Predation threat alters community structure and ecosystem function

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Abstract

Predators can have dramatic effects on food web structure and ecosystem processes. However, the total effect of predators will be a combination of prey removal due to consumption and non-consumptive effects (NCEs) mediated through changes to prey behavioural, morphological, or life history traits induced to reduce predation risk. In this study, we examined how consumptive and non-consumptive effects alter community structure and an ecosystem function using the aquatic ecosystem housed within tropical bromeliads. We exposed emptied bromeliads to no predators, caged predators (NCEs only), or uncaged predators (NCEs and consumptive effects) and recorded densities of all macro-invertebrates, microbial densities and in situ CO$_2$ concentrations after 40 days. We found that predators altered community structure and CO$_2$ concentrations largely through NCEs. The magnitude of the effects of NCEs was substantial, contributing > 50% of the total effects of predators on macro-invertebrate communities. The non-consumptive effects of predators were also strong enough to generate a trophic cascade, which significantly increased microorganisms and ecosystem respiration, leading to increased in situ CO$_2$ concentrations. The most likely mechanism behind the NCEs on macro-invertebrate density is detection of predator cues by ovipositing adult females, who actively choose to avoid bromeliads containing predators. Through this mechanism, predator NCEs modified community colonization, the structure of food webs, populations of lower trophic levels, and an ecosystem processes performed by the community. We therefore propose that quantification of the relative strength of predator NCEs in natural ecosystems is critical for predicting the consequences of predator loss from the world’s ecosystems.
Introduction

The total effect of predators on prey is a combination of direct consumption, and predator non-consumptive effects (NCEs). Predator NCEs may take several forms, such as changes in prey behavioural, morphological or life history traits (Tollrian and Harvell, 1999; Peacor and Werner, 2001). Predator NCEs are prevalent in a wide variety of ecosystems, but are particularly common and strong in ecosystems with sit-and-wait or sit-and-pursue predators (Preisser et al., 2007), as cues from sedentary predators provide a point source indicator of the risk of attack. The magnitude of the effect of predator NCEs can also be large, and has been shown to be greater than the effects of direct consumption (Trussell et al., 2006a).

Previous studies have demonstrated that predators can generate trophic cascades through NCEs alone (Schmitz et al., 1997; Forbes and Hammill, 2013), and affect ecosystem processes (Strickland et al., 2013). However, this past research into predator NCEs has tended to focus on simple, two or three trophic level food chains (Trussell et al., 2006b), often with each trophic level represented by a single species (although see (Peacor et al., 2012), and with species densities being determined by the investigators. The effects of predators may be enhanced by artificial manipulation of densities, and reduction of a complex food web to a simple food chain as effects transfer fairly linearly, whereas the reticulate nature of real food webs can dampen trophic cascades (Carpenter, 1996). As predators are currently being lost from the world’s ecosystems at an unprecedented rate (Estes et al., 2011), understanding the strength of predator NCEs is crucial to understanding the consequences of their loss.

Predator NCEs may take many forms, and may affect community dynamics through a variety of mechanisms. Prey species that cross ecosystem boundaries through ontogeny, such as insects and amphibians that have terrestrial adult stages, but oviposit in aquatic ecosystems, may avoid locations within a landscape that pose a predation risk (Berendonk
and Bonsall, 2002; Resetarits and Binckley, 2009; Vonesh and Blaustein, 2010). The decision to avoid ovipositing in locations containing predators can alter colonisation rates, leading to changes in the composition of communities (Kraus and Vonesh, 2010). In addition to altering colonisation rates, the presence of aquatic predators can also increase larval development rates, shortening the length of time individuals are exposed to predation as larvae (Hammill and Beckerman, 2010). Additionally, the threat of predation can reduce foraging rates of competitively dominant prey, influencing community dynamics (Werner and Anholt, 1996).

These pre- and post-colonisation processes act to determine the eventual community structure (Vonesh et al., 2009).

The structure of ecological communities affects the functions the ecosystem performs (Schulze and Mooney, 1994; Cadotte et al., 2011; Hooper et al., 2012). This relationship between community structure and ecosystem functioning implies predator NCE-mediated changes to communities may alter the functioning of ecosystems, including the production of CO₂ through ecosystem respiration. Previous studies have demonstrated that predators are able to alter community respiration to such an extent they change from being sources to sinks of CO₂ (Schindler et al., 1997), or vice versa (Atwood et al., 2013). Freshwater ecosystems globally emit similar levels of CO₂ (up to 1.65 Pg C yr⁻¹) as land use change (Cole et al., 2007). This relatively high level of CO₂ production, coupled with the bi-directional effects of predators, means understanding the mechanisms by which predators alter community respiration has relevance for ecological management actions undertaken to mitigate CO₂ production (Schmitz et al., 2013).

