

RESEARCH ARTICLE

High nitrogen loading impacts the temperature-size rule and heat tolerance in a nettle-feeding butterfly

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Abstract

1. Host-plant quality and ambient temperature are key environmental drivers of herbivorous insect performance, affecting growth, development, and survival. While temperature accelerates physiological processes in ectothermic insects, nutrient limitation in host plants can constrain insect herbivore fitness. Yet, few studies have examined how these two factors interact to shape insect herbivore performance.
2. We experimentally quantified the combined effects of host-plant stoichiometry and rearing temperature on the larval growth rate, development time, and adult heat tolerance of the Map butterfly (*Araschnia levana* L. 1758). Caterpillars were reared on stinging nettle (*Urtica dioica* L.) cultivated under three nutrient addition regimes differing in N:P ratios—to alter the nutrient stoichiometry of their host plants—and under two diurnally fluctuating temperature regimes simulating ambient (day–night: 21°C/12°C) and elevated (day–night: 24°C/16°C) conditions.
3. Both temperature and host plant stoichiometry affected butterfly performance. Warming and a balanced stoichiometry enhanced growth and development; under these favourable conditions, caterpillars exhibited rapid growth, faster development, higher survival, and increased adult heat tolerance. A trade-off between size and age at maturity—individuals either matured rapidly at smaller sizes or more slowly at larger sizes—was observed only under conditions that were favourable for growth, suggesting that developmental time was the main limiting factor under these conditions for the final butterfly body size.
4. Our results thus demonstrate that host plant nutrient imbalances can mediate how insect herbivores prioritize between size and age at maturity under warming conditions, influencing thermal tolerance too. This suggests that eutrophication and climatic warming may jointly reshape the allocation strategies, performance, and resilience of insect herbivores in Anthropocene ecosystems.

KEYWORDS

Araschnia levana, body size, climate change, eutrophication, host-plant quality

Dirk Maes and Wilco C. E. P. Verberk shared senior authorship.

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1 | INTRODUCTION

Host-plant quality and ambient temperature are important drivers of herbivorous insect performance (Clissold & Simpson, 2015). Consequently, anthropogenic stressors such as nitrogen enrichment and global warming are reshaping insect communities worldwide (Harvey et al., 2023; Wagner et al., 2021). Temperature has profound direct effects on physiological rates, accelerating growth and development in ectotherms (Brown et al., 2004; Meunier et al., 2024). Because these organisms depend on the thermal characteristics of their surroundings to sustain suitable body temperatures allowing activity (Kearney et al., 2009), even moderate warming can alter their life histories. Higher developmental temperatures typically accelerate development but produce smaller adults, as development rates increase more steeply than growth rates—a pattern known as the temperature-size rule (Atkinson, 1994; Forster et al., 2011; Semsar-Kazerouni et al., 2022; Verberk et al., 2021). This reduction in adult body size reflects the trade-off often observed between size and age at maturity: animals can either achieve maturation rapidly but at the cost of a smaller body size, as there is less time to grow, or they achieve maturity later at a larger size. Because insect fecundity usually scales positively with body size (Honěk, 1993), temperature thus emerges as a key driver of overall biological fitness (Bale et al., 2002; Bjørge et al., 2018; Ghosh et al., 2013; Lindroth et al., 1997).

Beyond its physiological effect, temperature can also limit activity patterns, as organisms must invest time and energy in behavioural thermoregulation, especially when it comes to tolerating temperature extremes (Hutchison & Maness, 1979). Rising ambient temperatures increase the risk of overheating, as metabolic and respiratory processes approach their critical thermal limits (Neven, 2000). Understanding the ability of species to tolerate heat is therefore central to predicting their response to ongoing and future climatic change (Klockmann et al., 2017). Acclimation—whether induced during development or in adults—can increase heat tolerance through physiological or developmental adjustments (Bowler & Terblanche, 2008; Morley et al., 2019). Such plasticity in thermal tolerance limits can extend the time window for activity (Barnes, 2021).

In addition to temperature, the nutritional environment during development affects metabolic efficiency, survival rates, and ultimately fitness (Morehouse & Rutowski, 2010). The elemental composition of host plants, particularly their nitrogen (N) and phosphorus (P) content, is a key determinant of food quality for insect herbivores (Hessen et al., 2013; Vogels et al., 2023). A higher quality diet promotes larval growth, increasing pupal mass and adult body sizes (Holmes et al., 2020). According to the N limitation hypothesis, higher dietary nitrogen concentrations generally promote insect growth, development and survival (White, 2012). Yet, nutrient enrichment can also disrupt the nutritional balance: excessive N deposition alters host-plant biochemical composition and can induce P limitation, thereby reducing overall food quality (Raharivololoniaina et al., 2023; Vogels et al., 2023; Zhu et al., 2023). Such stoichiometric shifts have been shown to impair larval development and fitness in several insects, including butterflies (Kurze et al., 2018; Lebigre

et al., 2018), although responses can vary even among closely related species (Zhu et al., 2023). The host plant N:P ratio determines the prevailing nutrient limitation—low ratios indicate N limitation and high ratios P limitation—which in turn affects herbivore fitness through multiple physiological and developmental pathways. The direction and strength of these effects likely depend on the herbivore's life-history strategy and trait composition (Stevens et al., 2018; Vogels et al., 2023).

Nettle-feeding butterflies have specialized on the nitrophilous stinging nettle (*Urtica dioica*) as their larval host plant (Pullin, 1986). Historically confined to relatively nitrogen-rich patches in woodlands and floodplains (Olsen, 1921), *U. dioica* has expanded markedly over recent decades due to human-induced land-use change and widespread nitrogen pollution (Taylor, 2009). Excessive amounts of anthropogenic N inputs from atmospheric deposition and intensive fertilization (Erismann et al., 2015; Galloway et al., 1995; Vitousek et al., 1997) have thus created persistently N-enriched habitats, within and outside woodlands.

