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 multitrophic 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 response-surface and factorial field experiment to determine effects of nutrient enrichment (multiple levels of NH_4NO_3, PO_4, and captured prey), top predator (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 NH_4NO_3 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 PO_4 or supplemental prey. Plants such as *S. purpurea* that create phytotelmata are more than inert containers and their inhabitants 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 various roles of plants in structuring both green and brown food webs have been studied extensively, but an additional role for plants in structuring food webs is much less appreciated. In tree holes and modified leaves, plants create habitats for a range of aquatic food webs. Whereas 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), 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 that 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 *Metriocnemus knabi* Coq., are mutualists. These two inquilines accelerate the breakdown and decomposition of arthropod prey captured by

S. purpurea (Heard, 1994). Ammonia (NH_4) and carbon dioxide (CO_2) released as bacteria mineralize nutrients in the prey (Butler *et al.*, 2008) and NO_3, NH_4, and PO_4 regenerated by rotifers (Błędzki and Ellison, 1998, 2002; Błędzki *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), which maintains homeostatic conditions for the inquiline food web and prevents a switch to anaerobic conditions within the phytotelmata, which can nonetheless occur if prey 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 with the food web itself 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.

Here, we describe how nutrient enrichment, top predators, and the living habitat of the plant itself affect the structure of the four trophic-level 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. Atmospheric deposition of N and P in northeastern North America provide an immediate, biologically reactive source of nutrients (as NO_3, NH_4, and PO_4) that can be used by both the plant and the microbial community at the base of its aquatic food web (Ellison and Gotelli, 2002). Increasing N, but not P, significantly increased the food web's "saturation" (a measure d indicating how many trophic levels and interactions were present in the web and described in detail in Materials and Methods), but saturation declined at highest levels of N addition. 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.

MATERIALS AND METHODS

STUDY SPECIES

Sarracenia purpurea (the northern, or purple, pitcher plant) is a long-lived (> 50 yr), rosette-forming, carnivorous perennial plant common to bogs throughout Canada and the eastern United States. 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 semi-permanent 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).

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 two–three weeks old; and then declines during 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 instars of *Fletcherimyia* also prey on rotifers and 1st and 2nd instars of *Wyeomyia* (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).

126 In all the experiments described below, *food-web saturation* was our response variable.
127 Each macrobial taxon (rotifer, mite, midge, mosquito, sarcophagid fly) in the food web was
128 assigned a binary value representing its presence (Table 1; Fig. 1); we assumed all food webs
129 had protozoa and bacteria. The saturation of the food web in a pitcher was defined as the sum of
130 the equivalent decimal values of each macrobial taxon present. There are 32 possible food webs
131 that can be assembled from these five taxa; the decimal value for each food web ranges from 0
132 (no macrobial taxon present) to 31 (all macrobial taxa present), with increasing numbers
133 indicating more "saturated" food webs. Food webs with higher saturation values had both more
134 trophic levels present and more trophic links present (Fig. 1).
135
136 STUDY SITE
137 The experiments reported here were conducted at Swift River Bog, a 1.9-ha glacial kettle
138 bog in Belchertown, Massachusetts, USA (42.27 °N, −72.34 °W, 124 m a.s.l.; Kearsley, 1999).
139 This bog has a lagg (moat) around it that never exceeds 2 m in width, but there is no open water
140 within the bog itself. Common *Sphagnum* species present at Swift River Bog were *Sp.*
141 *angustifolium* (C.E.O. Jensen ex Russow) C.E.O. Jensen*, Sp. magellanicum* Brid., and *Sp.*
142 *rubellum* Wilson (Ellison and Gotelli, 2016). The dominant vascular plants growing on the bog
143 mat were shrubs in the Ericaceae: *Chamaedaphne calyculata* (L.) Moench (leatherleaf)*, Kalmia*
144 *polifolia* Wangenheim (bog laurel), *K. angustifolia* L. (sheep laurel), *Rhododendron viscosum*
145 (L.) Torrey (swamp rhododendron) and *Vaccinium macrocarpon* Aiton (cranberry); scattered
146 *Picea mariana* (Miller) Britton (Pinaceae; black spruce) grow across the bog. The site has a large
147 population (1000s of plants) of *S. purpurea* (Ne'eman *et al.,* 2006; Ellison and Gotelli, 2016).
148 Total nitrogen deposition (NO_3 + NH_4) at the National Atmospheric Deposition Program