We quantified predator NCEs on community structure and community respiration using the natural ecosystems contained in the water filled leaf axils of Guzmania bromeliad phytotelmata. Bromeliad leaves are arranged in a rosette structure, and within the wells created by the leaf axils of tank bromeliads exists an aquatic, detrital-based ecosystem
Bromeliad communities experience periodic droughts that can lead to the loss of many members of the community, and are therefore in a seasonal cycle of drought and re-colonisation, with community structure being related to colonisation rates (Srivastava et al., 2008). Following the onset of rain, bromeliad ecosystems are colonised by macro-invertebrate insect larvae, including predatory Odonata, mosquitoes (Culicidae - filter feeders and browsers), as well as detritivorous Chironomidae, Tipulidae and Scirtidae. Throughout the rainy season, adult insects are continuously adding to the macro-invertebrate community though oviposition, and individuals are being lost as they emerge as adults or die. The number of insect larvae present in a bromeliad at any one time is therefore governed by the relative rates of oviposition vs emergence and death. Bromeliad ecosystems also contain a broad microbial community, including ciliates (subphylum Ciliophora), flagellates (subphylum Mastigophora), and rotifers. This microbial community consumes bacteria, fungi, and detritus. The community within the bromeliad therefore contains at least two different compartments, the microbial food web and a detritivore food web, with larvae of the damselfly *Mecistogaster modesta* (Selys, 1860) acting as top predators in both (Figure 1b).

The aquatic ecosystems within bromeliads are net-heterotrophic, fuelled predominantly by the mineralization of organic compounds from allochthonous detritus, resulting in the release of CO$_2$, methane (Martinson et al., 2010; Atwood et al., 2013) and dissolved nutrients (Ngai and Srivastava, 2006). Theoretically, Odonate predators may either increase or decrease community respiration, depending on which trophic pathways are most influenced by predation risk (Figure 1b). The effect of predators on CO$_2$ concentrations would depend on whether the dominant trophic pathway was even-numbered (detritus-microbes-mosquitoes-predator), in which case predators would increase CO$_2$ concentrations, or odd-numbered (detritus-macroinvertebrates-predator), where predators would decrease CO$_2$ concentrations. However, as both mosquitoes and protists exhibit omnivory within
species and variance in trophic levels between species (Figure 1b), it is difficult \textit{a priori} to establish the effective number of trophic levels in this pathway.

As \textit{M. modesta} are generalists able to consume a wide variety of macro-invertebrates, risk of consumption and a selection pressure to avoid bromeliads containing \textit{M. modesta} is likely to be felt by all species. We therefore made three hypotheses: 1 - the presence of predators would lead to lower densities of all macro-invertebrate species. 2 - The reduction in macro-invertebrates would create a trophic cascade, releasing the microbial community from the pressures of macro-invertebrate predation (especially from mosquitoes), increasing their densities. 3 - Predator NCEs on the macro-invertebrate and microbial communities significantly affect rates of community respiration [as approximated by CO$_2$ concentrations within the water column (Del Giorgio et al., 1999)].

\textbf{Materials and Methods}

We collected 30 bromeliads from the genus \textit{Guzmania} from a tropical mid-elevation rainforest (~700 m above sea level) within 5 km of Estación Biológica Pitilla, Área de Conservación Guanacaste, Costa Rica. Prior to being used in the experiment, all bromeliads were thoroughly washed and immersed in water in an inverted position for 24 hrs, then left to dry for 7 days. This method eliminated most if not all invertebrates and residual chemical cues from predators prior to use. The preparation process did not lead to mortality in any of the plants. Our study was conducted during the rainy season (October and November), a time when oviposition by intermediate trophic levels (mosquitoes, Chironomidae, Tipulidae and Scirtidae) is highest and \textit{M. modesta} are in mid-instars (Srivastava, 2006). Additionally, \textit{M. modesta} rarely oviposit during the rainy season, which helped ensure that predator free treatments remained free of \textit{M. modesta} throughout the study (Srivastava, 2006). At the start of the experiment, plants were moved to a 30 m x 30 m patch of secondary forest, and
suspended from trees that had a diameter at breast height greater than 10 cm. The wells of the plants were filled using commercially available mineral water and leaf litter from Conostegia xalapensis Bonpl. was distributed throughout the plant at a density of 200 mg (dry weight) per 100 ml (total plant volume) to act as food for the community. The leaves of C. xalapensis are highly abundant and easily recognisable, using a single species for leaf litter in all treatments minimised differences in nutrient composition.