Because specialist nettle feeders are adapted to a highly nitrophilous host plant, they are assumed to benefit from nitrogen-rich diets and to tolerate fertilization levels typical of (intensive) agricultural settings. Indeed, experimental work has shown that *Aglais* caterpillars fed on N-fertilized nettles display higher survival, shorter larval development times, and greater pupal mass (Kurze et al., 2017; see also Pullin, 1986; Lindroth et al., 1997). Nonetheless, despite the increase in host-plant presence and possible adaptation to high N content, some nettle feeders have experienced strong population declines in the past decades, notably the Map butterfly (*Araschnia levana* L. 1758).

Understanding how host-plant nutrient composition interacts with temperature to shape insect performance is therefore important. Both global warming and eutrophication are altering the nutritional resource landscape across ecosystems (Meunier et al., 2024; Peñuelas et al., 2013). Indeed, several studies have experimentally combined temperature and host plant or diet quality (e.g. *Pieris napi*—Bauerfeind & Fischer, 2013; *Pieris rapae*—Parker & Kingsolver, 2023). Nevertheless, integrative approaches explicitly addressing nutrient stoichiometry remain limited. Such integrative approaches are essential to understand and predict how species and communities respond to multiple, co-occurring drivers of global change (Meunier et al., 2024).

Here, we used *A. levana* to experimentally test how larvae respond in terms of growth, development time, and survival to the combined effects of host-plant nutritional quality and warming. Furthermore, we assessed how these developmental conditions affect adult heat tolerance. We predicted that (i) individuals reared on N-rich nettles and high N:P ratios would develop faster, survive better, and produce heavier pupae than those on N-poor nettles; (ii) higher temperatures would amplify the effects of nutrient limitation (during caterpillar development on N-poor nettles) by increasing metabolic demands; (iii) individuals reared at higher temperatures would show increased heat tolerance through larval thermal acclimation; and (iv) both acclimation to high developmental

temperatures and N-rich diet would enhance adult heat tolerance, as a higher quality, nitrogen-rich diet is expected to improve overall condition, which may in turn increase the ability to withstand heat stress. We also explored the relationship between pupal weight and larval development time for different fertilization treatments and rearing temperatures.

2 | MATERIALS AND METHODS

2.1 | Study species

The Map butterfly (*A. levana*) is a Eurasian species that is native to Europe and widely distributed, occurring from Western Europe to eastern Russia and Japan (Mitikka et al., 2008). Over the last decades, it has expanded its range very significantly towards the north and the west (Parmesan et al., 1999). *Araschnia levana* belongs to the nymphalid family, with its larvae feeding on stinging nettle *U. dioica* (Pullin, 1987). It is bivoltine and famous for showcasing a pronounced case of seasonal polymorphism, with distinct morphological phenotypes for spring versus summer imagoes. Due to recent warmer summers, an additional third generation in late summer has become more common in central Europe (Altermatt, 2010).

2.2 | Host-plant treatments

Urtica dioica seedlings were collected from a forest edge close to agricultural fields in Noord-Brabant, The Netherlands (51.594662, 5.608490) in March 2024. A total of 140 shoots were potted in garden soil (Table S1). After a month, the plants were transferred to bigger pots and kept under open greenhouse conditions and received water in ample supply. They were randomly assigned to three host-plant treatments: (i) a 'low nutrient' treatment (50 kgN/ha/year, 20 kgP/ha/year, hereafter referred to as np), (ii) an 'increased N' treatment (300 kgN/ha/year, 20 kgP/ha/year, hereafter referred to as Np), and (iii) an 'increased N and P' treatment (300 kgN/ha/year, 80 kgP/ha/year, hereafter referred to as NP) (Kurze et al., 2017; Müllerová et al., 2014; Taylor, 2009). The chosen applications of fertilizer are based on both commonly used quantities in agriculture, ranging from 0 to over 500 kgN/ha/year in different grasslands across Europe (Herzog et al., 2006) and on previous work by Kurze et al. (2017), and were calculated based on the pot volume. When the first larvae hatched, the plants received the first 20% of the fertilizer. The remaining 80% was given in four further equal doses, divided over the caterpillar's 2-month rearing period (May–June). Manipulating nitrogen additions should result in host plants with different N:P ratio's, and skewed N:P ratio's are expected to burden the growth rate and/or survival of the caterpillars. To determine whether the observed effects were due solely to increased N or to a change in the N:P ratio, a third treatment was added in which both N and P were increased.

A soil sample was taken at the moment of potting. This was analysed for elemental concentrations using an ICP-OES instrument, a type of optical emission spectrometer designed for multi-element analysis (ICP-OES iCAP 6000, Fischer Scientific). Additionally, leaf samples were taken right before fertilizer addition, after the first fertilizer event, and approximately every other day during the experiment. A random sample of leaf cuttings of each treatment was taken, dried and ground to a homogeneous powder and analysed with a C/N analyser (Vario Micro Cube, Elementar), as well as with the ICP-OES emission spectrometer to determine the nitrogen (N%) and phosphorus (P%) concentration, as well as the N:P ratio in reference to the dry weight (Kurze et al., 2017).

2.3 | Rearing experiment

Gravid adult females (i.e., carrying mature eggs) of *A. levana* ($n=7$) were captured in the Ooijpolder (51.854338, 5.924752), Nijmegen, The Netherlands on 29 April 2024. They were kept in individual boxes and transferred to Radboud University (Nijmegen), where they were kept in rearing cages (70 × 90 × 100 cm) under open greenhouse conditions, providing shelter from rain and extreme temperatures. For oviposition, females were provided with potted *U. dioica* plants. Females had access to freshly picked flowers and water for adult feeding, as previous experiments have shown the importance of high food quality for female fecundity in *A. levana* (Mevius-Schütz & Erhardt, 2005). Deposited eggs were collected daily during 7 days; host plants containing eggs were transferred to a separate cage until egg hatching. Hatching eggs from all females were pooled prior to allocation these across treatments; consequently, exact clutch sizes per female could not be determined and family identity was not retained.

