiak, M., Woyciechowski, M. & Czarnoleski, M. 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 (2021).
20. Drossart, M. & Gérard, M. Beyond the decline of wild bees: optimizing conservation measures and bringing together the actors. *Insects* **11**, 649 (2020).
21. Di Pasquale, G. et al. Variations in the availability of pollen resources affect honey bee health. *PLoS One* **11**, e0162818 (2016).
22. Wilson, R. S. et al. Many small rather than few large sources identified in long-term bee pollen diets in agroecosystems. *Agric. Ecosyst. Environ.* **310**, 107296 (2021).
23. Zu, P. et al. Pollen sterols are associated with phylogeny and environment but not with pollinator guilds. *New Phytol.* **230**, 1169-1184 (2021).

24. Kleijn, D. et al. Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. *Nat. Commun.* **6**, 7414 (2015).
25. Filipiak, M. A better understanding of bee nutritional ecology is needed to optimize conservation strategies for wild bees-the application of ecological stoichiometry. *Insects* **9**, 85 (2018).
26. Filipiak, M. & Weiner, J. Plant-insect interactions: the role of ecological stoichiometry. *Acta Agrobot.* **70**, 1-16 (2017).
27. Kay, A. D. et al. Toward a stoichiometric framework for evolutionary biology. *Oikos* **109**, 6-17 (2005).
28. Cherif, M. Biological stoichiometry: the elements at the heart of biological interactions in *Stoichiometry and Research - The Importance of Quantity in Biomedicine* (ed Innocenti, A.) 357-376 (InTech, Rijeka, 2012).
29. Peñuelas, J. et al. The bioelements, the elementome, and the biogeochemical niche. *Ecology* **100**, e02652 (2019).
30. González, A. L., Dézerald, O., Marquet, P. A., Romero, G. Q. & Srivastava, D. S. The multidimensional stoichiometric niche. *Front. Ecol. Evol.* **5**, 110 (2017).
31. Sobczyk, Ł., Filipiak, M. & Czarnoleski, M. Sexual dimorphism in the multielemental stoichiometric phenotypes and stoichiometric niches of spiders. *Insects* **11**, 484 (2020).
32. Morehouse, N. I., Nakazawa, T., Booher, C. M., Jeyasingh, P. D. & Hall, M. D. Sex in a material world: why the study of sexual reproduction and sex-specific traits should become more nutritionally-explicit. *Oikos* **119**, 766-778 (2010).
33. Gresty, C. E. A. et al. Flower preferences and pollen transport networks for cavity-nesting solitary bees: implications for the design of agri-environment schemes. *Ecol. Evol.* **8**, 7574-7587 (2018).
34. Wood, T. J., Holland, J. M. & Goulson, D. Pollinator-friendly management does not increase the diversity of farmland bees and wasps. *Biol. Conserv.* **187**, 120-126 (2015).
35. Wood, T. J., Holland, J. M. & Goulson, D. Providing foraging resources for solitary bees on farmland: current schemes for pollinators benefit a limited suite of species. *J. Appl. Ecol.* **54**, 323-333 (2017).
36. Vanderplanck, M., Gilles, H., Nonclercq, D., Duez, P. & Gerbaux, P. Asteraceae paradox: chemical and mechanical protection of taraxacum pollen. *Insects* **11**, 304 (2020).
37. Klaus, F., Tscharntke, T., Bischoff, G. & Grass, I. Floral resource diversification promotes solitary bee reproduction and may offset insecticide effects – evidence from a semi-field experiment. *Ecol. Lett.* **24**, 668-675 (2021).
38. Holzschuh, A., Dormann, C. F., Tscharntke, T. & Steffan-Dewenter, I. Mass-flowering crops enhance wild bee abundance. *Oecologia* **172**, 477-484 (2013).
39. Eckhardt, M., Haider, M., Dorn, S. & Müller, A. Pollen mixing in pollen generalist solitary bees: a possible strategy to complement or mitigate unfavourable pollen properties? *J. Anim. Ecol.* **83**, 588-597 (2013).
40. de Roos, A. M. & Persson, L. *Population and Community Ecology of Ontogenetic Development* (Princeton University Press, Princeton, 2002).

