Ecological Entomology (2009), **34**, 535–543 DOI: 10.1111/j.1365-2311.2009.01101.x

More is not necessarily better: the impact of limiting and excessive nutrients on herbivore population growth rates

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Abstract. 1. The body tissues of insect herbivores contain higher concentrations of nitrogen and phosphorus than do their host plants, leading to an elemental mismatch that can limit herbivore growth, fecundity and ultimately influence population dynamics. While low nutrient availability can limit herbivore growth and reproduction, nutrient levels that exceed an organism's nutritional requirements, i.e. an organisms' threshold elemental ratio, can also decrease performance.

2. We conducted a laboratory experiment to examine the impacts of nitrogen and phosphorus additions on population growth rates of a phloem-feeding insect herbivore.

3. Herbivore per capita population growth rates were highest at intermediate foliar nitrogen concentrations, indicating a performance cost on the highest nitrogen foliage. While there was no direct effect of foliar phosphorus concentration on insect performance, there was a strong and unexpected indirect effect. High soil phosphorus availability increased both foliar nitrogen concentrations and aphid tissue nitrogen, resulting in low population growth rates when both soil nitrogen and phosphorus availabilities were high.

4. In this study, experimental increases in foliar nitrogen levels led to a decrease in herbivore performance suggesting that excessive nutrient levels can limit herbivore population growth rates.

Key words. *Aphis nerii*, *Asclepisa*, ecological stoichiometry, growth rate hypothesis, threshold elemental ratio.

Introduction

Ecological stoichiometry examines the balance of macronutrients, usually nitrogen (N), carbon (C), and phosphorus (P), in ecological interactions, while also providing theory and mechanisms that link elemental composition to individual growth, population dynamics, food web structure, community composition, ecosystem function, and evolution (Elser *et al.*, 1996; Frost *et al.*, 2002; Sterner & Elser, 2002; Schade *et al.*, 2003; Andersen *et al.*, 2004; Elser, 2006). Theoretical population models that include stoichiometry have produced fascinating and, at times counterintuitive, results (Andersen *et al.*, 2004).

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Insect herbivores are considered nutrient limited, because they have a higher N and P content than their host plants and this mismatch leads to constraints on their growth and production (Mattson, 1980; Elser, 2000; Elser *et al.*, 2000; Huberty & Denno, 2006). N is considered the most limiting macronutrient for insect herbivores (Mattson, 1980; White, 1993) and it is the raw material of protein synthesis (Sterner & Elser, 2002). N addition has been linked with increased insect density, shorter development time, higher survival rates, increased body mass, higher fecundity, resistance to pathogens, and higher maximum population growth rates (R_{max}) (Mattson, 1980; Cisneros & Godfrey, 2001; Nevo & Coll, 2001; Tsai & Wang, 2001; Stiling & Moon, 2005; Huberty & Denno, 2006; Lee *et al.*, 2006; Zehnder & Hunter, 2008).

Relatively few studies have examined the impact of variation in host plant P on the growth and performance of terrestrial insect herbivores. However, P is an integral component of nucleic

acids, and necessary for the synthesis of both RNA and DNA (Weider *et al.*, 2005a). Plant per cent P is significantly lower than herbivore per cent P, suggesting that P could be limiting in terrestrial systems (Elser *et al.*, 2000). Variation in host plant P and concomitant changes in insect body P influence insect growth, behaviour, survival, and development time (Perkins *et al.*, 2004; Bertram *et al.*, 2006; Huberty & Denno, 2006; Watts *et al.*, 2006). Additionally, there is a rich literature examining the impact of phosphorus additions on aquatic organisms, especially *Daphnia*, for which low phosphorus levels lead to reduced growth rates (Sterner & Elser, 2002; Shimizu & Urabe, 2008) and variation in phosphorus concentrations influences competitive outcomes (Weider *et al.*, 2005b).

To date, most studies have assumed that nutrient additions will increase herbivore growth and reproduction. However, research examining the impact of nutrient ingestion on herbivore fitness has documented increased mortality caused by excess nutrient ingestion (Simpson *et al.*, 2004; Raubenheimer *et al.*, 2005). Boesma and Elser (2006) formalised the concept of high plant nutrient concentrations being energetically costly to herbivores. This occurs if nutrient additions lead to host plants that exhibit C/N/P ratios that exceed herbivore threshold elemental ratios (TER), i.e. host plant elemental content is higher than the level that satisfies herbivore requirements (Boersma & Elser, 2006). Most animals actively maintain homeostasis of their body elemental composition (Sterner & Elser, 2002), and must therefore excrete excess elements (Boersma & Elser, 2006). If excretion is energetically costly, then animals that consume foods which exceed TER levels will exhibit reduced growth, reproduction, and ultimately reduced population growth rates (Anderson *et al.*, 2005; Boersma & Elser, 2006).

