

Stoichiometric tracking of soil nutrients by a desert insect herbivore

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Abstract

Biogeochemistry and population biology have developed independently, with few attempts at linkage, almost none of which were mechanistically based. We hypothesize that biogeochemical cycling is linked to herbivore population dynamics through the influence of soil nutrient availability on foliar nutrient content, which constrains herbivore investment in phosphorus (P)-rich molecules necessary for growth. We show that variation in desert soil P availability is linked to abundance of an insect herbivore (*Sabimia setosa*) through the influence of soil P on the C:P ratio of the host plant (*Prosopis velutina*). Low P availability increases C:P ratio of *Prosopis* leaves, leading to a decline in body %P, %RNA and abundance of *Sabimia*. Tight association between soil, plant and herbivore P provides the first evidence of a mechanistic pathway linking P biogeochemistry to terrestrial food webs by altering the supply of dietary P to herbivores, thus limiting their capacity for growth by constraining the production of P-rich cellular ribosomal RNA (rRNA).

Keywords

Biogeochemistry, ecological stoichiometry, growth rate hypothesis, mesquite, phosphorus, population ecology, RNA.

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INTRODUCTION

Biogeochemistry and population biology have been developing along disparate paths for several decades, with few attempts at linkage, almost none of which have been mechanistically based (Hunter & Price 1992; Pomeroy 2001). The recent development of ecological stoichiometry, a relatively new body of theory concerning the balance of energy and multiple chemical elements in living systems (Reiners 1986; Elser *et al.* 1996; Elser *et al.* 2000a), has led to the development of testable hypotheses which have the potential to mechanistically link biogeochemical cycling with population dynamics and life-history evolution (Reiners 1986; Elser *et al.* 2000a; Vanni *et al.* 2002).

The growth rate hypothesis (GRH) (Elser *et al.* 1996), for example, suggests a mechanistic linkage between phosphorus (P) biogeochemistry and the growth and reproduction of individual organisms (a major determinant of population dynamics). Specifically, evidence is accumulating on the fact that variation in organismal C:N:P ratio is largely determined by variation in body P content (Elser *et al.* 2000b; Vanni *et al.* 2002). The GRH posits that this variation in body P arises, at least in part, from variation in

allocation to ribosomal RNA (rRNA) among organisms with different growth or development rates (Elser *et al.* 1996). As RNA is rich in P and is often a major contributor to organism biomass (Sutcliffe 1970; Dobberfuhl 1999), it contributes significantly to total P content in many organisms (Hessen & Lyche 1991). Thus, fast-growing organisms that are rich in RNA have higher P contents than do slow-growing organisms, leading to a higher demand for P from their environment, and a higher likelihood of limitation by low-P food (Hessen 1992; Sterner & Schulz 1998).

The GRH predicts that organisms grown on P-rich food should have higher P and RNA contents and faster growth rates. This prediction implies a potential chain of causation linking soil P availability – through its effects on plant growth and tissue P content – to herbivore P and nucleic acid content, growth and reproduction, and, ultimately, population dynamics. We evaluated this prediction of the GRH by examining several of the links in this chain in a desert ecosystem. Specifically, we related spatial and temporal variability in soil P availability to stoichiometry of velvet mesquite (*Prosopis velutina*) and one of its dominant insect herbivores (the curculionid weevil *Sabimia*

setosa). In addition, we studied these relationships during 2 years that differed considerably in the pattern and amount of precipitation, which allowed us to investigate the influence of interannual variation in precipitation on soil nutrient supply, plant tissue chemistry and the potential transmission of these effects to the herbivore trophic level.

METHODS

Leaves and adult insects were collected in early May and early September 2000, and late April 2001 from 25 mesquite trees in the Verde River watershed in the Sonoran Desert near Phoenix, Arizona. Collection dates corresponded to the end of the winter (January to April) or monsoon (June to September) rainy seasons. From each mesquite, insects and leaves were collected from four individual branches. Insect nets were placed over each branch, which was then pruned from the tree and shaken vigorously to remove insects. Insects were stored in plastic bags and frozen at -80°C until analysis. Branches were placed in plastic bags until they were returned to the laboratory, where all leaves were removed, dried at 60°C and weighed to obtain total foliar dry mass from each branch. A subsample of individual *Sabinia* ($n = 2\text{--}6$ per branch) were removed from the freezer and dried. The remaining insects were kept at -80°C until nucleic acid analyses were completed. All individual *Sabinia* collected from each branch were counted, and abundance per branch was standardized by dividing total number of individuals by total foliar mass.

