Regime shifts, alternative states, and hysteresis in the *Sarracenia* pitcher plant microecosystem

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Abstract

Changes in environmental conditions can lead to rapid shifts in ecosystem state ("regime shifts"), which subsequently returns slowly to the previous state ("hysteresis"). Large spatial, and temporal scales of dynamics, and the lack of frameworks linking observations to models are challenges to understanding, and predicting ecosystem responses to perturbations. The naturally-occurring microecosystem inside leaves of the northern pitcher plant (*Sarracenia purpurea*) exhibits oligotrophic, and eutrophic states that can be induced experimentally by adding insect "prey." This regime shift has been modeled previously with difference equations including parameters for pitcher photosynthesis, oxygen diffusion through the pitcher liquid, prey input, and oxygen demand of decomposition. Here, we simplify the model structure, test, and parameterize it using data from a prey addition experiment, and use sensitivity analysis to reveal different model outcomes that are related to plausible values of free parameters. Simulations clearly illustrate that the microecosystem model displays regime shifts, and subsequent hysteresis. Parallel results were observed in the plant itself after experimental enrichment with prey. Decomposition rate of prey was the main driver of system dynamics, including the time the system remains in an anoxic state, and the rate of return to an oxygenated state. Biological oxygen demand influenced the shape of the system's return trajectory. The combination of simulated results, sensitivity analysis, and use of empirical results to parameterize the model more precisely demonstrates that the *Sarracenia* microecosystem model displays behaviors qualitatively similar to models of larger ecological systems. Given its scalability, the *Sarracenia* microecosystem is a valuable experimental platform for rapidly studying ecological dynamics of major importance.

Introduction

Regime shifts in ecological systems are defined as rapid changes in the spatial or temporal dynamics of a more-or-less stable system. Ecological regime shifts are caused by slow, directional changes in one or more underlying state variables, such as species abundance, dissolved oxygen content, or nutrients (Scheffer *et al.* 2009, 2001). Regime shifts are of particular concern when the return rate to a previous (and perhaps more desirable) state is slow or requires a larger input of energy or resources relative to what initiated the state change (i.e.,, hysteresis). In the last several years, many researchers have suggested that a wide range of ecological systems are poised to "tip" into new regimes (Petraitis & Dudgeon 2016; Scheffer *et al.* 2009), or even that we are approaching a planetary tipping point (Barnosky *et al.* 2012; but see Brook *et al.* 2013). Because changes in the underlying state variables of most ecosystems occur over time spans of years to centuries, our understanding of the causes, and consequences of ecological regime shifts has progressed relatively slowly. More rapid progress could be achieved by working with well-understood model systems that can be described mathematically, and manipulated experimentally over shorter time scales.

It is rare to find an ecological system in which the occurrence of a regime shift, and its cause-and-effect relationship with one or more underlying environmental drivers is unambiguous (Bestelmeyer *et al.* 2011). This is primarily because long time series of observations collected at meaningfully large spatial scales are required to identify the environmental driver(s), its relationship to the response variable of interest, the stability of each state, the breakpoint between them, and hysteresis of the return time to the original state (Bestelmeyer *et al.* 2011; Petraitis & Dudgeon 2016). Detailed modeling, and decades of observations, and experiments have led to a thorough understanding of one canonical example of an ecological regime shift: the rapid shift from oligotrophic (low nutrient) to eutrophic (high nutrient) states in lakes (e.g., Carpenter & Brock 2006; Carpenter *et al.* 2011). The primary difficulties with using lakes as models for studying alternative states, and ecological regime shifts are their large size (which precludes

extensive replication: Carpenter 1998), and the long time scales (decades) required to observe a regime shift, subsequent ecosystem hysteresis, and eventual recovery (Contamin & Ellison 2009; Mittlebach *et al.* 1995). Models of lake ecosystems, and their food webs, and associated empirical data, have revealed that returning lakes from a eutrophic to an oligotrophic state can be very slow—on the order of decades to centuries—(Contamin & Ellison 2009), and depends not only on slowing or reversing directional changes in underlying state variables but also on the internal feedback dynamics of the system. Other aquatic systems, including fisheries (Biggs *et al.* 2009), rocky intertidal communities, and coral reefs (Petraitis & Dudgeon 2016) have provided additional empirical support for these model results, in terms of both dynamics, and duration (Dakos *et al.* 2012).