149 (NADP) Quabbin Reservoir monitoring station in the same watershed (MA08: 42.3925 °N,
150 −72.3444 °W) ranged from 1.25–1.60 mg/L during the years of this study, and has averaged 1.19
151 (range: 0.58–2.12) mg/L since measurements began in 1982 (National Atmospheric Deposition
152 Program, 2020). Phosphorus deposition is not monitored by NADP or other agencies in the US,
153 but P deposition may have significant effects in P-limited ecosystems or alter stoichiometric
154 ratios in otherwise N-limited ones (Mahowald *et al.*, 2008; Tipping *et al.*, 2014). Although *S.*
155 *purpurea* is stoichiometrically N- or N+P-limited (Ellison, 2006), field experiments have
156 suggested that *S. purpurea* preferentially takes up P from supplemental prey (Wakefield *et al.*,
157 2005).
158

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

160 The first experiment investigated the effects of inorganic N and P (as would be
161 accumulated in the phytotelmata by atmospheric deposition) on food-web saturation in situ. We
162 used a factorial response-surface design (Cottingham *et al*., 2005) in a "pulse" experiment (sensu
163 Bender *et al*., 1984) in which 5 levels each of NH_4NO_3 (4.687, 9.375, 18.75, 37.5, and 75.0 mM)
164 and PO_4 (0.625, 1.25, 2.5, 5.0, and 10.0 mM) and controls (distilled, deionized water: dH_2O)
165 were added biweekly to each pitcher of 130 non-flowering plants that were randomly assigned to
166 one of five groups of 26 plants. The levels of N used bracketed the observed deposition of N
167 measured by NADP in the years before the study, whereas the levels of P were chosen to span
168 the N:P ratios previously used by Ellison and Gotelli (2002) in other nutrient addition
169 experiments with *S. purpurea*.

170 Plants were located at least 10 m (linear distance) from the lagg surrounding the bog,
171 separated from one another by at least 50 cm, and atop the *Sphagnum* mat but not under shrubs

172 or trees. Each plant within a group was randomly assigned a nutrient treatment (one of the 25
173 possible N × P combinations or the dH_2O control). Beginning on 4 June 2001, 20 ml of nutrient
174 solution was added once to each pitcher 2-3 days after it had opened and fully hardened. Plants
175 were checked every three days for new leaf production. Every 3 wk (25 June, 16 July, 6 August,
176 27 August, and 17 September), a complete set of 26 plants was harvested. At each harvest,
177 pitcher fluid was pipetted into sterile tubes (one tube/leaf) and the presence or absence of each
178 member of the food web was determined.

179 We ran a parallel "press" experiment (sensu Bender *et al.*, 1984) in which we
180 haphazardly located 26 non-flowering plants and randomly assigned each of them to one of the
181 same 26 nutrient treatments as in the pulse experiment. 2-3 d after each pitcher had opened and
182 hardened, 20 ml of nutrient solution was added to it, and the 20-ml level was marked on the side
183 of the pitcher with indelible ink. Every two weeks, the food web was pipetted out of each pitcher
184 and into a petri dish. Presence of each macrobe was determined in the field, and all were returned
185 to the pitcher. Rotifers and protozoa were counted in the lab in 1-ml subsamples taken at the
186 same time. After field counts of invertebrates, the pitcher fluid was poured back into the pitcher
187 and additional nutrient solution was added to maintain the fluid level at 20 ml. This experiment
188 ran from 4 June–17 September 2001.