After drying, leaf subsamples were ground in a SPEX Certiprep 8000D ballmill (SPEX Centiprep, Metuchen, NJ). Carbon and nitrogen content of ground leaf material and individual *Sabinia* were measured on a Perkin–Elmer 2400 CHN analyzer (Perkin Elmer Inc., Wellesley, MA). Phosphorus content was measured on ground leaves, and individual insects by persulphate and sulphuric acid digestion followed by colorimetric analysis. Lignin was determined as the acid-insoluble fraction using acid detergent methods on an Ankom fibre analyzer (Ankom Technology, Fairport, NY).

Nucleic acids were measured on frozen individual insects using protocols modified from methods used for zooplankton (Wagner *et al.* 1998; Gorokhova & Kyle 2002). Dry weights of individual insects were estimated using a previously determined regression relationship between pixel area of individual *Sabinia* on a digital photograph and dry weight. The DNA and RNA were quantified by extraction with *N*-laurylsarcosine, followed by sonication and staining with Ribogreen[®] (Molecular Probes, Eugene, OR). Nucleases were used to determine DNA and RNA separately. The contribution of RNA to total body P content in *Sabinia* was estimated by assuming

that P contributes 9.5% of RNA. Due to limitations of methods used and small size of the insects (100–150 μg dry weight), we were not able to measure %P and %RNA on the same individual insect. Relationships between these variables required us to first calculate mean values for each variable. Some branches and/or trees did not provide enough individuals for all analyses, in which case only %P was measured. This, coupled with loss of some samples because of technical problems, resulted in reductions in sample sizes for some of the analyses.

In August to September 2000 and March to April 2001, Soil P availability was estimated using anion exchange resins (AER) (Lajtha 1988; Giblin *et al.* 1991) to measure PO_4 concentrations. Resin bags were constructed by weighing 4 g (dry weight) of AER into a small nylon bag. Before deployment in the soil, AER bags were rinsed three times and soaked overnight in 0.5 M NaHCO_3 . After soaking, bags were rinsed thoroughly with distilled water and spun dry before transportation to the field. At each tree, a single AER bag was buried in the soil to a depth of 10 cm by cutting a slit in the soil using a shovel to minimize soil disturbance. Bags were placed in the ground in early August 2000 and early March 2001. After 1 month bags were retrieved, returned to the laboratory and frozen for later analysis. Resin bags were then thawed and rinsed to remove soil particles. Resins were extracted in 100 mL of 0.5 M HCl. All extractant samples were adjusted to neutral pH and PO_4 was measured on a Lachat Quick Chem 8000 Flow Injection Analyzer (Lachat Instruments, Milwaukee, WI).

Statistical comparisons in which months are compared were performed on mean values of individual trees for each month. Analysis of variance was used to compare months for these variables. All multiple comparisons between months were performed using Tukey's test. Regression analyses used the mean of branches calculated for individual trees. Regressions reported here represent the models that best fit the data for each comparison of variables. Assumption tests indicated that transformations were not necessary. Soil resin P was log-transformed to simplify presentation.

RESULTS

The winter of 2000 was considerably drier than that in 2001 in central Arizona (8 cm vs. 13 cm of rain from January to April; National Weather Service). In addition, although both years showed above average winter rains (average = 5.9 cm), most of the rain in winter 2000 fell during a single storm in early March (7 cm between 5 and 7 March 2000), while precipitation was more evenly spread throughout the winter in 2001.

Mesquite leaf C:P was significantly negatively related to resin bag P concentration ($\mu\text{g bag}^{-1}$, ln-transformed) in both September 2000 ($n = 21$) and April 2001 ($n = 25$) (Fig. 1a).