We conducted a full factorial experiment to examine the effects of N and P additions on the population dynamics of *Aphis nerii*, the Milkweed-Oleander aphid. We hypothesised that both N and P additions would increase the aphid per capita population growth rate up to *A. nerii's* TER, and then beyond this point per capita population growth rates would decrease as nutrient additions increased. Additionally, we hypothesised that aphids would maintain elemental homeostasis.

Materials and methods

Study system

Our model herbivore was *Aphis nerii* (Hemiptera: Aphididae), a phloem feeding specialist of milkweed, *Asclepias* spp, and oleander, *Nerium oleander* L. (Apocynaceae). *Aphis nerii* undergoes parthenogenetic reproduction for most of its life cycle. *Asclepias syriaca*, the host plant species chosen for this experiment, is broadly distributed across eastern North America and grows in a wide variety of soil types generally associated with disturbance.

Experimental design

We used a full factorial experimental design with three levels of N addition (0, 5, and 10 g/m^2/year) and three levels of P addition (0, 5, and 10 g/m^2/year) for a total of nine treatment combinations with 10 replicates of each. These nutrient addition levels were chosen to create foliar N and P concentrations that fell within the natural range of *A. syriaca* (see below).

To control for genetic variation among insects in our study, we used a clonal aphid colony established from a single individual collected from Gainesville, Florida, and maintained at low densities on *A. syriaca* in a growth chamber. The experiment was conducted in a temperature- and light-controlled walk-in growth chamber. Metal halogen grow lights on timers (16:8 h, day:night) provided heat and light, and daytime and nighttime temperatures were 34 ± 2.4 °C and 24 ± 0.48 °C, respectively, well within the range experienced by Florida populations of *A. nerii*.

Asclepias syriaca seeds (Butterfly Encounters, Inc.) were planted in a nutrient-free potting medium (Rediearth), and watered as needed. Genotypes were unknown. Seedlings were grown in small pots (8 cm diameter) and were not root bound during the course of our experiment. Plants were randomly assigned to treatments and rotated daily to homogenise any environmental gradients within the growth chamber. At the start of the experiment, all seedlings were 4 weeks old, approximately the same size (10–15 cm in height), and composed of a single stem bearing 6–10 leaves.

For the three N treatments 0, 0.071, and 0.143 g of ammonium nitrate, NH_4NO_3, which is 35% N by weight, were dissolved in 80 ml of deionised water to produce a stock solution for each plant. Next, 20 ml was applied weekly with a pipette for 4 weeks prior to aphids being added to plants.

For the P treatments, 0, 0.055, and 0.111 g respectively of Triple Super Phosphate (TSP), which is 45% P by weight, was ground with a mortar and pestle and added to 80 ml of deionised water 2 weeks before the first treatment application. This TSP solution was shaken daily to ensure that P fully dissolved before it was applied to the experimental plants. The treatment was divided into 4 weekly applications. N and P treatments were applied on the same day.

One day after the final N and P applications, three plants were randomly selected from each treatment combination and aboveground plant biomass was harvested. Plants were dried at 64 °C in a drying oven (Lab-line, Dubuque, IA, U.S.A.). Aboveground plant biomass was determined using a Mettler balance (Mettler Toledo, Greifensee, Switzerland).

On the same day, two adult apterous *A. nerii* from the colony were placed on each of the remaining seven plants per treatment and left overnight to reproduce. The next morning, all adult aphids and all but three of the first instar offspring were removed from each plant. Each plant was caged. Cages were constructed from 710-ml Ziploc containers and organza netting over wire frames.

Aphids remained on the plants for 12 days (approximately two to three generations), and then all aphids were counted. Adult and nymphal aphids were not counted separately. All adult aphids were removed from the plants with a paintbrush and placed in a plastic scintillation vial. Nymphs were not collected because of potential variation in body nutrient concentration among life stages (Watts *et al.*, 2006). Adult aphids from each plant were pooled. Aphids were left alive in the vial for

24 h to void their gut contents, and then moved to a different vial before being oven dried.

After the aphids were removed, each plant was clipped at the base of its stem, rinsed with deionised water to remove aphid honeydew, and oven dried as above.

Field collected milkweeds

To ensure that our experimental manipulations generated foliar nutrient concentrations that fell within the natural range that *A. nerii* would likely encounter, leaf samples from native *A. syriaca* were collected from throughout Michigan ($n = 36$ plants). Both old and young leaves were collected to examine the widest possible range of N and P concentrations. Leaves were oven dried as above.

C/N/P analyses (plants and herbivores)

Both laboratory and field-collected plants were ground into a fine powder using a ball mill grinder (Spex Certiprep, Metuchen, NJ, U.S.A.). For C and N analysis, ground plant samples were weighed into tin capsules with a Mettler UMT2 microbalance (Mettler Toledo) and analysed with a Carlo Erba NA 1500 CHN analyser (Carlo Erba, Milan, Italy). For aphid C and N analysis, 10 dried adult aphids were weighed into tin capsules and analysed in the same way. For total per cent P, 0.5 g of plant sample or the remainder of the aphid sample was weighed into acid-washed, pre-ashed ceramic crucibles, ashed at 500 °C, acid digested, and analysed spectrophotometrically (Clesceri *et al.*, 1998).

