Terrestrial support of aquatic food webs depends on light inputs: a geographically-replicated test using tank bromeliads

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Abstract. Food webs of freshwater ecosystems can be subsidized by allochthonous resources. However, it is still unknown which environmental factors regulate the relative consumption of allochthonous resources in relation to autochthonous resources. Here, we evaluated the importance of allochthonous resources (litterfall) for the aquatic food webs in Neotropical tank bromeliads, a naturally replicated aquatic microcosm. Aquatic invertebrates were sampled in more than 100 bromeliads within either open or shaded habitats and within five geographically distinct sites located in four different countries. Using stable isotope analyses, we determined that allochthonous sources comprised 74% (±17%) of the food resources of aquatic invertebrates. However, the allochthonous contribution to aquatic invertebrates strongly decreased from shaded to open habitats, as light incidence increased in the tanks. The density of detritus in the tanks had no impact on the importance of allochthonous sources to aquatic invertebrates. This overall pattern held for all invertebrates, irrespective of the taxonomic or functional group to which they belonged. We concluded that, over a broad geographic range, aquatic food webs of tank bromeliads are mostly allochthonous-based, but the relative importance of allochthonous subsidies decreases when light incidence favors autochthonous primary production. These results suggest that, for other freshwater systems, some of the between-study variation in the importance of allochthonous subsidies may similarly be driven by the relative availability of autochthonous resources.

Key words: allochthonous carbon; allochthony; aquatic food webs; autochthonous carbon; autochthony; natural microcosms; stable isotopic analysis; tank bromeliads; tropics.

Introduction

Allochthonous resources provide subsidies of energy and organic matter to the food webs of recipient systems (see Polis et al. 1997). In aquatic lotic ecosystems, inputs of terrestrial detritus support most secondary production within upland forested streams (Finlay 2001, Mosisch et al. 2001), but diminishes in importance relative to aquatic algae in larger rivers (Finlay 2001, Collins et al., *in press*). More recently, the importance of terrestrial resources for subsidizing aquatic lentic ecosystems has

been recognized (e.g., Pace et al. 2004, Carpenter et al. 2005, Solomon et al. 2011, Berggren et al. 2014). Allochthonous resources accounted for more than half of total carbon flow to planktonic, benthic and fish communities after additions of carbon isotope tracers to experimental lakes (Pace et al. 2004, Carpenter et al. 2005). In these lakes, the importance of allochthonous sources for aquatic communities was positively related to both the concentration of allochthonous dissolved organic carbon and negatively related to the abundance of planktonic primary producers (Carpenter et al. 2005, Cole et al. 2011, Solomon et al. 2011). A similar pattern has been observed for other temperate humic lakes (Berggren et al. 2014, Kelly et al. 2014, Scharnweber

et al. 2014). In all these cases, the authors pointed out that external carbon inputs are several orders of magnitude higher than autochthonous production, and that the allochthony was sustained either through the uptake of allochthonous dissolved organic carbon by microbial food webs or through the consumption of particulate organic carbon (POC) by higher trophic levels.

Nevertheless, the nutritional quality of allochthonous terrestrial carbon as a food resource to aquatic organisms has been questioned. Terrestrial organic matter is usually humic-rich and nutrient-poor when compared to autochthonous carbon sources (Steinberg et al. 2006). Terrestrial organic matter also has very low content of ω-3 polyunsaturated fatty-acids (PUFA), particularly the longer carbon chains (eicosapentaenoic acid – EPA; docosahexaenoic acid – DHA), posing a challenge for animals which are restricted to obtaining such ω-3 PUFAs from their diet (Brett and Muller-Navarra 1997). On the other hand, some algal groups (e.g., diatoms, cryptophytes) synthesize and have high contents of EDA and DHA fatty acids (Brett and Muller-Navarra 1997), making these algae potentially preferred food resources for aquatic herbivores. In summary, the conflicting results obtained from *in situ* studies with stable isotopes and in vitro studies of the physiology of some aquatic invertebrates has led to an ongoing active debate about the relative importance of allochthonous carbon subsidies in aquatic lentic ecosystems. The small number of studied aquatic systems and model organisms currently limits broader generalizations. The current study overcomes these sample size limitations by evaluating the contribution of allochthonous resources for multiple taxa within a diverse food web, and for a large number of independent ecosystems located in different habitats over five distant sites within the New World tropics.

Bromeliaceae is a family of New World plants (except *Pitcairnia feliciana,* a South African species) whose distribution ranges from the south of the United States to the central part of Argentina and Chile. Bromeliads can be locally abundant, reaching densities of approximately 10 individuals m^{-2} in early succession stages of tropical forests (Cascante-Marin et al. 2006). Several bromeliad species form natural aquatic microcosms by holding rainwater in the rosettes formed by the bases of their leaves (hereafter tank bromeliads) and housing a specialized aquatic invertebrate biota. Thus, tank bromeliads combine high replicability and a rich aquatic fauna making them suitable natural microcosms for field-based ecological studies (Srivastava et al. 2004). Allochthonous detritus is often considered the main source of energy and matter to these aquatic food webs (*e.g.,* Richardson 1999, Srivastava 2006). Leaf litter is reduced by shredder and scraper macroinvertebrates and further processed by collector and filter-feeding macroinvertebrates (Srivastava 2006). More recently, it has been recognized that autochthonous primary production by aquatic microalgae could also fuel food webs in tank bromeliads (Brouard et al. 2011, 2012), but the contribution of microalgae

relative to detritus has yet to be evaluated. Both the input of allochthonous resources and autochthonous production are strongly related to the structure, size and location of the tank bromeliad within the terrestrial habitat (Richardson 1999, Brouard et al. 2011, 2012, Marino et al. 2011). For example, bromeliads located in low canopy cover areas show higher algae abundances than those under closed canopy (Brouard et al. 2011, 2012). Therefore, bromeliads sampled in closed versus open habitats are expected to encompass a natural gradient from higher to lower dependency on allochthonous carbon sources.

