Regulation by the Pitcher Plant Sarracenia purpurea of the Structure of its Inquiline Food Web

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ABSTRACT.—Phytotelmata, the water-filled habitats in pitcher plants, bromeliad tanks, and tree-holes, host multilithrophic food webs that are model experimental systems for studying food-web structure and dynamics. However, the plant usually is considered simply as an inert container, not as an interacting part of the food web. We used a manipulative field experiment with a response-surface design to determine effects of nutrient enrichment (multiple levels of NH₄NO₃, PO₄, and captured prey), top predators (removed or present), and the plant itself (with or without plastic tubes inserted into the pitchers to isolate the food web from the plant) on the macrobial food web within the modified leaves (“pitchers”) of the carnivorous pitcher plant Sarracenia purpurea. Connection to the plant, addition of NH₄NO₃, and removal of the top predator significantly increased the food web’s saturation, defined as its trophic depth and number of interactions. No effects on food-web saturation resulted from addition of PO₄ or supplemental prey. Plants such as S. purpurea that create phytotelmata are more than inert containers and their inquilines are more than commensal inquilines. Rather, both the plant and the inquilines are partners in a complex network of interactions.

INTRODUCTION

Understanding controls on food web structure has been an organizing focus for community ecology for well over a century (e.g., Pimm, 1982; Polis, 1994; Carpenter et al., 2001; McCann, 2012; Wokovich et al., 2014; Layman et al., 2015; Zou et al., 2016). As primary producers, plants are the bottom-up controllers of “green” food webs, whereas as “detritus” the decomposing leaf litter of plants is the resource base for “brown” food webs (Cebrian and Lartigue, 2004). The bottom-up roles of plants as producers of fresh biomass and necromass in structuring, respectively green and brown food webs, have been studied extensively, but other roles for plants in structuring food webs through habitat creation and indirect interactions are much less appreciated. For example, in tree holes and modified leaves, plants create habitats for a range of aquatic food webs. The complex, mostly detritus-based food webs in these phytotelmata—water-filled habitats in, for example, tree holes, bromeliads, and pitcher plants (Maguire, 1971)—have been used as model experimental...
systems for studying the dynamics of aquatic food webs (e.g., Kitching, 2000, 2001; Kneitel and Miller, 2002; Miller et al., 2002; Ellison et al., 2003; Srivastava et al., 2004). However, the phytotelmata itself normally is regarded simply as a container for its inquilines: animals that live commensally “within the nest or abode of another” (OED, 2019). It has been recognized only rarely that the plant itself can mediate trophic interactions of inquiline food webs.

Research with carnivorous pitcher plants has provided two notable examples of the importance of phytotelmata on inquiline food webs. Bradshaw and Creelman (1984) experimentally showed the carnivorous pitcher plant *Sarracenia purpurea* L. (Sarraceniaceae) and two of its obligate inquilines, larvae of the pitcher-plant mosquito *Wyeomyia smithii* (Coq.) and the pitcher-plant midge *Mettlicomnus knabi* Coq., are mutualists. These two inquilines accelerate the breakdown and decomposition of arthropod prey captured by *S. purpurea* (Heard, 1994). Ammonium (NH₄) and carbon dioxide (CO₂) released as bacteria mineralize nutrients in the prey (Butler et al., 2008) and NO₃, NH₄, and PO₄ regenerated by rotifers (Blędzki and Ellison, 1998, 2002; Blędzki et al., 2018) are absorbed by the plant and used for photosynthesis, subsequent growth, and reproduction (Bradshaw and Creelman, 1984; Joel and Gepstein, 1985; Butler and Ellison, 2007). The plant, in turn, oxygenates the pitcher fluid (Bradshaw and Creelman, 1984; Joel and Gepstein, 1985; Sirota et al., 2013), maintaining homeostatic conditions for the inquiline food web and preventing a switch to anaerobic conditions within the phytotelmata, which can nonetheless occur when prey species are superabundant (Krieger and Kourtev, 2012; Sirota et al., 2013; Northrop et al., 2017).

