



Land use influences the nutrient concentration and composition of pollen and nectar rewards of wildflowers in human-dominated landscapes

Emiliano Pioltelli^{a,b,1}, Lorenzo Guzzetti^{a,b,1}, Malika Ouled Larbi^{a,b}, Rita Celano^{b,c}, Anna Lisa Piccinelli^{b,c}, Andrea Galimberti^{a,b}, Paolo Biella^{a,*}, Massimo Labra^{a,b}

^a Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126, Milano, Italy

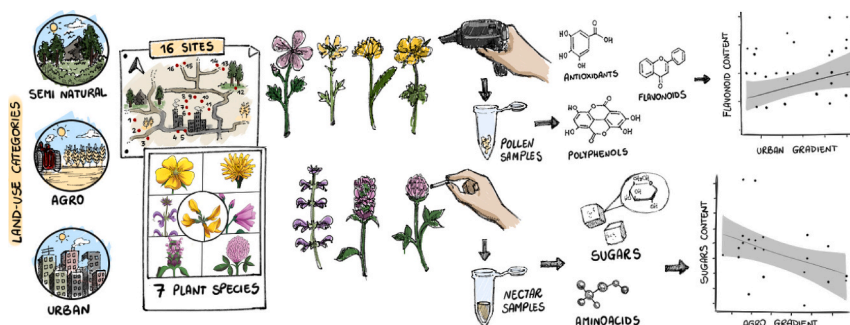
^b NBFC, National Biodiversity Future Center, Palermo, 90133, Italy

^c Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 132, 84084, Salerno, Italy

HIGHLIGHTS

- Few studies have explored the impact of land use on floral reward chemistry and nutritional value.
- 7 meadow species were studied for nutritional traits across both urbanization and agriculture gradients.
- Both plant primary and secondary metabolism were investigated by means of analytical chemistry.
- Both nectar sugar content and pollen antioxidant activity were influenced by land use.
- Results offer novel insights for restoration ecology strategies in human-modified environments.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Dr. Elena Paoletti

Keywords:

Nutritional ecology
Pollen and nectar
Pollinators diet
Landscape anthropization
Plant metabolism

ABSTRACT

Plant biodiversity is crucial to satisfy the trophic needs of pollinators, mainly through nectar and pollen rewards. However, a few studies have been directed to ascertain the intraspecific variation of chemical features and the nutritional value of nectar and pollen floral rewards in relation to the alteration of landscapes due to human activities. In this study, by using an existing scenario of land use gradients as an open air laboratory, we tested the variation in pollen and nectar nutrient profiles along gradients of urbanization and agriculture intensity, by focusing on sugar, aminoacids of nectar and phytochemicals of pollen from local wild plants. We also highlighted bioactive compounds from plants primary and secondary metabolism due to their importance for insect well-being and pollinator health. We surveyed 7 different meadow species foraged by pollinators and common in the main land uses studied. The results indicated that significant variations of nutritional components occur in relation to different land uses, and specifically that the agricultural intensification decreases the sugars and increases the antioxidant content of flower rewards, while the urbanization is positively associated with the total flavonoid content in pollen. These effects are more evident in some species than in others, such as *Lotus corniculatus* L. (Fabaceae) and *Malva sylvestris* L. (Malvaceae), as shown by the untargeted metabolomic investigation. This study is crucial for understanding the nutritional landscape quality for pollinators in association to different

* Corresponding author at: Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, Milan, 20126, Italy.

E-mail address: paolo.biella@unimib.it (P. Biella).

¹ The two authors contributed equally to this study and would like to share co-first authorship.

<https://doi.org/10.1016/j.scitotenv.2023.168130>

Received 24 August 2023; Received in revised form 12 October 2023; Accepted 24 October 2023

Available online 29 October 2023

0048-9697/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

land uses and sets a base for landscape management and planning of pollinator-friendly strategies by improving the quality of plant rewards to provide benefits to pollinator health in various environmental contexts.

1. Introduction

The progressive expansion of urban and agricultural areas is associated with a series of environmental modifications that greatly impact urban and periurban plants and pollinators biology (Biella et al., 2022; Tommasi et al., 2022). Since many pollinators base their nutrition on flower rewards, which have an impact on their development and population size, changes in the environment modifying nectar and pollen could also affect the conservation of pollinators and the pollination ecosystem service at several levels. Therefore, studying the intraspecific variation of flower resources composition is urgent (Venjakob et al., 2020), especially in relation to human-induced environmental alterations, to understand the magnitude of the ongoing impact and so to supply landscapes or mitigate situations of lack of nutrients for pollinators. This is particularly relevant within the process of ecological transition and intensification towards sustainable cities and agricultural areas, so as to plan actions capable of guaranteeing an adequate quality of floral resources for pollinators (Jones and Rader, 2022).

Flowering plants and pollinators are entangled by reproduction and foraging needs, respectively. Specifically, flower visitors mainly depend on pollen and nectar to satisfy their nutritional requirements (Nicolson et al., 2018). For instance, nectar is the main food for the adult forms of various pollinators and pollen is the main food for the larval stages of bees or some adult hoverflies and beetles (Faegri and Van Der Pijl, 1979). These resources display relevant differences in their chemical and nutritional composition, mainly in relative amounts of the main components. Nectar is a solution of mainly sugars that supports the essential needs of the energetic metabolism of pollinators, but it also contains several compounds, such as amino acids, and phytochemicals (Barberis et al., 2021; Roy et al., 2017). These latter compounds are relevant to multiple aspects of pollinators ecology as they can manifest as either attractive agents or deterrents, thereby exhibiting a substantial range of effects that are frequently contingent upon dosage (Stevenson et al., 2017; Manson et al., 2013). Pollen chemistry is mainly composed of lipids, proteins, amino acids, and it is characterized by a high diversity and concentration of secondary metabolites (Palmer-Young et al., 2019; Thakur and Nanda, 2020; Aylanc et al., 2021).

Pollinator diet on flower resources is an essential factor for them to counteract the negative impact of anthropogenic stressors through the provision of essential nutrients from plant primary and secondary metabolism (Barascou et al., 2021; Wong et al., 2018). In order to guarantee an adequate dietary intake in space and time for the different species of pollinators, it is essential to understand which are the environmental parameters that influence macro and micronutrients composition of pollen and nectar. Many environmental variables play a role on the floral reward chemical features, especially in the quantitative variation of phytochemicals (Zu et al., 2021; Palmer-Young et al., 2019). Indeed, the metabolism of plant secondary compounds is known to be highly responsive to environmental biotic and abiotic factors, among which light, temperature, and drought, as well as biotic agents, such as the damage produced by herbivores and parasites (Khare et al., 2020). However, a landscape-scale investigation of pollen and nectar quality is needed. This because most studies related to the investigation of pollinators diet quality as a function of the landscape took into account the variations in the food resources directly collected by a few model species (Pioltelli et al., 2023a; Vaudo et al., 2018; Donkersley et al., 2017). From the plant side, so far most of the studies focused on landscape induced changes in the structure of the flora by means of species composition and diversity (Hou et al., 2023), or indirectly via the pollen (Biella et al., 2022). However, regarding the rewards to pollinators, only a few studies focused on the modifications of the nutritional landscape represented by

the plant communities in different contexts and the variation of the chemical composition of community nectar in response to environmental pressures (Tew et al., 2021; Biella et al., 2022). Moreover, a conceptual link between the availability of specific pollen species in the environment and the dynamics of bee populations was previously detailed by highlighting the importance of pollen key nutrients in the landscape (Filipiak et al., 2022). In this context, still little is known on how land use gradients modify the intraspecific variation of the chemical composition of both the main flower rewards of nectar and pollen.

The general goal of this study is to investigate the changes in the nutritional - chemical profile of some representative species foraged by pollinator insects along gradients of human-altered landscapes. Specifically, based on what outlined above and on field and laboratory evidence that abiotic features shape flower rewards (Venjakob et al., 2020; Akter and Klečka, 2022), we expected to observe changes in nectar and pollen of wild flowering plants in relation to varying types of land-use. To investigate this aspect, we aimed to quantify the effect of the main land use gradients associated with human presence and activities (i.e., urbanization, agriculture intensification, air temperature) on pollen and nectar from wild plants. Furthermore, the second objective was to analyse the individual nutritional components of pollen and nectars, (i.e., macronutrients and micronutrients) with particular reference to bioactive molecules capable of promoting the insects well-being. This investigation is useful to better design the Nature-Based Solutions (NBS) suitable to promote functional biodiversity of future cities as claimed by the European Green Deal and the UN Agenda 2030. To address this issue, we conducted a field sampling, coupled with an array of chemical analyses to investigate the variations occurring in the flower rewards provided by some commonly visited meadows species.

2. Material and methods

2.1. Floral resources sampling

To study the floral resources composition of urban, peri-urban, semi-natural and rural areas, 16 sites (Fig. 1) with different environmental characteristics in terms of anthropization levels were selected. The sites were specifically selected to represent highly contrasting land use conditions. Half of the sites were located in areas predominantly characterized by seminatural hay meadows, while the other half exhibited a high degree of impervious surfaces (i.e., concrete, buildings, and asphalt). The selection of these sites was based on data obtained from regional land use cartography (DUSAF 6.0, available at <https://www.dati.lombardia.it/Territorio/Dusaf-6-0-Usa-del-suolo-2018/7rae-fng6>), and accessibility was verified prior to selection. Sites were located in the North of Italy and spread across different provinces. The sampling of pollen and nectar took place in June 2021.