Cardenolide analysis (plants)

Fertilisation treatments can cause complex changes in plant quality, including changes in chemical defences which could indirectly impact aphid population growth rates (Agrawal, 2004). Cardenolides, or cardiac glycosides, are a group of bitter-tasting, toxic, cardiac-active steroids found in various milkweed species including *A. syriaca*. Cardenolides have many negative effects on herbivores, even in those species adapted to feed on them (Brower *et al.*, 1984; Malcolm & Brower, 1989; Zalucki *et al.*, 2001). Cardenolides were analysed using methods modified from Malcolm and Zalucki (1996). Briefly, ground plant samples (above) were extracted with methanol and analysed using high-performance liquid chromatography (HPLC). Peaks were detected by diode array at 218 nm, and absorbance spectra were recorded from 200 to 300 nm with digitoxin as an internal standard. Peaks with symmetrical absorption maxima between 217 and 222 nm were recorded as cardenolides (Malcolm & Zalucki, 1996). Total cardenolide concentration was calculated as the sum of all individual cardenolide peaks.

Statistical analysis

Aphid per capita growth rate 'r' was calculated on each plant by subtracting the natural log of the initial density (N_1) from the natural log of the final density (N_2) divided by the number of days (t_2–t_1) that aphids were on the plants: $r = (\ln N_2 – \ln N_1)/(t_2 – t_1)$. Aphid 'r' was used instead of final population size to facilitate comparisons with other studies examining aphid population growth rates (e.g. Agrawal, 2004; Zehnder & Hunter, 2007a).

Aphid per capita population growth rate, N concentration and P concentration, as well as plant biomass, cardenolide concentration, foliar N, C and P were compared among treatments using two-way anova with N and P treatments as main effects. The effect of treatments on plant quality and quantity was analysed only for the control plants that did not receive aphids. Potential effects of plant nutrient concentrations, plant nutrient ratios, and cardenolide concentrations on aphid population growth were examined further using simple and multiple regression models, in which individual milkweed plants and their aphid populations were used as replicates. Residuals were checked for normality, and data were transformed when necessary (SAS version 8.2, SAS Institute Inc., Cary, NC, U.S.A.).

Results

Asclepias syriaca foliar N concentrations increased as rates of both N and P addition increased (Fig. 1a nitrogen $F_{2,18} = 10.14$, $P = 0.0014$; phosphorus $F_{2,18} = 10.69$ $P = 0.0011$; nitrogen*phosphorus $F_{4,18} = 1.59$, $P = 0.226$). Foliar P concentrations increased as rates of P addition increased; however, N addition had no influence on foliar P levels (Fig. 1b: nitrogen $F_{2,18} = 0.35$, $P = 0.711$, phosphorus $F_{2,18} = 41.08$, $P < 0.0001$, nitrogen*phosphorus $F_{4,18} = 0.88$, $P = 0.498$). Neither N nor P additions influenced foliar carbon concentrations (data not shown, nitrogen $F_{2,18} = 2.58$, $P = 0.107$; phosphorus $F_{2,18} = 1.77$, $P = 0.206$, nitrogen*phosphorus $F_{4,18} = 1.52$, $P = 0.243$). Increasing N addition led to an increase in aboveground plant biomass, whereas P addition had no impact on plant biomass (Fig. 1c: nitrogen $F_{2,18} = 5.02$, $P = 0.019$; phosphorus $F_{2,18} = 0.56$, $P = 0.579$, nitrogen*phosphorus $F_{4,18} = 0.68$, $P = 0.613$). Increasing P additions led to a decrease in total cardenolide concentrations, but N additions had no effect on *A. syriaca* cardenolide concentrations (Fig. 1d: nitrogen $F_{2,12} = 1.46$, $P = 0.270$; phosphorus $F_{2,12} = 7.60$, $P = 0.007$; nitrogen*phosphorus $F_{4,12} = 2.47$, $P = 0.101$).

Aphid per capita population growth rate was highest when intermediate rates of N and P addition were combined (Fig. 2a). Aphid population growth rates were also high when rates of nutrient addition were opposing (high N/low P or low N/high P). The combination of high addition rates of both N and P led to declines in aphid population growth rates (Fig. 2a: nitrogen*phosphorus $F_{4,54} = 3.12$, $P = 0.023$).

