Pollinator assemblage response to nutrient enrichment in grasslands

Tese de Doutoramento

Juan Pablo Cancela Vallejo

Doutoramento em

Biologia

Ponta Delgada
2024

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Tese especialmente elaborada para obtenção do grau de Doutor em Biologia

Title
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Institutions and Financial support

Financed by:
This thesis was funded by Fundação para a Ciência e a Tecnologia (FCT) through the PhD fellowship (SFRH/BD/145175/2019).

Developed at:
Centre for Ecology, Evolution and Environmental Changes (cE3c) & CHANGE – Global Change and Sustainability Institute, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

and

University of the Azores, cE3c- Centre for Ecology, Evolution and Environmental Changes/ Azorean Biodiversity Group, CHANGE – Global Change and Sustainability Institute, School of Agricultural and Environmental Sciences, Rua Capitão João d´Ávila, 9700-042 Angra do Heroísmo, Azores, Portugal.

With the support of:
EUCLIPO project (LISBOA-01-0145-FEDER-028360)
CAPES project (FCT-20191360996P)
cE3c Project UIDB/00329/2020 (DOI 10.54499/UIDB/00329/2020)

How to cite this publication:

Cancela J.P. (2024). Pollinator assemblage response to nutrient enrichment in grasslands. PhD dissertation, University of the Azores, Ponta Delgada, Portugal.

ACKNOWLEDGMENTS

Along this journey I have received the support of numerous friends, colleagues, family and partner.

First of all, I want to thank my supervisors all the support they gave throughout the years. They have been pure inspiration as scientists and I am glad they have been next to me in this journey.

All my friends from my hometown, Colmenar Viejo, showed support and listened to both frustrations and good news. All my love for you guys.

Colleagues and friends from Universidad Autónoma de Madrid and Museo Nacional de Ciencias Naturales, my former institutions before moving to Portugal, thank you for your company and support. Especially to Rob Wilson, my former supervisor and a true friend, thanks for always sharing ideas and advising me through the PhD process. Another special mention to Juan Modolell, who passed away in 2023 and was always open to share his experience as a former scientist and his precious advice for my PhD and life in general.

People from the University of Lisbon, University of the Azores, Federal University of Goiás and SLU, most of you were PhD or Postdoc students and shared the same challenges I had. Thanks for all the advice, PhD related stuff and, more importantly, for the rest of the time shared together.

To my family; they always showed respect and interest for what matters to me and that is all I can ask for.

To my girlfriend Sasha who had to suffer all the good and the bad of someone doing a PhD every day, even during our holidays. Thank you from the bottom of my heart.

ACKNOWLEDGMENTS PER CHAPTER

CHAPTERS 2 and 3

I want to thank Conceição Caldeira and Miguel Bugalho for providing the nutrient experimental site where our research was conducted; Maryse Vanderplank, Idoia Ariz and Gaëtan Glauser for their contribution to the pollen sample analysis and the subsequent discussion of the results. Also, I want to thank Thomas Wood for identifying the bees collected in the study site.

CHAPTER 4

I want to thank all NutNet collaborators involved in this work and especially Harry Olde Venterink, Elizabeth Borer and Eric Seabloom for their help and feedback throughout the process of analysing data and writing the chapter.

ABSTRACT

Insect pollinators are declining worldwide, with agricultural intensification as one of the main drivers. Besides land-use changes (including landscape and ecosystem simplification), and a massive use of pesticides, agricultural intensification is associated with elevated nutrient inputs or nutrient enrichment, which is a major threat to global biodiversity. In the last decades, studies mostly from temperate northern hemisphere regions enabled understanding the effects of nutrient enrichment on plant communities, identifying general trends such as increased productivity, decreased plant richness, and changes in plant chemistry. However, regions such as the Mediterranean ecoregion (e.g., southern Europe), and several subtropical and tropical parts of South America have not received enough attention in terms of nutrient effect on plants. Furthermore, knowledge on nutrient enrichment cascading effect on higher trophic levels, including on pollinators, is lacking worldwide.

This thesis' main objective is to assess the simultaneous effects of multiple nutrient addition treatments on pollinator assemblages and link them to changes in plant community composition, flower abundance and floral rewards (pollen quantity and quality). To achieve this, we used experimental sites from the Nutrient Network (NutNet – a coordinated research network of grassland sites implementing standardized nutrient addition treatments) and combined:

i) a local approach (using an ongoing nutrient manipulation field experiment located in a Mediterranean grassland in Portugal) to assess nutrient enrichment effects on floral rewards, and its effect on pollinators, and on plant-pollinator interacting patterns.

ii) a large scale approach (using data from 14 ongoing nutrient manipulation field experiments) to understand the effects of nutrient enrichment on different flowering plant and pollinator communities spread across three continents (in Europe and in North and South America), and to assess the effect of soil fertility and temperature on nutrient enrichment effect on plants and pollinators.

Using the local approach, we detected contrasting effects on flower abundance of three co-flowering dominant plant species (*Crepis capillaris*, *Raphanus raphanistrum* and

Echium plantagineum), with NP enrichment increasing flower abundance while P enrichment decreased it. Nutrient enrichment effects on pollen quantity and quality in terms of amino acids were plant species-specific, with strong, medium and weak effects on the three species. Phosphorus enrichment had a positive effect on *Raphanus* visitation rate at the flower scale driven by an increase in amino acid concentration, but a negative effect on pollinator visitation rate (considering the whole plant community) at plot level, due to a general reduction in flower abundance. On the other hand, plant-pollinator foraging patterns were affected by NP and P enrichment and the interaction networks: i) NP enrichment was associated with an increase in pollinator diversity and plant generality, resulting in higher network generalization; and ii) P enrichment was associated with a decrease in pollinator diversity, with those visitors showing specialized foraging patterns, increasing network specialization.

Using the large-scale approach, we found that regional temperature and soil fertility interacted with nutrient enrichment, mainly N enrichment (and also P enrichment), modulating the effect on plant and on pollinator diversity. In warmer regions, the effect of N enrichment was negative on plant and pollinator diversity, while it was positive in colder regions. Moreover, plant richness response was stronger on poor soils while flower abundance response was stronger on rich soils. Pollinator response to nutrient enrichment was more accentuated than plant response.

This thesis' results clearly show that nutrient enrichment is an important driver of floral resource availability, with bottom-up effects on pollinators. Data from our local approach show that NP enrichment may improve floral resources (flower abundance and pollen quantity and quality) in a Mediterranean grassland with positive effects on pollinator diversity; while the addition of P alone was related to negative effects on floral abundance, which negatively affected pollinator visitation rate and pollinator diversity. Besides affecting plant-pollinator diversity, we showed that nutrient enrichment affected pollinator foraging patterns, promoting network generalization (NP enrichment) or specialization (P enrichment). Changes in network generalization patterns could affect community tolerance to future perturbations. Data from our large-scale approach show that pollinators are particularly at risk in warmer regions, which are usually biodiversity hotspots and contribute greatly to global food production. Ongoing global warming in addition to the increasing use of these regions for intensive agriculture could compromise

crop pollination and food supply if policies aiming at controlling farming intensity (including nutrient loads) are not implemented.

Overall, this thesis provides crucial information regarding the effects of nutrient enrichment on pollinator assemblages, through the study of the different pathways through which nutrient-driven changes in plants may impact pollinators. In order to better understand the effects of nutrient enrichment on plants, and especially on pollinators, future research should prioritize understudied regions and include a wider range of plant and pollinator species in community-level studies.

Keywords:
Floral resources, grassland, insect pollinator assemblage, nutrient enrichment, plant-pollinator interactions, soil fertility, temperature.

RESUMO

Os insetos polinizadores estão globalmente em declínio, sendo a intensificação da agricultura um dos principais fatores promotores desse declínio. Além das alterações no uso do solo (incluindo a simplificação da paisagem e dos ecossistemas) e do uso massivo de pesticidas, a intensificação da agricultura também está associada a uma elevada adição de nutrientes, ou enriquecimento em nutrientes, a qual constitui uma grande ameaça para a biodiversidade global. Nas últimas décadas, estudos principalmente nas regiões temperadas do hemisfério norte possibilitaram conhecer os efeitos do enriquecimento em nutrientes em comunidades de plantas, identificando várias tendências como o aumento de produtividade, redução da riqueza de plantas e as mudanças na química destas. Não obstante, regiões como a ecorregião Mediterrânica (sul da Europa) e várias regiões tropicais e subtropicais da América do Sul não têm recebido suficiente atenção sobre o efeito do enriquecimento em nutrientes nas plantas. Além disso, existe uma lacuna no conhecimento do efeito em cascata do enriquecimento em nutrientes em níveis tróficos superiores, como os polinizadores.

O objetivo principal desta tese é avaliar os efeitos simultâneos de vários tratamentos de enriquecimento em nutrientes nos polinizadores, relacionando-os com as alterações na composição das comunidades de plantas, na abundância de flores e nos recursos florais (qualidade e quantidade de pólen). Contribuindo para colmatar as atuais lacunas de conhecimento, usámos locais experimentais da Nutrient Network (NutNet – uma rede de investigação coordenada de pradarias que implementam tratamentos estandardizados de adição de nutrientes) e combinámos:

i) uma abordagem local (usando uma experiência de campo de manipulação de nutrientes que está a ser desenvolvido numa pradaria Mediterrânica em Portugal) para avaliar os efeitos do enriquecimento em nutrientes nos recursos florais e seu efeito nos polinizadores e nos patrões de interação planta-polinizador.

ii) uma abordagem a larga escala (com dados de 14 experiências de campo de manipulação de nutrientes) para compreender os efeitos do enriquecimento em nutrientes em diferentes comunidades de plantas e polinizadores em três continentes (Europa e América do Norte e do Sul) e avaliar o efeito da

fertilidade do solo e da temperatura no efeito do enriquecimento em nutrientes nas plantas e polinizadores.

Com a abordagem local, detetámos efeitos contrastantes na abundância de flores de três espécies dominantes coexistentes (*Crepis capillaris*, *Raphanus raphanistrum* e *Echium plantagineum*), ou seja, o enriquecimento em NP aumentou a abundância de flores enquanto o enriquecimento em P diminuiu-a. Os efeitos do enriquecimento em nutrientes na qualidade (em termos dos aminoácidos) e quantidade do pólen foi específica para cada espécie, tendo-se observado efeito forte, médio e fraco no conjunto das três espécies. Nenhum tratamento teve efeito negativo sobre a taxa de visitação dos polinizadores (total ou nos grupos de polinizadores), o que foi explicado por compensações na qualidade e quantidade de pólen ao nível da flor e da parcela. O enriquecimento em P teve um efeito positivo na taxa de visitação de *Raphanus* devido ao aumento da concentração de aminoácidos no pólen, mas teve um efeito negativo na taxa de visitação da comunidade de plantas ao nível de parcela, devido a uma redução da abundância de flores. Finalmente, o enriquecimento em NP e em P afetou os padrões de procura de alimento, e consequentemente, as redes de interação: i) enriquecimento em NP aumentou a diversidade de polinizadores e a generalidade de plantas, resultando numa maior generalização da rede; e ii) o enriquecimento em P diminuiu a diversidade de polinizadores, sendo estes mais especializados na procura de alimento, o que aumentou a especialização da rede.

Com a abordagem a larga escala, observou-se que a temperatura regional e a fertilidade do solo interagem com o enriquecimento em nutrientes, principalmente em N (mais também em P), modulando o efeito na diversidade de plantas e polinizadores. Em áreas quentes, o efeito do enriquecimento em N foi negativo na diversidade de plantas e polinizadores, mas foi positivo em regiões frias. Adicionalmente, a resposta da riqueza de plantas foi mas forte em locais com solos pobres enquanto a abundância de flores foi a resposta foi mais forte em locais com solos ricos. A resposta dos polinizadores ao enriquecimento em nutrientes foi mais acentuada do que a resposta das plantas.

Os resultados desta tese claramente mostram que o enriquecimento em nutrientes é impulsor de alterações da disponibilidade de recursos florais, com efeitos ascendentes até os polinizadores. Os dados de nossa abordagem local claramente mostram que o

enriquecimento em NP pode melhorar os recursos florais (em quantidade e qualidade) numa pradaria Mediterrânica com efeitos positivos na diversidade de polinizadores; enquanto o enriquecimento em P pode ter efeitos negativos na abundância de flores, com efeitos negativos na taxa de visitação dos polinizadores e na sua diversidade. Além do efeito na diversidade, mostrámos que o enriquecimento em nutrientes afetou os padrões de procura de alimento dos polinizadores, para uma generalização (enriquecimento em NP) ou especialização (enriquecimento em P) das redes. Mudanças na generalização da redes poderia afetar a tolerância das comunidades a futuras perturbações. Os dados de nossa abordagem em larga escala mostram que os polinizadores estão em maior risco em regiões quentes, que geralmente são hotspots de biodiversidade e que contribuem para a produção global de alimentos. O aquecimento global em curso e o aumento do uso dessas regiões para a agricultura intensiva podem comprometer a polinização das culturas e o fornecimento de alimentos, caso políticas para controlar a intensidade agrícola (incluindo as quantidades de nutrientes adicionados) não sejam implementadas.

Em geral, esta tese fornece informações cruciais sobre os efeitos do enriquecimento em nutrientes nos polinizadores, por meio do estudo de diferentes vias através das quais as mudanças nas plantas causadas pelo enriquecimento em nutrientes, impactam os polinizadores. Para compreender melhor esses efeitos, as investigações futuras deveriam priorizar regiões pouco estudadas e incluir uma ampla variedade de espécies de plantas e de polinizadores em estudos ao nível da comunidade.

Palavras chave:
Recursos florais, pradaria, conjunto de insetos polinizadores, enriquecimento em nutrientes, interações planta-polinizador, fertilidade do solo, temperatura.

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CHAPTER 1. GENERAL INTRODUCTION

1.1 Insects and their ecosystem services

Insects form the most diverse and numerous group in the animal kingdom with more than 1 million described species (Stork, 2018) and possibly having ten times more (i.e., 10 million) undescribed species (Mora et al., 2011). Insects have conquered all ecosystems worldwide (e.g., aquatic ecosystems, deserts, extreme mountain conditions) and have adapted to multiple life styles: diurnal/nocturnal, solitary/social, above/below ground. They are highly diverse and have a wide range of ecological roles (including their influence in agriculture), with potential effects on human health. Despite this, there is relatively low awareness of their significance (Collen et al., 2012) and only a few insect orders have typically concentrated much of the interest or research due to their beauty (e.g., butterflies), proximity or interactions with humans (e.g., disease vectors, pests), or because of a certain exploitable service (e.g., honeybees). Therefore, we lack knowledge about thousands of insect species, including their basic biology and ecology (see Cardoso et al., 2011). As a result, we use limited knowledge from relatively few insect orders to make broad statements concerning other insect species, which may lead to inaccurate conservation decisions (see next section for more information).

Insects provide numerous ecosystem services, such as organic matter decomposition, contribution to soil structuring, pest control (e.g., predation and parasitism), seed dispersal, and pollination (Scudder, 2017). Pollination of wild and crop plant species is perhaps the ecosystem service provided by insects of which society is most aware and values the most. It is estimated that 87% of the 352,000 angiosperm species are pollinated by animals, mostly insects (Ollerton et al., 2011), while 75% of crops benefit from pollination services (Klein et al., 2007; Siopa et al., 2024). In the USA alone, ecological services provided by insects are valued at $57 billion annually (Losey and Vaughan, 2006). Approximately one third of all insect species described so far are pollinators (350,000 species) (Wardhaugh, 2015), of which 95% belong to the following groups (Fig. 1.1): Lepidoptera (butterflies and moths; ~140,000 species), Hymenoptera (wasps and bees; ~70,000 species), Coleoptera (scarab beetles, false blister beetles; ~77,000 species)

and Diptera (hoverflies; 6,000 species, bee flies; 5,000 species and other groups; ~55,000 species) (Rader et al., 2009; Wardhaugh, 2015). Insects of the orders Orthoptera (Micheneau et al., 2010), Blattodea (Pérez-Gómez et al., 2023), Hemiptera (Ishida et al., 2009), as well as some other arthropods (e.g., acari, spiders, etc.; Wardhaugh, 2015), are also known to pollinate. As outlined above, insects are central to ecosystem functioning, and thus important economically. Thousands of pollinator species contribute to crop and wild plant pollination. However, a growing number of publications report recent declines in pollinator populations, threatening crops and wild plant populations (e.g. Potts et al., 2010).

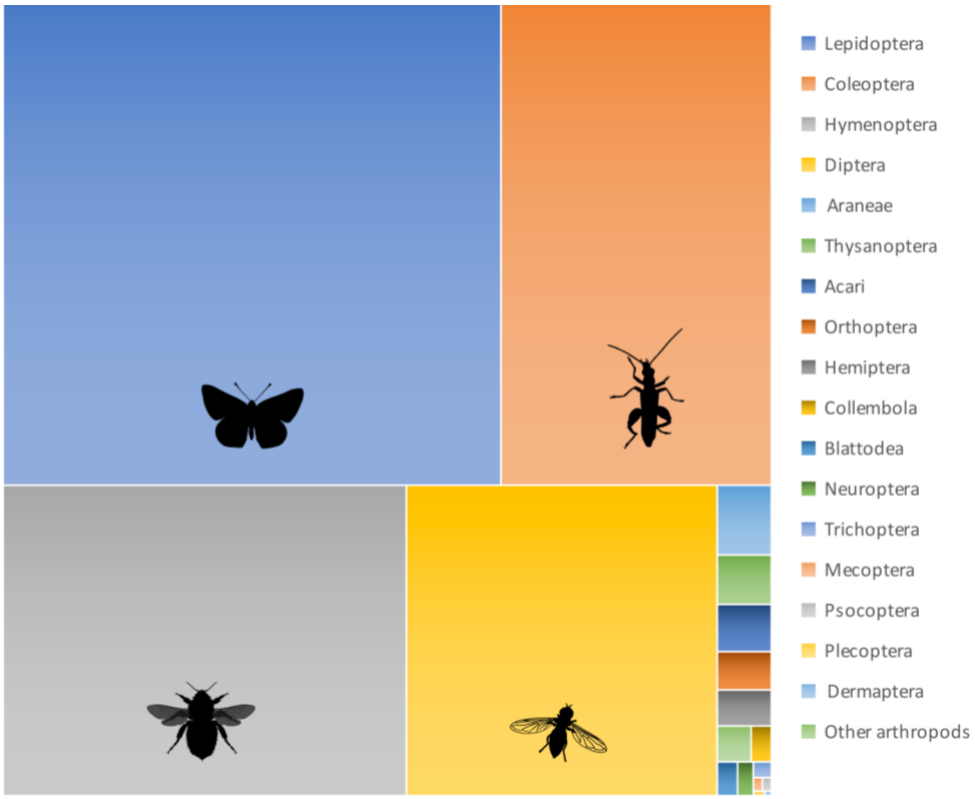


Figure 1.1. TreeMap showing the contributions of the different pollinator groups to the total number of described pollinator species (350,000 species).

1.2 Global changes cause pollinator declines

Our planet's stability during the past 10,000 years allowed human civilizations to arise, develop and thrive. Since the Industrial Revolution, however, human interference on Earth has increased to a point where we are threatening its stability. Increasing instability, mainly due to impacts on biodiversity, climate, and the nitrogen (N) and phosphorus (P)

cycles (Steffen et al., 2015), may render the planet unsafe for future generations. Adding another tier of complexity is the fact that these drivers are all interconnected, with changes in species distribution occurring due to changing regional climates (Thuiller et al., 2008) and N and P supplies (Bobbink et al., 2010; Ceulemans et al., 2014).

Due to the important ecological and economic roles of insect pollinators (hereafter, pollinators), concerns about their decline have spread from ecology and economy to decision-makers (e.g. the European Union), farmers and society in general. Although global pollinator declines have been reported (Cameron et al., 2011; Sánchez-Bayo et al., 2019; Dicks et al., 2020; Wagner et al., 2021; Zattara and Aizen, 2021; Blüthgen et al., 2022), most research stems from Europe (Beismeijer et al., 2006; Potts et al., 2010; Goulson et al., 2015; Powney et al., 2019; Wood et al., 2020; Müller et al., 2023). Hallmann et al. (2017) reported a 76% loss in flying insect biomass over 27 years in protected areas of Germany. The drastic results of this study (referred to as the "Insect Armageddon") attracted extensive media coverage (Carrington, 2017), but at the same time criticism of its methods and approach. The study of species richness rather than biomass would have been a more informative variable. The scale used was small and the sampling methodology uneven, which might have led to inaccurate conclusions (see Eggleton, 2020). Moreover, as shown by Borges et al. (2020), insect decline patterns are also associated with the increase in diversity of exotic and invasive species, particularly on island ecosystems. As most long-term studies have focused on a few insect orders (generally Hymenoptera and Lepidoptera: Bommarco et al., 2012; Warren et al., 2021), this introduces bias about general pollinator trends. Despite these gaps, it is clear that pollinators are declining. However, we lack sufficient evidence to confirm that all insect orders are declining or that these declines are occurring in all biomes. To address these issues, we need to focus on understudied areas (e.g. southern hemisphere) and collect information on a broad range of species from various pollinator orders, including Diptera and Coleoptera.

The studies reporting declines in bees (Wood et al., 2020; Zattara and Aizen, 2021), bumblebees (Cameron et al., 2011; Bommarco et al., 2012), flies (Barendregt et al., 2021) and butterflies (Wepprich et al., 2019; Warren et al., 2020) highlight various drivers, such as climate and land use changes, agricultural intensification, and the use of pesticides (Fig. 1.2). Climate change forces pollinator species to move in search of their thermal

optimum, which shifts species distributions in latitude and altitude (Parmesan, 2006; Devictor et al., 2012, Marshall et al., 2020). Agricultural intensification brings an extensive set of associated changes, such as land-use change, landscape and ecosystem simplification, increased use of pesticides (including herbicides, fungicides and insecticides) and elevated nutrient input or nutrient enrichment (Benton et al., 2002). Since pesticides (Goulson et al., 2015; Raine and Rundlöf, 2024), and insecticides in particular (Godfray et al., 2014), can impact pollinator populations, agencies such as the European Food Safety Authority (EFSA), have imposed some restrictions on their use (e.g., chloropyrifes). However, the effects of nutrient enrichment alone on pollinators, and on insects more generally, are not well understood. Therefore, this thesis examines the impact of nutrient enrichment on pollinators.

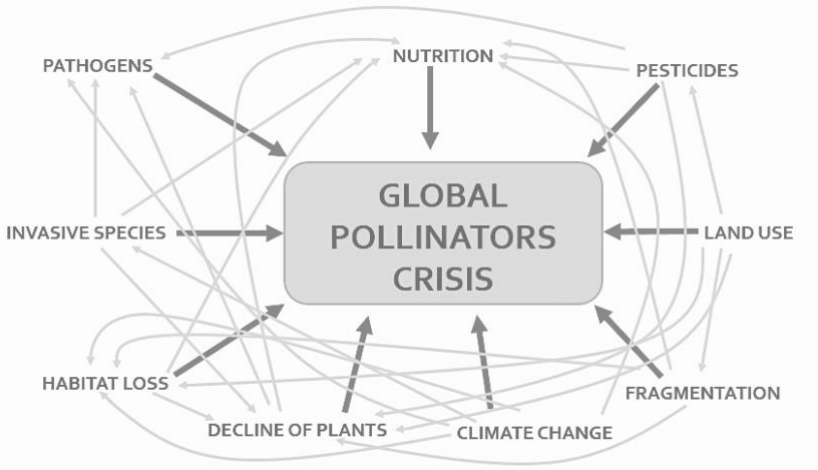


Figure 1.2. Global change drivers that contribute to the pollinator crisis (source: the www.campusbuzz.blog).

1.3 Nutrient enrichment

Essential nutrients such as nitrogen (N), the prime component of amino acids, proteins, nucleic acids, and chlorophylls and phosphorus (P), a fundamental component of cellular membranes, energy storing molecules such as ATP and PPi, and nucleic acids, play irreplaceable roles in all life forms. However, these nutrients are largely unavailable to most organisms, which greatly limits productivity in most ecosystems (Ågren et al., 2012). Industry, transportation and the widespread and intensive use of synthetic fertilizers (e.g., ammonia, nitric acid, triple superphosphate) in the 20th century (Erisman et al., 2008; Galloway et al., 2008) have greatly altered the biogeochemical cycles of N

and P (Fig. 1.2), increasing their availabilities. For example, N deposition increased by up to 10-fold from 1860 to 1990 (Galloway et al., 2004). As a result of ongoing anthropogenic nutrient inputs (e.g., N, P, and other nutrients such as potassium – K), ecosystems that evolved in oligotrophic (nutrient-poor) soils and are adapted to low nutrient availability, are at risk of losing their ecological integrity, which can negatively affect their biodiversity. It is widely acknowledged that nutrient enrichment from agro-industrial activities is a global threat to biodiversity (Sala et al., 2000; Galloway et al., 2003; Phoenix et al., 2006; Bobbink et al., 2010; De Schrijver et al., 2011; Ochoa-Hueso, et al., 2017; Ceulemans et al., 2017a; 2017b), ecosystem functioning (Phoenix et al., 2012; Southon et al., 2013), including nutrient cycling (Fields, 2004), and ecosystem services (Alcamo et al., 2005; Clark et al., 2017). Indeed, the magnitude of human interference in the global N and P cycles has crossed its planetary safety boundaries (Fig. 1.3), thus threatening the Earth's stability (Steffen et al., 2016; Rockström et al., 2023; Richardson et al., 2023).

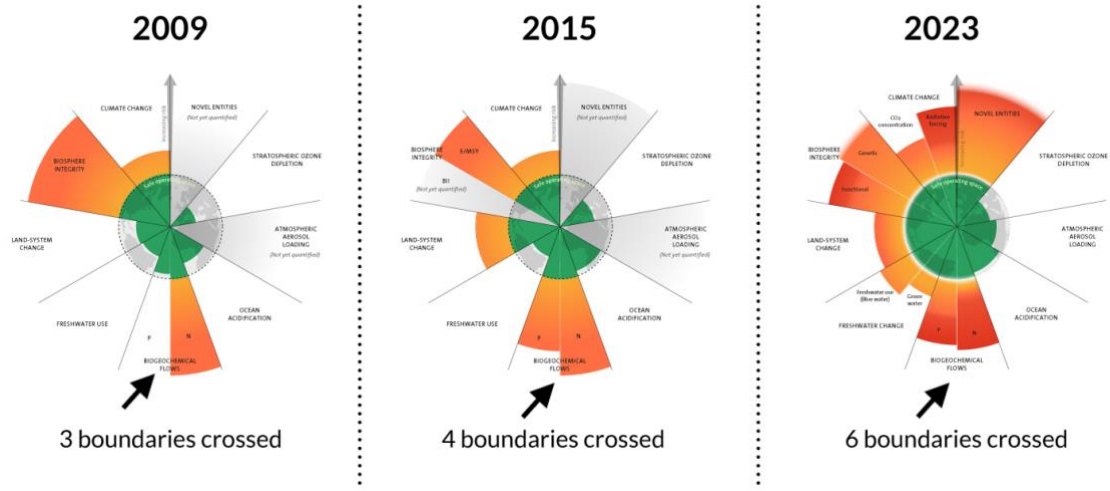


Figure 1.3. The evolution of planetary boundaries based on Steffen et al. (2016), Richardson et al. (2023) and Rockstöm et al. (2023). Credit: Azote for Stockholm Resilience Centre, Stockholm University.

In line with the global estimates (Galloway et al., 2004), N deposition in almost all biodiversity hotspots (33 out of 34 areas) is expected to double by 2050 (Phoenix et al., 2006; Bobbink et al., 2010), which will strongly impact plant and animal communities. For example, the Mediterranean Basin is a biodiversity hotspot limited by N and P availabilities (Dias et al., 2011; Sardans and Peñuelas, 2013), where the landscape is very patchy, i.e., areas of conservation interest are near urban, industrial, and intensive

agricultural areas. As these anthropogenic land uses are associated with higher inputs of nutrients and pollutants, the proximity to areas of conservation interest is very likely. Taking intensive agriculture as an example, nutrients applied in the form of synthetic fertilizers can impact natural ecosystems through: i) atmospheric deposition (both dry and wet); ii) run-off, especially in hilly areas where natural vegetation is close to crops; and iii) leaching, where nutrients from these activities percolate through the soil profile and reach the phreatic layer, polluting aquatic systems. Despite pollinators' important ecological and economic roles, and transversal concerns about their decline, the effects of nutrient enrichment on pollinators remains largely unknown and understudied. Therefore, this thesis brings new and innovative research on this "neglected topic".

1.4 Plant-mediated effects of nutrient enrichment on pollinators

Studies on the ecological effects of nutrient enrichment date back to the 1970s, and have focused mostly on the effects of N (and sulphur) deposition on plant communities in temperate ecosystems in Europe and North America (Stevens et al., 2004; Bobbink et al., 2010; Phoenix et al., 2012). This bias reflects the intense agro-industrial activities in these regions since 1970 (Galloway et al., 2008), which led to earlier and more severe impacts of nutrient enrichment than in other regions. This regional bias regarding knowledge about the ecological effects of nutrient enrichment can compromise extrapolations to biomes developed in low fertility soils, as the background soil fertility (i.e., without nutrient enrichment) can determine the strength of the impact of nutrient enrichment (Harpole et al., 2016). For example, Olsson and Kellner (2006) observed in three coniferous forest sites in Sweden that the effects of fertilization tended to be more profound when the plants were growing in nutrient-poor sites. Vogels et al. (2023) concluded the same after their review study. The effect of nutrient enrichment on plant communities can also be affected by regional temperature. For example, temperature affects soil properties and plant performance, such as plant nutrient uptake capacity and metabolism (Bassirirad, 2000; Boczulak et al., 2014). Moreover, N and P cycles are mostly controlled by microbial activity, which also depends on temperature. Clark et al. (2007) showed that the effects of nutrient enrichment were more negative on plant richness in sites with low temperature. On the other hand, high temperatures and nutrient addition increased flower abundance and pollinator visitation and development (Hoover

et al., 2012; Rahariviololoniaina et al., 2021). Therefore, soil fertility and temperature are relevant abiotic characteristics to consider when assessing the effects of nutrient enrichment.

Furthermore, by acknowledging the modulating effects of soil fertility and climate on the ecological effects of nutrient enrichment, the knowledge obtained in temperate ecosystems should not be extrapolated to other biomes. For example, Mediterranean ecosystems and tropical and subtropical parts of South America have received far less attention (Bobbink et al., 2010). Mediterranean ecosystems are biodiversity hotspots and are a global conservation priority (Myers et al., 2000; Klausmeyer and Shaw, 2009) particularly vulnerable to nutrient enrichment as they evolved, and are adapted to poor soils, i.e., naturally N and P limited. As Mediterranean ecosystems host very rich biota, they are strategic ecosystems to study the impacts of nutrient enrichment (Pinho et al., 2012; Dias et al., 2014; Ochoa-Hueso et al., 2017). In the last 15 years there has been a strong effort to understand the impacts of N enrichment on European Mediterranean ecosystems (e.g., Dias et al., 2011; Pinho et al., 2012; Dias et al., 2013; Dias et al., 2017; Ulm et al., 2017; Ochoa-Hueso et al., 2017; Dias et al., 2020), though research has focused mostly on plants and soil. Studies on the effects of nutrient enrichment on other trophic levels of European Mediterranean ecosystems are thus urgently needed. Recently, a growing number of studies in South America (Brazil and Argentina) have assessed the effects of nutrient enrichment on plant-insect communities (e.g., Ramos et al., 2018; Perez-Mendez et al., 2022). This is partly due to tropical regions, such as the Brazilian Cerrado, being increasingly used for agriculture and cattle production (Davidson et al., 2012). The highly diverse Brazilian Cerrado (Myers et al., 2000), which is particularly limited by P but also by N (Oliveira et al., 2002), is being exposed to large nutrient inputs (Lannes et al., 2012; Ramos et al., 2018). This is occurring at a rate much faster than science can track and mitigate its ecological impacts.

In the last decades, considerable efforts have been made to understand the impacts of nutrient enrichment worldwide, especially in non-temperate ecosystems that remain poorly understood. Global experimental networks have contributed to filling geographical gaps with respect to nutrient enrichment research. For example, the Nutrient Network or NutNet (https://nutnet.org/) is a coordinated research network of more than 130 grassland sites worldwide implementing standardized nutrient addition treatments.

This is a leading project addressing many research questions regarding nutrient enrichment (for more details, please see Chapter 4).

The following sections review the three main pathways through which nutrient enrichment can impact pollinators via changes in plant traits (Fig. 1.4): i) plant species diversity and composition; ii) flower abundance and morphology; and iii) floral rewards quantity and quality (see David et al., 2019). Although these effects of nutrient enrichment on plants occur simultaneously, most studies on the topic only cover one set of impacts and are restricted to one plant species. Therefore, in this thesis we aim to assess the multiple and simultaneous effects of nutrient enrichment on plant and pollinator communities and their interactions.

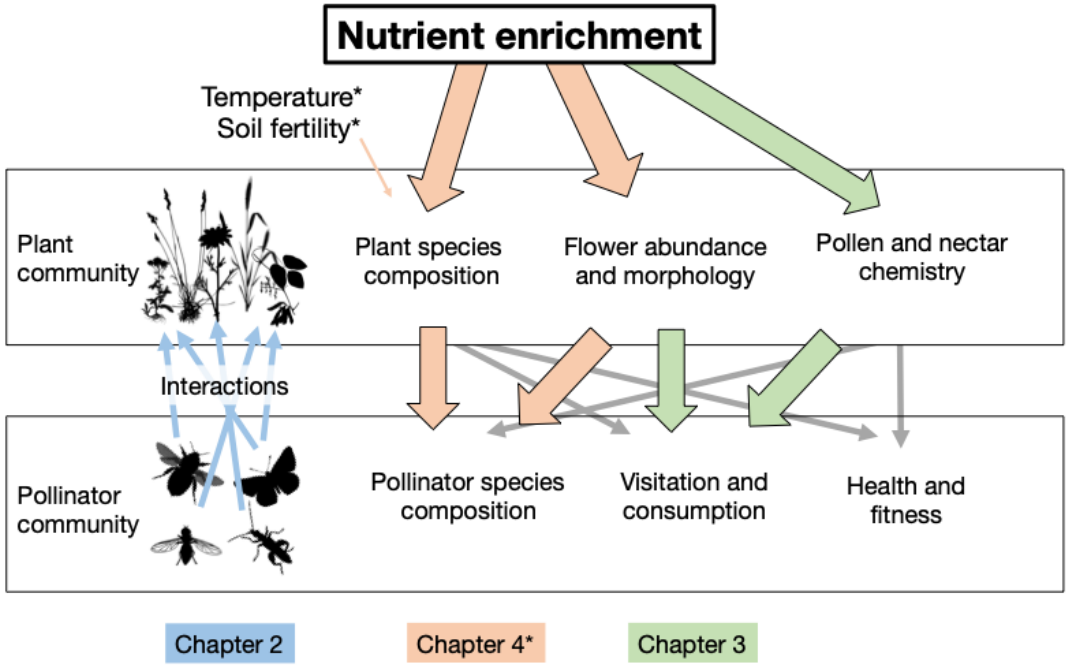


Figure 1.4. Pathways through which nutrient enrichment impacts pollinators. Grey arrows represent existing pathways that are not the objective of this thesis.

1.4.1 Changes in plant species diversity and composition

Nitrogen has been the most studied nutrient, and so most of our understanding comes from its effect on plants. In general, nutrient enrichment benefits fast-growing plant species that increase their above-ground biomass. These species can then outcompete smaller and/or slow-growing species for space and light, decreasing their abundance and eventually excluding them from the plant community. As a result, plant community

composition changes, and plant richness decreases (Fig. 1.5; Bobbink et al., 1998; Hautier et al., 2009; Lannes et al., 2012; Isbell et al., 2013; Borer et al., 2014; Eskalinen et al., 2022). Besides some forbs and woody plants (e.g., *Betula pubescens*, *Rubus* sp., *Urtica dioica*), the nutrient-benefited plant species are usually grasses (e.g., *Elymus repens*, *Holcus lanatus*, *Melinis minutiflora*, *Molinia caerulea*, *Urochloa decumbens*). Within the nutrient-affected plant species, Fabaceae (i.e., legumes) is one of the plant families that suffers most negative impacts of nutrient enrichment (Tognetti et al., 2021; Wang et al., 2022). Under N-limiting conditions, legume plant species have a competitive advantage due to their ability to form root nodules that host N fixing bacteria. However, when N is no longer limiting (i.e., upon N enrichment), this trait no longer confers a competitive advantage, and legumes tend to be excluded from the plant community. Other plant families like Asteraceae equally suffer the effect of all nutrients (Tilman et al., 1999). Since grasses are wind-pollinated (i.e., anemophilous) and legumes and Asteraceae are entomophilous, plant communities developing in nutrient enriched environments are poor resources for pollinators.

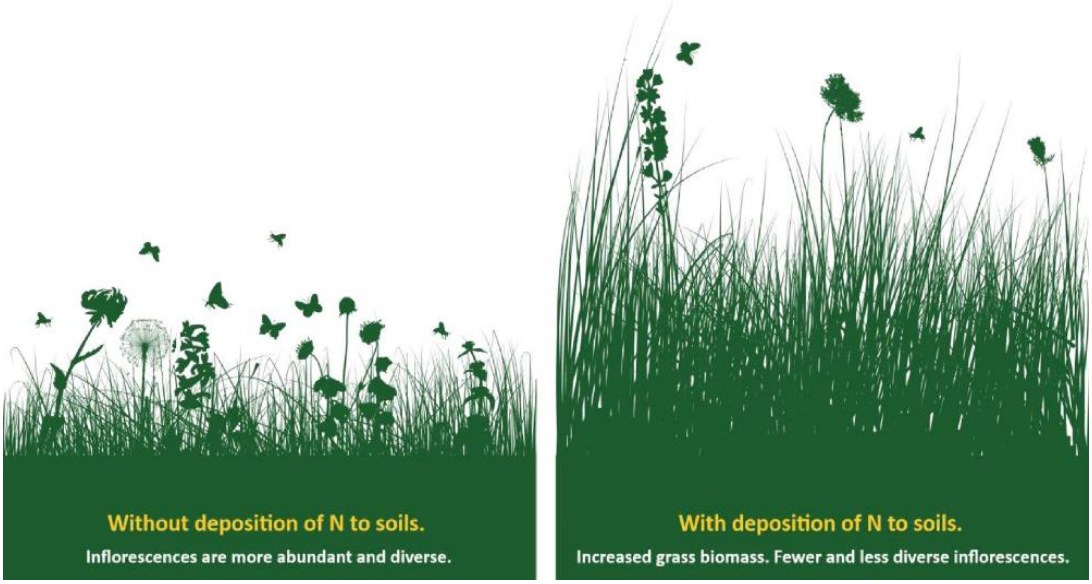


Figure 1.5. Plant-pollinator community response to N deposition. Source: David et al. (2019).

The effects of nutrient enrichment propagate to higher trophic levels such as herbivores or flower visitors (Haddad et al., 2000; Nijssen et al., 2017; Stevens et al., 2018). Haddad et al. (2000) detected an increase in total insect abundance although insect richness decreased following reductions in plant richness. Similarly, Cuesta et al. (2008) found

increases in beetle abundance in fertilization treatments and Wimp et al. (2010) found an increase in both arthropod abundance and richness. Conversely, Asmus et al. (2018) detected opposing responses of arthropod abundance to fertilization. Bishop et al. (2010) found that grasshoppers responded positively to P fertilization, which was correlated with the percentage of plant cover of preferred plants and their changed nutritional values.

The disappearance of certain plant species that are outcompeted by others under nutrient enrichment has the potential to impact plant-pollinator interaction networks. Spatial changes in nitrophobous (i.e., plant species adapted to low N availability) flowering plant species under N enrichment decreased the diversity of pollinators, bees and butterflies (Carvalheiro et al., 2019). Öckinger et al. (2006) reported butterfly species extinctions due to declines in their foodplants (also related to N enrichment and plant tolerance). Wang et al. (2022) linked declines in bumblebee richness to lower diversity of legumes in N addition treatments, as bumblebees were mainly foraging on them. Therefore, the change in plant community composition negatively impacted the pollinator community, as the plant species on which pollinators rely (i.e., nitrophobous plant species) disappear under nutrient enrichment. However, the effect could be positive if the plant species benefits from nutrient enrichment, i.e., if the nutrient-benefited plant species are nitrophilous.

1.4.2 Changes in flower abundance and morphology

Nutrient enrichment can also change plants' investment in aboveground biomass (i.e., leaf abundance and size), sexual reproduction (i.e., flower abundance, size and shape, Fig. 1.6A) and its timing and duration (i.e., phenology). In general, when plants grow in a fertilized or enriched environment, they have more resources to increase their biomass and to produce more flowers. Due to the relevance of flower abundance and morphology for plant reproductive success and pollinator attraction, there are more studies on the effects of nutrient enrichment on flower abundance and shape (Muñoz et al., 2004; Burkle and Irwin, 2009; Majetic et al., 2017), than on phenology (Cleland et al., 2006) or leaf size (Knops and Reinhart, 2000; Russo et al., 2020). Flower abundance in most of these studies increased at plot level under NPK enrichment (Burkle and Irwin, 2009; Hudewenz et al., 2012; Russo et al., 2020), although some studies showed a decrease (Villa-Galaviz

et al., 2021). When N was added alone, the general trend was an increase in flower abundance (Muñoz et al., 2005; Burkle and Irwin, 2010; Majetic et al., 2017). Wang et al. (2022) did not find an effect with N or P addition, but found a positive effect with the coaddition of both. As potential pollinators are attracted or deterred by different flower traits, a change in flower characteristics will impact them.

Higher flower abundance and larger flower size increase pollinator visitation rate (Burkle and Irwin, 2010; Majetic et al., 2017, Vaudo et al., 2022). Russo et al. (2020) detected contrasting effects on pollinator abundance depending on the study year, despite recording taller plants and higher flower display in fertilized plants. In contrast to the main trend, Villa-Galaviz et al. (2020) detected a decrease in flower abundance with negative consequences for bumblebees. When changes in flower abundance and shape are not connected to pollinator responses, other changes might be at play, such as changes in pollen or nectar chemistry. Thus, it is important to conduct studies that comprise as much of the spectrum of effects of nutrient enrichment on the plant community to better understand the impacts on pollinators.

1.4.3 Changes in floral rewards quality

Nutrient enrichment can also change the quantity and quality (i.e., chemical composition) of the floral rewards: nectar and pollen (Fig. 1.6B). Nectar is the most common reward plants offer to their pollinators. Nectar, which is pollinators' energy source, is mainly composed of sugars (up to 70% w/w of sucrose, fructose and/or glucose) and smaller amounts of amino acids, proteins, lipids, micronutrients and vitamins (Nicholson and Thornburg, 2007; Nicholson, 2022). Since nectar varies greatly in quantity and quality between plant families (i.e., interspecific variation) and between individuals of the same species (i.e., intraspecific variation), changes in the plant species community and in individual plant physiology due to nutrient enrichment, can affect the quantity and quality of the nectar available for pollinators. Pollen, which is the main N source for many adult pollinators and their offspring, is mainly composed of proteins and amino acids (2-60% depending on the plant family; Ruedenauer et al., 2019) and smaller amounts of sugars, lipids and vitamins (Roulston et al., 2000; Nicholson, 2011). The amount of proteins and amino acids determine pollen quality, which can be detected by some pollinators such as

honeybees and bumblebees (Cook et al., 2003), and presumably more bee species (Vaudo et al., 2024). Pollen quality and quantity affect bee nest provisioning, and some plant species provide higher quality pollen than others (Kuppler et al., 2023). In general, the volume and concentration of central compounds (i.e., protein, sugars, fatty acids) in nectar and pollen are key for pollinator resource selection, as pollinators complement their diets feeding on different plants based on their nutritional values (Vaudo et al., 2015; Wright et al., 2018).

Nutrient enrichment can change plants' primary metabolism, leading to changes in sugars, amino acids and lipids present in flower rewards. In general, nutrient enrichment increases central compound concentrations (i.e., sugars, amino acids and lipids). Sugar (Baude et al., 2011; Barber and Soper Gorden, 2015) and amino acid content (Gijbels et al., 2015; Ceulemans et al., 2017c) increased in the nectar produced by plants under nutrient enrichment. Gardener and Gillman (2001) detected an increase in total nectar amino acid concentration in fertilized treatments, in line with Gijbels et al. (2014; 2015). However, due to the different pathways of amino acids, it is more likely that certain amino acids increase while others decrease, although this is still poorly understood as responses are species specific. A study on the effect of nutrient enrichment on nectar and pollen amino acid concentration found negative responses in nectar and positive responses in pollen (Ceulemans et al., 2017c). Although there are relatively few studies on this topic, they exemplify the importance of plant species-specific responses to nutrient enrichment and highlight the difficulty in establishing general trends.