41. Loreau, M. *From Populations to Ecosystems: Theoretical Foundations for a New Ecological Synthesis* (Princeton University Press, Princeton, 2010).
42. Bukovinszky, T. et al. Effects of pollen species composition on the foraging behaviour and offspring performance of the mason bee *Osmia bicornis* (L.). *Basic Appl. Ecol.* **18**, 21-30 (2017).
43. Goos, J. M., Cothran, R. D. & Jeyasingh, P. D. Within-population variation in the chemistry of life: the stoichiometry of sexual dimorphism in multiple dimensions. *Evol. Ecol.* **31**, 635-651 (2017).
44. Blanco, G. & Mercer, R. W. Isozymes of the Na-K-ATPase: heterogeneity in structure, diversity in function. *Am. J. Physiol. Ren. Physiol.* **275**, F633-F650 (1998).
45. Bergwitz, C. & Jüppner, H. Phosphate sensing. *Adv. Chronic Kidney Dis.* **18**, 132-144 (2011).
46. Werner, A. & Kinne, R. K. H. Evolution of the Na-Picotransport systems. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **280**, R301-R312 (2001).
47. Kaspari, M. The seventh macronutrient: how sodium shortfall ramifies through populations, food webs and ecosystems. *Ecol. Lett.* **23**, 1153-1168 (2020).
48. Woodard, S. H. & Jha, S. Wild bee nutritional ecology: predicting pollinator population dynamics, movement, and services from floral resources. *Curr. Opin. Insect Sci.* **21**, 83-90 (2017).
49. Warzecha, D., Diekötter, T., Wolters, V. & Jauker, F. Attractiveness of wildflower mixtures for wild bees and hoverflies depends on some key plant species. *Insect Conserv. Divers.* **11**, 32-41 (2017).
50. Loladze, I. Hidden shift of the ionome of plants exposed to elevated CO<sub>2</sub> depletes minerals at the base of human nutrition. *eLife* **3**, e02245 (2014).
51. Ziska, L. H. et al. Rising atmospheric CO<sub>2</sub> is reducing the protein concentration of a floral pollen source essential for North American bees. *Proc. Biol. Sci.* **283**, 20160414 (2016).
52. Bosch, J., Sgolastra, F. & Kemp, W. P. Life cycle ecophysiology of Osmia Mason bees used as crop pollinators in *Bee Pollination in Agricultural Eco-systems* (eds James, R. & Pitts-Singer, T. L.) 83-105 (Oxford University Press, Oxford, 2008).
53. Giejdasz, K. & Wilkaniec, Z. Individual development of the red mason bee (*Osmia rufa* L., Megachilidae) under natural and laboratory conditions. *J. Apic. Sci.* **46**, 51-57 (2002).
54. Kim, J. Y. Female size and fitness in the leaf-cutter bee *Megachile apicalis*. *Ecol. Entomol.* **22**, 275-282 (1997).
55. Seidelmann, K. Optimal progeny body size in a solitary bee, *Osmia bicornis* (Apoidea: Megachilidae). *Ecol. Entomol.* **39**, 656-663 (2014).
56. Danforth, B. N., Minckley, R. L. & Neff, J. L. *The Solitary Bees: Biology, Evolution, Conservation* (Princeton University Press, Princeton, 2019).
57. Fisher, R. A., Corbet, A. S. & Williams, C. B. The relation between the number of species and the number of individuals in a random sample of an animal population. *J. Anim. Ecol.* **12**, 42 (1943).
58. Magurran, A. E. *Measuring Biological Diversity* (Wiley-Blackwell, Malden, 2013).
59. Harper, D. A. T. *Numerical Palaeobiology: Computer-based Modelling and Analysis of Fossils and their Distributions* (Wiley-Blackwell, 1999).

60. Hammer, Ø., Harper, D. A. T. & Ryan, P. D. PAST: paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **4**, 1-9 (2001).
61. Hammer, Ø. Past 4 - the past of the future.  
<https://www.nhm.uio.no/english/research/infrastructure/past/> (2021).
62. Smilauer, P. & Lepš, J. *Multivariate Analysis of Ecological Data using CANOCO 5* (Cambridge University Press, Cambridge, 2014).
63. Lowry, R. VassarStats: website for statistical computation. <http://vassarstats.net/index.html> (2021).