The plant species target for this study are detailed in Table 1 and belong to different families and flower morphology. Species were selected to provide for a wide range of plants foraged by pollinator insects and because they were representative, in terms of availability, in the study area. Some of the selected plant species were more suitable for the sampling of nectar due to floral zygomorphic symmetry which favors nectar accumulation in the corolla, while others displayed easier access to pollen grains, such as those radial-shaped.

The randomly selected floral units of each species were covered with a nylon mesh 24 h prior to the sampling to avoid possible depletion of the resources by pollinator visits (Biella et al., 2021). Pollen was sampled by using a specifically adapted portable vacuum (E-PoSa, Pioltelli et al., 2023b) modified to have a filtering unit consisting of a specifically perforated 5 mL tube and two filtering systems composed of

stainless steel mesh and filter paper. Nectar was sampled by using commercially available 5 μL and 10 μL glass capillary (Merck, Germany). Pollen and nectar collection was performed during the same day at each site in an hour range between 10 am and 12 am for all the species in order to minimise as much as possible biases due to the daytime. Samples were stored in a 1:1 v/v nectar/EtOH ratio to avoid microbial-mediated degradation of the occurring compounds (Power et al., 2018). Once in the laboratory, the nectar/EtOH solutions were dried in a tube under gaseous nitrogen and subsequently resuspended in 500 μL of ultrapure Milli-Q H_2O and stored at -80°C up to the analyses. Pollen samples were freeze-dried and stored at -80°C up to the analyses.

2.2. Landscape and local metrics

Land cover data were obtained from the regional land use cartography (DUSAF 6.0 <https://www.dati.lombardia.it/Territorio/Dusaf-6-0-Uso-del-suolo-2018/7rae-fng6>). This map is available at a scale of 1:10.000 with a minimum linear dimension of polygons of 20 m and was developed from AGEA orthophotos and SPOT 6/7 satellite images. The original level and sub-level of land use classification were grouped into 3 main classes: impervious cover (i.e. concrete, asphalt, buildings), semi-natural (i.e. hay meadows surrounded by forest), agricultural fields (i.e. agriculture margins). Detailed information on land use classification is reported in Text S1 and Table S1. Using QGIS v 3.10.11 we computed buffers of 1 km radius around each sampling location and the coverage percentage of the three different classes of land use were calculated.

Land surface temperature information were obtained from data retrieved through remote sensing imaging spectroradiometer (MODIS MOD11A2 from the NASA database (<https://modis.gsfc.nasa.gov/data/dataproduct/mod11.php>) with a resolution of 1 km. The original raster layer with a resolution of 1 km was downsampled to a finer resolution of 100 m with bilinear interpolation and the mean temperature in June

2021 was calculated starting from the mean daily registered temperature.

With the data regarding the landscape features (i.e., impervious cover, semi-natural and agricultural) and with those relative to the temperature, a Principal Component Analysis was performed in R 4.3.1. (R Core Team, 2022) in order to summarise the variability of the gradient across the different sampling sites. The principal component analysis on the landscape and climatic variables showed two main PCs complying with the two investigated land use gradients (Fig. 2). In particular, the PC1, which accounts for almost 80 % of the variability of the data, clearly defined the urbanization gradient with sites at higher impervious surface and higher temperatures associated with high values of the PC. The PC2 which accounts for 16 % of the variability in the data was associated with a gradient spanning from semi-natural areas (low values) to agricultural sites (high values).

2.3. Phytochemical analysis

Nectar and pollen were subjected to a set of analytical chemistry experiments aimed at evaluating the variations occurring in the small molecules (lower than 1200 Da) composition of flower rewards. Table 1 reports a synthetic overview of the metabolic investigations performed on nectar and pollen; further details are provided in the next paragraphs.

2.3.1. Nectar sugar composition

Once resuspended, nectars were 100-fold diluted and analysed for the content of free sugars (sucrose, glucose, and fructose) by an enzymatic kit provided by Megazyme, Ireland. For the analysis of sucrose 100 μL of β -fructosidase were added to 50 μL of sample (or H_2O for the blank) followed by an incubation of 5 min. Consequently, the analysis of free sugars (glucose and fructose) started by mixing 50 μL of sample/ H_2O to 1050 μL of H_2O , while for the sucrose analysis, 950 μL H_2O was

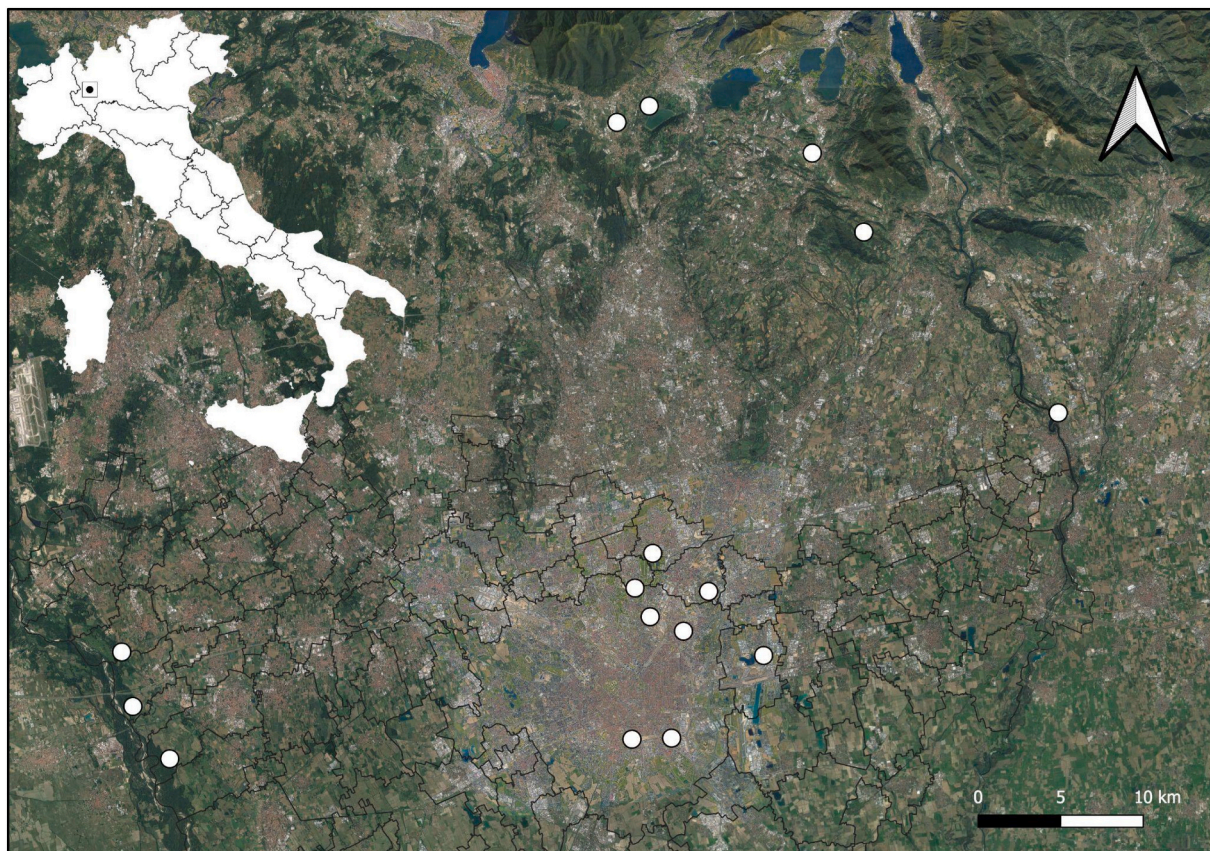


Fig. 1. Map of the sampling sites where pollens and nectars analysed within the present study were collected.

added. Then, for both analyses, we added 50 μL buffer pH 7.6 and 50 μL of a solution NADP^+/ATP followed by an incubation of 3 min.

At this point, the absorbance (A_1) was read at 340 nm to monitor the basal level of each sample (against the blank). Then the analysis continued with the addition of 10 μL of the enzymes hexokinase and glucose 6-phosphate dehydrogenase followed by an incubation of 5 mins. Then the absorbance was read at 340 nm (A_2). The difference value between A_2 and A_1 referred to the glucose content. Finally, 10 μL of phosphoglucose isomerase were added and each sample was incubated for 10 mins at room temperature. Then the absorbance was read again at 340 nm (A_3). The difference value between A_3 and A_1 referred to the fructose content. The content of sucrose was obtained by the difference between the absorbances of samples incubated without and with the enzyme β -fructosidase. Data on the concentration were normalized to the volume of nectar recovered and expressed as $\mu\text{g}/\mu\text{L}$ nectar. Total sugar content (TSC) was calculated as the sum of glucose, fructose, and sucrose.