Our experiment adopted a split-brood design. Broods of individual females were split 4 days after hatching and randomly distributed across treatments, such that larvae from different females were represented across treatment combinations. Given the number of females, containers, and treatment replicates, the probability that a treatment consisted of offspring from a single female was low. Accordingly, we did not explicitly account for potential family or parental effects in further analyses. Larvae were reared per 15 individuals in a plastic container (16 × 14 × 9 cm), and provided with an ample supply of fresh cuttings of *U. dioica* from the allocated fertilization treatment, either at a control temperature 21°C/12°C (day/night, resp.), or at an increased temperature treatment of 24°C/16°C. Both climate chambers were set to a photoperiod of L16D8 (light:dark). The applied photoperiod in this experiment results in direct development and produced the summer morph. In total, 180 larvae were divided over the fertilization × temperature treatments, resulting in 30 individuals per treatment combination.

To prevent any bias due to possible differences in ambient conditions among the two climate chambers, treatment settings and caterpillars were rotated between chambers twice during the experiment

(Figure S1). Similarly, all containers were moved around daily within the chambers to avoid biases due to minor within-chamber spatial heterogeneity in light and temperature conditions.

Growth rate, development time and survival were measured (Figure S1). Growth rate was quantified as the mean daily increase in larval body mass during larval development (from instar II to the final instar). Larvae were weighed every 3–4 days between Day 5 and 25 after hatching. At each time point, five larvae per container were weighed and averaged. Growth rate was calculated as the increase in body mass per day for each container. Individuals were weighed using an electronic balance (0.01 mg precision, Sartorius AG, LA310S). Larval development time was quantified as the time in days between egg hatching and pupation. Pupae were weighed within 1 day after pupation, as soon as the puparium was cured (Kurze et al., 2017), after which they were individually stored at their respective rearing temperatures at the same thermal regime experienced by the larvae, until emergence. Survival rate corresponds to the proportion of larvae that produced adults. After emergence, the fresh body mass of each adult individual was weighed. Adults were tested for heat tolerance (see further), and next stored in a freezer (−20°C) for further analyses. All adults were later dried in an incubator at 70°C for 24 h, and their total dry body mass was measured.

Percentage loss of body mass upon eclosion was calculated by comparing the fresh mass of the pupa before emergence to the fresh mass of the adult after emergence.

2.4 | Heat tolerance

Temperature-controlled water baths were used to test adult heat tolerance. All individuals were tested between 24 and 48 h after emergence. Depending on survival, 6–18 individuals were tested per treatment combination ($N_{\text{total}} = 88$). Butterflies were placed individually in a glass vial (15 mL), large enough for spreading the wings, but not for flying. Before measuring heat tolerance, all individuals experienced a short period at room temperature (c. 21°C) in the lab for a maximum of 30 min. To prevent potential confounding effects by desiccation (see Sinclair et al., 2024), wet Whatman paper was placed at the bottom of each vial to ensure 100% humidity. Next, the vials were placed in the temperature-controlled water baths, which were programmed at a set temperature. Initially, we explored a range of test temperatures (43°C, 45°C, 48°C, or 50°C) and showed that the time the butterflies could survive heat stress followed a log-linear relation (Figure S2), confirming previous studies (Jørgensen et al., 2019; Rezende et al., 2020). Based on this preliminary test, measurements were performed at 43°C and 45°C.

Two endpoints were recorded during the heat tolerance trials. As the trials progressed, butterflies entered a period of rapid movement, and the moment at which this movement stopped was recorded as a behavioural stress response endpoint. Further into the trial, body movement stopped completely (i.e., heat knockdown), which was our second endpoint. Although individuals may theoretically recover

from knockdown, this endpoint represents a proxy for acute thermal tolerance. Only the second endpoint was used for statistical analysis and is reported in the results. The full measuring period was video-recorded, to determine the exact duration until each endpoint. Heat tolerance measurements were done on different, consecutive days depending on emergence, in order to standardize the age of emerging butterflies between 1 and 2 days.

2.5 | Replication statement

Scale of inference	Scale at which the factor is applied	Number of replicates
Species	Species	Six treatment combinations, with 30 individuals each

2.6 | Animal ethics statement

This study involved the rearing of lepidopteran larvae and adult butterflies (invertebrates). Under Dutch legislation (Experiments on Animals Act), ethical approval is not required for research involving insects. No institutional ethical review was therefore necessary. All individuals were handled and maintained with due care to minimize stress and mortality.

2.7 | Statistical analyses

To test whether our nutrient additions affected leaf stoichiometry, we used linear models to test for differences in N concentration, P concentration and N:P ratios of *U. dioica* leaf material, using treatment as the fixed effect. For N- and P concentrations, percentage values were first converted to proportions and arcsine-square root transformed prior to analysis.

To assess the effects of rearing temperature and host-plant treatment on larval and pupal period and pupal and adult mass, we fitted linear mixed models (LMER, R-package *lme4*, version 1.1-35.5; Bates et al., 2015), including container number as a random effect. For all response variables, we initially fitted models including the interaction between rearing temperature and host-plant treatment. As the interaction term was not statistically significant for any response variable and model comparison based on AIC supported simpler additive models, we report additive models in the main text. Post hoc Tukey's tests (R-package *stats*, version 3.6.2b; R Core Team, 2022) were applied to the additive models to compare treatment levels (see model outputs Table S5).

To compare the developmental trajectories between treatments, we characterized a 'developmental slope' for each treatment combination. This slope was obtained by fitting linear models (LMs) with pupal weight plotted against time to emergence, thereby linking variation in growth to variation in development rate.