189

190 EXPERIMENT 2 – JOINT INFLUENCE OF THE PLANT, NUTRIENTS, AND PREDATION ON FOOD-WEB
191 STRUCTURE

192 The second experiment investigated how changes in food-web saturation resulted from
193 interactions between bottom-up effects of nutrient addition, top-down effects of predation, and
194 effects of the plant itself. We used a four-level factorial/response-surface experiment with 212

195 non-flowering plants located at least 10 m (linear distance) from the lagg surrounding the bog,
196 separated from one another by at least 50 cm, and atop the *Sphagnum* mat but not under shrubs
197 or trees. As in Experiment 1, plants were assigned randomly to unique combinations of
198 treatments that included physically isolating the inquiline food web within the phytotelmata.

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

205 The second treatment was a continuous (ordinal) effector that examined the effects of N
206 deposition. One of eight different concentrations of inorganic nitrogen (NH_4NO_3: 0, 1.56, 3.1,
207 6.25, 12.5, 25, 50, or 100 mg N/L) was added to a randomly selected plant; there were n = 8
208 replicates of each N concentration in each set of 64 tube/tube-control/no tube treatments.
209 Because there was a constant uptake of NH_4NO_3 in control and tube-control pitchers, we
210 supplied an equivalent NH_4NO_3-N load each wk into each pitcher. Plant liquid volume was
211 maintained at two-thirds full by adding nutrient solutions or deionized water as necessary. Plants
212 with intact tubes were not able to assimilate NH_4NO_3 supplied to the tubes; we assumed loss
213 from volatilization and bacterial utilization was small compared to plant assimilation. Therefore,
214 we simply topped off each tube to two-thirds full every week with NH_4NO_3 solution.

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

Finally, because of the importance of *Fletcherimyia* at multiple points in this food web (Błędzki and Ellison, 1998; Butler *et al.*, 2008; Baiser *et al.*, 2013), the fourth treatment 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 in the base package 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/3bd2c920cf431442d34227f3f5fc7c2a).

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 ($P = 0.001$), was significantly higher in leaves produced earlier in the season ($P = 0.025$), and in leaves harvested in mid-summer ($P = 0.033$) (Fig. 2). Although the amount of P added did not alter food web saturation ($P = 0.42$), there was a significant N × P interaction ($P = 0.036$).

Food-web saturation in the coincident press experiment also peaked at intermediate levels of N added ($P = 0.05$) and was highest in mid-summer ($P < 0.001$) in those leaves that were produced earliest in the season ($P = 0.04$) (Fig. 2). As in the pulse experiment, the amount of P added did not alter food web saturation ($P = 0.54$), but in contrast to the results of the pulse experiment, there was no significant N × P interaction in the press experiment ($P = 0.58$).

EXPERIMENT 2

Inquilines colonized all the plants, but *Wyeomyia* was observed less frequently in pitchers with intact tubes than in pitchers with perforated tubes or no tubes at all (Table 2). Similarly, *Fletcherimyia* colonized only two pitchers with intact tubes and the added *Fletcherimyia* survived in only one pitcher with an intact tube (Table 2). Occurrences of the other macrobial inquilines were unrelated to tube treatment or *Fletcherimyia* addition (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

tubes (Fig. 3, right panel; P = 0.0003). Food webs in tubes rarely had predators (*Wyeomyia* or *Fletcherimyia*), as these Diptera would not oviposit in intact tubes. Removal of *Fletcherimyia* from plants led to an increase in food web saturation (Fig. 3, left panel; P = 0.0003), as this predator consumes both rotifers and *Wyeomyia* larvae. Neither additions of prey nor inorganic nitrogen (NH_4NO_3-N) alone altered food-web saturation (P = 0.37; P = 0.36, respectively). However, there was a nutrient × *Fletcherimyia* interaction (P = 0.03): in the presence of *Fletcherimyia*, increasing NH_4NO_3 led to decreased saturation of the food web. In contrast, in the absence of *Fletcherimyia*, increasing NH_4NO_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.

