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Ruohan Ma

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THÈSE DE DOCTORAT

Effets *bottom-up* de la fertilisation sur des agents de biocontrôle

Ruohan MA

Institut Sophia Agrobiotech – MiB team

**Présentée en vue de l'obtention
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écologie **d'Université Côte d'Azur**
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Effets *bottom-up* de la fertilisation sur des agents de biocontrôle

(Bottom-up effects of fertilization on biocontrol agents)

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Résumé

Dans les agroécosystèmes, les communautés d'arthropodes peuvent être influencées par des forces *bottom-up* induites par des variations environnementales, telles que la fertilisation, qui modifient les caractéristiques des plantes. Alors que l'effet de ces forces ascendantes sur le deuxième niveau trophique est bien étudié, leur effet sur le troisième niveau trophique reste moins connu. La fertilisation altère le premier niveau trophique, influençant ainsi la qualité nutritionnelle des plantes. Ces changements affectent ensuite le deuxième niveau trophique *i.e.* les herbivores. L'extension des effets *bottom-up* jusqu'au troisième niveau trophique pourrait agir directement sur les ennemis naturels via la plante ou indirectement via l'herbivore. Or, si les forces *bottom-up* atteignent le 3^{ème} niveau trophique *i.e.* les agents de biocontrôle, elles peuvent modifier la qualité et/ou l'intensité de la régulation *top-down* de la lutte biologique contre les ravageurs. Dans ce contexte, cette thèse vise à élucider l'influence de la fertilisation sur l'interaction entre plantes, herbivores et ennemis naturels, à la fois au niveau individuel (traits d'histoire de vie et efficacité du biocontrôle) et au niveau de la population (dynamique de la population).

Nous avons étudié l'effet de différentes doses de fertilisation sur le troisième niveau trophique, tout en testant si la diversité du régime alimentaire des ennemis naturels (prédateurs omnivores *vs* carnivores) ou leur guildes fonctionnelle (prédateurs *vs* parasitoïdes) modifiaient l'intensité des effets *bottom-up*. Pour cela, nous avons évalué l'effet *bottom-up* de la fertilisation sur le système tri-trophique "tomate – ravageur herbivore - ennemis naturels" selon trois approches : (a) une méta-analyse pour obtenir une vue d'ensemble de l'état de l'art sur le sujet; (b) des analyses expérimentales au niveau individuel dans des conditions contrôlées en laboratoire (c) et au niveau de la population en serre.

Nos résultats suggèrent que les effets *bottom-up* de la fertilisation varient avec la dose de fertilisation et selon les espèces et la guildes fonctionnelle des organismes constituant le système tri-trophique. Globalement les parasitoïdes semblent moins affectés que les prédateurs. L'efficacité des prédateurs carnivores (et dans une moindre mesure omnivores) comme agents de biocontrôle semble quant à elle défavorisée par une forte fertilisation car les prédateurs consommeraient moins de proies car elles seraient de meilleure qualité nutritionnelle. L'effet *bottom-up* de la fertilisation est également modulé par les herbivores qui réagissent

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différemment à ces variations. Par exemple, la fécondité des pucerons uniquement augmente avec les doses de fertilisation (la dose optimale pour les aleurodes et *Tuta absoluta* serait intermédiaire) et, à l'échelle populationnelle, une fertilisation élevée favorise les populations de pucerons.

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Cette étude montre comment une approche multiple (bibliographique et expérimentale multi-échelles) permet d'aborder une même question selon plusieurs angles et suggère que la manipulation des forces *bottom-up* pourrait contribuer à la gestion de certaines espèces de ravageurs dans le cadre de la lutte intégrée.

Mots clés: Prédateur, parasitoïde, forces écologiques, interaction tri-trophique, agents de biocontrôle, ennemis naturels, réduction des intrants, engrais inorganiques, interaction plante-insecte

Abstract:

In agroecosystems, arthropod communities may be influenced by bottom-up forces induced by environmental variations (e.g., fertilization) through the modification of plant traits. The way bottom-up forces affect the second trophic level is well documented: Fertilization is considered a “bottom-up driver” as it influences the first trophic level (“bottom”) by modifying plant tissues macronutrient and defense content, affecting the plant nutritional quality for herbivores. This, in turn, leads to changes in the second trophic level (“up”). But less is known on their ability to affect the third trophic level. Plant-mediated bottom-up forces may affect natural enemies directly (plant-mediate) or indirectly (plant-herbivore-mediated). Furthermore, as bottom-up effect extend to the third trophic level, they could mediate the intensity of top-down regulation, i.e., biological control of pests. In this context, the objective of the PhD was to elucidate how the fertilization influence the interaction among plant, herbivores and their natural enemies at both individual (life-history traits and biocontrol efficiency) and population (population dynamics) levels.

We studied the plant-mediated bottom-up effect of different doses of fertilization on the third trophic level (natural enemies), to determine whether diversity in the natural enemy diet regime (carnivorous vs omnivorous predators) or functional guild (predator vs parasitoid) modifies the intensity of bottom-up effects. We evaluated the tri-trophic system “tomato plant – pest - natural enemies” following three different approaches: (a) meta-analysis to overview of the current state of the art; (b) experimental analysis at individual level under controlled conditions (lab experiments) to look for mechanisms and (c) population dynamic level experiment in greenhouses.

Our results suggest that the bottom-up effects of fertilisation vary with the dose of fertilisation and according to the species and functional guild of organisms making up the tri-trophic system. Overall, parasitoids appear to be less affected than predators. The effectiveness of carnivorous (and to a lesser extent omnivorous) predators as biocontrol agents seems to be adversely affected by high fertilisation, as the predators consume fewer prey because they are of better nutritional quality. The bottom-up effect of fertilisation is also modulated by herbivores, which react differently to these variations. For example, the fecundity of aphids alone increases with fertilisation doses (the optimum dose for whiteflies and *Tuta absoluta* would be intermediate) and, on a population scale, high fertilisation favours aphid populations.

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This study shows how a multiple approach (bibliographic and multi-scale experimental) makes it possible to tackle the same question from several angles and suggests that manipulating these bottom-up forces could contribute to the management of certain pest species as part of integrated pest management.

Keywords: Predator, parasitoid, tri-trophic interaction, biocontrol efficiency, biocontrol agents, agricultural transition, input reduction, inorganic fertilisers, plant-insect interaction

Declaration

French :

Je déclare par la présente que le travail présenté dans cette thèse n'a pas été soumis pour l'obtention d'un autre diplôme ou d'une qualification professionnelle, et qu'il est le résultat de mon propre travail indépendant.

" En présence de mes pairs. Avec l'obtention de mon doctorat en sciences biologiques de la vie, dans ma quête de connaissances, j'ai mené des recherches exigeantes, fait preuve de rigueur intellectuelle, de réflexion éthique et de respect des principes d'intégrité de la recherche. Dans la poursuite de ma carrière professionnelle, quel que soit le domaine choisi, je m'engage, dans toute la mesure de mes moyens, à continuer à faire preuve d'intégrité dans mon rapport au savoir, dans mes méthodes et dans mes résultats. "

English :

I hereby declare that the work presented in this thesis has not been submitted for any other degree or professional qualification, and that it is the result of my own independent work.

« In the presence of my peers. With the completion of my doctorate in biological life sciences, in my quest for knowledge, I have carried out demanding research, demonstrated intellectual rigor, ethical reflection, and respect for the principles of research integrity. As I pursue my professional career, whatever my chosen field, I pledge to the greatest of my ability, to continue to maintain integrity in my relationship to knowledge, in my methods and in my results. »

MA Ruohan

Date 03/04/2024

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1. **Ma, RH**, Gu JM, Jaworski CC, Xue ZX, Li XL, Wang PL, Han P, Desneux N. Life stage affects prey use with fitness consequences in a zoophytophagous mirid bug. *Phytoparasitica* (2023): 1-9.
2. **Ma RH**, LAVOIR AV, Jaworski CC, Desneux EA, Han P, Desneux, N. Bottom-up effects of reduced fertilization on natural enemies and biocontrol efficiency. *Entomologia Generalis* (under review).

Prepare papers for submission:

1. **Ma RH**, Valente M, LAVOIR AV, Jaworski CC, Boll R, Desneux, N. Are reduced fertilization and biological control compatible for pest regulation? In prep. for *Journal of Pest Science*.
2. **Ma RH**, LAVOIR AV, Desneux, N. Bottom-up effects of inorganic fertilizers on natural enemies: a meta-analysis. In preparation.
3. **Ma RH**, Wang PL, Han P, Jaworski CC, Desneux, N. Plant-mediated bottom-up effects of salinity stress on an omnivorous predator: implications for biological control. In prep. for *Pest management Science*.

Oral presentation

1. Journées des doctorants du département Santé des plantes et Environnement de l'INRAE - JUIN 2022 in CENTRE INRAE BORDEAUX-NOUVELLE AQUITAINE
2. **Does Bottom-up effect reach third trophic level i.e., natural enemies?** Journées de l'Ecole Doctorale de Nice - JEDNs (2022 sessions of Doctoral School Days) - May 2022 in Valrose Campus

3. **Does Bottom-up effect reach third trophic level i.e., natural enemies?** 2022 session of Academic Seminar of Chinese Students and Scholars in Germany – France, online (Most Popular Award)

4. **Does Bottom-up effect reach third trophic level i.e., natural enemies?** The Third International Conference on Insect Pest Management, Oct. 20-23, Nanjing, China (Best Oral Presentation Award)

5. **Does Bottom-up effect reach third trophic level i.e., natural enemies?** ENTOMOLOGICAL SOCIETY OF AMERICA, November 5-8 / National Harbor, MD – On-demand presentation

Poster presentation

1. **Does Nutrient Bottom-up effects Reach Natural enemies?** Journées de l'Ecole Doctorale de Nice - JEDNs (2021 session of Doctoral School Days) - May 2021 Online

2. **Does Fertilisation Bottom-up effects Reach Natural Enemies in the Tomato-Pest-Natural enemies tri-trophic system?** XII European Congress of Entomology, October 16-20 / Cultural Conference Center of Heraklion, Crete, Greece

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Sophia-Antipolis, France

Ruohan

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Abbreviations

IGP: Intraguid Predation

ESR: Efficiency-Substitution-Redesign

LICS: Low Input Cropping Systems

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General introduction

General introduction

In agroecosystems, arthropod communities may be influenced by bottom-up forces (BU) triggered by environmental variations, such as fertilization, that modify plant traits. The impact of these forces on the second trophic level is well-documented (Han et al., 2020; Becker et al., 2021; Han et al 2022). However, their ability to affect the third trophic level is less understood. Plant-mediated bottom-up forces may directly or indirectly, i.e., through herbivores, influence natural enemies.

While fertilizers play a crucial role in providing essential nutrients for crop growth, it is imperative not to overlook their environmental impact. The intensification of fertilization has given rise to new global environmental challenges, including nutrient imbalances (MacDonald et al., 2011; Penuelas et al., 2013; Lu & Tian, 2017), leaching of nutrients from crops leading to groundwater contamination, greenhouse gas emissions and associated impacts (Lee et al., 2020; Weihrauch & Weber, 2020; Romero et al., 2021). Additionally, the escalating cost of fertilizers poses economic challenges for food security (Obersteiner et al., 2013; Van der Velde et al., 2014; Bonilla-Cedrez et al., 2021). Based on these issues, Parr et al., (1990) propose low-input agricultural systems that minimize the demand for off-farm resources, optimize resource-use efficiency, and ensure both optimal agronomic outputs (biomass yield and quality) and potential benefits for ecosystem services. Such systems stand as a crucial pillar in the development of modern, sustainable agricultural practices (Pointereau et al., 2017).

Fertilization is considered a “bottom-up driver” as it influences the first trophic level (“bottom”) by modifying plant tissues macronutrient and defense content. This, in turn, affects the nutritional quality for herbivores and leads to changes in the second trophic level (“up”) concerning herbivore survival, development, or reproduction (Inbar et al., 2001; Hosseini et al. 2018, 2019; Han et al., 2019a, 2019b, 2020, 2022). Correspondingly, decreased N fertilization directly reduces herbivore fitness – the nitrogen limitation hypothesis (White 1983) – but it may have negative impact on plant yield/or quality (Daugherty et al., 2007; Hosseini et al., 2018, 2019).

As the fertilization bottom-up effect extend to the third trophic level natural enemies, bottom-up forces driving by fertilization could also indirectly alter the performance and abundance of natural enemies (arthropod predators and parasitoids) (Karungi et al., 2006; Dong et al., 2018; Hosseini 2018; Han et al. 2019). In contrast, several findings indicate parasitoids and

omnivorous predators are not affected by different nitrogen fertilization levels (Han et al., 2015; Zaller et al., 2009).

By modifying traits of natural enemies, bottom-up effects mediate also the intensity of top-down regulation, i.e., biological control of pests. Excessive nitrogen application to plants can reduce predation/parasitism rates and may result in an increased overall herbivore population load if natural enemies cannot cope with heightened pest population growth (Hosseini et al., 2018, 2019, 2010). Conversely, decreased N fertilization, by reducing herbivore fitness, could enhance predator efficiency (Couture et al., 2010; Aqueel & Leather, 2012; Aqueel et al., 2015). In contrast, several findings indicate that parasitoids and omnivorous predators are not affected by different nitrogen fertilization levels (Zaller et al., 2009; Dong et al. 2018).

In this context, the objective of the PhD was to elucidate how the fertilization may influence the interaction among plant, herbivores and their natural enemies at both individual (life-history trait and biocontrol ability) and population (population dynamics) levels. The thesis comprises two main parts: a literature review on the topic (Chapter 1 and 2) and experimentation research (chapter 3 and 4). In Chapter 1, we delve into the realm of natural enemies, predominantly predators and parasitoids, emphasizing the importance of comprehending their biology, diversity, foraging behavior, and biocontrol agents well. Subsequently, we outline the bottom-up effect on natural enemies, thoroughly describing bottom-up forces and their potential impacts, including examples of herbivorous arthropod pests and their natural enemies, particularly predators and parasitoids. Omnivorous predators could be impacted through bottom-up effects by consuming both plants and prey (Han et al., 2015), while parasitoids or strict predators appear to be primarily impacted through host prey consumption (Chesnais et al., 2016). Identified mechanisms of fertilization effects on the third trophic level include (a) alterations in nutritional prey (host) quality, (b) changes in defensive behaviors exhibited by prey (host), and (c) modifications in natural enemy foraging-feeding patterns (Chesnais et al., 2016; Hosseini et al., 2018; Han et al., 2020; Becker et al., 2021). Consequently, understanding these diverse effects may shed light on the variations observed in bottom-up effects. In Chapter 2, we conduct a comprehensive review of scientific literature exploring the bottom-up effect of fertilization on plant-mediated interactions with various natural enemies. We undertook this meta-analysis as it directly aligns with our research objectives. We amalgamated datasets on a global scale, providing a consolidated overview to facilitate connection with independent research efforts. In chapter 3 and 4, the study aims to explore the bottom-up effects of inorganic fertilizer application across various tri-trophic systems, specifically focusing on the “tomato

plant – herbivorous pest - natural enemies” interaction. The investigation seeks to determine whether diet regime (carnivorous versus omnivorous predators) or functional guild (predator versus parasitoid) modifies the intensity of bottom-up effects on the trophic level. The chapter 3 presents a series of bioassays conducted under laboratory conditions, focusing on key natural enemy life-history traits, including development, longevity, fertility, and their biocontrol ability (mainly parasitism or predation rate). Population dynamic bioassays are carried out under greenhouse conditions, assessing both insect population dynamics and natural enemies' ability to control pests. The final chapter, chapter 5, involves a general discussion of the PhD work and proposes future directions for the current studies.

These studies aim to enhance our understanding of the bottom-up effects of fertilization on “Plant-herbivorous insect-natural enemy” tri-trophic interactions. The insights gained provide a comprehensive understanding for biological control in the agricultural transition (ESR framework), offering options to enhance effective biological control strategies and improve sustainable agricultural systems.

Chapter 1: Bottom-up forces on herbivores and natural-enemies in agro-ecosystems

A trophic cascade is a modification propagating either up or down in a food web with negative and/or positive effects at successive levels (Ripple et al 2016). Ecological forces define consumer–prey interactions, in which a lower trophic level influences the upper one (bottom-up forces “BU”) or in which a higher trophic level influences the lowest one (top-down forces “TD”). These forces are triggered by environmental factors which modify one of the trophic levels (e.g., fertilisation modify the plant nutritional quality and by so, reach herbivores) (Han et al 2022).

In agro-ecosystems, arthropod communities can be largely structured by bottom-up forces driven by various intrinsic (i.e., plant traits in terms of insect resistance or environmental stress tolerance), or abiotic factors (i.e., resource/nutrition availability) (Hunter & Price, 1992). Bottom-up effects are major ecological forces in crop–arthropod pest–natural enemy multitrophic interactions, and notably, they are important in driving crop–arthropod community dynamics. They are triggered by soil and/or plant conditions and first-trophic-level variables can notably influence pest populations through altered multitrophic interactions (Fig. 1: See also Chen et al., 2010; Blundell et al., 2020; Han et al. 2019b).

Extensive theoretical and empirical research has explored the impact of both bottom-up and top-down forces on community structure (Costamagna et al., 2007; Costamagna & Landis, 2006; Denno et al., 2002, 2003; Dyer & Letourneau, 1999, 1999; Polis et al., 2000; Power, 1992; Spiller & Schoener, 1994; White, 1978). However, while top-down forces have been conceptualized for practices in agriculture (e.g., biocontrol services), bottom-up forces have received little attention in the framework of integrated pest management (IPM) (Han et al., 2019a, 2022).

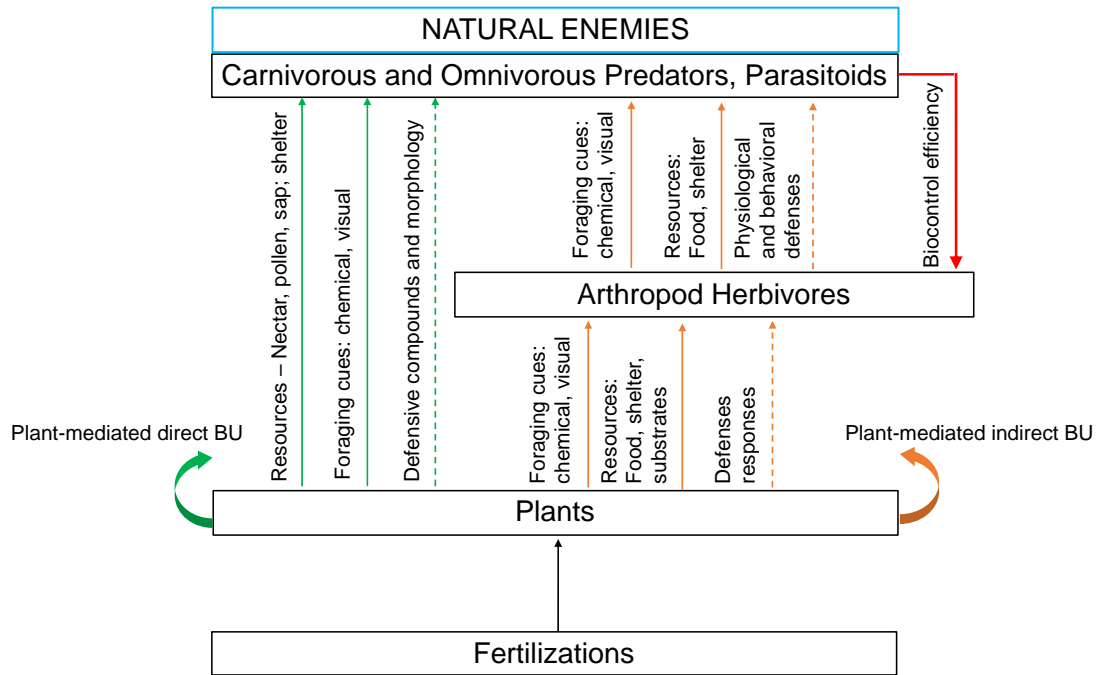


Fig. 1 Schematic representation of tri-trophic effects of nitrogen inputs to plants. Solid lines represent positive effects and dashed lines represent negative effects. The direction of arrow indicates the direction of energy flow (Adapted from Chen et al. 2010).

1.1 Plant-mediated bottom forces on arthropod herbivores, based on fertilization

Among the abiotic factors able to trigger bottom-up forces, fertilization have deserved a major historical focus (Han et al 2022). Fertilization is crucial resources required for optimal plant growth and reproduction (Schoonhoven et al. 2005). Thus, variations in fertilization (excessive or deficient) may induce physiological changes in plant traits, that are linked to alterations their nutritional value and chemical defenses mechanisms, (Inbar et al. 2001; Chen et al., 2010; Gutbrodt et al., 2011), resulting in bottom-up effects, either positive or negative, on arthropod communities, such as herbivores and their natural enemies (**Fig. 1**). The adequacy and appropriateness of resource inputs to plants play a crucial role in shaping plant physiology, which, in turn, has cascading effects on the dynamics of pest populations. Understanding these intricate relationships is essential for developing effective strategies in agriculture and ecosystem management.

Firstly, in plant-herbivorous insect interactions, changes in fertilizer application to plants can impact the survival, development, fecundity, and population dynamics of herbivorous arthropods (Blazhevski et al., 2018; Fallahpour et al., 2015; Han et al., 2019b). The availability of fertilization can influence plant nutritional quality from the herbivore's nutritional perspective through various mechanisms. For instance, fertilization can alter the plant's direct defenses against herbivorous insects by causing qualitative and quantitative changes in defensive compounds, such as digestibility reducers and toxins. The significance of these defenses to the plant is likely to depend on whether the benefits gained from deterring herbivory outweigh the nutritional advantages of increased nitrogen content in the plant (Inbar et al. 2001; Royer et al. 2013; Han et al., 2019b).

Foraging cues, such as chemical and visual signals, play a crucial role in plant-herbivore interactions. When herbivores damage plants, they release a variety of volatile chemical compounds throughout the plant tissue, either around the feeding site or distally and above the injured site. Thus, the plant's investment in toxins and digestibility-reducing agents may depend on the cost of producing of defensive compounds in relation to the metabolic needs of the plants and the action of herbivores and their natural enemies. Fertilization may also affect the plant's indirect defenses, i.e., the efficacy of killing natural enemies of herbivores that attack the plant. Fertilization levels can indeed alter the production and release of these volatiles, depending upon the plant species (Chen et al., 2010). These effects may be expressed via altering quantitatively and/or qualitatively the herbivore-induced plant volatiles that are critical to foraging success of natural enemies. Parasitoids were more attracted with higher N plants and exerted greater control on herbivores on such plants. For example, a parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) of the whitefly *Bemisia argentifolii*, significantly more whiteflies were parasitized per leaf in the higher N treatment than in the lower N treatment (Bentz et al. 1996). Fox et al. (1990) found more parasitoids *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) in the well-fertilized treatment and parasitism rates were lowest under regimes without application of fertilizer, although foliar N level and protein concentration were marginally positively correlated with parasitism rate. Additionally, parasitoids that had emerged from high N treatments were more female-biased which may explain its higher success on high N plants. Therefore, parasitoids and predators may increase their overall fitness developing in higher N hosts due to increased suitability. However, their ability to locate hosts will likely be reduced in higher N plant communities because of reduced

phytochemical cues and greater foraging areas in foliage. The fitness of predators may depend on the quality of the predator's prey, which in turn may depend on the quality of the prey's host. For example, when jumping spiders, *Portia fimbriata* (Araneae: Salticidae), were provided with prey composed of intraguild spiders, they had greater survival, in comparison to those supplied with N-poor phytophagous insects (Li and Jackson 1997). Compared to predaceous stink bugs (*Podisus maculiventris*) reared on caterpillars fed on diets made of mature-leaf powder, their conspecifics reared on caterpillars fed on diets made of new-leaf powder grew faster (Strohmeyer et al. 1998). The higher growth rate of *P. maculiventris* when feeding on caterpillars reared on a young leaf diet was attributed to higher nutrients in the caterpillars, even in the presence of higher amounts of iridoid glycosides, which are known feeding deterrents to generalist herbivores (Strohmeyer et al. 1998).

Plants serve as both food and shelter for arthropod herbivores and natural enemies. For instance, herbivorous insect development has been found positively correlated with increased plant nitrogen concentration, especially among Lepidoptera herbivores, enhancing their ability to feed and oviposit (Inbar et al. 2001; Chen et al., 2008; Han et al 2014).

Both predators and parasitoids feed on pollen, floral and extrafloral nectar, and various fitness correlates of many natural enemies, such as longevity, movement, and fecundity are increased by feeding on these plant foods as supplemental food sources (reviews in Wäckers 2005; Irvin and Hoddle 2007). Plants primarily serve as a source of nitrogenous compounds, such as proteins and amino acids, in pollen. With increasing fertilization levels, this food source becomes more valuable and/or abundant for species that feed upon it. However, the overall effects of fertilization on nectar production vary among plant species. Fertilization changes may also impact the amino acid content of nectar, thereby affecting the quality of herbivores for natural enemies (Mevi-Schütz and Erhardt, 2005). Additionally, nitrogen availability may influence the abundance of nectar by affecting changes in the number of available nectaries (e.g., through changes in flower abundance), potentially leading to either a reduction or an increase in competition for resources among natural enemies.

Furthermore, plants not only provide natural enemies with food but also shelter. Various plant structures, such as leaf domatia and leaf veins, can provide shelter and overwintering sites to various arthropod insects (Agrawal and Karban, 1997). Increased fertilization availability substantially increases density and decreases plant biodiversity, altering the composition of

plant communities at the plant community scale (Suding et al., 2005; Manning et al., 2009; Gough et al., 2000). This could remove necessary refuge habitats for herbivore species, making them more vulnerable to predation. Correspondingly, decreased nitrogen fertilization application directly reduces herbivore fitness, in accordance with the “*Nitrogen Limitation Hypothesis*” (White, 1993).

These effects may be expressed through (1) altering plant structure, which may affect natural enemy foraging efficiency, and (2) altering the quality of plant-associated food and shelter for natural enemies. All of these interactions are depicted in **Fig. 1**.

The lower nutritional quality of host plants may reduce an herbivore’s capacity for **defensive behavior** (Salt 1968; van Driesche and Bellows 1988), which, in turn, may be influenced by nitrogen availability (Chen et al., 2008). Many plant allelochemicals that serve as defensive compounds are sequestered by various herbivorous insects in their hemolymph. Predators and host-feeding parasitoids that feed on these insects, as well as the larval offspring of parasitic wasps that spend part of their life inside such insects, may experience adverse effects in terms of developmental time and survivorship in many cases (Chen et al., 2010). The effects of fertilization on herbivore defense and natural enemies may vary depending on the plant species or the type of allelochemical produced. Conversely, the quantities of many constitutive defensive plant secondary metabolites are negatively correlated with fertilization levels. Consequently, in such cases, predators and parasitoids that consume herbivores grown on host plants with higher fertilization levels may perform better.

The specificity of these effects can vary depending on the type and magnitude of the changes in resource input and the characteristics of the interacting plant - pest species. Additionally, the life-history strategy or the feeding guild in herbivorous insects has been considered another important mediator of the relative strengths of bottom-up and top-down forces (Denno et al., 2002; Becker et al 2021). The feeding guild has a major influence: sap-feeding herbivores primarily interact with compounds in the plant vascular system, while leaf chewers encounter various compounds in leaf tissue (Stam et al., 2014). Bottom-up effects were stronger on sap feeders, and nitrogen effects were stronger on sap feeders. Top-down effects of leaf chewer herbivory partly attenuated bottom-up effects and partly suppressed plant defenses (Becker et al 2021).

Considering the dual influence of fertilizer application, with both positive and negative impacts on both plants and their invertebrate herbivores, it is reasonable to anticipate effects on higher trophic levels (Fig. 1). In the plant–herbivores–natural enemies’ system, these bottom-up forces are mediated by fertilization regime, altering lower trophic levels in plant traits (plant quality or growing status), thereby triggering bottom-up effects on prey-predator or host-parasitoid interactions and consequently influencing the performance of natural enemies, including the efficacy of biocontrol services (Denno et al., 2002; Denno & Fagan, 2003; Pearson, 2010). Nitrogen limitation may also influence prey-predator or parasitoid interactions (Denno & Fagan, 2003), and the nutritional quality of host plants may significantly alter predator consumption or parasitoid parasitism by reorganizing the nitrogen imbalance between prey and predator/parasitoid. As for herbivores, these plant/herbivore mediated bottom-up effects might depend on several features of natural enemies which ask first for a strong knowledge on natural enemies’ biology.

1.2 A diversity of natural enemies

Natural enemies are mainly predators and parasitoids. Predators are upper trophic level consumers that eat lower trophic level consumers, called prey, and are usually fairly active because they have to hunt their prey. Yet, they are involved in a trophic interaction predator-prey. Parasitoids are defined as organisms that spend a significant portion of their feed, grow and larvae develop attached to or within a single host organism and ultimately kill the host (Godfray 1994; Eggleton & Gaston, 1990). Yet, they are involved in a trophic interaction parasite-host.

In agroecosystems, insect predators and parasitoids that attack and feed on other insects, particularly on insect pests of crop plants, are considered natural enemies of pests, also called biocontrol agents. Through this type of feeding, natural enemies contribute to pest population regulation referred to as natural biological control. Depending on the features of the target pests and the method of implementation, three types of pest biological control programs are widely accepted:

(1) Classical biological control: This method relies on the artificial introduction and augmentation of biocontrol agents originating from the area where invasive species originate.

(2) Conservation biological control: This approach involves regulating agro-ecosystems through habitat management, thereby enhancing the efficacy of natural enemies in controlling pests by increasing their survival, adaptability, and behavior.

(3) Augmentative biological control: This type refers to all forms of biological controls in which natural enemies are periodically reintroduced to increase the natural population.

1.2.1 Parasitoid biology, diversity and efficacy as biocontrol agents

Insect parasitoids, their phytophagous hosts, and their host plants compose a significant proportion of the world's biodiversity (Hawkins, 1994). They represent one of the most critical and best-investigated groups because of their vital role in biocontrol as natural enemies of pests.

Among these organisms, we intend to consider the order Hymenoptera because it is by far the most species-rich insect order concerning the number of parasitoids, 68,000 species have been described worldwide, representing up to 20% of all known insect species (LaSalle and Gauld 1991; Godfray 1994; Feener and Brown 1997). Among them, the subfamily Aphidiinae includes more than 400 described species worldwide, all of them parasitizing aphids (Hemiptera: Aphididae; Sanchis et al., 2001; Petrović et al., 2014). Parasitoids are typically more specialized in their host selection than predators (Memmott et al., 2000). Changes in the population density of parasitoids reflect changes in the population of insect hosts which in turn are affected by their host plants (Muller et al., 1999). Their development and physiology directly depend upon the host's vigor and indirectly upon the quality of host plants (Turlings & Benrey, 1998).

1.2.1.1 Parasitoid biology

Insect parasitoids exhibit a distinct life cycle characterized by parasitic larval stages and a free-living adult stage (**Fig. 2**). The importance of their role as natural enemies of insect pests is underscored by their unique reproductive strategy. During the adult stage, some parasitoids should maximize food consumption during larval stage and ultimately favor adult fitness (Harvey et al., 2002). Host-feeding parasitoids feed directly on the host for nourishment (Zhang et al., 2019). The female parasitoid mates and starts to search for hosts deposits an egg, either inside (Endoparasite) or outside (Ectoparasite) its host (**Fig. 3**), typically another insect or

arthropod. Subsequently, the emerging larva develops by consuming the host, ultimately leading to the host's demise.

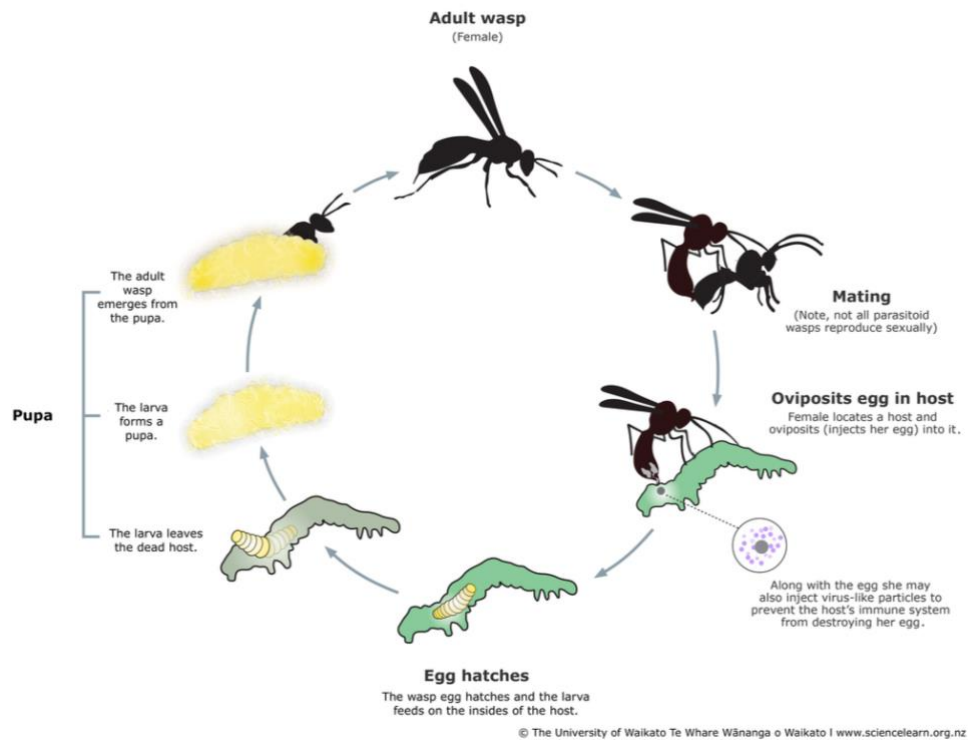


Fig. 2 A generalized diagram of a parasitoid wasp life cycle. Different species will differ in regards to the number of eggs laid and whether they lay eggs on the host or inject a single egg into the host (The University of Waikato Te Whare Wānanga o Waikato).

Parasitism has a great diversity of forms and it has been proposed to categorize parasitoids into two major groups - koinobionts and idiobionts - according to their effects on host development (Askew and Shaw 1986). Koinobionts allow the host to develop whereas idiobionts stop host development and larvae usually feed externally.



Fig. 3 Classification of parasitic wasps

1.2.1.2 The optimal foraging behavior of parasitoids: correlation preference-performance

The parasitism process includes several steps from host finding until successful adult emergence (Desneux et al. 2009a, 2009b). Regarding behavior traits that are relevant in parasitism, the first step is host detection (**Fig. 4**) which involves: habitat location (plants), patch location (plant parts where hosts locate) and host location (individual pests). Parasitoids locate patch and hosts based on physical, visual or chemical cues, both plant and pest. Host-induced plant volatiles are both detectable and dependable (Rehman and Powell 2010). Host specialist parasitoids show specificity to chemical cues from plants and aphids (Vet and Dicke 1992).

The second step is host evaluation, when the female encounters the potential host, evaluates it with antennae and ovipositor probing. The cues for host evaluation could be host color, shape, and texture, chemical and sensory signals (Rehman and Powell 2010). If herbivores are accepted as a host, the female parasitoid will decide to oviposit and deposit an egg at the third step of host acceptance. In the case of Aphelinidae family, the parasitoid will determine to feed or oviposit on hosts. Sometimes aphid movements or defense could trigger the attack of parasitoid (Desneux et al. 2009a, 2009b, **Fig. 4**).