In order to test for the effect of temperature and host-plant treatments on survival time of heat tolerance, linear mixed models (LMER) were constructed with test day and container as random factors, but since test day did not improve the models' fit, it was not retained in the model. Partial residuals were calculated to illustrate the relationship between the response variable (here, survival time when exposed to a high temperature) and a given independent variable, while accounting for the effects of other independent variables in the model.

3 | RESULTS

3.1 | Host-plant stoichiometry

The nutrient additions altered the stoichiometry of the nettle leaves (ANOVA: $F_{2,49}=3.29$, $p=0.046$; Figure 1). Leaf nitrogen concentrations were slightly higher following N addition (Np vs. np: $\beta=0.872\pm0.370$, $t=2.36$, $p=0.058$; NP vs. np: 0.727 ± 0.364 , $t=1.995$, $p=0.124$; Figure 1a, $p=0.049$; Table S2), while phosphorus concentrations remained similar across treatments (all pairwise contrasts $p>0.42$; Figure 1b, $p>0.05$; Table S2). As a result, leaf N:P ratios tended to be higher in the N-rich treatments (Np and NP, ~20) than in the N-poor treatment (np; ~16) (NP vs. np: $\beta=3.896\pm1.924$, $t=2.03$, $p=0.116$; Np vs. np: $\beta=3.025\pm1.949$, $t=1.55$, $p=0.275$; Figure 1c, $p>0.05$; Table S2). Although these differences were not statistically significant, they reflect the intended experimental contrasts and provide the nutritional context for the observed caterpillar responses.

3.2 | Body mass and larval development

Caterpillars reared on N-rich nettles (NP or Np) had shorter larval periods (NP vs. np: $\beta=-3.30\pm0.618$, $t=-5.34$, $p=0.0006$; Np vs. np: $\beta=-2.98\pm0.616$, $t=-4.83$, $p=0.0012$; Figure 2A,

$F_{2,5.98}=23.83$, $p<0.001$; Table S3). Higher nitrogen availability promoted faster larval growth, which allowed caterpillars to reach greater pupal mass despite the reduced development time (NP vs. np: $\beta=0.0314\pm0.00449$, $t=6.996$, $p=0.0001$; Np vs. np: $\beta=0.0248\pm0.00447$, $t=5.55$, $p=0.0006$; Figure 2B, $F_{1,5.81}=20.542$, $p=0.0034$; Table S3). Survival was higher in N addition treatments (Table 1). Temperature accelerated development (24°C vs. 21°C: $\beta=-5.58\pm0.50$, $t=-11.13$, $p<0.0001$; Figure 2A, $F_{1,6.03}=326.99$, $p<0.001$; Table S3) and increased growth rate equally, such that caterpillars reached the same pupal mass in a shorter time in the warmer treatments (24°C vs. 21°C: $\beta=0.00146\pm0.00363$, $t=0.401$, $p=0.699$; Figure 2B, $F_{1,7.76}=0.16$, $p=0.699$; Table S3).

Within treatments, the relationship between development time and pupal mass was variable (Figure 3). For caterpillars reared at 24°C and fed with nettles fertilized by a high amount of nitrogen (NP or Np), we observed a strong positive relationship between development time and pupal mass: individuals reaching the pupal stage more rapidly had lower mass (NP: $\beta=0.104\pm0.0227$, $t=4.61$, $p<0.001$, $R^2=0.46$; Np: $\beta=0.0679\pm0.0315$, $t=2.16$, $p=0.039$, $R^2=0.14$; Figure 3). Among the four remaining treatments, only caterpillars reared at 21°C on high-nitrogen and phosphorus (NP) host plants showed a non-significant positive trend between pupal mass and developmental time ($\beta=0.00987\pm0.00716$, $t=1.38$, $p=0.18$, $R^2=0.071$; Figure 3). In contrast, caterpillars reared on a N-poor diet (np) at either 21°C or 24°C, as well as those fed at one of the N-rich diets (Np) at 21°C, showed no such relationship, as longer development did not result in heavier pupae (24°C np: $\beta=-0.00501\pm0.0109$, $t=-0.46$, $p=0.65$, $R^2=0.010$; 21°C Np: $\beta=-0.00137\pm0.00402$, $t=-0.34$, $p=0.74$, $R^2=0.0048$; 21°C np: $\beta=-0.00283\pm0.00457$, $t=-0.62$, $p=0.54$, $R^2=0.018$; Figure 3).

Percentage loss of body mass differed across treatments, with higher retention in caterpillars reared at 24°C under N-rich conditions (NP: 53.5%, Np: 51.9%) compared with other treatments (np-24°C: 39.7%, NP-21°C: 43.3%, Np-21°C: 39.1%, np-21°C: 37.1%).

