

Regulation of community structure:
top-down effects are modified by omnivory,
nutrients, stress, and habitat complexity

An Abstract of a Dissertation

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Juan Manuel Jiménez Martínez

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Abstract

Biological communities are structured by a complex set of processes that have hindered the development of a general theory of community structure regulation. Current ideas and hypotheses have largely ignored the presence of interactions among the factors that regulate community structure. In this thesis I focused explicitly on the interactions among omnivory, nutrient availability, salinity stress levels, and habitat complexity. I examined these interactions using a combination of mesocosm and field factorial experiments in which I manipulated the levels of nutrients, salinity stress, and the presence (or absence) of top omnivores and common herbivores. These experiments demonstrated that, (1), nutrient addition increases the strength of top-down forces, but the effects of salinity stress are variable and species dependent, and (2), decreasing habitat complexity increases the strength of top-down forces and trophic cascades. In sum, my results suggest that the inherent complexity of natural communities and the interactions among the forces structuring them can lead to many different experimental outcomes that reflect the many ways in which species interactions are affected by environmental variables to alter the structure of these communities. Progress in understanding community structure will come from experiments that probe these interactive processes and seek to identify commonalities in responses based on consumer behavior, plant traits or habitat type.

GENERAL INTRODUCTION

Because food webs are structured by both a variety of top-down (predation), and bottom-up (plant quality and productivity) factors, obtaining a general theory of community structure has proven to be a complicated task. If we consider a simple food web with three trophic levels, top-down effects are those changes in the behavior and abundance of herbivores caused by predators, which may then cascade down through the food web until they affect primary producers. Bottom-up effects are those changes in the productivity, nutrient content, physiology and/or physical structure of primary producers caused by the environment, which may then cascade up from the plants through the food web, finally affecting the top predators. Both top-down and bottom-up effects can change the abundance or diversity of different trophic levels in a community.

Despite more than 19,000 papers published on the subject (Web of Science: community structure as topic search), we have not yet achieved a consensus about how top-down and bottom-up forces combine to structure biological communities (Hunter and Price 1992). Recent synthesis studies have shown that the strength of top-down effects and trophic cascades from predators vary greatly within and across ecosystems (Shurin et al. 2002); top-down effects on herbivores are large on all ecosystems but top-down effects on plants are larger on aquatic ecosystems. They have also shown that in aquatic ecosystems herbivores have a stronger bottom-up control than organisms in higher trophic levels (Brett and Goldman 1996, 1997). One likely reason for this lack of

consensus is that there are interactions among factors mediating top-down and bottom-up effects (Hunter and Price 1992). Four of the many factors that might regulate the relative strength of top-down and bottom-up effects are the presence of omnivores, nutrient availability, environmental stress, and habitat complexity. Although many studies have examined these factors in isolation, few have explored their interactions.

The relative strength of top-down forces may be regulated by the species diversity and food web complexity of a community. This hypothesis states that simpler food webs with a less diverse predator and herbivore community will have stronger top-down trophic cascades (Strong 1992, Polis and Strong 1996b). Increasing the complexity of the food web, by either increasing the number of species interacting or by including species that eat at multiple trophic levels (i.e. omnivores), will attenuate or stop the top-down effects through a diverse network of reticulate interactions (Polis 1991, Polis and Strong 1996b). Finke and Denno (2004), for example found that increasing the diversity of the predator guild dampened the strength of the top-down control. Few studies, however, have revealed the presence of trophic cascades in highly specious and complex systems such as tropical forests (Letourneau and Dyer 1998, Pace et al. 1999). Letourneau and Dyer (1998) showed that Strong top-down trophic cascades can be found on diverse systems, such as tropical rain forests, where they documented a trophic cascade in which the presence of a specialists predaceous beetle on ants released several species of herbivores from the ants top-down control and allowed them to reduce leaf tree area by half.

The levels of primary productivity may also influence the relative strength of the top-down factors. The ecosystem exploitation hypothesis proposes that, in systems with high nutrient input to primary producers, top-down effects from predators are expected to be stronger than on systems with low nutrient input; more productive systems will host a more diverse and abundant herbivore guild and, thus, a more diverse and abundant predator guild which will produce a stronger top-down control of the community (Oksanen et al. 1981, Hunter and Price 1992). Stenborg et al. (2005) found the top-down control of a Lepidoptera larvae and its damage on a boreal forest understory by birds was higher in fertilized plots. Other studies, however, have shown that this is not always the case (Forkner and Hunter 2000, Ritchie 2000, Chase 2003b) and that contrary to the predictions of this hypothesis, trophic cascades can be weak or non-existent in highly productive systems and strong in unproductive systems. For example, Chase (2003b) found strong top-down trophic cascades in ponds with low primary productivity and weak or non-existent trophic cascades in ponds with high primary productivity. Thus, support for this hypothesis is also equivocal.

We can expect interactions between food web complexity and primary productivity levels, in particular when omnivores are present, because nitrogen (nutrient) limitation is thought to mediate omnivory (Fagan et al. 2002). Omnivory might either enhance or attenuate top-down effects because eating at multiple trophic levels can either allow omnivores to maintain their populations at constant levels when preferred prey are scarce, or dissipate the omnivores' top-down effects among a complicated network of food-web interactions (Polis and Strong

1996b, Eubanks and Styrsky 2005). Increasing nutrient input can strengthen top-down effects from top consumers (Fretwell 1977, Oksanen et al. 1981) such as top omnivores. Increasing nutrient input into a system can increase the energetic yield of plants and herbivore density and, hypothetically, this could allow the omnivore to switch to a diet consisting only of herbivores (therefore increasing their top-down effect on herbivores) if plant and prey were substitutable diet choices for the omnivore (Van-Rijn and Sabelis 2005). That is, if the omnivore can completely exclude or greatly reduce the amount of plant material in its diet, then the strength of top-down effects from the omnivore on its herbivorous prey will increase.

The levels of environmental stress are also thought to affect the relative strength of top-down and bottom-up factors. The environmental stress hypothesis proposes that increasing levels of stress will strengthen bottom-up effects and weaken top-down effects (Connell 1975, Menge and Sutherland 1976) because as environmental stress increases the number of food web levels and the complexity of the food web decrease. Only a handful of studies have evaluated the effects of environmental stress on community structure (Moon and Stiling 2000, Moon and Stiling 2002a, Moon and Stiling 2004), with the general result that increasing environmental stress decreases top-down control of herbivores by predators and parasitoids. However, increasing nutrient availability can dampen the negative effects of environmental (salinity) stress on top-down control of communities (Moon and Stiling 2000).

The habitat complexity hypothesis proposes that increasing habitat complexity will either increase or decrease herbivore abundance, depending on how habitat complexity affects predator-prey interactions. If structure reduces intraguild predation (Finke and Denno 2002, Grabowski 2004, Langellotto and Denno 2004), it will tend to increase predator diversity and abundance. Increased predator abundance may then reduce herbivore abundance; alternatively, herbivore densities may increase if structure also provides refuges for prey (Warfe and Barmuta 2004). As far as I know, only one study has explored the *interactive* effects of nutrients and habitat complexity on the relative strength of top-down and bottom-up forces (Denno et al. 2002), finding that increased habitat complexity promoted top-down control of herbivorous planthoppers by spiders; however, increasing nutrient availability to plants eventually led to an increase in planthoppers which overwhelmed top-down effects of spiders.

Finally, based on bottom-up theory, a different set of hypotheses has been used to try to explain, in particular, when and how herbivore population outbreaks and attack on plants happen. When plants are exposed to optimal conditions, such as adequate levels of nutrients or water, the plant vigor hypothesis proposes that these faster growing and more palatable plants should support higher levels of herbivores than less vigorous plants (Price 1991). Alternatively, the plant stress hypothesis proposes that when plants are exposed to medium levels of stress, such as water stress, plants can react by increasing the concentration of more soluble nitrogenous molecules in their tissues, making

plants more palatable to herbivores; these conditions are also expected to lead to herbivore populations outbreaks (White 1969). Because in nature plants are exposed to several environmental gradients at the same time, each one of them potentially affecting plant vigor and stress, it is important to understand how different environmental gradients interact to affect the susceptibility of plants to herbivores.

The interactive effects of omnivory, nutrients, stress and habitat complexity on community structure are particularly important to understand in coastal ecosystems such as salt marshes. A number of important consumers in salt marshes are omnivores (Buck et al. 2003, Ho and Pennings 2008). Salt marshes contain natural gradients of salinity and nutrient availability (Bertness and Pennings 2000) that interact to mediate plant productivity and species composition. Moreover, these gradients are changing as growing human populations in coastal regions affect estuarine habitats by increasing eutrophication (Valiela et al. 1992, Nixon 1995) and changing hydrological and salinity regimes (Copeland 1966, Hoese 1967, Alber 2002). Although a number of studies have addressed how these anthropogenic impacts affect emergent wetland plants (Jefferies and Perkins 1977, Crain et al. 2004, Pennings et al. 2005), little is known about how they mediate food web interactions. Humans are also altering habitat complexity in salt marshes by building structures on or near marshes, by promoting the accumulation of trash and debris amid high-marsh vegetation, and by landscaping the terrestrial borders of saltmarshes.

In order to understand how omnivory, nutrients, environmental stress, and habitat complexity interact to affect the regulation of communities, I performed two mesocosm experiments (chapters 1 and 2) and two field experiments (chapters 3 and 4) using food webs common in salt marsh habitats.

In chapter 2 I describe a mesocosm experiment where I manipulated nutrients and salinity stress levels as well as the presence of a common omnivorous grasshopper and a common herbivorous planthopper on the cordgrass *Spartina alterniflora* to explore their interactive effects on community structure. In chapter 3 I describe a mesocosm experiment where I used a similar design as in chapter 2, but instead focused on the food web of the *Iva frutescens* shrub. I again manipulated nutrient and salinity stress levels and the presence of a common omnivore, but increased the complexity of the food web by including two common herbivores on *Iva*. As in chapter two, this set-up was used to explore the interactive effects of these factors on community structure. In chapter 4 I describe a field experiment where I focused on exploring the interactive effects of nutrient and salinity stress levels on the abundance of a third common herbivore and its damage to *Iva*. Finally, in chapter 5 I describe a field experiment where I manipulated nutrients, salinity stress, and habitat complexity levels in order to understand their interactive effects on the arthropod community living on *Iva frutescens*.

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CHAPTER 2

An omnivore's trophic cascade is independent of bottom-up salinity and nutrient conditions

Abstract

Despite intense research there are still no general rules as to how the structure of communities is mediated. Because of the complexity of the processes regulating community structure, there may be interactions among factors mediating top-down and bottom-up effects. I used a mesocosm study to examine how salinity stress (control and addition), nutrient levels (control and addition) and the presence (or absence) of a top omnivore (grasshopper *Orchelimum fidicinium*) and a common herbivore (planthopper *Prokelisia* sp.) interacted to alter top-down and bottom-up effects in a salt marsh trophic system. I hypothesized that nutrients would strengthen top-down forces but salinity stress would reduce the effects of nutrients on top-down forces. Both salinity and nutrient addition decreased leaf C:N and increased *Prokelisia* abundance. Additionally, both the herbivores and the omnivore reduced *Spartina alterniflora* (cordgrass) growth, but top-down effects from *Prokelisia* planthoppers and the omnivore *Orchelimum fidicinium* grasshopper on *Spartina alterniflora* were mediated by bottom-up conditions; i.e., *Orchelimum* grasshoppers reduced plant growth more when nutrients were added. In contrast, the top-down control by the omnivorous grasshopper of *Prokelisia* planthopper populations and their damage to *Spartina alterniflora* was independent of bottom-up conditions. Further trophic cascades involving other plant traits such as plant growth were masked by the omnivore because its plant consumption did not decrease in the presence of herbivores suggesting that *Orchelimum*'s diet is not flexible. These results

indicate the importance of knowing food preferences of omnivores in order to understand the effects of omnivores on community structure and illustrate that top-down effects on plants from consumers are likely to depend on both plant stress and nutrient status. Because a wide variety of effects are possible within a single, simple system, the complexity of these interactions caution against overly-simplistic generalizations about trophic controls in natural systems.

Introduction

Because food webs are structured by both a variety of top-down (predation), and bottom-up (plant quality and productivity) factors, obtaining a general theory of community structure is a complicated task. Both top-down and bottom-up effects can change the abundance or diversity of different trophic levels in a community. Despite intense research on several of these processes, I have not yet achieved a consensus about how they combine to structure biological communities (Hunter and Price 1992, Power 1992, Strong 1992). One likely reason for the lack of consistent results is that there are interactions among factors mediating top-down and bottom-up effects (Hunter and Price 1992). Three factors that might regulate the relative strength of top-down and bottom-up effects are the presence of omnivores, nutrient availability, and environmental stress. Although many studies have examined these factors in isolation, few have explored their interactions.

Omnivory might either enhance or attenuate top-down effects (Polis and Strong 1996a, Eubanks 2005, Eubanks and Styrsky 2005). Omnivory could enhance top-down effects because omnivores might be able to sustain their population levels when prey are scarce by eating at multiple trophic levels (Dayton 1984, Eubanks and Denno 2000, Ho and Pennings In Press). On the other hand, omnivory might also attenuate top-down effects because omnivores might become satiated faster by eating at multiple trophic levels, and therefore reduce their per-capita consumption of any particular prey (Eubanks and Denno 1999, Eubanks and Denno 2000). Despite the commonness of omnivory and its

potentially important effects on communities (Polis 1991), only a handful of experimental studies have explored the effects of true omnivory (eating at more than one trophic level including primary producers) on the strength of top-down forces in terrestrial habitats (Agrawal et al. 1999). Although the effects of omnivory on trophic cascades have received some attention in aquatic systems (Lodge et al. 1994, Pringle and Hamazaki 1998), interactions may be different in terrestrial environments (Polis 1991, Strong 1992, Shurin et al. 2002). Moreover, because variation in plant quality is likely to affect whether an omnivore prefers to feed on plants versus higher trophic levels, the impact of omnivores on food webs is likely to be contingent upon bottom-up factors that affect plant quality. Two well-known hypotheses discuss these factors.

The “Ecosystem Exploitation Hypothesis” (hereafter EEH) states that top-down effects from predators will be stronger with increasing nutrient input (Fretwell 1977, Oksanen et al. 1981). Recent studies, however, have shown that this is not always the case, and that trophic cascades can instead be weak in productive systems and strong in unproductive systems (Ritchie 2000, Chase 2003a). This counterintuitive result could be a consequence of interactions between nutrients and other factors, such as omnivory, that can potentially regulate the impact of nutrients on top-down forces. Because nitrogen limitation is thought to mediate omnivory (Eubanks and Denno 1999, Eubanks and Denno 2000, Fagan et al. 2002), intraguild predation and cannibalism (Polis and Strong 1996a, Denno and Fagan 2003, Matsumura et al. 2004), it is important to study their interactive effects on community structure.

The environmental stress hypothesis proposes that increasing levels of stress will strengthen bottom-up effects and weaken top-down effects (Connell 1975, Menge and Sutherland 1976, Menge and Sutherland 1987). Only a handful of studies have evaluated the effects of environmental stress on community structure (Moon and Stiling 2000, Moon and Stiling 2002a, Moon and Stiling 2004), with the general result that increasing environmental stress decreases top-down control of herbivores by predators and parasitoids. However, interactions with other factors such as nutrient levels can alter this result. In particular, increasing nutrient availability can ameliorate the negative effects of salinity stress on top-down control of herbivores (Moon and Stiling 2000). It is important to note that the salt marsh system studied by Moon and Stiling, as in the system studied here, the salinity stress treatment directly affected plants, but the herbivores and the parasitoid (the omnivore in our case) were not affected directly by salinity, but indirectly through changes in plant quality, plant physiology and plant physical structure (Moon and Stiling 2000, Moon and Stiling 2002a, Moon and Stiling 2004). To our knowledge no study has explored three-way interactions among omnivory, nutrients and stress on the relative strength of top-down and bottom-up forces.

The interactive effects of omnivory, nutrients and stress on community structure are particularly important to understand in coastal ecosystems such as salt marshes. A number of important consumers in salt marshes are omnivores (Buck et al. 2003, Ho and Pennings In Press). Salt marshes contain natural gradients of salinity and nutrient availability (Bertness and Pennings 2000) that

interact to mediate plant productivity and species composition. Moreover, these gradients are changing as growing human populations in coastal regions affect estuarine habitats by increasing eutrophication (Valiela et al. 1992, Nixon 1995) and changing hydrological and salinity regimes (Copeland 1966, Hoese 1967, Alber 2002). Although a number of studies have addressed how these anthropogenic impacts affect emergent wetland plants (Jefferies and Perkins 1977, Crain et al. 2004, Pennings et al. 2005), little is known about how they mediate food web interactions.

To examine the mechanisms regulating the relative roles of top-down and bottom-up factors and their interactions, I studied a food web consisting of a common salt marsh grass, *Spartina alterniflora* Loisel, the herbivores *Prokelisia marginata* Van Duzee and *P. dolus* Wilson, and the top omnivore *Orchelimum fidicinium* Rehn and Hebard (Plate 1). *Spartina alterniflora* (hereafter, *Spartina*) is the most common salt marsh plant on the Atlantic and Gulf coasts of the United States, typically dominating lower and middle marsh elevations (Adams 1963, Aberle 1990). *Spartina* stands are exposed to wide gradients of nutrient availability, flooding and salinity. These gradients are mostly responsible for the occurrence of different height forms of *Spartina alterniflora* (Gallagher et al. 1988), with a short-form (10 - 40 cm in height) occupying the middle marsh and a tall-form (1 – 2 m in height) with a higher nitrogen content occupying the lower marsh (Valiela et al. 1978, Richards et al. 2005).

The most common herbivores of *Spartina* are the delphacid planthoppers *Prokelisia marginata* and *P. dolus* (Denno et al. 1987). *Prokelisia marginata* and *P. dolus* (hereafter, *Prokelisia*) are multivoltine, with a summer generation time of 5-6 weeks (Denno et al. 1985, Stiling et al. 1991). They are phloem sap-feeders (Denno et al. 1987) that can reach densities of up to 1,000 adults and 100,000 nymphs per m² (Denno et al. 2000).

The most common true omnivore in the *Spartina* zone of southeastern salt marshes is the tettigoniid grasshopper *Orchelimum fidicinium* (Smalley 1960, Wason and Pennings In press). Although historically regarded as an herbivore (Teal 1962), *O. fidicinium* (hereafter, *Orchelimum*), like most tettigoniids, is omnivorous, and readily eats small arthropods; *Orchelimum* grasshoppers eat *Prokelisia* planthoppers at an average rate of 7.8 individuals per day (SE: 1.4; N = 10) when enclosed in a 1L jar for 48 hours (Wason and Pennings personal observations). *Orchelimum* is univoltine, with adult densities reaching 9.6 individuals per m² (Smalley 1960, Stiling et al. 1991).

In mesocosm experiments, I manipulated nutrient availability, soil salinity, and the presence of herbivores and top omnivores in a factorial design. Because *Orchelimum* is omnivorous, eating both plants and herbivores, I hypothesized that it would have both direct negative and indirect positive effects on plants, leading to weak net effects. I hypothesized that nutrient addition would directly increase *Prokelisia* herbivore populations, but would also mediate top-down control of *Prokelisia* by *Orchelimum* by increasing the nutrient contents of plants and increasing herbivore abundance. I hypothesized that salinity would decrease

Prokelisia populations and again mediate top-down control of *Prokelisia* by *Orchelimum* by altering plant quality and herbivore abundance. I further hypothesized that salinity stress would decrease the positive effects of nutrients on *Prokelisia* populations, and mediate the effects of nutrient addition on top-down control of *Prokelisia*.

Methods

Experimental work was done in mesocosms at the University of Georgia Marine Institute on Sapelo Island, GA (81.2699°W, 31.3929°N). Two hundred *Spartina* plants of intermediate height (ca. 120 cm tall) were collected from the Dean Creek salt marsh at the southern end of the island in late June, 2005, and potted in 3.5 L plastic pots using a 50/50 mixture of potting soil and sand. After 20 d, the 112 healthiest plants were randomly assigned to four combinations of nutrients (ambient, fertilized) and salinity (low, high), and acclimated for seven weeks (7 x 12 treatments = 112). Nutrient and salinity treatments were initiated 45 days prior to the start of the experiment with a low dose (5 g) of nitrogen-based fertilizer (20:10:5 N:P:K fertilizer pellets, Forestry Suppliers) and a low salinity concentration (10 ppt). Nutrient additions were progressively increased every two weeks to a final level of 21 g per month. The salinity treatment was progressively increased every two weeks to a final level of 25 ppt. Plants were watered daily with fresh or saline water and allowed to drain fully. The nutrient

and salinity additions were sufficient to affect plant traits and herbivore abundances (see Results).

Prokelisia and *Orchelimum* were captured at several salt marsh sites on Sapelo Island. To measure their individual and combined effects, I set up an omnivore treatment (present/absent) and an herbivore treatment (present/absent) and I combined them in a factorial design with the nutrient and salinity treatments. Treatments including *Orchelimum* contained a single grasshopper; plants were checked daily and grasshoppers that died were replaced. Treatments including *Prokelisia* were initiated with 30 nymphs and supplemented weekly with 40 adults to represent immigrants. Examination of a subset of the individuals indicated that I stocked ca. 80% *P. marginata* and 20% *P. dolus*. Plants and arthropods were enclosed in a 1.4 m high cage made of fine cloth mesh. Mesocosms were located outdoors under a plastic roof that sheltered them from direct rain.

Once arthropods were stocked, the experiment ran for one month (August 26 to September 27, 2005). At the end of the experiment I counted all the arthropods present on the plants. I measured several plant traits at the beginning and end of the experiment. As indicators of plant growth and herbivory, I measured plant height and the number of healthy and damaged leaves, scoring damage from both *Prokelisa* (visible as yellow spots against the normal dark green leaves) and *Orchelimum* (visible as missing leaf material). I calculated plant growth as the difference between the initial and the final plant height and

used it in the analysis instead of final plant height. As indicators of leaf quality, I measured chlorophyll content using an optical meter (Opti-Sciences CCM-200 chlorophyll meter) and leaf toughness using a penetrometer (balance model 516, Chatillon N.Y.) at the end of the experiment. Leaf chlorophyll content was negatively correlated with leaf C:N ratio ($n=80$, $r= 0.61$, $P< 0.0001$), based on a subset of leaves collected at the end of the experiment (Perkin-Elmer 2400 CHN analyzer). Because I fixed *Orchelimum* density at one individual per cage, replacing individuals that died, I did not examine bottom-up effects on *Orchelimum*. Data were analyzed with four-way ANOVA. Nutrients, salinity, herbivore addition, and omnivore addition were treated as fixed effects. C:N, chlorophyll content and *Prokelisia* abundance were log-transformed to meet assumptions of normality and homoscedasticity.

Results

Both nutrient addition and salinity stress had strong bottom-up effects. Nutrient addition significantly increased *Spartina* leaf chlorophyll and nitrogen content (Fig. 1a, b) and decreased leaf toughness, but leaf toughness only when *Orchelimum* was present (Fig. 1c). Either salinity stress or nutrient addition alone increased *Prokelisia* abundance by 75 or 76% (Fig. 1d) and decreased leaf C:N by 17 or 36% (Fig. 1e); however, when both salt and nutrients were added, leaf C:N did not decrease much further, and *Prokelisia* abundance did not increase further. Overall, nutrient addition decreased the number of leaves damaged by *Prokelisia* (Fig. 1f). Across all treatments with *Prokelisia*, the number of leaves

damaged by *Prokelisia* decreased with increasing chlorophyll content (Fig. 1g), suggesting that *Prokelisia* needed to feed less extensively on nutrient-rich plants. There was no interaction between salinity and nutrients for chlorophyll content, *Prokelisia* damage, or leaf toughness (Table 1).

Both *Prokelisia* and *Orchelimum* had strong top-down effects on plants. Also, *Orchelimum* had a strong top-down effect on *Prokelisia*. However only the top-down effects on plants were mediated by bottom-up conditions. *Prokelisia* reduced leaf toughness (Fig. 2a) and chlorophyll content (Fig. 1a) and increased C:N ratio (Fig. 1b); however, leaf chlorophyll content and C:N ratio were only affected by *Prokelisia* when nutrients were added. *Prokelisia* reduced plant growth but only when nutrients and salt were added (Fig. 2b). *Orchelimum* increased leaf toughness only in unfertilized treatments (Fig. 1c). Fertilization decreased all C:N ratios, but the largest fertilization effect occurred when *Orchelimum* was absent and salt was not added (Fig. 2c). Additionally, *Orchelimum* reduced plant growth more when nutrients were added (Fig. 2d). I do not show the *Orchelimum* damage results because all the leaves on *Spartina* plants were damaged when the grasshopper was present.