## Figures

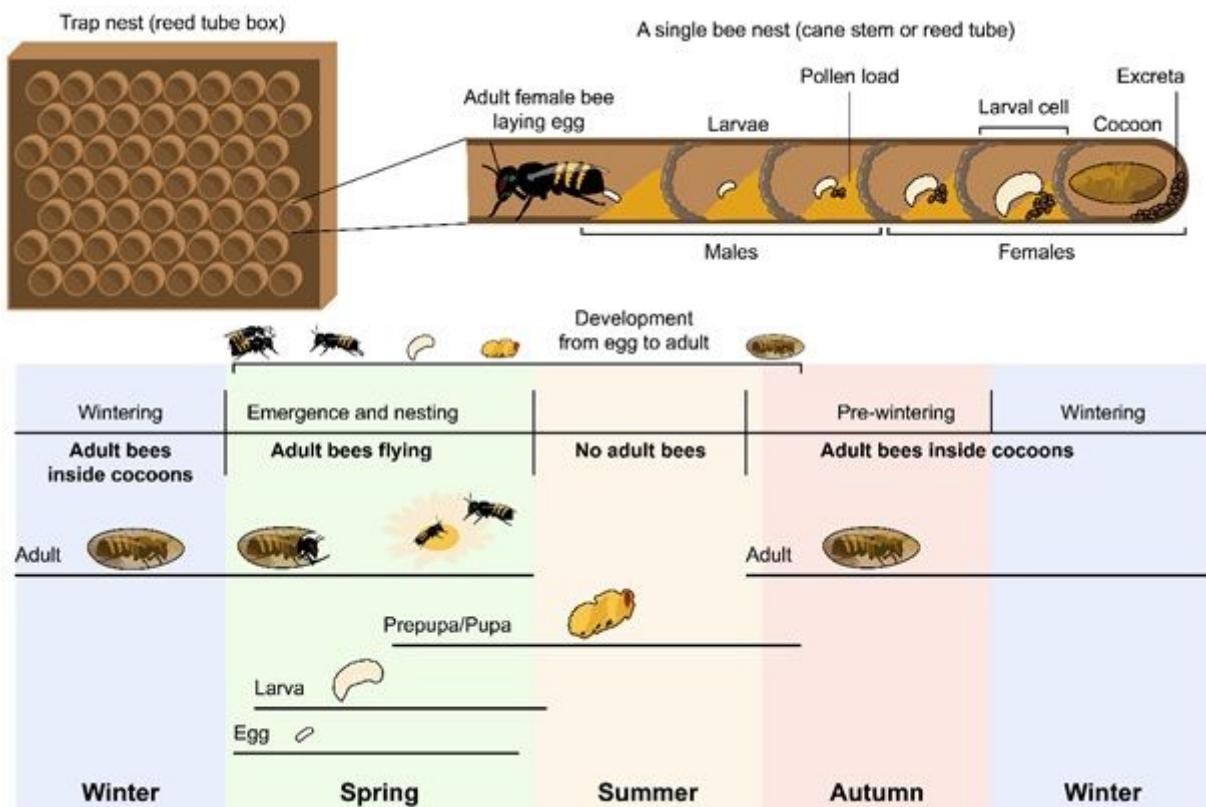
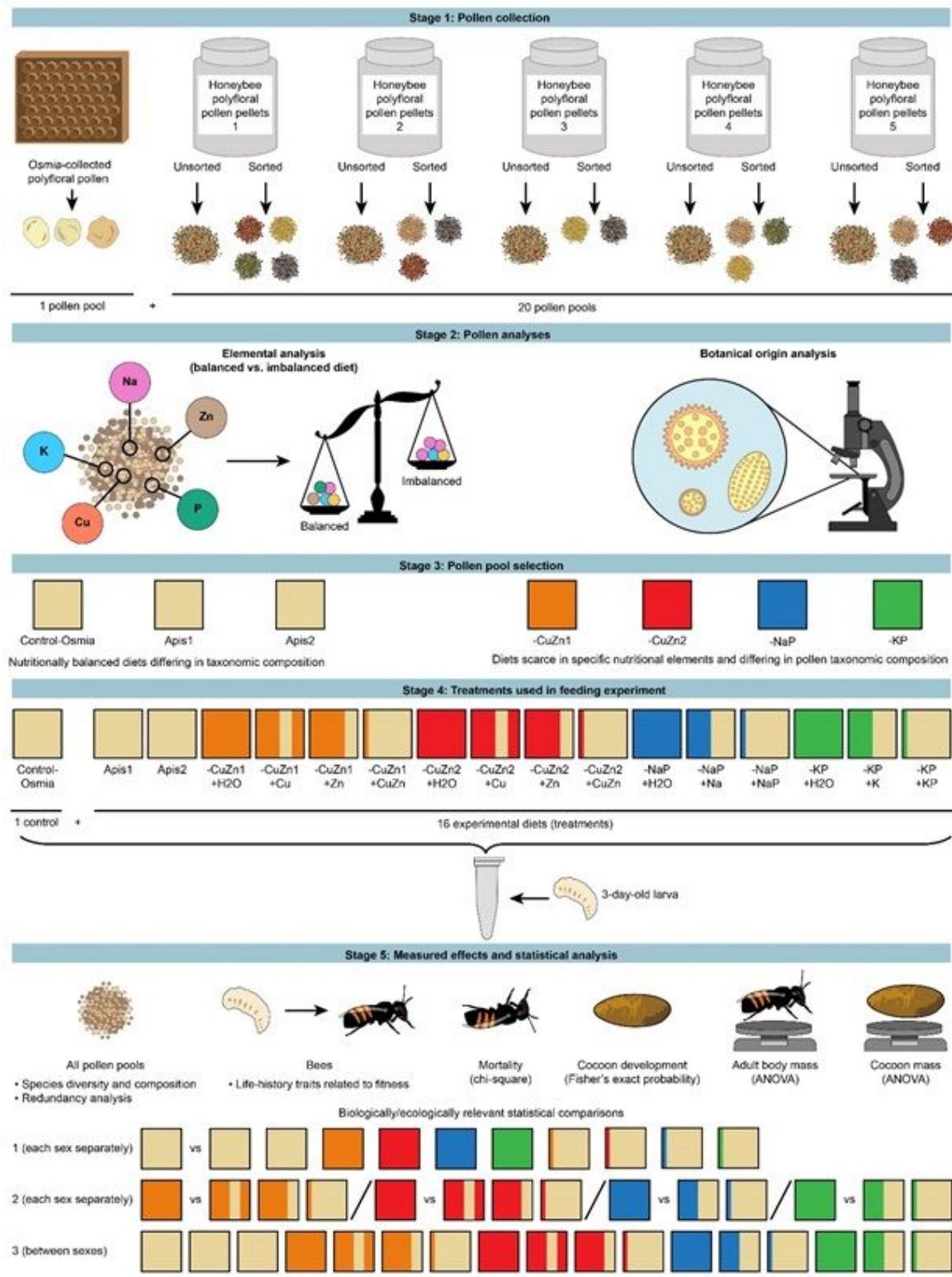