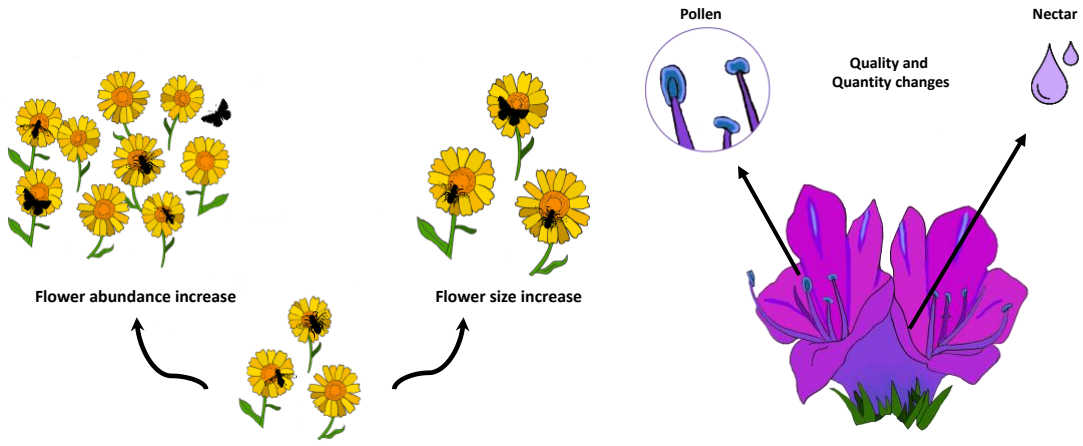


Figure 1.6. Changes experienced by flowers under nutrient enrichment include: A) changes in flower abundance and size and B) changes in floral rewards.

Despite the importance of nectar and pollen chemistry for pollinators, the number of studies on the topic is limited. One of the reasons is the difficulty in gathering sufficient material to be analysed. However, in recent years pollen protocols and equipment have improved, lowering the minimum required quantity for analysis (Vanderplanck et al., 2014). Few studies have addressed the effect of nutrient enrichment on floral rewards chemistry and its subsequent effect on pollinators. Ceulemans et al. (2017c) detected divergent responses in nectar and pollen essential amino acid concentration. The pollen of fertilized plants had a lower proportion of essential amino acids (lower "pollen quality"), which negatively affected *Bombus terrestris* workers and larval development. Similarly, Hoover et al. (2012) observed higher mortality in pollinators that consumed artificial nectar of "plants" growing in fertilized treatments, despite only detecting positive changes in nectar total sugar and amino acid concentrations and not in the amino acids relative proportions. Lastly, Vaudo et al. (2022) showed an increase in the quantity of floral rewards (nectar volume and pollen number) that increased bumblebee visitation rate.

Thus, high quality and quantity floral rewards appear to be important for bee pollinators in general (Vaudo et al., 2024), and particularly for bumblebees (Hanley et al., 2008) and honeybees (Cook et al., 2003; Hendriksma et al., 2014). Changes in floral rewards could impact visitation rate and in some cases pollinator health. Therefore, it is important to investigate the effect of nutrient enrichment on flower chemistry at the community level, and to understand how the changes eventually affect pollinator interactions.

1.5 Objectives

Several studies have addressed single aspects of the effect of nutrient enrichment on pollinators (e.g. Muñoz et al., 2004; Burkle and Irwin, 2010). In general, these studies use few plant and pollinator species, focus on one fertilization treatment (mostly N alone or a combination of N, P and K) and are conducted under controlled conditions (i.e., in greenhouses). Thus, studies focusing on the effects of increased availability of single and multiple nutrients, carried out under field conditions and including the entire co-flowering plant communities are rare and urgently needed. Furthermore, studies are needed in ecosystems that comprise important conservation areas worldwide, with poorer soils and

warmer temperatures compared to northern Europe and North America. In this thesis, we examine a wide range of effects of nutrient enrichment on pollinators in understudied areas like Mediterranean ecosystems, characterized by oligotrophic soils and warm temperatures. This is done through a set of experimental sites with multiple fertilization treatments, in which both plant and pollinator communities are studied.

The main objective of the thesis is to assess the simultaneous effects of multiple nutrient addition treatments on pollinator communities, linking them to changes in plant community diversity and composition, flower abundance and floral rewards' chemistry (pollen quantity and quality). To achieve this, we used sites from NutNet and combined: (i) a local approach, through the study of an ongoing nutrient manipulation field experiment located in Portugal (Fig. 1.7), with a community historically adapted to low levels of anthropogenic disturbance; and (ii) a large scale approach, using data from 14 ongoing nutrient manipulation field experiments located in Europe and in North and South America. Since all sites are part of the NutNet, they have all replicated identically a long-term nutrient experiment (Fig. 1.8), allowing us to experimentally separate the role of nutrients across ambient site conditions to determine whether the effects vary with environmental conditions (i.e., background soil fertility and climate).

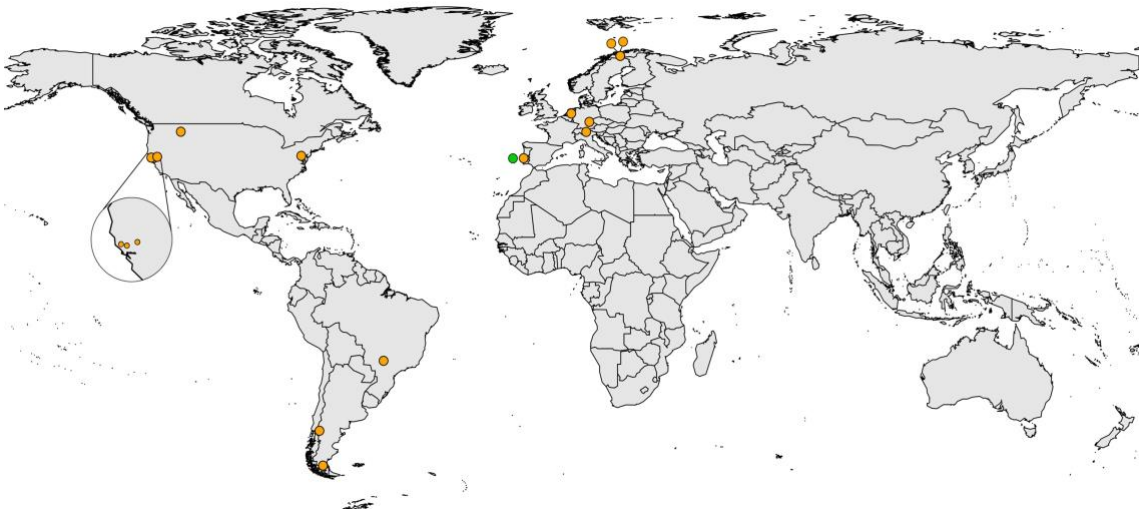


Figure 1.7. Sites participating in the large-scale approach (orange) and local approach (green) of the thesis.

The specific objectives of this thesis are:

- To determine the effects of nutrient enrichment on plant-pollinator interactions through the study of plant-pollinator networks in different nutrient enrichment treatments (**Chapter 2**).
- To understand the effects of nutrient enrichment on flower abundance and floral rewards' chemistry and the effect on pollinators by analysing pollen from dominant plants and surveying their visitors (**Chapter 3**).
- To examine the effects of nutrient enrichment on flowering plant and pollinator diversity through the study of different communities in three continents (**Chapter 4**).
- To assess the effect of abiotic variables such as soil fertility and regional temperature on nutrient enrichment and how this affects plants and pollinators (**Chapter 4**).

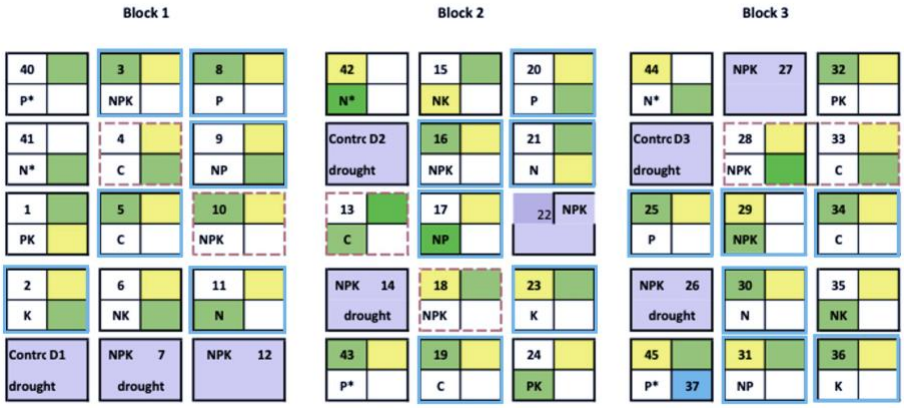


Figure 1.8. Example of experimental design shared by the participating sites in NutNet. Design from Companhia das Lezírias experiment, Portugal (Nogueira et al., 2019).

1.6 Thesis outline

The thesis is structured in five chapters, including a main introduction (**Chapter 1**) to the topic context and framework and the thesis goals, followed by three chapters in which the results of this thesis are compiled. The final chapter (**Chapter 5**) corresponds to the main discussion of the findings with recommendations for future research.

The contents of each chapter are:

Chapter 1 contextualizes the relationships between nutrient enrichment, plant community changes, and the impacts on pollinators. It also identifies research gaps and outlines the thesis goals.

Chapter 2 focuses on the effect of nutrient enrichment on plant-pollinator interaction networks, using two years of data from the Portuguese long-term experimental site also used in Chapter 3. We found an effect of nutrient enrichment on pollinator assemblage composition, with control treatment being the most homogeneous in terms of more species shared within plots. Plant and pollinator diversity were affected by different treatments: while NP reduced plant richness, it increased flower abundance and pollinator diversity and P and K addition generally reduced both flower abundance and pollinator diversity. We also found changes in network metrics, particularly under N and P coaddition and the addition of P alone. N and P coaddition increased generalization in plant-pollinator networks (increased plant generality), whereas P addition increased network specialization (lower interaction evenness, higher species specialization). Overall, this study shows that nutrient enrichment affects flower visitor perceptions of floral resources, altering community composition, diversity and interaction patterns, with NP and P having particularly important and contrasting effects.

Chapter 3 investigates the effects of nutrient enrichment on flower abundance and chemistry and the link with pollinator visitation. We conducted the study in a long-term nutrient experimental site in Portugal, where we selected three dominant co-flowering plant species with different floral traits (*Crepis capillaris*, *Echium plantagineum* and *Raphanus raphanistrum*, Fig. 1.9), collected their pollen and studied their visitors. We found varying responses among the plant species at flower level, from strong changes in pollen amino acid quality and quantity (*Crepis*), to moderate (*Raphanus*) and weak responses (*Echium*). Pollinator visitation only increased in *Raphanus* flowers in the P treatment, where we detected an increase in amino acid concentration. At plot level, we detected increases (NP) and decreases (P) in flower abundance in the three species. The addition of NP increased amino acid concentration but there was no effect on visitation rate of the three species or the whole plant community at plot level. The addition of P decreased pollen quantity and amino acid concentration. Additionally, we detected a marginal negative response in visitation rate to the full plant community in the P treatment. Although responses were species-specific, we highlight that among co-flowering species the general effects of nutrient eutrophication were dampened.

Figure 1.9. Flower visitors on the three plant species selected in Chapter 3; from left to right: *Raphanus raphanistrum*, *Echium plantagineum* and *Crepis capillaris*.

Chapter 4 investigates the effect of nutrient enrichment on plant and pollinator diversity. Using data from 14 plant and pollinator communities in three continents, we assessed the effect of different fertilization treatments on plant and pollinator abundance and richness. The 14 sites spanned a wide range of ambient temperature and soil fertility and replicated a long-term nutrient experiment identically (Fig. 1.10). This allowed us to separate the role of nutrients across ambient site conditions to determine whether effects vary with environmental conditions. We found an interactive effect of temperature and soil fertility with nutrient enrichment, with N input having a more negative impact on plants and pollinators in warmer climates. Moreover, the effects on flower abundance were more pronounced in high fertility soils, while plant richness was more affected in poor fertility soils. In general, the effect of nutrient enrichment in colder climates was positive, and pollinators were more responsive to nutrient enrichment than plants. These results highlight the high susceptibility of warmer regions and particularly their pollinators to nutrient enrichment. This chapter is presented in a format in which Results and Discussion sections are written together, to match the format of the Journal selected for future submission.

Figure 1.10. Experimental design of different NutNet sites participating in Chapter 4.

Chapter 5 presents the discussion of the findings reported in Chapters 2, 3 and 4 and their relevance within the broader framework of the topic. It also contains the concluding remarks and presents future research paths.

CHAPTER 2. Nutrient enrichment affects plant-flower visitor assemblage interaction patterns in a Mediterranean grassland

2.1 Introduction

Plants produce organic matter (with structural and physiological roles) from mineral elements absorbed from the soil and the atmosphere. Some of these mineral elements are crucial for plants to complete their life cycle and are therefore considered essential nutrients (e.g., nitrogen – N, phosphorus – P and potassium – K) (White and Brown, 2010). Therefore, soil nutrient availability, together with other abiotic factors, shape plant community composition and influence plants' investment in floral resources (Burkle and Irwin, 2010). Due to human activities, soil nutrient availabilities are increasing at unprecedent levels (Galloway et al., 2004; 2008), and such ongoing environmental eutrophication is recognized as a threat to biodiversity (Sala et al., 2000; De Schrijver et al., 2011; Ceulemans et al., 2017a; 2017b, Rockström et al., 2023) and ecosystem functioning (Phoenix et al., 2012; Southon et al., 2013). While the effects of nutrient enrichment on plant community composition are well understood (e.g. Bobbink et al., 2010), the few studies on the cascading effects on higher trophic levels focus mostly on foliar herbivores (Haddad et al., 2000; Asmus et al., 2018; Nessel et al., 2021). Although pollinators feed on floral resources and can be severely affected by nutrient enrichment, the number of studies on the topic is limited.

Although nutrient effects on plant-pollinator interactions are still poorly understood, there are three main non-exclusive pathways through which nutrient enrichment can have an effect on pollinators (David et al., 2019) namely: i) Changes in plant species community composition (and associated changes in floral resource assembly). Several studies show that plant-pollinator assemblages are affected by changes in plant communities, generally, losing pollinator species that rely on plant species that disappear under nutrient enrichment (Öckinger et al., 2006; Carvalheiro et al., 2019). ii) Changes in plant investment in sexual reproduction (i.e., flower production). In general, studies reporting increases in flower abundance also reported positive effects on flower visitation

frequency of pollinators (Muñoz et al., 2005; Burkle and Irwin, 2010; Majetic et al., 2017). iii) Changes in flower chemistry. Studies showing nutrient-driven changes in pollen and nectar concentration and composition, showed negative effects on pollinators increasing their mortality in the adult or larval stage (Hoover et al., 2012; Ceulemans et al., 2017c), although the results are species-specific (Chapter 3).

Changes in plant composition, flower production and quality, driven by nutrient enrichment, can affect the way pollinators perceive and visit flowering plants, affecting plant-pollinator network patterns, even if overall levels of pollinator and plant diversity remain unchanged. In a study run in experimental plots, Wang et al. (2022) reported that although nutrient-driven changes in plant composition had limited effects on pollinator diversity, network generality decreased under NP addition. Similarly, Villa-Galaviz et al. (2021) found that, although the decrease in flower richness and abundance caused by fertilization had no effect on total pollinator richness, it affected plant-pollinator network vulnerability. In both studies, there was an effect on plants (plant species composition or richness and/or flower abundance), no effect on total pollinator diversity but effects on plant-pollinator interactions (generality and vulnerability). Yet, these studies were conducted in ecosystems with rich soils, and it is unclear if similar effects are expected in other regions of the world where soil and climatic conditions are different, particularly areas with poor soils and warmer temperatures.

Given the knowledge gaps highlighted above, here we assess the nutrient enrichment effects on a plant-visitor community in a Mediterranean grassland, an ecosystem with poor soils (limited by N and P) and warm temperatures (Dias et al., 2012; Sardans and Peñuelas, 2013; Nogueira et al., 2019). As nutrient enrichment changes the composition of flowering plant species, flower abundance and quality, and flower visitation patterns (David et al., 2019), and in poor and warm ecosystems there is likely a larger number of flower visitors adapted to nitrophobous plant species, we expect that the composition of visitor assemblages will significantly differ between treatments. More specifically, we expect that increasing nutrient input will lead to higher homogeneity of visitor assemblages among plots with high nutrient input and lower diversity (expectation 1). Moreover, as the loss of nitrophobous plant species is associated with the loss of specialized flower visitors that prefer such resources (Carvalheiro et al., 2019), we expect that nutrient input will increase visitors' generalization level, increasing network

connectance (reducing network specialization), decreasing interaction evenness and affecting flower visitors and plant generality (plant generality) (expectation 2).

2.2 Material and methods

2.2.1 Study site and experimental design

The study site located at Companhia das Lezírias is a semi-natural Mediterranean grassland, formed by several habitats of which Montado is the predominant type. It is located north-east of Lisbon, Portugal (38º 49′ 45.13" N, 8º 47′ 28.61" W). The climate is Mediterranean with a mean annual rainfall of 537 mm and a mean annual temperature of 14.9ºC, ranging from 10ºC in January to 22.5ºC in August (Nogueira et al., 2019). Site topography is flat and the soil is a well-drained deep Haplic Arenosol (IUSS Working Groups WRB, 2006) with low soil water retention capacity. The grassland community is naturally composed by annual C3 species that grow in winter and early spring, and start senescing in late spring. The vegetation is predominantly composed by Asteraceae species like *Tolpis barbata* L. and *Crepis capillaris* L., the Poaceae *Agrostis pourretii* Willd. and *Avena barbata* Link, the legume *Ornithopus compressus* L., the Boraginaceae *Echium plantagineum* L. and the Brassicaceae *Raphanus raphanistrum* L. (Nogueira et al., 2017).

A nutrient addition field experiment was set up in 2013 and is part of the Nutrient Network (NutNet, a global network of field experiments that tests the effect of multiple nutrients on plant communities, Borer et al., 2014). Three nutrients N, P and K were tested alone and in combination, leading to a total of six treatments: addition of N (N), addition of P (P), addition of K (K), addition of N and P (NP), addition of N, P and K (NPK) and no nutrient added (designated as "Control"). N, P and K were added at a rate of 10 g m^{-2} yr^{-1}. N was applied as time-release urea, P as triple-superphosphate and K as potassium sulphate. In the first year of fertilization (2013), each plot that received K also received 100 g m^{-2} of a nutrient mixture containing iron (15%), sulphur (14%), magnesium (1.5%), manganese (2.5%), copper (1%), zinc (1%), boron (0.2%), and molybdenum (0.05%). Each treatment was replicated 3 times i.e., each treatment was applied to three 25 m^2 plots, arranged in blocks.

2.2.2 Flower and flower visitor surveys

Flower and visitor data were collected in 2021 and 2022 (9^{th} and 10^{th} years of the experiment, respectively), from March to June which corresponds to the main flowering peak. During the flowering season, we visited each plot every 15 to 20 days, in favourable weather conditions for insect activity, i.e., with no rain, wind between 0 and 2 (Beaufort wind scale), and air temperature between 13 and 35 degrees Celsius. In each survey, and within each plot, we identified all vascular plant species and counted all flowers present (excluding Poaceae). Then, visitation to the flowering species was studied using a time-based methodology. For each flowering plant species, a focal spot with 10 to 30 flowers (the exact number was registered) was selected and all the flowers were observed for 10 minutes. All flower visitation events (defined as an insect contacting the reproductive parts of the flower, feeding on nectar or pollen) were registered, and whenever a visitor escaped before being captured (net or entomological aspirator) a detailed description was added. Captured flower visitors were later separated into morphospecies and, whenever possible, identified to species level by a taxonomist (see acknowledgements). Non-captured individuals, whose description did not match any of the collected individuals, were kept in the richness analyses as a separate morphospecies.

2.2.3 Diversity and network measurements

To determine the effects of nutrient enrichment on flower visitation patterns, we used the data collected to build 34 local plant-visitor interaction networks (18 plots, 16 of which were repeated in two years; the remaining two were not sampled in 2021 because non Poaceae were outcompeted by *Avena barbata* in one plot of NP and one plot of NPK). For each plot network we calculated the following six metrics: i) Network size, which is the sum of species in the lower and higher levels of the network; ii) Weighted connectance, which is the number of links per species weighted on the basis of the network flux rate (a higher weighted connectance is usually related to a smaller network size; fewer nodes with better connection); iii) d', which is the degree of interaction specialization at species level based on dominance of species of the lower level (plants); iv) interaction evenness, which indicates how similar are link strength values between plants and flower visitors; v) plant generality, which corresponds to the average number of visitor species attracted to each plant (metric 'vulnerability' in bipartite, but we opted

to call it plant generality as vulnerability only makes sense for herbivores); and vi) pollinator generality, which is the average number of plant species visited by each pollinator species (called 'generality' in bipartite).

2.2.4 Data analysis

When analysing the effect of treatments on the different network metrics it is important to take into account the level of completeness of flower visitor assemblages of each plot. In order to do this, we calculated sampling coverage in each plot network with *iNext* R package (Hsieh et al., 2016) and checked if there were differences in sampling coverage between treatments. We ran a linear mixed model (LMM) for sampling coverage in which year was included as a fixed effect and plot within block as random effects.

As singletons (species with abundance equal to 1) can substantially alter the compositional results, we removed such records for the calculation of dissimilarity analyses. To test if homogeneity in the assemblages was greater in fertilization treatments than in control (expectation 1), we calculated plot-level dissimilarities in visitor species within and between treatments with the Bray Curtis index (vegdist, *vegan* package). Subsequently, we ran two linear models for within and between treatment dissimilarities with year as a fixed effect. Additionally, to know the contribution of each species to the general dissimilarity between two treatments, we ran the "percentage similarity test" (Simper) with *vegan* package. Last, we assessed changes in plant richness, flower abundance and flower visitor abundance and richness (considering all species) by running LMMs with the three nutrients (N, P and K) included as binomial explanatory variables (added vs non-added), year and sampling coverage as fixed effects and plot within block as random effects. Additionally, using the same model structure, we ran LMMs to detect changes in Diptera, Coleoptera and Hymenoptera diversity and proportion. Lepidoptera order was not consider for these analyses due to low diversity.

To test the effect of nutrient enrichment on network size, weighted connectance, degree of specialization, interaction evenness and pollinator and plant generality (expectation 2), we used all species recorded in the study. We first calculated the network metrics with *bipartite* R package and the function networklevel and specieslevel (Dormann et al., 2009). Treatment bipartite networks were plotted with *NetworkD3* package. Then, we ran

LMMs for all network metrics with the three nutrients (N, P and K) included as binomial explanatory variables (added vs non-added), year and sampling coverage included as fixed effect and plot within block as random effects.

All models were fitted using gaussian distribution with the package *lme4* R package (Bates et al., 2014), and *DHARMa* R package (Hartig 2022) was used to verify that residuals were normally distributed. Analyses were performed in R environment (version 4. 0. 3, R development core team). We used *gplots* R package to plot the graphs (Warnes et al., 2020).

2.3 Results

In total, we recorded flower visitation information for 13 flowering plant species (out of 21 flowering plants) and 81 flower visitor species or morphospecies. There were more flower visitor species and more flowering plant species in 2022 than in 2021: in 2021, we recorded 49 visitor species and seven visited plant species while in 2022 we recorded 71 visitor species and 13 visited plant species (Table S2.1). The total number of interaction events between the two years (flower visitation events) was 1271. Each plot-level network had an average of 15 visitor species (varying from 6 to 35 species) and five flowering plant species (varying from 3 to 8 species), with a higher diversity in 2022 than in 2021 (see Table S2.1, Fig S2.1 and S2.2). Plot level visitor sampling coverage varied from 53 to 93% (Table S2.2) with no significant differences detected between treatments (χ^2 test, p-value=0.44). Hymenoptera was the main flower visitor group in terms of abundance and richness. Hymenoptera and Coleoptera were significantly more diverse in 2022 than in 2021, while Diptera diversity did not change between years despite the proportion of this group being significantly higher in 2021 (Table S2.3, Fig. 2.1).

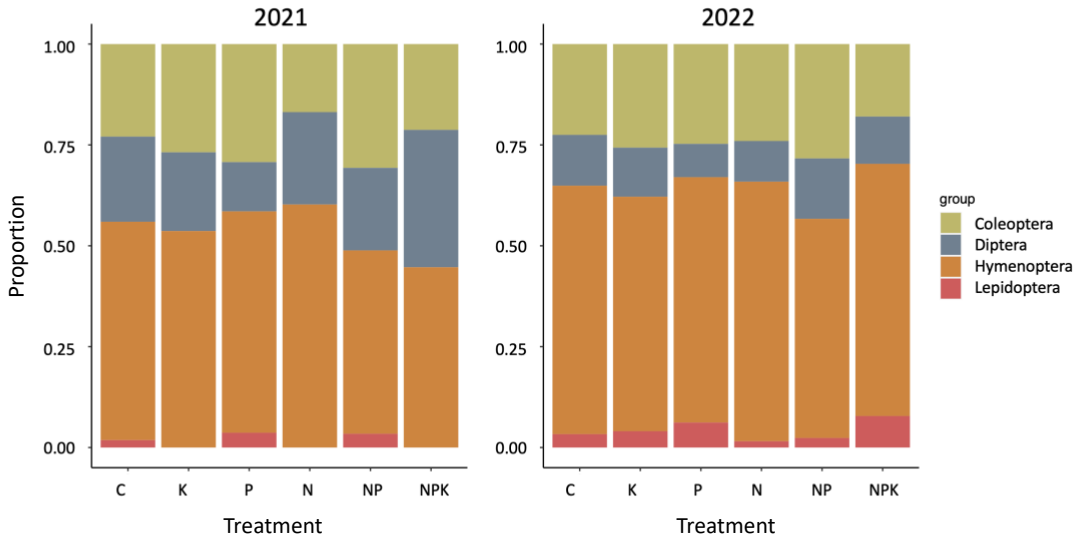


Figure 2.1. Proportions of flower visitor orders in each treatment per year. Colours represent the four orders studied (see legend). For statistical details see Table S2.3.

2.3.1 Effect of nutrient enrichment on flower visitor assemblage homogeneity and richness

Although eighteen visitor species were common to all treatments (Fig. 2.2), flower visitor assemblage composition varied between treatments (i.e. Bray Curtis similarity values between treatments were significantly different to zero, see Table 2.1, Fig 2.3, Table S2.4). On average, treatments where fertilizers were applied only shared 54% of the species with Control plots. However, this difference was mainly driven by P, with all treatments where P was added (NP, NPK and P) having greater differences from Control than other treatments (Fig. 2.3). Flower visitor assemblages were more homogeneous within Control plots (i.e. more species shared between plots) than within the other treatments' plots. Treatments with the greatest within-treatment dissimilarity were NPK and P plots (0.64 and 0.65) (i.e. fewer species shared between plots) (Fig. 2.3, Table 2.1), reporting even higher dissimilarity than comparisons between treatments. In treatments where only P was added, within-treatment dissimilarity was significantly higher than the control's, with P being the treatment with fewest shared species between plots. See the contribution of each species to treatment dissimilarity in Table S2.5.

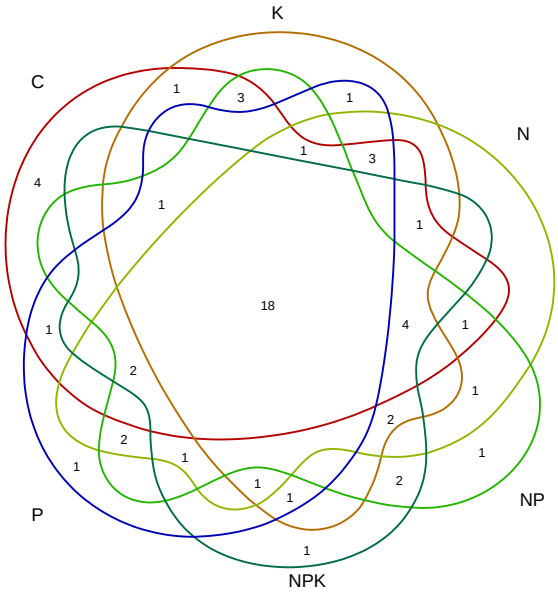


Figure 2.2. Venn diagram representing the shared visitor species between treatments. Each treatment is coded with one colour: red for Control (C), orange for K, lime green for N, mint green for NP, dark green for NPK and blue for P.

Table 2.1. Effect of nutrient enrichment on within (plots from the same treatment) and between (plots from different treatments) treatment dissimilarity.

	Treatment		Year	
	Sum sq	P-value	Sum sq	P-value
Within dissimilarity	0.172	0.04	0.067	0.028
Between dissimilarity	0.219	0.026	0.39	<0.001

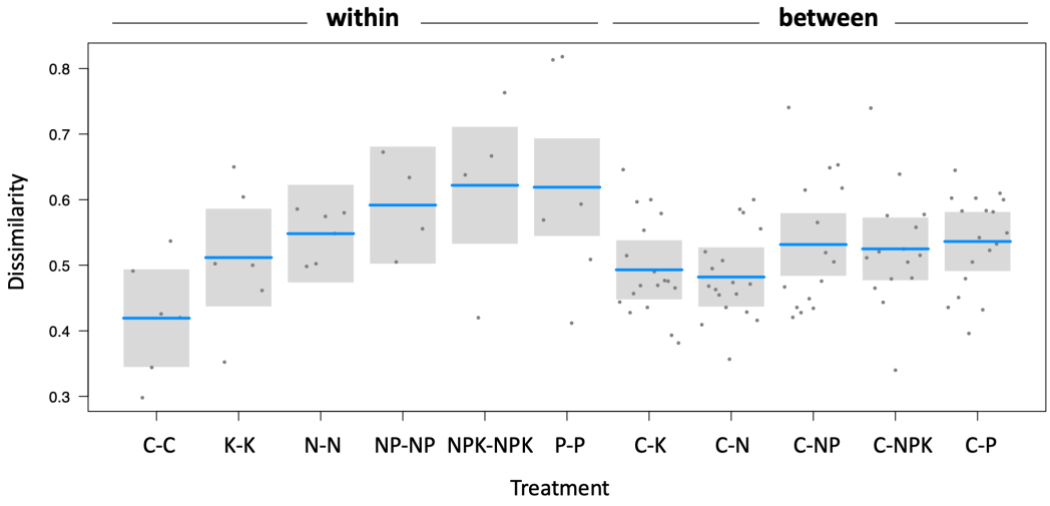


Figure 2.3. Within treatment and between treatment dissimilarities. For statistical details see table 2.1.

Although Control plots were more similar among themselves, this was the treatment with more unique species (four) in relation to other treatments. Other treatments had one (P, NP and NPK) or zero unique species (N, K). Some of the dominant visitor species present in the study region had clear preferences for treatments with N compared to treatments without N (Table S2.6). For instance, *Apis mellifera* (which made up 6% of the total number of visitation events) visited 1.8 times more the treatments with N than those without N, and other bees like *Eucera cineraria* (1.5%) and *Osmia* spp. (0.4%) visited the treatments with N 2.8 and 2.5 times more. The bee species *Andrena fulica* (0.2%) and *A. orana* (0.6%) and the butterfly *Pieris rapae* (0.1%) visited treatments with N almost exclusively. On the other hand, other flower visitor species preferred treatments without N (650 visits, 53%) such as the beetle *Chasmatopterus* sp. which visited the treatments without N 2.3 times more (which made up 8.7% of the total number of visitation events) or the bees *Lasioglossum malachurum* (1.2%) and *Panurgus perezi* (11.3%) with 1.5 and 1.8 times more visits to treatments without N. *Panurgus* calcaratus (2.3%), *L. villosulum* (1%) and the flies *Bombylius minor* (0.4%) and *Microchrysa* sp. (0.5%) only visited treatments without N.

Concerning alpha diversity metrics, the addition of nutrients led to generally fewer plant and flower visitor species (richness and abundance) in comparison with Control plots (Fig 2.4, Table S2.2). Plant richness decreased significantly when N and P were coadded while flower abundance, flower visitor abundance and richness decreased when P or K were added (Fig 2.4, Table S2.3), driven mainly by changes in Diptera diversity but also Hymenoptera (abundance) and Coleoptera (richness). In contrast, the co-addition of N and P increased flower abundance, pollinator abundance and pollinator richness. Network size was affected by the addition of nutrients, with Control networks being significantly bigger than K and P networks (Fig. 2.5, Table S2.2) which recorded fewer flower visitors. The co-addition of N and P significantly increased the size of the network.

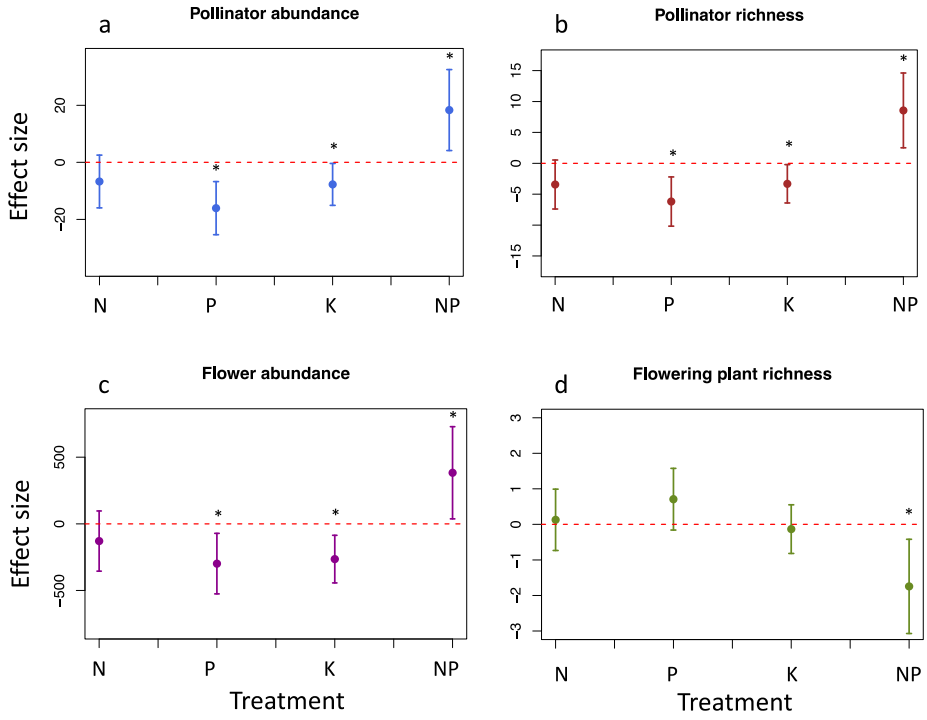


Figure 2.4. Pairwise comparisons between treatments. The values of mean difference between the treatments with fertilizers and control treatment and the associated confidence interval are presented for visitor abundance (a), visitor richness (b) and flower abundance (c) and plant richness (d). Control is represented by the red dotted line. Significant differences in relation to the control are marked (i.e. * p value < 0.05). See statistical details in Table S2.3.

2.3.2 Effect of nutrient enrichment on flower visitor specialization

Nutrient addition significantly affected flower visitation patterns (Table S2.2, S2.3). Despite the increases in richness and abundance, flower visitors that visited plots where N and P were coadded had a more generalized behaviour, these plots having significantly lower flower visitor specialization (d'). Plots within this treatment also had higher interaction evenness and plant generality than control plots. Yet, when P was added alone interaction evenness and plant generality decreased while flower visitor specialization increased (Table S2.2, S2.3, Fig. 2.5). When N and K were added alone no significant effects were detected on network metrics.

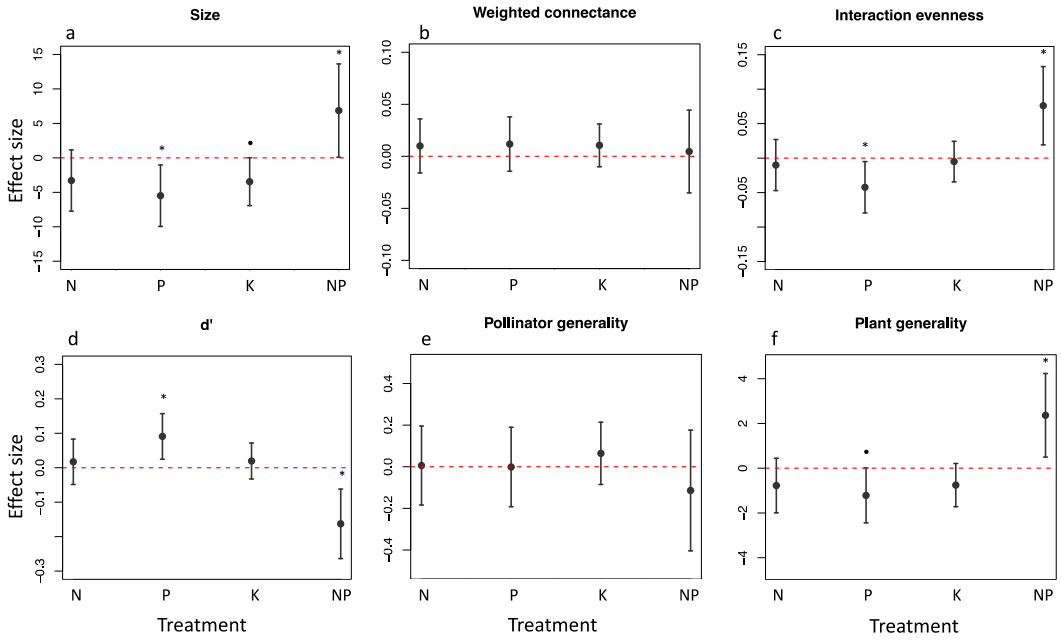


Figure 2.5. Effect size + CI plots of the six network metrics analysed. Treatments are on the x-axis and the effect on each metric on the y-axis. Control is represented in each plot by the red dotted line. Significant effects ($p<0.05$) are marked by (*) and marginally significant effects ($p<0.1$) are marked by (•). See statistical details in Table S2.3.

2.4 Discussion

Studies on the effects of environmental eutrophication on oligotrophic environments are rare and mostly focus on plants. Here we show that increasing NPK soil levels affects foraging patterns of flower visitors, with effects varying between insect orders. As discussed below these findings provide important insights for the understanding of the consequences of global changes in ecosystem functioning.

The fact that flower visitor assemblages detected visiting control plots were more similar (i.e. higher Bray-Curtis similarity values) than those detected within fertilized plots (particularly NPK and P) was not expected. Indeed, we expected that species with low tolerance to nutrient increases would not have visited plant species within fertilized plots, and that reduced sets of species visiting fertilized plots (only tolerant species which are likely wide range generalists) would result in more similar (homogeneous) assemblages. As the total number of species of visitors was similar or even slightly higher in control plots than in plots where nutrients were added (see Table S 2.1) and mean values per plot were higher in control plots (Fig 2.4), it is possible that the high similarity values detected

in control plots are due to high levels of nestedness (i.e. species present in plots that received less visits, e.g. due to lower numbers of flowers, are a subset of those with higher numbers of visits). Decomposing similarity into nestedness and turnover (Baselga, 2010), would help to detect if this last metric is higher in control plots than in plots under fertilization. However, as all plots are located in the same region (i.e. the pool of potential visitors is similar for all plots, which are all near each other) and many plant species occur in different treatments, even if with different frequencies, it is also possible that visitors approach flowers within fertilized plots (being detected as visitors) and dislike them due to changes in their chemistry (Ceulemans et al., 2017c; Vaudo et al., 2022). This may lead to future avoidance of plants with such chemistry, increasing turnover. Further analyses and detailed measurements of floral resource chemistry would be required to disentangle these findings.

When comparing effects of different nutrients and their effects when added in combination we detected that visitor assemblages from plots where only P was added showed the greatest within-treatment dissimilarity (suggesting high turnover) but also were most distinct when compared with other treatments (Table S2.4), suggesting that there is a set of species that was only detected in these treatments. Flower visitation patterns were also distinct for this treatment (higher specialization of plants and pollinators). This uniqueness of P assemblages could be due to a higher diversity of plant species that may include species that are not very attractive, revisitation being avoided by flower visitor species (see next paragraphs). Previous studies with similar methodology (Wang et al., 2022) found higher dissimilarity of plant-pollinator species within NP treatment and not the P treatment, contrasting with our results. This suggests differences in nutrient limitation between Mediterranean grasslands and Tibetan alpine grasslands.

The fact that some species, such as *Apis mellifera*, showed clear preferences for treatments where N was added was expected. Indeed, this species preference for N was also detected in previous studies (Ramos et al., 2018), and many bees are known to prefer to get larval resources from nitrophilous plant species (Carvalheiro et al., 2019). The preferences of *Eucera* spp. and *Pieris rapae* for nitrophilous plants detected in our study were previously reported in Carvalheiro et al. (2019). This choice for N treatments could be caused by inherent preference or by an increased plant generality (attraction) through increasing biomass, flower abundance or morphology (Muñoz et al., 2005; Burkle and

Irwin 2010; Majetic et al., 2017). The preferences could also be caused by changes flower reward chemistry (Cook et al., 2003; Vanderplanck et al., 2014; see Chapter 3). The visitor preference for treatments without N could be related to the presence of plant species that do not appear in fertilization conditions or are less abundant (low N tolerance or nitrophobous) such as certain Asteraceae (Tilman et al., 1999; Nicolson, 2022). Interestingly, some visitor species that in our study site showed preferences for visiting plants within plots with no N added, such as *Lasioglossum* or *Panurgus* species, were reported as oligolectic on nitrophilous plants in Carvalheiro et al. (2019). Our species were reported to prefer Asteraceae (Baldock et al., 2018).

While when added alone the nutrients had a negative impact on flower visitors, the co-addition of N and P increased visitor diversity (Fig 2.4 , leading to a bigger network size, Fig 2.5). Yet, this increase was associated with an increase in the generalization level of visitors (reduced d') and plants (Fig 2.4a, Fig. 2.5f). These results suggest that NP plots attracted a higher number of more generalist visitor species which may be interested in less diverse but more abundant flower assemblages detected in this treatment (Fig 2.4c, d). Such generalist visitor species could be replacing more specialized species. A higher plant generalization was also a result of losing certain plant species (e.g. *Coleostephus myconis* and *Mysopates orontium*, Table S2.7*)*. Indeed, we expected plant richness to decrease in agreement with other studies (Harpole et al., 2016; Villa-Galaviz et al., 2021; Seabloom et al., 2021; Wang et al., 2022), this decrease also affecting pollinators. The coaddition of N and P was also associated with an increased in interaction evenness (Fig. 2.5c, d). We observed a higher number of interactions between *E. plantagineum* and, especially, *R. raphanistrum* with flower visitors when NP was added (Figs. S2.1 and S2.2), leading to a more homogeneous distribution of interactions, increasing interaction evenness. At the same time, the attraction of more generalist species which visit abundant plant species decreased d'. An increase in interaction evenness and a generalized flower visitor assemblage was found in urban compared to agricultural ecosystems (Geslin et al., 2013), hinting that pollinator assemblages generalization is occurring as a result of different pressures (e.g. nutrient enrichment and urbanization).

The reduction of visitor diversity (decreasing network size) associated with P addition was related to a reduction in Diptera diversity (Table S2.3). Flower abundance of certain highly visited plant species such as *Echium plantagineum* and *Raphanus raphanistrum*

declined within P plots. This could explain the lower flower visitor diversity, especially Diptera. Phosphorous was the only nutrient whose addition led plant richness to increase (Fig. 2.4d). This was due to plant species such as *Lathyrus sp.* and *Ornithopus compressus* that were not present in control but occurred in P treatments (Figs. S2.1, S2.2, Table S2.7). This decreased the visitor-plant ratio, and thus plant generality. At the same time, P addition was associated with lower interaction evenness and higher visitor specialization (d', Fig. 2.5c, d). These results suggest that plants grown within P plots attracted fewer flower visitors, but those attracted were more specialized and visited both dominant and scarce plant species (compared to control and NP visitation patterns). As a consequence of this behaviour, interaction patterns were more heterogeneous and specialized, decreasing interaction evenness and increasing d'. A decreased interaction evenness is usually found in modified habitats (Tylianakis et al., 2007; Fisogni et al., 2021). Previous experimental studies done in other parts of the world (Wang et al., 2022), did not detect a negative effect of P on flower abundance or flower visitor diversity, probably because P is not as limiting as N in the system (Tibetan alpine grassland, Gao et al., 2018) but also because it was added in lower quantities (50 kg ha^{-1} vs 100 kg ha^{-1} in our study). However, Mediterranean oligotrophic soils are deficient in both N and P (Dias et al., 2012), so alleviating the limitation of either N or P alone may create stoichiometric imbalances, which may have major negative impacts on the plant community.