Orchelimum reduced *Prokelisia* abundance by about 50% (Fig. 3a) and thereby reduced the number of leaves damaged by *Prokelisia* by 25% (Fig. 3b). Top-down control of *Prokelisia* populations and the trophic cascade on the number of leaves damaged by *Prokelisia* were strong regardless of bottom-up

conditions, as shown by the absence of significant bottom-up x top-down interaction effects on table 1 for these two variables (Table 1).

Discussion

Although bottom-up effects in this experiment were strong, top-down effects at the herbivore level did not depend on bottom-up conditions. The omnivorous grasshopper produced a trophic cascade on the number of leaves damaged by *Prokelisia* planthoppers, but further trophic cascades on other plant traits were masked by the low flexibility of the omnivore's diet.

Nitrogen addition increased leaf chlorophyll content, decreased leaf C:N and toughness, and increased *Prokelisia* abundance. These effects are consistent with the E.E.H. (Fretwell 1977, Oksanen et al. 1981), and are similar to those that have been found in other salt marsh studies (Moon and Stiling 2000, Denno et al. 2002, Gratton and Denno 2003).

Salinity addition decreased leaf C:N and increased *Prokelisia* abundance. This result is consistent with the plant stress hypothesis, which predicts that environmental stresses to plants (water stress in particular) decrease resistance to insect herbivory by mobilizing nutrients and otherwise altering foliar chemistry (White 1969, White 1984, Mattson and Haack 1987). Increments in soluble nitrogen-rich molecules in the plant tissues as a response to the lower osmotic pressure of salty soils are well known in salt marsh plants which experience soils with low osmotic pressure that make it difficult for plants to absorb water and nutrients (Morris 1984). Huberty and Denno (2004), however, argued that

positive effects on sap-feeding arthropods from osmotic stress should only occur if the stress was pulsed as opposed to continuous, because only a pulsed stress would allow for turgor pressure in the plant to increase again such that sap-feeding arthropods could profit from the elevated nutrient concentrations produced by the water stress. Given that the high salinity treatment in our experiment (25 ppt) was well within the levels that *Spartina* can tolerate (Richards et al. 2005), it would be fair to say that our “high” salinity treatment was at best only a moderate stress and thus might have allowed *Prokelisia* to profit from the increased nutrient content of the plants. Moon and Stiling (2005) also obtained positive effects of salinity stress on a sap-feeding planthopper (*Pissonotus quadripustulatus*, Homoptera: delphacidae) but only at intermediate levels of salinity stress (ca. 10 ppt); at higher levels of salinity stress (ca. 14 ppt) the effects of salinity on plant traits and planthopper abundance became negative.

These positive effects of salinity on plant nutrient content and *Prokelisia* abundance are inconsistent with the environmental stress hypothesis (Connell 1975, Menge and Sutherland 1976, Menge and Sutherland 1987), which predicts that the effects of stress will be more severe on consumers than on producers, reducing the top-down control of the community. Based on past findings, I expected salinity to decrease *Prokelisia* abundance by reducing plant nutrient absorption (Smart and Barko 1980, Feller 1995), and increasing toughness (Moon et al. 2000). It is possible that at low to medium levels of salinity stress that are well within the range of plant tolerance plant-herbivore interactions in salt

marshes are better explained by the plant stress hypothesis, but that at high levels of salinity stress that severely stress plants they are better explained by the environmental stress hypothesis.

Because both nitrogen and salinity had positive effects on plant nitrogen content and *Prokelisia* abundance, one might expect that the combination of both factors would increase plant nitrogen and planthopper abundance even further, but this did not occur. Moon and Stiling (2000) and Stiling and Moon (2005) obtained a similar result for *Pissonotus quadripustulatus* feeding on the shrub *Borrichia* and proposed two possible explanations for this less than additive interaction. First, increments in soil interstitial salinity could have precluded plants from utilizing the extra available nitrogen. Second, increments in soil nitrogen content may have ameliorated plant stress, therefore decreasing the stress-related production of nitrogenous compounds. Whatever the mechanism, the fact that nitrogen and salinity have less than additive effects on herbivores is important, because it means that the effects of nitrogen addition on herbivores cannot be predicted without also considering the salinity regime experienced by the plants.

The omnivorous grasshopper *Orchelimum fidicinium* produced a strong top-down trophic cascade in this experiment. *Orchelimum* reduced *Prokelisia* abundance, which in turn reduced the number of leaves damaged by *Prokelisia*. However, although *Prokelisia* affected leaf chlorophyll content and toughness, it did not affect plant growth, and thus the beneficial effect of *Orchelimum* on *Spartina* (reduced leaf damage from *Prokelisia*) did not translate into a growth

benefit. In general, it has proven easier to demonstrate trophic cascades that affect herbivore damage to plants than cascades that affect traits such as plant growth (Schmitz et al. 2000b). Other studies have demonstrated a negative effect of *Prokelisia* on *Spartina* growth (Denno et al. 2002, Finke and Denno 2004), and it is likely that I would have found such an effect had the experiment continued longer than one month.

Omnivores can switch between food sources depending on availability. For example, in another salt marsh study, the omnivorous crab *Armases cinereum* consumed fewer *Iva frutescens* leaves when aphids were present as an alternative food source (Ho & Pennings in press). Accordingly, I expected *Orchelimum* to feed more on *Spartina* when *Prokelisia* were absent, and to reduce feeding on *Spartina* when *Prokelisia* were present. However, there was a strong negative effect of *Orchelimum* on *Spartina* growth whether or not *Prokelisia* was present. This suggests that *Orchelimum*'s diet is not completely flexible, and that it requires a large amount of plant material in its diet; that is, its food resources may be complimentary rather than substitutable (Van-Rijn and Sabelis 2005).

Whether omnivory should strengthen or weaken trophic cascades is unclear (Polis and Strong 1996b, Eubanks 2005, Eubanks and Styrsky 2005). The answer is likely to depend in part on the omnivore's feeding preferences and the strength of its negative effects on different trophic levels. In this case, the omnivore strongly suppressed both the herbivore and the plant, but the direct negative effects on the plant dominated the food web. The omnivore's presence

strongly reduced the herbivore's abundance and herbivore damage to the plant, but the omnivore's direct consumption of the plant masked the presence of further trophic cascades on other plant traits. In contrast, Ho and Pennings (In Press) found trophic cascades from an omnivorous crab to several plant traits, probably because the crab consumed less plant material when the herbivores were present as an alternative prey. Trophic cascades involving *Prokelisia*, but produced by intraguild predators (spiders) instead of a true omnivore have been demonstrated repeatedly (Denno et al. 2003b, Gratton and Denno 2003, Finke and Denno 2004). Even though these studies have shown that intraguild predation can dampen trophic cascades, it is very likely that trophic cascades originating from predators are stronger and easier to document than trophic cascades originating from omnivores that always include some plant material in their diet.

Top-down and bottom-up effects interacted at the plant level but these interactions were not reflected at the herbivore level. Top-down effects from both the omnivore and the herbivore on plant traits commonly increased when nutrients were added. The effects of nutrients on the top-down forces at the plant level in this study were in accord with EEH (Fretwell 1977, Oksanen et al. 1981, Hunter and Price 1992) because the strength of top-down forces increased when nutrients were added. Previous studies testing this hypothesis in a *Borrchia frutescens* system (Moon and Stiling 2002a, Moon and Stiling 2004, Stiling and Moon 2005) have also shown positive effects of nutrients on the direct top-down effects of herbivores on plants. A previous study using a *Spartina* grass trophic

system also supported EEH at the plant level because the direct top-down effects of *Prokelisia* planthoppers on dead *Spartina* biomass was increased by nutrient addition due to higher population densities of the planthoppers under these conditions (Denno et al. 2002). Only two previous studies, to our knowledge, have explored the effects of plant quality on the strength of top-down forces from a true omnivore (Eubanks and Denno 1999, Eubanks and Denno 2000) but they focused on how plant quality affected the omnivore's capacity to control their herbivore prey and did not present data on how plant quality affected plant consumption; it is therefore difficult to make any comparisons with the direct top-down effects from the omnivore in our study on plant traits.

Salinity stress also affected the direct top-down forces acting on *Spartina* plants. The effects of salinity stress on the direct top-down forces acting at the plant level, in this study, were opposed to the environmental stress hypothesis (Connell 1975, Menge and Sutherland 1976, Menge and Sutherland 1987) because salt addition by itself or in combination with nutrients increased top-down effects of the herbivore on certain plant traits. This could be due to the fact that our salinity stress levels were mild and allowed the sap-feeding planthoppers to profit from an increased nutrient content due to water stress as discussed above. It is unclear, however, how our salinity stress treatment could have increased the top-down effects from the omnivorous grasshopper.

Despite these interesting effects of nutrient addition and salinity stress on the direct top-down forces at the plant level, these same kinds of interactions were not present at the herbivore level. Top-down effects at the herbivore level

and trophic cascades from omnivores in this system were strong independent of bottom-up conditions. This finding is contrary to E.E.H. (Fretwell 1977, Oksanen et al. 1981) and the environmental stress hypothesis (Connell 1975, Menge and Sutherland 1976, 1987) because neither nutrients nor salinity stress affected the strength of top-down forces or the trophic cascade. Previous studies testing these hypotheses have found that nutrients can strengthen top-down forces on herbivores and salinity stress can weaken them (Denno et al. 2002, Moon and Stiling 2002a, Moon and Stiling 2004). One mechanism that might have reduced the importance of interactions between top-down and bottom-up factors at the herbivore level in this experiment is that the mesocosm cages prevented immigration and emigration of arthropods. In particular, migration of *Prokelisia* from low to high quality plants is known to mediate the strength of top-down interactions in the field (Denno et al. 2003a, Denno et al. 2005). It is likely that *Orchelimum* also moves across the marsh to follow high-quality plants or abundant prey, with similar effects, but this has not been documented.

We mention two other caveats to our results. First, the grasshopper densities that I used are at the high end of densities that I normally observe in the field. Thus, the top-down effects that I documented likely represent maximum levels, although the fact that I supplemented *Prokelisia* stocks weekly ameliorates this concern. Second, the lower salinity treatment (0 ppt) rarely occurs in the field except as a temporary condition following heavy rain, and both the salinity and nutrient treatments likely did not encompass the full range of

variation in these factors that occurs in the field. Had I used a wider range of bottom-up conditions, I might have seen stronger bottom-up effects.

Despite these caveats, our results are consistent with and shed new insight into past field studies. A number of studies have shown that fertilizing *Spartina* plants in the field decreases C:N ratio and increases *Prokelisia* abundance (Vince et al. 1981, Stiling and Rossi 1997, Gratton and Denno 2003, McFarlin et al. In press), and that higher salinities decrease *Spartina* C:N ratio (Moon et al. 2000, Moon and Stiling 2002a, Moon and Stiling 2004). Similarly, a number of studies have shown that predators (mostly spiders) can exert strong top-down control on *Prokelisia* populations (Denno et al. 2003b, Finke and Denno 2004, Denno et al. 2005). The role of *Orchelimum* and related tettigoniids as top-down consumers in salt marshes, however, has been overlooked since the foundational work of Teal (1962) and Smalley (1960) misidentified these species as strict herbivores. Following the assumption that *Orchelimum* is a strict herbivore, subsequent experimental work that detected decreases in *Prokelisia* when *Orchelimum* was present attributed the inverse density relationships to competition (Stiling et al 1991). However, our study revealed that *Orchelimum* is not simply excluding *Prokelisia*. By enclosing the system in mesocosms and controlling emigration, the *Orchelimum*-induced changes in *Prokelisia* were not due to emigration but due to mortality. *Orchelimum* grasshoppers have been observed eating *Prokelisia* at an average of 7.8 individuals per day (SE: 1.4; N = 10) when enclosed in a 1L jar for 48hours (Wason and Pennings personal observations). Also, periodical observations of *Orchelimum* grasshoppers inside

our cages showed that *Orchelimum* intensively hunted *Prokelisia* making them hide in between *Spartina* leaves.

In summary, I found that both top-down and bottom-up forces were strong in this salt marsh system. Salinity and nutrients, important bottom-up factors in salt marshes, interacted in a less than additive way to increase herbivore populations. In contrast to our expectations, top-down control of herbivores and the trophic cascade on the plant's herbivore damage was strong independent of bottom-up conditions. The low flexibility of the omnivore's diet seems to have masked further trophic cascades on other plant traits. Because omnivorous feeding habits are common in nature, re-examination of other well-studied systems for over-looked omnivores may shed new insight into old results. Wide varieties of outcomes are possible in a single simple system. The complexity of these interactions cautions against overly-simplistic generalizations about trophic controls in natural systems.

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Tables

Table 1: Summary table of the analysis of variance results for the effects of salinity stress (low or high), nutrient availability (ambient or addition), herbivore treatment (present or absent), omnivore treatment (present or absent) and their interactions on C:N ratio, chlorophyll content, leaf toughness, plant growth, *Prokelisia* abundance and *Prokelisia* damage. Full ANOVA results can be found in digital appendix B. Initial number of leaves was included as a covariate in tests of *Prokelisia* damage. NT = not tested. NS = not significant.

Source	<i>P</i>					
	C:N ratio	Chlorophyll content	Leaf toughness	Plant growth	<i>Prokelisia</i> abundance	<i>Prokelisia</i> damage
Salinity (S)	0.04	0.20	0.99	0.13	0.21	0.22
Nutrients (N)	<.0001	<.0001	0.045	0.57	0.04	0.02
S*N	0.02	0.10	0.51	0.65	0.01	0.47
Herbivore (H)	0.14	0.005	0.001	0.59	NT	NT
S*H	0.09	0.18	0.78	0.46	NT	NT
N*H	0.04	0.01	0.14	0.52	NT	NT

S*N*H	0.76	0.69	0.48	0.02	NT	NT
Omnivore (O)	0.03	0.89	0.37	<.0001	0.005	0.002
S*O	0.88	0.80	0.33	0.18	0.71	0.92
N*O	0.15	0.46	0.047	0.04	0.83	0.64
S*N*O	0.03	0.09	0.43	0.61	0.87	0.61
H*O	0.78	0.16	0.45	0.52	NT	NT
S*H*O	0.14	0.87	0.89	0.55	NT	NT
N*H*O	0.61	0.64	0.58	0.07	NT	NT
S*N*H*O	0.40	0.99	0.07	0.69	NT	NT
Initial number of leaves	NS	NS	NS	NT	NS	0.008

Figures

Figure 1: Interactive effects of nutrients, salinity stress and top-down forces from the herbivorous *Prokelisia* planthopper and the omnivorous *Orchelimum* grasshopper on several plant traits (**a**, **b**, and **c**). Interactive effects of nutrient and salt addition on *Prokelisia* abundance and C:N ratio(**d** and **e**). Bottom-up effects of nutrient addition of the number of leaves damaged by *Prokelisia* (**f** and **g**). Figure **g** shows the linear negative relationship between the numbers of leaves damaged by *Prokelisia* and leaf chlorophyll content. H+: herbivore present; H-: herbivore absent; O+: omnivore present; O-: omnivore absent; S+: salt added; S₀: no salt added; Black bars: no nutrient addition (N₀); white bar: nutrient addition (N+). ANOVA statistics represent the nutrient x herbivore interaction (**a** and **b**), the nutrient x omnivore interaction (**c**), the salinity x nutrient interaction (**d** and **e**) and the nutrient effects (**f** and **g**).. Data are means + 1 SE.

Figure 2: Interactive effects of nutrients, salinity stress and top-down forces from the herbivorous *Prokelisia* planthopper and the omnivorous *Orchelimum* grasshopper on several plant traits. H+: herbivore present; H-: herbivore absent; O+: omnivore present; O-: omnivore absent; S+: salt added; S₀: no salt added; Black bars: no nutrient addition (N₀); white bar: nutrient addition (N+). ANOVA statistics represent the effects of herbivores (**a**), the salinity x nutrient x herbivore interaction (**b**), the salinity x nutrient x omnivore interaction (**c**) and the nutrient x omnivore interaction (**d**). Data are means + 1 SE.

Figure 3: **a)** Top-down effects from the omnivorous *Orchelimum* grasshopper on the herbivorous *Prokelisia* planthopper. **b)** Trophic cascade from the omnivorous *Orchelimum* grasshopper to the number of leaves damaged by *Prokelisia*. O+: omnivore present; O-: omnivore absent. ANOVA statistics represent the omnivore's top-down effects. Data are means + 1 SE.