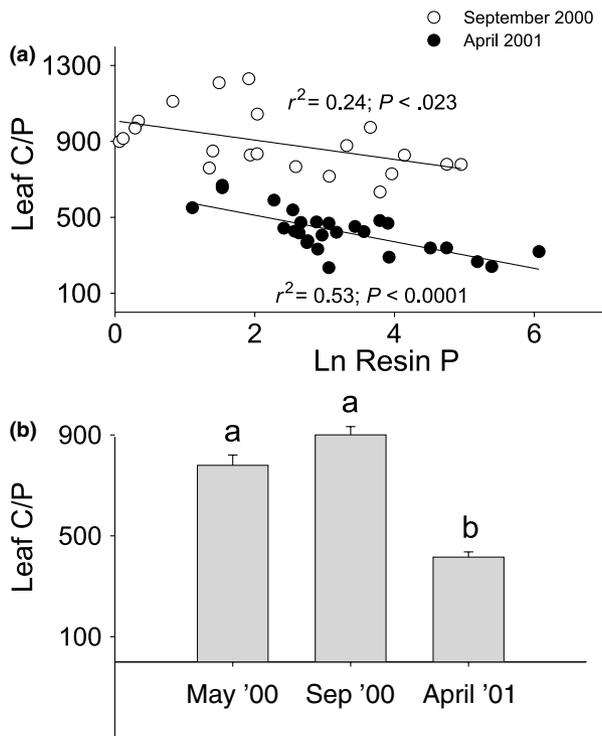


Figure 1 (a) Relationship between leaf C/P and soil P availability (using resin P as an index). We found a significant negative relationship between these variables in both months. Symbols represent mean values for single trees. (b) Leaf C/P ratios for mesquites collected in May and September 2000, and April 2001. Leaf C/P is significantly lower in April 2001 ($n = 25$) than in May ($n = 23$) and September ($n = 21$) 2000 (ANOVA followed by Tukey's test; $P < 0.01$). Error bars are SE.

Soil P concentrations were not significantly different between the 2 months (27 ± 8 vs. $38 \pm 6 \mu\text{g bag}^{-1}$ in September and April, respectively). Leaf C/P ratios were significantly lower in April 2001 than in both May and September 2000 (Fig. 1b). *Sabinia* exhibited significantly higher %P (Fig. 2a), and %RNA and RNA:DNA ratios (Fig. 2b) in April 2001 than in May and September 2000. The DNA content did not differ between months (Fig. 2b). A comparison of *Sabinia* %P with %RNA-P showed a significant positive relationship (Fig. 2c), with the slope of the relationship approximately equal to 1 (slope = 0.9996).

Abundance of *Sabinia* was significantly negatively related to leaf C:P in both September ($r^2 = 0.34$; $P = 0.036$) and April ($r^2 = 0.47$; $P = 0.001$) alone and when all data were combined (Fig. 3a). In contrast, *Sabinia* abundance was not significantly related to %lignin of mesquite leaves despite substantial seasonal variation in leaf lignin concentrations (Fig. 3b). *Sabinia* abundance and %P were also unrelated to %N in leaves (J.D. Schade, unpublished data).