As negative effects of the addition of N alone were not detected in our Mediterranean site, it is likely that P is more limiting in the study region, limiting N intake and any subsequent impact. Potassium (K) treatment reduced network size due to a decrease in flower abundance and pollinator diversity but similar to N it did not affect pollinator interactions with plants. Despite changes in flower abundance that affected pollinator diversity, plant richness and interaction networks did not change suggesting that keeping an unaltered plant richness could explain the absence of changes in interaction networks of these treatments.

In general, nutrient enrichment changed how flower visitor perceive flower assemblages. Coaddition of N and P increased plant generality as a result of increasing visitor diversity and reducing plant richness. As those visitors had a generalist preference for floral resources, interaction evenness increased and d' decreased. Higher interaction evenness could be associated with long-term sustainability of the plant-pollinator community

(Tylianakis et al., 2010). On the other hand, in P networks we detected a decrease in plant generality and interaction evenness, as a result of a decrease in visitor diversity, with visitors having a more specialist behaviour which interacted more heterogeneously with plants. This could potentially affect the network tolerance to disturbance (Memmott et al., 2004; Morán-López et al., 2017) as plant pollination depends on fewer insects. In contrast, in host-prey networks a reduced interaction evenness has been proved to increase ecosystem stability (Albrecht et al., 2007).

2.5 Conclusions

In this study we were able to test the effect of multiple nutrient treatments on plant-visitor assemblage diversity and network interactions in a Mediterranean grassland. Beyond changes in the flower-visitor assemblage diversity (quantitative variables that several other studies have assessed) important changes affecting visitor foraging patterns were detected. To improve the understanding of the mechanisms that regulate the additive and interactive effects of nutrient enrichment on flower visitor assemblages, studies on network structure are needed particularly in ecosystems with varying natural levels of nutrient availability. Our results contribute to a better understanding and wider representation of the changes observed; help to predict the impacts of the ongoing global changes of N and P biogeochemical flows on ecosystem functioning; and highlight the need to add environmental eutrophication to the list of important drivers of pollinator declines.

2.6 Supplementary Information

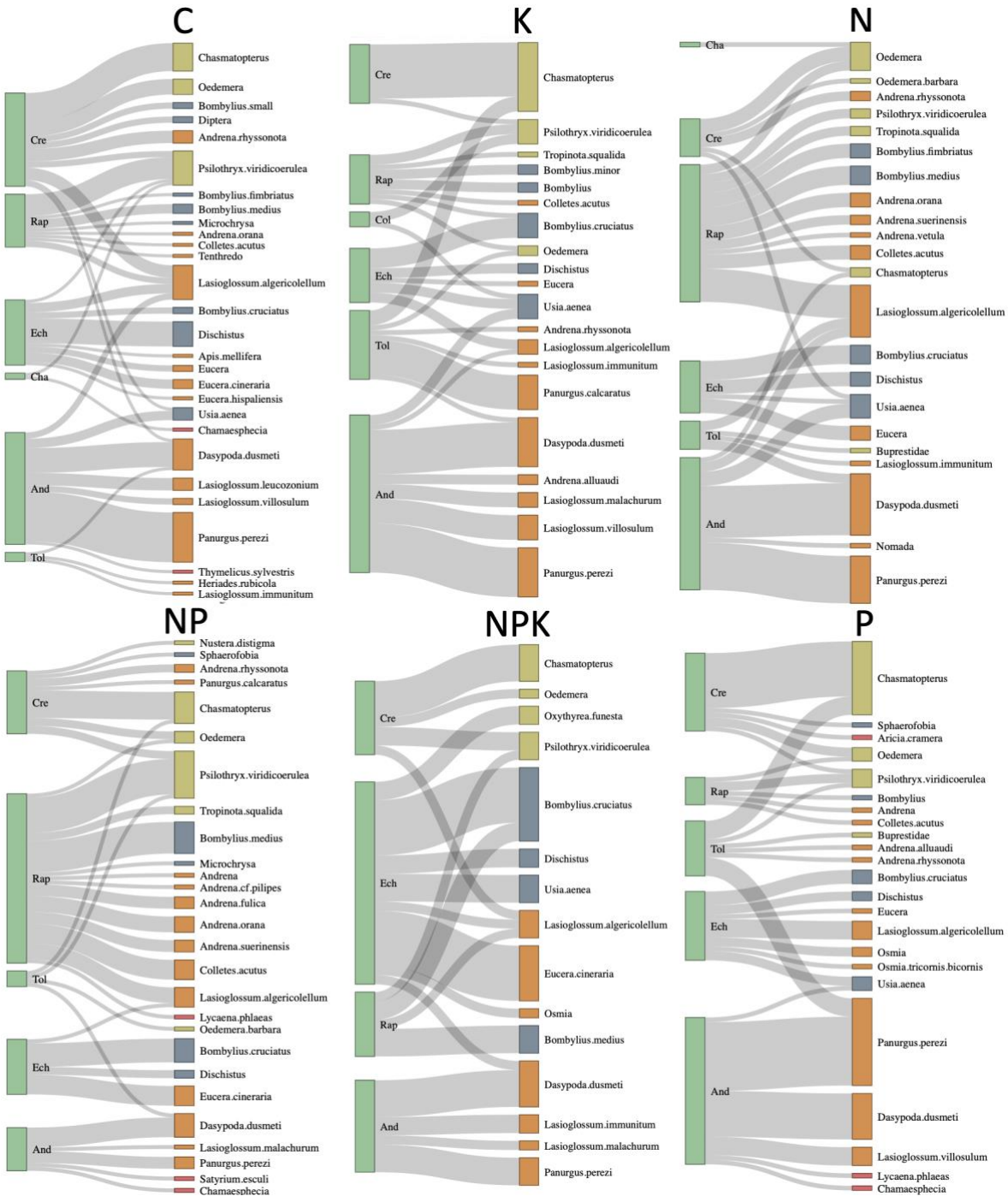


Figure S2.1. Plant-pollinator interaction networks resulting from merging all plots per treatment from 2021. Plant species are place on the left and pollinator species on the right of each network. Plant species were coded as: And= *Andryala integrifolia*. Ech= *Echium plantagineum*. Cre= *Crepis capillaris*. Rap= *Raphanus raphanistrum*. Tol= *Tolpis barbata*. Col= *Coleostephus myconis*. Visitors in orange are bees, in blue are flies, in yellow are beetles and in red are butterflies.

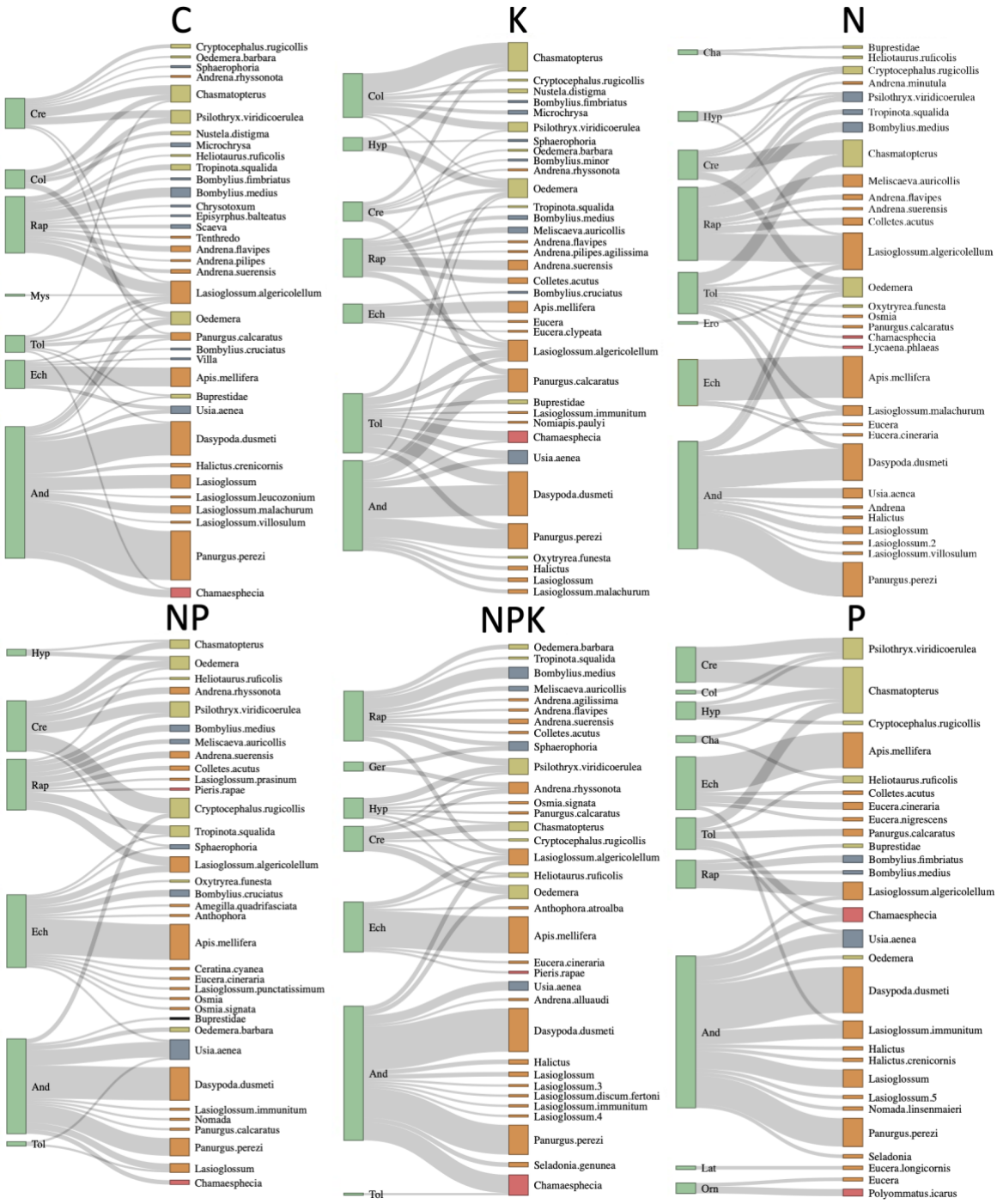


Figure S2.2. Plant-pollinator interaction networks from 2022 for each treatment. Plant species are placed on the left and pollinator species on the right of each network. Plant species were coded as: And= *Andryala integrifolia*. Ech= *Echium plantagineum*. Cre= *Crepis capillaris*. Rap= *Raphanus raphanistrum*. Tol= *Tolpis barbata*. Col= *Coleostephus myconis*. Lat= *Lathyrus sp.*. Mys= *Mysopates orontium*. Hyp= *Hypochaeris sp.*. Ero= *Erodium botrys*. Cha= Chamaemelum sp.. Ger= *Geranium molle* and Orn= *Ornithopus compressus*. Visitors in orange are bees, in blue are flies, in yellow are beetles and in red are butterflies.

Table S2.1. Absolute values (total number per treatment), mean richness values per plot and associated standard deviation (SD) values of plant and visitor species richness and abundance per treatment and year. Total plant richness, visitor richness and visitor abundance are presented per year.

	Plants			**Pollinators**					
	Richness	**Mean per plot**	**SD**	**Richness**	**Mean per plot**	**SD**	**Abundance**	**Mean per plot**	**SD**
Total 2021	7			49			**491**		
C	6	5	0	28	16	0	109	36.3	1.2
K	6	4.7	0.6	20	12.3	0.6	82	27.3	6.1
N	6	4.7	0.6	22	13.3	3.8	83	27.7	12.7
NP	5	4	0	27	17.0	4.2	88	38.5	12.0
NPK	4	3	0	15	10.0	4.2	47	23.5	10.6
P	5	4.3	0.6	23	9.7	3.2	82	27.3	5.5
Total 2022	13			72			**780**		
C	6	4.3	0.6	35	20.3	2.5	151	50.3	3.2
K	6	4.3	0.6	35	20.0	1.0	148	49.3	5.7
N	7	5.0	1.0	31	17.0	2.0	129	43.0	20.1
NP	5	3.7	0.6	35	20.0	4.0	127	42.3	16.8
NPK	6	4.0	1.0	34	16.7	5.1	128	42.7	16.4
P	10	6.7	1.5	28	15.7	4.7	97	32.3	7.0
Total	13			82			**1271**		

Table S2.2. Plot values for all diversity and network metric variables. Variables were coded as: Plant generality (Pla gen), pollinator generality (Poll gen), Sampling completeness (SC), Weighted connectance (Wei con), interaction evenness (Int eve), link density (Lin den), pollinator species (Sp poll), plant species (Sp pla), treatment (treat), flower abundance (flo abu).

Year	Plot	Treat	Block	SC	Size	d'	Pla gen	Poll gen	Wei con	Int eve	Lin den	Sp poll	Sp pla	Abu poll	Flo abu
2021	C1	control	1	0.82	21	0.41	3.79	1.14	0.12	0.61	2.46	16	5	37	1185
2021	C2	control	2	0.84	21	0.37	3.6	1.2	0.11	0.63	2.4	16	5	37	731
2021	C3	control	3	0.72	21	0.29	4.3	1.69	0.14	0.66	2.99	16	5	35	1082
2021	K1	K	1	0.74	18	0.4	4.06	1.28	0.15	0.61	2.67	13	5	26	761
2021	K2	K	2	0.83	17	0.48	2.41	1.39	0.11	0.55	1.91	12	5	34	798
2021	K3	K	3	0.74	16	0.44	3.27	1	0.13	0.6	2.13	12	4	22	833
2021	N1	N	1	0.53	21	0.25	4.14	1.2	0.13	0.64	2.67	16	5	23	747
2021	N2	N	2	0.8	14	0.49	2.62	1.16	0.14	0.57	1.9	9	5	18	850
2021	N3	N	3	0.89	19	0.29	4.94	1.4	0.17	0.67	3.17	15	4	42	883
2021	NP1	NP	1	0.77	18	0.32	3.57	1.08	0.13	0.62	2.33	14	4	30	1203
2021	NP3	NP	3	0.79	24	0.3	7.03	1.13	0.17	0.65	4.08	20	4	47	1250
2021	NPK1	NPK	1	0.88	16	0.29	5.2	1.17	0.2	0.67	3.21	13	3	31	498
2021	NPK3	NPK	3	0.82	10	0.29	2.81	1.42	0.21	0.68	2.12	7	3	16	234
2021	P1	P	1	0.77	17	0.42	3.42	1.24	0.14	0.57	2.34	12	5	30	809
2021	P2	P	2	0.91	10	0.54	1.68	1.49	0.16	0.52	1.6	6	4	31	548
2021	P3	P	3	0.83	15	0.38	3.95	1.12	0.17	0.63	2.54	11	4	21	554
2022	C1	control	1	0.74	26	0.33	3.84	1.2	0.1	0.56	2.52	20	6	49	618
2022	C2	control	2	0.82	28	0.27	6.98	1.29	0.15	0.65	4.14	23	5	54	713
2022	C3	control	3	0.86	23	0.33	5.32	1.42	0.15	0.65	3.38	18	5	48	624
2022	K1	K	1	0.68	24	0.26	4.25	1.34	0.12	0.58	2.8	19	5	43	281
2022	K2	K	2	0.8	26	0.33	5.17	1.42	0.13	0.66	3.3	21	5	54	571
2022	K3	K	3	0.83	26	0.28	4.71	1.7	0.12	0.67	3.21	20	6	51	593
2022	N1	N	1	0.55	22	0.36	3.73	1.09	0.11	0.59	2.41	17	5	29	788
2022	N2	N	2	0.93	26	0.34	4.34	1.35	0.11	0.61	2.85	20	6	66	482
2022	N3	N	3	0.74	22	0.37	3.67	1.58	0.12	0.58	2.63	15	7	34	677
2022	NP1	NP	1	0.79	24	0.28	5.16	1.13	0.13	0.63	3.15	20	4	51	738
2022	NP2	NP	2	0.76	29	0.24	6.13	1.3	0.13	0.65	3.72	24	5	53	552
2022	NP3	NP	3	0.53	21	0.27	4.04	1.23	0.13	0.65	2.64	16	5	23	498
2022	NPK1	NPK	1	0.86	24	0.37	4.61	1.26	0.12	0.6	2.93	18	6	49	800
2022	NPK2	NPK	2	0.84	26	0.33	4.7	1.13	0.11	0.6	2.92	21	5	55	657
2022	NPK3	NPK	3	0.85	15	0.22	4.31	1.62	0.2	0.7	2.97	11	4	24	192
2022	P1	P	1	0.64	29	0.36	6.16	1.2	0.13	0.57	3.68	21	8	39	618
2022	P2	P	2	0.85	18	0.42	2.8	1.48	0.12	0.59	2.14	12	6	33	428
2022	P3	P	3	0.82	22	0.48	2.82	1.35	0.09	0.6	2.09	14	8	25	433

Table S2.3. Effect of nutrient enrichment on plant and flower visitor diversity and network metrics. Linear mixed models were run for all variables. P-values were obtained with log-likelihood ratio tests. Nutrient treatments are represented in each column. SC refers to sampling completeness.

	N		**P**		**K**		**NP**		**YEAR**		**SC**	
	Chi sq	P-value	Chi sq	P-value	Chi sq	P-value	Chi sq	P-value	Chi sq	P-value	Chi sq	P-value
Flower abundance	1.3	0.2619	6.6	0.0100	8.5	0.0036	4.7	0.0299	13.4	0.0002	0.0	0.8883
Plant richness	0.1	0.7699	2.6	0.1100	0.1	0.7039	6.7	0.0099	21.6	0.0000	0.7	0.4097
Pollinator abundance	2.0	0.1552	11.4	0.0007	4.3	0.0392	6.4	0.0114	23.6	0.0000	12.1	0.0005
Pollinator richness	2.9	0.0897	9.3	0.0023	4.4	0.0363	7.7	0.0056	28.7	0.0000	0.0	0.8288
Hymenoptera proportion	0.2	0.6370	0.0	0.8295	0.1	0.8112	0.3	0.5618	4.2	0.0394	0.0	0.8408
Diptera proportion	0.0	0.8389	0.4	0.5029	0.4	0.5413	1.4	0.2410	11.4	0.0007	1.4	0.2410
Coleoptera proportion	0.1	0.7266	0.2	0.6607	0.0	0.9995	0.0	0.9754	0.0	0.8534	0.7	0.3963
Hymenoptera abundance	0.0	0.8345	3.5	0.0619	1.2	0.2789	0.9	0.3438	59.8	0.0000	20.8	0.0000
Diptera abundance	1.1	0.2905	8.4	0.0038	1.3	0.2603	7.6	0.0057	1.8	0.1782	0.6	0.4479
Coleoptera abundance	1.6	0.2130	2.2	0.1393	1.9	0.1732	1.8	0.1745	5.6	0.0179	4.2	0.0407
Hymenoptera richness	1.0	0.3190	1.9	0.1635	0.5	0.4725	1.5	0.2170	31.8	0.0000	0.1	0.7761
Diptera richness	7.5	0.0061	22.9	0.0000	4.8	0.0281	15.1	0.0001	0.7	0.4096	1.0	0.3181
Coleoptera richness	0.2	0.6714	5.6	0.0175	2.3	0.1333	3.4	0.0648	11.6	0.0007	0.1	0.7083
Network size	2.1	0.1461	5.8	0.0160	3.8	0.0510	3.9	0.0472	40.1	0.0000	0.0	0.9521
Weighted connectance	0.6	0.4500	0.8	0.3764	1.0	0.3137	0.1	0.8209	8.1	0.0045	1.1	0.2923
Interaction evenness	0.3	0.5919	5.0	0.0258	0.1	0.7375	6.9	0.0087	0.0	0.9687	0.2	0.6203
d’ species specialization	0.3	0.6075	7.3	0.0071	0.5	0.4642	10.0	0.0016	3.3	0.0696	1.7	0.1944
Pollinator generality	0.0	0.9500	0.0	0.9913	0.7	0.3986	0.6	0.4427	3.7	0.0543	1.2	0.2689
Plant generality	1.6	0.2126	3.8	0.0516	2.3	0.1255	6.2	0.0131	3.5	0.0606	0.0	0.9589

Table S2.4. Bray Curtis dissimilarity values (Diss.) obtained in between and within treatment comparisons (Com.) in 2021 and 2022. Within comparisons are coded with a "w" and between comparisons with a "b".

Year	Plot 1	Plot 2	Treatment	Diss.	Com.	Year	Plot 1	Plot 2	Treatment	Diss.	Com.
2021	C1	C2	C	0.556	w	2021	C2	N3	C-N	0.519	b
2021	C1	C3	C	0.362	w	2021	C2	NP1	C-NP	0.485	b
2021	C2	C3	C	0.408	w	2021	C2	NP3	C-NP	0.500	b
2021	K1	K2	K	0.567	w	2021	C2	NPK1	C-NPK	0.529	b
2021	K1	K3	K	0.417	w	2021	C2	NPK3	C-NPK	0.585	b
2021	K2	K3	K	0.714	w	2021	C2	P1	C-P	0.515	b
2021	N1	N2	N	0.650	w	2021	C2	P2	C-P	0.647	b
2021	N1	N3	N	0.563	w	2021	C2	P3	C-P	0.544	b
2021	N2	N3	N	0.567	w	2021	C3	K1	C-K	0.533	b
2021	NP1	NP3	NP	0.737	w	2021	C3	K2	C-K	0.618	b
2021	NPK1	NPK3	NPK	0.702	w	2021	C3	K3	C-K	0.500	b
2021	P1	P2	P	0.633	w	2021	C3	N1	C-N	0.571	b
2021	P1	P3	P	0.878	w	2021	C3	N2	C-N	0.500	b
2021	P2	P3	P	0.882	w	2021	C3	N3	C-N	0.421	b
2022	C1	C2	C	0.426	w	2021	C3	NP1	C-NP	0.492	b
2022	C1	C3	C	0.537	w	2021	C3	NP3	C-NP	0.679	b
2022	C2	C3	C	0.420	w	2021	C3	NPK1	C-NPK	0.508	b
2022	K1	K2	K	0.604	w	2021	C3	NPK3	C-NPK	0.640	b
2022	K1	K3	K	0.500	w	2021	C3	P1	C-P	0.460	b
2022	K2	K3	K	0.462	w	2021	C3	P2	C-P	0.569	b
2022	N1	N2	N	0.574	w	2021	C3	P3	C-P	0.667	b
2022	N1	N3	N	0.548	w	2021	K1	N1	K-N	0.500	b
2022	N2	N3	N	0.580	w	2021	K1	N2	K-N	0.591	b
2022	NP1	NP2	NP	0.505	w	2021	K1	N3	K-N	0.500	b
2022	NP1	NP3	NP	0.634	w	2021	K1	NP1	K-NP	0.455	b
2022	NP2	NP3	NP	0.556	w	2021	K1	NP3	K-NP	0.781	b
2022	NPK1	NPK2	NPK	0.420	w	2021	K1	NPK1	K-NPK	0.474	b
2022	NPK1	NPK3	NPK	0.667	w	2021	K1	NPK3	K-NPK	0.667	b
2022	NPK2	NPK3	NPK	0.763	w	2021	K1	P1	K-P	0.455	b
2022	P1	P2	P	0.412	w	2021	K1	P2	K-P	0.719	b
2022	P1	P3	P	0.593	w	2021	K1	P3	K-P	0.652	b
2022	P2	P3	P	0.509	w	2021	K2	N1	K-N	0.679	b
2021	C1	K1	C-K	0.508	b	2021	K2	N2	K-N	0.692	b
2021	C1	K2	C-K	0.710	b	2021	K2	N3	K-N	0.763	b
2021	C1	K3	C-K	0.579	b	2021	K2	NP1	K-NP	0.651	b
2021	C1	N1	C-N	0.474	b	2021	K2	NP3	K-NP	0.679	b
2021	C1	N2	C-N	0.585	b	2021	K2	NPK1	K-NPK	0.723	b
2021	C1	N3	C-N	0.532	b	2021	K2	NPK3	K-NPK	0.680	b
2021	C1	NP1	C-NP	0.531	b	2021	K2	P1	K-P	0.651	b
2021	C1	NP3	C-NP	0.805	b	2021	K2	P2	K-P	0.477	b
2021	C1	NPK1	C-NPK	0.576	b	2021	K2	P3	K-P	0.741	b
2021	C1	NPK3	C-NPK	0.804	b	2021	K3	N1	K-N	0.682	b
2021	C1	P1	C-P	0.500	b	2021	K3	N2	K-N	0.550	b
2021	C1	P2	C-P	0.667	b	2021	K3	N3	K-N	0.563	b
2021	C1	P3	C-P	0.709	b	2021	K3	NP1	K-NP	0.529	b
2021	C2	K1	C-K	0.492	b	2021	K3	NP3	K-NP	0.797	b
2021	C2	K2	C-K	0.521	b	2021	K3	NPK1	K-NPK	0.472	b
2021	C2	K3	C-K	0.661	b	2021	K3	NPK3	K-NPK	0.737	b
2021	C2	N1	C-N	0.559	b	2021	K3	P1	K-P	0.569	b
2021	C2	N2	C-N	0.527	b	2021	K3	P2	K-P	0.698	b

Year	Plot 1	Plot 2	Treatment	Diss.	Com.	Year	Plot 1	Plot 2	Treatment	Diss.	Com.
2021	K3	P3	K-P	0.762	b	2022	C2	N1	C-N	0.580	b
2021	N1	NP1	N-NP	0.608	b	2022	C2	N2	C-N	0.429	b
2021	N1	NP3	N-NP	0.652	b	2022	C2	N3	C-N	0.471	b
2021	N1	NPK1	N-NPK	0.585	b	2022	C2	NP1	C-NP	0.476	b
2021	N1	NPK3	N-NPK	0.632	b	2022	C2	NP2	C-NP	0.519	b
2021	N1	P1	N-P	0.725	b	2022	C2	NP3	C-NP	0.649	b
2021	N1	P2	N-P	0.774	b	2022	C2	NPK1	C-NPK	0.525	b
2021	N1	P3	N-P	0.524	b	2022	C2	NPK2	C-NPK	0.505	b
2021	N2	NP1	N-NP	0.702	b	2022	C2	NPK3	C-NPK	0.481	b
2021	N2	NP3	N-NP	0.692	b	2022	C2	P1	C-P	0.523	b
2021	N2	NPK1	N-NPK	0.673	b	2022	C2	P2	C-P	0.581	b
2021	N2	NPK3	N-NPK	0.765	b	2022	C2	P3	C-P	0.532	b
2021	N2	P1	N-P	0.702	b	2022	C3	K1	C-K	0.393	b
2021	N2	P2	N-P	0.673	b	2022	C3	K2	C-K	0.465	b
2021	N2	P3	N-P	0.684	b	2022	C3	K3	C-K	0.381	b
2021	N3	NP1	N-NP	0.577	b	2022	C3	N1	C-N	0.600	b
2021	N3	NP3	N-NP	0.551	b	2022	C3	N2	C-N	0.416	b
2021	N3	NPK1	N-NPK	0.507	b	2022	C3	N3	C-N	0.556	b
2021	N3	NPK3	N-NPK	0.690	b	2022	C3	NP1	C-NP	0.505	b
2021	N3	P1	N-P	0.606	b	2022	C3	NP2	C-NP	0.653	b
2021	N3	P2	N-P	0.699	b	2022	C3	NP3	C-NP	0.618	b
2021	N3	P3	N-P	0.581	b	2022	C3	NPK1	C-NPK	0.558	b
2021	NP1	NPK1	NP-NPK	0.333	b	2022	C3	NPK2	C-NPK	0.515	b
2021	NP1	NPK3	NP-NPK	0.600	b	2022	C3	NPK3	C-NPK	0.577	b
2021	NP1	P1	NP-P	0.448	b	2022	C3	P1	C-P	0.610	b
2021	NP1	P2	NP-P	0.700	b	2022	C3	P2	C-P	0.600	b
2021	NP1	P3	NP-P	0.714	b	2022	C3	P3	C-P	0.549	b
2021	NP3	NPK1	NP-NPK	0.769	b	2022	K1	N1	K-N	0.600	b
2021	NP3	NPK3	NP-NPK	0.651	b	2022	K1	N2	K-N	0.426	b
2021	NP3	P1	NP-P	0.868	b	2022	K1	N3	K-N	0.632	b
2021	NP3	P2	NP-P	0.769	b	2022	K1	NP1	K-NP	0.565	b
2021	NP3	P3	NP-P	0.642	b	2022	K1	NP2	K-NP	0.656	b
2021	NPK1	P1	NPK-P	0.633	b	2022	K1	NP3	K-NP	0.619	b
2021	NPK1	P2	NPK-P	0.742	b	2022	K1	NPK1	K-NPK	0.600	b
2021	NPK1	P3	NPK-P	0.569	b	2022	K1	NPK2	K-NPK	0.596	b
2021	NPK3	P1	NPK-P	0.733	b	2022	K1	NPK3	K-NPK	0.667	b
2021	NPK3	P2	NPK-P	0.830	b	2022	K1	P1	K-P	0.558	b
2021	NPK3	P3	NPK-P	0.556	b	2022	K1	P2	K-P	0.520	b
2022	C1	K1	C-K	0.600	b	2022	K1	P3	K-P	0.576	b
2022	C1	K2	C-K	0.490	b	2022	K2	N1	K-N	0.537	b
2022	C1	K3	C-K	0.469	b	2022	K2	N2	K-N	0.533	b
2022	C1	N1	C-N	0.474	b	2022	K2	N3	K-N	0.545	b
2022	C1	N2	C-N	0.456	b	2022	K2	NP1	K-NP	0.462	b
2022	C1	N3	C-N	0.585	b	2022	K2	NP2	K-NP	0.562	b
2022	C1	NP1	C-NP	0.449	b	2022	K2	NP3	K-NP	0.600	b
2022	C1	NP2	C-NP	0.434	b	2022	K2	NPK1	K-NPK	0.490	b
2022	C1	NP3	C-NP	0.565	b	2022	K2	NPK2	K-NPK	0.396	b
2022	C1	NPK1	C-NPK	0.479	b	2022	K2	NPK3	K-NPK	0.692	b
2022	C1	NPK2	C-NPK	0.340	b	2022	K2	P1	K-P	0.483	b
2022	C1	NPK3	C-NPK	0.639	b	2022	K2	P2	K-P	0.517	b
2022	C1	P1	C-P	0.542	b	2022	K2	P3	K-P	0.615	b
2022	C1	P2	C-P	0.432	b	2022	K3	N1	K-N	0.513	b
2022	C1	P3	C-P	0.583	b	2022	K3	N2	K-N	0.466	b
2022	C2	K1	C-K	0.579	b	2022	K3	N3	K-N	0.429	b
2022	C2	K2	C-K	0.477	b	2022	K3	NP1	K-NP	0.460	b
2022	C2	K3	C-K	0.476	b	2022	K3	NP2	K-NP	0.564	b

Year	Plot 1	Plot 2	Treatment	Diss.	Com.
2022	K3	NP3	K-NP	0.521	b
2022	K3	NPK1	K-NPK	0.571	b
2022	K3	NPK2	K-NPK	0.490	b
2022	K3	NPK3	K-NPK	0.514	b
2022	K3	P1	K-P	0.529	b
2022	K3	P2	K-P	0.566	b
2022	K3	P3	K-P	0.568	b
2022	N1	NP1	N-NP	0.538	b
2022	N1	NP2	N-NP	0.494	b
2022	N1	NP3	N-NP	0.469	b
2022	N1	NPK1	N-NPK	0.500	b
2022	N1	NPK2	N-NPK	0.450	b
2022	N1	NPK3	N-NPK	0.692	b
2022	N1	P1	N-P	0.524	b
2022	N1	P2	N-P	0.443	b
2022	N1	P3	N-P	0.615	b
2022	N2	NP1	N-NP	0.397	b
2022	N2	NP2	N-NP	0.419	b
2022	N2	NP3	N-NP	0.678	b
2022	N2	NPK1	N-NPK	0.456	b
2022	N2	NPK2	N-NPK	0.390	b
2022	N2	NPK3	N-NPK	0.622	b
2022	N2	P1	N-P	0.545	b
2022	N2	P2	N-P	0.434	b
2022	N2	P3	N-P	0.644	b
2022	N3	NP1	N-NP	0.619	b
2022	N3	NP2	N-NP	0.576	b
2022	N3	NP3	N-NP	0.564	b
2022	N3	NPK1	N-NPK	0.610	b
2022	N3	NPK2	N-NPK	0.605	b
2022	N3	NPK3	N-NPK	0.621	b
2022	N3	P1	N-P	0.623	b
2022	N3	P2	N-P	0.582	b
2022	N3	P3	N-P	0.621	b
2022	NP1	NPK1	NP-NPK	0.327	b
2022	NP1	NPK2	NP-NPK	0.373	b
2022	NP1	NPK3	NP-NPK	0.595	b
2022	NP1	P1	NP-P	0.388	b
2022	NP1	P2	NP-P	0.518	b
2022	NP1	P3	NP-P	0.595	b
2022	NP2	NPK1	NP-NPK	0.495	b
2022	NP2	NPK2	NP-NPK	0.456	b
2022	NP2	NPK3	NP-NPK	0.627	b
2022	NP2	P1	NP-P	0.558	b
2022	NP2	P2	NP-P	0.500	b
2022	NP2	P3	NP-P	0.627	b
2022	NP3	NPK1	NP-NPK	0.594	b
2022	NP3	NPK2	NP-NPK	0.644	b
2022	NP3	NPK3	NP-NPK	0.556	b
2022	NP3	P1	NP-P	0.607	b
2022	NP3	P2	NP-P	0.556	b
2022	NP3	P3	NP-P	0.600	b
2022	NPK1	P1	NPK-P	0.494	b
2022	NPK1	P2	NPK-P	0.481	b
2022	NPK1	P3	NPK-P	0.667	b
2022	NPK2	P1	NPK-P	0.494	b
2022	NPK2	P2	NPK-P	0.459	b
2022	NPK2	P3	NPK-P	0.553	b
2022	NPK3	P1	NPK-P	0.593	b
2022	NPK3	P2	NPK-P	0.754	b
2022	NPK3	P3	NPK-P	0.542	b

Table S2.5. Visitor species contribution to treatment dissimilarities. Visitors are listed from higher to lower contribution to dissimilarity. P values show if the contribution is significant (“**”<0.01, “*”<0.05, "•”<0.1). Standard deviation is coded as SD. Species average with 0 value were removed from each treatment comparison list. ava and avb are the average abundances per group. cumsum is the ordered cumulative contribution.

Comparison C and K

	average	sd	ratio	ava	avb	cumsum	p	
Chasmatopterus	0.058909	0.051997	1.1329	3.0000	4.8333	0.1041	0.310	
Panurgus.perezi	0.041542	0.030769	1.3501	7.0000	3.8333	0.1775	0.753	
Dasypoda.dusmeti	0.037027	0.033834	1.0944	4.6667	5.5000	0.2430	0.900	
Panurgus.calcaratus	0.033802	0.024561	1.3763	0.6667	3.1667	0.3027	0.006	**
Lasioglossum.algericolellum	0.033599	0.015988	2.1015	3.8333	2.3333	0.3621	0.406	
Apis.mellifera	0.025812	0.035173	0.7339	1.8333	1.0000	0.4077	0.925	
Oedemera.sp	0.025081	0.016069	1.5608	2.0000	2.0000	0.4521	0.579	
Usia.aenea	0.023131	0.019310	1.1979	1.3333	2.0000	0.4930	0.439	
Psilothryx.viridicoerulea	0.022854	0.016069	1.4223	3.0000	1.6667	0.5334	0.857	
Chamaesphecia	0.018914	0.022810	0.8292	1.0000	1.0000	0.5668	0.392	
Dischistus.sp.	0.018212	0.016400	1.1105	1.3333	0.3333	0.5990	0.108	
Bombylius.medius	0.015278	0.012493	1.2229	1.3333	0.3333	0.6260	0.805	
Lasioglossum	0.013826	0.014565	0.9493	1.1667	0.3333	0.6504	0.560	
Lasioglossum.villosulum	0.013589	0.015750	0.8628	0.5000	0.8333	0.6744	0.282	
Bombylius.cruciatus	0.011409	0.010318	1.1057	0.5000	1.0000	0.6946	0.944	
Lasioglossum.leucozonium	0.011390	0.015515	0.7341	0.8333	0.0000	0.7147	0.006	**
Andrena.rhyssonota	0.011170	0.013896	0.8038	0.8333	0.3333	0.7345	0.650	
Andrena.suerinensis	0.010606	0.013345	0.7948	0.3333	0.8333	0.7532	0.569	
Lasioglossum.malachurum	0.010411	0.010170	1.0237	0.6667	0.8333	0.7716	0.361	
Bombylius.minor	0.008507	0.010064	0.8453	0.3333	0.5000	0.7867	0.098	.
Colletes.acutus	0.008085	0.008425	0.9597	0.1667	0.6667	0.8009	0.805	
Microchrysa	0.007547	0.008763	0.8612	0.5000	0.3333	0.8143	0.058	.
Tropinota.squalida	0.007390	0.008250	0.8958	0.5000	0.3333	0.8273	0.740	
Eucera.cineraria	0.006837	0.015742	0.4343	0.5000	0.0000	0.8394	0.769	
Andrena.flavipes	0.006490	0.008561	0.7581	0.5000	0.1667	0.8509	0.206	
Black.buprestidae	0.006273	0.007775	0.8069	0.3333	0.3333	0.8620	0.468	
Eucera	0.005790	0.006808	0.8505	0.3333	0.3333	0.8722	0.626	
Meliscaeva.auricollis	0.005624	0.008605	0.6535	0.0000	0.5000	0.8822	0.740	
Nustera.distigma	0.005401	0.006208	0.8700	0.3333	0.3333	0.8917	0.073	.
Andrena.alluaudi	0.005248	0.011990	0.4377	0.0000	0.3333	0.9010	0.346	
Cryptocephalus.rugicollis	0.005132	0.008487	0.6047	0.3333	0.1667	0.9101	0.806	
Lasioglossum.immunitum	0.005120	0.006720	0.7619	0.1667	0.3333	0.9191	0.769	
Bombylius.fimbriatus	0.004893	0.006379	0.7670	0.3333	0.1667	0.9278	0.606	
Diptero	0.004690	0.010807	0.4340	0.3333	0.0000	0.9360	0.233	
Bombylius.sp	0.004670	0.006765	0.6903	0.0000	0.3333	0.9443	0.210	
Tenthredo	0.004208	0.006199	0.6788	0.3333	0.0000	0.9517	0.034	*
Halictus.crenicornis	0.003999	0.009176	0.4358	0.3333	0.0000	0.9588	0.259	
Scaeva	0.003862	0.005622	0.6871	0.3333	0.0000	0.9656	0.033	*
Halictus	0.003731	0.005396	0.6915	0.0000	0.3333	0.9722	0.567	
Oedemera.barbara	0.003225	0.005328	0.6053	0.1667	0.1667	0.9779	0.834	
Sphaerophoria	0.003171	0.005244	0.6046	0.1667	0.1667	0.9835	0.869	
Andrena.pilipes	0.003084	0.005080	0.6071	0.1667	0.1667	0.9890	0.282	
Andrena.orana	0.002379	0.005485	0.4338	0.1667	0.0000	0.9932	0.843	
Oxythyrea.funesta	0.001991	0.004535	0.4390	0.0000	0.1667	0.9967	0.761	
Heliotaurus.ruficolis	0.001863	0.004268	0.4365	0.1667	0.0000	1.0000	0.870	

Comparison C and N

	average	sd	ratio	ava	avb	cumsum	p	
Panurgus.perezi	0.044549	0.040620	1.0967	7.0000	4.0000	0.08099	0.666	
Apis.mellifera	0.041548	0.044898	0.9254	1.8333	2.8333	0.15652	0.531	
Dasypoda.dusmeti	0.040158	0.029837	1.3459	4.6667	4.6667	0.22953	0.823	
Chasmatopterus	0.039425	0.025379	1.5534	3.0000	2.1667	0.30120	0.753	
Lasioglossum.algericolellum	0.032592	0.028098	1.1599	3.8333	4.3333	0.36045	0.464	
Psilothryx.viridicoerulea	0.030391	0.019816	1.5337	3.0000	1.0000	0.41570	0.523	
Oedemera.sp	0.023065	0.017795	1.2962	2.0000	2.3333	0.45763	0.766	
Dischistus.sp.	0.019085	0.018239	1.0464	1.3333	0.5000	0.49233	0.088	.
Lasioglossum	0.017046	0.017735	0.9611	1.1667	0.6667	0.52332	0.235	
Bombylius.medius	0.014907	0.010035	1.4855	1.3333	1.3333	0.55042	0.815	
Usia.aenea	0.014065	0.011781	1.1939	1.3333	1.5000	0.57599	0.967	
Andrena.rhyssonota	0.013525	0.015652	0.8641	0.8333	0.3333	0.60058	0.483	
Chamaesphecia	0.012393	0.016488	0.7517	1.0000	0.1667	0.62311	0.768	
Colletes.acutus	0.012266	0.010371	1.1828	0.1667	1.0000	0.64541	0.491	
Lasioglossum.leucozonium	0.012076	0.016627	0.7263	0.8333	0.0000	0.66736	0.006	**
Lasioglossum.malachurum	0.011019	0.010408	1.0587	0.6667	0.6667	0.68740	0.288	
Bombylius.cruciatus	0.010838	0.011187	0.9688	0.5000	0.6667	0.70710	0.953	
Meliscaeva.auricollis	0.009892	0.010735	0.9215	0.0000	0.8333	0.72508	0.207	
Eucera	0.009784	0.010482	0.9334	0.3333	0.6667	0.74287	0.219	
Tropinota.squalida	0.009717	0.007582	1.2817	0.5000	0.6667	0.76054	0.470	
Eucera.cineraria	0.008788	0.015873	0.5536	0.5000	0.1667	0.77651	0.729	
Andrena.suerinensis	0.008590	0.011020	0.7795	0.3333	0.5000	0.79213	0.765	
Panurgus.calcaratus	0.008326	0.009469	0.8793	0.6667	0.1667	0.80727	0.896	
Bombylius.fimbriatus	0.008025	0.008918	0.8999	0.3333	0.5000	0.82186	0.179	
Andrena.orana	0.007772	0.013080	0.5942	0.1667	0.5000	0.83599	0.391	
Andrena.flavipes	0.007643	0.009887	0.7731	0.5000	0.3333	0.84988	0.118	
Cryptocephalus.rugicollis	0.007602	0.012076	0.6295	0.3333	0.5000	0.86370	0.636	
Lasioglossum.villosulum	0.007447	0.010953	0.6799	0.5000	0.1667	0.87724	0.655	
Microchrysa	0.006618	0.010221	0.6475	0.5000	0.0000	0.88927	0.155	
Black.buprestidae	0.006095	0.007223	0.8439	0.3333	0.3333	0.90035	0.494	
Bombylius.minor	0.005021	0.007413	0.6773	0.3333	0.0000	0.90948	0.363	
Diptero	0.004981	0.011559	0.4309	0.3333	0.0000	0.91854	0.140	
Tenthredo	0.004440	0.006600	0.6727	0.3333	0.0000	0.92661	0.017	*
Halictus.crenicornis	0.004202	0.009696	0.4334	0.3333	0.0000	0.93425	0.209	
Nustera.distigma	0.004175	0.006117	0.6826	0.3333	0.0000	0.94184	0.325	
Scaeva	0.004051	0.005935	0.6825	0.3333	0.0000	0.94920	0.023	*
Oedemera.barbara	0.003905	0.006557	0.5955	0.1667	0.1667	0.95630	0.752	
Lasioglossum.immunitum	0.003790	0.006408	0.5914	0.1667	0.1667	0.96319	0.883	
Heliotaurus.ruficolis	0.003514	0.005834	0.6024	0.1667	0.1667	0.96958	0.663	
Nomada	0.002624	0.005995	0.4377	0.0000	0.1667	0.97435	0.335	
Halictus	0.002395	0.005466	0.4382	0.0000	0.1667	0.97871	0.748	
Oxythyrea.funesta	0.002395	0.005466	0.4382	0.0000	0.1667	0.98306	0.650	
Lycaena.phlaeas	0.002203	0.005023	0.4386	0.0000	0.1667	0.98707	0.588	
Sphaerophoria	0.002074	0.004784	0.4336	0.1667	0.0000	0.99084	0.924	
Andrena.pilipes	0.001950	0.004489	0.4343	0.1667	0.0000	0.99438	0.572	
Andrena	0.001545	0.003514	0.4398	0.0000	0.1667	0.99719	0.708	
Osmia	0.001545	0.003514	0.4398	0.0000	0.1667	1.00000	0.849	