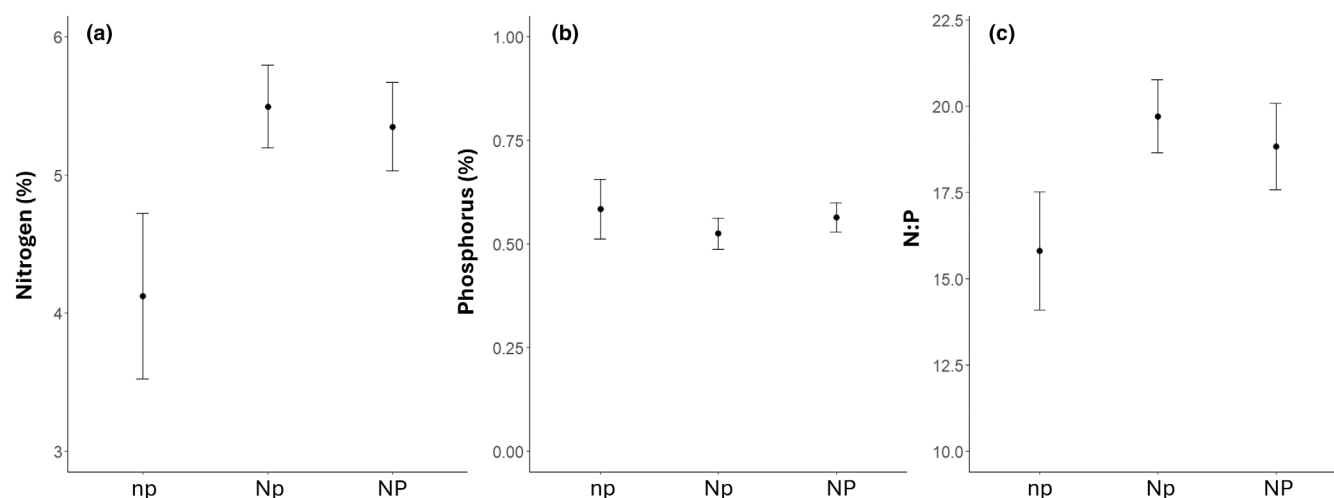


FIGURE 1 (a) Nitrogen concentration, (b) phosphorus concentration, and (c) N:P molar ratio of *Urtica dioica* host plants depending on the fertilization treatments (np [50 kg N/ha/year, 20 kg P/ha/year], Np [300 kg N/ha/year, 20 kg P/ha/year], and NP [300 kg N/ha/year, 80 kg P/ha/year]). Data expressed as mean $\pm$ SE.

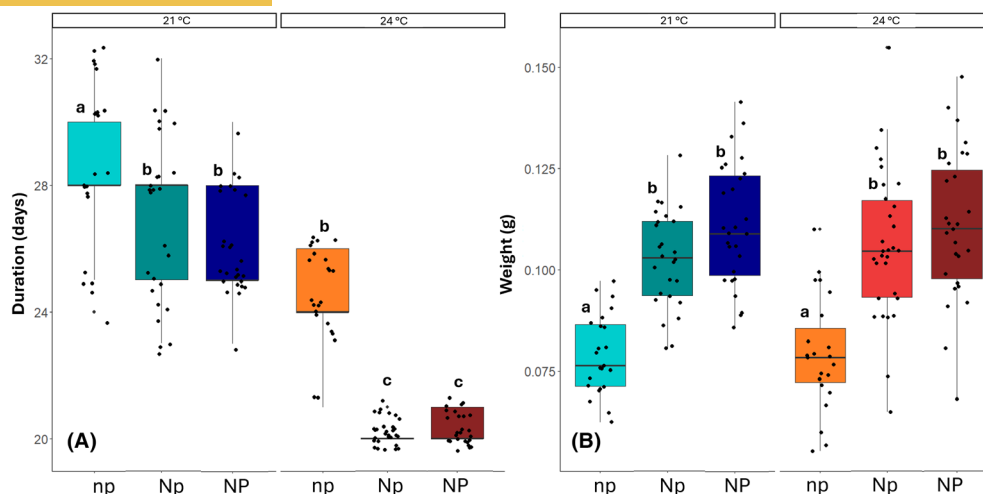


FIGURE 2 (A) Larval development time, and (B) pupal weight of *Araschnia levana* butterflies depending on the fertilization treatment, per rearing temperature. Data are expressed using boxplot graphs including SE. Colour hue indicates temperature treatment (blue-toned: 21°C; red-toned), while colour shade indicates nettle-fertilization treatments (light—np [50kg N/ha/year, 20kg P/ha/year], med—Np [300kg N/ha/year, 20kg P/ha/year], and dark—NP [300kgN/ha/year, 80kg P/ha/year]). Significant differences between treatments are indicated by letters above boxplots (ANOVA, $p < 0.05$).

TABLE 1 Survival of larvae per temperature (21D14N & 24D16N) and host-plant (np, Np and NP) treatment in percentage (%) and as a fraction of total individuals ($n = x/x$).

	Survival	
	21°C	24°C
np	80.00% $\pm$ 6.67 ($n = 24/30$)	76.67% $\pm$ 16.67 ($n = 23/30$)
Np	86.67% $\pm$ 0.00 ($n = 26/30$)	96.67% $\pm$ 3.33 ($n = 29/30$)
NP	90.00% $\pm$ 3.33 ($n = 27/30$)	93.33% $\pm$ 0.00 ($n = 28/30$)

3.3 | Heat tolerance

With increasing heat stress intensity, the time butterflies could withstand heat stress—defined as the point at which all body movement ceased—decreased (Figure 4A, $p < 0.001$). Heat tolerance was higher in individuals reared at 24°C compared with individuals reared at 21°C (21°C vs. 24°C: $\beta = 0.082 \pm 0.021$, $t = 3.89$, $p = 0.003$; Figure 4B; Table S5). In addition, heat tolerance was also modulated by diet; individuals reared on N-rich nettles (Np or NP) were more heat tolerant than individuals reared on N-poor nettles (np) (NP vs. np: $\beta = 0.126 \pm 0.078$, $t = 1.61$, $p = 0.139$, Np vs. np: $\beta = 0.152 \pm 0.074$, $t = 2.05$, $p = 0.073$; Figure 4C; Table S5).

4 | DISCUSSION

Nutritional imbalances can negatively affect the development and fitness of herbivorous insects (Vogels et al., 2023), while temperature strongly affects physiological rates in ectotherms, accelerating growth and development (Brown et al., 2004; Meunier et al., 2024).

Yet, few studies have examined the combined effects of temperature and host-plant stoichiometry.

The average optimum N:P ratio for terrestrial herbivores is estimated around 28:1 (Elser et al., 2000). Given that nettles are rich in nitrogen, we expected the optimum N:P ratio to be higher than this average for our nettle-feeding model species—the Map butterfly *A. levana*. Our findings support this prediction: performance was poorest in the N-poor treatment (np, N:P ~16), suggesting an imbalance towards nitrogen limitation. This resulted in slower growth, lower final weight, and lower heat tolerance, all of which improved for caterpillars fed N-rich nettles (Np and NP). Comparable effects of stoichiometric imbalance have been reported in crickets reared on heather (*Calluna vulgaris*)—a host-plant adapted to nutrient-poor soil conditions—where elevated N:P ratios (up to 42) caused phosphorus limitation and reduced cricket growth and reproduction (Vogels et al., 2024). Together, these studies highlight that herbivore performance peaks around species-specific N:P optima and that deviations reduce performance (Elser et al., 2000; Vogels et al., 2023).