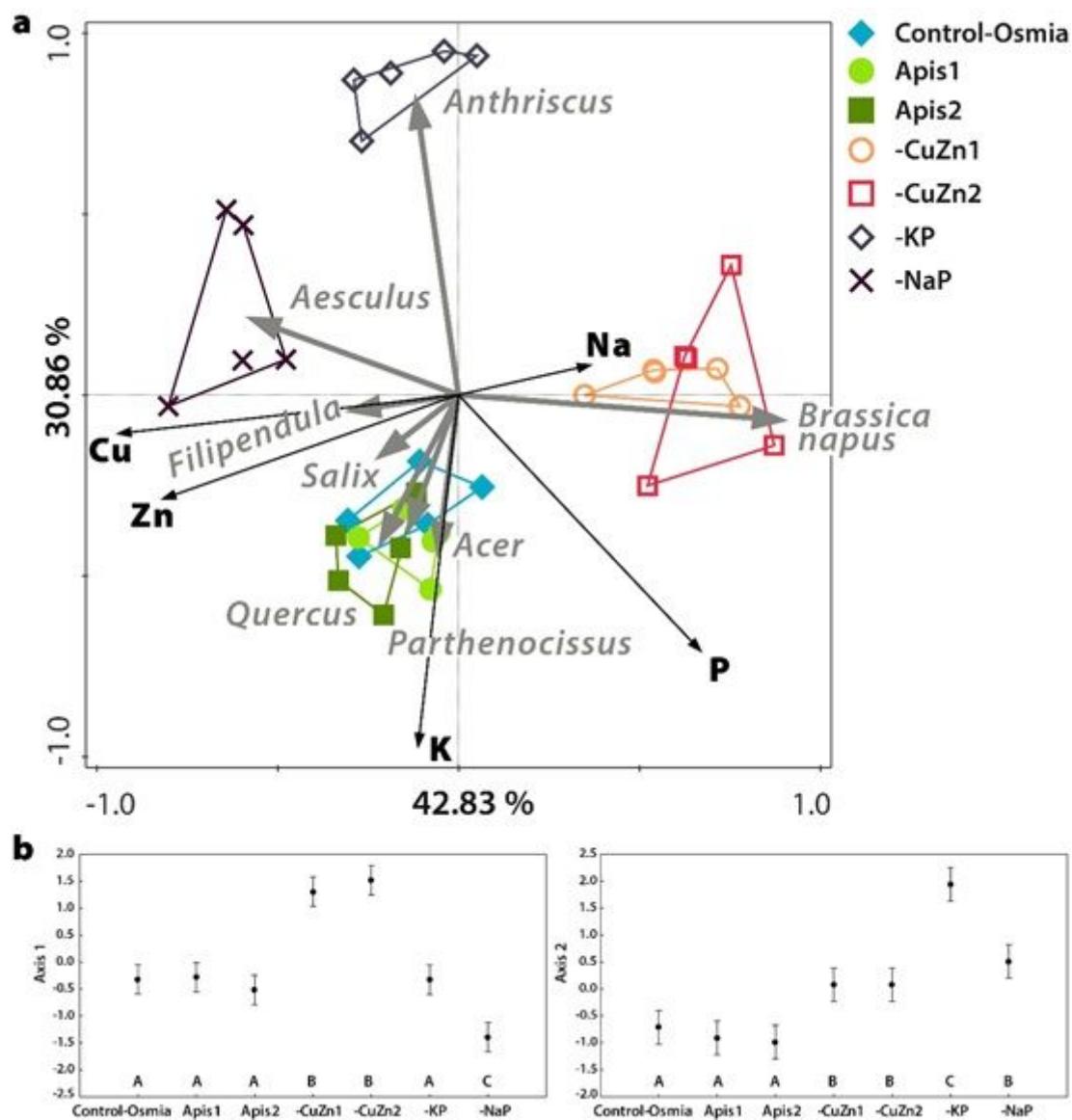
Figure 1

Nesting biology of solitary Osmia bees.