2.3.2. Nectar amino acids content

The nectar amino acids composition was evaluated by UHPLC (Ultimate Dionex 3000, ThermoFisher, USA) coupled with a UV and fluorescence detector. The analytes were separated with a Kinetex C-18 column (2.1×100 mm, $2.6 \mu\text{m}$) coupled to a column guard (Phenomenex, USA). The mobile phases were (A) NH_4COOH 5 mmol/L, pH 7.8 and (B) $\text{MeCN}/\text{MeOH}/\text{H}_2\text{O}$ 45:45:10 v/v/v. The chromatographic gradient was made as follows: 0–0.5 min 2 % B, 2 min 15 % B, 3 min 25 % B, 4 min 35 % B, 5 min 45 % B, 10 min 70 % B, 11–15 min 98 % B. The column was conditioned at 2 % B for 4 min before the injection. The amino acid were automatically derivatized as follows: 5 μL of borate buffer pH 10.2 (Agilent, USA), 1 μL of sample/blank/analytical standard, 1 μL of a solution 1 mg/mL o-phthalaldehyde (Merck, Germany) in 2 % (v/v) β -mercaptoethanol (Merck, Germany) or 2.5 mg/mL Fmoc chloride (Merck, Germany) in MeCN , 3 μL of a solution CH_3COOH 1 mol/L (Merck, Germany). The total injection volume was 10 μL . The elution was performed at a flow rate of 0.5 mL/min. The calibration curve for each amino acid (Merck, Germany) was made up in a range between 0.1 and 2 $\mu\text{g}/\text{mL}$. For the detection and quantification of the analytes, we mainly exploited the fluorescence detector. OPA-derivatized amino acids were excited at 338 nm and the emission

signal detected was 442 nm, while the Fmoc-derivatized one (proline) was excited at 262 nm and the emission was detected at 325 nm. As a further control, the UV detector was set at 338 nm for OPA-derivatized amino acids, while proline was detected as 262 nm. The integration of the chromatograms was performed by using the Chromeleon Software (ThermoFisher, USA) and the concentration values obtained were normalized on the volume of sampled nectar in order avoid sampling biases and expressed as $\text{ng}/\mu\text{L}$ nectar. Total aminoacids (TAA) content was calculated by adding the concentration of all the single aminoacids, while the essential aminoacids (EAA) content was obtained by adding the concentration of the aminoacids essential for bees (Jeannerod et al., 2022).

2.3.3. Pollen phytochemicals extraction and analysis

The extraction and the subsequent quantification of secondary metabolites occurring in pollen samples was carried out as follows. One mg pollen for each sample was weighed and extracted in a ratio 1:1000 w/v in MeOH 70 % v/v for two extraction cycles with the support of a bath sonicator (frequency: 37 Hz, temperature: 30 °C). At the end of each extraction cycle, the supernatant was collected and dried under gaseous nitrogen (N_2). The extracts were resuspended in 1 mL H_2O and analysed for the total phenol (TPC) and flavonoid content (TFC) and the total antioxidant capacity (TEAC) as reported in Guzzetti et al., 2017 with minor modifications. In detail, the quantification of flavonoids was made by using quercetin as analytical standard instead of catechin.

The extracts obtained from pollen samples were dried under gaseous nitrogen and analysed by HRMS for the untargeted metabolomic characterization. Both the negative and positive ionisation mode was considered for each sample analysed. The RP-HPLC-MS analysis was performed according to the same facilities and parameters provided in Pioltelli et al., 2023c. The chromatographic gradients were adjusted according to the profile occurring among the different species.

2.4. Statistical analysis

Data about pollen TPC, TFC, and TEAC and data on nectar TSC, TAA, and EAA were analysed to investigate putative response to landscape variables by using the software R (v. 4.3.1). Specifically, data on pollen TPC, TFC, and TEAC were normalized according to the min/max scaling

Table 1

Overview of the main chemical investigations performed in the present study on floral rewards, with details on their importance to the diet of pollinator insects, unit of measurement, sample size, statistical approach for their analysis, and species investigated.

Floral rewards	Species	Floral shape	Analytes	Unit of measurement	Value as a reward	N	Statistical analysis
Nectar	1. <i>Trifolium pratense</i> L. (Fabaceae) 2. <i>Prunella vulgaris</i> L. (Lamiaceae) 3. <i>Salvia pratensis</i> L. (Lamiaceae)	1. Keel/Flag flower 2. Gullet flower 3. Gullet flower	Sugars (intended as the sum of glucose, fructose, and sucrose)	$\mu\text{g}/\mu\text{L}$	Support to the insect energy metabolism	22	Untransformed data. Gaussian distribution
			TSC	$\text{ng}/\mu\text{L}$	Nutritional needs, fitness, attraction	22	Untransformed data. Gamma distribution (non normal data)
			Aminoacids (intended both as total content as well as essential aminoacid content)				
Pollen	1. <i>Potentilla reptans</i> L. (Rosaceae) 2. <i>Lotus corniculatus</i> L. (Fabaceae) 3. <i>Hypochaeris radicata</i> L. (Asteraceae) 4. <i>Malva sylvestris</i> L. (Malvaceae)	1. Bowl flower 2. Keel/Flag flower 3. Ray flower 4. Bowl flower	Phenolic compounds (TPC)	$\mu\text{g}/\text{mg}$	Defence against parasites infection and pesticides detoxifiers	58	Min-max rescaled data. Beta distribution.
			Antioxidant activity (TEAC)	$\mu\text{g}/\text{mg}$	Evaluation of the overall ability to counteract oxidative related stress phenomena	58	Min-max rescaled data. Binomial distribution.
			Flavonoids (TFC)	$\mu\text{g}/\text{mg}$	Defence against parasites infection and pesticides detoxifiers	58	Min-max rescaled data. Beta distribution.
			Untargeted metabolome	NA	Identification of specific phytochemicals endowed with different bioactive properties to pollinators	58	Ordination analysis (RDA) and random forest

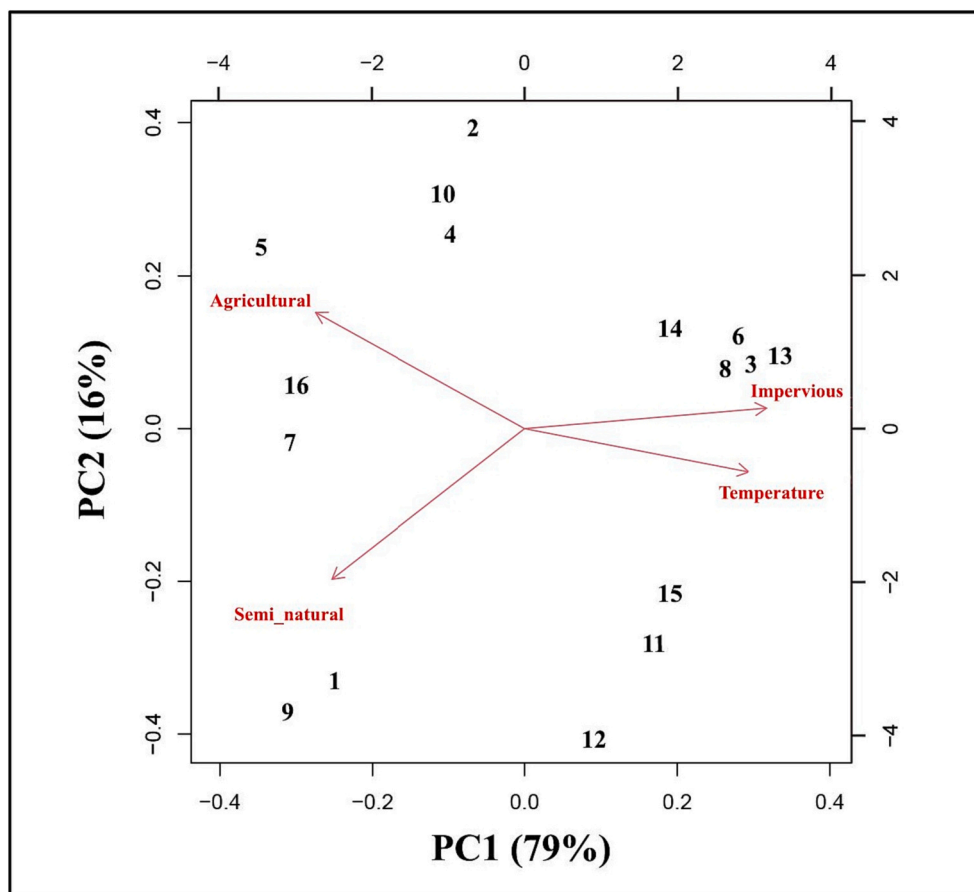


Fig. 2. Principal component analysis with the landscape and environmental features of the sampling sites. The arrows indicate the direction where a particular category is higher and let the definition of two different landscape gradients.

method to account for the largely different ranges observed among the species investigated. We fitted Generalised Linear Mixed effects Models (GLMMs) with a binomial distribution of the response variable for data regarding the phytochemical composition of pollen. The dispersion parameter was monitored to be around 1 (in case of values lower than 1, the models were run with a beta distribution to avoid type II errors). The fixed effects included into the models were the land use variables obtained by the abovementioned PCA, naturally uncorrelated, while the species were included as random components into the regression models. Data regarding nectar nutrition were analysed by GLMMs with a gaussian distribution of the response variable concerning TSC, while a Gamma distribution was implemented for TAA and EAA due to the violation of the assumption of normality.