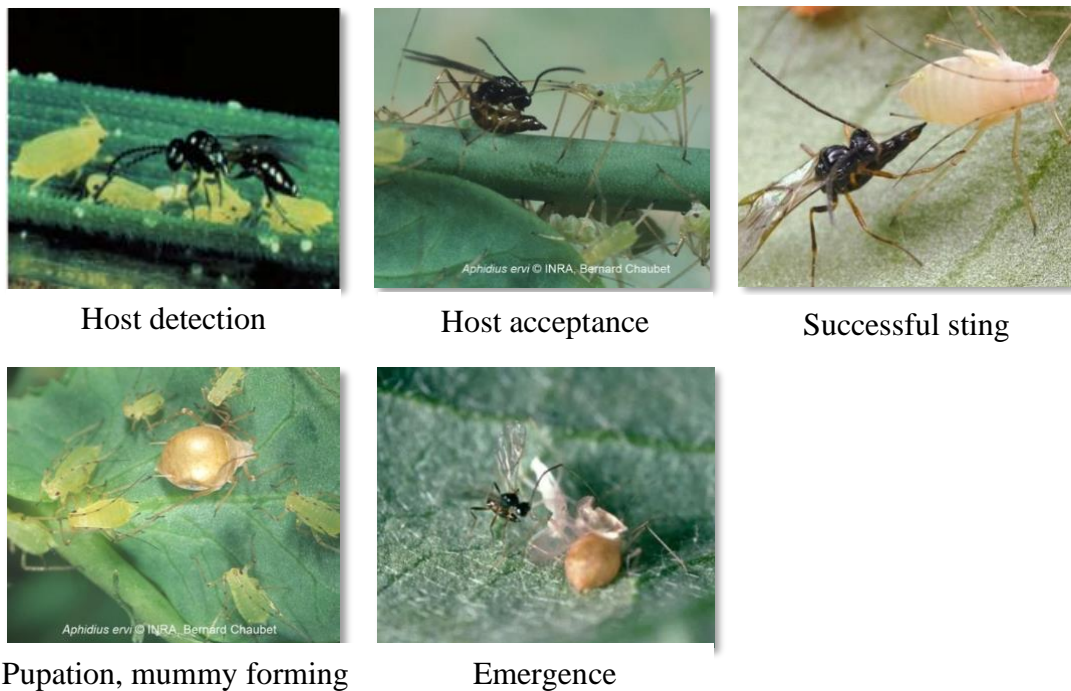


Fig. 4 Host selection process preference (host detection – acceptance – successful sting), and immature parasitoid inside hosts performance (pupation, mummy forming – emergence) (adapted from Desneux et al. 2009).

After parasitoids successfully deposit an egg inside hosts, the suitability of its offspring depends on the capacity of host manipulation (defenses against the host immune system) and regulation (the parasitoid development may affect its host development, behavior, physiology, and biochemistry). Among the proteins present in braconid venom, it is suggested that a GGT play an important role in the success of the parasitoid *A. ervi*: once injected, venom GGT1 can target the reproductive tract of the female aphid host, leading to ovarian degeneration and decreased fertility (Gatti et al., 2021; **Fig. 4**).

The success in the control of a pest is dependent upon parasitoid behavior of host selection i.e., the preference and their host suitability i.e., the performance. Overall, the ‘optimal foraging strategy’ stands as a central paradigm of host selection pattern (Jaenike 1978) and various factors might influence such pattern. For instance, plants are known to indirectly influence the foraging efficiency of parasitoids, as they directly influence host acceptance, suitability and regulation (Tumlinson et al., 1992; Poppy, 1997; Turlings and Benrey, 1998; Monticelli et al

2021). Similarly, parasitoid performance depends mainly on host quality, which in turns is modulated by the host plant and abiotic factors (Vinson, 1990; Godfray, 1994).

1.2.1.3 Specialist vs. generalist parasitoids

When we say that parasitoids are typically more specialized in their host selection than predators (Memmott et al., 2000), what does it mean exactly? The degree of specialization of one species, (as known as the specificity) is evaluated by its niche breadth, as known as or spectrum of resources they can use (Futuyma and Moreno 1988). As ecological niches are multidimensional, i.e., consisting of habitat, diet breadth, etc; the degree of specialization of one species could be different at each dimension. For example, one parasitoid could be habitat specialist and host generalist (Vet and Dicke 1992; Monticelli et al., 2021). The parasitoids in the subfamily Aphidiinae are specialists in parasitizing aphids, laying their eggs exclusively within aphids. Some species target a restricted range of aphid species, and in certain cases, only a single species. The immature parasitoid develops by consuming the aphid (Sidney et al., 2010). However, the concept of specialist and generalist is also relative and context-dependent. Among parasitoids, the one attacks over 20 host species is a generalist in compare to the one attack only one host species (Zikic et al. 2017). Monticelli et al. (2019) demonstrated that the preference-performance relationship exists for specialist parasitoids but not for intermediate specialist-generalists and true generalists. This is likely due to the combined effects of low selectivity and variable performance in generalist parasitoids (van Klinken, 2000). Generalists are less affected by specific aphid defenses against them, such as endosymbionts, whereas they are strongly affected by general defenses used against natural enemies, such as the aphid's ability to sequester toxic compounds. The preference of generalists is not an accurate proxy for the actual realized host range or performance of parasitoids.

1.2.1.4 The interaction of plant - pest-parasitoid in biological control

Parasitoids as biocontrol agents play a crucial role in controlling a diverse range of pest invertebrates (Heimpel & Mills, 2017), influencing key ecological processes like herbivore-plant interactions and predator-prey relationships. Additionally, the high host specificity exhibited by parasitoids is a crucial attribute that enhances the ecological safety and precision of their biocontrol activities. Therefore, the adaptability, specificity, and effectiveness of

parasitoids on pest populations make them essential components of biocontrol programs, contributing to sustainable and environmentally friendly pest management strategies. We considered modifying the foraging environment to increase the biological control efficiency on target pests by manipulating the resource inputs to the plants, such as altering fertilization regimes, may trigger bottom-up effects to parasitic wasps via its host, and thereby alter the top-down control forces provided by parasitoids.

1.2.2 Predator biology, diversity and efficacy as biocontrol agents

Predators play a crucial role in ecological systems by controlling the populations of potential pests, contributing to the overall balance of ecosystems. The most common insect predators are including ladybeetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae), hoverflies (Diptera: Syrphidae), dragonflies and damselflies (Odonata: Coenagrionidae), gall midges (Diptera: Cecidomyiidae), and predatory bugs (Hemiptera: Anthocoridae) (van Hemden, 2007). Other arthropods, such as spiders and predatory mites, are also important predators of insect pests (**Fig. 5**).

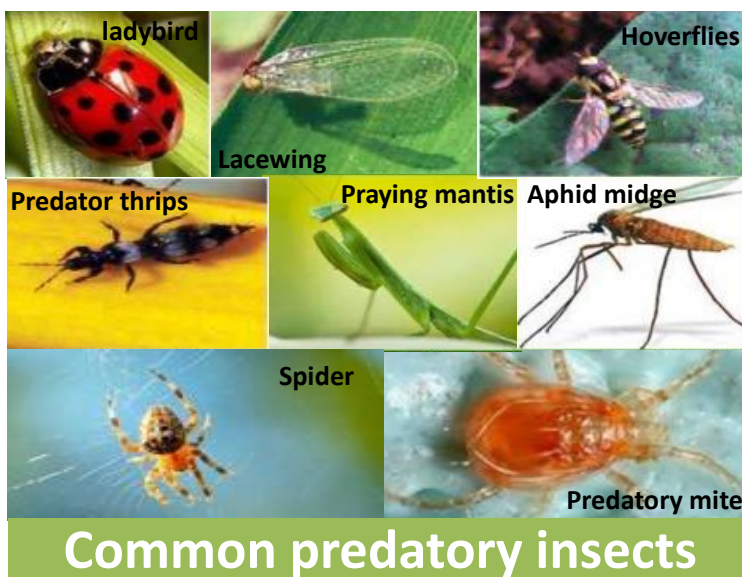


Fig. 5 Common predatory insects found in the agro-systems

1.2.2.1 Predator life cycles

Predator life cycles vary between taxa. For instance, ladybeetles progress through a four-stage (egg, larva, pupa, and adult) life cycle known as complete metamorphosis (**Fig. 6a**). On the opposite, all predatory bugs have 3 life stages - egg, nymph, and adult - where nymphs often resemble adults but are smaller with incompletely developed wings. They generally produce several generations each year (**Fig. 6b**).

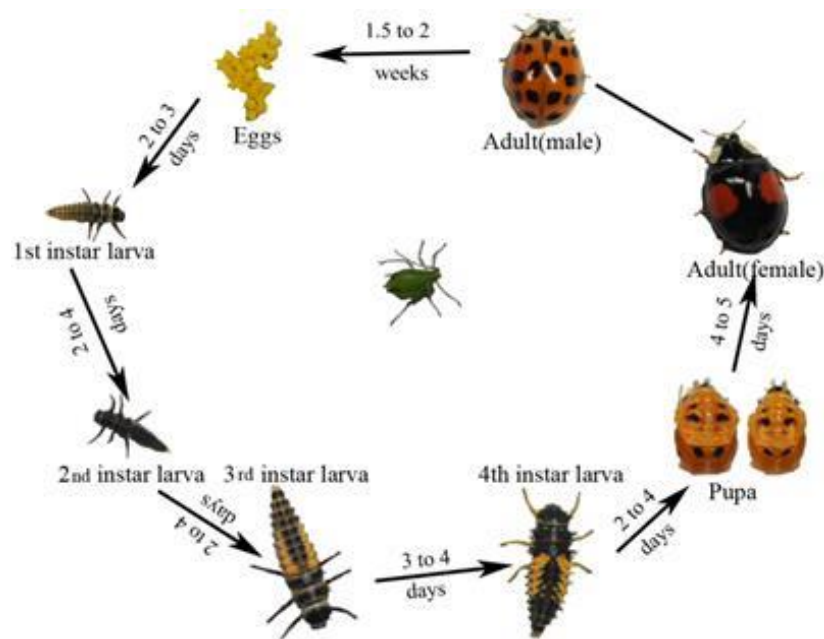


Fig. 6a Life cycle of *Adalia bipunctata*.

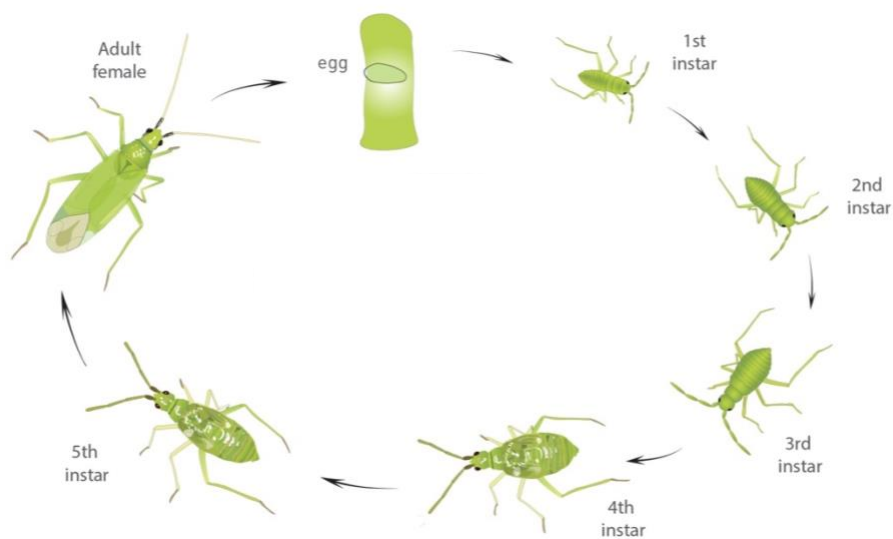


Fig. 6b Life cycle of *Macrolophus pygmaeus*.

1.2.2.2 Specialist vs. generalist predators

Predaceous insects exhibit great variation in their dietary range. Most arthropod predators are **generalist**, which means that they have a wide range of prey and even are polyphagous, feeding also on non-prey items such as plant fluid or pollen (e.g., the bug *Podisus maculiventris* and the ladybeetle, *Coleomegilla maculata*). **Specialist predators** feeding on only one or a few species of prey, such as vedalia beetle, *Rodolia cardinalis*, and the green lacewing, *Chrysopa slossonae*, are highly specific and feed on only one species of prey, whereas the aphid-feeding coccinellids, *Hippodamia convergens* and *Adalia bipunctata*, are stenophagous or oligophagous and restrict feeding to a range of related taxa.

1.2.2.3 Predator feeding behaviour and diet regime -

Carnivorous vs. omnivorous predators

Insect predators are large relative to their prey and require more than one prey individual to complete their development, they have free-living predatory immature stages, and many species of insect predators are predaceous in both immature and adult stages (Doutt, 1964, Hagen et al., 1976). They can be classified according to the life stage of prey they attack (e.g., egg, larval and adult predators). Some predators feed on prey during both their larval and adult stages (e.g., ladybeetles), while others are predaceous only during the larval stage (e.g., lacewings) as adults feed on nectar and pollen. Predators may feed on any or all life stages of their prey, including eggs, larvae (caterpillars, grubs, and maggots), nymphs, pupae, or adults. Predatory bugs feed by piercing their prey with narrow mouthparts and sucking out body fluid. They can be classified according to their foraging strategy (e.g., active searchers, ambush or filter feeders, web or bolas builders).

Predators that exclusively consume other arthropods are called **carnivores**, and herbivores are often their prey. While predators are typically associated with carnivorous behavior, it's important to note that many of these “carnivores” also rely on plant-derived foods for nutrients. The extent to which predators depend on primary consumption varies. Wäckers and van Rijn (2005) distinguish between several categories: ‘life-history omnivores,’ ‘temporal omnivores,’ and ‘permanent omnivores.’ **Life-history omnivores** are natural enemies that strictly depend on plant-derived food during a part of their life cycle, such as hoverflies. **Temporal omnivores** supplement their carnivorous diet at certain life stages, such as host-feeding parasitoids.

Permanent omnivores maintain a varied diet throughout their lifecycle, such as predatory mites and ladybeetles. Temporal or permanent omnivores can use foods like (extra-) floral nectar, pollen or honeydew as an alternative to prey. This diet extension therefore allows them to bridge periods of low prey availability (Limburg and Rosenheim 2001). Life-history omnivores fully depend on non-prey food, usually during their adult stage. For instance, most Hemiptera species are considered as omnivorous predators which need to acquire nutrients and energy from their host plants as well as from insect preys (Janssen et al. 2003). Omnivores that feed on multiple pest species such as whiteflies, aphids, thrips, leaf miners, spider mites and Lepidoptera species. Omnivores may also feed preferentially on a particular type of prey, for instance prey of high mobility, even if it is not the most suitable one (Maselou et al., 2018).

1.2.2.4 Prey-plant feeding balance for omnivorous predators

For omnivorous, it is essential to fully understand the functional relationships between prey and plant feedings. Three hypothetical models, namely the switching, facilitating and independence models were proposed to describe such relationship (**Fig. 7**; Gillespie and McGregor, 2000). In the switching model, plants and preys are alternative foods for predators. Thus, the prey feeding should increase when plant feeding decreases due to low plant quality, resulting in inverse relationships between plant and prey feedings (**Fig. 7a**). In the facilitating model, plants contain key components (nutrients or water) that facilitate prey feeding through digestion or assimilation and thus, the prey feeding rate increases as plant feeding rate increases. In omnivorous Hemipterans, for example, supplementing a plant diet with prey resulted in enhanced fitness (Coll 1998). Some of the enhancement of omnivore fitness may be due to synergistic effect of plant and prey feeding. It was suggested that omnivorous Hemiptera need plant water to produce the saliva required for the extra-oral digestion of preys (Cohen 1995, 1998; Sinia et al 2004). Thus, in the facilitating model, positive relationships between plant and prey feedings are expected (**Fig. 7b**). In the independence model, by definition, there is no relation between prey and plant feedings (**Fig. 7c**).

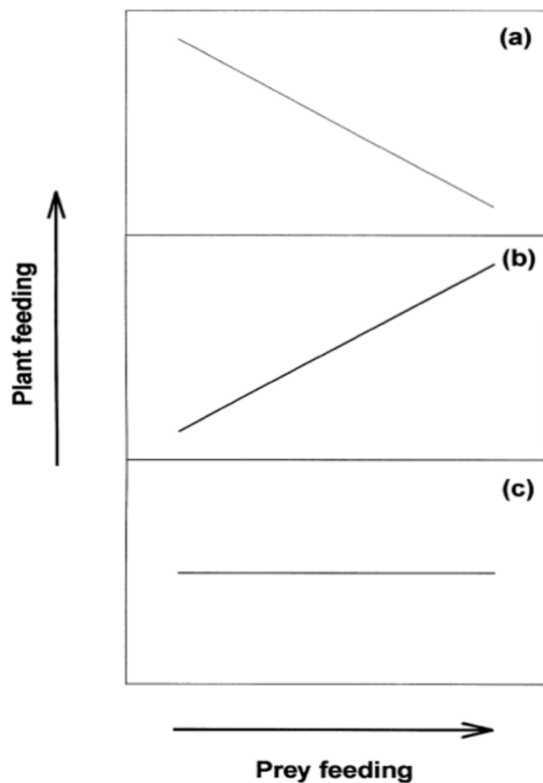


Fig. 7 Three models of feeding behaviour in predatory Hemiptera.

(a) The amount of plant feeding decreases with increased prey feeding. (b) The amount of plant feeding increases with increased prey feeding. (c) The amount of plant feeding is independent of the amount of prey feeding (Gillespie and McGregor, 2000).

In the **Article 1**, we examined the effects of fitness and feeding behavior of the omnivorous *Lygus pratensis* on different types of supplementary prey (*Aphis gossypii* and *Helicoverpa armigera* eggs) on green bean pods *Phaseolus vulgaris* L. (Fabales: Fabaceae) (**Fig. 8**). The study helps understand the feeding strategies of Hemiptera predators on prey types. Adding protein-rich eggs to a plant-based diet has been shown to increase fitness in other predatory bugs (Urbaneja et al., 2005; Jaworski et al., 2015; Maselou et al., 2018). In contrast, the nutritional quality of *A. gossypii* nymphs is lower compared to that of *H. armigera* eggs. We hypothesized that 1) omnivorous Hemipteran predators' fitness might be enhanced by supplementary prey, depending on prey nutritional quality; 2) the feeding behavior of omnivorous depends on the prey type and life stage (juvenile vs. adult). This aimed to help understand the feeding strategies of omnivore predators regarding the nutritional interest of both prey types. We found that the fitness and foraging behaviour of *L. pratensis* varied with the diet provided: *L. pratensis* nymphs were efficient at preying on immobile *H. armigera* eggs, which increased their time spent feeding on bean tissue, while *L. pratensis* adults spent similar time feeding on plant tissue versus prey no matter the prey type. This suggests life-history omnivory, that is *L. pratensis* incorporate more prey content in their diet once they reached adulthood – a phenomenon that was found to increase the stability of food webs. Practically,

the findings from the current study may help optimize the biological control efficiency of omnivorous predators. Practically, the findings from the current study may help optimize biological control efficiency of Hemiptera predators.



Fig. 8 Research model of fitness and feeding behavior of the omnivore *Lygus pratensis* on different types of supplementary prey (*Aphis gossypii* and *Helicoverpa armigera* eggs) and life stage (nymph vs. adult) on green bean pods *Phaseolus vulgaris* L.

1.2.2.5 The interaction of plant-pest-predator in biological control

Predators are commonly used for pest control. The direct impacts of carnivores on prey density or biomass, whether through consumptive or non-consumptive pathways, may indirectly affect lower trophic levels in a trophic cascade (Polis 1999; Shurin et al., 2006). Much of the research on trophic cascades has focused on the beneficial effects of carnivores on plants through the suppression of herbivores, consistent with the ‘green world hypothesis’ (Hairston et al., 1960). Generalist predators, which feed on various species, are undeniably effective against both indigenous and exotic pests (Rosenheim et al. 1993; Snyder and Ives 2003). However, as generalist predators may not be as sensitive to fluctuations in the density of any single prey species, they may not contribute significantly to the control of herbivore populations. Moreover, their tendency to attack not only herbivores but also the specialized natural enemies of

herbivores can disrupt the control of herbivore populations. Consequently, generalist predators may be less efficient in controlling biological control agents (Snyder et al., 2001).

Hemiptera omnivorous predators have been widely used as predators against various insect crop pests in biological control programs (Sinia et al. 2004; Kaplan and Thaler 2011; Chailleux et al., 2013; Thomine et al., 2020). They might put crops at risk, and the frequency of this behavior has been precisely assessed (Naranjo and Gibson 1996; Coll 1998; Kaplan and Thaler 2011; Puentes et al., 2018). Before the release and augmentation of omnivorous predators use in biological control programs, it is necessary to establish to which extent they may damage crops (Castañé et al. 2011). Using endophytic fungi might be an option to counteract crop damages (Garantonakis et al., 2018).

1.3 Aims and objectives – Bottom-up effects on natural enemies

Looking at the diversity of feeding behavior among other, bottom-up forces might not reach natural enemies in a similar way. Natural enemies inhabiting agro-ecosystems such as parasitoids may be largely affected by bottom-up forces because herbivore hosts represent the entire nutritional and physiological environment during immature parasitoid development, and such herbivores are often strongly impacted by bottom-up effects (Han et al. 2014, 2016). Environmental changes inducing bottom-up effects on hosts may, in turn, modulates parasitoid preference (host acceptance) and/or their performance (host suitability) as these are critical determinants of the parasitoid host range (**Fig. 1**; Desneux et al., 2009a; 2009b; 2012; Nguyen et al., 2023). For instance, plant vigor could modulate dispersal of parasitoids presumably through changes in parasitoid preference when selecting hosts (Kher et al. in 2014) and plant characteristics mediated by growing conditions can impact parasitoid ability to attack host aphids (Desneux and Ramirez-Romero 2009). Availability of critical nutrients to plants, e.g., nitrogen, could affect the development of aphid parasitoids as well (Chesnais et al. in 2016, Aqueel et al. 2015) but effects vary in function of host species considered.

Similarly, prey quality (nutritional suitability) is an important aspect of the predator-prey interaction that can have a cascade of effects on the life history parameters of a predator,

including its developmental time, juvenile survival, reproductive rate, and adult longevity, all primary determinants of fitness (Obrycki and Orr, 1990; Michaud, 2000; Michaud, 2005; Schmidt et al., 2012; Jensen et al., 2012). The prey is strongly affected by nutritional, allelochemical, and morphological characteristics of their host plants (see review Whitman 1994). Among the properties of plants that determine their quality for herbivores, nutritional constituents are primary factors. So, we hypothesized that altering the fertilization regimes to plants may trigger bottom-up effects to predators via its prey, and thereby alter the development trials, reproduction, and biocontrol efficacy of the predators.

Omnivorous species, which were similarly like herbivores, are strongly influenced by nutritional, allelochemical, and morphological characteristics of their host plants (Coll & Guershon 2002). In omnivorous Hemipterans, for example, supplementing a plant diet with prey resulted in enhanced fitness (Coll 1998). Similarly, supplementing a plant diet with various prey items had a strong positive effect on the reproduction. Some of the enhancement of omnivore fitness may be due to synergistic effect of plant and prey feeding and bottom-up forces triggered by fertilization might modify such effect.

In our study, we will address these hypotheses and tested whether inorganic fertilizer application might trigger or not bottom-up forces reaching natural enemies and we will compare such effects on various natural enemy types following a bibliographic approach (chapter 2) and an experimental one with both laboratory-controlled conditions (chapter 3) and a population dynamic approach in greenhouse conditions (chapter 4). We will comprehensively describe the biology and unique characteristics of parasitoids and predators as biological control agents. Understanding their distinctions is crucial, as it provides insights into why they respond differently to fertilization. This knowledge is fundamental for determining how fertilization might affect them divergently. This research aims to contribute to the scientific understanding of fertilizer regimes' impacts on plant-pest-natural enemy interactions across different scales. By doing so, it will provide valuable insights for making informed decisions regarding fertilizer strategies in agricultural settings, particularly within the context of integrated pest management (IPM) strategies.

The objectives of the research outlined in this thesis were as follows:

- 1) A quantitative review of the existing literature regarding the impacts of plant mediates on arthropod crop pests and natural enemies influenced by various fertilizers factors (chapter2).

- 2) To determine if fertilizer application modifies the intensity of bottom-up effects to reach the natural enemy on the trophic level between diet regime (carnivorous versus omnivorous predators) or functional guild (predator versus parasitoid) under laboratory conditions (chapter 3).
- 3) Explore the interaction between fertilization levels and natural enemies' type to record the population dynamic of both pest and natural enemies in greenhouse mesocosms (chapter 4).

Article 1

Life stage affects prey use with fitness consequences in a phytophagous mirid bug

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Life stage affects prey use with fitness consequences in a zoophytophagous mirid bug

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Abstract Omnivorous insects make foraging decisions between plant and prey resources depending on their accessibility, availability, and nutritional value. This shapes the stability and complexity of food webs, but also pest control services in agroecosystems. The mirid bug *Lygus pratensis* is a common cotton pest in China, but it also feeds on a variety of prey species. However, little is known about how different types of available resources affect its fitness and foraging behaviour. In laboratory experiments, we measured the fitness (survival, longevity and fecundity) of *L. pratensis* fed with bean pod only, bean and *Aphis gossypii* nymphs, or bean and *Helicoverpa armigera* eggs, and we also conducted focal observations of its foraging behaviour when provided the latter two. Adding *H. armigera* to its diet increased its fitness (both survival and fecundity), while adding

A. gossypii was marginally detrimental. The different diets did not affect the time spent walking (searching for food resources) or preying, but significantly affected the time spent sapping bean tissue depending on *L. pratensis* life stage. Nymphs spent more time sapping plant when provided with *H. armigera* than *A. gossypii* (possibly through higher efficiency of handling prey). In addition, adults spent less time sapping plant than did nymphs (possibly through good efficiency of handling *A. gossypii* mobile prey and due to higher protein requirements). This special case of life-history omnivory highlights the complexity of natural food webs, where a major crop pest at the juvenile stage may become a biological control agent at the adult stage.

Keywords Activity budget · Foraging behaviour · Mixed diet · Omnivory · Phytophagy

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Introduction

Omnivores are species that can feed on two or more trophic levels, e.g. omnivorous insects may prey on other insects and feed on plants (Jaworski et al., 2013; Pappas et al., 2018; Pérez-Hedo et al., 2021). Foraging for prey or feeding on the plant is largely context-dependent, depending on the availability and type of resources, and the life stage of the omnivore itself (Coll & Guershon, 2002; Han et al., 2020). Such a foraging pattern is common in insects, and is partly responsible for the observed complexity and high connectedness of plant–insect food webs (Han et al., 2015a, 2020; Sinia et al., 2004; Thompson et al., 2007).

Miridae are one of the major families of omnivorous insects (Thompson et al., 2007). For optimal fitness, such omnivorous predators acquire complementary nutrients and energy from host plants and animals, which greatly differ in nutritional value and chemical composition (Eubanks & Denno, 2000; Coll & Guershon, 2002; Lundgren et al., 2009; Desneux & O’Neil, 2008; Ren et al., 2022). Mirid bugs are widely used as biocontrol agents against various insect crop pests including whiteflies, thrips, aphids, and Lepidoptera (Chailleux et al., 2013; Jaworski et al., 2015; Thomine et al., 2020). For instance, *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) has been widely used as a biocontrol agent for the management of whiteflies and lepidopteran pests in greenhouses in Europe (Chailleux et al., 2020; Han et al., 2015a, b; Han et al., 2019). The high availability of plant material in a habitat also conditions the switching from prey to plant feeding, perhaps irrespective of prey density (Vankosky & Vanlaerhoven, 2015). However, most Miridae species are predatory throughout their life stage no matter whether plant food is available (Kaplan & Thaler, 2011).

Lygus pratensis (L.) (Hemiptera: Miridae) is a common pest species in cotton crops in Xinjiang, north-west China (Lu & Wu, 2011; Lu et al., 2008, 2010). It also infests many other crops including alfalfa, Chinese date, grape, and pear (Yang et al., 2004). It is a sap-sucking insect and both juveniles and adults can extract nutrients from plants by attacking various plant tender parts, causing plant stunting, abscission of squares and bolls (in cotton), and fruit malformation resulting in significant quality and yield losses

(Wang, 1996). However, it can also be beneficial to crops due to its pest control capacity. It attacks other insect pests, such as eggs of the cotton bollworm *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), and cotton aphids *Aphis gossypii* Glover (Hemiptera: Aphididae), the two main cotton pests in China (Wu et al., 2005; Yao et al., 2016; Zhang et al., 2021; Lu et al., 2022). A better understanding of the feeding behaviour of *L. pratensis* is necessary to assess its potential as biocontrol agent versus as a crop pest in agroecosystems.

One important characteristic for predators to select prey is prey mobility (Maselou et al., 2018). As primary insect pests in cotton, *H. armigera* and *A. gossypii* (Lu et al., 2012, 2022) are preyed upon by zoophytophagous mirid bugs including *L. pratensis* (Alvarado et al., 1997; Li et al., 2020). While *H. armigera* eggs are immobile, *A. gossypii* nymphs are mobile and able to defend. Besides, the nutritional value of *H. armigera* eggs may be higher than that of *A. gossypii* nymphs (higher lipid and protein content), and this could increase the preference and fitness of *L. pratensis* to feed on *H. armigera* eggs. Indeed, many studies have shown that Lepidopteran eggs are high in lipids and proteins and may thus best satisfy nutritional needs: generalist insect predators that fed on Lepidopteran eggs had higher survival, a shorter development time, and a higher fecundity than those fed on other prey species (Lumbierres et al., 2021; Ren et al., 2022; Siddique & Chapman, 1987). Here, we tested two hypotheses: (i) *L. pratensis* feeding on *H. armigera* eggs rather than *A. gossypii* nymphs may increase *L. pratensis* fitness; (ii) the feeding behaviour (predominantly plant feeding versus prey feeding) of *L. pratensis* depends on life stage (juvenile versus adult) and on the prey type.

Materials and methods

Study organisms

Lygus pratensis was originally collected from fields of alfalfa *Medicago sativa* (Fabales: Fabaceae) by sweep-netting in Shihezi (44°27’N, 85°94’E), Xinjiang Uyghur Autonomous Region of China, in August 2017. The species was identified following

procedures used in previous studies (Liang et al., 2013; Lu et al., 2008). It was reared in plastic rearing containers (20×13×8 cm) under controlled laboratory conditions (25±2 °C, 60±5% RH and 16L: 8D). The food resources provided were green bean pods *Phaseolus vulgaris* L. (Fabales: Fabaceae) commercially available in Shihezi, Xinjiang, China, and a 10% sucrose solution. Green bean pods were used as oviposition substrate and were renewed every other day. Bean pods with eggs were moved to individual Petri dishes, lined with filter paper, and kept in the incubator under the same climatic conditions. *Lygus pratensis* individuals of the first generation were used for the experiment. Aphids *A. gossypii* were collected from a cotton field in Shihezi, Xinjiang, China, while *H. armigera* adults were collected by light traps on the campus of Shihezi University. Both were reared on cotton seedlings (*Gossypium hirsutum* L.) in separate cages under the same climatic conditions as above.

Fitness traits of *L. pratensis*

We measured the effects of a mixed diet on *L. pratensis* fitness traits, including survival, longevity and fecundity. The methodology was similar to that described by Lu et al. (2008). *Lygus pratensis* individual females were held for 24 h in rearing boxes for oviposition (with fresh green beans as the oviposition substrate). Newly-hatched *L. pratensis* nymphs (within 12 h) were placed in microcosms made of a Petri dish covered by an upside-down plastic cup with ventilation on the top (Jaworski et al., 2013). The Petri dish was lined up with absorbent cotton, and a bean pod was provided on a pin through the cup wall. The food treatment was either: (a) one fresh bean pod only; (b) one fresh bean pod and 50 *A. gossypii* nymphs; or (c) one fresh bean pod and 50 *H. armigera* eggs. The exact number of prey was deposited in the microcosm using a fine brush to not damage them. 70 replicates of each treatment were prepared. Bean pods (mass~3 g; length~4.5 cm) were previously soaked in a 0.5% sodium hypochlorite solution for 10 min to remove any pesticide residue and then rinsed with water and dried with absorbent paper. Bean pods and prey were replaced every day to ensure

enough fresh food was available for the development of *L. pratensis*. Each *L. pratensis* nymph was checked daily to record emergence of the next instar, until they reached adulthood or died. The emerged adults were sexed and paired (female: male ratio 1: 1) before being placed in the same microcosm (N=30). After mating, the females lay eggs in the oblique section of the bean pod, and eggs can be easily observed under the microscope. Laid eggs were counted every day until the female died to calculate fecundity. Adult longevity was calculated as the total number of days before death occurred.

Foraging behaviour of *L. pratensis*

Focal observations of the foraging behaviour of *L. pratensis* were performed under the same laboratory conditions as above. Foraging behaviour is the process by which an animal searches for and feeds on food. It involves a series of activities including orientation, prey/host plant location, and prey handling/plant consumption (Schone, 2014). We used five behavioural categories (Rosenheim et al., 2004): (1) resting (insect staying still); (2) grooming (grooming antenna, stylet or wings with forefoot); (3) walking (moving but no contact of mouthparts with plant or prey); (4) sapping plant (inserting stylet into plant material for more than 5 s and with the head moving up and down); (5) preying (prey probing: stylet in contact with prey, or prey feeding: stylet inserted into prey for more than 5 s). Behaviours 3–5 are part of the foraging activity. After being starved for 24 h and for each diet treatment, 25 nymphs and 20 virgin adults including 10 females and 10 males (sex ratio 1:1) were individually placed in a Petri dish (diameter: 7.0 cm). The diet treatments were: (a) a fresh bean pod and 50 3rd-4th *A. gossypii* nymphs or (b) a fresh bean pod and 50 *H. armigera* eggs. Each individual was observed continuously for 20 min under a stereomicroscope (Nikon, at 3.0× magnification with a 10× ocular lens) and the time spent on each of the five behaviours was recorded. All focal observations were conducted by the same observer and between 10:00 and 20:00 during daylight hours (Rosenheim et al., 2004). For each individual, the total time spent on each behaviour was calculated.

Statistical analyses

All analyses were performed using the R software (R Core Team, 2022). When using linear models and generalised linear models (GLMs), the absence of residual heteroscedasticity and overdispersion was verified in the best model using the functions ‘simulateResiduals()’ and ‘testDispersion’ (R package ‘DHARMA’; Hartig, 2022). If fixed effects were significant, biologically relevant comparisons of means between groups were performed with a Tukey test for linear models with a single fixed effect (function ‘TukeyHSD’, R package ‘stats’; R Core Team, 2022) and otherwise with the ‘emmeans’ function (R package ‘emmeans’; Lenth, 2022).

Survival rate as a function of diet was analysed using a Cox proportional hazards regression model with diet as fixed effect on (i) the whole life span, and (ii) juvenile stage (survival until adult emergence; function ‘coxph’, R package ‘survival’; Therneau, 2022), and using the ‘relevel’ function (R package ‘stat’, R Core Team, 2022) to compare pairs of treatments. Survival curves were created with the ‘survfit’ function (R package ‘survival’) to model the fit, and the ‘ggsurvplot’ function to plot the fit (R package ‘survminer’; Kassambara et al., 2021). We also assessed how diet affected the proportion of nymphs reaching adulthood using a GLM with diet as fixed effect and a binomial distribution (function ‘glm’, R package ‘stats’; R Core Team, 2022). Then, we assessed how diet affected the longevity of adults and the fecundity of females using linear models and an ANOVA with diet as fixed effect.

We assessed the effect of diet and life stage (nymphs vs. adults) and the interaction between these two factors on the foraging activity budget time (spent walking, sapping plant or preying) of *L. pratensis* using independent regressions and adjusting *P*-values *a posteriori* (Huang, 2020) with the Benjamini and Hochberg (1995) correction to account for data non-independence (function ‘p.adjust’, R package ‘stats’; R Core Team, 2022). We used a linear model for sapping plant and GLMs with a negative binomial error distribution for the other two activities to account for data overdispersion (function ‘glm.nb’, R package ‘MASS’; Venables & Ripley, 2002). The significance of fixed effects for each test was estimated through a stepwise regressive type-II model comparison with an ANOVA.

