

LONG-TERM EFFECTS OF NITROGEN ENRICHMENT ON POLLEN CHEMISTRY OF A PLANT SPECIES FROM BRAZILIAN SAVANNAS, *PAVONIA ROSA-CAMPESTRIS*

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Abstract—Human activities have substantially increased soil nutrient availability during the past decades, affecting plant community composition and plants' nutritional content. Several amino acids found in pollen, the main source of larval diet for bees, affect the development, health, and behaviour of this important group of pollinators. A better understanding of the consequences of global changes on pollen amino acid content can help explain and predict future impacts on bee populations and diversity. This is particularly relevant in regions that are highly exposed to fertilizers due to their importance for global food production, such as the Brazilian savannas (Cerrado), where soils are mostly dystrophic.

Here, we use a long-term controlled fertilization experiment conducted in Cerrado and demonstrate that even after 10 years since the last fertilization addition, effects were still detectable on leaf and pollen chemical content. More specifically, pollen amino acid content of *Pavonia rosa-campestris* (Malvaceae), a species known to be important for the diet of several native bee species, changed because of nitrogen (N) addition. Not only did the overall amino acid content increase with N addition, but its profile was also affected, with the proportion of some amino acids increasing (e.g. isoleucine, leucine, serine, threonine), while decreasing for others (e.g. cysteine).

These amino acids can have important effects on larval development and flower visitor behaviour. Further studies evaluating the effects on a diverse set of plant species and the consequent impacts on flower visitation and bee fitness are essential to better understand the full consequences of increased nitrogen availability in nutrient-limited ecosystems such as Cerrado.

Keywords—Brazilian savannas, global changes, pollen chemistry, soil eutrophication

INTRODUCTION

Natural environments are increasingly more exposed to high inputs of nutrients, nitrogen (N) being one whose cycle has been most affected by human activity (Steffen et al. 2015; Bouwman et al. 2017). The effects of such ongoing environmental eutrophication on plant communities are well recognized, with evidence of long-term negative impacts on overall diversity worldwide (Midolo et al. 2019). For example, nutrient-driven changes in

richness and overall plant biomass are likely caused by the deregulation of competition relationships between plants (e.g. Isbell et al. 2013; Barbosa et al. 2014). These community-level changes in plant assemblages can have important effects on primary consumers and higher trophic levels. Yet, even when N increases are not sufficiently accentuated (or when other resources, such as phosphorous, are limiting N uptake) to affect plant biomass and community composition, effects may already be noticeable at the plant level

affecting plant chemistry and investment in floral resources (e.g. flower morphology, nectar, and pollen production, see David et al. 2019).

Several studies reported nutrient-driven changes in plant stoichiometry and total nutrient leaf content (e.g. Barbosa, Tomlinson, et al. 2014; Su et al. 2021). Such impacts on plant chemistry can substantially change how primary consumers interact with the plants, affecting ecosystem functioning, even when effects on plant diversity are still undetectable. Indeed, previous studies have reported effects on leaf herbivore development and population dynamics that were mediated by changes in plant chemistry (e.g. Haddad et al. 2000; Throop & Lerdau 2004; Pöyry et al. 2017). Bees can also perceive changes in floral resources' chemical composition (Linander et al. 2012; Ruedenauer et al. 2019) having gustatory receptors that responds to specific amino acids such as glutamate, aspartate, asparagine, arginine, lysine, and glutamine (e.g. *Apis mellifera*, Lim et al. 2019). Therefore, foraging can also be influenced by the composition of floral resources quality, such as amino acid or protein:lipid content (Cook et al. 2003; Nichols and De Ibarra 2006; Vaudo et al. 2016). Yet, very few studies have looked at the impacts of soil nutrient enrichment on floral resource chemistry (David et al. 2019), and those that exist mostly focused on a few European (Gardener & Gillman 2001b; Gosselin et al. 2013; Gijbels et al. 2014, 2015; Ceulemans et al. 2017) or North American plant species (Adler & Irwin 2005; Manson et al. 2012; Cook et al. 2013). Moreover, most focus on nectar (but see Ceulemans et al. 2017), whereas pollen is the main resource for bee larval development (e.g. Heil 2011; Cook et al. 2013; Majetic et al. 2017) also being essential for sexual maturation and ovary development (Human et al. 2007). This handful of published studies shows that overall amino acid (AA) content in floral resources can increase when plants are grown under increased nitrogen supply conditions and that the overall AA profile changes, with some AA increasing their dominance over others (Gardener & Gillman 2001b; Gijbels et al. 2014, 2015; Ceulemans et al. 2017). Within these few studies, there is a diversity of results in terms of which AAs increase or decrease. Moreover, as the strength and direction of such effects may likely depend on other environmental conditions such as climate, water availability, soil attributes, or

vegetation type (reviewed in Midolo et al. 2019), it is unclear if the results of these studies focused on temperate systems (mostly grasslands) can be an indicator of similar effects within tropical regions. This knowledge gap limits our ability to understand and predict the impacts of soil nutrient enrichment on flower visitors, many of whom are important pollinators for the vast majority of angiosperms (Ollerton et al. 2011; Rader et al. 2016). Such scarcity of studies is partially caused by the difficulties in extracting field samples and running detailed chemical analyses. Several protocols on pollen chemistry require large pollen amounts (some requiring a minimum of 50 mg, AOAC 1980, Vossler 2015), and samples can only be harvested during a very narrow time window (flowering season), within which their flower production may vary. Yet, recent methodological approaches made great advances, allowing quantification of AAs based on very low amounts of pollen (~1 to 5 mg, Vanderplanck et al. 2014; Stabler et al. 2018).

Here, we contribute to filling the gap of knowledge on the effects of soil eutrophication on floral resources by evaluating the impacts of increased availability of nutrients (nitrogen and phosphorous) on the pollen AA profile of a plant species from Cerrado (Brazilian savannas), *Pavonia rosa-campestris*. Cerrado soils are naturally nutrient-poor and acidic, so local flora species have evolved to adapted to these conditions (Haridasan 2008). Since impacts of nutrient input are likely stronger or more rapidly detected in regions with naturally nutrient-poor soils than in regions with fertile soils, especially in habitats dominated by perennial herbs and with acidic soils (Bobbink et al. 2010; Simkin et al. 2016) such as those of savannas, we expect that impacts found in our study are more accentuated than those found in previous studies run in temperate regions.

MATERIALS AND METHODS

This study was run in an experimental area of Cerrado *sensu stricto* (Brazilian savanna) established at the Ecological Reserve of the Brazilian Institute for Geography and Statistics (coordinates decimal degrees: -15.9284, -47.8367). The experimental area consists of 20 parcels (5x5 meters) subjected to a fertilization experiment, involving the manipulation of several

macronutrients, with each treatment replicated four times (full experimental design described in Bustamante et al. 2012). For this specific study, we focused on control parcels (no nutrients added) and on parcels subjected to N addition (100 kg of N per ha per year as $(\text{NH}_4)_2\text{SO}_4$), and NP addition (where in addition to N, further 100 kg of P per ha per year, as $\text{Ca}(\text{H}_2\text{PO}_4)_2 + \text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), totalling 12 parcels. Nutrient supplementation occurred twice a year, between 1998 and 2006, at the beginning and again at the end of the rainy season.