To investigate inter-specific differences in the nutrient content among the sampled species, we fitted (G)LM(M)s with the species as the fixed effect and the site as the random effect (in case of significant fluctuations of the nutrient of interest among sites). The dependent variables were assumed to be normally or Gamma distributed for nectar nutrients and binomially (or beta distributed in case of overdispersion parameters <1) for the analysis of the phytochemical composition of pollen.

The analysis of the metabolic profiles by HRMS was initially performed on the MS-DIAL software (Version 4.9) for peak peaking, deconvolution, noise setting and normalization. Normalized data were then analysed in R. Firstly, a Redundancy Analysis (RDA) was performed to test the impact of the land use variables (PC1 and PC2) on the overall metabolome of the pollen of the studied species. For the significant outputs, a random forest regression model was implemented to understand which were the most significant metabolic features responding to the land use variables. Packages exploited were TMB (Kristensen et al.,

2016), glmmTMB (Brooks et al., 2017), ggplot2 (Wickham, 2016), MuMIn (Bartoń, 2022), vegan (Oksanen et al., 2022), and rfPermute (Archer, 2022). The significant m/z were more deeply characterized by studying their fragmentation patterns in the MS/MS or DDA analysis both with literature research and using the UNIFI Software 1.9.4 EN (Waters, USA) with the library “Waters Traditional Medicine Library” provided by Waters, USA.

3. Results

3.1. Impact of the land use variables on nectar nutritional composition

The three analysed species presented differences in their nectar chemical profiles (Table S2). Concerning the analysis of nectar TSC in response to the landscape gradients, this was negatively influenced in a linear manner by the PC2 (Fig. 3) which describes a gradient moving from semi-natural to agriculture dominated habitat ($\chi^2 = 3.985$; $p = 0.046$). No significant effect of the urbanization gradient described by the PC1 was detected ($\chi^2 = 0.127$; $p = 0.722$). The TSC of nectar did not vary significantly across the analysed species (Table S2).

No significant effects of the two land use gradients were observed on TAA (PC1 $\chi^2 = 0.162$, $p = 0.687$; PC2: $\chi^2 = 0.017$, $p = 0.897$) and EAA (PC1 $\chi^2 = 0.683$, $p = 0.494$; PC2: $\chi^2 = 0.749$, $p = 0.454$) content of nectar. A significant effect of the species on nectar TAA and EAA (Table S2) was observed.

3.2. Impact of land use variables on pollen phytochemicals content

Among those analysed, the species richest in phytochemicals was *P. reptans* followed by *L. corniculatus*, *H. radicata*, and *M. sylvestris*, as

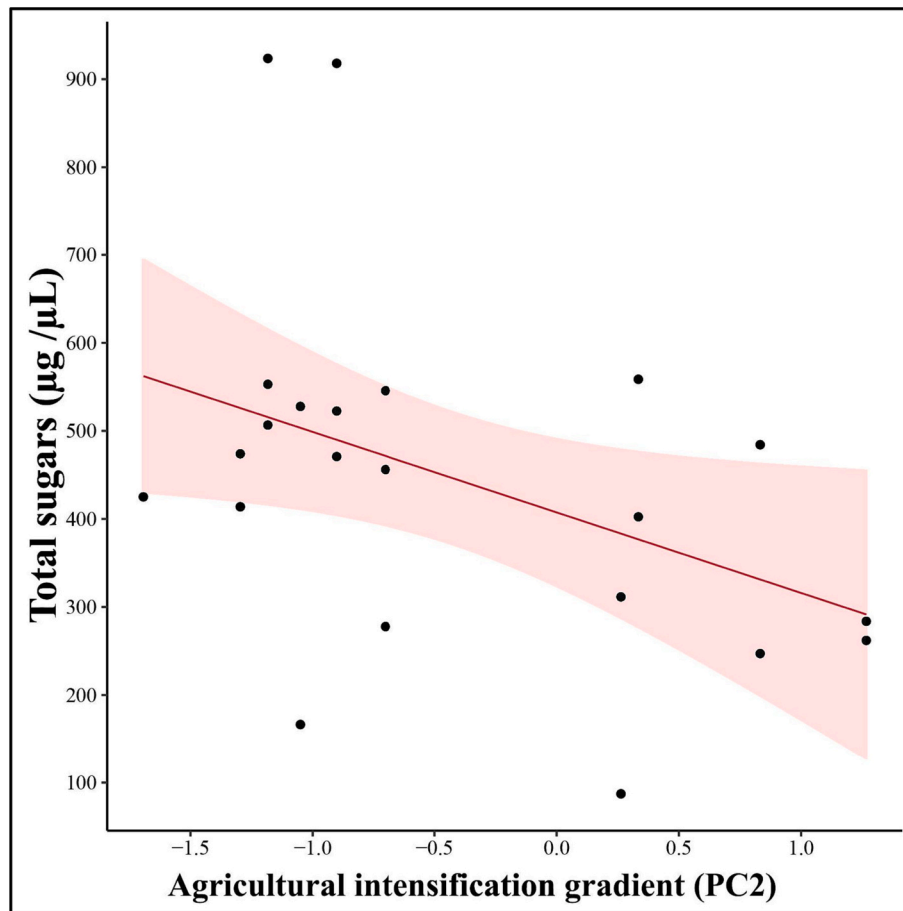


Fig. 3. Relationship between the PC2 (defining an agriculture intensification gradient) and the TSC of nectars expressed as µg sugars per µL of sampled nectar ($n = 22$). The R^2 value of the model is equal to 19.2 %.

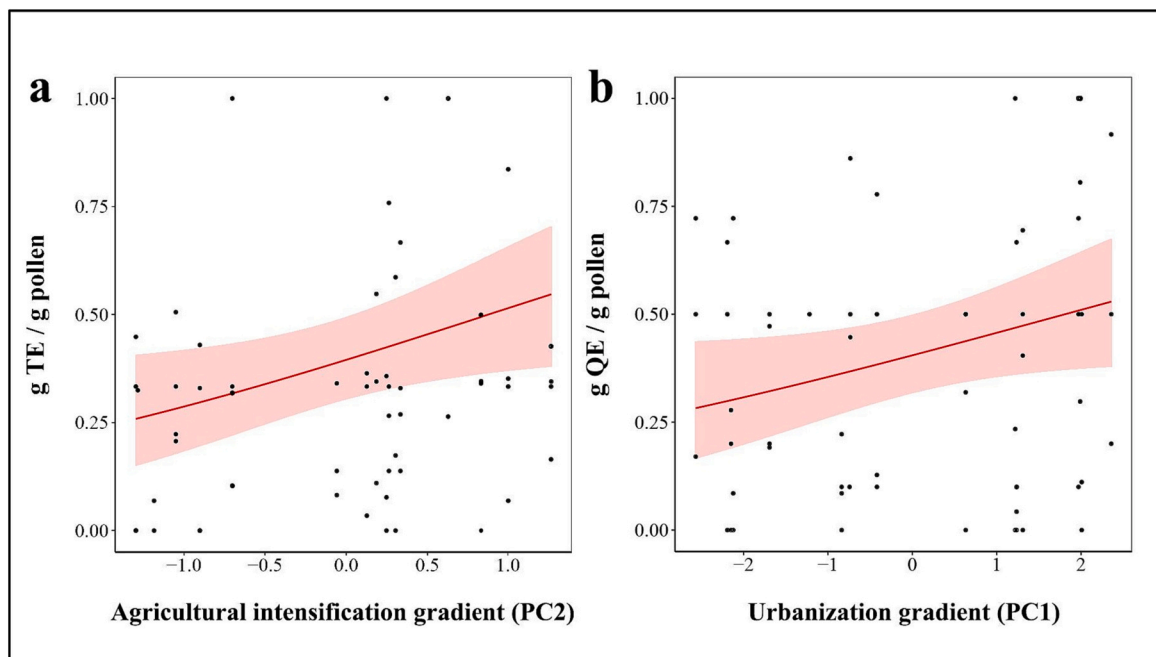


Fig. 4. Relationship between the total phytochemicals content of pollen from the analysed species and the landscape variables: (a) relation between the total antioxidant activity of pollen and the PC2 ($n = 58$; R^2 : 31.3 %); (b) relation between the total flavonoid content of pollen and the PC1 ($n = 58$; R^2 : 31.7 %).

reported in Table S2. The phytochemical profile of pollen was not significantly influenced by the land-use variables concerning the total phenol content (PC1 $\chi^2 = 1.364$, $p = 0.245$; PC2: $\chi^2 = 1.804$, $p = 0.179$). However, the antioxidant activity of pollen showed a positive relation with the agricultural intensification gradient ($\chi^2 = 4.981$, $p = 0.026$) and the total flavonoid content was positively related to the urbanization gradient ($\chi^2 = 3.86$, $p < 0.05$) as reported in Fig. 4a and b.

3.3. Metabolomic assessment of pollen phytochemicals

The metabolomic analysis of pollen from wildflower species showed different responses among species to the land use variables. The output of the RDA is reported in Tables 2 and 3. A significant response to the urbanization gradient (PC1) was found in the metabolomic investigation of the pollen of *L. corniculatus*, both in positive and negative ion current, while the metabolome of *M. sylvestris* displayed a significant response to the agriculture intensification gradient (PC2) in negative ionisation mode.