The thermal responses we observed in caterpillar growth and development conform to well-established patterns in ectotherms (Buckley, 2022; Honěk & Kocourek, 1990; Semsar-Kazerouni et al., 2022; Verberk et al., 2021). Also, as expected, developmental acclimation to higher rearing temperatures increased heat tolerance (Bowler & Terblanche, 2008; Morley et al., 2019). However, adult heat tolerance was also influenced by larval diet, with the highest tolerance observed in individuals reared on nutritionally balanced nettles. Previous studies have focused mainly on starvation (Semsar-Kazerouni et al., 2020) or specific nutrient constraints such as cholesterol limitation (Koussoroplis et al., 2023). Andersen et al. (2010) reported improved heat tolerance in adult fruitflies when reared on a protein-enriched medium and suggested that this may be related to the induction of heat shock

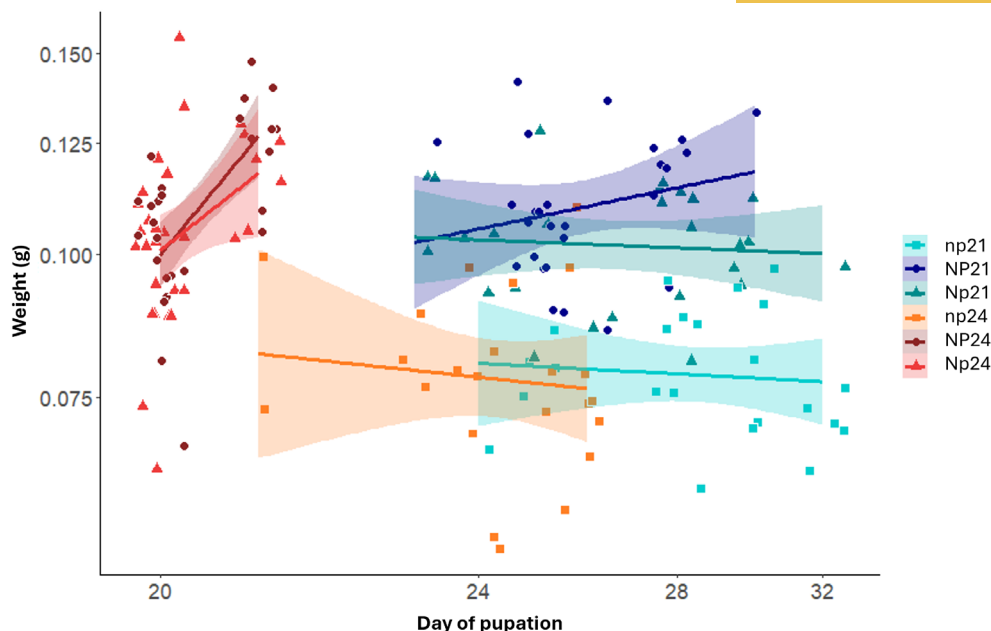


FIGURE 3 Relation between pupal weight and larval development time for different fertilization treatments and rearing temperatures. Lines represent fitted relationships from linear mixed models, and shaded areas indicate 95% confidence intervals. Colour hue indicates temperature treatment (blue-toned: 21°C; red-toned: 24°C), while colour shade indicates nettle-fertilization treatments (light, and squares—np [50 kg N/ha/year, 20 kg P/ha/year], med and circles—Np [300 kg N/ha/year, 20 kg P/ha/year], and dark and triangle—NP [300 kg N/ha/year, 80 kg P/ha/year]).

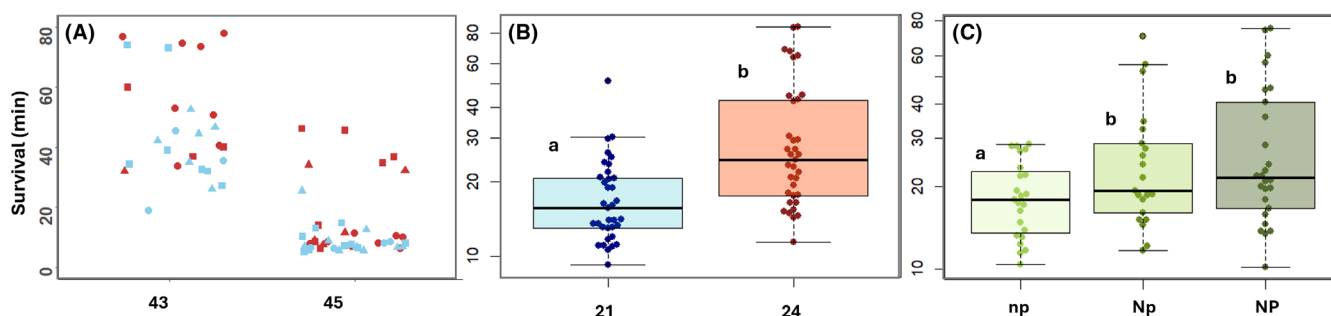


FIGURE 4 Heat tolerance plots depicting time (in minutes) until body movement ceased, for: (A) waterbath temperature at 43°C and 45°C, at both rearing temperature (21°C in blue, and 24°C in red) and at the fertilization treatment (square for np, circle for Np and triangle for NP), (B) rearing temperatures 21°C (in dark blue) and 24°C (in red), and (C) nettle-fertilization treatments np (in light green), Np (in green) and NP (in dark green). Panel (A) shows raw heat tolerance measurements at each assay temperature, whereas panels (B) and (C) show model-adjusted values (partial residuals). Panels (B) and (C) include measurements from both assay temperatures (43°C and 45°C), with assay temperature accounted for in the statistical model. Lowercase letters in panels (B) and (C) indicate significant differences between groups (based on Tukeys post hoc test ($p < 0.05$)). For (B) and (C) the partial residuals were calculated to illustrate the relationship between the response variable (here, survival time when exposed to a high temperature) and a given independent variable, while accounting for the effects of other independent variables in the model. Such plots are constructed by adding the residuals of the model to the fitted relationship of the independent variable of interest and plotting these values (on the y-axis) against the independent variable of interest (on the x-axis).

proteins or the use of proteins as an additional energy source. Our results demonstrate that the stoichiometric quality of larval food can also modulate adult heat tolerance, expanding our current understanding of how global change drivers—such as warming and nutrient enrichment—interact to affect insect physiology and behaviour (Harvey et al., 2023).

