

**FOREST FRAGMENTATION CHANGES MACROINVERTEBRATE COMMUNITY
COMPOSITION IN NEOTROPICAL TREEHOLES**

by

Angie Jhovanska Nicolás Stella

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Abstract

Habitat loss and fragmentation are the main drivers of biodiversity loss in terrestrial ecosystems, particularly in the tropics. Fragmented habitats can interfere with organism dispersion, population persistence and ecosystem functions but empirical studies report wide variation in the sensitivity of organisms and functions to fragmentation and the mechanisms operating behind observed responses remain poorly understood. In this study we examined the effects of forest fragmentation on the colonization of artificial treeholes in northwestern Costa Rica by measuring the responses at the community level. We explored four potential mechanisms driving differences in macroinvertebrate communities between continuous and fragmented forests: dispersal limitation, microclimate changes and bottom up or top down effects. Macroinvertebrate community composition differed significantly between continuous forests and forest remnants but not in the predicted direction. Our results suggest that treeholes in fragmented forest contain higher abundance of detritivores and experience changes in predator species identity consistent with increased nutrient input and a potential relaxation of predation pressure in small forest remnants. An overall resilience of treehole communities to forest fragmentation is interpreted with care as time-delayed responses to fragmentation continue to be a possibility. These findings advance our understanding of the response of biological communities to forest fragmentation and emphasize the value of preserving even small forest remnants for biodiversity conservation.

Preface

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A version of the research chapter will be submitted for publication under the co-authorship of A.N and D.S.

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*To the many park rangers and activists who
live and die protecting our tropical forests*

Chapter I. General Introduction

Increasing demand for land in agriculture and development drives the rapid loss of natural habitats at a global scale. In the case of forests, reduction in size is accompanied by the subdivision of remaining forest area into patches separated by roads and various combinations of land use types. The global extent of forest loss and fragmentation is remarkable. A recent analysis concludes that most forest remnants on Earth are now less than 10 Ha in area and are subject to fragmentation effects (Haddad et al. 2015). Deforestation rates are particularly high in the tropics (Hansen et al. 2013), where forests are simultaneously the oldest, most diverse and complex terrestrial ecosystems (Laurance and Bierregaard Jr. 1997, Myers et al. 2000).

Island biogeography theory predicts that more species should colonize and persist in large habitat fragments close to other patches than in small and isolated fragments (MacArthur and Wilson 1967). Foundational research on the effects of fragmentation considered mainly the size and connectivity of habitat patches to understand their colonization rates (Simberloff 1976). Decades of empirical studies show, however, that terrestrial systems do not always behave as oceanic islands separated by hostile matrices. Instead, both the spatial context of forest remnants (measured by fragment area, edge, shape complexity, patch connectivity and matrix contrast) and a series of species traits (such as life history, trophic position, body size, dispersal ability, niche breadth and rarity) modulate biological responses to fragmentation (Kareiva 1990, Laurance and Bierregaard Jr. 1997, Collinge and Forman 1998, Henle et al. 2004, Ewers and Didham 2006, Didham et al. 2012, Fahrig 2013). For example, area effects are very important for sedentary species while isolation has stronger effects on mobile species (Ewers and Didham 2006, Prugh et al. 2008, Öckinger et al. 2009).

In forest fragments, the increased exposure to forest edge is associated with higher solar radiation, wind exposure and warmer temperatures as well as with marked changes in plant community composition (Turton and Freiburger 1997, Laurance et al. 2000). These changes in microclimate and vegetation structure have been linked to the disappearance of some species (such as insectivorous birds and leaf litter or dung insects) from forest edges and small fragments (Linares 1990, Didham 1997, Kapos et al. 1997, Barbaro et al. 2012). In contrast, disturbance tolerant species are positively associated or unaffected by edge conditions (Kapos et al. 1997, Ries and Sisk 2010).

In addition to directly affecting species distributions, fragmentation can interfere with trophic interactions. For example, multiple studies document reduced herbivory (Valladares et al. 2006, Vásquez et al. 2007, Martinson and Fagan 2014) and decreased parasitism in fragments (van Nouhuys 2005, Elzinga et al.

2007, Holzschuh et al. 2010). Fragmentation interferes with important ecological and ecosystem processes as well. Despite idiosyncratic variation in the direction of change, we know that leaf litter decomposition rates are altered in forest remnants. Some studies show faster litter breakdown towards forest edges (Burghouts et al. 1992, Didham 1998) while others report decomposition declines in small fragments suggesting impaired nutrient recycling (Moreno et al. 2014). Fragmentation also interferes with pollinator visits, (Aizen and Feinsinger 1994, Steffan-Dewenter and Tschardt 2000), seed dispersal and seed predation (Herrerías-Diego et al. 2008), colonization and overall resilience (Gonzalez 2000) .

Understanding the response of entire biological communities to fragmentation is key for biodiversity preservation and for planning protected areas. The fragmented landscape of northwestern Costa Rica provides a natural setting for the study of biological responses to fragmentation. Forest conversion to agriculture and cattle ranching began in the 1950s and intensified towards the mid 1970s in the region. An area of 2500 square kilometers of dense and semi-dense forest on the west flanks of Cordillera Central in the 500-2000 m elevation range was cleared between 1966 and 1977 (Joyce 2006). Agriculture (sugar cane, cotton and rice) and especially cattle ranching expansion resulted in further depletion during the following decade, but the establishment of four protected areas between 1971 and 1975, including the nearby Santa Rosa National Park led to the protection and restoration of dry and pre-montane moist forest in the area (Allen 2001). The intensity of cattle grazing activities decreased in 1985 after a drop in beef market prices followed by the abandonment of large pasture areas. Many of these pastures were opportunely bought by Daniel Janzen and Winnie Hallwachs through a fundraising campaign that culminated in the designation of 82 000 Ha as Guanacaste National Park in 1989, and which extends to 165 000 Ha today (Janzen 2000, Allen 2001). Starting in the 1990s teak plantations and orange groves were established in Guanacaste. The latter belong to Grupo Del Oro (a company owned by British Aid Agency Commonwealth Development Corp) and were acquired through an agreement with the local Conservation Area that included the transfer of 1200 Ha of forested land for environmental services including pest control (Joyce 2006).

Common logging and clearing practices in Guanacaste result in incomplete deforestation. Cattle ranches often preserve ten percent of trees in pasture areas for cattle shading, though landowners are prone to remove further trees later when market wood prices are favourable. Trees are usually cut in stages: first, high grade logging removes only the largest high value trees; then, additional trees are felled as logging roads develop; finally the area is cleared and burned (Joyce 2006). Consequently, although the same tree species may be present in forest gaps as in primary forest, species composition often changes between gaps to create a forest mosaic typical of the structure and composition of Tropical Moist and Tropical Wet life zones. Furthermore, historic aerial imagery, although limited in resolution, suggests thousands of patches (median size 29-61 Ha) existed outside of protected areas by the year 2000 (Joyce 2006).

Monitoring the effects of fragmentation on ecological communities requires a system that can be easily surveyed and is widespread on the landscape. Phytotelmata are an ideal contained system for the analysis of entire foodwebs (Srivastava et al. 2004). In the tropics, where large water bodies are scarce, bromeliads, rock pools, inflorescences, pitcher plants and treeholes are the preferred breeding habitats for many macroinvertebrates with aquatic life stages (Williams 2006).

In this study we examine the colonization of treeholes in the fragmented landscape of Guanacaste National Park and its surroundings. The following chapter presents the results of a colonization experiment where we contrast communities of insects, protists and leaf litter decomposition rates in artificial treeholes from forest patches and continuous forest sites. Results of an accompanying natural tree hole survey are summarized in the appendix.



Figure 1. Examples of the natural and artificial treeholes surveyed in this study

Chapter II. Community composition and ecosystem function changes in treeholes in forest fragments

Introduction

Fragmentation of natural habitats occurs following division into increasingly isolated patches surrounded by a matrix of human modified landscape (Fahrig 2003). Together with habitat loss, fragmentation is a main driver of terrestrial biodiversity loss in the tropics (Sala et al. 2000, Haddad et al. 2015). Tropical forest cover has drastically decreased in the last decades and the current rate of deforestation is expected to continue in most tropical regions (Pereira et al. 2010, Hansen et al. 2013). An exception is the well-documented amelioration in Brazil (FAO 2010, Achard et al. 2014, Nepstad et al. 2014).

The three main components of forest fragmentation- decreased habitat area, increased isolation and increased exposure to edge habitat- exert enduring effects on biodiversity. For disturbance tolerant organisms, habitat fragmentation can increase local abundance and species richness via improved access to a combination of resources from multiple patches or via the establishment of prey refugia (Schneider 2001, Fahrig 2003, Ewers and Didham 2006, Brosi et al. 2007, Gavish et al. 2012). On the other hand, the negative effects of fragmentation are generally associated with biotic homogenization following the replacement of limited range species by widespread species), disruption of ecological functions such as pollination and dispersal, increased extinction risk of vulnerable species, reductions in nutrient retention, and overall ecosystem degradation within forest patches (Turner and Corlett 1996, Laurance and Bierregaard Jr. 1997, Krauss et al. 2010, Haddad et al. 2015).

At the community level, fragmentation effects on biodiversity will result from the simultaneous action of two sets of factors, those pertaining to the spatial arrangement of forest remnants in the landscape and those pertaining to the traits of organisms. In terms of spatial structure, important factors have been shown to include patch area, shape complexity of patches, distance to source habitat, proportion of forest versus matrix habitat in the landscape, patch history and differences between forest and the surrounding matrix (Ribas et al. 2005). In terms of organism traits, negative impacts of fragmentation are expected for organisms with low dispersal ability, narrow niche breadth, high trophic level, large body size and low local abundance (Ewers and Didham 2006).

Understanding the consequences of forest fragmentation for biological communities is a key step in planning the conservation of tropical biodiversity. One group of particular relevance to ecologists are tropical insects which are estimated to represent the majority of species of the world (May 2010, Bisby et al. 2013,

Novotny and Miller 2014, Stork et al. 2015). Most studies of arthropod responses to fragmented habitats have focused on entirely terrestrial communities, and contrasting results have been reported. For example, leaf litter and dung beetle communities show moderate to high sensitivity to fragmentation (Didham et al. 1998, Driscoll and Weir 2005, Nichols et al. 2007) whereas mixed results have been reported for termites (Francisco et al. 1994), ants (Carvalho and Vasconcelos 1999, Hill et al. 2011) and flying insects such as bees (Brosi 2009, Ferreira et al. 2015), butterflies and flies (Fowler et al. 1993). Data on the response of insects with aquatic life stages is scarcer in the literature. Studies on stream macroinvertebrates show that reduced forest cover can shift the composition of aquatic larval communities (Iwata et al. 2003, Kleine and Trivinho-Strixino 2005, Salmah, Md Rawi et al. 2013) but except for one recent study comparing insect communities in different land use types (Khazan et al. 2015) the response of on non-stream insect groups to fragmentation remains largely unexplored.

Treehole communities provide insight into the response of organisms with both aquatic and terrestrial life stages. Treeholes are cavities in tree trunks formed by normal trunk growth or in response to bark damage (Kitching 1971). In tropical forests, where lakes and permanent water bodies are rare, water-filled treeholes and other phytotelmata represent an important aquatic habitat and are colonized by insects as soon as the rainy season begins. The macroinvertebrates in treeholes –mainly mosquitoes, flies and beetles- are markedly distinct from those in surrounding aquatic habitats, with only 20% of community composition overlap (Blakely et al. 2012). In fact some species like predatory damselfly larvae (Pseudostigmatidae), rely exclusively on these cavities for breeding sites (Fincke 2006). Pseudostigmatids and other members of these temporary communities assemblages depend on the persistence of trees in forest remnants (Leonard et al. 1995, Yanoviak 1999), and therefore may be naturally vulnerable to deforestation and fragmentation when phytotelmata availability is compromised (Yanoviak et al. 2006a, Ngai et al. 2008).

Forest fragments may have fewer treehole specialists in their species pool than large, continuous areas of forest. Island biogeography theory (MacArthur and Wilson 1967) predicts that larger forest areas would contain more species if (a) treehole macroinvertebrates are dispersal limited, in which case, large patches and continuous forest would have higher colonization rates, (b) sampling effects are important, such that rare species are more likely to be sampled in larger areas, or (c) unlike the last two explanations, the species pool is sensitive not so much to forest patch area but to the total amount of forested area in the landscape. Although little is known about the dispersal of treehole macroinvertebrates, ovipositing adults are assumed to travel by either wind, phoresy (e.g. via amphibians or bird nest materials) or active aerial transport (Simberloff 1976). Mark-recapture studies of comparable aquatic Diptera families suggest treehole flies are generally weak fliers (Bilton et al. 2001) and active dispersal ranges are expected to be less than 2 km for ceratopogonids (Lillie et al 1981 and Kikerby et al 2013), culicids (Levi-Castillo 1951, Honório et al. 2003), phlebotomin psychodids

(Alexander 1987, Morrison et al. 1993), syrphids (Schweiger et al. 2007) and odonates (Conrad et al. 1999, Khazan 2014). We expect treehole macroinvertebrates to be both dispersal and habitat limited when forest patches are embedded in an agricultural matrix completely lacking treeholes. If the species pool of treehole macroinvertebrates is higher in large, continuous areas, this may be manifest at the treehole scale as either higher diversity per treehole or greater turnover in species composition between treeholes. Experimental studies in tropical islands provided early evidence for this patch-size mechanism (Simberloff and Wilson 1969, Simberloff 1976), and recent studies have demonstrated its utility for understanding effects of forest fragmentation in the Amazon (Saunders et al. 1992, Fahrig 2003, Hill et al. 2011)

Alternatively, the microclimate of fragmented forests may differ from that of continuous forests and result in fewer or different treehole macroinvertebrates. In particular, forest fragments tend to have warmer and more variable temperatures than continuous forests (Laurance and Bierregaard Jr. 1997), which may result in greater rates of treehole desiccation. Previous studies in temperate (Sota et al. 1994, Srivastava and Lawton 1998) and tropical treeholes (Bradshaw and Holzapfel 1988) suggest that treehole insects are largely drought limited. Species composition in bromeliads (a similar phytotelm system) is largely predictable from the drought tolerance of species (Amundrud and Srivastava 2015). We therefore predict that warmer, variable conditions in fragments would reduce diversity in treeholes and shift species composition towards drought-tolerant species.