3.4. Identification of the most responsive metabolites to land use variables

Phytochemicals most responsive to the urbanization gradient in *L. corniculatus* pollen were those belonging to the family of flavonoids (Table 4, also represented as the chromatographic traces in Fig. S1c, S2c, and S3c), and the majority of them was positively associated with the urbanization gradient described by the PC1 (Fig. S1b). Additional information on the correlation coefficient between the land use variables (PC1 and PC2) and the significant features are reported in Fig. S1b, S2b and S3b. The analysis performed on *M. sylvestris* pollen extract shows an impact of the agricultural intensification gradient on the phytochemical composition of pollen, in particular a high degree of correlation was associated with an antioxidant compound, identified as rosmarinic acid (see Table 4). Additionally, Fig. S1a, S2a and S3a show the output of the random forest regression analysis and report the significant features responsible for the variation of pollen metabolomic associated to land use variables in *L. corniculatus* (Fig. S1a, S2a) and *M. sylvestris* (Fig. S3a).

No significant effects of the land use gradient were detected in the metabolome investigation of *P. reptans* and *H. radicata* pollen, neither in negative nor in positive ionisation mode.

4. Discussion

The first important result of this study is the identification of a well-marked relationship between landscape composition and some aspects of the chemical profile of both pollen and nectar. The total sugar content of the nectar was negatively influenced by the agriculture intensification gradient, while no significant variations in nectar TAA and EAA were detected in response to any of the land use gradients considered. It is conceivable an effect of agrochemicals (i.e., herbicides) in the surrounding of the crops which may alter sugar metabolism, since many herbicides commercially available impair the photosynthesis efficiency (Oettmeier, 2003). However, a recent study by Russo et al., 2023 on a different panel of flowers did not find any significant variation on nectar sugars concentration based on the herbicide treatment, making this topic in need of further investigation, hopefully on a wider phylogenetic set of plants.

Concerning the phytochemical composition of pollen, secondary

metabolites are gaining increasing attention due to the important nutraceutical properties that some of them exhibit, for instance by acting as pesticide detoxifiers or by reducing the probability of parasite infections upon specific dosages (Đordjević et al., 2023; Riveros and Gronenberg, 2022; Mao et al., 2013). In this study we found that the overall antioxidant activity of pollen increases significantly along the agriculture intensification gradient, while along the urbanization gradient a specific increase in the flavonoid content was observed. It is arguably that these patterns may be explained by the higher stress levels experienced by the plants. The transition from natural to urban areas is likely to pose some pressures to plants, for instance by increasing the transpiration level due to higher temperature and lower humidity which may elicit the biosynthesis of stress-defence compounds, such as flavonoids (Qian et al., 2022; Innes et al., 2019), also at the pollen level (Rutley et al., 2021), as highlighted in the present study. Many flavonoids have recently been appointed as nutraceutical in the diet of pollinator insects (Riveros and Gronenberg, 2022; Fitch et al., 2022) and a higher occurrence in the pollen of species growing in urbanized areas may be helpful in mitigating stress phenomena, such as those related to oxidation and ageing (Berenbaum and Calla, 2021). Concerning the positive correlation observed between the pollen antioxidant activity and the agriculture intensification gradient, the occurrence of agrochemicals in agricultural areas (mainly herbicides) could act as a driver of stress for wild plants triggering the production of defence compounds (Cesco et al., 2021). Another important result of our study derived from the metabolomic investigations on the pollen extracts was the identification of the species more responsive to the land use gradient. We found that the chemical composition of the pollen of *L. corniculatus* was significantly impacted by the urbanization gradient, whilst the pollen of *M. sylvestris* responded to the agriculture intensification one. The most important metabolites influenced by the land use variables belong to the class of phenolics, mainly flavonoids, many of which were positively related to the PC1 in the pollen of *L. corniculatus*. However, this pattern was not associated with an increase in the antioxidant activity nor in the total phenol content of pollen, and this may be because some phenolic compounds other than flavonoids or belonging to different classes of phytochemicals may balance the flavonoids variation. Indeed, in the pollen of *L. corniculatus* we found that some phytochemicals highlighted by the random forest analysis were negatively associated with the PC1. Concerning the agriculture intensification gradient, the increase in the total antioxidant activity of pollen may be partly related with the metabolomic variations occurring in the pollen of *M. sylvestris*. We found that rosmarinic acid, a potent antioxidant compound (Chadni et al., 2023), was positively related to the PC2 and may be involved in the total antioxidant activity observed from pollen extracts. However, another identified compound endowed with antioxidant properties, a patuletin derivative, showed a negative correlation with the PC2. Nevertheless, the radical scavenging properties of the different metabolites may vary depending on their exact concentration which was not evaluated in the present research.

In a context where landscape anthropization can influence the chemical composition of floral rewards at different metabolic levels, providing an effective plant community able to sustain the diet of pollinator insects should be considered for the planning and definition of effective Nature based Solutions (NbS) in highly anthropized environments. In recent years, NbS are particularly attracting the interest of policy makers due to their potential contribution to biodiversity

Table 2
Output of the RDA considering the metabolomic analysis in negative ionisation mode.

Species	<i>P. reptans</i>		<i>H. radicata</i>		<i>L. corniculatus</i>		<i>M. sylvestris</i>	
	Model	<i>p</i>	Model	<i>p</i>	Model	<i>p</i>	Model	<i>p</i>
PC1	1.378	0.148	1.359	0.238	2.474	0.021	0.352	0.767
PC2	0.406	0.905	1.454	0.212	2.105	0.054	9.097	< 0.001

Table 3
Output of the RDA considering the metabolomic analysis in positive ionisation mode.

Species	<i>P. reptans</i>		<i>H. radicata</i>		<i>L. corniculatus</i>		<i>M. sylvestris</i>	
Ion current	Positive		Positive		Positive		Positive	
Model	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
PC1	1.261	0.286	1.939	0.08	4.952	0.003	1.95	0.136
PC2	0.39	0.799	0.975	0.422	1.051	0.389	2.733	0.066

Table 4
List of the identified metabolites resulted significant from the random forest analysis in the response to the land use variables. Rt = Retention time; *m/z* = mass/charge ratio.

Species	Peak	Adduct	Rt	<i>m/z</i>	MS/MS ions	Ontology	ID	Class	Ref
<i>Lotus corniculatus</i>	1	[2M-Na] ⁺	2.9	144	143, 128, 127, 117, 91	C ₁₀ H ₁₄ N ₂ O ₅	Thymidine	Nucleoside	Waters traditional Medicine Library
<i>Lotus corniculatus</i>	2	[M-H] ⁺	5.6	463	301	C ₂₃ H ₂₆ O ₁₀	9,10-DiMP-3-O-Glc	Flavonoid	Zhang et al., 2015
<i>Lotus corniculatus</i>	3	[M-H] ⁺	7.3	317	302, 285, 168, 158, 140, 134, 107	C ₁₆ H ₁₂ O ₇	5,6,7,3'-Tetrahydroxy-4'-methoxyisoflavone	Flavonoid	Waters traditional Medicine Library
<i>Lotus corniculatus</i>	4	[M-H] ⁺	7.5	301	286, 269, 241, 229, 153	C ₆ H ₁₂ O ₆	5- methyl kaempferol	Flavonoid	Waters traditional Medicine Library
<i>Lotus corniculatus</i>	5	[M-H] ⁺	8	549	301	C ₂₆ H ₂₈ O ₁₃	9,10-DiMP-3-O-malonyl -Glc	Flavonoid	Zhang et al., 2015
<i>Lotus corniculatus</i>	6	[M-H] ⁺	10	331	316, 301, 298, 168	C ₁₇ H ₁₄ O ₇	3,5,6-Trihydroxy-4',7-dimethoxyisoflavone	Flavonoid	Waters traditional Medicine Library
<i>Lotus corniculatus</i>	7	[M-H] ⁻	5.6	387	369, 207, 163	C ₁₈ H ₂₈ O ₉	Tuberonic acid glycoside	Hormone derivative	Waters traditional Medicine Library
<i>Lotus corniculatus</i>	8	[M-H] ⁻	6	593	446, 430, 299, 285, 151	C ₂₇ H ₃₀ O ₁₅	Kaempferol 3-O-L-rhamnopyranosyl-(1 → 2)-glucopyranoside	Flavonoid	Waters traditional Medicine Library
<i>Lotus corniculatus</i>	9	[M-H] ⁻	7	649	503, 460, 446, 315, 313, 113	C ₃₁ H ₃₈ O ₁₅	Tubuloside E	Phenylpropanoid	Waters traditional Medicine Library
<i>Lotus corniculatus</i>	10	[M-H] ⁻	7.2	463	301, 286	C ₂₂ H ₂₄ O ₁₁	Hesperetin-7-O-glycoside	Flavonoid	Waters traditional Medicine Library
<i>Lotus corniculatus</i>	11	[M-H] ⁻	10	329	229, 211, 183, 171	C ₁₈ H ₃₄ O ₅	Sanleng acid	Fatty acid	Waters traditional Medicine Library
<i>Lotus corniculatus</i>	12	[M-H] ⁻	12	982	941, 923, 879, 615, 597, 247, 205, 163, 139	C ₄₉ H ₈₀ O ₂₀	Agrostragaloside IV	Saponin	Waters traditional Medicine Library
<i>Malva sylvestris</i>	13	[M-H] ⁻	0.8	282	150, 133, 108	C ₁₀ H ₁₃ N ₅ O ₅	Guanosine	Nucleoside	Waters traditional Medicine Library
<i>Malva sylvestris</i>	14	[M-H] ⁻	3	359	197, 153, 123	C ₁₈ H ₁₆ O ₈	Rosmarinic acid	Phenolic acid	Waters traditional Medicine Library
<i>Malva sylvestris</i>	15	[M-H] ⁻	5.4	655	493, 330	C ₂₈ H ₂₂ O ₁₈	Patuletin diglucoside	Flavonoid	Boukhris et al., 2016