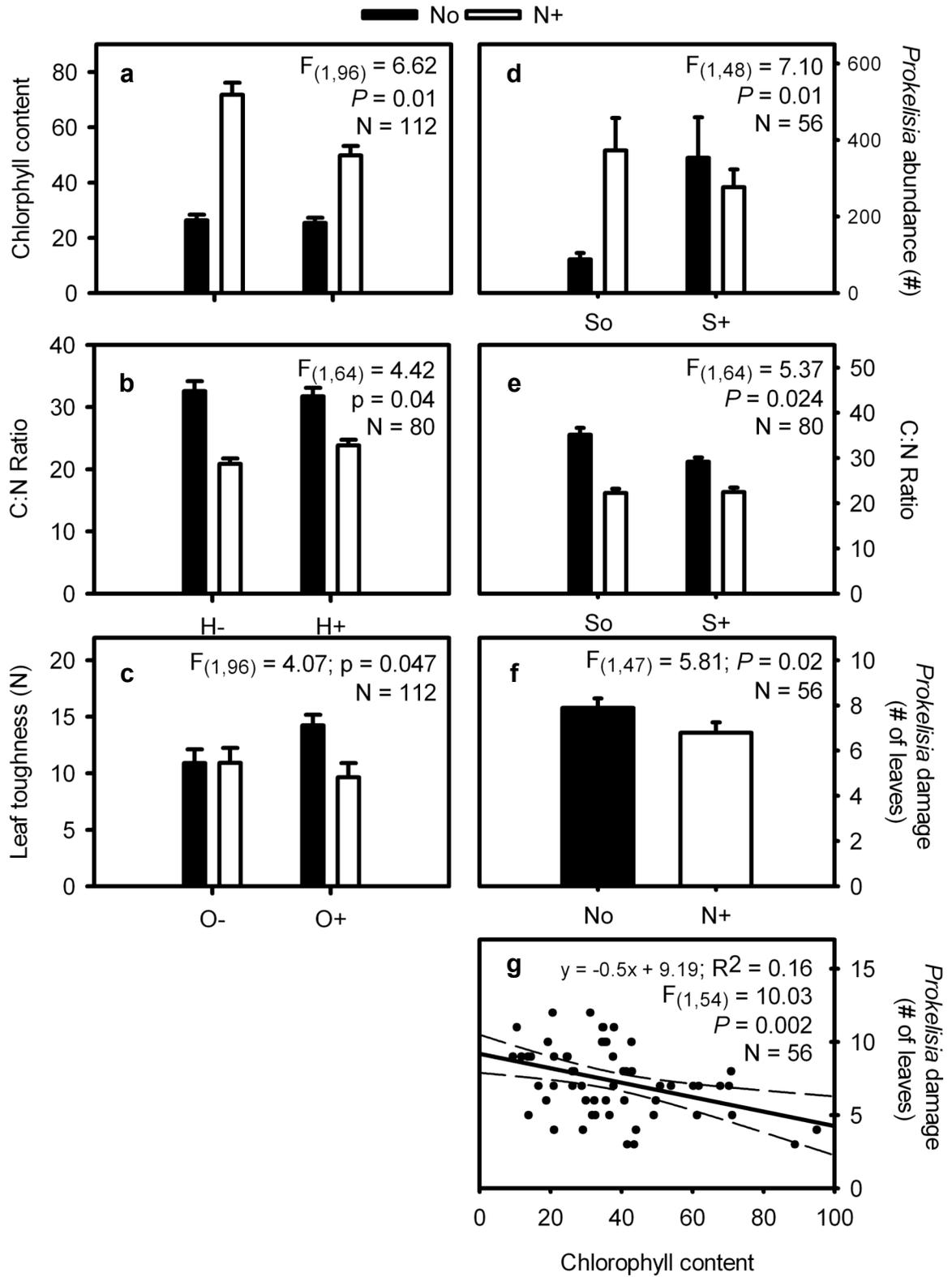


Figure 1

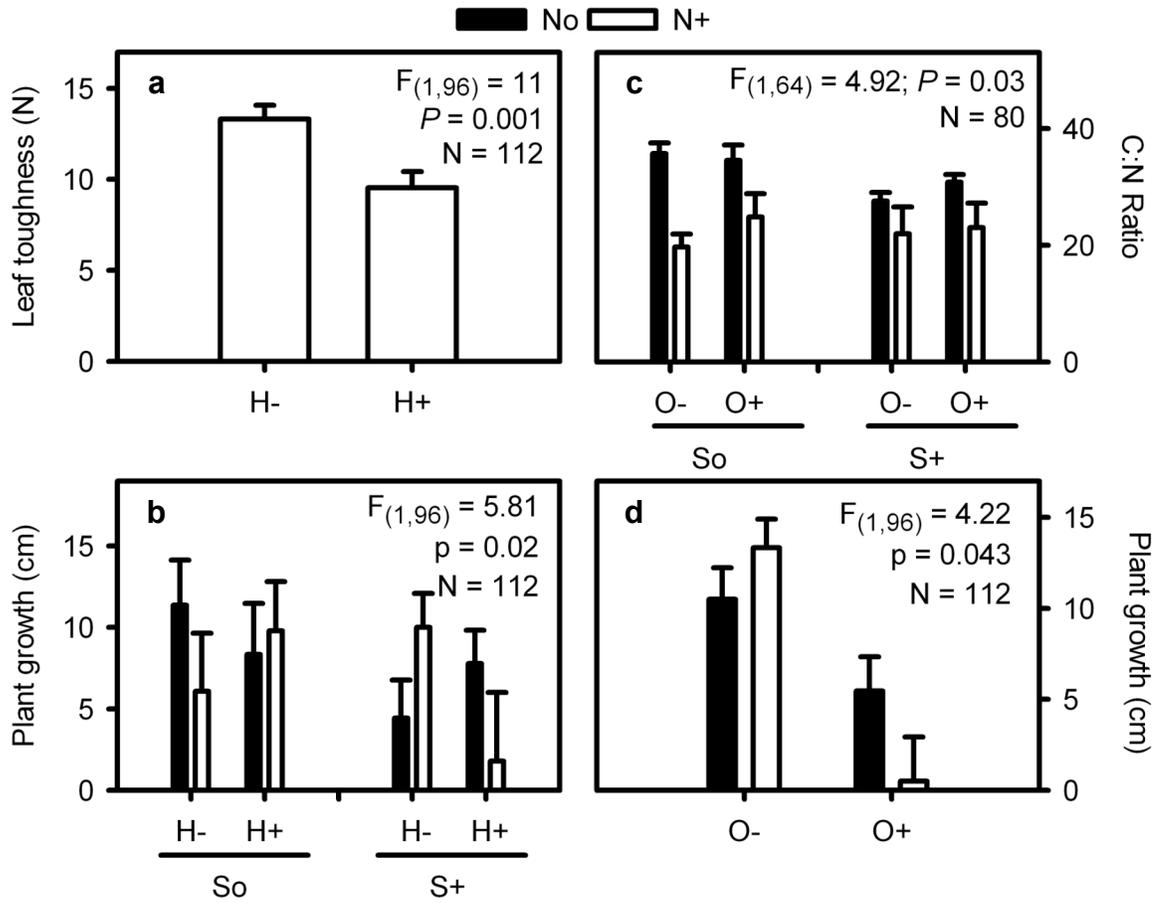


Figure 2

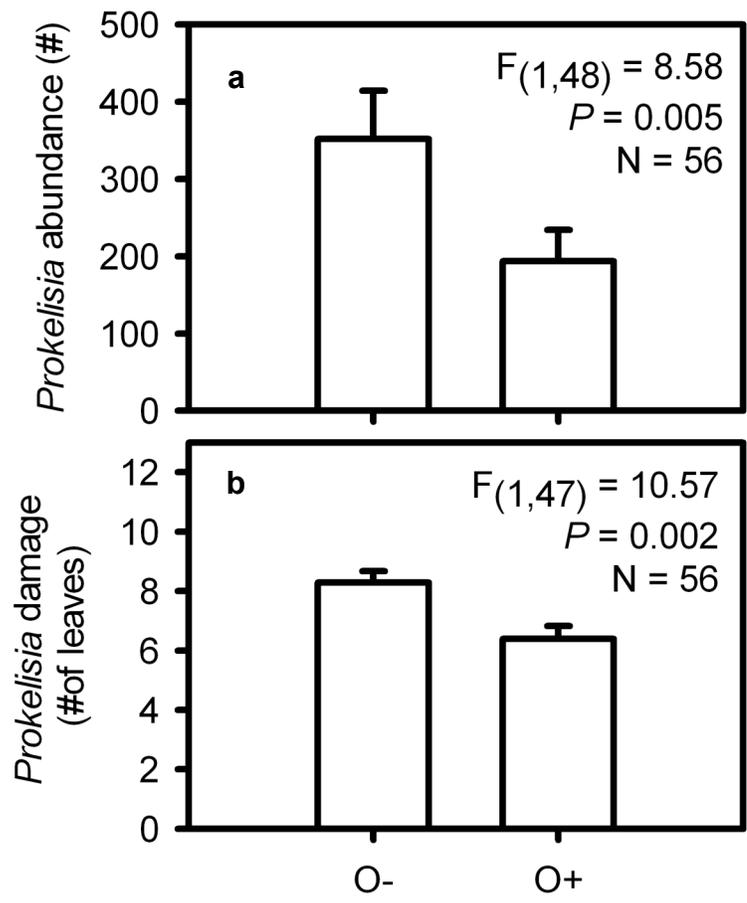
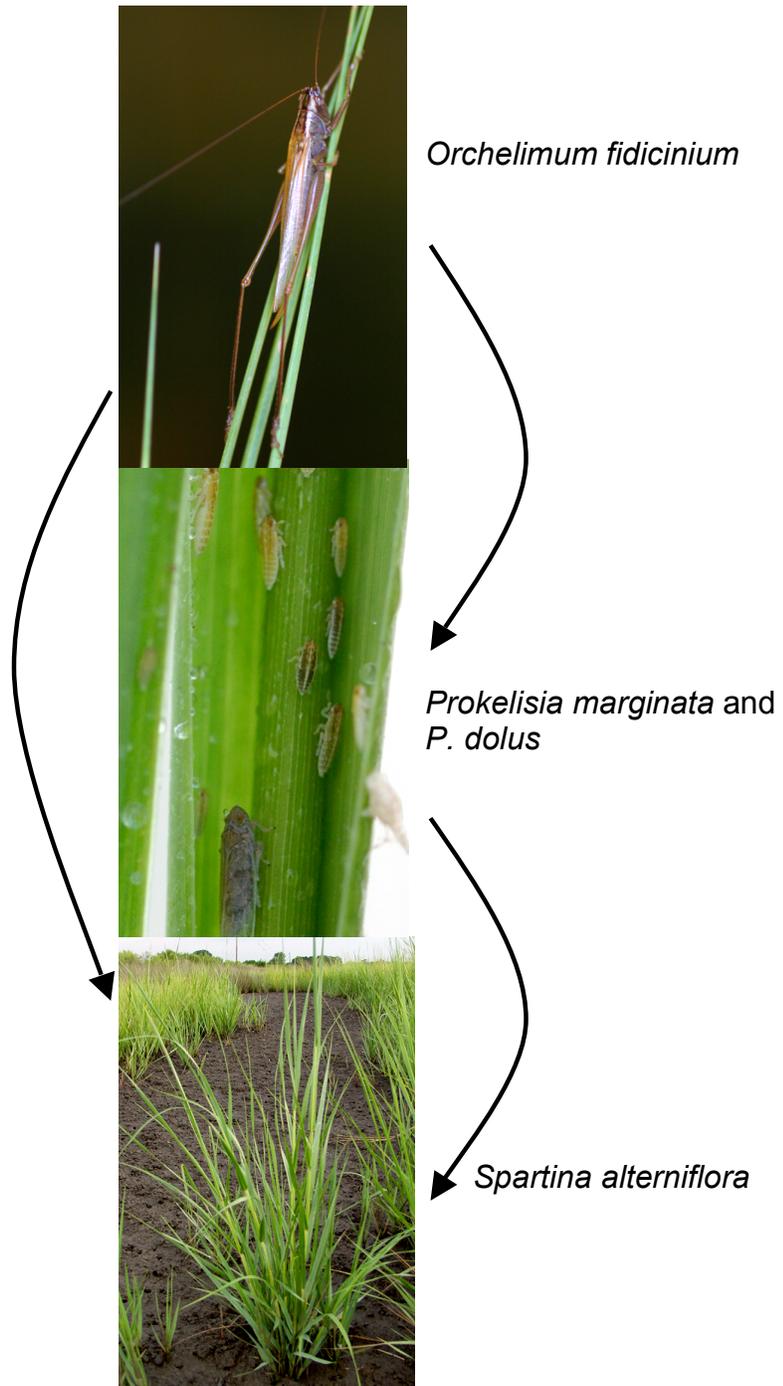


Figure 3

Plate 1: *Spartina alterniflora* food web used in the experiment: Grasshopper: *Orchelimum fidicinium*; Planthoppers: *Prokelisia marginata* and *P. dolus*. Plant: *Spartina alterniflora*. Photos taken by Chuan-Kai Ho.



APPENDIX A:

Table 1A: Experimental design for the *Spartina alterniflora* salt marsh plant trophic system. The levels of the omnivore treatment were completely crossed with the levels of the herbivore treatment, nutrient addition and salinity stress (Factorial design: 2 x 2 x 2 x 2). The omnivore and herbivore treatments are illustrated at the top of the figure and the nutrients and salinity stress levels are illustrated in the left margins of the figure. Nomenclature: O: omnivore (*Orchelimum* grasshopper); H: herbivore (*Prokelisia* planthopper); P: plant (*Spartina*).

		Omnivore					
		Present (O+)		Absent (O-)			
		Herbivore		Herbivore			
		Present (H+)	Absent (H-)	Present (H+)	Absent (H-)		
		O	O				
		↓	↓				
		H		H			
		↓	↓	↓			
		P	P	P	P		
Salinity	Ambient	Ambient	n = 7	n = 7	n = 7	n = 7	n = 28
		Addition	n = 7	n = 7	n = 7	n = 7	n = 28
	Addition	Ambient	n = 7	n = 7	n = 7	n = 7	n = 28
		Addition	n = 7	n = 7	n = 7	n = 7	n = 28
		n = 28	n = 28	n = 28	n = 28	N = 112	

APPENDIX B:

Table 1B: Analysis of variance results for the effects of salinity stress (low or high), nutrient availability (ambient or addition), herbivore treatment (present or absent), omnivore treatment (present or absent) and their interaction on (A) Log C:N ratio, (B) Log chlorophyll content, (C) leaf toughness, (D) plant growth, (E) Log *Prokelisia* abundance and (F) *Prokelisia* damage. Only a subset of plants were included in the C:N ANOVA (A).

A) log C:N Ratio

Source	DF	SS	MS	F	P
Model	15	2493.97	166.27	7.93	<.0001
Error	64	1341.59	20.96		
C. Total	79	3835.55			
Salinity (S)	1	91.74	91.74	4.38	0.04
Nutrients (N)	1	1762.31	1762.31	84.07	<.0001
S*N	1	112.49	112.49	5.37	0.02
Herbivore (H)	1	46.70	46.70	2.23	0.14
S*H	1	61.98	61.98	2.96	0.09
N*H	1	92.61	92.61	4.42	0.04
S*N*H	1	1.95	1.95	0.09	0.76
Omnivore (O)	1	107.25	107.25	5.12	0.03
S*O	1	0.50	0.50	0.02	0.88
N*O	1	44.45	44.45	2.12	0.15

S*N*O	1	103.13	103.13	4.92	0.03
H*O	1	1.65	1.65	0.08	0.78
S*H*O	1	46.32	46.32	2.21	0.14
N*H*O	1	5.55	5.55	0.26	0.61
S*N*H*O	1	15.33	15.33	0.73	0.40

B) Log Chlorophyll content

Source	DF	SS	MS	F	<i>P</i>
Model	15	34009.12	2267.27	10.95	<.0001
Error	96	19882.19	207.11		
C. Total	111	53891.31			
Salinity (S)	1	347.85	347.85	1.68	0.20
Nutrients (N)	1	28445.21	28445.21	137.35	<.0001
S*N	1	566.91	566.91	2.74	0.10
Herbivore (H)	1	1692.50	1692.50	8.17	0.005
S*H	1	370.57	370.57	1.79	0.18
N*H	1	1370.33	1370.33	6.62	0.01
S*N*H	1	32.23	32.23	0.16	0.69
Omnivore (O)	1	4.13	4.13	0.02	0.89
S*O	1	13.18	13.18	0.06	0.80
N*O	1	113.04	113.04	0.55	0.46
S*N*O	1	595.90	595.90	2.88	0.09
H*O	1	405.87	405.87	1.96	0.16

S*H*O	1	5.58	5.58	0.03	0.87
N*H*O	1	45.81	45.81	0.22	0.64
S*N*H*O	1	0.01	0.01	0.00	0.99

C) Leaf toughness

Source	DF	SS	MS	F	<i>P</i>
Model	15	10.95	0.73	1.94	0.03
Error	96	36.20	0.38		
C. Total	111	47.15			
Salinity (S)	1	0.00	0.00	0.00	0.99
Nutrients (N)	1	1.56	1.56	4.13	0.045
S*N	1	0.16	0.16	0.43	0.51
Herbivore (H)	1	4.15	4.15	11.00	0.001
S*H	1	0.03	0.03	0.08	0.78
N*H	1	0.83	0.83	2.21	0.14
S*N*H	1	0.19	0.19	0.50	0.48
Omnivore (O)	1	0.31	0.31	0.82	0.37
S*O	1	0.36	0.36	0.94	0.33
N*O	1	1.53	1.53	4.07	0.047
S*N*O	1	0.24	0.24	0.63	0.43
H*O	1	0.22	0.22	0.58	0.45
S*H*O	1	0.01	0.01	0.02	0.89
N*H*O	1	0.11	0.11	0.30	0.58

S*N*H*O	1	1.26	1.26	3.34	0.07
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D) Plant growth

Source	DF	SS	MS	F	P
Model	15	4309.11	287.27	2.85	0.001
Error	96	9662.57	100.65		
C. Total	111	13971.68			
Salinity (S)	1	234.32	234.32	2.33	0.13
Nutrients (N)	1	32.14	32.14	0.32	0.57
S*N	1	20.57	20.57	0.20	0.65
Herbivore (H)	1	30.04	30.04	0.30	0.59
S*H	1	54.32	54.32	0.54	0.46
N*H	1	41.29	41.29	0.41	0.52
S*N*H	1	585.14	585.14	5.81	0.02
Omnivore (O)	1	2232.14	2232.14	22.18	<.0001
S*O	1	185.14	185.14	1.84	0.1782
N*O	1	424.32	424.32	4.22	0.043
S*N*O	1	26.04	26.04	0.26	0.61
H*O	1	41.29	41.29	0.41	0.52
S*H*O	1	36.57	36.57	0.36	0.55
N*H*O	1	350.04	350.04	3.48	0.07
S*N*H*O	1	15.75	15.75	0.16	0.69

E) Log *Prokelisia* abundance

Source	DF	SS	MS	F	P
Model	7	570952.20	81564.60	3.14	0.008
Error	48	1247532.80	25990.30		
C. Total	55	1818485.10			
Salinity (S)	1	41678.10	41678.10	1.60	0.21
Nutrients (N)	1	116010.21	116010.21	4.46	0.04
S*N	1	184480.48	184480.48	7.10	0.01
Omnivore (O)	1	223102.22	223102.22	8.58	0.005
S*O	1	3708.51	3708.51	0.14	0.71
N*O	1	1234.94	1234.94	0.05	0.83
S*N*O	1	737.76	737.76	0.03	0.87

F) *Prokelisia* damage

Source	DF	SS	MS	F	P
Model	8	109.47	13.68	3.23	0.005
Error	47	199.08	4.24		
C. Total	55	308.55			
Salinity (S)	1	6.50	6.50	1.53	0.22
Nutrients (N)	1	24.62	24.62	5.81	0.02
S*N	1	2.20	2.20	0.52	0.47
Omnivore (O)	1	44.77	44.77	10.57	0.002
S*O	1	0.04	0.04	0.01	0.92
N*O	1	0.95	0.95	0.22	0.64

S*N*O	1	1.09	1.09	0.26	0.61
I.L. [£]	1	32.06	32.06	7.57	0.008

£: Initial number of leaves

CHAPTER 3

Interactive effects of nutrients, stress, and omnivory on community structure are
species-specific

Juan M. Jiménez

Abstract

Despite intense research there are still no general rules as to how the structure of communities is mediated. Because of the complexity of the processes regulating community structure, there may be interactions among factors mediating top-down and bottom-up effects. I used a mesocosm study to test a series of hypotheses about how salinity stress (control and addition), nutrient levels (control and addition) and the presence (or absence) of a top omnivore (crab: *Armases cinereum*) and two common herbivores (the beetle *Paria aterrima* and the aphid *Uroleucon ambrosiae*) interacted to alter top-down and bottom-up control of a salt-marsh trophic system.

We found that bottom-up effects interacted with top-down effects but the results of these interactions were species specific. Nutrient addition increased the strength of top-down effect on plants. However, salinity stress either cancelled the positive effects of nutrient addition on the top-down forces from herbivorous beetles and the omnivorous crab, or it increased the strength of top-down forces from the omnivorous crab. Nutrient addition increased aphid populations, but top-down effects from the crab on aphids overwhelmed nutrients effects. In contrast, the presence of the omnivorous crabs decreased the number of leaves damaged by the beetles independent of bottom-up conditions. Finally, interactions between herbivores (side-to-side effects) affected the strength of top-down forces coming from the omnivore and from the herbivorous beetle. The results from our study show how bottom-up, top-down and side-to-side factors can interact in complicated ways to determine the structure of a

community, and illustrate how the development of a predictive model of community structure can be complicated by the presence of interactions among regulating factors.

Introduction

Because food webs are structured by both a variety of top-down (predation), and bottom-up (plant quality and productivity) factors, obtaining a general theory of community structure is a complicated task. Both top-down and bottom-up effects can change the abundance or diversity of different trophic levels in a community. Despite intense research on several of these processes, I have not yet achieved a consensus about how they combine to structure biological communities (Hunter and Price 1992, Power 1992, Strong 1992). One likely reason for the lack of consistent results is that there are interactions among factors mediating top-down and bottom-up effects (Hunter and Price 1992). Four factors that might regulate the relative strength of top-down and bottom-up effects are the presence of omnivores, nutrient availability, environmental stress and side-to-side interactions (e.g., competition) between herbivore species. Although many studies have examined these factors in isolation, few have explored their interactions.

Omnivory might either enhance or attenuate top-down effects (Polis and Strong 1996a, Eubanks 2005, Eubanks and Styrsky 2005). Omnivory could enhance top-down effects because omnivores might be able to sustain their population levels when prey are scarce by eating at multiple trophic levels (Dayton 1984, Eubanks and Denno 2000, Ho and Pennings In Press). On the other hand, omnivory might also attenuate top-down effects because omnivores might become satiated faster by eating at multiple trophic levels, and therefore reduce their per-capita consumption of any particular prey (Eubanks and Denno

1999, Eubanks and Denno 2000). Despite the commonness of omnivory and its potentially important effects on communities (Polis 1991), only a handful of experimental studies have explored the effects of true omnivory (eating at more than one trophic level including primary producers) on the strength of top-down forces in terrestrial habitats (Agrawal et al. 1999). A number of studies have examined the effects of omnivory on trophic cascades in aquatic systems (Lodge et al. 1994, Pringle and Hamazaki 1998), but interactions in aquatic and terrestrial environments are likely to differ in several ways (Polis 1991, Strong 1992, Shurin et al. 2002). Moreover, because variation in plant quality is likely to affect whether an omnivore prefers to feed on plants versus higher trophic levels, the impact of omnivores on food webs is likely to be contingent upon bottom-up factors that affect plant quality.

The “Ecosystem Exploitation Hypothesis” (hereafter, EEH) states that top-down effects from predators will be stronger with increasing nutrient input (Fretwell 1977, Oksanen et al. 1981). Recent studies, however, have shown that this is not always the case, and that trophic cascades can instead be weak in productive systems and strong in unproductive systems (Ritchie 2000, Chase 2003a). This counter-intuitive result could be a consequence of interactions between nutrients and other factors, such as omnivory, that can potentially regulate the impact of nutrients on top-down forces. Because nitrogen limitation is thought to mediate omnivory (Eubanks and Denno 1999, Eubanks and Denno 2000, Fagan et al. 2002), intraguild predation and cannibalism (Polis et al. 1989,

Polis and Strong 1996a, Denno and Fagan 2003), it is important to study their interactive effects on community structure.

The environmental stress hypothesis proposes that increasing levels of stress will strengthen bottom-up effects and weaken top-down effects (Connell 1975, Menge and Sutherland 1976, Menge and Sutherland 1987). Only a handful of studies have evaluated the effects of environmental stress on community structure (Moon et al. 2000, Moon and Stiling 2000, Moon and Stiling 2002a, Moon and Stiling 2004), with the general result that increasing environmental stress decreases top-down control of herbivores by predators and parasitoids. However, interactions with other factors such as nutrient levels can alter this result. In particular, increasing nutrient availability can dampen the negative effects of salinity stress on top-down control of herbivores (Moon and Stiling 2000). It is important to note that in the system studied by Moon and Stiling, as in the work reported here, the salinity stress treatment directly affected plants, but consumers were affected indirectly by salinity through changes in plant quality, plant physiology and plant physical structure rather than directly (Moon and Stiling 2000, Moon and Stiling 2002a, Moon and Stiling 2004). Thus, these tests of the environmental stress hypothesis address scenarios slightly different from those envisioned by the original proponents of the hypothesis.

Side-to-side interactions within the herbivore trophic level and their effects on bottom-up and top-down regulation of communities are recently receiving increasing attention (Denno et al. 2000, Moon and Stiling 2002b, Vos et al. 2004,

Kaplan et al. 2007). Competition for resources among herbivore species can affect the performance of the weaker competitor species in the next generation (Denno et al. 2000), can affect the density of some herbivore species and alter their response to bottom-up forces (Moon and Stiling 2002b) and can increase the susceptibility of some species to predation due to developmental delays produced by induced plant defenses earlier in the season (Vos et al. 2004, Kaplan et al. 2007). Despite these important effects on community structure, only one study has explored the interaction between side-to-side effects on herbivores and bottom-up and top-down forces acting on communities (Moon and Stiling 2002b). To our knowledge no study has explored four-way interactions among omnivory, nutrients, stress and herbivores' side-to-side effects on the relative strength of top-down and bottom-up forces. Because herbivore arthropods are affected in different ways by environmental variables (Goranson et al. 2004), it is important to understand how changes in these variables affect the interactions among herbivores and the structure of the communities they live on.

The interactive effects of omnivory, nutrients, stress and side-to-side effects among herbivores on community structure are particularly important to understand in coastal ecosystems such as salt marshes. A number of important consumers in salt marshes are omnivores (Buck et al. 2003, Ho and Pennings In Press) and a number of herbivore species co-occur and potentially compete in saltmarshes (Goranson et al. 2004). Salt marshes contain natural gradients of salinity and nutrient availability (Bertness and Pennings 2000) that interact to mediate plant productivity and species composition. Moreover, these gradients

are changing as growing human populations in coastal regions affect estuarine habitats by increasing eutrophication (Valiela et al. 1992, Nixon 1995) and changing hydrological and salinity regimes (Copeland 1966, Hoese 1967, Alber 2002). Although a number of studies have addressed how these anthropogenic impacts affect emergent wetland plants (Jefferies and Perkins 1977, Crain et al. 2004, Pennings et al. 2005), little is known about how they mediate food web interactions. Because humans are stressing communities and ecosystems in many different ways at the same time, it is important to understand how the effects of a particular stress factor modify the effects of others.

To address these issues, I studied a food web consisting of a common salt marsh shrub, the marsh elder *Iva frutescens* L. (Asteraceae), the herbivores *Uroleucon ambrosiae* Thomas (Aphididae) and *Paria aterrima* Olivier (Chrysomelidae), and the top omnivore *Armases cinereum* Bosc (= *Sesarma cinereum*; Grapsidae) (Plate 1). *Iva frutescens* (hereafter, *Iva*) is a common salt marsh shrub on the Atlantic and Gulf coasts of the United States, typically dominating higher marsh elevations (Bertness et al. 1992a, Bertness et al. 1992b, Bertness and Hacker 1994). *Iva frutescens* roots are only flooded during the higher tides of the month followed by prolonged periods of time when the roots are exposed. During these periods the soil surrounding *Iva* can either dry out or be flushed by rainfall, and therefore plants can experience a wide range of soil interstitial salinities (Levine et al. 1998). *Iva* shrubs are also exposed to nutrients from terrestrial runoff, groundwater and tidal waters.

The most abundant herbivore on *Iva frutescens* is the aphid *Uroleucon ambrosiae* (hereafter, *Uroleucon*) (Hacker and Bertness 1995). Another common herbivore on *Iva* is the beetle *Paria atterrима* (hereafter, *Paria*) (Ho and Pennings In Press).

A common true omnivore in the *Iva frutescens* zone of southeastern salt marshes is the crab *Armases cinereum* (Teal 1958, Abele 1992, Buck et al. 2003). *Armases cinereum* eats both *Iva* leaves and small herbivore arthropods like the aphids *Uroleucon* and the beetle *Paria*, and can strongly suppress herbivore populations in the field (Ho and Pennings in press).

In a factorial mesocosm experiment, I manipulated nutrient availability, soil salinity, and the presence of herbivores and top omnivores. Because *Armases* is omnivorous, eating both plants and herbivores, I hypothesized that it would have both direct negative and indirect positive effects on plants, leading to weak net effects. I hypothesized that nutrient addition would directly increase *Uroleucon* and *Paria* herbivore populations, but would also alter top-down control of these herbivores by *Armases* by altering plant quality and herbivore abundance. I hypothesized that salinity would decrease *Uroleucon* and *Paria* populations and again alter top-down control of these herbivores by *Armases* by altering plant quality and herbivore abundance. I further hypothesized that salinity would decrease the positive effects of nutrients on *Uroleucon* and *Paria* populations, and mediate the effects of nutrient addition on top-down control of both herbivores. Finally, I hypothesized that *Uroleucon* would reduce plant damage by *Paria* by reducing the nutrient content of the plant.

Methods

Experimental work was done in mesocosms at the University of Georgia Marine Institute (UGAMI) on Sapelo Island, GA (31.3929°N, 81.2699°W). Our experiment crossed two levels each of nutrients, salinity, aphids, beetles and crabs, for a 5-way orthogonal design with a total of 160 replicates. *Iva* plants ($n=210$) were germinated from seeds collected in October 2002 from marshes in South Carolina, Georgia and Florida and maintained in 3.5 L pots in an open-air (roof but no sides) green house at UGAMI. A subset ($n=44$) of these plants was used in a 4-week mesocosm experiment in July of 2004 (Ho and Pennings In Press). These plants had 11 months to recover before being used in the current experiment, and any that appeared stressed were excluded from the study as described below, such that plants that had and had not been used in the earlier experiment were visually indistinguishable.

In early June 2005, *Iva* plants were repotted in a 60/40 mixture of potting soil and sand in 3.5 L plastic pots. After 7 days, 160 healthy and similarly-sized (ca. 64 cm tall) plants were randomly assigned to four combinations of nutrients (ambient, fertilized) and salinity (low, high) and acclimated to these treatments for 30 days. Nutrient and salinity treatments were initiated with a low dose (5 g) of nitrogen-based fertilizer (20:10:5 N:P:K fertilizer pellets, Forestry Suppliers) and a low salinity concentration (5 PSU). Nutrient additions were progressively increased every week to a final level of 21 g every two weeks. The salinity

treatment was progressively increased every two weeks to a final level of 20 PSU. Plants were watered daily with fresh or saline water and allowed to drain fully. The nutrient and salinity additions were sufficient to affect plant traits, herbivore abundance and herbivore damage (see Results).

Arthropods were captured at several salt marsh sites around Sapelo Island. To examine the individual and combined effects of the two herbivores, I added aphids and beetles alone and in combination. To examine the effects of the omnivorous *Armases* crab, I set up treatments with and without crabs present. Mesocosms including *Armases* contained a single crab. Mesocosms including aphids were initiated with 20 individuals. Mesocosms including beetles (but not aphids) contained 3 beetles, but mesocosms including both herbivores were stocked with only 2 beetles. This was done to try to keep the total herbivore biomass as constant as possible. I did not similarly adjust initial aphid numbers because I expected aphid numbers to be highly dynamic and were interested in how they would respond to different treatment combinations. Plants were checked daily to replace crabs and beetles that died. Beetles were replaced to keep the treatments constant throughout the experiment; *Paria* beetles did not reproduce under mesocosm conditions and were eaten within a few days of being exposed to the *Armases* crab. Therefore, it is likely that the top-down control of the *Armases* crab on the *Iva* beetle's damage included a large trait-mediated (behavioral) component. Plants and arthropods were enclosed in a 1 m high cage made of fine cloth mesh.

