

**TROPHIC EFFECTS ON NUTRIENT CYCLING**

by

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## Abstract

The top-down effects of consumers and bottom-up effects of resource availability are important in determining community structure and ecological processes. I experimentally examined the roles of consumers -- both detritivores and predators -- and habitat context in affecting nutrient cycling using the detritus-based insect community in bromeliad leaf wells. I also investigated the role of multiple resources in limiting plant productivity using meta-analyses.

The insect community in bromeliads only increased nitrogen release from leaf detritus in the presence of a predator trophic level. When only detritivores were present, the flow of stable isotope-labeled nitrogen from detritus to bromeliads was statistically indistinguishable from that in bromeliads lacking insects. I suggest that emergence of adult detritivores constitutes a loss of nitrogen from bromeliad ecosystems, and that predation reduces the rate of this nutrient loss. Hence, insects facilitate nutrient uptake by the plant, but only if both predators and detritivores are present. Moreover, predators can affect nutrient cycling by influencing the spatial scale of prey turnover. This mechanism results in a pattern opposite to that predicted by classic trophic cascade theory.

Increasing habitat complexity can have implications for nutrient cycling by decreasing the foraging efficiency of both predators and their prey, and by affecting the vulnerability of predators to intraguild predation. Along a natural gradient in bromeliad size, I found that, depending on the relationship between community composition and habitat size, habitat complexity interacts with the changing biotic community to either complement or counteract the impact of predators on nutrient uptake by bromeliads.

In contrast to the existing emphasis on single-resource limitation of primary productivity, meta-analyses of a database of 653 studies revealed widespread limitation by multiple resources, and frequent interaction between these resources in restricting plant growth. A framework for analyzing fertilization studies is outlined, with explicit consideration of the possible role of multiple resources. I also review a range of mechanisms responsible for the various forms of resource limitation that are observed in fertilization experiments.

These studies emphasize that a wider range of predator and nutrient impacts should be considered, beyond the paradigm of single resource limitation or classic trophic cascades.

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## **Co-authorship statement**

I designed the experiments outlined in Chapter 2 in consultation with D.S. Srivastava. I performed the research and statistical analyses, and wrote the manuscript, with editorial and statistical advice from my co-author.

I also designed the experiments detailed in Chapter 3, analyzed and interpreted the data, and wrote the manuscript. D.S. Srivastava carried out the experiment in the field and provided comments on the drafts. J.B. Shurin helped to interpret the results and provided input on the writing of the paper.

The research detailed in Chapter 4 originated during discussions with E.E. Cleland and W.S. Harpole. E.E.C., W.S.H., E.W. Seabloom and I jointly designed the research and performed the analysis using data we collected as part of the Trophic Structure Comparisons Working Group at the National Center for Ecological Analysis and Synthesis. I was responsible for determining the conceptual direction of the review, interpreting the results and for writing the manuscript, with editorial input from my co-authors. E.T. Borer, M.E.S. Bracken, J.J. Elser, D.S. Gruner, H. Hillebrand, J.B. Shurin and J.E. Smith contributed to the database used in the analyses and commented on the manuscript.

## Chapter 1 - General Introduction

The top-down effects of consumers and the bottom-up effect of resources are among the most important forces structuring ecological communities (Polis 1994). The balance of herbivory (Hairston et al. 1960) and the availability of nutrients (Aerts & Chapin 2000), for instance, determines the amount of primary productivity that is present in a habitat. The diversity of communities is also strongly influenced by consumers (Paine 1966, Yanoviak 2001, Schmitz 2003, Kneitel & Chase 2004, Hillebrand et al. 2007) and resources (Interlandi & Kilham 2001, Kneitel & Miller 2002, Grover & Chrzanowski 2004, Harpole & Tilman 2007, Hillebrand et al. 2007). Similarly, ecosystem processes such as decomposition and nutrient cycling can be altered by resource availability or consumer activity. Faster decomposition of leaf litter, for example, can occur when nutrients are added to a system (e.g. Hobbie 2000, Hobbie & Vitousek 2000, Bärlocher & Corkum 2003) and Perring et al. (2008) have shown that greater nitrogen availability can affect phosphorus cycling in terrestrial systems. Both herbivores and higher-order consumers can also determine rates of nutrient flux (e.g. Vanni 2002, Frost & Hunter 2004, Stief & Hölker 2006) or mineralization rates (e.g. Schröter et al. 2003).

When the top-down effect of predators has indirect effects on trophic levels other than that of their prey, a trophic cascade can result, whereby an increase in predators decreases herbivory and allows more autotrophs to persist (Strong 1992, Polis 1994, Pace et al. 1999, Schmitz et al. 2000, Halaj & Wise 2001). The effects on the autotroph population have been attributed to the direct reductions in herbivore numbers due to predation (Power 1990, Hambäck et al. 2004) and to behavioural changes in the herbivore population (Schmitz et al. 1997, Werner & Peacor 2003, Křivan & Schmitz 2004, Schmitz et al. 2004, Creel et al. 2005). For instance, reductions in foraging rate or activity of consumers due to predator presence are common (e.g. Short & Holomuzki 1992, Schmitz et al. 1997, Juliano & Gravel 2002, Werner & Peacor 2003, Křivan & Schmitz 2004, Stief & Hölker 2006) and would also result in decreased herbivore pressure.

Both herbivorous and predaceous consumers can affect the rates of nutrient turnover. The relative abundances of carbon, nitrogen and phosphorus differ considerably between autotrophs and herbivores (Elser et al. 2000). The stoichiometry of consumer tissue relative to that of their prey determines which elements are retained and which are excreted (Urabe 1993, Sterner & Elser 2002, Vanni et al. 2002). Excretion rates, and hence the rates at which nutrients are recycled back into the system, are themselves determined by consumer characteristics such as size and taxonomy (Vanni 2002, Vanni et al. 2002). The release of excess nutrients by

herbivores can have consequences for the nutrient availability for autotrophs (Elser et al. 1988, Sterner 1990, Urabe et al. 1995, Elser & Urabe 1999, Urabe et al. 2002). The activity of predators can similarly result in changes to herbivore stoichiometry and to nutrient concentrations in an ecosystem (Vanni et al. 1997, Elser et al. 2000).

In Chapters 2 and 3, I examine the roles of predation and habitat context (in this case, habitat size and complexity) in determining nutrient cycling. The aquatic insect community contained in tropical bromeliad plants was used as the study system for the field experiments detailed in this thesis. Tropical bromeliads of the genera *Vriesea* and *Guzmania* can be epiphytic or soil-bound, possessing interlocking leaves that collect water and falling leaf litter (Benzing 2000). As the plants grow and their ability to capture leaf litter correspondingly increases, this detrital resource becomes progressively more important relative to inputs via atmospheric deposition (Reich et al. 2003). Plant growth is restricted by the availability of water (Laube & Zotz 2003) and nutrients (Laube & Zotz 2003, Ngai & Srivastava 2006), while light availability can limit survivorship (Winkler et al. 2007).

Insects with aquatic larvae oviposit in the leaf wells of bromeliads and form a detritus-based food web (Benzing 2000). Because bromeliads can hold the majority of standing water during the dry season in the Costa Rican wet tropical forests where my studies were conducted (J.T.N, personal observation), they are an important resource for a large number of invertebrates, reptiles and amphibians (Richardson 1999, Armbruster et al. 2002). Bromeliad characteristics such as size and complexity, and resource availability are important in determining the species richness of the invertebrate community (Armbruster et al. 2002), as are local environmental conditions, such as temperature and bromeliad density (Ngai et al., in press).

The bromeliad is thought to benefit from the invertebrate community as its leaves have specialized trichomes that can absorb the nutrients released by detrital breakdown by the invertebrates and microorganisms (Benzing 2000). However, the facilitative effect of the detrital invertebrate community has never been demonstrated, although deposition of spider-associated debris provides a source of nutrients for their host plants (Romero et al. 2006). In Chapter 2, I examine whether the community of detritivorous insects facilitates nitrogen availability for their host bromeliads. I answer this question by manipulating the insect community in bromeliad leaf wells and, using stable nitrogen isotopes, determine the effect of detritivorous insects on the nitrogen nutrition and growth of bromeliads. I then determine how predation affects nitrogen cycling (Chapters 2 and 3) both experimentally with manipulated insect communities and using a shift in predator abundance that occurs over a habitat size gradient.

The role of predators can be modified by the environmental context of the interaction. The presence of habitat structure, for instance, can decrease an organism's vulnerability to predation (e.g. Persson & Eklöv 1995, Almany 2004, Janssen et al. 2007, Piko & Szedlmayer 2007) and can also influence a predator's behaviour, with consequences for the effectiveness of predation (e.g. Grabowski & Powers 2004, Warfe & Barmuta 2004). In chapter 3, I also investigate how habitat complexity influences the role of predation in nutrient flux. Using a natural habitat size gradient, I relate the relationship between predator abundance and nitrogen cycling to changing habitat complexity in two bromeliad genera (*Guzmania* and *Vriesea*).

In the final section of the thesis, I consider the role of bottom-up forces in limiting primary productivity. The availability of resources, including nutrients (Aerts & Chapin 2000) and water (Field et al. 1998), is widely recognized to restrict autotroph growth. In practice, limiting nutrients are identified as those that increase plant growth when their supply to autotrophs is supplemented (Chapin et al. 1986). The result of these studies is that nitrogen has been identified as the main limiting resource in terrestrial systems (Vitousek & Howarth 1991, White 1993) and phosphorus in freshwaters (Heckey & Kilham 1988 but see Elser et al. 1990). The productivity of marine ecosystems is historically thought to be limited by nitrogen in coastal habitats (Howarth 1988, Downing 1997) and iron in the open ocean (Martin et al. 1994).

The dominance of nitrogen limitation on land has been attributed in part to the ease with which nitrate is lost from soils in comparison to phosphate (Vitousek & Howarth 1991). However, the extent to which nitrogen limits productivity relative to phosphorus can change depending on site succession: a shift from nitrogen-limitation at young sites to phosphorus-limitation of plant growth at older, more weathered sites has been observed (Vitousek et al. 1993, Crews et al. 1995, Vitousek & Farrington 1997). As succession proceeds, microbial activity and bacterial nitrogen fixation increase nitrogen levels in soils (Ohtonen et al. 1997). In contrast, phosphorus is weathered from rocks (Schlesinger 1997) and will therefore be most abundant when the site is first established. With losses over time due to soil leaching and the formation of insoluble phosphorus compounds, the supply of phosphorus to plants will be depleted (Walker & Syers 1976). Nitrogen can also be lost due to fire (Raison 1979) and microbial denitrification (Sprent 1987).

The mechanisms determining the nature of nutrient limitation in freshwater and marine systems are less clear. In lakes, nitrogen- or phosphorus-limitation can be driven by characteristics of the surrounding watershed, such as land use and nutrient sources, or different denitrification rates (Downing & McCauley 1992). Rates of nitrogen fixation help to determine

the prevalence of nitrogen versus phosphorus limitation in oceans (Karl et al. 2001), as do rates of denitrification and the supply of trace metals that limit nitrogen fixation (Falkowski et al. 1998).

In spite of the commonly accepted view of single resource limitation, recent empirical (e.g. Interlandi & Kilham 2001, Gleeson & Good 2003, Grover & Chrzanowski 2004, Arrigo 2005, James et al. 2005, Harpole & Tilman 2007), theoretical (e.g. Gleeson & Tilman 1992, van den Berg 1998) and meta-analytic (Elser et al. 2007) studies have found that multiple resources may in fact limit primary productivity and may interact to determine autotroph biomass. In Chapter 4, I determine the prevalence of multiple resource limitation of primary productivity and to explore the mechanisms responsible for this type of limitation. I address this question using a meta-analytic approach, with a database of 653 factorial fertilization studies from marine, terrestrial and freshwater ecosystems. I also review a range of mechanisms responsible for the various forms of resource limitation that are observed in fertilization experiments.

The overall aims of this thesis are to investigate the effects of predation and habitat context on nutrient cycling and to examine the role of multiple resources in limiting plant productivity.

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## Chapter 2 - Predators accelerate nutrient cycling in a bromeliad ecosystem<sup>1</sup>

### Introduction

The availability of nutrients in ecosystems is determined by resource supply and recycling rates and affects important ecosystem properties (Aerts & Chapin 2000, Hobbie & Vitousek 2000, Tewfik et al. 2005). The relative roles of abiotic supply and food web configuration in determining resource-processing rates remain contentious and poorly understood. Under anthropogenic pressure, ecosystems are predicted to lose predators disproportionately, affecting ecosystem processes (Duffy 2003). Current ecological theory predicts that predator loss will affect nutrient cycling by changing prey abundance (density-mediated effects, as in a trophic cascade) (Power 1990) or prey foraging efficiency (trait-mediated effects) (Werner & Peacor 2003). These changes can further affect nutrient cycling by altering the species composition or size structure of the prey community. In this study, we examined the effects of predators on nutrient cycling by using the detritus-based insect community in bromeliads. We demonstrate that predation can have counterintuitive effects on nutrient cycling.

Leaves of tank-forming bromeliads (e.g., *Vriesea* and *Guzmania* genera) are tightly interlocking, forming wells that collect water and leaf litter and provide habitat for aquatic insect larvae. The detritus not only supports the insect community but also provides a source of nutrients for the bromeliad. A natural gradient also exists in predation where the major predator, a damselfly larva (*Mecistogaster modesta*), becomes more abundant as the plant grows. Although it has been hypothesized that aquatic insects increase nutrient flux to the bromeliad, this relationship has never been documented.

### Methods

#### Fertilization experiments

Experiments were conducted in Costa Rica (10°59' N, 85°26' W). First, we ran fertilization experiments to determine whether nitrogen (N) or phosphorus (P) limit the productivity of the plant and insect components of this ecosystem. A factorial fertilization experiment was conducted on the bromeliad *Guzmania scherzeriana* (n = 8), with two levels each of N (0N: no N added, 1N: 12 g L<sup>-1</sup> KNO<sub>3</sub>) and P (0P: no P added, 1P: 12 g L<sup>-1</sup> NaH<sub>2</sub>PO<sub>4</sub>).

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Nutrient solutions (10 ml 4 d<sup>-1</sup>) were added to bromeliad wells for 32 days, with leaf demography measured six months afterwards.

For the insect experiment, potted *Conostegia xalapensis* Bonpl. (Melastomataceae) seedlings were fertilized (200 ml 2 wk<sup>-1</sup>) for seven months with either nitrogen (12 g L<sup>-1</sup> KNO<sub>3</sub>), phosphorus (12 g L<sup>-1</sup> NaH<sub>2</sub>PO<sub>4</sub>) or water (control) to produce leaf litter that is relatively enriched with either N or P. A factorial design of litter treatment (N, P, water) by species (one of the three major detritivore species: Tipulidae, Scirtidae, Chironomidae) was conducted (n = 10). One individual of standard length was placed in an artificial bromeliad (plastic leaf glued into a 50 ml centrifuge tube filled with water and covered with mesh) and provisioned with 0.7 g dry weight of leaf litter (conditioned in water for 4 d). Water was changed (10 ml 2 d<sup>-1</sup>) to simulate rain flushing and insect survivorship was monitored over 35 days.