FOCAL PLANT SPECIES

We focused on the perennial species *Pavonia rosa-campestris* (Malvaceae), the only non-Poaceae flowering species that was detected flowering in at least three parcels of each treatment over the sampling period. Species within the genus *Pavonia* have self-pollination through delayed style curvature (Buttrose et al. 1977; McDade & Davidar 1984; Goodwillie & Weber 2018), which means that if there is no deposition of pollen on the stigma until a certain point in time, and if weather conditions are favourable (Domingos-Melo et al. 2020), the styles curve towards the anthers, coming in contact with the pollen and promoting self-pollination. While Malvaceae tend to have lower amino acid content in their pollen in comparison to species from other families (Jeannerod et al. 2022), *Pavonia* spp. are known to contribute to the pollen diet of several bee species (e.g. Ribeiro et al. 2016), some Brazilian species even being specialized on this genus (e.g. *Ptilothrix plumate*, Hymenoptera: Apidae: Emphorini, Schlindwein & Martins 2000; Schlindwein et al. 2009).

LEAF AND POLLEN ANALYSES

While our study focused on floral resources, as a preliminary evaluation we checked if the foliar chemical composition was still affected by nutrient composition nine years after fertilization treatments had ceased. To evaluate foliar carbon and nitrogen (CN) content, within each treatment, six samples of mature leaves were collected in August 2015, dried at 60°C for 72h, and ground. Samples used to run the chemical analyses had ca. 0.1g (0.098-0.101g). Carbon and nitrogen analyses were then done using a CN elemental analyser (Truspec CHN628, Leco Instruments Ltda, St Joseph, Michigan, USA 2013) through combustion (950°C), which detects CO_2 and N_2 through infrared and thermal conductivity.

Pollen samples were collected in August 2017 from all available fully developed flowers with no signs of fertilization (no signs of petal senescence) found during field surveys. A total of 77 flowers were collected. Stamens from each flower were removed and placed in a sterilized Eppendorf tube. Samples were later dried at room temperature, and pollen grains were extracted from anthers with help of a vortex. We repeated the process using a combination of vibration within sterilized water (vortex) and centrifugation (12 mil rpm, for ca. 3 min). After the removal of all non-pollen fragments, samples were dried at 40°C. As for most flowers pollen extracted was very low (frequently below 1mg), up to 10 flowers were combined within a parcel in a single sample. This led to a total of 31 samples: 18 were from control plots, 10 from plots that received N addition, and three from plots that received NP addition. Amino acid analyses were performed using HPLC (High-performance liquid chromatography) after hydrolysis using the Stein & Moore method, applying the protocol described by Vanderplanck and collaborators (2014). Previous studies (Gardener & Gillman 2001a) have shown that HPLC can produce accurate and precise data for the AA composition of floral resources.

DATA ANALYSES

To evaluate the effect of fertilization treatments on N and C leaf concentration of *Pavonia rosa*, we used a Linear Mixed Model (LMM) with treatment as a fixed variable and parcel ID as a random variable. All analyses were run using R version 4.2.0 (R core team, 2022).

For each pollen sample, we calculated the total amount of each AA (mg/g of pollen, a standardized unit used for comparison across plant species), and the relative abundance of each AA (% of total amino acids). As the amount of pollen greatly varied among samples, we first used samples from control plots (no nutrient addition) to analyse the effect of sample weight on the reliability of AA quantification and define a threshold above which sample weight no longer had an effect. To do that, we used Generalized Linear Mixed Models (GLMM) (Gamma distribution, log link function) using sample weight as a fixed variable and plot ID as a random variable. For each AA, z-score values (a standardized metric of variability, centred at zero)

were calculated and its absolute value (i.e. distance from the mean) was used as a response variable. We then repeated the procedure using samples with pollen weights above different values (0.5, 0.75, 1, 1.25 mg).

To evaluate the effects of fertilizer treatment on the variability of the AA pollen profile of the studied species, we used all samples with pollen weights greater than the threshold value identified (see results) and calculated the dissimilarity (Bray-Curtis index) on the pollen profile between each pair of pollen samples using the vegan R package (Oksanen et al. 2022). We then ran a GLMM (Gamma distribution, log link function). As each sample was compared with all others, the IDs of both samples within the pair were included as random variables.

To evaluate the effects of fertilizer treatment on the absolute concentration (mg/g of pollen) and relative abundance (% of total AA amount) of each AA, we used all samples with pollen weights greater than the threshold value. As the number of samples in fertilized plots above the threshold was reduced (only 3 samples for NP), we merged N and NP treatments and used as explanatory variable (GLMM, Gaussian distribution, with plot ID as a random effect) the presence (or absence) of nutrient addition. As a sensitivity test, we repeated the analyses after removing the three pollen samples from the NP treatment.

RESULTS

Our results show that after ca. 10 years of the last fertilization event, foliar and pollen chemical

compositions are still affected by fertilization treatments. More specifically, foliar analyses showed that while effects on foliar N content in treatments were not significant, foliar C content was significantly affected (ca. 1.6% higher) when nutrients (N or NP) were added, leading to a mild but non-significant increase on the C:N ratio in leaves (Fig. 1, Appendix 1). Results were similar when NP treatment was removed from data analyses.

Regarding pollen, effects were detected on AA absolute content, as well as on AA profile composition and variability. A total of 17 AAs were detected in the pollen of *Pavonia rosacampestris*. The most abundant AAs were glutamic acid (reaching 40 mg/g), aspartic acid (up to 30 mg/g), and lysine (up to 20 mg/g). Stabilization of AA relative abundances (% of total AAs) in control plots only occurred for samples with more than 1mg of pollen (Appendices 1 and 2), so this was defined as the threshold value for all subsequent pollen analyses. Based only on the samples with more than 1mg (eight samples from three control plots and nine samples from five plots with nutrient addition), we found that nutrient addition greatly reduced variability in pollen AA profile between plant individuals (Fig. 2). Moreover, while absolute content increased for all AAs in response to fertilization, not all AAs increased in same proportion leading to changes in AA relative abundance (Fig. 3). Some AAs had higher average relative abundance under fertilization, namely leucine, isoleucine, serine, threonine, alanine, and lysine, while other AAs had reduced average relative abundance under fertilization. Among