Here, we evaluated the relative importance of allochthonous resources (litterfall) to the aquatic food webs of Neotropical tank bromeliads. We sampled more than 100 tank bromeliads within either open or closed habitats, within five geographically distinct sites located in four different countries. We determined the relative importance of allochthonous resources for all major groups of invertebrates in each site using stable isotope mixing models based on $\delta^{13}C$ and $\delta^{15}N$ data. We also analyzed detrital density in and canopy cover over each tank bromeliad as predictors of the relative importance of allochthonous resources to the aquatic food webs. We hypothesized that: (1) invertebrates in open habitats would have a lower contribution of allochthonous sources to their diet than invertebrates in closed habitats; (2) canopy cover and detrital density would predict the shift between food webs based on allochthonous and autochthonous sources; and (3) the extent of the shift from one source to the other would differ between invertebrate taxa, depending on their functional feeding group.

METHODS

Sample collection and analyses

We sampled bromeliads and their aquatic food webs in a single site in each of three countries (Costa Rica, French Guiana and USA/Puerto Rico) and from two sites in Brazil (Macae and Ilha do Cardoso, hereafter Cardoso). Cardoso and Macae are *restinga* ecosystems, a type of coastal vegetation located on nutrient-poor sand deposits from the Quaternary period. This vegetation is characterized by a mix of patches of medium-sized trees where the water table is near the surface, and shrub patches where the water table is deeper in the soil (Magnago et al. 2012). Costa Rica, French Guiana and Puerto Rico sites are dominated by primary and secondary tropical rain forests with sparse open clearings for roads and pasture. In the Costa Rica, French Guiana, and Cardoso sites, we sampled tank bromeliads in open and closed habitats, while in the Macae and Puerto Rico sites, we sampled bromeliads only in open or closed habitats, respectively, due to lack of the other habitat in the location. We considered a habitat "open" if it receives direct sunlight during most of the daylight hours, and

"closed" if it is shaded for most of the day by overhead vegetation. Within each habitat type in each site, we sampled a minimum of 10 healthy adult bromeliads, not in the flowering stage. All sampling was performed within 2 weeks in each site, between January 2011 (Puerto Rico) and September 2012 (Costa Rica). Details of each sampling site, such as the site description, bromeliad genera and the number of tank bromeliads sampled are summarized in Appendix S1: Table S1.

For each bromeliad, we recorded the species of bromeliad, the number of green leaves holding water, bromeliad diameter (the average of two orthogonal measurements), and bromeliad height (a measurement from the base of its leaves to the highest point on the plant). We estimated canopy cover (%) for each bromeliad using a Lemmon spherical densitometer or from photographs taken while holding the camera towards the sky just a few inches above the center of the bromeliad. The photos were analyzed with ImageJ 1.48v software (http://imagej.nih.gov/ij/). We collected samples of dead leaves from trees overhanging the bromeliads, from at least three different locations at both the open and closed habitats from each site, to set the isotope baseline for allochthonous inputs in the site (see herein). We were careful to select leaves that, although dead, had not yet begun to be biotically decomposed. For each bromeliad, we collected the water accumulated in the plant (with a siphon, or by removing the plant and pouring the water into a funnel), measured the volume, and took it to the laboratory for invertebrate sampling. Once the bromeliad was empty of water, we measured maximum water holding capacity, the most common measurement of bromeliad size (e.g., Petermann et al. 2015), by slowly filling the bromeliad with a known volume of water until it overflowed. We collected all detritus contained in each plant. The amount of fine and coarse detritus trapped in each bromeliad was summed and used as a direct measurement of the allochthonous input of organic matter to the bromeliad system. We washed the detritus collected in each bromeliad through 150 μm sieves, oven-dried (40–50°C) it until it reached constant mass, and recorded this mass. We calculated the detrital density of each plant by dividing the amount of detritus trapped in each bromeliad by its maximum water holding capacity. Resource availability in aquatic ecosystems is usually expressed as a concentration, for example limnological studies of autochthony typically report chlorophyll-a in units of $g\,m^{-3}$. Detritus density (total mass of detritus/ maximum water volume) similarly represents the concentration of allochthonous resources in the habitat.

We collected aquatic macroinvertebrates from both the water sampled from the bromeliads and from the detritus trapped inside the plants. In all cases, collections included the six most abundant taxa in every bromeliad. Aquatic invertebrates were identified, grouped into taxonomic families (within classes Insecta and Ostracoda), orders (within class Insecta), or sub-classes (within class Clitellata), and classified into functional groups (i.e.,

shredders, collector-gatherers, filter-feeders, predators). This procedure allowed us to overcome differences in insect species composition among sites and generalize our results with regard to the main functional groups (see Appendix S1: Table S1, for invertebrate composition). We kept the collected organisms in distilled water for 24 h to allow the evacuation of the gut contents, then rinsed them with tap water and oven-dried them at 60°C for 72 h. At least 0.5 mg dry mass of each invertebrate taxonomic group from each functional group was processed for isotopic analyses. If sample mass did not reach that recommended for isotopic analyses, samples were then composed by a combination of individuals from similar taxonomic groups without mixing different functional feeding groups (e.g., the filter-feeding mosquitoes of the several genera of the Culicidae family could be mixed with each other, but not with predatory mosquitoes from the genus *Toxorhynchites*). The three dead leaf samples collected from each habitat were oven-dried (60°C for 72 h), ground, and homogenized with a mortar and pestle, and sub-samples of ~3 mg were separated for isotope analyses. $\delta^{13}C$ and ^{15}N were determined using a NC2500 elemental analyzer interfaced to a Thermo Delta V isotope ratio mass spectrometer (IRMS) at the Cornell University, or using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 IMS at the Davis Stable Isotope Facility at the University of California, Davis. Analytical errors were 0.11‰ for $\delta^{13}C$ and 0.13‰ for $\delta^{15}N$ based on internal lab standards (animal standard).