### **Predator manipulations**

Leaf detritus enriched in <sup>15</sup>N was used to trace the movement of N through the food web in bromeliads containing either no insects, detritivores only, or detritivores and predators. Potted *Conostegia* seedlings were fertilized with K<sup>15</sup>NO<sub>3</sub> (200 ml of 0.4 g L<sup>-1</sup> K<sup>15</sup>NO<sub>3</sub> pot<sup>-1</sup> 2 wk<sup>-1</sup>). Bromeliads (*Vriesea gladioliflora* H.Wendl.) were rinsed six months prior to the experiment, removing insect larvae and litter from the leaf wells, then exclosed with netting to prevent insect colonization. <sup>15</sup>N-enriched litter (δ<sup>15</sup>N = 856 versus δ<sup>15</sup>N = 1.8 in unmanipulated leaves) was added at the start of the experiment (4 g dwt per bromeliad). Detritivorous insects were added to a third of the bromeliads, while detritivores and predatory damselflies were added to another third and the remaining bromeliads were left without insects (n = 10 per treatment). Simulating oviposition, insects were again added three weeks later. After 40 days, the youngest leaves were collected from each bromeliad for isotopic analysis. Although different bromeliad genera were used for the fertilization and food web experiments, *Vriesea* and *Guzmania* have similar ecophysiologicals, relationship with the insect community and use of leaf litter (Benzing 2000, Reich et al. 2003), and would be expected to respond similarly to these manipulations.

### **N:P ratios of detritivores and leaf litter**

Over two months, detritivores were collected from bromeliads, pooling larvae within a bromeliad for each species (n = 6 for chironomid sp. A and scirtids, n = 4 for chironomid sp. B, n = 3 for tipulids). Chironomids were mostly of the genus *Polypedilum* (Diptera) and tipulids from

the *Trentepohlia* species (Diptera). Falling leaf litter ( $n = 6$ ) was collected for two weeks in mesh trays from positions formerly occupied by arboreal bromeliads.

## Analyses

Phosphorus content was determined using the ammonium molybdate method after digestion with acid, while N content was measured using a LECO CHN analyzer. Isotopic analyses were conducted at the Environmental Isotope Lab, University of Waterloo. Data were transformed as necessary to fit statistical assumptions. GLMs used a log-link function and were based on Poisson errors (R statistical package, <http://www.r-project.org/>). Scale parameters were used to correct for overdispersion.

## Results

The N:P ratio of unfertilized bromeliads is extremely low ( $8.49 \pm 1.28$  by atom, mean  $\pm$  SEM,  $n = 8$ , c.f. Elser et al. 2000), suggesting that the bromeliads are N-limited (Koerselman & Meuleman 1996). Experiments showed that addition of both N and P tended to increase the net production of leaves (Table 2.1). However, the two nutrients had different effects on leaf demography: addition of N increased the number of new leaves produced relative to the other treatments while P additions reduced the number of dead leaves. Bromeliad growth is therefore more strongly N-limited, although P affected leaf senescence. Nitrogen increased the survivorship of scirtid larvae (Table 2.2), but did not affect survivorship of tipulid or chironomid larvae. Phosphorus increased survivorship of both scirtid and, marginally, tipulid larvae, but not chironomid survivorship. Hence, while both N and P can limit insect productivity, there is no clear indication of overall N or P limitation for the detritivore community as a whole.

Both tissue nutrient ratios and fertilization experiments showed that N, rather than P, primarily limits productivity of bromeliads and can limit insect productivity, so we focused on the effects of trophic structure on N cycling. Detritivores alone do not increase the amount of N moving from leaf litter to bromeliad leaves (Fig. 2.1, Bonferroni-corrected t-test, detritivores alone versus control,  $z = 0.478$  and  $P = 0.63$ ), but the addition of predators to the bromeliads does significantly enhance this N-flow (detritivores plus predators versus detritivores alone,  $z = 2.36$  and  $P = 0.018$ ). The survey of bromeliad-associated insect species shows that larger detritivores (chironomid A, scirtids, and tipulids) have N:P ratios higher than that of leaf litter [Fig. 2.2,  $F_{1,20} = 5.05$ ,  $P = 0.04$  for linear contrast following significant analysis of variance ( $F_{4,20} = 3.66$ ,  $P = 0.02$ )].

## Discussion

The presence of detritivores alone did not affect the amount of N entering bromeliads from the enriched detritus (Fig. 2.1). However, in the presence of both detritivores and predators, there was a significant enrichment in  $^{15}\text{N}$  in bromeliad leaves compared with plants containing detritivores alone, indicating that the presence of predators increased the flow of N from litter to bromeliads. This is surprising given that previous studies, consistent with the predictions of density or trait-mediated effects, have shown that predators decrease litter decomposition by reducing detritivore abundance (Ruetz et al. 2002) or by decreasing the foraging rate (Short & Holomuzki 1992) of detritivorous arthropods.

We hypothesize that the detritivorous insects, which pupate relatively rapidly, constitute a loss of litter-derived N for bromeliads when they emerge. A survey indicated that detritivorous insects generally have higher N:P ratios than those found in typical litter (Fig. 2.2), suggesting that, as leaf litter is consumed, the insects will preferentially retain N in their body tissues and release P. Predation by longer-lived damselfly larvae converts the mobile pool of N contained in detritivores into fecal pellets that can be decomposed by microbes or leached to release N in a form available to the bromeliad. Thus, insects facilitate nutrient uptake by the plant, but only if both predators and detritivores are present.

These results emphasize the importance of the temporal and spatial scales of dispersal for nutrient flux. The emergence of adult insects means that, although detritivores increase resource flux over larval time scales by releasing nutrients from litter, these insects act as a nutrient sink for bromeliads over their entire life span. The faster emergence rate of detritivores compared with that of predators allows predation to reduce the loss of N from the bromeliad. Although we use insects in bromeliads to examine biotic effects on nutrient cycling, our results can give insights into other systems where mobility differs between trophic levels. Some trophic interactions, for instance, involve migratory and non-migratory species or species that undergo ontogenetic niche shifts. This mechanism may also apply if the prey species has a very different range size than its predator. Given the increased extinction risk of higher trophic levels, understanding the mechanisms whereby predators drive important ecosystem processes is critical in predicting anthropogenic impacts on natural systems.

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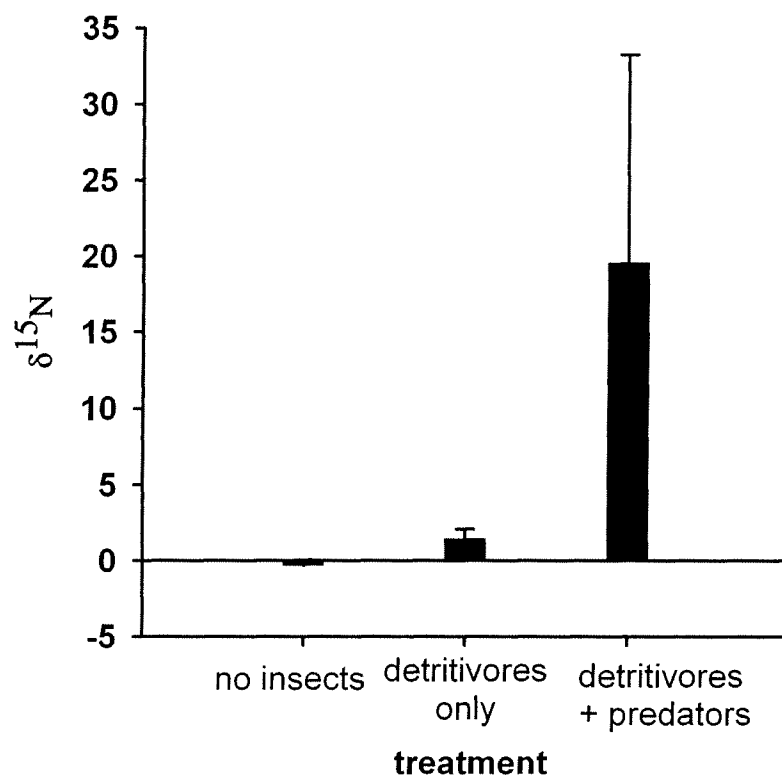
**Table 2.1.** Results of two-way analyses of variance for the plant fertilization experiment.

Degrees of freedom = 1, 27 for all F-values. The production of new leaves in bromeliads is more nitrogen-limited, while leaf senescence is more phosphorus-limited.

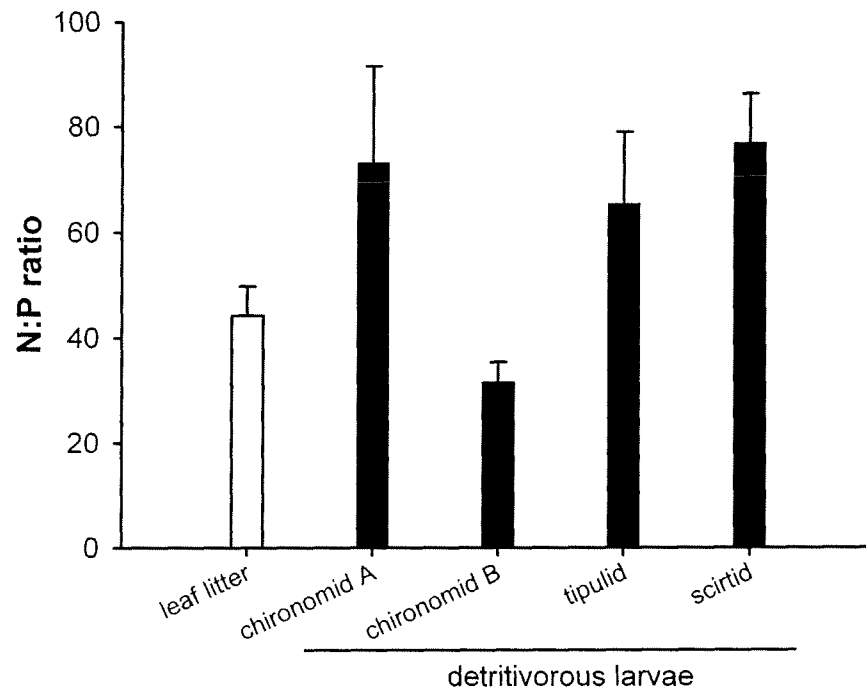
	Effect	F	p-value
<b>Net change in number of leaves</b>	N	3.86	0.06
	P	3.42	0.07
	N x P	0.202	0.66
<b>New leaves</b>	N	8.79	0.006
	P	0.452	0.51
	N x P	1.08	0.31
<b>Dead leaves</b>	N	0.218	0.64
	P	5.26	0.03
	N x P	0.135	0.72

**Table 2.2.** Results of GLM for insect fertilization experiment. Survivorship of the three most common detritivore insects was measured. Both nitrogen- and phosphorus-limitation of insect survivorship are observed, but no overall trend in nutrient limitation was observed for the detritivore community.

	<u>Scirtid</u>		<u>Tipulid</u>		<u>Chironomid</u>	
	z	p-value	z	p-value	z	p-value
<b>N</b>	-2.34	0.02	-0.86	0.39	-0.86	0.39
<b>P</b>	-2.28	0.02	1.85	0.06	0.01	0.99



**Figure 2.1.**  $\delta^{15}\text{N}$  in new bromeliad leaves for plants containing no insects, detritivorous insects only, or detritivore and predatory insects (mean + SEM).



**Figure 2.2.** Comparison of N:P ratios (by atom) for detritivore larvae and for leaf litter (mean + SEM). Chironomid B is a smaller detritivore that accounts for only a small proportion of detritivore biomass in bromeliads.

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## **Chapter 3 - Habitat size and complexity mediate predator effects on nitrogen cycling <sup>2</sup>**

### **Introduction**

The top-down effects of predators often percolate through networks of community interactions to influence the functioning of ecosystems (Hairston et al. 1960, Pace et al. 1999). Studies of trophic cascades have demonstrated that predators can impact the abundance and composition of trophic levels below them (e.g. Power 1990, Hambäck et al. 2004), as well as the ecosystem processes carried out by these organisms. For instance, decomposition rates are often reduced by predator consumption of detritivores (e.g. Yanoviak 2001, Ruetz et al. 2002, Grieg and McIntosh 2006) and by reductions in detritivore foraging in the presence of consumers (e.g. Short and Holomuzki 1992). Other ecosystem functions, such as nutrient cycling, have also been shown to be influenced by the activity of predators (e.g. Vanni and Layne 1997, Vanni et al. 1997, Beard et al. 2002).

Ecological research has focused on the effects of predators via direct reductions in the numbers of prey or the indirect, trait-mediated impacts on prey populations and processes (Power 1990, Hambäck et al. 2004, Schmitz et al. 2004). By either mechanism, increases in predator abundance in a three trophic-level system is expected to increase the abundance of autotrophs through reductions in herbivory. In detritus-based system, an increase in predators would decrease the breakdown of detrital resources and the resulting release of nutrients. However, we recently documented the opposite effect of insect predators on nutrient recycling in bromeliad phytotelmata (Ngai and Srivastava 2006). Bromeliad leaves capture falling detritus from the tree canopy (Benzing 2000), which support bromeliad growth (Reich et al. 2003) and a diverse food web of insect detritivores and predators, other invertebrates and bacteria. We found that predators can change the location or timing of prey turnover and hence, the spatial distribution of nutrients released by prey. By consuming prey that would otherwise mature and emerge from a patch, predators increase the local cycling of nutrients and prevent export of nutrients via prey movement. This result indicates that the effects of predators of reduced nutrient export by prey are stronger than those of reduced detrital processing, resulting in positive effects of predators on plant nutrient acquisition.

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<sup>2</sup> A version of this chapter will be submitted for publication: Ngai, JT, DS Srivastava and JB Shurin. Habitat size and complexity mediate predator effects on nitrogen cycling.

Since the effect of predators on nutrient cycling was demonstrated in an experimental setting and with a manipulated community, it is not clear whether this mechanism applies under natural conditions. The dominant aquatic predator, a damselfly larvae (*Mecistogaster modesta*, Pseudostigmatidae), becomes increasingly abundant as bromeliads grow and the habitat for insects increases (Srivastava et al. 2005). Hence, plants experience natural variation in predator abundance along a gradient of bromeliad size. In this study, we distinguish between the effects of a conventional trophic cascade and the effects of predators on prey turnover by determining the relationship between detrital nutrient uptake by bromeliads and insect composition along a range of habitat sizes. If predators reduce decomposition via a trophic cascade, we predict that nutrient uptake will be negatively related to the ratio of predator to detritivore density. If the spatial effect of predators on prey emergence and nutrient export is dominant then nutrient should be positively related to the predator: detritivore ratio. We again focus on N as it is known to be an element that commonly limits the productivity of terrestrial plants (Vitousek and Howarth 1991), and we have previously found that bromeliad growth is restricted by the availability of this nutrient (Ngai and Srivastava 2006). We also expand our examination of the predator community to include invertebrate predators other than damselflies as they also increase the predation pressure on detritivores.

In addition, habitat complexity may be important for influencing the magnitude and nature of predator impacts on their prey and therefore the effects of predators on detrital processing and nutrient recycling. More complex habitats, for instance, can increase the richness and abundance (Lassau and Hochuli 2005) of predatory species, with implications for other trophic levels (e.g. Finke and Denno 2004). Habitat complexity can also decrease predation rates by creating refuges (Almany 2004, Janssen et al. 2007) or affecting predator behaviour (Warfe and Barmuta 2004). Alternatively, prey capture could change due to shifts in intraguild interactions that are mediated by habitat structure (Grabowski and Powers 2004, Finke and Denno 2006).

To investigate the relationship between predation and N cycling in the context of changing predator abundance and habitat complexity using a bromeliad-insect ecosystem, we used field experiments to ask: (1) How do changes in the trophic structure of an insect community along a habitat size gradient affect the flow of N from litter to bromeliads?; and (2) How does habitat complexity affect this relationship? We use two genera of bromeliads that have varying leaf morphologies, from many narrow leaves in *Guzmania* to fewer wide leaves in *Vriesea*, to examine a range of habitat complexities.