In a previous study, we experimentally demonstrated that organic-matter loading (i.e.,, the addition of insect biomass ["prey"] to pitchers) can cause a shift from oligotrophic to eutrophic conditions in a naturally-occurring microecosystem: the water-filled leaves of the northern (or purple) pitcher plant, *Sarracenia purpurea* L. (Sirota *et al.* 2013). Because bacteria that reproduce rapidly drive the nutrient-cycling dynamics of the five-trophic level *Sarracenia* microecosystem (Butler *et al.* 2008), prey additions cause shifts from oligotrophic to eutrophic states in hours or days rather than years or decades. Further, the comparatively small volume of individual pitchers, the ease of growing them in greenhouses, and the occurrence of large, experimentally manipulable populations in the field (Srivastava *et al.* 2004) has allowed for replicated studies of trophic dynamics, and regime shifts in a whole ecosystem. Here, we extend, and mathematically simplify Sirota et al.'s (2013) mathematical model of the *Sarracenia* microecosystem. We estimate parameter values using new empirical data, and introduce more realism into the underlying environmental drivers of the model. We then use sensitivity analysis to identify the model parameters that control most strongly the dynamics of the system. We illustrate that—as in large lakes—once organic-matter input is stopped, the *Sarracenia* microecosystem can eventually overcome the hysteresis in the system, and return to an oligotrophic state. We conclude that the mathematical model illustrates dynamic behaviors that are qualitatively similar to models of

regime shifts in lakes, and other ecosystems, and we suggest that the *Sarracenia* microecosystem is a scalable model for studying ecological regime shifts in real time.

Methods

The pitcher-plant microecosystem

The eastern North American pitcher plants (*Sarracenia* spp.) are perennial carnivorous plants that grow in bogs, poor fens, seepage swamps, and sandy out-wash plains (Schnell 2002). Their leaves are modified into "pitchers" (Arber 1941), tubular structures that attract, and capture arthropods, and occasionally small vertebrate prey (e.g., Butler *et al.* 2005; Ellison & Gotelli 2009). In the pitchers, prey are shredded by obligate pitcher-inhabiting arthropods, including histiostomatid *Sarraceniopus* mites, and larvae of sarcophagid (*Fletcherimyia fletcheri*), and chironomid flies (*Metrocnemius knabi*) (Addicott 1974; Heard 1994; Jones 1923). The shredded organic matter is further decomposed, and mineralized by a diverse assemblage of microbes, including protozoa (Cochran-Stafira & von Ende 1998), yeasts (Boynton 2012), and bacteria (Peterson *et al.* 2008).

Unlike other species of *Sarracenia* that also secrete, and use digestive enzymes to extract nutrients from their captured prey, *S. purpurea* secretes digestive enzymes at most for only a few days during development (Gallie & Chang 1997). Instead, *Sarracenia purpurea* relies on its aquatic food web to decompose the prey, and mineralize their nutrients (Butler & Ellison 2007). As a result, the rainwater-filled pitchers of *S. purpurea* are best considered a detritus (prey)-based ecosystem in which bacterially-mediated nutrient cycling determines whether it is in an oligotrophic or eutrophic state (Bradshaw & Creelman 1984; Butler *et al.* 2008; Sirota *et al.* 2013).