Similarly, one of the Asian pitcher plants, *Nepenthes bicalcarata* Hook.f. (Nepenthaceae) hosts the ant *Colobopsis schmitzi* (Stärcke) (Formicidae) in the plant’s hollow tendrils (Clarke and Kitching, 1995). The ants move out of the tendrils to forage within the pitchers on large prey that the plant captures. The ants also clean the pitcher’s peristome, reducing fungal colonization and increasing prey-capture efficiency (Thornham et al., 2011), which in turn leads to greater nutrient acquisition, photosynthesis, and growth of this perennial plant (Bazile et al., 2012).

The lack of attention to linkages between food-web structure or dynamics and the habitat in which the food web itself assembles is not restricted to phytotelmata. Although spatiotemporal dynamics of food webs have been studied, documented, and modeled (e.g., Poisot et al., 2015; Pilosof et al., 2017; Piovia-Scott et al., 2017; Hutchinson et al., 2019; Schiaffino et al., 2019), how complete food webs assemble in a habitat that itself is changing through time and interacts directly with the food web remains unexplored. We are unsure whether this lack of attention results from the rarity of such situations, our failure to perceive them, or the difficulty in studying them. However, living phytotelmata, such as pitcher plants, provide opportunities to study experimentally the dynamics of a food web that assembles in a habitat that itself is a living, growing organism. The experiments we describe here were designed to test whether pitcher plants are more than simply containers for inquilines and how the interactions between plants, the habitat it creates, and the food web that assembles within it change through time.

Here, we describe how nutrient enrichment, top predators, and the living habitat of the plant itself affect the structure of the four trophic-level macrobial food web that inhabits the modified pitcher-shaped leaves (Arber, 1941) of *S. purpurea*. In a set of controlled field experiments, we first examined the impact of nutrient deposition on the food web that assembles in *S. purpurea* pitchers. Second, we severed the connection between the plant and its food web by inserting closed plastic tubes (or appropriate controls) in between pitchers.
and their food webs to determine if effects of experimental nutrient enrichment through additions of prey and inorganic nutrients resulted from direct effects on the food web or indirect effects mediated by the plant. We recognize that a focus on the macrobes will overlook many important processes and interactions of the microbes present in S. purpurea food webs. This is a general issue with studies of food webs (e.g., Pringle and Hutchinson, 2020) that will be able to be addressed as the microbial assemblages of S. purpurea continue to be better characterized (e.g., Peterson et al., 2008; Miller and terHorst, 2012; Paise et al., 2014; Canter et al., 2018; Boynton et al., 2019; Zhang et al., 2020a, 2020b).

**MATERIALS AND METHODS**

**STUDY SPECIES**

*Sarracenia purpurea* (the northern, or purple, pitcher plant) is a long-lived (>50 y), rosette-forming, carnivorous perennial plant common to bogs throughout Canada and the eastern United States. Pitcher plants flower early in the growing season (May in Massachusetts) before any new leaves are produced; flower buds for the following season are set in August–September (Shreve, 1906). Pitcher plants have weakly developed root systems; nutrients are obtained principally from insect prey attracted to the brightly colored pitchers and extrafloral nectaries (Bennett and Ellison, 2009). Unlike the other 10 species of *Sarracenia* (Mellichamp and Case, 2009), *S. purpurea* pitchers fill with rainwater and these semipermanent pools (phytotelmata) are colonized by a suite of specialized invertebrates and more generalist protists and bacteria (Addicott, 1974; Cochran-Stafira and von Ende, 1998; Błędzki and Ellison, 2003; Ellison et al., 2003; Peterson et al., 2008; Miller and terHorst, 2012; Paise et al., 2014; Canter et al., 2018; Boynton et al., 2019; Zhang et al., 2020a, 2020b).