## Methods

### Field site

Field work was conducted in the Area de Conservación Guanacaste, in northwestern Costa Rica (10°59' N, 85°26' W). Although the site consists of wet tropical forests, it experiences a brief dry season from February to April; this study was run during the rainy season when bromeliad-bound insect larvae are most abundant. This mid-elevation location (700 m a.s.l.) consists of a mixture of primary forest, which has not been logged for at least 50 years, secondary forest that was cleared over 16 years ago and open pasture. The dominant vegetation in the primary forest consists of *Calophyllum brasiliense*, *Sloanea faginea*, *Rinorea* sp. and *Pourouma bicolor* trees. *Hedyosmum bonplandianum*, *Clethra mexicana*, *Vernonia triflosculosa* and *Siparuna andina* predominate in the secondary forest, while pastures have scattered small trees, including *Conostegia xalapensis*, *Nectandra hihua*, *Hampea appendiculata* and *Vochysia ferruginea* and forested hedgerows. See Ngai et al. (in press) for a more detailed site description.

### Study organisms

While tropical Bromeliaceae can exist as soil-bound plants, this study focused on the epiphytic, tank-forming members of this family (*Vriesea* and *Guzmania* genera). These genera produce tightly interlocking leaves, forming leaf wells that collect water and falling leaf litter (Benzing 2000). As soon as the bromeliad is large enough to capture detritus, this leaf litter comprises the dominant source of N for the plant compared to atmospheric deposition (Reich et al. 2003). These leaf wells also serve as an oviposition site for many insects with aquatic larval stages, including chironomids (most commonly *Polypedilum* species, Diptera), tipulids (*Trentepholia* species, Diptera), mosquitoes (*Anopheles*, *Culex* and *Wyeomyia* spp., Diptera) and scirtid beetles (Coleoptera); together with other invertebrates and microorganisms, these insect larvae form a detritus-based food web consisting of over 70 species at this site.

The dominant predator in this system is a damselfly larva (*Mecistogaster modesta* Selys, Pseudostigmata, Odonata), which is most common in bromeliads greater than 100 ml in capacity. Where it occurs, this species generally comprises more than 85% of the predator biomass in bromeliads at our site. Other predators include larvae of tabanids, tanypodine chironomids, ceratopogonids and, more rarely, *Toxorhynchites* mosquitoes and adults of hydrophilid beetles. The activity of the invertebrate community may benefit the bromeliad by increasing the

availability of nutrients within the leaf wells (Ngai and Srivastava 2006) that plants can take up via specialized trichomes on the leaf surface (Benzing 2000).

### **Experimental size gradient**

To test the effect of bromeliad size and genus on the transfer of N from detritus to the plant, we examined N uptake using an isotopic-labeling study. Bromeliads (*Vriesea sanguinolenta* and *Guzmania scherzeriana*) ranging in capacity from 64 ml to 1520 ml were collected from primary and secondary forests in Costa Rica, and established in a common garden in the secondary forest by hanging the plants from rope strung between trees. The bromeliads were left for five months to allow for natural insect colonization. *Vriesea* and *Guzmania* bromeliads (n = 15 for each genus) have different leaf morphologies that may lead to different insect communities establishing in their leaf wells (e.g. Armbruster et al. 2002).

Potted *Conostegia xalapensis* Bonpl. (Melastomataceae) seedlings were fertilized with  $K^{15}NO_3$  (200 ml of  $0.4 \text{ g L}^{-1} K^{15}NO_3 \text{ pot}^{-1} 2 \text{ wk}^{-1}$ ) every two weeks over five months to produce leaf litter enriched in  $^{15}N$  (c.f. Ngai and Srivastava 2006). Senesced leaves were collected as they fell by keeping seedlings wrapped in garden netting (mesh size 2 cm) during the months when the seedlings were fertilized; this labeled leaf litter was used to trace the movement of N from detritus to the bromeliad. The capacity of the bromeliads was determined directly by filling the plant wells with water and measuring the amount of liquid used. After conditioning the leaves in water for 48-60 hours, 4.34 mg dwt of leaves per ml well capacity was added to bromeliads.

After 33 days, the newest bromeliad leaves were collected and dried. A subsample of bromeliads (n = 7 for each genus) of a range of volumes (62 to 1520 ml) was dissected leaf by leaf to collect and identify insect larvae, with all invertebrate individuals being sorted to family and to morphospecies. Classification of feeding type (predator versus detritivore) was based on Merritt and Cummins (1996), and Thompson and Townsend (2003).

### **Analyses**

Leaf tissue was ground using an amalgamator (Henry Schein, Melville, New York) and sent to the University of California Davis Stable Isotope Facility for isotopic analysis. Habitat complexity was calculated as the number of leaves per ml of bromeliad well volume (c.f. Srivastava 2006). This is a reasonable measure of habitat complexity as insects must move between leaf wells to access resource patches. More leaves per unit volume means that insects

experience a more finely sub-divided habitat while foraging and therefore need to travel through more leaf wells to access the same volume of water. Biomass was calculated from insect length using regression equations established from surveys at our site (D.S.S., unpublished data). Data were transformed as necessary to fit statistical assumptions and analyzed using linear regression (JMP ver. 5.1, SAS Institute).

## Results

### Nitrogen uptake

The relationship between N uptake, as represented by enrichment in  $^{15}\text{N}$  of bromeliad leaves, and well volume differed between bromeliad genera. There was a non-significant trend of increasing  $\delta^{15}\text{N}$  with bromeliad volume in *Guzmania* (Fig. 3.1a,  $R^2 = 0.086$ ,  $F_{1,13} = 1.22$ ,  $p = 0.29$ ). In contrast,  $\delta^{15}\text{N}$  and hence uptake of litter-derived N strongly declined with size in *Vriesea* (Fig. 3.1b,  $R^2 = 0.59$ ,  $F_{1,13} = 19.0$ ,  $p = 0.0008$ ). The  $\delta^{15}\text{N}$  signature of bromeliad leaves also tended to increase with the ratio of predator: detritivore densities, significantly for *Vriesea* (Fig. 3.2b,  $R^2 = 0.67$ ,  $F_{1,5} = 10.03$ ,  $p = 0.02$ ) but not *Guzmania* (Fig. 3.2a,  $R^2 = 0.17$ ,  $F_{1,4} = 0.80$ ,  $p = 0.42$ ).

### Insect community

While the density of predator individuals generally increased with plant volume for insect communities in *Guzmania* (Fig. 3.3a,  $R^2 = 0.28$ ,  $F_{1,5} = 1.99$ ,  $p = 0.22$ ) and decreased for those in *Vriesea* plants (Fig. 3.3b,  $R^2 = 0.42$ ,  $F_{1,5} = 3.57$ ,  $p = 0.12$ ), in neither case was this relationship significant. In contrast, detritivore density decreased non-significantly in *Guzmania* (Fig. 3.3c,  $R^2 = 0.44$ ,  $F_{1,5} = 3.89$ ,  $p = 0.10$ ) and tended to increase in *Vriesea* (Fig. 3.3 d,  $R^2 = 0.23$ ,  $F_{1,5} = 1.48$ ,  $p = 0.28$ ).

The ratio of predator density (number of individuals per ml of bromeliad volume) to detritivore density increased with bromeliad size in *Guzmania* (Fig. 3.3e,  $R^2 = 0.44$ ,  $F_{1,5} = 3.91$ ,  $p = 0.10$ ). In the case of *Vriesea*, the density of predators relative to detritivores declined significantly with bromeliad volume (Fig. 3.3f,  $R^2 = 0.63$ ,  $F_{1,5} = 8.42$ ,  $p = 0.03$ ).

In the experimental bromeliads, damselfly and tabanid larvae comprised  $90.25\% \pm 8.32\%$  (mean  $\pm$  SEM) of the biomass of predators.

## Habitat complexity

Habitat complexity for the insects, as measured by the number of leaves per unit of well volume, declined significantly with bromeliad size. This was true for both *Guzmania* (Fig. 3.4,  $R^2 = 0.87$ ,  $F_{1, 14} = 96.57$ ,  $p < 0.0001$ ) and *Vriesea* (Fig. 3.4,  $R^2 = 0.80$ ,  $F_{1, 14} = 55.60$ ,  $p < 0.0001$ ).

## Discussion

### Predation increases nitrogen flow to plants

Our results indicate that predatory insects increase N flow from detritus to bromeliads mainly by preventing nutrient export in the bodies of emerging detritivorous insects. Nitrogen flow from detritus to phytotelm leaves declined with plant size in *Vriesea* bromeliads and was independent in *Guzmania* (Fig. 3.2). The ratio of predators to detritivorous insects was greatest in smaller *Vriesea* plants, possibly as a result of reduced habitat complexity and spatial refugia in larger plants. This suggests that the greater ability of an abundant predator population to consume their detritivorous prey and control the emergence of adult detritivores from bromeliads results in more N remaining in the bromeliad ecosystem and being taken up by the plant. This is consistent with previous work showing that predators increase N-flow within the insect-bromeliad system (Ngai and Srivastava 2006), yet contrary to the predictions based on trophic cascade theory. The non-significant relationship between  $\delta^{15}\text{N}$  and the insect community in *Guzmania* may be explained by the effect of habitat characteristics on predation, as will be discussed below.

Although we found no support for the classic trophic cascade pattern where predators reduce decomposition by suppression detritivore populations, this is not to say that the predators do not affect either the numbers or behaviour of their prey. Other food webs of aquatic insects have shown strong effects of predation (Power 1990, Rosemond et al. 1998, Yanoviak 2001). Within the insect-bromeliad system, predation by damselflies significantly reduces the numbers of detritivores (Srivastava 2006, B. Starzomski, D. Suen and D.S.S, unpublished data). It may simply be that when there is a considerable imbalance in the spatial or temporal scale of prey movement with respect to their predators, the effects of predators on prey turnover overwhelm the classic trophic cascade.

An alternate explanation for the change in  $^{15}\text{N}$ -uptake over the size gradient is that the physiological ability or requirement of bromeliads for nitrogen changes as a function of plant age. Although changes in plant nutrient relations are commonly observed with growth and ontogeny (Aerts and Chapin 2000, Wanek et al. 2002, Reich et al. 2003), it is unlikely that this

could explain the patterns we observed, as this mechanism would require uptake ability to increase with size in *Guzmania* but decrease with size in *Vriesea*.

### **Effects of habitat complexity on predation**

The effects of predators on nutrient cycling may covary with plant size as a result of the reduced complexity of the foraging arena in large bromeliads. For both *Guzmania* and *Vriesea*, habitat complexity declined with bromeliad volume, while complexity at all volumes was lower for *Vriesea* plants compared to that of *Guzmania* (Fig. 3.4). The difference in complexity between the genera is further amplified by the fact that *Vriesea* reach much larger sizes than *Guzmania* (Fig. 3.4). The smallest *Guzmania* have many leaves with little water volume, rendering them vulnerable to desiccation. However, as *Guzmania* increase in size, leaf wells deepen. The two largest and most common predators, damselfly and tabanid larvae, are more vulnerable to periodic desiccation than their prey because of their long larval stages, and so it is not surprising that the predator: detritivore ratio tends to increase with bromeliad size in *Guzmania*. *Vriesea*, on the other hand, have generally much lower drought risk as they have wider, deeper leaf wells (Fig. 3.4) than all but the largest *Guzmania* (D.S.S., unpublished data). We therefore do not expect to see as strong a drought limitation of the predator community in *Vriesea*.

In *Vriesea*, the predator: detritivore ratio is highest in the smallest, most complex bromeliads. This may reflect effects of complexity on foraging efficiency. Increased habitat complexity affects the foraging ability or rate of a diverse suite of organisms by creating refuges for prey (e.g. Almany 2004, Warfe and Barmuta 2004). In bromeliads, predators and detritivores both move between leaf wells in search of resource-rich patches and greater habitat complexity decreases foraging rate by dividing a given patch into more compartments, necessitating a larger allocation of time to searching for food. However, in a previous experiment with bromeliads, increased habitat complexity affected the detritivores and damselflies similarly by decreasing their per capita foraging efficiency (Srivastava 2006). The predator community, however, must contend with another constraint in their environment besides simply finding food. Conspecifics can act not only as competitors but also as intraguild predators, where cannibalism is an extreme form of interference competition (Polis 1981). Odonates -- damselflies and dragonflies -- are frequently cannibalistic (e.g. van Buskirk 1989, Johansson and Suhling 2004), and intraguild predation has been observed both in damselflies of the same family (Fincke 1994) and in *M. modesta* (J.T.N., personal observation). Predation by larger larvae on smaller individuals is a

significant source of mortality in some odonates (e.g. Polis 1981, Johnson et al. 1985). Intraspecific predation is also an important source of mortality in tabanids (e.g. Meany et al. 1976). As with the predator-detritivore interactions, habitat complexity could decrease rates of intraguild predation by increasing the availability of refuges or reducing encounter rates with intraguild predators (Fox 1975, Grabowski and Powers 2004, Finke and Denno 2006). The reduction of intraguild predation due to increased habitat structure has also been found by a recent meta-analysis (Janssen et al. 2007).

The presence of intraguild predators may have consequences for the foraging rate or activity levels of predators. Predators, in general, often cause their prey to increase time spent in refuges, and reduce foraging rates and activity (e.g. Short and Holomuzki 1992, Schmitz et al. 1997, Werner and Peacor 2003). This reduction in foraging and activity has also been observed for damselflies, both in the presence of predaceous fish (e.g. Johansson et al. 2001, Dmitriew and Rowe 2005) and other odonates (e.g. Stocks and McPeck 2003, Brodin and Johansson 2004). Thus, the shift in the insect community toward more detritivores and fewer predators in large plants may also be due to intraspecific antagonism among predators.

Our study indicates that there are four components to the effects of habitat complexity on interactions between insects: (1) complexity reduces predator foraging efficiency on detritivores, as well as (2) detritivore foraging efficiency on detritus; (3) predators may experience higher cannibalism in larger, less complex habitats and (4) reduce their foraging in response to increased vulnerability to intraguild predators. In this study, we observed a consistent density of predators as bromeliad volume increased (Fig. 3.3a and Fig. 3.3b) but a decline in habitat complexity with volume (Fig. 3.4), suggesting that intraspecific predators are more frequently encountered by predators in larger bromeliads. In *Vriesea*, habitat complexity dampens the effects of predators on detritivores (Fig. 3.3b), resulting in reduced N transfer from detritus to the plant along the *Vreisea* size gradient (Fig. 3.1b). These results suggest that predation on detritivores is affected by the interactive effects of bromeliad size and complexity, and that the shifts in top-down control in turn influence nutrient processing and acquisition by the plant.