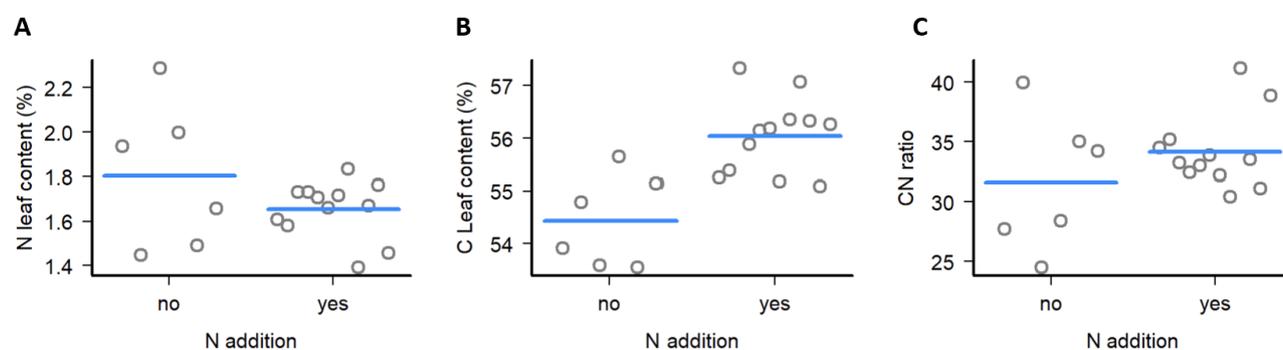


Figure 1. Effect of fertilization treatments on N leaf content (A), C leaf content (B), and leaf C:N ratio of *Pavonia rosa* in 2015 (ten years after fertilization ceased). Data were analysed using Linear Mixed Model with parcel ID as a random variable, and dots represent the partial residuals (i.e. after removing the effect of parcel ID). Blue lines represent estimated means and circles represent residuals. Significant effects were detected for C content, but not for N content and C:N ratio (for statistical details see Appendix 1).

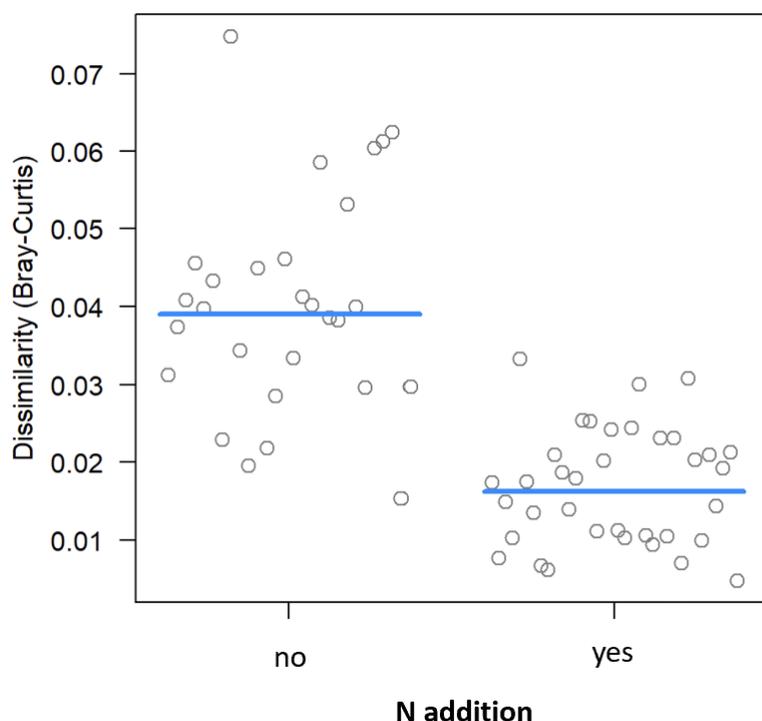


Figure 2. Effect of fertilization treatments on the dissimilarity (Bray-Curtis index calculated between individuals) of the amino acid composition of *Pavonia rosa* pollen in 2015 (ten years after fertilization ceased). Data were analysed using Linear Mixed Model with parcel ID as a random variable, and dots represent the partial residuals (i.e. after removing the effect of parcel ID). Blue lines represent estimated means and circles represent residuals. For statistical details see Appendix 1.

those that had their relative abundance reduced were two sulfurated amino acids (cysteine, methionine), as well as valine and proline. After the sensitivity test removing the three samples from NP treatment from the analyses all trends were maintained (Appendix 1).

DISCUSSION

Despite the importance of plant chemistry for the regulation of plant-animal interactions, there is a scarcity of studies evaluating the impacts of the ongoing increase of nutrient availability on floral resources. Our results show that human-driven input of nutrients can substantially affect plant chemistry, especially floral resources. For leaves we only detected significant changes in C content and not on N content, and the fact that such changes did not reflect in changes in C:N ratio was unexpected. Future studies providing information on similar compounds on both leaves and pollen would be important to better understand plant physiological dynamics, and check if leave chemistry data (which is much easier to obtain) could be used as a proxy of pollen chemistry. Nevertheless, such results agree with previous studies on foliar chemistry run in the same study site in 2004-2005 (eight years after the start of fertilization, which ceased in 2006) that show that

N, P, S (mg g^{-1}), total phenol and tannin foliar concentrations (% dry leaf) of several plant species differed between fertilization treatments (Jacobson & Bustamante 2019). Yet, such data were collected while fertilization treatments were still being applied annually, and here we show that effects are still detectable more than 10 years after nutrient input flux has ceased. Previous studies run in the same experimental site after fertilization treatments ceased show that NP input reduced plant richness, this being partly driven by invasion of the exotic grass *Melita minutiflora* (de Mello et al. 2014, Silveira et al. 2021), and that soil chemistry, organic matter dynamics and soil microbial community differed between treatments (Silveira et al. 2021; Pompermaier et al. 2022). These differences in soil may partly explain the long-term effects on the vegetative and reproductive parts of plants, since soil microbiota release several enzymes involved in the cycle of several nutrients such as C and N, P, S (Falkowski et al. 2008; Kaiser et al. 2016). Yet, while N deposition is known to be a dominant factor driving leaf C:N ratio change (Sheng et al. 2021), and C leaf content and AA pollen content of our study species were altered, N leaf content remained similar, and changes in the C:N leaf ratio was not statistically different (note that for other plant species from the same experimental plot, significant C:N leaf ratio were