A third mechanism by which fragmentation may affect treehole macroinvertebrates concerns resource quality. Leaf toughness tends to increase as tropical forest succession progresses (Craven et al. 2015). As fragmented forests have a higher proportion of fast growing pioneer trees, such forests produce leaf litter which is less defended and has higher nutrient content than that in continuous forest (Ewel 1976, Dantas and Phillipson 1989, Taylor et al. 1989). In leaf litter-based systems like treeholes, changes in leaf litter quality can shift community composition of leaf processing insects (Paradise and Kuhn 1999, Paradise 2000, Yanoviak 1999) as well as insect emergence (Richardson 1991, Compson et al. 2013). These effects of leaf litter are particularly well documented for tree hole mosquitoes (Leonard et al. 1995, Walker et al. 1997, Yanoviak 1999). Increases in detrital nutrient content, relative to carbon, have also been shown to accelerate invertebrate growth in bromeliads (González et al. 2014). Therefore, treeholes in fragmented forests that contain high quality leaf litter may be expected to have greater abundances, species number, and a shift in macroinvertebrate composition to faster-growing detritivores. The predictions are opposite to those originating from the previous two mechanisms.

Finally, fragmentation may affect treehole communities via top-down effects. Although response of predators to habitat fragmentation can vary depending on their feeding specialization, body size and ability to move through the matrix (Crooks 2002, Gehring and Swihart 2003, Ryall and Fahrig 2006), in general,

predators have been found to be more sensitive to reductions in habitat size (Hoyle 2004, Srivastava et al. 2008) and fragmentation (Zabel and Tschardtke 1998, van Nouhuys 2005, Ewers and Didham 2006) than their prey. Previous studies suggest that some species of treehole predators (Odonata: Pseudostigmatidae) are very sensitive to forest conversion (Fincke and Hedström 2008), so we expect them to be absent or rare in small fragments. A reduced odonate presence could induce trophic cascades down the treehole foodweb. As the top predators in tropical treeholes, odonates reduce the number of mosquitoes and other macroinvertebrates surviving to pupation (Fincke et al. 1997) Furthermore, these predator-induced changes in insect abundance and community composition could cascade down to protists, decomposing microbes and therefore leaf litter decomposition rates (Srivastava and Bell 2009). Previous evidence of this trophic cascade comes from laboratory experiments where grazing by treehole mosquitoes *Aedes triseriatus* (Fish and Carpenter 1982) and *Culex mollis* (Yanoviak 1999a) accelerated leaf litter breakdown.

This study answers the following questions: 1) What are the effects of fragmentation on tropical tree hole macroinvertebrates, protists and detrital decomposition? 2) What are the relative roles of forest patch area and landscape processes driving any observed effects? 3) Which of the four proposed mechanisms explain observed effects of patch area and landscape composition?

Methods

Study site

This study was conducted in mid-elevation (311-535 m a.s.l.; mean= 420 m a.s.l) tropical semi deciduous and tropical moist forests of Northwestern Costa Rica (Hedström and Sahlén 2001, Joyce 2006). We sampled tree holes in an 11 by 13 km area north of the Orosí Volcano and east of Area de Conservación Guanacaste (ACG). The area experiences two distinct seasons: a dry period at the beginning of the year (Jan-April 2011: 150.8±58.3 mm/month) and a rainy season that extends through most of the remaining months (May-December 2011: 479.4 ±170.1 mm/month; data from Pitilla Biological Station, ACG).

The majority of forest fragments used in this study were formed in mid 1990s (Freddy Vargas, DelOro, S.A., pers. comm.). The area surrounding these patches comprises a combination of land uses: orange and pineapple groves managed either at an industrial scale by an international fruit company (DelOro, S.A.) or at a small scale by independent farmers; human settlements (population < 7000) and extensions of primary and secondary tropical forest that were preserved presumably for potential bio control purposes but that are currently subject to opportunistic logging. Forest fragments include a range of forest succession stages characteristic of the landscape.

We sampled macroinvertebrate communities from twenty study sites (Figure 2). Eight sites were located in continuous forest (>160 Ha) and were used as controls, while twelve sites were located in forest fragments classified into two size categories: six small (0.8-6.8 Ha) and six large (8.4-32.6 Ha) patches. We set up transects in a straight line from the forest edge to the center of the patch, a point estimated from satellite imagery. Transects in intact sites were established at least 250 m into the forest to minimize potential edge effects (Laurance et al. 2002, Harper et al. 2005)

A minimum distance of 130 m separated sites in different patches and sites within same habitat category were separated by at least 366 m. All forest sites except three were surrounded by orange plantations (patch L6 was surrounded by vegetable fields and regenerating pasture; while intact sites I5 and NIC were bordered by teak plantations). Two of our forest patches showed signs of moderate human disturbance (trails in S4, L4) and one showed signs of incipient logging at the time of final sampling (L6).

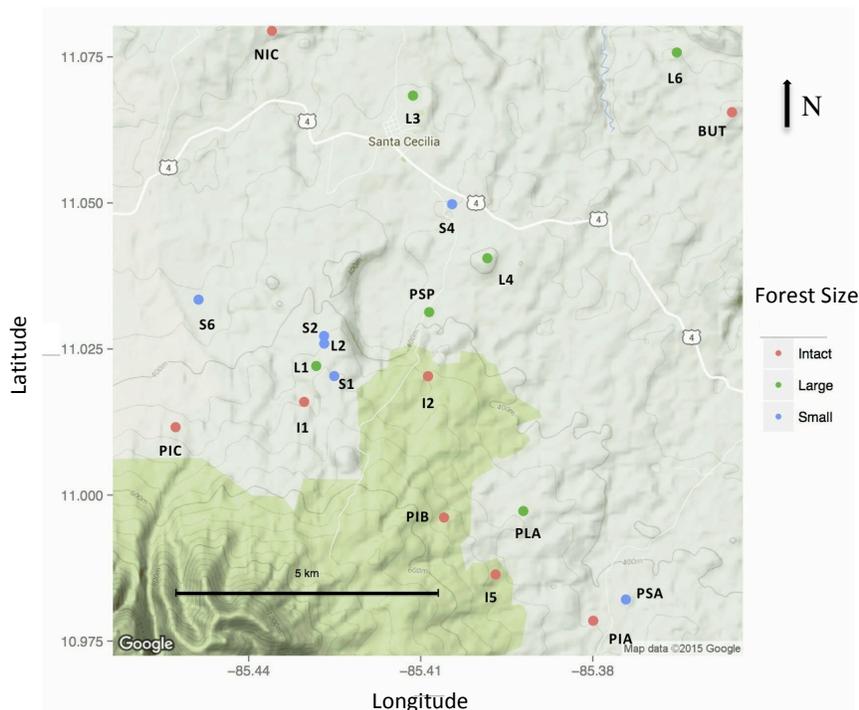


Figure 2. Study area and sampling sites. Map of north section of Guanacaste Province showing sites surveyed in this study grouped into size categories. Mean site elevation is 420 m asl (311-535 m asl). Boundaries of Area de Conservación Guanacaste are shown in darker green.

Artificial treeholes: colonization and detrital decomposition

In each of our study sites we set up twenty artificial tree holes by attaching plastic cups to trees of at least 5 cm of diameter at breast height (henceforth DBH). We used round black cups (opening diameter of 10.8cm, height 8.4 cm, manufactured by Advanced Plastics Inc., Burnaby BC, Canada) in which we drilled a draining

hole (1.3 cm diameter, located 6 cm above the bottom of cup and covered with 2.2 mm mesh) to allow the exit of excess water and keep the cup from overflowing. Cups were fixed to trees using steel wire at 1.5 m above ground.

We added four grams (± 0.1 g) of dried leaf litter to all cups. Using a random number table, two leaf litter treatments were randomly assigned to treeholes: half the cups contained a mixture of dried leaf litter from that site, and half contained a standard leaf litter treatment to act as control (*Conostegia xalapensis*, a pasture tree commonly found in secondary growth areas). Within each treehole, 2g of the 4g of litter was loose in the cup, to create habitat structure, and the other 2g were in litter bags to measure decomposition. Our litter bags were designed to measure decomposition while allowing macroinvertebrates to access the leaf litter, and so consisted of coarse mesh bags (size 10.5 x 9.5 cm; mesh opening 0.6 x 1 cm) sewed shut with string. We followed the same procedure with the standard leaves of *C. xalapensis*. All leaf litter was dried in a conventional oven at 70°C until mass stabilized for determinations of dry mass.

We filled each artificial tree hole with 400 ml of mineral water and added a wooden stick to facilitate insect oviposition. Leaf litter, rainwater and stemflow were allowed to fall naturally into treeholes. Four hundred cups were set up between June 24th and July 6th 2011. After one month (June 24th to July 25th) we destructively sampled 50% of our artificial treeholes (10 from each site). The entire cup's content was transferred to closeable plastic containers and transported to Pitilla Biological Station. At the station, we filtered the cup's contents through a 250 μ m sieve and measured water volume. We rinsed surfaces of intact leaves, including that in litter bags, to wash all organisms into the sieves, and then examined all material retained on the sieve for macroinvertebrates. Animals were counted and preserved in 80% ethanol for later identification. After macroinvertebrates were removed from the leaf litter in litter bags, we dried the litter bag detritus at 70°C to constant mass and recorded the dry mass; loss of detrital dry mass over time was used as an estimate of decomposition rate.

In the field, we also pipetted 1.5 ml samples of water from each artificial tree hole and added two drops of Lugol's solution for preservation (an iodine-based solution that binds to organisms making them lose buoyancy). Subsamples of 100 μ l from 85 day samples were processed in the laboratory at the Biodiversity Research Center, University of British Columbia, at 20X magnification (Nikon TE-2000S). Photographic keys and general references (Patterson 1996) were used to estimate protist and rotifers abundance and diversity.

For the final sampling, 85 days after the experiment started (September 18th -27th) the remaining cups were processed following the same procedure. All organisms were collected under a scientific research permit issued by the ACG (ACG PI-023-2011. Scientific passport #02766).

Abiotic differences among sites

We placed a data logger temperature sensor (TRIX-8 Temperature Data Recorder by MicroDAQ) at the end of each transect, to monitor temperature variation across sites. Data loggers were attached to trees at about 1.5 meters aboveground and were scheduled to measure temperature every 30 minutes. Temperature analysis was constrained to sites where measurements were made at overlapping dates (Aug 5-Sept 2). This excluded one Large (L6), two Small (S6, S2) and three Intact sites (PIB, PIA and BUT) but their incorporation into the analysis did not change trends or significance levels. We measured trunk circumference at breast height (later transformed to DBH). We photographed the foliage cover directly above each treehole (Sony Cybershot G camera with 24-120 mm lens). To estimate the fraction of sky obscured by canopy vegetation above each treehole, we calculated the number of white pixels in binary transformations of color photographs (Image J. NIH US, 2013).

Natural Tree hole Survey

In order to test if our artificial treeholes were attracting macroinvertebrates typical of natural treeholes, we surveyed natural treeholes along transects in 14 of our study sites. Two small (S1, S4, L2) and one large (PLA) site could not be surveyed for natural treeholes due to logistic constraints. In each site we established ten transects, each 20m long by 3 meters wide and perpendicular to the edge-center transects used for artificial treeholes. On one side of each transect, we recorded all treeholes and sampled those less than 1.8 meters above ground. We recorded physical attributes (height from the ground, opening diameter and water volume) for each tree hole, and sampled the tree hole with a turkey baster. We collected all leaf litter and water present, removed and preserved macroinvertebrates and determined the mass of dry leaf litter content following the same procedures as in artificial tree holes.

Macroinvertebrate identification

Preserved immature macroinvertebrates were identified under a dissecting microscope with the help of specialized references (Brown et al. 2009, 2010). For mosquitoes we used an unpublished key to the mosquitoes of Costa Rica (L.G.Chaverri). We used general guides to aquatic insects to classify organisms into functional groups (Merritt and Cummins 1988).

Leaf litter nutrient content

We analyzed a subset of the site-specific litter for nutrient content. Detrital samples were dried at 60°C for 72 h, ground and homogenized with a mortar and pestle, and put into tin capsules. We determined C and N content from five-milligram subsamples using a CN2500 elemental analyzer at the University of Cornell. The P content of leaf litter was determined using a persulfate digestion method and ascorbic acid method (APHA 1992). Dried leaf material was ground in a ball mill prior to measuring the mass of a homogenized subsample on a digital microbalance ($\pm 0.1 \mu\text{g}$). Elemental contents were expressed on a percent dry mass basis.

Although all treeholes initially contained the same amount of leaf litter, 82 out of 379, or 21% of cups, received additional inputs from leaves or sticks that naturally fell into the cups during the experiment. However this additional matter represented less than 11% of total leaf litter mass on average, therefore, we did not consider this a significant addition of nutrients.

Landscape metrics:

We analyzed satellite imagery (Google Earth, 2011-2013) to estimate patch size and connectivity. Fragment area was estimated using ArcGIS 10.0. We calculated the length of shortest straight-line between the focal patch and the nearest forest patch (Euclidean nearest neighbor, ENN), as well as the minimum distance to a continuous forest. We calculated a Proximity Index (PI) for each patch (Gustafson and Parker 1994), as the summed area of all other patches (S) within 1km of the focal patch weighted by the inverse square of their distance (Z) from the focal patch: $PI = \sum_{i=1}^n S_i/Z_i$. Patches with many large patches in close proximity have large index value, therefore, low isolation.

Statistical Analysis

We used generalized linear mixed effect models to test the null hypothesis that community attributes do not change with fragmentation. We first examined the effects of Time, Leaf litter (standard vs. site-specific) and Forest size (small patch, large patch and continuous forest) on total macroinvertebrate abundance, total species richness, detrital decomposition and filter feeder abundance. Data were restricted to the final sampling period for cases where too few organisms had colonized treeholes by first sampling date, (i.e. shredder-scraper abundance, collector abundance, predator abundance) or organisms were not sampled (protist community). We removed one outlier from statistical analysis (tree hole #395, from intact site NIC, which contained extremely high abundance of oligochaetes in various degrees of segmentation). For protist data we additionally included sorter identity as a covariate as protist identification is a learnt skill.

We used the proportion of dry mass loss to calculate daily mean decomposition rates and we log-transformed temperatures of each site before analyzing it. We used generalized linear mixed effect models (glmmADMB) with negative binomial distributed errors to analyze total macroinvertebrate abundance and species richness as well as for protist abundance. These models had appropriate dispersion ratios so no correction for overdispersion was needed. We used log likelihood and AIC to compare between candidate models. For models where overdispersion was present or convergence was not possible, we analyzed univariate responses along fragmentation gradients using either generalized linear mixed effect models (glmer: lme4) or glmmADMB model builder for zero inflated models with negative binomial errors in the case of abundance data and binomial errors for presence-absence data (Bolker et al. 2009) . Community-level responses at the morphospecies level were analyzed with PERMANOVA (R, Vegan package) using Site as random variable and 10,000 iterations.