Data analyses

We analyzed the relative contribution of allochthonous and autochthonous sources to the diet of invertebrates using Stable Isotope Analysis in R (SIAR package, v. 4.1.1, Parnell et al. 2010). This mixing model method uses a Bayesian approach to estimate the proportional contribution of food sources to a consumer diet, while accounting for uncertainty in the carbon and/or nitrogen isotope composition of sources and consumer tissues as well as in the trophic enrichment factors (Parnell et al. 2010). Stable isotope values were entered for individual invertebrate groups (families, orders or sub-classes). While the stable isotope composition of allochthonous litter could be estimated from samples taken from each studied site, we could not directly sample for autochthonous producer $\delta^{13}C$ and $\delta^{15}N$ in the bromeliads, because periphytic and free-living algae could not be properly separated from particulate organic carbon for analyses. Instead, we used two different methods to estimate the stable isotope composition of autochthonous producers (see Table 1). For the Macae site, we used unpublished $\delta^{13}C$ and $\delta^{15}N$ data for autochthonous producers (mean $\delta^{13}C$ −16.60 ‰ ± SE: 1.45 ‰, and mean $\delta^{15}N$ 4.94 ‰ ± SE: 4.85 ‰) obtained from cleaned bromeliads (washed to remove organisms and detritus) left filled with water in a full open area for 2 months to

TABLE 1. Mean and standard error (in parenthesis) of δ^{13}C and δ^{15}N values of allochthonous and autochthonous resources for open and closed habitats of all sampled sites. Allochthonous isotopic values for all sites and autochthonous isotopic values for Macae were estimated from samples taken from each studied site. Autochthonous isotope values for Puerto Rico, Costa Rica, French Guiana and Cardoso were obtained in the literature (Doi et al. 2010). Values are shown in ‰.

| | Puerto Rico | Costa Rica | | French Guiana | | Cardoso | | Macae |
	Closed	Closed	Open	Closed	Open	Closed	Open	Open
δ^{13}C allochthonous end-member	−31.1 (0.13)	−32.1 (1.47)	−30.3 (1.15)	−31.9 (1.97)	−31.6 (1.56)	−29.6 (0.62)	−29.7 (1.56)	−24.3 (1.08)
δ^{15}N allochthonous end-member	−1.76 (0.86)	−0.74 (1.08)	−1.41 (1.05)	4.00 (0.86)	−3.04 (1.83)	−3.72 (1.01)	−3.69 (1.83)	−0.34 (0.26)
δ^{13}C autochthonous end-member	−16.80 (4.42)							−16.60 (1.45)
δ^{15}N autochthonous end-member	5.10 (4.56)							4.94 (4.85)

aquatic primary producer colonization and growth. This algal cultivation procedure was not possible in the other sites, so for these sites, we used previously published δ^{13}C and δ^{15}N data for periphytic algae from freshwater systems ($n = 49$): mean δ^{13}C − 16.80 ‰ (±SE: 4.42 ‰) and mean δ^{15}N 5.10 ‰ (±SE: 4.56 ‰) (Doi et al. 2010). We were able for Macae to conduct analyses using either the cultivated algal samples for this site or the literature values, and the similarity of these results (see Appendix S1: Fig. S1) indicates that the literature values are a reasonable approximation. We report in the main text results for Macae based on the cultivated algae as this is the more direct measure.

Trophic enrichment factor values for invertebrates were obtained from the literature (δ^{13}C 1.0 ± 0.5‰, δ^{15}N 2.5 ± 0.5‰: Caut et al. 2009). We ran each mixing model using 500,000 iterations. We conducted these mixing model analyses at two different levels: (1) all invertebrates grouped together within a bromeliad, to determine overall effects of habitat within each site; and (2) each taxonomic group examined separately, to examine taxon specific effects of habitat within each site. To compare resource use between taxonomic groups of invertebrates, habitats or sites, we examined the 95% confidence intervals of each source. If confidence intervals between the groups being compared do not overlap, the groups significantly differed in their use of that source.

In the aforementioned analyses, the habitat was categorized into open and closed. However, if taxa use resources in relation to their availability in the bromeliad, we would expect that as the ratio of autochthonous to allochthonous sources gradually changes, the isotope values of specific invertebrate taxa would show a similar shift. In further analysis, we used both the detrital density in bromeliads and the canopy cover over bromeliads to predict the relative contribution of allochthonous resources to the diet of aquatic invertebrates. Detrital density is a direct measurement of the amount of allochthonous resources availability in the bromeliad, while canopy cover reflects the potential for either reduced autochthonous production (high cover blocks light

transmission) or high allochthonous entry of detritus (high cover is associated with litterfall). In this analysis, we considered the overall invertebrate food web in each of the eight datasets (as determined in the stable isotope mixing models), and asked whether canopy cover, detrital density or habitat category (open vs. closed) was the best predictor of the allochthonous contribution (nlme package, v. 3.1-120, Pinheiro et al. 2015). We compared the predictive power of models with the adjusted R-squared. All statistical analyses were performed in R (R Core Team 2015).