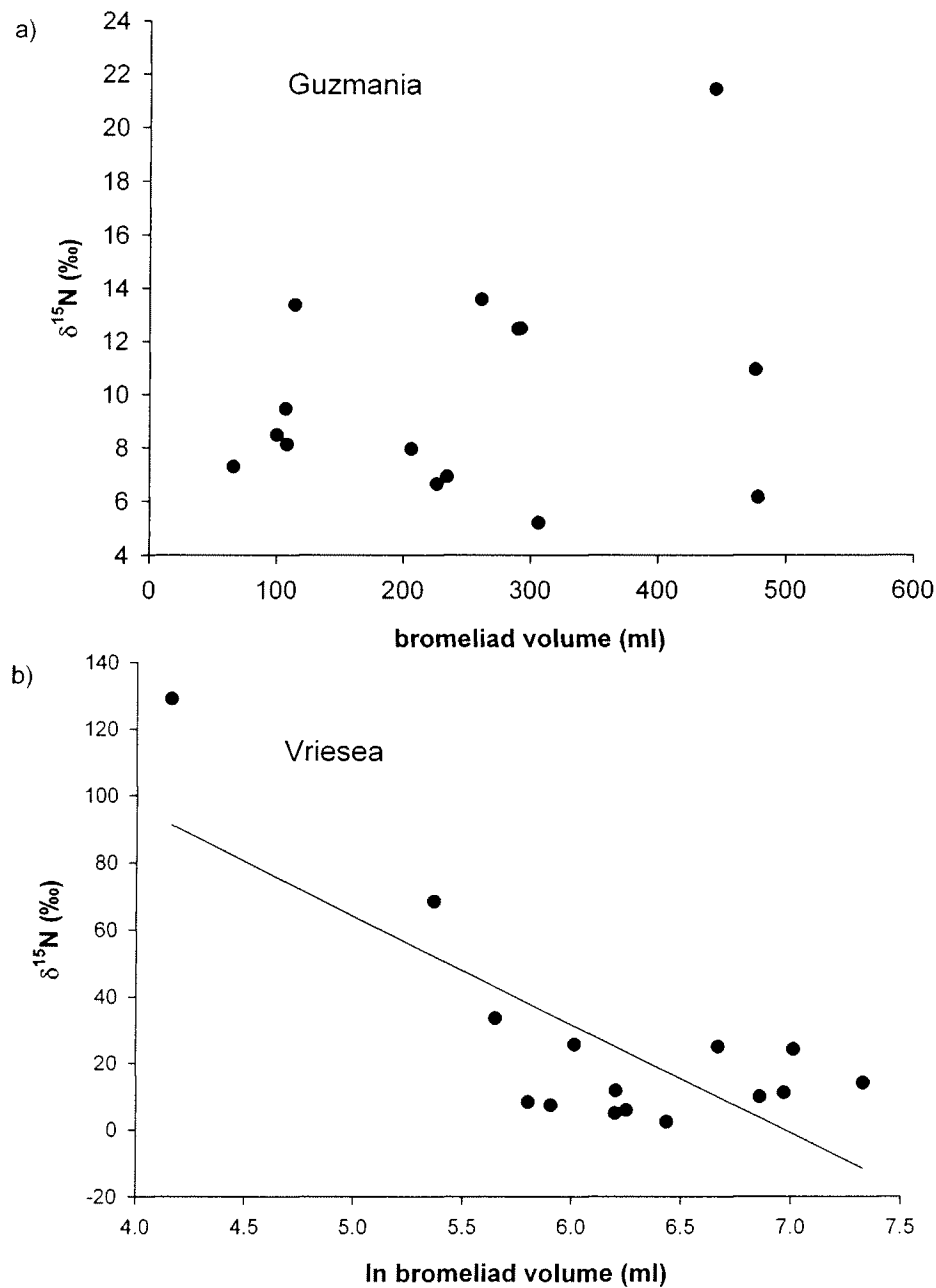
## Conclusions

Along a gradient of changing trophic structure, we show that nitrogen cycling is determined by the effect of predators on the export of nutrients from bromeliads by emerging detritivorous insects, rather than by a classic trophic cascade through reduction in decomposition in the presence of predators. We also find that this shifting trophic structure interacts with

habitat complexity to determine N cycling in this system. Our results emphasize the importance of considering the scale of organism dispersal when examining nutrient movement, as well as the habitat context of these interactions. Although we demonstrate these effects in a bromeliad microcosm, our findings should have implications for other systems. Habitats are expected to be greatly changed by human activities, causing considerable loss of species (Vitousek et al. 1997) and likely altering dispersal patterns. Predators in particular are vulnerable to extinction, with expected consequences for ecosystem functioning (Duffy 2003, Petchey et al. 2004). Given the role of predation in determining nutrient cycling within an ecosystem, it will be important to determine how changing habitat variables and trophic structure will interact to affect ecosystem processes.

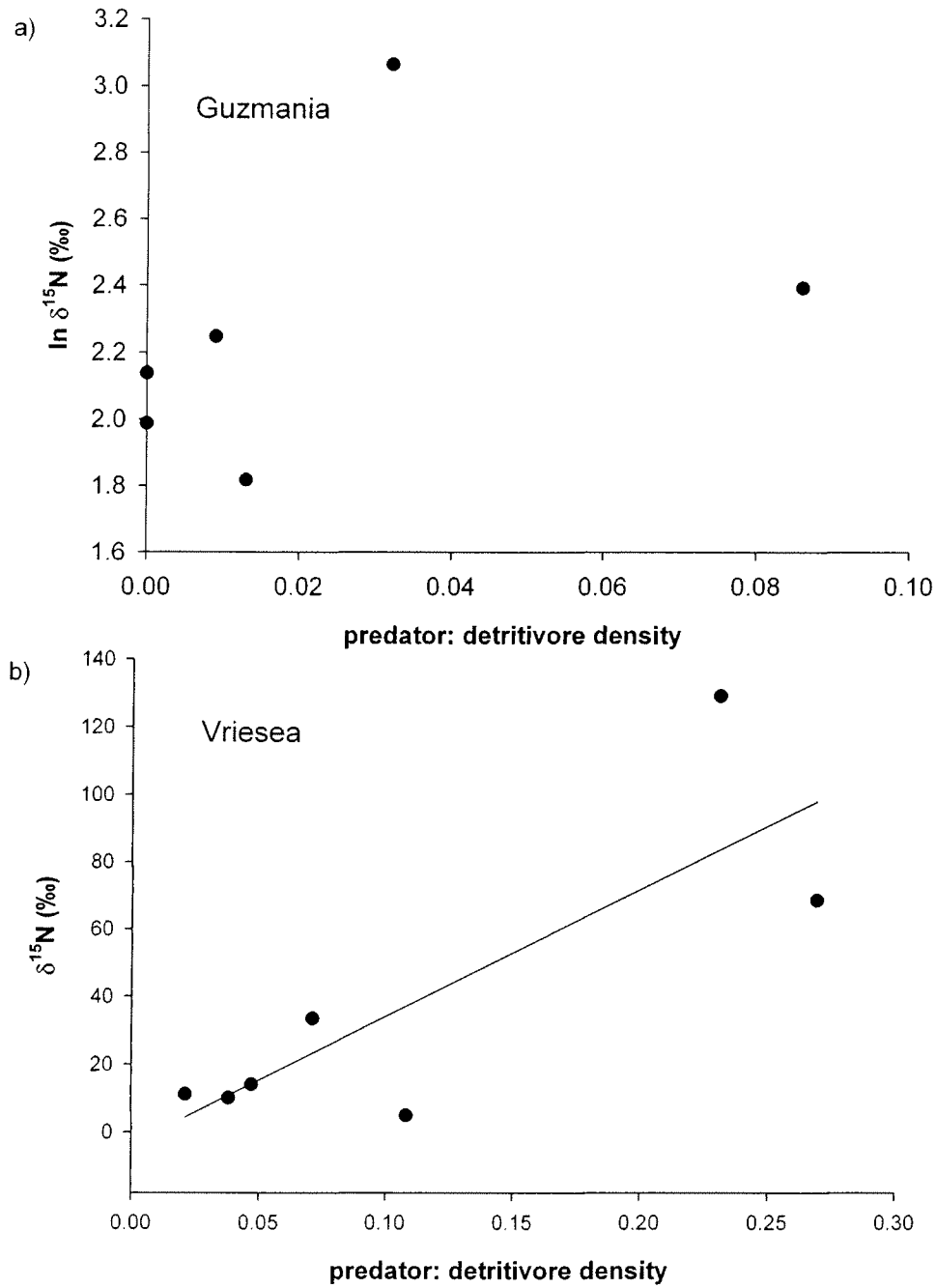
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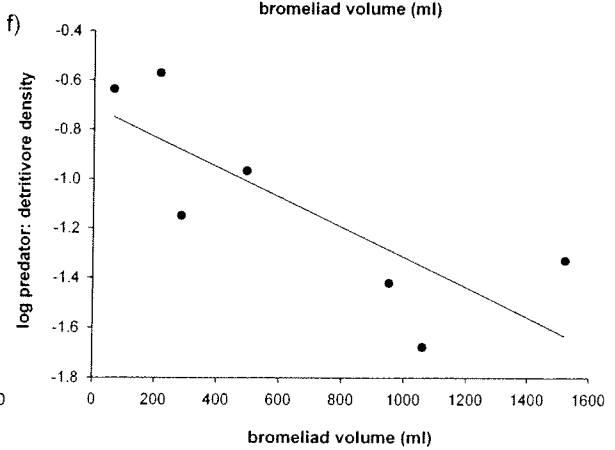
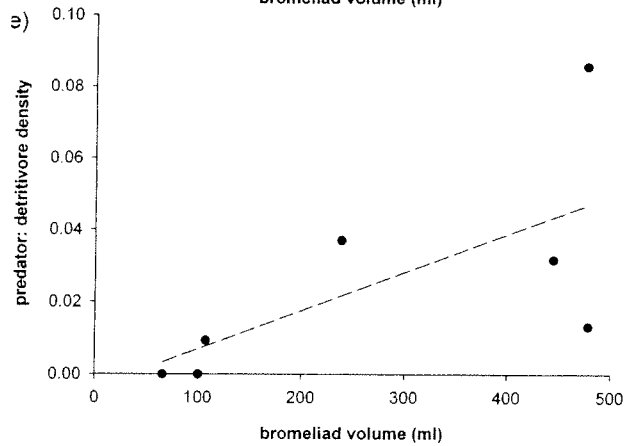
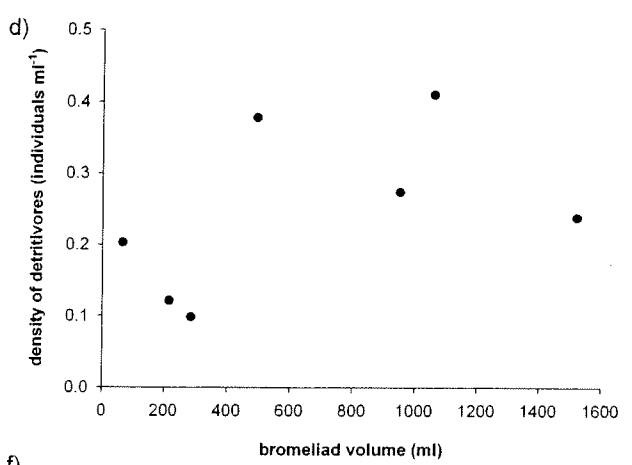
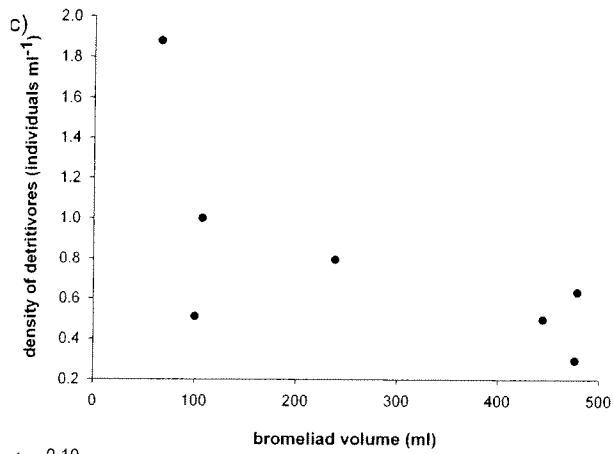
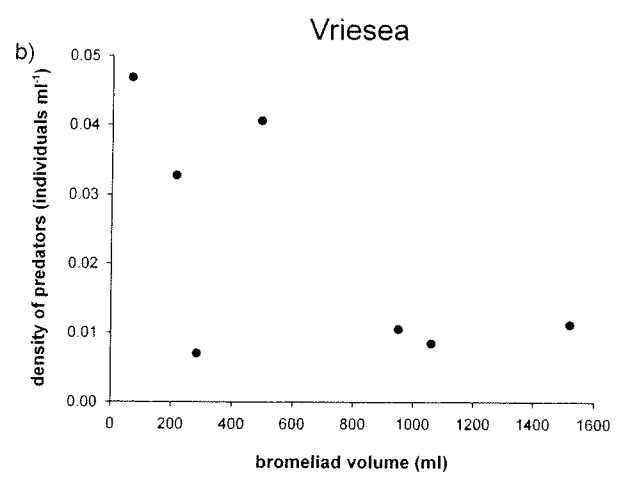
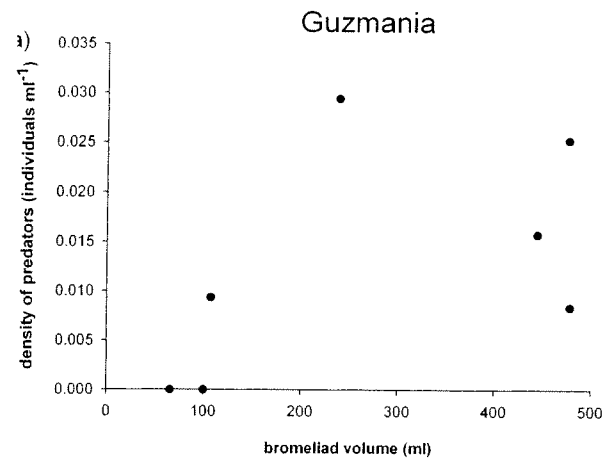


**Figure 3.1.**  $\delta^{15}\text{N}$  (‰) in new bromeliad leaves for plants along a bromeliad size gradient. a) *Guzmania*:  $\delta^{15}\text{N} = 7.84 + 0.00890 \times \text{volume}$  ( $R^2 = 0.086$ ,  $F_{1, 13} = 1.22$ ,  $p = 0.29$ ); b) *Vriesea*:  $\delta^{15}\text{N} = 226.27 - 32.45 \times \ln \text{volume}$  ( $R^2 = 0.59$ ,  $F_{1, 13} = 19.0$ ,  $p = 0.0008$ ). The line is a best-fit regression model.  $n = 15$  for each genus.

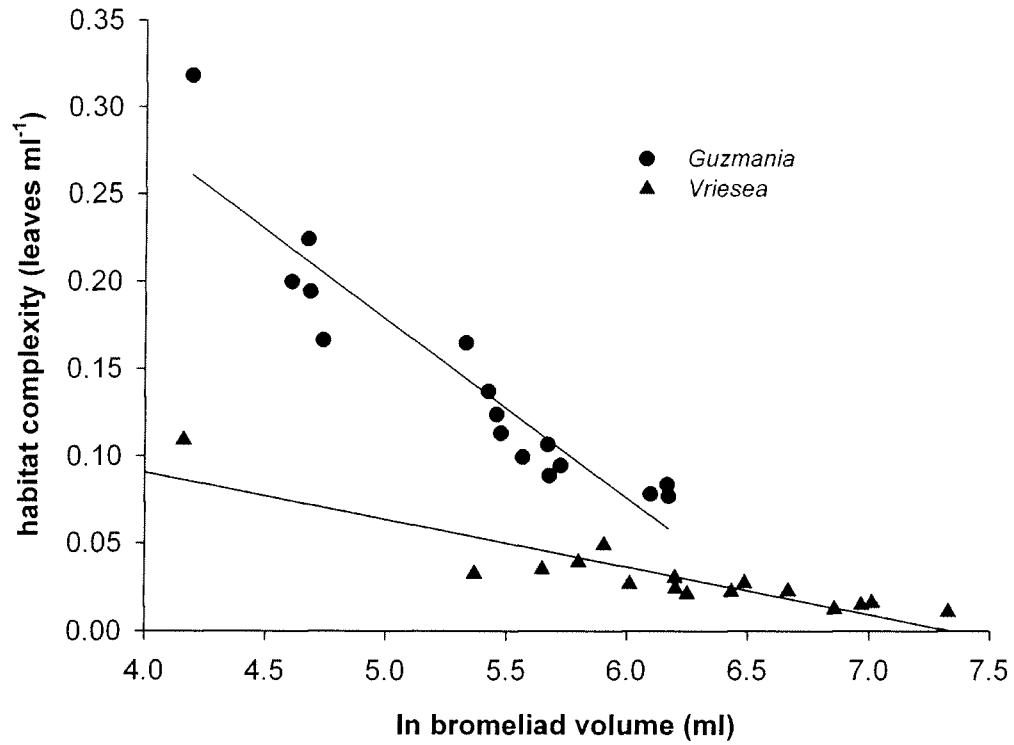




**Figure 3.2.** Changes in  $\delta^{15}\text{N}$  (‰) in new bromeliad leaves with the ratio of predator: detritivore density. a) *Guzmania*:  $\ln \delta^{15}\text{N} = 2.15 + 5.42 \times \text{predator: detritivore ratio}$  ( $R^2 = 0.17$ ,  $F_{1,4} = 0.80$ ,  $p = 0.42$ ); b) *Vriesea*:  $\delta^{15}\text{N} = -3.42 + 376.34 \times \text{predator: detritivore ratio}$  ( $R^2 = 0.67$ ,  $F_{1,5} = 10.03$ ,  $p = 0.02$ ). All predators are included in counts. The line is a best-fit regression model.  $n = 6$  for *Guzmania* and 7 for *Vriesea*.