According to the temperature-size rule, caterpillars grow and develop more rapidly when reared under warmer conditions yet

typically yield smaller adult butterflies as development rate increases more than growth rate (Atkinson, 1994; Verberk et al., 2021; Verspagen et al., 2020). In butterflies, however, this pattern is frequently weaker or absent, as accelerated development under warming is almost fully compensated by higher growth rates, resulting in comparable adult mass (Blanckenhorn & Demont, 2004; Semsar-Kazerouni et al., 2022). Across arthropods, variation in the magnitude of temperature-size responses has been linked to voltinism and

habitat type, with butterflies, multivoltine insects and terrestrial ectotherms showing weak body-size responses (Horne et al., 2015; Verberk et al., 2021). Indeed, across temperatures, we did not observe a consistent trade-off between size and age at maturity (Abrams & Rowe, 1996).

However, within certain treatments characterized by rapid growth (Np and NP treatment at 24°C), a trade-off emerged, that is, larvae gained substantial mass by delaying maturation. Similar interactive effects of temperature and host-plant quality on growth have been reported for the butterfly *Melitaea cinxia* (Verspagen et al., 2020). Our results extend these patterns, as trade-offs were observed only under the combination of warm rearing conditions and a balanced food stoichiometry. Moreover, our results not only report interactions for growth rates but also include responses in development time, whose effects combine to determine pupal mass. All in all, these results suggest that a warming world may enable some insects to better compensate for stoichiometric imbalances.

Pupal mass declined on average by 57% upon adult emergence, consistent with water loss during eclosion (Molleman et al., 2011). Losses exceeding 50%, however, indicate that during growth, larvae accumulated water rather than functional biomass (Molleman et al., 2011), making variation in pupal mass a poor predictor of adult fitness (Fischer et al., 2005). In our study, mass loss (47%–63%) correlated strongly with the strength of the size–age trade-off (Figure 5, $R^2 = 0.84$), suggesting that under conditions of rapid growth, functional biomass was accumulated, which is likely relevant for reproduction or overwintering (Boggs & Freeman, 2005). Such plasticity in growth and energy allocation in response to stoichiometry and temperature could influence population dynamics and seasonal interactions in multivoltine insects under climate change.

Our nutrient manipulations successfully altered the N:P ratio of host plants, with N-rich treatments (Np, NP) reaching a higher N:P ratio (~20), compared with the ratio of ~16 in the N-poor treatment (np). The concentrations of N in the stinging nettle leaves correspond to those recorded in previous studies using similar N

fertilization levels (50 and 300 kgN/ha/year) (Kurze et al., 2017) and fall within the natural range for nettles (Biesiada et al., 2009; Merckx et al., 2015; Serruys & Van Dyck, 2014). P-fertilization did not significantly change leaf P concentration, indicating that our low P treatment (20 kg P/ha/year) was not limiting for nettle growth.

While *A. levana* served as a nettle-feeding butterfly model species, similar experiments on other nettle feeders would be valuable (Bryant et al., 1997). Comparative eco-evolutionary studies could help contextualize some of our current results and explain interspecific differences. Past work has largely focused on nitrogen effects (e.g. Kurze et al., 2017), though some studies have explored N:P imbalances (Audusseau et al., 2015) or temperature impacts (Bryant et al., 2013). Increased nitrogen enhanced development and benefitted biomass in *Aglais io* and *Aglais urticae*, consistent with the N limitation hypothesis, but effects of N:P imbalance were not tested (Kurze et al., 2017). Temperature and degree-day requirements were also found to influence development, voltinism, and distribution patterns (Bryant et al., 2013), suggesting that extending such comparative experiments across multiple factors and species would thus provide broader insights into how global change drivers jointly shape life-history variation in nettle-feeding butterflies. In addition to expanding to other nettle-feeding species, comparative work across different populations within the distributional range of *A. levana* could reveal potential local adaptation or population-specific responses (Parker & Kingsolver, 2023). This is especially interesting given that *A. levana* has expanded its range towards the north and the west over the last decades.

A limitation of our study is that we could not determine the sex for all individuals. Larvae cannot be reliably sexed, and even among adults, sex could be assigned for only ~41% of individuals. Within this subset, males were smaller than females, consistent with previous studies (Freitak et al., 2019). Sex may also influence other traits, including pupal and adult weight loss (Molleman et al., 2011), heat tolerance (Verspagen et al., 2025), and growth and developmental trajectories (Stillwell et al., 2014; Teder, 2014). Because sex was unknown for many individuals, it could not be included in our analyses,