Prior to the start of the experiment, we recorded the maximum volume of water each plant could hold, and randomly assigned them to three experimental treatments: no predators, caged predators (predator NCEs only) and uncaged predators (NCE’s and consumptive effects of predators, hereafter referred to as total predator effects). Each treatment was represented by 10 replicates, and plants did not significantly differ in size between treatments (volume range 500 – 1500 ml, mean 1013.5ml, F(1,28) = 2.17, p = 0.15). The strength of predator NCEs can be related to predator biomass, with larger predators generating stronger effects (Hill and Weissburg, 2013). To minimise the confounding effect of predator size, all predators used in the experiment were mid-instar M. modesta larvae with body lengths, 12 – 15 mm body. A single M. modesta larva was added inside the cage for caged (NCE only) replicates, and one outside the cage for uncaged (total predator effect) replicates. Using a single predator per plant mimics natural M. modesta densities for the size of bromeliads used in our study (Srivastava et al., 2005). Predator cages consisted of 50 ml clear centrifuge tubes. Two 15 mm diameter holes were drilled in the sides of the tubes and covered with 80 µm mesh. These mesh-covered holes allowed water inside and outside the cages to mix, facilitating diffusion of predator chemical cues. We used 80 µm mesh as it allowed the transfer of chemical cues without clogging with detritus, but did not allow the passage of small macro-invertebrate prey into predator cages. We also added empty cages to no predator and uncaged predator treatments to ensure differences between treatments were not due to the
presence/absence of cages. All cages were placed inside a well in the second row of leaves from the centre of the plant. Placing the cage relatively close to the centre ensured that chemical cues from a caged predator could diffuse down through the rest of the plant, as water cascades down through the bromeliad during rain showers. Caged predators were fed a single mosquito and chironomid larvae every other day, whereas uncaged predators consumed insects within the bromeliads. We mimicked the feeding procedure in the uncaged predator and no predator treatments to ensure adequate control, in case adult mosquitoes were attracted to the plant by our presence.

The experiment was run for 30 days, during which time the insect community accumulated through natural colonisation. After the 30 day colonisation period, we compared differences in macro-invertebrate community structure and density, microbial density, and in situ CO$_2$ concentration. A 30 day study period was used as it allowed for multiple colonization events, while minimizing loss from emergence. Chironomidae, Tipulidae and Scirtidae colonizing the bromeliads have larval stages that are typically greater than one month in duration (Srivastava, 2006). Although 30 days may have exceeded the hydroperiod of mosquito species in this study, oviposition by adult mosquitoes is ongoing throughout the rainy season.

At the end of the experiment, we randomly selected a well that had not contained the cage, and collected a 1 ml water sample, which was preserved with Lugols media and later used to calculate protist density. In a different well, 6 ml of water was extracted using a 50 ml Pressure-Lok® syringe (VICI Precision Sampling Corp., Baton Rouge, LA), injected in a gas tight vacutainer (Labco Limited High, Wycombe, UK.), chilled and transported within 72 h to the Department of Civil Engineering, Environmental Laboratory at the University of British Columbia for analysis of dissolved CO$_2$ gas concentrations. Two CO$_2$ samples were compromised during transit, and thus not included in the analysis. Collections and
calculations of CO$_2$ concentrations from sample water followed procedures from Hope et al.
(Hope et al., 1995).

Following CO$_2$ collections we removed all water, insects and detritus from the plant. Insect larvae were sorted and preserved in ethanol within six hours. We identified mosquito larvae to species, while other insects (largely Chironomidae, Tipulidae and Scirtidae) were sorted to family level. For each plant, we calculated macro-invertebrate density (the total number of individuals of each species divided by plant volume) in order to directly compare organisms from plants of different sizes. Data are available on the Knowledge Network for Biocomplexity (http://knb.ecoinformatics.org/knb/metacat/knb.302.1/knb). To estimate densities of micro-organisms, all protists and rotifers were counted in a 50 µm sub-sample of the original Lugols-preserved sample. Five micro-organism samples were compromised during transit to the University of British Columbia, and removed from the analysis.