**Figure 3.3.** Changes in the insect community along a habitat size gradient. All predators are included in counts. Lines are from best-fit regression models ( $n = 7$  for each genus, solid line:  $p < 0.05$ , dashed line:  $0.10 > p > 0.05$ ). **(a, b)** Predator density (number of individuals  $\text{ml}^{-1}$ ) along a bromeliad size gradient. a) *Guzmania*: density =  $0.0037 + 0.000033 \times \text{volume}$  ( $R^2 = 0.28$ ,  $F_{1,5} = 1.99$ ,  $p = 0.22$ ); b) *Vriesea*: density =  $0.036 - 0.000021 \times \text{volume}$  ( $R^2 = 0.42$ ,  $F_{1,5} = 3.57$ ,  $p = 0.12$ ). **(c, d)** Detritivore density (number of individuals  $\text{ml}^{-1}$ ) along a bromeliad size gradient. c) *Guzmania*: density =  $1.30 - 0.0018 \times \text{volume}$  ( $R^2 = 0.44$ ,  $F_{1,5} = 3.89$ ,  $p = 0.10$ ); d) *Vriesea*: density =  $0.18 + 0.00011 \times \text{volume}$  ( $R^2 = 0.23$ ,  $F_{1,5} = 1.48$ ,  $p = 0.28$ ). **(e, f)** Ratio of predator: detritivore density (individuals  $\text{ml}^{-1}$ ) along a bromeliad size gradient. e) *Guzmania*: ratio =  $-0.0038 + 0.00011 \times \text{volume}$  ( $R^2 = 0.44$ ,  $F_{1,5} = 3.91$ ,  $p = 0.10$ ); f) *Vriesea*: log ratio =  $-0.71 - 0.00061 \times \text{volume}$  ( $R^2 = 0.63$ ,  $F_{1,5} = 8.42$ ,  $p = 0.03$ ).



**Figure 3.4.** Habitat complexity (number of bromeliad leaves ml<sup>-1</sup>) along a bromeliad size gradient. a) *Guzmania* (circles): complexity =  $0.69 - 0.10 \times \log \text{volume}$  ( $R^2 = 0.87$ ,  $F_{1,14} = 96.57$ ,  $p < 0.0001$ ); b) *Vriesea* (triangles): complexity =  $0.20 - 0.027 \times \ln \text{volume}$  ( $R^2 = 0.80$ ,  $F_{1,14} = 55.60$ ,  $p < 0.0001$ ). Lines are from best-fit regression models.  $n = 16$  for each genus.

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## **Chapter 4 - Multiple resource limitation of primary production: embracing the interaction<sup>3</sup>**

### **Introduction**

Industrial mobilization and deposition of mineral nutrients have greatly altered nutrient supply and cycling across ecosystems. Globally, anthropogenic nitrogen (N) and phosphorus (P) additions to ecosystems have more than doubled in the past 50 years (Vitousek et al. 1997; Falkowski et al. 2000; Tilman et al. 2001), increasing production (Elser et al. 2007) and altering species diversity of many communities (Stevens et al. 2004; Harpole and Tilman 2007; Hillebrand et al. 2007; Clark and Tilman 2008). Despite these global impacts, our understanding of how these nutrients interact remains rooted in a conceptual framework that has remained relatively unchanged and untested for over 150 years (Liebig 1842). Here we present a synthetic overview of the possible ways in which multiple nutrients interact to affect primary production and use a series of simple statistical tests to discriminate between these types of interactions using a database of over 600 factorial N and P fertilization experiments from marine, freshwater, and terrestrial ecosystems (Elser et al. 2007).

Liebig and Sprengel's Law of the Minimum, which suggests that the resource in shortest supply limits plant growth (Liebig 1842; van der Ploeg et al. 1999), has been central to our understanding of the restrictions to ecosystem productivity. Emphasis on the role of single resources has been reinforced by the focus on P-limitation of autotrophs in freshwater ecosystems (Hecky and Kilham 1988), N-limitation in terrestrial habitats (White 1993; Vitousek and Howarth 1991), and some combination of N- or iron-limitation in marine waters (Vitousek and Howarth 1991). However, recent empirical (e.g. Interlandi and Kilham 2001; Gleeson and Good 2003; Grover and Chrzanowski 2004; Mills et al. 2004; Arrigo 2005; James et al. 2005, Harpole and Tilman 2007), theoretical (e.g. Gleeson and Tilman 1992; van den Berg 1998) and meta-analytic (Elser et al. 2007) studies increasingly demonstrate that there is widespread limitation of primary productivity by multiple resources and that these resources often have interactive effects on primary production.

Both observations and theory suggest that a paradigm of multiple resource limitation may more accurately reflect the conditions in most autotroph communities. Fifty years ago, Redfield

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(1958) observed that the atomic ratios of carbon (C), N and P were remarkably stable in marine seston, and that the same ratio characterized the availability of dissolved nutrients in those waters (discussed in Sterner and Elser 2002). This suggested that marine phytoplankton used these nutrients in the same balance in which they were supplied, such that their growth was simultaneously limited by multiple nutrients. Recent work has demonstrated predictable element ratios in terrestrial plants and soil microbes that, like Redfield's phytoplankton, broadly appear to track global patterns of soil nutrient availability (e.g. Hedin 2004; Kerkhoff et al. 2004; McGroddy et al. 2004; Reich and Oleksyn 2004; Cleveland and Liptzin 2007; Townsend et al. 2007).

Economic models that consider plant growth in the context of resource budgets also indicate that autotroph fitness should be maximized when growth is equally limited by all resources (Bloom et al. 1985). Assuming that resources are at least partially substitutable (e.g. either C or N can be used in plant secondary defenses), the increase of any limiting resource can stimulate a number of autotroph processes. Autotrophs should therefore allocate their supplies of abundant resources to obtain those that are limiting, resulting in co-limitation of plant growth by multiple nutrients (Bloom et al. 1985; Chapin et al. 1987).

Experimentally, multiple resource limitation has been shown both at the individual- and community-levels. Gleeson and Good (2003) demonstrated that shortleaf pine are co-limited by multiple nutrients, and that interactions among limiting resources determine plant growth and the pattern of root allocation. Rubio et al. (2003) found that plants could simultaneously follow both Liebig and economic models of limitation depending on the set of nutrients examined; in either case, they found that multiple nutrients limited plant growth. More recently, a meta-analysis comparing the prevalence of N- and P-limitation of autotroph community biomass production in marine, freshwater and terrestrial ecosystems found that limitation by both nutrients was prevalent in all three habitats (Elser et al. 2007). Moreover, in terrestrial and freshwater habitats, N- and P-limitation were equally important, with strong non-additive responses to additions of both nutrients in all three environments.

In spite of this shifting paradigm, much confusion remains about the nature and mechanisms behind the limitation of productivity by multiple resources, and there is a need for a unified conceptual framework for such studies of nutrient limitation. The identity of limiting resources for plant species has traditionally been determined using fertilization experiments where the supply of resources is supplemented and plant growth monitored. A resource is considered limiting if addition of that resource stimulates plant growth relative to unfertilized

control plots (Aerts and Chapin 2000; Chapin et al. 1986). These experiments are typically analyzed statistically in an analysis of variance (ANOVA) framework, with significant main effects indicating a limiting role for a particular resource. When resources are manipulated factorially, interactions between nutrients can also be examined, but these interactions are seldom interpreted or investigated beyond these statistical tests. The nature of these interactions, however, can provide valuable information about the mechanisms influencing nutrient limitation.

Here, we propose a comprehensive categorization of types of nutrient limitation (Fig. 4.1) and outline a series of tests (Fig. 4.2) through which responses to fertilization can be separated into these eight categories. Mechanisms related to biogeochemistry, physiology, community interactions, experimental limitations and statistical artifacts can lead to this variation in responses to resource addition. We then demonstrate the interpretive power of this framework by analyzing and interpreting over 650 published NP factorial studies.

### **Empirical testing for multiple resource limitation**

Factorial resource addition experiments are required to test for multiple resource limitation and interactions among resources. Although they are often overlooked, a thorough examination of these interactions can be enormously informative about the mechanisms at play in controlling plant biomass responses to nutrient addition. Here we provide a framework for testing and interpreting the possible interactions, using planned statistical tests. Given a factorial fertilization experiment, there are five general categories of nutrient limitation (Fig. 4.1, Cases D-H) that display a variety of interactions, beyond the three categories of possible results that lack nutrient interactions (Fig. 4.1, Cases A-C). We restrict our theoretical framework to interactions between two resources (e.g. N and P) but the framework we develop here can readily be extended to consider different numbers or sets of resources.

Here we consider a hypothetical experiment in which experimental units are fertilized with N and P in a factorial design with replication. The results would be analyzed using an appropriate ANOVA statistical model that might include blocking, repeated measures if the experiment was sampled through time, and any other aspects of the experimental design. The presence of significant main effects, interaction terms and significant planned contrasts can identify limitation of biomass production by single or multiple nutrients, as well as the specific behaviour of multiple resource interactions (Fig. 4.1). Given the results of this hypothetical fertilization experiment, we designed a series of three statistical tests to diagnose each of eight categories of resource limitation.

The initial step in our framework is to determine whether there is a significant interaction term in the ANOVA (Fig. 4.2). If not, the number and identity of significant main effects will indicate the *lack of nutrient limitation* by the resources examined (Case A, no significant main effects), *single nutrient limitation* (Case B, one significant main effect) or *additive dual nutrient limitation* (Case C, two significant main effects). In the latter case, both resources independently limit autotroph biomass.

When the ANOVA identifies a significant interaction term, the next step is to determine whether the resources interact to cause a super-additive or sub-additive response to fertilization relative to addition of each resource alone (Fig. 4.2). In the case of experiments exhibiting a super-additive response to fertilization, this involves conducting statistical contrasts between the single addition treatments and the controls. The presence of only one significant contrast indicates *sequential co-limitation* (Case E), where response to the second limiting nutrient depends on addition of the first nutrient. This corresponds to Liebig-type limitation where addition of one nutrient shifts the system to limitation by another. This response is exhibited when resources are essential and non-substitutable. When both contrasts are significant, there are two co-limiting nutrients, or *synergistic co-limitation* by both resources (Case F). Lack of significant contrasts indicates *strict co-limitation*, where simultaneous addition of both nutrients is needed for a growth response (Case D).

In the case of sub-additive results, significant and positive contrasts between treatments and controls indicate that the system is limited by one or both of the added nutrients, but the response is *constrained* (Case G) such that some other factor, such as another nutrient, abiotic conditions or herbivory, limits productivity once both nutrients are added. Alternatively, significant and negative contrasts indicate an *antagonistic* response to nutrient addition (Case H) where one nutrient has a positive effect in the presence of the other but a negative effect when alone.

## Methods

In the previous section we presented a procedure that allows a researcher to identify the nature of limitation by multiple resources in an experimental study system, given raw data from a factorial fertilization experiment. Next, we determine the prevalence of the various categories of resource limitation observed in experiments across different kinds of ecosystems. To determine this, one would ideally analyze raw data and have appropriate statistical models to reflect

experimental designs from each of many studies; in practice this is not feasible. Instead we have chosen to take a meta-analytic approach.

Recently, Elser et al. (2007) published a meta-analysis of the effects of N and P addition on community-level primary production in marine, freshwater and terrestrial ecosystems and reported significant interactions between N and P in many ecosystems. However, this analysis included many non-factorial additions of N and P, and so the true nature of these interactions was not explored. Here we conduct a full exploration of the factorial experiments in this database. Of the 1069 experiments in the database used for the Elser et al. (2007) meta-analysis, 653 studies performed factorial N and P fertilizations, and 147 of these reported error estimates. For criteria used in study selection and response units, see the Methods section and Appendices for Elser et al. (2007). The original data can be obtained via the public data repository of the National Center for Ecological Analysis and Synthesis ([knb.ecoinformatics.org/knb/style/skins/nceas/](http://knb.ecoinformatics.org/knb/style/skins/nceas/)). Here, we used two metrics to classify each of these 653 studies into one of eight categories of resource limitation based on the means and standard errors (when available) for the control ( $N_0P_0$ ), N addition ( $N_1P_0$ ), P addition ( $N_0P_1$ ) and N+P addition ( $N_1P_1$ ) treatments.

### **A meta-analytic test for resource interactions**

As a first step, we extend the analysis of Elser et al. (2007) using published methods for testing for interactions in factorial meta-analysis (Guerevitch et al. 2000; Hawkes and Sullivan 2001; Borer et al. 2006). This analysis was performed using the 653 factorial studies in an updated and expanded version of the Elser et al. (2007) database to determine the prevalence of resource interactions. Using this method, the main effect of N addition is calculated as

$$LRR_N = (\ln(N_1P_1) + \ln(N_1P_0)) - (\ln(N_0P_1) + \ln(N_0P_0)) \quad (\text{eq. 1})$$

the main effect of P addition is calculated as

$$LRR_P = (\ln(N_0P_1) + \ln(N_1P_1)) - (\ln(N_0P_0) + \ln(N_1P_0)) \quad (\text{eq. 2})$$

and the N by P interaction is

$$LRR_{NP} = (\ln(N_0P_0) + \ln(N_1P_1)) - (\ln(N_0P_1) + \ln(N_1P_0)). \quad (\text{eq. 3})$$

We note that this method tests for additivity in natural log space, such that an interaction of zero would still be a super-additive response in the original units. This meta-analytic approach only tests for an overall interaction and for significant main effects in the dataset. In the following sections, we classify individual studies according to their type of nutrient limitation.