Oxygen dynamics in lakes, and pitchers

Trophic state (i.e.,, oxygen dynamics) in both lakes, and *Sarracenia* pitchers can be described using a simple model that yields alternative oligotrophic, and eutrophic states, and hysteresis in the shift between them (Scheffer *et al.* 2001):

$$\frac{dx}{dt} = a - bx + rf(x) \tag{1}$$

In Scheffer's model, the observed variable x (e.g., oxygen concentration) is positively correlated with state variable a (e.g., rate of nutrient input or photosynthesis), and negatively correlated with state variable b (e.g., rate of nutrient removal or respiration). The function $rf(x)$ defines a positive feedback that increases x (e.g., the rate of nutrient recycling between the sediment in lakes or mineralization-immobilization by bacteria of shredded prey in a water-filled *Sarracenia* pitcher). If $r > 0$ and the maximum of $\{rf(x)\} > b$, there will be more than one equilibrium point (i.e.,, stable state) (Scheffer *et al.* 2001); the function $f(x)$ determines the shape of the switch between the states and the degree of hysteresis.

Following Scheffer *et al.* (2001), we use a Hill function for $f(x)$:

$$f(x) = \frac{x^p}{x^p + h^p} \tag{2}$$

The Hill function provides a simple model that can produce threshold behavior. The inflection point is determined by the parameter h (Fig. 1A). Given that the other parameters are set so that there are alternative states (i.e.,, $rf(x) > b$), h determines the threshold for the transition between the alternative states. When viewed in a phase-space (Fig. 1B), the transition between states can be seen as a path traversed by the system between distinct regions (i.e.,, phases). In part because of this threshold property, the Hill function has been applied to systems ranging from biochemistry, and microbiology, and through to ecology, wherein system dynamics depend on a limiting resource (e.g., Mulder & Hendriks 2014).

We model the dynamics of the trophic state of the *Sarracenia* microecosystem using a difference equation of the same underlying form as Eq. 1:

$$x_{t+1} = \underbrace{a_t A_t}_{\text{Photosynthesis}} - \underbrace{\left\{ m + a_t \left[\frac{w_{t-1}}{w_{t-1} + K_w} \right] \right\}}_{BOD} + \underbrace{D_t(x_t)}_{Diffusion} \tag{3}$$

Each model term is described below, and summarized in Table 1.

The model (Eq. 3) of the *Sarracenia* microecosystem (Fig. 2A) is made up of the two main terms: production of oxygen by photosynthesis, and use of oxygen (respiration) during decomposition (BOD: biological oxygen demand). The pitcher fluid is oxygenated (x) at each discrete time step (t) as the plant photosynthesizes (A_t). The value of A_t is determined by sunlight, which we model as a truncated sine function producing photosynthetically active radiation (PAR) (Fig. 2B), and by the maximum photosynthetic rate (A_{max}), (Fig. 2C), which leads to the production of dissolved oxygen in the pitcher fluid (Fig. 2D).

Decomposition of shredded prey by bacteria requires oxygen. The oxygen demand from respiration is modeled by the BOD term in Eq. 3. The parameter m is the basal metabolic respiration of the food web with no prey to decompose in the system. Adding prey (w) induces decomposition, which we model as a negative exponential function with rate parameter β, and a constant W (maximum prey mass decomposed over 48 hours) using Eq. 4, and illustrated in Figure 2E.

$$w_{t+1} = w_t \cdot e^{-\beta \cdot W} \tag{4}$$

Bacterial populations increase at a rate determined by a half-saturation function with parameter K_w (Eq. 3), which increases BOD, and the depletion of oxygen from the pitcher fluid (Fig. 2F). Food web demand for oxygen (i.e.,, BOD) depends on the decomposition rate (β), and the shape parameter (K_w), but only when prey is present in the system ($w_{t-1} > 0$ in Eq. 3). When prey is absent (i.e.,, $w_{t-1} = 0$), BOD terms simplify by multiplication to the basal metabolic rate (m).

The other impact of prey addition, and subsequent decomposition by the food web is the release of nutrients into the pitcher fluid. The mineralization variable n_t (Eq. 3), which is modeled as proportional to the product of the amount of oxygen, and prey in the system (i.e.,, $n_{t+1} = c \cdot (w_t \cdot x_t)$ where c is a constant of proportionality), creates a feedback from decomposition to oxygen production by the plant (i.e.,, the path in Fig. 2A from the food web to nutrients to pitcher to oxygen, and back to the food web).