As our macro-invertebrate community composition data required the analysis of multiple response variables (i.e. the density of each species), and a single explanatory variable (predator treatment), we opted to use a multivariate approach. The “adonis” function from the package “vegan” (Oksanen et al., 2012), built using the R statistical language (R Development Core Team, 2013) can be used to carry out permutational analysis of variance (PERMANOVA) using distance matrices, and is a generally robust method to investigate differences in multivariate data. We initially ran a PERMANOVA comparing differences in macro-invertebrate community structure among the three experimental treatments (no predator, caged predator, uncaged predator, n = 10 for each treatment). To establish which treatments differed from each other, we carried out post-hoc pair-wise comparisons of each treatment pair, and applied a Bonferroni correction to avoid inflating the chance of finding significant results (Holm, 1979). Differences in macro-invertebrate community structure between the experimental treatments were visualised using multidimensional scaling plots.
Multidimensional scaling uses ordination techniques to display the information within a distance matrix. Each replicate is assigned a co-ordinate in each of n-dimensions, by setting n = 2, data can be plotted in 2-dimensional space. Within this space, replicates that are close together are similar to each other, while replicates that are far apart are different. MDS plots therefore represent a method to easily illustrate similarities of difference between replicates in terms of densities of multiple different species (Garpe et al., 2006). We subsequently used ANOVAs and post-hoc Tukey’s tests to look at the differences in populations of community members, giving a biological explanation for the community differences expressed in the MDS plots, and to investigate differences in community respiration (dissolved CO₂ concentrations in the water). In order to account for non-normality of the data, mosquito, macro-invertebrate detritivore, and micro-organismal densities were log transformed prior to analysis. In order to avoid inflating the chance of finding significant differences due to running multiple tests, we applied a Holm-Bonferroni correction to the P-values generated from the ANOVAs, and report the corrected P-values in the results.

After we used formal statistical analysis to demonstrate the differences between experimental treatments in consumer densities and dissolved CO₂ concentrations, we used randomised bootstrap methods to quantify the proportion of total predator effects accounted for by NCEs. We randomly sampled, with replacement, 10 replicates within each treatment and calculated a mean. We then calculated the difference between the caged predator mean and the no predator mean (NCEs of predators only), and the difference between the uncaged predator mean and the no predator mean (total predator effect). Dividing the NCEs by total predator effects then gave us the relative size of the NCEs (as a percentage of total predator effects). To generate a distribution, this method was repeated 10,000 times for each parameter. Randomised bootstraps are generally more accurate and robust than other methods of analysing the magnitude of differences between treatments (Adams and Anthony, 1996).
Additionally, as randomised bootstraps generate a distribution of differences between treatment means, confidence limits around the estimate can be reported (Forbes and Hammill, 2013). In several instances the difference between no predator treatments and caged predator treatments was greater than the difference between no predator and uncaged predator treatments, resulting in the median reported size of NCEs being greater than 100% of total predator effects. However for all response variables the lower 95% confidence limit was <100%, demonstrating that NCEs were not significantly greater than total predator effects, and values >100% are likely statistical noise.

Results

Macro-invertebrate community structure differed significantly among no predator, caged predator, and uncaged predator treatments ($f_{(2,27)} = 14.1$, $p < 0.001$, PERMANOVA, Figure 2). Pair-wise comparisons showed that community structure in control treatments was different from caged predator treatments ($f_{(1,18)} = 29.4$, adjusted $p = 0.003$, PERMANOVA, Figure 2) and uncaged predator treatments ($f_{(1,18)} = 20.6$, adjusted $p = 0.003$, PERMANOVA, Figure 2). Caged and uncaged predator treatments also differed from each other ($f_{(1,18)} = 5.6$, adjusted $p = 0.006$, PERMANOVA, Figure 2).

The effects of predator treatment on community composition appeared to be due to significant density changes among treatments in both mosquitoes and macro-invertebrate detritivores (Table 1, Figure 3a). Compared to no-predator controls, densities of all mosquito genera were significantly reduced in the presence of caged predators (Table 1, Figure 3a), and uncaged predators (Table 1, Figure 3a). Densities of Culex and Wyeomyia were significantly lower in uncaged compared to caged predator treatments, while Anopheles densities were not different between caged and uncaged treatments (Table 1, Figure 3a). The majority of the predator-associated reductions in mosquito density for all species were due to...
NCEs, as caged predators (NCEs only) caused a reduction all mosquito species > 50% that of uncaged predators (Figure 4). Compared to no predator controls, all macro-invertebrate detritivore densities were significantly reduced by both caged predators and uncaged predators (Table 1, Figure 3b-d); although we found no evidence of significant differences between caged and uncaged predator treatments for any of the benthic detritivore families (Table 1, Figure 3b-d). Therefore, NCEs appeared to account for the vast majority (~100%) of the total predator effects on detritivore densities (Figure 4).