Once arthropods were stocked, the experiment ran for one month (July 20th to August 20th 2005). At the end of the experiment I counted all the arthropods present on the plants. I measured several plant traits at the beginning and end of the experiment, as follows. As an indicator of plant growth and herbivory, I measured plant height and the number of healthy and damaged leaves, scoring both damage from *Paria* beetles (visible as elliptical areas missing from the interior of the leaf) and damage from *Armases* crabs (visible as irregular areas missing from the leaf margin). As indicators of leaf quality, I measured chlorophyll content as a proxy for leaf nitrogen content (Opti-Sciences CCM-200 chlorophyll meter) and leaf toughness (Penetrometer type 516, Chatillon N.Y.) at the end of the experiment. Because I fixed *Armases* and *Paria* densities, replacing individuals that died, and neither species reproduced during the experiment, I examined effects on *Armases* and *Paria* damage, but not their populations.

Data were analyzed with 5-way ANOVA, with all factors treated as fixed effects. Variables were transformed when necessary to meet assumptions of normality and homoscedasticity. Aphid abundance and beetle damage were log transformed and crab damage, chlorophyll content, number of leaves, number of healthy leaves and change in number of leaves were square root transformed. Initial plant height or initial number of leaves, as most appropriate, were included as covariates in some models. Tuckey-HSD tests were performed when necessary. Figures show untransformed data for clarity.

Results

Both nutrient addition and salinity stress had strong bottom-up effects (Fig. 1, Table 1). Nutrient addition significantly increased *Iva* leaf chlorophyll content, number of leaves, number of healthy leaves (data not shown), *Iva* height, growth, and change in number of leaves; however, nutrient effects on *Iva* height and growth and on the change in number of leaves depended on an interaction with salinity. Salinity stress dampened the positive effects of nutrient addition on *Iva* height (Fig. 1c), growth (Fig. 1d), and change in number of leaves (Fig. 1e). Nutrient addition also increased aphid abundance (Fig. 2a) and crab leaf damage (Fig. 2b), but decreased beetle leaf damage; however, beetle leaf damage depended on an interaction between nutrients and salinity stress; nutrients tended to stimulate beetle damage when salt stress was low, but decrease it when salt stress was high (Fig. 2 c). Salinity stress did not affect leaf chlorophyll content, number of leaves, the number of healthy leaves, aphid abundance, or crab leaf damage (data not shown).

Omnivorous crabs had strong top-down effects on both aphids and beetles (Fig. 3, Table 1). When the crab was present, aphid numbers were reduced from over 100 per plant to close to zero (Fig. 3A); this decrease was somewhat greater when beetles were absent, leading to a significant crab x beetle interaction (Fig. 3a). In addition, the crab reduced beetle damage by 60 % (Fig. 3b). Although I replaced beetles that were eaten by the crab, there was a time lag between when they were eaten and when they were replaced. As a result, the crab was able to somewhat decrease the average number of beetles present

per plant. In addition, the effect of the crab on beetle damage may include effects on beetle behavior: Beetles may have spent less time feeding when crabs were present, although I did not document this.

The crab also had direct top-down effects on *Iva* plants, but these effects were mediated by interactions with aphids and beetles (Fig. 3, Table 1). When present without other arthropods, the crab intensively fed on *Iva* leaves; but when aphids or beetles were also present, the crab reduced its leaf consumption, presumably because it was increasingly feeding on arthropods rather than plants (Fig. 3c). When both herbivores were present, however, the crab leaf consumption was only marginally reduced (Fig. 3c). Because the crab, on average, fed less on leaves when beetles or aphids were present, they reduced leaf chlorophyll content only when beetles and aphids were both absent (Fig. 3d). Finally, Beetle also had top-down effects on plant traits by reducing *Iva* height, but only when crabs were absent (Fig. 3ef).

Bottom-up effects interacted with top-down forces from the crab to affect chlorophyll content and aphid numbers (Fig. 4, Table 1). The crab reduced leaf chlorophyll content but only when either salt or nutrients were added (Fig. 4ab). Nutrients strongly increased aphid numbers, but only when the crab was absent (Fig. 4c). When the crab was present, it strongly suppressed aphids regardless of nutrient levels.

Bottom-up effects also interacted with each other and with top-down forces from beetles to affect plant traits (Fig. 5, Table 1). Nutrients increased plant height and growth, but only when salinity was low and beetles were absent (Fig.

5ab). Nutrients also increased leaf chlorophyll content more when aphids were present or beetles were absent (Fig. 5cd).

Side-to-side effects also changed the strength of top-down forces (Table 1). *Paria* beetles reduced the number of leaves (Fig. 5e), number of healthy leaves (Fig. 5f) and change in number of leaves (Fig 5g), but only when aphids were absent and salinity stress was low. These results must be evaluated with caution because the interaction with aphids could be due to the fact that I stocked only two beetles when aphids were present instead of three. If this difference in beetle loads was the only factor responsible for the differences, I would expect beetles to reduce plant traits by 33% more when aphids were absent versus when they were present. However, plant traits were reduced 55%, 84% and 84% respectively. Therefore, there are indeed side-to-side effects altering the top-down forces from the beetles.

Discussion

Bottom-up effects were strong and predictable at the plant level but their effects at the herbivore level were variable. The omnivorous crab had weak direct top-down effects on plants but strong direct top-down effects on herbivores and strong indirect top-down effects on plants due to its flexible diet. While bottom-up forces did not alter trophic cascades, direct top-down forces on plants and herbivores were altered in complicated ways by bottom-up forces. Finally, side-to-side interactions affected the strength of top-down forces coming from the omnivore and from the herbivorous beetle. Below, I discuss these findings in

turn, and consider how the complicated interactions illustrated here can affect the regulation of community structure.

Nutrient addition increased *Iva* plant fitness, aphid abundance and crab leaf damage but decreased the number of leaves with beetle damage (Fig. 1, Table 1). It is not immediately clear why nutrient addition should decrease the number of leaves damaged by *Paria* beetles. In a previous study with a similar design, Jiménez and Pennings (In preparation) found that nutrient addition decreased the number of *Spartina alterniflora* leaves damaged by a sap-feeding planthopper (*Prokelisia marginata*), and suggested that herbivores might move about on plants less when nutrient content was higher, leading to less dispersed damage. Overall, however, the effects of nutrient addition on the plant and its consumers was consistent with E.E.H (Fretwell 1977, Oksanen et al. 1981) because nutrient addition stimulated plants and herbivores. Previous studies in salt marsh systems have also found that nutrient addition to *Spartina alterniflora* (Denno et al. 2002, Gratton and Denno 2003, Denno et al. 2005) and *Borrchia frutescens* (Bowdish and Stiling 1998, Moon and Stiling 2000, Moon and Stiling 2002a) has consistently had positive effects on the plants and their consumers.

Salinity stress decreased plant fitness and *Paria* leaf damage but did not affect either *Uroleucon* abundance or *Armases* leaf damage. The effects on *Iva* plants and *Paria* beetles were consistent with the environmental stress hypothesis (Connell 1975, Menge and Sutherland 1976, Menge and Sutherland 1987). It is unclear why *Uroleucon* abundance and *Armases* leaf damage were not affected by salinity stress, because the level of salinity stress used in the

experiment (20‰) is considered fairly high for *Iva* plants (Bertness et al. 1992a), and sharply reduced plant growth. Most past findings would have suggested that salinity would decrease *Uroleucon* aphid abundance and *Armas* crab leaf damage by reducing plant nutrient absorption (Smart and Barko 1980, Feller 1995) and increasing toughness (Moon et al. 2000, Moon and Stiling 2000, Moon and Stiling 2002a). However, two recent studies suggest that at low or medium levels of salinity stress you can expect positive effects on consumers, but at high levels of salinity stress you can expect negative effects on the system (Moon and Stiling 2005, Jiménez and Pennings In preparation). It is possible, then, that our levels of stress were high enough for *Iva* plants and *Paria* beetles to be negatively affected but not high enough to negatively affect *Uroleucon* aphids or *Armas* crabs. This suggests that a given level of stress might have different effects depending on the kind of consumer. Because in the *Iva* system salinity stress affects consumers indirectly through the plant by changes in the plant's physiology and physical structure, it is very likely that these changes have different effects on different consumer types. Goranson et al. (2004) found that herbivore species reacted differently to natural gradients of salinity stress experienced by plants, with some herbivores preferring plants from high salinity habitats and others from low salinity habitats.

Because nitrogen addition had positive effects on plant traits and consumers, and salinity stress had negative effects on plant traits and *Paria* beetles, one might expect that combining nutrient addition and salinity stress would largely cancel out both effects. This was true for plant traits but not for

Paria beetles: nutrients and salinity stress synergistically decreased *Paria* leaf damage (Fig. 2). The salinity stress levels used in our study are considered high (20‰) for *Iva* plants (Bertness et al. 1992a). Previous studies in salt marshes have found less than additive interactions between nutrients and medium levels of salinity stress (Moon and Stiling 2005, Jiménez and Pennings In preparation) with both nutrients and salinity stress having independent positive effects on plants and sap-feeding herbivores but having no further effects when combined. The difference between our current results and these past results could be due to differences in the level of salinity stress -low, medium or high- (Jiménez and Pennings In preparation) or to the fact that chewing herbivores like *Paria* and sucking herbivores like those studied previously respond differently to plant traits (Goranson et al. 2004).

The omnivorous crab *Armases cinereum* produced a strong top-down trophic cascade involving *Paria* leaf-chewing beetles but not one involving *Uroleucon* sap-feeding aphids. *Armases* reduced *Paria* leaf damage, which in turn significantly increased *Iva* height and marginally increased *Iva* growth (Fig. 4 F and table 1). In contrast, although *Armases* strongly reduced *Uroleucon* aphid populations, this did not benefit plants (Fig. 3 B and table 1). It is possible that if I had been able to measure *Uroleucon* aphid's damage to plants I would have been able to detect a trophic cascade. These aphids do not leave visible marks when they feed, making it difficult to directly measure their damage; instead I relied on indirect measures such as effects on plant traits. Jiménez and Pennings (In preparation) in a similar study found strong top-down effects of the omnivore

Orchelimum grasshopper on *Prokelisia* planthopper damage (sap-feeding herbivore), but failed to detect any trophic cascades on plant traits. Schimtz et al. (2000a) showed that in general it is easier to demonstrate trophic cascades that affect herbivore damage than plant traits such as plant growth. Since our experiment ran for only one month, it is likely that I would have detected a stronger cascading effect of *Armases* on plants had the duration of the experiment been extended. Ho and Pennings (In Press) found a modest trophic cascade from *Armases* to *Iva* via aphids in mesocosm experiments, but they studied a simpler food web than I did, with no variation in bottom-up conditions. Ho and Pennings (In Press) also found a strong trophic cascade from *Armases* to *Iva* via a diverse herbivore assemblage in the field over a 24 month study, indicating that the trophic cascades observed by us and them in the greenhouse also occur in the field.

By comparing our study with others examining top-down effects of omnivores, I can see that the nature of top-down effects from omnivores depends in part on how omnivores treat plant versus animal foods. The omnivorous crab *Armases cinereum* had weak direct negative effects but a strong indirect effect (trophic cascade) on *Iva*. The crab fed on *Iva* leaves and reduced leaf chlorophyll content, but only when aphids or beetles were absent. When aphids or beetles were present, the crab reduced its consumption of leaves and ate more prey. Ho and Pennings (In Press) found similar results for the *Iva* food web in a previous study. They suggested that the crab's diet is highly flexible and that it prefers and performs better on an animal than a plant diet when given the choice (Buck

et al. 2003). In contrast, Jiménez and Pennings (In preparation) found strong top-down effects of the omnivore *Orchelimum* grasshopper on *Spartina* plants independent of the presence or absence of alternative prey in the system, suggesting that *Orchelimum*'s diet is not as flexible, and that it requires a large amount of plant material in its diet. Most studies exploring top-down effects from omnivores have mainly focused on the effect of plant food on the strength of the omnivore's top-down control of herbivores (Eubanks 2005, Eubanks and Styrsky 2005) and therefore, it is difficult to make comparisons about how the diet flexibility of their omnivores affected the top-down control of their communities. Despite the scarcity of studies addressing the effects of complimentary and substitutable food resources on omnivore's top-down control, results from our lab agree with Van-Rijn and Sabelis' (2005) prediction that when plant and prey food resources are substitutable, that is when the omnivores readily switch between prey and plant diets, omnivores exert strong top-down control over herbivores, but when plant and prey food resources are complimentary (omnivores cannot survive without certain nutrients provided by plants), top-down effects and trophic cascades driven by omnivores are significantly reduced. In this study, as in Ho and Pennings (In Press), I found strong trophic cascades from an omnivorous crab on plant traits because the crab consumed less plant material and ate more prey when the herbivores were present.

Top-down and bottom-up forces interacted in this experiment. The effects of nutrients on top-down forces in this study were in accord with EEH (Fretwell 1977, Oksanen et al. 1981). In general, nutrient addition increased the strength

of top-down forces because plants and herbivores did better when fertilized if their consumers were absent, but less so if consumers were present. Most previous studies addressing these hypotheses in salt marshes have also found stronger top-down control of communities when nutrients are added to food webs on *Spartina alterniflora* (Denno et al. 2002, Gratton and Denno 2003, Denno et al. 2005) and *Borrchia frutescens* (Bowditch and Stiling 1998, Moon and Stiling 2000, Moon and Stiling 2002a). Only Jiménez and Pennings (In preparation) have found strong top-down effects from an omnivore independent of bottom-up conditions. As discussed above, this probably occurred because plants and herbivores did better when their consumers were absent irrespective of whether or not nutrients were added.

The effects of salinity stress on top-down forces in this study were generally in accord with the environmental stress hypothesis (Connell 1975, Menge and Sutherland 1976, Menge and Sutherland 1987, Menge et al. 1996, Thompson et al. 2004), with one exception (Fig. 5 A). As expected, salinity weakened top-down effect of *Paria* beetles on plants. In contrast, salinity stress strengthened top-down effect of *Armases* crabs on plants. The reason for different effects of salinity on *Paria* and *Armases* is unclear, because both consumers prefer to eat *Iva* plants experiencing high salinities (Goranson et al. 2004). Although previous studies in salt marshes have found weaker top-down forces and trophic cascades with increasing salinity stress (Moon and Stiling 2002a, Moon and Stiling 2004, Stiling and Moon 2005), our study suggests that bottom-up effects on top-down forces depends on how the plants react to the bottom-up factors

and how consumers react to the changes produced on the plants (Goranson et al. 2004).

The interactive effects of nutrients and salinity stress on top-down forces were in accord with previous studies in salt marshes (Moon and Stiling 2002a, Moon and Stiling 2004, Stiling and Moon 2005) in that when both treatments were applied together, salinity stress cancelled the positive effects of nutrient addition on top-down forces.

Finally, side-to-side effects in this study changed the strength of top-down forces. First, beetles had a negative effect on top-down control of aphid populations by crabs, and therefore had a positive indirect effect on aphids. Second, aphids had a negative effect on top-down control of plants by beetles and therefore had a positive indirect effect on plants. Our study did not investigate in detail the mechanisms involved in these side-to-side interactions, and more detailed studies specifically addressing these interactions would be needed to fully understand the mechanisms involved. However, these results add to the growing number of studies in the last few years that show how important side-to-side interactions can be on the regulation of community structure (Denno et al. 1995, Denno et al. 2000, Moon and Stiling 2002b, Vos et al. 2004, Kaplan et al. 2007).

In summary, bottom-up effects from nutrients and salinity stress had positive and negative effects on plant traits, respectively. At the herbivore level, however, nutrients and salinity stress had variable effects, suggesting that the nature of bottom-up effects on herbivores depends on differences in how

different herbivores react to the physiological changes produced in the plants. Crabs had weak direct top-down effects on plants, but strong direct top-down effects on herbivores, and strong indirect top-down effects on plants due to their flexible diet. While bottom-up forces did not alter trophic cascades, direct top-down forces on plants and herbivores were altered in complicated ways by bottom-up forces. Nutrient additions in general increased the strength of direct top-down forces coming from herbivores and the omnivore. Salinity stress, in contrast, had variable and opposite effects on direct top-down forces on the plants. Finally, side-to-side interactions affected the strength of top-down forces coming from the omnivore and from the herbivore beetle. These results from our study show how bottom-up, top-down and side-to-side factors can interact in complicated ways to determine the structure of a community and illustrate how the development of a good predictive model of community structure can be complicated by the presence of interactions among regulating factors.

Acknowledgments

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Tables

Table 1: Summary table of *P*-values from analysis of variance tests of the effects of salinity stress (low or high), nutrient availability (ambient or addition), omnivorous crabs (presence or absence) aphids (presence or absence), beetles (presence or absence) and their interactions on crab damage, aphid abundance, beetle damage, chlorophyll (Chlo.) content, number of leaves (# of L.), number of healthy leaves (# of H.L.), change in number of leaves (Δ # of L.), plant height and plant growth. Complete ANOVA tables are presented in digital appendix B. Significant *P*-values are presented in bold for clarity. NT=not tested; treatments for which a variable was always zero were not included in ANOVA models to avoid problems with heterogeneity of variances.

Source	<i>P</i>								
	Crab damage	Aphid abundance	Beetle damage	Chlo. content	# of L. # of L.	Δ # of L.	# of H. L.	Plant height	Plant growth
Salinity (S)	0.60	0.15	<.0001	0.13	0.30	0.04	0.10	<.0001	<.0001
Nutrients (N)	0.007	0.03	0.003	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
S*N	0.46	0.20	<.0001	0.25	0.07	0.003	0.11	0.005	0.02
Aphid (A)	0.45	NT	0.33	0.08	0.39	0.06	0.62	0.92	0.87

S*A	0.07	NT	0.61	0.32	0.93	0.96	0.95	0.33	0.72
N*A	0.49	NT	0.24	0.004	0.49	0.81	0.65	0.79	0.63
S*N*A	0.44	NT	0.06	0.63	0.51	0.86	0.71	0.12	0.09
Beetle (B)	0.74	0.85	NT	0.64	0.001	0.0006	<.0001	<.0001	0.0004
S*B	0.11	0.83	NT	0.82	0.31	0.27	0.32	0.03	0.06
N*B	0.28	0.08	NT	0.047	0.87	0.45	0.68	0.21	0.28
S*N*B	0.91	0.39	NT	0.99	0.54	0.08	0.38	0.002	0.02
A*B	0.006	NT	NT	0.38	0.30	0.84	0.37	0.87	0.89
S*A*B	0.07	NT	NT	0.64	0.02	0.04	0.02	0.25	0.15
N*A*B	0.80	NT	NT	0.42	0.86	0.41	0.71	0.22	0.40
S*N*A*B	0.28	NT	NT	0.26	0.69	0.48	0.44	0.47	0.74
Crab (C)	NT	<.0001	<.0001	0.01	0.56	0.27	0.39	0.96	0.72
S*C	NT	0.41	0.70	0.01	0.67	0.21	0.50	0.47	0.90
N*C	NT	0.006	0.25	0.008	0.26	0.50	0.26	0.80	0.69
S*N*C	NT	0.06	0.06	0.85	0.52	0.84	0.50	0.64	0.76
A*C	NT	NT	0.14	0.85	0.52	0.92	0.51	0.86	0.71

S*A*C	NT	NT	0.14	1.00	0.70	0.25	0.57	0.41	0.68
N*A*C	NT	NT	0.10	0.42	0.89	0.54	0.79	0.57	0.36
S*N*A*C	NT	NT	0.82	0.78	0.83	0.34	0.98	0.40	0.46
B*C	NT	0.02	NT	0.03	0.13	0.23	0.22	0.003	0.07
S*B*C	NT	0.70	NT	0.84	0.07	0.28	0.07	0.86	0.50
N*B*C	NT	0.68	NT	0.82	0.75	0.22	0.62	0.98	0.99
S*N*B*C	NT	0.18	NT	0.28	0.44	0.47	0.56	0.49	0.75
A*B*C	NT	NT	NT	0.02	0.35	0.14	0.22	0.47	0.94
S*A*B*C	NT	NT	NT	0.84	0.85	0.97	0.86	0.23	0.49
N*A*B*C	NT	NT	NT	0.95	0.94	0.42	0.84	0.28	0.31
S*N*A*B*C	NT	NT	NT	0.55	0.46	0.09	0.57	0.52	0.99
I.H [§]	0.0006	NT	NT	0.02	NT	NT	NT	<.0001	NT
I.L [£]	NT	NT	0.0004	NT	<.0001	NT	<.0001	NT	NT

§: Initial plant height

£: Initial number of leaves

Figures:

Figure 1: Bottom-up effects of nutrient addition and salinity stress on chlorophyll content (**a**), number (#) of leaves (**b**), Plant height (**c**), plant growth (**d**), and change in number of leaves (**e**). N₀: no nutrient addition; N₊: nutrient addition; S₀: no salt addition; S₊: salt addition. ANOVA statistics represent the main effects of nutrients (**a** and **b**) or nutrients x salinity stress interaction (**c**, **d**, and **e**). Figures show means + 1 SE. Shared letters indicate means that are not significantly different.

Figure 2: Bottom-up effects of nutrient addition and salinity stress on aphid abundance (**a**), the number of leaves with crab damage (**b**), and the number of leaves with beetle damage (**c**). N₀: no nutrient addition; N₊: nutrient addition; S₀: no salt addition; S₊: salt addition. ANOVA statistics represent the main effects of nutrients (**a** and **b**) or the nutrients x salinity stress interaction (**c**). Figures show means + 1 SE. Shared letters indicate means that are not significantly different.

Figure 3: Top-down effects of *Armases* crab presence at the herbivore (**a** and **b**) and plant (**c** and **d**) levels, and top-down interactive effects of *Paria* beetle and *Armases* crab presence on plant height (trophic cascade of *Armases* presence; **e** and **f**). B₋: beetle absent; B₊: beetle present; A₋: aphid absent; A₊: aphid present; C₋: crab absent; C₊: crab present. ANOVA statistics represent the crab x beetle interaction (**a**), the effects of crab presence (**b**), the beetle x aphid interaction (**c**), the beetle x aphid x crab interaction (**d**), and the beetle x crab interaction (**f**).

Figures show means + 1 SE. Shared letters indicate means that are not significantly different.

Figure 4: Interactive effects of bottom-up ($S_0/S+$ or $N_0/N+$) and top-down forces (crab presence/absence) on chlorophyll content (**a** and **b**) and aphid abundance (**c**). S_0 : no salt addition; $S+$: salt addition; N_0 : no nutrient addition; $N+$: nutrient addition; $C-$: crab absent; $C+$: crab present. ANOVA statistics represent the salinity stress x crab interaction (**a**) and the nutrient x crab interaction (**b** and **c**). Figures show means + 1 SE. Shared letters indicate means that are not significantly different.

Figure 5: Interactive effects of bottom-up ($S_0/S+$ or $N_0/N+$) and top-down factors (beetle and aphid presence/absence) on plant traits. S_0 : no salt addition; $S+$: salt addition; N_0 : no nutrient addition; $N+$: nutrient addition; $B-$: beetle absent; $B+$: beetle present; $A-$: aphid absent; $A+$: aphid present. ANOVA statistics represent the salinity stress x nutrients x beetle interaction (**a** and **b**), the nutrients x aphids interaction (**c**), the nutrients x beetle interaction (**d**), or the salinity stress x aphids x beetles interaction (**e**, **f**, and **g**). Figures show means + 1 SE. Shared letters indicate means that are not significantly different.