### Identifying the type of nutrient limitation: a statistical approach

We proceed beyond this overall test for any nutrient interaction by classifying each study into one of eight categories of nutrient limitation, using the flow diagram outlined in Figure 4.2. To identify main effects and interactions we used a method developed by Altman and Bland (2003). Their method is based on the standard statistical principle that if  $E_1$  and  $E_2$  are two estimates (in our case, means), with standard errors  $SE(E_1)$  and  $SE(E_2)$ , then the difference of the two estimates ( $E_1 - E_2$ ) has a standard error equal to the square root of  $SE(E_1)^2 + SE(E_2)^2$ . The difference of the estimates divided by the standard error of the difference follows a z-distribution which allows a test of the null hypothesis that the difference is not significantly different from zero. A value that differs significantly from zero indicates that there is a significant effect (N, P or interaction). Building on this approach, we designed the following tests for significant main effects of N and P addition, and the interaction term. An effect or interaction was considered statistically significant if the value from the equations below exceeded the critical z-score of 1.65, corresponding to  $p < 0.05$ .

$$\text{Main effect of added N: } \frac{(N_1P_0 + N_1P_1) - (N_0P_0 + N_0P_1)}{\sqrt{(SE_{N_1P_0}^2 + SE_{N_1P_1}^2) + (SE_{N_0P_0}^2 + SE_{N_0P_1}^2)}} \quad (\text{eq. 4})$$

$$\text{Main effect of added P: } \frac{(N_0P_1 + N_1P_1) - (N_0P_0 + N_1P_0)}{\sqrt{(SE_{N_0P_1}^2 + SE_{N_1P_1}^2) + (SE_{N_0P_0}^2 + SE_{N_1P_0}^2)}} \quad (\text{eq. 5})$$

$$\text{Interaction: } \frac{(N_1P_1 + N_0P_0) - (N_1P_0 + N_0P_1)}{\sqrt{(SE_{N_1P_1}^2 + SE_{N_0P_0}^2) + (SE_{N_1P_0}^2 + SE_{N_0P_1}^2)}} \quad (\text{eq. 6})$$

This method for classifying studies according to types of nutrient limitation also involved the following calculations for contrasts between control and the single nutrient addition treatments. Again, the contrast was considered statistically significant if the ratio exceeded the critical z-score of 1.65, corresponding to  $p < 0.05$ .

$$\text{Contrast for N addition only: } \frac{N_1P_0 - N_0P_0}{\sqrt{SE_{N_1P_0}^2 + SE_{N_0P_0}^2}} \quad (\text{eq. 7})$$

$$\text{Contrast for P addition only: } \frac{N_0P_1 - N_0P_0}{\sqrt{SE_{N_0P_1}^2 + SE_{N_0P_0}^2}} \quad (\text{eq. 8})$$

Finally, given a significant interaction term, we used the following test for super- or sub-additivity of the N + P response.

$$\text{Additivity: } \frac{N_1P_1 - N_0P_0}{(N_1P_0 - N_0P_0) + (N_0P_1 - N_0P_0)} \quad (\text{eq. 9})$$

A ratio value greater than 1 indicated a super-additive response and a value less than one indicated sub-additivity. The classification scheme used to discern the type of nutrient limitation is presented in Table 4.1, along with the number of studies that fell into each category, listed separately for marine, freshwater and terrestrial ecosystems. We note that this categorization method tests for significance and additivity in un-transformed units, as opposed to the traditional meta-analysis performed in the previous section using log-transformed values.

### **Identifying the type of nutrient limitation: an approach based on biological significance**

Low replication or statistical power (i.e. Type II error) in experiments may prevent detection of statistically significant responses even with clear and observable biological responses. Therefore, we used another approach for classifying studies according to their type of nutrient limitation, in this case based on the effect of each treatment in terms of biomass production. This method is based on “biological significance” rather than statistical significance, and allowed us to utilize a broader dataset for meta-analysis because it does not require an error estimate. We used the 653 factorial N and P addition studies from Elser et al. (2007, see above) to categorize responses according to the following metrics:

$$\text{Nitrogen response: } 100\% \times \frac{N_1P_0 - N_0P_0}{N_0P_0} \quad (\text{eq. 10})$$

$$\text{Phosphorus response: } 100\% \times \frac{N_0P_1 - N_0P_0}{N_0P_0} \quad (\text{eq. 11})$$

Interaction response:

$$100\% \times \frac{(N_1P_1 - N_0P_0) - [(N_1P_0 - N_0P_0) + (N_0P_1 - N_0P_0)]}{N_0P_0} \quad (\text{eq. 12})$$

We categorized combinations of biological responses according to the classification scheme presented in Table 4.1, in the same way we categorized the statistical responses above. We additionally explored how the distribution of biological response categories depended on the

threshold criterion value used to determine a “biologically significant” nutrient response by repeating the above categorization process over a range of values representing 0-100% minimum treatment response greater than the control. This allowed the quantitative assessment of criteria used to categorize responses. This is in contrast to previous work, which has defined “biological significance” according to somewhat arbitrary criteria, such as a treatment that produces a response 10% or greater than the control (e.g. Elser et al. 1990; Dixon and Garrett 1994; Dixon and Pechman 2005).

## Results

In our analysis of the literature of fertilization experiments, we found that marine, freshwater and terrestrial ecosystems all, on average, showed significant main effects of adding N and P on biomass production (Table 4.2, Fig. 4.3, based on 653 studies). Moreover, in all systems, the N-by-P interaction was significantly positive indicating super-additive responses. We then classified the type of interaction on a study-by-study basis for the 147 studies for which we were able to extract estimations of variance.

The results of our statistical approach for classifying studies according to their type of nutrient limitation are presented in Table 4.1. Our method categorized each of the 147 experiments into one of the types of nutrient limitation A-H. While the above method is conservative because interactions were tested for in log-space, this method is also conservative because of meta-analytic power issues: our results found that 20% of the studies fell into Case A, indicating that biomass production did not respond to additions of either nutrient. However, the authors of the original study reported at least one statistically significant result in every case. Authors’ original analyses of raw data and our method using only means and standard errors may differ because of blocking or other ANOVA terms which increased statistical power to detect significant effects, or it could indicate that our method for variance propagation from the difference of two means inflates this variance and substantially lowers our statistical power. In spite of our conservative estimates, the results in Table 4.1 indicate that 50% of studies displayed limitation by more than one nutrient (Cases C-H), and that one third had a significant interaction in the biomass response to N and P (Cases D-H). Given these conservative estimates, we conclude that multiple nutrient limitation occurs in the majority of studies, consistent with the findings of Elser et al (2007), in spite of our differing response metrics.

Fertilization increased primary production by 10% or more (our arbitrary criterion for biological significance) in virtually all (96%) studies, consistent with the results reported in the



original publications (Fig. 4.4). Only 8% of the studies were consistent with single nutrient limitation -- a greater than 10% response to the addition of either N or P, but not to both or to N and P together (Case B). Similarly, fertilization with both N and P rarely resulted in an antagonistic response (Case H). The majority of studies (70%) showed a super-additive effect of N and P such that the result of adding both nutrients was at least 10% greater than expected from adding N or P separately (Cases D-F).

Increasing the cut-off value for identifying a “significant” biological response resulted in a greater proportion of Case A, as expected: 50% of studies were categorized as “no response” when a 100% increase in autotroph production in treatment plots relative to the control was used at the cut-off. However, 37% of the studies showed a super-additive response to combined N and P that was at least 100% greater than the additive expectation of fertilization with N and P separately (Fig. 4.4).

## **Discussion**

### **Mechanisms of resource interaction**

We have developed a conceptual framework that allows us to distinguish between eight possible effects of multiple resource additions on producer biomass. We used this framework to evaluate the frequency of interactions between limiting nutrients in experimental studies and hence, the potential for multiple resource limitation. In this analysis, we have shown that limitation of primary production by multiple resources is more common than previously appreciated and there is a wide range in the behaviour of these interactions between limiting nutrients. The results of meta-analyses, supported by our conceptual model, demonstrate that the presence and form of statistical interactions in factorial fertilization experiments can provide insights into the mechanisms driving nutrient limitation. Although our statistical approach was conservative, we found a statistical interaction in the response of production to combined N and P addition in more than one third of studies. In addition, when the response of primary production to limiting nutrients was viewed relative to control plots or additive expectation, the majority of studies showed super-additive co-limitation of autotroph production by N and P.

However, there was considerable variation in the type of interaction between limiting nutrients. At the 10% threshold of “biological significance”, Liebig-type limitation (i.e. sequential co-limitation, or Case E) was common (one third of studies), but by no means the only type of co-limitation: strict and synergistic co-limitation (Cases D and F) together represented 37% of all studies (Fig. 4.4). As the minimum relative response threshold used for categorizing

“biological significance” increased, fewer studies showed responses to the additions of one or more nutrients, as expected. Despite this, autotroph communities in almost half of the 653 studies more than doubled in biomass with fertilization with N or P relative to the control (i.e. the biological response cutoff set at 100%; Fig. 4.4). Of those studies, 74% showed very strong N-by-P interactions (Cases D-F) with the response to both nutrients being twice as great as expected from responses to single nutrients. Studies with the greatest biomass increases in response to fertilization were dominated by strict co-limitation (Case D), while studies demonstrating Liebig-type and synergistic co-limitation (Cases E and F) tended to have somewhat smaller increases in biomass with fertilization. Hence, the magnitude of responses to N and P together was much greater than responses to either nutrient individually for studies showing strong nutrient limitation response.

These results emphasize that careful examination of the data -- beyond simply noting the presence of statistically significant interaction terms -- can generate deeper understanding of resource controls on primary production. Our conceptual model (Fig. 4.1), coupled with explicit statistical tests and interpretations (Fig. 4.2), provides a standardized method for investigators to determine the type of nutrient limitation. The potential mechanisms underlying each observed type of nutrient limitation fall into five categories discussed in the following sections: 1) biogeochemical and 2) physiological mechanisms, 3) community interactions, 4) experimental limitations and 5) statistical artifacts.

### **Biogeochemical mechanisms**

At the most basic level, supplies of resources other than light are regulated by biogeochemical processes. Inorganic nitrogen levels, for instance, are determined by the balance between N-fixation and denitrification, while P is controlled by various mineralogical processes. In terrestrial systems, the levels of these resources can vary with substrate age (Walker and Syers 1976; Vitousek 2004), with little accumulated biological N-fixation on young soils and increasing depletion or occlusion of P in older substrates. Hence, at the extremes of this developmental continuum, single-resource limitation of plant production is more likely (e.g. Vitousek and Farrington 1997; Case B). Internal processes of nutrient supply, such as differential recycling of N and P by consumers, can also affect the relative importance of N and P limitation (Elser et al. 1988). Similarly, in situations where one resource is already saturated, as in areas with high anthropogenic nutrient inputs, limitation by a single resource might more likely occur (e.g. LeBauer and Treseder 2008). Single-nutrient limitation may also be more

common when communities are dominated by producers that obtain atmospheric N via bacterial associations, such as legumes, a few diatoms and some macroalgae, or can fix nitrogen themselves as is the case with some cyanobacteria (e.g. Niklaus et al. 1998). In contrast, a relatively balanced supply of resources would lead to a synergistic (Case F) or additive response (Case C), whereby addition of one resource induces limitation by the other. In this case, large increases in biomass appear possible only when supplies of multiple resources are supplemented (Elser et al. 2007).

It is also important to consider whether added resources actually become available for uptake by the autotrophs. In instances where there is no biomass response to fertilization (Case A), the nutrients may be immobilized in substrates or by other organisms, or leached away prior to autotroph uptake (e.g. Shaver and Chapin 1995). Phosphorus, for instance, is often sequestered in soil (Vance et al. 2003) or adsorbed to particles in aquatic systems, while nitrate is mobile and subject to leaching (Vitousek and Howarth 1991) or loss to trace-gas production via microbial denitrification processes (Firestone and Davidson 1989). When more than one nutrient occurs at levels too low for uptake (Arrigo 2005), strict co-limitation (Case D) may result, as both nutrients must be supplemented before any growth response is observed.

### **Physiological mechanisms**

Beyond biogeochemical constraints on resource availability, variation in the physiology of primary producers will determine the extent of resource acquisition and use. In environments where autotroph growth is strongly limited by abiotic stressors, such as temperature or salinity (Larcher 1995), no growth response (Case A) or a constrained response (Case G) would be predicted. Similarly, these responses would be expected if physiological limits to plant growth are reached; for instance, a predominantly herbaceous plant community may reach an upper capacity to respond to fertilization as constraints in the production of support tissues are reached. Alternatively, an excess of nutrient supplementation may cause negative or toxic effects on autotrophs (e.g. N: Gutierrez and Whitford 1987; Larcher 1995), which can produce negative main effects and antagonistic interactions (Case H).

Producers adapted to low resource environments, such as arctic or desert habitats, may be restricted in their ability to uptake and utilize the added nutrients and little to no response to fertilization may be observed (Case A). Under these conditions, the autotrophs generally have low maximum growth rates, limited phenotypic plasticity and a restricted ability to respond to a large pulse of added resources (Chapin 1980). For autotrophs with limited storage capacity, such

as some phytoplankton or other small primary producers, the simultaneous availability of multiple resources in the environment might be particularly important in promoting growth; we would expect strict co-limitation (Case D) to be more common when storage capacity is minimal.

Interactions between resources can also affect the nature of nutrient limitation for producers. If the nutrients are not exchangeable and have independent roles, and supplies of both nutrients are at limiting levels, we would see strict co-limitation (Case D) in which producers require addition of both limiting resources before an increase in biomass is seen. Biologically, this may be due to compounds or enzymes that require more than one element for their formation, or elements that are needed for the same biochemical pathway. Antagonistic responses (Case H) can also occur if increases in the availability of one nutrient inhibits uptake of another resource, possibly by competing for binding sites during acquisition.

Many primary producers display flexibility in their use of resources, in which they allocate non-limiting resources in order to gain those that are scarce. For instance, terrestrial plants can allocate N to produce phosphatase enzymes in roots to acquire limiting P (Sinsabaugh and Moorhead 1994; Treseder and Vitousek 2001). Increases in N availability could also allow greater allocation to root production, such that a greater volume of soil could be exploited for P. These shifts in allocation would allow plants to respond with increased growth even when they have insufficient supplies of a particular nutrient. In this case, a growth response is observed when either resource is provided to the primary producers in a factorial fertilization experiment (Cases C, F and G). These cases appeared with relatively high frequency in our dataset using either statistical or biological criteria, suggesting high plasticity of plants to allocate among different physiological functions.

### **Community interactions**

Although Liebig and Sprengel's Law of the Minimum (Liebig 1842; van der Ploeg et al. 1999) was developed to explain the biomass production of individual crop plants, it has been applied to whole community responses to fertilization. This discrepancy may underlie confusion in the literature regarding nutrient limitation, where many authors invoke Liebig's Law when discussing ecosystem- or community-level responses to enrichment. Tilman's resource ratio theory (Tilman 1980) predicts that limitation of autotroph growth by a single resource would be uncommon in diverse communities where coexistence depends on different  $R^*$  values -- the minimum level of a resource that can support a consumer population -- for different resources.

Moreover, a combination of species limited by different single nutrients (Case B) will result in multiple resource limitation of growth for the community as a whole. Both lines of reasoning suggest that, at a community level, production should most often be limited by more than one resource. If an autotroph community becomes rapidly dominated by a single or few species after resource fertilization, single resource limitation may be more likely. In contrast, if species turnover is slower or if herbivores graze dominant plants (Hillebrand et al. 2007) and a constant level of species diversity is maintained, limitation of producer growth by multiple resources may be expected.

Interactions of autotrophs with consumers can limit the increase in biomass that is observed with nutrient addition. In the most extreme case, treatment plots will not differ in biomass from control areas if herbivory (Hawkes and Sullivan 2001) or pathogens (Mitchell 2003) are the main limitations to growth and also respond to autotroph production (resulting in Case A). Thus, alleviation of resource limitation may result in no net change in production (Case A) or may lead to only minimal increases (Case G) if additional growth is consumed by herbivores, if herbivores disproportionately consume producers that have received nutrient enrichment due to altered food quality (Hawkes and Sullivan 2001; Gruner et al. 2008), or if herbivores selectively remove producer species (or portions of species) that are disproportionately responsible for nutrient uptake (Chapin and Slack 1979; Brown 1994; Bracken and Stachowicz 2007).

Competition with other community members may even cause a decrease in biomass as a result of resource addition. For example, stimulation of the microbial community with addition of C can cause a decline in plant production because of producer-microbe competition for shared soil nutrients (e.g. Dunn et al. 2006). Alternatively, C additions can stimulate C-limited bacteria, which then increase denitrification and reduce N availability for plants (e.g. Alpert and Maron 2000). In contrast, interactions with symbionts could lead to a greater than expected biomass response. Excess photosynthetic C, for example, can be exchanged for nutrients via mycorrhizal associations and can potentially allow a synergistic response (Case F). Fertilization with P can increase the rate of N-fixation by symbionts and stimulate a larger biomass response by producer communities (Pearson and Vitousek 2002; Uliassi and Ruess 2002).