Effects of predators on mosquito and detritivore densities appeared to differentially alter densities of the microbial community (Table 1, Figure 3e). Compared to bromeliads without predators, Ciliophora densities were increased by the presence of uncaged, but not caged predators, while Mastigophora densities were significantly higher in the presence of caged predators, and higher still when predators were uncaged (Table 1, Figure 3e). Rotifera densities were unaffected by any experimental treatment (Table 1, Figure 3e). Predator NCEs accounted for the minority of total predator effects (< 50%) for all micro-organism Phyla (Figure 4).

Community respiration also significantly differed among predator treatments (Table 1, Figure 3f). Compared to bromeliads without predators, dissolved CO$_2$ concentrations were significantly higher in caged and uncaged predator treatments (Table 1, Figure 3f). Furthermore, dissolved CO$_2$ concentrations in uncaged predator treatments were significantly higher than caged predator treatments (Table 1, Figure 3f). The size of predator NCEs on CO$_2$ concentrations was a relatively large (61.4%) percentage of total predator effects (95% CI = 34.8% - 96.2%, Figure 4).

Discussion
We have demonstrated that predator NCEs are strong enough to generate changes in community composition, leading to altered food web structure and ecosystem processes in a natural bromeliad ecosystem. Within our experiment, the threat of predation alone substantially reduced macro-invertebrate densities, generating a trophic cascade that increased microbial densities. We believe that higher microbial densities led to the increased dissolved CO$_2$ concentrations in the water through greater community respiration. Our study suggests that predator NCEs may play a crucial role in determining community composition, and differences in community composition alter ecosystem respiration.

Our results showed that all macro-invertebrate species decreased in the presence of caged predators (NCEs only). This result provides clues as to the mechanisms by which predator NCEs affected prey densities. NCEs can alter communities through changes in competitive interactions between prey. Prey may induce defences to predation that affect their ability to compete with other species, reducing some prey species densities and increasing others (Mowles, Rundle & Cotton, 2011). However, our data do not support this as all macro-invertebrate species decreased in the presence of caged predators, suggesting no species gained an advantage over its competitors following predation risk. Although we are unable to determine the exact mechanism by which caged predator treatments reduced macro-invertebrate densities, predator NCEs could have been mediated through changes in; i) oviposition behaviour, affecting how the community was assembled, and/or ii) changes in larval development rate, increasing the rate at which individuals left the community through emergence. Many insect species have the ability to detect the presence of predators in an ecosystem and choose to oviposit elsewhere (Brodin et al., 2006; Vonesh and Blaustein, 2010), reducing the number present in the community. Diptera species have also been shown to increase larval development rates in response to the threat of predation (Hammill and Beckerman, 2010), which would reduce the density within the community through faster
emergence rates. Although the most parsimonious explanation for predator NCEs on larval macro-invertebrate densities is reduced oviposition rates and/or increased development rates, we cannot discount the possibility that predator NCEs also operate via indirect means. In pitcher plants, larval mosquito growth is facilitated by detrital breakdown by detritivore larvae (Heard 1994), but it is unknown if similar effects occur in bromeliads. As a result, negative effects of NCEs on detritivore oviposition rates could have reduced facilitative effects of detritivores on larval mosquitoes.

The threat of predation alone was sufficient to cause an increase in the density of protists, presumably related to the decrease in mosquito densities, but not rotifers, suggesting they are unaffected by changes in other trophic levels. It is unlikely that protist densities were affected by predator NCEs on detritivores, as previous experiments have shown no effect of free roaming odonates on protozoan (ciliates, flagellates) or rotifer densities in the absence of mosquitoes (Srivastava and Bell, 2009). By contrast, increased protist densities following a decrease in mosquito abundance (a trophic cascade) are well-documented in aquatic systems (Eisenberg et al., 2000; Kneitel and Miller, 2002).