Results

Effects of diet on fitness: Survival, longevity, and fecundity

The survival rate of *L. pratensis* over their entire life span was affected by their diet, although survival on a bean + *A. gossypii* diet was only marginally lower than survival on a bean + *H. armigera* eggs diet (Table 1; Fig. 1). However, differences were stronger during juvenile development (until day 18–25). Survival was marginally higher on a bean-only diet than a bean + *A. gossypii* diet, but nymph survival was significantly higher on a bean + *H. armigera* eggs diet than on the two other

Table 1 Effect of diet on survival rate as a function of life span (adult vs. juvenile). Significant effects after *P*-value correction for multiple testing are shown in bold, and if they are significant, comparisons of means between groups are shown

AG: bean + *A. gossypii* diet, HA: bean + *H. armigera* eggs diet
: *P* < 0.01; *: *P* < 0.001

Fixed effect: diet	LR	d.f	<i>P</i>	Adjusted <i>P</i>
Entire life span	11.4	2	0.0033 **	0.0033 **
Juvenile life stage	30.0	2	<0.001 ***	<0.001 ***
Comparisons of survival between food diets		Coefficient ± SE	<i>P</i>	Adjusted <i>P</i>
<i>Entire life span</i>				
bean vs. bean + AG	0.213 ± 0.127		0.095	0.095
bean vs. bean + HA	-0.290 ± 0.145		0.045 *	0.089
bean + AG vs. bean + HA	-0.363 ± 0.150		0.015 *	0.062
<i>Juvenile life stage</i>				
bean vs. bean + AG	0.615 ± 0.160		<0.001 ***	<0.001 ***
bean vs. bean + HA	-0.513 ± 0.230		0.026 *	0.077
bean + AG vs. bean + HA	-1.128 ± 0.230		<0.001 ***	<0.001 ***

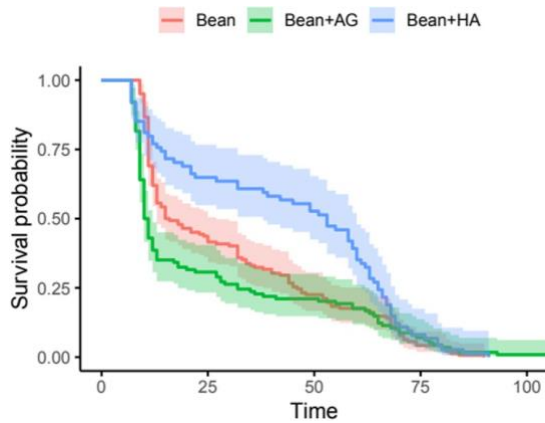


Fig. 1 Survival rate of *L. pratensis* through time reared on three different diet ('Bean': bean pod only; 'Bean + AG': bean pod + *A. gossypii*; 'Bean + HA': bean pod + *H. armigera* eggs). Shaded areas show the 95% confidence intervals

diets (Dev = 23.26, df = 2, $P < 0.001$; Fig. 2A). Significantly more nymphs reached the adult stage when reared on a bean + *H. armigera* eggs diet than on a bean-only diet or a bean + *A. gossypii* diet (Table 2). However, the diet did not significantly affect the longevity of adults ($F_{2,143} = 2.39$, $P = 0.095$; Fig. 2B). Finally, female fecundity

was significantly affected by diet ($F_{2,97} = 9.10$, $P < 0.001$; Table 2; Fig. 2C), and was twice and 1.4 times as high on a bean + *H. armigera* eggs diet as compared to a bean-only diet and a bean + *A. gossypii* diet, respectively.

Foraging behaviour

The time spent walking and preying was not significantly affected by the diet nor life stage and neither by the interaction between the two (Table 3; Fig. 3). However, the diet in interaction with life stage significantly affected the time spent sapping plant: the time spent sapping plant by nymphs was longer when provided with a bean + *H. armigera* eggs diet than compared to a bean + *A. gossypii* diet (mean \pm SE: nymphs, bean + *H. armigera*: 820 ± 70 ; nymphs, bean + *A. gossypii*: 544 ± 90 ; adults, bean + *H. armigera*: 585 ± 81 ; adults, bean + *A. gossypii*: 738 ± 69).

Discussion

Despite their omnivorous behaviour, many mirid bug species have a pest status, but the associated crop damage may depend on the lifestage.

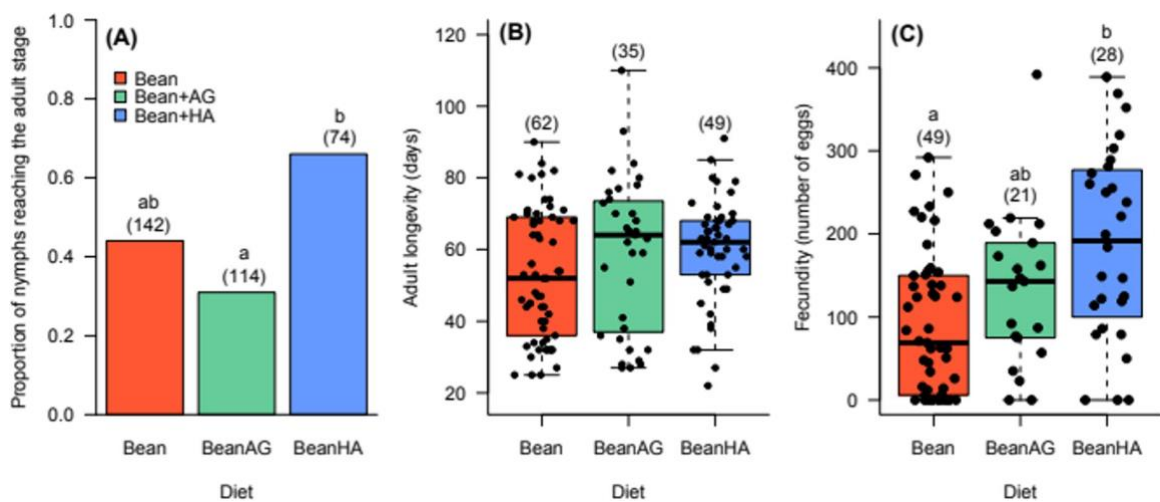


Fig. 2 Proportion of nymphs reaching adult stage (A); Adult longevity (boxplot; B); and female fecundity (boxplot; C) as a function of diet. 'Bean': bean-only diet; 'Bean + AG': bean + *A. gossypii* diet; 'Bean + HA': bean + *H. armigera* diet. Numbers in parentheses show sampling sizes for each group.

Significant differences between diets are shown with different letters above bars (mean comparisons; Table 2). The scale in (B) starts from the earliest observed emergence time of adults (day 18)

Table 2 Comparison of means between diets for the proportion of nymphs reaching the adult stage (emmeans test) and for female fecundity (Tukey test). Significant differences are shown in bold

emmeans test	estimate ± SE (on log scale)	P
<i>Proportion of nymphs reaching the adult stage</i>		
bean + AG – bean	-0.559 ± 0.264	0.087
bean + HA – bean	0.928 ± 0.298	0.0053 **
bean + HA – bean + AG	1.487 ± 0.319	< 0.001 ***
Tukey test		
Difference [95CI]		
<i>Fecundity</i>		
bean + AG – bean	41.0 [-30.0; 112]	0.22
bean + HA – bean	95.6 [29.8; 161]	< 0.001 ***
bean + HA – bean + AG	54.6 [-16.4; 126]	0.13

** : $P < 0.01$; *** : $P < 0.001$

In this study we investigated how the presence of prey affected the foraging behaviour and fitness of the omnivorous but mostly phytophagous mirid bug *L. pratensis*. We found that feeding on a bean + *H. armigera* eggs diet increased fitness (higher survival and fecundity), while a bean + *A. gossypii* diet had marginally detrimental effects, compared to a bean-only diet. We also found that the foraging behaviour was altered by diet: nymphs spent more time sapping plant on a bean + *H. armigera* diet compared to a bean + *A. gossypii* diet, and they also spent more time than adults sapping plants on a bean + *H. armigera* diet.

Supplementing *H. armigera* eggs in addition to green bean pods enhanced *L. pratensis* survival and

fecundity (but not longevity). Adding protein-rich eggs to a plant-based diet has been shown to increase fitness in other predatory bugs (Jaworski et al., 2015; Maselou et al., 2018; Ren et al., 2022; Siddique & Chapman, 1987; Urbaneja et al., 2005). This is evidence that omnivorous mirid bugs may need prey as part of their diet to achieve optimal reproduction (Han et al., 2015a). Here, we observed that *L. pratensis* was attracted to and very often preyed on *H. armigera* eggs when provided in food mixtures.

In contrast, the supplement of *A. gossypii* aphids to a bean diet had marginally detrimental effects on *L. pratensis* fitness. A lower fitness on a bean + *A. gossypii* diet compared to a bean + *H. armigera* diet could be due to the lower nutritional quality of *A. gossypii* nymphs compared *H. armigera* eggs, but this does not explain why

Table 3 Effect of diet and life stage on time spent on each foraging behaviour: walking, sapping plant or preying (independent GLMMs). Significant effects after *P*-value correction for multiple testing are shown in bold, and if they are significant, comparisons of means between groups are shown

Fixed effect	LR	d.f	P	Adjusted P
<i>Walking</i>				
Diet*life stage	2.41	1	0.12	0.12
Diet	1.08	1	0.30	
Life stage	0.274	1	0.60	
<i>Sapping plant</i>				
Diet*life stage	7.67	1	0.0067 **	0.020 *
<i>Preying</i>				
Diet*life stage	3.03	1	0.082	0.12
Diet	0.00218	1	0.96	
Life stage	0.130	1	0.72	
Mean comparisons between groups				
<i>Sapping plant (s)</i>				
Adults: AG – HA	153 ± 107	0.16		
Nymphs: AG – HA	-276 ± 112	0.016 *		
HA: adults – nymphs	194 ± 109	0.079		
AG: adults – nymphs	-235 ± 110	0.035 *		

AG bean and *A. gossypii* diet, HA bean and *H. armigera* eggs diet
* : $P < 0.05$; ** : $P < 0.01$

supplementing *A. gossypii* was marginally detrimental to *L. pratensis*. One reason could be the difficulty to attack prey, leading on significant time and energy loss and therefore poorer fitness, as most predators select food resources based simultaneously on availability, accessibility (e.g., prey size), and nutritional quality (Woodward & Hildrew, 2002). Third and fourth instar aphid nymphs were used in the experiments and their individual sizes were larger than those of *L. pratensis* juveniles. In addition, aphids show various mechanisms to defend themselves against predatory attacks (e.g. see Desneux et al., 2009; Luo et al., 2022). Rosenheim et al. (2004) observed that most often, *Lygus hesperus* ignored aphid prey or retreated upon contact. By contrast, *L. pratensis* nymphs provided with bean+*H. armigera* eggs could have better optimized their foraging activity by quickly feeding on *H. armigera* eggs, allowing more time sapping bean tissue.

Conversely to *L. pratensis* nymphs, adults spent a similar time sapping bean tissue when *H. armigera* eggs were provided compared to when *A. gossypii* nymphs were provided. This could be because they were more efficient at attacking *A. gossypii* than nymphs were, and therefore could have been able to feed on prey and plant tissue at equivalent rates no matter what prey type was provided. It is likely that handling *H. armigera* eggs was as easy for *L. pratensis* nymphs as for adults since egg prey are immobile. Also in average, *L. pratensis* adults spent less time

sapping bean tissue than did *L. pratensis* nymphs. This was in part compensated (although not significantly) by spending more time preying. This may be related to a higher protein requirement by the time of reaching sexual maturity, especially under a sub-optimal diet during the juvenile stage (Barrett et al., 2009). Bean tissue-mediated indirect interactions between *L. pratensis* and *A. gossypii*, both feeding on bean tissue, were likely minor in explaining *L. pratensis* behavioural changes here, since plant defences in in vitro plant parts are lower (Heil & Ton, 2008).

In conclusion, the fitness and foraging behaviour of *L. pratensis* varied with the diet provided: *L. pratensis* nymphs were efficient at preying on immobile *H. armigera* eggs, which increased their time spent feeding on bean tissue, while *L. pratensis* adults spent similar time feeding on plant tissue versus prey no matter the prey type. This suggests life-history omnivory, that is *L. pratensis* incorporate more prey content in their diet once they reached adulthood – a phenomenon that was found to increase the stability of food webs (Kratina et al., 2012). With regards to the pest status of *L. pratensis*, our study suggests that mostly juveniles are crop pests since they spend more time than adults feeding on plant tissue. While adults still considerably feed on plant tissue, they may also reduce plant damage by preying on alternative herbivorous pest species.

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Authors' contributions Pei-ling Wang and Ruo-han Ma secured the funding; Pei-ling Wang, Peng Han and Nicolas Desneux designed the study; Ruo-han Ma, Jia-min Gu, Zheng-xuan Xue and Xue-ling Li acquired the data; Ruo-han Ma and Coline C. Jaworski performed the data analysis; Ruo-han Ma, Coline C. Jaworski, Peng Han and Nicolas Desneux wrote the manuscript. All authors agreed to the publication.

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Declarations

Competing interests The authors declare no competing interests.

Conflicts of interests All authors declared to have no conflicts of interests.

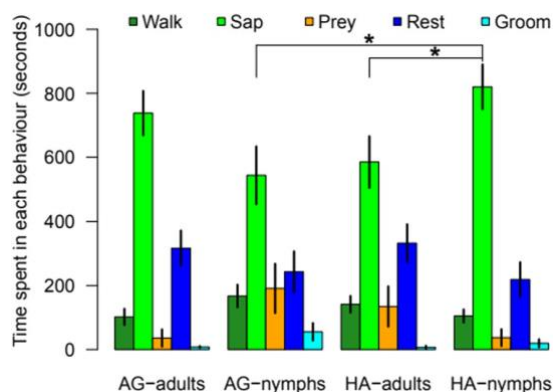


Fig. 3 Mean (\pm SE) time spent on five different behaviours of *L. pratensis* nymphs and adults feeding on bean and *A. gossypii* (AG) or bean and *H. armigera* eggs (HA) for 20 min. ‘Walk’: walking; ‘Sap’: sapping plant; ‘Prey’, preying; ‘Rest’: resting; ‘Groom’: grooming. Significant differences between treatments for the three foraging behaviours (walking, sapping plant or preying) are shown with ‘*’ (see Table 1)

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Chapter 2: Literature review on fertilizers and natural enemies: a meta-analysis

In the previous chapter, we explored the realm of natural enemies, predominantly predators and parasitoids, emphasizing the importance of comprehending their biology, diversity, foraging behavior, and role as biocontrol agents well. Subsequently, we discovered that bottom-up forces (BU) induced by environmental variations, such as fertilization, can indeed reach the third trophic level by modifying plant traits.

Inorganic fertilization plays a crucial role in agriculture, as vast amounts are used to boost crop yields. However, a significant portion of this fertilizer is leached into the surrounding environment, posing risks to natural ecosystems (Erisman et al., 2008). Therefore, it is essential to fully understand the effects of fertilizers on ecosystems at all levels to ensure their safe and effective use. The concept of reducing fertilizer application and transitioning toward low-input sustainable agricultural systems has gained momentum (Parr et al., 1990), highlighting the importance of better understanding their effects on pests and natural enemies associated with crop plants to ensure sustainability and widespread adoption.

While the response of plants to fertilizers has been extensively characterized and understood, the impact of fertilizers on terrestrial insect populations remains a subject of intense debate and ongoing research. A quantitative review of the existing literature regarding the impacts of plant mediates on arthropod crop pests and natural enemies influenced by various fertilizers factors. Inorganic fertilisers, particularly nitrogen, have been found to increase the occurrence of pests, particularly sap feeding insects (Hasken and Poehling, 1995, Bethke et al., 1998, Bi et al., 2001). This is due in part to an increased availability of nitrogen, aiding protein production and insect development (Awmack and Leather, 2002). Fertilizer effects on host plant morphological characteristics such as increased leaf growth have also been shown to influence pest infestation (Helenius, 1990, Honek, 1991). Despite numerous studies attempting to elucidate these effects, a comprehensive understanding of how fertilizers influence insect communities, their dynamics, and interactions within terrestrial ecosystems is still in its nascent stages. As such, further research is warranted to unravel the

complexities of this relationship and inform more nuanced and ecologically agricultural practices.

The previous meta-analysis studies on the impact of the synthetic fertilizer on insect population dynamics present in different ecosystems (Butler et al., 2012) or comparing organic and conventional fertilizer effects on natural enemies (Garratt et al., 2011) involved datasets with a low number of studies and collected only few traits on natural enemies (Garratt et al., 2011; Butler et al., 2012). In the context of this thesis, our comprehensive meta-analysis aimed to elucidate the intricate interplay between environmental factors of fertilization practices, with a focus on natural enemy predators and parasitoids. We also considered the diet regime of predators, which are omnivorous and carnivorous. Omnivorous predators could be impacted through bottom-up effects by consuming both plants and prey (Han et al., 2015), while parasitoids or strict predators appear to be primarily impacted through host prey consumption (Chesnais et al., 2016). Our study addresses these hypotheses and tested whether inorganic fertilizer application might trigger or not bottom-up forces reaching tritrophic level.

In our meta-analysis, we examined the contrasting effects of four levels (low, medium, high, and excess) of full NPK with only N-modification fertilization solution on key life history traits of natural enemies (development, survival, abundance, reproduction, biocontrol, and survival), at both individual (lab, climate chamber) and population (greenhouse, field, forest) level. We included the main taxonomic orders of parasitoids (Hymenoptera and parasitoid mixture) and predators (Chrysopidae, Coleoptera, Diptera, Odonata, and predator mites). Our findings revealed that fertilization triggered a tri-trophic nutritional cascade affecting natural enemy fitness, with both types of fertilizers influencing natural enemies' development, survival, abundance, and reproduction positively. The bottom-up effects of fertilization varied across natural enemy functional guilds, with parasitoids, though less impacted than predators, still exhibit sensitivity to fertilization levels. Additionally, the diet regime of predators influences the intensity of bottom-up effects, with carnivorous predators showing less impact compared to

omnivorous predators. Despite the overall positive influence of higher fertilizer levels on natural enemies' development and reproduction, there was a negative impact on biocontrol efficacy. Importantly, our study underscored the significance of study conditions, with large-scale field experiments showing potential buffering effects of nitrogen fertilizers. Additionally, diverse taxonomic orders of natural enemies respond differently to fertilizers, with Hymenoptera and Hemiptera exhibiting significant positive responses.

In the literature review, our meta-analysis drew upon a wealth of existing literature to synthesized data from diverse studies investigating the effects of fertilizers on insect communities across various ecosystems. This comprehensive approach enhances our understanding of how changes in fertilizer influence plant-mediated bottom-up effects and their impact on natural enemies. While much of the previous research has focused on understanding the impact of fertilizer application on tritrophic interactions with bottom-up effects, specifically in the context of a single natural enemy attacking the same herbivore, the great strength of the presented study lies in providing a comprehensive overview of the current state of knowledge in this field. by uncovering potential disparities in the responses of natural enemies to fertilization. Our study offer insights that transcend individual study boundaries.

Article 2

Bottom-up effects of inorganic fertilizers on natural enemies: a meta-analysis

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Abstract

In the pursuit of sustainable agriculture, particularly under low-input conditions, the impact of reduced fertilizer resources on agro-ecosystems becomes crucial. This comprehensive study combines a bibliographic review and a categorical random-effects meta-analysis to globally assess the potential of fertilizers in enhancing conservational biological control services. The focus is on key traits of natural enemies (Parasitoids & Predators) affected by fertilizers, including biocontrol efficiency, development, reproduction, abundance/richness, and survival. Analyzing data from 36 papers, the study reveals contrasting effects of full NPK with only N-modification fertilization solution on natural enemies. We demonstrated that fertilization triggered a tri-trophic nutritional cascade to natural enemy fitness, both types of fertilizers influence natural enemies' development, survival, abundance and reproduction positively. The bottom-up effects of fertilization vary across natural enemy functional guilds. Parasitoids, though less impacted than predators, still exhibit sensitivity to fertilization levels. Additionally, the diet regime of predators influences the intensity of bottom-up effects, with carnivorous predators showing less impact compared to omnivorous predators. Despite the overall positive influence of higher fertilizer levels on natural enemies' development and reproduction, there is a negative impact on biocontrol efficacy. Importantly, the study underscores the significance of study conditions, with large-scale field experiments showing potential buffering effects of nitrogen fertilizers. Additionally, diverse taxonomic orders of natural enemies respond differently to fertilizers, with Hymenoptera and Hemiptera exhibiting significant positive responses.

This study not only contributes insights into the nuanced relationship between synthetic fertilizers and natural enemies but also serves as a critical reference for ongoing research investigating the effects of fertilizers on ecosystems and insect taxa. It emphasizes the need for

a balanced approach in fertilizer application to ensure sustainable agricultural practices while preserving conservational biological control services.

Keywords: nitrogen, NPK fertilizer, parasitoid wasp, predator, low-input

1 Introduction

Cropping production increased in many countries over the last decades of the 20th century through the use of high-yielding varieties, fertilizer inputs, high-density planting and pesticide uses. While fertilizers play a crucial role in providing essential nutrients for crop growth, their impact on the environment should not be overlooked. The intensification of fertilization has given rise to new global environmental challenges, such as nutrient imbalances (MacDonald et al., 2011; Penuelas et al., 2013; Lu & Tian, 2017; Romero et al., 2021), the leaching of nutrients from crops leading to groundwater contamination, as well as greenhouse gas emissions, and associated environmental repercussions (Sardans & Peñuelas, 2015; Lee et al., 2020; Weihrauch & Weber, 2020; Romero et al., 2021; Tedengren, 2021). Moreover, the escalating costs associated with fertilizers have posed economic challenges, particularly concerning food security (Obersteiner et al., 2013; Van der Velde et al., 2014; Khabarov & Obersteiner, 2017; Bonilla-Cedrez et al., 2021). Consequently, a transition toward low-input agricultural systems has been suggested (Parr et al., 1990).

Sustainable agricultural development should be cost-effective, simple to adopt, healthy and environmentally friendly (Biala et al., 2007). Low-input practices that minimize the demand for off-farm resources and optimize resource-use efficiency, while ensuring both optimal agronomic outputs (biomass yield and quality) and potential benefits for ecosystem services, are one pillar of the modern sustainable agricultural systems (Pointereau et al., 2017). Among the low-input practices, reducing fertilizer and pesticide inputs are particularly targeted. Plant

fertilization and crop protection should be conducted in a way that maximizes biological prevention of pests and diseases, in particular promoting integrated pest management (IPM).

In conventional agriculture, inorganic fertilizers contain high concentrations of specific nutrients, such as nitrogen (N), phosphorus (P) and potassium (K), in forms readily available for plant uptake. Applying in high concentrations to meet the perceived nutrient demands of crops. However, this practice can lead to excess nutrient application, where plants cannot fully utilize all the nutrients provided. The surplus nutrients can leach into groundwater or runoff into surface water bodies, causing pollution and eutrophication (Galloway et al., 2008; Khabarov & Obersteiner, 2017; Penuelas et al., 2023). Organic fertilization and bio-fertilization are often considered more environmentally friendly alternatives to synthetic fertilizers. Many agricultural areas are adopting organic fertilization due to the significant issue of groundwater pollution caused by synthetic fertilizers, as well as other environmental concerns. Consequently, there is a growing trend towards reducing or completely discontinuing the use of inorganic fertilizers in the future. Before transitioning to organic agriculture, it is essential to understand the potential effects of organic fertilizers in agroecosystems.

Plant and pest responses to fertilization are well characterized and understood and numerous studies have shown that fertilizers can promote plant growth. Fertilization influences the plant, by altering their macronutrient and defense content. Therefore, reducing N fertilization application directly impacts herbivore fitness – the “*Nitrogen limitation hypothesis*” (White, 1993) – but it could have negative influence on the plant yield (Hosseini et al., 2018). Waring & Cobb (1992), followed by Butler et al., (2012), used a meta-analysis approach to merge related literature and found fertilizers NPK have positive effect on insects.

However, the potential bottom-up effect of fertilization on natural enemies (3rd trophic level) through plants and pests is less clear. Natural enemy - predators and parasitoids that prey on or parasitize pests - may be indirectly affected by changes in pest quality and/or abundance

(Chesnais et al., 2016; Winter & Rostás, 2010). The responses of arthropod natural enemies to different types and doses of fertilizers are also variable (Aziz et al., 2015; Bodo et al., 2002; Karungi et al., 2006; Ponti et al., 2007). Garratt et al., (2011) highlighted, for instance, a positive impact of organic fertilizers, based on a few papers. But an overall understanding is still missing and there is a need to review more available research on the fertilizers mediated bottom-up effects on natural enemies.

The present study used both vote-counting and meta-analysis techniques to investigate the effects of fertilizer types and doses on various natural enemies' types and their life-history traits. We will focus on inorganic fertilization, specifically comparing full NPK with only N-modification fertilization solution, because they were using full solution or they were only changed N. In fact, that we have many papers on nitrogen and NPK. This is why we focus on. Indeed, N is a key element for insects and have hypothesis of nitrogen. Many insects are primary herbivores that strip nitrogen from plants; the nitrogen amassed in insects can comprise a substantial amount of the total nitrogen flux in the ecosystem (Whittaker, 2001). Additionally, we will explore various doses of inorganic fertilization, including low, medium, high, and excess levels. This testing aims to optimize the use of inorganic fertilizers, minimize environmental impact, enhance pest control efficiency, and effectively manage costs in agricultural systems. Both the types and doses of fertilization will be examined concerning natural enemies related to their taxonomy of their functional roles (parasitoids vs carnivorous/omnivorous predators), and different life history traits (development, longevity, survival, abundance, fecundity, and biocontrol efficacy). Furthermore, studies investigating effects of fertilizers on higher trophic levels are conducted either in lab, climate chamber, greenhouse or field setting and we will test if conclusions from controlled conditions are similar to the field.

In the context of Low Input Cropping Systems (LICS) aimed at sustainable agriculture, the focus is on optimizing on-farm resources and minimizing the use of purchased off-farm resources, including fertilizers, pesticides, mineral fertilizers, and fuel, to reduce production costs and prevent surface and groundwater pollution. Consequently, we aim to investigate the effects of reducing fertilization inputs, specifically transitioning from high to medium fertilization levels, on biocontrol efficacy.

2 Materials and Methods

2.1 Literature search and compilation of dataset

A systematic review of primary literature published in peer-reviewed journals up to 2023 was conducted. Web of science was used to identify the relevant papers by applying combination of search strings (Fig. 1): (ferti* AND NPK OR Phosphor* OR Potassium OR Nitrogen OR nitrate OR nutri* OR ammoni* OR N addition* OR N deposition* OR N input) AND (bio* control OR predat* OR Prey OR parasit* OR natural en* OR spider OR beetle OR wasp OR ladybird* OR ladybug* OR pest control OR pest).

Published literature which observed the response of a natural enemy species or group of natural enemy species to fertilizer inputs were included in this meta-analysis. Up to now, only nitrogen (including ammonia), potassium, phosphorus and NPK inorganic synthetic fertilizers were incorporated. Each fertilization mediated plant - natural enemy combination tested in those papers was considered and called as one “effect” later. In studies which monitored the response of various insects’ species to fertilizers or various doses of fertilizers, each species or doses were considered independent and included as a separate “effect” i.e. data point.

The criteria for inclusion in the present meta-analysis were as follows (Fig. 1):

- (1) Studies should have been published in peer-reviewed papers;
- (2) Only experimental papers were considered; reviews excluded;

- (3) Studies should concern fertilization mediated plant - natural enemy combination;
- (4) Studies reported should have used control treatments (no fertilizer) compared to fertilized treatments (any types or doses of fertilization inputs);
- (5) Studies should report the effect of fertilization on at least one natural enemy species on at least one life-history trait. We recorded the variables which includes development (weight change, elytra length), longevity/survival rate, fecundity/fertility, sex ratio, abundance/diversity, and biocontrol efficiency.
- (6) the replicates of treatment should be more than three. Mean or median, SD or any measures of variance and sample size should be available.

When extracting information, any study with ambiguity in its description of a variable was discarded.

2.1.2 Data extraction and monitoring

To investigate whether different types of fertilizers have different effects on natural enemies, a categorical meta-analysis was applied. Several explicative variables were considered: (1) Fertilizer types were classed as nitrogen, NPK; (2) The same type of fertilizers was grouped at the level of doses (low, medium, high and excess fertilizers): The information on the amount of fertilizer were taken from the recommended use of fertilizers for each crop as mentioned in the literature, and we defined the recommended amount as “high fertilizer”, the amount of fertilizer applied as 25% of the recommended amount as “low fertilizer”, 50-75% as a “medium fertilizer”, and more than the recommended fertilizer 120-200% as “excess fertilizer”. No fertilization is considered as “control” group. (3) For natural enemies, we first separate as functional groups: predators vs parasitoids, (4) then according to taxa (Coleoptera, Diptera...).

All the scientific name of natural enemies were checked and corrected regarding the website <https://inpn.mnhn.fr> respectively. Species were then gathered following orders. Finally, (5) study sites were also categorized into lab, climate chamber, greenhouse, field or forest.

The responding variables were the various NE life history traits, such as development, longevity, survival, abundance, fecundity, and biocontrol efficacy. Within a study, the mean or median, SD or SE or quartiles and sample size of experimental (i.e., fertilized) and control (i.e., not fertilized) groups can be used to calculate the individual effect size representing the difference between two variables. Often a paper would provide the standard error rather than the SD, in which case the following equation was used to calculate the SD:

$$SD = SE \times \sqrt{(n)}$$

The majority of the data were extracted from the tables and texts directly, while data were presented in graph form, Web Plot Digitizer software was used to extract the information.

2.1.3 Data analysis

The following method was inspired from Butler et al (2012).

Effect size - Hedges coefficient, *Hedge's d*, was used to calculate effect sizes as it accounts for small sample sizes (Rosenberg, 2022), which was often the case for the studies included. Individual effect sizes and the variance of *d* were calculated for each effect i.e., fertilization mediated plant - natural enemy combination. No fertilization situations (control group) were compared to any types and doses of fertilizers (treated groups).

The studies used in meta-analysis are, in general, highly heterogeneous. A random-effects model was used for the meta-analysis as it accounts for variation in effect sizes (Hedges & Vevea, 1996). Average effect size, 95% confidence intervals (CI) and a Q statistic for each

category were calculated. An average effect size above 0 indicates that, overall, there was a positive response of the insects within the category to fertilizer type and/or doses. Likewise, below 0 would represent a negative response to fertilizers and a value of 0 would suggest no response. In addition, if CIs do not cross 0 then the effect size is considered statistically significant.

A Q test uses a measure of variance of each study to compare its contribution to the overall effect estimate (Huedo-Medina et al., 2006). Both a between and within category Q statistic were calculated. The former indicates that the different categories responded differently to fertilizers, and the latter indicates there is residual variance within categories not explained by the model (Hedges, 1984). So, a significance between Q statistic suggests that there is significant difference between the different categories in the meta-analysis (and that the categories are justified). Whereas a significance within Q statistic suggests that heterogeneity still exists in the data that is not explained by the categories. All calculations were performed using MetaWin version 3 (Rosenberg, 2022).

Vote count - The analysis involved recording the response direction for each data point, irrespective of statistical significance. Subsequently, a binomial sign test was utilized to compare the ratio of positive and negative responses, determining whether they occurred randomly. A *P*-value below 0.05 indicated that the responses were not random, suggesting a significant impact of fertilizers on natural enemies, whether positive or negative. In cases where the sample size was ≤ 5 for a specific variable, no binomial sign test was conducted due to the low statistical power it would entail.

While a sign test proves effective in evaluating a diverse array of studies (Borenstein et al., 2021) and is particularly valuable in complementing a more quantitative meta-analysis, it is

crucial to note its limitations. The sign test does not consider the magnitude of effect sizes or the quantity of evidence within each study.

3 Results

3.1 Overview the dataset

Applying these criteria, a total of 1045 effect from 36 studies were recorded regarding the responses of natural enemies to fertilizers (details of the literature search are shown in Fig. 1). We extracted 849 observations from these 36 papers extended over 15 countries and included 2 fertilizer types and 2 categories of natural enemies (307 data points of predators and 542 of parasitoids).

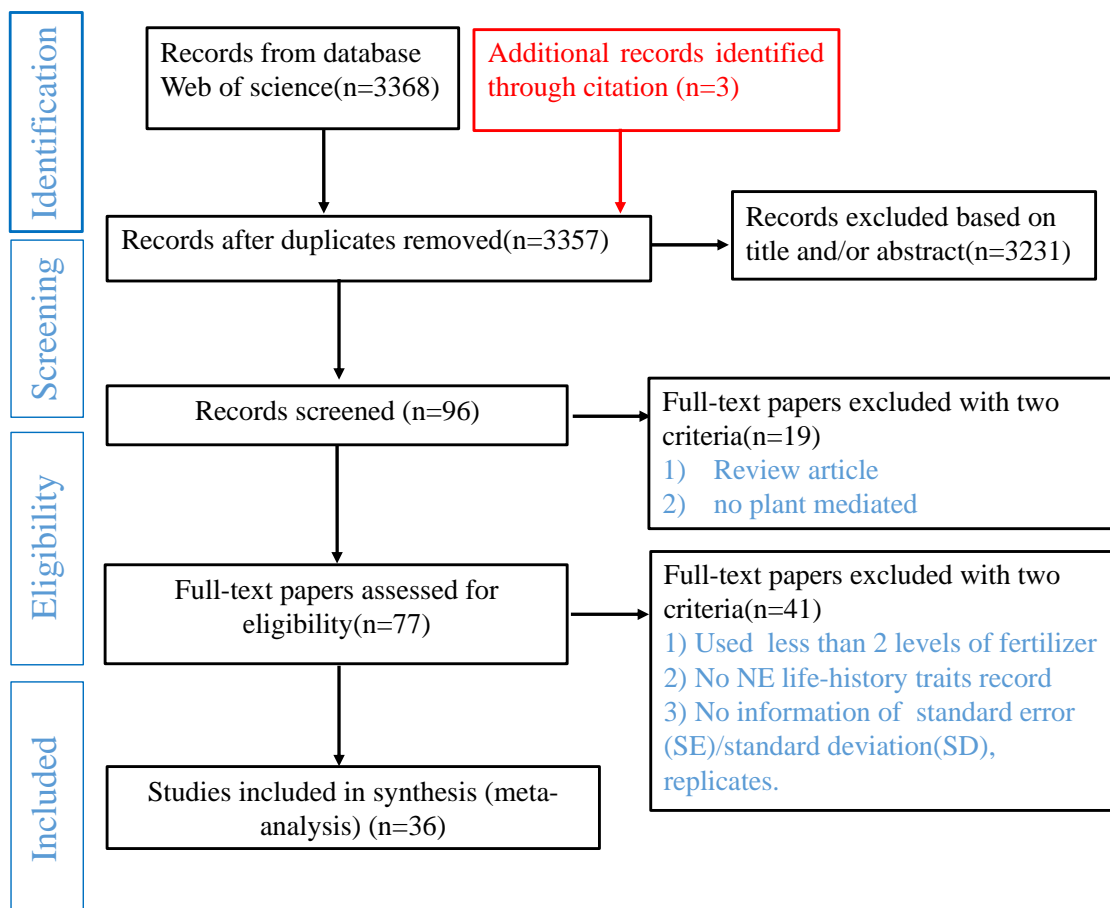


Fig. 1 PRISMA (Preferred Reporting Items for Systematic Meta-Analysis) flow diagram showing the procedure used for the selection of papers for meta-analysis.

Upon examining the datasets (Fig. 2A), it becomes apparent that the majority of studies are concentrated in North America, South Asia, and Northern Europe, indicating a well-organized spatial distribution worldwide. Additionally, the diversity of natural enemies was well-demonstrated, encompassing 11 taxonomic orders, which are equivalent to the main orders of natural enemies (Hajek & Eilenberg, 2018). To further understand the richness and diversity of crops, we plan to incorporate the information on the species and number of crops in future analyses.

We found that only a few papers per year are published on this topic (Fig. 2B), and there is no exponential increase in research output over time. This suggests that the subject is not receiving significant attention from the scientific community. Such limited output indicates that the research area may be underexplored or overlooked, highlighting the potential for further exploration in this area.

Finally, our investigation revealed a trend toward conducting more experiments in field conditions (Fig. 2C). This enables us to draw conclusions based on real growing conditions and make meaningful comparisons between controlled and uncontrolled environments. The shift towards field-based research signifies a recognition of the importance of studying phenomena within their natural context, where factors such as climate, soil conditions, and biological interactions can exert significant influence on outcomes.