Photosynthesis is limited by available nutrients (primarily nitrogen, and phosphorus, see Ellison 2006; Givnish *et al.* 1984) that are mineralized by bacteria from the prey (Butler *et al.*

2008). Photosynthesis is augmented (a_t) by nutrient mineralization rate (s). We model a_t as a saturating function with bounds determined by the range terms (a_{min}, and a_{max}), s, and the point of saturation (d):

$$a_{t+1} = a_t \times \left\{ \frac{a'_{max} - a'_{min}}{1 + e^{-s \cdot n_t - d}} + a'_{min} \right\} \tag{5}$$

Thus, the mineralization term couples respiration (oxygen depletion) to photosynthesis (oxygen production) when prey is introduced to the system, and the food web begins to decompose the prey and release nutrients into the pitcher fluid. Finally, a small amount of oxygen diffuses into the pitcher fluid directly from the atmosphere ($D(x)$), which is unlikely to influence the fluid oxygen at a rate that is negligible in comparison to photosynthesis or BOD.

Estimating decomposition rate

We used data from a greenhouse pitcher-feeding experiment to estimate the decomposition parameter. The experiment was conducted over 35 days, starting on July 6, 2015, in a temperature-controlled greenhouse at the University of Vermont's Biological Research Complex (Burlington, Vermont, USA). Eighteen newly-formed pitchers > 8 ml in volume were rinsed with deionized water, and randomly assigned to one of three organic matter addition treatments: 0.0, 0.5, or 5.0 mg/ml of pitcher fluid. Pitcher fluid was collected from randomly-selected pitchers at Molly Bog (Morristown, Vermont, USA: 44.4997562 N,-72.641978 W) on the morning of 6 July 2015. The fluid was transported to the greenhouse, filtered through the 30-micron frit bed of a chromatography column (BioRad, Hercules, California, USA) to remove macrobes, homogenized, and added to experimental pitchers in the greenhouse.

Pitchers were loaded with a single pulse of organic matter every morning for the first four days. In this experiment, the organic matter was bovine serum albumin (BSA), DNA from salmon testes at a concentration of 1.5 micrograms per milligram of BSA, and trace elements potassium, calcium, sodium, magnesium, and manganese in a ratio of 1:0.115:0.044:0.026:0.0001. In a pilot study, this organic matter "cocktail" yielded similar changes in dissolved oxygen as we had

obtained previously using ground wasps as organic matter (Sirota et al. 2013). Three 100-μL aliquots of pitcher fluid were sampled from each pitcher twice a day from day 0 to day 20 at 8:30am, and 5:00pm ($\pm$ 2 hrs), once per day from day 20 to day 28 at 8:30am ($\pm$ 2 hrs), and once each on days 30, 31, 33, and 35 (8:30am $\pm$ 2 hrs). Samples were centrifuged at 13,000g, after which the supernatant containing soluble BSA was removed, placed in a sterile tube, and frozen at -80 C until analyzed. A simple Bradford assay (Bradford 1976) was used to determine the concentration of BSA in each of the pitcher fluid samples. The assay was done using Bradford reagent (VWR), and the absorbance of each sample was measured on a Biophotometer Plus spectrophotometer (Eppendorf) at an optical density of 600 nm. Samples were read randomly on the spectrophotometer to avoid reaction time as a confounding variable. Standard curves were created, and sample concentrations were determined using the R software system (R Core Team 2016).

We used an empirical least-squares estimator (LSE) approach to generate a "best fit" value for the decomposition parameter (β) in Eq. 4, given the quantity of prey added in the experiment, and the duration of the prey addition. As K_w is not a part of the decomposition term, it was set it to 2, and we did not vary it during parameter estimation, and model fitting. We then ran a series of 35-day simulations (equivalent to the run-time of the prey-addition experiment) in which β was sampled from a grid of values between ranging from 1E-8 to 0.0007, and the amount of prey in the simulation was recorded at each simulated minute. For each run, the sum of squared errors (SSE) was recorded as $\sum (sim - obs)^2$. The β that minimized the SSE in each simulation was considered to be the best-fit value for each replicate pitcher (n = 12).