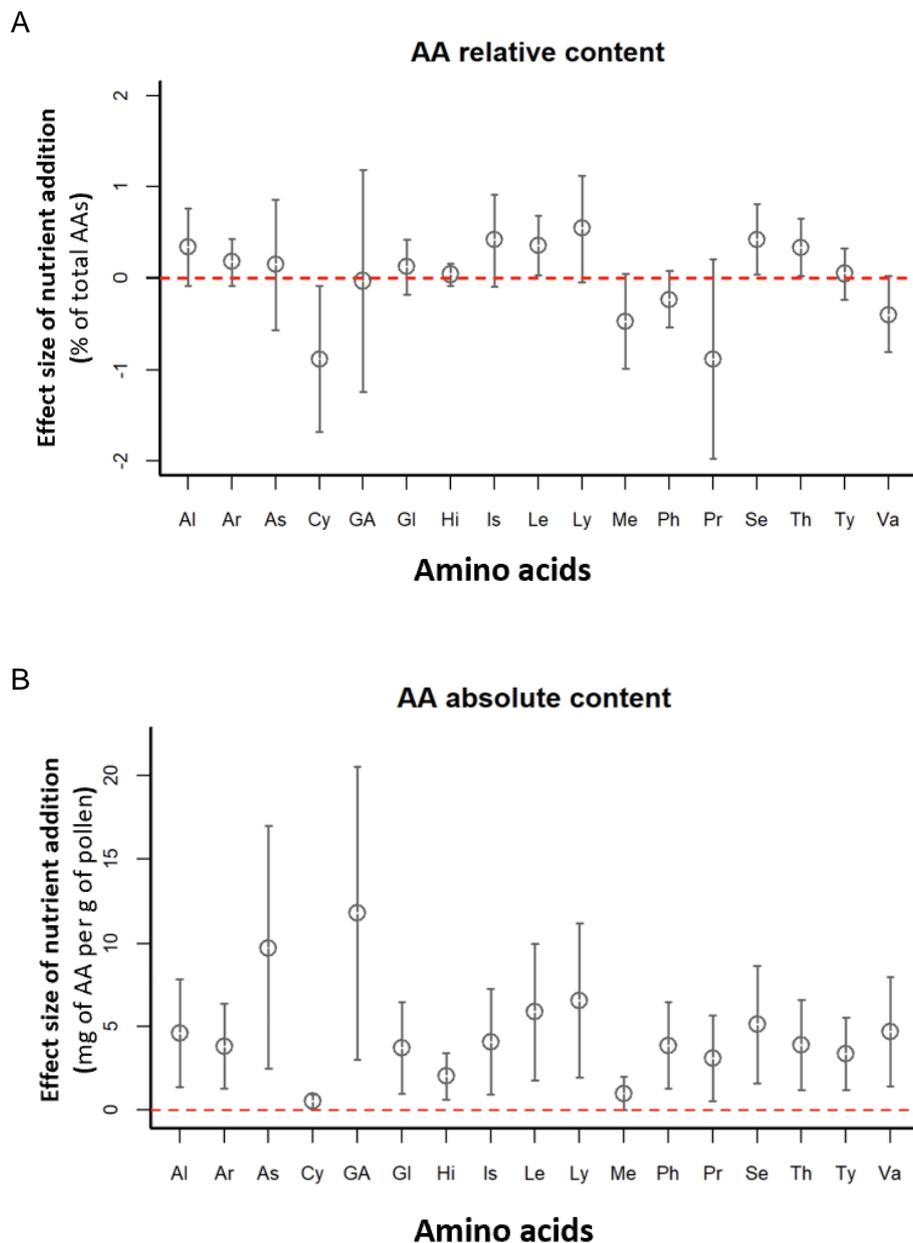


Figure 3. Effect of increased nutrient availability (either nitrogen or nitrogen and phosphorus combined) on pollen (A) amino acid relative content (% of total AAs) and (B) absolute content (mg of AA per g of pollen) of *Pavonia rosacampestris*. For each AA, data were analysed using a linear mixed model (nutrient addition as a fixed term and plot ID as a random effect). Error bars represent 95% CI. For detailed statistical results see Appendix 1.

detected, unpublished data). Future studies involving the analysis of other nitrogenated components of leaves and pollen (e.g. AAs and alkaloids) and floral resource components with a high carbon (e.g. nectar carbohydrates, such as sugars) would help to better understand the full range of impacts on plant chemistry. Below we discuss in more detail the changes in pollen chemical composition and compare the patterns detected in our study in Brazilian Cerrado with those detected by the handful of studies on European plants.

EFFECTS OF NUTRIENT ENRICHMENT ON THE AMINO ACID PROFILE OF FLORAL RESOURCES

Here, we detected that nitrogen addition affects the content and relative abundance of several amino acids that are important for bees. At least ten of those are essential for insects (EAAs) as they cannot be synthesized *de novo*, namely arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (de Groot 1952; Brodbeck & Strong 1987). In addition, some non-essential AAs are also important as a source of energy for flight metabolism (e.g. proline, Teulier et al. 2016), larval

development (de Groot 1952; Hrasnigg et al. 2003; Chang 2004) and also being key determinants of longevity (e.g., due to their role in immune function, Glavinic et al. 2017; Bonoan et al. 2020, and pathogen resistance, Huang 2012). Since nitrogen is an essential nutrient for the synthesis of AAs, an overall positive effect of N addition on the concentration of AAs detected in floral resources (Fig. S3) was expected. Among the AAs that had their content increased following nutrient addition, glutamic and aspartic acids are involved in the metabolism of glutamate and aspartate, respectively. Since honey bee contact sensory hairs are known to respond strongly to these two AAs (Lim et al. 2019), effects on bee visitation patterns are possible. Despite the importance of all AAs for adequate physiological functioning in insects, and while plant tissues concentrations of essential AAs are often below the intermediate "adequate" concentrations (Brodbeck & Strong 1987), it is possible that above a certain threshold, AA intake can lead to toxicity, due to accumulation of ammonia when specific metabolic pathways are overloaded (Brodbeck & Strong 1987). Indeed, an increased level of essential amino acids (EEA) in the insect diet can reduce bee lifespan, the strength of such effects depending on EEA:C ratio (e.g., Paoli et al. 2014). Moreover, several AAs are precursors of alkaloids that can also have toxic effects. Detailed studies that combine information on AA and alkaloid levels in floral resources would be needed to better understand the interlinked effects of these two important groups of compounds as well as their impact on plant-pollinator interactions, and pollinator health.

The results here presented show that certain AAs are more likely to be positively affected than others by increased nutrient availability, affecting the relative abundance of the various AAs. As different AAs have different effects on chemoreceptors of insects, some stimulating salt or sugar cells, others inhibiting them (Shiraishi & Kuwabara 1970; Nepi 2014; Ruedenauer et al. 2019; Lim et al. 2019; Carlesso et al. 2021), such changes in relative abundance can affect how floral visitors perceive and interact with plant species, especially the selective foraging behaviour of generalist bees (Hanley et al. 2008; Ruedenauer et al. 2015). Indeed, bees can perceive differences in AAs in pollen (Ruedenauer et al. 2019) and among the AAs that are known to stimulate sugar taste

receptors in insects (Shiraishi & Kuwabara 1970), two had their relative abundances increased by nitrogen (leucine, isoleucine), and two had their relative abundance reduced (methionine, valine). Moreover, one AA which is known to affect the salt cell of some insects (e.g. Shiraishi & Kuwabara 1970), and consequently the way bees perceive floral resources (Finkelstein et al 2022), also had its abundance reduced (proline). While tests of AAs on insect chemoreceptors were only run for some species of insects (Shiraishi & Kuwabara 1970; Nepi 2014; Ruedenauer et al. 2019; Carlesso et al. 2021) and there may be interspecific variability, such changes can help understand (i) why changes in AA profile have been shown to affect flower visitation patterns of bees (Alm et al. 1990; Petanidou et al. 2006), butterflies (Alm et al. 1990), and flies (Woodcock et al. 2014), and (ii) why some AAs act as attractants for some species and repellent for others (Inouye & Waller 1984; Petanidou et al. 2006). Further studies are still needed to understand whether such changes in AA profile are part of the mechanisms behind the changes in flower visitation rates detected for several species of pollinators in flowers exposed to an environment enriched with nutrients (e.g., Hoover et al. 2012; Marini et al. 2015; Ramos et al. 2018).