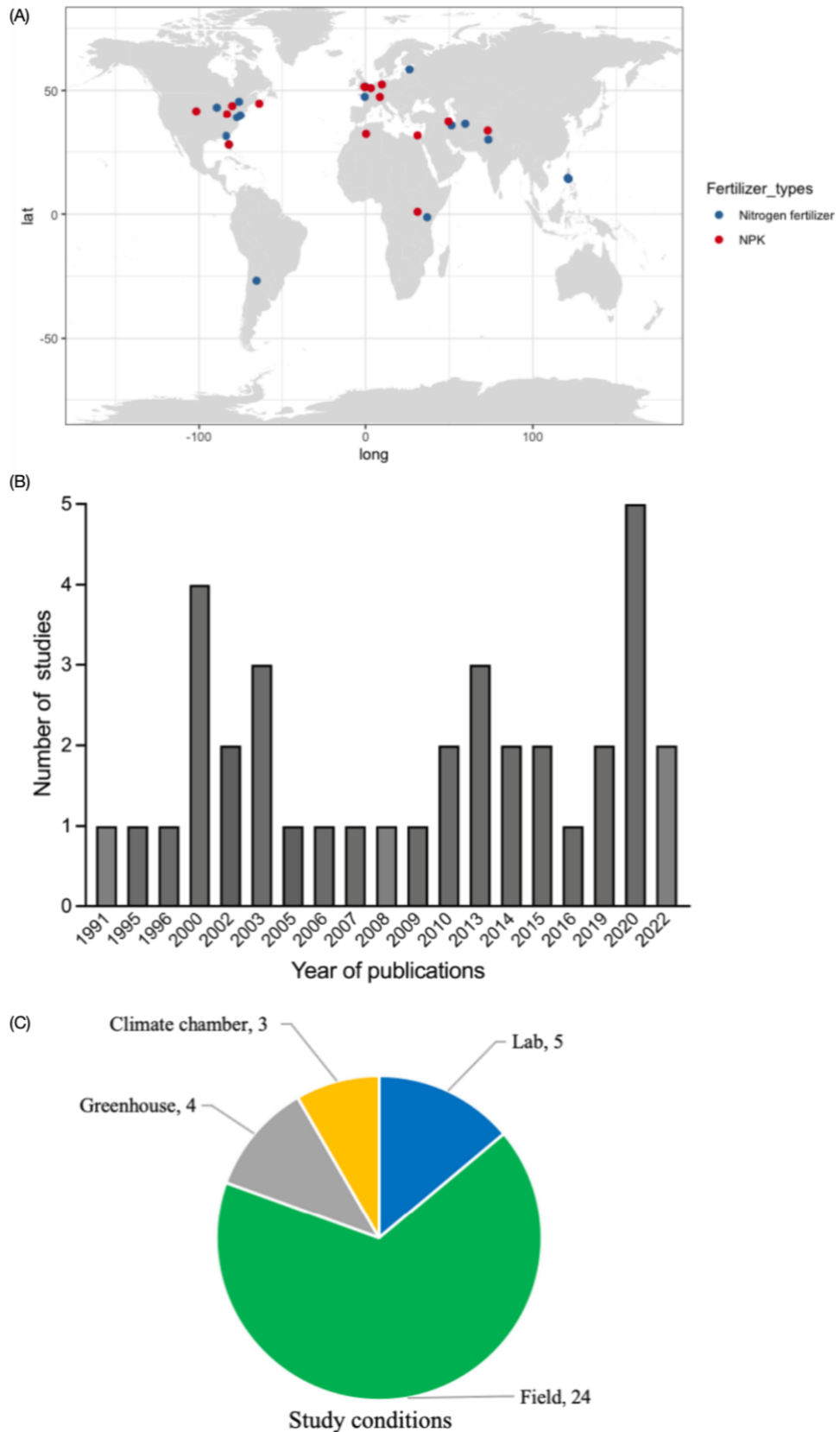


Fig. 2 Summary of the review dataset. (A) Distribution of study locations in fertilizer regions providing data for our meta-analyses (N = 36). Note that points may overlap; (B) studies by the year of publication; (C) studies by experimental conditions.

3.2 Do we have the same effect of fertilization types on different natural enemies?

The influence of crossing fertilizers types and functional groups of natural enemies - When all studies were included in the meta-analysis and a vote was counted, of the 849 responses regarding overall fertilization types (566 responses were positive, 266 were negative, and 17 were neutral), the 613 responses regarding nitrogen fertilization (395 responses were positive, 208 were negative, and 10 were neutral), and the 236 responses regarding NPK fertilization (171 responses were positive, 58 were negative, and 7 were neutral) (Table 1). When all studies on carnivorous predators were included in the meta-analysis, a significant positive effect of fertilizers on carnivorous predators was observed (Fig. 3), although there was significant heterogeneity ($I^2 = 28.67$, $Q = 222.92$, $df = 159$, $P < 0.001$). After subdivision of responses into different fertilizer types, carnivorous predators showed a significant positive association with the application of both N and NPK fertilizers (N fertilizer: $I^2 = 24.59$, $Q = 157.80$, $df = 119$, $P = 0.01$; NPK fertilizer: $I^2 = 39.96$, $Q = 64.96$, $df = 39$, $P = 0.006$). Heterogeneity was significant within the categories ($Q = 222.76$, $df = 158$, $P < 0.001$), but no effect was observed between the categories ($Q = 0.16$, $df = 1$, $P = 0.69$). This suggests that N fertilizers could have a global positive effect on carnivorous predators.

For omnivorous predators, a significant positive effect of global fertilizers on omnivorous predators were observed (Fig. 3), showing significant heterogeneity ($I^2 = 28.13$, $Q = 64.00$, $df = 46$, $P = 0.04$). After subdivision of responses into different fertilizer types, omnivorous predators exhibited a significant positive association with N fertilizers ($I^2 = 33.79$, $Q = 63.43$, $df = 42$, $P = 0.02$), but no effect was observed with NPK fertilizer ($I^2 < 0.0001$, $Q = 0.286$, $df = 3$, $P = 0.96$). Heterogeneity was significant within the categories ($Q = 63.71$, $df = 45$, $P = 0.03$), but no effect was found between the categories ($Q = 0.29$, $df = 1$, $P = 0.60$). This suggests that N fertilizers could have a global positive effect on the omnivorous predators.

This was in accordance with the vote count where, out of 307 responses of predators, 198 were positive, 106 were negative, and 3 were neutral (Table 1). Predators include the 10 responses of Coleoptera (198 were positive, 106 were negative, and 3 were neutral), Diptera (8 were positive), Hemiptera (30 were positive, 10 were negative, and 1 were neutral), Neuroptera (48 were positive, 42 were negative, and 1 were neutral), Odonata (9 were positive and 3 were negative), Predator mix (66 were positive, 33 were negative, and 1 were neutral), and Spider (30 were positive and 10 were negative).

When all parasitoid studies were included in the meta-analysis, a significant positive effect of fertilizers on parasitoids was observed (Fig. 3), although significant heterogeneity was noted ($I^2 = 47.51$, $Q = 1030.61$, $df = 541$, $P < 0.0001$). After subdivision of responses into different fertilizer types, parasitoids exhibited a significant positive association with the application of both N and NPK fertilizers (N fertilizer: $I^2 = 30.82$, $Q = 575.27$, $df = 398$, $P < 0.0001$; NPK fertilizer: $I^2 = 66.20$, $Q = 420.13$, $df = 142$, $P < 0.0001$). Furthermore, the effect size of NPK fertilizer was nearly three times that of N fertilizer (0.56 compared with 0.20; Fig. 3). Significant heterogeneity was observed both between or within the categories (between $Q = 35.21$, $df = 1$, $P < 0.0001$; within $Q = 995.40$, $df = 540$, $P < 0.0001$), indicating that fertilizers could have a global impact on parasitoids. The vote count resulted in 325 positive and 112 negative responses out of 448 parasitoid responses, and all were from Hymenoptera (Table 1).

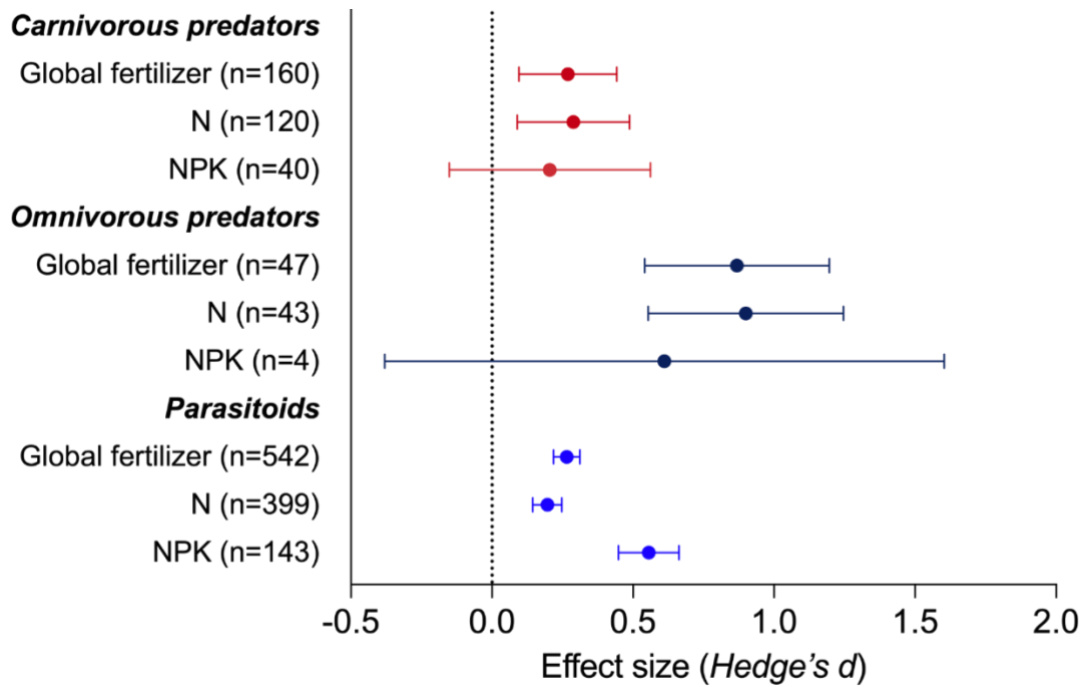


Fig. 3 Mean effect sizes of fertilizer types responses for all functional groups of carnivorous predators, omnivorous predators, as well as parasitoids. Error bars represent 95% confidence intervals. Overall response to fertilizers calculated using a random-effects model. Responses categorized by fertilizer type were calculated using a categorical random-effects model.

Table 1 Summary of the data used for the vote-count showing the number of positive and negative responses to fertilizer treatments. In all cases, the control group is a “no fertilization” situation.

		n	+	-	0	P values
Natural enemies	Predator	307	198	106	3	< 0.0001
	Parasitoid	542	368	160	4	< 0.0001
Functional group	redators					
	Coleoptera	10	3	7	0	0.34
	Diptera	8	8	0	0	0.008
	Hemiptera	41	30	10	1	0.004
	Neuroptera	91	48	42	1	0.68
	Odonata	12	9	3	0	0.15
	Predator mix	100	66	33	1	0.002
Parasitoids	Spider	40	30	10	0	0.002
	Hymenoptera	448	325	112	11	< 0.0001
	Parasitoid mix	94	43	48	3	0.47
Study conditions	lab	242	202	38	2	< 0.0001
	Climate chamber	71	50	17	4	0.001
	Greenhouse	42	31	10	1	0.003
	Field	482	272	200	10	0.005
	Forest	12	11	1	0	0.006
Fertilizer types	Overall	849	566	266	17	< 0.0001

	Nitrogen	613	395	208	10	< 0.0001
	NPK	236	171	58	7	< 0.0001
Fertilizer levels	Low	209	128	75	6	0.001
	Medium	97	65	30	2	0.001
	High	402	268	126	8	< 0.0001
	Excess	141	105	35	1	< 0.0001
Nitrogen	Low	159	91	65	3	0.081
	Medium	72	45	25	2	0.044
	High	283	187	91	5	< 0.0001
	Excess	99	27	72	0	< 0.0001
NPK	Low	50	10	37	3	< 0.0001
	Medium	25	20	5	0	0.004
	High	119	81	35	3	0.0001
	Excess	42	8	33	1	< 0.0001

3.3 Do we have the effect depend on fertilization doses on different natural enemies?

The influence crossing of fertilizers doses and functional groups of natural enemies –

Carnivorous predators, on the other hand, shows a significant response only to high and excess fertilizer applications (high fertilizer: $F^2 = 38.80$, $Q = 99.68$, $df = 61$, $P = 0.001$; excess fertilizer: $F^2 = 50.27$, $Q = 98.54$, $df = 49$, $P < 0.0001$), with no statistically significant response observed for other fertilizers (low fertilizer: $F^2 < 0.0001$, $Q = 8.81$, $df = 11$, $P = 0.64$; medium fertilizer: $F^2 < 0.0001$, $Q = 13.58$, $df = 35$, $P = 0.10$; Fig. 4).

Conversely, the response of omnivorous predators to all fertilizer levels is not significant, and data for excess fertilizer are unavailable (low fertilizer: $F^2 = 0.03$, $Q = 9.00$, $df = 9$, $P = 0.44$; medium fertilizer: $F^2 = 56.91$, $Q = 13.93$, $df = 6$, $P = 0.03$; high fertilizer: $F^2 = 26.96$, $Q = 39.70$, $df = 29$, $P = 0.09$; Fig. 4).

Specifically, parasitoids also demonstrate a positive response with increasing fertilizer levels (low fertilizer: $Q = 183.33$, $df = 144$, $P = 0.01$; medium fertilizer: $Q = 117.83$, $df = 49$, $P < 0.0001$; high fertilizer: $Q = 464.97$, $df = 259$, $P < 0.0001$; excess fertilizer: $Q = 224.7$, $df = 860$, $P < 0.0001$; Fig. 4).

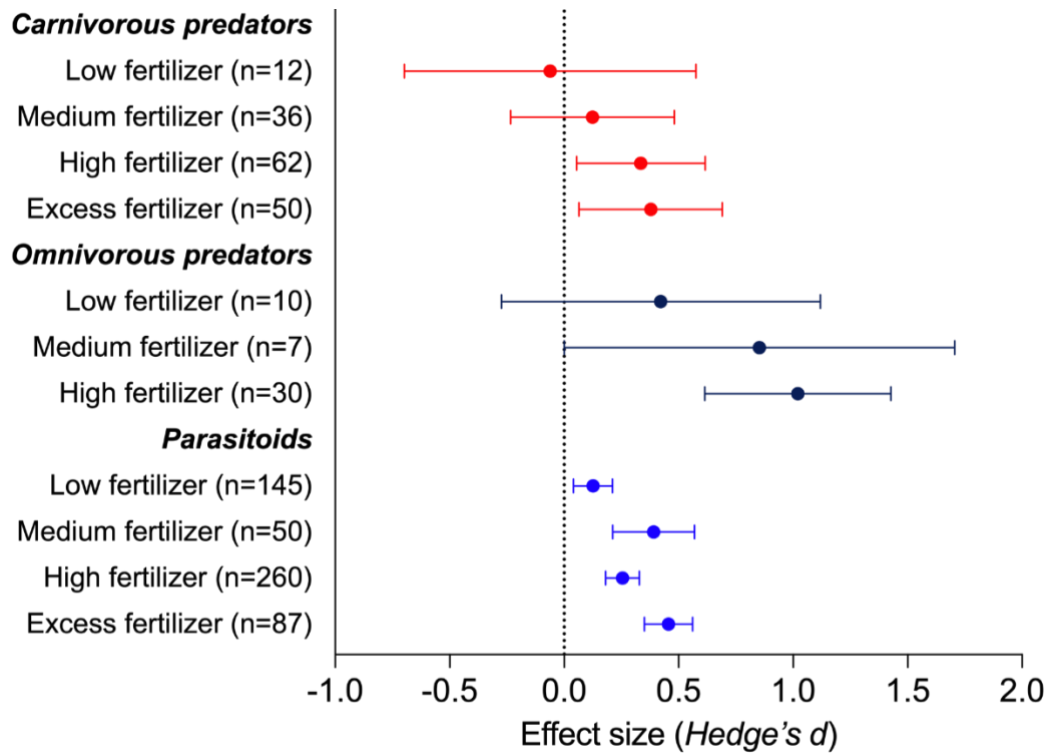


Fig. 4 Mean effect sizes of fertilizer levels responses for all functional groups of natural enemies, parasitoids, carnivorous predators, omnivorous predators, as well as predator mix. Error bars represent 95% confidence intervals. Overall response to fertilizer levels calculated using a random-effects model.

The influence crossing of fertilizers doses and traits of natural enemies – When all studies were included in the meta-analysis and a vote was counted, of the 209 responses regarding low fertilizer level (128 responses were positive, 75 were negative, and 6 were neutral), 72 responses regarding medium fertilizer level (65 responses were positive, 30 were negative, and 2 were neutral), 402 responses regarding high fertilizer level (268 responses were positive, 126 were negative, and 8 were neutral), and 141 responses regarding excess fertilizer level (105 responses were positive, 35 were negative, and 1 were neutral) (Table 1). Carnivorous predators abundance significant association observed with low and high fertilizer (low fertilizer: $Q = 30.80$, $df = 11$, $P < 0.0001$; high fertilizer: $Q = 114.66$, $df = 26$, $P < 0.0001$), while no effect is seen for medium fertilizer levels ($Q = 7.19$, $df = 5$, $P = 0.21$; Fig. 5). Carnivorous predators' biocontrol does not exhibit a significant association with medium and high fertilizer levels

(medium fertilizer: $Q = 6.50$, $df = 13$, $P = 0.93$; high fertilizer: $Q = 23.67$, $df = 16$, $P = 0.10$), but significant with excess fertilizer ($Q = 248.43$, $df = 33$, $P < 0.0001$; Fig. 5). Carnivorous predators development significant association observed with high fertilizer ($Q = 35.33$, $df = 11$, $P < 0.001$), while no effect is seen for medium and excess fertilizer levels (medium fertilizer: $Q = 11.40$, $df = 11$, $P = 0.41$; excess fertilizer: $Q = 7.06$, $df = 11$, $P = 0.79$; Fig. 5). Carnivorous predators' reproduction does not exhibit a significant association with any fertilizer levels (medium fertilizer: $Q = 0.11$, $df = 3$, $P = 0.99$; high fertilizer: $Q = 0.47$, $df = 5$, $P = 0.99$; excess fertilizer: $Q = 3.28$, $df = 3$, $P = 0.35$; Fig. 5).

Parasitoids abundance significant association observed with high fertilizer ($Q = 46.37$, $df = 25$, $P < 0.01$), while no effect is seen for low and medium fertilizer levels (low fertilizer: $Q = 22.01$, $df = 22$, $P = 0.46$; medium fertilizer: $Q = 0.80$, $df = 3$, $P = 0.85$; Fig. 6). Parasitoids biocontrol significant association with all fertilizer levels (low fertilizer: $Q = 118.75$, $df = 62$, $P < 0.0001$; medium fertilizer: $Q = 86.13$, $df = 19$, $P < 0.0001$; high fertilizer: $Q = 323.47$, $df = 155$, $P < 0.0001$); excess fertilizer ($Q = 61.09$, $df = 21$, $P < 0.0001$; Fig. 6). Parasitoids development also significant association with all fertilizer levels (low fertilizer: $Q = 121.47$, $df = 28$, $P < 0.0001$; medium fertilizer: $Q = 64.31$, $df = 20$, $P < 0.0001$; high fertilizer: $Q = 193.59$, $df = 46$, $P < 0.0001$); excess fertilizer ($Q = 196.13$, $df = 34$, $P < 0.0001$; Fig. 6). Parasitoids reproduction significant association with high and excess fertilizer levels (high fertilizer: $Q = 51.92$, $df = 28$, $P < 0.01$; excess fertilizer: $Q = 83.75$, $df = 27$, $P < 0.0001$), while no effect is seen for low and medium fertilizer levels (low fertilizer: $Q = 30.79$, $df = 27$, $P = 0.28$; medium fertilizer: $Q = 0.04$, $df = 2$, $P = 0.98$; Fig. 6). The survival of parasitoids was not affected by any fertilizer levels (low fertilizer: $Q = 0.58$, $df = 1$, $P = 0.45$; medium fertilizer: $Q = 0.22$, $df = 1$, $P = 0.64$; high fertilizer: $Q = 2.35$, $df = 1$, $P = 0.13$) other than excess fertilizer ($Q = 7.51$, $df = 1$, $P < 0.01$; Fig. 6).

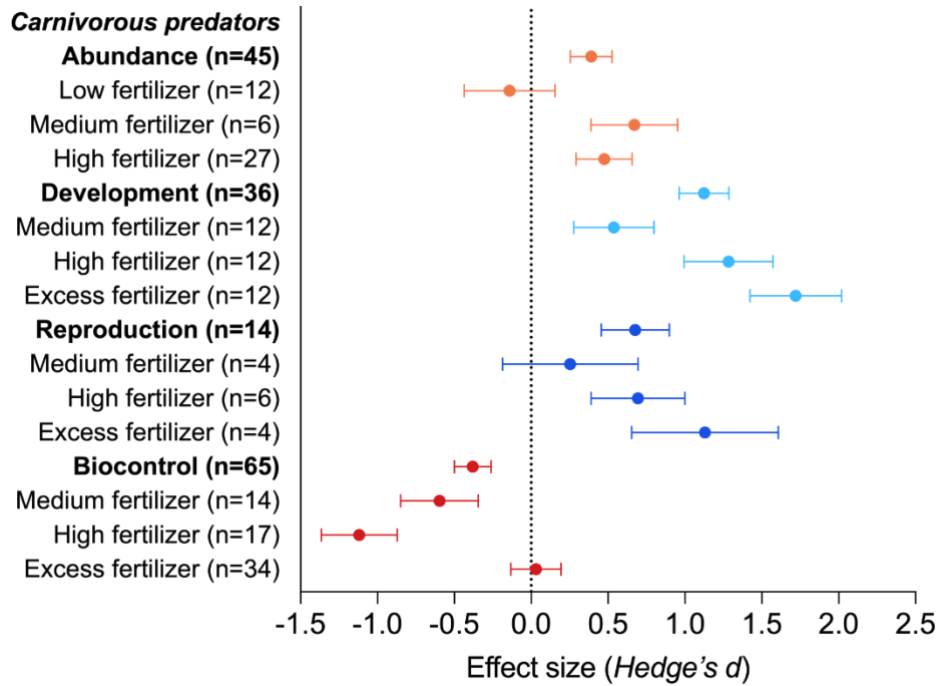


Fig. 5 Mean effect sizes of fertilizer levels on metric traits responses for carnivorous predators. Error bars represent 95% confidence intervals. Overall response to fertilizer levels calculated using a random-effects model.

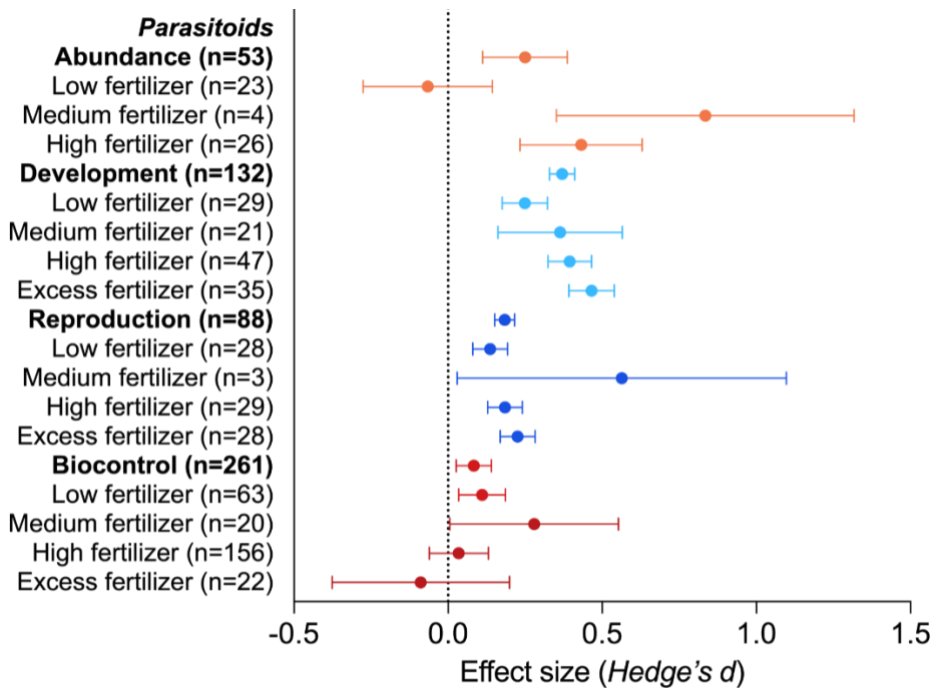


Fig. 6 Mean effect sizes of fertilizer levels on metric traits of parasitoids. Error bars represent 95% confidence intervals. Overall response to fertilizers levels calculated using a random-effects model.

3.4 Does fertilization bottom-up effect depend on experimental conditions?

The influence crossing natural enemies and experimental conditions – Carnivorous predators show a significant response to lab and field conditions (lab: $I^2 = 25.35$, $Q = 111.18$, $df = 83$, $P = 0.02$; field: $I^2 = 34.87$, $Q = 113.63$, $df = 74$, $P < 0.01$; Fig. 7).

Parasitoids show a significant response to climate chamber, greenhouse, and field conditions (climate chamber: $I^2 = 76.69$, $Q = 300.33$, $df = 70$, $P < 0.0001$; greenhouse: $I^2 = 69.95$, $Q = 123.13$, $df = 37$, $P < 0.0001$; field: $I^2 = 24.12$, $Q = 345.28$, $df = 262$, $P < 0.001$; Fig. 8). The conditions under parasitoids were not affected by lab and forest conditions (lab: $I^2 = 11.58$, $Q = 177.57$, $df = 157$, $P = 0.012$; forest: $I^2 < 0.0001$, $Q = 4.29$, $df = 11$, $P = 0.96$; Fig. 8).

This was in accordance with the vote count where, include the 242 responses of lab (202 were positive, 38 were negative, and 2 were neutral), 71 responses of climate chamber (50 were positive, 17 were negative, and 4 were neutral), 42 responses of greenhouse (31 were positive, 10 were negative, and 1 were neutral), 482 responses of field (31 were positive, 10 were negative, and 1 were neutral), and 12 responses of forest (11 were positive and 1 were negative (Table 1).

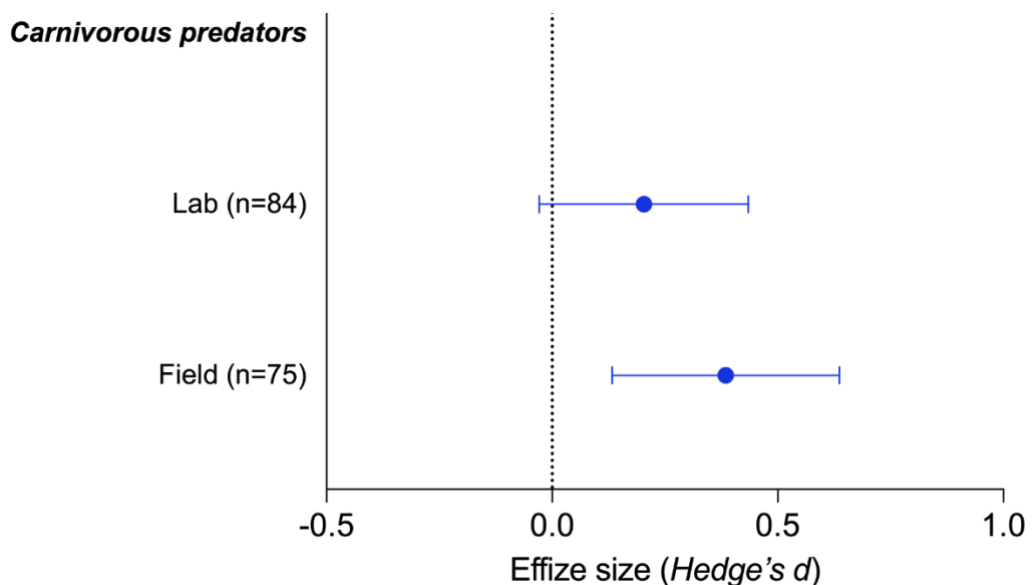


Fig. 7 Mean effect sizes of carnivorous predators on different conditions. Error bars represent 95% confidence intervals. Overall response to conditions calculated using a random-effects model.

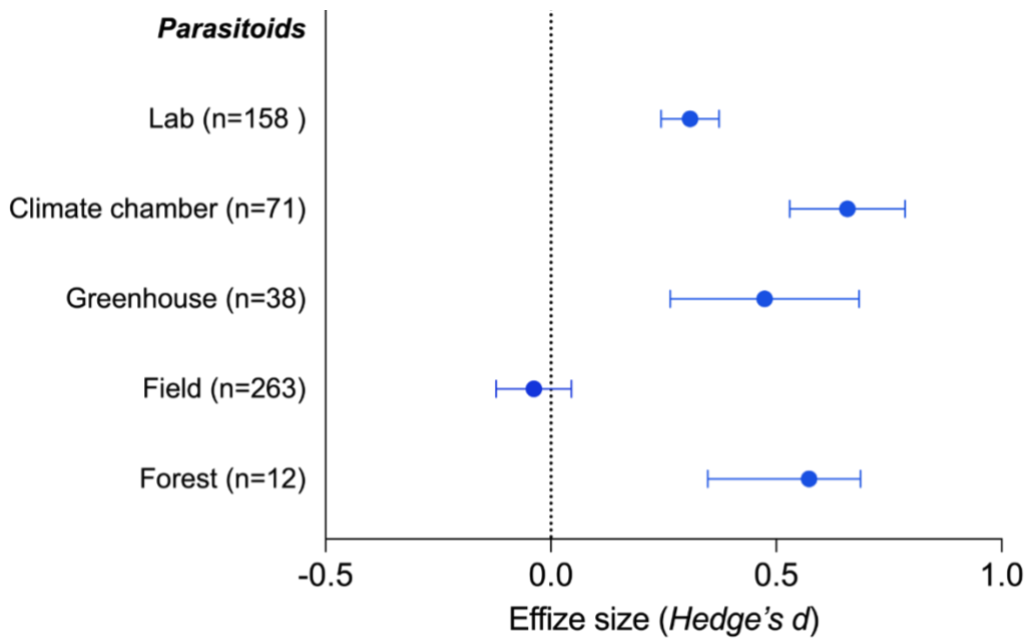


Fig. 8 Mean effect sizes of parasitoids on different conditions. Error bars represent 95% confidence intervals. Overall response to conditions calculated using a random-effects model.

4 Discussion

This article builds on previous literature reviews looking at the effects of the addition of plant nutrients and the response of natural enemies. We used natural enemy types in separate articles, natural enemies can be divided into predators and parasitoids according to their functional groups. And the predators can be divided into carnivorous and omnivorous according to their diet regime. Synthetic fertilizers have a positive effect on natural enemies' abundance, development, reproduction, and biocontrol efficiency, and these are shown by both the vote count and meta-analysis. And we compare 2 different situations, with or without inorganic fertilizations. We did the separations under nitrogen-only modification and compared full NPK

solutions. Because they were using full solution or they were only changed N, and they have hypothesis of nitrogen. There was however, significant heterogeneity across the studies included in this review thus justifying the use of a categorical meta-analysis to identify other factors influencing this strong natural enemy's response to fertilizers.

Fertilization bottom-up effects on natural enemies.

In the general way, the global fertilization increases all the traits of carnivorous predators and parasitoids, and is therefore positive for them. And we also look for all the traits with different type of fertilization. The fertilization types we consider are nitrogen-only modify and full NPK solutions. Parasitoids were more affected by NPK fertilization compared to N fertilization.

Natural enemy traits responded positively to increased fertilization doses between low fertilization and excess fertilization. In fact, for carnivorous predators, when at low fertilization did not affect the traits of the natural enemies. And for medium fertilization, the number of repetitions between carnivorous predators and parasitoids is not too far and we don't have the same answer. Carnivorous predators were not affected in medium fertilization and parasitoids respond positively to medium fertilization. It seems parasitoids are more clearly impacted by fertilization change, even if the change in fertilization dose was very small. This was not the case for carnivorous predators, and in fact was not enough to see a clear effect between predators and parasitoids, since we did not have the same number of replicates. So, we go for each trait of natural enemies. Fertilization had positive effects on the traits of both natural enemies, except for the biocontrol effects of carnivorous predators and we also worked on each trait of natural enemies in different fertilization doses. Fertilization doses had less impact on parasitoids biocontrol compared to carnivorous predators. So, both the type of fertilization and the dose of fertilization have an impact on natural enemies. The bottom-up effect is different between carnivorous predators and parasitoids. Carnivorous predators had more effect on N-modified fertilization, while parasitoids had more effect on the full NPK solution. For different

fertilization doses, carnivorous predators were less affected by changes in fertilization dose compared to parasitoids.

Overall natural enemies respond positively to increased fertilization doses between low fertilization and excess fertilization. However, they responded more strongly affected by NPK fertilizers compared to N fertilization, especially for parasitoids. Application of nitrogen fertilizer had a significant positive effect on natural enemies, as is evident from both the vote count and the meta-analysis. This supports the studies of White (1993) and Douglas (1993), which found nitrogen to be the limiting factor for insect populations. As the level of nitrogen fertilizer increased, the positive response effect of natural enemies gradually increased, especially in comparison with the low fertilizer level. These findings have important implications in agricultural environments where nitrogen fertilization application is high and the response of natural enemies' species is of concern to farmers.

Interestingly, NPK fertilizer had a stronger effect on natural enemies than N fertilizers. NPK fertilizer has been shown to have a larger effect on plant growth than any of the individual fertilizers. Consequently, this would increase the available food for phytophagous insects and thus increase prey feeding by natural enemies. In addition, studies have shown that, in nutrient rich conditions, plants reduce production of secondary metabolites in order to increase growth. Therefore, this research provides potential support for the 'plant vigour hypothesis' (Price, 1991) and the growth/differentiation balance hypothesis (Herms, 2002). Finally, the increased growth of plants can alter the insect's habitat (such as increasing shade), making the environment more hospitable. All these factors could account for insect populations responding more strongly to NPK fertilizer. In agreement with Butler et al (2012), insects population showed a positive response to nitrogen (N) and NPK fertilizers. However, the effects of NPK fertilization levels on natural enemies is not the same as for nitrogen fertilization. Natural enemies responded more

strongly to excess levels than medium NPK fertilizers. Therefore, it is not true that more fertilizer is better for natural enemies, it depends on the type of fertilizer.

Fertilization bottom-up effects on predators – In the general way, the global fertilization increases all the traits of carnivorous predators, and is therefore positive for them. The fertilization types we consider are nitrogen-only modify and full NPK solutions.

Omnivorous predators need to acquire nutrients from both their host plants and their prey (Janssen et al., 2003). Their fitness is strongly dependent on characteristics of the environment, notably the plant and prey qualities (Agrawal et al., 1999; Eubanks et al., 2000; Janssen et al., 2003) and therefore of abiotic factors such as fertilization (Gillespie et al., 2000; Sinia et al., 2004). We found that omnivorous predators showed a strong response to fertilizer. This may be linked to host quality change by plant fertilization (Stiling et al., 2005). Contrary to omnivorous predators, fertilization bottom-up effects have less impact on carnivorous predators.

Fertilization bottom-up effects on parasitoids - The results for parasitic wasps were consistent with the overall findings, potentially attributed to the relatively large sample size of parasitic wasps examined. Parasitoids were more affected by NPK fertilization compared to N fertilization. Additionally, we observed no substantial change in the impact of parasitic wasps irrespective of the fertilizer doses employed. Our findings align with Becker et al. (2021), who proposed a diluted bottom-up effect reaching the third trophic level. This implies that significant alterations in the second trophic level may be necessary to affect the third trophic level.

It seems parasitoids are more clearly impacted by fertilization change, even if the change in fertilization dose was very small. This was not the case for carnivorous predators, and in fact was not enough to see a clear effect between predators and parasitoids, since we did not have the same number of replicates. In fact, the traits of the carnivorous predators were not affected when at low fertilizer. And for medium fertilizer, the number of repetitions between carnivorous predators and parasitoids is not too far and we don't have the same answer, in which carnivorous

predators were not affected in medium fertilizer and parasitoids respond positively to medium fertilizer.

Then we took on each trait of natural enemies and found fertilization had positive effects on the traits of both natural enemies, except for the biocontrol effects of carnivorous predators. And fertilization doses had less impact on parasitoids biocontrol compared to carnivorous predators.