Sensitivity Analysis

We used a sensitivity analysis in which we varied the prey addition rate (w), decomposition rate (β), and the half-saturation constant K_w to explore the behavior of the microecosystem model across a wide range of parameter space. Rather than set combinations of fixed values for the three parameters of interest, we sampled the model parameter space by drawing values independently

from a uniform distribution: $K_w \sim$ U(0.0001, 3), $\beta \sim$ U(1.0E-6, 2.0E-3) and $w \sim$ U(0, 100). To characterize baseline (oligotrophic) oxygen concentrations, for each combination of β, and K_w we ran one simulation in which no prey was added to the system (w = 0). In all simulations (n = 15000) variables were initialized as 0 with the exception of oxygen (x), which was initialized using the photosynthesis term (x_0 = 7.55). Prey additions occurred at mid-day on days 4-6 (i.e., $t = 6480$ to $t = 9360$), each simulation ran for 30 simulated days (43,200 minutes), and output was saved for each simulated minute. The simulations were initialized using a random sample of parameter values, and run in parallel. Because the model is completely deterministic, the resulting runs can be reproduced by starting the simulations with the exact values used to initialize, and parameterize the models, which are available via the Harvard Forest Data Archive (`http://harvardforest.fas.harvard.edu/harvard-forest-data-archive/`.

to aid in the detection of the impact of the most important parameters and variables, in all simulations we set some parameter values to zero, which altered the model in the following two ways. First, we ignored the $D(x_t)$ term because we assumed that the amount of oxygen diffusing directly into the pitcher fluid from the atmosphere would be orders of magnitude lower than oxygen produced by pitcher photosynthesis (Kingsolver 1979). Second, we noted that since the basal metabolic respiration of the food web parameter (m) is an additive constant, any change in the value of the constant m, (basal respiration of the microbial community) would result only in a proportional change in value of x, not in the shape (i.e., dx/dt), of the oxygen production over time. Thus, we could set $m = 0$ without loss of generality.

By setting $m = 0$, we also observed that the photosynthetic augmentation term (a_t) influenced photosynthesis (A_t), and BOD ($\frac{w_{t-1}}{w_{t-1}+K_w}$) identically. Therefore, the parameters s, and d in Eq. 5 could be set as constants in the sensitivity analysis. By ignoring diffusion, setting $m = 0$, and fixing s, and d, we reduced the dimensionality of the sensitivity analysis to three (w, β, and K_w), which increased the interpretability of the results.

We calculated two measures of the state of the system from the time series of oxygen concentration (x_t): hypoxia, and return rate. We defined hypoxia in the model to be an oxygen

concentration of $\leq$ 1.6 mgL^{-1}, which is the median lethal O_2 concentration ([O_2]) for aquatic animals (Vaquer-Sunyer & Duarte 2008). We measured the return rate of the system as the linear trend in [O_2] (i.e., after removing the daily cycle in oxygen resulting from photosynthesis) across the entire simulation using Pearson's correlation coefficient. Although the return trajectory can be non-linear, the linear trend measures the gross trends of returning (positive), little impact of feeding (zero) or remaining at depressed oxygen levels.

Code Availability, and Execution

The model was coded in the **R** programming language (R Core Team 2016). The 15,000 model runs for the sensitivity analysis were run on the Odyssey Supercomputer Cluster at Harvard University (Research Computing Group, FAS Division of Science, Cambridge, Massachusetts). Data, code for the simulations, and output of analyses are available in the Harvard Forest Data Archive (`http://harvardforest.fas.harvard.edu/harvard-forest-data-archive`).

Results

The equation representing decomposition, and BOD resembles the Hill function in a general model of state changes with hysteresis (Eqs. 1, and 2). In general, when a Hill function is used in a basic alternative states model (e.g., $rf(x) > b$ in Eq. 1), the inflection point (e.g., half-saturation constant K_w) determines the threshold (Fig. 1A). Thus, modeling decomposition, and BOD using a Hill function provided us with sufficient flexibility to yield a variety of state changes.