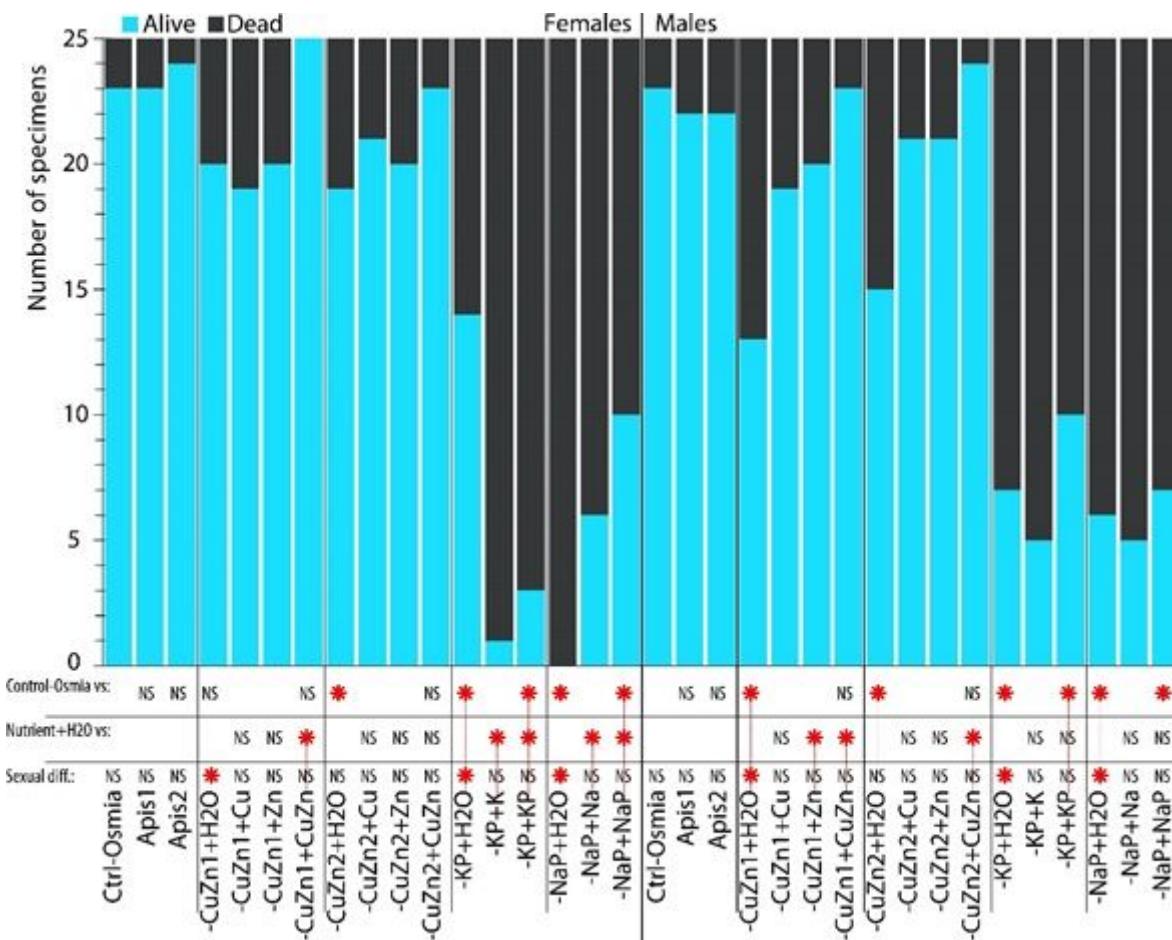
**Figure 2**

Experimental setup and measured effects.