protection and human well-being (Laforteza et al., 2018). However, their reliability at the plant-pollinators interaction level is still poorly evaluated and requires to disentangle which plant species has to be prioritised to support the trophic needs of pollinators. In the present study, we found a higher TAA and EAA content in the nectar of *T. pratense* compared with *P. vulgaris* while no significant differences were shown in the sugar content among the studied species (Table S2). Previous studies have already suggested that the nectar of species belonging to the Fabaceae family represent an important source of amino acids (Gardener and Gillman, 2001) whose content is an important factor influencing the foraging preference of pollinators (Venjakob et al., 2020). Conversely, the phenolic composition of pollen and consequently the related antioxidant activity was significantly higher in *P. reptans*, followed by *L. corniculatus*, and ultimately by *H. radicata* and *M. sylvestris* (Table S2). However, the secondary metabolites composition needs to be specifically defined since not all of them exert beneficial effects in the diet of pollinators. For instance, tannins are one of the major constituents of the phytochemical composition of *P. reptans* (Tomczyk and Latté, 2009) and may act as anti-nutrients, affecting the longevity of bees (Sagona et al., 2021). In the present study, our focus was posed on the small molecules and phytochemicals composition of flower rewards, since these compounds, differently from macronutrients (e.g., proteins and lipids), are the most frequently involved in short-term responses to changes in environmental variables, acting as osmolytes or

defence/stress compounds (Egan et al., 2021; Ghosh et al., 2021; Arathi et al., 2018). Furthermore, these compounds are known to play a significant role in pollinators' diet, acting as essential nutrients or as nutraceuticals (Koch et al., 2017; Richardson et al., 2015). The integration of these results with those arising from the investigation of the nutritional profiles of foraged pollen may be of help to disentangle at a deeper scale the effect of land use management on plant-pollinators trophic interactions.

It is important to highlight that local abiotic factors, such as solar radiation, temperature, and evapotranspiration might display great effects on the relative content of sugars in nectar (Plos et al., 2023), and also polypeptides in pollen (Descamps et al., 2021). The magnitude of these microclimatic variables may be relevant, especially concerning short-term variations in the concentration of small molecules similar to those analysed in the present study (Qian et al., 2022; Innes et al., 2019). Such modifications may even impact floral traits and pollinator efficiency (Aker and Klečka, 2022). At the individual level, the genotype and the stage of development of the flower may impact significantly on the chemical composition of nectar (Clearwater et al., 2018). For instance, in *Leptospermum scoparium*, the content of sugar in nectar is very low at the second floral stage, then it increases up to a peak at the third stage and then it tends to decline during the fourth floral stage (Clearwater et al., 2018). Furthermore, also the sexual floral stage was found to significantly impact the chemistry of nectar: for example, in

Echium vulgare, the nectar sugar content is significantly higher during the female phase compared to the male one mainly due to an increase in the volume of secreted nectar than sugar concentration, while the content of phenylalanine was shown to be significantly higher during the male stage than the female one (Barberis et al., 2021). Although these factors acting more at the individual- or even flower-level were not quantified in our study, they likely constitute a strong source of variation deserving future scientific insights.

From the applicative point of view, this investigation provides some practical implications for the management of urban and peri-urban green areas and the design of ecological restoration strategies in human-dominated landscapes. For example, the reduction in the sugar content of nectar observed in agricultural areas has strong implications for the potential of these sites to sustain the local pollinator communities. Indeed, if we convert the concentration of sugars observed in energetic terms (J/ μ L) and considering that an average 500 mg bee requires 600 J per hour of flight (Wilmer, 2011), we can calculate that this representative energetic requirement can be satisfied by foraging 11 μ L nectar in agricultural areas while only 5 μ L in semi-natural areas. Thus, the halving of the sugar content along the agriculture intensification gradient suggests the risk to increase the time devoted to foraging for pollinator insects. This result highlights the critical importance of boosting flower availability in these habitats in order to sustain the energetic needs of the local insect pollinators community. A more detailed study of the environmental variables influencing sugar content could also make it possible to plan tailored interventions across different areas, allowing for example a more nuanced control over the application of herbicides and other management actions. The increase in the flavonoids and antioxidant compounds in disturbed areas poses the attention on the impact of anthropogenic stressors on plant secondary metabolism. As previously highlighted, many of these metabolites have shown promising bioactivity against pesticides and parasites at the physiological level, which hints at a potential avenue for ecological fortification. However, it is essential to exercise caution, given that many of these compounds could exceed toxicity thresholds (Palmer-Young et al., 2019). Then, the seedling of species characterized by higher resilience to anthropogenic stressors in terms of secondary metabolism variations emerges as a prudent course of action. This approach can mitigate the potential hazard of exposing pollinator insects to toxic concentration of phytochemicals. Furthermore, providing for a diversified panel of plant species in a long lasting seasonal context can be crucial as a rich plant community affords pollinators the flexibility to selectively exploit resources based on their foraging needs and preferences (Blüthgen and Klein, 2011).

5. Conclusions

With this study we have deepened some aspects dealing with the nutritional ecology of pollinators focusing specifically on the resources they forage on. The results highlighted that some variations occurring in bee pollen nutrients and related to different land use managements could not be only due to the choice of foragers, but also depend on the plant resource itself. The variations highlighted in the chemical composition of pollen and nectar may impact the health status of pollinator insects in different environments, attenuating and/or exacerbating stress phenomena occurring at the insect physiological level. The present study suggests that the definition of a proper nutritional landscape for pollinator insects requires not only to identify the most nutritionally relevant species for pollinators, but also to understand to which extent habitat anthropization may modify their nutritional uptake by acting on plant metabolic pathways, in order to promote reliable actions for the planning of mitigation strategies in urban and agricultural contexts, such as the Nbs, by selecting species able to offer a proper nutrient composition regardless of the environment in which they occur.

CRediT authorship contribution statement

Emiliano Pioltelli: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Lorenzo Guzzetti:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Malika Ouled Larbi:** Investigation, Data curation, Visualization. **Rita Celano:** Investigation, Resources. **Anna Lisa Piccinelli:** Investigation, Resources. **Andrea Galimberti:** Supervision, Writing – review & editing. **Paolo Biella:** Conceptualization, Validation, Writing – original draft, Writing – review & editing. **Massimo Labra:** Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data are available at doi:<https://doi.org/10.6084/m9.figshare.24026328.v1>

Acknowledgments

The authors thank Fausto Ramazzotti, Nicola Tommasi, Andrea Decimi, and Luca Toniatti for their help with the sampling activities, Maura Briosci and Simona Serio for their support with the mass spectrometry and amino acids analyses, respectively.

Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU, Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP H43C22000530001, Project title “National Biodiversity Future Center - NBFC”. PB acknowledges support from Italian Ministry of Universities and Research (with resources from the PONRI FSE REACT-EU 2014–2020 – “Azione IV.4 - Dottorati e contratti di ricerca su tematiche dell’innovazione, Azione IV.6 - Contratti di ricerca su tematiche Green”).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.168130>.

References

- Akter, A., Klečka, J., 2022. Water stress and nitrogen supply affect floral traits and pollination of the white mustard, *Sinapis alba* (Brassicaceae). *PeerJ* 10, e13009. <https://doi.org/10.7717/peerj.13009>.
- Arathi, H.S., Bjostad, L., Bernklau, E., 2018. Metabolomic analysis of pollen from honey bee hives and from canola flowers. *Metabolomics* 14 (6), 86. <https://doi.org/10.1007/s11306-018-1381-5>.
- Archer, E., 2022. *rFPermute*: estimate permutation p-values for random Forest importance Metrics. R package version 2.5.1. <https://CRAN.R-project.org/package=rFPermute>.
- Aylanc, V., Falcão, S.L., Ertosun, S., Vilas-Boas, M., 2021. From the hive to the table: nutrition value, digestibility and bioavailability of the dietary phytochemicals present in the bee pollen and bee bread. *Trends Food Sci. Technol.* 109, 464–481. <https://doi.org/10.1016/j.tifs.2021.01.042>.
- Barascou, L., Sene, D., Barraud, A., Michez, D., Lefebvre, V., Medrzycki, P., Di Prisco, G., Strobl, V., Yañez, O., Neumann, P., Le Conte, Y., Alaux, C., 2021. Pollen nutrition fosters honeybee tolerance to pesticides. *R. Soc. Open Sci.* 8 (9), 210818 <https://doi.org/10.1098/rsos.210818>.
- Barberis, M., Bogo, G., Bortolotti, L., Conte, L., Alessandrini, M., Nepi, M., Galloni, M., 2021. Gender-biased nectar targets different behavioural traits of flower visitors. *Plant Ecol.* 222 (2), 233–246. <https://doi.org/10.1007/s11258-020-01101-5>.