### **Experimental limitations**

The design and experimental context of studies are obvious constraints on the observed response of autotrophs to fertilization. If some other resource not included in the treatments

limits producer growth, or if the amount of nutrients added is insufficient to overcome resource limitation, responses in producer biomass may not be observed (Case A). Limitation by additional resources could also lead to a constrained response by the autotrophs (Case G): it may be that another resource, which is not included in any of the treatments, becomes limiting to growth once the nutrients added become abundant. For example, carbon dioxide or a trace nutrient such as iron can become limiting when either N or P is applied, such that no additional growth response can be achieved when the two nutrients are added together. Water (Field et al. 1998) or light (Gibson 1988), are also often directly or secondarily limiting to growth but are generally not manipulated in nutrient enrichment experiments.

In factorial experiments, the relative amounts of resources added in each of the nutrient treatments may also be critical in determining the response seen. In fertilization experiments, researchers generally add nutrients in excess to completely alleviate nutrient limitation. However, if only one nutrient is added in sufficient quantities, we may only see a single nutrient response (Case B) when other nutrients play important roles in limiting production. Secondary resource limitation might also emerge only when the primary limiting nutrient is added in excess, as in Liebig-type limitation (Case E).

The timing of treatments and type of response that is measured can also influence the presence of significant treatment effects and the shape of any interactions. One consideration is the effect of applying press versus pulse treatments in fertilization experiments. When nutrients are added in a single large dose, a considerable portion of the resource may be lost due to leaching or immobilization in the soil, particularly if the supply vastly overwhelms autotroph uptake ability. This may be especially true for N, which is easily lost via leaching, and could affect the relative abundance of nutrients experienced by producers, decreasing the likelihood of observing N-limitation. In the long-term, press additions of nutrients may decrease the number of limiting resources in a system and depress community diversity (e.g. Suding et al. 2005; Harpole and Tilman 2007). Additive dual limitation (Case C), resulting from differential responses to nutrients within the producer community, may become less prevalent in press experiments as diversity is lost. Community interactions may also have larger effects within long-term experiments as species replacements take place and composition changes. Such responses may be more likely in aquatic communities with unicellular producers that undergo many generations during the course of an experiment, although terrestrial studies are usually longer in absolute terms. The long-term addition of nutrients can lead to species turnover (Crawley et al. 2005), causing a shift in nutrient limitation during the course of the experiment.

The choice and timing of response metric can also influence the response seen. Responses measured at the end of the growing season will average over the demands for nutrients at different phenological and developmental stages and may result in more frequent limitation by multiple resources. Moreover, if the added resources limit some process other than production, such as flowering, seed or spore set or senescence, no response may result if only producer growth is measured.

Given the ubiquity of co-limitation, there is a continuing need for the expanded use of factorial experiments. Single-factor experiments will underestimate limitation as they are unable to detect several categories of co-limitation (e.g. strict, sequential or antagonistic co-limitation).

### **Statistical artifacts**

While factorial experiments represent a powerful and efficient design for understanding interaction between multiple nutrients, their analyses come with caveats. Although two-factor ANOVA is designed to test for interactions, emphasis is often placed on interpretation of the main effects. This practice reflects an historical emphasis on identifying “the main limiting nutrient” for a given ecosystem. For example, Vince and Valiela (1973) interpreted a significant response of marine phytoplankton to N but not P to indicate that marine systems were primarily N-limited, despite a large N+P response (i.e. a strong NxP interaction); this interpretation has propagated through various literature reviews (e.g. Howarth 1988).

A similar situation may underlie the case for P in freshwaters. The experimental fertilization of Lake 226 at the Experimental Lakes Area in Canada -- a pivotal study in lake eutrophication research -- may require re-interpretation in the light of multiple nutrient limitation. One side of the partitioned lake received enrichment by inorganic N and organic C while the other side showed massive responses to inorganic P, added with N and C (Schindler 1974). Given the common observation of strong super-additive responses in many lakes, it is possible that the response on the P-addition side of the lake may not have been observed if only P had been added, as suggested by Elser et al. (1990). The approach outlined here considers more explicitly these additional resource interactions by carefully examining interactions with pre-planned contrasts.

The common practice of log-transforming biomass data is an additional consideration, as this would result in significant statistical interactions only on a multiplicative scale. For example, Elser et al. (2007) present log response ratios in their meta-analysis, in which case an additive response of N and P addition actually represents a super-additive response in the

original units. The general effect of examining log responses will be to underestimate the frequency of nutrient co-limitation. For this reason, we have devised tests which explicitly test for additivity in linear space.

## **Conclusions**

We find that limitation of biomass production by multiple nutrients is at least as common as single nutrient limitation across freshwater, marine and terrestrial systems. Moreover, interactive effects of N and P are frequent and varied in their features. This suggests that factorial fertilization experiments using multiple primary limiting nutrients are necessary to understand nutrient limitation. In addition, much useful information is lost when interactive effects are detected statistically but not interpreted mechanistically. Our taxonomy of interactive effects allows researchers to discriminate among a number of possible roles that nutrients can play in plant physiology, community interactions and biogeochemistry. Analysis of the existing literature shows that interactions in general are common, and most often take the form of super-additivity. Our results indicate that continued simultaneous increases in both nitrogen and phosphorus inputs from human activity will have super-additive effects on primary producers across many different ecosystems.

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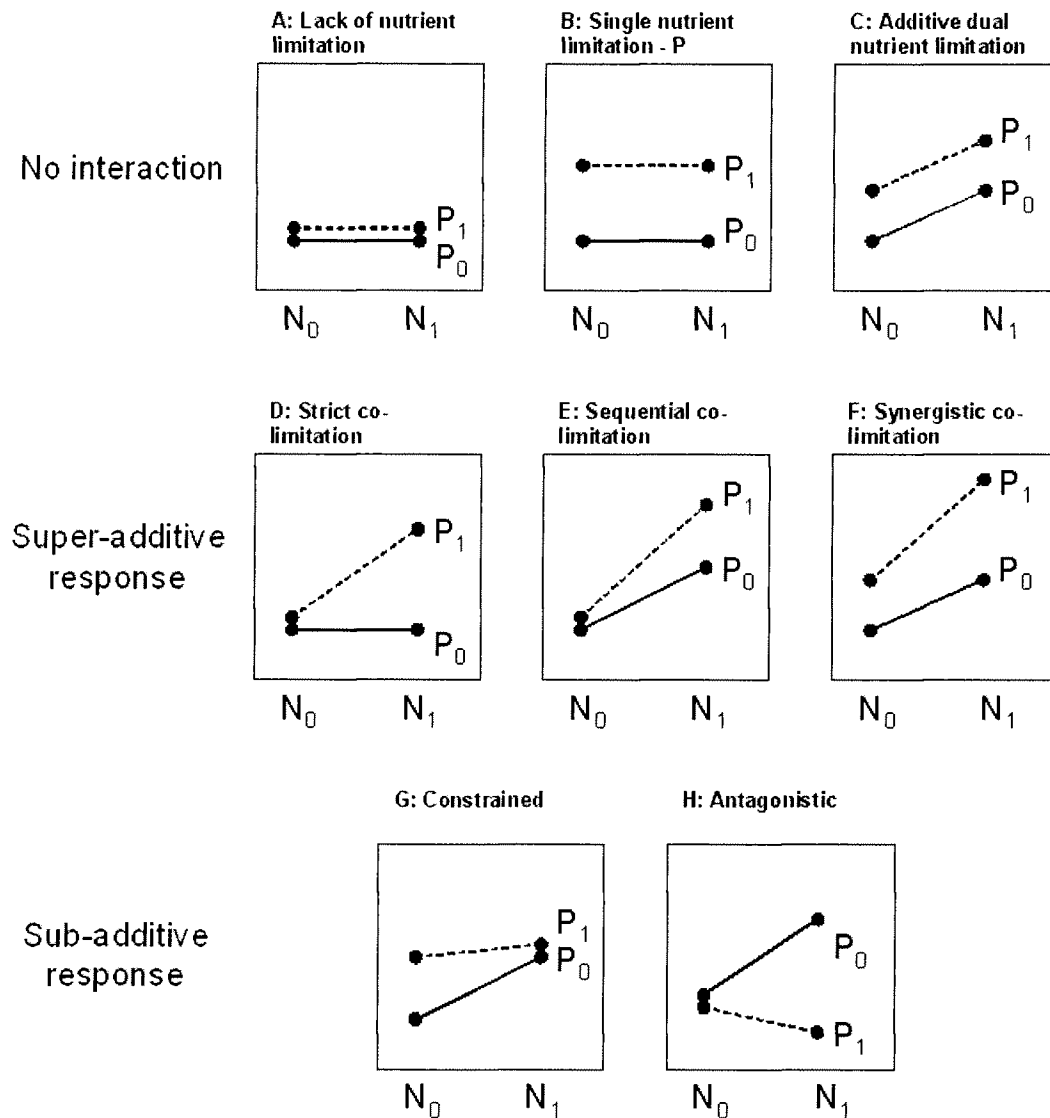


**Table 4.1.** Eight types of resource limitation are defined (columns to the left of vertical line). Categories D, E and F can be distinguished from each other by the number of significant contrasts between single resource treatments and controls (0, 1 and 2 respectively). The distribution amongst these eight categories of 147 factorial fertilization studies from three ecosystem types (freshwater - 55 studies, marine - 55, terrestrial - 37) is shown (columns to right of vertical line). The eight categories are outlined in detail in the text and in Figures 4.1 and 4.2.

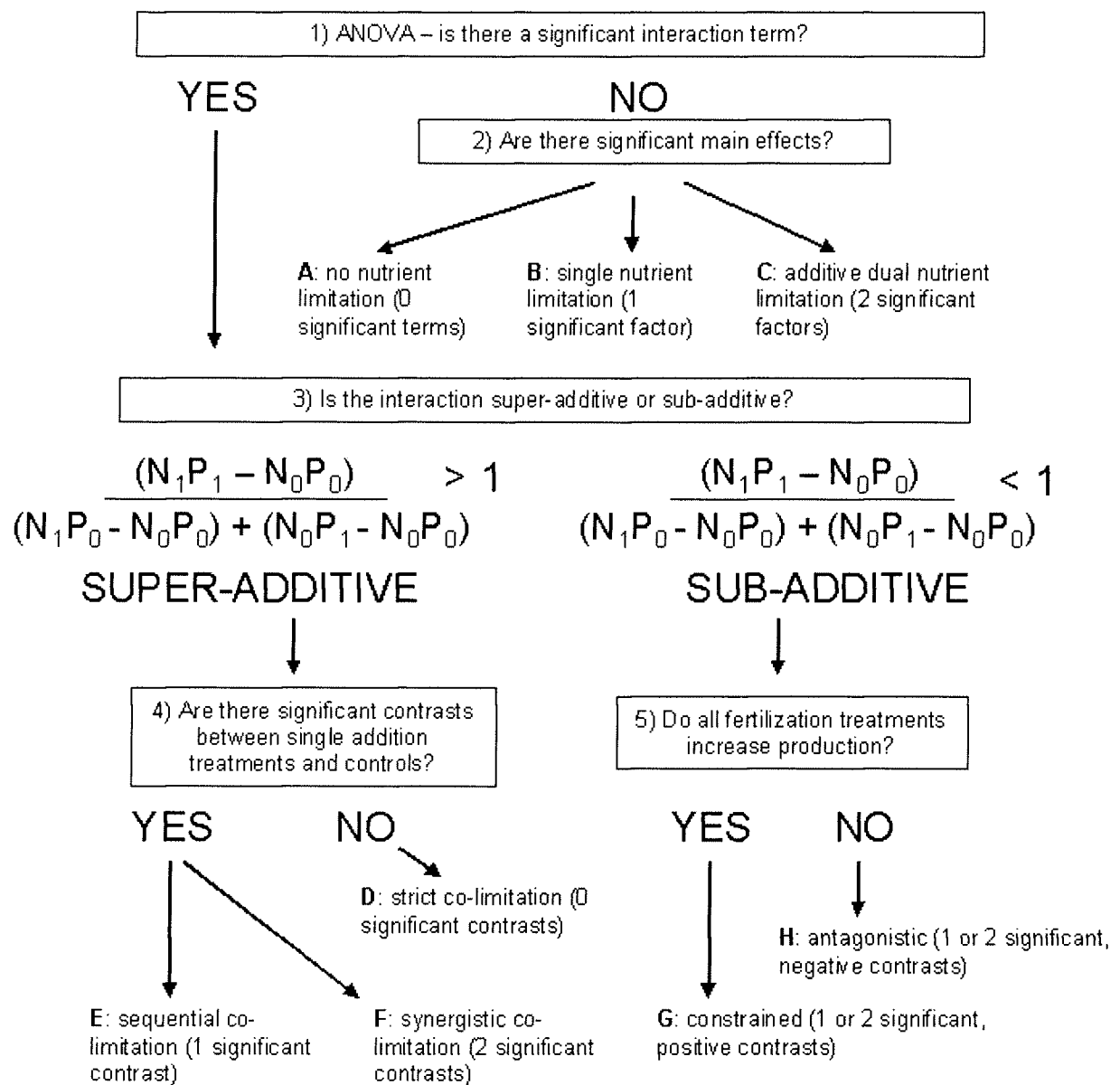
Type of limitation	Significant interaction?	Number of significant main effects	Super or sub additive	Treatment biomass < control?	% of freshwater studies	% of marine studies	% of terrestrial studies
A no limitation	No	0	.	No	20	24	14
B single limitation	No	1	.	No	33	27	30
C additive dual lim.	No	2	.	No	25	13	14
D strict co-lim.	Yes	.	Super	No	2	2	0
E sequential co-lim.	Yes	.	Super	No	13	15	24
F synergistic co-lim.	Yes	.	Super	No	5	18	19
G constrained	Yes	.	Sub	No	2	0	0
H antagonistic	Yes	.	Sub	Yes	0	2	0

**Table 4.2.** Results of t-tests on a factorial meta-analysis testing main effects of nitrogen and phosphorus addition and their interaction in 653 studies. T-tests compare mean response to zero (no response). Results are presented for all studies and for each of three ecosystem types.