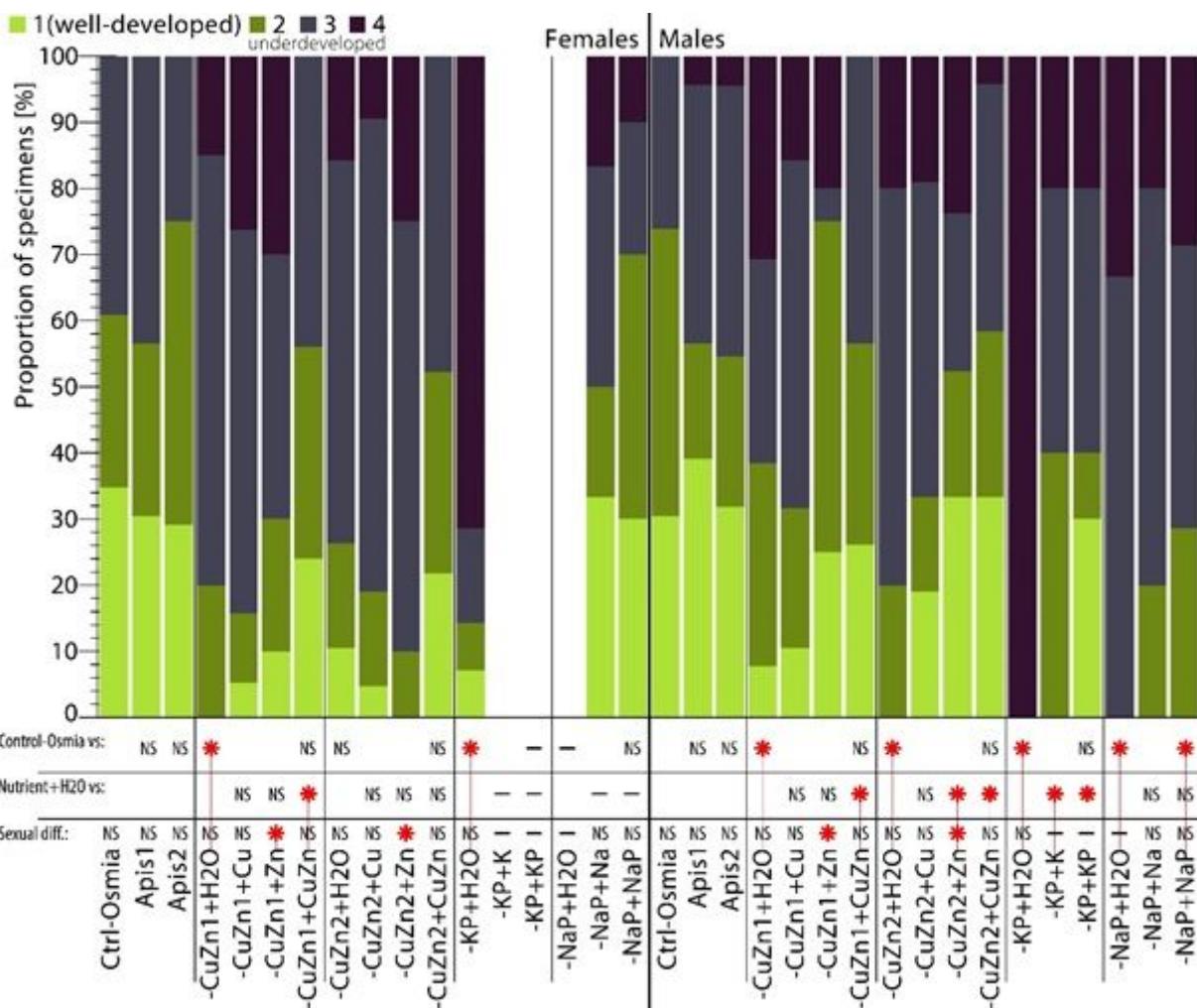
**Figure 3**

Multivariate analysis (RDA) of the relationship between the concentrations of the studied elements (P, Na, K, Zn and Cu) and the proportions of pollen from the dominant taxa (Aesculus, Anthriscus, B. napus, Filipendula, Parthenocissus, Quercus, and Salix) in the pollen pools used in the feeding experiment (Control-Osmia, Apis1, Apis2, -CuZn1, -CuZn2, -KP and -NaP). (a) RDA plot showing the first two axes and the corresponding percentages of the variance explained by these axes (73.69% in total); (b) ANOVA performed for the scores for both axes; the different letters denote significant differences between pollen pools, the black dots represent mean values, and the bars denote the confidence intervals. (Axis 1:  $F=61.37$ ,  $p=8 \times 10^{-15}$ ; Axis 2:  $F=45.75$ ,  $p=3 \times 10^{-13}$ ). The pollen pools differed in terms of their elemental composition, which was correlated with the proportion of specific plant taxa composing the pollen pool. The exact concentrations of the studied elements are given in Supplementary Table S2, and the species composition of each pollen pool is shown in Supplementary Table S1.