We then examined the additional response of total macroinvertebrate abundance and richness to environmental variables at two spatial scales: the tree scale and the landscape scale. At the tree scale we examined the role of tree size, distance to forest edge, percentage of forest canopy closure above the tree hole and tree hole water volume at the time of sampling. Variables at the landscape scale included patch size, minimum distance to a forest fragment, minimum distance to a continuous forest, and the focal patch proximity index. Due to the intrinsic shape and connectivity properties of continuous forest relicts, determination of total area and isolation metrics for intact forests was not possible. As area and isolation variables could be calculated just for forest patches, sites in continuous forests were excluded from landscape scale analysis. For this patch data set, patch size was analyzed both as a continuous and as a categorical variable.

In order to reduce the number of potentially explanatory variables included in this model, we used multiple stepwise regression on the residuals of a generalized linear model (GLM) with negative distributed and Poisson errors for Abundance and Richness respectively, considering Time and Leaf litter as fixed effects. Variables were automatically selected using the stepAIC function of the MASS Package. After model simplification, at the tree scale, residuals of macroinvertebrate abundance responded initially only to tree trunk diameter (Table A2) and at the landscape scale, only to patch proximity index (Table A2). Residuals of morphospecies richness responded at the tree scale only to trunk diameter and, at the landscape scale, only to minimum distance to a nearest forest (Table 2). These results were robust to model construction; for example, additional multiple stepwise regressions with all variables from both spatial scales analyzed simultaneously resulted in the exclusion of the same variables for Abundance (Appendix Table A2). For richness, proportion of forest cover replaced distance to nearest forest, and patch area was not removed from the simultaneous model (Appendix Table A2). Although distance to edge was not selected in these stepwise models, we included it in the final covariates list as it has frequently been cited as a driver of fragmentation effects.

We then combined the relevant variables in generalized linear mixed effect models (glmmADMB package, R) with negative binomial errors and selected between hierarchical models. To take into account the structure of our experimental design, we included forest fragment identity (henceforth “Site”) as a random effect in all of our analysis (Oksanen et al 2015).

For all GLMMs, the significance of each fixed effect (Time, Forest size, Leaf litter and their interactions) was tested by comparing model deviances fitted with and without each effect with a χ^2 test. All analysis were performed in R version 3.2.1 (R Core Team (2013) except for one multivariate NMDS plot built with Primer 6 (2014 PRIMER-E Ltd).

Results

1. Community responses to reduction in forest habitat

The composition of macroinvertebrate communities in our artificial tree holes differed with colonization time and between fragmented and intact forest: as colonization progressed, the community shifted in composition and increased in the abundance and diversity of individuals (Table 1, Fig 3). By 85 days, communities from forest patches contained higher numbers of species than those from intact forest (Table 1). Within patches, abundance and diversity was better predicted by tree size and isolation in the landscape than by patch size. Overall, the type of leaf litter present in tree holes affected decomposition rates and protist abundance but was not an important factor for most macroinvertebrates.

a. Effects of forest fragmentation on macroinvertebrate abundance and richness

Intact versus fragmented forest: effects on abundance and richness

A total of 7396 larval macroinvertebrates from forty-three taxa were collected from artificial tree holes and identified to the lowest possible taxonomic level. Additionally, 522 insect pupae were encountered but could not be identified (most of them presumed to be culicids) and were excluded from analysis. Overall, Diptera larvae (flies) comprised 89% of total insect abundance with mosquito larva (Culicidae) as the predominant group (62%). Unique to this study is the identification of Elmidae beetle larvae and isopod larvae and adults in artificial tree holes (Table 4). Throughout the following sections “Forest size” refers to the categorical distinction between small fragments, large fragments and continuous forest.

By 30 days of colonization, the abundance of macroinvertebrates in tree holes was slightly higher in continuous forest than in patches, and primarily consisted of mosquitoes. This trend had reversed by 85 days, when additional taxa colonized the treeholes and the average abundance was 19% higher in tree holes from forest patches than from intact forest (Forest size and Time by Forest size interaction: Table 1). As with total abundance, the number of morphospecies present in a tree hole also increased with time. At 30 days there were no differences in mean morphospecies richness between patches and intact forest, but by 85 days, tree hole species richness was 28% higher in patches than in continuous forest (‘Forest size’ and ‘Time’ significant: Table 1). Leaf litter type did not significantly affect either total macroinvertebrate abundance or richness (Table 1).

Forest patches: determinants of macroinvertebrate abundance and richness

After determining the effects of our experimental variables, our next aim was to build a more comprehensive model that incorporated additional covariates at the tree and landscape level. As some of our landscape variables could only be calculated for forest patches (e.g. patch area, proximity index) this analysis is

necessarily constrained to the forest fragments. We first used stepwise multiple regressions to reduce the large number of potential covariates at each spatial scale to the most important ones (see Methods). Once the important covariates had been selected, we built a model that explained macroinvertebrate abundance in forest patches in terms of our experimental variables (Time, Forest size, Leaf litter) as well as the covariates selected (tree size, patch proximity index) and of interest (distance to edge). Macroinvertebrate abundance increased significantly with Time, Tree size (DBH, see Fig. A6-e) and marginally with Proximity Index (Table 2). There was no effect of Leaf litter or Distance to forest edge, nor significant interaction terms (Table 2).

We repeated this process for richness of macro-invertebrate species in treeholes. Here the selected environmental covariates were again tree size and now distance to nearest forest and proportion of forest cover in 1 km radius. In the final model macroinvertebrate richness increased significantly with Time and tree size and decreased with Distance to nearest forest (Table 2). No effect of Distance to forest edge, Leaf litter type nor interactions on species richness were detected.

Species pool of intact versus fragmented forests

The above analyses concern the number of species in an individual treehole. As there is turnover in composition between treeholes, the number of species in a site increases as more and more treeholes are sampled. Rarefaction curves (Figure 9) suggest that patches contained on average more treehole-dwelling species than intact forest sites when less than 150 treeholes were sampled, but given the intersection of the curves at about 150 treeholes, this trend may reverse as sample effort increased. Indeed, site diversity is predicted to asymptote at approximately 67.4 species (± 26.2 , Chao estimator \pm SE) for the intact sites but at only about 38 and 41.7 (± 7.0 -5.8) species for large and small patches respectively.

b. Fragmentation effects on functional groups

Filter feeders were entirely represented by mosquito (Culicidae) larvae. Although mosquito larvae were always the most abundant group in our tree holes, their relative abundance decreased with colonization time from an initial 90% of individuals in 30-day-old tree holes to 45% at the end (85 days) of the experiment (Fig.6; Appendix Fig. A2). Their dominance decreases further in mature communities as represented by natural tree holes (Appendix Fig. A1).

In addition to decreasing between sample periods (Time: $\chi^2_{1, N=378} = 4.47$, $p = 0.035$), mosquito abundance per tree hole at 30 days was three times higher in continuous forest than in fragments (Fig.6; Intacts versus Patches $\chi^2_{1, N=180} = 4.79$, $p = 0.029$). *Limatus asulleptus* mosquitoes dominated in the early samples but by 85 days were supplanted by *Aedes* and *Haemagogus* spp. mosquitoes (Appendix Fig. A2). There was no significant effect of Leaf litter type or any interactions. Indicator species analysis (Table 4) suggests that the

decrease in mosquito abundance in small fragments was largely due to *Culex spp.* and *Limatus asulleptus* rather than *Aedes* or *Haemagogus spp.*

The abundance of all detritivores (i.e. collectors plus shredder-scrapers) increased dramatically from 30 to 85 days, especially in treeholes with native litter in small fragments (Fig. A7. Time x Leaf litter x Forest size interaction: $\chi^2_{2,365} = 11.14, p=0.004$. Because detritivore and predator larvae were so much slower to colonize treeholes than mosquitoes (Appendix A2), there were insufficient data to analyse the individual functional feeding groups (collectors, shredder-scrapers, predators) for the 30 day sampling period; therefore for the remainder of this section we analyse functional group results for the 85 day sampling period only.

In contrast to mosquitoes, the abundance of the detritivorous functional groups (Fig 6) tended to increase as forest size decreased from intact forest to large patches to small patches. For example, collector gatherers (mainly Ceratopogonidae, Chironomidae, Cecidomyiidae, Psychodidae, Syrphidae) were also more frequent and had marginally ($\chi^2_{2,N=182} = 5.05, p=0.08$) higher abundances in small patches, regardless of detrital type. Analysis of indicator species suggests that forest size effects amongst collectors were largely the results of Dipteran larvae in the families Psychodidae, Ceratopogonidae, Stratiomyidae, Phoridae and Syrphidae (see Table 4). One exception was Chironomidae larvae, which includes some of the most common collector species, and which were evenly distributed along the forest size gradient (indicator analysis: Table 4). Shredders and scrapers, represented mainly by marsh beetle larvae (Scirtidae) were most abundant and marginally more frequent in small patches (Size: Table 3). This pattern appeared especially strong in scirtids in treeholes with native leaf litter (Fig. 6), although no Leaf litter by Forest size interaction was found ($\chi^2_{1,N=184} = 2.47, p= 0.29$). We suspect that the Leaf litter signal was swamped by high variation in abundance in the native treeholes as compared to the standard treeholes. If we separated treeholes by detrital type to deal with this heterogeneity in variances, tree holes with native leaf litter mixtures in small fragments had 7-fold more shredder-scrapers than tree holes in intact forest (Size: $\chi^2_{2,N=94} = 8.35, p=0.015$), but tree holes with standard leaf litter had uniformly low abundance across the forest size gradient (Size: $\chi^2_{2,N=94} = 0.231, p=0.89$). There was no association between the occurrence of odonates in treeholes and detritivore abundance ($\chi^2_{1,N=183} = 0.97, p=0.78$).

Finally predators as a group were equally distributed across forest size categories ($\chi^2_{(2,N=192)} = 0.2281, p=0.892$), but were marginally more abundant in treeholes with standard detritus ($\chi^2_{(1,N=192)} = 3.73, p=0.054$). The lack of a forest size effect on the entire trophic group reflects an opposing pattern in how the two larger predators responded to the forest size gradient (Fig. 6). Damselfly larvae, the top predator in the system were found marginally less often in small forest fragments (Smalls $\chi^2 = 3.71, p = 0.054$). In contrast, the second

largest predator, the larvae of *Toxorhynchites sp* followed an opposite trend in treeholes with native leaf litter: its presence in small fragments was triple that in intact forest (in treeholes with native leaf litter). *Toxorhynchites* abundances were similar between Forest sizes in treeholes with standard detritus (Size: $\chi^2_{2,N=91} = 0.5$, $p=0.78$). Consequently, indicator species analysis identified *Toxorhynchites* as indicative of forest patches and *Mecistogaster spp.* as indicative of intact forests (Table 4). Overall, I found twice as many odonates ($n=42$) as *Toxorhynchites* ($n=20$) but these two predators were found to co-occur only once (in a small patch).

c. Protist communities

Flagellates were the most abundant protists in our tree hole water samples, which also contained lower densities of algae, amoeba, ciliates, diatoms and rotifers. Treeholes in small patches had roughly 3x higher protist densities than those in intact forest, and intact forests had slightly higher densities in treeholes with standard as opposed to native leaf litter leaf litter (Fig. 10; Size x Leaf litter: $X^2_{2, 141} = 7.56$, $p = 0.023$). These patterns in protist density could reflect top-down control from filter-feeding mosquitoes or bottom-up control through detrital decomposition. The abundance of filter feeder mosquitoes, although lower in small fragments where protists increase, did not explain variation in protist density ($X^2_{1,148}=1.54$, $p= 0.214$).

2. Environmental differences between fragments and forest

The daily mean air temperature at the center of fragments was on average an insignificant 0.3 °C warmer than at continuous sites ($\chi^2_{(2,14)} = 1.65$, $p=0.44$; Table 5), but daily maximum temperatures averaged 1°C higher in small fragments, significantly warmer than other size categories ($\chi^2_{(2,14)}=9.48$, $p=0.002$; Table 5, Fig A6-a). Differences in temperature between sites were also explained by small differences in elevation (daily mean: $\chi^2_{(1,14)} = 15.92$, $p<0.001$; daily maximum $\chi^2_{(1,14)}=7.98$, $p=0.018$). Variation in forest canopy cover and water loss in tree holes was small and surprisingly homogenous among forest size categories (Table 5; Appendix A6-c).

Edge effects

Distance of tree holes to forest edge did not exert a detectable effect on total macroinvertebrate abundance, ($\chi^2_{1,N=205} = 0.21$, $p=0.64$) or on species richness ($\chi^2_{1,N=205}= 0.88$, $p=0.35$) with the patch only data set. As Pseudostigmatid damselfly larvae have been found to be sensitive to disturbance (Fincke 2006, Khazan 2014), we specifically tested for edge avoidance in the occurrence of this family but found no support (Distance to forest edge: $\chi^2_{(1,101)} = 0.093$, $p= 0.760$).

3. Leaf litter decomposition in artificial treeholes

Leaf litter type, time and forest size had significant effects on leaf decomposition rates. As expected, leaf litter of the pasture species *C. xalapensis* from the standard Leaf litter treatment decomposed at a significantly faster rate than site-specific leaf litter mixtures, especially in the first 30 days (Leaf litter x Time interaction: Table 1, Fig. 8). Decomposition rates of both Leaf litter types decreased with time, confirming the well-known nonlinear decay in decomposition rates (Figure 8). Decomposition rates decrease with forest size in the first 30 days, but over 85 days the rates were similarly low across all forest size categories (Forest size x Time: Table 1, Fig. 8). We did not detect forest edge effects on leaf litter decomposition rates ($F_{1,282}=0.045$, $p=0.83$).

Table 1. Effects of experimental variables on treehole macroinvertebrate community composition, abundance, species richness, leaf litter decomposition and protist abundance. Multivariate analysis (1) shows results of Permanova. Univariate responses (2,3,5) were analyzed with generalized mixed effects models with negative binomial errors. Decomposition (4) was analyzed with linear mixed effect models.

Factor	Community Composition (1)			Macroinvertebrate Abundance (2)			Species Richness (3)			Leaf litter Decomposition (4)		
	df	F	p-value	df	X ²	p-value	df	X ²	p-value	df	X ²	p-value
Forest size	2	8.18	< 0.001	1	247.7	0.008	2	10.70	0.005	2	4.85	0.088
Leaf litter	1	0.96	0.390	2	9.77	0.666	1	0.60	0.440	1	86.4	<0.001
Time	1	29.3	<0.001	1	0.19	0.223	1	56.31	0.000	1	284.	<0.001
Forest size x Leaf litter	2	0.87	0.540	1	1.48	0.184	2	0.31	0.858	2	0.18	0.914
Forest size x Time	2	2.94	<0.001	2	3.39	0.000	2	0.80	0.670	2	14.3	0.001
Leaf litter x Time	1	1.07	0.301	2	18.93	0.723	1	1.97	0.161	1	4.74	0.029
Forest size x Leaf litter	2	1.25	0.140	1	0.13	0.403	2	5.29	0.071	2	1.62	0.446
Protist abundance (5)												
Factor	df	X ²	p value									
Forest size	2	22.1	0.000									
Leaf litter	1	11.8	0.001									
Sample sorter	2	4.72	0.094									
Leaf litter x Forest size	2	7.62	0.022									
Leaf litter x Sample sorter	2	3.99	0.136									
Forest size x Sample sorter	4	7.88	0.096									
Leaf litter x Forest Size x Sample	4	4.61	0.330									

Notes: (1) Permanova, 10⁵ iterations. Bray Curtis dissimilarity. Hellinger-transformed data. N=332. F-test degrees of freedom denominator= 320 (2) GlimmADMB. N=378. Overdispersion ratio =0.91. Type III Anova. Intercept 1,363 X²=247.66, p<0.001. Residuals 363.