COMPARISON WITH PREVIOUS STUDIES

Despite our low number of pollen samples, we detected significant changes in the absolute content of most AA analysed. Other studies done in regions of Europe where soils are naturally richer than those of our study region in Cerrado (no or slight constraints vs severe to very severe constraints on nutrient availability, FAO/IIASA/ISRIC/ISS-CAS/JRC, 2012) detected significant changes in a much lower number of AAs (4 out of 23 AAs for Gardener & Gillman 2001b, *Agrostemma githago*, Caryophyllaceae, study run in the south of England). Also in the relative content of AAs, we found effects for most AAs (10 out of 16), while others found effects in a lower number of AAs (4 in nectar and 5 in pollen out of 21 in Ceulemans et al. 2017 led on *Succisa pratensis*, Dipsacaceae, study run in mesocosms in Belgium; 8 out of 23 AAs in Gardener & Gillman 2001b led on *Agrostemma githago*, Caryophyllaceae, study run in south of England; 8 out of 18 in Gijbels et al. 2015 led on *Gymnadenia conopsea*, Orchidaceae,

study run in Belgium). This gives strength to our expectation that impacts of increased N availability may be more accentuated for native plants of Cerrado than for plants that evolved in regions where soils are naturally richer. Yet, studies with more plants or comparing phylogenetically closer plant species (e.g. same plant family or genus) would be needed to better evaluate if such differences are consistent. The type of fertilizer used, and the presence or absence of other plants in the experiment may also have an influence. Mineral or organic forms of nutrients can be applied directly to the soil to evaluate changes, either in a plant community under field conditions, with the focal species competing with other naturally occurring species (e.g. our study and (Gijbels et al. 2015)), or in (isolated) plants in a mesocosm under experimental conditions, ruling out effects of competition with other plants (e.g. Gardener & Gillman 2001b; Ceulemans et al. 2017). While the latter allows for higher control of the mechanisms involved, the former better represents the reality of ecosystems, with results being influenced by the complex network of interactions between plants and soil microorganisms. Moreover, while some fertilizers are based on sulphur (such as those used for the experiment in Cerrado), others are not. Since sulphur can affect nitrogen content in plants and is an essential element for some AAs, such differences in fertilizer formula may influence the effects on plant AA profile. Indeed, sulphur can also affect nitrogen content in plants as well as enhance the effects of nitrogen on AA composition (Lošák et al. 2011) and other nitrogen-based compounds, which could potentially be related with the changes in two sulphurated AAs, cysteine and methionine, detected in our study. All these methodological differences may influence the results and need to be considered when analysing and comparing studies. Nevertheless, independently of the method used, changes in soil nutrients need to consider the natural level of nutrients in the soil used in the experiment and despite the great diversity of AA profile across plants (Gardener & Gillman 2002), the results here presented indicate that floral chemistry of certain plants of Cerrado may be more susceptible to changes in nutrient availability than other plants previously studied in temperate regions.

CONCLUDING REMARKS

Understanding how ongoing global changes are affecting plant chemistry can contribute to plan management actions aiming to reduce impacts of such changes and predict associated impacts. Our study makes an important contribution to this field of research, being one of the few studies evaluating impacts on pollen quality and the first, to our knowledge, focusing on plants from tropical regions with highly oligotrophic soils. Moreover, it shows that the impacts of soil eutrophication can be long-lasting, with potential long-term effects on the interactions between plants and their consumers. While difficulty in extracting large samples of pollen is one of the constraints for expanding this field of research, our findings reinforce the evidence that AA chemical results are reliable with samples with as little as 1 mg of pollen (Appendices 2 and 3). It would also be important to better understand if the AA composition of vegetative tissues (which are easier to sample and analyse) can be used as a proxy of the AA composition of floral resources, as results from previous studies (Lohaus & Schwerdtfeger 2014) suggest. Overall, we call for more investment in studies about the impact of global changes on the chemistry of floral resources. More specifically, further studies on a diverse set of plant species, covering different phylogenetic lineages and ecological strategies for nutrient acquisition are needed to adequately predict the effects of environmental eutrophication on plant-pollinator interactions, and ideally, these evaluations should be accompanied by evaluations of flower visitation patterns.

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AUTHOR CONTRIBUTION

Concept and design LGC and MMCB, data collection LGC and MV, writing of first version of manuscript

LGC, edits and approval for publication LGC, MV, MMCB.

DISCLOSURE STATEMENT

The authors declare to have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data used in this paper is available here: <https://github.com/lgcarvalho/Pavonia2024/>

APPENDICES

Additional supporting information may be found in the online version of this article:

Appendix 1. Statistical details for analysis of the chemical composition of leaves and pollen.

Appendix 2. Effect of pollen weight on the variability of amino acid relative content in samples not exposed to fertilization experiments.

Appendix 3. Effect of pollen weight on the variability of results obtained for pollen amino acids relative content.