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

291 Independent of addition of inorganic nutrients, supplemental prey, or presence of top
292 predators, the plant itself had a net positive effect on inquiline food-web saturation. Food webs in
293 containers isolated from the plant were 10–20% less saturated than food webs in perforated tubes
294 or unmanipulated controls of comparable size. This result agrees with previous observations of,
295 and experiments with, *S. purpurea* and its inquilines, in which the plant oxygenated the pitcher
296 fluid, maintaining homeostatic conditions for the inquiline food web and preventing a switch to
297 anaerobic conditions (Bradshaw and Creelman, 1984; Joel and Gepstein, 1985; Sirota *et al.*,
298 2013).

299 Further addition of inorganic N, as is occurring through atmospheric deposition,
300 increased food-web saturation at intermediate levels, but saturation declined as N addition was
301 increased further (Fig. 2). We hypothesize that this result may reflect bottom-up effects of N on
302 microbial productivity (Moorhead and Sinsabaugh, 2006), shifts in how *S. purpurea* uses
303 different forms of N (Karagatzides *et al.*, 2009), or interactions between the plant and the
304 bacteria that mineralize nitrogen for it (Butler *et al.*, 2008; Mouquet *et al.*, 2008). Furthermore, N
305 addition alters pitcher morphology, reducing pitcher size (volume) while increasing the area of
306 its photosynthetic keel (Ellison and Gotelli, 2002; Bott *et al.*, 2008). Although smaller pitchers
307 have less complex food webs (Gotelli and Ellison, 2006), this effect was not observed or
308 measured here because we worked only with pitchers that were fully opened, not those that were

309 produced after nutrients were added to early-season pitchers and that would have expressed
310 morphological differences following nutrient translocation and use in new pitcher production
311 (Butler and Ellison, 2007).

312 Top-down effects of predation in the inquiline food web of *S. purpurea* have been
313 described repeatedly (*e.g.,* Addicott, 1974; Kneitel and Miller, 2002; Hoekman, 2007, 2011;
314 Peterson *et al.*, 2008), but in our experiments, these effects were manifest only when the food
315 web was isolated from the plant. Food webs without the predator *Fletcherimyia* were more
316 saturated (up to the level below *Fletcherimyia*) than food webs with this predator. The
317 interactions that we observed between top-down effects of *Fletcherimyia* and bottom-up effects
318 of inorganic N additions are mirrored by modelled trade-offs in overall system nitrogen
319 mineralization modulated by interactions between plants and mineralizing bacteria and between
320 plants and higher trophic levels (Mouquet *et al.*, 2008).

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

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502 **Table 1** Values assigned to each inquiline taxon for calculation of a food-web saturation index.
503 Occurrence of taxa in a given food web is given by the sum of the binary values of all taxa
504 present. The saturation index is the sum of the decimal values of all taxa present. See Fig. 1 for
505 an illustrative example of these calculations
506

Taxon	**Binary value**	**Decimal value**
Metriocnemus	1	1
Habrotrocha	10	2
Sarraceniopus	100	4
Wyeomyia	1000	8
Fletcherimyia	10000	16

507
508

509 **Table 2** Inquilines collected from pitchers in the four-level full factorial experiment in which
510 food webs were isolated from the plant with polyethylene tubes, inorganic and organic nutrients
511 were added to assess bottom-up effects, and the top predator *Fletcherimyia fletcheri* was added
512 to assess top-down effects. This table illustrates the effects on inquiline occurrence of the two
513 factors—the polyethylene tubes and the addition of the top predator—that had significant effects
514 on food-web saturation (Fig. 3). Values in each cell are the number of pitchers in which each
515 inquiline was collected, pooled over inorganic and organic nutrient addition treatments and both
516 harvests. The maximum possible number of occurrences for any given inquiline in a Tube ×
517 *Fletcherimyia* treatment combination is 32. Because cell entries within each column are
518 occurrences summed over each Tube × *Fletcherimyia* treatment combination, all entries within a
519 single row do not imply co-occurrences. Occurrences of the other macrobial inquilines within a
520 tube treatment were unrelated to the addition of *Fletcherimyia*
521