The magnitude and sometimes the direction of this response, however, is dependent on the type of fertilizer, application rates, and biology of the study organism, with predators and parasitoids sometimes showing contrasting responses. Importantly, the environment in which a study is conducted can have a marked effect on insect responses to fertilizer, with natural environments showing the potential for buffering effects of nitrogen fertilizers in particular. As well as highlighting the potential shortfall in the amount of research investigating particularly the fertilizers effects of predators and parasitoids, this study provides an invaluable flag post in the ongoing research investigating fertilizer effects on ecosystems. It demonstrates some clear patterns in the finding from the latest research whilst also providing an important bibliography of relevant literature. Finally, the idea further is to try to identify the best doses/types of fertilizer which can help to manage the pests more effectively *i.e.*, a fertilization regime that might have direct negative effects on pest and positive or at least neutral effects on their natural enemies.

For the condition in which a study was conducted has a large influence on the result of each study, the contrasting effect of study condition is reflected by significant between category Q values. Furthermore, both the magnitude and direction of mean effect sizes varied greatly between the different condition. There are many possible reasons as to why insects in different environments responded differently to fertilizers, one is the impact of experimental spatial scale. When an experiment is carried out on field and forest scale, a small positive mean effect size was observed in nitrogen and NPK fertilizers. When experiments are carried out on a smaller scale (lab, climate chamber and greenhouse), however, this trend to strongly response to

fertilizers. Vegetation in natural environments is much more diverse than in glasshouse and agricultural environments. Andow's (1991) natural enemy hypothesis proposes that more complex habitats contain more niches and, therefore, support a higher number of natural enemies which can also respond positively to fertilizer application (Bentz et al., 1996; Garratt et al., 2010; Krauss et al., 2007). In addition to being more diverse, the application of fertilizers to natural environments is more likely to result in changes in species composition that is normally absent in greenhouse or agricultural conditions (Olsson & Kellner, 2006). A change in host species composition can change available niches for both herbivorous insects and natural enemies, varying responses by insects to fertilizers. Furthermore, perhaps diverse plant communities act to buffer the effects of fertilizers thus dramatic effects on individual species of insect are not seen. Therefore, both alteration of host species composition, natural diversity, and the natural enemy hypothesis, provide some explanation for the different responses of insects in different environments particularly when comparing the effects of nitrogen in natural and greenhouse environments.

It is clear from both the meta-analysis and the vote count that the environment in which an experiment is performed influences the effect of fertilizer on natural enemies' responses. The variation seen between study environments also heeds caution and that conclusions drawn from a study in one environment will not necessarily apply to other conditions.

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Contributions

N. Desneux found the project. AV. Lavoir conceived the idea. AV. Lavoir designed and monitored the data collection. RH MA collected the data, did the statistics analysis and wrote the initial draft of the manuscript. AV Lavoir edited the manuscript. All authors approved final version of the manuscript.

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Chapter 3: Does Bottom-up effects reach parasitoid or predator in the same way, at the same degree? Studies at individual scale under laboratory condition

Regarding our bibliographic work (Chapter 2), we found that the bottom-up forces (BU) induced by fertilization, through the modification of plant traits, can indeed reach the third trophic level. In the literature review, through meta-analysis, we are beginning to observe a directional synthesis that enhances our understanding of how changes in fertilizer influence plant-mediated bottom-up effects and their impact on natural enemies. Much of the previous research has focused on understanding the impact of fertilizer application on tritrophic interactions with bottom-up effects, specifically in the context of a single natural enemy attacking the same herbivore. The great strength of the presented study lies in our investigation of bottom-up effects across trophic levels on plants of the same culture, studying the same herbivores and different natural enemy species (parasitoid, carnivorous, and omnivorous predator) under identical conditions. This experimental approach allows for more practically meaningful results.

We aim to conduct a comparative analysis in this regard. To achieve that, in this chapter we conducted a series of bioassays under controlled laboratory conditions where variations in fertilization regime were tested as bottom-up factors (Article 2). The aim of the present study was to investigate the bottom-up effects of reduced inorganic fertilization (from standard commercial levels to intermediate to no fertilization) across a variety of tri-trophic “tomato plant - pest - natural enemies” systems. We aimed to determine how the diet regime (carnivorous versus omnivorous predators) or the functional guild (predator versus parasitoid) modified the intensity of bottom-up effects using seven systems.

The studies were carried out in three different “tomato plant-herbivores-natural enemies” systems (aphid - or whitefly- or leaf miner-based systems), was conducted to explore the effects of decreasing fertilization on aphids *Macrosiphum euphorbiae* (Hemiptera: Aphididae), parasitoid *Aphidius ervi* (Hymenoptera: Braconidae: Aphidiinae), carnivorous predator *Adalia bipunctata* (Coleoptera: Coccinellidae), and omnivorous

predator *Macrolophus pygmaeus* (Hemiptera: Miridae); whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae), parasitoid *Encarsia formosa* (Hymenoptera: Aphelinidae), and omnivorous predator *Macrolophus pygmaeus* (Hemiptera: Miridae); leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae), carnivorous predator *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and omnivorous predator *Dicyphus errans* (Hemiptera: Miridae) on (1) key life-history traits, that is, development, longevity and fertility, and (2) their biocontrol ability (i.e., parasitism rate or predation rate). Table S1 and S2 summarizes experimental microcosms and conditions used for each pest-natural enemy system. Fig. S1 depicts the time of intervention for three treatments of fertilization and data measurements. The study model was presented in Fig. S2.

Table S1. Description of the experimental microcosms (Figures created with BioRender.com).




Experimental device	Picture	Description
Double-cup microcosm		Two cups on top of each other (bottom cup 350 mL, height 11 cm; top cup 600 mL, height 13 cm, diameter 6 cm). The top of each double-cup was covered with mesh secured with an elastic band. A tomato leaflet from a plant maintained in one of the three fertilizer treatments was placed in the top cup with the stem placed through a hole at the bottom of the top cup and dipped into the corresponding fertilization solution in the bottom cup.
Plastic box		A plastic box (diameter 10 cm, height 7 cm) with a 3 cm-diameter hole on the lid, covered with nylon mesh to allow air renewal and to decrease moisture inside. A tomato leaflet is placed inside the box and its vigor is maintained using floral foam moisturized with fertilization solution from its corresponding treatment.
Plastic box on an entire plant		The plastic box (same as above) is fixed on a leaf attached to its live plant.

Table S2. A summary of the experiments performed on different insect models.

Pest	Natural enemies	Development & Longevity	Reproduction	Biocontrol efficiency	Experimental device
<i>Macrosiphum euphorbiae</i> Fecundity (double-cup microcosm; Fig. 2A)	Parasitoid: <i>Aphidius ervi</i>	1. Developmental duration: egg to pupa (Fig. S3A) 2. Adult longevity (Fig. S3B)	Adult emergence rate (Fig. S3D)	Parasitism rate (Fig. S3C)	double-cup
	Carnivore predator: <i>Adalia bipunctata</i>	1. Developmental duration: egg to pupa (Fig. S3E) egg to adult (Fig. S3F) 2. Weight: female adult (Fig. 3G), male adult (Fig. S3H)	Fertility (Fig. S3J)	Predation rate (Fig. S3I)	double-cup
	Omnivore predator: <i>Macrolophus pygmaeus</i>	1. Developmental duration: nymph to adult (Fig. S3K) 2. Female adult weight (Fig. S3L)	NA	Predation rate (Fig. S3M)	plastic box
<i>Bemisia tabaci</i> fecundity (double-cup microcosm; Fig. 2B)	Parasitoid: <i>Encarsia formosa</i>	Development time: egg to pupa (Fig. S4A) egg to adult (Fig. S4B)	Adult emergence rate (Fig. S4E)	1. Parasitism rate (Fig. S4C) 2. Host feeding (Fig. S4D)	plastic box on entire plant
	Omnivore predator: <i>Macrolophus pygmaeus</i>	1. Developmental time: nymph – adult (Fig. S4F) 2. Adult weight (Fig. S4G) 3. Adult longevity (Fig. S4H)	Nymph emergence rate (Fig. S4J)	Predation rate (Fig. S4I)	plastic box plastic box on an entire plant (fecundity and longevity)
<i>Tuta absoluta</i> fecundity (double-cup microcosm; Fig. 2C)	Carnivore predator: <i>Chrysoperla carnea</i>	NA	NA	Predation rate (Fig. S5F)	plastic box
	Omnivore predator: <i>Dicyphus errans</i>	1. Development time: nymph -adult (Fig. S5A) 2. Adult weight (Fig. S5B) 3. Adult longevity (Fig. S5C)	Nymph emergence (Fig. S5E)	Predation rate (Fig. S5D)	plastic box

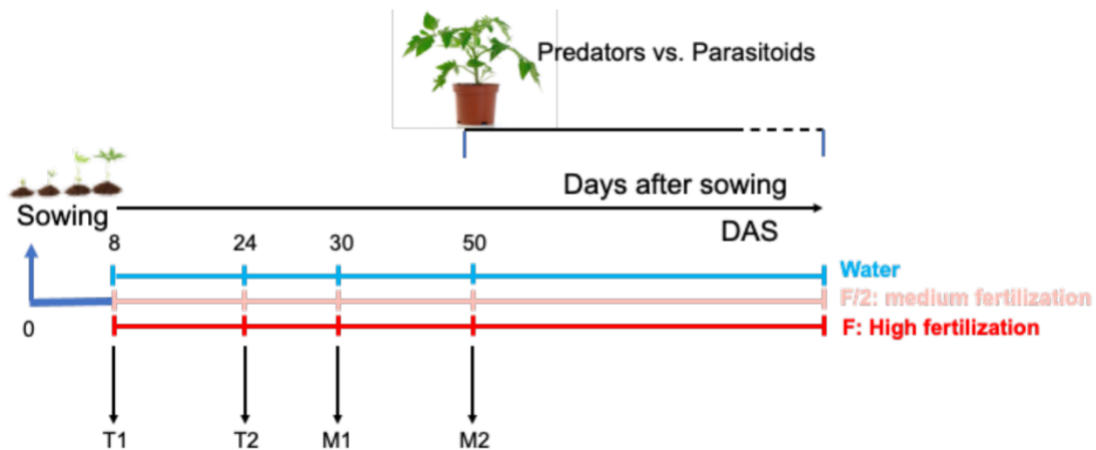


Fig S1. Timing of interventions (number of days since tomato sowing) for the application of fertilization treatment and data measurements on plants, pests, and natural enemies. The color code shows the fertilization treatments (blue = control i.e., tap water only; pink = intermediate fertilization; and red = high fertilization). T1 and T2 show plant transplantation times; T1 also coincides with the start of the fertilization treatment; M1 and M2 show the timing of plant measurements; M1 also coincides with the start of the fertilization treatment for pest rearing, while M2 shows the start of the experiments with natural enemies. DAS: Days After Sowing.



Fig. 1 Tomato plant growth under climate chamber conditions assigned to high fertilization (HF, commonly used fertilization level), medium fertilization (MF, a 50% dilution of HF), or control (W: water only, no nutrient).

Research models

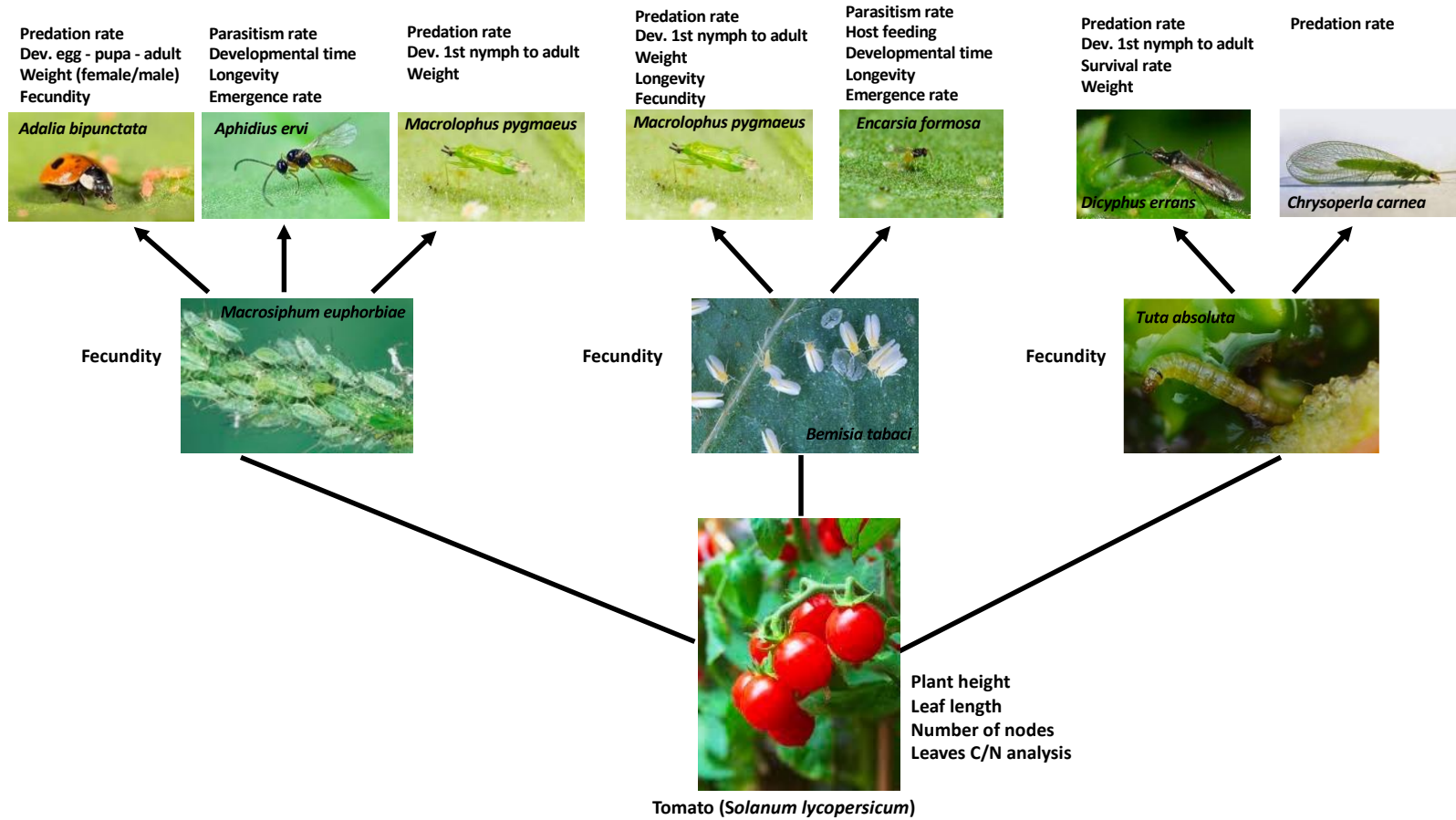


Fig S2. Research model: Trophic interactions between plants, pests and natural enemies.

We found strong evidence that reduced fertilization triggered a tri-trophic nutritional cascade, with changes in plant and herbivore nutritional quality cascading to natural enemy fitness, although with variation across natural enemy functional guilds. We first confirmed alterations at the first trophic level, where reduced fertilization led to decreased growth. This step was mandatory to investigate potential bottom-up effects on upper trophic levels. Leaf N content decreased with reduced fertilization levels, while leaf C/N ratio increased as expected. We expected modifications at the first trophic level would lead to changes at the second trophic level (herbivores). Indeed, reduced fertilization resulted in decreased pest fecundity as expected. However, this was not observed for *B. tabaci* from high to intermediate fertilization. Finally, we demonstrated that reduced fertilization triggered a tri-trophic nutritional cascade, where changes in plant and herbivore nutritional quality cascaded to natural enemy fitness, although with variation across natural enemy functional guilds. Reduced fertilization had the most pronounced effects on carnivorous predator traits compared to parasitoids or omnivorous predators.

Overall, we found that reducing fertilization to 50% of standard (optimal) application levels was marginally positive for biocontrol efficiency, which is promising. Considering our data, reducing fertilization from high to medium regime seems without consequences for biocontrol efficacy or slightly positive which is in line with Hosseini et al. (2018) conclusion. Therefore, we believe that we should pay attention to fertilizer use in agricultural systems when designing programs to utilize biological control, especially when using predatory natural enemies, which should provide avenues for optimizing pest control in IPM as long as there are no strong negative effects on the third trophic level (natural enemies).

But our laboratory set-up, only allowed to conclude on the effect of the quality of prey/host on natural enemies and not on the effect of the quantity. Yet, pest fecundity bioassays demonstrated a strong bottom-up effects on pest number of offspring/eggs which have to be considered when looking at the effect on the 3rd trophic level. Furthermore, the effects on fruit yield and quality had not been considered in our lab experiments, which prevents us from

concluding that reducing fertilization is valuable for improving biological control in tomato agricultural systems. Indeed, reducing fertilization application is expected to lead to a slight decrease in yield, this is at least partially offset by the reduced cost of fertilizer application and aphid control. To answer these questions, more comprehensive work on arthropod population dynamics and crop yields at larger scales (greenhouse or field) is required to assess the effects of different fertilizer regimes on arthropod communities and biological control services (Chapter 4).

Article 3

Bottom-up effects of reduced fertilization on natural enemies and biocontrol efficiency

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Abstract

In agroecosystems, arthropod communities may be influenced by bottom-up forces induced by environmental variations (e.g., fertilization) through the modification of plant traits. The way bottom-up forces affect the second trophic level is well documented, but how these effects cascade to the third trophic level is less understood. We aimed to understand: 1) how bottom-up effects vary between natural enemies with contrasted ecology, i.e., parasitoids and predators; and 2) how the diet regime of the predators affected the intensity of bottom-up effects. We set-up a lab experiment measuring the effects of reduced fertilization (from standard rates to no fertilization) on tri-trophic systems in tomato. The selected herbivores are frequent pests on tomato systems (*Macrosiphum euphorbiae*, *Bemisia tabaci* and *Tuta absoluta*). The respective parasitoids were *Aphidius ervi* and *Encarsia formosa*, and the respective predators *Adalia bipunctata* (carnivorous), *Macrolophus pygmaeus* (omnivorous), *Dicyphus errans* (omnivorous) and *Chrysoperla carnea* (carnivorous). We recorded plant growth and leaf carbon and nitrogen content, herbivores fecundity as well as various parasitoid/predator traits - development, longevity, reproduction and biocontrol efficiency.

We found evidence of diluted bottom-up forces through trophic levels depending on the herbivore types, with variable but overall marginally positive effects of reducing fertilization from high to intermediate levels on host quality and biocontrol efficiency. Parasitoids were overall less affected than predators. This work offers perspectives in the framework of

Integrated Pest Management where reduced fertilization may help better control pest populations without significant reductions in yields.

Keywords: Predator, parasitoid, tri-trophic interaction, nutritional quality, biocontrol

1 Introduction

In conventional agriculture, scientists and consumers are concerned about the large-scale use of chemical external inputs such as inorganic fertilizer. Applying excessive amounts of inorganic fertilizer at global scales leads to nutrient imbalances, groundwater contamination, greenhouse gas emissions and eutrophication, with consequences that also threaten ecosystem and human health (Galloway et al., 2008; Khabarov et al., 2017; Penuelas et al., 2023). Over the last decades, agriculture has transitioned from a purely profit-oriented activity to incorporate sustainability into productivity (Wezel et al., 2014). The “Efficiency-Substitution-Redesign” (ESR) framework captures the progression from shallower agroecological approaches to full system redesign in order to reach the goals of the agricultural transition (Hill, 1985, 2014; Vanbergen et al., 2020). Based on this, scientists are currently testing to reduce the amount of fertilizers needed without reducing food production (corresponding to the “efficiency” step), as recommended for sustainable agriculture transition (Altieri, 1995; Wezel et al., 2014).

Fertilization is considered as a “bottom-up driver” as it influences the first trophic level i.e., the plant, by modifying plant tissues macronutrient and defense content. This affects plant nutritional quality to herbivores (the second trophic level), leading to changes in their survival,

development, and reproduction (Han et al., 2020; Inbar et al., 2001; Schoonhoven et al., 2005).

Decreased N fertilization application directly reduces herbivore fitness – the “*Nitrogen limitation hypothesis*” (White, 1993) – but it could have negative influence on the plant yield/or quality (Daugherty et al., 2007; Hosseini et al., 2018, 2019).

By affecting herbivore nutritional quality and/or abundance, fertilization-driven bottom-up forces could also indirectly alter the performance and abundance of natural enemies such as arthropod predators and parasitoids (Daugherty, 2011; Chesnais et al., 2016; Han et al., 2022).

Such effect could in turn affect the intensity of top-down regulation of pests by natural enemies (biological control). For instance, excessive nitrogen application to plants can reduce predation/parasitism rates and/or result in increased overall herbivore population load if natural enemies cannot cope with increased pest population growth (Moon et al., 2000; Zaller et al., 2009; Hosseini et al., 2010). Correspondingly, decreased N fertilization, by reducing herbivore fitness, could increase predator efficiency (Couture et al., 2010; Aqueel et al., 2012). In contrast, several studies indicate that parasitoids and omnivorous predators are not affected by different nitrogen fertilization levels (Dong et al., 2018; Han et al., 2015; Zaller et al., 2009). However, maximum suppression of pests by carnivorous predators was observed at intermediate nitrogen levels (Fallahpour et al., 2020; Hosseini et al., 2018). These contrasted results may be due to specific responses of different natural enemy guilds to changes in fertilization levels, in terms of development, longevity, reproduction and biocontrol efficiency.

The aim of the present study was to investigate the bottom-up effects of reduced inorganic fertilization (from standard commercial levels to intermediate to no fertilization) across a

variety of tri-trophic “tomato plant - pest - natural enemies” systems, to determine how the diet regime (carnivorous versus omnivorous predators) or the functional guild (predator versus parasitoid) modified the intensity of bottom-up effects using seven systems, we measured: (1) key natural enemy life-history traits, that is, development, longevity and fertility, and (2) their biocontrol efficiency (i.e., parasitism rate or predation rate). We also recorded plant and herbivore traits to confirm the presence of bottom-up forces in these lower trophic levels.

2 Materials and Methods

2.1 Plant materials and fertilization treatments

The tomato plants (*Solanum lycopersicum* L. cv. Nano, Solanaceae) were grown from seeds in a climatic chamber (L:D 12:12, $24 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH) as reported by Han et al. (2014; 2020), at INRAE ISA, Sophia-Antipolis, France. Eight days after sowing, the seedlings were transplanted to individual pots (7 x 7 x 6.5 cm) containing 1:1 potting soil (Tonusol, Draguignan, France) and perlite grains (Perlite Italiana srl, Corsico, Italy). 24 days after sowing, plants were transferred to larger pots (diameter: 10 cm, height: 9 cm) filled with the same substrate (Fig. S1). From the first transplantation onwards, a full nutrient solution adjusted to pH 5.5. was supplied daily to tomato seedlings randomly assigned to high fertilization (HF, commonly used fertilization level in commercial conditions, formulation described in Han et al. 2014), medium fertilization (MF, a 50% dilution of HF), or control (W: water only, no nutrient). Each treatment was applied to 24 plants, making it 72 plants in total for each trophic system. The amount of nutrient solution supplied to plants increased daily to match plant needs.

The quality of the fertilization treatments was monitored by controlling nutrient solution electric conductivity, which was around 1.7 and 0.9 mS for the HF and MF treatments, respectively.

To confirm the effect of fertilization treatments on tomato seedlings, plant growth (plant height and number of nodes) was monitored twice in 12 randomly selected plants per treatment (30 and 50 days after sowing; Fig. S1). In addition, 50 days after sowing, the first four leaves from the base of each plant were sampled and immediately frozen into liquid nitrogen and stored at -80°C before analyses. To measure the nitrogen (N) and carbon (C) content, the sampled leaves were lyophilized then ground into fine powder using a ball mill MM301 (Retsch, Germany) and stored individually in small tubes. Leaf N and C content (gram per 100 g of dry mass) were measured with an elemental analyser (Flash EA1112 Series, ThermoFinnigan, Milan, Italy) at INRAE-PSH Avignon, France.

2.2 Insect models

Pest and natural enemy colonies were maintained in separated climatic chambers (L:D 16:8, 25 ± 1 °C, 70 ± 10 % RH) at INRAE ISA

Seven different tri-trophic systems were studied, where the plant was always tomato: (1) the aphid sap-feeder *Macrosiphum euphorbiae* (Hemiptera: Aphididae) with the parasitoid *Aphidius ervi* (Hymenoptera: Braconidae); (2) the whitefly sap-feeder *Bemisia tabaci* (Hemiptera: Aleyrodidae) with the parasitoid *Encarsia formosa* (Hymenoptera: Aphelinidae); (3) *M. euphorbiae* with the carnivorous ladybeetle predator *Adalia bipunctata* (Coleoptera:

Coccinellidae); (4) the leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) with the carnivorous lacewing predator *Chrysoperla carnea* (Neuroptera: Chrysopidae); (5) *M. euphorbiae* with the omnivorous mirid predator *Macrolophus pygmaeus* (Hemiptera: Miridae); (6) *B. tabaci* with *M. pygmaeus*; and (7) *T. absoluta* with the omnivorous mirid predator *Dicyphus errans* (Hemiptera: Miridae).

Pests – Colonies of *M. euphorbiae*, *B. tabaci* and *T. absoluta* were initially started from field-collected materials. They were reared in cages on 30-day-old tomato plants treated with one of the three fertilization levels (i.e., high fertilization, medium fertilization, or water) for at least five generations before use in the experiments.

Natural enemies – All natural enemies were provided by the Bioplanet company (Italy), and then reared in the laboratory. Parasitoids *A. ervi* were reared on the pea aphid *Acyrtosiphon pisum* (Hemiptera: Aphididae) maintained on potted broad bean plants (*Vicia faba* L.; Fabaceae). The *E. formosa* colony was established on *B. tabaci*, reared on tobacco plants (*Nicotiana tabacum* L. Solanaceae).

A colony of *A. binpunctata* was maintained on *M. euphorbiae* reared on tomato plants. Adults of *M. pygmaeus* and *D. errans* were reared on tomatoes with eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). *C. carnea* larvae were maintained in Petri dishes (3 cm diameter) with *E. kuehniella* eggs and a tomato leaf with mesh lids for ventilation.

2.3 Experimental design

All experiments were conducted in laboratory conditions from 50 days after plant sowing (around 10 leaves per tomato plants; Fig. S1) using either entire plants or leaves or leaflets, maintained in their respective fertilization regime for the whole experiment duration. Table S1 and S2 summarizes experimental microcosms and conditions used for each pest-natural enemy system. We first determined how tomato plants submitted to the three fertilization treatments influenced herbivore fecundity. For this, five 3rd-4th nymphs of *M. euphorbiae*, ten couples of *B. tabaci* adults or five couples of *T. absoluta* adults were placed inside a double-cup microcosm containing a fresh tomato leaflet to produce offspring and oviposition (7, 2, 2 days respectively). Then, we determined how herbivores reared on tomato plants under the three different fertilization regimes influenced the development (from egg-pupa-adult or nymph-adult), longevity (from female adulthood to death), reproduction (until female adults die) and biocontrol efficiency (2 days respectively) of natural enemies in each of the seven systems.

2.3.1 *Macrosiphum euphorbiae* Aphid-based experiments

Aphid reproduction - Five 3rd-4th nymphs of *M. euphorbiae* were randomly collected from the rearing cages with the different fertilization treatments (high fertilization, intermediate fertilization, and water) and carefully introduced to a tomato leaflet in a double-cup microcosm (Table S1). Aphids were allowed to reproduce for seven days, then the number of offspring was counted through visual inspection of the whole tomato leaflet. 15 replicates were prepared per fertilization treatment.

***M. euphorbiae* – *A. ervi* experiments** – A first experiment measured parasitoid **biocontrol efficiency** and **development** (Table S2). Mummies of *A. ervi* were randomly selected from the rearing cages and were kept individually in plastic capsule (24 x 10.5mm) and emergence was checked daily. After emergence, adult parasitoids were examined under a binocular microscope to determine their sex. From the emerged parasitoids, 1 male and 1 female were kept together in test tubes for 24 h (diameter 2 cm, length 7 cm) closed with a cotton plug soaked in an 80:20 water: honey mixture as food source. The next day, the mated pair of *A. ervi* wasps was introduced in a double-cup microcosm for 24 h containing a tomato leaflet with 50 *M. euphorbiae* 3rd-4th instar nymphs coming from one of the three colonies differing in fertilization matching the treatment of the tomato leaflet. The tomato leaves were changed every 5 days. Aphids were observed daily until formation of mummies. The numbers of mummies were counted and each mummy was kept individually in a Petri dish. Emerged parasitoids were counting and the sex ratio was determined. The following parasitoid life-history parameters were then calculated: (1) the parasitism rate (no. of mummies/50 initial aphid individuals); (2) the development time (from mummification to adult emergence) in days; (3) the emergence rate expressed as a percentage (no. of emerged parasitoids/no. of mummies × 100). 25 replicates were used per fertilization treatment.

Second, 10 female adults of *A. ervi* (unmated) were placed in a double-cup microcosm with tomato leaves and 50 *M. euphorbiae* of 3rd-4th instar nymph to measure parasitoid **longevity**. The tomato leaves and aphids were replaced every 5 days. We checked the number of live

parasitoid individuals daily until the last one died and recorded the number of days from adult emergence to death (adult longevity). 15 replicates were used per fertilization treatment.

***M. euphorbiae* – *A. bipunctata* experiments** - In the first experiment, we determined how *M. euphorbiae* reared on *S. lycopersicum* with different fertilizer inputs influenced the **development** time of *A. bipunctata* from egg to pupa and from egg to adult. One *A. bipunctata* egg was introduced in a double-cup microcosm along with 50 *M. euphorbiae* 3rd-4th instar nymphs. The newly emerged *A. bipunctata* adults were kept individually in Petri dishes (diameter 3 cm) to determine their sex and their weight. 18, 22 and 20 replicates were used for the water, intermediate fertilization and high fertilization treatments, respectively.

In a second experiment measuring *A. bipunctata*'s **predation rate**, the newly emerged *A. bipunctata* female adults were transferred into a new double-cup microcosm with 100 *M. euphorbiae* of mixed developmental stages, representing *ad libitum* resource (since there was always remaining aphids at the end of the feeding trial). The cups were checked 48 h after starting the feeding trial, and all remaining aphids were counted. 25 replicates were used per treatment.

Fecundity was measured in a third experiment, in which pairs of *A. bipunctata* adults (one male, one female) were selected from the colony cages under the different fertilization treatments and transferred into a double-cup microcosm. Each pair was then provisioned daily with 100 *M. euphorbiae* of mixed stages, obtained from the corresponding fertilization treatments. After one week allowing for repeated mating events, pairs were separated and females were isolated in the microcosm and fed daily with 100 aphids. We recorded the number of eggs produced

(**fecundity**), until the female stopped laying eggs (about 20 days). 10 replicates were used per treatment.

***M. euphorbiae* – *M. pygmaeus* experiments**

For the first experiment, one *M. pygmaeus* at 1st nymphal stage was placed in a plastic box with 50 *M. euphorbiae* 3rd or 4th instars from one of the treated rearing and leaflets obtained from the respective fertilization treatments tomato leaflets and *M. euphorbiae* were replaced every 3 days. The predator was observed daily to record its **development** time from 1st nymphal stage to adult stage. New *M. pygmaeus* adults were transferred into test tubes for sex identification and female adults were weighed. 10 replicates were used per treatment.

In a second experiment measuring **predation rate**, a female adult starved for a 24 h-period was transferred into a plastic box with 50 *M. euphorbiae* 3rd-4th instar nymphs on a tomato leaflet, and the number of remaining aphids after 48 h was counted. 20 replicates were used per treatment.

2.3.2 *Bemisia tabaci* whitefly-based experiments

Whitefly-based experiments with carnivorous predators were not performed because the identified carnivorous species (*Delphastus pusillus*) was not available for rearing during the experimental period.

Whitefly fecundity - Ten pairs of *B. tabaci* adults were randomly selected from the rearing cages under different fertilization treatments and carefully introduced in a double-cup

microcosm with a tomato leaflet. After 48 h, the number of laid eggs was recorded by examining the entire tomato leaflet under a microscope. 20 replicates were used per treatment.

***B. tabaci* – *E. formosa* experiments** - Ten *B. tabaci* first instar nymphs (mobile stage) were distributed equally among the leaflet of tomato plant with a fine brush, and nymph survival was checked 2 hours later under a microscope to ensure effective settlement of the nymphs.

The infested leaflet, still fixed on the plant, was then enclosed into a plastic box. Plants were then maintained for 7 days under their respective fertilization treatment, allowing sufficient time for *B. tabaci* nymphs to reach the 3rd instar. *Encarsia formosa* parasitoids at the pupal stage were added from the rearing cages of the corresponding fertilization treatment, and were kept individually in a plastic capsule where parasitoid emergence was checked daily. One newly emerged (less than 24h-old) *E. formosa* adult was then released in each box. After 24 h, wasps were removed from the boxes and the nymph were left on the leaflet. On the following days, the whitefly nymphs were incubated until all wasp progeny had pupated. We recorded the number of wasp pupae of *E. formosa*, and of unparasitized and dead *B. tabaci* nymphs.

Then, the parasitoid progeny was moved into another plastic box and we recorded the number of parasitoid wasps hatched and the duration from egg to pupal stage, and from pupa to adult emergence. The following parasitoid life-history parameters were then calculated: (1)

Parasitism rate and **host feeding rate** were calculated by determining the number of whitefly nymphs that were parasitized compared to unparasitized, and the number of nymphs consumed by the parasitoid, respectively. If whitefly hosts were killed by host-feeding, their bodies became flat and desiccated, making them visible under a stereoscopic microscope (Zang et. al.,

2008); (2) the **development time** (from cocoon to adult emergence) in days; and (3) the **emergence rate** expressed as a percentage (no. of emerged parasitoids/no. of cocoons \times 100).

10 replicates were used per fertilization treatment.

***B. tabaci* – *M. pygmaeus* experiments** - This omnivorous predator experiment is similar to the *M. euphorbiae* – *M. pygmaeus* experiment. Instead of aphids, 40 *B. tabaci* 1st instar nymphs were provided. We replaced the leaflets and the 40 *B. tabaci* every 3 days and we observed *M. pygmaeus* every day until the adult stage to measure *M. pygmaeus* **development time** from the 1st nymphal stage to the adult stage. New *M. pygmaeus* adults were transferred into test tubes for sex identification and weighed. 15 replicates were used per fertilization treatment.

After emergence, a female adult starved for a 24 h period was transferred into a plastic box with 40 *B. tabaci* 1st instar nymphs on a tomato leaflet (representing *ad libitum* food source to the predator) to measure the **predation rate**. The leaflets were checked 48 h later, and all remaining whiteflies were counted. 15 replicates were used per fertilization treatment.

The **fertility** of *M. pygmaeus* was measured by confining them in a plastic box on an entire plant. A pair of two *M. pygmaeus* adults (one female, one male) were enclosed in the plastic box and supplied with 40 *B. tabaci* nymphs provisioned at 2-day intervals. After 48 h of confinement allowing for repeated mating events, pairs were separated and males were removed, while females were fed every 2 days with 40 *B. tabaci* nymphs. Emerging *M. pygmaeus* nymphs within the microcosms were counted and removed daily until the female adults died, thereby also providing a measure of female adult **longevity**. 20 replicates were used per fertilization treatment.

2.3.3 *T. absoluta* Leaf miner-based experiments

Leaf miner-based experiments with parasitoids were not performed because the identified species (*Necremnus tutae*) was not available for rearing during the experimental period.

***T. absoluta* fecundity** – Five couples of *T. absoluta* adults were placed inside a double-cup microcosm containing a fresh tomato leaflet to enable mating and oviposition (adapted from Chailleux et al 2013). After 2 days of laying, adults were removed and eggs were counted. An average number of eggs per females for each replicate was performed. 15 replicates were used per fertilization treatment.

***T. absoluta* – *C. carnea* experiments** - To estimate the **predation rate** of *C. carnea* larvae on *T. absoluta* eggs, *C. carnea* 2nd-3rd instar larvae were starved for 12 h and then placed individually in plastic boxes provided with 100 *T. absoluta* eggs on tomato leaflets. The number of remaining *T. absoluta* eggs was counted after 48 h (Ismoilov et al 2020). 10 replicates were used per fertilization treatment.