Comparison C and NP

	average	sd	ratio	ava	avb	cumsum	p	
Panurgus.perezi	0.071009	0.041420	1.7144	7.0000	1.8333	0.1131	0.080	.
Dasypoda.dusmeti	0.045220	0.038685	1.1689	4.6667	3.5000	0.1851	0.663	
Apis.mellifera	0.039668	0.044704	0.8874	1.8333	2.6667	0.2483	0.623	
Psilothryx.viridicoerulea	0.035980	0.032338	1.1126	3.0000	3.1667	0.3056	0.367	
Chasmatopterus	0.032903	0.027091	1.2145	3.0000	2.0000	0.3580	0.857	
Lasioglossum.algericolellum	0.030241	0.025647	1.1791	3.8333	2.0000	0.4061	0.533	
Oedemera.sp	0.022515	0.019545	1.1519	2.0000	1.5000	0.4420	0.783	
Bombylius.medius	0.021099	0.018940	1.1140	1.3333	1.8333	0.4756	0.359	
Usia.aenea	0.020898	0.014052	1.4871	1.3333	1.5000	0.5089	0.575	
Dischistus.sp.	0.019691	0.019629	1.0031	1.3333	0.3333	0.5402	0.058	.
Cryptocephalus.rugicollis	0.018066	0.024171	0.7474	0.3333	1.5000	0.5690	0.147	
Bombylius.cruciatus	0.017561	0.016618	1.0568	0.5000	1.5000	0.5970	0.671	
Eucera.cineraria	0.016632	0.021453	0.7753	0.5000	1.0000	0.6234	0.411	
Lasioglossum	0.016152	0.016341	0.9884	1.1667	0.6667	0.6492	0.312	
Andrena.rhyssonota	0.015807	0.016352	0.9667	0.8333	0.8333	0.6743	0.304	
Tropinota.squalida	0.015430	0.018784	0.8214	0.5000	1.1667	0.6989	0.179	
Colletes.acutus	0.014135	0.020152	0.7014	0.1667	1.1667	0.7214	0.371	
Andrena.suerinensis	0.013631	0.014813	0.9202	0.3333	1.0000	0.7431	0.284	
Chamaesphecia	0.012647	0.015678	0.8066	1.0000	0.5000	0.7633	0.769	
Lasioglossum.leucozonium	0.012222	0.017101	0.7147	0.8333	0.0000	0.7827	0.002	**
Andrena.orana	0.008991	0.012303	0.7308	0.1667	0.6667	0.7971	0.234	
Panurgus.calcaratus	0.008574	0.009300	0.9219	0.6667	0.3333	0.8107	0.901	
Lasioglossum.malachurum	0.008365	0.009040	0.9253	0.6667	0.1667	0.8240	0.628	
Microchrysa	0.007306	0.010020	0.7292	0.5000	0.1667	0.8357	0.078	.
Lasioglossum.villosulum	0.007007	0.011586	0.6047	0.5000	0.0000	0.8468	0.627	
Oedemera.barbara	0.006495	0.006874	0.9448	0.1667	0.5000	0.8572	0.409	
Sphaerophoria	0.006215	0.006497	0.9566	0.1667	0.5000	0.8671	0.467	
Andrena.flavipes	0.006016	0.009455	0.6363	0.5000	0.0000	0.8766	0.290	
Andrena.fulica	0.005634	0.012827	0.4392	0.0000	0.5000	0.8856	0.335	
Bombylius.minor	0.005086	0.007626	0.6669	0.3333	0.0000	0.8937	0.377	
Eucera	0.005086	0.007626	0.6669	0.3333	0.0000	0.9018	0.731	
Diptero	0.005045	0.011851	0.4257	0.3333	0.0000	0.9099	0.094	.
Nustera.distigma	0.004911	0.006423	0.7646	0.3333	0.1667	0.9177	0.136	
Black.buprestidae	0.004785	0.006243	0.7665	0.3333	0.1667	0.9253	0.717	
Bombylius.fimbriatus	0.004485	0.006753	0.6642	0.3333	0.0000	0.9324	0.664	
Tenthredo	0.004485	0.006753	0.6642	0.3333	0.0000	0.9396	0.013	*
Meliscaeva.auricollis	0.004463	0.006633	0.6728	0.0000	0.3333	0.9467	0.845	
Halictus.crenicornis	0.004236	0.009849	0.4302	0.3333	0.0000	0.9534	0.195	
Scaeva	0.004081	0.006031	0.6767	0.3333	0.0000	0.9599	0.017	*
Lasioglossum.immunitum	0.003719	0.006424	0.5788	0.1667	0.1667	0.9659	0.905	
Andrena.pilipes	0.003285	0.005452	0.6025	0.1667	0.1667	0.9711	0.215	
Heliotaurus.ruficolis	0.003240	0.005379	0.6022	0.1667	0.1667	0.9762	0.696	
Andrena	0.003184	0.007298	0.4362	0.0000	0.1667	0.9813	0.428	
Osmia.signata	0.002666	0.006094	0.4376	0.0000	0.1667	0.9856	0.307	
Lycaena.phlaeas	0.001878	0.004276	0.4392	0.0000	0.1667	0.9886	0.741	
Nomada	0.001797	0.004089	0.4394	0.0000	0.1667	0.9914	0.563	
Osmia	0.001797	0.004089	0.4394	0.0000	0.1667	0.9943	0.757	
Oxythyrea.funesta	0.001797	0.004089	0.4394	0.0000	0.1667	0.9971	0.826	
Pieris.rapae	0.001797	0.004089	0.4394	0.0000	0.1667	1.0000	0.556	

Comparison C and NPK

	average	sd	ratio	ava	avb	cumsum	p	
Panurgus.perezi	0.061203	0.045485	1.3456	7.0000	3.2	0.1031	0.240	
Dasypoda.dusmeti	0.049282	0.034701	1.4202	4.6667	4.8	0.1861	0.482	
Apis.mellifera	0.044335	0.046668	0.9500	1.8333	3.2	0.2607	0.433	
Chasmatopterus	0.028679	0.024550	1.1682	3.0000	1.6	0.3090	0.900	
Lasioglossum.algericolellum	0.027098	0.017363	1.5607	3.8333	2.0	0.3547	0.674	
Bombylius.cruciatus	0.026258	0.029519	0.8895	0.5000	1.6	0.3989	0.280	
Psilothryx.viridicoerulea	0.026005	0.015816	1.6442	3.0000	2.0	0.4427	0.704	
Oedemera.sp	0.024453	0.018361	1.3318	2.0000	1.4	0.4839	0.631	
Chamaesphecia	0.022514	0.016958	1.3276	1.0000	1.8	0.5218	0.205	
Eucera.cineraria	0.022325	0.031344	0.7123	0.5000	1.4	0.5594	0.180	
Dischistus.sp.	0.019435	0.020016	0.9709	1.3333	0.4	0.5921	0.077	.
Bombylius.medius	0.019181	0.015801	1.2139	1.3333	1.6	0.6244	0.522	
Andrena.rhyssonota	0.017020	0.018387	0.9256	0.8333	1.0	0.6531	0.200	
Lasioglossum	0.015611	0.014601	1.0691	1.1667	0.6	0.6794	0.399	
Usia.aenea	0.013227	0.010398	1.2721	1.3333	1.4	0.7016	0.973	
Lasioglossum.leucozonium	0.012160	0.016811	0.7233	0.8333	0.0	0.7221	0.012	*
Sphaerophoria	0.009543	0.012670	0.7532	0.1667	0.8	0.7382	0.187	
Panurgus.calcaratus	0.008594	0.009295	0.9246	0.6667	0.2	0.7527	0.882	
Lasioglossum.immunitum	0.008446	0.010469	0.8068	0.1667	0.6	0.7669	0.461	
Lasioglossum.malachurum	0.008382	0.008895	0.9423	0.6667	0.2	0.7810	0.632	
Andrena.flavipes	0.007267	0.009118	0.7970	0.5000	0.2	0.7932	0.177	
Oedemera.barbara	0.007263	0.011897	0.6105	0.1667	0.4	0.8055	0.343	
Andrena.suerinensis	0.007146	0.011306	0.6320	0.3333	0.4	0.8175	0.833	
Lasioglossum.villosulum	0.006978	0.011431	0.6104	0.5000	0.0	0.8293	0.648	
Tropinota.squalida	0.006838	0.009027	0.7575	0.5000	0.2	0.8408	0.769	
Microchrysa	0.006659	0.010315	0.6456	0.5000	0.0	0.8520	0.157	
Heliotaurus.ruficolis	0.005591	0.008618	0.6488	0.1667	0.4	0.8614	0.449	
Oxythyrea.funesta	0.005508	0.011273	0.4887	0.0000	0.4	0.8707	0.324	
Cryptocephalus.rugicollis	0.005489	0.008887	0.6177	0.3333	0.2	0.8799	0.771	
Bombylius.minor	0.005057	0.007496	0.6746	0.3333	0.0	0.8884	0.360	
Eucera	0.005057	0.007496	0.6746	0.3333	0.0	0.8970	0.724	
Diptero	0.005017	0.011685	0.4293	0.3333	0.0	0.9054	0.185	
Bombylius.fimbriatus	0.004469	0.006666	0.6704	0.3333	0.0	0.9129	0.648	
Tenthredo	0.004469	0.006666	0.6704	0.3333	0.0	0.9205	0.019	*
Seladonia.genunea	0.004457	0.009102	0.4896	0.0000	0.4	0.9280	0.155	
Halictus	0.004266	0.008709	0.4898	0.0000	0.4	0.9351	0.471	
Meliscaeva.auricollis	0.004266	0.008709	0.4898	0.0000	0.4	0.9423	0.841	
Halictus.crenicornis	0.004227	0.009780	0.4322	0.3333	0.0	0.9495	0.252	
Nustera.distigma	0.004200	0.006170	0.6806	0.3333	0.0	0.9565	0.291	
Black.buprestidae	0.004074	0.005985	0.6807	0.3333	0.0	0.9634	0.786	
Scaeva	0.004074	0.005985	0.6807	0.3333	0.0	0.9702	0.030	*
Colletes.acutus	0.003816	0.006166	0.6189	0.1667	0.2	0.9767	0.989	
Osmia	0.002754	0.005636	0.4887	0.0000	0.2	0.9813	0.548	
Andrena.orana	0.002548	0.005940	0.4290	0.1667	0.0	0.9856	0.758	
Andrena.alluaudi	0.002228	0.004551	0.4896	0.0000	0.2	0.9894	0.599	
Osmia.signata	0.002228	0.004551	0.4896	0.0000	0.2	0.9931	0.412	
Pieris.rapae	0.002133	0.004354	0.4898	0.0000	0.2	0.9967	0.332	
Andrena.pilipes	0.001960	0.004525	0.4332	0.1667	0.0	1.0000	0.604	

Comparison C and P

	average	sd	ratio	ava	avb	cumsum	p	
Panurgus.perezi	0.069623	0.043053	1.6171	7.0000	4.5000	0.1182	0.103	
Chasmatopterus	0.044812	0.041314	1.0847	3.0000	4.8333	0.1943	0.656	
Dasypoda.dusmeti	0.040830	0.033255	1.2278	4.6667	3.8333	0.2636	0.794	
Apis.mellifera	0.035589	0.036599	0.9724	1.8333	1.6667	0.3240	0.718	
Psilothryx.viridicoerulea	0.033814	0.021549	1.5692	3.0000	1.6667	0.3814	0.394	
Lasioglossum.algericolellum	0.033531	0.017577	1.9077	3.8333	1.5000	0.4384	0.423	
Oedemera.sp	0.025701	0.019926	1.2898	2.0000	0.6667	0.4820	0.524	
Dischistus.sp.	0.020366	0.019945	1.0211	1.3333	0.3333	0.5166	0.046	*
Lasioglossum	0.017574	0.014618	1.2022	1.1667	0.8333	0.5464	0.203	
Bombylius.medius	0.017533	0.013695	1.2803	1.3333	0.1667	0.5762	0.638	
Usia.aenea	0.016433	0.011946	1.3756	1.3333	1.3333	0.6041	0.905	
Chamaesphecia	0.015227	0.014772	1.0308	1.0000	0.8333	0.6299	0.612	
Lasioglossum.villosulum	0.014352	0.019804	0.7247	0.5000	0.6667	0.6543	0.252	
Lasioglossum.leucozonium	0.012771	0.017182	0.7433	0.8333	0.0000	0.6760	0.001	***
Bombylius.cruciatus	0.012728	0.014594	0.8722	0.5000	0.5000	0.6976	0.893	
Andrena.rhyssonota	0.012577	0.016321	0.7706	0.8333	0.1667	0.7189	0.540	
Lasioglossum.immunitum	0.011975	0.018393	0.6510	0.1667	0.8333	0.7393	0.325	
Panurgus.calcaratus	0.010688	0.011598	0.9215	0.6667	0.3333	0.7574	0.841	
Eucera.cineraria	0.010506	0.015616	0.6728	0.5000	0.3333	0.7753	0.667	
Lasioglossum.malachurum	0.008496	0.009479	0.8963	0.6667	0.0000	0.7897	0.640	
Microchrysa	0.006982	0.010582	0.6598	0.5000	0.0000	0.8015	0.104	
Eucera	0.006489	0.007513	0.8637	0.3333	0.3333	0.8126	0.536	
Black.buprestidae	0.006422	0.007476	0.8590	0.3333	0.3333	0.8235	0.443	
Heliotaurus.ruficolis	0.006414	0.011223	0.5715	0.1667	0.3333	0.8343	0.343	
Andrena.flavipes	0.006283	0.009649	0.6511	0.5000	0.0000	0.8450	0.280	
Tropinota.squalida	0.006283	0.009649	0.6511	0.5000	0.0000	0.8557	0.821	
Bombylius.fimbriatus	0.006205	0.007141	0.8689	0.3333	0.3333	0.8662	0.430	
Halictus.crenicornis	0.005924	0.009894	0.5987	0.3333	0.1667	0.8763	0.072	.
Colletes.acutus	0.005730	0.007385	0.7759	0.1667	0.3333	0.8860	0.947	
Cryptocephalus.rugicollis	0.005642	0.009277	0.6082	0.3333	0.1667	0.8956	0.777	
Osmia	0.005421	0.012391	0.4375	0.0000	0.3333	0.9048	0.357	
Bombylius.minor	0.005315	0.007666	0.6933	0.3333	0.0000	0.9138	0.354	
Diptero	0.005272	0.012008	0.4390	0.3333	0.0000	0.9228	0.049	*
Polyommatus.icarus	0.005086	0.011615	0.4379	0.0000	0.3333	0.9314	0.319	
Tenthredo	0.004685	0.006830	0.6860	0.3333	0.0000	0.9393	0.007	**
Andrena.suerinensis	0.004427	0.010070	0.4396	0.3333	0.0000	0.9469	0.934	
Nustera.distigma	0.004398	0.006331	0.6946	0.3333	0.0000	0.9543	0.250	
Scaeva	0.004263	0.006142	0.6940	0.3333	0.0000	0.9616	0.009	**
Sphaerophoria	0.003824	0.006298	0.6071	0.1667	0.1667	0.9681	0.797	
Bombylius.sp	0.002710	0.006196	0.4375	0.0000	0.1667	0.9727	0.531	
Andrena.orana	0.002679	0.006102	0.4390	0.1667	0.0000	0.9772	0.722	
Andrena	0.002361	0.005387	0.4383	0.0000	0.1667	0.9812	0.552	
Andrena.alluaudi	0.002361	0.005387	0.4383	0.0000	0.1667	0.9852	0.539	
Lycaena.phlaeas	0.002295	0.005235	0.4384	0.0000	0.1667	0.9891	0.559	
Oedemera.barbara	0.002184	0.004968	0.4397	0.1667	0.0000	0.9928	0.876	
Halictus	0.002174	0.004957	0.4387	0.0000	0.1667	0.9965	0.798	
Andrena.pilipes	0.002049	0.004660	0.4398	0.1667	0.0000	1.0000	0.434	

Table S2.6. List of all visitor species and their abundance in each treatment. Treatments are separated in two groups based on the presence or absence of nitrogen. Total abundance per treatment is also presented.

ORDER	INSECT ID	C	K	P	N	NP	NPK
Hymenoptera	*Amegilla quadrifasciata*					1	
Hymenoptera	*Andrena*			1	1		
Hymenoptera	*Andrena agilissima*						1
Hymenoptera	*Andrena alluaudi*		2	1			1
Hymenoptera	*Andrena flavipes*	3	1		2		1
Hymenoptera	*Andrena fulica*					3	
Hymenoptera	*Andrena minutula*				1		
Hymenoptera	*Andrena orana*	1			3	4	
Hymenoptera	*Andrena pilipes*	1	1			1	
Hymenoptera	*Andrena rhyssonota*	5	2	1	2	5	5
Hymenoptera	*Andrena suerinensis*	2	5		3	6	2
Hymenoptera	*Andrena vetula*				1		
Hymenoptera	*Anthophora*					1	
Hymenoptera	*Anthophora atroalba*						1
Hymenoptera	*Apis mellifera*	11	6	10	17	16	16
Lepidoptera	*Aricia cramera*			1			
Coleoptera	*Black buprestidae*	2	2	2	2	1	
Diptera	*Bombylius cruciatus*	3	6	3	4	9	8
Diptera	*Bombylius fimbriatus*	2	1	2	3		
Diptera	*Bombylius medius*	8	2	1	8	9	8
Diptera	*Bombylius minor*	2	3				
Diptera	*Bombylius sp*		2	1			
Hymenoptera	*Ceratina cyanea*					1	
Lepidoptera	*Chamaesphecia sp*	6	6	5	1	3	9
Coleoptera	*Chasmatopterus sp*	18	29	29	13	12	8
Diptera	*Chrysotoxum sp*	1					
Hymenoptera	*Colletes acutus*	1	4	2	6	7	1
Coleoptera	*Cryptocephalus rugicollis*	2	1	1	3	9	1
Hymenoptera	*Dasypoda dusmeti*	28	33	23	28	21	24
Diptera	*Diptera*	2					
Diptera	*Dischistus sp*	8	2	2	3	2	2
Diptera	*Episyrphus balteatus*	1					
Hymenoptera	*Eucera sp*	2	2	2	4		
Hymenoptera	*Eucera cineraria*	3		2	1	6	7
Hymenoptera	*Eucera clypeata*		1				
Hymenoptera	*Eucera hispaliensis*	1					
Hymenoptera	*Eucera longicornis*			1			
Hymenoptera	*Eucera nigrescens*			1			

Hymenoptera	*Halictus sp*		2	1	1		2
Hymenoptera	*Halictus crenicornis*	2		1			
Coleoptera	*Heliotaurus ruficolis*	1		2	1	1	2
Hymenoptera	*Heriades rubicola*	1					
Hymenoptera	*Lasioglossum sp*	7	2	5	4	4	3
Hymenoptera	*Lasioglossum algericolellum*	23	14	9	26	12	10
Hymenoptera	*Lasioglossum discum fertoni*						1
Hymenoptera	*Lasioglossum immunitum*	1	2	5	1	1	3
Hymenoptera	*Lasioglossum leucozonium*	5					
Hymenoptera	*Lasioglossum malachurum*	4	5		4	1	1
Hymenoptera	*Lasioglossum prasinum*					1	
Hymenoptera	*Lasioglossum punctatissimum*					1	
Hymenoptera	*Lasioglossum small shiny*			1			
Hymenoptera	*Lasioglossum thin body*						1
Hymenoptera	*Lasioglossum villosulum*	3	5	4	1		
Lepidoptera	*Lycaena phlaeas*			1	1	1	
Diptera	*Meliscaeva auricollis*		3		5	2	2
Diptera	*Microchrysa sp*	3	2			1	
Hymenoptera	*Nomada sp*				1	1	
Hymenoptera	*Nomada linsenmaieri*			1			
Hymenoptera	*Nomiapis paulyi*		1				
Coleoptera	*Nustera distigma*	2	2			1	
Coleoptera	*Oedemera barbara*	1	1		1	3	2
Coleoptera	*Oedemera sp*	12	12	4	14	9	7
Hymenoptera	*Osmia sp*			2	1	1	1
Hymenoptera	*Osmia signata*					1	1
Hymenoptera	*Osmia tricornis/bicornis*			1			
Coleoptera	*Oxythyrea funesta*		1		1	1	2
Hymenoptera	*Panurgus calcaratus*	4	19	2	1	2	1
Hymenoptera	*Panurgus perezi*	42	23	27	24	11	16
Lepidoptera	*Pieris rapae*					1	1
Lepidoptera	*Polyommatus icarus*			2			
Coleoptera	*Psilothryx viridicoerulea*	18	10	10	6	11	10
Lepidoptera	*Satyrium esculi*					1	
Diptera	*Scaeva sp*	2					
Hymenoptera	*Seladonia sp*			1			
Hymenoptera	*Seladonia genunea*						2
Diptera	*Sphaerophoria sp*	1	1	1		3	4
Hymenoptera	*Tenthredo sp*	2					
Lepidoptera	*Thymelicus sylvestris*	1					
Coleoptera	*Tropinota squalida*	3	2		4	7	1
Diptera	*Usia aenea*	8	12	8	9	9	7
Diptera	*Villa sp*	1					
Total		**260**	**230**	**179**	**212**	**204**	**175**

Table S2.7. List of plant species present in each treatment.

Plant species	C	K	N	NP	NPK	P
Andryala integrifolia	x	x	x	x	x	x
Chamaemelum sp			x			x
Coleostephus myconis	x	x				x
Crepis capillaris	x	x	x	x	x	x
Echium plantagineum	x	x	x	x	x	x
Erodium botrys			x			
Geranium molle					x	
Hypochaeris sp		x	x	x	x	x
Lathyrus sp						x
Mysopates orontium	x					
Ornithopus compressus						x
Raphanus raphanistrum	x	x	x	x	x	x
Tolpis barbata	x	x	x	x	x	x

CHAPTER 3. Trade-offs in the responses on floral resources of three Mediterranean grassland plant species to nutrient enrichment buffer bottom-up effects on pollinators

3.1 Introduction

Since 87% of angiosperm species (Ollerton et al., 2011) and 75% of crop species (Klein et al., 2007) are pollinated by animals (mostly insects), insect pollinators provide crucial ecosystem services to natural and agricultural ecosystems. Although most studies come from Europe (Beismeijer et al., 2006; Potts et al., 2010; Goulson et al., 2015; Powney et al., 2019; Wood et al., 2020), evidence of pollinator declines worldwide (Cameron et al., 2011; Dicks et al., 2020; Zattara and Aizen, 2021) are attracting the attention and efforts of researchers, farmers, policy makers and society in general. Besides climate change, the main driver of pollinator declines is agricultural intensification (Raven and Wagner, 2021). Agricultural intensification brings a set of associated changes, such as increased pesticide and insecticide use, landscape simplification and high nutrient inputs, especially of the macronutrients nitrogen (N), phosphorous (P) and potassium (K) (Sala et al., 2000; Sánchez-Bayo et al., 2019; Wagner et al., 2021), which impact agroecosystems, and the surrounding natural ecosystems.

Effects of nutrient inputs (or nutrient enrichment) on plants have been extensively studied (e.g., Bobbink and Hettelingh, 2010; Harpole et al., 2016; Payne et al., 2017) with solid evidence of changes in plant community composition, and alterations at plant level, namely on plant chemistry and floral display that are less understood. Both changes in plant community composition and in plant traits can affect plant interactions with herbivores and pollinators (Muñoz et al., 2005; Hoover et al., 2012), as evidenced by cascading effects of nutrient enrichment on insect communities (e.g., Haddad et al., 2000; Stevens et al., 2018). In agreement, studies have shown that nutrient enrichment can affect plant-pollinator interactions through changes in the synthesis of central compounds in plants (see David et al., 2019), which are important for pollinator nutrition.

In general, plant-pollinator interactions are mutualistic, as plant sexual reproduction, especially cross pollination, is promoted, and pollinators collect floral rewards, i.e., nectar and/or pollen. Nectar is mainly used by pollinators as an energy source because it is a sugar-rich solution (up to 70% w/w) containing sucrose, fructose and/or glucose. Nectar also contains smaller amounts of amino acids (aa), proteins, fatty acids and vitamins (Nicholson and Thornburg, 2007; Nicholson, 2022). By contrast, pollen is the main N source for many adult pollinators and their offspring, since it is mainly composed of proteins (up to 60%), with small concentrations of sugars, lipids and vitamins (Roulston et al., 2000; Nicholson, 2011). The presence, composition, and quantity of central compounds (i.e., proteins, aa, sugars, fatty acids) in nectar and pollen are significant for pollinator selection of the resource, who complement their diets feeding on different plants based on their nutritional values (Vaudo et al., 2015; Wright et al., 2018). For example, floral rewards quality (e.g., quantity of essential aa) seems important for pollinators such us *Bombus* spp. (Hanley et al., 2008), honeybee (Cook et al., 2003; Hendriksma et al., 2014) and presumably other bee (and other pollinator) species, although there is a big knowledge gap. As nutrient enrichment may change plants' synthesis of important compounds for pollinator nutrition, this adds another tier of complexity to predicting changes in plant-pollinator interactions under nutrient enrichment. Despite the importance of nectar and pollen chemistry for pollinators, and the unpredictability of the effects of nutrient enrichment on plant-pollinator interactions, the number of studies on the topic is very limited. One of the reasons for this scarcity of studies is the difficulty in collecting enough material (i.e., pollen and/or nectar) to be analyzed. However, technological advancements in recent years allow accurate analysis of much lower quantities of plant material, so that the low quantity of pollen and nectar collected is no longer an obstacle to its analysis (Vanderplanck et al., 2014).

There are a few studies on the effect of nutrient enrichment on floral rewards quality in terms of aa concentration, and there is no consensus on their observations. In some cases, nutrient enrichment was shown to have:

i) Positive effects. For example, adding NPK increased nectar aa concentration in *Agrostemma githago* (Caryophyllacea) (Gardener and Gillman, 2001), *Ipomopsis aggregata* (Polemoniaceae) (Burkle and Irwin, 2009) and *Gymnadenia conopsea* (Orchidaceae) (Gijbels et al., 2015). Adding NPK also increased pollen aa concentration in *Succisa pratensis* (Caprifoliacea) (Ceulemans et al., 2017c). In the

case of *Gymnadenia conopsea*, adding N alone also increased nectar aa concentration (Gijbels et al., 2015).

ii) Neutral effects. For example, adding NPK did not influence nectar aa concentration in *Linum lewisii* (Linaceae) (Burkle and Irwin, 2009).

iii) Negative effects. For example, adding NPK decreased nectar aa concentration in *Succisa pratensis* (Ceulemans et al., 2017c).

Therefore, the effect of nutrient enrichment on floral rewards quality seems largely to depend on plant species and may differ within the same plant species, as shown in the case of *S. pratensis*, where adding NPK had a negative effect on its nectar aa concentration and a positive effect on its pollen aa concentration (Ceulemans et al., 2017c). Furthermore, Ceulemans et al. (2017c) also reported lower pollen quality due to a stronger reduction in essential aa (i.e., the aa that are not synthesized by insects and therefore need to be present in their diet), which was suggested to have impacted negatively *Bombus terrestris* workers and larval development. Similarly, Hoover et al. (2012) observed that adding N increased pollinators' mortality when consuming *Cucurbita maxima* (Cucurbitaceae) nectar, despite the positive effects, i.e., increases in nectar sugars and aa concentrations, and not in the aa relative proportions.

The main objective of this chapter was to understand the effects of nutrient enrichment (derived from a nearby intensive agricultural area) on plant-pollinator interactions in a semi-natural ecosystem, focusing on flower resources (pollen quantity and quality in terms of aa) of co-flowering species, and on its insect visitors. Plant species differ in their: i) flower abundance, ii) floral traits (e.g., flower colour and shape), which shape pollinator attraction to flowering plants (Carvalheiro et al., 2014; Wang et al., 2024) and varies between pollinator taxa (Rader et al., 2020); iii) capacity to acquire nutrients (with consequences for their growth), N preference, and investment in sexual reproduction (i.e., flower production); and iv) demand for specific nutrients (e.g., *Brassica* crops have a higher demand for sulphur than other crops in general; Marsic et al., 2021). Therefore, we tested the simultaneous effects of multiple nutrient addition treatments on flower resources (pollen quantity and quality in terms of aa) of three co-flowering Mediterranean grassland plant species, and on their visitors, at flower and at plot scale. Differences at flower and plot scale will depend on plant community diversity and composition. Based on the unpredictability of the effects of nutrient enrichment on floral rewards quantity

and quality, we expect that in biodiverse rich plant communities such as Mediterranean grasslands, nutrient enrichment can have positive, neutral, and negative effects on pollen quantity and quality depending on the plant species. Because of such a gradient of responses to nutrient enrichment, we anticipate the possibility of trade-offs in the responses of the studied plant species, which may buffer bottom-up effects on pollinators and therefore increase the ecosystem's resilience to nutrient enrichment. By testing the simultaneous effects of multiple nutrient addition treatments, we expect that the treatments that alleviate the ecosystem's limitation(s) and do not trigger nutritional imbalances may improve floral resources for insect pollinators and *vice versa*. Last, we expect different results at flower and at plot scale taking into account that nutrient enrichment derived changes at flower scale can be diluted or increased at plot level due to changes in flower abundance.

To test our hypotheses, we used a nutrient manipulation (N, P and K added alone, and in the following combinations, NP and NPK) field experiment running in a Mediterranean grassland since 2012, which belongs to the Nutrient Network (https://nutnet.org/) (Borer et al., 2014). We focused on three native grassland plant species that are widespread, differ in plant family and in floral traits (flower color and shape), and are frequently visited by pollinators in the study area: *Raphanus raphanistrum* L. (Brassicaceae), *Echium plantagineum* L. (Boraginaceae) and *Crepis capillaris* (Asteraceae). The novelty of our study includes the study of several co-flowering plant species belonging to three different families and growing in their habitat under natural conditions; and the link of the effects of our nutrient addition treatments on floral resources (quantity and quality) and on pollinators, at the flower and plot scale. Our study provides a better understanding of the effects of nutrient enrichment on Mediterranean ecosystems and particularly in wild co-flowering plant species subjected to different nutrient addition treatments, and their pollinator visitors. The plant-pollinator interactions approach we adopted to study the effects of nutrient enrichment from patchy landscapes (i.e., areas of conservation interest nearby areas of intensive agriculture) provides novel and critical knowledge of ecosystem functioning, which may be used as a tool in biodiversity conservation strategies, ecosystem functions and services, and ecological modelling.

3.2 Material and methods

3.2.1 Study site and experimental design

The study site is located at Companhia das Lezirias, approximately 50 km north-east of Lisbon, Portugal (38º 49′ 45.13”N, 8º 47′ 28.61”W), and corresponds to a semi-natural Mediterranean grassland within the Montado. The climate is Mediterranean with a mean annual rainfall of 537 mm and a mean annual temperature of 14.9ºC, ranging from 10ºC in January to 22.5ºC in August (Nogueira et al., 2019). Site topography is flat and the soil is a well-drained deep Haplic Arenosol (IUSS Working Groups WRB, 2006) with low soil water retention capacity. The grassland community is mainly composed of annual species that grow in winter and early spring and senesce in late spring. The dominant plant species include the: i) Asteraceae *Tolpis barbata* L. and *Crepis capillaris* L.; ii) Poaceae *Agrostis pourretii* Willd. and *Avena barbata* Link; iii) Fabaceae *Ornithopus compressus* L.; iv) Boraginaceae *Echium plantagineum* L.; and v) Brassicaceae *Raphanus raphanistrum* L. (Nogueira et al., 2017). We focused on the following three co-flowering plant species which are native, widespread, frequently visited by insect pollinators, and belong to different plant families and consequently their flowers have distinct features: the Brassicaceae *Raphanus raphanistrum* (designated as *Raphanus*), the Boraginaceae *Echium plantagineum* (designated as *Echium*), and the Asteraceae *Crepis capillaris* (designated as *Crepis*). They also have different N preferences (see Tyler et al., 2021; Midolo et al., 2023): *E. plantagineum* (6.8) is a nitrophilous species and *Raphanus raphanistrum* (5) and *Crepis capillaris* (3.5) are species less tolerant to N. All three species are ruderals (Flora-On) which means they are adapted to disturbance although their tolerance to disturbance may differ.

Our nutrient addition field experiment has been running since 2012 and is part of the Nutrient Network (NutNet - https://nutnet.org/), which is a research network comprised of more than 130 grassland sites worldwide testing the ecological effects of adding multiple nutrients, especially on plant communities (Borer et al., 2014). Control plots received no added nutrients, while there were five nutrient treatments: addition of N (designated as “N”), addition of P (designated as “P”), addition of K (designated as “K”), addition of N and P (designated as “NP”), and addition of N, P and K (designated as “NPK”). Each nutrient (N, P and K) was added at a rate of 100 kg ha^{-1} yr^{-1}. N was applied as time-release urea, P as triple-superphosphate and K as potassium sulphate. In the first

year of fertilization (2012), each plot that received K also received 1000 kg ha^{-1} of a nutrient mixture containing iron (15%), sulphur (14%), magnesium (1.5%), manganese (2.5%), copper (1%), zinc (1%), boron (0.2%), and molybdenum (0.05%). Each treatment was replicated 3 times i.e., each treatment was applied to three 25 m^2 plots, arranged in blocks.

3.2.2 Pollen sampling and extraction

In spring, during the flowering peak, pollen was sampled, and flower abundance and pollinator visitations were recorded, for each of the three studied plant species. *Raphanus* and *Echium* pollen was sampled from all six treatments in 2021, while *Crepis* pollen was sampled from only three treatments (control, N and NP) in 2020. *Crepis* was not sampled in the P treatment because its flower abundance was too low to collect enough pollen (see below). In each plot, 3 to 5 plants per species were randomly selected, i.e., the number of plants depended on the number of suitable (i.e., non-pollinated) flowers in each plant. For each of the three plant species, we hand-collected 5 to 15 flowers per plant (30-40 flowers per plot), to ensure we had enough pollen to be analyzed (10 flowers contained approximately 1 mg of pollen). All collected flowers were young, with no sign of pollination. The sampled flowers were stored in a cool box at 4ºC in the field and later in a freezer at -80ºC until analysis.

Due to the characteristics of each plant species' flowers (i.e., *Crepis* produces many small flowers arranged to form their unique inflorescence, while *Raphanus* and *Echium* produce bigger and easily separated flowers), the pollen extraction protocols were slightly different. To extract *Raphanus* and *Echium* pollen, flowers were dried in the oven for 3 hours at 60ºC. The filaments from *Raphanus* and *Echium* flowers and anthers were removed from each flower just leaving the anthers. We released *Raphanus* and *Echium* pollen from their anthers through vibration using a vortex for 2 minutes or, when the anthers did not open, using a Retsch MM400 mixer mill (Retsch GmbH, Haan, Germany) for 10 seconds. Anther fragments were removed, leaving pollen samples ready for further analyses. To extract *Crepis* pollen, flowers were also dried in the oven for 3 hours at 60ºC, after which external petals, sepals and other unwanted parts were removed. The mature flowers inside each *Crepis* inflorescence were collected and its pollen was extracted using distilled water and vibration (vortex for 3 minutes). *Crepis* pollen was

obtained after removing the water using a vacuum concentrator (Savant DNA 120 SpeedVac Concentrator) for 4 hours (depending on the amount of liquid used in the first step). Samples from each plant species were weighed and kept at -80 ºC until further analysis.

3.2.3 Pollen amino acids analysis

Raphanus, *Echium* and *Crepis* pollen samples were analyzed for their amino acid (aa) composition and concentration. Pollen samples of around 1 mg were analyzed (Vanderplanck et al., 2014), while pollen samples with less than 1 mg were pooled with samples from the same plot (different plant) to reach the minimum weight (c.a. 1 mg) to be analyzed. The aa composition, and their respective concentrations were determined by HPLC (Biochrom 20 Plus Amino Acid analyzer) after hydrolysis using the Stein & Moore method, following the protocol described by Vanderplanck et al. (2014).

Given pollen's importance as a N source for pollinators, the aa that were detected in the three plant species' pollen samples were classified as essential or non-essential (Table S3.1). For each pollen sample, we calculated: i) the pollen concentration of each aa, expressed as µmol g^{-1} of pollen; ii) the concentration of all aa, expressed as µmol g^{-1} of pollen; iii) total pollen weight, expressed as mg $flower^{-1}$.

3.2.4 Flower abundance and pollinator visitation

In each plot, during 2020 and 2021 we surveyed all flowering species and their visitors using a standardized time-based methodology. In each survey, and within each plot, we identified all plant species and counted all flowers present. For each flowering plant species, we selected a focal spot with 10 to 30 flowers and all the flowers were observed during 10-minute periods. During this period, we registered all flower visitation events (defined as an insect contacting the reproductive parts of the flower) and in cases when a visitor escaped before being captured (net or entomological aspirator) a detailed description was added. Captured flower visitors were later separated into morphospecies and, whenever possible, identified to species level by a taxonomist (see acknowledgements). Non-captured individuals, which a description did not match to any of the collected individuals, were kept as separate morphospecies.

3.2.5 Data analyses

The effect of the nutrient addition treatments per flower on pollen weight and on total amino acid concentration, and on total essential amino acid concentration, was tested using linear mixed-models (LMMs). Plot nested in block was added as a random effect in all models. The effect of the nutrient addition treatments on these four variables at plot level was analyzed using plot flower abundance and log-transforming the four variables. To analyze nutrient effect on flower abundance per plot we used the same model structure but using a Poisson distribution. We used the flower abundance values per plot for the entire season when pollen samples were collected. The effect of the nutrient addition treatments on pollinator visitation rate in 2020 and 2021 was analyzed with LMMs. Year was added as a covariate and plot within block as random effects. We log-transformed visitation rate in all models. Butterfly visitation was included in "Total visitation rate" but not analyzed separately due to their low diversity. All models were fitted using Gaussian distribution (expect for flower abundance) with the *lme4* R package (Bates et al., 2014). *DHARMa* R package (Hartig 2022) was used to verify that residuals were normally distributed. We ran Anova tests for each LMM (car R package) and post Hoc tests with *multcomp* R package and the function glht. Analyses were performed in R environment version 2023.3.0.386. We performed a Redundancy Analysis (RDA) to test the effect of plant species and nutrient addition treatments on pollen amino acid concentrations with rda function of *Vegan* R Package (Oksanen et al., 2016). Subsequently, we ran an Anova with the function anova.cca and plotted the results with ordiplot function.

3.3 Results

3.3.1 Effects of the nutrient additions on flower resources at flower scale

The plant species whose flowers produced more pollen was *Raphanus* (0.36 ± 0.16 mg flower^{-1}), followed by *Echium* (0.19 ± 0.06 mg flower^{-1}), and *Crepis* was the species that produced least pollen (0.09 ± 0.04 mg inflorescence^{-1}) (Table S3.2). Despite a tendency for lower pollen yield per flower upon P and NPK additions in *Raphanus*, and for a higher pollen yield per flower upon all nutrient additions in *Echium*, we only observed significant differences in *Crepis*' pollen yield per flower; i.e., adding N alone and in

combination with P (i.e., NP) increased *Crepis*' pollen yield per flower (p=0.006 for N; p=0.0005 for NP) (Fig. 3.1a).

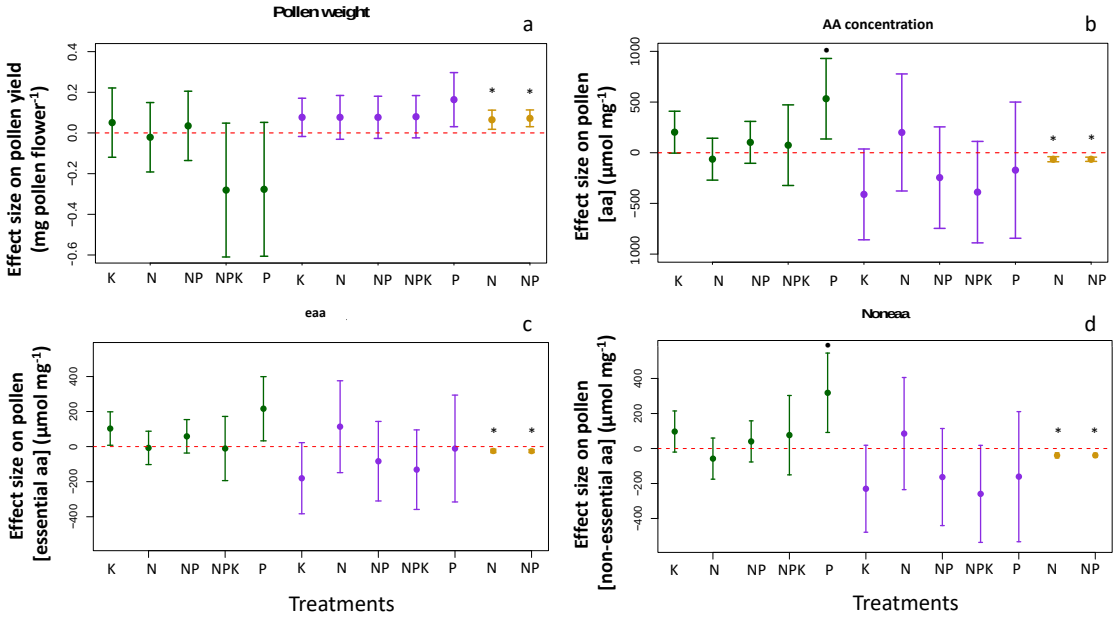


Figure 3.1. Effect size plots of the nutrient addition treatments on pollen production (a=pollen weight, b=amino acid concentration per flower, c=essential amino acids and d=non-essential amino acids). The estimates ± confidence interval for all the treatments are presented. Red dotted line represents control treatment. Significant effects (p<0.05) are marked by (*). See statistical details in Table S3.3.

Three aa (i.e., aspartate, glutamine and tryptophan) were not detected in any pollen sample from the three plant species. Therefore, we detected 17 aa in the pollen samples from the three plant species (Table S3.1). Following the same pattern observed for the pollen yield per flower, *Raphanus* was the plant species with higher pollen total aa concentrations (1374 ± 217 µmol g^{-1} of pollen), followed by *Echium* (1269 ± 431 µmol g^{-1} of pollen), and *Crepis* was the species whose pollen contained lower concentrations of total aa (65 ± 39 µmol g^{-1} of pollen). Upon the applied nutrient addition treatments, not all plant species changed their pollen total aa concentration, and not in response to all treatments. Compared with the control: i) adding P alone marginally increased the total aa concentration in *Raphanus'* pollen (ANOVA p=0.023: P p=0.08); ii) despite a tendency for lower values, no nutrient addition treatment changed *Echium*'s pollen total aa concentration; and iii) adding N alone and together with P (i.e., NP) reduced total aa concentration in *Crepis'* pollen (ANOVA p<0.001; p<0.001 for N and NP) (Fig. 3.1b). Except for *Crepis*, where we observed that the increase in pollen yield per flower due to

the nutrient addition treatments was accompanied by a reduction in total aa concentration, in *Raphanus* and *Echium* there was no clear pattern of response to the treatments (Fig. 3.1a-b).

From the 17 aa that were detected in the three plant species' pollen samples, nine aa were classified as essential and eight aa were classified as non-essential (Table S3.1). The pollen produced by the three plant species contained mostly the non-essential aa aspartic acid and glutamic acid, each representing around 11% of all aa. The essential aa valine was also very abundant in *Raphanus*' pollen (11%). The aa that were present at lower concentrations in the pollen produced by the three plant species (concentrations < 5%) were the essential aa histidine and methionine, and the non-essential aa cysteine and tyrosine (Table S3.1). The reduction of *Crepis* total aa concentration was due to a reduction of both essential aa and non-essential aa (Fig. 3.1). By contrast, the increase in *Raphanus* total aa concentration was due to stronger increase in non-essential aa than in essential aa, so *Raphanus* pollen in P contained a higher proportion of non-essential aa (Fig. 3.1). The results for the ANOVAs were significant for *Raphanus* and *Crepis* but not for *Echium* (Table S3.3). There was no clear effect of K and NPK treatments, which contained sulphur, on amino acids with sulphur in their formula such as Met and Cys. Moreover, the effect on *Raphanus* samples, a species with high sulphur demand, was not particularly stronger compared to *Echium* or *Crepis*.

The RDA (Fig. 3.2, Table S3.4) showed that pollen aa concentrations were influenced by both the plant species (*Raphanus*, *Echium* and *Crepis*) (ANOVA; p=0.001) and by the nutrient addition treatments (ANOVA; p=0.019) (Fig. 3.2). In total, plant species and nutrient addition treatments explained 89% of the observed variation in pollen aa composition (adjusted R^2=0.79). RD1, which was significant (p=0.006), clearly separated the aa composition of *Crepis*' pollen from that of *Raphanus* and *Echium*. The aa composition from these latter two species did not differ as RD2 was not significant (p>0.05). The nutrient addition treatments created a gradient along RD1, and P and K were the treatments that changed pollen aa composition the most while NP was the closest to Control, thus, being the most similar in terms of aa composition.