For macro-invertebrates, the proportion of total predator effects accounted for by NCEs was large, >50% for all mosquito species and ~100% for Anopheles and benthic detritivores. Predatory M. modesta are voracious, generalist predators, able to consume all other bromeliad-dwelling insects (Srivastava et al., 2005), meaning all species will be under selection pressure to avoid them. However, we postulate that differences in the contribution of NCEs between prey of M. modesta are related to the life-histories of the species involved, and the mechanisms used to avoid predator encounters. Prey may avoid M. modesta in two ways, adults may avoid ovipositing in bromeliads containing M. modesta, or larvae avoid encounters with M. modesta within the bromeliad wells. As M. modesta are found predominantly in the leaf litter at the base of bromeliad tanks (Srivastava et al., 2005), they
inhabit the same micro-habitat as benthic detritivores. This micro-habitat sharing will mean
benthic detritivores have a high chance of encountering predators. Anopheles larvae lack a
breathing siphon and are therefore constrained to the water surface, meaning when viewed
from underneath they are silhouetted and easily detected by M. modesta. Conversely to
detritivores and Anopheles, Culex and Wyeomyia larvae may move through the water column,
allowing them to efficiently avoid contact with predators. As it would appear benthic
detritivores and Anopheles have a lower ability to reduce predator encounters in the water,
they may experience a relatively higher pressure to avoid ovipositing in predator locations.
This increased pressure to avoid ovipositing in predator locations potentially explains why
NCEs accounted for such a large portion of total predator effects on Anopheles and benthic
detritivores, but not Culex and Wyeomyia.

The risk of odonate predation also affected community respiration, as measured by
concentrations of dissolved CO$_2$ in the water. Community respiration increased in the
presence of both caged and uncaged predatory odonates, even though these bromeliads
contained fewer macro-invertebrates. A probable explanation for higher CO$_2$ concentrations
in predator treatments was the increase in microbial densities. Bacterivorous protists have
important consequences for rates of detrital decomposition (Ribblett et al., 2005), and these
rates of decomposition are correlated with rates of community respiration (Young et al.,
2008). It would appear that the higher CO$_2$ concentrations generated through respiration and
decomposition by the microbial community were greater than the amount lost through
reductions in the macro-invertebrate community, resulting in an overall net increase in
community respiration. We believe it is unlikely that the presence of predators themselves
was enough to increase CO$_2$ concentrations. Based on the densities of micro-organisms we
found and respiration estimates from previous investigations (Lawton, 1971; Glazier, 2009)
we estimate predatory damselflies contributed <0.1% of the CO$_2$ generated by the micro-
organisms (full calculation in Appendix 1). The increase in CO$_2$ following addition of a predator is also contrary to previous studies in the same ecosystem that showed predators decrease CO$_2$ (Atwood et al., 2013; Atwood et al., 2014). The previous investigations by Atwood et al (2013, 2014) used a 3-tier food chain (Damselfly-macroinvertebrates-detritus), whereas the present investigation contained a 4-tier component (Damselfly-mosquito-protists and rotifers-detritus). Our results therefore agree with earlier investigations showing top predators increase CO$_2$ production in 4-tier food chains (Schindler et al., 1997). The results we present, in conjunction with the earlier work of Atwood (2013, 2014) and Schindler (1997) demonstrate the relationship between predator effects on CO$_2$ production and food chain length. Encouraging top predators has been proposed as a management initiative to reduce atmospheric CO$_2$ (Schmitz et al., 2013), we propose that without knowing food chain length, encouraging predators may increase rather than decrease CO$_2$ concentrations.

The use of cages to isolate predators from the remainder of the community allows chemical cues to disperse through the ecosystem (Hettyey et al., 2010), but means that prey are unable to use visual or tactile cues to detect predators. There is also growing evidence that prey species choose locations within a landscape based not only on the quality of the location itself, but also the quality of nearby locations (Resetarits and Binckley, 2009). Previous studies have shown prey avoid predator-free ecosystems that are located close to ecosystems containing predators (Resetarits and Binckley, 2009). Within our experiment, all plants were ~ 1.5 m apart, and arranged randomly (to avoid “clumping” of treatments). However, we cannot rule out that the proximity of bromeliads containing predators made our predator-free control plants more or less desirable to ovipositing females than “isolated” predator-free plants would have been.