***T. absoluta* - *D. errans* experiments** - This omnivorous predator assay is similar to the one described for aphids in plastic boxes but with another mirid species, *D. errans*. The predator nymph was provisioned with 30 *T. absoluta* eggs on tomato leaflets. Both leaflets and *T. absoluta* eggs were replaced every 3 days. *D. errans* **development time** was recorded daily until nymphs reaching adulthood. Adults were then weighed individually and transferred into test tubes for sex identification. 10 replicates were used per fertilization treatment.

Then, female adults, starved for 24 h, were kept in plastic boxes with 100 *T. absoluta* eggs on tomato leaflets. The leaflets were checked 24 h later and the number of preyed eggs were

counted as depleted egg shells. The **predation rate** was calculated as the number of preyed eggs /100 initial leaf miner egg individuals. 13 replicates were used per fertilization treatment. Finally, 3 days-old female and male *D. errans* adults were enclosed in a plastic box during 48 h with three terminal tomato leaflets allowing for repeated mating events. Then the female was moved in a new plastic box supplied with 100 *T. absoluta* eggs on tomato leaflets every 2 days, until the mirid female died. **Fecundity** was measured as the number of newly hatched *D. errans* nymphs for each mated female. **Longevity** was measured as the number of days until the female died. 6 replicates were used for each fertilization treatment.

2.5 Statistical analysis

All analyses were performed using the R software (R Core Team, 2023). We used linear mixed models (LMMs; function ‘lmer’; R library ‘lme4’; Bates et al., 2014) for plant traits (plant height and number of nodes), with the fertilisation treatment, time and their interaction as fixed effects. To analyse leaf C and N content, we performed Beta regression models with categorical independent variables, i.e., generalized linear models (GLMs) with a Beta error structure (function “glm” with link function “betareg”; R library “stats”; R Core Team 2023). The leaf C/N ratios were converted to logarithmic values to fit a general linear model (GLM) with a Gaussian family. The offspring numbers of *M. euphorbiae*, and the egg production of *B. tabaci* and of *T. absoluta* were analyzed using GLMs with a Poisson error structure. The absence of residual heteroscedasticity and overdispersion was verified in the best model using the functions ‘simulateResiduals’ and ‘testDispersion’ (library ‘DHARMA’; Hartig, 2022). We

also assessed how the fertilization treatment affected natural enemies' development time (i.e., eggs reaching pupa or adulthood) and longevity using Cox Proportional Hazards models (function 'coxph'; R Core Team, 2023). Then, we assessed how the fertilization level affected the emergence rate, parasitism rate or predation rate using generalized linear mixed-effect models (GLMMs) with a Poisson error structure. Finally, to compare across trophic systems, we calculated the effect sizes using Hedge's d between medium and high fertilization treatments, between water and medium fertilization treatments, and between water and high fertilization treatments for each natural enemy functional guild (parasitoids, carnivorous predators and omnivorous predators) and each natural enemy trait (Rosenberg, 2022).

3 Results

3.1 First trophic level: bottom-up effects of reduced fertilization effect on plant traits

Absence of fertilization significantly reduced plant height and node number compared to the intermediate and high fertilization treatments, and this became more pronounced at DAS 50 compared to DAS 30, leading to a significant fertilization * time interaction (plant height: $F_{2,66} = 38.6$, $P < 0.001$; number of nodes: $F_{2,66} = 32.1$, $P < 0.001$; Table S3, Fig. 1A, B). However, the MF and HF treatments did not differ significantly.

Leaf N content (dry mass percent) was significantly reduced with reduced fertilization ($z = 5.99$, $P < 0.001$; Table S4, Fig. 1C). Leaf C content (dry mass percent) was significantly reduced with reduced fertilization ($z = 6.01$, $P < 0.001$). This led to the leaf C/N ratio to be significantly higher in water control plants than in fertilized plants ($t = 44.6$, $P < 0.001$), although it was not different between medium and high fertilization treatments (Fig. 1D).

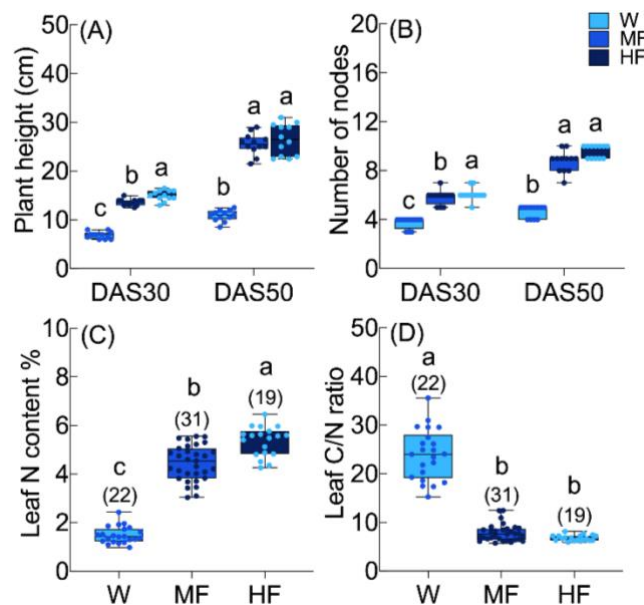


Fig 1. Plant height (A), and number of nodes (B) of tomato seedlings 30 (DAS30) and 50 (DAS50) days after sowing under different fertilization levels (‘W’: water treatment; ‘MF’: medium fertilization; ‘HF’: high fertilization). n = 12 replicates per date and fertilization level. (C) Leaf N content (in dry mass percent: g/100g MS) and (D) Leaf C/N ratio (unit-less) of tomato leaf at DAS 50 before the artificial infestation of pests. “*” indicated significant difference at $P < 0.05$.

Table S3. The effect of fertilization, time, and fertilization x time interaction on plant height and number of nodes per plant. General linear models (GLMs) with a Gaussian (plant height) or a Poisson (number of nodes) family were used. Significant differences are shown in bold. ‘W’: water treatment; ‘MF’: medium fertilization; ‘HF’: high fertilization.

GLMER test	Plant height		Plant nodes	
	<i>F</i> value	<i>P</i> value	<i>F</i> value	<i>P</i> value
<i>Plant growth</i>				
Fertilization	334.2	< 0.001 ***	267.19	< 0.001 ***
Time	488.1	< 0.001 ***	313.18	< 0.001 ***
Fertilization×Time	38.6	< 0.001 ***	32.07	< 0.001 ***

** $: P < 0.01$; *** $: P < 0.001$.

Table S4. Bottom-up effects of fertilization on plant nutritional quality of non-infested leaves (in dry mass percent: g/100g MS, means \pm SEM). ‘W’: water treatment; ‘MF’: medium fertilization; ‘HF’: high fertilization. Leaf C, leaf N content by fit a Beta regression model with categorical independent variables, link function "betareg". Transform leaf C/N ratio into logarithmic values and fit a general linear model (GLM) with a Gaussian family.

Treatments	N	Leaf N (% Dry mass)	Leaf C (% Dry mass)	Leaf C/N
W	22	1.50 \pm 0.07 c	34.37 \pm 0.31 b	23.96 \pm 1.11 b
MF	31	4.45 \pm 0.13 b	34.80 \pm 0.46 b	7.84 \pm 0.31 b
HF	19	5.36 \pm 0.14 a	36.18 \pm 0.37 a	6.81 \pm 0.14 a
<i>P-value (comparison between fertilization inputs)</i>		< 0.001 ***	< 0.001 ***	< 0.001 ***
<i>Statistics results</i>		<i>z-value</i> = 5.987	<i>z-value</i> = 6.005	<i>t-value</i> = 44.605

: $P < 0.01$; *: $P < 0.001$.

3.2 Second trophic level: effects of reduced fertilization on pest fecundity

Reduced fertilization significantly reduced aphid offspring number ($\chi^2 = 81.2$, $df = 2$, $P < 0.001$; Table S5; Fig. 2A). The fertilization treatment also significantly affected egg production in *B. tabaci* ($\chi^2 = 44.6$, $df = 2$, $P < 0.01$). The number of *B. tabaci* eggs was the highest in the intermediate fertilization treatment, while numbers in the high fertilization versus water treatments were not different (Table S5; Fig. 2B). Similarly, the fertilization treatment significantly affected egg production in *T. absoluta* ($\chi^2 = 18.9$, $df = 2$, $P < 0.01$), and the number of *T. absoluta* eggs was the highest in the intermediate fertilization level, but egg numbers in the high fertilization versus water treatments were not different (Table S5; Fig. 2C).

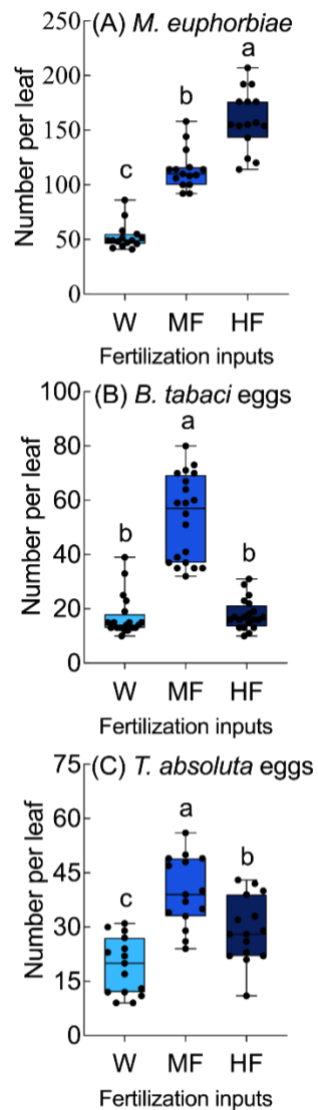


Fig 2. Effects of reduced fertilization on pest fecundity: (A) number of *M. euphorbiae* offsprings per leaf (n = 15); (B) number of *B. tabaci* eggs per leaf (n = 20), and (C) number of *T. absoluta* eggs per leaf (n = 15). ‘W’: water treatment; ‘MF’: Medium fertilization; ‘HF’: high fertilization. Significant differences between fertilization inputs are shown with different letters (a, b) above bars (mean comparisons; Table S4).

Table S5. Comparison of means between fertilization treatments for *M. euphorbiae*, *B. tabaci* and *T. absoluta* fecundity experiments (Tukey tests). Significant differences are shown in bold.

Tukey test	Difference [95CI]	P
<i>M. euphorbiae</i> fecundity		
W - MF	-61.3 [-79.2; -43.4]	< 0.001 ***
W - HF	-107.1 [-125.0; -89.2]	< 0.001 ***
MF - HF	-45.7 [-63.6; -27.8]	< 0.001 ***
<i>B. tabaci</i> fecundity		
W - MF	- 34.5 [- 44.0; -43.4]	< 0.001 ***
W - HF	- 0.8 [-11.0; 9.3]	0.978
MF - HF	33.7 [24.8; 42.5]	< 0.001 ***
<i>T. absoluta</i> fecundity		
W - MF	18.0 [10.4; 25.7]	< 0.001 ***
W - HF	10.3 [0.9; 19.6]	< 0.05 *
MF - HF	7.8 [-1.0; 16.5]	0.090

*: 0.05; **: $P < 0.01$; ***: $P < 0.001$

3.3 Third trophic level: Fertilization bottom-up effects on natural enemies

3.3.1. Parasitoids

Fertilization significantly influenced *A. ervi*'s development time from egg to pupa ($\chi^2 = 64.1$, $df = 2$, $P < 0.001$) and longevity ($\chi^2 = 170$, $df = 2$, $P < 0.001$). The development of *A. ervi* offspring was slowed down in the water treatment compared to the medium and high fertilization treatments (Fig. S3A). Fertilization also significantly affected longevity ($\chi^2 = 170$, $df = 2$, $P < 0.001$, Fig. S3B). Adults lived the longest in the medium fertilization treatment, followed by the high fertilization then water treatment. However, the parasitism rate was not affected by fertilization levels ($\chi^2 = 0.787$, $df = 2$, $P = 0.67$; Fig. S3C), and neither was the adult emergence rate ($\chi^2 = 4.47$, $df = 2$, $P = 0.11$; Fig. S3D).

Fertilization significantly decreased *E. formosa*'s development time (egg to pupa: $\chi^2 = 9.88$, $df = 2$, $P < 0.01$, Fig. S4A; egg to adult: $\chi^2 = 23.1$, $df = 2$, $P < 0.001$, Fig. S4B). However, fertilization did not influence the adult emergence rate ($\chi^2 = 4.47$, $df = 2$, $P = 0.11$; Fig. S4E) and neither the parasitism rate ($\chi^2 = 2.82$, $df = 2$, $P = 0.24$; Fig. S4C). Finally, the host feeding was similar across the three treatments, although there was a tendency for increased predation under intermediate fertilization conditions, i.e., when more whitefly offsprings were produced (Fig. S4D).

3.3.2. Carnivorous predators

The absence of fertilization significantly increased *A. bipunctata*'s development time (egg to pupa: $\chi^2 = 12.2$, $df = 2$, $P < 0.01$, Fig. S3E; egg to adult: $\chi^2 = 9.19$, $df = 2$, $P < 0.01$, Fig. S3F) and significantly decreased the weight of females and males' adults when compared with the high fertilization treatment. The predation rate of *A. bipunctata* females was highest in the water treatment (82%), lowest in the high fertilization treatments (64%), and intermediate but highly variable in the medium fertilization treatment (73%; $\chi^2 = 0.117$, $df = 2$, $P < 0.001$; Fig. S3I). Finally, the absence of fertilization significantly reduced *A. bipunctata*'s fertility compared to higher and intermediate fertilization levels ($\chi^2 = 22.0$, $df = 2$, $P < 0.001$; Fig. S3J). *Chrysoperla carnea*'s predation rate was significantly higher in the high fertilization treatment (95%) compared to the water treatment (88%), while the predation rate was intermediate in the intermediate fertilization treatment (71%; $\chi^2 = 9.07$, $df = 2$, $P = 0.011$; Fig. S5F).

3.3.3 Omnivorous predators

Reduced fertilization tended to increase *M. pygmaeus*' development duration from nymph to adulthood when provided with *M. euphorbiae*, although this was not significant (Water 19.0 ± 0.5 days, intermediate fertilization 18.0 ± 0.5 days, high fertilization 17.7 ± 0.3 days; $\chi^2 = 0.602$, $df = 2$, $P = 0.74$, Fig. S3K). The fertilization treatment did not either affect *M. pygmaeus* adult weight, despite a high variability observed in the high fertilization treatment ($\chi^2 = 0.0640$, $df = 2$, $P = 0.968$, Fig. S3L). *M. pygmaeus*' predation rate was not affected by the fertilization level ($\chi^2 = 0.0265$, $df = 2$, $P = 0.987$, Fig. S3M).

The fertilization treatment significantly affected *M. pygmaeus*' development time from nymph to adult when provided with *B. tabaci*, with a longer development time in water and intermediate fertilization treatments compared to the high fertilization treatment ($\chi^2 = 32.8$, $df = 2$, $P < 0.001$, Fig. S4F). In addition, the water treatment significantly increased *M. pygmaeus*' female adult longevity compared to intermediate fertilization ($\chi^2 = 4.24$, $df = 2$, $P = 0.006$; Fig. S4H). However, the fertilization treatment did not affect the weight of the *M. pygmaeus* adults (females and males' mixture) ($\chi^2 = 2.41$, $df = 2$, $P = 0.30$; Fig. S4G) and neither the fertility ($\chi^2 = 2.53$, $df = 2$, $P = 0.28$; Fig. S4J). Finally, female *M. pygmaeus*' predation rate was significantly higher in the intermediate fertilization treatment (53%), followed by the water (42%) and high fertilization treatments (37%; $\chi^2 = 0.797$, $df = 2$, $P = 0.018$; Fig. S4I).

Reduced fertilization significantly increased *D. errans*' development time between the high fertilization and water treatments ($\chi^2 = 3.92$, $df = 2$, $P = 0.007$; Fig. S5A). The high fertilization treatment also increased *D. errans* adults' weight ($\chi^2 = 14.1$, $df = 2$, $P < 0.01$; Fig. S5B). However, the fertilization treatment did not affect female adult longevity, fertility nor predation

rate (longevity: $\chi^2 = 0.264$, $df = 2$, $P = 0.88$, Fig. S5C; fertility: $\chi^2 = 1.50$, $df = 2$, $P = 0.47$, Fig.

S4D; predation rate: $\chi^2 = 1.29$, $df = 2$, $P = 0.53$, Fig. S5E).

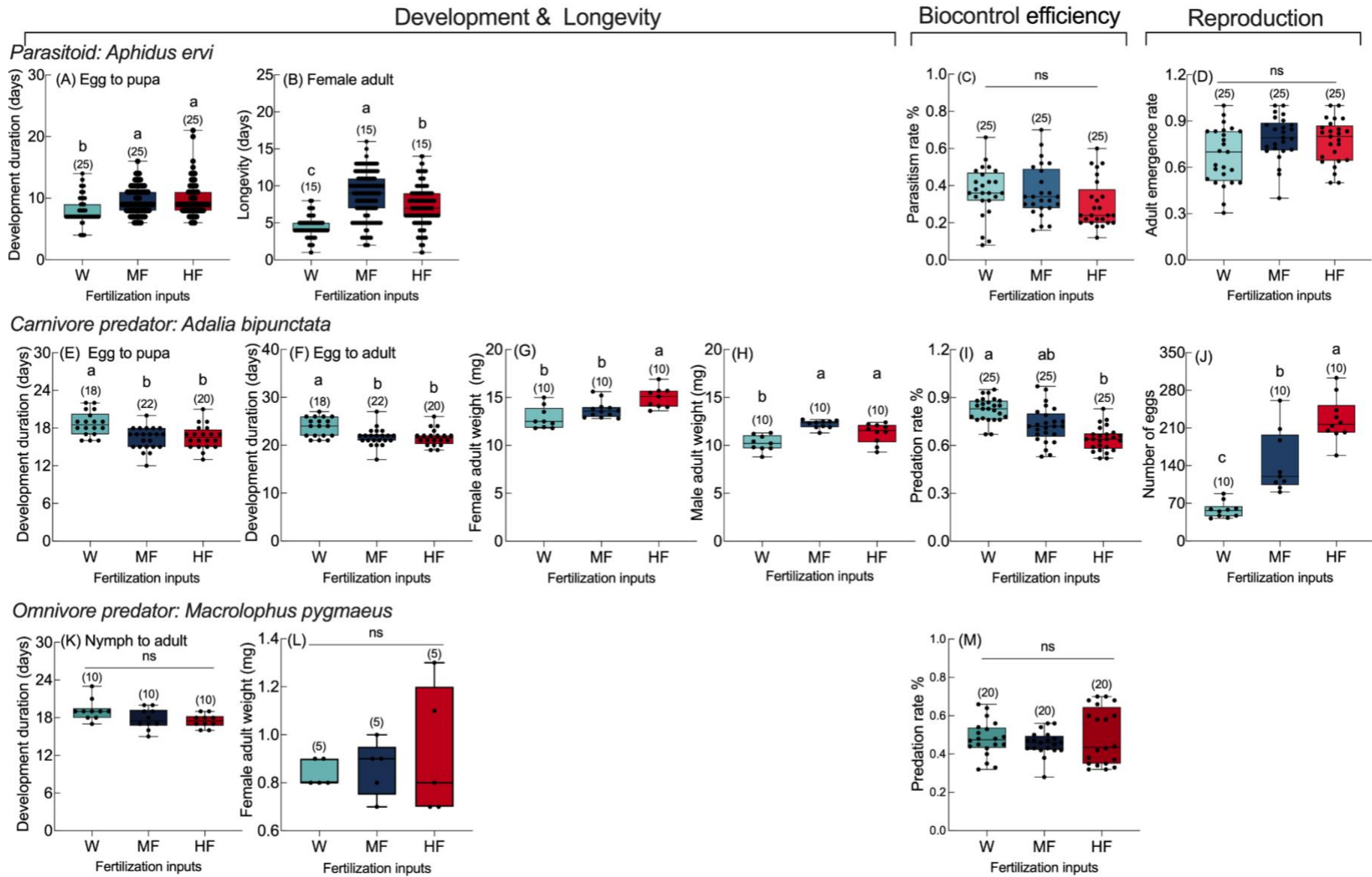


Fig S3. Effects of fertilization on natural enemy life history traits and biocontrol efficiency on aphids. Parasitoid: the development duration of *A. ervi* from egg to pupa stage (A), *A. ervi* adult longevity (B), parasitism rate of *Aphidius ervi* on *Macrosiphum euphorbiae* (C), and *A. ervi* adult emergence rate (D); Carnivore predator: the development duration of *A. bipunctata* from egg to pupa stage (E), the development duration of *A. bipunctata* from egg to adult stage (F), *A. bipunctata* female adult weight (G), *A. bipunctata* male adult weight (H), *Adalia bipunctata* female adult predation rate (I), *A. bipunctata* female fecundity (J); Omnivore predator: The development duration of *M. pygmaeus* from 1st nymphal stage to adult stage (K), and *M. pygmaeus* female adult weight (L) *Macrolophus pygmaeus* female adult predation rate (M). ‘W’ : water treatment; ‘MF’ : Medium fertilization; ‘HF’ : high fertilization. Numbers in parentheses show sampling sizes for each group. Significant differences between fertilizations are shown with different letters above bars.

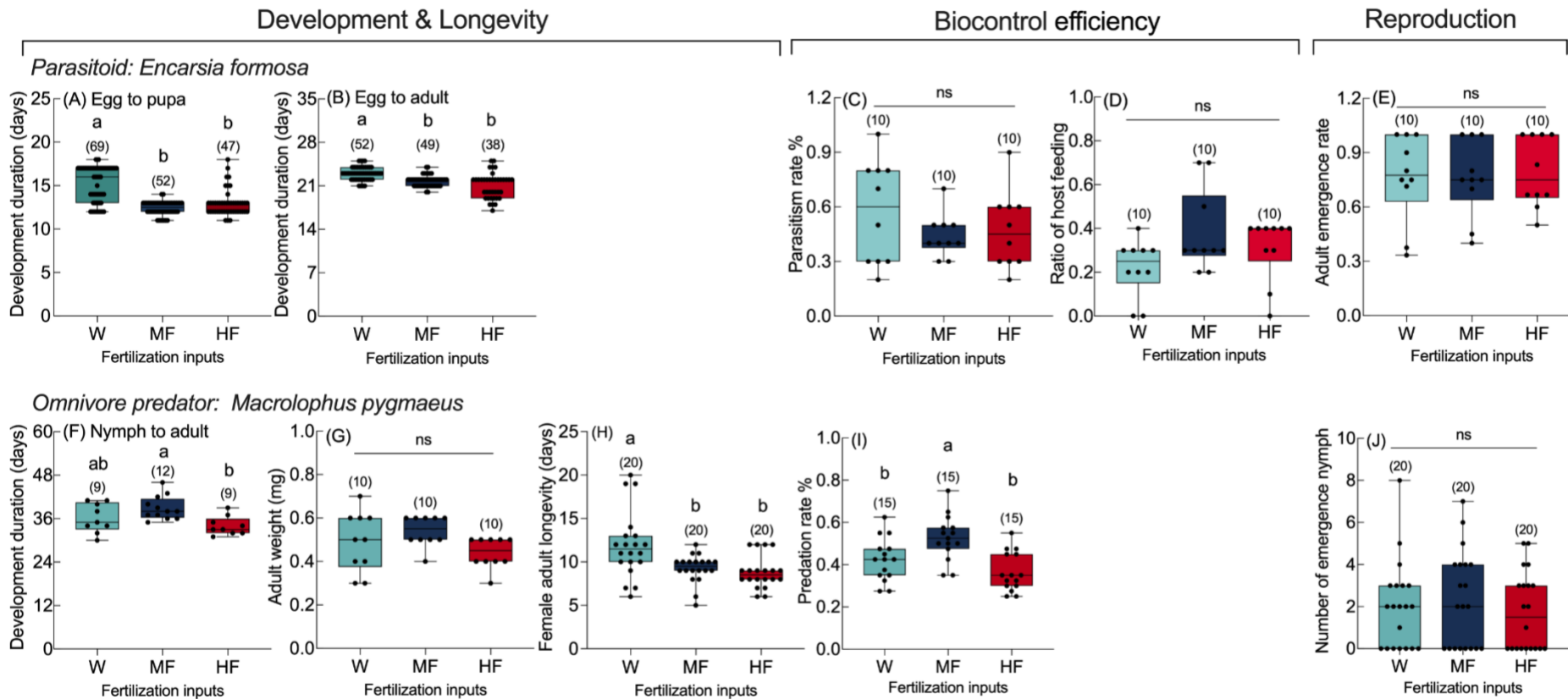


Fig S4. Effects of fertilization on natural enemy life history traits and biocontrol efficiency on whiteflies. Parasitoid: the development time of *E. formosa* from egg to pupa stage (A), the development time of *E. formosa* from egg to adult stage (B), parasitism rate of *Encarsia formosa* on *Bemisia tabaci* (C), the percentage of *B. tabaci* nymph consumed by *E. formosa* (D), *E. formosa* adult emergence rate (E); Omnivore predator: the development time of *Macrolophus pygmaeus* from 1st nymphal stage to adult stage (F), *M. pygmaeus* adult weight (G), *M. pygmaeus* female adult longevity (H), and *M. pygmaeus* female adult predation rate (I), number of *M. pygmaeus* 1st stage nymphs emerged (female adult fecundity) (J).

‘W’: water treatment; ‘MF’: Medium fertilization; ‘HF’: high fertilization. Numbers in brackets show sampling sizes for each group. Significant differences between fertilizations are shown with different letters above bars.

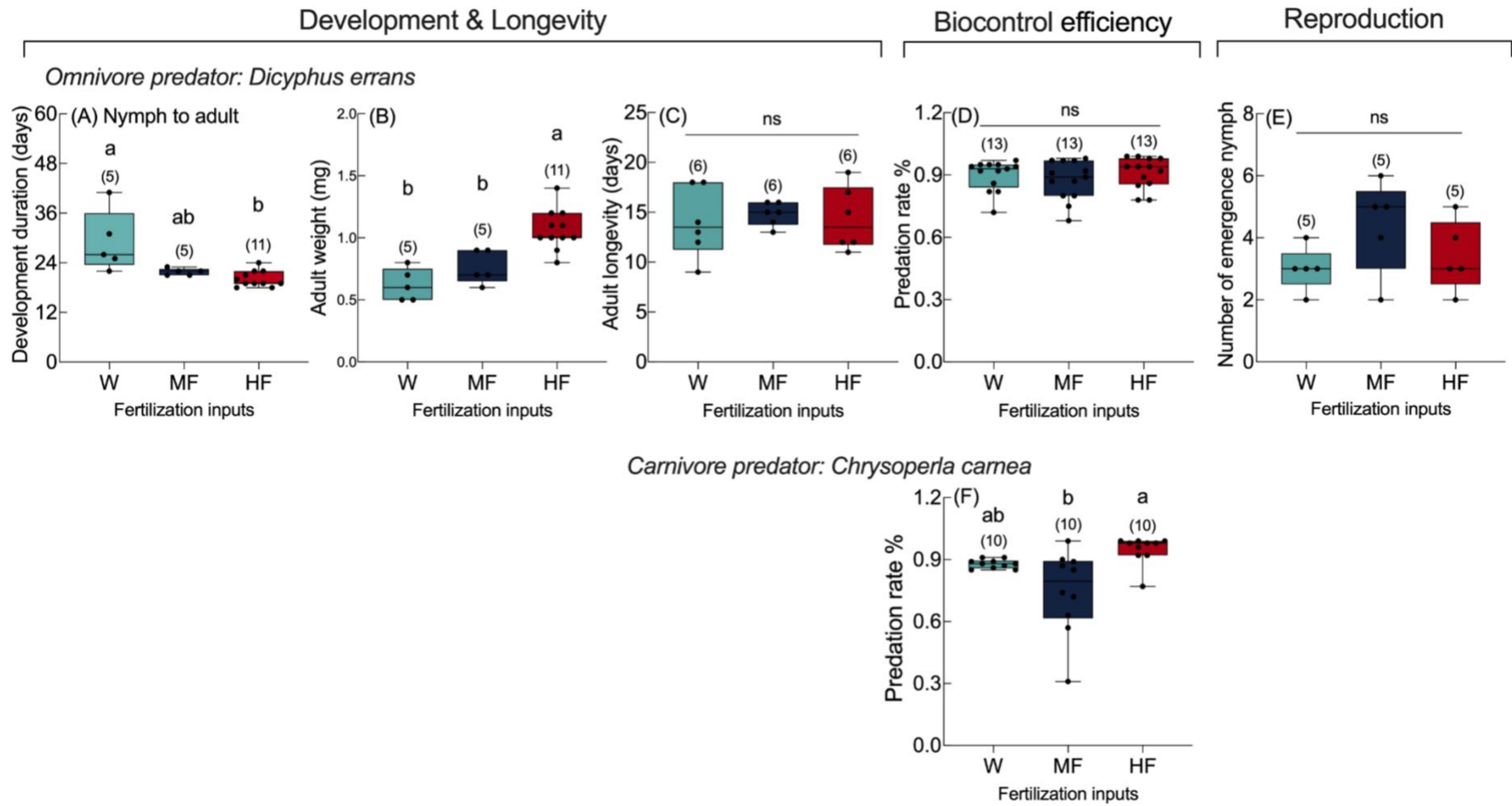


Fig S5. Effects of fertilization on natural enemy life history traits and biocontrol efficiency on *Tuta absoluta* eggs. Omnivore predator: the development duration of *Dicyphus errans* from nymph to adult stage (A), *D. errans* adult weight (B), *D. errans* female adult longevity (C), the predation rate of *D. errans* female adult on *T. absoluta* eggs (D), number of *D. errans* 1st stage nymphs emerged (female adult fecundity) (E); Carnivore predator: the predation rate of *Chrysoperla carnea* (Stephens) on *T. absoluta* eggs (F). ‘W’: water treatment; ‘MF’: Medium fertilization; ‘HF’: high fertilization. Numbers in parentheses show sampling sizes for each group. Significant differences between fertilizations are shown with different letters above bars.

3.4 Overall comparisons

The bottom-up effects of fertilization depended on the natural enemy fitness trait considered. Despite the large number of pest-natural enemy tested, the overall sampling size of effect sizes for some fitness traits and functional groups is too small to draw firm conclusions. Despite this, a number of trends are visible. First, fertilization bottom-up effects on natural enemy development were never different from zero (i.e., no effect) however the functional guild considered (Fig. 3A, D). Conversely, increasing fertilization always led to positive effects on natural enemy reproduction (Fig. 3B, E). Bottom-up effects of fertilization on biocontrol efficiency were the least consistent. Reducing fertilization from an intermediate level to no fertilization led to a response overall not different from zero (no effect) on the biocontrol efficiency of parasitoids and omnivorous predators, while it negatively affected the carnivore predator (but this would need to be replicated on other systems to allow a more robust conclusion; Fig. 3C). Reducing from high to intermediate fertilization led to an overall more consistent response, which tended to be marginally positive (Fig. 3F): in other words, reducing fertilization from a commonly used level to a 50% reduction should have a slightly positive impact on the reproduction and biocontrol efficiency of natural enemies of all functional guilds (Fig. 4H, I).

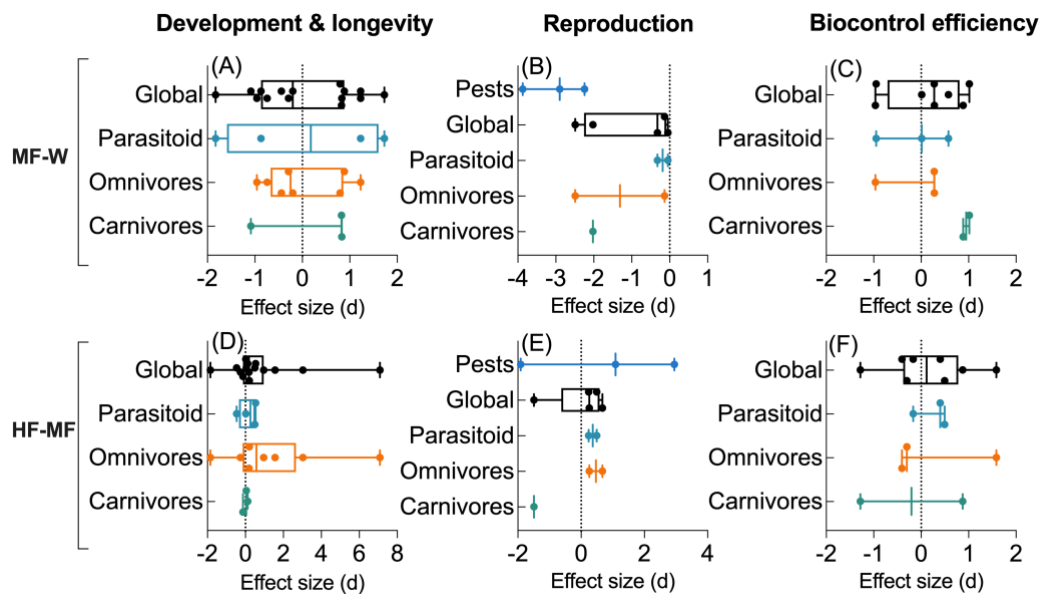


Fig 3. Mean effect sizes of fertilization on the seven pest-natural enemy systems. The effect of reducing fertilization from intermediate level (MF) to no fertilization (W), with MF serving as the reference on: (A) development and longevity traits, (B) reproduction, (C) biocontrol efficiency; the effect of reducing fertilization from high (HF) to intermediate (MF) level, with HF serving as the reference on: (D) development and longevity, (E) reproduction and (F) biocontrol efficiency.

4 Discussion

We investigated the bottom-up effects of reduced fertilization on a variety of tri-trophic plant-herbivore-natural enemy systems. We first confirmed alterations at the first trophic level, where reduced fertilization caused reduced tomato plant growth (Han et al., 2014, 2015, 2020). Leaf N content decreased with reduced fertilization levels while leaf C/N ratio increased as expected (Becker et al., 2021; Han et al., 2014, 2019). We expected modifications at the first trophic level to cause changes to the second trophic level (herbivores), which should provide avenues

for optimizing pest control in IPM as long as there are no strong negative effects on the third trophic level (natural enemies).

Fertilization bottom-up effects on herbivores. Overall, reduced fertilization decreased pest fecundity as expected. This was likely caused by the observed associated reduction in leaf nitrogen content, as found in previous studies (Scriber et al., 1981; Nevo et al., 2001; Davies et al., 2004). However, this was not true for *B. tabaci* from high to intermediate fertilization. Crafts-Brandner (2002) showed that the free amino acid content was markedly higher in *B. tabaci* adults feeding on cotton plants receiving high levels of nitrogen fertilization (as compared with plants receiving low levels of nitrogen fertilization). They found that *B. tabaci*'s fecundity was negatively correlated with plant total amino acid content due to increased excretion of excess amino acids through honeydew, which was suggested as evidence that whiteflies can adjust their metabolism in response to plant nitrogen content (Crafts-Brandner, 2002).

In an IPM perspective, where the manipulation of fertilization is a tool to optimize pest control, reducing pest fecundity through reduced crop plant fertilization is an encouraging avenue as long as it does not compromise yields. However, our results show that reduced fertilization does not always achieve positive outcomes in terms of reduced pest population dynamics.

Fertilization bottom-up effects on parasitoids. We found that reduced fertilization did not modify parasitoid biocontrol efficiency and neither reproduction (Fig. S3; S4). Our results are

in line with Becker et al (2021) who suggested a diluted bottom-up effect reaching the third trophic level, meaning that only major alterations in the second trophic level can influence the third trophic level.

Reduced fertilization from an intermediate level to no fertilization reduced development duration and reduced longevity in *A. ervi*. Conversely, the development duration of *E. formosa* was increased under reduced such fertilization. Plants indirectly influence parasitoids through the nutritional suitability of their host which is important for their development, survival, sex ratio, longevity, fecundity, and size (Vinson et al., 1980; Krauss et al., 2007; Winter et al., 2010; Chesnais et al., 2016). For koinobiont parasitoids, such as *A. ervi* and *E. formosa*, host quality is determined both by host resources at the time it is parasitized and resources acquired by the host during parasitoid development (Vinson et al., 1987; Mackauer et al., 1997). Therefore, reduced *A. ervi* development time and longevity under reduced fertilization is probably due to reduced host plant quality. However, *E. formosa* development duration was increased with reduced fertilization, but different parasitoid species can have different influences on their hosts by manipulating the amount of food consumed by parasitized hosts, their oral secretion, etc., which in turn affects the plant's ability to recognize the herbivore species and changes its defensive response (Tan et al., 2018; Poelman et al., 2011). Note that our natural enemy experiments tested the effects of host quality – not quantity – on biocontrol efficiency. In a study by Bentz et al. (1996), parasitism of whiteflies by the parasitoid *E. formosa* was higher on fertilized plants than on unfertilized plants. In field and laboratory studies the parasitoid

Diadegma insulare preferred hosts, diamondback moth (*Plutella xylostella* (L.)) from fertilized plants compared to hosts on unfertilized plants (Fox et al., 1996).