The simulations with the model produced dynamics observed in the empirical pitcher plant microecosystem. Because photosynthesis is nutrient-limited in *Sarracenia* (Ellison 2006), addition of prey increased modeled photosynthesis (Fig. 3A) relative to oligotrophic, prey-free pitchers. In the oligotrophic state, and when no prey was added, BOD remained low throughout the entire simulation (black line in (Fig. 3B). After prey was added on, for example, days 4-6 (t = 6480 to t = 9360 minutes), the system jumped into its alternative state: BOD increased rapidly then

declined slowly as prey was mineralized (grey line in Fig. 3B). The combination of the smooth, slow recovery response of photosynthesis to prey addition, and the abrupt shift in BOD following prey addition (Fig. 3A & B) resulted in an abrupt shift in the system from an oxygenated state into an anoxic state, and a very slow (hysteretic) recovery (Fig. 3C). The hysteresis of the system is clearly apparent when oxygen concentration is plotted as a time-lagged phase plot (lag = 1440 minutes starting at t=720), which shows the change in oxygen following addition of prey at t = 6480, and the slow return due to high BOD (Fig. 3D). These results accord with observations from field, and greenhouse experiments in which oxygen was observed to decline with the capture or addition of insect prey to the pitcher (Sirota *et al.* 2013), and demonstrate the presence of both state changes, and hysteresis (i.e., Fig. 3D) for at least some parameterizations of the model.

The parameter fitting, and sensitivity analysis revealed several key effects of the parameters that we varied. First, the LSE model-fitting procedure resulted in an estimate of β of $\overline{x} = 0.00041 \pm 0.0004$ [SE] (Fig. 4, vertical lines). Second, varying β had a large effect on the percent time spent in an hypoxic state, and the return rate (steeper contours with increasing β in Fig. 4). Last, varying the amount of prey by two orders of magnitude produced a sharp threshold for the effect of varying β on hypoxia, and return rate (Fig. 4).

Although varying β has a potentially larger effect on the dynamics of the microecosystem than varying K_w, the latter plays an important role in determining the return trajectory of the oxygen. For simulations with lower values of K_w, the oxygen concentration was still exponentially increasing when the simulation ended (Fig. 5A). Relative to simulations with higher K_w, the return rate was faster when β was low enough, and there was prey (i.e., w_t) remaining in the pitcher at the last observed time (Fig. 5B). Thus, in this part of the parameter space, if another round of feeding were to occur at a similar level of prey input, the system would never recover, and would remain in or near an hypoxic state.

Discussion

General theoretical work in complex systems has suggested that the definition of system boundaries is arbitrary, and carries the potential for systems dynamics to be mechanistically connected to, but unpredictable from, lower levels (or scales) of organization (Levine *et al.* 2016; Ulanowicz 2012). However, others have argued that food web dynamics of whole ecosystems can be inferred from the components (i.e., motifs, and modules) of these ecosystems (McCann 2012). Overall, our model of the *Sarracenia* microecosystem supports the latter assertion: a focus on particular pathways (e.g., photosynthesis, decomposition) reproduced the non-linear behavior of its oxygen dynamics, including state changes, and hysteresis. The results of the sensitivity analysis also revealed that the carrying capacity of the bacterial community (as it was simulated by the effect of K_w) could contribute to observed non-linear state-changes of the *Sarracenia* microecosystem.

Although the dynamics of the *Sarracenia* microecosystem are very similar to those of lakes, and streams, there are several differences. First, oxygen levels in the pitcher plant are dynamically controlled both by photosynthesis of the plant that serves as a strong driver of oxygen levels. In lakes, the primary oxygen production is carried our by phytoplankton, which are emersed in the aquatic system. Second, lake food webs are "green" (i.e., plant-based); whereas pitcher plant food webs are "brown", or detritus-based (Butler *et al.* 2008). In lakes, the shift to a eutrophic state noccurs through addition of limiting nutrients (usually N or P), accumulation of producer biomass that is uncontrolled by herbivores (see Wood *et al.* 2016), and subsequent decomposition that increases biological oxygen demand (Carpenter *et al.* 1995; Chislock *et al.* 2013). The *Sarracenia* microecosystem's "brown" food web also experiences an increase in oxygen demand, and microbial activity; however, this occurs during the breakdown of detritus that is characteristic of its shift from an oligotrophic to a eutrophic state (Sirota *et al.* 2013). Even though the source of the nutrients in the *Sarracenia* microecosystem is "brown", the functional shape of the pathways involved in its nutrient cycling are similar to those in lakes with "green" food webs, and are likely to lead to similar qualitative dynamical behavior of both systems.