(3) Glmer, Poisson, n=378, overdispersion = 0.91. Type II Anova. Residual d.f. =366.

(4) Lmer. Rate (g mass loss/day) ^0.5 .N= 311

(5) GlmmADMB, negative binomial. 85 days data only. N=162. overdispersion ratio = 1.42. Residuals =142.

Table 2. Effects of landscape and tree scale variables on macroinvertebrate abundance and richness. Analysis was restricted to forest fragments (N=205) and modeled negative binomial-distributed errors using variables pre-selected by multiple regression. DBH is tree trunk diameter at breast height. No over dispersion was detected for either Abundance (ratio 0.93) or Richness (ratio 0.95). As no interactions were present, type II estimates are shown.

Factor	Abundance (1)			Factor	Richness (2)		
	Df	Chi sq.	p-value		Df	Chi sq.	p-value
Leaf litter	1	0.11	0.732	Leaf litter	1	0.256	0.613
Time	1	10.95	<0.001	Time	1	14.09	<0.001
Distance to Forest Edge	1	0.21	0.644	Distance to Forest Edge	1	0.883	0.347
Tree size (DBH)	1	3.14	0.076	Tree size (DBH)	1	9.257	0.002
Proximity Index	1	3.38	0.066	Forest cover in 1Km radius	1	0.969	0.325
Leaf litter x Time	1	0.49	0.484	Patch Area	1	0.518	0.472
				Distance to nearest forest	1	4.688	0.030
				Leaf litter x Time	1	0.006	0.937

Table 3. Functional feeding group response to Leaf litter and Forest size at the end of the experiment. Except Filter Feeders, all models correspond to 85 day data (N=192) and use negative binomial (abundance) or binomial (presence- absence) errors.

Response	Factor	df	Shredder-Scrapers ¹		Filterer feeders ²		Collector-Gatherers ³		Predators ⁴	
			X ²	Pr (>X ²)	X ²	Pr (>X ²)	X ²	Pr (>X ²)	X ²	Pr (>X ²)
Abundance	Forest size	2	6.63	0.036	2.45	0.294	5.05	0.080	0.23	0.892
	Leaf litter	1	0.04	0.846	0.18	0.670	1.29	0.256	3.74	0.053
	Leaf litter x Forest size	2	2.26	0.323	1.48	0.476	0.23	0.892	3.35	0.187
Presence/Absence	Forest size	2	5.59	0.061	2.340	0.310	9.13	0.010	0.33	0.848
	Leaf litter	1	0.60	0.438	0.652	0.4196	0.35	0.555	1.49	0.222
	Leaf litter x Forest size	2	2.13	0.345	0.608	0.738	2.27	0.321	2.82	0.244

Overdispersion ratios (Abundance; Presence/Absence): ¹S:0.6; 0.75. ²FF: 1.03; 0.82. ³C-G: 0.79; 0.47. ⁴P: 0.89; 0.65.

Table 4. Observed frequency per habitat type and indicator value of artificial tree hole taxa.
Taxonomic class is indicated in captions. FG are functional feeding groups (CG: collector-gatherer, FF=Filter feeder, S=Shredder-scrappers, P= predator, Par=parasite) Gr = Indicator Value Group, indicates greater abundance and frequency in either intact forests (group I), forest patches (group P) or no association (N).

Family	FG	Taxon	Observed frequency (%)			Gr.	Indicator Value	p-value
			Total	Intact forest	Forest patch			
ANNELIDA								
Oligochaete	CG	Oligochaete spp.	1.6	2.7	0.9	I	0.0319	0.194
CRUSTACEA								
Isopoda	S	Isopoda sp 1	1.1	0.7	1.3	P	0.0089	0.838
INSECTA								
Coleoptera								
Scirtidae	S	Prionocyphon	10.8	8.0	12.7	P	0.0737	0.331
Elmidae	S	Elmidae sp.1	0.5	0.7	0.4	P	0.0057	0.54
Diptera								
Cecidomyiidae	CG	Cecidomyiid sp.1	5.0	2.7	6.6	N	0.0357	0.411
	CG	Cecidomyiid sp.2	2.6	1.3	3.5	P	0.0417	0.089
	CG	Cecidomyiid sp3	0.8	1.3	0.4	I	0.0099	0.786
Ceratopogonidae	CG	Bezzia sp.	17.7	9.3	23.1	P	0.1962	0.001
Chironomidae	CG	Chironomid sp. 1	4.0	5.3	3.1	I	0.0448	0.142
	CG	Chironomid sp. 2	9.5	8.0	10.5	P	0.0466	0.839
	CG	Chironomid sp. 3	2.1	3.3	1.3	P	0.0123	0.855
	P	Chironomid sp. 4	0.5	0.7	0.4	I	0.0067	1
Corethrellidae	P	Corethrella sp 1	0.3	0.0	0.4	N	0.0102	0.285
Culicidae	FF	<i>Aedes</i> sp. 1	26.4	21.3	29.7	N	0.1641	0.104
	FF	<i>Aedes</i> sp. 2	1.8	0.7	2.6	P	0.0261	0.231
	FF	<i>Anopheles argyritarsis</i>	4.0	4.7	3.5	N	0.0238	0.708
	FF	<i>Culex alogistus</i>	0.3	0.7	0.0	I	0.0081	1

Family	FG	Taxon	Observed frequency (%)			Gr.	Indicator Value	p-value
			Total	Intact forest	Forest patch			
	FF	<i>Culex bihaicolus</i>	1.6	1.3	1.7	N	0.0159	0.512
	FF	<i>Culex sp. 1</i>	0.3	0.7	0.0	I	0.0081	1
	FF	<i>Culex urichii</i>	2.4	4.7	0.9	I	0.0385	0.08
	FF	<i>Culex mollis</i>	3.2	2.7	3.5		0.0402	0.139
	FF	<i>Haemagogus chalcospilans</i>	16.1	10.0	20.1	N	0.1061	0.126
	FF	<i>Haemagogus equinus</i>	10.0	4.0	14.0	P	0.0705	0.261
	FF	<i>Haemagogus janthinomis</i>	3.7	2.7	4.4	N	0.0381	0.177
	FF	<i>Limatus asulleptus</i>	47.2	58.7	39.7	I	0.3772	0.001
	P	<i>Toxorhynchites hypoptes</i>	4.5	3.3	5.2	P	0.0561	0.039
	FF	<i>Trichoprosopon digitatum</i>	0.3	0.0	0.4	N	0.0091	0.623
Dolichopodidae	P	Dolichopodid sp.1	0.3	0.7	0.0	I	0.0081	1
Ephidridae	CG	Ephidrid sp.1	0.5	0.7	0.4	P	0.0057	0.541
Phoridae	CG	Phorid sp.1	4.5	0.7	7.0	P	0.0807	0.004
Psychodidae*	CG	Psychodid sp.1	6.6	2.0	9.6	P	0.1544	0.001
Psychodidae	CG	Psychodid sp.2.	1.1	0.0	1.7	P	0.0408	0.007
Stratiomyidae *	CG	Odontomyia sp.1	1.3	0.0	2.2	P	0.0510	0.003
Syrphidae *	CG	Copestylum sp 1	3.2	0.4	2.4	P	0.0485	0.03
Tipulidae	S	Tipulid sp. 1	0.5	0.0	0.9	P	0.0204	0.103
Unknown	CG	Diptera sp.1	0.8	0.0	1.3	P	0.0141	0.289
	CG	Diptera sp.2	0.3	0.7	0.0	I	0.0081	1
	CG	Diptera sp.3	0.3	0.7	0.0	I	0.0081	1
Lepidoptera	CG	Lepidoptera sp.	0.3	0.0	0.4	N	0.0091	0.614
Zygoptera								
Pseudostigmatidae	P	<i>Mecistogaster</i> sp.1 and sp. 2	9.5	12.0	7.9	I	0.0638	0.228

Family	FG	Taxon	Observed frequency (%)			Gr.	Indicator Value	p-value
			Total	Intact forest	Forest patch			
NEMATODA								
Mermitidae	Par	Nematoda sp.1	0.8	0.7	0.9	P	0.0038	1

Table 5. Environmental differences between fragmented and non-fragmented forest. Stars indicate variables that are significantly different among three categories. Air temperature was measured at the center of forest patches and at the furthestmost point from entrance at intact sites.

Variable	Intact forest	Large fragment	Small fragment
Treeholes (n)	150	118	111
Mean distance to forest edge (m)	325 ± 43	79 ± 52	38 ± 31
Mean tree trunk size or DBH (cm) *	15.4 ± 13.24	12.09 ± 9.19	12.1 ± 10.06
Mean Canopy cover (%)	82.5 ± 9.04	81.7 ± 12.81	82.5 ± 10.17
Mean air temperature (°C)	23.1 ± 0.46	23.4 ± 0.4	23.4 ± 0.36
Mean maximum air temperature (°C) *	24.8 ± 0.71	25.4 ± 0.64	25.8 ± 0.69
Maximum air temperature recorded (°C)	29.1	29.2	32.1
Mean proportion of initial tree hole water lost (%)	6 ± 14	3 ± 14	4 ± 13

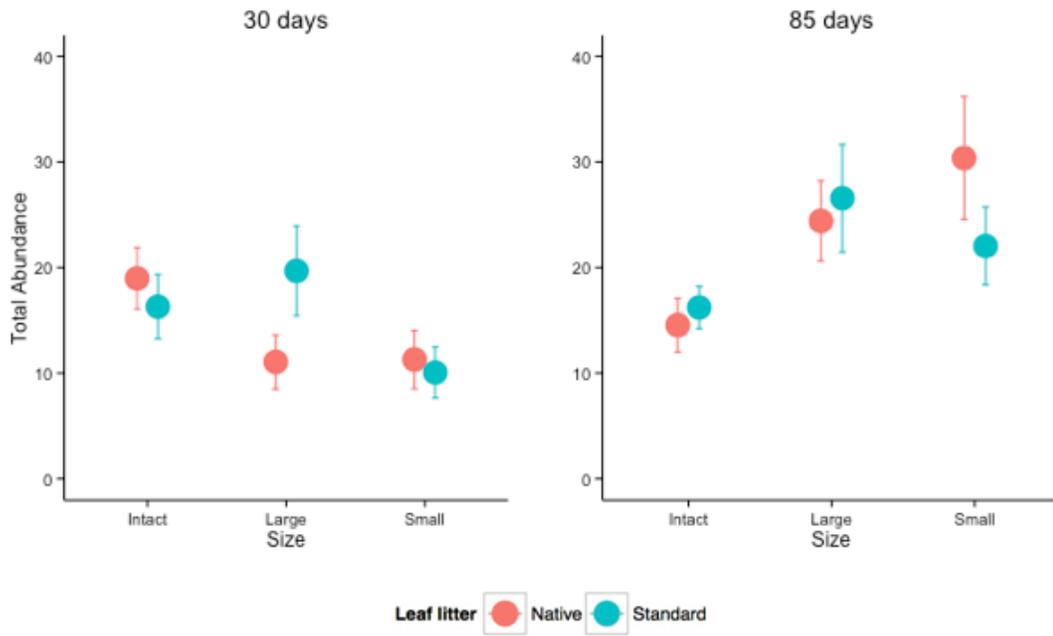


Figure 4. Effect of experimental variables on macroinvertebrate abundance. Figure shows data from both sampling sites. Forest size categories include fragments of small (n=6) and large (n=6) size as well as continuous forest sites (n=8). Standard detritus refers to treeholes with *Conostegia xalapensis* leaves, while Native refers to site specific leaf litter. Bars are standard errors of the mean.

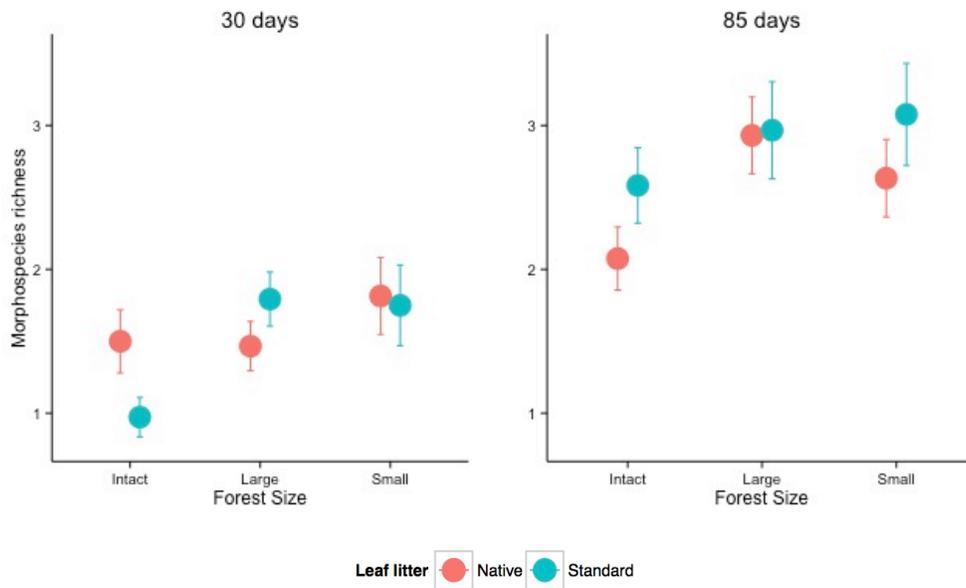


Figure 5. Effects of experimental variables on macroinvertebrate morphospecies richness. Bars are standard errors of the mean. Figure shows data from both sampling sites. Forest size categories include fragments of small (n=6) and large (n=6) size as well as continuous forest sites (n=8). Standard detritus refers to treeholes with *Conostegia xalapensis* leaves, while Native refers to site specific leaf litter. Bars are standard errors of the mean.