Finally, for *A. ervi*, the reduced development duration from intermediate fertilization to no fertilization (shorter generation time), coinciding with a reduced aphid fecundity, would be advantageous for biocontrol at the population dynamics level, but this is unclear how biocontrol would change over multiple generations in *E. formosa*. However, no adverse changes in either parasitoid species were observed when reducing fertilization from high to intermediate levels, therefore this would likely be beneficial for biocontrol efficiency in terms of population dynamics.

Fertilization bottom-up effects on omnivorous predators. Omnivorous predators need to acquire nutrients from both their host plants and their prey (Janssen et al., 2003). Their fitness (and their biocontrol efficiency to some extent) is strongly dependent on characteristics of the environment, notably the plant and prey qualities (Agrawal et al., 1999; Eubanks et al., 2000; Janssen et al., 2003) and therefore of abiotic factors such as fertilization (Gillespie et al., 2000; Sinia et al., 2004). We found that reduced fertilization increased development duration in all tri-trophic systems including omnivorous predators; it also reduced weight in *D. errans* feeding on *T. absoluta*. This may be linked to a reduced host quality through to reduced plant fertilization (Stiling et al., 2005).

As for parasitoids, omnivorous predator fecundity and biocontrol efficiency (except for *M. pygmaeus* on whiteflies) were not affected by fertilization, also matching the hypothesis of a

diluted bottom-up effects (Becker et al., 2021) despite the double interaction of the omnivorous predators with both the plants and the preys.

Finally, reducing fertilization from high to intermediate levels was beneficial for *M. pygmaeus* feeding on *B. tabaci*, which had a higher predation rate, but there was no effect on the other prey-omnivore systems. This coincided with the highest fecundity of *B. tabaci*, and such potentially more suitable fertilization conditions to *B. tabaci* may result in whiteflies of better quality to *M. pygmaeus*, increasing its predation rate. However, the effects on biocontrol efficiency at a population dynamics level would be difficult to extrapolate from these laboratory conditions. Similarly, the increased development time under reduced fertilization found in all omnivore-based systems could potentially slow down omnivore population growth, but may also result in increased total predation, through the predation of juveniles.

Fertilization bottom-up effects on carnivorous predators. Contrary to parasitoids and omnivorous predators, fertilization bottom-up effects modified various carnivorous predator traits (development, reproduction and biocontrol efficiency). For *A. bipunctata* feeding on aphids, reducing fertilization from high to intermediate levels increased the development time and predation rate, and slightly decreased adult weight and fecundity. Hosseini et al. (2019) suggested that, as beetles in the high fertilization treatment became satiated, they fed less and devoted more time to searching. In contrast, *A. bipunctata* in the intermediate fertilization treatment may have experienced delayed satiation (Bayoumy, 2011), as shown by an increased

aphid consumption, possibly to compensate for their lower energy content, and/or because of increased digestion time (Wang et al., 2024). At high fertilization, *A. bipunctata* also had access to prey of likely higher energy content which could have benefited their fecundity, which was strongly reduced under reduced fertilization.

Conversely to *A. bipunctata*, *C. carnea* feeding on *T. absoluta* eggs had the lowest predation rate under intermediate fertilization, although variation due to the fertilization treatment remained weak. This slightly reduced predation rate coincided with a significantly increased fecundity of *T. absoluta* under intermediate fertilization. An increased number of eggs laid by *T. absoluta* females could be a result of a host plant considered to be a more suitable host (Pérez-Hedo et al., 2021), but this would likely have only weak effects on egg nutritional quality to the predator, conversely to *T. absoluta* actively feeding on host plants of varying quality. In this case, the extrapolated biocontrol efficiency of *D. errans* over multiple generation times would be lower than in either high fertilization and water treatments.

Conversely, reducing plant fertilization would not only reduce the fitness and nutritional quality of *M. euphorbiae* directly, but it would also improve *A. bipunctata*'s predation efficiency. At the same time, if we extrapolate our results to population dynamics, *A. bipunctata* population growth might be strongly decreased with reduced fertilization, due to reduced fecundity, and this may result in an overall decrease in aphid biocontrol efficiency.

We demonstrated that reduced fertilization triggered a tri-trophic nutritional cascade, where changes in plant and herbivore nutritional quality cascaded to natural enemy fitness, although with variation across natural enemy functional guilds. Reduced fertilization had the highest

effects on carnivorous predator traits compared to parasitoids or omnivorous predators. Although reducing fertilizer application is expected to lead to a slight decrease in crop yields, this may be at least partially offset by the reduced cost of fertilizer application and by increased pest control (Hosseini et al., 2018). Overall, we found that reducing fertilization to 50% of standard (optimal) application levels was marginally positive for biocontrol efficiency, which is promising. It seems that the response varies depending on the specific species, with some natural enemy species showing a positive response to reduced fertilization, resulting in improved biocontrol efficiency from a population perspective. However, for some other natural enemy species, the response appears to be negative. Therefore, it is necessary to replicate the current results to each new plant-pest-natural enemy system. In addition, to improve the generality of our results, future research should seek to reproduce the results across a wider range of tri-trophic systems, as well as at larger, more realistic spatial scales (e.g., greenhouse or field scale). This is notably important to measure effects on pest and natural enemy population dynamics, as well as effects on crop yield and quality.

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Chapter 4: Does reduced fertilization bottom-up effects modify the biocontrol efficiency of natural enemies in controlling aphids under real growing conditions? Studies at population dynamic scale under greenhouse conditions

In the previous chapter on lab experiments, the bottom-up effects of fertilization on “tomato plant-herbivores-natural enemies” interactions have been examined at individual level. We found strong bottom-up effects of fertilization inputs on the performances of the natural enemies in the aphid-based system, between the carnivorous predator *Adalia bipunctata* and parasitoid *Aphidius ervi* (conclusion of chapter 3). However, bottom-up effects by resource inputs and top-down forces by the natural enemies on pest observed at the individual level does not always translate into population effects (Zaugg et al., 2013). Thus, to completely understand how bottom-up and top-down forces interact on the pest, it is necessary to design experiments at larger scales which allowed monitoring the population dynamics of both herbivorous insect and natural enemies.

During the first generation, regardless of the type of natural enemies, the aphid population increased less under medium fertilization conditions compared to high fertilization. Aphid abundance decreased with an increase in natural enemies, specifically during the second and third generations of natural enemies. In our experimental results, the number of aphids in the high fertilization treatment was higher than in the medium fertilization treatment and did not start to decline until the eighth week, corresponding to a significant increase in predator numbers in the high fertilization treatment. Nevertheless, aphid abundance was also suppressed in the high fertilization treatment due to high parasitism rates at the end of the experiment (weeks 8 to 9). Therefore, we predict a significant positive response in parasitoid wasps in the next generation. Additionally, reducing fertilization to 50% of standard (optimal) application levels was marginally positive for controlling pests early and limiting pest outbreaks without decreasing the yield of tomato fruits.

In chapter 4, we presented our studies that examined the bottom-up effects of tomato plant fertilization inputs and the top-down effects of natural enemies on a target herbivorous pest, the aphid *Macrosiphum euphorbiae*, on tomato (*Solanum lycopersicum* L) at the population level under realistic conditions for tomato production (**Article 3**). Based on the results of our previous laboratory experiments (Chapter 3), we predicted that modifications at the first trophic level would lead to changes at the second trophic level (herbivores) and would not have a strong negative effect on the third trophic level (natural enemies). To test this hypothesis, we utilized the tritrophic model of “tomato plant – aphid pest - predator/parasitoid” and established a larger experimental set-up in greenhouse mesocosms to monitor the population dynamic of both the pest and natural enemies. Our hypotheses were:

- (1) Reducing fertilization will decrease the performance of both aphids and their natural

enemies as nutritional quality of plant and prey/host will decrease.

(2) We expect distinct responses for both natural enemies as carnivorous predators were more affected by fertilization regimes compared to parasitoids in our previous lab experiments. The study was performed under greenhouse conditions, tomato plants assigned to high fertilization (HF, commonly used fertilization level), medium fertilization (MF, a 50% dilution of HF). The herbivorous insect and predators/or parasitoids were introduced into the system with tomato plants under high or medium fertilization inputs. Then the population dynamics of pest as well as both types of natural enemies can be monitored for predicting the relative strength of bottom-up forces vs. top-down forces.

As mandatory for any “bottom-up” studies, we first confirmed alterations at the first trophic level. Reduced fertilization resulted in decreased tomato plant growth and nutritional quality, with a decrease in leaf N content observed as fertilization levels decreased, while the leaf C/N ratio increased as expected. But interestingly, we saw no effect of fertilization regime on our yield proxy (tomato weight).

To discuss the pest and natural enemy’s population data, we identified two different time periods. During the first period, reduced fertilization led to a decrease in pest abundance. Throughout the initial 2/3 of the experiment, the number of natural enemies was low and similar for both types and fertilization levels, rendering the impact of natural enemies on aphid populations negligible. Therefore, **fertilization levels directly influenced aphid populations through a plant-mediated bottom-up effect (individual effect)**. Plant physiology is altered, which, in turn, influences the nutritional quality of plant tissue consumed by herbivores. Aphid populations showed less increase under medium fertilization compared to higher fertilization.

In the second period, natural enemies reduce pest abundance – At the end of the experiment duration, aphid abundance decreases with the increase in natural enemies. These results suggest that **fertilization levels indirectly influenced aphid populations through a plant-pest-mediated bottom-up effect (population level effects)**. Fertilization bottom-up effects are transferred to higher trophic levels and may affect population-regulating processes like parasitism, predation, and competition, thereby altering the composition and biomass of plants. Such ecosystem changes may, in turn, impact the growth of herbivore populations on the plants and subsequently influence the populations of generalist predators and parasitoids, which in turn would affect the plant-feeding herbivore populations.

Finally, for parasitoid *A. ervi*, an increased number of mummies was produced when reducing fertilization from high to intermediate levels, with no adverse changes observed in the number

of emerging mummies. This coincided with a reduction in aphid fecundity, suggesting potential advantages for biocontrol at the population dynamics level. Conversely, based on the results of our lab experiments in Chapter 3, reducing plant fertilization would not only reduce the fitness and nutritional quality of *M. euphorbiae* directly, but it would also improve *A. bipunctata*'s predation efficiency. At the same time, our population dynamics results showed that the population growth of *A. bipunctata* decreased with reduced fertilization, attributed to reduced fecundity. However, this reduction did not have an impact on the overall efficiency of aphid biocontrol. Moreover, reduced fertilizer application did not lead to a decrease in fruit yield.

We demonstrated that reduced fertilization triggered a tri-trophic nutritional cascade, where changes in plant and herbivore nutritional quality cascaded to natural enemy population dynamic, although with variation across natural enemy functional guilds. Overall, our findings indicate that reducing fertilization to 50% of standard (optimal) application levels had a marginally positive or no effect on biocontrol efficiency. The response appears to vary depending on the specific species and generations. Based on the observed trend of natural enemy population growth at the end of our experiment, we anticipate a notable increase in the next generation of natural enemies, especially parasitic wasps. However, for *A. bipunctata*, low aphid populations may no longer be sufficient for the reproduction of their offspring. This approach will not only provide us with a comprehensive understanding of insect population dynamics and biological control of aphids, but also offers options for enhancing effective biological control strategies.

Article 4

Are reduced fertilization and biological control compatible for pest regulation?

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Abstract

This study investigates tritrophic interactions (plant-herbivore-natural enemy) influenced by bottom-up forces, specifically fertilizer factors. Tomato (*Solanum lycopersicum* L.) plants were provided with two levels of fertilization and examined the responses of the parasitoid *Aphidius ervi* and coccinellid predator *Adalia bipunctata* to potato aphids *Macrosiphum euphorbiae* were examined. While a moderate decrease in fertilization did not significantly affect parasitoid mummification, emergence, or parasitism rates, the temporal interplay of fertilizer regimes revealed the potential to impact aphid and predator populations on tomato plants. Significant variations in nitrogen concentration and carbon-to-nitrogen ratios among fertilizer treatments were identified in tomato leaf analyses, influencing aphid nutritional quality. Consequently, we conclude that a moderate reduction in fertilizer application to tomato plants can effectively control aphid outbreaks without reducing tomato fruit yields. This not only improve the fitness of the natural enemies, contributing to the biological control of aphids, but also concurrently reduces production costs.

Keywords: Predator, parasitoid, tri-trophic interaction, biocontrol, fertilization, Bottom-up factors

1 Introduction

Current conventional agriculture is using excessive fertilization that threatens ecosystems and human health and increases greenhouse gas emissions (Elliott et al., 2014; Galloway et al., 2008;

Savci, 2012). In the framework of the agricultural transition, the first step of the ESR model (Hill, 1985, 2014; Vanbergen et al., 2020) is to reduce fertilizer inputs (“E step for Efficiency”) to avoid the negative consequences of an excessive use. Insufficient fertilizer inputs could be obviously negative for crop health and yield (Hartmann et al., 2015; Hosseini et al., 2018) means that we can’t reduce below a certain level. The questions are if intermediate level of fertilization is sustainable for crop health and yield and what will be the consequences of such practices on pest regulation?

The fertilization regime acts as a "bottom-up driver" by influencing plant traits (e.g., leaf biochemistry and physical properties) which in turns impact the survival, development, fecundity and the population dynamic of herbivorous arthropods (Blazhevski et al., 2018; Fallahpour et al., 2015; Han, Desneux, et al., 2019). Resource limitation may cause herbivore performance and population growth to rapidly decline as intraspecific competition intensifies, and thus can potentially strengthen density dependence (Denno et al., 1995). Decreased N fertilization application directly reduces herbivore fitness – the “*Nitrogen limitation hypothesis*” (White, 1993). For instance, reducing fertilization from conventional nitrogen level to the lowest nitrogen level significantly reduced the population of cereal aphids at both flowering and milking (Qiao et al., 2021). *Aphis nerii* population growth was lower on plants with low nitrogen availability (Couture et al., 2010). *Spodoptera frugiperda* larvae fed with deficiency supply of N leaves gained less weight during their development and had reduced pupal and adult weights compared to larvae reared on full supply of N leaves (Winter & Rostás, 2010). Similar results were reported from *Spodoptera exigua* developing on N deficient cotton (Y.

Chen et al., 2008) or larvae of other Lepidoptera feeding on several host plants (D. Coley et al., 2006).

As fertilisation bottom-up forces can affect the herbivore at the second trophic level, they might reach also the third trophic level, i.e., the natural enemies which are the agents of biological control in cropping system. Yet, modifications at the lower trophic levels can cascade up, influencing higher ones and altering top-down influences i.e., the efficacy of biocontrol services (Denno & Fagan, 2003; Hosseini et al., 2018; Han et al., 2022). But natural enemies may be influenced differently by bottom-up forces due to their inherent characteristics.

In the intricate web of predator-prey interactions, the nutritional suitability of prey emerges as a pivotal factor influencing the predator's life history parameters. These parameters including its developmental time, juvenile survival, reproductive rate, and adult longevity, stand as fundamental determinants of overall fitness (Jensen et al., 2012; Michaud, 2000, 2005; Obrycki & Orr, 1990; Schmidt et al., 2012). Predator may also benefit from an increase in prey density (Abrams & Matsuda, 1996; Taylor, 1988). Finally, the nutritional, allelochemical, and morphological characteristics of their host plants (see review Whitman, 1994) could also play a role, particularly for omnivorous predators (Han et al. 2015). Previous research by Hosseini et al. (2018) showed that the leaf and aphid nutrient content increased with increasing nitrogen fertilizer, which led to a decrease in the rate of aphid consumption by *Hippodamia variegata* at higher aphid *Aphis gossypii* densities and altered foraging and feeding behaviors of the predator. Couture et al. (2010) found that increased host-plant nitrogen availability improved prey quality by reducing C:N ratios and observed *Harmonia axyridis* consumed 30% fewer aphids from

high-nitrogen plants, compared with low-nitrogen plants. In agreement with Chen et al. (2005) found increased consumption by the coccinellid predator *Leis axyridis* as the soluble protein concentrations of an aphid prey decreased.

Similarly, the parasitoid *Cotesia marginiventris* had significantly lower pupal and adult weights when their hosts had fed on N-deprived soybean leaves (Winter & Rostás, 2010). However, the development duration, parasitism, and offspring size of the parasitoid *Necremnus tutae* were not affected by optimal and limited nitrogen (Dong et al., 2018). Additionally, added nitrogen resulted in a decrease in the number of mummies produced by female parasitoids but an increase in the proportion and the size of female offspring (Mace & Mills, 2016). Parasitoids are organisms that feed, grow and develop within the bodies of their arthropod hosts, ultimately leading to the host's demise (Eggleton & Gaston, 1990). Parasitoid performance primarily hinges on host quality, influenced by both the host plant and abiotic factors (Godfray, 1994; Vinson & Williams, 1991). They are typically more specialized in their host selection compared to predators (Memmott et al., 2000). Consequently, the nutrition status of the host is a critical factor influencing its suitability for parasitoid development. Parasitoids may respond to an increased biomass in the herbivore community by selecting larger size and high-quality hosts (de Sassi et al., 2012; Stiling & Moon, 2005).

In this context, we tested the compatibility of reducing fertilization level and using natural enemies as biocontrol agents for pest regulation. Based on previous lab experiments (Ma et al, in prep), we used the tritrophic model of “tomato plant – aphid pest - predator/parasitoid” and

built a larger experimental set-up in greenhouse mesocosms, in order to record the population dynamic of both pest and natural enemies. Our hypotheses were:

- (3) Reducing fertilization will decrease the performance of both aphids and their natural enemies as nutritional quality of plant and prey/host will decrease.
- (4) We expect distinct responses for both natural enemies as carnivorous predators were more affected by fertilization regimes compared to parasitoids in our previous lab experiments.

This approach will not only provide us with a comprehensive understanding of insect population dynamics and biological control of aphids, but also offers options for enhancing effective biological control strategies.

2 Materials and Methods

2.1 Biological materials: a tritrophic study system

As previously mentioned, we choose a tritrophic system model based on tomato plants, aphid pest, and natural enemies, comparing one predator and one parasitoid species.

The chosen herbivore is the aphid *M. euphorbiae* (Hemiptera: Aphididae). Colonies are coming from field-collected material and were reared on potato and tomato plants in cages, in a climate chamber (L:D 16:8, 25 ± 1 °C, 70 ± 10 % RH).

The predator *A. bipunctata* (Coleoptera: Coccinellidae) and the parasitoid *A. ervi* (Hymenoptera: Braconidae) were bought regularly from Bioplanet company (Italy) then introduced in rearing

cages. *A. bipunctata* was reared on tomato plants supplemented with aphids *M. euphorbiae*.

The parasitoid *A. ervi* mummies were kept in an insect rearing cage (Dimensions: W60 x D60 x H60 cm) and provided with cotton plugs soaked in water: honey mixture (80:20) as food for newly emerged adults.

The host plant, tomato *Solanum lycopersicum* L. Solanaceae, (cultivar San Marzano Nano; BAVICCHI S.P.A.), was grown from seeds in a growth chamber (L:D 12:12, $24 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH) at INRAE Sophia Antipolis station to DAS 7 (Days After Seeding), then the seedlings were transferred in the greenhouse to new pots ($7 \times 7 \times 6.5$ cm, TEKU, Rixheim, France) filled with perlite grains (Perlite Italiana srl, Corsico, Italy) (see schedule Fig. S1A).

Fertilization (MF level) was applied until DAS 14, then the tomato seedlings were transferred to larger pots (volume: 2L, diameter: 12.5 cm, height: 13.5 cm), filled with the same substrate, and randomized into two groups under high or medium fertilization (HF or MF) treatments, supplied by an automated system. The stock fertilization solution was prepared by mixing and diluting stock solutions containing (per 100 L) 12.4 kg of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 4 kg of $\text{KNO}_3 \cdot \text{H}_2\text{O}$, 1.6 kg of KH_2PO_4 , 4 kg of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.9 kg of KCl, 9.4 kg of CaCl_2 , 2.25 L of HNO_3 and 0.5 L of H_3PO_4 . Micronutrients were supplied as commercial solutions of Kanieltra (3 L per 100 L) with iron provided as Masquolate-Fe (3 L per 100 L). The high fertilization is common concentration fertilization in tomato greenhouse production (HF - vh) and medium fertilization is half of the full fertilization solution (MF - vh/2). The quality of nutrient solution was controlled by measuring the electric conductivity as approx. 1.7 and 0.9 mS for the HF and MF levels respectively.

2.2 Experimental design

Experiments were conducted in an environment-controlled greenhouse (T=25 °C and RH=60%) at INRAE Sophia Antipolis experimental station in South France from March to early June 2023. Ambient temperature, relative humidity inside the greenhouse and solar radiation outside the greenhouse were monitored throughout the experiment (see Fig. S2).

The experiment followed a 2×2 factorial design, organized through a randomized block design, within four 40 m²-compartments comprising four treatments each (Fig. 1B). The first factor was the fertilization treatment supply to tomato plants (HF vs MF). The second factor consisted of the presence of natural enemy types (predator *A. bipunctata* vs parasitoid *A. ervi*). The following four treatments - HF+ *A. bipunctata*; MF+*A. bipunctata*; MF+*A. ervi*; HF+ *A. ervi* - were replicated in the four above-mentioned compartments (Fig. S1B).

A total of 16 tunnels were set up, with four tunnels (replicates) for each treatment (Fig. S1B). Each tunnel was made by covering a double-row of tomato plants with a fine nylon mesh (2-m height, 1-m width and 5-m length). Sixteen plants were cultivated in two rows (5-m length and 0.3-m width for one row) with 8 plants per row in each tunnel on which all the potted plants were placed about 30 cm apart.

Each plant was manually infested with 10 *M. euphorbiae* adults in all tunnels (i.e., 160 aphids per tunnel) at DAS 40 (Fig. 1A). The three front leaflets of the first fully expanded leaf from the top were infested with aphid individuals randomly selected from rearing cages. The predator *A. bipunctata* (density of 5 couples per tunnel) or the parasitoid *A. ervi* (density of two couples

per plant) were released 3 times (once a week from DAS 50) into each tunnel. The density of released insects was chosen based on previous studies (Han et al., 2020; de Campos et al., 2020).

2.3 Data recording

Plant measurements - We measured plant height (cm) on all plants, one day before aphid infestation and collected leaf samples for chemical analysis to confirm the effect of the fertilization treatments on the first trophic level (tomato plants) and to assess their nutritional quality for herbivores. At the end of the experiment, we collected and weighted (kg) all fruits produced inside each tunnel as a proxy for tomato yield.

Leaf samples collected at DAS 39 were used for plant chemistry analyses. The three front leaflets of the first fully expanded leaf from the top were harvested from 8 randomly selected plants in each tunnel and immediately stored at -80°C pending analysis. The leaves were then lyophilized. Dried leaf samples were ground into fine powder using a ball mill MM301 (Retsch, Germany) and stored individually in small tubes. Leaf nitrogen and carbon contents were measured with an elemental analyzer (Flash EA1112 Series, ThermoFinnigan, Milan, Italy) at INRAE-PSH Avignon, France. The leaf C/N ratio was calculated from the leaf N and C contents (percent of the leaf dry mass).

Insect population dynamic - We started monitoring the population dynamics of all insect species in each tunnel weekly from DAS 50 i.e., from the NE release, for 60 days. At DAS 40, the number of aphids per plant is also recorded as the number of introduced individuals. For

each survey, 8 of 16 plants were sampled in each tunnel using a zigzag pattern between both plant rows. To avoid bias in counting over time, the starting point (of the zigzag pattern) of plant sampling alternated from one survey date to the next.

On each plant, we counted *A. bipunctata* eggs, larvae, pupa, and adults on the entire plant and the number of *A. ervi* mummies and emerged mummies as well as the number of *M. euphorbiae* nymphs and adults on six tomato leaves, two on the top, two in the middle and two at the bottom of tomato plants.

2.4 Statistical analyses

All statistical analyses were done using the R software program (R Core Team 2023, version 4.2.3).

We used Generalized linear mixed-effect models (GLMMs) for plant traits (i.e., plant height family Gaussian; leaf N content and leaf C/N ratio by Beta regression model), and the interactions between factors: fertilisation treatments and compartment. For fruit weight, we tested the fertilization supply effect crossed with the type of NE.

The population dynamics of total aphids and natural enemies in all time periods data were fitted in a GLMMs (Generalized Linear Mixed-Effects Models, Function *glmer* in the package “lme4”) for a binomial regression model distributed response with repeated measurements (‘sampling date’ as repeated factor, ‘compartment’ as random effect). The selection of error structures was based on the AIC/BIC criteria to best accommodate the data distribution in which

error structures were chosen to best account for data distribution. Function *emmeans* in the package “emmeans” was performed for pairwise comparisons when the parameter estimates of the most complete model including significant effects only. This model enabled us to compare the tendency of the complete temporal set of data between treatments. Experiment duration vary with the recorded data: DAS 40-DAS 99 for aphid population; DAS 57-DAS 99 for predator eggs and larvae, and parasitoid mummies or parasitism rate; DAS 64-DAS 99 for emerged mummies, and DAS 71-DAS 99 for the number of predator pupa and adults.

3 Results

3.1 Plant trait measurements

Fertilization treatments (i.e., MF, HF) affected plant height significantly (fertilization: $\chi^2 = 36.398$, $df = 1$, $p < 0.001$; compartment: $\chi^2 = 13.636$, $df = 3$, $p = 0.003$, fertilization \times compartment: $\chi^2 = 36.398$, $df = 3$, $p < 0.001$; Fig. 1C). Supplying the MF treatment to tomato plants decreased the leaf N content (fertilization: $\chi^2 = 67.45$, $df = 1$, $p < 0.001$; compartment: $\chi^2 = 11.57$, $df = 3$, $p = 0.009$; fertilization \times compartment: $\chi^2 = 4.54$, $df = 3$, $p = 0.209$; Fig. 1A, Table S1) and increased the C/N ratio (fertilization: $\chi^2 = 93.544$, $df = 1$, $p < 0.001$; compartment: $\chi^2 = 11.84$, $df = 3$, $p = 0.008$; fertilization \times compartment: $\chi^2 = 5.42$, $df = 3$, $p = 0.144$; Fig. 1B, Table S1).

However, at the end of the experiment, fruit yield was not affected by fertilization supply or natural enemy type (Fig. 1D).

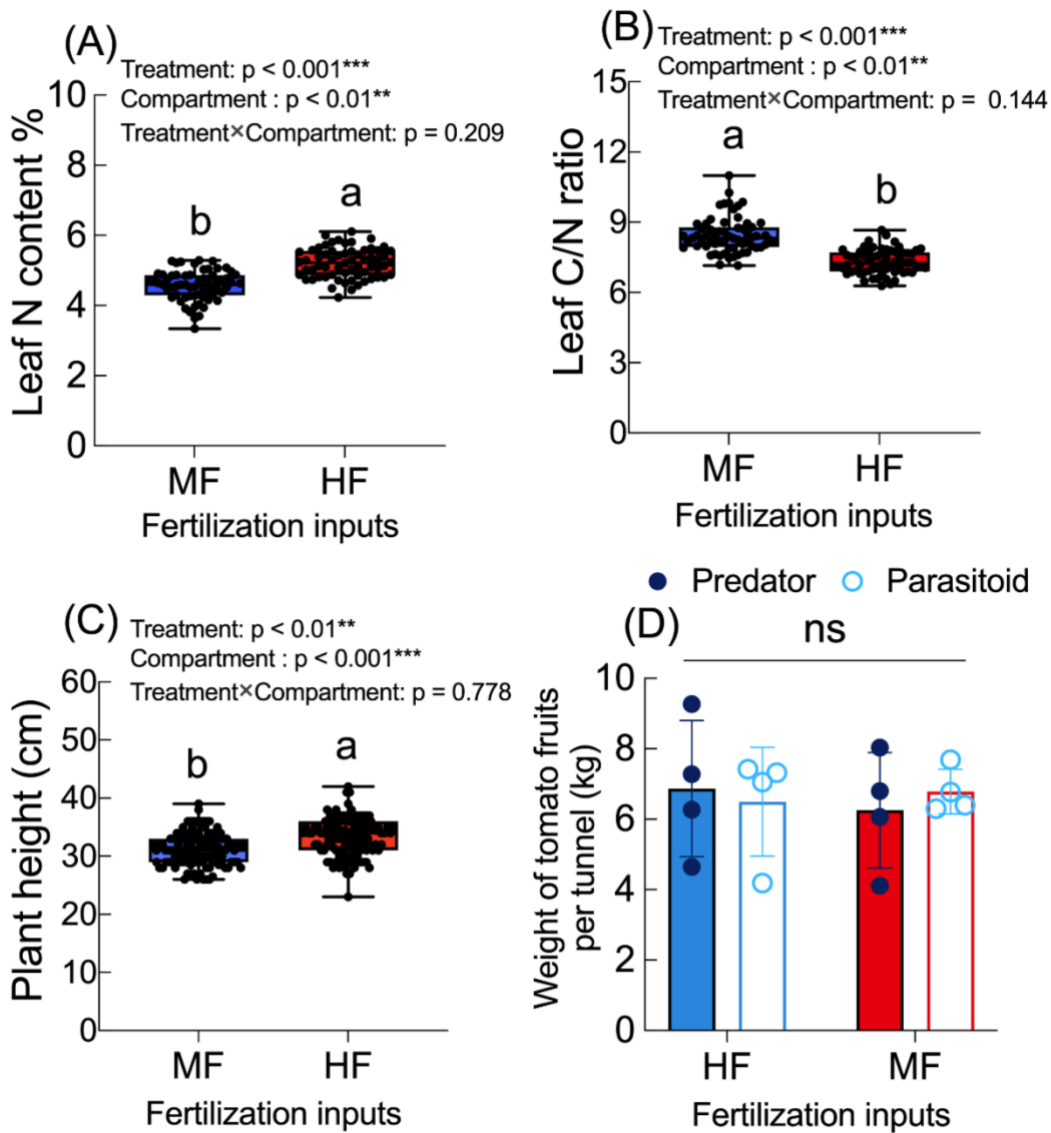


Fig. 1 Effects of the fertilization supply to tomato plants on (A) leaf N content (g/100 g leaf dry mass, $n = 64$), (B) leaf C/N ratio (unit-less, $n = 64$), (C) plant height at DAS 39 ($n = 64$), (D) tomato fruits yield per tunnel (mean \pm SE, $n = 4$) according to the treatments.

3.2 Insect population dynamics

3.2.1 Impact of fertilization treatments in the “Tomato Plant-Aphid-Parasitoid” system

Aphid abundance increased from the introduced individuals and peaked in week 8 then started declining for both treatments (Fig. 2A, significant time effect: $\chi^2 = 899$, $df = 8$, $p < 0.001$). Fertilizer supply significantly influenced the overall abundance (fertilizer: $\chi^2 = 5.17$, $df = 1$, $p = 0.023$), more particularly in interaction with time as the abundance of aphids is higher in HF between DAS 71 and DAS 85 then reverse from DAS 92 (treatment \times time: $\chi^2 = 38.5$, $df = 8$, $p < 0.001$) (Table S2A; Fig. 2A).

In parallel, parasitoid mummy abundance peaked at DAS 92-99, following by the emergence of adults (Table S2A; Fig. 2B, C; significant time effect: $\chi^2 = 971.11$, $df = 5$, $p < 0.001$). Plant fertilization supply had significant effect on the number of mummies (fertilizer: $\chi^2 = 4.74$, $df = 1$, $p = 0.03$) with sometimes slightly less mummies in the HF treatment (treatment \times time: $\chi^2 = 23.5$, $df = 6$, $p < 0.001$).

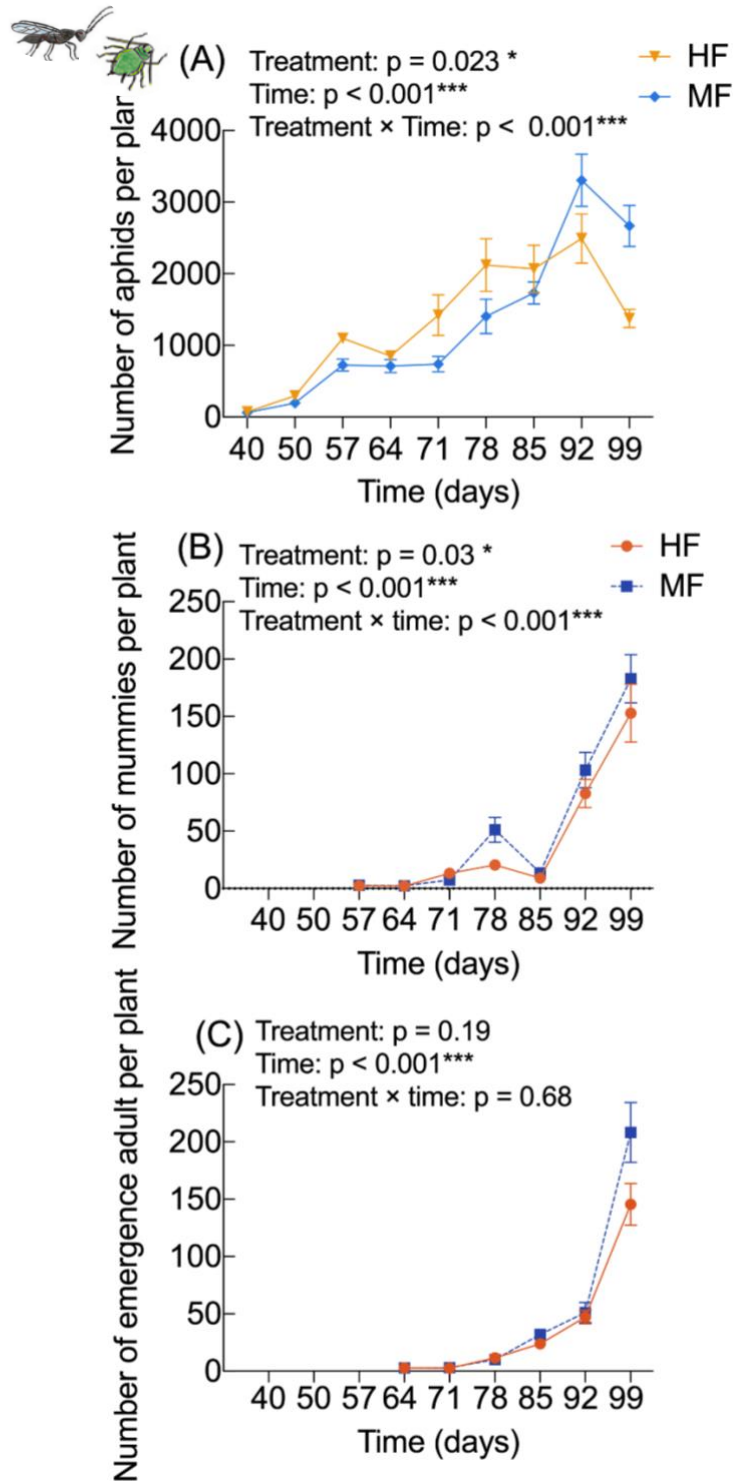


Fig. 2 Population abundance (mean number of individuals per plant \pm SE, $n = 4$) of the aphid *Macrosiphum euphorbiae* (A), the parasitoid *Aphidius ervi* mummies ($n = 8$) (B), emerged mummies ($n = 8$, repeated 4 times) (C) and parasitism rate ($n = 4$) (D); HF, high fertilization supply; MF, medium fertilization supply, each experiment unit repeated 4 times. The community dynamics were monitored from DAS50 - DAS106.

3.2.2 Impact of fertilization treatments in the “Tomato Plant-Aphid-Predator” system

Aphid abundance increased from the introduced individuals and peaked at DAS 85-92 then declined to a relatively low level by the DAS 99 for both treatments (Table S2B; Fig. 3A: Significant time effect: $\chi^2 = 645.0$, $df = 8$, $p < 0.001$), with more aphids on HF treatments from DAS 57 to 85 (treatment \times time: $\chi^2 = 19.1$, $df = 8$, $p = 0.015$) (Table S2B; Fig. 3A).