Predictions based on the model are highly sensitive to changes in the parameterization of β. In the initial parameterization of this model, we started with an empirical estimate of decomposition rate in which $>$ 99% of the average amount of prey captured could be decomposed in a single day (Baiser *et al.* 2011; Sirota *et al.* 2013). This is extremely rapid decomposition relative to a set of 58 previously published food webs (Lau *et al.* 2016), in which 1.27% to 66.2% of available detritus or organic matter is decomposed each day. When we set the decomposition parameter (β) equal to 2.57E-6, the overall decomposition rate approached the mean of the published food webs (24.22% $\pm$ 2.79 S.E.). This value for β (0.0004) is within the parameter space that we observed experimentally, and used in our sensitivity analysis, and suggests that insights gained from the *Sarracenia* microecosystem should be scalable to larger systems.

The results of our model, and sensitivity analyses, combined with empirical data from Sirota *et al.* (2013), suggest that the *Sarracenia* microecosystem should be a powerful system with which to develop new understanding of the dynamics of complex ecosystems. Both the abiotic environment, and the biotic components of the *Sarracenia* microecosystem are comparable in complexity to large lakes (Baiser *et al.* 2016; Kitching 2000; Srivastava *et al.* 2004), and it features similar critical transitions, and non-linear dynamical behavior, both of which are of broad interest for theoretical ecologists. The food web of *Sarracenia purpurea* consists of species that share an evolutionary history, organized into five trophic levels, and with interactions that have been shaped by both environmental, and co-evolutionary forces (Bittleston *et al.* 2016; Ellison & Gotelli 2009).

Mesocosm studies have been critiqued for lacking any or all of these characteristics (Carpenter 1998) but a recent meta-analysis of the scaling relationships of the half-saturation constant (K_w) provides evidence that uptake of nutrients such as nitrogen, phosphorus by food webs, and inter-trophic nutrient transfers, likely are invariant to spatial scale (Mulder & Hendriks 2014). At the same time, the dynamics of the *Sarracenia* microecosystem play out over days, rather than years, decades, centuries, or even longer. Thus, it provides an experimental, and computational model with which to study the linkages between "green", and "brown" food webs (e.g., Sitvarin *et al.* (2016); Wolkovich *et al.* (2014)), and identify early warning signals of state changes

in ecosystems that are of crucial importance for environmental management (Abbott & Battaglia 2015; Hoekman 2010).

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Table 1: Terms, units, and interpretation for the model of oxygen dynamics in pitcher plant fluid.

Term	Units	Interpretation
t	minutes	time (model iteration)
f	$1/t$	Constant adjusting sine wave of diurnal PAR for frequency of measurements
x_t	mg/L	$[O_2]$: concentration of oxygen in the pitcher fluid
Photosynthesis		
A_t	mg/L	Production of oxygen by photosynthesis, and infused from the plant into the fluid during the day
a_t	mg/L	Photosynthetic rate augmentation by microbial nutrient mineralization
a_{min}, a_{max}	mg/L	Min or max possible photosynthetic augmentation
PAR	$\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$	Photosynthetically active radiation
Respiration		
w_t	mg	Mass of prey remaining at time t
W	mg	Maximum mass of decomposable prey (set at 75μ g)
K_w	mg/min	Half-saturation constant for bacterial carrying capacity
m	mg/L	Basal metabolic oxygen used by bacteria (respiration)
Nutrients		
n_t	mg/L	Quantity of nutrients mineralized by decomposition; a function of w_t, and x_t
s	dimensionless	Sigmoidal curve steepness relating nutrient mineralization to augmentation
d	mg	Inflection point of sigmoidal curve relating mineralization to augmentation
β	dimensionless	The rate of prey decomposition

Figure legends

Figure 1: The threshold dynamics of the Hill function are determined in part by the inflection parameter h. A) Plotted output of the Hill function for different values of h (different lines shaded darker for lower values), ranging from 0.1 to 150 with $p = 10$. B) Lagged ($k = 1$ lag term) phase plot of the Hill function with $h = 71.11$, showing the state transition (lower-left to upper-right). A small amount of random variation was introduced to the series to reveal overlapping points within the two states.