Arthropod prey captured by the plant is the basal resource for this detritus-based food web, which shreds the prey and mineralizes the available nutrients (Butler and Ellison, 2007; Butler et al., 2008). Prey capture initially increases as pitchers open, expand, and harden; reaches a peak when the leaves are 2–3 wk old; and then declines to near zero by the end of the growing season (Fish and Hall, 1978; Ellison et al., 2003). Drowned carcasses of captured prey (primarily ants and flies; Ellison and Gotelli, 2009) are shredded by larvae of the pitcher-plant midge *Metriocnemus knabi* and larvae of the sarcophagid fly *Fletcherimyia fletcheri* (Aldrich); the shredded prey are broken down further by bacteria (Cochran-Stafira and von Ende, 1998; Heard, 1994; Baiser et al., 2011). The bacteria, organic matter, protozoa, and algae in turn are consumed by the bdelloid rotifer *Habrotrocha rosa* Donner and a variety of protozoa, which, along with the pitcher-plant mite *Sarraceniopus gibsoni* (Nesbitt), also consume detritus directly (Wallace et al., 2006). Protozoa also consume bacteria (Cochran-Stafira and von Ende, 1998). These primary consumers are preyed on by larvae of the pitcher-plant mosquito *W. smithii* (all these macrobes will be referred to below using their generic epithets). Late instar larvae of *Fletcherimyia* also prey on rotifers and 1st and 2nd instars of *Wyemyia* (Błędzki and Ellison, 1998; Butler et al., 2008; Baiser et al., 2013). Not only does *Fletcherimyia* complete its larval development within pitchers, but the adult flies are pollinators of *S. purpurea* (Ne’eman et al., 2006).

**STUDY SITE**

The experiments reported here were conducted at Swift River Bog, a 1.9 ha glacial kettle bog in Belchertown, Massachusetts, U.S.A. (42.27 °N, -72.34 °W, 124 m a.s.l.; Kearsley, 1999). This bog has a lagg (moat) around it that never exceeds 2 m in width, but there is no open water within the bog itself. Common *Sphagnum* species present at Swift River Bog were
**FOOD-WEB SATURATION: DEFINITION OF THE RESPONSE VARIABLE**

In all the experiments described below, *food-web saturation* was our response variable. We defined food-web saturation as follows: each macrobial taxon (rotifer, mite, midge, mosquito, sarcophagid fly) in the food web was assigned a binary value representing its presence (Table 1; Fig. 1); we assumed all food webs had protozoa and bacteria. The saturation of the food web in a pitcher was defined as the sum of the equivalent decimal values of each macrobial taxon present. There are 32 possible food webs that can be assembled from these five taxa; the decimal value for each food web ranges from 0 (no macrobial taxon present) to 31 (all macrobial taxa present), with increasing numbers indicating more saturated food webs. Food webs with higher saturation values had both more trophic levels present and more trophic links present (Fig. 1), but the food-web saturation index does not reflect any particular trophic relationship. Further, the index can be easily extended, if any other taxa (*e.g.*, microbes) were to be included.

**EXPERIMENT 1 – IMPACTS OF N AND P ON FOOD-WEB SATURATION**

The first experiment investigated the effects of inorganic N and P (as would be accumulated in the phytotelmata by atmospheric deposition) on food-web saturation in situ.
We used a factorial response-surface design (Cottingham et al., 2005) in a "pulse" experiment (sensu Bender et al., 1984) in which five levels each of NH$_4$NO$_3$ (4.687, 9.375, 18.75, 37.5, and 75.0 mM) and PO$_4$ (0.625, 1.25, 2.5, 5.0, and 10.0 mM) and controls (distilled, deionized water: dH$_2$O) were added biweekly to each pitcher of 130 nonflowering plants that were randomly assigned to one of five groups of 26 plants. The levels of N used bracketed the observed deposition of N measured by NADP in the years before the study, whereas the levels of P were chosen to span the N:P ratios previously used by Ellison and Gotelli (2002) in other nutrient addition experiments with S. purpurea without any evidence of nutrient toxicity (sensu Camargo et al., 2005).