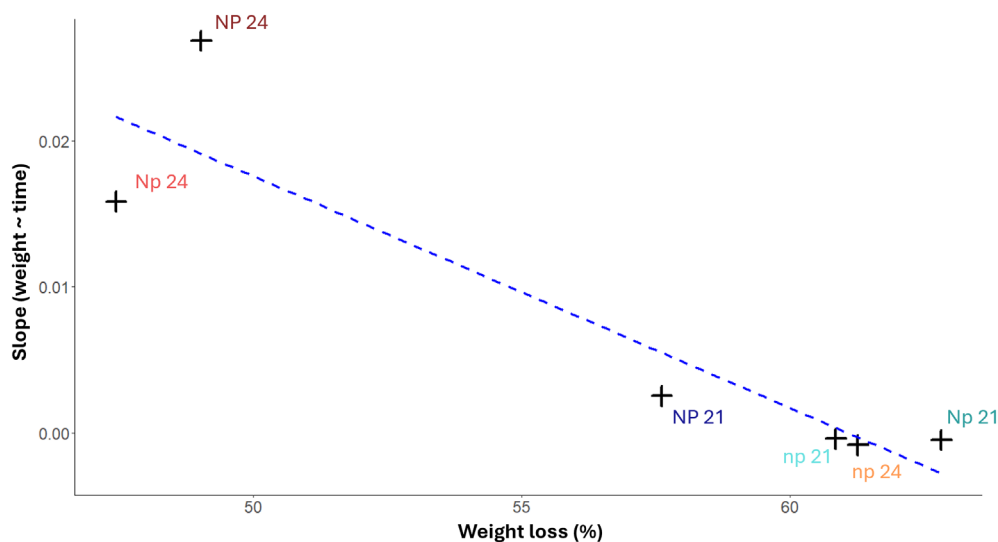


FIGURE 5 Relation between the development slope (pupal weight ~ time until pupation; Figure 3) and mass lost during emergence.

meaning sexual dimorphism contributed unaccounted-for variation. However, we found no evidence that treatment groups differed in sex ratios, suggesting that the reported treatment effects are robust to sex differences.

Here we have focused on nitrogen loadings and temperature during larval development and the consequences for adult phenotype and thermal tolerance performance. To assess the impact on adult fitness, yet another factor may also be at play. This factor has actually been tested in *A. levana* with a focus on adult-stage compensatory feeding (Mevi-Schütz & Erhardt, 2005), rather than larval stoichiometric effects. Uptake and utilization of nectar amino acids by Map butterflies are a compensatory mechanism enabling them to override impacts of poor larval nutrition on adult fitness traits. However, if females are reared on high-quality larval food, it could make them in turn less dependent on certain types of nectar, and thus the presence of particular wild flowers that are rich in amino acids and other food components. Hence, this could be an asset in anthropogenic landscapes where nectar abundance and diversity of wild flowers have declined significantly (Wallisdeveries et al., 2012). Thus, studies linking larval food quality and adult compensatory feeding would be timely. In conclusion, high temperature and balanced host-plant stoichiometry consistently accelerated *A. levana*'s development and improved its growth and heat tolerance. Our findings reveal a trade-off between size and age at maturity in certain treatments only, suggesting that development trajectories can differently interact with diet quality under warmer conditions. Adult heat tolerance was modulated by larval diet, as individuals reared under N-rich conditions were more tolerant than individuals reared under N-poor conditions, highlighting an important developmental carry-over effect. This life-history response showed a close association with weight loss, further indicating that growth performance and biomass accumulation are interactively affected. Thus, the balance between size and age at maturity may shift under stoichiometric imbalances in a warming world. Together, these results underscore how interacting global change drivers—warming and nutrient enrichment—can reshape insect life histories through their effects on growth, energy allocation, and thermal performance.

AUTHOR CONTRIBUTIONS

Berber M. J. W. Meulepas, Dirk Maes, Wilco C. E. P. Verberk, and Thomas Merckx designed the study. Berber M. J. W. Meulepas performed the research and collected the data. Berber M. J. W. Meulepas, Dirk Maes, and Wilco C. E. P. Verberk analysed the data. Berber M. J. W. Meulepas, Dirk Maes, Wilco C. E. P. Verberk, Hans Van Dyck, and Thomas Merckx contributed to the methodology. Berber M. J. W. Meulepas wrote the first manuscript draft, with all authors contributing critically to the manuscript and giving final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data and code are available in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.jsxksn0r1> (Meulepas et al., 2026).

STATEMENT OF INCLUSION

Our study was conducted in the greenhouse facilities of Radboud University (Nijmegen, the Netherlands), in a region where the study species naturally occurs. The research team includes authors based in both the Netherlands and Belgium, representing institutions and organizations from the broader region in which the study was carried out. As this work formed part of a PhD project, most data collection was performed by the lead author, with conceptual and methodological input from supervisors across institutions. The study species, the Map butterfly (*A. levana*), is locally common but declining in parts of Western Europe, making the research relevant to understanding regional ecological change. While no formal engagement with external stakeholders or community groups was required for this type of experimental work, findings contribute to local and regional discussions on insect responses to environmental change and have been shared informally through academic presentations and seminars.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Visual representation of the weight (g) during the run of the experiment, over the three life stages (caterpillars in red, pupae in blue, and adults in yellow), with vertical dashed lines indicating the moments when containers were relocated between chambers.

Figure S2. Heat tolerance for all temperatures measured (43°C, 45°C, 47°C, 48°C, and 50°C).

Table S1. Elemental composition of garden soil used during experiment, before the start of the experiment.

Table S2. Exact values (mean $\pm$ SD) of nitrogen (%), phosphorus (%) and N:P molar ratio values.

Table S3. ANOVA tables of LMER testing the effect of temperature and nitrogen enrichment on duration of larval and pupal period, and pupal and adult weight. Models contained container as a random factor.

Table S4. Life-history values per temperature and nitrogen enrichment treatment. Values given as mean $\pm$ SD.

Table S5. Results of LMER testing the effect of temperature and N ($\pm$ P) enrichment on duration of larval period. Models contained container as a random factor.

Table S6. Results of LMER testing the effect of temperature and N ($\pm$ P) enrichment on duration of pupal weight. Models contained 'container' as a random factor.

Table S7. Results of LMER testing the effect of temperature and N ($\pm$ P) enrichment on butterfly heat tolerance. Models contained 'container' as a random factor.

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