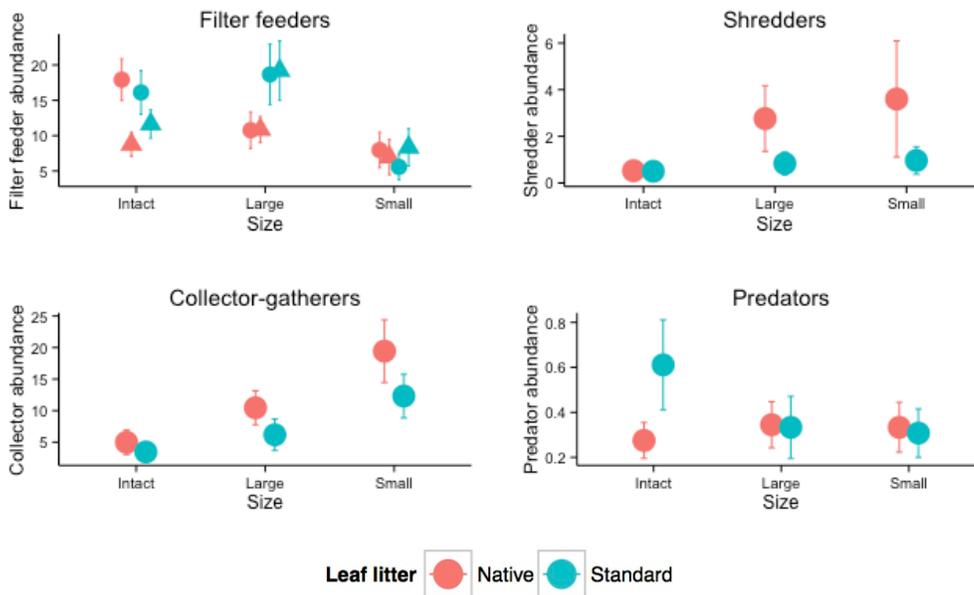
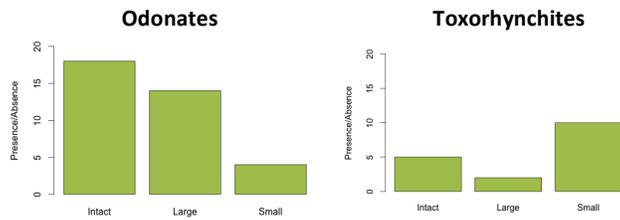


Figure 6. Effects of Forest Size and Leaf litter type on functional groups. Filter feeder abundances are shown at 30 (circles) and 85 days (triangles). Only 85-day results are shown for Shredder-Scrapers, Collectors and Predators, as there were minimal colonizations by these groups at 30 days. Forest size categories include fragments of small (n=6) and large (n=6) size as well as continuous sites (n=8). Standard detritus refers to treeholes with *C. xalapensis* leaves, while Native refers to site specific leaf litter. Bars are standard errors of the mean.

7a) Predator Presence-Absence



7b) Predator Abundance

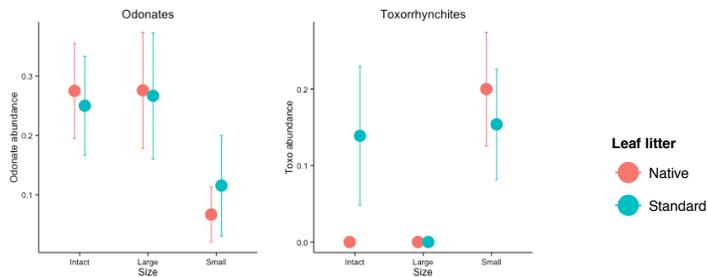


Figure 7. Tree hole predator presence/absence (7a) and abundance (7b) across a forest fragmentation gradient at the end of the experiment. Forest categories include fragments of small (n=6) and large (n=6) size as well as continuous sites (n=8). Standard detritus refers to treeholes with *Conostegia xalapensis* leaves, while Native refers to treeholes with site-specific leaf litter. Bars are standard errors of the mean.

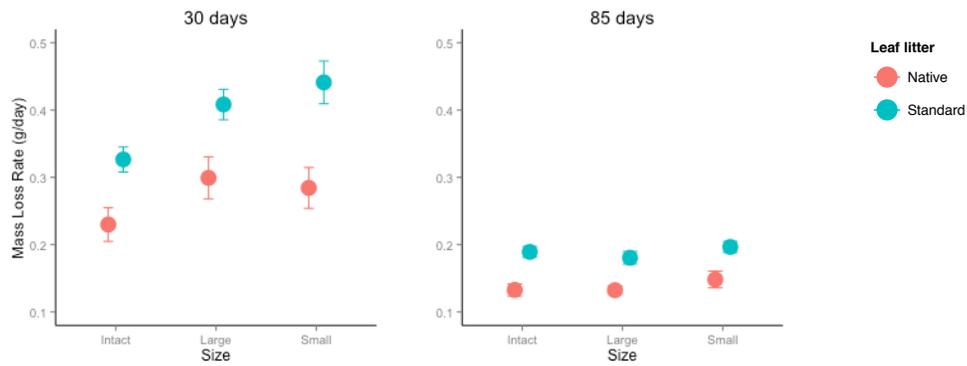


Figure 8. Effects of experimental variables on leaf litter decomposition. Bars are standard error of the mean. Forest size categories include fragments of small (n=6) and large s (n=6) and size as well as continuous sites (n=8). Standard detritus refers to treeholes with *Conostegia xalapensis* leaves, while Native refers to treeholes with site-specific leaf litter.

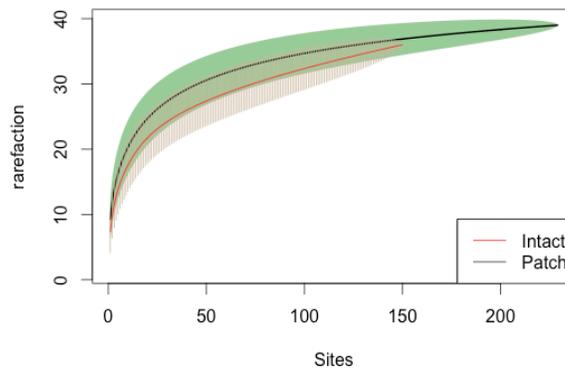


Figure 9. Macroinvertebrate species accumulation curves in forest patches (green, top) and intact forest (red, bottom). Site-based rarefaction curve, showing expected values with standard deviation error bars. The close to overlapping curves show slightly higher species richness in forest patches before an intersection point.

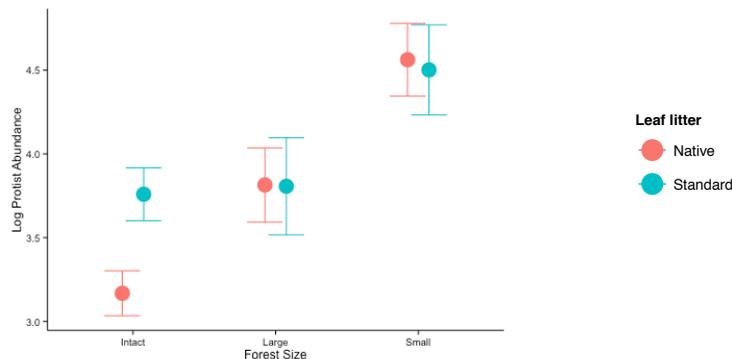


Figure 10. Tree hole protist abundance across a forest fragmentation gradient at the end of the experiment. Figure shows results of 90 day old artificial treeholes. Bars are standard error of the mean.

Discussion

In this study, we documented effects of forest fragmentation on treehole food webs, including the composition, abundance and richness of macroinvertebrates, densities of protists and detrital decomposition. We quantified fragmentation effects as both a gradient in forest size, from intact forests to large patches to small patches, as well as by the landscape configuration surrounding patches. Total abundance, richness, and taxonomic and functional composition of the macroinvertebrate community were generally sensitive to the forest size gradient. Within patches, abundance and richness increased with time and tree size, but abundance decreased with the proximity index. Our results suggest that both top predators and detritivores are likely to change in species identity and in abundance respectively. Fragmentation also affected protist densities, both directly as well as indirectly through modifying leaf litter quality. Litter decomposition was faster in small fragments than intact forest in the first 30 days, but by 85 days was similar across habitat types.

We had predicted that forest fragmentation would induce changes in treehole macroinvertebrate communities because of changes in the species pool as a result of dispersal limitation, in top-down effects of predator abundance, in bottom-up effects of detrital quality, or in the microclimate. Our results suggest that, of these four potential mechanisms, dispersal limitation and microclimate effects may be minor whereas bottom-up effects of detrital quality and potential top down effects explain most effects of fragmentation on treehole food webs, as we now examine in detail.

Species pool and dispersal limitation effects

Forest fragmentation is predicted to reduce the species pool for forests, through decreased colonization (reduced fragment size or connectivity reduces the likelihood of intercepting reproductive adults or propagules) or decreased persistence (smaller habitats contain fewer species, due either to sampling effects or low persistence of small populations) (Ewers and Didham 2006). However, in this study, there were at most subtle differences in the species pools between fragmented and intact forests. Even after sampling 150 treeholes in intact forest and 220 treeholes in fragmented forest, the total number of species encountered was similar between habitat type but still increasing within each type. The Chao estimates suggested that, with complete sampling, the species pool of intact forests could eventually be 50% higher than that of the fragmented forests.

If the number of species in a treehole is limited by the size of the local species pool, we would expect sites with small species pools to have fewer species per treehole. Instead we found the highest number of species per treehole in small patches, which are predicted to have similar or lower species pools than intact

forests. Indeed, in the indicator species analysis, many more species were identified as having an affinity to forest patches rather than having an affinity to intact forests (Table 4). Species that are indicative of forest patches might be originating from the matrix, as suggested by a recent study in Mexico where higher macroinvertebrate species richness was found in artificial treeholes in orange groves than in forests (Khazan et al. 2015). Orange and teak trees in our area do not tend to contain treeholes; although they could support other container habitats, such as bromeliads, overlap in species composition with treeholes is limited (Frank and Lounibos 1987, Fincke 2006, Blakely et al. 2012). Furthermore treehole richness in patches did not decrease with distance to nearby forest, as would be expected from classic dispersal limitation.

Beetles (Coleoptera) are often considered to be dispersal limited because of weak flight capabilities (den Bouer 1970, Desender et al. 1998, Horgan and Chávez 2004). Therefore, we expected the abundance and frequency of marsh beetle larvae (Scirtidae) would decrease with fragmentation. However, there was no negative effect of forest size on either abundance or frequency of scirtids in treeholes. Only in treeholes with native leaf litter, did scirtid abundance respond to forest size, and here the response was opposite to that predicted from dispersal limitation: scirtid abundance was three times higher in small fragments than in intact forest. These results more likely reflect bottom-up effects of litter quality as we discuss shortly. In general, shredder-scrapers and collectors were most frequent and abundant in small patches.

One possible explanation for these results is that small isolated patches act as a sink for populations of species with poor dispersal abilities, which tend to avoid dispersal related mortality by staying within a fragment (Thomas 2000, Ewers and Didham 2006). As a consequence of such potential “crowding effects”, the organisms that were able to survive habitat loss concentrate over the few resources available in patches (Debinski and Holt 2000, Ewers and Didham 2006, Grez et al. 2008). An alternative explanation is that slight differences in temperature over the forest size gradient, which we argue later to affect litter decomposition and protist densities, fuel the base of the food web and result in greater numbers of shredder-scrapers and collectors.

Only a few taxa show affinity for intact forests. Dispersal limitation probably restricted Pseudostigmatid damselflies to intact forests, as they are known from previous studies to avoid crossing gaps of more than 50 m (Khazan 2014). Further taxonomic resolution is required however to understand how the different odonate species (*M. linearis* and *M. ornata*) colonize treeholes in a fragmentation gradient. For more sedentary organisms like oligochaetes, which can only disperse between treeholes via phoretic vectors (Yanoviak 2001), it is possible that intact forests contained either more natural treeholes or more vectors (e.g. frogs, birds) moving between natural and artificial treeholes.

Treehole detritivores do not yet benefit from relaxed top pressure in fragments

As treehole predators are reported to be sensitive to forest loss and degradation (Gehring and Swihart 2003, Yanoviak et al. 2006a, Fincke and Hedström 2008) we expected overall reduced predator presence in tree holes in fragmented forest and, correspondingly, signals of release from predation pressure in the rest of the invertebrate community (as in Burks et al. 2001, Terborgh et al. 2001). Although fragmentation had no overall effect on the prevalence of predators, it did shift the identity of the top predator: the frequency of damselfly larvae decreased from large to small fragments just as that of the predatory mosquito larvae, *Toxorhynchites*, increased. This pattern could either reflect different habitat preferences of odonate larvae and *Toxorhynchites*, or it could result from only odonates being susceptible to fragmentation but consuming *Toxorhynchites* larvae whenever they co-occur (Fincke 1999).

Regardless of the underlying mechanism, this shift in predator identity with fragmentation was expected to reduce predation pressure on other invertebrates, given that *Toxorhynchites* larvae are less efficient at catching prey than odonate larvae (Fincke 1999). Our results suggest an increase of dipteran larvae in forest fragments, a trend that has been previously reported (Sekercioglu et al. 2002), however, even though small forest patches had fewer odonates and more detritivores, there was no significant association between the occurrence of odonates in treeholes and detritivore abundance. Thus, we did not detect cascading effects of odonate presence on overall metrics of prey abundance. We believe this reflects the length of time for odonate development in treeholes, which can take over 185 days in treeholes with <1L of water (Fincke 1992a). At the end of our experiment, odonate larvae may simply have been still too small to exert measurable top down effects. With time, as communities advance towards a climax, trophic cascades triggered by the changes in the predator community (Crooks and Soulé 1999) are expected to act synergistically with other forest fragmentation effects in shaping community structure.

Treehole detritivores benefit from higher detrital quality in fragments

Forest fragmentation can affect detrital quality because compared to intact forest, fragments typically have fast-growing, poorly defended trees whose leaves likely have higher nutrient content (Laurance et al. 1998). Indeed, leaf litter in small fragments had lower Carbon to Nitrogen ratios than intact forests (Appendix A3). We predicted that such nutritious litter would have bottom-up effects on rates of decomposition and the invertebrate food web. Indeed, detrital decomposition was accelerated in tree holes in small forest fragments, at least in the most rapid (<30 day) phase of decomposition. However, higher decomposition in small fragments could also be driven by higher temperatures that accelerate microorganism growth and leaf litter

breakdown (Didham 1998). We can distinguish between litter quality and site microclimate as potential explanations by comparing decomposition between standard and native litter. Differences in palatability are unlikely to be the main mechanism behind forest size effects on decomposition, as the same effect of fragmentation was observed for the litter for a standard tree species *C. xalapensis* as for site-specific litter.