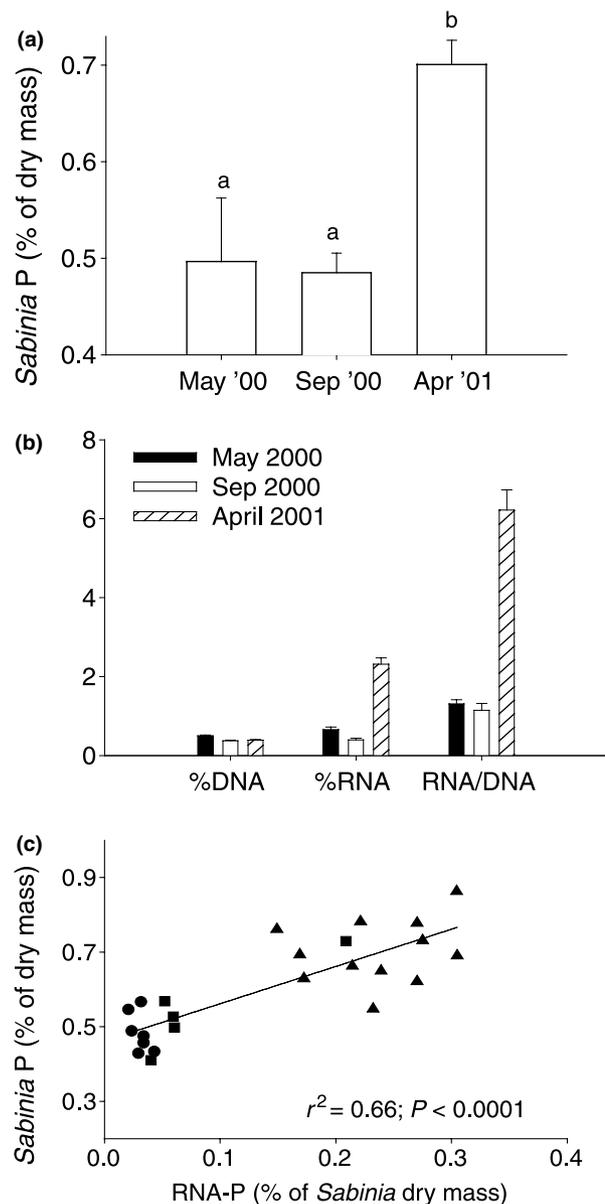


Figure 2 Temporal variation in, and relationship between, *Sabinia* body P and RNA content. (a) P content as percentage of dry mass of individual *Sabinia setosa*. Letters indicate significant differences (ANOVA followed by Tukey's test; $P < 0.01$). (b) DNA and RNA as percentage of dry mass and RNA/DNA in individual *Sabinia*. Only %RNA and RNA/DNA are significantly higher in April 2001 (ANOVA followed by Tukey's test; $P < 0.001$). (c) Relationship between *Sabinia* %P and %RNA-P. ■: May 2000; ●: September 2000; ▲: April 2001. Error bars in (a) and (b) are SE.

DISCUSSION

Patterns of variation in leaf C:P ratio and soil P indicate that mesquite leaf C:P ratios were strongly influenced by

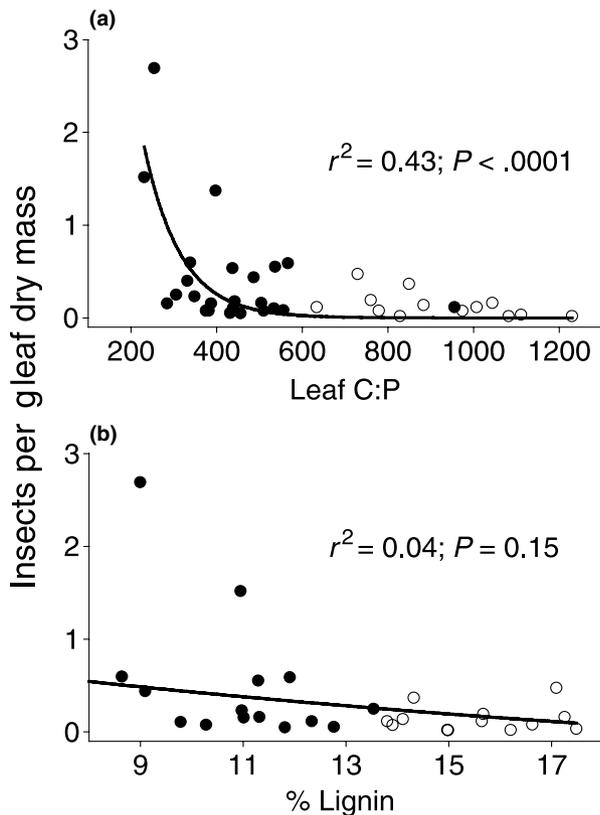


Figure 3 Relationship between abundance of *Sabinia* and leaf chemical characteristics. (a) Mesquite leaf C/P and (b) %lignin of mesquite leaves. For both C/P and %lignin, regression lines were determined using an exponential decay model, which showed the best fit to the data for both variables. Open circles are September 2000, closed circles are April 2001.

both spatial and temporal variation in soil P availability. Negative correlations between leaf C:P and soil P within each season suggest that mesquite trees responded strongly to spatial variation in soil P with changes in foliar C:P. Lower leaf C:P at a given level of available P in April compared with September (Fig. 1a) likely occurred, in part, because leaves sampled in April were young and not yet fully expanded, while leaves sampled the previous September were mature and fully expanded. Furthermore, low C:P ratios in spring 2001 followed a fairly wet winter, whereas higher C:P ratios in spring 2000 (Fig. 2) occurred following a winter when most of the precipitation fell in a single rain event. Indeed, monthly measurements of AER showed an increase in available P between July and September 2000, and February and March 2001 following increases in precipitation (J.D. Schade, unpublished data). Taken together, these results clearly show that mesquite leaf C:P responded to spatial variation in soil P, and suggest a response to interannual and seasonal variation in

soil P, driven by variation in pattern and amount of precipitation.