		Number of pitchers in which inquiline occurred				
Tube treatment	***Fletcherimyia* treatment**	***Metriocnemus***	***Habrotrocha***	***Sarraceniopus***	***Wyeomyia***	***Fletcherimyia***
Intact	Added	27	6	15	13	1
	Not added	26	8	11	20	2
Perforated	Added	31	7	8	28	13
	Not added	29	6	12	28	10
None	Added	24	2	11	28	14
	Not added	28	1	6	24	6

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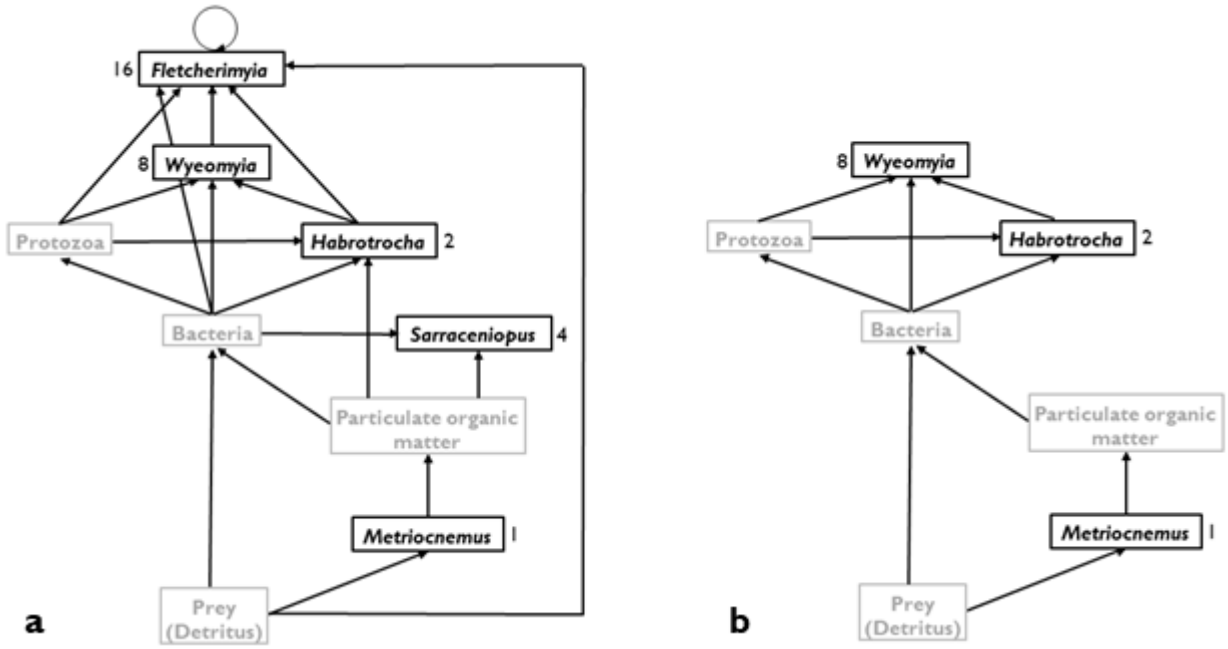
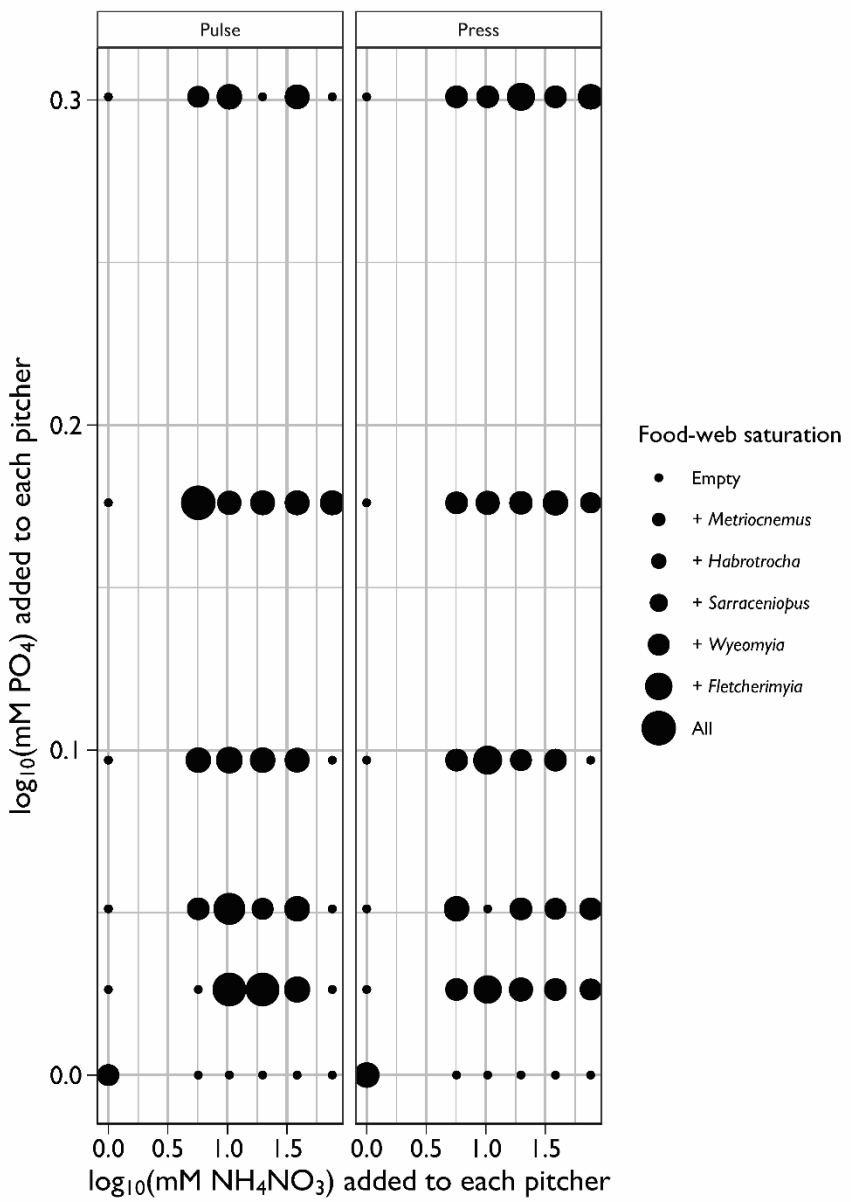