We found some evidence of bottom-effects of fragmentation on the food web. If higher litter quality in small fragments supported more organisms, we would expect forest size effects on abundance to be restricted to (or at least greater for) native litter. Consistent with this mechanism, we found that poor native litter quality in intact sites depressed protist densities relative to the standard litter. Other differences between forest sizes must also be important, though, because regardless of litter type, protist densities increased as forest size decreased. Here, we again suspect microclimate difference may be important. Although other systems have shown strong top-down effects of mosquitoes on protist densities (Hoekman 2007), mosquitoes were poorly correlated with protist densities in our study. Indeed a recent manipulative study of resources and mosquitoes in bromeliads, in the same study site, also showed stronger bottom-up than top-down control on protist communities (Petermann et al. 2015).

We also found evidence that high litter quality in small sites increased detritivore (collectors plus shredder-scrapers) abundances: forest size effects were greatest for native litter. The effect of this increase in detrital quality on the invertebrate community was particularly evident in marsh beetle larvae (Scirtidae), the most efficient shredder-scrapers of treeholes at the time of sampling. Scirtids had significantly higher abundance in fragments than intact forest, but only in those artificial treeholes containing native as opposed to the standard litter, strongly suggesting a resource-driven response.

We did not detect evidence of these bottom up effects extending beyond scirtids to the rest of the foodweb, but we anticipate that with time detritivores presence is likely to increase in small patches via two mechanisms: 1) an increased abundance of shredder-scrapers in treeholes from small patches containing more nutritious leaf litter triggering a resource processing chain that has been reported to facilitate emergence of other detritivores in bromeliads and treeholes (Starzomski et al. 2010, Pelz-Stelinski et al. 2011) or 2) bottom-up effects exerting changes only on the first leaf litter consumer level (scirtids and tipulids, the most efficient detritivores) while higher levels are more directly affected by a release of top predation pressure. A similar mechanism was reported in an omnivore ant-plant tropical system by Dyer and Letourneau (1999) who found that increased nutrient availability affects only direct consumers, while higher levels are more strongly affected by predation than nutrient availability. Although *Toxorhynchites hypoptes* also showed a Forest size by Leaf litter interaction, it is a predator so it should not be directly affected by litter quality. It is possible that this species is instead using a by-product of litter decomposition in intact forests as an oviposition cue (Srivastava and Lawton 1998). Future work could disentangle the proximate driver of bottom-up effects of

fragmentation on litter quality for different species, whether mediated through larval survivorship or by adult oviposition behaviour.

Microclimate but not edge effects partially explain food web changes

We have already argued that microclimate differences along the forest size gradient may affect decomposition and protist densities, potentially even flowing up to detritivores. Here, the main microclimatic difference responsible is likely maximum temperature, as Forest sizes were surprisingly similar in the other microclimatic conditions, including canopy cover and water loss in treeholes. Previous studies suggest that variation in the structure of temperate treehole metacomunities is strongly affected by environmental factors such as amount of detritus (leaf litter) and water volume (Paradise et al 2008). While in our study we standardised both of these factors at the start of the experiment and neither diverged between habitat types in the next 85 days, it is possible that for natural treeholes moderately higher temperatures are associated with increased leaf litter decomposition rates and eventually increased desiccation risk. A warmer climate is predicted to increase microbial decomposition rates while reducing detritivore mediated decomposition in aquatic systems (Boyero et al. 2011).

Fragmentation effects on microclimate are often associated with the creation of forest edges and reduced canopy cover. However, in this study we found no significant change in canopy cover from the edges to centers of our patches (Appendix A5), similar to a study by (Sekercioglu et al. 2002). It is therefore perhaps not surprising that we did not detect any effect of distance from edge on the abundance and richness of treehole invertebrates, nor on decomposition rates. Other researchers have found contrasting edge effects on detrital decomposition, for example none in continuous forests but strong edge effects for forest fragments (Didham 1998). Two previous studies of ground litter decomposition in the same landscape found no evidence of edge effects, with the explanation being that vines and lianas effectively sealed the edges of fragments in these forests (Noble 2013, Cuke 2015 submitted). This may reflect the age (> 30 years) of fragments in our study, as older edges are reported to show more complex and variable effects on fauna (Kapos et al. 1997). Although edge effects have been widely reported (Fahrig 2003), other studies show no effect or variable effects on abundance or species composition (Williams Linera 1990, Didham et al. 1998).

Predictors of change between fragments

If we restrict our analyses of invertebrate abundance and richness just to the fragmented forest, we find evidence for processes operating at the fine spatial scales (tree-level) and landscape scales (up to 1km away),

but the size of the fragment itself was relatively unimportant. We caution that this conclusion holds just for the fragments, as we could not include intact forests in our analysis.

At the scale of individual trees, we found an association between tree diameter and macroinvertebrate abundance and richness. This was not because larger trees had larger treeholes, as by using artificial treeholes we had carefully controlled for treehole water volume. We interpret the tree diameter effect as an indication of adult insects' oviposition preference for cavities located in larger trees, as normally such treeholes hold larger water volumes and are less prone to desiccation. It has been reported previously that adult insects choose phytotelmata based on cues of low drought risk (Bradshaw and Holzapfel 1988, Fincke 1992b, Srivastava and Lawton 1998, Amundrud and Srivastava 2015) but we are not aware of other studies that suggest that tree diameter is used as a cue for treehole quality. Populations of organisms for which larger tree size is an important factor for adults selecting breeding sites, are expected to eventually decrease in small patches where large trees are uncommon (Laurance et al. 2000).

Comparisons between categories of forest size assume that patch size determines dispersal irrespective of landscape context, whereas effects of proximity assume that dispersal within a landscape is determined by connectivity rather than area. The literature makes a clear distinction between area and connectivity effects of fragmentation, with some studies suggesting that area effects dominate (Aizen and Feinsinger 1994, Hill and Curran 2003), others that connectivity effects dominate (Tockner et al. 1999, Gonzalez 2000) and others that both area and isolation are poor predictors for some species for which the permeability of surrounding matrix to species movements exerts a more important effect on occupancy (Prugh et al. 2008). In our study, isolation effects (as approximated by the proximity index and distance to nearest forest) were less important than expected. Although dispersal distances of treehole insects have never been directly quantified with genetic markers, previous studies suggest distances under 2 km for many of the Diptera families that are common in treeholes (Levi-Castillo 1951, Lillie et al. 1981, Alexander 1987, Morrison et al. 1993, Honório et al. 2003, Schweiger et al. 2007) and particularly short dispersal distances for the damselflies (Conrad et al. 1999, Khazan 2014). However neither the abundance nor richness increased with proximity to forest in our treeholes.

Our results suggest that the adults of treehole insects can move easily in and out of even the smallest fragments (Table 4), but their ability to encounter other suitable treeholes in the surrounding landscape could be limited by the distance between forested areas. The isolation of a forest fragment is not only determined by proximity of other sources of suitable habitat but also by matrix suitability. A recent study in southern Mexico found most treehole detritivores can in fact disperse through a matrix habitat of orange groves (Khazan et al. 2015) similar to that of our study. However, a complementary study found that primary forest may be irreplaceable for the largest treehole damselfly *Megaloprepus caeraulatus*, given that adults can disperse but

do not breed in corridors (Khazan 2014). For pseudostigmatids and other species that depend on a ‘*non-random array of plant species*’ (Fincke 2006) such as cavity forming trees, instead of how far the nearest patch is, a more relevant question may be which of the patches in a fragmented landscape retained viable populations of cavity forming trees.

Resilience to fragmentation

So far, we have examined potential reasons behind effects of fragmentation on treehole food webs. However, a number of attributes of tree hole food webs were surprisingly resilient to forest fragmentation, especially the total abundances of filter feeders and predators (even though the composition of these groups responded). Most of the macroinvertebrate species found in intact forests were also found in patches, although the pattern in species accumulation suggests that complete sampling would have started to detect differences. Certainly, many studies have not found strong negative effects of fragmentation on arthropod communities when compared to continuous forest (Klein 1989, Sekercioglu et al 2001, Quintero and Rosling 2005, Brosi et al 2008, Damborsky et al 2015).

There are two potential explanations for these patterns in resilience. First, an extinction debt may not have been realized (Tilman et al. 1994, Vellend et al. 2006, Krauss et al. 2010) In the case of treehole communities, long-lived trees may continue to exist and provide treehole habitat in fragments despite not being reproductively viable (Turner and Corlett 1996). For example, in a companion study of natural treeholes in our study area (Appendix 1), we found that odonate larvae were associated with patches where at least one of the trees *Inga sertulifera* and/or *Swartzia cubensis* (both Fabaceae) was present. These trees (and others not identified) have a gnarled or divided trunk architecture more prone to form cavities that collect water (Derraik 2005). Over time, as these types of trees are lost from fragments, we expect that the treehole-associated pseudostigmatids will be progressively lost.

Alternatively, it is possible that most components of the food web can persist in the long term in fragmented habitats, as long as forest habitat quality and minimum connectivity requirements are met (Hill 1995) and as long the matrix remains permeable. This landscape is under ongoing pressure from increases in human settlement, and some of the larger surrounding orange groves have recently been converted to small-scale mixed agriculture under a government land ownership program. The effect of these dynamic changes in the landscape on insect dispersal remains to be seen. For now, it seems that most but not all macroinvertebrates can still find their way in and out of forest fragments, even small ones. Although Didham (1997, in Rivas 2005) suggests that forest areas of 300-500 Ha are needed for the viability of arthropod populations, our results show that for treehole insects fragments smaller than 10 Ha can still support most

macroinvertebrate detritivore species in treeholes communities for at least three decades after fragmentation occurs.

Temporal dynamics

Our study followed the trajectory of succession in artificial treeholes along a gradient of forest fragmentation, and so it also demonstrated large temporal turnover. Data from these two sampling periods show evidence of substantial changes in composition as colonization progresses. This fast turnover is consistent with reports from previous studies in both treeholes (Williams 2006) and artificial containers (Murrell et al. 2014). The mosquito-dominated 30-day-old communities are replaced by more even communities as the more diverse collector-gatherer group becomes prevalent at 85 days.

Even inside the filter feeder guild we found signs of a rapid species turnover. The initial dominance of *Lasulleptus* decreases as they are gradually replaced by *Aedes*, *Haemagogus* and eventually *Culex* species. A comparable pattern was previously reported by Murrell and Juliano (2013) who suggest that at initial stages of artificial container colonization *Aedes* larvae competitively suppress *Culex* species. Laboratory experiments also found the later to be more vulnerable to predation by the mosquito *Toxorhynchites* (Murrell and Juliano 2013). The observed low prevalence of *Limatus* larvae (and *T.digitatum*) in mature natural treeholes as opposed to our artificial treeholes is consistent with their reported closer association with containers habitats (Yanoviak 1999b). Our results lead us to speculate about the presence of a colonization-competitive trade-off as predicted by theory (Tilman et al. 1994), which could have *Limatus* species as the strongest colonizers and *Aedes* and *Haemagogus* , as more efficient competitors.

Caveats

The main limitation of our study is that it is an early snapshot of ecological succession. The studied organisms have fast generational times and colonization was well under way by the time of our second sampling, but we cannot exclude the possibility that the dynamics would have been changed with a longer colonization phase that might include later colonizers and larger instars. In particular, we suspect that the early instars of tipulid and odonate larvae found in our second sampling time were still too few and too small to exert detectable effects on leaf litter breakdown and on prey respectively. In the case of early protist communities, adding an inoculum of filtered mature treehole water to speed community development would be recommended for future studies.

Another limitation was taxonomic resolution. It is possible that some of our morphospecies lump more than one species thereby we may be underestimating species richness. It is also possible that some of our unknown Diptera larvae are early instars of tipulids, so a few shredders may be underrepresented in our analysis. In the case of top predators, there are at least 5 species of pseudostigmatids in Costa Rica: one colonizes bromeliads exclusively (*Mecistogaster modesta*) while the other 4 are treehole specialists (*M. ornata*, *M. linearis*, *P.aberrans* and *Megaloprepus caeraulatus*). The four have been reported to emerge from artificial treeholes (Fincke 1999). *Pseudostigma accedens*, may also occur in the region but very little is known about its distribution. The larvae cannot be reliably separated to species, even by experts, and is very possible that these species respond differently to fragmentation. For example, *Megaloprepus caeraulatus* has slightly longer development times and emerges from larger (>1L) treeholes than the two *Mecistogaster* species (*M. linearis*, and *M.ornata*) (Fincke 1992a). *Megaloprepus caeraulatus* larvae are reported to be more sensitive to desiccation (Fincke and Hedström 2008). Consistently, they are associated with continuous forest where large trees persist, while *Mecistogaster* larvae, on the other hand are more tolerant to secondary or disturbed forests (Fincke 2006). Rearing larvae to the adult stage or making use of molecular tools is necessary to disentangle fragmentation effects at the species level for these predators.

Our study also uses a space-for-time substitution design. Although this is a common design for fragmentation studies, it assumes that fragments are randomly located on the landscape and have reached an equilibrium, neither of which may be true. In both fragments and continuous forest disturbance and logging tend to aggregate in space and expand with time. In fact, fragmented landscapes dynamics usually involve continuous forest gradually retreating, fragments shapes and sizes varying as secondary growth expands around the edges and changes patch isolation. Furthermore, time for extinction debts to realize can vary substantially between species (Ewers and Didham 2006). Our study also cannot completely separate the effects of habitat connectivity from those of habitat loss per se, since these co-vary in our landscape. However, our goal was not to partition the effects of fragmentation but to establish the strength of the net effects of habitat fragmentation.

Although our artificial containers were successfully colonized (94% of our them contained macroinvertebrates) and the majority of taxa from natural treeholes were represented, our inferences are restricted to species that can colonize artificial treeholes and that can survive in small treeholes. Artificial treeholes may also sometimes attract species more commonly associated with containers than treeholes, such as *Limatus asulleptus* (G. Chaverri com.pers and Yanoviak 1999b).

Implications

This study shows that forest fragmentation can affect the invertebrate and protist communities in treeholes, as well as initial rates of detrital decomposition. Although our work focused entirely on insect larvae, we justify this by noting that microsite and food availability for larvae are often key limiting factors in insect distribution (Fincke 2006, Schweiger et al. 2007, Khazan 2014). Treehole insects eventually emerge as adults and can be a substantial contribution to the terrestrial food web. Thus effects on treehole insect abundance and composition can represent a change in the food resources for insectivorous birds, amphibians and bats, some of which could be potentially already affected by habitat loss and fragmentation.

On the positive side, the discovery of diverse tree hole communities in large fragments, resembling those in intact forest, highlights the value of some forest fragments for biodiversity conservation. We conclude that large forest fragments in particular can be a very valuable habitat for treehole-dwelling damselfly and dipteran larvae, but only if those tree species associated with tree hole formation are preserved and only if enough connectivity between forests is allowed.