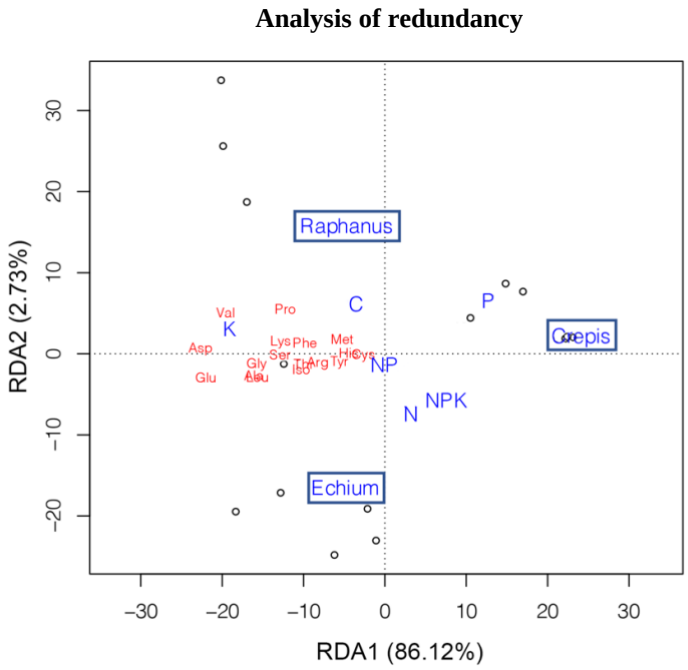


Figure 3.2. Redundancy analyses (RDA) for the amino acid absolute concentration per treatment and species. Pollen samples are represented by black dots, amino acids are in red, treatments in blue. See further details in Table S3.4.

3.3.2 Effects of the nutrient additions on flower resources at plot scale

Adding NP increased the flower abundance of the three plant species (Fig. 3.3) and adding P decreased it. *Raphanus* flower abundance also increased in N and decreased in NPK. The plot scale effects of nutrient enrichment on pollen weight and aa concentration were assessed by combining these variables with flower abundance data per plot. In terms of pollen yield per plot, NPK and P decreased it in *Raphanus*, no treatment changed it in *Echium*, and NP increased it in *Crepis* (Fig 3.4). In terms of pollen aa per plot, NP marginally increased it in *Raphanus*, while NPK and P decreased it, and no treatment changed it in *Echium* and *Crepis*. Additionally, NP marginally increased *Raphanus* pollen essential aa (Fig. 3.4c-d), while NPK decreased essential and non-essential aas. *Echium* non-essential aas decreased marginally under P addition.

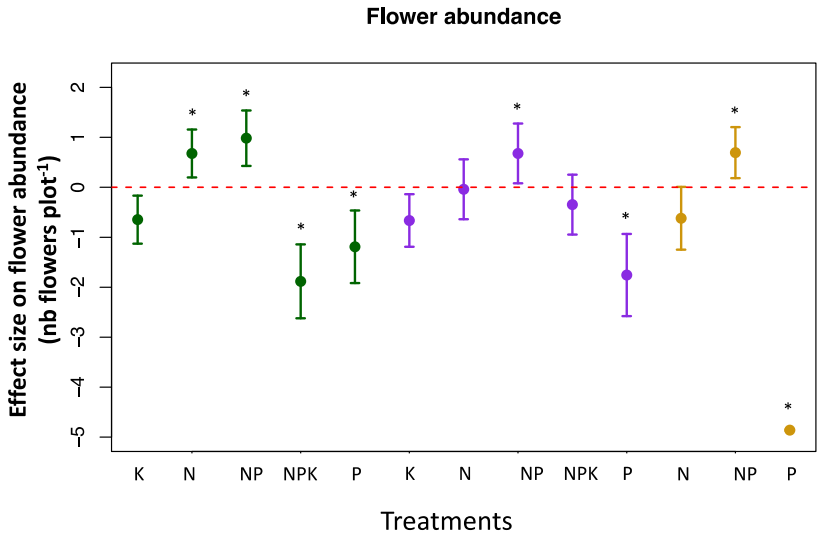


Figure 3.3. Effect size plot of the fertilization treatments on flower abundance. The estimates ± confidence interval across all the treatments are presented. Red dotted line represents control treatment. Species are coded by colours: green for *Raphanus*, purple for *Echium* and yellow for *Crepis*. Significant effects ($p<0.05$) are marked by (*). See statistical details in Table S3.3. For *Crepis* flower abundance, data from K and NPK were not collected in 2020.

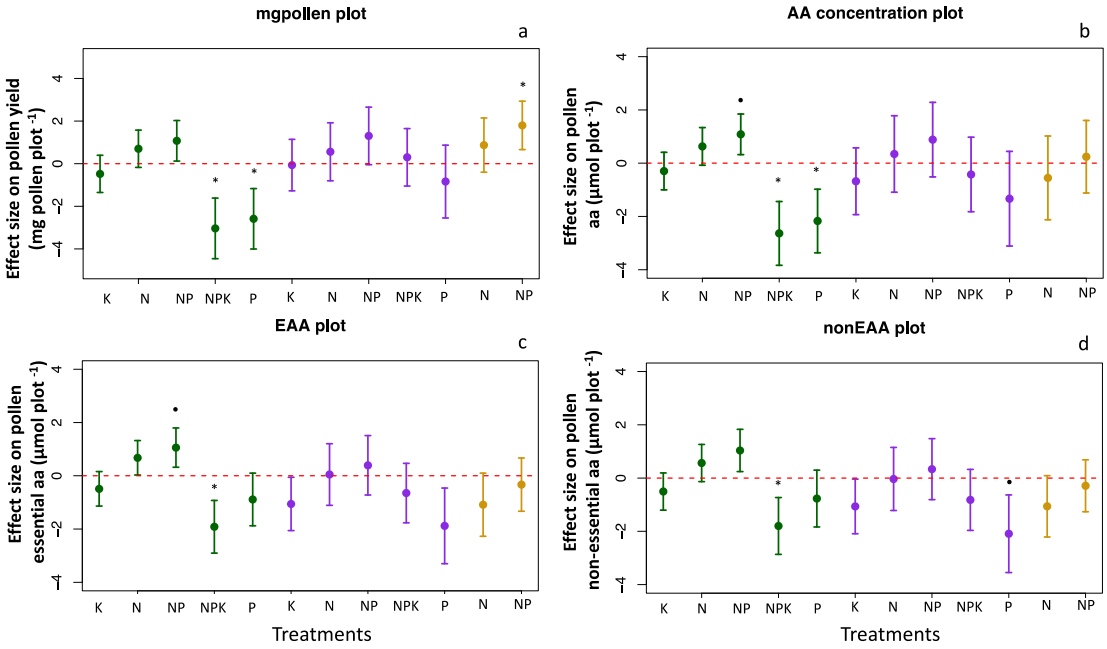


Figure 3.4. Effect size plot of the fertilization treatments on pollen production (pollen weight, amino acid concentration, essential and non-essential amino acids) per plot. The estimates ± confidence interval across all the treatments are presented. Red dotted line represents control treatment. Species are coded by colours: green for *Raphanus*, purple for *Echium* and yellow for *Crepis*. Effects ($p<0.05$) are marked by (*) and marginally significant effects ($p<0.1$) are marked by (•). See statistical details in Table S3.3.

3.3.3 Linking nutrient-driven effects on flower resources and pollinators

Raphanus was the most visited plant species of the three plants with a total of 156 visits, followed by 121 in *Echium* and 98 in *Crepis*, which together accounted for ca. 60% of the plant community visits (Table S3.5). Despite *Crepis*' low flower abundance and poor quality pollen, its flowers were visited by insect pollinators at a similar rate as *Raphanus* (i.e., 0.22 visits/flower in *Raphanus* and 0.19 visits/inflorescence in *Crepis*). *Echium*'s flowers received the fewest insect pollinators (0.15 visits/flower) of the three. For the nutrient addition treatments, we only detected changes in total pollinator visitation rates in *Raphanus* flowers present in the P treatment, with these flowers receiving more pollinator visits than those growing in control plots (Fig. 3.5a). No nutrient addition treatment changed the visitation rate of *Echium* and *Crepis* flowers or the whole plant community visitation rate at flower level, but one treatment (P) marginally decreased visitation rate when considering the whole plant community (Fig. 3.5d). NP marginally increased bee visitation of *Raphanus* flowers and P increased fly visitation also of *Raphanus* flowers (Fig. 3.6 a,b). At plot scale, no nutrient addition treatment changed the visitation rates of total pollinators, bees, flies and beetles (Fig 3.6d-f).

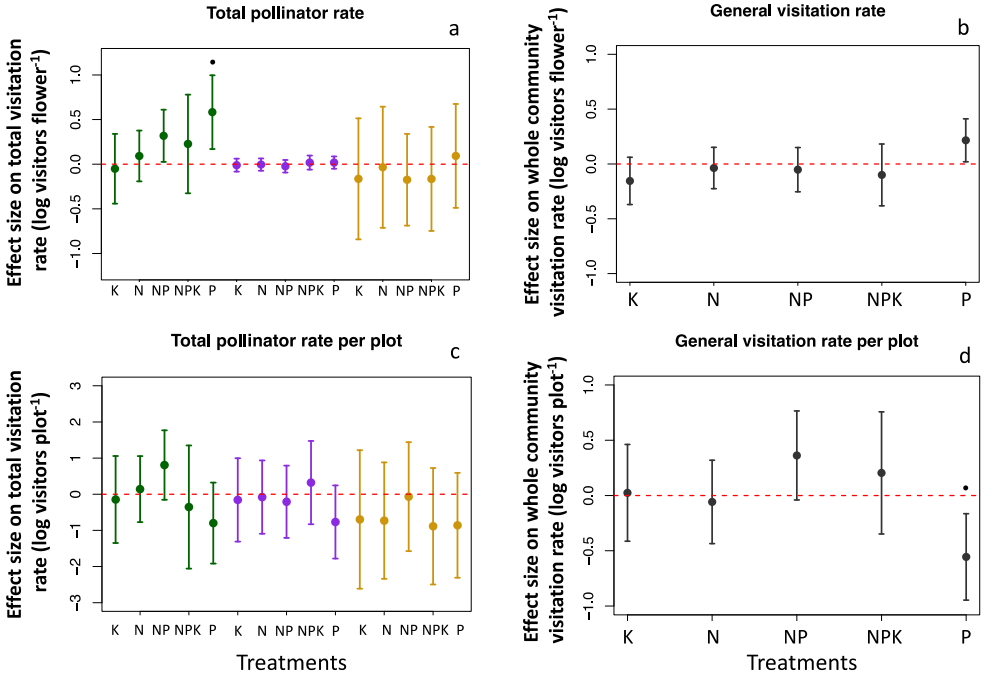


Figure 3.5. Effect size of the nutrient addition treatments on total pollinator visitation rate in each of the three plant species and in the whole plant community per flower (a, b) and per plot (c, d). Red dotted line represents control treatment. Species are coded by colours: green for *Raphanus*, purple for *Echium*, yellow for *Crepis* and black for whole plant community. Significant effects ($p<0.05$) are marked by (*) and marginally significant effects ($p<0.1$) are marked by (•). See statistical details in Table S3.3.

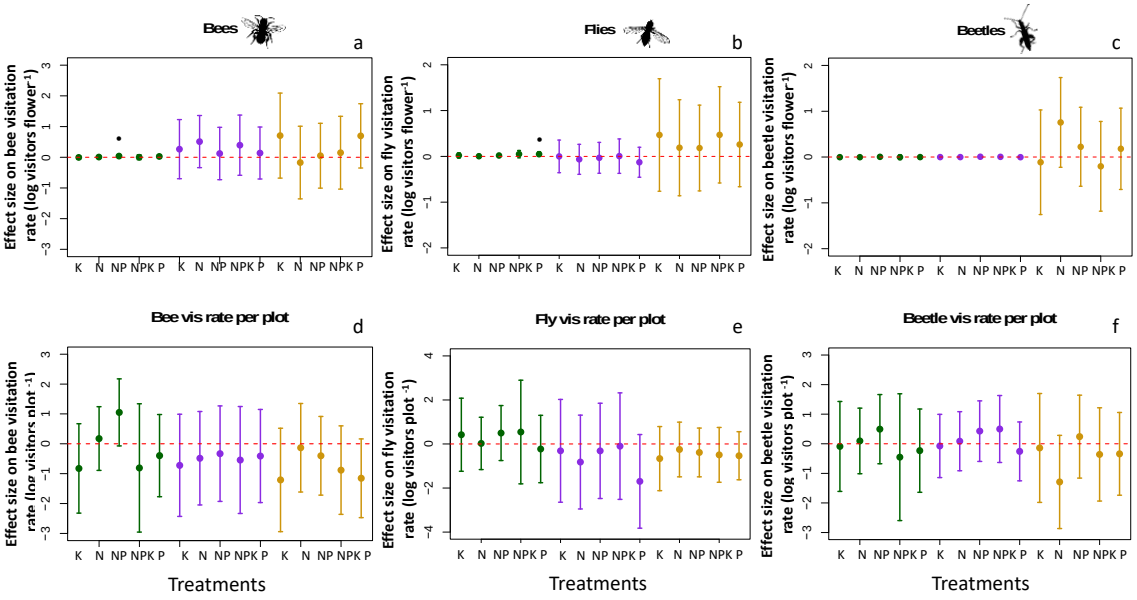


Figure 3.6. Effect size of the nutrient addition treatments on bee, fly and beetle visitation rate in each of the three plant species per flower (a, b, c) and per plot (d, e, f). Red dotted line represents control treatment. Species are coded by colours: green for *Raphanus*, purple for *Echium*, yellow for *Crepis*. Marginally significant effects ($p<0.1$) are marked by (•). See statistical details in Table S3.3.

3.4 Discussion

Using a unique dataset with a nine year nutrient-manipulation field experiment, we assessed, for the first time, the simultaneous effects of multiple nutrient addition treatments on flower resources (pollen quantity and quality) of three co-flowering Mediterranean grassland plant species, and on their visitors at the flower and plot scale. Despite a marginal negative effect on visitation rate (whole plant community) in the P treatment (Figs 3.5d), our study clearly shows that, for this level of nutrient additions (i.e., 100 kg ha^{-1} yr^{-1}), no treatment had a negative effect on total pollinator visitation rate (Figs 3.5a, b and 3.6 a-c), which was explained by trade-offs in the effects on pollen quantity and quality.

3.4.1 Effects of the nutrient additions on flower resources at flower scale

As expected, our nutrient addition treatments changed pollen quantity and quality in the three co-flowering Mediterranean plant species. However, the strength and the direction of the response to the nutrient addition treatments was plant species-specific, and there was no consistent effect of the nutrient addition treatments across plant species (Fig. 3.1).

For example, although adding N alone and together with P (i.e., N and NP) increased pollen yield per flower in *Crepis*, these nutrient addition treatments had no effect in the two other plant species, *Raphanus* and *Echium* (Fig. 3.1a). Therefore, our observations in *Crepis* corroborate those by Lau and Stephenson (1993), Atasay et al. (2013) and Vaudo et al. (2022), who reported increases in pollen yield in respectively *Cucurbita pepo* flowers, apple tree flowers and *Cucumis sativus* under N and NP addition.

The responses observed in our three plant species in terms of pollen aa concentration at flower scale (including for essential and non-essential aa – Fig. 3.1) further support that nutrient-driven changes are plant species-specific. While the marginal increase in *Raphanus*' pollen aa concentration (upon P addition, Fig. 3.1b) is in line with results from other studies (e.g., Gardener and Gillman 2001; Ceulemans et al., 2017), the decrease in *Crepis*' pollen aa concentration (upon N and NP additions) contradict it, and the lack of effect in *Echium*'s pollen aa concentration is in line with what was observed in *Linum lewisii* (Burkle and Irwin, 2009). The fact that we observed similar responses in essential and non-essential aa in the three plant species (Fig 3.1c-d), except for *Raphanus* upon P addition, contrasts with other studies on pollen chemistry reporting more changes in essential than in non-essential aa (Ceulemans et al., 2017c), or more changes in non-essential aa (Carvalheiro et al., 2023), further supporting the specificity of the plant species-specific responses to nutrient enrichment. Such plant species-specificity may be explained by non-exclusive factors:

i) plant species differ in their capacity to acquire nutrients and their relative growth rate. Since fast-growing species have a greater capacity to acquire nutrients (Lambers and Poorter, 1992), it is likely that they will not display their potential growth rate when they are limited by one or more nutrients. However, when nutrient limitation was alleviated by our nutrient addition treatments, the plant species with higher capacity to acquire nutrients displayed higher growth rates. The fact that *Raphanus* and *Echium* have Ellenberg scores higher than *Crepis*, suggests that these plant species have higher capacity to acquire nutrients, allowing them to benefit more from our nutrient addition treatments and compete with other plant species, including *Crepis*.

ii) different plant species or families may have higher demand for specific nutrients. For example, *Brassica* crops, which belong to the same plant family as *Raphanus*, display a high sulphur demand due to the production of glucosinolates that contain

at least two sulphur atoms (Marsic et al., 2021). Despite *Raphanus*' higher demand for sulphur, we did not observe a strong effect of adding K or NPK (i.e., K is added in the form of potassium sulphate) in the pollen concentrations of the aa containing sulphur [i.e., the essential aa methionine (Met) and the non-essential aa cysteine (Cys), Table S3.1 and S3.2, Fig. 3.1] or changes in growth or biomass as observed in treatments with N (data not shown).

By analysing pollen aa composition, the RDA clearly grouped our three plant species in two groups (Fig. 3.2) showing that the aa composition in *Crepis* pollen differed from that in *Raphanus* and *Echium*. This separation of *Crepis* from the group formed by *Raphanus* and *Echium* may be explained by non-exclusive factors such as: i) phylogenetic differences in the pollen aa composition in these three plant species (Roulston et al., 2000); ii) *Crepis* responded differently to the nutrient addition treatments when compared with *Raphanus* and *Echium*, as evidenced by the pollen aa concentration and quantity per plot (Fig. 3.4); iii) the fact that it was not possible to survey and sample the three plant species in the same year (*Crepis* was sampled in 2020 and *Raphanus* and *Echium* in 2021) added confounding factors such as differences in temperature and water availability, which may affect plant development and floral rewards (Descamps et al., 2021).

Since Mediterranean ecosystems are co-limited by N and P (Dias et al., 2011, Sardans and Peñuelas, 2013), it is not surprising that NP changed pollen aa composition the least in relation to the control in our grassland (Fig. 3.2), i.e., NP was the nutrient addition treatment that stoichiometrically resembled the control the most. Other studies have shown that addition of NPK increases nectar and/or pollen aa concentration and induces changes in aa composition (Gardener and Gillman 2001; Gijbels et al., 2015; Ceulemans et al., 2017c; N: Gijbels et al., 2014). Vaudo et al. (2022) showed that NP increased nectar volume and concentration and pollen concentration. However, since those studies only tested the effect of one treatment with two or three nutrients together, it is impossible to tease out which nutrient(s) is/are driving the response in terms of flower reward chemistry. Testing the addition of multiple nutrients alone and in combination is a clear advantage of our approach.

Furthermore, the fact that K and P were the most contrasting nutrient addition treatments and those that differed more from the control in terms of pollen aa composition, probably

reflects greater nutrient imbalances caused by these two nutrient addition treatments. Although the ecosystem is most likely co-limited by N and P, alleviating only the P limitation probably increased that of N. On the other hand, the K addition treatment corresponds to the annual addition of two nutrients (i.e., potassium and sulphur) and of other nutrients in the first year of experiment (i.e., iron, magnesium, manganese, copper, zinc, boron, and molybdenum), which are not likely to limit this ecosystem, and most probably increased the N and P co-limitation.

3.4.2 Effects of the nutrient additions on flower resources at plot scale

Despite several plant species-specific responses (Figs 3.1 and 3.2), the response to the NP treatment in terms of flower abundance of our three plant species was similar, with higher investment in sexual reproduction (flower abundance) in the NP treatment (Fig. 3.3), similar to what was observed in a Tibetan alpine grassland (Wang et al., 2022). Also, the response to P treatment was similar in the three species, negatively affecting flower abundance. Since most studies do not test the effect of different nutrient addition treatments alone and in combinations, it is difficult to compare our results with more studies. Nevertheless, other studies reported positive effects of adding N alone (Muñoz et al., 2004, Cuesta et al., 2008, Burkle and Irwin, 2010) similar to what we observed for *Raphanus*, or together with more nutrients (e.g. NPK – Burkle and Irwin, 2009).

By integrating the effects of our nutrient addition treatments on flower abundance (Fig. 3.3) and on pollen quantity and quality (Fig. 3.1), we observed a common pattern across our three plant species, i.e., the simultaneous addition of N and P (i.e., NP) increased or tended (e.g., *Raphanus* pollen quantity per plot, Fig. 3.4a) to increase pollen quantity and aa (including of essential and nonessential aa) at the plot scale (Fig. 3.4), but not at the flower scale (Fig. 3.1). Indeed, besides water, Mediterranean ecosystems are co-limited by both N and P (Dias et al., 2011; Sardans and Peñuelas, 2013; Nair et al., 2019). Therefore, alleviating the limitation of either N or P alone (or other nutrients, such as in the K addition treatment) triggers a greater depletion of the least available nutrient(s) as observed by the increased P limitation upon adding N in a Mediterranean shrubland (Ulm et al., 2017), and probably explains why the addition of P alone decreased *Raphanus* and *Echium* pollen yield and aa concentration at plot scale (Fig. 3.4). Alleviating the limitation of either N or P alone may create stoichiometric imbalances, which may have major

impacts on plant functioning (including plant drought tolerance (Nair et al., 2019)), and even change plant community composition (Fanelli, Lestini and Sauli, 2008; Dias et al., 2011). In agreement, we observed changes in plant vegetative growth (i.e., changes in biomass, data not shown) and in plant investment in sexual reproduction (i.e., changes in flower abundance – Fig. 3.3) resulting from our nutrient addition treatments. A clear example of the changes in plant investment in sexual reproduction is the insufficient number of *Crepis* flowers for pollen analysis in most treatments except N and NP (and control plots). Although plant investment in sexual reproduction can be affected by changes in nutrient stoichiometry (especially NP) in different ways (Wang, van Dijk and Wassen, 2022), flower abundance increased in the three studied co-flowering Mediterranean grassland plant species receiving NP, further supporting the co-limitation by N and P in this grassland ecosystem. However, in the case of *Crepis*, despite the positive tendency of adding NP on flower abundance (Fig. 3.3), this was levelled out by the trade-offs in pollen yield and quality at the flower scale (Fig. 3.1) and therefore we did not observe a positive effect of adding NP on pollen quantity and aa concentration (including of essential aa) at the plot scale (Fig. 3.4).

3.4.3 Linking nutrient-driven effects on flower resources and pollinators

At the flower scale, no nutrient addition treatment changed pollinator visitation rates for our flowering plant community composed of 21 plant species, although P addition tended to increase it (Fig. 3.5b). Furthermore, the only case of significant changes in pollinator visitation rates at flower scale was P addition, which increased *Raphanus* flower visitation rate (Fig. 3.5a), including of flies (Fig. 3.6b) and bees (NP treatment, Fig. 3.6a). The visitation rates of beetles at flower scale did not change with any nutrient addition treatment on *Raphanus*, or any other plant species and the entire flowering community, which separates the foraging strategies of flies and bees from those of beetles. The P-driven increase in *Raphanus* flower visitation rate may be related to the simultaneous increase in its pollen aa concentration (with a higher increase of non-essential aa compared to essential aa – Fig. 3.1). A similar link between improved aa concentration and increased pollinator visitation was reported by Hoover et al. (2012) for N fertilization. However, the P-driven decrease in flower abundance per plot we observed (Fig. 3.3) could also have influenced pollinator visitation rates since pollinators tend to visit more flowers in smaller flower patches compared to bigger patches (Goulson, 2000), i.e., in

low flower abundance patches, each flower has a higher probability of being visited than in high flower abundance patches. Therefore, the lower flower abundance of *Raphanus* (and of other plant species such as *Echium*) in P treatments could have increased the visitation of the few plants and flowers (Tadey 2015). Contrary to our results, other studies clearly show a link between increased flower abundance and increased visitation rate (e.g., Muñoz et al., 2005; Majetic et al., 2016).

Pollinator visitation rates of our three plant species remained unchanged at plot scale (Fig. 3.5-c). Bee, fly and beetle visitation rate did not change at plot scale (Fig. 3.6d-f). Similar results at plot scale were found by Burkle and Irwin (2010). However, when considering the full plant community, insect visitation showed a marginal decrease in visitation in P treatment at plot level (Fig. 3.5d). Although not significant, NP tended to increase pollinator visitation rates of the flowering plant community at the plot scale, which probably reflects the generalized NP-driven increase in flower abundance (Fig. 3.3), further reinforcing the ecosystem's co-limitation by N and P.

The fact that we did not observe a negative effect of our nutrient addition treatments on total pollinator visitation rates may be explained by the following non-exclusive factors:

i) the nutrient dose (i.e., 100 kg ha^{-1} yr^{-1}) we applied may be below the threshold for negative effects in Mediterranean ecosystems. Indeed, Millard et al., (2021) modelled the effect of land-use type and intensity (i.e., fertilizer application rate) on global pollinator biodiversity and showed that very high levels of intensity (1,000 kg ha^{-1}) negatively impacted Hymenoptera and Lepidoptera and but positively affected Diptera, while no effect was detected on Coleoptera; although levels around 250 kg ha^{-1} could be beneficial for some groups. Vaudo el al. (2022) found that higher doses of NP (>200 ppm) added in different ratios involved an asymptotic or negative effect on flower abundance and flower reward chemistry, while lower doses (75 ppm) brought positive effects. Similarly, Ramos et al. (2018) found that N doses between 60 to 72 kg ha^{-1} were optimum for crop pollinator density in the Brazilian Cerrado, while N doses $\geq$ 100 kg ha^{-1} decreased pollinator abundance. Therefore, pollinator responses to nutrient additions seem to depend on the nutrient being added, the dose, the ecosystem and the pollinator group.

ii) Although we did not have another system with lower diversity to compare, our grassland ecosystem's biodiversity (21 plant species and 58 insect visitor species) is likely to contribute to its resilience. Several studies corroborate the positive link between biodiversity and ecosystem resilience (Loreau, 2000; Isbell et al., 2015; Oliver et al., 2015; Mori, 2016), including the resilience of plant-pollinator systems (Huang, Tu and D'Odorico, 2021), while low diversity communities experience more changes (Isbell et al., 2015).

If pollinator visitation rates only reflected flower abundance (Fig. 3.3) (i.e., pollinators do not select the flowers they visit), what is known as neutral process (interactions are random and strongly dependent on flower abundance, Pizante et al., 2023), the plant species whose flowers would be more visited would be *Raphanus*, followed by *Echium*, and *Crepis* flowers would be least visited. Furthermore, the fact that *Raphanus* flowers produced more pollen with more aa (Fig. 3.1), followed by *Echium* and *Crepis*, would reinforce a potential pollinator choice based on probabilities alone. Indeed, *Raphanus* flowers received the higher visitation rate (0.22 visits flower^{-1}), but *Crepis* flowers received almost the same visits (0.19 visits inflorescence^{-1}), and much more than *Echium* flowers (0.15 visits flower^{-1}). This shows that the *Crepis* visitation rate was higher than would be expected based on flower abundance, pollen yield and aa concentration alone. Therefore, pollinators were attracted to *Crepis* flowers by other factors. Indeed, floral traits shape pollinator attraction to flowering plants (Carvalheiro et al., 2014; Wang et al., 2024), varying between pollinator taxa (Rader et al., 2020). Flower colour and shape can help explain the pollinator visitation rates we observed since: (i) Diptera, which accounted for ~20% of all visitors (data not shown), prefer white and yellow flowers (such as *Raphanus* and *Crepis*, respectively) over blue/violet flowers (such as *Echium*), (ii) solitary bees, which accounted for approximately half of the pollinators (data not shown), have a wide range of floral preferences, visiting all three plant species; and (iii) beetles prefer disk/bowl horizontal flowers (such as *Crepis*). Furthermore, other factors such as phenology, especially in fertilization treatments that could change it, also affect visitor preference to flowers (Peralta et al., 2020). Altogether, plant-visitor interactions respond to a combination of floral traits, floral chemistry and neutral processes (e.g., abundance of the plants and their flowers).

3.5 Conclusions

We assessed, for the first time, the simultaneous effects of multiple nutrient addition treatments on flower resources (pollen quantity and quality) of three co-flowering Mediterranean grassland plant species and their visitors, at the flower and plot scale. However, we identified the following limitations of our study: i) the difference in pollen extraction between *Echium* and *Raphanus* with accessible flower stamens and *Crepis* with less accessible inflorescences, ii) the small, or insufficient number of flowers sampled in some treatments (e.g., no *Crepis* samples in K, NPK and P; and low numbers of *Raphanus* flowers in NPK and P); iii) while pollinators were surveyed in two consecutive years, pollen quantity and quality was only analysed in one year, which differed between plant species (i.e., 2020 for *Crepis* and 2021 for *Raphanus* and *Echium*); iv) pollen may contain other important compounds for pollinators (e.g., proteins, sugars, fatty acids, alkaloids), whose quantity and composition may be influenced by our nutrient addition treatments, which were not analyzed; and v) while pollinators can collect pollen and/or nectar when visiting flowers, we did not analyse nectar quantity and quality, and vi) the site at Companhia das Lezírias has a plant and pollinator community historically adapted to low-management intensity levels (Sucena-Paiva et al., 2022).

Despite these limitations, our data suggest that effects at flower scale are plant-species specific, while those at plot scale are driven by community nutrient limitations. Our results highlight the varied responses of flowering plants to nutrient enrichment. Co-existing plants were affected to different degrees by nutrient enrichment, with the species *Echium* and its pollinators relatively unaffected. On the other hand, and following the expectations based on other studies, *Raphanus* increased aa concentration and visitation rate, although it was not related to higher flower abundance. Lastly, *Crepis*, against our predictions, increased pollen weight per flower but decreased aa concentration, with no effect on total visitation. A diverse plant community composed by flowering species that respond differently to nutrient enrichment has the potential to dilute the nutrient enrichment effects on the pollinator community. Further analysis is required to tease apart the relative effects of fertiliser and pesticide application at the global scale (Millard et al.,. 2021) as well as other global drivers of biodiversity loss.

3.6 Supplementary Information

Table S3.1. List of the amino acids (aa) detected in the pollen samples, and their respective abbreviations. Each aa was classified as essential or non-essential to animals, and the presence or absence of sulphur (S) was indicated.

Essential or non-essential	Amino acid (aa)	Aa symbol	Aa contains S
Essential	Arginine	Arg	no
	Histidine	His	no
	Isoleucine	Ile	no
	Leucine	Leu	no
	Lysine	Lys	no
	Methionine	Met	yes
	Phenylalanine	Phe	no
	Threonine	Thr	no
	Valine	Val	no
Non-essential	Alanine	Ala	no
	Aspartic acid	Asp	no
	Cysteine	Cys	yes
	Glutamic acid	Glu	no
	Glycine	Gly	no
	Proline	Pro	no
	Serine	Ser	no
	Tyrosine	Tyr	no

Table S3.2. Pollen amino acid (aa) concentration. The concentration of each aa is presented per sample separated by year, plant species, treatment and plot.

Plot	Treatment	Year	Species	Aspartic acid	Threonine	Serine	Glutamic acid	Proline	Glycine	Alanine	Cysteine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Histidine	Lysine	Arginine
C1A	C	2021	Raphanus	115.89	45.96	65.50	107.88	85.40	78.58	76.42	17.74	125.48	31.88	47.51	71.93	26.09	55.49	20.79	70.23	39.01
C1B	C	2021	Raphanus	130.10	51.68	70.05	111.21	99.70	80.45	77.43	10.29	125.95	33.49	50.42	76.69	28.46	58.82	23.04	71.88	42.22
C2A	C	2021	Raphanus	156.31	73.83	91.63	134.32	116.54	100.61	106.19	17.10	165.84	44.34	73.02	100.12	34.82	73.02	29.01	91.52	50.60
C2B	C	2021	Raphanus	186.06	70.74	100.75	172.40	126.06	117.60	119.34	20.21	181.55	48.92	70.86	107.50	40.88	82.80	35.63	111.37	61.07
C2C	C	2021	Raphanus	88.71	40.97	56.21	93.55	80.81	70.15	64.54	19.60	109.89	34.10	41.77	61.71	22.42	48.98	17.88	63.73	33.53
C3A	C	2021	Raphanus	162.55	63.87	85.82	139.34	133.77	105.60	98.11	20.27	168.72	46.62	65.88	99.78	35.89	75.90	29.36	100.10	55.87
C3B	C	2021	Raphanus	144.76	65.41	80.99	125.84	103.94	92.28	89.99	15.33	148.81	38.83	64.61	90.28	31.83	68.95	24.79	81.78	45.06
K1A	K	2021	Raphanus	154.35	57.20	80.86	138.68	116.81	99.91	88.19	14.98	153.44	41.17	60.99	92.86	32.78	68.56	28.09	93.90	50.23
K2A	K	2021	Raphanus	173.10	70.19	96.26	147.22	127.80	114.91	110.04	21.20	180.67	54.21	72.10	108.71	40.79	85.61	33.23	110.84	58.86
K2C	K	2021	Raphanus	165.27	63.83	88.87	146.31	114.36	106.65	100.02	15.91	162.18	45.00	65.39	98.21	35.64	76.34	28.64	95.77	54.45
K3A	K	2021	Raphanus	171.40	74.11	98.15	173.31	123.00	115.96	111.13	16.87	177.21	49.20	73.13	110.15	40.85	85.74	32.59	111.92	61.69
K3B	K	2021	Raphanus	175.12	71.98	100.79	158.14	134.62	117.72	112.00	20.46	183.71	50.49	72.96	109.83	40.91	84.90	32.20	102.65	59.97
K3C	K	2021	Raphanus	158.58	60.90	87.17	147.57	0.00	102.61	94.70	32.16	156.36	40.15	61.51	93.23	35.51	70.48	28.48	88.94	50.43
N1A	N	2021	Raphanus	140.10	51.78	73.99	113.17	0.00	84.19	87.22	0.00	135.06	34.01	53.47	80.64	28.48	58.11	24.52	78.64	43.29
N1C	N	2021	Raphanus	128.77	60.87	82.90	134.42	108.76	102.01	94.48	19.17	157.68	44.03	63.04	95.07	34.85	75.24	26.65	95.39	51.71
N2B	N	2021	Raphanus	127.90	54.90	74.39	126.69	0.00	91.37	85.51	0.00	142.36	36.91	56.46	85.07	30.47	66.59	23.70	78.87	45.57
N3A	N	2021	Raphanus	177.76	52.82	75.96	134.48	0.00	92.56	85.13	0.00	157.53	43.79	58.34	88.17	32.79	66.99	25.49	77.68	49.58
N3B	N	2021	Raphanus	158.50	61.25	85.81	155.15	110.76	101.59	94.98	14.36	152.23	37.45	60.46	92.17	33.03	69.44	28.46	91.86	52.31
N3C	N	2021	Raphanus	159.46	63.71	86.34	139.54	0.00	99.78	96.10	32.16	65.56	36.97	65.13	97.47	29.98	74.03	101.94	0.00	51.65
NP2A	NP	2021	Raphanus	159.75	68.85	91.11	148.55	0.00	110.38	107.81	0.00	170.34	44.83	68.53	104.76	39.21	81.17	30.25	101.93	58.31
NP2B	NP	2021	Raphanus	158.18	63.73	89.15	129.20	126.09	103.33	101.67	20.70	169.21	46.78	64.21	98.23	35.97	75.56	27.50	93.18	53.09
NP2C	NP	2021	Raphanus	159.96	59.64	86.68	123.12	0.00	96.63	97.90	32.16	152.19	37.77	60.13	92.99	34.44	70.30	25.73	86.05	49.42
NP3A	NP	2021	Raphanus	118.82	55.92	77.95	110.81	110.79	81.37	83.89	13.14	114.04	34.30	50.44	80.36	23.50	60.41	22.15	73.73	41.12
NP3B	NP	2021	Raphanus	171.55	81.94	113.66	157.42	176.31	121.77	126.26	9.70	175.83	50.11	76.28	121.94	43.65	90.62	36.06	114.32	66.61
NP3C	NP	2021	Raphanus	155.22	63.60	94.45	163.96	116.43	101.38	97.18	17.11	137.18	39.06	55.10	92.10	26.04	71.31	29.17	98.57	51.44
NPK3B	NPK	2021	Raphanus	190.25	54.08	87.11	146.48	122.66	93.60	84.97	22.33	145.87	35.74	56.16	85.67	21.23	68.76	27.87	86.33	43.13
P2A	P	2021	Raphanus	220.56	81.95	120.07	181.88	170.28	129.21	127.12	11.38	183.05	51.22	75.28	123.57	39.62	94.58	37.89	119.64	63.53
C1	C	2021	Echium	130.62	60.65	76.64	140.79	76.36	98.32	92.78	12.01	83.98	17.92	63.67	96.61	35.20	47.96	24.75	72.28	49.82
C1	C	2021	Echium	121.02	55.03	67.30	123.07	58.82	87.34	82.95	16.51	75.98	13.54	55.29	84.09	31.76	43.54	22.74	68.01	41.82
C2	C	2021	Echium	205.65	96.45	120.74	217.55	100.61	146.79	144.88	18.67	128.03	17.63	103.83	152.23	47.85	76.46	39.45	114.23	76.64
C3	C	2021	Echium	220.94	100.44	122.89	235.10	101.89	156.77	150.90	22.01	136.56	35.39	103.91	157.23	51.96	79.93	42.15	125.84	83.74
C3	C	2021	Echium	168.47	78.98	99.60	193.42	78.34	130.35	120.50	18.17	109.83	29.56	80.29	122.84	41.24	62.06	32.29	94.78	64.42
K1	K	2021	Echium	42.25	22.55	20.55	14.96	39.74	23.13	83.00	27.70	45.92	0.00	22.51	47.04	14.87	18.23	9.00	23.56	26.38
K1	K	2021	Echium	91.14	44.49	55.25	108.36	41.10	66.80	63.96	12.61	61.03	8.09	44.52	68.00	23.97	35.78	17.17	43.49	34.62
K1	K	2021	Echium	103.14	50.36	36.82	89.27	90.15	67.61	149.08	29.96	88.98	18.25	63.23	106.84	33.70	47.92	21.56	57.50	51.05
K2	K	2021	Echium	136.62	66.91	82.19	155.60	65.62	104.52	100.44	16.10	93.91	17.04	71.36	106.63	37.84	51.82	24.77	70.65	53.68
K2	K	2021	Echium	183.56	90.89	109.82	204.71	86.56	135.62	135.28	17.75	120.33	28.31	95.38	144.27	51.35	71.43	34.48	90.24	71.94
K3	K	2021	Echium	46.07	25.83	20.15	8.44	53.18	21.30	88.47	38.45	49.69	4.65	25.78	51.26	17.92	18.42	7.63	20.36	29.70
K3	K	2021	Echium	135.69	63.50	80.88	152.99	68.89	102.01	98.47	21.50	95.12	13.47	69.19	104.33	36.46	51.91	23.92	59.16	50.66
K3	K	2021	Echium	178.27	87.72	107.09	204.99	85.64	133.50	130.76	20.27	119.93	43.68	105.61	143.97	46.75	69.19	33.29	82.74	69.33
N1	N	2021	Echium	221.15	109.30	135.56	257.43	0.00	168.91	165.90	0.00	144.67	44.00	113.22	174.96	64.18	87.25	40.82	111.81	89.68
N3	N	2021	Echium	224.13	107.45	136.52	269.79	119.12	175.70	165.30	25.10	144.34	43.81	112.58	170.06	69.13	85.47	43.73	117.75	89.40
N3	N	2021	Echium	119.65	57.97	72.09	135.57	0.00	91.57	87.25	0.00	78.35	23.92	59.93	91.26	36.05	46.49	22.26	62.65	47.52
NP1	NP	2021	Echium	64.58	31.74	39.92	72.99	25.07	48.37	47.12	13.90	46.54	5.13	29.86	48.80	17.90	23.77	11.61	30.69	22.88
NP1	NP	2021	Echium	174.75	86.61	106.97	192.91	80.45	131.13	128.71	23.23	121.72	34.51	85.37	133.05	43.21	68.54	32.14	89.09	68.90
NP3	NP	2021	Echium	133.20	65.59	66.13	100.22	0.00	114.18	125.87	32.16	97.07	35.27	85.90	122.19	43.39	54.21	25.52	86.41	60.86
NP3	NP	2021	Echium	181.36	88.87	111.00	213.67	0.00	140.27	133.16	0.00	119.02	31.28	92.43	139.56	53.31	70.96	35.26	94.43	72.52
NP3	NP	2021	Echium	123.31	60.94	77.27	144.32	61.08	98.40	93.20	16.13	83.56	17.43	62.97	97.61	36.79	49.73	24.01	66.91	51.02
NPK1	NPK	2021	Echium	97.54	48.16	59.63	116.59	0.00	75.12	71.18	0.00	67.21	19.21	49.76	75.53	28.69	38.11	17.75	47.69	39.07
NPK1	NPK	2021	Echium	179.67	88.86	110.79	202.26	0.00	136.96	132.06	32.16	119.31	34.07	91.21	139.77	52.78	71.10	33.28	94.42	72.81
NPK1	NPK	2021	Echium	99.50	48.69	61.36	115.16	48.02	78.35	73.30	15.34	67.31	16.33	50.42	75.38	30.16	39.38	18.79	54.12	40.01
NPK3	NPK	2021	Echium	84.23	46.03	30.69	65.83	0.00	30.28	129.06	32.16	90.46	20.49	64.73	94.00	31.93	40.37	18.24	32.68	49.11
NPK3	NPK	2021	Echium	111.60	52.39	66.51	122.31	60.80	80.47	78.54	16.31	135.45	37.43	55.54	85.17	27.04	63.19	20.60	58.09	42.03
P3	P	2021	Echium	140.72	68.07	84.00	155.07	76.50	104.03	99.72	9.80	166.29	5.36	72.75	108.73	40.90	82.09	27.12	76.04	55.54
P3	P	2021	Echium	130.94	63.15	77.44	141.67	69.41	94.98	91.95	15.92	165.41	34.60	82.40	107.39	31.21	75.53	24.31	71.65	50.71
C1	C	2020	Crepis	13.78	6.30	9.11	14.23	11.30	11.40	11.66	4.81	8.44	0.93	4.97	9.10	3.48	5.11	2.28	8.71	4.98
C2	C	2020	Crepis	9.35	4.44	6.43	9.84	5.66	7.71	7.65	4.19	6.51	0.57	4.45	6.86	3.04	3.47	1.33	5.80	3.11
C3	C	2020	Crepis	12.70	5.68	8.05	12.31	9.77	9.75	10.29	5.63	8.04	0.50	4.49	8.10	2.91	4.68	1.92	7.56	4.20
N1	N	2020	Crepis	8.54	3.96	5.77	8.73	6.29	6.96	6.86	3.41	5.42	0.59	3.66	6.05	2.22	3.17	1.36	5.47	3.14
N3	N	2020	Crepis	1.61	0.67	1.35	1.65	1.45	1.20	1.14	2.49	1.65	0.12	0.54	0.75	0.34	0.56	0.17	0.89	0.34
N3	N	2020	Crepis	5.93	2.70	3.74	5.90	2.59	4.79	4.66	2.97	4.16	0.36	2.11	3.85	1.59	2.24	1.05	3.65	2.19
NP1	NP	2020	Crepis	10.12	4.64	6.68	10.78	9.34	7.77	8.14	3.49	6.28	0.63	3.51	6.77	2.52	3.55	1.58	6.54	3.46
NP1	NP	2020	Crepis	8.81	3.91	5.24	8.21	6.52	5.93	6.04	2.95	5.21	0.25	2.88	5.38	2.04	2.99	1.32	5.06	2.67
NP2	NP	2020	Crepis	3.64	1.60	2.59	3.96	1.30	2.86	2.72	2.97	3.12	0.20	1.22	2.33	0.69	1.06	0.51	2.15	1.09
NP2	NP	2020	Crepis	2.07	0.89	1.40	2.15	1.39	1.59	1.66	2.48	1.84	0.14	0.68	1.33	0.58	0.69	0.18	1.14	0.52
NP3	NP	2020	Crepis	3.76	1.54	2.48	3.79	2.34	2.72	2.46	1.91	2.47	0.09	1.33	2.38	0.91	1.53	0.46	2.26	1.12
NP3	NP	2020	Crepis	3.01	1.32	2.08	3.19	1.93	2.05	2.09	2.11	2.13	0.16	0.98	1.81	0.57	1.07	0.35	1.79	0.90

Table S3.3. Summary of the Linear Mixed Models (LMMs) per flower and plot of pollen weight, aa concentration, EAA and non-EAA concentration, flower abundance and visitation rate (total, bee, fly and beetle) of the three plant species.