In parallel, predator egg, larva, pupa and adult numbers (Table S2B; Fig. 3B-D) increased with time ($p < 0.001$) with respective delays. Number of eggs and adults (and with a lighter pattern pupa) were higher in HF conditions only at the end of the experiment (from DAS 85) (eggs: treatment \times time: $\chi^2 = 20.33$, $df = 6$, $p = 0.002$; adults: treatment \times time: $\chi^2 = 13.16$, $df = 4$, $p = 0.011$).

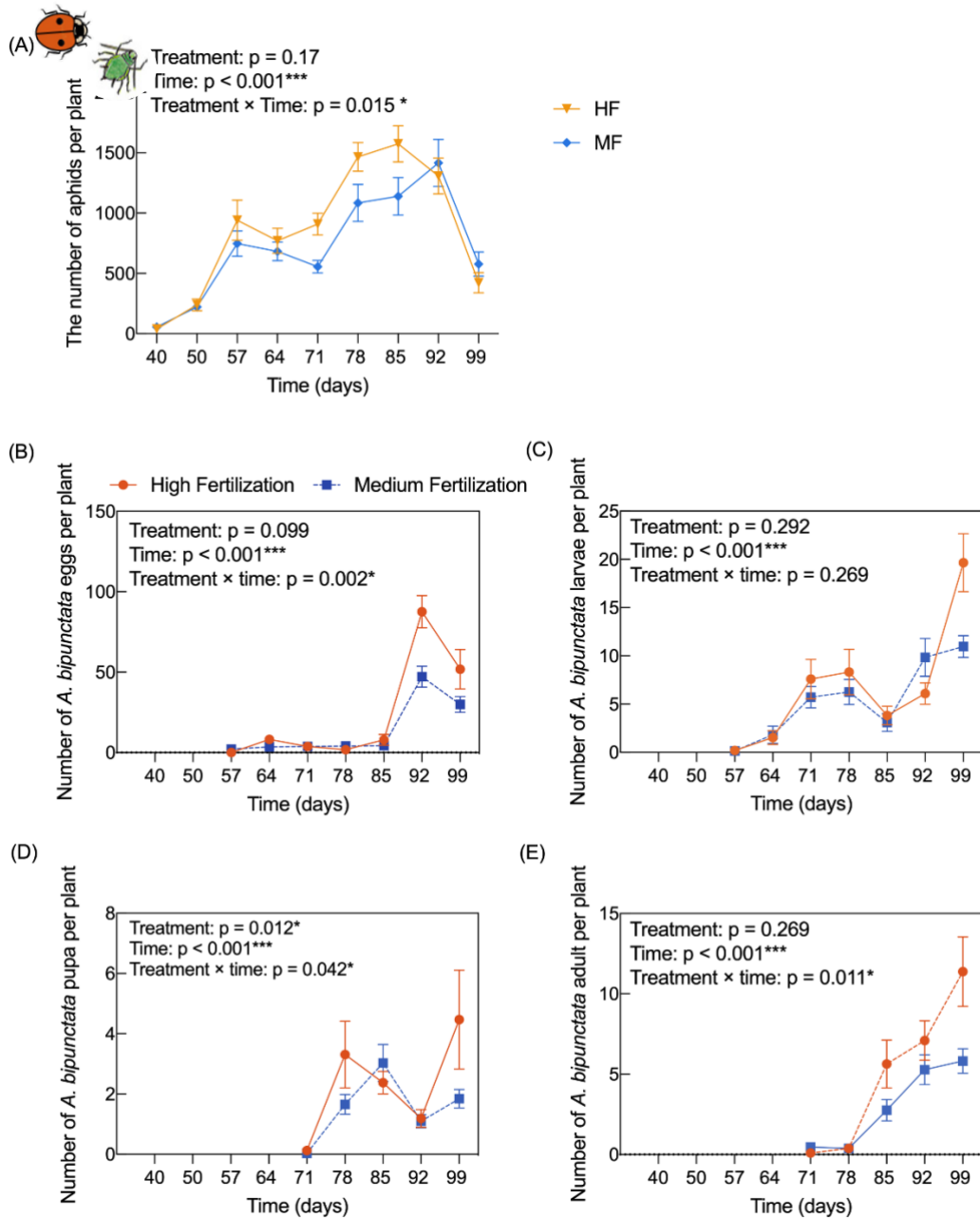


Fig. 3 Population abundance (mean number of individuals per plant \pm SE, $n = 4$) of the aphid *Macrosiphum euphorbiae* (A), the predator *Adalia bipunctata* eggs ($n = 8$) (B), larvae ($n = 8$) (C), pupa ($n = 8$) (D) and adults ($n = 8$) (E); HF, high fertilization supply; MF, medium fertilization supply, each experiment unit repeated 4 times. The community dynamics were monitored from DAS50 - DAS106.

4 Discussion

As mandatory for any “bottom-up” studies, we first have to check if fertilization regime indeed impacted the first trophic level, means the tomato plants. As expected from previous experiments (Han et al., 2020; Becker et al., 2021), fertilization treatments indeed modify plant growth and nutritional quality. Based on these data, we might look for potential bottom-up effects. However, we have to keep in mind the compartment effect, which indeed was significant, even so it did not change the global picture with plants with a higher C/N ratio and lower plant height on MF treatment, compared to HF. This suggests variation in dietary nitrogen availability for aphids.

First period, reducing fertilization reduce pest abundance – During the first 2/3 of the experiment duration, disregarding the type of natural enemies, aphid population increased less under MF conditions compare to HF. This phenomenon could not be link to natural enemies’ population as, for both NE types, the population was low and similar between both treatments during this time. Then, pest abundance was slightly regulated by fertilization decrease.

Fertilization plays a pivotal role in plant-herbivore interactions, and our data support the ‘Nitrogen limitation hypothesis’ (White, 1993). Similarly, a study on *Aphis nerii* (Fonscolombe) on the milkweed found higher aphid N concentration with higher N concentration plants, and population growth was lower on plants with low nitrogen availability (Couture et al., 2010). Lowest nitrogen level significantly reduced the population of cereal aphids at both flowering

and milking (Qiao et al., 2021). Additionally, fertilization alters the microclimate of crops, contributing to aphid population growth (Duffield et al., 1997; Throop & Lerdau, 2004).

Second period, natural enemies reduce pest abundance – In the last 1/3 of the experiment duration, aphid abundance decrease with natural enemies increase, more precisely the 2nd then 3rd generation of natural enemies. During this phase, temperatures stayed relatively stable then could not explain this decrease of aphid abundance.

Specifically, fertilizer limitations on tomato plants did trigger slightly positive effects on the biocontrol efficiency of parasitoid associated with fertilizer input. These findings suggest a buffered effect of fertilizer at the third trophic level (Chesnais et al., 2016). The reduced fitness of aphids feeding on plants supplied with lower fertilization dose even translated into a slightly increased number of parasitoid mummies. In our experiment, the emerged mummies and parasitism rate remained unchanged with fertilizer input. Similar findings were observed in a walnut aphid-Aphidiinae parasitoid system and cereal-aphid-parasitoid system, indicating that fertilizer levels did not affect the parasitism rate. Dong et al. (2018) also found that regulating the negative effects of nitrogen and water inputs to plants did not result in negative consequences for parasitic wasps. Densities of aphids, insect immune defenses, and their parasitoids increased synchronously with the amount of fertilizer (Forkner & Hunter, 2000; Garratt et al., 2010; Mace & Mills, 2016). Nevertheless, aphid abundance was also suppressed in the HF treatment due to high parasitism rates at the end of the experiment (weeks 8 to 9). Therefore, we predict a significant positive response in parasitoid wasps in the next generation.

In contrast, fertilizer limitations on tomato plants trigger negative effects on the predator egg reproduction, the average number of pupae, and adults per plant. The application of different fertilizer regimes to tomato plants may influence the macronutrients levels in *M. euphorbiae* reared on these plants, subsequently affecting the population dynamics of *A. bipunctata*. Predators might enhance their overall fitness by developing in hosts with higher fertilization, in contrast to parasitic wasps. While an improvement in the nutritional quality of prey driven by plant conditions enhances the fitness of predators, it also leads to decreased prey consumption (Calabuig et al., 2018; Couture et al., 2010; Gratton & Denno, 2003; Hosseini et al., 2018). As a probable compensatory response to the decline in aphid trophic quality, the coccinellid predator *Harmonia axyridis* consumed more low quality *A. nerii* (Couture et al., 2010). In our experimental results, the number of aphids in the HF treatment was higher than in the MF treatment and did not start to decline until the eighth week, corresponding to a significant increase in predator numbers in the HF treatment.

A. bipunctata in the HF treatment matured quickly, exhibiting high egg production, pupa and adults' numbers. Similarly, the fecundity of *Hippodamia variegata* in the 210 N treatment had high fecundity over their lifetime compared to 110 N treatment (Hosseini et al., 2019). *Harmonia axyridis* reared on *Lepidoptera* eggs had higher fecundity than those beetles reared on *Acyrtosiphon pisum*, attributed to the higher protein and lipid content of *Lepidoptera* eggs (Specty et al., 2003). Although the HF treatment resulted in high reproduction, there was no significant difference in the average number of larvae between the treatments over time. This is evidence by the high mortality of first instar larvae in the HF treatment, likely contributing

to the lower intrinsic rate of increase (r) and might be a consequence of their rapid growth rate (Hosseini et al., 2019). Similarly, Jalali and Michaud (2012) found that mortality of first instar *A. bipunctata* (L.) was negatively correlated with r when the beetles were fed on *Myzus spp.* reared on both pepper and tobacco. In high-nitrogen treatments, carbohydrate was kept constant as the percentage of protein content was increased, which tends to shorten developmental time and longevity of the beetles, a predictable life-history trade-off (Stearns, 1998) Wang 2018). Accordingly, even though the HF treatment would increase beetles' reproduction, it would also reduce their longevity, thus impacting their biological control following release. The application of high fertilization levels of plants used to result in a lower predation rate of beetles, while more aphids were consumed by beetles on lower nitrogen fertilizer treatments to achieve the same nutrition level (Aqueel & Leather, 2012; Couture et al., 2010; Hosseini et al., 2019). At the end of experiment, aphid populations were controlled in the HF treatment due to high predator populations. This result is also consistent with the predation rate measurements from our lab experiments. Coccinellid contributed to a longer generation time in nutrient limitation, with females attempting to compensate for low prey quality by feeding longer in low fertilization treatment to increase their reproductive success (Hosseini et al., 2019; Jalali & Michaud, 2012; Vargas et al., 2012). This could be interpreted by our findings as *A. bipunctata* takes more time to establish populations in the MF treatment. We infer that the MF would be optimal for rearing *A. bipunctata* and maximizing its predation on *M. euphorbiae*. Further increases in host plant fertilization applications leads to increased aphid populations and reduced predation potential.

In the context of biological control, reducing fertilization application from high to intermediate levels decreased pest fecundity as expected and lowered production costs. However, reducing fertilization will also decrease predator populations, potentially increasing the predation efficiency of predators upon release. Although the level of fertilizer application may have less effect on parasitoids directly, plants may initially suffer from aphid infestation. Additionally, it is generally assumed that reduced fertilization application to plants decreases plant nitrogen content and reduces insect damage or populations. Our results supported that reducing fertilization can help control pests early and limit pest outbreaks without decreasing the yield of tomato fruits. However, determining the most appropriate fertilization level remains unclear. Investigating the trade-off between the degree of reduced fertilization inputs negatively impacting plants and pest suppression is essential for maintaining a balance between essential yields and effective, sustainable pest control strategies.

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Supplementary Materials for the article:

Are reduced fertilisation and biological control compatible for pest regulation?

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Table S1 Bottom-up effects of plant nutrition on quality of non-infested leaves (in dry mass percent: g/100g MS). Additionally, p-values, degrees of freedom, and Chisq-values comparisons among different “fertilization input” treatments, “compartment”, and the interaction of “fertilization input × compartment” by GLMMs model with Beta structure. ‘MF’: medium fertilization; ‘HF’: high fertilization.

Explanatory variables	N	Leaf N			Leaf C/N		
		<i>df</i>	χ^2	<i>P</i> value	<i>df</i>	χ^2	<i>P</i> value
Treatment	64	1	67.45	< 0.001 ***	1	93.544	< 0.001 ***
Compartment	64	3	11.57	0.009 **	3	11.844	0.008 **
Treatment × Compartment	64	3	4.5351	0.209	3	5.4185	0.144

: P* < 0.01; *: P* < 0.001.

Table S2 *Aphidius ervi* mummies and emergency mummies, the number of *Adalia bipunctata* eggs, larvae, pupa, and adults per plant as well as *Macrosiphum euphorbiae* population per plant, according to the treatments and the times (days) as well as all the pairwise interaction (A, B - by GLMMs model) (all the data was using family = binomial distribution).

(A)

Explanatory variables	Number of <i>Macrosiphum euphorbiae</i>			Number of <i>Aphidius ervi</i> mummies			Number of <i>Aphidius ervi</i> emergence mummies		
	df	χ^2	P value	df	χ^2	P value	df	χ^2	P value
Treatment	1	5.17	0.023 *	1	4.74	0.03 *	1	1.6915	0.1934
Time	8	899	< 0.001 ***	6	1055	< 0.001 ***	5	971.11	< 0.001 ***
Treatment × Time	8	38.5	< 0.001 ***	6	23.5	< 0.001 ***	5	3.1234	0.681

(B)

Explanatory variables	Number of <i>Macrosiphum euphorbiae</i>			Number of <i>Adalia bipunctata</i> eggs			Number of <i>Adalia bipunctata</i> larva			Number of <i>Adalia bipunctata</i> pupa			Number of <i>Adalia bipunctata</i> adults		
	df	χ^2	P value	df	χ^2	P value	df	χ^2	P value	df	χ^2	P value	df	χ^2	P value
Treatment	1	1.88	0.17	1	0.00	0.099	1	1.111	0.292	1	6.35	0.012 *	1	1.224	0.269
Time	8	645	< 0.001 ***	6	107.44	< 0.001 ***	6	263.61	< 0.001 ***	4	129.75	< 0.001 ***	4	338.31	< 0.001 ***
Treatment × Time	8	19.1	0.015 *	6	20.33	0.002 **	6	7.601	0.269	4	9.921	0.042 *	4	13.16	0.011 *

** : $P < 0.01$; *** : $P < 0.001$.

Effets bottom-up de la fertilisation sur des agents de biocontrôle
Chapter 4

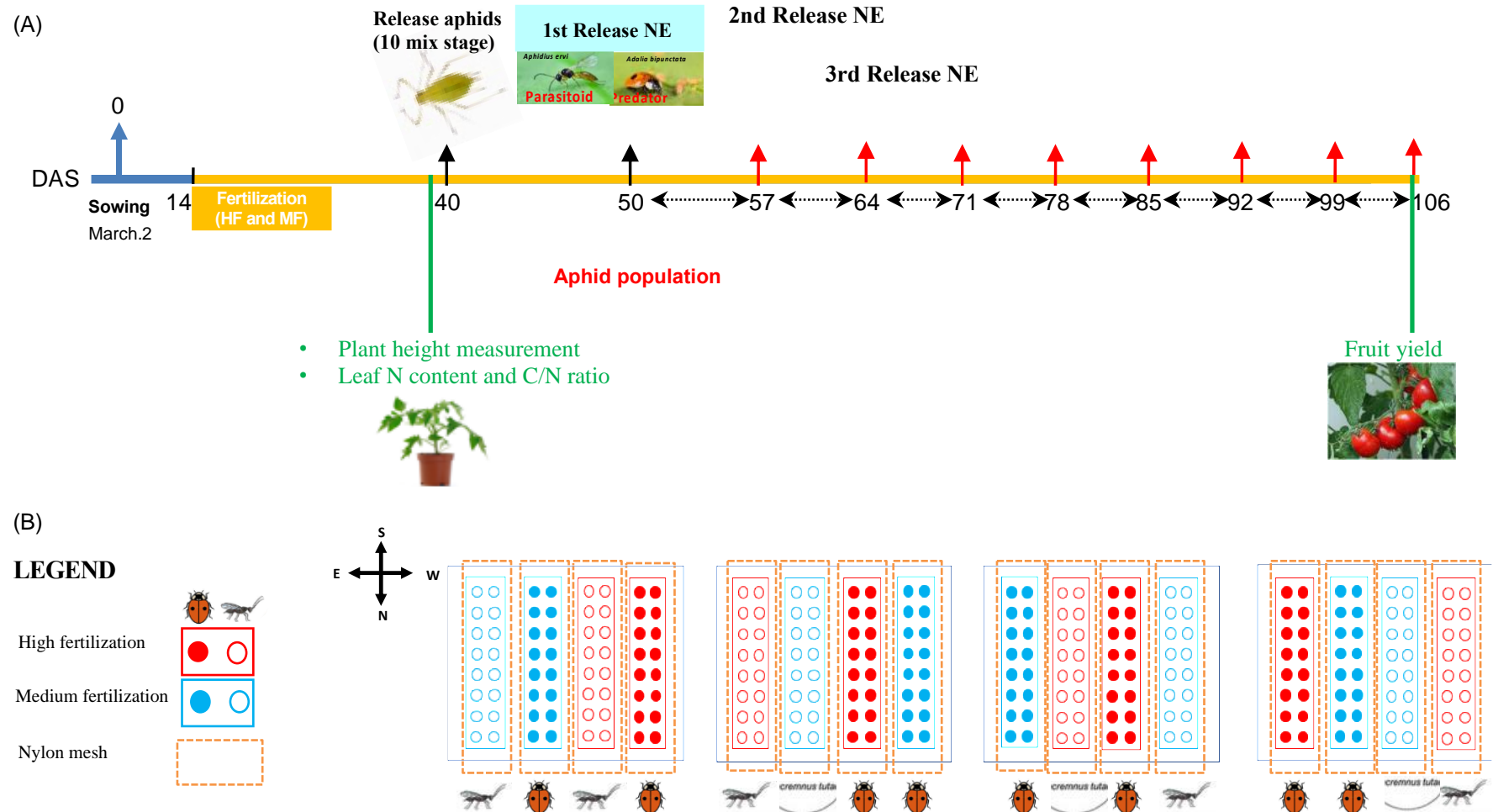


Fig. S1 The experimental schedule: DAS: days after sowing; HF/MF: high and medium fertilization inputs (A); Greenhouse experimental setup (B).

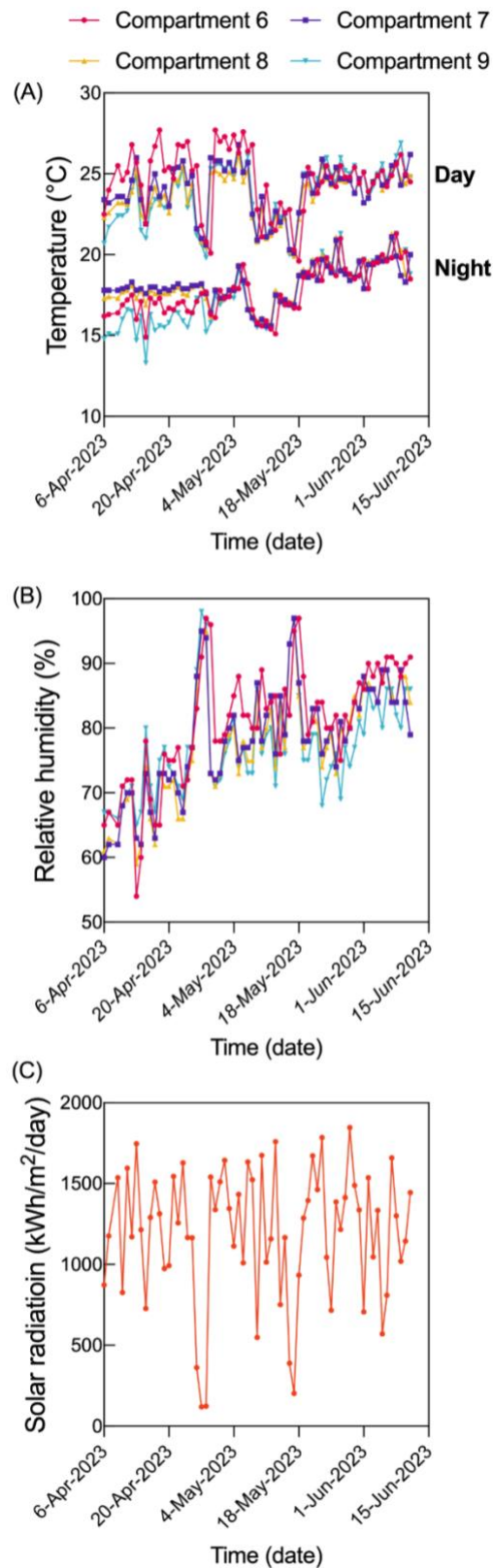


Fig. S2 Ambient temperature inside the compartment (A), relative humidity inside the compartment (B) and solar radiation outside the greenhouse (C) during the experiment period from 6th April to 15th June in 2023, INRAE Sophia-Antipolis, France.

Chapter 5: General discussion

With increasing concerns over the environmental costs and sustainable agriculture, my PhD research in the field of plant-herbivore-natural enemy interactions focused on investigating the impact of reduced fertilization in plants on natural enemies' life-history traits and biocontrol services. We applied both (a) meta-analysis; (b) experimental analysis at both individual level (lab experiment) and population level (greenhouse experiment). In our study, bottom-up effects we assessed by manipulating fertilization regimes (e.g., plant resources) on a natural enemy under laboratory conditions. Bottom-up effects were evaluated by examining the impact of various tri-trophic systems "tomato plant – pest - natural enemies". This involved comparing key life-history traits of natural enemies (development, longevity, and fertility) as well as their biocontrol abilities (parasitism rate or predation rate) across different natural enemy types. We aimed to determine whether diversity in the natural enemy diet regime (carnivorous versus omnivorous predators) or functional guild (predator versus parasitoid) modifies the intensity of bottom-up effects to reach the natural enemy on the trophic level. In conclusion, our bibliographic and experimental studies, at both individual and population levels, provided various fundamental and practical implications. Our current study presents a comprehensive evaluation of the impact of bottom-up forces (fertilizer input) on the individual performance and populations of natural enemies and herbivorous insects. Additionally, we explore the combined effects of both bottom-up and top-down forces on herbivorous insects.

Firstly, the meta-analysis with the 849 observations from 36 papers (**Article 2**) showed a global overview of whether fertilization can influence traits and biocontrol efficiency of parasitic wasps (542 data points) and predators (307 data points) through bottom-up effects. The most commonly used fertilizers are nitrogen (N) and NPK fertilizer. The natural enemies of study showed a significant positive association with the application of both nitrogen and NPK fertilizers. This suggests that fertilizers could have a global impact on the traits of natural enemies. Interestingly, we discovered that 1) natural enemies exhibited a significant positive association with NPK fertilizers, nearly three times that of nitrogen fertilizers and globally

impact both on predator and parasitoid; 2) NPK fertiliser more substantial impact on populations than on individual of natural enemies.

Secondly, through different levels of NPK fertilizer, we found that bottom-up effects are not equivalent in natural enemies (i.e., parasitoids and predators) with contrasting ecologies, and that predator dietary habits influence the strength of bottom-up effects through the study of lab experiments (**Article 3**). Considering our comprehensive findings regarding high fertilization (HF, a commonly used fertilization level), medium fertilization (MF, representing a 50% dilution of HF), or control (W: water only, no nutrient), the bottom-up effects of fertilization on biocontrol efficiency were relatively inconsistent. Reducing fertilization from a commonly used level to a 50% reduction should potentially have a slightly positive impact on the biocontrol efficiency of natural enemies across all functional guilds.

Thirdly, we selected one of the sets of models from the lab experiment to apply to the greenhouse to explore the bottom-up effects of population dynamics (**Article 4**). We obtained strong evidence that a half dilution of fertilizer application not only improves the adaptability of natural enemies and facilitates the biological control of aphids, but also does not affect fruit yield. In particular, a moderate reduction in fertilizer on tomato plants did not induce negative effects on various traits of *A. ervi* population dynamics and even resulted in a slight increase in the number of parasitoid mummies. However, high fertilization led to *A. bipunctata* maturing quickly, displaying high egg production, pupa and adult numbers; nevertheless, it also contributed to an increase in the pest population. However, the long-term bottom-up effects of fertilization on aphid natural enemies at the population level remain unknown. Studies on effects beyond the second-generation cycle are also needed.

The “Efficiency-Substitution-Redesign” (ESR) framework captures the progression from shallower agroecological approaches to full system redesign to reach the goals of the

agricultural transition (Hill, 1985, 2014; Vanbergen et al., 2020). Following this framework, the objective is to reduce the amounts of current fertilizers (mainly inorganic) without reducing food production (“efficiency” step), as recommended for sustainable agriculture transition (Altieri, 1995; Wezel et al., 2014). Therefore, we examined by comparing the impact of high and medium fertilization levels between carnivorous predators and parasitoids on a target herbivorous pest, the aphid, in greenhouse conditions. Overall, our findings indicate that reducing fertilization to 50% Notably, the effects were marginally positive for biocontrol efficiency, presenting a promising outcome. From an applied perspective, the manipulation of fertilization regimes may help optimize agricultural practices by promoting negative effects on herbivorous insects (often pests) and enhancing the performance of their natural enemies, all without compromising crop yields.

These studies have the potential not only to provide insights into basic ecological questions but also to contribute to a comprehensive understanding of the development of optimized biological control in the agricultural transition (ESR framework).

The concept of low-input resources (i.e., fertilization) has been widely studied as it is crucial for the transition to sustainable agriculture. This fact is underscored by its extensive use in studies aimed at comprehending the bottom-up forces driven by fertilization, which modulate the fitness of natural enemies (i.e., arthropod predators and parasitoids) (Daugherty, 2011; Chesnais et al., 2016; Han et al., 2022). Although reducing fertilizer application is expected to lead to a slight decrease in yield, this may be at least partially offset by the reduced cost of fertilizer application and by increased pest control (Hosseini et al., 2018).

1. The effects of reduced fertilization inputs on parasitoid

In our parasitoid-based meta-analysis, full NPK with only N-modification fertilization solution of four levels of (low, medium, high, and excess) on parasitoids were involved. The largest orders of Hymenoptera parasitoids showed positive response to fertilization. Based on the

results of our laboratory experiment, reduced fertilization from an intermediate level to no fertilization reduced development duration and reduced longevity in *A. ervi*. Conversely, the development duration of *E. formosa* was increased under reduced such fertilization. However, reduced fertilizations did not modify parasitoid wasps' biocontrol efficiency or their reproduction (**Article 3**). However, no adverse changes in either parasitoid species were observed when reducing fertilization from high to intermediate levels, therefore this would likely be beneficial for biocontrol efficiency in terms of population dynamics. Yet, it has to be noted that it was the effects of host quality – not quantity on biocontrol efficacy that was tested in our lab experiments.

Additionally, we selected *A. ervi* for the study and reproduced the findings across a wider range of greenhouse tri-trophic systems. The population of 2nd and 3rd generation *A. ervi* increased during the final third of the experiment. Specifically, reducing fertilization from high to intermediate levels has a positive effect on the biocontrol efficiency (number of mummies) of *A. ervi* and does not negatively affect parasitoid reproduction (number of emergence adults). This correlates with the results of the development and longevity of *A. ervi* in our laboratory experiment, where the reduction of fertilizers led to a shorter developmental duration of parasitoid wasps (shorter generation times). This coincided with a decrease in aphid fecundity, which would be advantageous for biological control at the population dynamics level.

2. The effects of reduced fertilization inputs on carnivorous predators

In our meta-analysis, carnivorous predators showed a significant response to fertilization, and this effect significantly across to high and excess fertilizer applications. Based on the results of our laboratory experiment, fertilization bottom-up effects modified various carnivorous predator traits (development, reproduction and biocontrol efficiency). For *A. bipunctata* feeding on aphids, reducing fertilization from high to intermediate levels increased the development time and predation rate, and slightly decreased adult weight and fecundity. *A. bipunctata* in the intermediate fertilization treatment may have experienced delayed satiation (Bayoumy, 2011),

as evidenced by an increased aphid consumption. This could be due to the lower energy content of the aphids and/or increased digestion time (Wang et al., 2024). Additionally, *A. bipunctata* fecundity was strongly reduced under reduced fertilization. Conversely to *A. bipunctata*, *C. carnea* feeding on *T. absoluta* eggs had the lowest predation rate under intermediate fertilization, although variation due to the fertilization treatment remained weak. The slight decrease in predation rates coincided with a significant increase in *T. absoluta* fecundity under intermediate fertilization. The cause of the increase in *T. absoluta* fecundity may be due to the fact that the host plant is perceived to be a more suitable host at intermediate levels of fertilizers (Pérez-Hedo et al., 2021). This indirectly affects predator biocontrol efficiency might by weakly affecting the nutritional quality of the eggs. Conversely, reducing plant fertilization would not only reduce the fitness and nutritional quality of aphids directly, but it would also improve *A. bipunctata*'s predation efficiency. However, if we extrapolate our results to population dynamics level, *A. bipunctata* population might be strongly decreased with reduced fertilization, due to reduced fecundity. This may result in an overall decrease in aphid biocontrol efficiency. Therefore, to test our inferences, we conducted a study on *A. bipunctata* within a greenhouse population dynamic experiment. The population of 2nd and 3rd generation *A. bipunctata* increased during the final third of the experiment. Specifically, reducing fertilization from high to intermediate levels on tomato plants trigger negative effects on the predator egg reproduction, the average number of pupae, and adults per plant. This coincided with a decrease in aphid fecundity, subsequently affecting the population dynamics of *A. bipunctata*. At the end of experiment, aphid populations were controlled under the high treatment due to the high number of predators, but did not differ significantly from the intermediate level of nitrogen fertilization. This result is also consistent with the predation rate measurements from our lab experiments. We infer that the medium fertilization would be optimal for rearing *A. bipunctata* and maximizing its predation on *M. euphorbiae* at both the individual and population dynamics level.

3. The effects of reduced fertilization inputs on omnivorous predators

From our meta-analysis results, we observed that omnivorous predators exhibited a strong response to fertilization, although this effect did not significantly across different fertilizer levels. Laboratory experiments focusing on the life-history of omnivore predator *L. pratensis* have demonstrated that supplementary prey based on green pea pods with protein-rich *H. armigera* eggs enhances the fitness (survival and fecundity) of omnivorous predator compared to adding aphids with lower nutritional quality. The omnivore *L. pratensis* nymphs were efficient at preying on immobile *H. armigera* eggs, which increased their time spent feeding on bean tissue, while *L. pratensis* adults spent similar time feeding on plant tissue versus prey no matter the prey type (**Article 1**). This study helps understand the feeding strategies of omnivorous predators on the varying quality of prey types. Then based on the characteristics of omnivorous predators, which require nutrients from both host plants and prey, and their significant reliance on the quality of plants and prey for their adaptations. We found that reduced fertilization increased development duration of omnivorous predators; it also reduced weight in *D. errans* feeding on *T. absoluta* eggs (**Article 3**). This may be linked to reduced host quality through reduced plant fertilization (Stiling et. al., 2005). Reducing plant fertilization from high to intermediate levels was beneficial for *M. pygmaeus* feeding on *B. tabaci*, which had a higher predation rate, but there was no effect on the other prey-omnivore predator systems. This coincided with the highest fecundity of *B. tabaci*, and such potentially more suitable fertilization conditions to *B. tabaci* may result in whiteflies of better quality to *M. pygmaeus*, increasing its predation rate. However, the effects on biocontrol efficiency at a population dynamics level would be difficult to extrapolate from these laboratory conditions. Similarly, the increased development time under reduced fertilization found in all omnivore-based systems could potentially slow down omnivore population growth, but may also result in increased total predation, through the predation of juveniles.

We demonstrated that reduced fertilization triggered a tri-trophic nutritional cascade, wherein alterations in plant and herbivore nutritional quality influenced natural enemy fitness, albeit

with variations among natural enemy functional guilds. Notably, reduced fertilization had a greater impact on carnivorous predator traits compared to parasitoids or omnivorous predators. It seems that the response varies depending on the specific species, with some natural enemy species showing a positive response to reduced fertilization, resulting in improved biocontrol efficiency from a population perspective. However, for some other natural enemy species, the response appears to be negative.

Perspectives

Our investigation into potential mechanisms underlying the observed plant-arthropod interactions is still ongoing. Future studies in the plant-mediated indirect interactions in a tri-trophic system may focus on:

At short term - The meta-analysis database requires further expansion, and we plan to incorporate a broader range of fertilizers beyond inorganic fertilizations. Adding organic fertilization and bio-fertilization expands the scope of the database, offering a more comprehensive understanding of various fertilization methods. This diversification is crucial for researchers and practitioners seeking a holistic view of agricultural practices.

Organic fertilization and bio-fertilization are often considered more environmentally friendly alternatives to synthetic fertilizers. Many agricultural areas are adopting organic fertilization due to the significant issue of groundwater pollution caused by synthetic fertilizers, as well as other environmental concerns. Consequently, there is a growing trend towards reducing or completely discontinuing the use of inorganic fertilizers in the future. Before transitioning to organic agriculture, it is essential to understand the potential effects of organic fertilizers in agroecosystems.

At middle term - For the greenhouse experiment, we observed a second-generation effect (time effect). To delve deeper into this, we aim to investigate the subsequent generations of natural enemies to understand their potential outcomes. While we've only just begun to uncover the narrative, we're noticing deviations from the initial observations (1st generation), prompting our curiosity about what lies ahead.

For the laboratory experiment, we need to delve deeper into the mechanisms underlying the observed differences in population dynamics over time. Specifically, we need to investigate how changes in development and reproduction contribute to these differences. Currently, we lack a comprehensive understanding of which traits drive these temporal variations. In the greenhouse experiment, we observed that the second generation of natural enemies developed in response to the aphids subjected to the fertilization regime. However, it's important to note that in our experiment, the natural enemies were sourced from commercial suppliers and may not have developed in the same conditions as the aphids they were meant to control. This

discrepancy could explain the differences observed over time. To truly understand what's happening, we may need to conduct a revised laboratory experiment where we rear the natural enemies under different fertilization conditions alongside the aphids. By doing so, we can observe how the generation of natural enemies develops under varying fertilization levels, providing insights into the observed differences over time. It's also crucial to explore these mechanisms further to determine the direct and indirect effects of omnivore predators on both plants and pests.

At long term - Large-Scale Field Experiments:

Firstly, expanding the breadth of my research endeavors involves embarking on extensive, multi-year field experiments that span a wide array of ecosystems, crop varieties, and geographic settings. This entails conducting meticulously designed studies on a grand scale, traversing diverse landscapes, climatic conditions, and agricultural contexts to comprehensively investigate the intricate interplay of variables influencing ecological dynamics and agricultural outcomes. Secondly, delving into the intricacies of Intraguild Predation (IGP) dynamics involves a thorough examination of the complex interactions and mutual influences occurring among natural enemies, namely predators and parasitoids, alongside potential pests belonging to the same guild. By scrutinizing these interactions at various levels, from individual behaviors to population dynamics, this research seeks to unravel the underlying processes shaping community structure and trophic dynamics, thereby providing valuable insights into the ecological mechanisms governing predator-prey relationships and pest regulation within ecosystems. Thirdly, advanced mechanistic studies entail the utilization of cutting-edge technologies and methodologies to delve deep into the molecular, genetic, or physiological facets of these interactions. Through rigorous experimentation and analysis, this research endeavor aims to enhance our understanding of the intricate web of relationships between plants and associated organisms, ultimately contributing to the development of more effective strategies for managing ecological interactions and promoting sustainable agricultural practices. Finally, promoting community and stakeholder engagement involves cultivating partnerships with farmers and agricultural organizations to facilitate the implementation and assessment of the practical relevance of research outcomes. By fostering open communication channels and knowledge-sharing platforms, researchers aim to bridge the gap between scientific insights and on-the-ground realities, ensuring that research outcomes directly benefit agricultural communities. Through active engagement and participatory approaches, this collaborative

endeavor strives to empower farmers with the necessary tools, knowledge, and support to enhance productivity, resilience, and sustainability within their agricultural systems.

All these studies provide valuable insights into the trophic dynamics of plant-arthropod interactions, offering potential solutions for optimizing IPM programs.

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