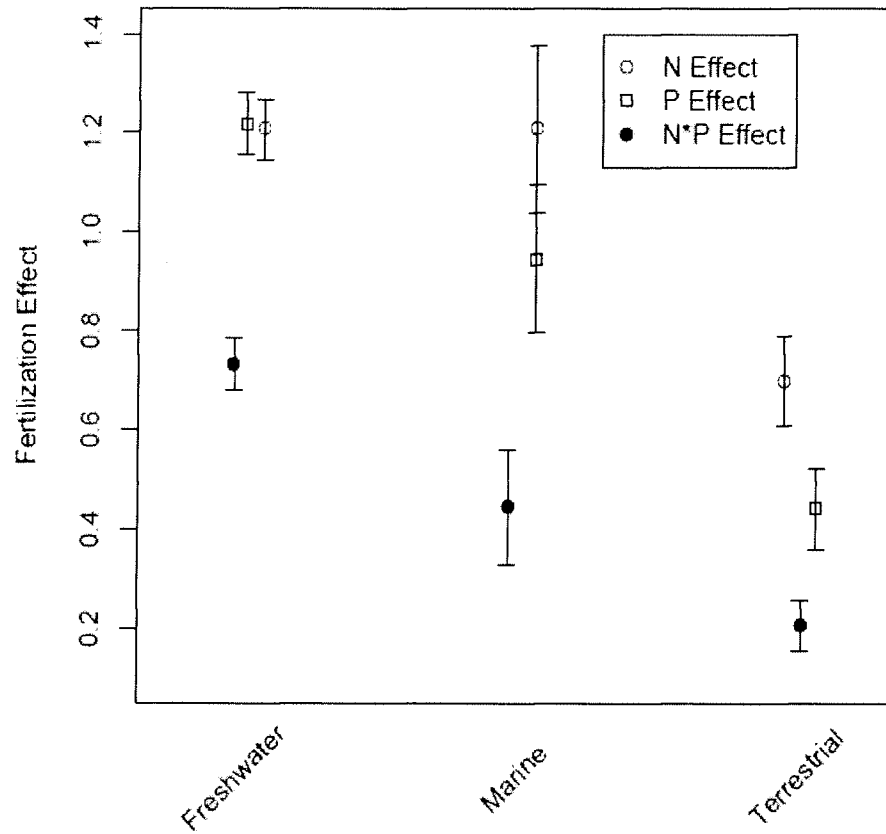
System	Response	df	Mean	t	p (mean $\neq$ 0)
All	N	652	1.147	21.706	< 0.0001
All	P	652	1.082	20.556	< 0.0001
All	NxP	652	0.624	14.302	< 0.0001
Freshwater	N	470	1.207	19.812	< 0.0001
Freshwater	P	470	1.218	19.586	< 0.0001
Freshwater	NxP	470	0.732	13.733	< 0.0001
Marine	N	104	1.207	7.244	< 0.0001
Marine	P	104	0.946	6.323	< 0.0001
Marine	NxP	104	0.445	3.874	< 0.0001
Terrestrial	N	76	0.700	7.820	< 0.0001
Terrestrial	P	76	0.442	5.446	< 0.0001
Terrestrial	NxP	76	0.206	4.095	< 0.0001



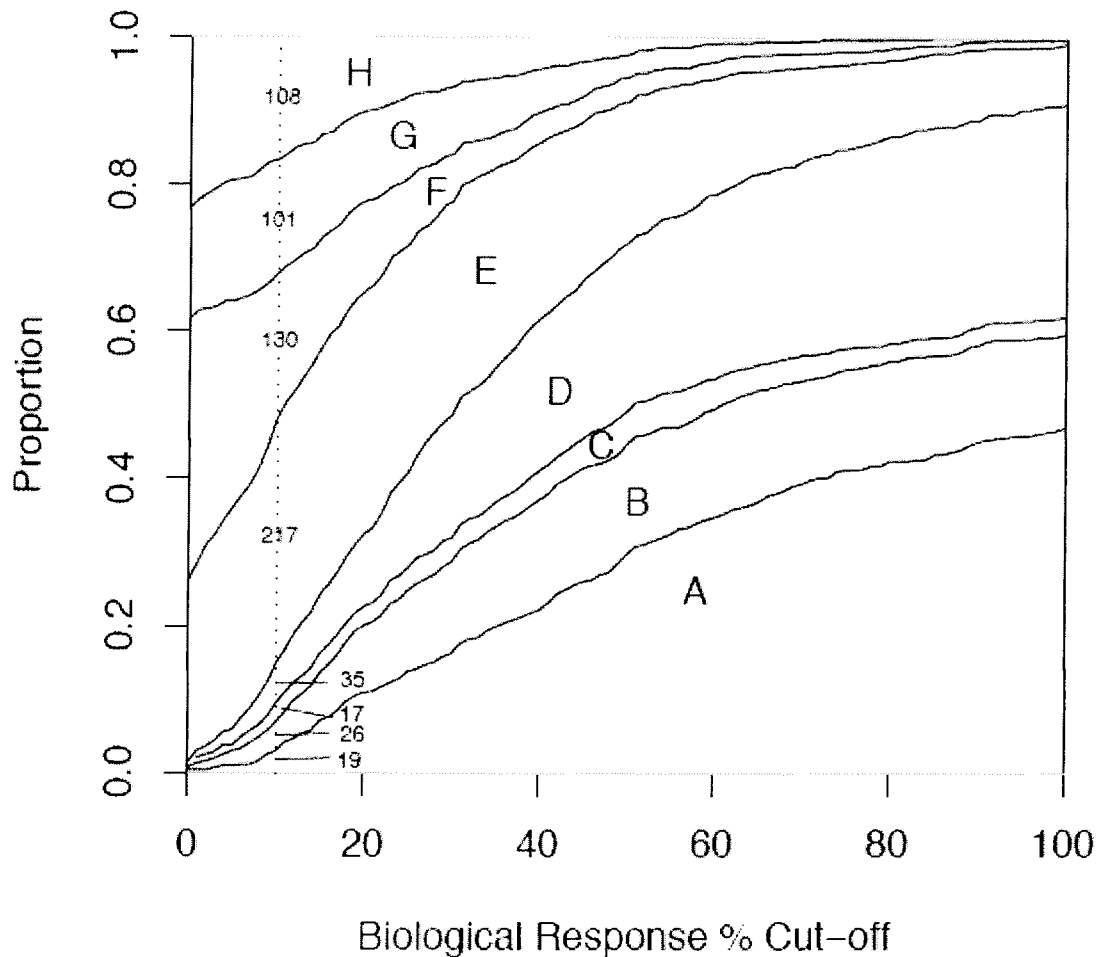
**Figure 4.1.** Types of nutrient limitation and co-limitation by two nutrients, here using nitrogen (N) and phosphorus (P) for the purposes of illustration. Y-axis represents growth, biomass or production responses to resource addition. Subscripts denote treatments, 0 = not added, 1 = nutrient added. Intact lines connect treatments where P is not added, dashed lines connect treatments where P is added.



**Figure 4.2.** Flow diagram of statistical tests to determine the type of nutrient limitation, using input data from a hypothetical experiment with factorial nitrogen and phosphorus fertilization treatments.



**Figure 4.3.** Results of a meta-analysis of 653 factorial fertilization studies. The fertilization effect size (y-axis) is defined in the text. Note that the NP effect tests for the effect of N+P additions above that of fertilization with N alone and P alone (i.e. a super-additive effect). Error bars represent  $\pm 1$  SEM.



**Figure 4.4.** Proportions of response categories as a function of the minimum observed percent biological response required for “significance”. Cumulative proportion of each category that is found using each level of biological response cut-off, from bottom to top: A) No response; B) Single nutrient, C) Two nutrients; D) Strict co-limitation; E) Sequential co-limitation; F) Synergistic co-limitation; G) Constrained; H) Antagonistic. A-C: no interactions; D-F: super-additive interactions; G-H: sub-additive interactions. Vertical dotted line shows arbitrary 10% cut-off for “significant” biological response, with number showing the numbers of studies belonging to each category; total number of studies was 653. See text for category descriptions.

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## **Chapter 5 - General Conclusions**

### **Summary**

It is well established that nutrient supply plays a fundamental part in determining productivity and community composition in ecosystems (Tilman 1985, Aerts & Chapin 2000). In turn, the biotic community can drive nutrient flux, but this role has been less explored in the scientific literature. What is known, however, is that consumers can affect the flow of elements via differences between their tissue stoichiometry and that of their food source (e.g. Elser & Urabe 1999, Daufresne & Loreau 2001, Vanni et al. 2002) or changes in prey quality (e.g. Urabe et al. 2002). Impacts of predation on ecosystem functions, including nutrient cycling, have generally been explored within the context of trophic cascades (e.g. Short & Holomuzki 1992, Vanni & Layne 1997, Vanni et al. 1997, Beard et al. 2002, Ruetz et al. 2002, Grieg & McIntosh 2006) but the effect of the spatial context of these interactions on nutrient cycling remains unclear.

Studies of resource subsidies, however, have found that consumers can transport considerable amounts of resources across ecosystem boundaries, with consequences for the nutrient cycling or productivity within recipient systems (Polis et al. 1997, Anderson & Polis 1999). Consumer biomass, in addition, can serve as an important nutrient subsidy (e.g. Danell et al. 2002). The effects of predators on the distribution of prey biomass, therefore, may constitute an important mechanism of predator impacts on nutrient cycling, beyond the classic direct and trait-mediated indirect effects on prey.

In the first parts of the thesis, I investigated the interaction between consumers and nutrient cycling. I then explored the more general issue of nutrient limitation and the role of multiple resources in limiting autotroph productivity. The main findings of this thesis are that:

#### **Presence of only detritivores does not increase nitrogen uptake by bromeliads**

In the presence of only the detritivore community, there is no significant effect on the flow of nitrogen from plant litter to bromeliad tissue (Chapter 2). This is unexpected because of the facilitative relationship between detritivores and tank-forming bromeliads that has been suggested previously.

### **Predators can increase nutrient cycling by restricting prey emigration**

In the insect-bromeliad system, predators increased nutrient cycling by their prey, a result contrary to the predictions of classical ecological theory. In bromeliads containing only detritivore insects, there was only a modest increase in the amount of leaf litter-derived nitrogen that was released and incorporated into plant tissue. It was only in the presence of the higher trophic level that there was a significant increase in nitrogen flow from detritus to the phytotelm plants (Chapter 2). This is true both in the manipulated insect communities and along a natural gradient of shifting trophic structure (Chapter 3).

These experiments show that the usual effects of trophic cascade can be overwhelmed by a previously undescribed but broadly-applicable mechanism: the effects of predators on the spatial location of prey turnover. In demonstrating that spatial scale affects the relationship between trophic structure and nutrient cycling in a manner not predicted by current ecological theory, our results have important implications for food web and ecosystem ecology. Since predators are globally more threatened than their prey, our results also have important ramifications for conservation.

### **Habitat complexity influences the role of predators in nitrogen cycling**

Increasing habitat complexity, while decreasing the foraging efficiency of both predators and their prey, also affects the vulnerability of predators to intraguild predation. Depending on the relationship between community composition and habitat size, this effect interacts with the changing biotic community to either complement or counteract the impact of predators on the detritivore community, and hence on nitrogen flux in bromeliads (Chapter 3).

The most important implication of these findings is that changes in habitat characteristics interact with trophic structure to determine ecosystem functioning. This is of particular interest because of anthropogenic declines in both habitat size and complexity that are occurring concurrently with high rates of species loss (Vitousek et al. 1997). Predators are especially vulnerable to extinction (Duffy 2002, Duffy 2003, Petchey et al. 2004, Raffaelli 2006), so it is becoming increasingly important to assess how altered habitat variables and predation pressure affects the remaining biotic community.

### **Limitation of primary productivity by multiple resources is common**

In spite of the existing emphasis on single resource limitation of primary productivity (e.g. Hecky & Kilham 1988, Vitousek & Howarth 1991), we found that limitation of autotroph

growth by multiple resources across terrestrial, aquatic and marine ecosystems is more common than previously thought. We suggest that different mechanisms underlie the various resource interactions that we observed and that the nature of the resource interactions provides important information about the biology of the ecosystem. The major contribution of this study (Chapter 3) is to outline a framework for analyzing fertilization studies, with explicit consideration of the possible role of multiple resources.

Even in systems with one primarily limiting resource, the role of other resources is not negligible (e.g. Sterner & Elser 2002). Moreover, the importance of secondary limiting resources in affecting productivity would be expected to increase in the face of rapidly changing nutrient cycles due to human activities. For instance, nitrogen and phosphorus are being added in large quantities to a wide range of systems (Jefferies & Maron 1997, Vitousek et al. 1997, Carpenter et al. 1998). Long considered the primary limiting nutrients on land (Vitousek & Howarth 1991) and in freshwaters (Hecky & Kilham 1988), respectively, additions of these nutrients could shift ecosystems to limitation by other resources. Hence, the interactive effects of multiple nutrients on community and ecosystem ecology are areas of research that bear further exploration.

## **Future directions**

Based on the results outlined in the thesis, it might be interesting to pursue the following questions:

- What imbalance in the spatiotemporal scale of herbivore versus predator movement is required for an effect on the nutrient pools? In Chapter 2, I determined that the role of predators in reducing prey emigration was important in determining the amount of nitrogen being retained by the ecosystem. However, the magnitude of this effect may depend on the scale of herbivore migration relative to that of predators. This question might be best addressed using modeling, to determine how the amount of nutrients in a patch changes when herbivores migrate much more so than predators, and vice versa. A meta-analysis may also be helpful in exploring the role of imbalance in migration rates in determining rates of nutrient cycling and sizes of nutrient pools in different systems.
- What is the role of stoichiometric imbalance in determining the effects of predators on nutrient cycling? The stoichiometric content of body tissue has strong effects on the rates at which an animal excretes nutrients (Vanni et al. 2002). Moreover, the maintenance of

elemental homeostasis means that nutrient excretion should reflect the imbalance in nutrients between an organisms and its food (Sterner & Elser 2002). We might expect, therefore, that predation might have stronger effects on nutrient cycling when prey and predators are stoichiometrically very different. Again, this question might be best addressed using mathematical modeling, either by incorporating an efficiency term or by modeling stoichiometry explicitly. Use of an efficiency term would assume that prey that have very different stoichiometry from their predators would be lower quality food sources.

- When do the facilitative effects outweigh the competitive effects of the insect community in bromeliads? In Chapters 2 and 3, I found that the presence of insects in bromeliad leaf wells increased nitrogen availability for uptake by the phytotelm plants. However, it is unclear whether the insects always play a facilitative role in this system, given that they also require these nutrients. Further work is needed to determine the conditions under which insects might be competing with the plants for resources, with possible negative consequences for plant growth. In particular, high predation rates may increase the rate of nutrient turnover and hence, the facilitative effects of the insect community. Similarly, lower emergence rates might allow more nutrients to remain in the bromeliad wells, with expected benefits for the plants. It may also be interesting to consider the role of the microbial community: if mineralization rates via microbial action are high, the effects of the insect community on nutrient release from litter may be minimal.
- What is the prevalence of intraguild predation in bromeliads? In Chapter 3, I suggest that intraguild antagonism among the predators affects their impact on nutrient cycling. This question could be addressed by determining the natural  $\delta^{15}\text{N}$  signatures of the various members of the bromeliad food web via an extensive survey, and mapping the feeding relationships that exist in this system using dietary mixing models. Fecal analysis may also help to answer this question.
- What is the role of habitat structure in determining rates of intraguild predation? Similar to the set-up of the experiments described in Srivastava (2006), the effects of habitat complexity could be explored experimentally to determine its effects on intraguild predation in damselflies or tabanids. This could be crossed with a resource level treatment to assess how prey density might modify this relationship.
- What is the relative importance of cannibalism versus interference competition in the intraguild interactions among the predators in this system? It is unclear the extent to

which direct as opposed to trait-mediated effects among predators determine the magnitude of their impact on detritivores and nitrogen cycling. However, odonates, for instance, are known to reduce foraging activity in the presence of predators (Stocks & McPeck 2003, Brodin & Johansson 2004), while cannibalism can be a substantial source of mortality for both odonates and tabanids (Meany et al. 1976, Benke 1978, Polis 1981, Johnson et al. 1985). Similar to the experiments of Schmitz et al. (1997), risk and predation treatments could be created to disentangle the effects of behaviour and direct consumption.

- Based on the distribution of the different categories of nutrient limitation in terrestrial, freshwater and marine ecosystems, it may be possible to more closely examine the mechanisms responsible for restricting autotroph productivity in the different systems. In Chapter 4, a number of possible mechanisms are outlined, but their relative importance in different systems is unknown.

## **General implications**

These studies focus on relatively neglected aspects of two well-studied ecological processes. Both the consumer effects on nutrient cycling and resource limitation of productivity are expected to be important in the context of current environmental change, and a wider range of possible effects and mechanisms, as well as the habitat context of these changes, needs to be considered if predictions are to be made about shifts in ecosystem functioning. Nutrient limitation, in particular, needs to be examined more systematically as the importance of multiple resources for ecological communities is becoming increasingly clear (e.g. Sunding et al. 2005, Bergstrom & Jansson 2006, Harpole & Tilman 2007, Clark & Tilman 2008). Conservation and management of habitats and species may depend expanding our understanding of the roles of nutrients and predators beyond the paradigm of single resource limitation or classic trophic cascades.



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