	Flower level				**Plot level**			
	TREATMENT		YEAR		TREATMENT		YEAR	
	Chi sq	p-value	Chi sq	p-value	Chi sq	p-value	Chi sq	p-value
Raphanus								
Pollen weight	7.3	0.190			55.18	<0.001		
Aa concentration	12.96	0.023			62.16	<0.001		
EAA	11.03	0.051			51.6	<0.001		
nEAA	14.12	0.02			38.98	<0.001		
Flower abundance					102.30	<0.001		
Total vis rate	12.38	0.030	0.392	0.531	18.21	0.002	1.399	0.230
Bee vis rate	10.11	0.072	0.79	0.374	19.65	0.001	0.226	0.634
Fly vis rate	10.46	0.063	9.46	0.002	2.92	0.712	1.83	0.175
Beetle vis rate	0.82	0.976	6.34	0.012	3.48	0.627	5.447	0.019
Echium								
Pollen weight	6.82	0.230			7.22	0.2041		
Aa concentration	7.48	0.190			8.40	0.1353		
EAA	7.35	0.190			14.9	0.011		
nEAA	7.83	0.165			15.37	0.008		
Flower abundance					41.50	<0.001		
Total vis rate	1.85	0.869	0.20	0.656	8.92	0.112	0.11	0.739
Bee vis rate	4.13	0.531	0.42	0.515	1.71	0.887	0.0017	0.966
Fly vis rate	1.88	0.87	12.190	<0.001	6.94	0.220	12.837	<0.001
Beetle vis rate	5.23	0.38	6.300	0.012	6.11	0.295	9.590	0.002
Crepis								
Pollen weight	13.70	0.001			9.64	0.008		
Aa concentration	40.74	<0.001			1.04	0.5931		
EAA	37.03	<0.001			3.29	0.193		
nEAA	42.83	<0.001			3.36	0.186		
Flower abundance					331703	<0.001		
Total vis rate	1.11	0.952	4.62	0.032	5.01	0.415	1.04	0.307
Bee vis rate	6.20	0.287	0.28	0.593	9.75	0.082	0.039	0.843
Fly vis rate	2.49	0.778	3.08	0.079	3.55	0.615	3.51	0.060
Beetle vis rate	7.10	0.213	0.21	0.643	7.12	0.212	0.448	0.503
Whole community								
Flower abundance					47.72	<0.001		
Visitation rate	12.47	0.028	30.58	<0.001	19.99	0.001	0.325	0.568

Table S3.4. RDA results including partitioning of the variance, Anovas by term and by axis and the coordinates of the points represented in the graph (Fig. 3.2).

VARIANCE

Partitioning of variance:

	Inertia	Proportion
Total	910956	1.0000
Constrained	816368	0.8962
Unconstrained	94588	0.1038

ANOVA BY TERM

Model: rda(formula = tab ~ Species + Treatment, data = tab1)

	Df	Variance	F	Pr(>F)	
Species	2	442673	16.3800	0.002	**
Treatment	5	373695	5.5311	0.026	*
Residual	7	94588			

ANOVA BY AXIS

Model: rda(formula = tab ~ Species + Treatment, data = tab1)

	Df	Variance	F	Pr(>F)	
RDA1	1	784477	58.0553	0.005	**
RDA2	1	24831	1.8376	0.909	
RDA3	1	5608	0.4150	1.000	
RDA4	1	963	0.0713	1.000	
RDA5	1	273	0.0202	1.000	
RDA6	1	161	0.0119	1.000	
RDA7	1	56	0.0041	1.000	
Residual	7	94588			

COORDINATES (AAs, rows, species and treatments)

	RDA1	**RDA2**
Aspartic acid	-22.716993	0.5916782
Threonine	-9.999035	-1.2342405
Serine	-12.876270	-0.1083983
Glutamic acid	-21.993766	-2.9136605
Proline	-12.259571	5.5351209
Glycine	-15.773148	-1.2949837
Alanine	-16.122954	-2.6466503
Cysteine	-2.665734	-0.2258503
Valine	-19.562603	5.0887501
Methionine	-5.273012	1.7957930
Isoleucine	-10.312171	-1.9136832
Leucine	-15.684786	-2.8942466
Tyrosine	-5.582433	-1.0864225
Phenylalanine	-9.827419	1.3459944
Histidine	-4.426708	0.1061421
Lysine	-12.819918	1.4374014
Arginine	-8.312950	-1.1703107

	RDA1	**RDA2**
row1	22.181793	1.885057
row2	23.070661	2.021629
row3	22.423545	2.100632
row4	-12.819445	-17.136414
row5	-18.332566	-19.461543
row6	-1.085596	-23.029541
row7	-6.204881	-24.808768
row8	-2.136406	-19.113188
row9	10.519691	4.420289
row10	-20.137997	33.728419
row11	-19.890566	25.609276
row12	-12.429990	-1.253070
row13	-16.976725	18.708783
row14	16.976441	7.672328
row15	14.842041	8.656110

	RDA1	**RDA2**
Crepis	22.5587	2.002
Echium	-5.0099	-16.522
Raphanus	-6.2695	15.520
Treatment C	-3.5919	6.159
Treatment K	-19.1116	3.074
Treatment N	3.1850	-7.420
Treatment NP	-0.2527	-1.333
Treatment NPK	7.4200	-5.720
Treatment P	12.6809	6.538

Table S3.5. List of flower visitors per year. Each column represent the plant species visited.

Year	Species	*Raphanus raphanistrum*	*Echium plantagineum*	*Crepis capillaris*	Total
2020	*Andrena fulica*	2			2
2020	*Andrena pilipes*	1			1
2020	*Andrena suerensis*	4			4
2020	*Anthophora atroalba*		1		1
2020	*Apis mellifera*	2	6		8
2020	*Bombylius fimbriatus*	1			1
2020	*Bombylius medius*	12			12
2020	*Bombylius minor*	3			3
2020	*Bombylius sp*	2			2
2020	*Chasmatopterus*			2	2
2020	*Dasypoda dusmeti*		1	2	3
2020	*Dischistus*		2		2
2020	*Eucera codinai*		3		3
2020	*Eucera collaris*	1			1
2020	*Eucera sp*		1		1
2020	*Heliotaurus ruficolis*	1	10		11
2020	*Hymenoptera*			1	1
2020	*Lasioglossum algericolelum*	4	1		5
2020	*Oedemera*	4	1	2	7
2020	*Panurgus*			1	1
2020	*Pieris rapae*	3			3
2020	*Sirphidae*	1			1
2020	*Sphaerophoria*	1			1
2020	*Tropinota*	2	1		3
2021	*Andrena sp1*	2			2
2021	*Andrena cf pilipes*	1			1
2021	*Andrena fulica*	3			3
2021	*Andrena orana*	8			8
2021	*Andrena rhyssonota*			8	8
2021	*Andrena suerinensis*	5			5
2021	*Andrena vetula*	1			1
2021	*Apis mellifera*		1		1
2021	*Aricia cramera*			1	1
2021	*Bombylius cruciatus*	3	25		28
2021	*Bombylius fimbriatus*	4			4
2021	*Bombylius medius*	18			18
2021	*Bombylius minor*	2		2	4
2021	*Bombylius sp*	3			3
2021	*Chasmatopterus sp*			44	44
2021	*Colletes acutus*	11			11
2021	*Dasypoda dusmeti*		1	1	2
2021	*Diptera*			2	2
2021	*Dischistus sp*		19		19
2021	*Eucera sp*		7		7
2021	*Eucera cineraria*		14		14
2021	*Eucera hispaliensis*		1		1
2021	*Lasioglossum algericolellum*	14	11	5	30
2021	*Lycaena phlaeas*	1			1
2021	*Microchrysa sp*	2			2
2021	*Nustera distigma*			1	1
2021	*Oedemera barbara*			1	1
2021	*Oedemera sp*	5		13	18
2021	*Osmia sp*		3		3
2021	*Osmia tricornis/bicornis*		1		1
2021	*Oxythyrea funesta*		2		2
2021	*Panurgus calcaratus*			1	1
2021	*Psilothryx viridicoerulea*	23	1	9	33
2021	*Sphaeroforia sp*			2	2
2021	*Tropinota squalida*	5			5
2021	*Usia aenea*		8	2	10
2021	*Tenthredo sp*	1			1

CHAPTER 4. Negative effects of nitrogen input on flowers and pollinators are greater in warmer climates

4.1 Introduction

Grasslands and shrublands are highly diverse ecosystems that cover 40% of the global land area and provide important ecosystem services (White et al., 2000; Habel et al., 2013; Bengtsson et al., 2019). Human activities have profoundly transformed these ecosystems worldwide, and nutrient enrichment derived from agro-industrial activities is recognized as a major threat to global biodiversity (Sala et al., 2000; De Schrijver et al., 2011; Ceulemans et al., 2017a; 2017b) and ecosystem functioning (Phoenix et al., 2012; Southon et al., 2013). Nutrient enrichment decreases plant species diversity as it generally promotes fast-growing tall species (e.g. grasses and other nitrophilous plants) which outcompete slow-growing short species (e.g. forbs) for light (Bobbink et al., 1998, 2010; Hautier et al., 2009; Borer et al., 2014; Eskelinen et al., 2022). Such shifts in plant community composition may influence the abundance and diversity of pollinators, since some pollinators prefer nitrophilous plant species while others prefer nitrophobous species (Öckinger et al., 2006; Carvalheiro et al., 2019). Nutrient enrichment also affects the investment of plants in the production, morphology, and chemical composition of flowers (David et al., 2019); characteristics that influence pollinator foraging choices (Vanbergen, 2013; Wagner et al., 2021) and for which investments likely vary among plant species. Understanding the effects of nutrient enrichment on floral resources and pollinators is, consequently, fundamental to better assess the impacts of global changes.

The effect of nutrient enrichment on plant communities and pollinators may vary across regions and local soil conditions. For example, plant species from oligotrophic environments tend to be more sensitive to soil nutrient enrichment (Olsson and Kellner 2006; Dise et al., 2011; Vogels et al., 2023). Consequently, nutrient-driven changes in the floral resources and pollinators of oligotrophic environments may be more accentuated. Also, variation in regional temperature affects plants and pollinators (Totland, 1994; Holland and Bourke, 2015) and, hence, may influence how plant-pollinator interactions are affected by nutrient enrichment. While in tundra low temperatures and low nutrient

availability are the main plant stressors, in regions with warmer climates the constraints come mostly from nutrient availability (De Deyn et al., 2008).

To compare the effect of nutrient enrichment on floral resources and pollinators across regions, we gathered data from 14 grassland and shrubland sites spread through three continents, covering a diversity of climatic conditions and soil fertility levels. These sites have identically replicated a long-term nutrient manipulation experiment, as part of the global Nutrient Network Experiment (Borer et al., 2014, see further methodological details below). Overall, we expect that the effect of nutrient enrichment on floral resources will differ across regions, depending on regional natural soil fertility and annual mean temperature (H1). More specifically, we expect more frequent and accentuated plant losses at lower temperatures. Also, as N input is a powerful driver of plant diversity loss (Stevens et al., 2004; Tognetti et al., 2021) due to its limitation in the soils, we expect stronger effects of N addition compared to P or K. N also increases flower abundance (Muñoz et al., 2005; Cuesta et al., 2008) so we expect to record higher flower abundance when N is added. Last, the addition of more nutrients is linked to stronger negative changes in plant richness (Harpole et al., 2016) so we expect to see higher losses in plant richness in treatments with more nutrients combined. On the other hand, whilst we expect that pollinator response to nutrient enrichment depends on regional natural soil fertility and mean annual temperature, as effects of nutrient enrichment on pollinators are driven both by quantitative and qualitative changes in flower resources, the effects will be more accentuated for pollinators than for flower resources (H2).

4.2 Material and Methods

4.2.1 *Study site and experimental design*

We conducted a study at 14 experimental sites in grasslands and shrublands (Table S4.1, Fig S4.1) spread through three regions: North America (5), South America (3) and Europe (6). All but one (Argentina-rnegro.ar) participate in the Nutrient Network (NutNet, Borer et al., 2014) and all use a similar experimental design, with a full-factorial combination of nutrients (N, P, K) to 25 m^2 plots arranged in randomized blocks. The resulting treatments were N, NP, NK, NPK, K, PK, P, and Control (Control = no nutrient added). Each treatment had 3 to 8 replicates depending on the site. N, P and K were added at a

rate of 10 g m^{-2} yr^{-1}. N was applied as time-release urea, P as triple-superphosphate and K as potassium sulphate. In the first year of fertilization of every site, each plot that received K also received 100 g m^{-2} of a nutrient mixture containing iron (15%), sulphur (14%), magnesium (1.5%), manganese (2.5%), copper (1%), zinc (1%), boron (0.2%), and molybdenum (0.05%) (Borer et al., 2014). Thus, K treatment represents the addition of K and also macro and micronutrients. In Argentina-rnegro.ar, N was supplied at a rate of 10 g $m^{-2}.yr^{-1}$, P at 7.5 g $m^{-2}.yr^{-1}$ and K at 5.6 g.$m^{-2}.yr^{-1}$without supplementing a mixture of micronutrients.

4.2.2 Flower and pollinator community surveys

In each of the 14 study sites, we surveyed flower and pollinator communities during the local peak flowering season. We collected data between 2017 and 2022, some sites having data for more than one year (see details in Table S4.1). Within each survey, all flower units present in each plot were counted (excluding Poaceae plants that are not entomophilous). To standardize measurements of flower abundance between species, we defined a floral unit as 1 cm^2 with at least one open flower (see Carvalheiro et al., 2014 for more details). For some species an inflorescence corresponded to one floral unit (e.g. *Ornithopus compresus* or *Trifolium campestre*) while for other species single flowers such as *Balsamorhiza sagittata* or *Carduus acanthoides* contained several floral units per inflorescence. When flower abundance was very high, we estimated the total number of flower units based on randomly selected subsamples, (i.e. two 1 m^2 plots were randomly placed within the plot), and counted all floral units within to then estimate flower abundance at plot level (25 m^2). We identified all vascular plants to species level.

For seven study sites (four in North America, one in Europe and two in South America) we sampled pollinators (see details in Table S4.1). We conducted surveys only under favourable weather conditions for insect activity, with no rain, no wind and air temperatures between 13 and 35 degrees Celsius. In each plot, we surveyed all flowering species (excluding Poaceae plants that are not entomophilous) using a time-based methodology. For each flowering plant species, we selected a focal spot with 10 to 30 flower units and all the flower units were observed during a fixed time period. To standardize effort, we calculated pollinator abundance per plot. We registered all flower visitation events (defined as an insect contacting the reproductive parts of the flower) and

in cases when a visitor escaped before being captured (net or entomological aspirator) a detailed description was added. All captured flower visitors were later separated into morphospecies and, whenever possible, identified to species level by a taxonomist (see acknowledgements). Non-captured individuals with a description that did not match any of the collected individuals were kept in the richness analyses as separate morphospecies.

4.2.3 Climate and soil information

For each study site we extracted information on air temperature and soil variables that characterize the region where plants and pollinators evolved. For temperature, we extracted all values of monthly minimum and maximum temperatures from 2014 to 2018, and calculated mean average air temperature. We used information from multiple years to control for exceptional weather events (Table S4.1). In most sites, temperature information was obtained from in-site weather stations. In sites without local climate stations, information was extracted using WorldClim (Fick and Hijmans, 2017).

Information on soil fertility metrics was obtained in control plots between 2016 and 2021. In each site we collected soil samples of around 100g. We opted to use soil total concentrations of N (in %) and of P (in ppm) as a proxies of soil fertility (Table S4.5). Nitrogen concentration was obtained using dry combustion gas chromatography on an Elemental Analyzer (Costech ECS 4010 CHNSO Analyzer, Valencia, California, USA) and phosphorous concentration, using the Mehlich-3 extraction method.

4.2.4 Data analyses

For H1 and H2 we ran general linear mixed models (GLMM, assuming negative binomial distribution). For H1 we ran models for flower unit abundance and plant richness using data from all sites (14) while for H2 we ran models for pollinator abundance and pollinator richness using data from seven sites that had pollinator data. All models from the two hypotheses had three nutrients (N, P and K) included as binomial fixed explanatory variables (added vs not added), as well as temperature, and soil N and P concentrations. Year and plot within block within study site were included as random structure. To control for potentially long-term effects, “years since start of fertilization”

was included as a covariate. Analyses were performed in R using the lme4 R package (Bates el al 2015). DHARMa R package (Hartig, 2020) was used to verify the model residual distribution, outliers and dispersion. The full models for flower abundance and plant richness (H1) and pollinator abundance and richness (H2) included all possible interactions between nutrients, temperature and soil N (up to quadruple interactions). Soil P was added as a fixed factor as model AICc was lower when it was excluded from the interactions with nutrients and temperature. Additionally, for each of the variables in H1 and H2 we ran the dredge function (MuMIn R package, Burnham and Anderson, 2002) with three models that contained all the possible interactions between the variables in order to obtain the model with the lowest AICc (Table S4.2). These models were used to identify the significant effects of variables and interactions in the models as well as the importance (weight) of each nutrient.

4.3 Results and Discussion

A total of 306 flowering plant species belonging to 56 families and 251 pollinator species were recorded in the study sites (Table S4.4). Hymenoptera made up 54 % of the visitor species, followed by Diptera (18%), Lepidoptera (16%), and Coleoptera (11%). Flies and butterflies were recorded in all sites, while bees were absent in one site in South America (Argentinian southern site) and beetles were absent from two sites in South America (Argentinian southern site and Brazilian Cerrado) (Table S4.1). Overall, we show that the effect of N enrichment on floral resources differs across regions, depending on regional natural soil fertility and annual mean temperature, also depending to a lesser extent on P and K input. Warmer regions were particularly sensitive to negative impacts on floral resources, which apart from having reduced densities also had a lower number and diversity of potential pollinators.

4.3.1 Influence of climate on floral resources

The impact of nutrient input on floral resources depended on local climate and natural soil conditions (Table S4.2). As N input is known to be an important driver of plant diversity loss (Stevens et al., 2004; Tognetti et al., 2021) we expected stronger effects of N addition compared to P or K. Indeed, P and K had much lower importance than N

explaining the variability of flower abundance and diversity, with very mild positive effects on flower abundance (Tables S4.2 and S4.3). Nevertheless, P did have an important impact on plant richness (e.g. increasing P availability reduced richness) and mediated the effects of N (i.e. interactive effects were detected, see Fig 4.1, Table S4.2).

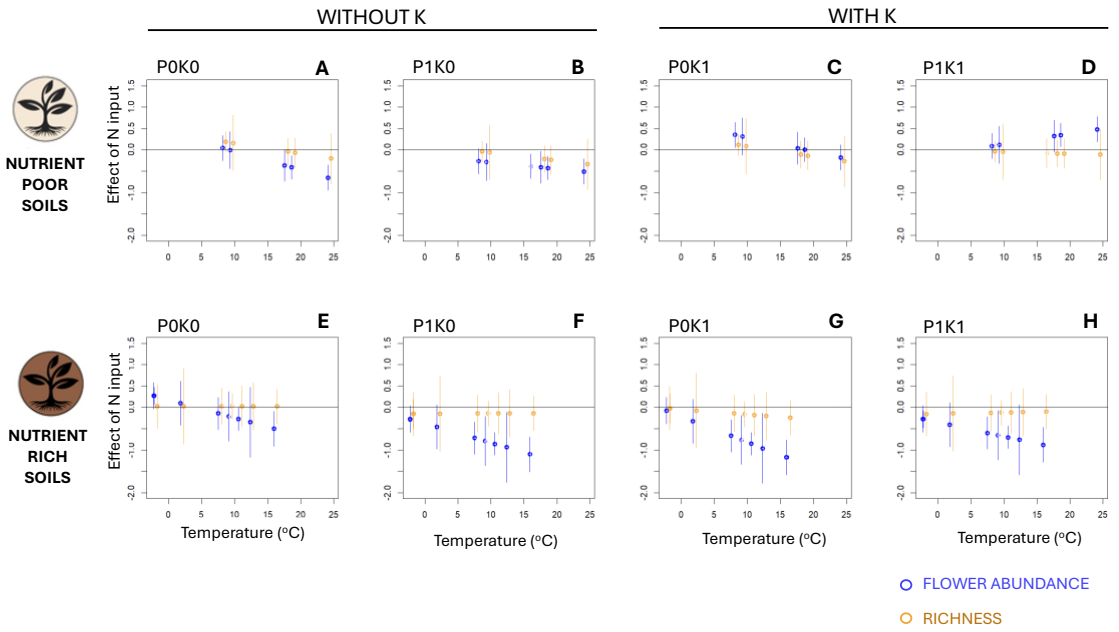


Figure 4.1. Effect of nutrient input on flower abundance (blue) and flowering plant richness (orange). Data on flowers were collected from the 14 experimental sites. Each dot (site) represents the difference between the estimated values obtained with N input and without N (black horizontal line), under the indicated conditions of P and K. Estimates were extracted from the full model (see full partial residual plot in Fig S4.2, and statistical details in Table S4.2). For visualization purposes, we considered the N concentration of naturally nutrient-poor soils to be 0.17 % (25% quantile, sites plotted had values below or equal to the median) and for naturally rich soils we used the value 0.34 % (75% quantile, sites plotted had values above the median).

Previous studies showed that adding N increased flower abundance (e.g. Muñoz et al., 2005 and Cuesta et al., 2008 run in alpine, subalpine regions) and plant richness (Dias et al., 2014, Mediterranean region) while negative effects on plant richness are more frequent (Harpole et al., 2016, Seabloom et al., 2021). Our results suggest that differences in climate and soil characteristics can help explain this diversity of results. Indeed, we show that increases in flower abundance driven by N input are more likely to occur in colder environments, more frequently and accentuated in regions with nutrient-poor soils and if K or PK are added as well (NK or NPK) (Fig 4.1C-D). The fact that flower abundance increases (in NPK) were not accompanied by increases in plant species

richness (Fig. 4.1D) suggests that few species benefit from N input. However, even though plant richness (at the time of sampling) was little affected, it is possible that overall richness is affected.

Our observation that the negative impact of nutrient input on flower resources was more accentuated in warmer regions (Fig. 4.1) contrasts with Clark et al. (2007) who detected a stronger negative effect of N input on plant communities in regions with low temperature and low soil Cation Exchange Capacity. Our observation that a negative impact of nutrient enrichment on flowering plant diversity occurred less frequently and less markedly in colder climates suggests that low temperatures could buffer the negative effect of nutrient enrichment. Yet, nitrophilous plants in cold regions could be benefiting from nutrient enrichment, increasing overall plant richness despite the loss of nitrophobous plants under the same conditions (Hautier et al., 2009). Flores-Moreno et al. (2016) analysed vegetative data also in NutNet experimental sites and detected that although the negative effects of several nutrient treatments on native plant richness were less accentuated in regions with warmer temperatures, the losses of native plant species driven specifically by N enrichment (treatments N and NP) were more accentuated in warmer climates (Table S2.17 in Flores-Moreno et al., 2016). More detailed analyses would be needed to compare the strength of the effect on vegetative metrics and flower metrics, to better understand if impacts on flower resources could be more accentuated than those expected based on vegetative cover.

4.3.2 Influence of soil fertility levels on floral resources

In warmer regions, the effect of N on plant species richness tended to be more accentuated in sites with low soil fertility (Fig 4.1). These effects were even more accentuated when N was added in combination with P or K. In contrast, the negative impact of N input on flower abundance was less accentuated when P and K were also added (Fig. 4.1B, C), and even turned into a positive effect (NPK; Fig. 4.1D).

N input also led to a widespread decline in flower abundance in warmer regions, particularly in sites with a N-rich soil (Fig. 4.1). Moreover, the negative impact of adding N became more accentuated either when P (NP) or K (NK) were also added (Fig. 4.1F, G). These differences in results highlight the importance of these other major essential

macronutrients (P and K) for flowering, and match with previous studies showing more accentuated effects when multiple nutrients are supplied, reducing niche dimensionality (Harpole et al., 2016, Seabloom et al., 2021). Other studies also detected that N-driven negative impacts on plant richness were more frequent and pronounced in nutrient-poor soils (Olsson and Kellner 2006, Dise et al., 2011, Vogels et al., 2023). This may be related to the fact that plant communities in such regions are mainly formed by species adapted to low nutrient levels, having evolved energetically expensive strategies to acquire the scarce nutrients available (e.g. symbiosis with bacteria or fungi, e.g., Dias et al., 2017, Mahmoudi et al., 2021) and, hence, are not able to adjust quickly and take advantage of an increase in nutrient availability. The dose of nutrients added (i.e., addition of 100 kg ha^{-1} of nutrients) compared with the quantity of these nutrients present in the soil, is disproportionally higher in poor soils compared to rich soils, thus stronger effects are expected.

4.3.3 Effects of nutrient enrichment on pollinators

As detected for plants, the impact of nutrient input on pollinators, both in terms of overall visit frequency (abundance) and richness of species, depended on local climate and natural soil conditions (Fig. 4.2, Table S4.2). Yet, the impact of nutrient application on flower resources was not always a good indicator for the impact on pollinators.

In colder regions, N input almost always increased pollinator abundance and richness (except for the pattern of NK supply in Fig 4.2G). Such positive effects were detected in both low and high fertility soils, despite the general decline in flower abundance. In colder regions, the positive effect of N on pollinator abundance and richness was stronger when P and/or K were also added. These general positive results contrast with similar studies conducted in cold conditions reporting no effects on pollinator abundance for NPK (Villa-Galaviz et al., 2021) and N, P and NP (Wang et al., 2022) but are in agreement with Muñoz et al. (2005) and Burkle and Irwin (2010) for N addition. These positive effects could be related to nutritional advantages to pollinators e.g. N input leading to higher sucrose concentration in nectar (Baude et al., 2011) or higher pollen amino acid and lipid concentration (Vaudo et al., 2015) which could benefit adult foraging, reproduction and larval development, relevant in colder regions. Further studies evaluating how nectar and pollen properties change with nutrient availability under different climatic conditions are needed to better understand the mechanisms behind the detected effects.

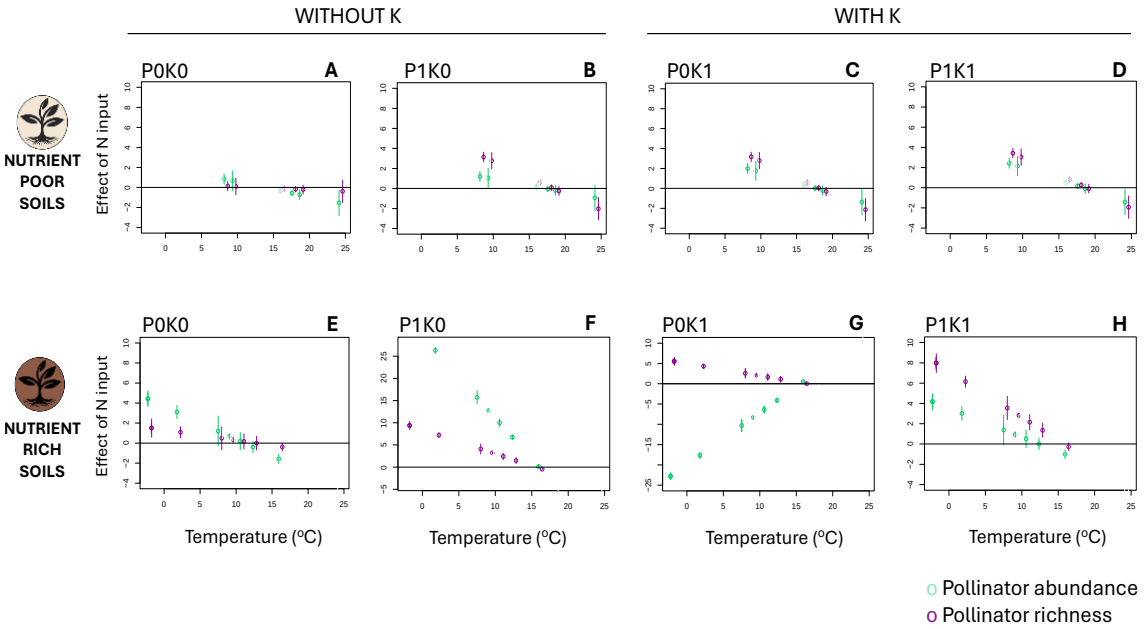


Figure 4.2. Effect of nutrient input on pollinator abundance (green) and richness (purple). Data on pollinators were collected in seven sites. Each dot (site) represents the difference between the estimated values obtained without and with N input, under the indicated conditions of P and K. Estimates were extracted from the full model (see full partial residual plot in Fig S4.3, and statistical details in Table S4.2). For visualization purposes, we considered the N concentration of naturally nutrient-poor soils to be 0.17 % (25% quantile, sites plotted had values below or equal to the median) and for naturally rich soils we used the value 0.34 % (75% quantile, sites plotted had values above the median).

In warmer regions, results greatly depended on soil fertility. In sites with poor fertility soils, the negative effects of N input on pollinator abundance and richness were in accordance with those detected for plants. Only when N was added together with P and K (NPK, Fig. 4.2D) negative effects of N input were clearly more accentuated for pollinators. In sites with nutrient rich soils, negative effects of high N input were more frequent and accentuated on plants than on pollinators (compare patterns in Figures 4.1 and 4.2). Negative effects on the visitation frequency of native pollinators were also reported by Ramos et al. (2018) in the Brazilian Cerrado, a region with a nutrient poor soil. Notably, in this region a positive effect of N addition was detected for *Apis mellifera*, an exotic species in Cerrado but native in several of our sites with nutrient rich soils. Other studies have reported similar negative effects of N on insect abundance in warm regions (Gallego-Zamorano et al., 2023) or N and NP effect (Nessel et al., 2023). Increasing the soil N concentration decreases the relative availability of other nutrients (e.g. P) which are essential for insects with complete metamorphosis (i.e. bees, beetles,

butterflies and flies). This may explain why N input negatively affects pollinators compared to insects with partial metamorphosis (Gallego-Zamorano et al., 2023). This P limitation is likely higher in regions with nutrient-poor soils. Yet, this would likely equally affect pollinators in warm and cold climates. The fact that the negative effects were more accentuated in warmer regions could be a direct response to the negative effects detected in plant communities.

Our observation that in warmer and colder regions we detected more accentuated changes in pollinator abundance compared to flower abundance (Fig 4.1 vs Fig 4.2), suggests that effects of nutrient enrichment on pollinators are stronger, probably driven not only by changes in flower abundance and composition, but quantitative and qualitative changes in flower rewards (Vaudo et al., 2024). Similarly, pollinator richness had more accentuated responses than plant richness at both high and low temperatures. These findings are similar to Carvalheiro et al. (2010), who detected bigger bottom-up effects of an invasive species on herbivores and parasitoids than on plants, and Villa-Galaviz et al. (2021), who detected a stronger fertilization effect on bumblebees than on flowers.

4.4 Conclusions

This study is the first large scale assessment of the effects of nutrient input on floral resources and pollinators covering a wide range of soil fertility and climate conditions. Our results indicate that nutrient enrichment is an important driver of floral resource availability with bottom-up effects on pollinators, sites with warm temperatures being particularly at risk. These trends may be aggravated under future warming climate scenarios (IPCC, 2007), which could exacerbate the negative effects of ongoing eutrophication. Biodiversity hotspots in Tropical and Mediterranean regions can be particularly susceptible (Myers et al., 2000). These regions are increasingly used for agriculture (Davidson et al., 2012; Lapola et al., 2013) and have suffered intensification in recent decades, hence exposing these ecosystems to large nutrient inputs (Lannes et al., 2012; Ramos et al., 2018). As most crops rely on insects to be pollinated (75% of crops relay on insect pollination, Siopa et al., 2024), the negative effects of N input reported also highlight an important threat to crop pollination and global food production (Millard et al., 2023). Policy and management plans aiming at controlling nutrient loads must be

implemented worldwide, taking into account interacting effects such as regional temperature or soil fertility that can strengthen the negative effects of nutrient enrichment on biodiversity.

4.5 Supplementary Information

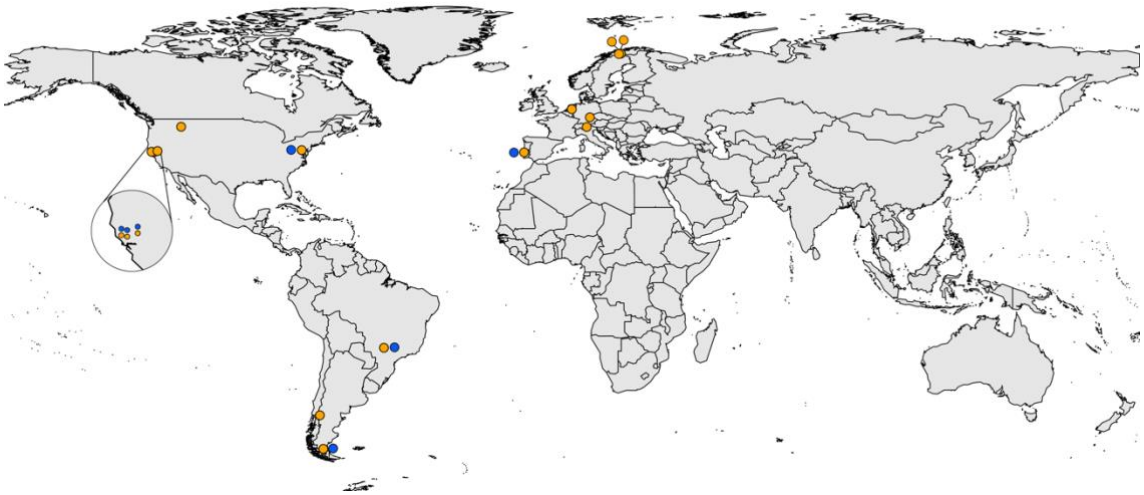


Figure S4.1. Location of the NutNet sites used in this study. Orange circles are sites with plant (flower abundance and plant richness) data. Blue circles indicate those sites where pollinator diversity was also recorded.

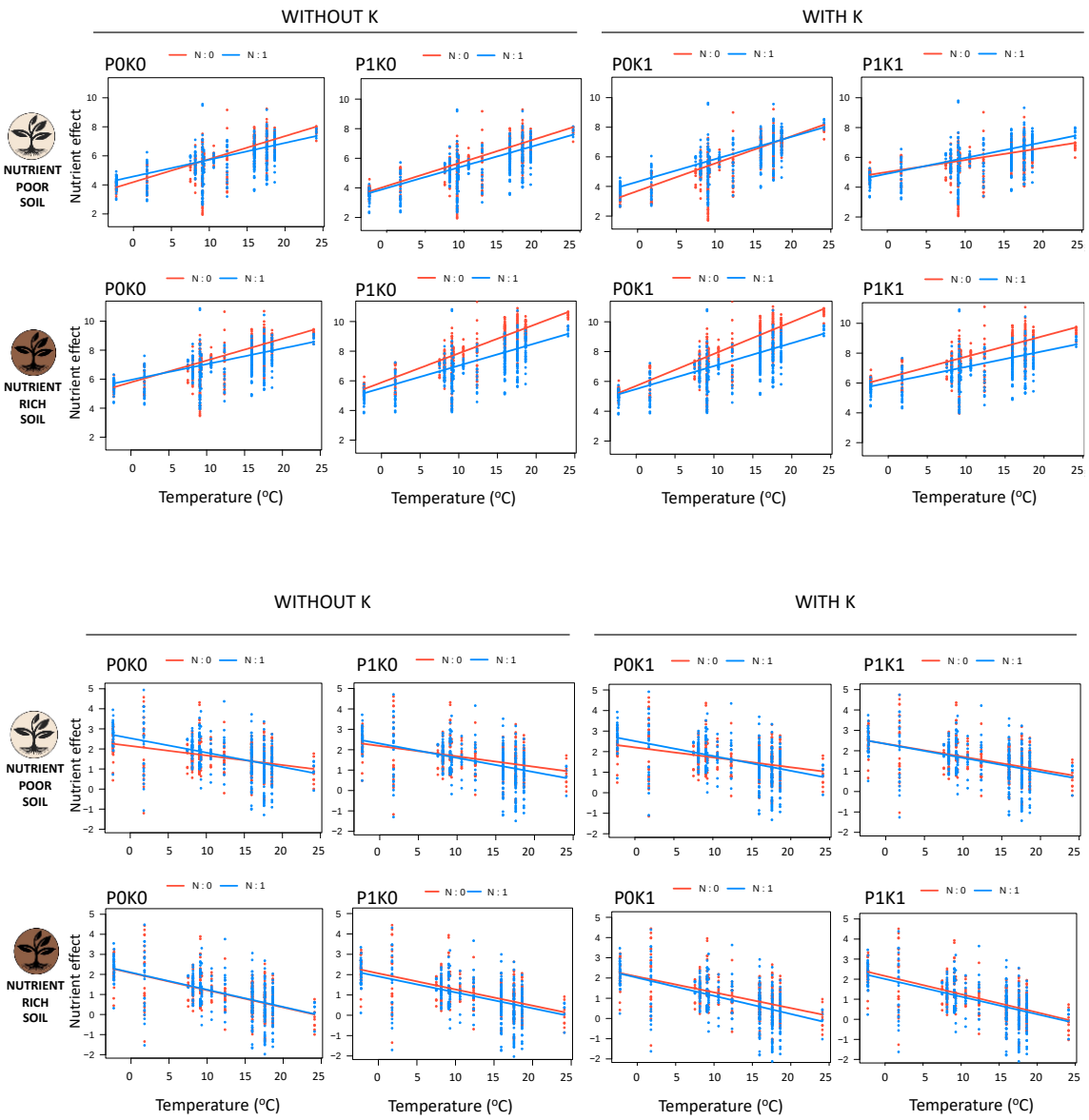


Figure S4.2. Effect of nutrient input on flower abundance (top) and plant richness (down), under different climatic and soil conditions. Each plot represents two treatments: one without N (red line) and one with N as indicated on the left top corner in each plot. The graphs were based on the full model (see statistical details of model selection in Table S4.2).

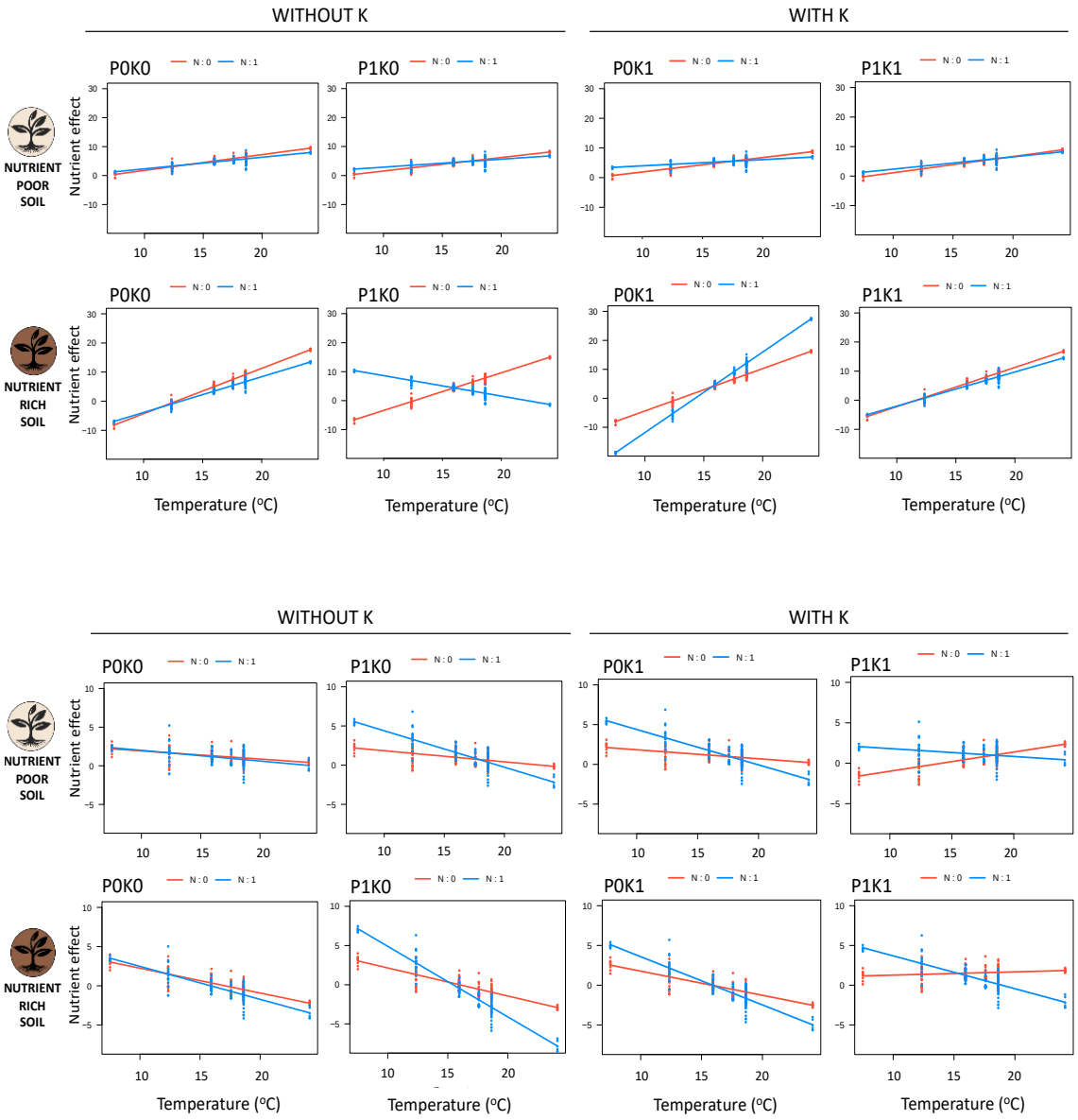


Figure S4.3. Effect of nutrient enrichment on pollinator abundance (top) and richness (down) from the experimental sites. Each plot represents two treatments: one without N (red line) and one with N as indicated on the left top corner in each plot. The graphs were based on the full model (see statistical details of model selection in Table S4.2).

Table S4.1. Variable information for each study site. “Poll” refers to pollinator, “fert” to fertilization, "Pct N" corresponds to N % in the soil, "ppm P" corresponds to parts per million of P in the soil and “Temp” refers to temperature.

SITE	Code	Survey year	Plant survey	Poll survey	1st year fert	Nb plant surveys	Treatments	N pct	P ppm	Temp Weather station	Temp World-Clim
MARYLAND	mcdan.us	2021	yes	yes	2018	1	C, K, N, NPK, P	0.22	26.5	-	12.35
BRAZIL	lagoas.br	2018	yes	yes	2015	1	C, K, N, NPK, P	0.03	29.3	-	24.12
ARGENTINA PAT	potrok.ar	2019	yes	yes	2015	2	C, K, N, NK, NP, NPK, P, PK	0.21	326.3	-	7.51
PORTUGAL	comp.pt	2020	yes	yes	2012	1	C, N, NP, P	0.11	34	18.63	
		2021			2012	2	C, N, NP, P, NPK, K				
		2022			2012	2	C, N, NP, P, NPK, K				
GERMANY	bayr.de	2020	yes	no	2015	1	C, N, NP, P, NPK, K	0.16	148.75	9.29	
ARGENTINA RNEG	rnegro.ar	2018	yes	no	2016	1	C, N, NP, P, NPK, K, NK, PK	0.39	13.38	-	9.11
FINLAND saana	saana.fi	2020	yes	no	2014	1	C, K, N, NK, NP, NPK, P, PK	0.43	67.75	-2.23	
FINLAND kilp	kilp.fi	2020	yes	no	2013	1	C, K, N, NK, NP, NPK, P, PK	0.54	74.5	-2.27	
NETHERLANDS	veluwe.nl	2018	yes	no	2017	1	C, K, N, NK, NP, NPK, P, PK	0.21	127	10.59	
SWITZERLAND	valm.ch	2019	yes	no	2008	2	C, K, N, NK, NP, NPK, P, PK	0.34	23	1.78	
MONTANA	msla.us	2019	yes	no	2017	1	C, K, N, NK, NP, NPK, P, PK	0.17	18.3	8.15	
		2020			2017	1	C, K, N, NK, NP, NPK, P, PK				
		2021			2017	1	C, K, N, NK, NP, NPK, P, PK				
MCLAUGHLIN	mcla.us	2023	yes	yes	2007	3	C, K, N, NK, NP, NPK, P, PK	0.26	28.6	15.93	
HOPLAND	hopl.us	2023	yes	yes	2007	3	C, K, N, NK, NP, NPK, P, PK	0.20	31.3	15.95	
SIERRA FOOTHILLS	sier.us	2023	yes	yes	2007	3	C, K, N, NK, NP, NPK, P, PK	0.17	18.3	17.58	

Table S4.2. Model selection for each plant and pollinator diversity variable. Model selection used the dredge function of the package *MuMIn* from three models that contained all the possible interactions between the variables. Models in each variable are ordered from lower AIC to higher. The first model has Delta 0 until the cut-off model with delta 2.7 or a maximum number of 55 models per variable.