The N content of aphid body tissue increased as both N and P additions increased (Fig. 2b: nitrogen $F_{2,54} = 8.56$, $P = 0.007$; phosphorus $F_{2,54} = 5.35$, $P = 0.008$; nitrogen*phosphorus $F_{4,54} = 1.78$, $P = 0.148$). At low rates of P addition, increasing rates of N addition led to an increase in aphid P content (Fig. 2c). At intermediate and high rates of P addition, variation in N addition did not influence aphid P content (Fig. 2c: nitrogen*phosphorus $F_{4,54} = 4.96$, $P = 0.002$). Effects of nutrient addition on aphid population growth rates and body nutrient concentrations were

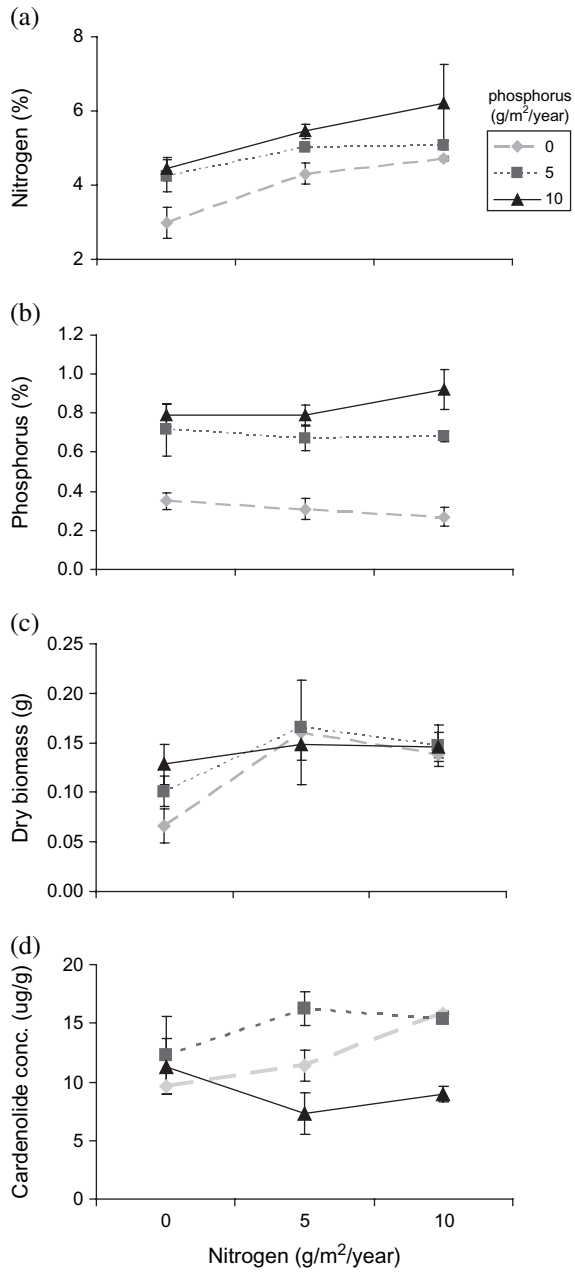

Fig. 1. Mean (±SE) (a) foliar nitrogen concentration, (b) foliar phosphorus concentration, (c) aboveground biomass accumulation and (d) total cardenolide concentration of 4–week-old *Asclepias syriaca* seedlings treated with three levels each of nitrogen and phosphorus ($n = 3$ for each treatment combination).

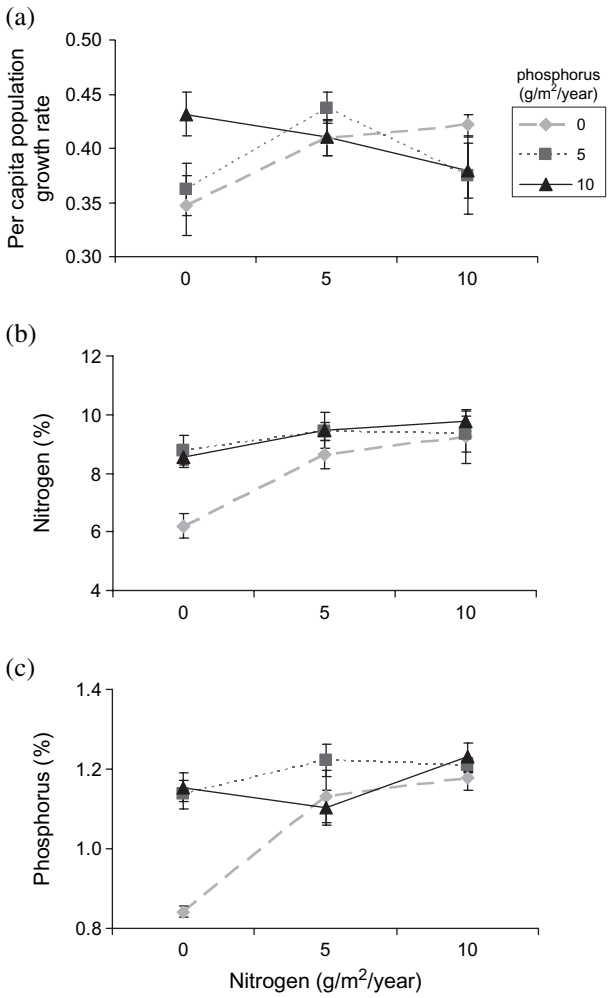

Fig. 2. Mean (±SE) (a) per capita population growth rate, (b) body nitrogen concentration and (c) body phosphorus concentration of *Aphis nerii* reared on *Asclepias syriaca* treated with three levels of nitrogen and three levels of phosphorus ($n = 7$ for each treatment combination).

not apparently linked to crowding of aphids. Almost all adult aphids produced were apterous (wingless) with only four alate (winged) aphids produced during the entire experiment. Alate production is an indicator of crowding in *A. nerii* (Zehnder & Hunter, 2007a). The four alates were not included in the N and P analyses.