REFERENCES

- Adler LS, Irwin RE (2005) Ecological costs and benefits of defenses in nectar. *Ecology* 86:2968–2978. <https://doi.org/10.1890/05-0118>
- Alm J, Ohnmeiss TE, Lanza J, Vriesenga L (1990) Preference of cabbage white butterflies and honey bees for nectar that contains amino acids. *Oecologia* 84:53–57. <https://doi.org/10.1007/BF00665594>
- AOAC - Association of Official Analytical Chemists (1980) *Official Methods of Analysis*, AOAC, Washington, DC, USA.
- Barbosa ERM, van Langevelde F, Tomlinson KW, Carvalho LG, Kirkman K, de Bie S, Prins HHT (2014) Tree species from different functional groups respond differently to environmental changes during establishment. *Oecologia* 174:1345–1357. <https://doi.org/10.1007/s00442-013-2853-y>
- Barbosa ERM, Tomlinson KW, Carvalho LG, Kirkman K, de Bie S, Prins HHT, van Langevelde F (2014) Short-Term Effect of Nutrient Availability and Rainfall Distribution on Biomass Production and Leaf Nutrient Content of Savanna Tree Species Bond-Lamberty B (ed). *PLoS ONE* 9:e92619. <https://doi.org/10.1371/journal.pone.0092619>
- Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore M, Bustamante M, Cinderby S, Davidson E, Dentener F, Emmett B, Erisman J-W, Fenn M, Gilliam F, Nordin A, Pardo L, De Vries W (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications* 20:30–59. <https://doi.org/10.1890/08-1140.1>
- Bonoan RE, Gonzalez J, Starks PT (2020) The perils of forcing a generalist to be a specialist: lack of dietary essential amino acids impacts honey bee pollen foraging and colony growth. *Journal of Apicultural Research* 59:95–103. <https://doi.org/10.1080/00218839.2019.1656702>
- Bouwman AF, Beusen AHW, Lassaletta L, van Apeldoorn DF, van Grinsven HJM, Zhang J, Ittersum van MK (2017) Lessons from temporal and spatial patterns in global use of N and P fertilizer on cropland. *Scientific Reports* 7:40366. <https://doi.org/10.1038/srep40366>
- Brodbeck BV, Strong DR (1987) Amino acid nutrition of herbivorous insects and stress to host plants. In: Barbosa P, Schultz JC (eds) *Insect Outbreaks*. Academic Press, New York, pp 347–364.
- Buttrose M, Grant W, Lott J (1977) Reversible curvature of style branches of *Hibiscus trionum* L., a pollination mechanism. *Australian Journal of Botany* 25:567. <https://doi.org/10.1071/BT9770567>
- Carlesso D, Smargiassi S, Pasquini E, Bertelli G, Baracchi D (2021) Nectar non-protein amino acids (NPAAs) do not change nectar palatability but enhance learning and memory in honey bees. *Scientific Reports* 11:11721. <https://doi.org/10.1038/s41598-021-90895-z>
- Ceulemans T, Hulsmans E, Vanden Ende W, Honnay O (2017) Nutrient enrichment is associated with altered nectar and pollen chemical composition in *Succisa pratensis* Moench and increased larval mortality of its pollinator *Bombus terrestris* L. Nascimento FS (ed). *PLOS ONE* 12:e0175160. <https://doi.org/10.1371/journal.pone.0175160>
- Chang CL (2004) Effect of Amino Acids on Larvae and Adults of *Ceratitis capitata* (Diptera: Tephritidae). *Annals of the Entomological Society of America* 97:529–535. [https://doi.org/10.1603/0013-8746\(2004\)097\[0529:EOAAOL\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2004)097[0529:EOAAOL]2.0.CO;2)
- Cook SM, Awmack CS, Murray DA, Williams IH (2003) Are honey bees' foraging preferences affected by pollen amino acid composition? *Ecological Entomology* 28: 622–627. <https://doi.org/10.1046/j.1365-2311.2003.00548.x>
- Cook D, Manson JS, Gardner DR, Welch KD, Irwin RE (2013) Norditerpene alkaloid concentrations in tissues and floral rewards of larkspurs and impacts on pollinators. *Biochemical Systematics and Ecology* 48:123–131. <https://doi.org/10.1016/j.bse.2012.11.015>
- David TI, Storkey J, Stevens CJ (2019) Understanding how changing soil nitrogen affects plant–pollinator interactions. *Arthropod-Plant Interactions* 13:671–684. <https://doi.org/10.1007/s11829-019-09714-y>
- de Mello TRB, Munhoz CBR, Bustamante MMC (2014) Effects of nutrient additions on the diversity of the herbaceous-subshrub layer of a Brazilian savanna (Cerrado). In: Sutton MA, Mason KE, Sheppard LJ,

- Sverdrup H, Haeuber R, Hicks WK (eds) Nitrogen deposition, critical loads and biodiversity. Springer, Dordrecht, pp 147–154. https://doi.org/10.1007/978-94-007-7939-6_16
- Domingos-Melo A, Bezerra SM da S, Nadia T de L, Machado IC (2020) The dark side of the rain: self-pollination setbacks due to water exposure in *Pavonia varians* Moric (Malvaceae), a species with rain-dependent flowering. *Acta Botanica Brasiliica* 34:437–441. <https://doi.org/10.1590/0102-33062020abb0023>
- Falkowski PG, Fenchel T, Delong EF (2008) The microbial engines that drive Earth's biogeochemical cycles. *Science* 320:1034–1039. <https://doi.org/10.1126/science.1153213>
- FAO/IIASA/ISRIC/ISS-CAS/JRC, 2012. Harmonized World Soil Database (version 1.2). FAO, Rome, Italy and IIASA, Laxenburg, Austria
- Finkelstein CJ, CaraDonna PJ, Gruver A, Welti EAR, Kaspari M, Sanders NJ (2022) Sodium-enriched floral nectar increases pollinator visitation rate and diversity. *Biology Letters* 18:20220016. <https://doi.org/10.1098/rsbl.2022.0016>
- Gardener MC, Gillman MP (2001a) Analyzing Variability in Nectar Amino Acids: Composition Is Less Variable Than Concentration. *Journal of Chemical Ecology* 27:2545–2558. <https://doi.org/10.1023/A:1013687701120>
- Gardener MC, Gillman MP (2001b) The effects of soil fertilizer on amino acids in the floral nectar of corncockle, *Agrostemma githago* (Caryophyllaceae). *Oikos* 92:101–106. <https://doi.org/10.1034/j.1600-0706.2001.920112.x>
- Gardener MC, Gillman MP (2002) The taste of nectar - a neglected area of pollination ecology. *Oikos* 98:552–557. <https://doi.org/10.1034/j.1600-0706.2002.980322.x>
- Gijbels P, Ceulemans T, Van den Ende W, Honnay O (2015) Experimental fertilization increases amino acid content in floral nectar, fruit set and degree of selfing in the orchid *Gymnadenia conopsea*. *Oecologia* 179:785–795. <https://doi.org/10.1007/s00442-015-3381-8>
- Gijbels P, Van den Ende W, Honnay O (2014) Landscape scale variation in nectar amino acid and sugar composition in a Lepidoptera pollinated orchid species and its relation with fruit set. *Journal of Ecology* 102:136–144. <https://doi.org/10.1111/1365-2745.12183>
- Glavinic U, Stankovic B, Draskovic V, Stevanovic J, Petrovic T, Lakic N, Stanimirovic Z (2017) Dietary amino acid and vitamin complex protects honey bee from immunosuppression caused by *Nosema ceranae* Jadhao SB (ed). *PLOS ONE* 12:e0187726. <https://doi.org/10.1371/journal.pone.0187726>
- Goodwillie C, Weber JJ (2018) The best of both worlds? A review of delayed selfing in flowering plants. *American Journal of Botany* 105:641–655. <https://doi.org/10.1002/ajb2.1045>
- Gosselin M, Michez D, Vanderplanck M, Roelants D, Glauser G, Rasmont P (2013) Does *Aconitum septentrionale* chemically protect floral rewards to the advantage of specialist bumblebees?: Impact of toxic rewards on visitors. *Ecological Entomology* 38:400–407. <https://doi.org/10.1111/een.12032>
- de Groot AP (1952) Amino acid requirements for growth of the honeybee (*Apis mellifica* L.). *Experientia* 8:192–194. <https://doi.org/10.1007/BF02173740>
- Haddad NM, Haarstad J, Tilman D (2000) The effects of long-term nitrogen loading on grassland insect communities. *Oecologia* 124:73–84. <https://doi.org/10.1007/s004420050026>
- Hanley ME, Franco M, Pichon S, Darvill B, Goulson D (2008) Breeding system, pollinator choice and variation in pollen quality in British herbaceous plants. *Functional Ecology* 22:592–598. <https://doi.org/10.1111/.1365-2435.2008.01415.x>
- Haridasan, M (2008) Nutritional adaptations of native plants of the cerrado biome in acid soils. *Brazilian Journal of Plant Physiology* 20:183–195. <https://doi.org/10.1590/S1677-04202008000300003>
- Heil M (2011) Nectar: generation, regulation and ecological functions. *Trends in Plant Science* 16:191–200. <https://doi.org/10.1016/j.tplants.2011.01.003>
- Hoover SER, Ladley JJ, Shchepetkina AA, Tisch M, Gieseg SP, Tylianakis JM (2012) Warming, CO₂, and nitrogen deposition interactively affect a plant-pollinator mutualism: Disruption of plant-pollinator mutualism. *Ecology Letters* 15:227–234. <https://doi.org/10.1111/j.1461-0248.2011.01729.x>
- Hrassnigg N, Leonhard B, Crailsheim K (2003) Free amino acids in the haemolymph of honey bee queens (*Apis mellifera* L.). *Amino Acids* 24:205–212. <https://doi.org/10.1007/s00726-002-0311-y>
- Huang Z (2012) Pollen nutrition affects honey bee stress resistance. *Terrestrial Arthropod Reviews* 5:175–189. <https://doi.org/10.1163/187498312X639568>
- Human H, Nicolson SW, Strauss K, Pirk CWW, Dietemann V (2007) Influence of pollen quality on ovarian development in honeybee workers (*Apis mellifera scutellata*). *Journal of Insect Physiology* 53: 649–655. <https://doi.org/10.1016/j.jinsphys.2007.04.002>
- Inouye DW, Waller GD (1984) Responses of Honey Bees (*Apis Mellifera*) to Amino Acid Solutions Mimicking Floral Nectars. *Ecology* 65:618–625. <https://doi.org/10.2307/1941424>
- Isbell F, Reich PB, Tilman D, Hobbie SE, Polasky S, Binder S (2013) Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. *Proceedings of the National Academy of Sciences*