Flower abundance model abundance

Model ID	(Int)	N	P	K	pctN	ppm_P	Tmp	Yearfert	K:N	N:pctN	N:Tmp	P:pctN	P:Tmp	pctN:Tmp	N:pctN:Tmp	N:ppm_P	df	logLik	AICc	delta	Weight
12395	4.078	1.104			10.340		0.153	-0.223		-3.677	-0.041						12	-5.769.019	11562.4	0.0	0.278
20587	4.078	1.104			10.340		0.153	-0.223		-3.677	-0.041						12	-5.769.014	11562.4	0.0	0.257
6251	4.079	1.104			10.340		0.153	-0.223		-3.677	-0.041						12	-5.769.051	11562.5	0.1	0.259
12396	4.052	1.096		0.048	10.340		0.154	-0.224		-3.650	-0.041						13	-5.768.875	11564.2	1.8	0.115
20588	4.052	1.096		0.048	10.340		0.154	-0.224		-3.650	-0.041						13	-5.768.853	11564.2	1.8	0.108
6252	4.052	1.096		0.048	10.330		0.154	-0.224		-3.650	-0.041						13	-5.768.888	11564.2	1.8	0.108
12411	3.645	1.100			11.010	0.002	0.167	-0.220		-3.671	-0.041						13	-5.768.931	11564.3	1.9	0.109
20603	3.645	1.100			11.010	0.002	0.167	-0.220		-3.670	-0.041						13	-5.768.907	11564.3	1.9	0.102
4203	4.561	0.181			9.388		0.132	-0.222		-1.869							11	-5.771.044	11564.4	2.0	0.104
2155	4.561	0.181			9.388		0.132	-0.222		-1.869							11	-5.771.062	11564.4	2.0	0.097
12399	4.068	1.101	0.018		10.340		0.154	-0.224		-3.665	-0.041						13	-5.768.982	11564.4	2.0	0.101
6267	3.645	1.100			11.010	0.002	0.167	-0.220		-3.671	-0.041						13	-5.768.944	11564.4	2.0	0.103
282731	4.169	1.105			10.060		0.139	-0.225		-3.678	-0.041			0.077			13	-5.768.950	11564.4	2.0	0.097
39019	4.170	1.105			10.050		0.139	-0.225		-3.679	-0.041			0.077			13	-5.768.987	11564.5	2.1	0.098
6255	4.069	1.101	0.018		10.340		0.154	-0.224		-3.665	-0.041						13	-5.769.026	11564.5	2.1	0.094
20591	4.068	1.101	0.018		10.340		0.154	-0.224		-3.665	-0.041						13	-5.768.989	11564.5	2.1	0.094
45167	3.966	1.124	0.201		10.370		0.161	-0.224		-3.721	-0.041		-0.016				14	-5.768.244	11565.0	2.6	0.08
28783	3.966	1.124	0.201		10.380		0.161	-0.224		-3.721	-0.041		-0.016				14	-5.768.228	11565.0	2.6	0.08
151663	3.965	1.124	0.201		10.380		0.161	-0.224		-3.721	-0.041		-0.016				14	-5.768.224	11565.0	2.6	0.07
22639	3.966	1.124	0.201		10.370		0.161	-0.224		-3.722	-0.041		-0.016				14	-5.768.256	11565.1	2.7	0.07
12524	4.080	1.016		-0.041	10.390		0.154	-0.224	0.180	-3.690	-0.041						14	-5.768.285	11565.1	2.7	0.07
6380	-4.081	1.016		0.041	10.380		0.154	-0.224	0.180	-3.690	-0.041						14	-5.768.298	11565.1	2.7	0.07
20716	-4.080	1.016		0.041	10.390		0.154	-0.224	0.180	-3.690	-0.041						14	-5.768.265	11565.1	2.7	0.07

Model ID	(Int)	N	P	K	pctN	ppm_P	Tmp	Yearfert	N:P	N:pctN	N:Tmp	P:pctN	P:Tmp	pctN:Tmp	N:P:pctN	N:pctN:Tmp	df	logLik	AICc	delta	weight
2137151	2.19	0.5591	-0.02185		-0.4762	0.002346	-0.01311		-0.09674	-1.719	-0.03716			-0.2021		0.1037	16	-1615.07	3262.9	0	0.133
2136127	2.208	0.5149	-0.06654		-0.4692	0.002336	-0.01327			-1.725	-0.03724			-0.2011		0.1037	15	-1.616.249	3263.1	0.2	0.116
2145343	2.234	0.5557	-0.1059		-0.6375	0.002339	-0.0138		-0.09354	-1.714	-0.03645	0.3143		-0.1997		0.1012	17	-1.614.201	3263.2	0.3	0.111
2144319	2.253	0.5151	-0.1531		-0.6344	0.002331	-0.01398			-1.726	-0.03652	0.3285		-0.1987		0.1011	16	-1615.3	3263.3	0.4	0.105
2153535	2.167	0.5573	0.02545		-0.4779	0.00235	-0.01121		-0.09528	-1.716	-0.03641		-0.005131	-0.1996		0.1006	17	-1614.29	3263.4	0.5	0.102
2137215	2.084	0.5595	-0.02223		-0.7223	0.002516	-0.01855	0.02771	-0.09624	-1.721	-0.03724			-0.1933		0.1039	17	-1.614.346	3263.5	0.6	0.096
2152511	2.188	0.5128	-0.0175		-0.477	0.002337	-0.01149			-1.722	-0.03639		-0.005256	-0.1984		0.1006	16	-1.615.433	3263.6	0.7	0.092
2136191	2.1	0.5156	-0.0667		-0.7179	0.002508	-0.0188	0.02806		-1.726	-0.03732			-0.1918		0.1039	16	-1.615.513	3263.7	0.8	0.085
2145407	2.129	0.5555	-0.1067		-0.8871	0.002511	-0.01927	0.02779	-0.09309	-1.713	-0.03648	0.3155		-0.191		0.1013	18	-1.613.472	3263.8	0.9	0.081
2144383	2.144	0.5154	-0.1534		-0.8839	0.002502	-0.01951	0.02814		-1.727	-0.03658	0.3293		-0.1895		0.1013	17	-1614.56	3263.9	1	0.078
2153599	2.06	0.5578	0.02518		-0.7255	0.00252	-0.01668	0.02787	-0.09469	-1.718	-0.03647		-0.00515	-0.1907		0.1007	18	-1.613.561	3264	1.1	0.043
2152575	2.078	0.5134	-0.01747		-0.7227	0.00251	-0.01699	0.02813		-1.723	-0.03644		-0.005278	-0.1891		0.1008	17	-1.614.691	3264.2	1.3	0.039
2137135	2.908	0.5575	-0.02243		-1.735		-0.04002		-0.09615	-1.718	-0.03713			-0.183		0.1039	15	-1.616.914	3264.5	1.6	0.034
2136111	2.925	0.5134	-0.06689		-1.727		-0.04017			-1.722	-0.0372			-0.1818		0.1038	14	-1618.08	3264.7	1.8	0.03
2145327	2.951	0.5534	-0.1069		-1.895		-0.04067		-0.09295	-1.71	-0.03638	0.3157		-0.1807		0.1012	16	-1.616.037	3264.8	1.9	0.029
2137152	2.182	0.5597	-0.02093	0.01111	-0.4735	0.002348	-0.01294		-0.09935	-1.718	-0.03713			-0.202		0.1036	17	-1.615.009	3264.8	1.9	0.029
2669631	2.215	0.5954	-0.06939		-0.5647	0.002344	-0.01347		-0.1767	-1.867	-0.03655	0.1782		-0.201	0.3165	0.1019	18	-1.613.986	3264.9	2	0.028
2144303	2.966	0.513	-0.1538		-1.886		-0.04073			-1.722	-0.03645	0.3301		-0.1796		0.1012	15	-1.617.123	3264.9	2	0.028
2153519	2.887	0.5552	0.02475		-1.74		-0.03819		-0.09467	-1.713	-0.03636		-0.005119	-0.1805		0.1007	16	-1.616.138	3265	2.1	0.026
2152495	2.904	0.511	-0.01795		-1.733		-0.03835			-1.719	-0.03633		-0.005243	-0.1794		0.1008	15	-1.617.266	3265.2	2.3	0.024
2145344	2.224	0.5556	-0.105	0.01122	-0.6306	0.002343	-0.01357		-0.09617	-1.71	-0.03637	0.3141		-0.1996		0.1009	18	-1.614.139	3265.2	2.3	0.024
2136128	2.206	0.5149	-0.06668	0.00534	-0.4712	0.002336	-0.01326			-1.725	-0.03724			-0.2009		0.1036	16	-1.616.235	3265.2	2.3	0.024
2161727	2.213	0.5557	-0.06428		-0.59	0.002343	-0.0129		-0.0939	-1.713	-0.03638	0.2223	-0.001852	-0.1995		0.1008	18	-1.614.173	3265.2	2.3	0.023
2153536	2.161	0.5574	0.02631	0.01109	-0.4791	0.002349	-0.01111		-0.09782	-1.714	-0.03636		-0.005129	-0.1994		0.1004	18	-1614.23	3265.4	2.5	0.022
2160703	2.234	0.5142	-0.1185		-0.5932	0.002334	-0.0132			-1.724	-0.03642	0.2519	-0.001542	-0.1985		0.1008	17	-1.615.282	3265.4	2.5	0.022

Pollinator abundance model selection

Model ID	(Int)	N	P	K	pctN	ppm_P	Tmp	Yearfert	N:P	N:pctN	N:ppm_P	N:Tmp	K:pctN	P:pctN	P:Tmp	N:P:pctN	pctN:Tmp	N:pctN:Tmp	df	logLik	AICc	delta	weight
12331	13.88	3.14			-28.94		-0.37			-6.75		-0.10							11	-1.115.671	2254.4	0	0.157
6187	13.84	3.15			-28.86		-0.37			-6.76		-0.10							11	-1.115.707	2254.5	0.1	0.135
20523	13.85	3.15			-28.90		-0.37			-6.77		-0.10							11	-1.115.688	2254.5	0.1	0.13
32891	13.18	0.34			-91.59	0.02	-0.41	-0.41									5.87		12	-1.114.628	2254.5	0.1	0.132
262267	13.2	0.34			-91.71	0.02	-0.41	-0.42									5.88		12	-1.114.601	2254.5	0.10	0.128
282747	12.07	3.11			-87.84	0.02	-0.37	-0.41		-6.63		-0.10					5.78		14	-1.112.382	2254.5	0.1	0.127
39035	12.05	3.11			-87.79	0.02	-0.37	-0.41		-6.62		-0.10					5.78		14	-1112.4	2254.6	0.2	0.132
43	15.08	0.34			-31.83		-0.42												9	-1.118.006	2254.7	0.3	0.113
270459	13.27	0.23			-91.67	0.02	-0.41	-0.41			0.00						5.87		13	-1.113.749	2255.0	0.6	0.099
14383	14.03	3.02	-0.35		-28.88		-0.37		0.50	-7.12		-0.11							13	-1.113.788	2255.1	0.7	0.113
22575	14.01	3.03	-0.34		-28.86		-0.37		0.49	-7.11		-0.11							13	-1.113.846	2255.2	0.8	0.09
7215	14	3.03	-0.34		-28.86		-0.37		0.48	-7.10		-0.11							13	-1.113.884	2255.3	0.9	0.092
51	-0.5014	0.35				0.01	0.20												9	-1.118.334	2255.4	1	0.082
47151	13.68	3.02	0.39		-28.73		-0.35		0.52	-7.38		-0.10			-0.04				14	-1.112.833	2255.4	1	0.081
284799	12.25	3.00	-0.34		-87.95	0.02	-0.37	-0.41	0.48	-6.97		-0.10					5.79		16	-1110.55	2255.4	1	0.095
12395	11.67	3.13			-21.15		-0.27	-0.08		-6.72		-0.10							12	-1.115.123	2255.5	1.1	0.09
40063	12.25	3.00	-0.34		-87.89	0.02	-0.37	-0.41	0.48	-6.98		-0.10					5.78		16	-1.110.587	2255.5	1.1	0.084
153647	13.67	3.02	0.39		-28.74		-0.35		0.51	-7.35		-0.10			-0.04				14	-1.112.884	2255.5	1.1	0.077
6251	11.66	3.15			-21.14		-0.27	-0.08		-6.76		-0.10							12	-1.115.159	2255.6	1.2	0.078
20587	11.66	3.14			-21.15		-0.27	-0.08		-6.75		-0.10							12	-1.115.141	2255.6	1.2	0.075
23599	13.66	3.02	0.39		-28.69		-0.35		0.51	-7.36		-0.1034-			0.04				14	-1.112.919	2255.6	1.2	0.079
14896	14.44	2.84	-0.42	-0.72	-30.54		-0.38		0.63	-7.21		-0.10	4.11						15	-1.111.859	2255.7	1.3	0.082
107	12.88	0.34			-24.03		-0.31	-0.08											10	-1.117.427	2255.8	1.4	0.081
7472	14.43	2.861	-0.4077	-0.7132	-30.53		-0.3774		0.614	-7.208		-0.09969	4.079						15	-1111.97	2255.9	1.5	0.04
12335	13.86	3.24	-0.11		-28.78		-0.37			-6.94		-0.11							12	-1.115.323	2255.9	1.5	0.074
2095	15.24	0.14	-0.31		-31.93		-0.42		0.46										11	-1.116.435	2256.0	1.6	0.074
6191	13.83	3.249	-0.1116		-28.7		-0.3686			-6.957		-0.1066							12	-1.115.351	2256.0	1.6	0.073
33919	13.35	0.1537	-0.3086		-91.8	0.02097	-0.4109	-0.4139	0.4492								5.88		14	-1.113.129	2256.0	1.6	0.04
4139	14.98	0.6267			-31.1		-0.4171			-1.778									10	-1.117.594	2256.1	1.7	0.04
34939	13.07	0.6207			-90.38	0.02081	-0.4088	-0.412		-1.707							5.83		13	-1.114.283	2256.1	1.7	0.04
39039	12.03	3.22	-0.1159		-88.31	0.02076	-0.3646	-0.4118		-6.83		-0.1057					5.839		15	-1.112.023	2256.1	1.7	0.04
40320	12.7	2.816	-0.4093	-0.7271	-86.58	0.01947	-0.3715	-0.3932	0.6102	-7.052		-0.09799	4.167				5.516		18	-1.108.621	2256.1	1.7	0.04
1071	15.23	0.1487	-0.3045		-31.91		-0.4169		0.449										11	-1.116.547	2256.2	1.8	0.04
2091	14.99	0.6249			-31.14		-0.4175			-1.766									10	-1.117.654	2256.2	1.8	0.04
2136187	12.86	1.327			-91.99	0.02053	-0.4053	-0.4093		2.53		-0.01486					5.995	-0.463	15	-1112.09	2256.2	1.8	0.04
14447	11.9	3.017	-0.3409		-21.38		-0.2723	-0.07886	0.4949	-7.101		-0.1054							14	-1.113.271	2256.3	1.9	0.03
30767	14.19	3.062	-0.6751		-29.78		-0.3741		0.513	-7.421		-0.1052		2					14	-1.113.275	2256.3	1.9	0.03
47	15.12	0.3565	-0.09022		-31.83		-0.4165												10	-1.117.747	2256.4	2	0.03
12844	14.18	3.034		-0.4913	-30.41		-0.3762			-6.861		-0.09766	3.282						13	-1.114.437	2256.4	2	0.03
91	5.33	0.3386			-9.027	0.007358		-0.134											10	-1.117.822	2256.5	2.1	0.03
8235	14.94	0.634			-31.82		-0.4071					-0.01813							10	-1.117.821	2256.5	2.1	0.03
12332	13.87	3.125		0.05433	-28.98		-0.3717			-6.792		-0.1026							12	-1.115.591	2256.5	2.1	0.03
34863	14.91	0.1396	0.3827		-31.88		-0.3979		0.4653						-0.042				12	-1.115.596	2256.5	2.1	0.03
45103	13.55	3.164	0.6087		-28.61		-0.3524			-7.124		-0.0999			-0.04369				13	-1.114.502	2256.5	2.1	0.03
47664	14.09	2.914	0.2229	-0.6935	-30.23		-0.3589		0.654	-7.59		-0.09979	3.863		-0.03924				16	-1.111.113	2256.5	2.1	0.03
556	15.37	0.3117		-0.4899	-33.39		-0.4189						3.249						11	-1.116.754	2256.6	2.2	0.03
12347	13.57	3.147			-28.46	0.000419	-0.3591			-6.763		-0.1039							12	-1.115.669	2256.6	2.2	0.03
44	15.1	0.3302		0.05396	-31.99		-0.417												10	-1.117.894	2256.7	2.3	0.02
2608	15.64	0.06326	-0.375	-0.7099	-33.67		-0.4209		0.5918				4.03						13	-1.114.599	2256.7	2.3	0.02
47215	11.59	3.006	0.3871		-21.32		-0.2542	-0.07788	0.5161	-7.342		-0.103			-0.04463				15	-1.112.329	2256.7	2.3	0.02
59	14.68	0.3362			-31.23	0.0005795	-0.4001												10	-1.117.969	2256.8	2.4	0.02
14960	12.28	2.833	-0.4126	-0.7385	-22.9		-0.2746	-0.08146	0.632	-7.18		-0.09885	4.211						16	-1.111.268	2256.8	2.4	0.02
1079856	15.02	2.153	-1.274	-1.013	-33.36		-0.3825		1.863	-3.835		-0.0926	5.598	4.998		-7.088			17	-1.110.114	2256.8	2.4	0.02
27	4.461	0.3406			-9.173	0.008919													9	-1.119.063	2256.9	2.5	0.02

Pollinator richness model selection

ModelID	(Int)	N	P	K	pctN	ppm_P	Temperature	Yearfert	N:P	N:K	N:pctN	N:Temperature	P:K	P:pctN	P:Temperature	K:pctN	K:Temperature	pctN:Tmp	df	logLik	AICc	delta	Weight
16463	2.903	0.226	-0.498		-3.561			-0.103						2.461					11	-530.8	1084.7	0	0.22
8271	2.903	0.226	-0.498		-3.561			-0.103						2.461					11	-530.8	1084.7	0	0.153
41007	-1.485	0.227	-0.487		22.860		0.195							2.385				-1.662	12	-529.8	1084.9	0.2	0.136
49199	-2.048	0.224	0.443		24.400		0.216								-0.034			-1.672	12	-529.9	1085.1	0.4	0.12
18511	2.934	0.152	-0.585		-3.578			-0.103	0.167					2.467					12	-530.1	1085.5	0.8	0.141
9295	2.934	0.152	-0.585		-3.578			-0.103	0.167					2.467					12	-530.1	1085.5	0.8	0.098
41023	-0.209	0.227	-0.493		21.160	-0.002	0.150							2.447				-1.715	13	-529.1	1085.7	1.0	0.093
42031	-1.428	0.152	-0.576		22.760		0.194		0.171					2.393				-1.659	13	-529.1	1085.7	1.0	0.089
41071	0.119	0.228	-0.489		13.040		0.128	-0.047						2.401				-1.041	13	-529.2	1085.8	1.1	0.085
50223	-1.983	0.147	0.357		24.260		0.215		0.175						-0.035			-1.667	13	-529.2	1085.9	1.2	0.081
32871	1.589	0.223	0.448				0.050	-0.108							-0.035				11	-531.5	1086.1	1.4	0.106
16487	1.589	0.223	0.448				0.050	-0.108							-0.035				11	-531.5	1086.1	1.4	0.073
49263	-0.473	0.224	0.444		14.840		0.150	-0.045							-0.034			-1.069	13	-529.3	1086.2	1.5	0.072
16479	2.847	0.226	-0.500		-3.735	0.001		-0.100						2.473					12	-530.6	1086.4	1.7	0.091
32887	1.132	0.224	0.445			0.002	0.070	-0.109							-0.035				12	-530.6	1086.5	1.8	0.089
20559	2.885	0.275	-0.509		-3.438			-0.103			-0.333			2.534					12	-530.7	1086.7	2.0	0.077
16495	3.091	0.226	-0.500		-3.996		-0.008	-0.101						2.474					12	-530.8	1086.8	2.1	0.074
16464	2.901	0.225	-0.499	0.013	-3.569			-0.103						2.453					12	-530.8	1086.8	2.1	0.074
34919	1.618	0.150	0.367				0.050	-0.108	0.167						-0.035				12	-530.9	1087.0	2.3	0.068
18527	2.879	0.152	-0.587		-3.758	0.001		-0.100	0.168					2.481					13	-529.9	1087.3	2.6	0.059

Table S4.3. Nutrient importance per plant and pollinator diversity model. Importance was obtained from the term "weight" in every model (Table S4.2). The weight from every model where the nutrient appeared was combined.

	Model 1 Flower abundance	**Model 2 Plant richness**	**Model 3 Pollinator abundance**	**Model 4 Pollinator richness**
N	2.63	1.42	3.06	1.99
P	0.59	1.42	1.71	1.99
K	0.54	0.10	0.43	0.07

Table S4.4. Total plant and flower visitor species list. Plant species list and visitor list per site are also provided.

List of plant species

Species	Family
Achillea atrata	Asteraceae
Achillea millefoilum	Asteraceae
Achyrachaena mollis	Asteraceae
Adesmia lotoides	Fabaceae
Agoseris heterophylla	Asteraceae
Ajuga pyramidalis	Lamiaceae
Alchemilla xantochlora	Rosaceae
Amaranthaceae sp	Amaranthaceae
Amsinckia menziesii	Boraginaceae
Anagallis arvensis	Primulaceae
Andromeda polifolia	Ericaceae
Andryala integrifolia	Asteraceae
Anemone multifida	Ranunculaceae
Antennaria alpina	Asteraceae
Antennaria dioica	Asteraceae
Anthriscus sylvestris	Apiaceae
Anthyllis vulneraria	Fabaceae
Arabis holboellii	Brassicaceae
Arctostaphylos uva-ursi	Ericaceae
Arenaria capillaris	Caryophyllaceae
Arenaria congesta	Caryophyllaceae
Arnica montana	Asteraceae
Aster alpinus	Asteraceae
Asteraceae	Fabaceae
Astragalus alpinus	Fabaceae
Astragalus frigidus	Fabaceae
Astragalus inflexus	Poaceae
Baccharis spp	Asteraceae
Balsamorhiza sagittata	Asteraceae
Barbarea vulgaris	Brassicaceae
Bartsia alpina	Orobanchaceae
Bellardia trixago	Orobanchaceae
Berberis darwinii	Berberidaceae
Berberis microphylla	Berberidaceae
Bistorta vivipara	Polygonaceae
Boechera pendulocarpa	Brassicaceae
Borreria tenella	Rubiaceae
Brodiaea sp.	Liliaceae
Calandrinia menziesii	Portulacaceae
Calceolaria uniflora	Calceolariaceae
Calochortus venutus	Liliaceae
Campanula barbata	Campanulaceae
Campanula rotundifolia	Campanulaceae
Campanula scheuchzeri	Campanulaceae
Cardamine oligosperma	Brassicaceae
Carduus acanthoides	Asteraceae
Carduus defloratus	Asteraceae
Carduus nutans	Asteraceae
Carduus pycnocephalus	Asteraceae
Centaurea jacea	Asteraceae
Cerastium arvense	Caryophyllaceae
Cerastium fontanum	Caryophyllaceae
Cerastium glomerium	Caryophyllaceae
Cerastium holosteoides	Caryophyllaceae
Chamaecrista flexuosa	Fabaceae
Chamaemelum sp	Asteraceae
Chloraea alpina	Orchidaceae
Chlorogalum pomeridianum	Asparagaceae
Cichorium intybus	Asteraceae
Cirsium arvense	Asteraceae
Cirsium helenioides	Asteraceae

Species	Family
Clarkia purpurea	Onagraceae
Claytonia perfoliata	Montiaceae
Coeloglossum viride	Orchidaceae
Coleostephus myconis	Asteraceae
Collinsia linearis	Plantaginaceae
Collomia linearis	Polemoniaceae
Convolvulus arvensis	Convolvulaceae
Coronilla varia	Fabaceae
Crepis biennis	Asteraceae
Crepis capillaris	Asteraceae
Croton glandulosus	Euphorbiaceae
Cryptantha celesoides	Boraginaceae
Daphne striata	Thymelaeaceae
Daucus carota	Apiaceae
Delphinium hesperium	Ranunculaceae
Dianthus armeria	Caryophyllaceae
Dianthus deltoides	Caryophyllaceae
Dianthus sylvestris	Caryophyllaceae
Dichelostemma multiflorum	Asparagaceae
Dichelostemma capitatum	Asparagaceae
Dichelostemma congestum	Asparagaceae
Echium plantagineum	Boraginaceae
Embothrium coccineum	Proteaceae
Erigeron alpinus	Asteraceae
Erigeron annuus	Asteraceae
Erigeron divergens	Asteraceae
Erigeron patagonicus	Asteraceae
Erigeron pumilus	Asteraceae
Erigeron uniflorus	Asteraceae
Erodium botrys	Geraniaceae
Erodium cicutarium	Geraniaceae
Euphorbia cyparissias	Euphorbiaceae
Euphorbia spathulata	Euphorbiaceae
Euphrasia frigida	Scrophulariaceae
Euphrasia minima	Scrophulariaceae
Fabiana imbricata	Solanaceae
Fallopia convolvulus	Polygonaceae
Filipendula ulmaria	Rosaceae
Fragaria chiloensis	Rosaceae
Fragaria vesca	Rosaceae
Gaillardia aristata	Asteraceae
Galeopsis tetrahit	Lamiaceae
Galium album	Rubiaceae
Galium anisophyllon	Rubiaceae
Galium mollugo	Rubiaceae
Galium parisiense	Rubiaceae
Gaura coccinea	Onagraceae
Gavilea spp	Orchidaceae
Gentiana acaulis	Gentianaceae
Gentiana campestris	Gentianaceae
Gentiana nivalis	Gentianaceae
Gentiana verna	Gentianaceae
Geranium carolinianum	Geraniaceae
Geranium dissectum	Geraniaceae
Geranium molle	Geraniaceae
Geranium pusillum	Geraniaceae
Geranium sylvaticum	Geraniaceae
Geum montanum	Rosaceae
Geum triflorum	Rosaceae
Gnaphalium norvegicum	Asteraceae
Gnaphalium supinum	Asteraceae
Helianthemum nummularium	Cistaceae
Hieracium alpinum	Asteraceae
Hieracium aurantiacum	Asteraceae
Hieracium pilosella	Asteraceae
Hieracium staticifolium	Asteraceae
Hieracium sylvaticum	Asteraceae
Hippocrepis comosa	Fabaceae
Homogyne alpina	Asteraceae
Hypericum perforatum	Hypericaceae

Species	Family	Species	Family
Hypochaeris glabra	Asteraceae	*Melilotus officinalis*	Fabaceae
Hypochaeris incana	Asteraceae	*Micropus californicus*	Asteraceae
Hypochaeris radicata	Asteraceae	*Minuartia biflora*	Caryophyllaceae
Iris macrosiphon	Iridaceae	*Minuartia verna*	Caryophyllaceae
Jacobaea vulgaris	Asteraceae	*Myosotis alpestris*	Boraginaceae
Junellia odonellii	Verbenaceae	*Myosotis arvensis*	Boraginaceae
Knautia arvensis	Caprifoliaceae	*Myosotis decumbens*	Boraginaceae
Lagophylla minor	Asteraceae	*Myosotis micrantha*	Boraginaceae
Lasthenia californica	Asteraceae	*Mysopates orontium*	Plantaginaceae
Lathyrus angulatus	Fabaceae	*Nardophyllum bryoides*	Asteraceae
Lathyrus magellanicus	Fabaceae	*Nassauvia aculeata*	Asteraceae
Leontodon autumnalis	Asteraceae	*Navarretia pubescens*	Polemoniaceae
Lepidium nitidum	Brassicaceae	*Nemophila pedunculata*	Boraginaceae
Leucanthemum adustum	Asteraceae	*Nigritella nigra*	Orchidaceae
Leucanthemum ircutianum	Asteraceae	*Oenothera biennis*	Onagraceae
Leucheria purpurea	Asteraceae	*Ornithopus compressus*	Fabaceae
Lewisia rediviva	Montiaceae	*Osmorhiza berteroi*	Apiaceae
Linanthus bicolor	Polemoniaceae	*Oxalis enneaphylla*	Oxalidaceae
Linaria vulgaris	Plantaginaceae	*Oxalis stricta*	Oxalidaceae
Linum bienne	Linaceae	*Packera cana*	Asteraceae
Lithospermum ruderale	Boraginaceae	*Parentucellia viscosa*	Orobanchaceae
Lobelia inflata	Campanulaceae	*Paris quadrifolia*	Melanthiaceae
Lomatia hirsuta	Proteaceae	*Parnassia palustris*	Parnassiaceae
Lomatium hooverii	Apiaceae	*Pedicularis tuberosa*	Scrophulariaceae
Lotus alpinus	Fabaceae	*Perezia recurvata*	Asteraceae
Lotus corniculatus	Fabaceae	*Petrorhagia dubia*	Caryophyllaceae
Lupinus bicolor	Fabaceae	*Pfaffia glomerata*	Amaranthaceae
Lupinus microcarpus	Fabaceae	*Phyllodoce caerulea*	Ericaceae
Lupinus sericeus	Fabaceae	*Physalis heterophylla*	Solanaceae
Lysimachia arvensis	Primulaceae	*Phyteuma hemisphaericum*	Campanulaceae
Madia sativa	Asteraceae	*Phytolacca americana*	Phytolaccaceae
Maytenus chubutensis	Celastraceae	*Pilosella caespitosa*	Asteraceae
Medicago lupulina	Fabaceae	*Pilosella piloselloides*	Asteraceae
Medicago polymorpha	Fabaceae	*Plagiobothrys nothofulvus*	Boraginaceae
Melampyrum sylvaticum	Scrophulariaceae	*Plantago bellardi*	Plantaginaceae

Species	Family
Plantago lanceolata	Plantaginaceae
Policarpea corymbosa	Caryophyllaceae
Polygala alpestris	Polygalaceae
Polygonum aviculare	Polygonaceae
Portulaca amilis	Portulacaceae
Potentilla aurea	Rosaceae
Potentilla crantzii	Rosaceae
Potentilla grandiflora	Rosaceae
Potentilla recta	Rosaceae
Praxelis pauciflora	Asteraceae
Pulsatilla alpina	Ranunculaceae
Pulsatilla vernalis	Ranunculaceae
Purshia tridentata	Rosaceae
Pyrola minor	Ericaceae
Pyrola rotundifolia	Ericaceae
Ranunculus acris	Ranunculaceae
Ranunculus bulbosa	Ranunculaceae
Ranunculus californicus	Ranunculaceae
Ranunculus montanus	Ranunculaceae
Ranunculus nivalis	Ranunculaceae
Raphanus raphanistrum	Brassicaceae
Rhinanthus groenlandicus	Orobanchaceae
Rhinanthus minor	Orobanchaceae
Rhodiola rosea	Rosaceae
Ribes spp	Grossulariaceae
Rubus phoenicolasius	Rosaceae
Rubus saxatilis	Rosaceae
Rumex acetosella	Polygonaceae
Rumex crispus	Polygonaceae
Rumex sp	Polygonaceae
Salix herbacea	Salicaceae
Sanicula bipinnata	Apiaceae
Satureja darwinii	Lamiaceae
Saussurea alpina	Asteraceae
Saxifraga paniculata	Saxifragaceae
Schinus patagonicus	Anacardiaceae
Sempervivum arachnoideum	Crassulaceae
Sempervivum montanum	Crassulaceae
Senecio doronicum	Asteraceae
Senecio inaequidens	Asteraceae
Senecio jacobaea	Asteraceae
Senecio miser	Asteraceae
Senecio vulgaris	Asteraceae
Serapias lingua	Orchidaceae
Sherardia arvensis	Rubiaceae
Sibbaldia procumbens	Rosaceae
Sidalcea sp.	Malvaceae
Silene dioica	Caryophyllaceae
Silene nutans	Caryophyllaceae
Silene rupestris	Caryophyllaceae
Silene vulgaris	Caryophyllaceae
Sisymbrium altissimum	Brassicaceae
Sisyrinchium bellum	Iridaceae
Solanum carolinense	Solanaceae
Solidago virgaurea	Asteraceae
Sonchus asper	Asteraceae
Sonchus oleraceus	Asteraceae
Stachys arvensis	Lamiaceae
Stellaria media	Caryophyllaceae
Taraxacum officinale	Asteraceae
Taraxacum sp	Asteraceae
Thalictrum alpinum	Ranunculaceae
Thesium alpinum	Santalaceae
Thymus polytrichus	Lamiaceae
Tolpis barbata	Asteraceae
Torilis arvensis	Apiaceae
Tragopogon dubius	Asteraceae
Trientalis europaea	Primulaceae
Trifolium albopurpureum	Fabaceae
Trifolium arvense	Fabaceae

Species	Family
Trifolium badium	Fabaceae
Trifolium bifidum	Fabaceae
Trifolium campestre	Fabaceae
Trifolium ciliolatum	Fabaceae
Trifolium dubium	Fabaceae
Trifolium fucatum	Fabaceae
Trifolium gracilentum	Fabaceae
Trifolium hirtum	Fabaceae
Trifolium microdon	Fabaceae
Trifolium pratense	Fabaceae
Trifolium repens	Fabaceae
Triphysaria eriantha	Orobanchaceae
Triteleia hyacinthina	Asparagaceae
Triteleia laxa	Asparagaceae
Trollius europaeus	Ranunculaceae
Tuberaria guttata	Cistaceae
Vaccinium myrtillus	Ericaceae
Vaccinium vitis-idaea	Ericaceae
Verbascum blattaria	Scrophulariaceae
Vernonia chamaedrys	Plantaginaceae
Veronica alpina	Plantaginaceae
Veronica chamaedrys	Plantaginaceae
Veronica fruticans	Plantaginaceae
Vicia americana	Fabaceae
Vicia cracca	Fabaceae
Vicia hirsuta	Fabaceae
Vicia sativa	Fabaceae
Vicia tetrasperma	Fabaceae
Vicia villosa	Fabaceae
Viola arvensis	Violaceae
Viola reichei	Violaceae
Viola rupestris	Violaceae
Waltheria indica	Malvaceae
Zornia sp	Fabaceae

List of visitor species

Species	**Order**
Anthrenus sp.	Coleoptera
Black tiny beetle	Coleoptera
Buprestidae	Coleoptera
Buprestidae 2	Coleoptera
Cerambycidae	Coleoptera
Chasmatopterus	Coleoptera
Chauliognathus marginatus	Coleoptera
Coleoptera	Coleoptera
Cryptocephalus rugicollis	Coleoptera
Cycloneda munda	Coleoptera
Diachus auratus	Coleoptera
Heliotaurus ruficolis	Coleoptera
Hippodamia convergens	Coleoptera
Larinus planus	Coleoptera
Microrhopala vittata	Coleoptera
Miridae	Coleoptera
Mordellistena cervicalis	Coleoptera
Nustela distigma	Coleoptera
Odontocorynus umbellae	Coleoptera
Oedemera barbara	Coleoptera
Oedemera sp	Coleoptera
Oxytryrea funesta	Coleoptera
Popillia japonica	Coleoptera
Psilothryx viridicoerulea	Coleoptera
Sehirus cinctus	Coleoptera
Sphaerophoria	Coleoptera
Tropinota squalida	Coleoptera
Bombyliidae sp.1	Diptera
Bombyliidae sp.2	Diptera
Bombyliidae sp.3	Diptera
Bombylius cruciatus	Diptera
Bombylius fimbriatus	Diptera
Bombylius major	Diptera
Bombylius medius	Diptera

Species	**Order**
Bombylius minor	Diptera
Bombylius sp	Diptera
Copestylum sp.1	Diptera
Diptera	Diptera
Diptera 1	Diptera
Diptera 2	Diptera
Diptera 3	Diptera
Diptero	Diptera
Dischistus sp.	Diptera
Eristalis tenax	Diptera
Euaresta bella	Diptera
Leucopodella bigoti	Diptera
Microchrysa	Diptera
Musca domestica	Diptera
Muscidae	Diptera
Ocyptamus sp.1	Diptera
Palpada sp.1	Diptera
Paragus haemorrhous	Diptera
small dark fly	Diptera
small fly 2	Diptera
small long thin fly	Diptera
Sphaerophoria contigua	Diptera
Sphaerophoria novaeangliae	Diptera
Syrphidae	Diptera
Syrphidae 1	Diptera
Syrphus sp	Diptera
Syrphus torvus	Diptera
tiny fly	Diptera
Tipuloidea	Diptera
Toxomerus geminatus	Diptera
Toxomerus marginatus	Diptera
Toxomerus sp.1	Diptera
Usia aenea	Diptera
Villa sp	Diptera

Species	**Order**
yellow and orange fly	Diptera
Diptera 1 (Trichoceronia sp)	Diptera
Diptera 2 (Dasyuromyia sp)	Diptera
Diptera 3 (Nemestrínidos sp)	Diptera
Heteroptero (Neocaulotops sp.)	Hemiptera
Aphid 1	Homoptera
Agapostemon virescens	Hymenoptera
Ancyloscelis sp.1	Hymenoptera
Andrena 1	Hymenoptera
Andrena 3	Hymenoptera
Andrena agilissima	Hymenoptera
Andrena alluaudi	Hymenoptera
Andrena fulica	Hymenoptera
Andrena orana	Hymenoptera
Andrena pilipes	Hymenoptera
Andrena rhyssonota	Hymenoptera
Andrena sp.	Hymenoptera
Andrena suerinensis	Hymenoptera
Andrena vetula	Hymenoptera
Anthophila	Hymenoptera
Anthophora californica	Hymenoptera
Anthophora sp.	Hymenoptera
Apis mellifera	Hymenoptera
Apis mellifera	Hymenoptera
Apis mellifera	Hymenoptera
Apis mellifera	Hymenoptera
Atalopedes campestris	Hymenoptera
Augochloropsis aff. Illustris	Hymenoptera
Augochloropsis smithiana	Hymenoptera
Augochloropsis sp 1	Hymenoptera
big orange bee with black stripes	Hymenoptera
big orange wasp	Hymenoptera
black and yellow wasp	Hymenoptera
black bee	Hymenoptera

Species	**Order**
black/yellow wasp with dark wings 1	Hymenoptera
black/yellow wasp with dark wings 2	Hymenoptera
Bombus bimaculatus	Hymenoptera
Bombus californica	Hymenoptera
Bombus crotchii	Hymenoptera
Bombus impatiens	Hymenoptera
Bombus melanopygus	Hymenoptera
Bombus sp	Hymenoptera
Bombus sp.	Hymenoptera
Bombus vosnesenskii	Hymenoptera
Centris (Melacentris) dorsata	Hymenoptera
Centris (Trachina) cf. fuscata	Hymenoptera
Centris sp 1	Hymenoptera
Centris sp2	Hymenoptera
Ceratina 2	Hymenoptera
Ceratina sp	Hymenoptera
Colletes acutus	Hymenoptera
Colletes sp	Hymenoptera
Dasypoda dusmeti	Hymenoptera
Dolichovespula maculata	Hymenoptera
Eucera 1	Hymenoptera
Eucera 2	Hymenoptera
Eucera cineraria	Hymenoptera
Eucera hispaliensis	Hymenoptera
Exomalopsis analis	Hymenoptera
Exomalopsis fulvofasciata	Hymenoptera
Exomalopsis sp.1	Hymenoptera
Florilegus (Floriraptor) melectoides	Hymenoptera
Formicidae 1	Hymenoptera
Formicidae 2	Hymenoptera
Formicidae 3	Hymenoptera
Halictidae	Hymenoptera
Heriades rubicola	Hymenoptera
Hymenoptera 1	Hymenoptera

Species	**Order**
Hymenoptera 2	Hymenoptera
Hymenoptera 3	Hymenoptera
Hymenoptera 4	Hymenoptera
Hymenoptera 5	Hymenoptera
Hymenoptera 6	Hymenoptera
Hymenoptera 7	Hymenoptera
large black bee	Hymenoptera
large black bee with green thorax	Hymenoptera
large yellow wasp	Hymenoptera
Lasioglossum 1	Hymenoptera
Lasioglossum 2	Hymenoptera
Lasioglossum 3	Hymenoptera
Lasioglossum 7	Hymenoptera
Lasioglossum 8	Hymenoptera
Lasioglossum algericolellum	Hymenoptera
Lasioglossum immunitum	Hymenoptera
Lasioglossum incompletum	Hymenoptera
Lasioglossum leucozonium	Hymenoptera
Lasioglossum malachurum	Hymenoptera
Lasioglossum villosulum	Hymenoptera
long thin black and yellow wasp	Hymenoptera
Megachile sp	Hymenoptera
Megachile sp.1	Hymenoptera
Megachile sp.2	Hymenoptera
Melipona (Melikerria) quinquefasciata	Hymenoptera
Nomada edwardsii	Hymenoptera
Nomada sp	Hymenoptera
Osmia 1	Hymenoptera
Osmia cara	Hymenoptera
Osmia sp 1	Hymenoptera
Osmia sp.	Hymenoptera
Osmia tricornis/bicornis	Hymenoptera
Oxaea alvarengai	Hymenoptera
Panurgus calcaratus	Hymenoptera
Panurgus perezi	Hymenoptera
small dark wasp	Hymenoptera
small orange bee	Hymenoptera
small shiny bee	Hymenoptera
small wasp 1	Hymenoptera
small wasp 2	Hymenoptera
small wasp 3	Hymenoptera
Tenthredo	Hymenoptera
Thalestria spinosa	Hymenoptera
tiny orange bee	Hymenoptera
wasp sp.1	Hymenoptera
wasp sp.10	Hymenoptera
wasp sp.11	Hymenoptera
wasp sp.12	Hymenoptera
wasp sp.13	Hymenoptera
wasp sp.14	Hymenoptera
wasp sp.15	Hymenoptera
wasp sp.16	Hymenoptera
wasp sp.17	Hymenoptera
wasp sp.18	Hymenoptera
wasp sp.19	Hymenoptera
wasp sp.2	Hymenoptera
wasp sp.21	Hymenoptera
wasp sp.22	Hymenoptera
wasp sp.23	Hymenoptera
wasp sp.24	Hymenoptera
wasp sp.25	Hymenoptera
wasp sp.26	Hymenoptera
wasp sp.27	Hymenoptera
wasp sp.28	Hymenoptera
wasp sp.29	Hymenoptera
wasp sp.3	Hymenoptera
wasp sp.30	Hymenoptera
wasp sp.4	Hymenoptera
wasp sp.5	Hymenoptera

Species	**Order**
wasp sp.6	Hymenoptera
wasp sp.7	Hymenoptera
wasp sp.8	Hymenoptera
wasp sp.9	Hymenoptera
Agraulis vanillae maculosa	Lepidoptera
Aricia cramera	Lepidoptera
big hairy butterfly	Lepidoptera
brown to light white medium sized butterfly	Lepidoptera
Butterfly sp1	Lepidoptera
Butterfly sp2	Lepidoptera
Chamaesphecia	Lepidoptera
Chiomara basigutta	Lepidoptera
Chiomara sp1	Lepidoptera
Coenonympha tullia	Lepidoptera
Danaus gilippus gilippus	Lepidoptera
Epargyreus clarus	Lepidoptera
Euptoieta claudia	Lepidoptera
Euptoieta hegesia meridiania	Lepidoptera
Eurema elathea flavescens	Lepidoptera
Glaucopsyche lygdamus	Lepidoptera
Helioipyrgus domicella willi	Lepidoptera
Heliopetes macaira orbigera	Lepidoptera
Heliothodes diminutivus	Lepidoptera
Hemiargus hanno hanno	Lepidoptera
Hylephila phyleus	Lepidoptera
Hylephila phyleus phyleus	Lepidoptera
Lepidoptera	Lepidoptera
Lepidotero 1 (Argyrophorus chiliensis)	Lepidoptera
Lepidotero 2 (Hylephila signata)	Lepidoptera
Lycaena phlaeas	Lepidoptera
Phyciodes tharos	Lepidoptera
Phycoides mytilla	Lepidoptera
Pieris rapae	Lepidoptera
Pieris rapae	Lepidoptera

Species	**Order**
Polites peckius	Lepidoptera
Pyrgus orcus	Lepidoptera
Pyrisita leuce leuce	Lepidoptera
Satyrium esculi	Lepidoptera
small light orange butterfly 1	Lepidoptera
small light orange butterfly 2	Lepidoptera
small white/grey butterfly with black spots	Lepidoptera
Stalachtis phlegia phlegitonta	Lepidoptera
Thymelicus sylvestris	Lepidoptera
Urbanus proteus	Lepidoptera
Vanessa cardui	Lepidoptera
Trichoptera	Trichoptera