Using individual aphid population-host plant combinations as replicates, we developed simple and multiple regression models (Proc GLM, SAS version 8.2, SAS Institute Inc., Cary, NC, U.S.A.) to examine linear and quadratic effects of N, P, and cardenolides, and their interactions, on the per capita growth rate of aphids. Quadratic terms were included in models as specific tests of the Boesma and Elser (2006) hypothesis of declining herbivore performance at highest nutrient availabilities. In no case did the multiple regression models, or their interaction terms, provide additional information over the simple regression models that we present here.

Highest aphid population growth rates were found on *A. syriaca* plants with intermediate nitrogen concentrations (Fig. 3a: quadratic model $R^2 = 0.325$, $P < 0.001$). There was no evidence for a linear relationship between *A. syriaca* N and aphid population growth rates (linear model $R^2 = 0.054$, $P = 0.080$). There was no relationship between *A. syriaca* P and aphid population growth rates (Fig. 3b: linear model $R^2 = 0.015$, $P = 0.350$, quadratic model $R^2 = 0.016$, $P = 0.648$) and no relationship

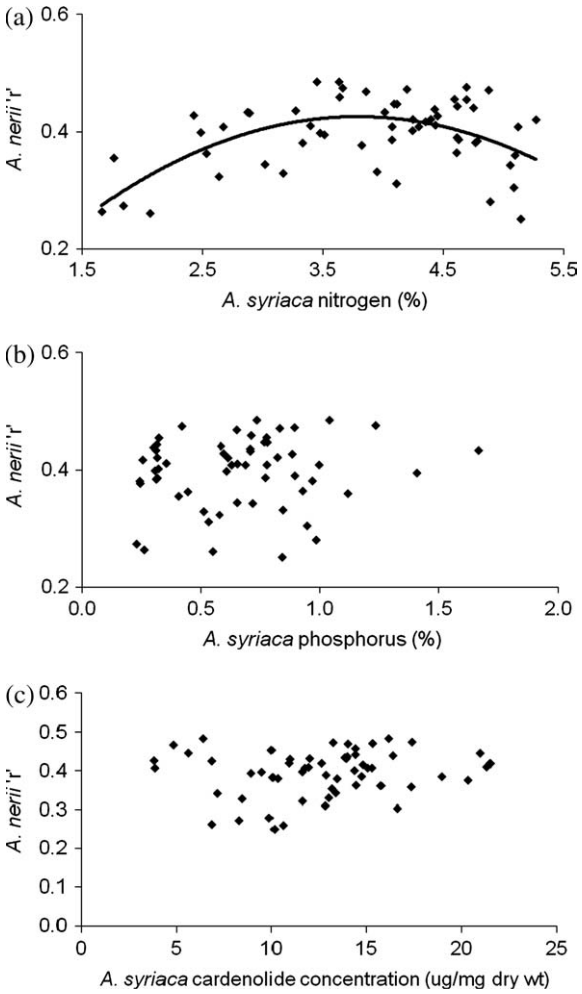

Fig. 3. Relationships between *Aphis nerii* per capita population growth rate (r) and *Asclepias syriaca* (a) foliar nitrogen concentrations, (b) foliar phosphorus concentrations, and (c) foliar cardenolide concentrations. Each point represents a single aphid-host plant pair [$n = 63$ in (a) and (b) and 57 in (c)]. *Asclepias syriaca* values are from the plants upon which the aphids were feeding.

between aphid population growth rates and plant cardenolide concentrations (Fig. 3c: linear model $R^2 = 0.020$, $P = 0.293$, quadratic model $R^2 = 0.04$, $P = 0.299$).

Increases in host plant foliar N concentrations led to concomitant increases in aphid N concentrations (Fig. 4a: $R^2 = 0.294$ $P < 0.001$). However, increases in plant P concentrations had much weaker effects on aphid P concentrations (Fig. 4b: $R^2 = 0.084$, $P = 0.034$). Examining nutrient ratios uncovers a similar pattern; increases in host plant foliar C/N led to concomitant increases in aphid C/N levels (Fig. 4c: $R^2 = 0.596$, $P < 0.001$). However, increases in host plant C/P had no effect on aphid C/P (Fig. 4d: $R^2 = 0.007$, $P = 0.217$).