RESULTS

Tank bromeliads differed in their morphological and water tank characteristics among sites and between habitats (Appendix S1: Table S2). In general, bromeliads from open habitats had smaller diameters, but could accumulate more water. These differences between bromeliads from open and closed habitat were especially strong at the Cardoso site. As expected, detrital density and canopy cover were higher in closed than open habitats, confirming that closed and open habitats differed in their potential for allochthonous subsidies and autochthonous production. Among open habitats, canopy cover was lower at *restinga* sites (Cardoso and Macae) than at sites with tropical rain- or moist-forests (Costa Rica, French Guiana and Puerto Rico).

Allochthonous and autochthonous sources had different isotopic values (Table 1). Mean δ^{13}C values of allochthonous litter for all sites varied from −32.1 ‰ (±SE: 1.47 ‰) for the closed habitat in Costa Rica to −24.3 ‰ (±SE: 1.08 ‰) for the open habitat in Macae, whereas the mean δ^{15}N values of allochthonous litter varied from −3.72 ‰ (± SE: 1.01 ‰) for the closed habitat in Cardoso to 4.00 ‰ (±SE: 0.86 ‰) for the closed habitat in French Guiana. Mean δ^{13}C and δ^{15}N values of autochthonous sources for all sites except Macae were, respectively, −16.80 ‰ (±SE: 4.42 ‰) and 5.10 ‰ (±SE: 4.56 ‰), whereas mean δ^{13}C and δ^{15}N values of autochthonous sources for Macae site were
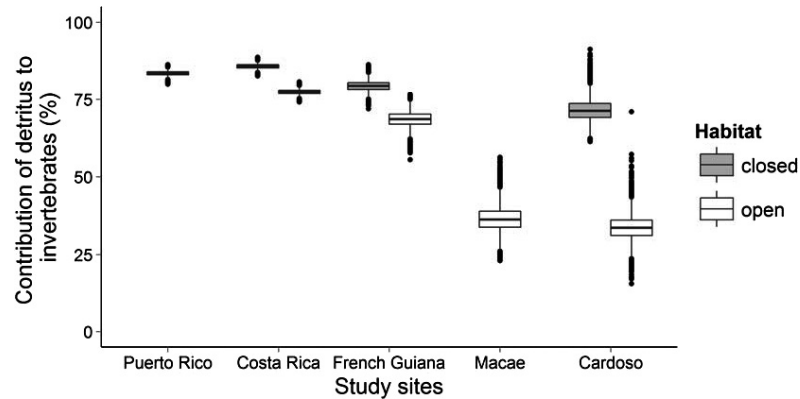
FIG. 1.   Proportion of allochthonous source contribution to the diet of aquatic invertebrate communities in closed and open habitats of five different sampling sites. Sites are sorted by latitude started from the northernmost (Puerto Rico) to the southernmost (Cardoso) site. The upper and lower whiskers correspond to the 25th and 75th percentile; dots show the range of feasible solutions at 95% as determined by SIAR models.

−16.60 ‰ (±SE: 1.45 ‰) and 4.94 ‰ (±SE: 4.85 ‰), respectively (Table 1).

Allochthonous sources contributed most to the carbon sources of bromeliad invertebrates in all closed habitats, and autochthonous sources contributed most to invertebrate communities in some, but not all, open habitats (Fig. 1). For open habitats, the contribution of autochthonous sources to the diet of aquatic invertebrates notably increased from the northerly forested sites to the southerly *restinga* sites (Fig. 1). Allochthonous detritus contributed between 69 and 86% (95th percentile confidence intervals) to the diet of aquatic invertebrates from closed habitat bromeliads and between 23 and 78% (95th percentile confidence intervals) to the diet of aquatic invertebrates from open habitat bromeliads. In three sites, we sampled bromeliads in both open and closed habitats allowing comparisons between habitats within sites. Cardoso showed the greatest difference between closed and open habitats in terms of the contribution of allochthonous detritus to the elemental composition of aquatic invertebrates (Fig. 1). Cardoso is also the southernmost site sampled and was the only site in this group characterized by *restinga* vegetation.

An important question is whether differences between habitats in the contribution of allochthonous versus autochthonous sources to the invertebrate community as a whole reflected shifts in the presence of particular taxonomic groups, or changes in resource use within taxonomic groups. Our results support the latter. The overall pattern between habitats and sites was maintained even when we zeroed in on particular taxonomic groups (Table 2). Furthermore, in each dataset, different taxonomic groups showed similar contributions of allochthonous detritus to the diet (Table 2).

Our analysis so far has treated open and closed habitats as discrete categories. However, there is considerable variance within these habitat categories in terms

of both canopy cover and detrital density (Appendix S1: Table S2). By considering continuous gradients of canopy cover and detrital density, we could perform more powerful analyses of how habitats determine allochthonous resource use in aquatic invertebrates. We found that the relative contribution of allochthonous sources to aquatic invertebrates was strongly related to canopy cover, but not to detrital density or the interaction between both factors (linear regression model, $F_{3,4} = 54.53$, Adjusted $R^2 = 0.915$, $P < 0.001$, Fig. 2). The contribution of allochthonous sources increased from approximately 35% in the low canopy cover-open habitats of Macae and Cardoso sites to approximately 65% in the intermediate canopy-cover-open habitat of French Guiana, and to approximately 80% in the high canopy-cover-closed habitat of all sites and open habitat of Costa Rica (Fig. 2A). The addition of the categorical habitat descriptors (open, closed) did not improve the fit of models, indicating that there were no additional effects of open vs. closed habitats that were not directly related to canopy cover.