Fig. 1 Complete, fully saturated inquiline food web of *Sarracenia purpurea* (**a**; modified from Baiser *et al.*, 2013) and an example of incomplete, unsaturated one (**b**). Values adjacent to each of the macrobial taxa are those used to compute food-web saturation (see Table 1). Prey, bacteria, protozoa, and particulate organic matter were assumed to occur in all sampled food webs, and each of the possible combinations of macrobes gives a unique saturation value. In these two examples, the saturation value of the complete web (a) = 31, whereas that of the food web lacking *Fletcherimyia* and *Sarraceniopus* (b) = 11

533



534

535 **Fig. 2** Response surface of food-web saturation (defined in Table 1) in pitchers fed one of 25
536 combinations of nitrogen (N) and phosphorus (P) or distilled water once, three days after the
537 pitcher opened (the "pulse" experiment; left panel); or three days after the pitcher opened and
538 every two weeks thereafter (the "press" experiment; right panel). This figure illustrates data from
539 the first leaf of the season (fed on or beginning the week of 4 June 2001) at the mid-summer

harvest (6 August 2001 for the pulse experiment, 23 July 2001 for the press experiment), when saturation was highest

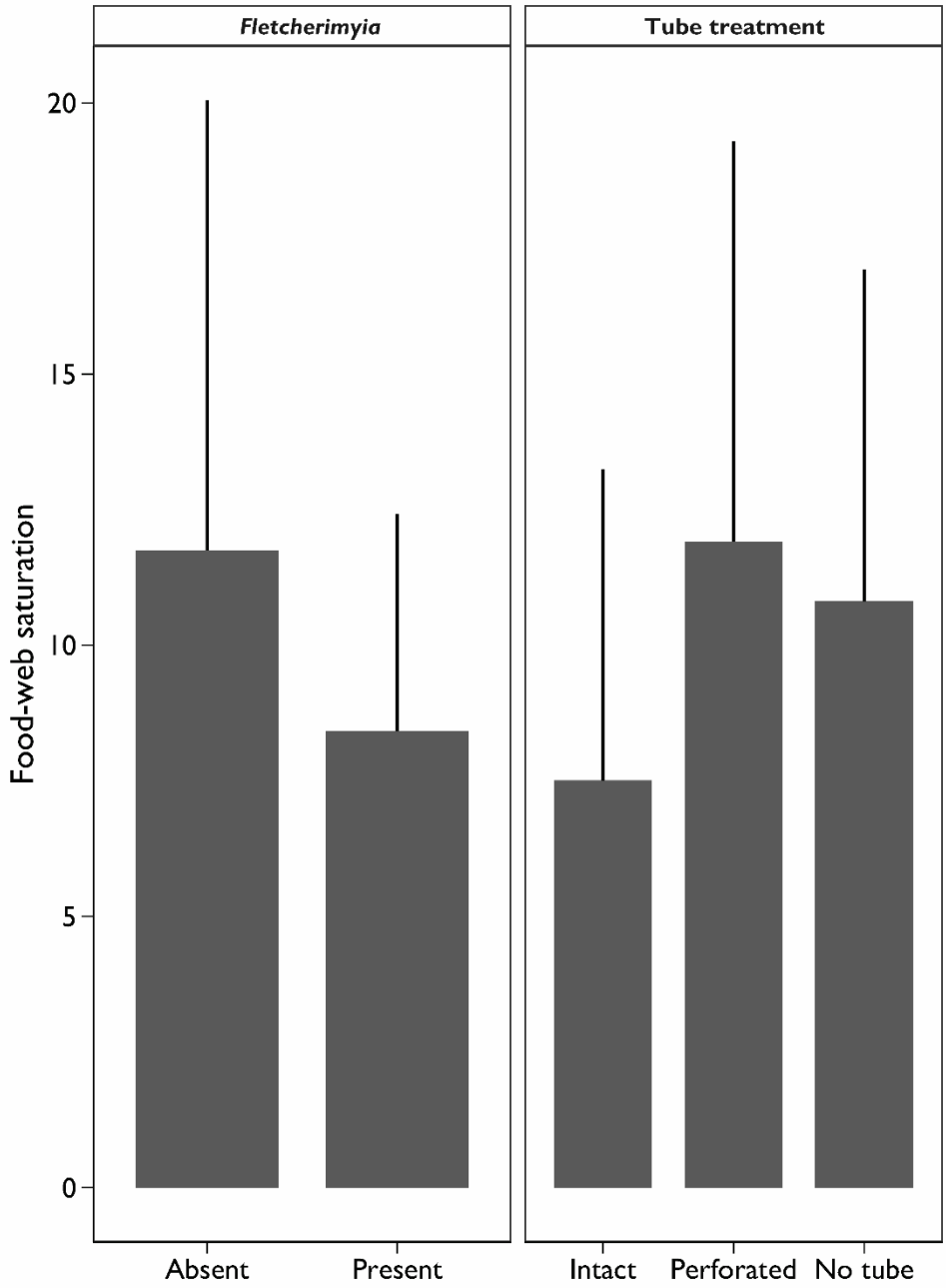


Fig. 3 Saturation in the *Sarracenia purpurea* macrobial food webs in the four-level tube × inorganic nitrogen × fruit-fly × *Fletcherimyia* manipulation. The right panel shows the effects of the plant itself on the food web, whereas the left panel shows the effects of the top predator, *Fletcherimyia*, on the food web. Bars are means ± one standard deviation of the mean of data from all leaves and both harvests pooled over all other treatments.