There was no relationship between aphid per capita population growth rates and aphid N or P concentrations (data not shown aphid N: $R^2 = 0.018$, $P = 0.336$; aphid P: $R^2 = 0.011$, $P = 0.969$). There was a significant, albeit weak, negative linear relationship

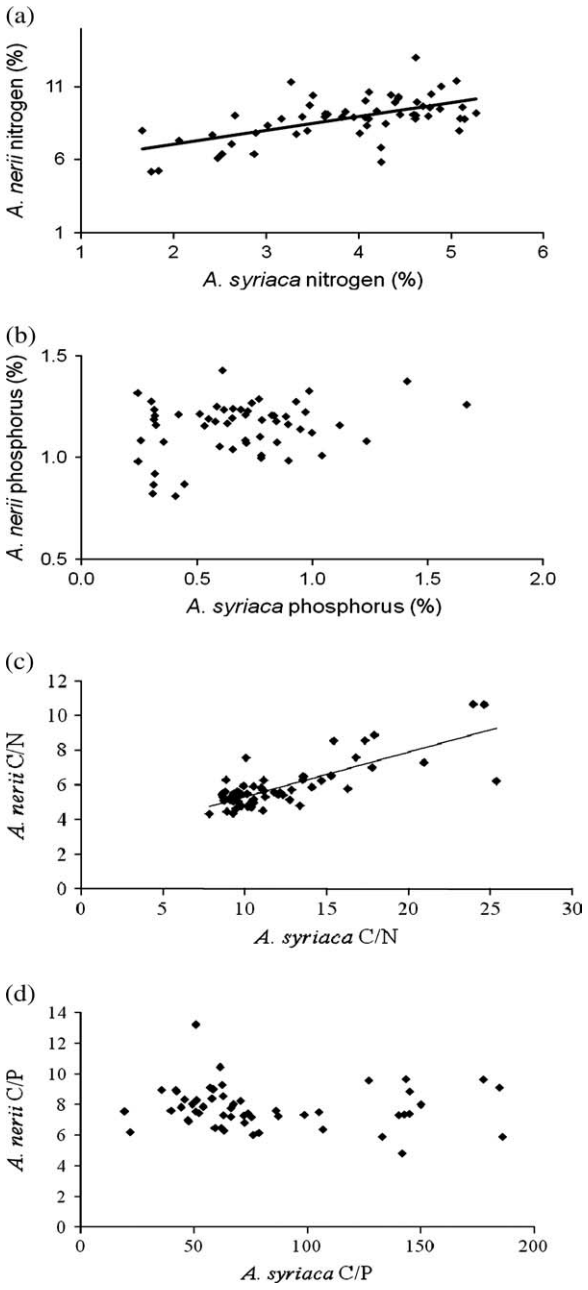

Fig. 4. Relationship between *Aphis nerii* body concentrations and *Asclepias syriaca* foliar quality (a) nitrogen concentration, (b) phosphorus concentration, (c) C/N ratio and (d) C:P ratio. Each point represents a single aphid-host plant pair ($n = 63$). *Asclepias syriaca* values are from the plants upon which the aphids were feeding.

between aphid per capita growth rates and aphid C/N, and there was no relationship between aphid per capita population growth rate and aphid C/P ratios (data not shown aphid C:N: $R^2 = 0.086$, $P = 0.027$, aphid C:P: $R^2 = 0.005$, $P = 0.591$).

The foliar C/N/P ratios resulting from our experimental manipulations fall within the range of elemental ratios that aphids are likely to encounter under natural conditions (Table 1).

Table 1. Foliar nitrogen, phosphorus, and carbon concentrations (% dry mass) in field-collected ($n = 36$) and experimental ($n = 27$) *Asclepias syriaca* plants

		Nitrogen	Phosphorus	Carbon
Field collected	Minimum	1.93	0.17	40.37
A. syriaca	Maximum	5.82	1.61	47.38
	Mean	3.31	0.41	43.55
	Se	0.15	0.05	0.29
Experimental	Minimum	2.23	0.21	42.89
A. syriaca	Maximum	6.6	1.11	52.72
	Mean	4.64	0.62	45.38
	Se	0.18	0.05	0.35

Compared with other terrestrial invertebrate herbivores (published in Elser *et al.*, 2000), aphids have low C/P and N/P ratios (Table 2), consistent with the hypothesis that high tissue phosphorous concentrations are associated with high rates of population growth (Elser *et al.*, 2003).

Discussion

Constraints on herbivore growth and reproduction can result from low nutrient concentrations or a mismatch in their relative availability (Elser *et al.*, 2000; Raubenheimer *et al.*, 2005; Huberty & Denno, 2006). Here we show that there exist negative effects of high nutrient concentrations in support of the threshold elemental ratio hypothesis (Boersma & Elser, 2006). Aphid population growth rates were highest at intermediate N and P levels and high when nutrient levels were opposing. In contrast, the combination of high levels of both N and P led to declines in aphid 'r'. Additionally, we found that high plant N concentrations can result from both high soil N and high soil P. This leads to an indirect effect of soil P availability on aphids, mediated by increasing plant N.