DISCUSSION

Aquatic biologists have debated for over a decade the importance of allochthonous subsidies for fueling freshwater food webs. Part of the reason for this protracted debate has been the variation between studies in the calculated contribution of allochthonous detritus to food webs. Here, using data from over 100 independent replicates from contrasting habitats in five sites across the New World tropics, we show that although the contribution of allochthonous subsidies can be variable over space, it is also predictable from canopy cover. Our study, in conjunction with analyses of sets of lakes and rivers (Carpenter et al. 2005, Cole et al. 2011, Solomon et al. 2011, Berggren et al. 2014, Kelly et al. 2014,

TABLE 2. Allochthonous contribution (proportion) to the diet of aquatic invertebrate groups in open and closed habitats estimated by Bayesian isotope mixing models (SIAR).

Taxonomic Group	Puerto Rico Closed	Costa Rica Closed	Costa Rica Open	French Guiana Closed	French Guiana Open	Macae Open	Cardoso Closed	Cardoso Open
(Insecta, Diptera) Culicidae	0.85[a]	0.89[a]	0.81[a]	0.86[a]	0.83[a]	0.65	0.73	0.37
(Insecta, Diptera) Chironomidae	0.81[a]	0.86[a]	0.76[a]	0.85[a]	0.67	0.13	0.78[a]	0.48
(Insecta, Coleoptera) Scirtidae	0.85[a]	0.91[a]	0.80[a]	0.85[a]		0.12[b]	0.66[a]	0.29[b]
(Insecta, Diptera) Tipulidae	0.84[a]	0.84[a]	0.77[a]	0.78[a]	0.72	0.31[b]	0.54	0.36
(Insecta) Odonata		0.77[a]	0.75[a]	0.77[a]	0.66[a]	0.34[b]	0.69[a]	0.23[b]
(Insecta, Diptera) Tabanidae				0.72[a]	0.68		0.71[a]	0.50
(Insecta, Coleoptera) Hydrophilidae								0.42
(Insecta, Trichoptera) Calamoceratidae							0.80[a]	
(Clitellata) Hirudinea							0.63	
(Insecta, Diptera) Dolychopodidae							0.91[a]	
(Clitellata) Oligochaeta				0.96[a]	0.72	0.29[b]		
(Ostracoda, Podocopida) Limnocytheridae				0.57	0.51	0.52		
(Insecta, Hemiptera) Veliidae				0.80[a]	0.57			
(Insecta, Diptera) Ceratopogonidae						0.19[b]		
All	0.80	0.82	0.74	0.74	0.64	0.16	0.70	0.34

Note: Values reported are the mode, which represent the most likely proportion of diet source. We examined the 95% confidence intervals of both allochthonous and autochthonous source contributions for each taxonomic group diet within habitats. If those intervals did not overlap, the group showed a significantly greater reliance on allochthonous or autochthonous carbon to its diet. Significant results are shown with different letters (a – for greater reliance on allochthonous source or b – for greater reliance on autochthonous source). Sites are sorted by latitude started from the northernmost (Puerto Rico) to the southernmost (Cardoso) site.

Scharnweber et al. 2014, Collins et al., *in press*), argues that the importance of allochthonous vs. autochthonous resources in aquatic systems is driven by their relative availability. Our study also delves into the mechanisms behind this result, showing that even within particular taxonomic families the importance of allochthony is under environmental control.

Autochthonous algae are of higher nutritional value than allochthonous detritus, both because of the lower C:N and C:P ratio and because of higher PUFA content (Brett et al. 2009, Taipale et al. 2014). Algal primary production is usually limited by light incidence in shaded headwater streams and forested small ponds (e.g., Von Schiller et al. 2011, Thrane et al. 2014). We suspect the same holds for algae in tank bromeliads, as bromeliads are usually located in partial shade. Nutrients may only be a secondary limitation in this system as nutrients are provided by the falling detritus to the system. We therefore expected that, once the light incidence increases over the bromeliads (open habitats, plants under low canopy cover), the availability of algal biomass would increase, and the aquatic invertebrates would have preferably consumed nutritionally favorable autochthonous sources or would change in community composition toward algivores. Surprisingly, a greater consumption of autochthonous sources was only

partially observed in the open habitats, i.e., this shift from allochthony to autochthony in open habitats was much greater at Cardoso and Macae than at the other sites (Fig. 1, Table 2). That is primarily because open habitats of different sites greatly differed in the canopy cover over tank bromeliads (Appendix: Table S2). Using canopy cover as a continuous predictor of the light intensity over the tank bromeliads, we observed that allochthonous support of aquatic food webs strongly depends on light inputs, i.e., the greater light input, the smallest contributions of allochthonous sources to aquatic food webs of tank bromeliads (Fig. 2A). It is yet to be determined – both for bromeliads and for other freshwater systems – whether this relation is linear or there is a threshold of autochthonous carbon input above which the system changes from allochthonous to autochthonous-carbon based. The observed support of aquatic food webs of tank bromeliads by allochthonous carbon sources seems to be more related to the low availability of autochthonous carbon sources in some systems than to any preference for the consumption of allochthonous detritus.