- 110:11911–11916. <https://doi.org/10.1073/pnas.1310880110>
- 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:e0269992. <https://doi.org/10.1371/journal.pone.0269992>
- Kaiser K, Wemheuer B, Korolkow V, Wemheuer F, Nacke H, Schöning I, Schrupf M, Daniel R (2016) Driving forces of soil bacterial community structure, diversity, and function in temperate grasslands and forests. *Scientific Reports* 6:33696. <https://doi.org/10.1038/srep33696>
- Linander N, Hempel de Ibarra N, Laska M (2012) Olfactory detectability of L-amino acids in the european honeybee (*Apis mellifera*). *Chemical Senses* 37: 631–638. <https://doi.org/10.1093/chemse/bjs044>
- Lim S, Jung J, Yunusbaev U, Ilyasov R, Kwon HW (2019) Characterization and its implication of a novel taste receptor detecting nutrients in the honey bee, *Apis mellifera*. *Scientific Reports* 9:11620. <https://doi.org/10.1038/s41598-019-46738-z>
- Lohaus G, Schwerdtfeger M (2014) Comparison of Sugars, Iridoid Glycosides and Amino Acids in Nectar and Phloem Sap of *Maurandya barclayana*, *Lophospermum erubescens*, and *Brassica napus* Kusano M (ed). *PLoS ONE* 9:e87689. <https://doi.org/10.1371/journal.pone.0087689>
- Lošák T, Hlusek J, Martinec J, Vollmann J, Peterka J, Filipcik R, Varga L, Ducsay L, Martensson A (2011) Effect of combined nitrogen and sulphur fertilization on yield and qualitative parameters of *Camelina sativa* [L.] Crtz. (false flax). *Acta Agriculturae Scandinavica, Section B – Soil & Plant Science* 61:313–321. <https://doi.org/10.1080/09064710.2010.490234>
- Majetic CJ, Fetters AM, Beck OM, Stachnik EF, Beam KM (2017) *Petunia* floral trait plasticity in response to soil nitrogen content and subsequent impacts on insect visitation. *Flora* 232:183–193. <https://doi.org/10.1016/j.flora.2016.08.002>
- Manson JS, Rasmann S, Halitschke R, Thomson JD, Agrawal AA (2012) Cardenolides in nectar may be more than a consequence of allocation to other plant parts: a phylogenetic study of *Asclepias* Johnson M (ed). *Functional Ecology* 26:1100–1110. <https://doi.org/10.1111/j.1365-2435.2012.02039.x>
- Marini L, Tamburini G, Petrucco-Toffolo E, Lindström SAM, Zanetti F, Mosca G, Bommarco R (2015) Crop management modifies the benefits of insect pollination in oilseed rape. *Agriculture, Ecosystems & Environment* 207:61–66. <https://doi.org/10.1016/j.agee.2015.03.027>
- McDade LA, Davidar P (1984) Determinants of fruit and seed set in *Pavonia dasypetala* (Malvaceae). *Oecologia* 64:61–67. <https://doi.org/10.1007/BF00377544>
- Midolo G, Alkemade R, Schipper AM, Benítez-López A, Perring MP, De Vries W (2019) Impacts of nitrogen addition on plant species richness and abundance: A global meta-analysis Xu X (ed). *Global Ecology and Biogeography* 28:398–413. <https://doi.org/10.1111/geb.12856>
- Nepi M (2014) Beyond nectar sweetness: the hidden ecological role of non-protein amino acids in nectar. *Journal of Ecology* 102:108–115. <https://doi.org/10.1111/1365-2745.12170>
- Nicholls E, de Ibarra NH (2017). Assessment of pollen rewards by foraging bees. *Functional Ecology* 31:76–87. <https://doi.org/10.1111/1365-2435.12778>
- Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szocs 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, McGlenn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J (2022). *vegan: Community Ecology Package*. R package version 2.6-2, <https://CRAN.R-project.org/package=vegan>.
- Ollerton J, Winfree R, Tarrant S (2011) How many flowering plants are pollinated by animals? *Oikos* 120:321–326. <https://doi.org/10.1111/j.1600-0706.2010.18644.x>
- Paoli PP, Wakeling LA, Wright GA, Ford D (2014) The dietary proportion of essential amino acids and Sir2 influence lifespan in the honeybee. *AGE* 36:9649. <https://doi.org/10.1007/s11357-014-9649-9>
- Petanidou T, Van Laere A, N. Ellis W, Smets E (2006) What shapes amino acid and sugar composition in Mediterranean floral nectars? *Oikos* 115:155–169. <https://doi.org/10.1111/j.2006.0030-1299.14487.x>
- Pompermaier VT, Campani AR, Dourado E, Coletta LD, Bustamante MM da C, Nardoto GB (2022) Soil mesofauna drives litter decomposition under combined nitrogen and phosphorus additions in a Brazilian woodland savanna. *Austral Ecology* 47:26–34. <https://doi.org/10.1111/aec.13082>
- Pöyry J, Carnevalheiro LG, Heikkinen RK, Kühn I, Kuussaari M, Schweiger O, Valtonen A, van Bodegom PM, Franzén M (2017) The effects of soil eutrophication propagate to higher trophic levels: Effects of soil eutrophication on herbivores. *Global Ecology and Biogeography* 26:18–30. <https://doi.org/10.1111/geb.12521>
- R Core Team (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>