We suspect that high levels of N and P additions increased plant N concentrations above the aphid threshold elemental ratio (TER, Boersma & Elser, 2006), leading to a decrease in aphid population growth rates. Aphids on plants receiving the low N/low P treatments had the lowest N and P body concentrations, and the lowest per capita population growth rates (Fig. 2). The lack of stoichiometric regulation at low nutrient additions is interesting, because consumers are predicted to maintain overall elemental homeostasis (Anderson *et al.*, 2005). Stoichiometric regulation by *A. nerii* is strong as N and P supplementation increases, but population growth rates drop at high N/high P treatments meaning that decreased 'r' is not related to aphid body composition (Fig. 2). Therefore, aphids are processing and excreting these excess nutrients and the excretion costs lead to decreased population growth rates. Future work will examine whether the quantity and composition of aphid honeydew varies in response to variation in host plant quality, and we predict that honeydew N concentration will increase when plant N levels exceed aphid TER. Phloem feeders are predicted to be more sensitive to dietary nutrient intake than are herbivores that feed on higher quality food, because they are adapted to live on low nutrient foods (Nevo & Coll, 2001; Boersma & Elser, 2006). Declines in growth at high N concentrations have been documented for other herbivores, including grasshoppers (Raubenheimer & Simpson, 2004). As nutrient excess incurs physiological costs, it is expected that decreased growth, survival, and reproduction of consumers will occur whenever there is a stoichiometric imbalance in the diet.

Table 2. A comparison of *Aphis nerii* elemental ratios from this study ($n = 63$) with terrestrial invertebrate data from Elser *et al.* (2000) (C/N $n = 124$, C/P $n = 27$, N/P $n = 22$ studies). *A. nerii* values are averages across all treatments

	C/N	C/P	N/P
A. nerii	5.89 ± 0.18	44.78 ± 1.22	7.83 ± 0.18
Terrestrial invertebrate Herbivores	6.5 ± 0.17	116 ± 13.93	26.4 ± 2.15

Most insect herbivores are considered to be N limited (Mattson, 1980), and previous work in this system has shown that low levels of N addition (up to 4 g/m^2/year) increase aphid maximum per capita population growth rates (Zehnder & Hunter, 2008). Additionally, there is a rich literature of fertilisation experiments showing positive effects of N addition on insect performance (reviewed in Kyto *et al.*, 1996). High levels of N application, usually 20 g/m^2/year or greater (Jansson & Smilowitz, 1986), are used in many fertilisation experiments and often lead to increases in herbivore growth and fecundity (Kyto *et al.*, 1996). Our results do not contradict these past experiments. Instead, our results highlight the importance of P availability in addition to N in affecting herbivore population dynamics; only at high P levels was there evidence for a negative affect of high N levels on aphid performance. Our data suggest that high combinations of nutrients, rather than high concentrations of individual nutrients, are most deleterious to insect growth rates. Similarly, nitrogen, phosphorus, and sulfur ratios influence soybean looper and two-spotted spider mite growth and development. The influence of any one of these nutrients on herbivore performance depends upon nutrient ratios, and herbivore responses to nutrient availability are non-linear (Busch & Phelan, 1999; Raubenheimer *et al.*, 2005). The average concentrations of N and P in *A. nerii* tissue (Fig. 2b,c) were considerably higher than those in field- and lab-collected *A. syriaca* (Table 1), suggesting that low levels of both macronutrients may limit aphid performance and reduce population growth rates. Our work is consistent with previous research, highlighting the imbalance in nutrient concentrations between plant and insect biomass (Elser *et al.*, 2000).

While our study was not designed to elucidate physiological mechanisms, the nutritional ecology literature illustrates a number of possible mechanisms explaining how high N concentrations result in decreased population growth rates. For example, high plant N concentrations could potentially decrease aphid feeding rates, lead to compensatory feeding or alter assimilation efficiencies (Raubenheimer, 1992; Simpson *et al.*, 2004). Excess N could limit carbohydrate assimilation (Clissold *et al.*, 2006), although this is unlikely in this system because plant phloem has high carbohydrate levels. Additionally, aphids

contain symbiotic bacteria, *Buchnera*, and secondary symbionts (Douglas, 1998), and plant nutrient concentrations could impact these symbionts and alter their relationship with the aphids.

There was an indirect effect of P additions on aphid performance mediated by increasing plant N, meaning that plant N concentrations are influenced by soil N and soil P. Soil P could increase plant N acquisition by increasing the rate of nitrate reduction. Nitrate reductase catalyses this reaction, and the activity of this enzyme is controlled by phosphorylation (Lambers *et al.*, 1998). Additionally, if plants increased root hair density or root mass ratio in response to soil P availability, then this could promote N uptake as well (Lambers *et al.*, 1998). Similarly, *Spartina alterniflora* exhibited increased growth in response to N but not P additions, and P facilitated N uptake (Huberty & Denno, 2006).