Our study demonstrated habitat-induced shifts in the contribution of allochthonous resources to invertebrate food webs. This shift could have been realized in two ways, either as a turnover in species composition from
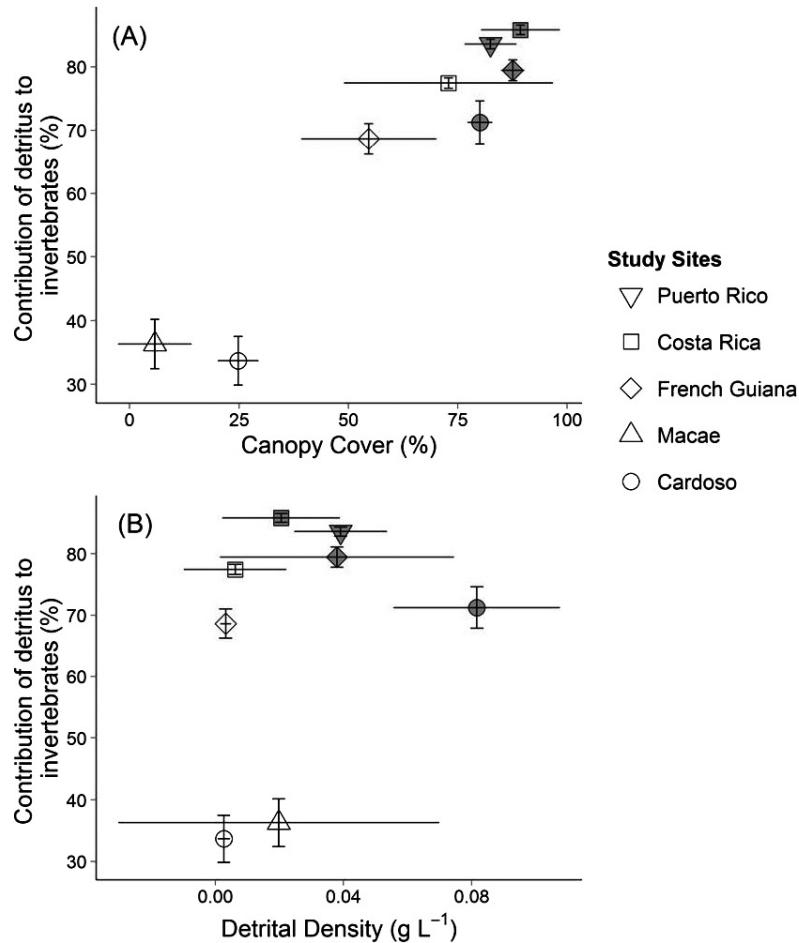
FIG. 2.   The relation between the allochthonous source contribution to the diet of aquatic invertebrate communities and (A) the percentage of canopy cover or (B) the detrital density of tank bromeliads at open and closed habitats of five different sampling sites. Open habitats are shown with open symbols while closed habitats are shown with solid symbols. Note that we have just sampled in open habitats at the Macae site and in closed habitats in Puerto Rico site.

detrivorous to algivorous functional groups, or a shift in diet within taxonomic groups (omnivory). There are well-documented changes in species composition along gradients of habitat openness within sites (Ngai et al. 2008, Dézerald et al. 2013), and our study similarly found a paucity of Tabanidae, Dolichopodidae (both Diptera), Hirudinea and Trichoptera in open habitats. Evidence of consumption of different resources within taxonomic groups has accumulated in the literature, indicating that this phenomenon may be more the rule rather than the exception (Lancaster et al. 2005), and jeopardizing the use of the functional feeding group to infer resource assimilation in aquatic invertebrates (Mihuc 1997). Here, we also documented changes in diet within most taxonomic groups, indicating that compositional shifts cannot be the only mechanism underlying the changes in allochthonous contribution (Table 2). A deeper examination of these taxonomic groups and their feeding behaviors may help explain this plasticity.

Bromeliad invertebrate food webs are composed of several trophic functional groups, including filter feeders (e.g., Culicidae), scrapers (e.g., Scirtidae), shredders (e.g., Tipulidae) and collector-gathers (e.g., Chironomidae, Oligochaeta), which are preyed upon by intermediate and top predators (e.g., Tabanidae, Odonata). Filter feeders consume a variety of microorganisms, from rotifers to bacteria, and either detritus or algae can form the base of such microbial communities (Merritt et al. 1992). Scrapers like scirtids forage on the surface layers of detritus, but are probably largely consuming the attached microorganisms as they have low impacts on detrital decomposition (Srivastava and Bell 2009). Collector-gatherers rely on the mixture of fine material accumulated in the bottom of ecosystems. Thus, all three groups – filter feeders, scrapers and collector-gatherers – have low reliance on coarse detritus and could potentially obtain energy directly or indirectly from free-living and bromeliad-attached algae in open habitats (Brouard et al. 2012). Indeed,

taxa in these functional groups uniformly showed shifts in their diet from closed to open habitats (Table 2). By contrast to the aforementioned functional groups, shredders use their mandibles to chop up and consume coarse detritus with high impacts on detrital decomposition (e.g., Srivastava and Bell 2008). It is therefore surprising that allochthonous resources did not dominate the diet of Tipulidae, the primary shredder in most bromeliads, equally in open and closed habitats (Table 2). However, shredders feed preferentially on benthic algae in some acid stream (Dangles 2002) and consume periphytic algae in high light intensity experimental conditions (Franken et al. 2005), and the same phenomena could also occur in some open habitat bromeliads. There are also accounts of predation in some aquatic Tipulidae (Merritt and Cummins 1995) and this has recently been confirmed in Costa Rican bromeliads (S. Amundrud, pers. comm.). Predators consume all of filter feeders, scrapers, collector-gatherers and shredders, and so are expected to integrate any changes in the diet of their prey. Indeed, the predators that occurred in both open and closed habitats (Odonata, Tabanidae) show shifts in their isotopic signature similar to the shifts in their prey (Table 2). Note that extrapolations of the Tabanidae data should be taken with care as only two individuals were collected in open canopy sites, indicating that this group, in fact, prefers closed, detritus-rich ecosystems.