List of plant species per site

ARGENTINA RNEG	ARGENTINA PAT	BRAZIL	GERMANY	MONTANA
Anemone multifida	*Adesmia lotoides*	*Amaranthaceae spp*	*Achillea millefoilum*	*Achillea millefoilum*
Baccharis spp	*Calceolaria uniflora*	*Borreria tenella*	*Centaurea jacea*	*Arabis holboellii*
Berberis darwinii	*Cerastium arvense*	*Chamaecrista flexuosa*	*Cerastium holosteoides*	*Arenaria capillaris*
Berberis microphylla	*Erigeron patagonicus*	*Croton glandulosus*	*Crepis biennis*	*Arenaria congesta*
Cerastium arvense	*Hypochaeris incana*	*Lamiaceae spp*	*Dianthus deltoides*	*Astragalus inflexus*
Chloraea alpina	*Junellia odonellii*	*Pfaffia glomerata*	*Fragaria vesca*	*Balsamorhiza sagittata*
Embothrium coccineum	*Leucheria purpurea*	*Policarpea corymbosa*	*Galium album*	*Boechera pendulocarpa*
Fabiana imbricata	*Nardophyllum bryoides*	*Portulaca amilis*	*Hieracium aurantiacum*	*Collinsia linearis*
Fragaria chiloensis	*Nassauvia aculeata*	*Praxelis pauciflora*	*Hypochaeris radicata*	*Cryptantha celesoides*
Gavilea spp	*Oxalis enneaphylla*	*Vernonia chamaedrys*	*Knautia arvensis*	*Erigeron divergens*
Lathyrus magellanicus	*Perezia recurvata*	*Waltheria indica*	*Leucanthemum ircutianum*	*Erigeron pumilus*
Lomatia hirsuta	*Satureja darwinii*	*Zornia sp*	*Lotus corniculatus*	*Gaillardia aristata*
Maytenus chubutensis	*Senecio miser*		*Pilosella piloselloides*	*Gaura coccinea*
Osmorhiza berteroi			*Ranunculus acris*	*Geum triflorum*
Ribes spp			*Ranunculus bulbosa*	*Lewisia rediviva*
Schinus patagonicus			*Rhinanthus minor*	*Lithospermum ruderale*
Taraxacum sp			*Senecio jacobaea*	*Lupinus sericeus*
Viola reichei			*Taraxacum officinale*	*Myosotis micrantha*
			Trifolium dubium	*Packera cana*
			Trifolium repens	*Purshia tridentata*
			Veronica chamaedrys	*Sisymbrium altissimum*
			Vicia cracca	*Taraxacum officinale*
			Vicia hirsuta	*Tragopogon dubius*
			Vicia tetrasperma	
			Viola arvensis	

FINLAND KILP	FINLAND SAANA	HOLPLAND	MCLAUGHLIN	NETHERLANDS
Andromeda polifolia	*Anthriscus sylvestris*	*Achillea millefolium*	*Achyrachaena mollis*	*Achillea millefoilum*
Antennaria alpina	*Astragalus alpinus*	*Agoseris heterophylla*	*Agoseris heterophylla*	*Crepis capillaris*
Antennaria dioica	*Astragalus frigidus*	*Amsinckia menziesii*	*Calandrinia menziesii*	*Fallopia convolvulus*
Astragalus alpinus	*Bartsia alpina*	*Asteraceae*	*Calochortus venutus*	*Galeopsis tetrahit*
Bartsia alpina	*Bistorta vivipara*	*Brassicaceae*	*Carduus pycnocephalus*	*Geranium pusillum*
Bistorta vivipara	*Campanula rotundifolia*	*Calandrinia menziesii*	*Chlorogalum pomeridianum*	*Hypericum perforatum*
Campanula rotundifolia	*Cerastium fontanum*	*Carduus pycnocephalus*	*Clarkia purpurea*	*Hypochaeris radicata*
Erigeron uniflorus	*Cirsium helenioides*	*Cerastium glomerium*	*Convolvulus arvensis*	*Jacobaea vulgaris*
Euphrasia frigida	*Coeloglossum viride*	*Claytonia perfoliata*	*Delphinium hesperium*	*Myosotis arvensis*
Gnaphalium supinum	*Euphrasia frigida*	*Dichelostemma capitatum*	*Erodium cicutarium*	*Polygonum aviculare*
Hieracium alpinum	*Filipendula ulmaria*	*Dichelostemma congestum*	*Euphorbia spathulata*	*Rumex acetosella*
Leontodon autumnalis	*Geranium sylvaticum*	*Erodium botrys*	*Geranium dissectum*	*Senecioinaequidens*
Minuartia biflora	*Gnaphalium norvegicum*	*Erodium cicutarium*	*Hypochaeris glabra*	*Trifolium arvense*
Phyllodoce caerulea	*Hieracium sylvaticum*	*Euphorbia spathulata*	*Lasthenia californica*	*Vicia hirsuta*
Potentilla crantzii	*Melampyrum sylvaticum*	*Galium parisiense*	*Lupinus microcarpus*	*Viola arvensis*
Pyrola minor	*Myosotis decumbens*	*Geranium dissectum*	*Medicago polymorpha*	
Ranunculus acris	*Paris quadrifolia*	*Geranium molle*	*Ranunculus californicus*	
Ranunculus nivalis	*Parnassia palustris*	*Hypochaeris glabra*	*Senecio vulgaris*	
Rhodiola rosea	*Pyrola minor*	*Iris macrosiphon*	*Sidalcea sp.*	
Rumex acetosella	*Pyrola rotundifolia*	*Lomatium hooverii*	*Sonchus asper*	
Salix herbacea	*Ranunculus acris*	*Lupinus bicolor*	*Taraxacum officinale*	
Saussurea alpina	*Rhinanthus groenlandicus*	*Micropus californicus*	*Tragopogon dubius*	
Sibbaldia procumbens	*Rubus saxatilis*	*Petrorhagia dubia*	*Trifolium fucatum*	
Solidago virgaurea	*Rumex acetosella*	*Plagiobothrys nothofulvus*	*Trifolium hirtum*	
Taraxacum sp	*Saussurea alpina*	*Ranunculus californicus*	*Triphysaria eriantha*	
Thalictrum alpinum	*Silene dioica*	*Sidalcea sp.*	*Vicia villosa*	
Trientalis europaea	*Solidago virgaurea*	*Sisyrinchium bellum*		
Trollius europaeus	*Taraxacum sp*	*Trifolium albopurpureum*		
Vaccinium myrtillus	*Thalictrum alpinum*	*Trifolium bifidum*		
Vaccinium vitis-idaea	*Trollius europaeus*	*Trifolium ciliolatum*		
Veronica alpina	*Vaccinium myrtillus*	*Trifolium dubium*		
	Vaccinium vitis-idaea	*Trifolium gracilentum*		
		Trifolium hirtum		
		Trifolium microdon		
		Vicia americana		
		Vicia sativa		
		Vicia villosa		

PORTUGAL	SIERRA FOOTHILLS	SWITZERLAND		MARYLAND
Andryala integrifolia	*Agoseris heterophylla*	*Achillea atrata*	*Potentilla grandiflora*	*Achillea millefoilum*
Bellardia trixago	*Amsinckia menziesii*	*Ajuga pyramidalis*	*Pulsatilla alpina*	*Barbarea vulgaris*
Chamaemelum sp	*Anagallis arvensis*	*Alchemilla xantochlora*	*Pulsatilla vernalis*	*Carduus acanthoides*
Coleostephus myconis	*Brodiaea sp.*	*Anthyllis vulneraria*	*Ranunculus montanus*	*Carduus nutans*
Crepis capillaris	*Cardamine oligosperma*	*Arctostaphylos uva-ursi*	*Rhinanthus minor*	*Cichorium intybus*
Echium plantagineum	*Carduus pycnocephalus*	*Arnica montana*	*Saxifraga paniculata*	*Cirsium arvense*
Erodium botrys	*Clarkia purpurea*	*Aster alpinus*	*Sempervivum arachnoideum*	*Coronilla varia*
Geranium molle	*Convolvulus arvensis*	*Campanula barbata*	*Sempervivum montanum*	*Daucus carota*
Hypochaeris sp	*Dichelostemma capitatum*	*Campanula scheuchzeri*	*Senecio doronicum*	*Dianthus armeria*
Lathyrus angulatus	*Dichelostemma multiflorum*	*Carduus defloratus*	*Silene nutans*	*Erigeron annuus*
Linum bienne	*Erodium botrys*	*Cerastium arvense*	*Silene rupestris*	*Galium mollugo*
Mysopates orontium	*Erodium cicutarium*	*Cerastium fontanum*	*Solidago virgaurea*	*Hypericum perforatum*
Ornithopus compressus	*Euphorbia spathulata*	*Daphne striata*	*Taraxacum officinale*	*Linaria vulgaris*
Parentucellia viscosa	*Galium parisiense*	*Dianthus sylvestris*	*Thesium alpinum*	*Lobelia inflata*
Plantago bellardi	*Geranium carolinianum*	*Erigeron alpinus*	*Thymus polytrichus*	*Lysimachia arvensis*
Raphanus raphanistrum	*Geranium dissectum*	*Erigeron uniflorus*	*Trifolium badium*	*Medicago lupulina*
Rumex sp.	*Geranium molle*	*Euphorbia cyparissias*	*Trifolium pratense*	*Oenothera biennis*
Serapias lingua	*Hypericum perforatum*	*Euphrasia minima*	*Trifolium repens*	*Oxalis stricta*
Silene sp	*Hypochaeris glabra*	*Galium anisophyllon*	*Vaccinium myrtillus*	*Physalis heterophylla*
Stachys arvensis	*Lagophylla minor*	*Gentiana acaulis*	*Vaccinium vitis-idaea*	*Phytolacca americana*
Tolpis barbata	*Lepidium nitidum*	*Gentiana campestris*	*Veronica chamaedrys*	*Pilosella caespitosa*
Tuberaria guttata	*Linanthus bicolor*	*Gentiana nivalis*	*Veronica fruticans*	*Plantago lanceolata*
	Lupinus bicolor	*Gentiana verna*	*Viola rupestris*	*Potentilla recta*
	Madia sativa	*Geum montanum*		*Rubus phoenicolasius*
	Navarretia pubescens	*Helianthemum nummularium*		*Silene vulgaris*
	Nemophila pedunculata	*Hieracium pilosella*		*Solanum carolinense*
	Petrorhagia dubia	*Hieracium staticifolium*		*Trifolium campestre*
	Plagiobothrys nothofulvus	*Hippocrepis comosa*		*Trifolium pratense*
	Sanicula bipinnata	*Homogyne alpina*		*Trifolium repens*
	Senecio vulgaris	*Leucanthemum adustum*		*Verbascum blattaria*
	Sherardia arvensis	*Lotus alpinus*		
	Stellaria media	*Minuartia verna*		
	Torilis arvensis	*Myosotis alpestris*		
	Trifolium dubium	*Nigritella nigra*		
	Trifolium hirtum	*Parnassia palustris*		
	Triteleia hyacinthina	*Pedicularis tuberosa*		
	Triteleia laxa	*Phyteuma hemisphaericum*		
	Vicia sativa	*Polygala alpestris*		
	Vicia villosa	*Potentilla aurea*		
		Potentilla crantzii		

List of visitor species per site

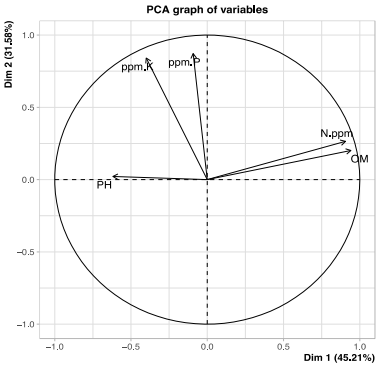
SIERRA FOOTHILLS	HOLPLAND	MCLAUGHLIN	ARGENTINA PAT
Andrena sp.1	*Andrena sp1*	*Andrena sp1*	*Aphid 1*
Anthophila	*Anthophila*	*Anthophila*	*Diptera 1 (Trichoceronia sp)*
Anthophora sp.	*Anthophora californica*	*Anthophora sp.*	*Diptera 2 (Dasyuromyia sp)*
Anthrenus sp.	*Apis mellifera*	*Anthrenus sp.*	*Diptera 3 (Nemestrínidos sp)*
Apis mellifera	*Bombus melanopygus*	*Bombus californica*	*Heteroptero (Neocaulotops sp.)*
Bombus californica	*Bombus vosnesenskii*	*Bombus crotchii*	*Lepidotero 1 (Argyrophorus chiliensis)*
Bombus crotchii	*Bombylius major*	*Bombus vosnesenskii*	*Lepidotero 2 (Hylephila signata)*
Bombus melanopygus	*Buprestidae*	*Coleoptera*	*Trichoptera*
Bombus sp.	*Cerambycidae*	*Diptera*	*wasp sp.29*
Bombus vosnesenskii	*Coenonympha tullia*	*Heliothodes diminutivus*	*wasp sp.30*
Bombylius major	*Coleoptera*	*Hippodamia convergens*	
Buprestidae 1	*Diptera*	*Lasioglossum incompletum*	
Coleoptera	*Hippodamia convergens*	*Miridae*	
Diptera	*Lepidoptera*	*Syrphidae*	
Glaucopsyche lygdamus	*Miridae*	*Tipuloidea*	
Halictidae	*Osmia cara*		
Heliothodes diminutivus	*Osmia sp.*		
Hippodamia convergens	*Phycoides mytilla*		
Lasioglossum incompletum	*Syrphidae*		
Miridae			
Muscidae			
Nomada edwardsii			
Osmia cara			
Syrphidae			
Tipuloidea			

PORTUGAL	MARYLAND	BRAZIL	
Andrena 2	*Agapostemon virescens*	*Agraulis vanillae maculosa*	*small white/grey butterfly with black spots*
Andrena 3	*Apis mellifera*	*Ancyloscelis sp.1*	*Stalachtis phlegia phlegitonta*
Andrena agilissima	*Atalopedes campestris*	*Apis mellifera*	*Thalestria spinosa*
Andrena alluaudi	*Bombus bimaculatus*	*Augochloropsis aff. Illustris*	*tiny fly*
Andrena fulica	*Bombus impatiens*	*Augochloropsis smithiana*	*tiny orange bee*
Andrena orana	*Bombus sp1*	*Augochloropsis sp 1*	*Toxomerus sp.1*
Andrena pilipes	*Ceratina sp1*	*big hairy butterfly*	*wasp sp.1*
Andrena rhyssonota	*Chauliognathus marginatus*	*big orange bee with black stripes*	*wasp sp.10*
Andrena suerinensis	*Colletes sp1*	*big orange wasp*	*wasp sp.11*
Andrena vetula	*Cycloneda munda*	*black and yellow wasp*	*wasp sp.12*
Apis mellifera	*Diachus auratus*	*black bee*	*wasp sp.13*
Lasioglossum immunitum	*Mordellistena cervicalis*	*black/yellow wasp with dark wings 1*	*wasp sp.14*
Lycaena phlaeas	*Paragus haemorrhous*	*black/yellow wasp with dark wings 2*	*wasp sp.15*
Aricia cramera	*Diptera 1*	*Bombyliidae sp.1*	*wasp sp.16*
Black tiny beetle	*Diptera 2*	*Bombyliidae sp.2*	*wasp sp.17*
Bombylius cruciatus	*Diptera 3*	*Bombyliidae sp.3*	*wasp sp.18*
Bombylius fimbriatus	*Dolichovespula maculata*	*brown abd white medium sized butterfly*	*wasp sp.19*
Bombylius medius	*Epargyreus clarus*	*Butterfly sp1*	*wasp sp.2*
Bombylius minor	*Eristalis tenax*	*Butterfly sp2*	*wasp sp.21*
Bombylius sp	*Euaresta bella*	*Centris (Melacentris) dorsata*	*wasp sp.22*
Buprestidae 2	*Euptoieta claudia*	*Centris (Trachina) cf. fuscata*	*wasp sp.23*
Ceratina sp2	*Formicidae 1*	*Centris sp 1*	*wasp sp.24*
Lasioglossum villosulum	*Osmia sp1*	*Centris sp2*	*wasp sp.25*
Chamaesphecia	*Formicidae 2*	*Chiomara basigutta*	*wasp sp.26*
Chasmatopterus	*Formicidae 3*	*Chiomara sp1*	*wasp sp.27*
Colletes acutus	*Hylephila phyleus*	*Copestylum sp.1*	*wasp sp.28*
Cryptocephalus rugicollis	*Hymenoptera 1*	*Danaus gilippus gilippus*	*wasp sp.3*
Dasypoda dusmeti	*Hymenoptera 2*	*Euptoieta hegesia meridiania*	*wasp sp.4*
Diptera 2	*Hymenoptera 3*	*Eurema elathea flavescens*	*wasp sp.5*
Dischistus sp.	*Hymenoptera 4*	*Exomalopsis analis*	*wasp sp.6*
Eucera	*Hymenoptera 5*	*Exomalopsis fulvofasciata*	*wasp sp.7*
Eucera 2	*Hymenoptera 6*	*Exomalopsis sp.1*	*wasp sp.8*
Eucera cineraria	*Hymenoptera 7*	*Florilegus (Floriraptor) melectoides*	*wasp sp.9*
Eucera hispaliensis	*Larinus planus*	*Helioipyrgus domicella willi*	*Urbanus proteus*
Heliotaurus ruficolis	*Lasioglossum 1*	*Heliopetes macaira orbigera*	*yellow and orange fly*

PORTUGAL	MARYLAND	BRAZIL
Heriades rubicola	*Lasioglossum 2*	*Hemiargus hanno hanno*
Lasioglossum 1	*Lasioglossum 3*	*Hylephila phyleus phyleus*
Lasioglossum 4	*Megachile sp*	*large black bee*
Lasioglossum algericolellum	*Microrhopala vittata*	*large black bee with green thorax*
Lasioglossum leucozonium	*Musca domestica*	*large yellow wasp*
Lasioglossum malachurum	*Odontocorynus umbellae*	*Leucopodella bigoti*
Microchrysa	*Phyciodes tharos*	*long thin black and yellow wasp*
Nomada sp	*Pieris rapae*	*Megachile sp.1*
Nustela distigma	*Polites peckius*	*Megachile sp.2*
Oedemera barbara	*Popillia japonica*	*Melipona (Melikerria) quinquefasciata*
Oedemera sp	*Sehirus cinctus*	*Ocyptamus sp.1*
Osmia sp2	*Sphaerophoria contigua*	*Oxaea alvarengai*
Osmia tricornis/bicornis	*Sphaerophoria novaeangliae*	*Palpada sp.1*
Oxytryrea funesta	*Syrphidae 1*	*Pyrgus orcus*
Panurgus calcaratus	*Syrphus sp1*	*Pyrisita leuce leuce*
Panurgus perezi	*Syrphus torvus*	*small dark fly*
Pieris rapae	*Toxomerus geminatus*	*small dark wasp*
Psilothryx viridicoerulea	*Toxomerus marginatus*	*small fly 2*
Satyrium esculi	*Vanessa cardui*	*small light orange butterfly 1*
Sphaerophoria		*small light orange butterfly 2*
Tenthredo		*small long thin fly*
Thymelicus sylvestris		*small orange bee*
Tropinota squalida		*small shiny bee*
Usia aenea		*small wasp 1*
Villa sp		*small wasp 2*
		small wasp 3

Table S4.5. PCA of the main soil variables measured in each of the study sites. Due to the correlation between ppm P with ppm K and pct N with Organic matter, we selected for the analyses ppm P and pct N as variables representing soil fertility.

Site	N ppm	ppm P	ppm K	OM	PH
ARG-Potrok	0.21	326.3	487.66	4.03	6.23
BRA-Abts01	0.03	29.33	23.67	0.8	5.73
BT_GER	0.16	148.75	152.5	3.68	5.08
FIN-Virt01	0.54	74.5	78.5	13.8	4.67
FIN-Virt02	0.43	67.75	48.75	9.33	6.15
hopl.us	0.20	31.3	130.3	4.7	6.47
mcla.us	0.26	28.66	284.66	5.87	6.7
NET-Veen01	0.21	127	62.33	5.83	5.8
POR-CLEZ	0.11	34	74	2.1	5.83
sier.us	0.17	16	76	3.98	6.22
SWI-valmusta	0.34	23	138.66	7.47	6
USA-lekb	0.17	18.3	295.66	3.8	6.6
USA-Mcda02	0.22	26.5	138.17	5.4	6.38



CHAPTER 5. GENERAL DISCUSSION

Several studies have addressed single aspects of nutrient enrichment effects on pollinators such as changes in flower abundance (Burkle and Irwin, 2009, Majetic et al., 2017) or changes in floral rewards (Ceulemans et al., 2017c). However, these studies usually focus on a few plant and pollinator species exposed to one fertilization treatment (mostly N or P alone, or a combination of N, P and K), and are conducted under controlled conditions (i.e., in greenhouses). Instead, this thesis focused on the effects of increased availability of single and multiple nutrients, carried out under field conditions, and including the entire communities of co-flowering plants and insect pollinators. Indeed, as the knowledge on the few insect orders (e.g., Hymenoptera, Lepidoptera) and species (e.g., *Apis* or *Bombus* species) studied so far may not be applicable to other species, in this study nutrient enrichment effects were assessed on entire insect pollinator communities (including Diptera and Coleoptera) reaching a total of 251 pollinator species.

Due to fundamental differences in edapho-climatic characteristics and biodiversity, lessons learned from nutrient enrichment effects on temperate ecosystems may not be applicable to other bioregions. To compare the effect of nutrient enrichment on pollinators and plant-pollinator interactions across regions, this thesis gathered data from 14 sites (13 grasslands and 1 shrubland) spread through three continents (six in Europe, five in North America and three in South America), covering diverse climatic conditions and soil fertility levels (Fig. S4.1). These sites have identically replicated a long-term nutrient experiment, as part of the global Nutrient Network Experiment (Borer et al., 2014). By integrating data from these 14 nutrient manipulation field experiments, this thesis focused on nutrient enrichment effects in understudied biomes/ecosystems, including Mediterranean (Europe and California) and Tropical ecosystems (Brazilian Cerrado), biodiversity hotspots but also Tundra and temperate ecosystems.

Finally, despite the importance of floral rewards' (i.e., nectar and pollen) quantity and quality in shaping plant-pollinator interactions, the number of studies on the topic is limited (Ceulemans et al., 2017c, Vaudo et al., 2022). Therefore, using an ongoing

nutrient manipulation field experiment located in Portugal, we assessed the simultaneous effects of multiple nutrient addition treatments on: i) floral resources (pollen quantity and quality) of three co-flowering Mediterranean grassland plant species, and their visitors, at the flower and plot scale; and ii) plant-pollinator network interactions, and iii) plant and pollinator diversity.

Thus, this is the first study to explore several of the nutrient enrichment effects on plant-pollinator communities in a Mediterranean ecosystem and to compare the effects detected with other regions.

5.1 Changes in plant and pollinator diversity and composition

5.1.1 Local scale – Mediterranean grassland

By focusing on the ongoing nutrient manipulation field experiment located in a Mediterranean grassland in Portugal (**Chapter 2**) we observed that: i) both P and K enrichment had a negative effect on pollinator richness; ii) N enrichment alone had no effect on plant-pollinator diversity, in contrast with other studies (e.g. Öckinger et al., 2006; Cuesta et al., 2008) carried out in sites with higher soil fertility; iii) N and P when added together had contrasting effects on plants and pollinators, i.e., NP enrichment had a negative effect on plant richness (Fig. 2.4 **Chapter 2**), but a positive effect on pollinator abundance and richness, increasing network plant generality (higher visitor to plant ratio, Fig. 2.5). Therefore, NP enrichment may be negative for plants as it reduces their richness (although it increased flower abundance), but positive for pollinators, at least in this Mediterranean ecosystem (in section 5.3 we further discuss the effects of NP on species interactions).

In terms of beta diversity, we detected an effect on pollinator assemblages (Fig. 2.3, **Chapter 2**) in which control plots had more species in common (more homogeneity) than other treatments. However, we expected that in fertilization treatments species with low tolerance to fertilization would have disappeared, leaving only tolerant species (generalists) and resulting in more homogeneous assemblages, which is opposite to our results. A deeper analysis of species similarity, separating nestedness and species

turnover, could give a clear answer to this unexpected pattern (Baselga, 2010). On the other hand, the P treatment was the one with fewest shared species within plots (more heterogeneous), attracting different pollinator species (not shared) in each plot despite reducing pollinator richness. This was possible as P slightly increased plant diversity (e.g. presence of *Ornithoppus compressus*) which could have attracted a different set of visitors in each plot. I provide further discussion of this point in section 5.3.

Given the different effect each treatment has in the large and the local scale approach, it is important to highlight that:

i) other Mediterranean ecosystems may respond differently to nutrient enrichment. For example, if the plant community was dominated by perennial (e.g., a shrubland or a forest) instead of annual plant species as was the case in our study, the negative effects of nutrient enrichment could have been smaller and/or take longer to manifest due to the difference in life span between the N-benefited (with short life cycles, including annuals) and the N-affected species (perennials), which would allow the detection of the incomers but only drastic impacts on the losers (Dias et al., 2014).

ii) not all plant species are entomophilous, and grasses, which are anemophilous, are usually benefited by nutrient enrichment. This means that nutrient enrichment may reduce plant richness and reduce the relative abundance of entomophilous plant species, which may not influence overall pollinator richness of abundance, but affect specific pollinator groups.

iii) plant communities such as the one studied here dominated by annual plant species which germinate each year from the seed bank, are frequently characterized by high temporal variability in species composition driven by asynchronous fluctuations among populations of coexisting species. While year-to-year variability may result from unpredictable differences among years in environmental conditions (e.g. precipitation, temperature), long-term community changes are often caused by trends in environmental conditions or management practices such as nutrient enrichment (DeMalach et al., 2021). Therefore, results obtained here may reflect the influence of environmental conditions when data were gathered and may change with the continuation of the nutrient additions.

5.1.2 Large scale –14 NutNet sites

By gathering data from 14 long-term nutrient manipulation field experiments covering a wide range of climatic conditions and soil fertility levels, we showed that nutrient enrichment effects on plants and pollinators depended on soil fertility and temperature (**Chapter 4**). This modulating effect of soil fertility and temperature can explain contrasting results found in the literature. For example, negative responses in plant species composition that negatively affect (Carvalheiro et al., 2019) or have no effect (Wang et al., 2022) on pollinator diversity.

The strongest negative effects on plant richness were observed in sites with low fertility soils (i.e., soil total N $\leq$ 0.17%; Fig. 4.1), in agreement with Olsson and Kellner (2006), and Vogels et al. (2023). By contrast, positive effects on plant richness were observed in response to N enrichment in sites with low temperatures, disagreeing with Clark et al. (2007). Although our results further support the negative effect of nutrient enrichment on plant richness (Stevens et al., 2004; Bobbink et al., 2010; Harpole et al., 2016; Tognetti et al., 2021), they also corroborate the existence of positive effects (e.g. Dias et al., 2011, 2014), which are rare in the literature. The low number of reports of positive effects of nutrient enrichment on plant richness may be explained by the following three non-exclusive factors:

i) regions outside Europe and North America have not received enough attention and include ecoregions in eastern and southern Asia (China, India), an important part of the Mediterranean (California, southern Europe), and several subtropical and tropical parts of South America and Africa (Bobbink et al., 2010). By including sites from these understudied regions (i.e., the sites located in Southern Europe, Brazil, Argentina), we may be observing the results of different ecological processes triggered by nutrient enrichment.

ii) nutrient enrichment in low-fertility ecosystems may promote plant richness (Dias et al., 2011, 2014; Köbel et al., 2024), at least in the short-term, and/or when nutrient inputs are moderate, as shown for example for epiphytic lichens (Pinho et al., 2011) and biocrust communities (Dias et al., 2020). Assuming low nutrient availability as a severe environmental limitation, the nutrient-driven increase in richness may be explained by the revised Grime's humped-back model (Grime, 1973). Accordingly, the richness increment would reflect the alleviation of the

stress condition (nutrient limitation), allowing the coexistence of the characteristic site species with the incoming exploitative ones.

iii) the use of low or reduced loads of fertilizers, that has been shown to bring positive changes to plant communities and pollinators (e.g. Burkle and Irwin, 2010; Hudewenz et al., 2012; Ramos et al., 2018) compared to higher doses which are associated with (stronger) negative changes.

Nutrient enrichment effects on pollinator richness followed a similar pattern, showing negative effects in sites with warm temperatures, and positive effects in response to N and also P enrichment, in sites with low temperatures (Fig. 4.2). Negative effects of N on insects at warmer temperatures were also reported by Gallego-Zamorano et al. (2023), particularly for insects with full metamorphosis such the pollinator groups studied here. The hypotheses for explaining the positive effects of nutrient enrichment on plant richness, may also apply to pollinator richness, although this needs further research.

Besides providing evidence of bottom-up effects of nutrient enrichment on pollinators, we show that the magnitude of the nutrient enrichment effect on pollinators is bigger than that on plants (both in abundance and richness), which agrees with Carvalheiro et al. (2010) and Villa-Galaviz et al. (2021). Therefore, our data show that pollinators are more vulnerable to nutrient enrichment than plants, being particularly at risk in warmer regions.

Taking the world's top four food-producing countries as an example (China, India, USA and Brazil - https://www.investopedia.com/articles/investing/100615/4-countries-produce-most-food.asp), and overlaying them on the global cropping intensity map (Fig. 5.1), it becomes clear that world food production depends greatly on agriculture in warmer regions, which is often highly intensive, as shown by the excessive use of fertilizers in China (Van Wesenbeeck et al., 2021) or of pesticides in Brazil (Panis et al., 2022). Although this thesis only focused on nutrient enrichment effects, agricultural intensification is also associated with land-use change, landscape and ecosystem simplification, and massive use of pesticides (including insecticides) (Benton et al., 2002). With the additional threat that climate change poses to local and global food security (Kompas et al., 2024), farmers may increase their chemical inputs (i.e., fertilizers and pesticides) to secure food production. Therefore, pollinators in warmer regions with intensive agriculture are probably at even higher risk if nutrient inputs become higher

than those tested here (i.e., 100 kg ha^{-1}), alongside increased pesticide use in extensive areas used for farming (Ferreira Filho et al., 2016), with little landscape heterogeneity.

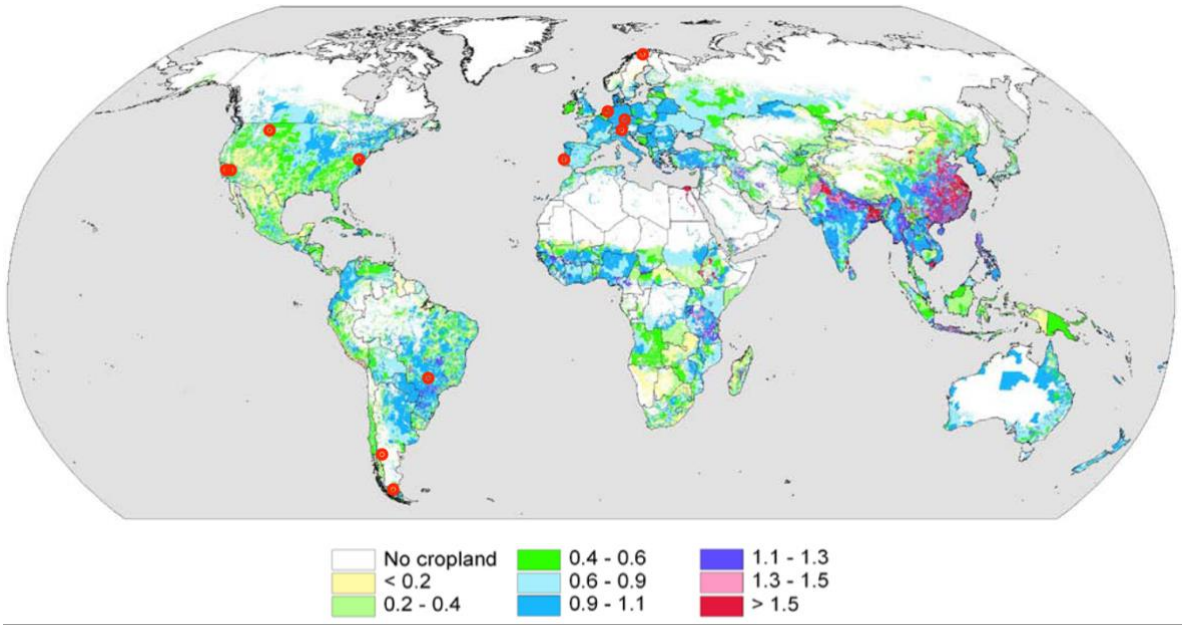


Figure 5.1. Global cropping intensity (yr^{-1}) when excluding fallow lands from the calculation of cropping intensity (Siebert et al., 2010). The 14 sites used in Chapter 4 were overlapped to relate them to cropping intensity.

5.2 Linking nutrient enrichment effects in floral rewards and pollinators

5.2.1 Local scale – Mediterranean grassland

By assessing the simultaneous effect of nutrient enrichment on three co-flowering plant species (**Chapter 3**) in terms of pollen quantity and quality, and pollinator visitors at the flower scale we observed that the effect on:

i) pollen traits (quantity and quality in terms of aa) differed between species, suggesting species-specific responses (Burkle and Irwin, 2009). N and NP enrichment increased pollen yield per flower in *Crepis*, similar to what was reported by Lau and Stephenson (1993). P enrichment increased pollen quality (i.e., increased aa concentration) in *Raphanus*, while N and NP enrichment decreased pollen quality in *Crepis*, contradicting what was reported by Gardener and Gillman (2001).

ii) pollinator visitation rate increased in *Raphanus* flowers only with P enrichment (the same treatment that increased pollen quality), similarly to Hoover et al. (2012). Considering specific pollinator groups, NP and P enrichment had a positive effect on bees and flies (respectively) visiting *Raphanus* flowers.

It is important to highlight that the nutrient enrichment effects of some treatments (i.e., P) were so negative on *Crepis* cover and flower production that it was only possible to sample pollen from some treatments. This drastic reduction in *Crepis* flowers may have affected specific pollinator groups such as beetles which mainly rely on open flowers like *Crepis*. Nutrient enrichment effects on pollinator visitation rates differed between species, being positive for *Raphanus* and neutral for *Echium* and *Crepis* despite the very low flower abundance in some treatments. Altogether, our results suggest that nutrient enrichment effects on pollinators are species-specific and differ across pollinator groups.

However, considering that one honeybee can visit 600-12,000 flowers per day (each honeybee visits 50-100 flowers during each collection trip, making 12 or more trips per day - https://www.apexbeecompany.com/honey-bee-facts/), the effects at the flower scale may differ from those at plot scale. Indeed, when assessing the simultaneous effect of nutrient enrichment on three co-flowering plant species at plot scale (including on flower abundance) we observed that the effect on:

i) flower abundance was similar in the three plant species, with NP enrichment increasing plants' investment in sexual reproduction, while P enrichment decreased it (Fig. 3.3).

ii) pollen traits (quantity and quality in terms of aa) were similar in the three plant species, with NP enrichment increasing, or tending to increase, pollen quantity and quality while P tended to decrease them.

iii) pollinator visitation rate of the three plant species showed no nutrient enrichment effect, not even considering specific pollinator groups. However, when considering the visitation rate to the whole plant community, a marginal negative effect was detected in the P treatment. The fact that we did not observe a negative effect of our nutrient addition treatments on total pollinator visitation rates may be explained by the fact that the nutrient dose (i.e., 100 kg ha^{-1} yr^{-1}) we applied may be below the threshold for negative effects, which

may vary with ecosystem type (Nessel et al., 2021) and group (Gallego-Zamorano et al., 2023; Millard et al., 2021). Non-exclusively, our grassland ecosystem's biodiversity (21 plant species and 58 insect visitor species) is likely to improve its resilience as showed by many studies (Isbell et al., 2015; Oliver et al., 2015; Mori, 2016).

The positive effect of NP enrichment on floral rewards quantity and quality at the plot scale together with the smaller change in pollen aa composition (Fig. 3.2), probably reflects the co-limitation by N and P of Mediterranean ecosystems (Dias et al., 2011, Sardans and Peñuelas, 2013). Therefore, alleviating the limitation of either N or P alone (or other nutrients, such as in the K addition treatment) triggers a greater depletion of the least available nutrient(s) as observed by the increased P limitation upon adding N in a Mediterranean shrubland (Ulm et al., 2017), and probably explains why the addition of P alone decreased *Raphanus* and *Echium* pollen yield and aa concentration at plot scale (Fig. 3.4). Despite the positive effect of NP enrichment on flower abundance, visitation rate remained unchanged, contrary to other studies reporting a positive relation between flower abundance and visitation rate (Muñoz et al., 2005; Majetic et al., 2017). On the other hand, P addition negatively affected flower abundance and pollinator visitation rate at plot scale (considering visitation to the whole plant community). Altogether, nutrient enrichment effects on the three co-flowering plant species (*Raphanus*, *Echium* and *Crepis*) in terms of pollen quantity and quality, and pollinator visitors at flower scale, differed from those at plot scale (Figs 3.1 and 3.4), reflecting trade-offs (including significant effects on flower abundance – Fig. 3.3).

Besides the effects of NP and P enrichment detected on flower abundance of the three co-flowering plant species in the Mediterranean grassland (**Chapter 3**), we detected a coupled response between changes in flower abundance and changes in pollinator abundance (increased with NP enrichment but decreased with P and K enrichment) in **Chapter 2**. This could be related to the study of the full plant community in **Chapter 2** compared with the study of three dominant plant species in **Chapter 3** that might not suitably represent changes at community level (the study of the whole community in **Chapter 3** showed a decrease in visitation rate in P but no effect in NP following changes in flower abundance). Also, data from different years were used: in **Chapter 3** data from

2020 and 2021 were used while in **Chapter 2** data from 2021 and 2022 were selected, adding inter year variability to the data. Additionally, the use of different variables e.g. visitation rate and pollinator abundance, could also explain the different results. Changes in pollinator abundance were driven mainly by changes in Diptera abundance while Hymenoptera or Coleoptera did not change. Our results show a positive effect of NP on certain flower resource variables (flower abundance) that in total did not have a negative effect on pollinator visitation rate (**Chapter 3**). However, the combination of results from **Chapter 2** and **3** suggests that NP certainly improves floral resource availability for pollinators, increasing pollinator abundance and richness (**Chapter 2**), but species assemblages were increasingly formed by generalists. The increase in generalist species may be replacing specialist species, what could have important consequences for ecosystem functioning.

5.2.2 Large scale – 14 NutNet sites

By integrating data from 14 long-term nutrient manipulation field experiments covering a wide range of climatic conditions and soil fertility levels, we showed that the effect of N enrichment on flower abundance depended on soil fertility and temperature, being: i) positive at low temperatures, particularly in low fertility sites (soil total N < 0.17%), and ii) negative at higher temperatures, especially in rich soils (soil total N > 0.34%), in contrast with results presented by Clark et al. (2007).

Surprisingly, when analysed together, NP enrichment had a negative effect on flower abundance in low fertility warmer sites (Fig. 2.1), such as the Mediterranean grassland in Portugal where we observed the opposite (Figs. 3.3) (see **Chapter 2** and **Chapter 3**), with positive changes detected. These apparent contrasting results are probably related to the way soils were classified as low and high fertility in the large scale study for visualization purposes (Fig 4.1 and 4.2), i.e., only soil N % was used to categorise sites. This means that in each soil fertility class there were soils with different nutrient limitations, for example, low fertility soils included the Mediterranean grasslands which are N and P limited, Cerrado ecosystems limited mainly by P (Oliveira et al., 2002; da Silva et al., 2016), and other temperate grasslands in Central Europe where N is the main limiting nutrient. As we are mixing sites with different limitations the response will reflect the nutrient limitation of the majority of the sites. One way to refine results would be to

add a second criterion to assess soil fertility including a soil N:P ratio to be able to distinguish between soils that are N limited, P limited and NP limited. However, more sites would be necessary in each category to achieve this.

5.3 Changes in plant and pollinator interaction patterns

5.3.1 Local scale – Mediterranean grassland

By assessing the effect of nutrient enrichment on plant and visitor assemblages (**Chapter 2**) in a field experiment located in Mediterranean grassland in Portugal we observed that:

i) N and P coaddition increased plant generality, attracting a higher number of more generalist visitor species which may respond to less diverse but more abundant flower assemblages detected in this treatment. Moreover, this treatment decreased plant richness, in agreement with other studies (Villa-Galaviz et al., 2021; Wang et al., 2022). A more generalized visitor assemblage was found in urban compared to agricultural ecosystems (Geslim et al., 2013).

ii) P addition decreased plant generality and interaction evenness, attracting a lower number of more specialist visitors, which visited a more diverse but less abundant flower assemblage. A decreased interaction evenness is usually found in modified habitats (Tylianakis et al., 2007; Fisogni et al., 2021).

Effects on pollinator abundance were reflected in plant generality, which increased when N and P were co-added and decreased when P was added, contrasting with the results in Wang et al. (2022). Pollinator generality did not change compared to control, which is consistent for N and P treatments (Wang et al., 2022) and NPK (Villa-Galaviz et al., 2020). The positive effect of NP enrichment on pollinator diversity probably reflects the co-limitation by N and P of Mediterranean ecosystems (Dias et al., 2011; Sardans and Peñuelas, 2013), as shown in **Chapter 3**. This suggests as presented in section 5.2.1 that N and P coaddition has an overall positive effect on pollinator assemblage diversity in Mediterranean ecosystems. However, NP enrichment shifts plant and pollinator interaction networks to be more generalist, where pollinators are visiting less diverse flower assemblages. In contrast, P treatment in Mediterranean ecosystems has been associated with negative effects on flower abundance and pollinator diversity (and

marginal negative effects on the full plant community visitation rate). Interestingly, the interaction patterns among the species recorded in this treatment were more specialized. Changes in generalization network patterns could affect community tolerance to future perturbations. Hence, these results highlight the need to add environmental eutrophication to the list of important drivers of pollinator declines.

5.4 Caveats, gaps and future work

Our results indicate that nutrient enrichment is an important driver of floral resource availability affecting pollinators. However, some caveats need to be raised. In **Chapter 3**, we identified several limitations. First of all, the site at Companhia das Lezírias has a plant and pollinator community historically adapted to low-management intensity levels. Second, there was an insufficient number of flowers sampled in some treatments, and pollen quantity and quality were only analysed in one year, which differed between plant species. Third, pollen contains other important compounds for pollinators besides amino acids (e.g., sugars, fatty acids, secondary compounds) whose quantity and composition may be influenced by our nutrient addition treatments and were not analyzed. Also, changes in relative abundance of these primary and secondary compounds could change the perception of the floral resources by flower visitors. Last, nectar quality and quantity was also not analysed, even though pollinators visiting flowers could have been attracted to this reward. In **Chapter 4**, while in temperate regions surveying flowers and pollinators only during the few months of the flowering peak gives a good indication of the full picture (Fjellheim et al., 2014), for warmer tropical regions (e.g. Brazil) where flowering occurs throughout the year (Sakai & Kitajima, 2018) the results for a specific season may not be representative of floral availability and visitation over the full year. Also, despite having 14 sites to assess the effect on plants, only 7 sites contributed with pollinator data. A better temporal cover in tropical areas and greater cover of pollinator data would be important to better assess geographical patterns.

5.5 Future research

Future research on nutrient enrichment effects should fill the following knowledge gaps:

i) Effects on understudied pollinator groups; within pollinators, more priority should be given to studies focusing on solitary bees, flies, beetles and butterflies, compared to the few well studied species such as honeybee or bumblebees.
ii) Effects on poorly studied ecosystems such as Mediterranean or Tropical ecosystems; these understudied areas have warmer regional temperatures and poorer soils compared to the majority of the studies on the topic.
iii) Studies should be conducted in areas with low or minimal disturbance.
iv) Standardized protocols for nutrient loads are needed, applying similar fertilizers to be able to replicate methodologies and compare results more easily.
v) Assessment of more than one plant trait (e.g. flower abundance or plant richness) as nutrient enrichment affects plants through several pathways.
vi) Effects on flowers and floral rewards quantity and quality should include a wide range of plant species and families as responses are usually very specific.
vii) Addition of pollinator traits to future studies in the topic such as foraging (nectar and pollen) preferences, body size, nitrophily degree and other ecological and biological characteristics.

CONCLUSIONS

Overall, the results of this thesis indicate that nutrient enrichment is an important driver of floral resource availability with bottom-up effects on pollinators. At a local scale, we showed that a diverse plant community composed of flowering species that react differently to nutrient enrichment (species-specific responses) has the potential to dilute the nutrient enrichment effects on pollinator visitation. Moreover, we showed that nutrient enrichment besides affecting plant-pollinator diversity also affected pollinator foraging patterns, promoting network generalization or specialization under different treatments. Particularly, NP was associated with positive responses in flower abundance and pollinator diversity, yet these pollinators were more generalist. However, P was associated with contrasting responses in pollinator visitation rate and negative effects on

diversity, but floral visitors were more specialist. At a large scale, we showed that the effect of nutrient enrichment was influenced by temperature and soil fertility, with pollinators more affected than plants. This puts warm regions' susceptibility to nutrient enrichment in the spotlight, including biodiversity hotspots located in warm climatic regions such as the Mediterranean and the Tropics. Ongoing global warming in addition to the increasing use of these regions for (intensive) agriculture could compromise crop pollination and food supply if policies aiming at controlling nutrient loads are not implemented. To better understand the effects of nutrient enrichment on plants and especially on pollinators, future research should prioritize understudied regions and include a wider range of plant and pollinator species in community-level studies.

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Pollinator assemblage response to nutrient enrichment in grasslands

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