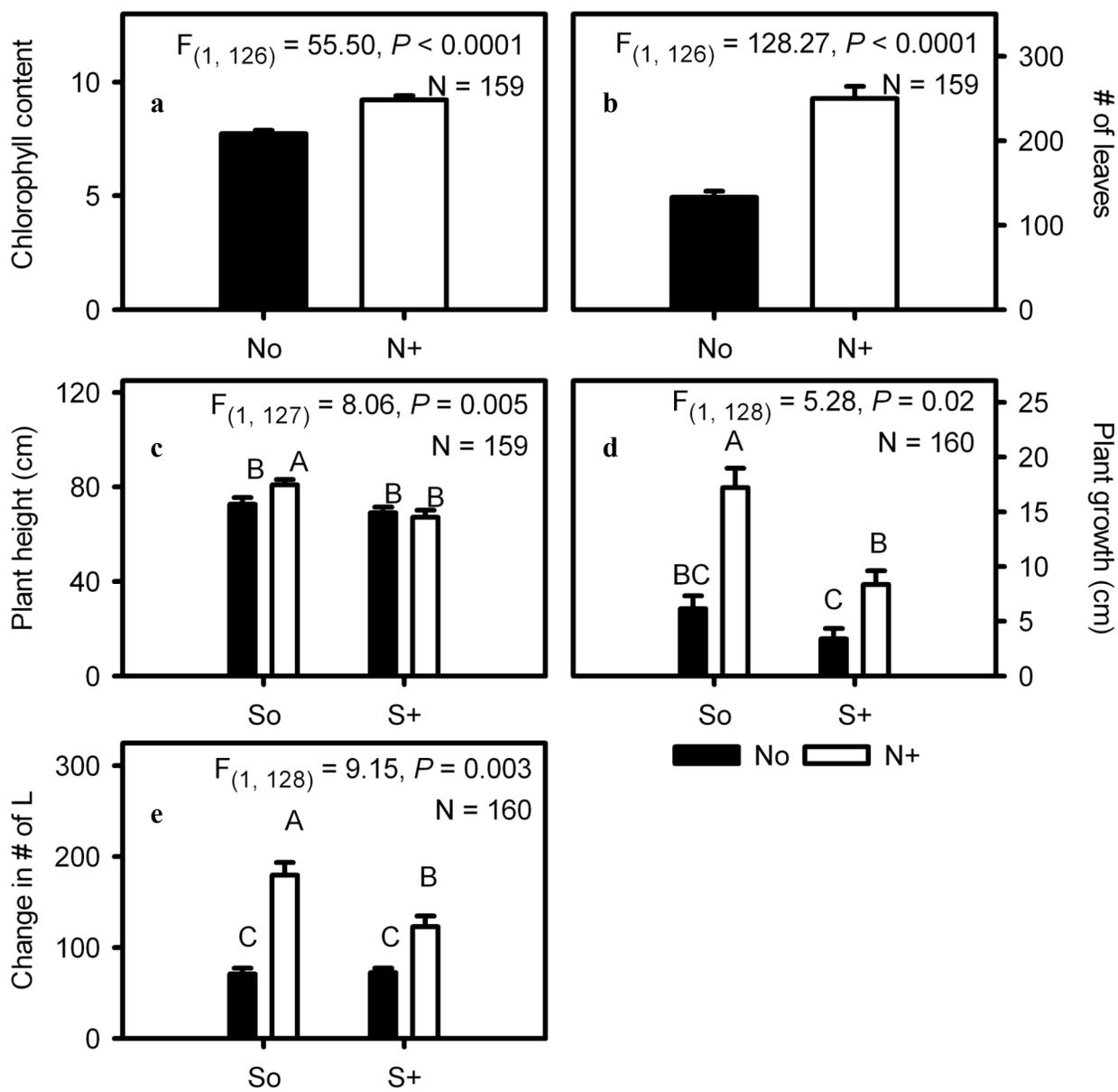


Figure 1

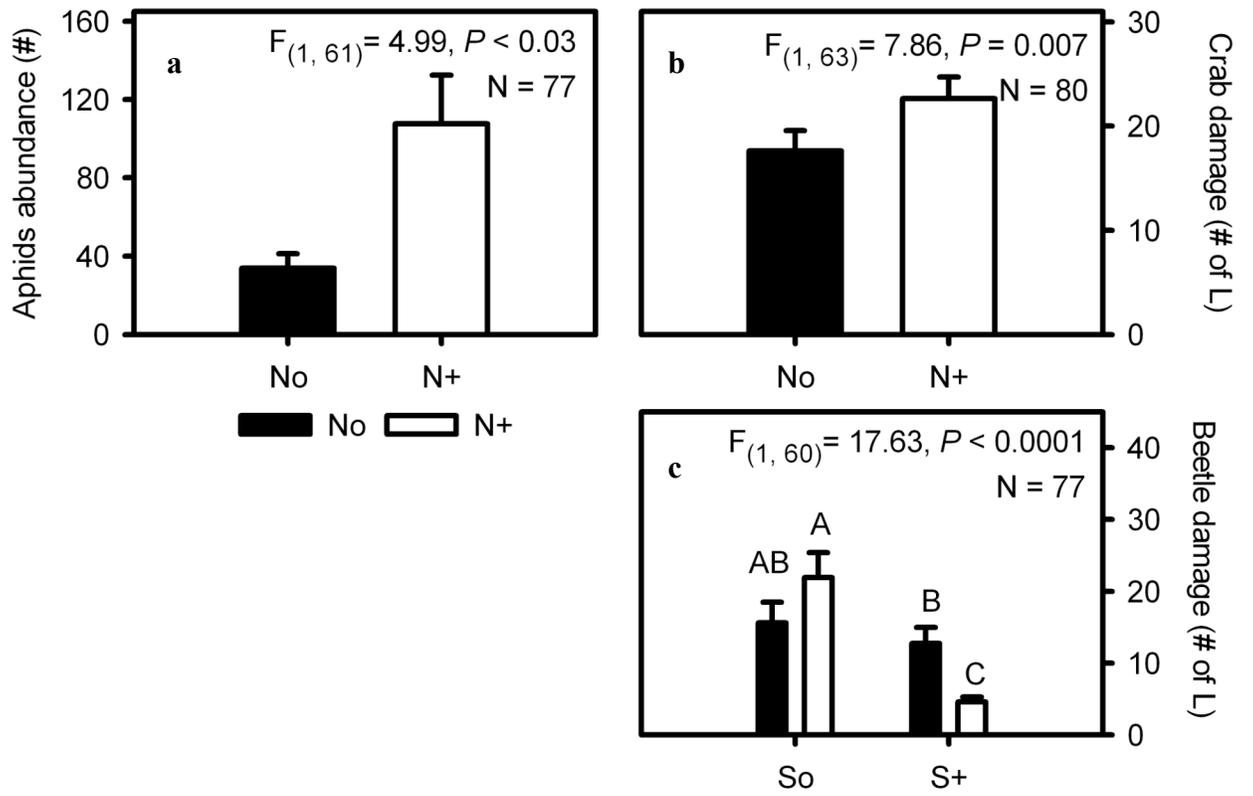


Figure 2

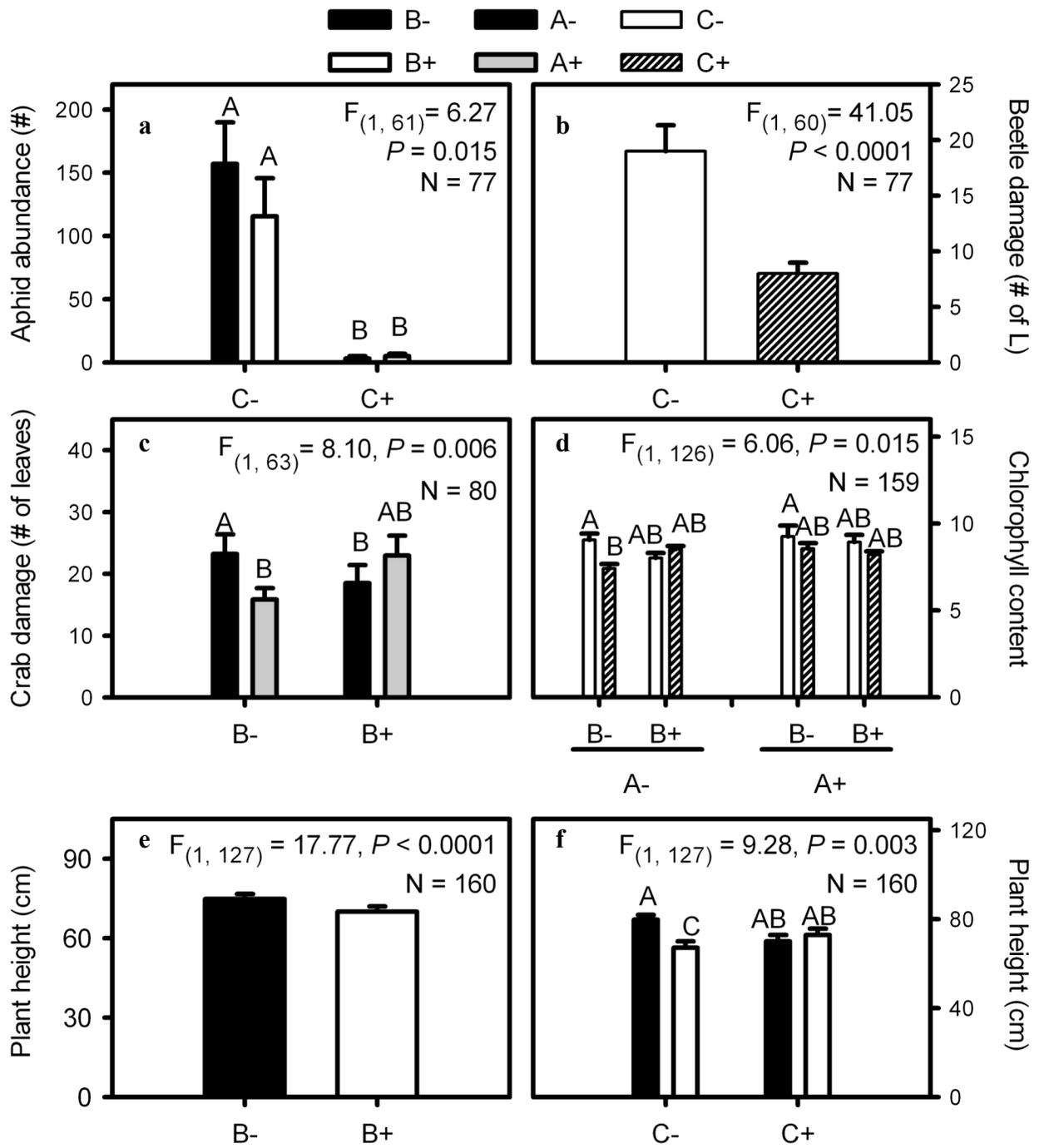


Figure 3

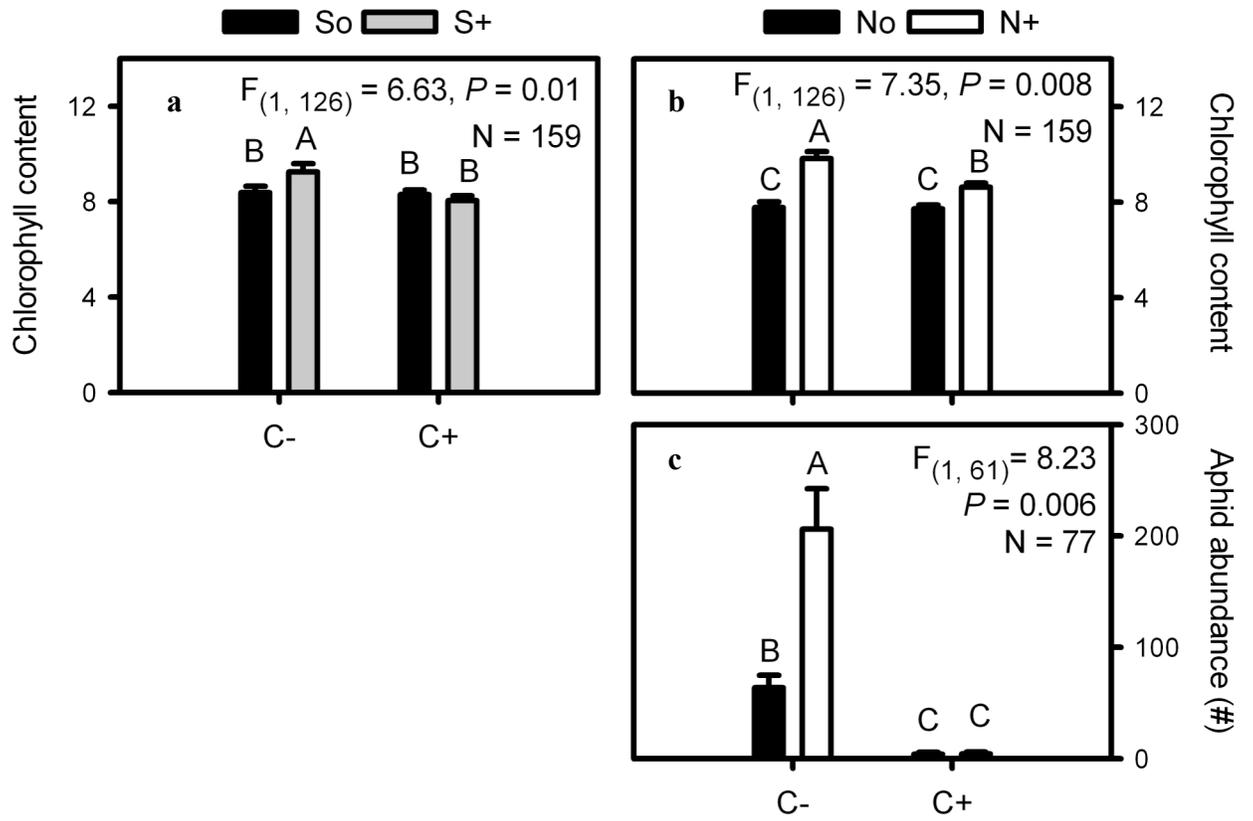


Figure 4

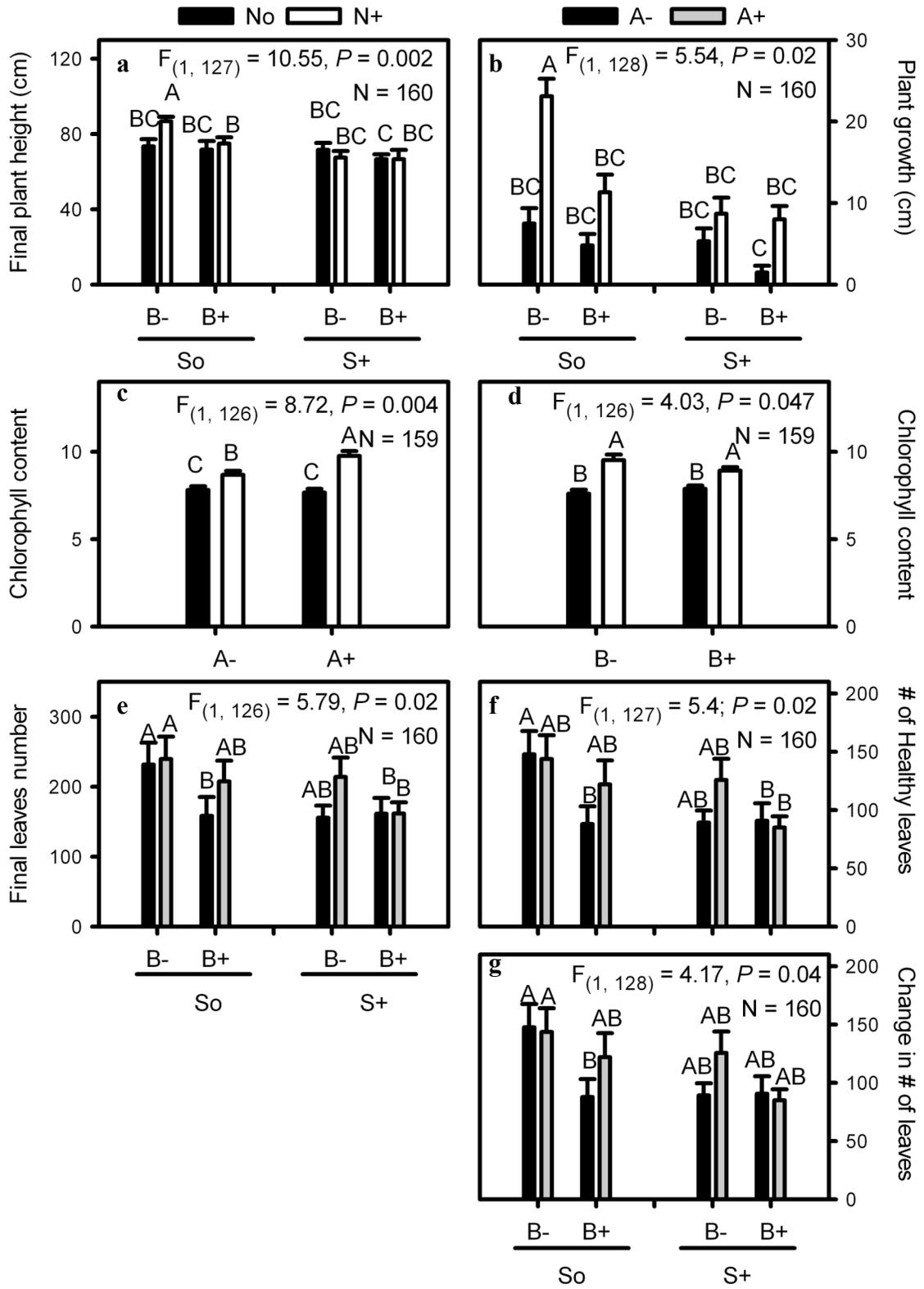


Figure 5

APPENDIX A:

Table 1A: Experimental design for the *Iva frutescens* salt marsh shrub trophic system. The levels of the omnivore treatment were completely crossed with the levels of the two herbivore treatments, and the levels of the nutrient addition and salinity stress treatments (Factorial design: 2 x 2 x 2 x 2 x 2). The omnivore and herbivore treatments are illustrated at the top of the figure and the nutrients and salinity stress treatments are illustrated in the left margins of the figure.

Nomenclature: C: crab (*Armases cinereum* omnivore); A: aphid (*Uroleucon ambrosiae* sap-feeder); B: beetle (*Paria atterrима* leaf-chewer); *Iva*: *Iva frutescens* Shrub.

		Iva system															
		Omnivore treatment															
		Crab present (C+)				Crab absent (C-)											
		Aphid treatment				Aphid treatment											
		Aphid present (A+)		Aphid absent (A-)		Aphid present (A+)		Aphid absent (A-)									
		Beetle treatment		Beetle treatment		Beetle treatment		Beetle treatment									
B+		B-		B+		B-		B+		B-							
Salinity	Ambient	Nutrients		Ambient		Addition		Ambient		Addition		n = 40					
		n = 5		n = 5		n = 5		n = 5		n = 5		n = 5					
	Addition	Nutrients		Ambient		Addition		Ambient		Addition		n = 40					
		n = 5		n = 5		n = 5		n = 5		n = 5		n = 5					
		n = 20		n = 20		n = 20		n = 20		n = 20		n = 20		N = 160			

APPENDIX B:

Table 1B: Analysis of variance results for the effects of salinity stress (low or high), nutrient availability (ambient or addition), crab treatment (present or absent), aphid treatment (present or absent), beetle treatment (present or absent), and their interaction on (A) square root crab damage, (B) Log aphid abundance, (C) Log beetle damage, (D) Square root chlorophyll content, (E) Square root number of leaves, (F) Square root number of healthy leaves, (G) Square root change in number of leaves (H) plant height, and (I) plant growth. Initial plant height and initial number of leaves were included as covariates in some of the ANOVAs.

A: Square root crab damage					
Source	DF	SS	MS	F	<i>P</i>
Model	16	3964.91	247.81	2.51	0.005
Error	63	6212.50	98.61		
C. Total	79	10177.41			
Salinity (S)	1	27.37	27.37	0.28	0.60
Nutrients (N)	1	775.12	775.12	7.86	0.007
S*N	1	54.04	54.04	0.55	0.46
Aphid (A)	1	56.85	56.85	0.58	0.45
s*A	1	331.92	331.92	3.37	0.07
N*A	1	48.38	48.38	0.49	0.49
s*N*A	1	58.80	58.80	0.60	0.44
Beetle (B)	1	11.07	11.07	0.11	0.74
s*B	1	253.40	253.40	2.57	0.11
N*B	1	115.64	115.64	1.17	0.28
S*N*B	1	1.39	1.39	0.01	0.91
A*B	1	798.65	798.65	8.10	0.006
S*A*B	1	334.27	334.27	3.39	0.07
N*A*B	1	6.62	6.62	0.07	0.80
S*N*A*B	1	115.05	115.05	1.17	0.28
i Plant height	1	1276.92	1276.92	12.95	0.0006

B: Log aphid abundance

Source	DF	SS	MS	F	<i>P</i>
Model	15	66205.02	4413.67	19.10	<.0001
Error	61	14098.42	231.12		
C. Total	76	80303.43			
S	1	492.94	492.94	2.13	0.15
N	1	1153.92	1153.92	4.99	0.03
S*N	1	379.71	379.71	1.64	0.20
Beetle (B)	1	8.82	8.82	0.04	0.85
S*B	1	10.81	10.81	0.05	0.83
N*B	1	752.49	752.49	3.26	0.0761
S*N*B	1	174.89	174.89	0.76	0.3878
Crab (C)	1	57585.13	57585.13	249.16	<.0001
S*C	1	156.53	156.53	0.68	0.41
N*C	1	1903.10	1903.10	8.23	0.006
S*N*C	1	825.18	825.18	3.57	0.06
B*C	1	1449.48	1449.48	6.27	0.02
S*B*C	1	34.36	34.36	0.15	0.70
N*B*C	1	39.29	39.29	0.17	0.68
S*N*B*C	1	419.20	419.20	1.81	0.18

C: Log beetle damage

Source	DF	SS	MS	F	<i>P</i>
Model	16	4566.20	285.39	8.92	<.0001
Error	60	1920.46	32.01		
C. Total	76	6486.65			
Salinity (S)	1	1279.62	1279.62	39.98	<.0001
Nutrients (N)	1	306.63	306.63	9.58	0.003
S*N	1	564.36	564.36	17.63	<.0001
Aphid (A)	1	30.43	30.43	0.95	0.33
S*A	1	8.22	8.22	0.26	0.61
N*A	1	46.01	46.01	1.44	0.24
S*N*A	1	117.24	117.24	3.66	0.06
Crab (C)	1	1314.08	1314.08	41.06	<.0001
S*C	1	4.72	4.72	0.15	0.70
N*C	1	42.73	42.73	1.33	0.25
S*N*C	1	116.30	116.30	3.63	0.06
A*C	1	72.58	72.58	2.27	0.14
S*A*C	1	72.04	72.04	2.25	0.14
N*A*C	1	91.73	91.73	2.87	0.10
S*N*A*C	1	1.73	1.73	0.05	0.82
i Leaf #	1	457.52	457.52	14.29	0.0004

D: Square root chlorophyll content

Source	DF	SS	MS	F	P
Model	32	198.24	6.19	3.72	<.0001
Error	126	209.78	1.66		
C. Total	158	408.01			
Salinity (S)	1	3.96	3.96	2.38	0.13
Nutrients (N)	1	91.92	91.92	55.21	<.0001
S*N	1	2.24	2.24	1.34	0.25
Aphid (A)	1	5.13	5.13	3.08	0.08
S*A	1	1.65	1.65	0.99	0.32
N*A	1	14.52	14.52	8.72	0.004
S*N*A	1	0.39	0.39	0.24	0.63
Beetle (B)	1	0.37	0.37	0.23	0.64
S*B	1	0.09	0.09	0.05	0.82
N*B	1	6.72	6.72	4.03	0.047
S*N*B	1	0.00	0.00	0.00	0.99
A*B	1	1.29	1.29	0.78	0.38
S*A*B	1	0.36	0.36	0.22	0.64
N*A*B	1	1.10	1.10	0.66	0.42
S*N*A*B	1	2.13	2.13	1.28	0.26
Crab (C)	1	10.18	10.18	6.11	0.01
S*C	1	10.42	10.42	6.26	0.01
N*C	1	12.24	12.24	7.35	0.008
S*N*C	1	0.06	0.06	0.04	0.85
A*C	1	0.06	0.06	0.04	0.85
S*A*C	1	0.00	0.00	0.00	1.00
N*A*C	1	1.10	1.10	0.66	0.42
S*N*A*C	1	0.13	0.13	0.08	0.78
B*C	1	7.68	7.68	4.61	0.03
S*B*C	1	0.07	0.07	0.04	0.84
N*B*C	1	0.08	0.08	0.05	0.82
S*N*B*C	1	1.97	1.97	1.18	0.28
A*B*C	1	10.10	10.10	6.07	0.02
S*A*B*C	1	0.07	0.07	0.04	0.84
N*A*B*C	1	0.01	0.01	0.00	0.95
S*N*A*B*C	1	0.60	0.60	0.36	0.55
i Plant height	1	9.45	9.45	5.67	0.02

E: Square root number of Leaves

Source	DF	SS	MS	F	P
Model	32	1527464.10	47733.30	34.58	<.0001
Error	127	175322.10	1380.50		
C. Total	159	1702786.20			