The addition of mosquitoes and chironomids to our caged treatment as food items would have introduced nutrients into the ecosystem, potentially providing extra resources for
the microbial community. While we cannot rule out that these extra nutrients may have had an effect, the number of prey items introduced as food was relatively low compared to the densities of individuals that naturally colonised (<8% in any one treatment). In terms of carbon additions, we estimate the total carbon content added as food in the caged predator treatments to be <5% of what was originally added as leaf litter (full calculation given in Appendix 2). This estimation is based on length-weight regressions of the food items (Sabo et al., 2002), and estimations of the carbon content of aquatic insects (Kraus and Vonesh, 2012) and leaf litter (Martin and Thomas, 2011). The estimation of percentage carbon introduced as food does not account for leaf litter falling into bromeliads naturally through the experiment, suggest the estimated 5% contribution may be high. Our results also suggest that nutrients being introduced as food items did not substantially affect the ecological community as micro-organism densities were lower in our caged predator treatments than in our uncaged predator treatments (increased nutrient additions should generate the opposite effect). The low amount of nutrients contributed by food additions, and the relatively low density of micro-organisms (compared to uncaged predators treatments) suggests the addition of prey into our caged predators treatments did not bias our results.

In many complex natural systems, predator NCEs may be overlooked due to difficulties associated with the quantification of their relative contribution. However, we show that failing to quantify and account for NCEs may lead to misunderstandings of the mechanisms by which predators affect community assembly, food web structure and ecosystem function. Here we show that predator NCEs altered prey densities, and produced trophic cascades that affected ecosystem processes. Global predator densities are in serious decline (Estes et al., 2011), and management strategies designed to replace predators ecologically must account for both consumptive and non-consumptive predator effects. In a changing world, failing to understand the consequences of predator NCEs may have serious
implications for the structure of natural communities, and the ecological functions they
perform.

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In addition to the above calculation, our conclusion that community respiration is attributable mainly to the microbial increase is justified on the basis of two previous studies. These previous investigations used the exact same system, but without the mosquito-microbe component of the food web (Atwood et al., 2013; Atwood et al., 2014). In both of these studies, predator presence reduced CO$_2$ of three-tier bromeliad food webs. The difference in the results of the current investigation and these previous papers is attributed to differences in food chain length. In the previous papers the authors study a simplified odd-numbered food chain, while the current investigation looks at a more reticulate even-numbered food chain. If the increase in community respiration we observe in the present study were due mainly to the addition of predators, we would have expected to see an increase in community respiration (and therefore CO$_2$) in these earlier studies, when in fact we see the opposite.
Appendix 2. Calculation of the potential for macroinvertebrates offered as food to act as a nutrient subsidy.

The possibility exists that increased CO$_2$ concentrations in the caged predator treatments (NCEs alone) may have been due to nutrient subsidies offered to the predator as food. To estimate the importance of the carbon added as food, we calculated the total mass of carbon added as food, and compared this value to the amount of carbon added to all bromeliads as leaf litter.

Leaf litter from tropical forests is composed of ~47% carbon (Martin and Thomas, 2011). In each bromeliad, we added 200mg (dry weight) leaf litter per 100ml plant volume. The mean volume across plants was 1013.5ml, meaning we added 2027 mg dry weight leaf litter, which equates to 952.7mg C. We added a total of 30 food items (mosquito and chironomid larvae) to each of the caged treatments over the course of the experiment. These food items were all ~5mm total length, we therefore used the length of 5mm to calculate dry mass using length-weight regression values for aquatic insects (Sabo et al., 2002). Using these values, we calculate we added a maximum of 98.6mg of food, which equates to 44.37mg C according to estimates of the carbon composition of aquatic insects (Kraus and Vonesh, 2012). This therefore means that the amount of carbon we introduced as food in the caged treatments was < 5% of what was initially introduced as leaf litter. This value of 5% is around 1/10$^\text{th}$ of the size of the error bars around the CO$_2$ estimate in figure 4, suggesting that the contribution to CO$_2$ concentrations made by the addition of food items has not substantially affected the results. This value of 5% would also be an over-estimate as it does not include carbon introduced to all replicates as leaf litter falling naturally from the trees during the experiment. For these reasons we believe that although adding food items to the caged predator treatments may have slightly increased CO$_2$ concentrations, the observed difference in CO$_2$ concentrations between our no predator and caged predator treatments is
primarily due to changes in community composition, rather than the introduction of food items.
Table 1. Results from ANOVAs performed on all species and CO₂ concentrations, with Tukey tests where applicable. “Corrected P” denotes the P-value following Holm-Bonferroni correction (Holm, 1979). Bolded values indicate significant differences at 0.05 level.
Figure legends

Figure 1. (a) Illustration of the structural nature of *Guzmania* bromeliads, and the technique used to generate experimental treatments, damselfly larvae and tubes enlarged 4x relative to bromeliads to improve clarity. (i) No predator treatments, (ii) Caged predator treatments, non-consumptive effects (NCEs) only, (iii) Uncaged predator treatments, both consumptive effects and NCEs. (b) Simplified bromeliad food web demonstrating proposed energy flow between trophic groups.