The mechanisms proposed here to explain the effects of fragmentation on treehole communities are not exclusive, and in real life are likely to operate simultaneously. Our results suggest that in the long term, forest fragmentation would increase detritivore biomass as a result of two synergistic effects: increased quality of resources and (eventually) reduced top down control from odonates. Specifically, the biomass pyramid in small fragments is likely to get more bottom heavy (i.e. increased detritivore biomass) as the nutritious leaf litter of such fragments is shredded and grazed by more abundant scirtid beetles, initiating a processing chain that facilitates the growth of fly and mosquito larvae. This will occur simultaneously with the release of top down pressure as less efficient predators like *Toxorhynchites* (Fincke 1999) become more frequent than voracious damselfly larvae. Fragmentation may therefore ultimately increase midge and mosquito populations, some of which are potential vectors of dengue fever, yellow fever, chikungunya and malaria (Galindo et al. 1950, Patz et al. 2000, Yanoviak et al. 2006b). Therefore we join other researchers (Langlois et al. 2001, LoGiudice et al. 2003, Yanoviak et al. 2006b, Huang et al. 2015) in anticipating changes in prevalence of vector-transmitted diseases with forest fragmentation.

Chapter III. General Conclusions

The main finding of this study was that treeholes in fragmented forests continue to preserve a large proportion of the macroinvertebrate community found in continuous tracts of forests even decades after fragmentation occurs. In general, small habitats contain fewer resources and are expected to support smaller populations which are prone to extinction due to stochastic and deterministic effects (Thomas 2000, Ribas et al. 2005). However, we did not detect major extirpations of taxa that were predicted to be sensitive to fragmentation, nor obvious reductions in the overall number of species (with the caveat that our taxonomic resolution was not always fine-grain).

The area and connectivity of forest fragments were less robust predictors of macroinvertebrate abundance or diversity at the patch scale than expected. As noted by previous studies, each habitat remnant is a random sample of the original forest, so - in addition to size and location - history and random effects can influence the resulting species richness and composition (Gonzalez 2000, Ribas et al. 2005). Other factors not measured in our study, such as tree community composition in fragments, and especially the presence of cavity-forming and large tree species, could play important roles explaining site variation in macroinvertebrate abundance and species richness.

We attributed the seemingly positive effect of fragmentation on treehole detritivores to crowding effects. These have been reported in insects, frogs, birds and mammals before (reviewed by Debinski and Holt 2000) and could arise from invasion of species from the matrix, use of patches as corridors by transient species, or disruption of the ability of predators and parasites to aggregate rapidly enough at outbreaks of prey or hosts (Debinski and Holt 2000, Grez et al. 2004). In any case, our results add to previous studies highlighting the value of heterogeneity in small forest fragments in the preservation of biodiversity and ecosystem function at landscape levels (Daily and Ehrlich 1995, Ricketts et al. 2001, Roesch et al. 2015).

We interpret with caution the resilience to fragmentation observed in treehole communities. Despite their fast generation times, the distribution of tree hole macroinvertebrates may show both immediate and time-delayed responses to fragmentation (Haddad et al. 2015). Their long-term persistence in fragmented landscapes depends on that of cavity-forming trees, and by extension, that of pollinators and seed dispersers of those tree species. The fate of metapopulations is determined by the balance between local extinction and recolonization and by how that balance interacts with random disturbances (Kareiva 1990). However, even populations on the verge of extinction can survive for long periods before going locally extinct (Bruce et al 1999, Hanski et al 2002) and, particularly in plant communities, extinction debt can take a hundred years

before being realized (Vellend et al. 2006). Therefore, we cannot confidently comment on the long-term persistence of these tree hole macroinvertebrate communities.

Our finding that artificial treeholes in larger trees attract more macro-invertebrates suggests that ovipositing females might normally use tree diameter as a cue for tree holes with more resources or reduced desiccation risk. In natural tree holes, mean tree hole water volumes and the length of time a tree hole holds water is likely to be affected by the altered precipitation regimes predicted for the tropics for a climate-changed future (Magrin et al. 2014). In such a scenario, the synergistic interaction of habitat fragmentation and other anthropogenic stressors might result in different plant and insect community structure in fragments, perhaps favouring drought-tolerant treehole species. Warmer temperatures, however, could have more severe detrimental effects on treehole macroinvertebrates. As the warming tolerance and thermal safety margin of tropical insects is substantially more constrained than that of their temperate counterparts, some studies predict intrinsic population growth rates of tropical insects could decrease by up to 20% with global warming by late 21st century (Deutsch et al. 2008). Although treeholes in small fragments experience only slight increases in mean temperature, the observed increased in maximum daily temperatures may have more direct effects on fitness of treehole insects in small fragments than in continuous forests in the warmer years to come.

Future research on the top-down effects of fragmentation in this system would benefit from longer colonization times to increase detection of odonate occurrence. For example, depending on resource abundance, the larvae of *Mecistogaster* spp. can emerge in as fast as 4 months or take up to 7 months to pupate and emerge (Fincke et al. 1997) Four month-old artificial treeholes combined with more extensive natural treehole surveys could provide further insight on their prey consumption effects. The relaxation of predation pressure suggested by reduced presence of damselfly larvae in small fragments requires further investigation. Morphological identification of adult insects or DNA barcoding of immature stages is needed to disentangle the response of each of the three *Megaloprepus* and *Mecistogaster* species to fragmentation. It is still an open question whether the increased prevalence of *Toxorhynchites* mosquitoes in small patches responds to release from odonate predation. One way to test this would be to experimentally manipulate colonization times and co-occurrence, and measure adult oviposition preferences and larval survival. Further experiments could also examine the relative role of these predators in reducing mosquito populations in fragmented landscapes. An alternative method to speed up microbial and protist community assembly in artificial tree holes would be to add an initial aliquot of natural treehole water (Chase et al. 2009). Additionally, to enhance the likelihood of colonization by the largest damselfly species *M. caerulatus*, the use of larger volume tree hole analogs (>1 L) is suggested (Fincke 1992a). In summary, this study illustrates the potential use of artificial treeholes in monitoring fragmentation effects on food web structure and advances our understanding of how tropical macroinvertebrate communities persist and shift in changing landscapes.

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Appendices

A1. Natural tree hole communities

We surveyed 60 natural tree hole cavities, 36 of which contained water at the moment of sampling. From them we collected a total of 451 macroinvertebrates from 13 families (28 morphospecies). Despite low sample size treehole abundance tended to decrease with habitat size: 47% of macroinvertebrates were collected from 17 treehole in intact sites, 44% from 16 treeholes in large patches and only 8% from 3 treeholes in small fragments. Natural treehole observations are limited by a smaller site pool, with two small and two large patches not sampled due to logistic constraints.

As opposed to artificial treeholes, where mosquito larvae dominated the biomass, in natural tree holes earthworms (Oligochaeta), marsh beetles (Scirtidae), biting midges (Ceratopogonidae), crane flies (Tipulidae) and damselflies (Zygoptera) were the most abundant groups. *Limatus asulleptus*, the most common mosquito species in artificial containers, was rare in natural treeholes, while the second most abundant mosquito (*Aedes* sp 1), was common both in artificial and natural treeholes. Some species sampled in artificial treeholes were not detected in natural ones (e.g. phorids, five of six *Culex* species except for *Culex rejector*, Chironomids (morphospecies 3 and 4) all unknown diptera larvae and isopods). Their absence from artificial containers could indicate (a) a sampling effect, suggesting sample size is under threshold for detection, (b) an exclusive association to artificial containers, or (c) that they are mid-to late succession colonists.

On the other hand, a few species (Tanypodinae chironomids, late instar tipulids and mature oligochaetes) were only found in natural tree holes, suggesting that our results from 85 day artificial treeholes underestimate their abundance. Although our small sample size limits our analysis of natural cavities, our observations suggest higher prevalence of damselfly larvae in natural treeholes than in artificial treeholes and a higher proportion of treeholes with odonates present in Intact forest (0.24) and Large patches (0.31) than in small patches (0). Interestingly, scirtid beetles followed a similar pattern with 80% of the abundance sampled and a highest proportion of treehole presence in Intact sites (0.41) than in Large or (0.25) Small patches (0.0).

Table A1. Characteristics of natural versus artificial treeholes

Tree hole type	Sample size	Sites sampled	Mean final water vol. (ml)	Total Abundance	Mean abundance per treehole.	Total Richness	Mean Richness	% of tree holes with odonates present
Artificial	379	20 (12 patches + 8 intact)	381	7396	19.5 (1-442)	43	2 (1-10)	9.5%
Natural	36	16 (8 patches + 8 intact)	160 (5 ml-2 l)	451	7.5(1-85)	28	1.5 (1-9)	28% (10/36)

A2. Temporal species turnover in treehole colonization

We found strong signal of temporal turnover of species in treeholes. Comparisons with long term established natural treeholes suggest artificial treeholes were in transition towards mature and more even communities, which, once colonized by damselflies, exhibit low abundance of prey species. This potentially successional pattern is evident even inside the Culicidae, with *Limatus* showing up as early colonizer gradually replaced by *Aedes* and *Haemagogus* in more mature communities. Although we can not rule out phenological differences in colonization, successional patterns of mosquito larvae in artificial containers have been experimentally verified by previous studies (Murrell et al. 2014) and are the most likely process underlying the observed temporal turnover in our study.

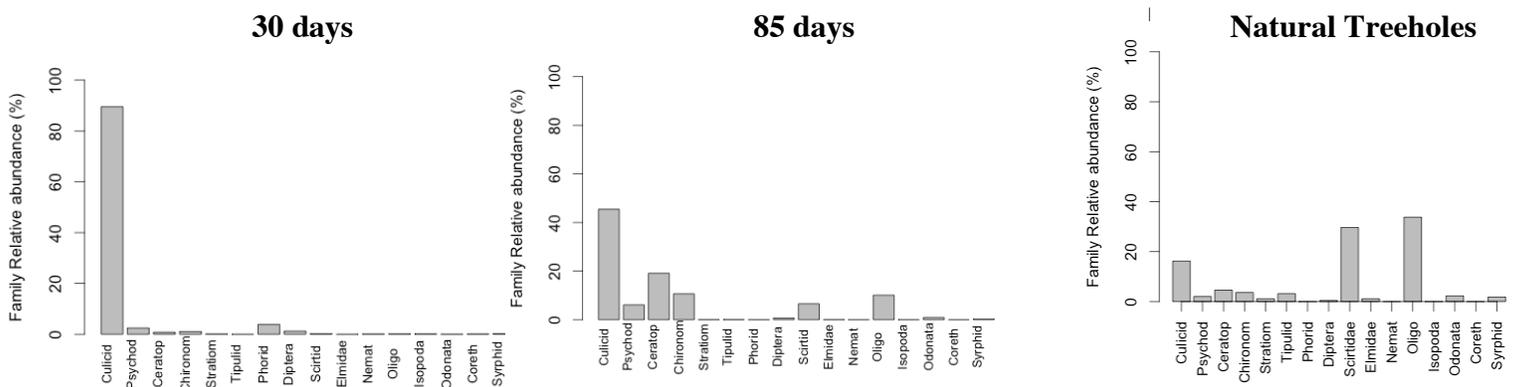


Figure A1. Community composition changes at the family level along a time gradient in artificial and natural tree holes. a) 30 days b) 85 days c) Natural (long term) treeholes.

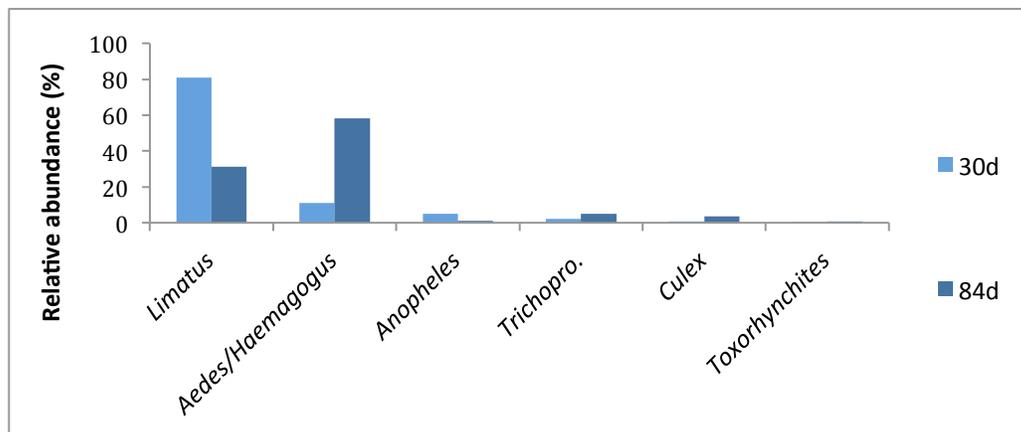


Figure A2. Relative abundance of Culicidae genera in one and three month old treeholes. Genus *Aedes* and *Haemagogus* are shown combined as morphological identification was not possible for some early instar specimens.

A3. Leaf litter nutrient content analysis

Samples of leaf litter from intact forest sites analyzed contained a higher carbon to nitrogen ratio than Leaf litter from fragmented sites ($\chi^2_{(3,13)}=6.35$, $p=0.017$). The latter ones sometimes contained more phosphorous although there was no significant trend in the mean ($\chi^2_{(3,13)}=0.05$, $p=0.997$). The nutrient content of standard leaves of *C. xalapensis* was less similar to that of small patches than expected, with the highest carbon-nitrogen ratio of all categories (Standard: 81.2) and a high phosphorous content.

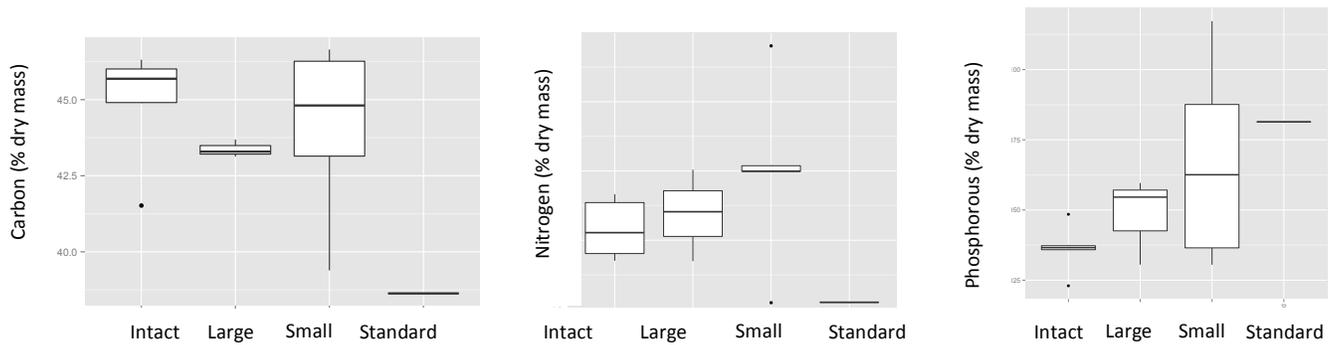


Figure A3. Leaf litter nutrient content across a forest size gradient. a) Carbon b) Nitrogen c) Phosphorous. Intact, Small and Large categories in this boxplots indicate leaves collected from leaf litter traps placed in five continuous sites (I2,BUT,PIA, PIC and NIC), three large sites (L1,L6, PSP) and four small sites (L2,S1,S2, S4,S6). Standard indicates leaves of common pasture species *Conostegia xalapensis*.