Plants were located at least 10 m (linear distance) from the lagg surrounding the bog, separated from one another by at least 50 cm, and atop the Sphagnum mat, but not under shrubs or trees. Each plant within a group was randomly assigned a nutrient treatment (one of the 25 possible N × P combinations or the dH$_2$O control). On 4 June 2001, 20 ml of nutrient solution was added once to each open pitcher on each plant. Plants were monitored every 3 d for new pitcher production, and nutrient solutions were added (again, only once) to each new pitcher 2–3 d after it had opened and fully hardened. Every 3 wk (25 June, 16 July, 6 August, 27 August, and 17 September), a complete set of 26 plants was harvested. At each harvest pitcher fluid was pipetted into sterile tubes (one tube/leaf), the leaves further rinsed to ensure that all rotifers and mites were collected, and the presence or absence of each member of the food web was determined.

We ran a parallel "press" experiment (sensu Bender et al., 1984) in which we haphazardly located 26 nonflowering plants and randomly assigned each of them to one of the same 26 nutrient treatments as in the pulse experiment. Two to 3 d after each pitcher had opened
and hardened, 20 ml of nutrient solution was added to it, and the 20 ml level was marked on
the side of the pitcher with indelible ink. Every 2 wk the food web was pipetted out of each
pitcher and into a petri dish; the volume of the fluid was recorded. Presence of each
macrobe was determined in the field, and all were returned to the pitcher. Rotifers and
protozoa were counted in the lab in 1 ml subsamples taken at the same time; total counts of
rotifers and protozoa were extrapolated to the full volume by simple multiplication. After
field counts of invertebrates, the pitcher fluid was poured back into the pitcher and
additional nutrient solution was added to maintain the fluid level at 20 ml. This experiment

EXPERIMENT 2 – INTERACTIONS BETWEEN THE PLANT, NUTRIENTS, AND PREDATION ON FOOD-WEB STRUCTURE

The second experiment investigated how changes in food-web saturation resulted from
interactions between bottom-up effects of nutrient addition, top-down effects of predation,
and effects of the plant itself. We used a four-level factorial/response-surface experiment
with 212 nonflowering plants located at least 10 m (linear distance) from the lagg
surrounding the bog, separated from one another by at least 50 cm, and atop the Sphagnum
mat but not under shrubs or trees. As in Experiment 1, plants were assigned randomly to
unique combinations of treatments that included physically isolating the inquiline food web
within the phytotelmata.

The first treatment (factor) assessed the influence of the plant itself on the food web and
had three levels. Pitcher interiors were: [1] fitted with intact polyethylene tubes (2.2-cm
diameter) (inquilines and their fluid isolated from effects of pitchers; n = 64 plants, 1
pitcher/plant); [2] fitted with perforated (ten 6.35 mm diameter drilled holes) polyethylene
tubes (polyethylene tube control; n = 64 plants); or [3] left without polyethylene tubes
(unmanipulated control; n = 64 plants).

The second treatment (ordinal factor used in the response surface) examined the effects
of N deposition. One of eight different concentrations of inorganic nitrogen (NH$_4$NO$_3$: 0,
1.56, 3.1, 6.25, 12.5, 25, 50, or 100 mg N/L) was added to a randomly selected plant; there
were n = 8 replicates of each N concentration in each set of 64 tube/tube-control/no tube
treatments. Because we assumed a constant uptake of NH$_4$NO$_3$ by pitcher tissue in control
and tube-control pitchers, and volatilization and uptake by bacteria in all pitchers, we
replaced fluid and nutrients with the appropriate NH$_4$NO$_3$ solution every 2 wk.

The third treatment (factor) examined effects of prey availability (organic nutrients).
Supplemental prey (10 lab-raised fruit flies/wk) were added to one-half of each inorganic N
× tube combination.

Finally, because of the importance of *Fletcherimyia* at multiple points in this food web
(Błedzki and Ellison, 1998; Butler *et al*., 2008; Baiser *et al*., 2013), the fourth treatment
(factor) assessed the influence of this top predator on the development of the pitcher-plant
food web in the context of bottom-up effects of prey and inorganic N addition and habitat
effects of the pitcher itself. Second instar *Fletcherimyia* larvae were either removed from
pitchers (one-half of each N × tube × fruit-fly treatment combination) or added to pitchers
(one-half of each N × tube × fruit-fly treatment combination).