One of the caveats of our study is that we did not (and often could not) measure the autochthonous primary production or the autochthonous stable isotope composition in the sampled tank bromeliads. Instead, we opted to evaluate the potential for autochthonous production in the system by the using the canopy cover data, and used previously collected data of periphyton stable isotopes within lentic systems. The tanks of bromeliads are usually detritus-rich, even in some open habitats (see Fig. 2B). High concentrations of dissolved colored carbon and fine particulate organic matter impose severe restrictions to the use of traditional spectrophotometer- or spectrofluorometer-based methods for analyzing chlorophyll-a (Carlson and Shapiro 1981), and some further difficulties to precise estimations to autochthonous isotopic values (see Marty and Planas 2008). We are aware that $\delta^{13}C$ and ^{15}N of algae vary spatially and temporally along changes in environmental conditions (Doi et al. 2010) and values of $\delta^{13}C$ and ^{15}N for benthic algae in lenthic systems are imperfect approximations of bromeliad tank conditions. However, both benthic regions of lenthic ecosystems and tank bromeliads are characterized by low water turbulence. The lower the turbulence of the water column the greater boundary layer thickness around primary producers are found, which causes substantial $\delta^{13}C$ enrichment in the primary producers (France 1995). This suggests that site variation in the values of $\delta^{13}C$ and ^{15}N for bromeliad algae may pale in comparison to the difference between detrital and algal signatures. In addition, the algae $\delta^{13}C$ of tank bromeliads of Macae was evaluated in sun-exposed bromeliads without any detritus additions, and the values (-16.6 ‰ $\pm$ 1.25 ‰, unpublished results) fall into the range used in the calculations for the other sites.

Despite these limitations, some important conclusions can be drawn from our results. Although the importance of allochthonous subsidies to bromeliad invertebrates is highly variable over space, much of this variation can be predicted by light penetration through the canopy and therefore presumably algal productivity. This suggests that some of the variation between studies of other aquatic systems may similarly be driven by the relative availability of autochthonous resources. Our study also demonstrates that compositional changes in food webs cannot uniquely determine shifts in the importance of allochthonous resources over environmental gradients; instead, shifts within the diet of individual taxa also play a key role. Useful avenues for future research will be determining whether food webs have linear or non-linear responses to changes in relative resource availability, determining if the detritus quality influences the dependence on allochthony by aquatic invertebrates, and determining the behavioral and physiological mechanisms behind the documented shifts in invertebrate diets.

Literature Cited

Brett, M. T., and D. Muller-Navarra. 1997. The role of highly unsaturated fatty acids in aquatic food web processes. Freshwater Biology 38:483–499.
Brett, M. T., M. J. Kainz, S. J. Taipale, and H. Seshan. 2009. Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. Proceedings of the National Academy of Sciences 106:21197–21201.
Brouard, O., A.-H. Lejeune, C. Leroy, R. Céréghino, O. Roux, L. Pelozuelo, A. Dejean, B. Corbara, and J.-F. Carrias. 2011. Are algae relevant to the detritus-based food web in tank-bromeliads? PLoS One 6:e20129.
Brouard, O., R. Céréghino, B. Corbara, C. Leroy, L. Pelozuelo, A. Dejean, and J.-F. Carrias. 2012. Understory environments influence functional diversity in tank-bromeliad ecosystems. Freshwater Biology 57:815–823.

Carlson, R. E., and J. Shapiro. 1981. Dissolved humic substances: a major source of error in fluorometric analysis involving lake waters. Limnology and Oceanography 26:785–790.

Carpenter, S. R., J. J. Cole, M. L. Pace, M. Van de Bogert, D. L. Bade, D. Bastviken, C. M. Gille, J. R. Hodgson, J. F. Kitchell, and E. S. Kritzberg. 2005. Ecosystem subsidies: terrestrial support of aquatic food webs from ^{13}C addition to contrasting lakes. Ecology 86:2737–2750.

Cascante-Marin, A., J. H. D. Wolf, J. G. B. Oostermeijer, J. C. M. den Nijs, O. Sanahuja, and A. Durán-Apuy. 2006. Epiphytic bromeliad communities in secondary and mature forest in a tropical premontane area. Basic and Applied Ecology 7:520–532.

Caut, S., E. Angulo, and F. Courchamp. 2009. Variation in discrimination factors (δ^{15}N and δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. Journal of Applied Ecology 46:443–453.

Cole, J. J., S. R. Carpenter, J. Kitchell, M. L. Pace, C. T. Solomon, and B. Weidel. 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. Proceedings of the National Academy of Sciences 108:1975–1980.

Collins, S. M., T. J. Kohler, S. A. Thomas, W. W. Fetzer, and A. S. Flecker. In press. The importance of terrestrial subsidies in stream food webs varies along a stream size gradient. Oikos 125:674–685. doi: 10.1111/oik.02713

Dangles, O. 2002. Functional plasticity of benthic macroinvertebrates: implications for trophic dynamics in acid streams. Canadian Journal of Fisheries and Aquatic Sciences 59:1563–1573.

Dézerald, O., C. Leroy, B. Corbara, J.-F. Carrias, L. Pélozuelo, A. Dejean, and R. Céréghino. 2013. Food-web structure in relation to environmental gradients and predator-prey ratios in tank-bromeliad ecosystems. PLoS One 8:e71735.

Doi, H., E. Kikuchi, S. Shikano, and S. Takag. 2010. Differences in nitrogen and carbon stable isotopes between planktonic and benthic microalgae. Limnology 11:185–192.