- Bartoń K., 2022. *MuMIn: multi-model Inference*. R package version 1.47.1, <https://CRAN.R-project.org/package=MuMIn>.
- Berenbaum, M.R., Calla, B., 2021. Honey as a functional food for *Apis mellifera*. *Annu. Rev. Entomol.* 66 (1), 185–208. <https://doi.org/10.1146/annurev-ento-040320-074933>.
- Biella, P., Akter, A., Muñoz-Pajares, A.J., Federici, G., Galimberti, A., Jersáková, J., Labra, M., Mangili, F., Tommasi, N., Mangili, L., 2021. Investigating pollination strategies in disturbed habitats: the case of the narrow-endemic toadflax *Linaria tonzigii* (Plantaginaceae) on mountain screes. *Plant Ecol.* 222 (4), 511–523. <https://doi.org/10.1007/s11258-021-01123-7>.
- Biella, P., Tommasi, N., Guzzetti, L., Pioltelli, E., Labra, M., Galimberti, A., 2022. City climate and landscape structure shape pollinators, nectar and transported pollen along a gradient of urbanization. *J. Appl. Ecol.* 59 (6), 1586–1595. <https://doi.org/10.1111/1365-2664.14168>.
- Blüthgen, N., Klein, A.M., 2011. Functional complementarity and specialisation: the role of biodiversity in plant–pollinator interactions. *Basic Appl. Ecol.* 12 (4), 282–291. <https://doi.org/10.1016/j.baae.2010.11.001>.
- Boukhris, M.A., Destandau, É., El Hakmaoui, A., El Rhaffari, L., Elfakir, C., 2016. A dereplication strategy for the identification of new phenolic compounds from *Anvillea radiata* (Coss. & Durieu). *C. R. Chim.* 19 (9), 1124–1132. <https://doi.org/10.1016/j.crci.2016.05.019>.
- Brooks, M.E., Kristensen, K., Benthem, K.J., van Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Mächler, M., Bolker, B.M., 2017. GlmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* 9(2), 378. <https://doi.org/10.32614/RJ-2017-066>.
- Cesco, S., Lucini, L., Miras-Moreno, B., Borruso, L., Mimmo, T., Pii, Y., Puglisi, E., Spini, G., Taskin, E., Tiziani, R., Zangrillo, M.S., Trevisan, M., 2021. The hidden effects of agrochemicals on plant metabolism and root-associated microorganisms. *Plant Sci.* 311, 111012. <https://doi.org/10.1016/j.plantsci.2021.111012>.
- Chadni, M., Isidore, E., Lagalle, F., Langlait, M., Dosso, A., Ioannou, I., 2023. Optimization of the supercritical extraction of rosmarinic acid from clary sage residue and the antioxidant activity of the extracts. *J. Supercrit. Fluids* 193, 105830. <https://doi.org/10.1016/j.supflu.2022.105830>.
- Clearwater, M.J., Revell, M., Noe, S., Manley-Harris, M., 2018. Influence of genotype, floral stage, and water stress on floral nectar yield and composition of mānuka (*Leptospermum scoparium*). *Ann. Bot.* 121 (3), 501–512. <https://doi.org/10.1093/aob/mcx183>.
- Descamps, C., Quinet, M., Jacquemart, A.L., 2021. Climate change–induced stress reduce quantity and alter composition of nectar and pollen from a bee-pollinated species (*Borago officinalis*, Boraginaceae). *Front. Plant Sci.* 12, 755843. <https://doi.org/10.3389/fpls.2021.755843>.
- Donkersley, P., Rhodes, G., Pickup, R.W., Jones, K.C., Power, E.F., Wright, G.A., Wilson, K., 2017. Nutritional composition of honey bee food stores vary with floral composition. *Oecologia* 185 (4), 749–761. <https://doi.org/10.1007/s00442-017-3968-3>.
- Dordievski, S., Vukašević, E.L., Čelić, T.V., Pihler, I., Kebert, M., Kojić, D., Purać, J., 2023. Spermidine dietary supplementation and polyamines level in reference to survival and lifespan of honey bees. *Sci. Rep.* 13 (1), Article 1. <https://doi.org/10.1038/s41598-023-31456-4>.
- Egan, P.A., Muola, A., Parachnowitsch, A.L., Stenberg, J.A., 2021. Pollinators and herbivores interactively shape selection on strawberry defence and attraction. *Evol. Lett.* 5 (6), 636–643. <https://doi.org/10.1002/evl3.262>.
- Faegri, K., Van Der Pijl, L., 1979. *Principles of Pollination Ecology*. Pergamon Press, Oxford, UK.
- Filipiak, Z.M., Denisow, B., Stawiarz, E., Filipiak, M., 2022. Unravelling the dependence of a wild bee on floral diversity and composition using a feeding experiment. *IJP-PAW.* 820, 153326. <https://doi.org/10.1016/j.scitotenv.2022.153326>.
- Fitch, G., Figueroa, L.L., Koch, H., Stevenson, P.C., Adler, L.S., 2022. Understanding effects of floral products on bee parasites: mechanisms, synergism, and ecological complexity. *International Journal for Parasitology: Parasites and Wildlife* 17, 244–256. <https://doi.org/10.1016/j.ijppaw.2022.02.011>.
- Gardener, M.C., Gillman, M.P., 2001. Analyzing variability in nectar amino acids: composition is less variable than concentration. *J. Chem. Ecol.* 27 (12), 2545–2558. <https://doi.org/10.1023/A:1013687701120>.
- Ghosh, U.K., Islam, Md.N., Siddiqui, Md.N., Khan, Md.A.R., 2021. Understanding the roles of osmolytes for acclimating plants to changing environment: a review of potential mechanism. *Plant Signal. Behav.* 16 (8), 1913306. <https://doi.org/10.1080/15592324.2021.1913306>.
- Guzzetti, L., Galimberti, A., Bruni, I., Magoni, C., Ferri, M., Tassoni, A., Sangiovanni, E., Dell’Aglì, M., Labra, M., 2017. Bioprospecting on invasive plant species to prevent seed dispersal. *Sci. Rep.* 7 (1), Article 1. <https://doi.org/10.1038/s41598-017-14183-5>.
- Hou, Y., Li, J., Li, G., Qi, W., 2023. Negative effects of urbanization on plants: a global meta-analysis. *Ecol. Evol.* 13 (4), e9894. <https://doi.org/10.1002/ece3.9894>.
- Innes, S.N., Arve, L.E., Zimmermann, B., Nybakken, L., Melby, T.L., Sollhag, K.A., Olsen, J.E., Torre, S., 2019. Elevated air humidity increases UV mediated leaf and DNA damage in pea (*Pisum sativum*) due to reduced flavonoid content and antioxidant power. *Photochem. Photobiol. Sci.* 18 (2), 387–399. <https://doi.org/10.1039/c8pp00401c>.
- Jeannerod, L., Carlier, A., Schatz, B., Daise, C., Richel, A., Agnan, Y., Baude, M., Jacquemart, A.L., 2022. Some bee-pollinated plants provide nutritionally incomplete pollen amino acid resources to their pollinators. *PLoS One* 17 (8), e0269992. <https://doi.org/10.1371/journal.pone.0269992>.
- Jones, J., Rader, R., 2022. Pollinator nutrition and its role in merging the dual objectives of pollinator health and optimal crop production. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 377 (1853), 20210170. <https://doi.org/10.1098/rstb.2021.0170>.
- Khare, S., Singh, N.B., Singh, A., Hussain, I., Niharika, K., Yadav, V., Bano, C., Yadav, R. K., Amist, N., 2020. Plant secondary metabolites synthesis and their regulations under biotic and abiotic constraints. *J. Plant Biol.* 63 (3), 203–216. <https://doi.org/10.1007/s12374-020-09245-7>.
- Koch, H., Brown, M.J., Stevenson, P.C., 2017. The role of disease in bee foraging ecology. *Curr. Opin. Insect. Sci.* 21, 60–67. <https://doi.org/10.1016/j.cois.2017.05.008>.
- Kristensen, K., Nielsen, A., Berg, C.W., Skaug, H., Bell, B.M., 2016. TMB: automatic differentiation and Laplace approximation. *J. Stat. Softw.* 70 (5) <https://doi.org/10.18637/jss.v070.i05>.
- Laforteza, R., Chen, J., van den Bosch, C.K., Randrup, T.B., 2018. Nature-based solutions for resilient landscapes and cities. *Environ. Res.* 165, 431–441. <https://doi.org/10.1016/j.envres.2017.11.038>.
- Manson, J.S., Cook, D., Gardner, D.R., Irwin, R.E., 2013. Dose-dependent effects of nectar alkaloids in a montane plant–pollinator community. *J. Ecol.* 101 (6), 1604–1612. <https://doi.org/10.1111/1365-2745.12144>.
- Mao, W., Schuler, M.A., Berenbaum, M.R., 2013. Honey constituents up-regulate detoxification and immunity genes in the western honey bee *Apis mellifera*. *PNAS* 110 (22), 8842–8846. <https://doi.org/10.1073/pnas.1303884110>.
- Nicolson, S.W., Neves, Da Silva Das, S., Human, H., Pirk, C. W. W., 2018. Digestibility and nutritional value of fresh and stored pollen for honey bees (*Apis mellifera* scutellata). *J. Insect Physiol.* 107, 302–308. <https://doi.org/10.1016/j.jinsphys.2017.12.008>.
- Oettmeier, W., 2003. Herbicides. *Encyclopedia of Agrochemicals*. John Wiley & Sons, Ltd., Inhibitors of Photosynthesis at Photosystem II <https://doi.org/10.1002/047126363X.agr129>.
- Oksanen J., Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O’Hara R, Solymos P, Stevens M, Zoecis E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J. 2022. *vegan: community ecology Package*. R package version 2.6-4. <https://CRAN.R-project.org/package=vegan>.
- Palmer-Young, E.C., Farrell, I.W., Adler, L.S., Milano, N.J., Egan, P.A., Irwin, R.E., Stevenson, P.C., 2019. Secondary metabolites from nectar and pollen: a resource for ecological and evolutionary studies. *Ecol* 100 (4), Article 4. <https://doi.org/10.1002/ecy.2621>.
- Pioltelli, E., Guzzetti, L., Ouled Larbi, M., Labra, M., Galimberti, A., Biella, P., 2023a. Landscape Fragmentation Constrains Bumblebee Nutritional Ecology and Foraging Dynamics. *Authorea Preprints*. <https://doi.org/10.22541/au.168989255.56800434/v1>.
- Pioltelli, E., Guzzetti, L., Tonietti, L., Copetta, A., Biella, P., Campone, L., Galimberti, A., 2023b. E-PoSa: a novel and effective tool for sampling pollen directly from flowers. *bioRxiv*. <https://doi.org/10.1101/2023.06.30.547178>, 2023-06.
- Pioltelli, E., Sartirana, C., Copetta, A., Brioschi, M., Labra, M., Guzzetti, L., 2023c. *Vigna unguiculata* L. Walp. leaves as a source of phytochemicals of dietary interest: optimization of ultrasound-assisted extraction and assessment of traditional consumer habits. *Chem. Biodivers.*, e202300797 <https://doi.org/10.1002/cbdv.202300797>.
- Plos, C., Stelbrink, N., Römermann, C., Knight, T.M., Hensen, I., 2023. Abiotic conditions affect nectar properties and flower visitation in four herbaceous plant species. *Flora* 303, 152279. <https://doi.org/10.1016/j.flora.2023.152279>.
- Power, E.F., Stabler, D., Borland, A.M., Barnes, J., Wright, G.A., 2018. Analysis of nectar from low-volume flowers: a comparison of collection methods for free amino acids. *Methods Ecol. Evol.* 9 (3), 734–743. <https://doi.org/10.1111/2041-210X.12928>.
- Qian, R., Hu, Q., Ma, X., Zhang, X., Ye, Y., Liu, H., Gao, H., Zheng, J., 2022. Comparative transcriptome analysis of heat stress responses of *Clematis lanuginosa* and *Clematis crassifolia*. *BMC Plant Biol.* 22 (1), 138. <https://doi.org/10.1186/s12870-022-03497-w>.
- R Core Team, 2022. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Richardson, L.L., Adler, L.S., Leonard, A.S., Andicochea, J., Regan, K.H., Anthony, W.E., Manson, J.S., Irwin, R.E., 2015. Secondary metabolites in floral nectar reduce parasite infections in bumblebees. *Proc. Royal Soc. B.* 282 (1803), 20142471. <https://doi.org/10.1098/rspb.2014.2471>.
- Riveros, A.J., Gronenberg, W., 2022. The flavonoid rutin protects the bumble bee *Bombus impatiens* against cognitive impairment by imidacloprid and fipronil. *J. Exp. Biol.* 225 (17), jeb244526. <https://doi.org/10.1242/jeb.244526>.
- Roy, R., Schmitt, A.J., Thomas, J.B., Carter, C.J., 2017. Nectar biology: from molecules to ecosystems. *Plant Sci.* 262, 148–164. <https://doi.org/10.1016/j.plantsci.2017.04.012>.
- Russo, L., Ruedenauer, F., Gronert, A., Vreken, I.V., Vanderplanck, M., Michez, D., Klein, A., Leonhardt, S., Stout, J.C., 2023. Fertilizer and herbicide alter nectar and pollen quality with consequences for pollinator floral choices. *PeerJ* 11, e15452. <https://doi.org/10.7717/peerj.15452>.
- Rutley, N., Miller, G., Wang, F., Harper, J.F., Miller, G., Lieberman-Lazarovich, M., 2021. Enhanced reproductive Thermotolerance of the tomato high pigment 2 mutant is associated with increased accumulation of Flavonols in pollen. *Front. Plant Sci.* 12 <https://doi.org/10.3389/fpls.2021.672368>.
- Sagona, S., Minieri, S., Coppola, F., Gatta, D., Casini, L., Palego, L., Betti, L., Giannaccini, G., Felicioli, A., 2021. Effects of chestnut hydrolysable tannin enrichment in the artificial diet of forager bees. *Apis mellifera*. *J. Apic. Res.* 0 (0), 1–7. <https://doi.org/10.1080/00218839.2021.1960744>.
- Stevenson, P.C., Nicolson, S.W., Wright, G.A., 2017. Plant secondary metabolites in nectar: impacts on pollinators and ecological functions. *Funct. Ecol.* 31 (1), 65–75. <https://doi.org/10.1111/1365-2435.12761>.
- Tew, N.E., Memmott, J., Vaughan, I.P., Bird, S., Stone, G.N., Potts, S.G., Baldock, K.C., 2021. Quantifying nectar production by flowering plants in urban and rural