Salinity (S)	1	1484.39	1484.39	1.08	0.30
Nutrients (N)	1	43031.89	43031.89	31.17	<.0001
S*N	1	4592.04	4592.04	3.33	0.07
Aphid (A)	1	1015.29	1015.29	0.74	0.39
S*A	1	10.04	10.04	0.01	0.93
N*A	1	648.97	648.97	0.47	0.49
S*N*A	1	611.10	611.10	0.44	0.51
Beetle (B)	1	15508.22	15508.22	11.23	0.001
S*B	1	1439.81	1439.81	1.04	0.31
N*B	1	37.88	37.88	0.03	0.87
S*N*B	1	518.60	518.60	0.38	0.54
A*B	1	1488.26	1488.26	1.08	0.30
S*A*B	1	7995.28	7995.28	5.79	0.02
N*A*B	1	40.61	40.61	0.03	0.86
S*N*A*B	1	221.91	221.91	0.16	0.69
Crab (C)	1	470.18	470.18	0.34	0.56
S*C	1	253.80	253.80	0.18	0.67
N*C	1	1797.66	1797.66	1.30	0.26
S*N*C	1	586.02	586.02	0.42	0.52
A*C	1	578.34	578.34	0.42	0.52
S*A*C	1	202.31	202.31	0.15	0.70
N*A*C	1	27.37	27.37	0.02	0.89
S*N*A*C	1	66.19	66.19	0.05	0.83
B*C	1	3289.05	3289.05	2.38	0.13
S*B*C	1	4530.83	4530.83	3.28	0.07
N*B*C	1	141.81	141.81	0.10	0.75
S*N*B*C	1	823.77	823.77	0.60	0.44
A*B*C	1	1190.81	1190.81	0.86	0.35
S*A*B*C	1	51.07	51.07	0.04	0.85
N*A*B*C	1	7.08	7.08	0.01	0.94
S*N*A*B*C	1	763.18	763.18	0.55	0.46
i Leaf #	1	746587.62	746587.62	540.81	<.0001

F: Square root number of Healthy leaves

Source	DF	SS	MS	F	P
Model	32	525393.61	16418.60	16.12	<.0001
Error	127	129334.84	1018.40		
C. Total	159	654728.45			
Salinity (S)	1	2866.76	2866.76	2.82	0.10
Nutrients (N)	1	35963.41	35963.41	35.31	<.0001
S*N	1	2670.67	2670.67	2.62	0.11
Aphid (A)	1	247.71	247.71	0.24	0.62

S*A	1	3.65	3.65	0.00	0.95
N*A	1	210.37	210.37	0.21	0.65
S*N*A	1	145.83	145.83	0.14	0.71
Beetle (B)	1	16954.56	16954.56	16.65	<.0001
S*B	1	1013.89	1013.89	1.00	0.32
N*B	1	171.30	171.30	0.17	0.68
S*N*B	1	777.11	777.11	0.76	0.38
A*B	1	838.44	838.44	0.82	0.37
S*A*B	1	5525.45	5525.45	5.43	0.02
N*A*B	1	138.25	138.25	0.14	0.71
S*N*A*B	1	601.67	601.67	0.59	0.44
Crab (C)	1	751.22	751.22	0.74	0.39
S*C	1	475.46	475.46	0.47	0.50
N*C	1	1313.56	1313.56	1.29	0.26
S*N*C	1	457.38	457.38	0.45	0.50
A*C	1	434.92	434.92	0.43	0.51
S*A*C	1	335.47	335.47	0.33	0.57
N*A*C	1	71.73	71.73	0.07	0.79
S*N*A*C	1	0.80	0.80	0.00	0.98
B*C	1	1576.61	1576.61	1.55	0.22
S*B*C	1	3311.92	3311.92	3.25	0.07
N*B*C	1	248.65	248.65	0.24	0.62
S*N*B*C	1	350.06	350.06	0.34	0.56
A*B*C	1	1548.40	1548.40	1.52	0.22
S*A*B*C	1	33.86	33.86	0.03	0.86
N*A*B*C	1	40.38	40.38	0.04	0.84
S*N*A*B*C	1	324.39	324.39	0.32	0.57
i Leaf #	1	196721.57	196721.57	193.17	<.0001

G: Square root change in number of leaves

Source	DF	SS	MS	F	P
Model	31	328672.04	10602.30	4.16	<.0001
Error	128	326056.41	2547.30		
C. Total	159	654728.45			
Salinity (S)	1	11071.52	11071.52	4.35	0.04
Nutrients (N)	1	185701.90	185701.90	72.90	<.0001
S*N	1	23311.04	23311.04	9.15	0.003
Aphid (A)	1	9397.50	9397.50	3.69	0.06
S*A	1	7.79	7.79	0.00	0.96
N*A	1	154.99	154.99	0.06	0.81
S*N*A	1	83.61	83.61	0.03	0.86
Beetle (B)	1	31152.01	31152.01	12.23	0.0006

S*B	1	3145.36	3145.36	1.23	0.27
N*B	1	1457.99	1457.99	0.57	0.45
S*N*B	1	7710.25	7710.25	3.03	0.08
A*B	1	107.31	107.31	0.04	0.84
S*A*B	1	10625.75	10625.75	4.17	0.04
N*A*B	1	1726.48	1726.48	0.68	0.41
S*N*A*B	1	1283.62	1283.62	0.50	0.48
Crab (C)	1	3126.11	3126.11	1.23	0.27
S*C	1	4075.49	4075.49	1.60	0.21
N*C	1	1189.13	1189.13	0.47	0.50
S*N*C	1	99.12	99.12	0.04	0.84
A*C	1	28.35	28.35	0.01	0.92
S*A*C	1	3358.93	3358.93	1.32	0.25
N*A*C	1	984.78	984.78	0.39	0.54
S*N*A*C	1	2314.86	2314.86	0.91	0.34
B*C	1	3651.19	3651.19	1.43	0.23
S*B*C	1	2999.14	2999.14	1.18	0.28
N*B*C	1	3886.02	3886.02	1.53	0.22
S*N*B*C	1	1308.54	1308.54	0.51	0.47
A*B*C	1	5552.91	5552.91	2.18	0.14
S*A*B*C	1	2.75	2.75	0.00	0.97
N*A*B*C	1	1691.39	1691.39	0.66	0.42
S*N*A*B*C	1	7466.20	7466.20	2.93	0.09

H: Plant Height

Source	DF	SS	MS	F	P
Model	32	41171.52	1286.61	26.06	<.0001
Error	127	6270.38	49.37		
C. Total	159	47441.90			
Salinity (S)	1	1687.16	1687.16	34.17	<.0001
Nutrients (N)	1	1995.47	1995.47	40.42	<.0001
S*N	1	397.84	397.84	8.06	0.005
Aphid (A)	1	0.55	0.55	0.01	0.92
S*A	1	46.32	46.32	0.94	0.33
N*A	1	3.69	3.69	0.07	0.79
S*N*A	1	119.50	119.50	2.42	0.12
Beetle (B)	1	877.55	877.55	17.77	<.0001
S*B	1	248.52	248.52	5.03	0.03
N*B	1	79.83	79.83	1.62	0.21
S*N*B	1	520.94	520.94	10.55	0.00
A*B	1	1.26	1.26	0.03	0.87
S*A*B	1	65.91	65.91	1.34	0.25

N*A*B	1	74.52	74.52	1.51	0.22
S*N*A*B	1	26.01	26.01	0.53	0.47
Crab (C)	1	0.13	0.13	0.00	0.96
S*C	1	26.37	26.37	0.53	0.47
N*C	1	3.35	3.35	0.07	0.80
S*N*C	1	10.91	10.91	0.22	0.64
A*C	1	1.45	1.45	0.03	0.86
S*A*C	1	33.85	33.85	0.69	0.41
N*A*C	1	16.23	16.23	0.33	0.57
S*N*A*C	1	35.37	35.37	0.72	0.40
B*C	1	457.97	457.97	9.28	0.003
S*B*C	1	1.46	1.46	0.03	0.86
N*B*C	1	0.02	0.02	0.00	0.98
S*N*B*C	1	23.13	23.13	0.47	0.49
A*B*C	1	25.52	25.52	0.52	0.47
S*A*B*C	1	72.96	72.96	1.48	0.23
N*A*B*C	1	58.22	58.22	1.18	0.28
S*N*A*B*C	1	20.31	20.31	0.41	0.52
i Plant height	1	26922.07	26922.07	545.28	<.0001

I: Plant growth

Source	DF	SS	MS	F	P
Model	31	6331.12	204.23	3.28	<.0001
Error	128	7958.42	62.18		
C. Total	159	14289.54			
Salinity (S)	1	1255.18	1255.18	20.19	<.0001
Nutrients (N)	1	2419.49	2419.49	38.91	<.0001
S*N	1	328.45	328.45	5.28	0.02
Aphid (A)	1	1.73	1.73	0.03	0.87
S*A	1	7.85	7.85	0.13	0.72
N*A	1	14.17	14.17	0.23	0.63
S*N*A	1	177.42	177.42	2.85	0.09
Beetle (B)	1	837.15	837.15	13.46	0.0004
S*B	1	222.61	222.61	3.58	0.06
N*B	1	74.09	74.09	1.19	0.28
S*N*B	1	344.26	344.26	5.54	0.02
A*B	1	1.23	1.23	0.02	0.89
S*A*B	1	128.01	128.01	2.06	0.15
N*A*B	1	43.98	43.98	0.71	0.40
S*N*A*B	1	6.87	6.87	0.11	0.74
Crab (C)	1	8.10	8.10	0.13	0.72
S*C	1	1.01	1.01	0.02	0.90

N*C	1	10.09	10.09	0.16	0.69
S*N*C	1	5.96	5.96	0.10	0.76
A*C	1	8.38	8.38	0.13	0.71
S*A*C	1	10.97	10.97	0.18	0.68
N*A*C	1	51.82	51.82	0.83	0.36
S*N*A*C	1	33.44	33.44	0.54	0.46
B*C	1	208.84	208.84	3.36	0.07
S*B*C	1	28.53	28.53	0.46	0.50
N*B*C	1	0.00	0.00	0.00	0.99
S*N*B*C	1	6.40	6.40	0.10	0.75
A*B*C	1	0.39	0.39	0.01	0.94
S*A*B*C	1	30.10	30.10	0.48	0.49
N*A*B*C	1	64.58	64.58	1.04	0.31
S*N*A*B*C	1	0.02	0.02	0.00	0.99

CHAPTER 4

Unexpected effects of predators overwhelm effects of soil nutrients and salinity on a salt marsh shrub and its beetle herbivore.

Juan M. Jiménez

Abstract

To test the interactive effects of plant-stress and plant-vigor on plant (*Iva frutescens*) traits and herbivore (*Ophraella notulata*) performance, I manipulated nutrient levels (ambient and addition) and salinity stress (ambient and addition) levels and excluded predators using cages in a factorial field experiment. I also performed three paired feeding assays in the laboratory using leaves collected from the experimental plants in the field experiment to determine if *Ophraella* beetles preferred to eat leaves from plants treated with nutrients (N+), salt (S+) or both (N+S+) versus control (S₀N₀) plants. Paired feeding assays showed that *Ophraella* beetles preferred to eat leaves from untreated plants (control) than from salinity stressed plants because salinity stress decreased their nutrient content (chlorophyll content used as proxy). In contrast, *Ophraella* beetles did not show a preference between either nutrients treated and untreated plants or between nutrients treated-salinity stressed and untreated plants. Finally, in the field experiment, I found no evidence supporting either the plant-vigor or the plant-stress hypothesis due to overwhelming reproduction of the beetles under mesocosm conditions. This suggests that *Ophraella* beetles are under strong predator/parasitoid control under field conditions.

Introduction

Understanding patterns of herbivore population outbreaks and attacks on plants has long been a topic of research in agricultural, silvicultural and ecological sciences. Two hypotheses about plant-herbivore interactions that have received major attention are the plant stress hypothesis and the plant vigor hypothesis. Despite extensive research, however, tests of these two hypotheses have produced conflicting results (Waring and Cobb 1992, Koricheva et al. 1998). A possible explanation for the lack of consistent results is that in nature plants are exposed to several environmental gradients at the same time, each one of them potentially affecting plant vigor and stress. It is therefore logical to expect interactions among gradients that affect the conditions of the plants. Such interactions are not taken into account in univariate studies of plant-herbivore interactions along stress or vigor gradients, and might confuse the results of such studies.

The plant vigor hypothesis (Price 1991) predicts that herbivores will perform better on vigorous plants or plant parts with a higher nutrient content and a higher growth rate. Most of the support for this hypothesis comes from studies on galling insects (Price et al. 1990, Price 1997) and vertebrate herbivores (Danell and Hussdanell 1985, Danell et al. 1985). A recent study on vertebrate herbivores, however, showed that vertebrate herbivory can have a unimodal relationship with plant shoot vigor (Makhabu et al. 2006). In addition, a recent study with mangrove herbivores found a variable relationship between herbivory and plant vigor that differed among herbivore species (Feller and Chamberlain

2007). Finally, a recent study with cicadas found no relationship between herbivory and plant vigor (Yang 2006). Because of the variability in these results, more studies are needed to better understand when and how herbivore species are affected by plant vigor.

The plant stress hypothesis (White 1969, White 1984, Mattson and Haack 1987) predicts that environmental stresses to plants (and in particular water stress) decrease resistance to insect herbivory by mobilizing nutrients and otherwise altering foliar chemistry. Studies testing this hypothesis, however, have yielded contradictory results because 1) different plant species react different to a particular stress, and 2) different feeding guilds of herbivorous insects respond in different ways to stressed plants (Koricheva et al. 1998, Goranson et al. 2004, Scheirs and De Bruyn 2005). In addition, Hubberty and Denno (2004) have suggested that variation in the results of plant stress studies can be partly explained by whether stresses are pulsed or chronic. Again, more experimental studies are needed to clarify how plant stress affects herbivorous insects.

Because in natural environments plants and herbivores are exposed to a variety of environmental gradients at the same time, each one affecting vigor and stress, it is logical to suspect that there might be interactions between these gradients that could affect the palatability of plants to herbivores. For example, a given plant might be exposed to soils with high nutrient concentrations but also high soil interstitial salinities, and therefore the interaction of these two factors would determine the condition of the plant and its palatability to herbivores. To date, however, only a relatively small number of studies have examined how

interactions between environmental gradients affect the palatability of plants to herbivores (Preszler and Price 1995, Moon and Stiling 2002a, Moon and Stiling 2004); these studies have commonly found that interactions between environmental gradients can be quite important in mediating plant palatability. For example, Preszler and Price (1995) found that water-stress increased the effect of plant vigor because water-stress increased the slope of the linear relationship between miner oviposition and shoot length on wild *Salix*.

In salt marsh habitats there are strong gradients of nutrient supply and physical stress (salinity and waterlogging) that have important consequences for plant growth. For example, *Spartina alterniflora* grasses (Valiela et al. 1978) and *Iva frutescens* bushes (Levine et al. 1998) both vary in size depending on the environment in which they grow. Taller *Spartina alterniflora* plants are found near creeks due to more frequent tidal inundation lowering salinity stress and increasing nitrogen availability (Mendelsohn and Morris 2002). Similarly, taller *Iva frutescens* bushes are found on the upper edge of the high marsh. At lower elevations, *Iva* bushes are stunted due to more frequent inundation and salinity stress. Salinity stress and nitrogen availability interact in this habitat because salt blocks the uptake of ammonia by plants from soils, and because plants increase the production of nitrogen-rich compounds to maintain turgor pressure in the face of the low soil water potentials caused by dissolved salts (Morris 1984). It is therefore important to understand how these environmental gradients interact to affect plant palatability to herbivores. In this study I manipulated soil nutrient

availability and soil salinity in a factorial field experiment to evaluate their direct and interactive effects on plant fitness and herbivore performance.

Methods

I studied a salt marsh shrub, the marsh elder *Iva frutescens* L. (Asteraceae), and one of its herbivores, the beetle *Ophraella notulata* (Futuyma and McCafferty 1990). *Iva frutescens* (hereafter, *Iva*) is a common salt marsh shrub on the Atlantic and Gulf coasts of the United States, typically dominating the terrestrial border of the marsh (Bertness et al. 1992a, Bertness et al. 1992b, Bertness and Hacker 1994). The high-marsh soils, and therefore *Iva frutescens* roots, are only flooded during the higher tides of the month followed by prolonged intervals between flooding. During these periods of emergence the soil surrounding *Iva* can either dry out or be flushed by rainfall, and therefore plants can experience a wide range of soil interstitial salinities (Levine et al. 1998). *Iva* shrubs are exposed to nutrients from terrestrial runoff, groundwater and tidal waters.

A common and abundant herbivore on *Iva frutescens* is the beetle *Ophraella notulata* (Futuyma and McCafferty 1990) (hereafter, *Ophraella*). *Ophraella* lays its eggs on *Iva* leaves, and both larvae and adults are specialist feeders on *Iva frutescens* (Futuyma and McCafferty 1990, Ho and Pennings In Press). *Ophraella* can complete a generation in less than a month in warm temperatures (Ho personal communication).

Field experiment

In a factorial field experiment I manipulated soil nutrient content and salinity. I hypothesized that nutrient addition would benefit *Ophraella* by increasing *Iva* growth rate, number of leaves and leaf nutrient content. I also hypothesized that salt addition would benefit *Ophraella* by increasing *Iva* leaf nutrient content, although it might also reduce *Iva* growth rate and number of leaves. Finally, I hypothesized that salt addition would interact with nutrient addition by lowering the nitrogen and water absorption capacity of *Iva* plants.

Experimental work was done in the field at salt marsh near Matagorda, TX (28° 38.47'N and 95° 57.95' W). The experiment crossed two levels of nutrients (nutrient addition and ambient) with two levels of salinity stress (salt addition and ambient) in a two-way orthogonal design. *Iva* plants ($n = 80$) of similar size (ca. 125 cm) were selected in early March of 2005, tagged and randomly assigned to treatment combinations ($n = 20/\text{treatment}$).

In late April 2006 I initiated nutrient and salinity treatments and enclosed plants in cages. The nutrient treatment was initiated with a dose of 63g of nitrogen-based fertilizer (20:10:5 N:P:K fertilizer pellets, Forestry Suppliers) and were progressively increased by adding 42g of the fertilizer every subsequent month for a total of 231g over a period of 4 months. Fertilizer pellets were pressed 15 cm deep into the soil around the plant within a circle defined by the plant canopy. The salinity treatment was initiated with a dose of 750g of salt pellets (Morton's Solar salt pellets) and was progressively increased to a final level of 1500g of salt pellets every two to four weeks, for a total of ca 9000g over

a period of 4 months. Salt pellets were dispersed within a circle defined by the plant canopy. Plants were enclosed within a fabric cage that was 2 m tall, closed at the bottom around plant stems with plastic cable ties and closed at the top with elastic bands to allow easy access to the plants. Plants were vigorously shaken and visually examined before enclosing them to ensure no arthropods were left inside the cages. Plants were checked every 2-4 weeks and any unwanted arthropods found inside the cages were removed. It was very unlikely that there were unseen *Ophraella* eggs on the plants since the beginning of the experiment. *Ophraella notulata* beetles were rarely seen at the study site. This is the general case for the Texas coast; to collect the number of beetles necessary for the study I had to visit 10 different sites repeatedly for a period of three months.

Opraella beetles were collected from nearby marshes from early April through mid July, 2006, and maintained in Petri dishes in the laboratory at ca. 20°C. Beetles were fed with freshly collected *Iva* leaves. On July 11-13, 2006, I started the experiment by adding seven *Opraella* beetles to each cage. The experiment was stopped after one month on August 14, 2006.

As indicators of plant size and herbivory, I measured plant height, the total number leaves and number leaves with beetle damage (*Ophraella* feeding damage was visible as elliptical areas missing from the interior of the leaf) at the beginning and end of the experiment. Herbivore damage was essentially zero at the beginning of the experiment. As indicators of leaf quality, I measured chlorophyll content as a proxy for leaf nitrogen content (Opti-Sciences CCM-200 chlorophyll meter) and leaf toughness (Penetrometer type 516, Chatillon N.Y.).

Leaf traits were measured only at the end of the experiment, on healthy, undamaged leaves. If all the leaves on a plant were damaged by herbivores, chlorophyll content and leaf toughness were not scored (6 plants). I also counted the number of adults, larvae and pupae of the *Ophraella* beetle per plant at the end of the experiment. Data were analyzed with 2-way ANOVAs, with all factors treated as fixed effects. The number of adults, larvae, and pupae of *Ophraella* and the sum of their abundances were log transformed to meet assumptions of normality and homoscedasticity. Because the results of the analyses of the abundances of adult, larvae, and pupae were the same as the results of the analysis of their combined abundances, I only show the results of the later. Figures show untransformed data for clarity.

Beetle feeding preferences

To determine whether *Ophraella* beetles preferred to feed on plants treated with nutrients (N+), salt (S+) or both (N+S+) versus control (S_0N_0) plants, I performed three, two-choice feeding assays ($n = 20/\text{assay}$). *Ophraella* beetles were collected from the field and kept in Petri-dishes under laboratory conditions (2 weeks) and fed fresh *Iva* leaves collected from nearby salt marshes. Individual beetles were used only once. For the assays, beetles were housed individually within Petri dishes (100 x 15 mm), and offered a choice between a treated leaf (N+, S+ or N+S+) and a control leaf (S_0N_0). I used undamaged leaves collected from experimental plants; each plant contributed only one leaf to each assay. To reduce any effect of leaf age on herbivore preference, I collected leaves in

standard positions (second fully expanded leaf). Feeding assays were started on August 4th 2006. Individual replicates were checked twice a day and were terminated when substantial feeding (30%) on at least one leaf had occurred or at the end of the third day. Each feeding assay began with 20 replicates. Replicates in which both leaves were completely consumed or in which neither leaf was eaten after a period of 72 hours provided no information on plant palatability and were omitted (1 replicate). Data were analyzed with paired t-tests.

Results

Field experiment

Nutrient addition increased chlorophyll content by 9.7% and salt addition decreased chlorophyll content by 11.6% (Fig. 1). Nutrient and salt addition did not affect any other plant trait measured, and did not affect the abundance of *Ophraella* beetles or beetle damage to plants (Table 1). When I checked the experiment after two weeks, herbivore damage to plants was modest; however, when I checked it after four weeks, the *Ophraella* population had increased sharply (to a maximum of 93 and an average of 13.45 ± 2.43 beetles plus larvae per plant; mean \pm SE), a high proportion of leaves were damaged, and some plants were essentially defoliated. The average number of beetles (adults plus larvae) does not reflect the sharp population increase of beetles because many plants had almost no healthy leaves left and therefore most of the beetles in these plants had died when the experiment finished.

Beetle feeding preferences

Ophraella beetles preferred control (S_0N_0) leaves over salinity-stressed ($S+$) leaves (Fig. 2). However, *Ophraella* beetles did not show a preference between nutrient addition ($N+$) leaves and controls (S_0N_0) or between nutrient addition-salinity stressed ($N+, S+$) leaves versus controls (S_0N_0) (Fig. 2).

Discussion

The results of this experiment must be interpreted with caution due to the massive increase in beetle numbers and severe defoliation of plants. In retrospect, it would have been better to have stocked the cages with fewer beetles, or to have stopped the experiment after less time had passed. Unfortunately, because the field site was at a remote location, I could only check it periodically, and the rapid increase in *Ophraella* numbers caught me by surprise.

Both nutrient addition and salinity stress had significant but weak effects on the chlorophyll content of the *Iva* leaves. However, while the effects of nutrients on chlorophyll content were in accord with the plant-vigor hypothesis (Price 1991), because it increased chlorophyll content (by ca 10%), the effects of salinity stress were opposed to the predictions of the plant-stress hypothesis (White 1969, White 1984, Mattson and Haack 1987), because it decreased chlorophyll content (by ca 12%). The effects of salinity stress on chlorophyll content are intriguing because in a previous mesocosm experiment of ours

(chapter 2) salinity stress increased *Iva* chlorophyll content (by ca 9%). The difference in the time of exposure to the salinity stress treatment between the two experiments could potentially explain the contrasting results; in the present field experiment I exposed the plants to the salinity treatments for 14 weeks. In contrast, in the previous mesocosm experiment I exposed the plants to the salinity stress treatment for only 8 weeks. It is logical to expect that the longer the plants be exposed to the salinity treatments the higher the stress suffered by the plants. Huberty and Denno (2004) have argued that when water-stress (induced by our salinity treatment) is pulsed, plants can respond by increasing the concentration of soluble nitrogen-rich molecules in the plant tissues which in turn could affect the chlorophyll content of the leaves making them more palatable for herbivores. In contrast, when water-stress is chronic plant quality and palatability decrease.

The effects of salinity stress on chlorophyll content explain the results from the paired feeding choice assay between salinity stressed (S+) and control plants (not treated with salt or nutrients: S₀N₀). *Ophraella* beetles preferred to feed on control plants twice as much as on salinity stressed plants. In contrast, despite the fact that nutrient addition increased leaf chlorophyll content *Ophraella* beetles did not show a preference between nutrient treated (N+) and control plants (S₀N₀). It is probable the weak positive effect of nutrient addition on chlorophyll content was not big enough (ca 10%) to impact the feeding preference of the beetle. In our previous mesocosm experiment (Chapter 2) nutrient addition

increased *Iva* chlorophyll content by 16% and that increased the damage by the beetle *Paria aterrima* (ca 27%).