Finlay, J. C. 2001. Stable-carbon-isotope ratios of river biota: Implications for energy flow in lotic food webs. Ecology 82:1052–1064.

France, R. L. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: food web implications. Marine Ecology Progress Series 124:307–312.

Franken, R. J. M., B. Waluto, E. T. H. M. Peeters, J. J. P. Gardeniers, J. A. J. Beijer, and M. Scheffer. 2005. Growth of shredders on leaf litter biofilms: the effects of light intensity. Freshwater Biology 50:459–466.

Kelly, P. T., C. T. Solomon, B. C. Weidel, and S. E. Jones. 2014. Terrestrial carbon is a resource, but not a subsidy, for lake zooplankton. Ecology 95:1236–1242.

Lancaster, J., D. C. Bradley, A. Hogan, and S. Waldron. 2005. Intraguild omnivory in predatory stream insects. Journal of Animal Ecology 74:619–629.

Magnago, L. F. S., S. V. Martins, C. E. G. R. Schaefer, and A. V. Neri. 2012. Restinga forests of the Brazilian coast: richness and abundance of tree species on different soils. Anais da Academia Brasileira de Ciências 84:807–822.

Marino, N. A. C., R. D. Guariento, V. Dib, F. D. Azevedo, and V. F. Farjalla. 2011. Habitat size determine algae biomass in tank-bromeliads. Hydrobiologia 678:191–199.

Marty, J., and D. Planas. 2008. Comparison of methods to determine algal δ^{13}C in freshwater. Limnology and Oceanography Methods 6:51–63.

Merritt, R. W., and K. W. Cummins. 1995. An introduction to the aquatic insects of North America. Third Edition. Kendall Hunt Pub Co., Dubuque, Iowa, USA., 862 p.

Merritt, R. W., R. H. Dadd, and E. D. Walker. 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. Annual Review of Entomology 37: 349–376.

Mihuc, T. B. 1997. The functional trophic role of lotic primary consumer: generalist versus specialist strategies. Freshwater Biology 37:455–462.

Mosisch, T. D., S. E. Bunn, and P. M. Davies. 2001. The relative importance of shading and nutrients on algal production in subtropical streams. Freshwater Biology 46:1269–1278.

Ngai, J. T., K. R. Kirby, B. Gilbert, B. M. Starzomski, A. J. D. Pelletier, and J. C. Ross-Conner. 2008. The impact of land-use change on larval insect communities: testing the role of habitat elements in conservation. Ecoscience 15:160–168.

Pace, M. L., J. J. Cole, S. R. Carpenter, J. F. Kitchell, J. R. Hodgson, M. C. Van De Bogert, D. L. Bade, E. S. Kritzberg, and D. Bastviken. 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature 427:240–243.

Parnell, A., R. Inger, S. Bearhop, and A. L. Jackson. 2010. Stable isotope analysis in R (SIAR). Available: http://cran.r-project.org/web/packages/siar/index.html. Accessed 2013 April 23.

Petermann, J. S., et al. 2015. Dominant predators mediate the impact of habitat size on trophic structure in bromeliad invertebrate communities. Ecology 96:428–439.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2015. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-120, http://CRAN.R-project.org/package=nlme.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics 28:289–316.

R Core Team 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Richardson, B. A. 1999. The bromeliad microcosm and the assessment of faunal diversity in a Neotropical forest. Biotropica 31:321–336.

Scharnweber, K., J. Syväranta, S. Hilt, M. Brauns, M. J. Vanni, S. Brothers, J. Köhler, J. Knežević-Jarić, and T. Mehner. 2014. Whole-lake experiments reveal the fate of terrestrial particulate organic carbon in benthic food webs of shallow lakes. Ecology 95:1496–1505.

Solomon, C. T., S. R. Carpenter, M. K. Clayton, J. J. Cole, J. J. Coloso, M. L. Pace, M. J. Zanden, and B. C. Weidel. 2011. Terrestrial, benthic, and pelagic resource use in lakes: results from a three-isotope Bayesian mixing model. Ecology 92:1115–1124.

Srivastava, D. S. 2006. Habitat structure, trophic structure and ecosystem function: interactive effects in a bromeliad-insect community. Oecologia 149:493–504.

Srivastava, D. S., and T. Bell. 2009. Reducing horizontal and vertical diversity in a foodweb triggers extinctions and impacts functions. Ecology Letters 12:1016–1028.

Srivastava, D. S., J. Kolasa, J. Bengtsson, A. Gonzalez, S. P. Lawler, T. E. Miller, P. Munguia, T. Romanuk, D. C. Schneider, and M. K. Trzcinski. 2004. Are natural microcosms useful model systems for ecology? Trends in Ecology & Evolution 19:379–384.

Steinberg, C. E. W., et al. 2006. Dissolved humic substances – ecological driving forces from the individual to the ecosystem level? Freshwater Biology 51:1189–1210.

Taipale, S. J., M. T. Brett, M. W. Hahn, D. Martin-Creuzburg, S. Yeung, M. Hiltunen, U. Strandberg, and P. Kankaala. 2014. Differing Daphnia magna assimilation efficiencies for terrestrial, bacterial, and algal carbon and fatty acids. Ecology 95:563–576.

Thrane, J.-E., D. O. Hessen, and T. Andersen. 2014. The absorption of light in lakes: negative impact of dissolved organic carbon on primary productivity. Ecosystems 17: 1040–1052.

Von Schiller, D., E. Marti, J. L. Riera, and F. Sabater. 2011. Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses. Freshwater Biology 52:891–906.

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