Figure 2. Multidimensional scaling plot (MDS) illustrating how predators alter the macro-invertebrate community composition in bromeliads. Within the plot, each point represents a single bromeliad community. Treatments containing predators are represented by circles, either caged (NCEs) $\bigcirc$, or uncaged (total predator effects $\bullet$). No predator controls (no predator effects) are represented by crosses ($\oplus$). The distance between points is proportional to the similarity in community composition, meaning nearby points represent similar communities.

Figure 3. Predator effects on densities of (a), mosquitoes (filter-feeders) (b), Chironomidae (e), Tipulidae, (d), Scirtidae, (e), Micro-organisms and (f) in situ CO$_2$ concentrations of bromeliad ecosystems. Caged predator treatments were only exposed to the non-consumptive effects of predators, while uncaged predator treatments were exposed to both the non-consumptive and consumptive effects of predators. Different letters denote treatments that differ significantly from each other according to post-hoc Tukeys testing within a genus (a), family (b-d), or phyla (f). Bars represent means ± standard errors.
Figure 4. Relative magnitude of non-consumptive effects (NCEs), compared to total predator effects, on species and families of organisms, as well as CO₂ concentrations, within bromeliad communities. For parameters where the bar height is greater than 100% the difference between caged predator and no predator treatments was greater than the difference between uncaged predator and no predator treatments. Data are means ± 95% confidence limits.
### Table 1

<table>
<thead>
<tr>
<th></th>
<th>f-statistic</th>
<th>corrected P</th>
<th>Tukey results (predator treatment)</th>
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<tr>
<td></td>
<td></td>
<td>none vs. uncaged</td>
<td>none vs. caged</td>
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<tr>
<td><strong>Mosquitoes</strong></td>
<td></td>
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<tr>
<td><em>Culex</em></td>
<td>$f_{(2,27)} = 102.6$</td>
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<td>$0.003$</td>
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<td><strong>Benthic detritivores</strong></td>
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<td>$f_{(2,27)} = 6.94$</td>
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</tr>
<tr>
<td>Tipulidae</td>
<td>$f_{(2,27)} = 6.17$</td>
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<td>Scirtidae</td>
<td>$f_{(2,27)} = 4.19$</td>
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<td><strong>Micro-organisms</strong></td>
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<td>Ciliphora</td>
<td>$f_{(2,22)} = 9.755$</td>
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<td>$0.61$</td>
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<td>Mastigophora</td>
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<td>Rotifera</td>
<td>$f_{(2,22)} = 1.75$</td>
<td>0.20</td>
<td>NA</td>
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<tr>
<td><strong>Respiration</strong></td>
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<tr>
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</tbody>
</table>

1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31
Figure 1

(a) (i) (ii) (iii)

(b)

- Odonates
- Mosquitoes
- Protists and rotifers*
- Bacteria, fungi, algae

*Multiple trophic levels, omnivory frequent

Detritus and nutrients leached from detritus

Detritivorous macroinvertebrates
Figure 3

(a) Log (mosquito larvae\(^{-1}\))

(b) Log (Chironomidae larvae\(^{-1}\))

(c) Log (Tipulidae larvae\(^{-1}\))

(d) Log (Scirtidae larvae\(^{-1}\))

(e) Log (micro-organisms\(^{\mu L^{-1}}\))

(f) CO\(_2\) concentration (ppm)

Mosquito genus: Culex, Wyeomyia, Anopheles

Microbe phylum: Ciliophora, Mastigophora, Rotifera
Figure 4

Relative magnitude of NCEs (as a percentage of total predator effects)

<table>
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<th>Mosquitoes</th>
<th>Detritivores</th>
<th>Micro-organisms</th>
</tr>
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<tbody>
<tr>
<td>Culx jenningii</td>
<td>Anopheles spp</td>
<td>Spirotarsus</td>
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<tr>
<td>Wyomyia aedebola</td>
<td>Chironomidae</td>
<td>Sciididae</td>
</tr>
<tr>
<td>Tipulidae</td>
<td>Ciliophora</td>
<td>Mastigophora</td>
</tr>
<tr>
<td>Rotifera</td>
<td>CO₂</td>
<td>Not significant</td>
</tr>
</tbody>
</table>


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References for Supplementary material