The lack of effects of nutrient and salinity additions on herbivore damage during the field experiment, despite their effects on chlorophyll content, can be explained by the strong population increase of *Ophraella* beetles inside the mesocosms. The mesocosms were stocked with 7 beetles each. I had not previously conducted field mesocosm experiments with *Ophraella*, and did not know whether their populations would increase or decrease. *Ophraella* beetles reproduced intensively, producing a maximum of 93 *Ophraella* (adults plus larvae) in a single mesocosm by the end of the experiment. As a result, most plants were heavily damaged, and many were essentially defoliated. This rapid population increase overwhelmed any effects on beetle population size that might have been caused by subtle differences in plant quality, and created a strong top-down pressure on plants that might have masked any bottom-up effects of nutrient levels and salinity stress on plant traits and herbivore damage.

Although this rapid increase in beetle numbers prevented us from testing our hypotheses about plant vigor and plant stress, it suggests the interesting possibility that in the field *Ophraella* beetles are strongly controlled by predators. *Ophraella* beetles were rare at all the sites visited in the Texas Gulf Coast (Pers. Obs). In addition I have never observed *Ophraella* beetle populations increase to the point that they defoliate plants at any site during extensive surveys along the Atlantic and Gulf coasts. Thus, it is likely that predators and parasites are strongly controlling *Ophraella* populations. Little is known about predation on salt

marsh beetles. However, Ho and Pennings (In Press) found that the omnivorous crab *Armases cinereum* had a strong top-down control on *Ophraella* under mesocosm conditions but not under field conditions. It is probable that the top-down effects on *Ophraella* on the field were masked by the presence of predators other than *Armases*, such as predatory insect, spiders, lizards and birds because the experimental design only excluded *Armases* from the plants. It would be productive to conduct further experiments exploring the importance of predators in regulating *Ophraella* populations.

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Tables

Table 1: A: Summary table of ANOVA results ($P < 0.05$) for the effects of salinity and nutrient treatments and their interaction on plant height, number of leaves, number of leaves with beetle damage, number of *Ophraella* beetles per plant (including adults and larvae), chlorophyll content and leaf toughness. Significant effects are indicated in bold. **B:** Means and standard errors of the variables measured per treatment combination. S*N: Salinity by nutrient interaction; S₀: no salt added; S₊: salt added; N₀: no nutrients added; N₊: nutrients added.

A: ANOVAs summary table

Source	<i>P</i>					
	Plant Height	# of Leaves	Beetle damage	Beetle abundance	Chlorophyll content	Leaf toughness
Salinity	0.26	0.52	0.93	0.51	0.01	0.66
Nutrient	0.40	0.68	0.83	0.98	0.04	0.25
S*N	0.62	0.64	0.90	0.93	0.46	0.84

B: Means of variables measured

		Mean ± SE					
		Plant Height	# of Leaves	Beetle damage	Beetle abundance	Chlorophyll content	Leaf toughness
S	N	126.9 ± 3.2	256.4 ± 35.0	99.0 ± 20.9	12.2 ± 3.5	9.4 ± 0.4	161.4 ± 7.7
S	N+	127.9 ± 3.5	258.6 ± 37.4	97.0 ± 20.7	12.6 ± 5.6	10.8 ± 0.4	154.0 ± 8.1
S+	N	121.9 ± 2.0	250.6 ± 30.2	103.4 ± 22.5	14.1 ± 5.7	8.6 ± 0.4	166.5 ± 6.8
S+	N+	126.0 ± 3.3	219.4 ± 37.8	96.1 ± 19.9	15.3 ± 4.6	9.2 ± 0.7	155.8 ± 7.9

Figures

Figure 1: Effect of nutrient addition and salinity stress on chlorophyll content of *Iva frutescens* leaves. ANOVA statistics represent main factor effects. Data are means + 1 SE.

Figure 2: Consumption of *Iva frutescens* leaves by *Ophraella notulata* beetles in laboratory paired feeding assays. Leaves used in the experiment came from plants treated with salt (S+), nutrients (N+), nutrients and salt (N+S+) or not treated at all (S₀N₀: control plants). Data are mean + 1 SE.

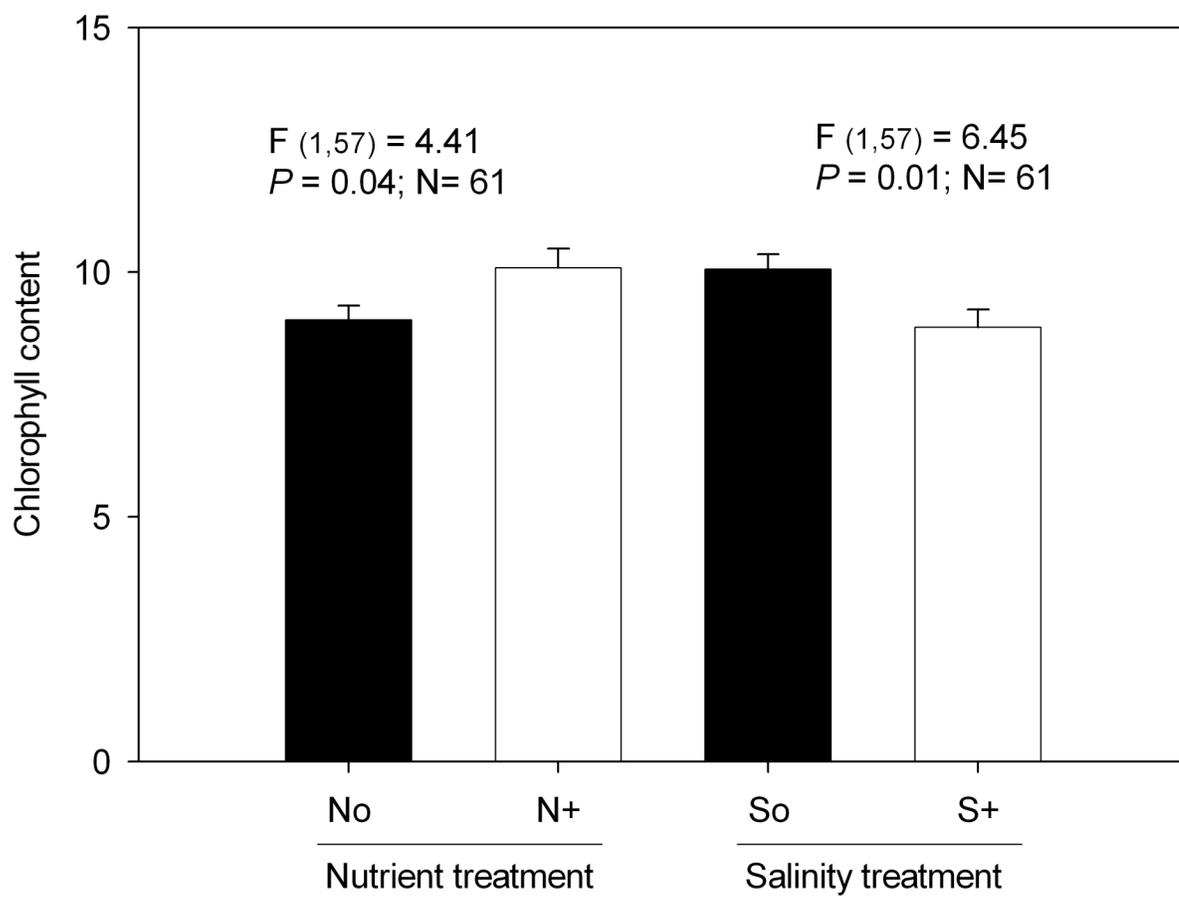


Figure 1

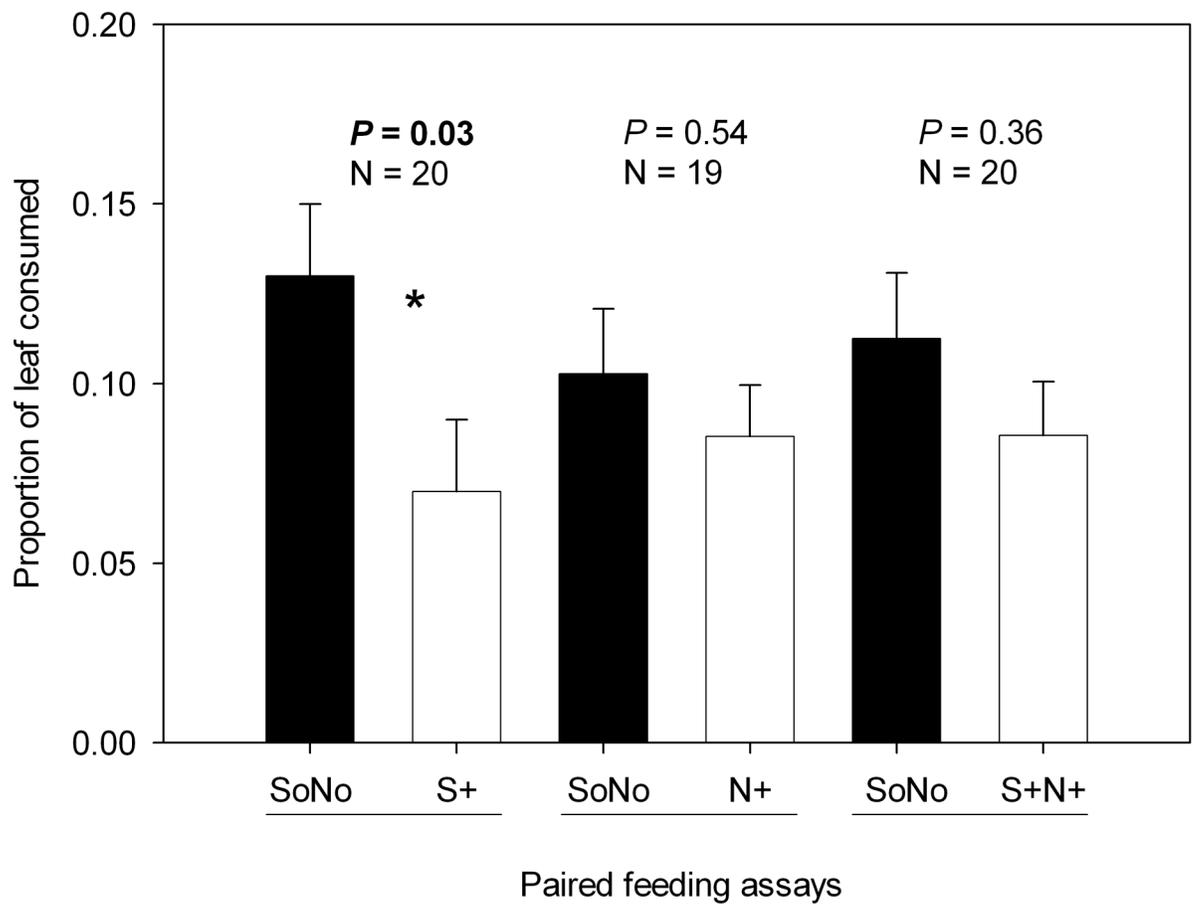


Figure 2:

CHAPTER 5

Decreasing habitat complexity increases top-down control of the *Iva frutescens* food web

Juan M. Jiménez

Abstract

Despite intense research there are still no general rules as to how the structure of communities is mediated. Because of the complexity of the processes that regulate community structure, there may be interactions among the factors that regulate top-down and bottom-up effects. In a field factorial experiment, I examined how the levels of nutrients (addition, control and subtraction), salinity stress (addition and control) and habitat complexity (low and control) interacted to affect the structure of the arthropod community living on the *Iva frutescens* shrub. Overall the three treatments interacted to affect community structure but the effects depended on the predator and herbivore guild being measured. In contrast with previous studies, which have usually found that decreasing habitat complexity reduces top-down effects, decreasing habitat complexity in this system increased the abundance of parasitoid wasps and intraguild predators (IGP; mostly jumping spiders) and decreased the number of leaves damaged by herbivorous *Ophraella notulata* beetles and acridid grasshoppers. However, the top-down effects of grasshoppers on leaves damage were only present when nutrients were added to the plants. Thus, in this system, decreasing habitat complexity increased top-down control of herbivore damage but only when nutrients were added to the plants. Finally, salinity stress cancelled the effects of nutrients and habitat complexity on IGP and increased the positive effects of nutrients on flies. These results indicate that habitat complexity does not always increase top-down control. Further studies focusing on the behavioral mechanisms leading to different effects of habitat complexity

on top-down effects in different systems may help explain these different results. Because the effects of habitat complexity may interact with nutrients and abiotic stress, these factors must be studied in integrated experiments.

Introduction

Because food webs are structured by both a variety of top-down (predation), side-to-side (competition), and bottom-up (plant quality and productivity) factors, obtaining a general theory of community structure is a complicated task. Despite intense research on several of these processes, we have not yet achieved a consensus about how they combine to structure biological communities (Hunter and Price 1992, Power 1992, Strong 1992).

Three factors that might regulate the structure of a community are soil nutrient levels, environmental stress levels and habitat complexity. Past studies exploring these factors have not identified consistent effects across studies. Because in nature none of these factors happens in isolation, it is possible that the lack of consistent results is due to interactions between these factors. Only one study, however, has explored the interaction between nutrient levels and habitat complexity (Denno et al. 2002) and only a handful of studies have explored the interaction between nutrient levels and environmental stress (Moon and Stiling 2000, 2002a, 2004). Therefore, the objective of this study was to explore how nutrients, salinity stress and habitat complexity interact to affect the structure of a food web.

The “Ecosystem Exploitation Hypothesis” (EEH) proposes that the abundance and diversity of herbivores and predators will be higher with increasing nutrient input eventually leading to stronger control of herbivores by predators (Fretwell 1977, Oksanen et al. 1981). Recent studies, however, have shown that this is not always the case, and that trophic cascades can instead be

weak in productive systems and strong in unproductive systems (Ritchie 2000, Chase 2003a). These contrasting results could be a consequence of interactions with other unaccounted factors, such as environmental stress and habitat complexity.

The environmental stress hypothesis proposes that increasing environmental stress will decrease the abundance and diversity of herbivores and predators, because only a few tolerant species will survive (Connell 1975, Menge and Sutherland 1976, Menge and Sutherland 1987). Only a handful of studies have evaluated the effects of environmental stress on community structure (Moon et al. 2000, Moon and Stiling 2000, 2002a, 2004), with the general result that increasing environmental stress increases the abundance of herbivores due to fewer and less effective predators and parasitoids. However, interactions with other factors such as nutrient levels can alter this result. In particular, increasing nutrient availability can dampen the negative effects of salinity stress on the abundance and the effectiveness of predators and parasitoids (Moon and Stiling 2000). It is important to note that in the system studied by Moon and Stiling, as in the work reported here, the salinity stress treatment directly affected plants, but consumers were affected indirectly by salinity through changes in plant quality, plant physiology and plant physical structure rather than directly (Moon and Stiling 2000, 2002a, 2004). Thus, these tests of the environmental stress hypothesis address scenarios slightly different from those envisioned by the original proponents of the hypothesis.

The habitat complexity hypothesis proposes that increasing habitat complexity will either increase or decrease herbivore abundance, depending on how habitat complexity affects predator-prey interactions. If structure reduces intraguild predation (Finke and Denno 2002, Grabowski 2004, Langellotto and Denno 2004), it will tend to increase predator diversity and abundance. Increased predator abundance may then reduce herbivore abundance; alternatively, herbivore densities may increase if structure also provides refuges for prey (Warfe and Barmuta 2004). As far as I know, only one study has explored the *interactive* effects of nutrients and habitat complexity on the relative strength of top-down and bottom-up forces (Denno et al. 2002), finding that increased habitat complexity promoted top-down control of herbivorous planthoppers by spiders; however, increasing nutrient availability to plants eventually led to an increase in planthoppers which overwhelmed top-down effects of spiders.

The interactive effects of nutrients, stress and habitat complexity on community structure are particularly important to understand in coastal ecosystems such as salt marshes. Salt marshes contain natural gradients of salinity and nutrient availability (Bertness and Pennings 2000) that interact to mediate plant productivity and species composition. Moreover, these gradients are changing as growing human populations in coastal regions affect estuarine habitats by increasing eutrophication (Valiela et al. 1992, Nixon 1995) and changing hydrological and salinity regimes (Copeland 1966, Hoese 1967, Alber 2002). Although a number of studies have addressed how these anthropogenic

impacts affect emergent wetland plants (Jefferies and Perkins 1977, Crain et al. 2004, Pennings et al. 2005), little is known about how they mediate food web interactions. Humans are also altering habitat complexity in salt marshes by building structures on or near marshes, by promoting the accumulation of trash and debris amid high-marsh vegetation and by landscaping the terrestrial borders of saltmarshes.

In order to arrive at a better understanding of the mechanisms regulating the structure of food webs, I used a factorial experiment to ask how soil nutrient levels, soil salinity levels and habitat complexity interact to affect the structure of the arthropod community on the shrub *Iva frutescens* and herbivore damage to the shrub.

The experiment tested the following overarching hypotheses: 1) nutrient addition to plants will increase the abundance of herbivores, leading to an increase in the abundance of predators; 2) increasing salinity stress will decrease the abundance of arthropods leading to lower herbivore damage, leading to a decrease in predators; 3) decreasing habitat complexity will reduce predator densities, leading to higher herbivore abundances and damage; 4) salinity stress will decrease the positive effects of nutrient addition on herbivores and predators; 5) nutrient addition will diminish the negative effects of lower complexity on herbivores by making plants more palatable and worthy of predation risk.

Methods:

To investigate the interactive effects of nutrients, salinity stress and habitat complexity on the *Iva frutescens* shrub, on the arthropod community living on *Iva* and on damage done to *Iva* by the herbivorous arthropods, I conducted a factorial field experiment in which I manipulated the amount of nutrients available to plants (low, control and high), soil salinity levels (control and high) and the degree of vegetation complexity (control and low) on the ground immediately surrounding the plant.

I marked 108 plants in late February 2007 and randomly assigned them to treatment combinations (12 treatments x 9 replicates=108 plants total). I increased nutrient availability to plants (N+) by initially adding eight 21g nitrogen based fertilizer pellets to each plant (April 16 2007) and then adding three extra pellets per plant every month thereafter. I decreased nutrient availability to plants (N-) by adding 400g of granular sucrose to a 1m² area surrounding each plant every two to four weeks starting April 16 2007. I increased soil salinity levels (S+) by adding 1500g of salt pellets to a 1m² area surrounding each plant every two to four weeks starting April 16 2007. I decreased vegetation complexity (L) by mowing the vegetation from a 1m² area surrounding a plant by using a weedwacker in April 15 2007. To document the natural variation of vegetation complexity surrounding the plants in our study site, I sampled the vegetation surrounding our control (N = 56) plants using 0.24 x 0.24 m quadrats at the end of the experiment, in September 15th 2007. I measured the height of the two tallest plants in each quadrat and then harvested all vegetation inside the

quadrats, dried it to a constant mass at 60°C, and weighed it. The vegetation surrounding *Iva* at this site was dominated by *Spartina patens*, *Juncus gerardii*, *Borrchia frutescens*, and *Phragmites australis* with a height of 120 ± 5.06 cm (mean \pm SE) and biomass of $112.13 \text{ g} \pm 6.25$ per 0.06 m^2 . The *Iva* plants were 188 ± 2.67 tall (mean \pm SE); thus, the adjacent vegetation surrounded about the lower half of each plant.

I also took initial and final measurements of plant height, number of leaves, number of leaves with beetle damage (specifically from *Ophraella notulata*; (Futuyma and McCafferty 1990), number of leaves with grasshopper damage, number of leaves with other damage (mostly miner damage), and leaf chlorophyll content. These measurements were taken on marked branches on each plant. At the end of the experiment, I took seven leaf samples at random per plant to measure the number of galls on each leaf. Also, a final sample of the arthropods present on the experimental plants was taken using a D-vac (N= 36; 3 replicates x 12 treatment combinations). For this the mouth of the D-vac (0.34 m in diameter) was swept vertically for 1 m at the same time that the vegetation was being beaten with the hand to dislodge as many arthropods from the plant as was possible. This was done on four sides per plant for a total swept area of 1.36 m^2 per plant. Each arthropod collected was assigned to one of the following feeding guilds: predators, intraguild predators (IGP), parasitoids, leaf chewers, flies and sap-feeders. The abundance of each arthropod guild was then calculated. Because *Ophraella* beetles are almost absent after the month of June in south Texas marshes, the number of leaves with *Ophraella* damage was a

better estimate of its abundance throughout the experiment. It is very likely that the damage scored at the end of the experiment was originally done between the months of February and June of 2006; in south Texas, *Ophraella* beetles start emerging from their eggs in February are most abundant between March and early June and are very rare after June (personal observation.).

Data on Plant traits and herbivore damage to *Iva* bushes were analyzed with a 3-way MANCOVA followed by individual ANOVAs for each variable measured. The logarithm of the initial number of leaves in the marked branch was included as the covariate. The densities of six arthropod guilds on *Iva* bushes were analyzed with a 3-way MANOVA followed by individual ANOVAs. All factors (nutrients, salinity, and vegetation complexity) were treated as fixed effects. While leaf number, beetle's damage, grasshopper's damage, miner's damage, predator's guild abundance, intraguild predator's abundance, parasitoid's abundance, leaf chewer's abundance, flies' abundance and sap-feeders' abundance were log-transformed, chlorophyll content was inversely transformed to meet the normality and homocedasticity assumptions. Tukey-HSD tests were performed when necessary.

Results

In total 1736 arthropods were collected and assigned to one of six guilds based on results of previous studies in the literature. The predator guild contained 4 assassin bug species, 1 pseudoscorpion species, 3 dragonfly species and 36 orbweaver species, but one orbweaver spider accounted for 45%

of the individuals. The IGP contained 10 jumping spider species, 2 crab spider species, and 1 mantid species, but one jumping spider accounted for 55% of the individuals. The parasitoid guild was composed of 27 wasp species but one of them accounted for 45% of the individuals. The leaf chewer guild was composed of 12 chrysomelid beetle species, 2 acridid grasshopper species and 2 lepidoptera larvae but one beetle accounted for 80% of the individuals. The sap-feeder guild was composed of 16 planthopper species and 2 thysanopteran species, but 2 planthopper species accounted for 53% of the individuals. The final guild consisted of flies that were difficult to assign to feeding mode, but were probably a mixture of folivores, gall makers, detritivores and fungivores.

While neither of the treatments in this experiment (nutrients, salinity stress or vegetation complexity) affected the abundance of predators, leaf chewing herbivores, or sap-feeders, the treatments did affect the number of parasitoids, intraguild predators (IGP), and flies on the plants (Table 1A and B). While decreasing complexity reduced the number of parasitoids present on the plants (Fig. 1a), increasing soil nutrients increased the number of parasitoids (Fig. 1b). Soil nutrient, salinity stress and vegetation complexity interacted to affect IGP abundance and fly abundance; decreasing complexity increased IGP abundance but only when nutrient were added (N+) and plants were not stressed by salinity (S) (Fig. 2a). Also, nutrient addition increased IGP abundance but only when vegetation complexity was low and plants were not stressed by salinity (Fig. 2a). Finally, nutrient addition increased fly abundance but only at ambient levels of vegetation complexity and when plants were stressed by salinity (Fig. 2b).

None of the treatments in this experiment (nutrients, salinity stress or vegetation complexity) significantly affected any of the plant traits measured (because natural variability masked treatment effects) but they did affect the amount of damage done by three herbivore guilds (Table 2a and b). Decreasing vegetation complexity reduced the number of leaves with beetle damage (Fig. 3a). Also, vegetation complexity interacted with nutrient levels to affect the number of leaves with grasshopper damage; decreasing vegetation complexity decreased the number of leaves with grasshopper damage, but only when nutrients were added to the plants (Fig. 3c). Decreasing soil nutrient levels reduced the number of leaves with miner damage, but increasing soil nutrient levels had no effect on miner damage (Fig. 3b). Finally, vegetation complexity interacted with salinity stress levels to affect the number of leaves with miner damage; increasing salinity stress reduced the number of leaves with miner damage when vegetation complexity was at ambient levels (control), but had no effect when vegetation complexity was lowered (Fig. 3d).

Discussion

Despite the fact that the three treatments (nutrients, salinity stress and vegetation complexity) used in this experiment did not significantly affect any of the plant traits measured (likely because environmental variation masked the effects of the treatments on plant traits), they did affect the number of leaves damaged by three types of herbivores (beetles, grasshoppers and leaf miners) and the abundance of one herbaceous and two predaceous arthropod guilds

(Tables 1 and 2). Overall, the three treatments interacted in complicated ways, altering each other's effects, and the effects of the treatments depended on the arthropod guild being measured.