- Rader R, Bartomeus I, Garibaldi LA, Garratt MPD, Howlett BG, Winfree R, Cunningham SA, Mayfield MM, Arthur AD, Andersson GKS, Bommarco R, Brittain C, Carvalho LG, Chacoff NP, Entling MH, Foully B, Freitas BM, Gemmill-Herren B, Ghazoul J, Griffin SR, Gross CL, Herbertsson L, Herzog F, Hipólito J, Jaggar S, Jauker F, Klein A-M, Kleijn D, Krishnan S, Lemos CQ, Lindström SAM, Mandelik Y, Monteiro VM, Nelson W, Nilsson L, Pattemore DE, de O. Pereira N, Pisanty G, Potts SG, Reemer M, Rundlöf M, Sheffield CS, Scheper J, Schüepp C, Smith HG, Stanley DA, Stout JC, Szentgyörgyi H, Taki H, Vergara CH, Viana BF, Woyciechowski M (2016) Non-bee insects are important contributors to global crop pollination. *Proceedings of the National Academy of Sciences* 113:146–151. <https://doi.org/10.1073/pnas.1517092112>
- Ramos D de L, Bustamante MMC, Silva FD da S e, Carvalho LG (2018) Crop fertilization affects pollination service provision – Common bean as a case study Saunders ME (ed). *PLOS ONE* 13:e0204460. <https://doi.org/10.1371/journal.pone.0204460>
- Ribeiro MHM, de Albuquerque PMC, da Luz CFP (2016) Pollen profile of Geopropolis samples collected of “Tiúba” (*Melipona* (*Melikierria*) *fasciculata* Smith 1854) in areas of Cerrado and flooded fields in the state of Maranhão, Brazil. *Brazilian Journal of Botany* 39:895–912. <https://doi.org/10.1007/s40415-016-0280-0>
- Ruedenauer FA, Leonhardt SD, Lunau K, Spaethe J (2019) Bumblebees are able to perceive amino acids via chemotactile antennal stimulation. *Journal of Comparative Physiology A* 205:321–331. <https://doi.org/10.1007/s00359-019-01321-9>
- Ruedenauer FA, Spaethe J, Leonhardt SD (2015) How to know which food is good for you: bumblebees use taste to discriminate between different concentrations of food differing in nutrient content. *Journal of Experimental Biology* 218:2233–2240. <https://doi.org/10.1242/jeb.118554>
- Schindwein C, Martins CF (2000) Competition between the oligolectic bee *Ptilothrix plumata* (Anthophoridae) and the flower closing beetle *Pristimerus calcaratus* (Curculionidae) for floral resources of *Pavonia cancellata* (Malvaceae). *Plant Systematics and Evolution* 224:183–194. <https://doi.org/10.1007/BF00986342>
- Schindwein C, Pick RA, Martins CF (2009) Evaluation of oligolecty in the Brazilian bee *Ptilothrix plumata* (Hymenoptera, Apidae, Emphorini). *Apidologie* 40:106–116. <https://doi.org/10.1051/apido/2008067>
- Sheng M, Tang J, Yang D, Fisher JB, Wang H, Kattge J (2021) Long-term leaf C:N ratio change under elevated CO₂ and nitrogen deposition in China: Evidence from observations and process-based modeling. *Science of The Total Environment* 800:149591. <https://doi.org/10.1016/j.scitotenv.2021.149591>
- Shiraishi A, Kuwabara M (1970) The Effects of Amino Acids on the Labellar Hair Chemosensory Cells of the Fly. *Journal of General Physiology* 56:768–782. <https://doi.org/10.1085/jgp.56.6.768>
- Silveira R, de Mello T de RB, Silva MRSS, Krüger RH, Bustamante MM da C (2021) Long-term liming promotes drastic changes in the composition of the microbial community in a tropical savanna soil. *Biology and Fertility of Soils* 57:31–46. <https://doi.org/10.1007/s00374-020-01504-6>
- Simkin SM, Allen EB, Bowman WD, Clark CM, Belnap J, Brooks ML, Cade BS, Collins SL, Geiser LH, Gilliam FS, Jovan SE, Pardo LH, Schulz BK, Stevens CJ, Suding KN, Throop HL, Waller DM (2016) Conditional vulnerability of plant diversity to atmospheric nitrogen deposition across the United States. *Proceedings of the National Academy of Sciences* 113:4086–4091. <https://doi.org/10.1073/pnas.1515241113>
- Stabler D, Power EF, Borland AM, Barnes JD, Wright GA (2018) A method for analysing small samples of floral pollen for free and protein-bound amino acids Carvalho L (ed). *Methods in Ecology and Evolution* 9:430–438. <https://doi.org/10.1111/2041-210X.12867>
- Steffen W, Richardson K, Rockström J, Cornell SE, Fetzer I, Bennett EM, Biggs R, Carpenter SR, de Vries W, de Wit CA, Folke C, Gerten D, Heinke J, Mace GM, Persson LM, Ramanathan V, Reyers B, Sörlin S (2015) Planetary boundaries: Guiding human development on a changing planet. *Science* 347:1259855. <https://doi.org/10.1126/science.1259855>
- Su Y, Ma X, Gong Y, Li K, Han W, Liu X (2021) Responses and drivers of leaf nutrients and resorption to nitrogen enrichment across northern China’s grasslands: A meta-analysis. *CATENA* 199:105110. <https://doi.org/10.1016/j.catena.2020.105110>
- Teulier L, Weber J-M, Crevier J, Darveau C-A (2016) Proline as a fuel for insect flight: enhancing carbohydrate oxidation in hymenopterans. *Proceedings of the Royal Society B: Biological Sciences* 283:20160333. <https://doi.org/10.1098/rspb.2016.0333>
- Throop HL, Lerdau MT (2004) Effects of Nitrogen Deposition on Insect Herbivory: Implications for Community and Ecosystem Processes. *Ecosystems* 7 <https://doi.org/10.1007/s10021-003-0225-x>
- Vanderplanck M, Leroy B, Wathélet B, Wattiez R, Michez D (2014) Standardized protocol to evaluate pollen polypeptides as bee food source. *Apidologie* 45:192–204. <https://doi.org/10.1007/s13592-013-0239-0>
- Vaudo AD, Patch HM, Mortensen DA, Tooker JF, Grozinger CM (2016) Macronutrient ratios in pollen shape bumble bee (*Bombus impatiens*) foraging strategies and floral preferences. *Proceedings of the National Academy of Sciences* 113:E4035. <https://doi.org/10.1073/pnas.1606101113>

Vossler FG (2015) Broad Protein Spectrum in Stored Pollen of Three Stingless Bees from the Chaco Dry Forest in South America (Hymenoptera, Apidae, Meliponini) and Its Ecological Implications. *Psyche: A Journal of Entomology* 2015:1–7. <https://doi.org/10.1155/2015/659538>

Woodcock TS, Larson BMH, Kevan PG, Inouye DW, Lunau K (2014) Flies and Flowers II: Floral Attractants and Rewards. *Journal of Pollination Ecology* 12:63–94. [https://doi.org/10.26786/1920-7603\(2014\)5](https://doi.org/10.26786/1920-7603(2014)5)

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