Figure 2: A) The pitcher plant model shown as a network diagram. The nodes in the graph, and their corresponding variables in the model are Prey (w), the microbially-dominated Food Web (controlled by K_w), Nitrogen (n), Oxygen (x), and the Pitcher Plant itself, which is included to show the fluxes of nitrogen, and oxygen as they relate to the plant. B) Photosynthetically Active Radiation (PAR) from the sun or other light source modeled as a sine wave. Although negative values of PAR are set equal to zero in our model's photosynthesis function, we show here the full sine-wave to illustrate the mathematical function from which PAR is derived. C) The relationship between PAR , and photosynthesis in the pitcher plant. D) The output of dissolved oxygen in the pitcher fluid as a function of pitcher-plant photosynthesis. E) The decomposition of prey over time as affected by the bacteria within the food web. F) The impact of prey addition (t = 2160) on pitcher plant dissolved oxygen.

Figure 3: The addition of prey impacts both photosynthetic oxygen production via augmentation from nutrients mineralized from prey, and oxygen depletion through the biological oxygen demand (BOD) of microbial metabolism. (A) shows how photosynthesis increases when prey is added (grey) on days 4-6 (t = 5040 to t = 7920 minutes; indicated by open circles), relative to when no prey was added (black). (B) Shows the quantity of oxygen used via the BOD of microbial decomposition. The net impact in this parameterization was a decrease in dissolved oxygen when prey was added to the system; (C) shows oxygen present in the pitcher at mid-day. (D) A time-lagged phase plot (t_0 = 720, lag = 1440 min) showing the change in oxygen production during the prey addition simulation. Beginning, and end points of the simulation are indicated by closed circles. When prey was added at t = 5040, t = 6480, and t = 7920 (open circles), it was decomposed rapidly by the microbially-dominated food web, resulting in oxygen depletion. The altered return trajectory (i.e., hysteresis) resulting from the biological oxygen demand in the system is shown by the arrows indicating the direction of the change in oxygen through time.

Figure 4: Sensitivity analysis of the pitcher-plant model revealed non-linear effects of varying the parameters β, and K_w (n = 15000 simulations). Contour plots show the percent time the system spent in an hypoxic state (top row), and the Pearson correlation coefficient for the decycled trend (bottom row). The sensitivity simulations were repeated for additions of prey corresponding to 1 mg$mL^{-}1$ (left column), and 100 mg$mL^{-}1$ (right column) of prey added to the microecosystem. The LSE estimate for β ($\pm$ 1 SE) is plotted in each contour plot as vertical solid, and dashed lines, respectively.

Figure 5: Oxygen dynamics in three simulations using different levels of K_w (light-grey = 0.1, dark-grey = 0.5, and black = 1) with the same rate of decomposition (β = 2.0E-6) illustrating hysteresis (i.e., altered return trajectory) of the system. (A) Lower levels of K_w produce slower return rates over the course of the simulation. Prey addition (open circles) depressed mid-day oxygen curves at lower values of K_w. Closed circles indicate the first, and last mid-day prey addition points. (B) A time-lagged (t = 1440) phase plot for the same simulations showing that lower values of K_w led to the oxygen being at lower levels for more time following prey addition (open circles), but followed a similar return trajectory as prey was decomposed by the food web (closed circles also indicate the beginning, and end of each series). Although all three series ran for the same amount of time, the lengths of the trajectories are different in phase space because lower values of K_w resulted in the system spending more time with the same amount of oxygen (i.e., $x_t = x_{t+1440}$).