Patterns of P and RNA concentrations in *Sabinia* reflected patterns in soil available P and mesquite leaf C:P ratios as predicted by the GRH. *Sabinia* exhibited higher %P, %RNA and RNA:DNA ratios in April 2001 when foliar C:P was low than in May and September when foliar C:P was high, consistent with a strong effect of food quality on *Sabinia* biochemistry. That *Sabinia* showed large variation in %P and %RNA between springs of two different years (May 2000 and April 2001) is an evidence of the fact that the variation arose from differences in diet rather than in the developmental stage of the insect. Furthermore, the significant relationship between *Sabinia* %P and %RNA-P (Fig. 2c) is a strong evidence that variation in body P content of *Sabinia* resulted from changes in RNA content. The fact that the slope of that relationship is not significantly different from 1 suggests, in fact, that all the variation in P is explained by changes in RNA content. These data indicate a strong influence of foliar P content on the primary molecule (RNA) that determines the growth capacity of *Sabinia*.

We also found evidence that improvement in stoichiometric food quality influenced *Sabinia* population dynamics in our study plots. The negative relationship between *Sabinia* abundance and leaf C:P (Fig. 3a) strongly suggests that the ecological success of this herbivore is controlled, at least in part, by the availability of P in its food resource. Although a large body of literature on insect nutrition has shown a strong influence of changes in structural materials, particularly lignin content, on insect performance (Mattson 1980; Scheirs *et al.* 2002), we found no relationship between *Sabinia* abundance and %lignin of mesquite leaves despite substantial seasonal variation in leaf lignin concentrations (Fig. 3b). We conclude from these results that the strong negative associations of foliar C:P ratio with *Sabinia* abundance, %P and %RNA are strongly indicative of P limitation of *Sabinia* in our study site. However, a controlled manipulation of dietary P supply (*sensu* Elser *et al.* 2001), along with measurements of insect growth, fecundity, mortality and migration is necessary to directly confirm the existence of P limitation of *Sabinia* performance.

Our results highlight a chain of interactions linking population size of *Sabinia* to pattern and amount of precipitation, mediated by effects of rainfall on soil P cycling and C:P ratio of mesquite leaves. Considering the overriding influence of water on productivity in the desert (Lajtha & Schlesinger 1988), such linkages are not particularly surprising. What is unique about our study is that effects on herbivores arise from the influence of soil P availability on stoichiometric food quality (measured as C:P of leaf tissue) rather than on total primary production *per se*. Furthermore, in contrast to past studies emphasizing

dietary N as a key constituent for herbivorous insects (Mattson 1980; Mattson & Scriber 1987; White 1993), our results are consistent with P limitation of this taxon. Our findings do not necessarily represent a fundamental challenge to previous work emphasizing the importance of N-limitation of herbivorous insects, as P-limitation of *Sabinia* may arise because of the relatively high concentrations of leaf N in mesquite (C/N ranged from 15 to 20; J.D. Schade, unpublished data), which is capable of N fixation.

A recent review comparing element ratios in terrestrial foliage and herbivorous insects shows that mean C/P and N/P of terrestrial foliage is higher than mean values of these ratios for terrestrial herbivores (Elser *et al.* 2000b). This suggests that terrestrial plant tissue is both C and N rich compared with herbivore tissue and suggests the possibility that P content of terrestrial foliage may often be low enough to limit terrestrial herbivores, and that P-limitation might be more prevalent than previously assumed (Elser *et al.* 2000b). Our findings clearly show that the effects of variation in the P content of food resources were manifested as variation in the P content of consumers driven by changes in RNA content. These relationships are consistent with a positive relationship between herbivore population growth and rates of ecosystem P supply. An alternative explanation, however, is that *Sabinia*, if mobile enough, may be aggregating in sites that are richer in P, leading to a relationship which is unrelated to a growth response to high P levels in food supplies. From our knowledge about their life-history habits, however, they are not mobile, and in fact live out their entire life cycle on a single tree. Larvae develop in the spring, with each individual larva completing development in a single individual flower bud, while the adults spend their lives feeding in a single individual tree. Movement of adults between trees is likely to be very rare (Clark 1978). These life-history characteristics suggest that movement of individuals is unlikely to explain the relationship between P supply and abundance. Coupled with data reported here on P and RNA content of *Sabinia*, it suggests that this relationship does, in fact, reflect increased population growth rates at sites with high P supply, mediated by a cellular mechanism, the elevation in individual *Sabinia* of cellular ribosomal RNA necessary for increased growth. Thus, stoichiometric mechanisms link cell biology, population dynamics and biogeochemistry.

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