**Figure 4**

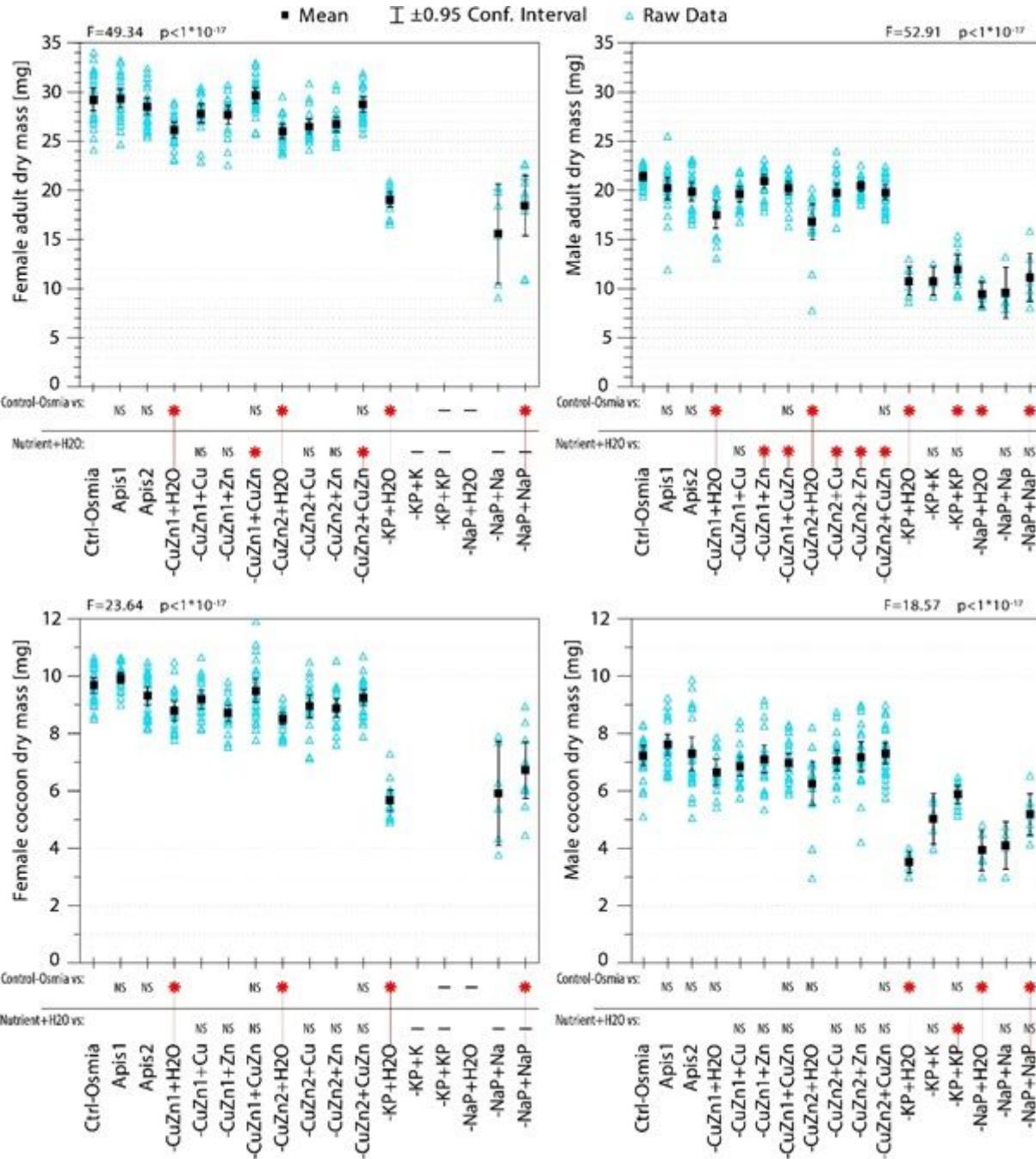
Mortality of growing and developing bees fed on various diets. Nutritional scarcity and supplementation affected bee mortality in a sex-dependent manner. Alive vs Dead = number of specimens that either survived to the adult stage or died before maturation; N=25 per treatment and sex; chi-square test, p<0.05. For all diets, the measured effects were compared to maintain the ecological relevance of the study: Control-Osmia was compared with every pollen pool that was not supplemented with nutrients as well as with the same pollen pool supplemented with all scarce nutrients for each sex separately; every nonsupplemented pollen pool was compared with its supplemented counterpart for each sex separately; and every treatment was compared between sexes. Statistically significant differences are indicated with red asterisks, and NS indicates no significant difference.