- landscapes. *J. Ecol.* 109 (4), 1747–1757. <https://doi.org/10.1111/1365-2745.13598>.
- Thakur, M., Nanda, V., 2020. Composition and functionality of bee pollen: a review. *Trends Food Sci Technol.* 98, 82–106. <https://doi.org/10.1016/j.tifs.2020.02.001>.
- Tomczyk, M., Latté, K.P., 2009. Potentilla—a review of its phytochemical and pharmacological profile. *J. Ethnopharmacol.* 122 (2), 184–204. <https://doi.org/10.1016/j.jep.2008.12.022>.
- Tommasi, N., Pioltelli, E., Biella, P., Labra, M., Casiraghi, M., Galimberti, A., 2022. Effect of urbanization and its environmental stressors on the intraspecific variation of flight functional traits in two bumblebee species. *Oecologia* 199 (2), 289–299. <https://doi.org/10.1007/s00442-022-05184-x>.
- Vaudo, A.D., Farrell, L.M., Patch, H.M., Grozinger, C.M., Tooker, J.F., 2018. Consistent pollen nutritional intake drives bumble bee (*Bombus impatiens*) colony growth and reproduction across different habitats. *Ecol. Evol.* 8 (11), 5765–5776. <https://doi.org/10.1002/ece3.4115>.
- Venjakob, C., Leonhardt, S., Klein, A.-M., 2020. Inter-individual nectar chemistry changes of field scabious. *Knautia arvensis*. *Insects.* 11 (2), 75. <https://doi.org/10.3390/insects11020075>.
- Wickham, H., 2016. Programming with ggplot2. In: Wickham, H. (Ed.), *Ggplot2: Elegant Graphics for Data Analysis*. Springer International Publishing, pp. 241–253. https://doi.org/10.1007/978-3-319-24277-4_12.
- Wilmer, P., 2011. *Pollination and Floral Ecology*. Princeton University Press, Princeton, New Jersey, USA. <https://doi.org/10.1515/9781400838943>.
- Wong, M.J., Liao, L.-H., Berenbaum, M.R., 2018. Biphasic concentration-dependent interaction between imidacloprid and dietary phytochemicals in honey bees (*Apis mellifera*). *PLoS One* 13 (11), e0206625. <https://doi.org/10.1371/journal.pone.0206625>.
- Zhang, J., Xu, X.J., Xu, W., Huang, J., Zhu, D.Y., Qiu, X.H., 2015. Rapid characterization and identification of flavonoids in *Radix Astragali* by ultra-high-pressure liquid chromatography coupled with linear ion trap-orbitrap mass spectrometry. *J. Chromatogr. Sci.* 53 (6), 945–952. <https://doi.org/10.1093/chromsci/bmu155>.
- Zu, P., Koch, H., Schwery, O., Pironon, S., Phillips, C., Ondo, I., Farrell, I.W., Nes, W.D., Moore, E., Wright, G.A., Farman, D.I., Stevenson, P.C., 2021. Pollen sterols are associated with phylogeny and environment but not with pollinator guilds. *New Phytol.* 230 (3), 1169–1184. <https://doi.org/10.1111/nph.17227>.