In total this factorial/response-surface design resulted in N = 2 plants in each tube × N ×
fruit-fly × *Fletcherimyia* treatment combination. We also haphazardly chose 20 additional
plants growing nearby in the bog as unmanipulated controls. One treated plant from each
treatment combination (96 pitchers) and half the unmanipulated controls were harvested
after 4 wk and the remainder after 8 wk. Food-web saturation in this experiment was
computed based on all inquilines present including *Fletcherimyia*, except in the *Fletcherimyia* addition treatments (to avoid simply documenting the addition in our calculation).

### Statistical Analysis and Data Availability

Data were analyzed using standard linear regression, analysis of variance, or analysis of covariance using functions for generalized linear models (glm) in the base and stats packages of the R statistical software system, version 3.6.3 (R Core Team, 2020). All data from this experiment are available from the Harvard Forest Data Archive, dataset HF193 (https://harvardforest.fas.harvard.edu/exist/apps/datasets/showData.html?id=hf193) and the Environmental Data Initiative (https://dx.doi.org/doi:10.6073/pasta/3bd2c920cf4312d3427f3e1442d34227f3f5fc7e2a).

Experiment 1 (pulse and press experiments) were analyzed using a linear regression model (*i.e.*, linear in the regression parameters) with which we fit a polynomial surface of food-web saturation as function of N and P concentrations and their interaction. Experiment 2 (four treatment factorial/response-surface design) was analyzed using a glm with main effects of the tubes (three levels), supplemental prey (two levels), *Fletcherimyia* addition (two levels), inorganic N addition (eight ordered levels treated as a continuous variable in the model), and all of their two- or three-way interactions. Because initial analyses revealed no significant interaction effects among the three discrete factors (*P* > 0.20 all cases), we report only the results of the main effects and the interactions with the inorganic N additions. Throughout, *F* statistics and associated *P* values are based on Type III sums of squares.

### Results

#### Experiment 1

In the pulse experiment, which established the baseline response of the inquiline food web to bottom-up control by inorganic nutrient additions, food-web saturation peaked at intermediate levels of N added (*F*$_{1,227}$ = 10.7, *P* < 0.01), was significantly higher in pitchers produced earlier in the season (*F*$_{1,227}$ = 5.0, *P* = 0.03), and in leaves harvested in mid-summer (*F*$_{1,227}$ = 4.4, *P* = 0.04) (Fig. 2). Although the amount of P added did not alter food web saturation (*F*$_{1,227}$ = 0.7, *P* = 0.42), there was a significant N × P interaction (*F*$_{1,227}$ = 4.4, *P* = 0.04).

Food-web saturation in the coincident press experiment also peaked at intermediate levels of N added (*F*$_{1,292}$ = 3.7, *P* = 0.05) and was highest in mid-summer (*F*$_{1,292}$ = 26.9, *P* < 0.01) in those pitchers that were produced earliest in the season (*F*$_{1,293}$ = 4.1, *P* = 0.04) (Fig. 2). As in the pulse experiment, the amount of P added did not alter food web saturation (*F*$_{1,292}$ = 0.4, *P* = 0.54), but in contrast to the results of the pulse experiment, there was no significant N × P interaction in the press experiment (*F*$_{1,292}$ = 0.3, *P* = 0.58). We did not observe any pitcher mortality from the higher levels of N additions.

#### Experiment 2

Inquilines colonized all the plants, but *Wyeomyia* was observed somewhat less frequently in pitchers with intact tubes than in pitchers with perforated tubes or no tubes at all ($\chi^2_{2}[2] = 28.5$, *P* < 0.01; Table 2). Similarly, *Fletcherimyia* colonized only two pitchers with intact tubes and the added *Fletcherimyia* survived in only one pitcher with an intact tube ($\chi^2_{1}[1] = 20.6$, *P* < 0.01; Table 2). Occurrences of the other macrobial inquilines were unrelated to tube
treatment or *Fletcherimyia* addition (respectively $\chi^2_{[2]} = 3.4, P = 0.18$ and $\chi^2_{[1]} = 0.03, P = 0.87$; Table 2).