**Figure 5**

Effect of diet on cocoon development. Zn, Cu and K scarcity negatively affected cocoon development in both sexes, but this effect was stronger on males than on females. For males, a similar negative effect of Na scarcity was also observed. Significant effects were determined according to Fisher's exact probability test,  $p<0.05$ . For all diets, the measured effects were compared to maintain the ecological relevance of the study: Control-Osmia was compared with every pollen pool that was not supplemented with nutrients as well as with the same pollen pool supplemented with all scarce nutrients for each sex separately; every nonsupplemented pollen pool was compared with its supplemented counterpart for each sex separately; and every treatment was compared between sexes. Statistically significant differences are indicated with red asterisks, minus signs indicate that a statistical comparison was not possible (too few replicates), and NS indicates no significant difference. The degree of cocoon development was assessed by qualitative analysis, and four stages of cocoon development were distinguished: (1) a fully developed cocoon that covered the whole bee body and consisted of a hard material; these cocoons were impossible to tear with bare hands but could be cut with a knife because they were sufficiently hard; (2) an almost-developed cocoon that covered the whole bee body but consisted of a soft material; these cocoons were impossible to cut with a knife because they were too soft, but they could be torn by hand;

(3) an underdeveloped cocoon that covered only part of the bee body and was soft; and (4) a very underdeveloped cocoon that did not cover any part of the bee body and consisted only of “woolly” matter.



**Figure 6**

The effect of diet on adult mass and cocoon mass. ANOVA,  $p < 0.05$ , calculated separately for each sex. A negative effect of Cu and Zn scarcity on body mass was observed for both sexes and was stronger in males than in females. In females, cocoon mass was also negatively affected by Cu and Zn scarcity, but Cu and Zn supplementation did not reverse the effect. K scarcity resulted in lower cocoon masses in both sexes, and in males, the effect was mitigated when the pollen was supplemented with K and P. For all diets, the measured effects were compared to maintain the ecological relevance of the study: Control-Osmia was compared with every pollen pool that was not supplemented with nutrients as well as with the

same pollen pool supplemented with all scarce nutrients for each sex separately; every nonsupplemented pollen pool was compared with its supplemented counterpart for each sex separately; and every treatment was compared between sexes. Statistically significant differences are indicated with red asterisks, minus signs indicate that a statistical comparison was not possible (too few replicates), and NS indicates no significant difference.

- pollen allowing to achieve an appropriate nutritional balance by *Osmia bicornis*
- pollen impairing larval development of *Osmia bicornis* via stoichiometric mismatch



Figure 7

Impact of pollen species available in the environment on the functioning of bee populations. (a) – diverse flora and (b) – poor flora. Stoichiometric mismatches experienced by bees feeding on unbalanced pollen

(b) drive the decline of the bee population. Nutritionally balanced pollen (a) drives success in the bee population. Specific key plant species that allow bees to achieve an appropriate nutritional balance may occur more frequently in diverse floras than in monotonous floras, so the bees that inhabit areas with diverse floras may be more likely to have balanced diets; this would increase their individual fitness and the success of their populations.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplementaryinformation.pdf](#)