Decreasing habitat complexity increased the abundance of two predaceous guilds but decreased the level of damage done by two herbaceous guilds. In my study, decreasing habitat complexity increased the abundance of parasitoid arthropods (mostly wasps), but decreased the number of leaves damaged by *Ophraella* beetles. Parasitoid wasps are known to lay their eggs inside other arthropods as well as inside arthropods' eggs and larvae (including those of beetles). These results could be explained by the dampening of chemical cues used by parasitoids to find their host in complex habitats: when host plants exist in mixed stands the chemical cues used by parasitoids to locate their host can be dampened; however when host-plants exist in pure stands the time to find their host is reduced (Gols et al. 2005). In contrast to previous studies, our results suggest that decreasing habitat complexity can increase the top-down control of communities. Previous studies have shown that increasing habitat complexity increases the diversity and abundance intraguild predators, decreases intraguild predation (Finke and Denno 2004, Grabowski 2004, Langellotto and Denno 2004) and therefore reduces herbivore abundance (sap-feeders). It is very likely that the contrasting results between my studies and those of other's are a product of different mechanisms involved community regulation by habitat complexity, which might be guild-dependent. Therefore, more studies looking into how habitat complexity affects the regulation of

communities of different arthropod guilds might prove useful in order to develop better predictive models.

In a similar way, habitat complexity also affected top-down control of grasshopper damage, but only when nutrients were present. Decreasing habitat complexity increased the abundance of IGP (mostly jumping spiders) but decreased the number of leaves damaged by grasshoppers; however, this pattern was only present when nutrients were added to the plants. Jumping spiders are known to actively hunt other arthropods including grasshoppers (Schmitz 2008). A possible mechanism for this result is that when habitat complexity is high jumping spiders have a larger vertical distribution than when habitat complexity is reduced; mowing the vegetation adjacent to *Iva* plants takes away other potential prey and reduces the amount of refuge, concentrating jumping spiders on the *Iva* bushes. This effect, however, was only present when nutrients were added to the plant. The interactive effects of habitat complexity and nutrient levels on IGP densities and grasshopper damage suggest that in our study system nutrient addition increases top-down control of communities but only in low complexity habitats.

In contrast, a previous study (Denno et al. 2002) showed that increasing habitat complexity promoted top-down control of herbivore planthoppers by spiders; however increasing nutrient availability to plants eventually led to an increase in the planthopper's population which overwhelmed the top-down effects of spiders. These contrasting results show that in order to develop good predictive models of community structure we need to first understand the

different mechanisms by which habitat complexity can affect the regulation of arthropod communities and then understand how each mechanism is affected by other factors such as nutrients.

The interactive effects of salinity stress, nutrients and habitat complexity on community structure are more complex and difficult to explain and changed depending on the feeding guild. Salinity stress either 1) canceled the effects of other interacting factors (nutrients and habitat complexity) or 2) it allowed for the positive effects of nutrients to be expressed. Salinity stress cancelled the interactive effects of nutrients and habitat complexity on the IGP's abundance. Previous studies have shown that salinity stress can either cancel/reduce the positive effects of nutrients on herbivores and their predators (Moon and Stiling 2004) or can have no effects on them (Albarracin and Stiling 2006). In contrast, salinity stress allowed the effects of nutrient addition on the abundance of the fly guild to be detectable, but only when habitat complexity was at ambient levels. As of yet, I do not know of a possible mechanism to explain this interaction. In addition to the interactions described above, salinity stress also interacted with habitat complexity alone; salinity stress decreased the number of leaves with miner damage but only at ambient levels of habitat complexity. When habitat complexity was reduced, the effect of salinity stress was cancelled. The increase in parasitoids and IGP could potentially explain this interaction if they tended to suppress miners overall regardless of salinity. Overall, the interactive effects of nutrients, salinity stress and habitat complexity were variable and were guild-dependent. Our study confirms previous results that show that the effects of

salinity on the regulation of arthropod communities and their damage on plants can vary depending on the level of salinity stress and on the arthropods involved (see chapters 1 and 2). To our knowledge, no previous studies have studied the interactive effects of nutrients, salinity stress and habitat complexity on communities.

In conclusion, our results suggest that nutrient availability to plants, environmental (salinity) stress and habitat complexity alter each other's effects, but the effects of these interactions on the regulation of communities depend highly on the arthropod guilds involved. In particular, I showed that decreasing habitat complexity could increase top-down control of the plant damage done by certain herbivore guilds but only when nutrients were added to plants.

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Tables

Table 1: A. Results from MANOVA tests of the effects of nutrient availability (reduction, ambient or addition), salinity stress (low or high), habitat complexity (ambient or low) and their interactions on the densities of the six arthropod guilds shown on table 1B. **B.** Summary table of *P*-values from analysis of variance tests of the effects of nutrient availability, salinity stress, habitat complexity and their interactions on the densities of six arthropod guilds: predators, intraguild predators, parasitoids, leaf chewers, flies and sap-feeders. Significant *P*-values ($P < 0.05$) are presented in bold for clarity. N = 36.

A: MANOVA results for arthropod's guild densities

Source	Degrees of freedom		Wilk's lambda	F	<i>P</i>
	Numerator	Denominator			
Model	66	107.12	0.02	1.66	0.01
Intercept	6	19	38.20	120.98	<.0001
Complexity (Cx)	6	19	0.71	2.24	0.08
Nutrients (N)	12	38	0.16	4.79	<.0001
Cx*N	12	38	0.60	0.91	0.55
Salinity (S)	6	19	1.02	3.22	0.02

Cx*S	6	19	0.13	0.42	0.85
N*S	12	38	0.40	1.81	0.08
Cx*N*S	12	38	0.61	0.89	0.56

B: Summary table for individual ANOVAs on arthropod's guild densities

Source	<i>P</i>					
	Intraguild		Leaf	Other	Sap	
	Predator	predator	Parasitoid	chewer	herbivores	feeder
Complexity (Cx)	0.47	0.25	0.02	0.08	0.01	0.91
Nutrients (N)	1.00	0.15	0.02	0.34	0.0004	0.05
Cx*N	0.21	0.47	0.30	0.88	0.66	0.99
Salinity (S)	0.06	0.71	0.35	0.96	0.01	0.60
Cx*S	0.38	0.76	0.55	0.40	0.49	0.54
N*S	0.06	0.48	0.16	0.42	0.63	0.62
Cx*N*S	0.78	0.02	0.86	0.57	0.04	0.49

Table 2: Results from MANOVA tests of the effects of nutrient availability (reduction, ambient or addition), salinity stress (low or high), habitat complexity (ambient or low) and their interactions on three plant traits and three types of herbivores damage shown on table 2B. **B.** Summary table of *P*-values from analysis of variance tests of the effects of nutrient availability, salinity stress, habitat complexity and their interactions on plant traits and herbivore damage: plant height, leaf number, chlorophyll content, *Ophraella* beetle damage, grasshopper damage and miner damage. Significant *P*-values ($P < 0.05$) are presented in bold for clarity. N = 107.

A: MANOVA results for plant traits and herbivore damage

Source	Degrees of freedom		Wilk's lambda	F	<i>P</i>
	Numerator	Denominator			
Model	72	490.02	0.31	1.64	0.002
Intercept	6	89	4.61	68.41	<.0001
Complexity (Cx)	6	89	0.19	2.84	0.01
Nutrients (N)	12	178	0.86	1.16	0.32
Cx*N	12	178	0.78	1.99	0.03
Salinity (S)	6	89	0.05	0.68	0.67
Cx*S	6	89	0.19	2.81	0.01

N*S	12	178	0.85	1.23	0.27
Cx*N*S	12	178	0.97	0.24	1.00
Log iL#	6	89	0.22	3.20	0.01

B: Summary table for individual ANOVAs on plant traits and herbivore damage

Source	<i>P</i>					
	Plant Height	Leaf number	Chlorophyll content	Beetle damage	Grasshopper damage	Miner damage
Complexity (Cx)	0.58	0.53	0.12	0.01	0.01	0.80
Nutrients (N)	0.77	0.57	0.70	0.25	0.28	0.04
Cx*N	0.49	0.10	0.38	0.76	0.02	0.95
Salinity (S)	0.75	0.42	0.36	0.60	0.67	0.10
Cx*S	0.67	0.30	0.23	0.08	0.62	0.01
N*S	0.11	0.60	0.05	0.43	0.29	0.21
Cx*N*S	0.93	0.87	0.79	0.68	0.84	0.99
Log iL#	0.46	0.005	0.28	0.01	0.001	0.06

Figures:

Figure 1: Bottom-up effects of vegetation complexity (**a**) and nutrients (**b**) on parasitoid's abundance. C: control (ambient levels of vegetation complexity); L: low vegetation complexity; N-: nutrient subtraction (by adding sucrose); N₀: control; N+: nutrient addition. Data are means + 1 SE. Shared letters indicate means that are not significantly different.

Figure 2: Interactive effects of nutrients, salinity stress and vegetation complexity on intraguild predator's (**a**) and herbivore's (**b**) abundance. S₀: no salt addition; S+: salt addition; N-: nutrient subtraction (by adding sucrose); N₀: control; N+: nutrient addition; C: control (ambient levels of vegetation complexity); L: low vegetation complexity. ANOVA statistics represent the nutrients x salinity stress x vegetation complexity interaction. Figures show means + 1 SE. Shared letters indicate means that are not significantly different.

Figure 3: Bottom-up effects of vegetation complexity and nutrients on the number of leaves damaged by *Ophraella* beetles (**a**) and the number of leaves damaged by miners (**c**), respectively. Also, interactive effects of nutrients and habitat complexity on the number of leaves damaged by grasshopper (**b**), and interactive effects of salinity stress and vegetation complexity on the number of leaves damaged by miners (**d**). S₀: no salt addition; S+: salt addition; N-: nutrient subtraction (by adding sucrose); N₀: control; N+: nutrient addition; C: control (ambient levels of vegetation complexity); L: low vegetation complexity. ANOVA

statistics represent the vegetation complexity effects (**a**), the nutrient effects (**c**), the nutrients x vegetation complexity interaction (**b**) and the salinity stress x vegetation complexity interaction (**d**). Figures show means + 1 SE. Shared letters indicate means that are not significantly different.

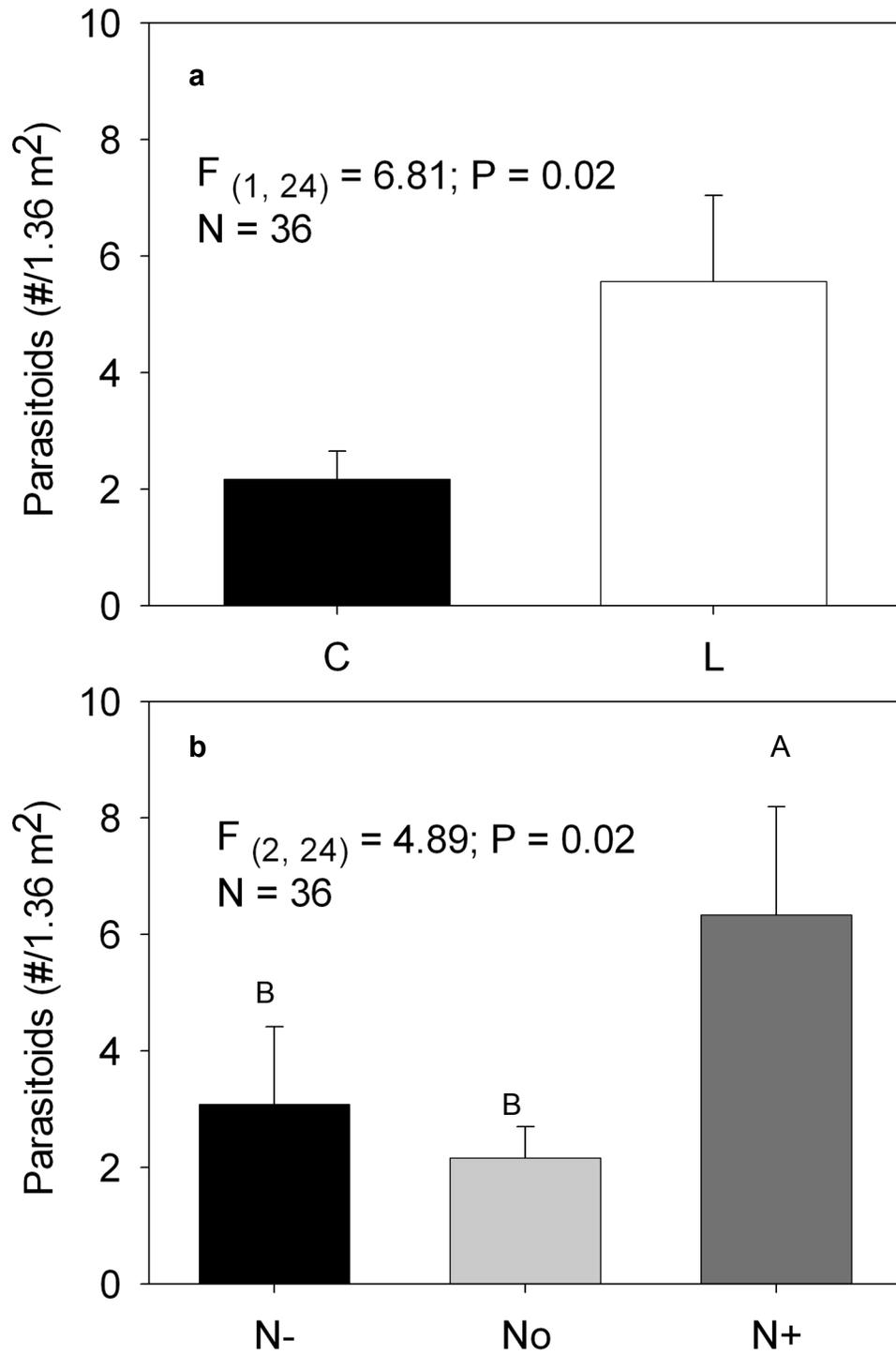


Figure 1:

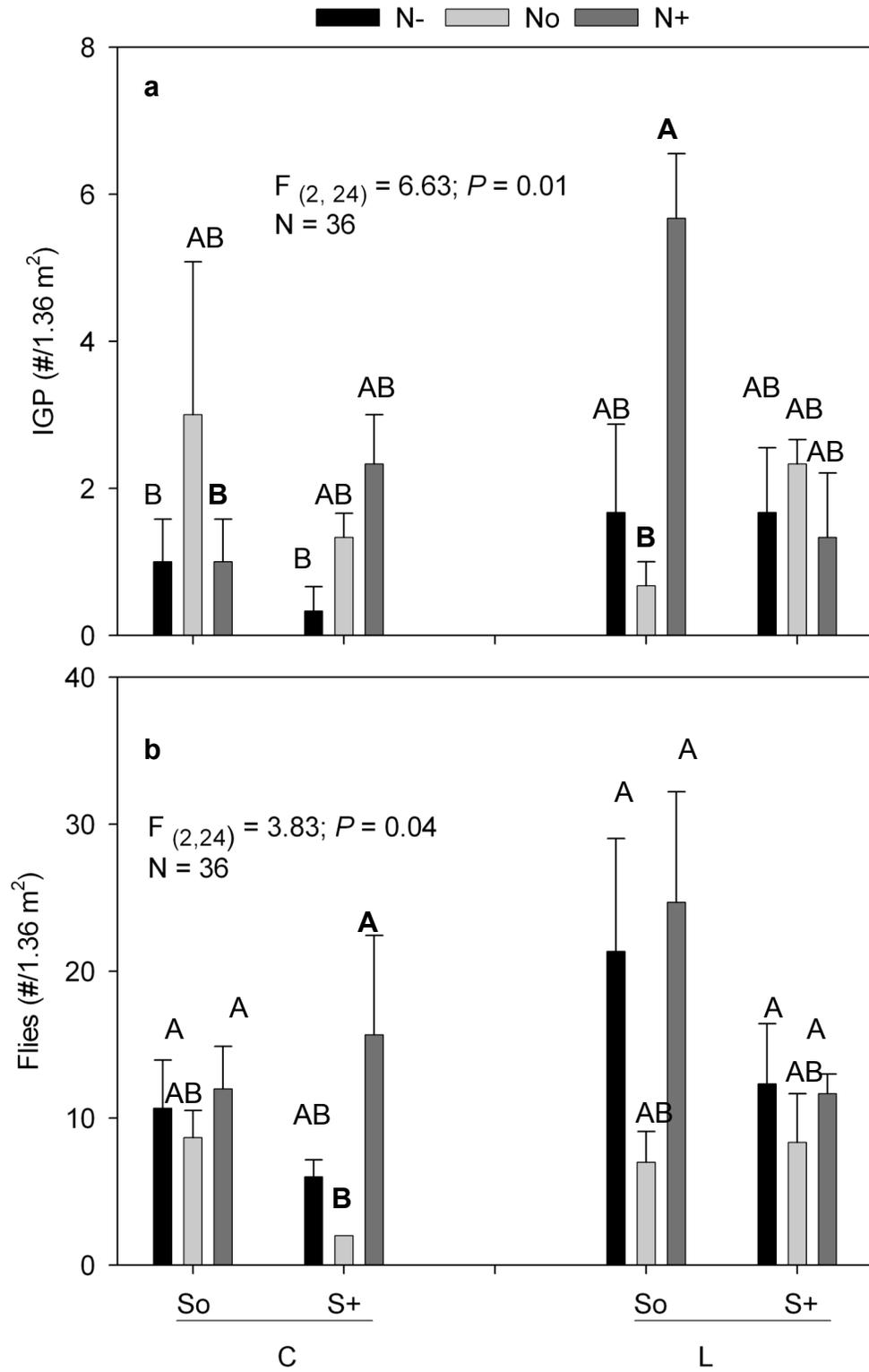


Figure 2:

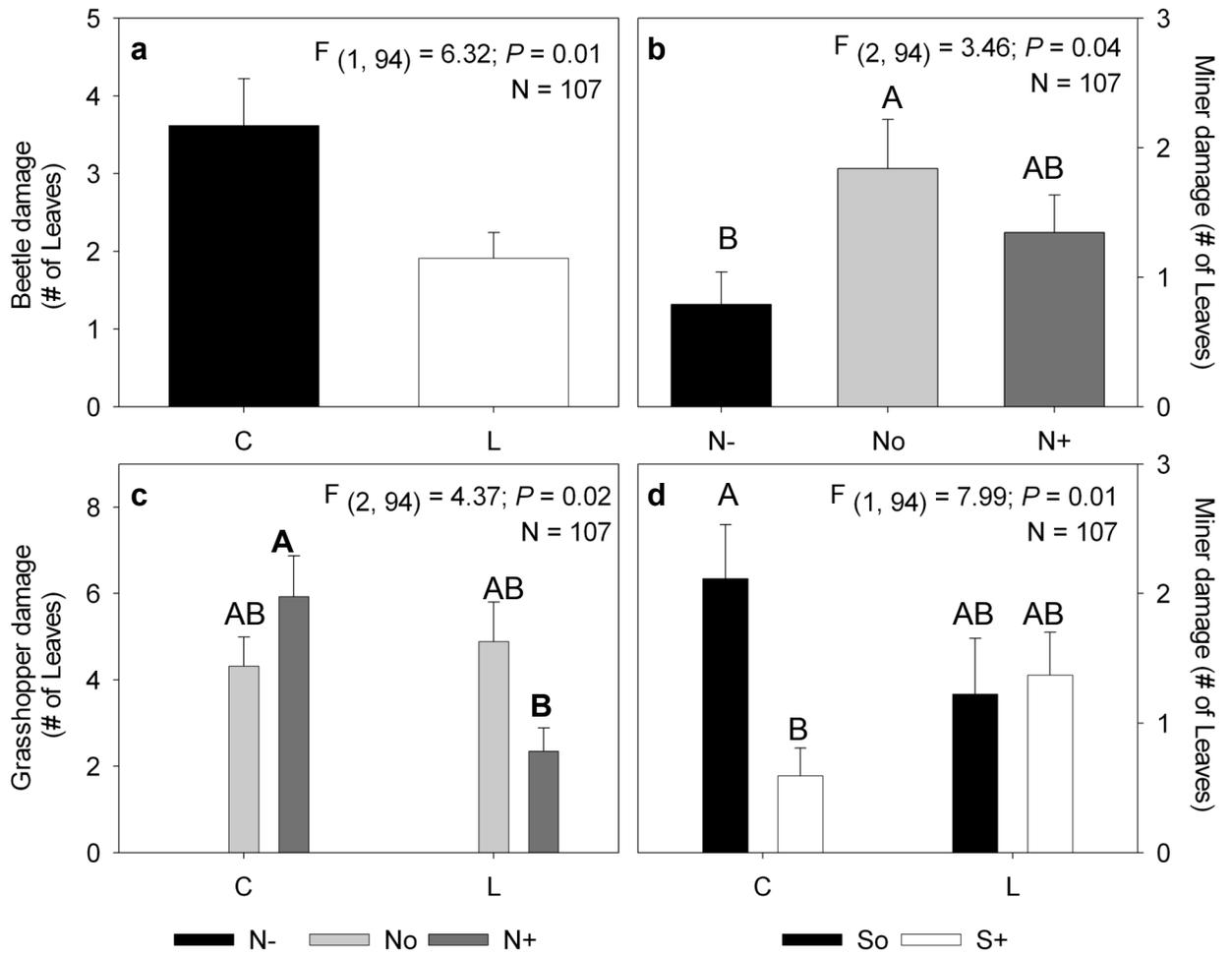


Figure 3:

GENERAL CONCLUSIONS

Biological communities and food webs are structured by a complex set of processes and have precluded ecologists to arrive at a general theory of community structure regulation. Many ideas and hypotheses have been proposed but have largely ignored the presence of interactions among the factors that regulate community structure. My studies are an attempt to fill this void and explore how omnivory, nutrient availability, salinity stress levels, and habitat complexity interact to structure communities. Two main conclusions that arise from my experiments are as follow. First, nutrient addition predictably increased the strength of top-down forces, but the effects of salinity stress were variable and species dependent. And second, decreasing habitat complexity increased the strength of top-down forces and trophic cascades.

First, as suggested by previous studies, both nutrient additions and salinity stress interacted to affect the strength of top-down forces. However, while the effects of nutrient addition were predictable, increasing the strength of top-down forces, the effects of salinity addition were variable and were species-dependent. For example, increasing salinity stress decreased the damage done to plants by a herbivorous beetle; in contrast an omnivorous crab reduced leaf chlorophyll content when plants were stressed with salt. My results also suggest that the level of salinity stress (low or mild vs. high) can also affect experimental results; while a mild salinity stress can increase herbivore damage, a higher level of salinity stress can reduce herbivore damage.

Second, while previous studies have suggested that increasing habitat complexity strengthens top-down control of communities, my results showed the opposite; decreasing habitat complexity strengthened top-down control of *Iva* communities. The differences between my results and previous studies likely come from differences in the mechanisms by which habitat complexity affects the arthropods and their behavior. In addition, nutrient addition and salinity stress altered in opposite ways the effects of habitat complexity on top-down control of communities. While salinity stress cancelled the effects of habitat complexity on top-down forces, nutrient addition increased the effect of habitat complexity on top-down forces. These kinds of interactions had not been documented before and highlight the importance of experiments exploring the interactions among the factors that regulate community structure.

In sum, my results suggest that the complexity of natural communities can lead to many different outcomes in experiments that reflect the many ways in which species interactions are affected by environmental variables to alter the structure of these communities. Environmental variables can affect some plant traits but not others on the same plant species. Also, because herbivores exploit plant resources in different ways the effects of plant traits on herbivores are diverse. The diversity of mechanisms involved in altering species interactions was reflected in the results of my experiments (table 1). The results of the tests of the omnivory hypothesis, the ecosystem exploitation hypothesis, environmental stress hypothesis, and the habitat complexity hypothesis depended on the plant traits measured or the herbivore species studied. The

results either supported the hypotheses, did not support the hypotheses or were opposed to the predictions of the hypotheses (table 1). Progress in understanding community structure will come from experiments that probe these interactive processes and seek to identify commonalities in responses based on consumer behavior, plant traits or habitat type. At the moment we are in the early stages of this process, but here is hope in finding general patterns in the future as more studies are conducted.

Table 1: Summary table with the numbers of results that support (yes) or not (no) the omnivory hypothesis (food web complexity), nutrient hypothesis (EEH), salinity stress hypothesis (ESH), and the habitat complexity (cx) hypothesis. Under the “Yes” columns the numbers represent significant P-values for main factors and/or interactions testing the corresponding hypotheses. Under “No” columns the numbers represent either non-significant P-values or significant P-values but from results opposite to the predictions of the corresponding hypothesis (n = 5).*: one result opposite to the predictions of the hypothesis. **: two results opposite to the predictions of the hypothesis.

	Omnivory		EEH		ESH		Habitat Cx	
	Yes	No	Yes	No	Yes	No	Yes	No
Herbivores	4	16	0	14	0	14	-	-
Primary producers	10	122	12	113*	6	119**	0	10**