A4. Increased leaf litter fall in forest patches than intact forest.

We placed leaf litter traps (810 cm² nets) in all of our sampling sites and collected them after approximately 30 days. Collected leaves were dried and weighed following the same protocol as leaves for decomposition analysis. Traps in fragmented forest collected 19% higher leaf litter input than those in primary forest (Dry mass per month (g): Intact: 25.5, Large patch: 31.2; Small patch: 29.7). Similar trends have been reported by studies in Guatemala and Brazil (Ewel 1976, Francisco et al. 1994) while contrasting results of higher leaf litter input in primary than secondary forest by other authors (Dantas and Phillipson 1989). In our study, together with leaf nutrient content analysis (see Fig. A3), these results appear to add to the evidence supporting a proposed increase in nutrient input in treeholes form small patches forests.

A5. Landscape and patch scale model simplification

Table A2. Model simplification of tree and landscape scale variables simultaneously. Results of multiple stepwise regressions on treeholes from forest fragments (N=205). Automated backward elimination based on Akaike Information Criteria (AIC) using R MASS package. Variables were regressed on the residuals of Time x Leaf litter general linear model with negative binomial errors and 192 degrees of freedom. Only the first and last step of variable elimination are shown. DBH= Tree trunk diameter at breast height.

ABUNDANCE (1)				RICHNESS (2)			
Full model		Start AIC=1535.45		Full model		Start AIC=672.9	
<i>Factor removed</i>	<i>Df</i>	<i>Deviance</i>	<i>AIC</i>	<i>Factor removed</i>	<i>Df</i>	<i>Deviance</i>	<i>AIC</i>
Canopy cover	1	224.77	1533.5	Distance to nearest forest	1	163.64	670.9
Distance to nearest forest	1	224.77	1533.5	Distance to continuous forest	1	163.65	
Final tree hole water volume	1	224.92	1533.6	Time x Leaves	1	163.66	670.9
Patch Area	1	224.97	1533.7	Final tree hole water volume	1	163.66	670.9
Distance to continuous forest	1	225	1533.7	Canopy	1	163.66	670.91
Proportion of forest cover in a1Km radius.	1	225.45	1534.2	Proximity index	1	163.8	670.92
Time x Leaves	1	226.07	1534.8	Proportion of forest cover in a1Km radius.	1	164.14	670.92
<none>		224.74	1535.5	Patch Area	1	164.69	671.06
Tree size (DBH)	1	226.89	1535.6	<none>		672.9	671.39
Proximity Index	1	227.82	1536.5	Tree size (DBH)	171	678.26	671.95
Reduced model	1524			Reduced model	657		
<none>		224.83	1523.9	<none>		164.4	659.66
DBH	1	227.99	1525	Proportion of forest cover in a1Km radius.		166.66	659.92
Proximity Index		229.72	1526.7	Patch Area	1	167.23	660.49
Time		239.99	1537	DBH	1	171.83	665.09
				Time	1	196.73	689.99
Null Deviance		251.2.		Null Deviance		208.8	
Residual Deviance		224.8.		Residual Deviance		164.4	

A6. Abiotic conditions in fragmented and non fragmented forest

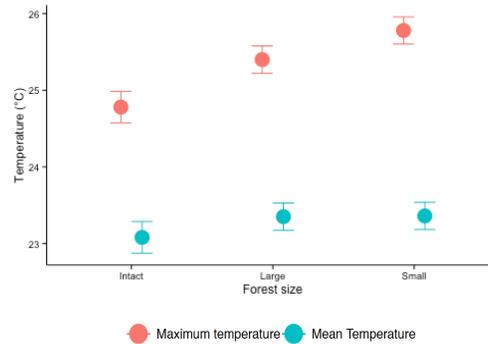


Figure A6-a. Mean and maximum daily air temperatures at the center of sampling sites. Temperatures were measured every thirty minutes between August 5 and September 2, 2011. This figure shows results only from sites where temperature was measured simultaneously during that interval (n=14). The inclusion of sites where temperature was measured in different dates (n=6) did not significantly change values or trends (not shown).

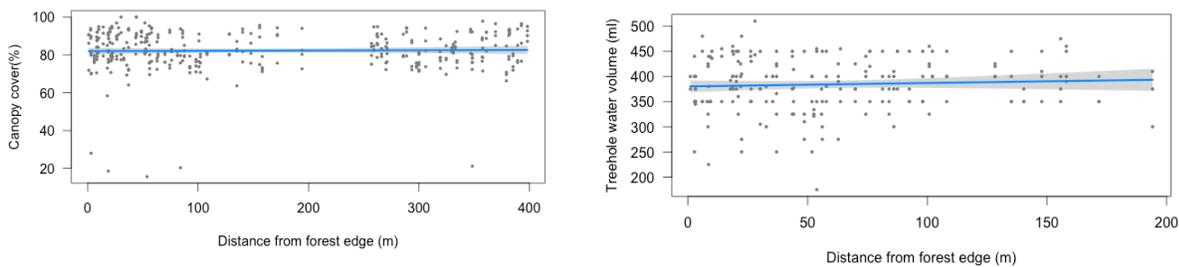


Figure A6-b. Effects of distance to forest edge on canopy cover (left) and final tree hole water volume (right). Shaded areas are confidence bands. No decline in habitat quality or water loss were evident with increased proximity to forest edge.

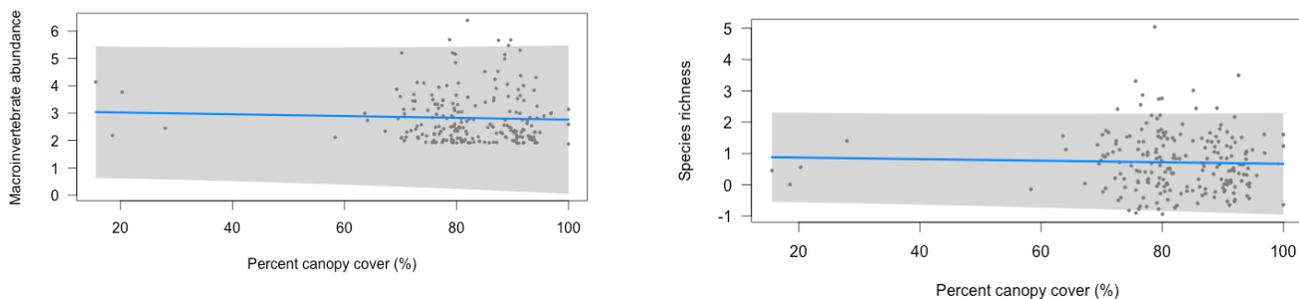


Figure A6-c. Effect of canopy cover on (a) Macroinvertebrate abundance and (b) Species richness in artificial treeholes from both samplings times. Shaded areas are confidence bands.

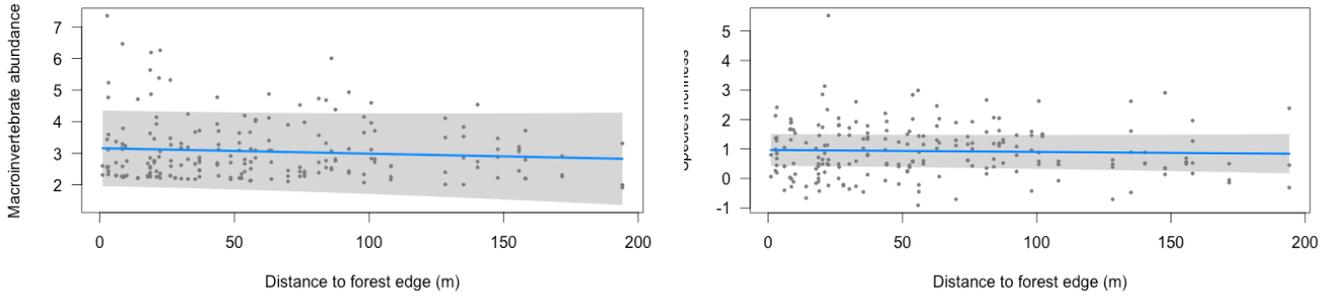


Figure A6-d. Forest edge effects on (left) Total macroinvertebrate abundance and (right) species richness. Figure shows data from forest patches in both sampling periods.

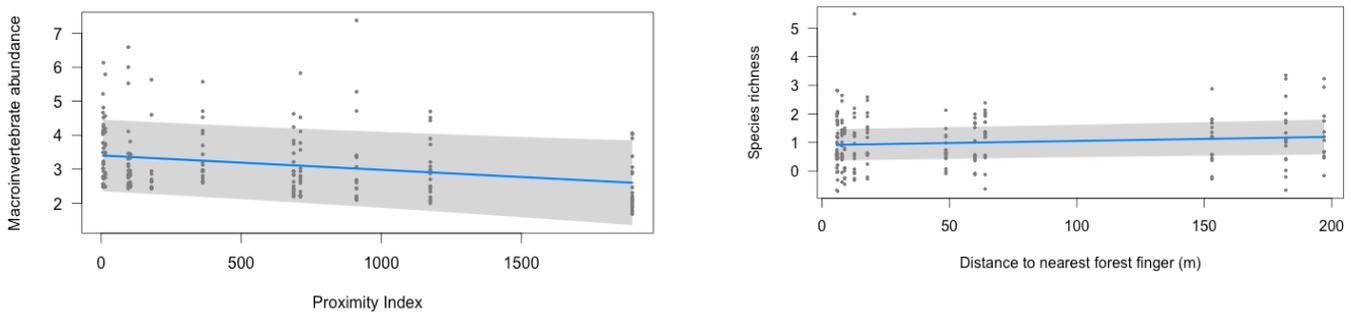


Figure A6-e. Effect of patch isolation metrics originally pre-selected by stepwise model simplification (Table X). Left: Proportion of forest-covered area in 1 Km radius effects on macroinvertebrate abundance. Right: Distance to nearest forest effects on species richness. Includes patch data from both sampling periods.

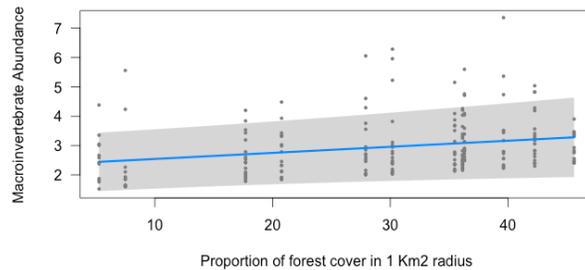


Figure A6-f. Effect of the only patch isolation metric with significant effect on macroinvertebrate abundance in forest patches in final model. Patches with lots of other large patches in close proximity have large Proximity Index value, therefore, low isolation. Species richness did not seem affected by any of the considered isolation metrics (not shown).

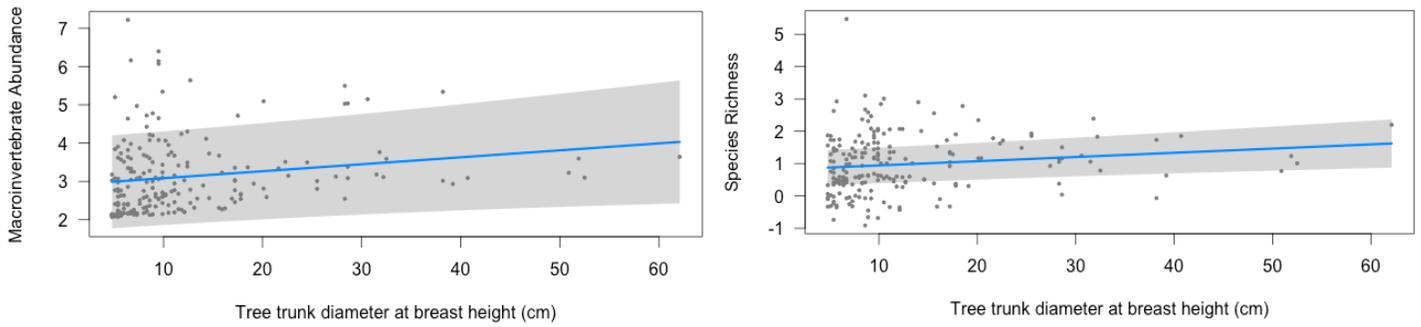


Figure A6-g. Effect of tree size (trunk diameter at breast height) on total macroinvertebrate abundance (left) and morphospecies richness and (right). Figure includes data from both sampling times and shows increased number of insects and species with increased tree size.

A7. Effect of experimental variables on total detritivore abundance

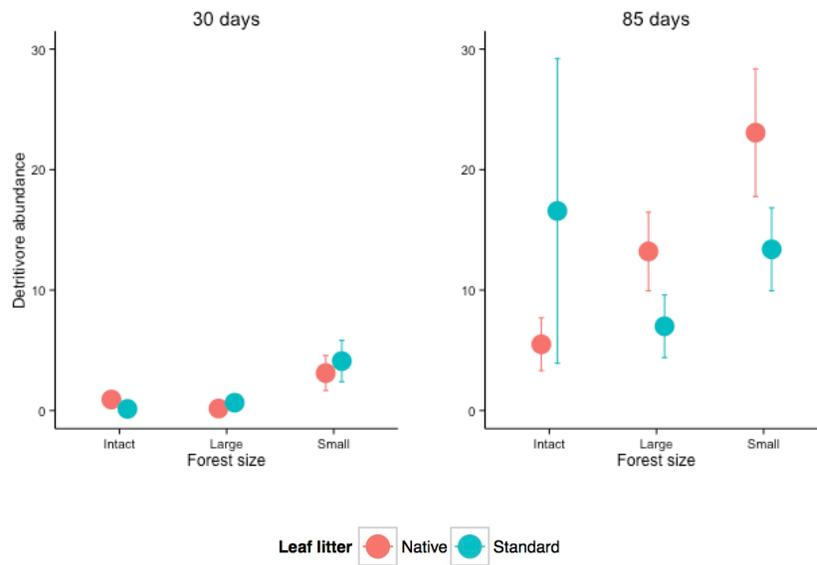


Figure A7. Effect of forest size, leaf litter type and sampling time on total treehole detritivore abundance. In this figure Detritivore pools all non-predatory and non-filter feeder larvae (shredders-scrappers and collector gatherers).