Connections with the plant increased food-web saturation: food webs in pitchers without tubes or with perforated tubes were significantly more saturated than food webs in pitchers with intact tubes (Fig. 3, right panel; $F_{2,173} = 8.4, P < 0.01$). Removal of *Fletcherimyia* from plants led to an increase in food web saturation (Fig. 3, left panel; $F_{1,173} = 13.2, P < 0.01$), as this predator consumes both rotifers and *Wyeomyia* larvae. Neither additions of prey nor inorganic nitrogen (NH$_4$NO$_3$-N) alone altered food-web saturation ($F_{1,173} = 0.8, P = 0.37$; $F_{1,173} = 0.0, P = 0.88$, respectively). However, there was a nutrient $\times$ *Fletcherimyia* interaction ($F_{1,182} = 4.9, P = 0.03$): in the presence of *Fletcherimyia*, increasing NH$_4$NO$_3$ led to decreased saturation of the food web. In contrast in the absence of *Fletcherimyia*, increasing NH$_4$NO$_3$...
led to increased saturation of the food web. None of the other two-way, three-way, or four-way interaction terms was significant (P > 0.18, all cases).

**DISCUSSION**

Plant productivity, expressed as new biomass eaten by herbivores or shed litter decomposed by detritivores, exerts bottom-up control of most food webs (McCann, 2012; Wokovich et al., 2014; Zou et al., 2016). As phytotelmata (Maguire, 1971), plants produce habitat for aquatic food webs (Kitching, 2000, 2001; Ellison et al., 2003; Srivastava et al., 2004), but other effects of phytotelmata on food webs have been studied only rarely (Bradshaw and Creelman, 1984; Joel and Gepstein, 1985; Clarke and Kitching, 1995; Thornham et al., 2011; Bazile et al., 2012). The experiments described here illustrate that the phytotelmata of northern pitcher plants are not simply inert containers in which complex aquatic food webs assemble and thrive. Rather, the plant itself interacts with, and determines the strength of, the bottom-up and top-down controls of the food web.

Unlike pitchers in other species of *Sarracenia* that are covered by a reflexed hood, pitchers of *S. purpurea* are open to the sky and accumulate water from precipitation. This has two consequences. First, as pitchers accumulate water, they create the habitat for the inquiline food web. Second, any nutrients that accompany precipitation can enter the food web through bacterial mineralization and immobilization.

Independent of addition of inorganic nutrients, supplemental prey, or presence of top predators, the plant itself had a net positive effect on inquiline food-web saturation. Food webs in containers isolated from the plant with polyethylene tubes were 10–20% less saturated than food webs in perforated tubes or unmanipulated controls of comparable size. This result agrees with previous observations of, and experiments with, *S. purpurea* and its inquilines, in which the plant oxygenated the pitcher fluid, maintaining homeostatic conditions for the inquiline

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**Table 2.—** Inquilines collected from 64 pitchers in the four-level full factorial experiment in which food webs were isolated from the plant with polyethylene tubes, inorganic and organic nutrients were added to assess bottom-up effects, and the top predator *Fletcherimyia fletcheri* was added to assess top-down effects. This table illustrates the effects of inquiline occurrence of the two factors—the polyethylene tubes and the addition of the top predator—that had significant effects on food-web saturation (Fig. 3). Values in each cell are the number of pitchers in which each inquiline was collected, pooled over inorganic and organic nutrient addition treatments and both harvests. The maximum possible number of occurrences for any given inquiline in a Tube × *Fletcherimyia* treatment combination is 32. Because cell entries within each column are occurrences summed over each Tube × *Fletcherimyia* treatment combination, all entries within a single row do not imply co-occurrences. Occurrences of the other macrobial inquilines within a tube treatment were unrelated to the addition of *Fletcherimyia*.