The relationship between plant N and aphid population growth rates ('r') is stronger than the relationship between plant P and aphid 'r' (Fig. 3). P additions influence aphid population growth rates indirectly by increasing foliar N concentrations (Fig. 1a), meaning it is not the P addition *per se*, but the effect that P has on *A. syriaca* foliar N that influences aphids. Similarly, nitrogen additions had a larger impact on *Prokelisia* planthopper growth and density than did phosphorus additions (Huberty & Denno, 2006). Like planthoppers, N may be more limiting than P for aphids. Additionally, there is a lot of variation in aphid 'r' within similar nitrogen concentrations, indicating that its more than just foliar nitrogen levels that influence aphid population growth rates. If the phosphorus additions altered the types of amino acids in plant phloem, then this variation in amino acid quality could influence aphid population growth rates.

While P additions led to a decrease in *A. syriaca* cardenolide concentrations (Fig. 1d), there was no relationship between *A. syriaca* cardenolide concentration and *A. nerii* population growth rates (Fig. 3c). This indicates that the decrease in cardenolide concentration did not influence aphid population growth rates. It is always possible that nutrient additions could have altered plant quality in ways that we did not measure, with subsequent effects on aphid population growth rates. For example, oviposition by sweet potato whitefly is deterred by plant P deficiency. P deficiency decreases plant water potential, which alters leaf sucrose concentrations and whitefly oviposition preference (Skinner & Cohen, 1994).

As a result of the difficulties in collecting and measuring phloem, we measured leaf N and P to estimate phloem quality for aphids. Phloem N and leaf N are generally related in other systems (Youssefi *et al.*, 2000), and multiple studies have found relationships between leaf foliar N and aphid performance (Jansson & Smilowitz, 1986; Kyto *et al.*, 1996; Bethke *et al.*, 1998). Unfortunately, there are no studies to date that have correlated phloem P with foliar P. Here, we assume that foliar N and P concentrations provide reasonable estimates of phloem N and P concentrations.

Only a single herbivore genotype on one host plant species was examined in this study. Previous work in this system has found no variation in aphid survival, fecundity, developmental rate or alate formation among aphid clones collected from different geographic locations (Zehnder & Hunter, 2007a). Additionally, all aphid clones exhibited density-dependent survival and fecundity on the four different milkweed species we examined, and milkweed species showed no variation in response to herbivory by the different aphid clones (Zehnder & Hunter, 2007a, b).

Fast-growing organisms, like aphids, should have higher per cent P than slow-growing organisms, because they contain more RNA for protein synthesis (Elser *et al.*, 2003). RNA is high in P and is a major contributor to organism biomass (Sutcliff, 1970). The Growth Rate Hypothesis proposes that variation in body P arises from variation in allocation to ribosomal RNA (Elser *et al.*, 1996; Elser *et al.*, 2003). *Aphis nerii* has higher tissue P concentrations (lower C:P or N:P) than those reported for other terrestrial invertebrate herbivores (Table 2) in support of the Growth Rate Hypothesis. However, we found no evidence for any correlation between intra-specific variation in P and aphid per capita population growth rate, perhaps because homeostasis limited intra-specific variation in P. As the concentrations of N in *A. syriaca* increased, so did *A. nerii* N concentrations, indicating that *A. nerii* does not maintain strict homeostasis with regards to N (Fig. 4a). Elemental homeostasis was apparently stricter with respect to P concentrations than N concentrations (Fig. 4b). Plant-mediated variation in aphid tissue N could influence higher trophic levels, such that aphids feeding on high-quality plants are high-quality prey items (Kagata & Ohgushi, 2006, 2007). However, it is currently unknown if the variation in aphid N or C/N is biologically important. Unfortunately, we did not measure the effect of variation in plant quality on aphid body mass, and both N and P additions have been shown to increase phloem-feeder body size (Huberty & Denno, 2006).

Ecological stoichiometry provides a framework whereby an organism's elemental constituents can be linked to its growth, reproduction, and population dynamics. In this study, excessive N levels led to a decrease in herbivore performance, indicating that excessive nutrient levels can limit herbivore population growth rates. These high plant N levels were caused by both high soil N and high soil P leading to a strong, indirect effect of P additions on plant and aphid performance. We suggest that deleterious effects of high nutrient concentrations on herbivores should receive further attention, and that the interactive, indirect effects of nutrient additions on herbivore performance should be studied.

Acknowledgements

We thank Jeremy Castings and Paige Marvin for helpful laboratory assistance, and Tom Maddox and Lisa Dean at the University of Georgia Analytical Chemistry Laboratory for analyses. Previous versions of this manuscript were significantly improved by the comments of A. Huberty and anonymous reviewers. This work was supported in part by NSF grant DEB 0342750 to MDH.

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Accepted 30 March 2009
First published online 26 May 2009