<table>
<thead>
<tr>
<th>Tube treatment</th>
<th>Fletcherimyia treatment</th>
<th>Number of pitchers in which inquiline occurred</th>
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<tbody>
<tr>
<td></td>
<td><em>Fletcherimyia</em></td>
<td><em>Metriocnemus</em></td>
</tr>
<tr>
<td>Intact</td>
<td>Added</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Not added</td>
<td>26</td>
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<tr>
<td>Perforated</td>
<td>Added</td>
<td>31</td>
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<td></td>
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</tr>
<tr>
<td>None</td>
<td>Added</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Not added</td>
<td>28</td>
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</table>
food web and preventing a switch to anaerobic conditions (Bradshaw and Creelman, 1984; Joel and Gepstein, 1985; Sirota et al., 2013).

Further addition of inorganic N, as is occurring through atmospheric deposition, increased food-web saturation at intermediate levels, but saturation declined as N addition was increased further (Fig. 2). We hypothesize this result may reflect bottom-up effects of N on microbial productivity (Moorhead and Sinsabaugh, 2006), shifts in how S. purpurea uses different forms of N (Karagatzides et al., 2009), or interactions between the plant and the bacteria that mineralize nitrogen for it (Butler et al., 2008; Mouquet et al., 2008). These studies, and others with excess prey addition show shifts in the macrobial food web or bacterial functional groups (see Northrop et al., 2021 for the latter), but no evidence of
nutrient toxicity (sensu Camargo et al., 2005). Furthermore, N addition alters pitcher morphology, reducing pitcher size (volume) while increasing the area of its photosynthetic keel (Ellison and Gotelli, 2002; Bott et al., 2008). Although smaller pitchers have less complex food webs (Gotelli and Ellison, 2006), this effect was not observed or measured here given we worked only with pitchers that were already fully opened, and not newly developed pitchers that would have expressed these morphological differences after nutrients were added (Butler and Ellison, 2007).

Top-down effects of predation in the inquiline food web of S. purpurea have been described repeatedly (e.g., Addicott, 1974; Kneitel and Miller, 2002; Hoekman, 2007, 2011; Peterson et al., 2008), but in our experiments, these effects were manifest only when the food web was isolated from the plant. Food webs in tubes rarely had predators (Wyeomyia or Fletcherimyia), as these Diptera would not oviposit in intact tubes and food webs without the predator Fletcherimyia were more saturated (up to the level below Fletcherimyia) than food webs with this predator. The interactions we observed between top-down effects of Fletcherimyia and bottom-up effects of inorganic N additions are mirrored by modelled trade-offs in overall system nitrogen mineralization as modulated by interactions between plants and mineralizing bacteria and between plants and higher trophic levels (Mouquet et al., 2008; Ellison and Gotelli, 2021). Finally, the potential importance of digestive enzymes in mediating any of these produced by the plant remains unknown. When digestive enzymes are produced during pitcher development and at what concentrations remain open questions (e.g., Gallie and Chang, 1977; Luciano and Newell, 2017). However, our results do illustrate that the pitcher itself does more than simply maintain a habitat for symbionts (cf. Luciano and Newell, 2017).

Overall, the results presented here together with those from other studies on pitcher-plant food webs illustrate that top-down and bottom-up effects on food-web structure are dependent on the habitat context in which they occur. Anthropogenic processes, such as fossil-fuel combustion leading to atmospheric deposition of inorganic nutrients, can alter habitat structure and shift control of food web structure from strictly bottom-up control to a mixture of top-down and bottom-up controls. These consequences are especially evident in habitats that themselves are living structures that respond to the same anthropogenic processes at similar time scales. Plants that create phytotelmata, such as S. purpurea, are more than inert containers and their inhabitants are more than commensal inquilines. Rather, both the plant and the inquilines are partners in complex networks of interactions. Our work and that of others illustrate clearly that the microecosystem created by S. purpurea can provide a model experimental system for exploring such interactions (Ellison and Gotelli, 2021).

Acknowledgments.—We thank Clarisse Hart, Callan Ordoyne, and Ali Rosenberg for significant assistance with the fieldwork, and two reviewers for excellent comments on an early version of the ms. This research was supported by NSF grants DEB 98-05722, 98-08504, 02-35128, and 02-34710 to AME, and permitted by the Massachusetts Department of Hatcheries.

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