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Vertical stratification of invertebrate assemblages in water-filled treeholes of a temperate deciduous forest

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Abstract

Water-filled treeholes provide temporal habitats and resources to detritus-based aquatic organisms in the above-ground forest strata. Treeholes are found at different vertical positions, and are often surrounded by dense understory vegetation, which may affect water volume and litter weight. Differences in water volume and litter weight are indicative of the habitat quantity and the quality of treehole communities. Therefore, in the present study, we used containers as artificial treeholes to examine the effects of these characteristics on treehole invertebrate assemblages in a broad-leaf deciduous forest in central-eastern Japan. We first generated two models: a direct-effect model that reflected the physical and chemical properties of treeholes (water volume, litter weight, and other measurable properties that might directly influence invertebrate survival and fitness); and an indirect-effect model that reflected the differences in vertical position and surrounding understory vegetation *per se*. We compared these models and found that species richness is better explained by the direct-effect model, whereas the indirect-effect model plausibly explains the differences in invertebrate abundances. Further analyses revealed that some species such as *Tripteroides bambusa* utilized lower treeholes, while higher treeholes had a greater abundance of Ceratopogonidae sp. A within dense understory vegetation. Our study demonstrates that treehole invertebrates are not only influenced by simple physicochemical properties, but also by ambient conditions. However, the response patterns were highly variable across species. Our approach provides insight for elucidating the key drivers of treehole detritivore diversity in vertically stratified environments.

Keywords: Mosquito; Phytotelmata; Treehole height; Understory vegetation

Introduction

The vertical positions of microhabitats determine the spatial distribution patterns of invertebrate assemblages (Basset, Hammond, Barrios, Holloway, & Miller 2003; Shaw 2004; Ulyshen 2011). In arboreal environments, habitat patches such as bryophytes, epiphytes, and treeholes (Shaw 2004; Sillett & Antoine 2004) are distributed across different vertical positions, and these patches provide important habitats for several vertebrate and invertebrate species (McCune 1993; Lindenmayer, Cunningham, Pope, Gibbons, & Donnelly

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2000; Whitford 2002; Krömer, Kessler, & Gradstein 2007; Fayle, Chung, Dumbrell, Eggleton, & Foster 2009). Biotic and abiotic factors vary along the vertical gradients of forests (Parker 1995; Ulyshen 2011), consequently influencing the distribution patterns of terrestrial organisms inhabiting arboreal habitat patches (Rodgers & Kitching 1998; Yoshida & Hijii 2005; Lindo & Winchester 2013). In the aboveground forest strata, water-filled treeholes provide habitats for detritus-based aquatic invertebrates by retaining rainwater and food resources (e.g., litter) (Kitching 1971). Treehole communities depend on the quality, quantity, and temporal fluctuations of allochthonous resources (i.e., food and water), which may be affected by forest structure characteristics (e.g., foliage availability) and the vertical position of the treeholes.

Although several studies have examined the vertical distribution of aquatic invertebrates (Scholl & DeFoliart 1977; Sinsko & Grimstad 1977; Lounibos 1981; Copeland & Craig 1990; Yanoviak 1999; Derraik, Snell, & Slaney 2005), little attention has been paid to the community structure in relation to environmental factors associated with vertical gradients (but see Blakely & Didham 2010). Species richness and the abundance of aquatic invertebrates are known to be influenced by abiotic factors such as drought susceptibility at higher strata (Yanoviak 1999; Blakely & Didham 2010) and the incursion of soil from rain splashes at or near the forest floor (Kitching 1971). Moreover, the understory vegetation may provide extra litter (Gilliam 2007; Welch, Belmont, & Randolph 2007) and intercept sunlight (Messier, Parent, & Bergeron 1998; Aubin, Beaudet, & Messier 2000), which could potentially affect treehole habitat conditions and the resulting structure of treehole faunal communities.

The effects of these abiotic factors associated with vertical gradients can be quantified using two models. A direct effect model reflects the physicochemical properties of treeholes (e.g., water volume, litter weight, and other measurable properties), which may directly influence the survival and fitness of individuals. In contrast, an indirect effect model reflects the differences in vertical position and surrounding understory vegetation *per se*, which encompasses variation in physicochemical (including variables in the direct-effect model) plus other unmeasured characteristics (e.g., habitat detectability and predation rate) that may influence the reproductive success, immigration, and extinction of treehole communities.

The direct effect model is related to the more individuals hypothesis (MIH) proposed by Srivastava and Lawton (1998), as treehole productivity is a parameter of the direct effect model. This hypothesis postulates that a habitat with higher productivity supports a large number of individuals, which reduces extinction risks of individual species, thereby resulting in higher species richness. The MIH was, however, rejected by the same authors (Srivastava & Lawton 1998), as their study found that treehole productivity was correlated with species richness, but not total abundance of aquatic insects. In contrast, another study by Yee and Juliano (2007) supported the MIH, as they found a significant indirect effect of treehole productivity through abundance on species richness of aquatic invertebrates.

In the present study, we used cylindrical containers as artificial treeholes to examine the effects of treehole height and understory vegetation on the aquatic assemblages. Our aim was to determine whether physicochemical properties, which have often been measured in previous studies, are sufficient to explain the variation in invertebrate community structures (direct effect model), or whether such community dynamics can be explained by unmeasured or unmeasurable properties related to habitat differences (indirect effect model). Artificial treeholes were used to standardize environmental factors such as habitat history, size, and initial resource quality and quantity, while attracting a similar set of species as natural systems (Yanoviak & Fincke 2005). We set these artificial treeholes at different vertical positions within habitats with dense or sparse understory vegetation to test the following hypotheses: (1) species richness and total abundance are explained by the different models; (2) habitat type (dense/sparse understory vegetation), height, and seasonality all, but differently, affect community composition and spatial distribution patterns of invertebrate species because of differences in habitat requirements and the life cycles of individual species.

Materials and methods

Site description

A field experiment was conducted in a broad-leaf deciduous forest in Karasawayama (36°21′N, 139°36′E), in the Kanto Plain of central Japan. This area is categorized as a monsoon Asian climatic zone with relatively high and low precipitation occurring June–September and December–January, respectively. Annual air temperature and precipitation in the study area averaged 13.9 °C and 1244.7 mm, respectively (1981–2010, Japan Meteorological Agency 2017). During the study period, the amount of precipitation was notably higher from July to September, but was very low in mid-October (see Supplementary Appendix A: Fig. 1). The forest is dominated by *Quercus serrata* Thunb., with a mean tree height of approximately 20 m. Dwarf bamboo was approximately 2 m high, and it was patchily distributed as understory vegetation. For comparison with the present study (in which we used artificial treeholes), we conducted preliminary surveys of natural treeholes in the Kanto Plain (see Supplementary Appendix B for more details).

Vertical stratification and presence of understory vegetation

On June 1, 2011, we selected 27 and 24 *Q. serrata* trees in dense and sparse understory vegetation habitats, respectively. Tree diameters ranged between 177 and 104 cm, and

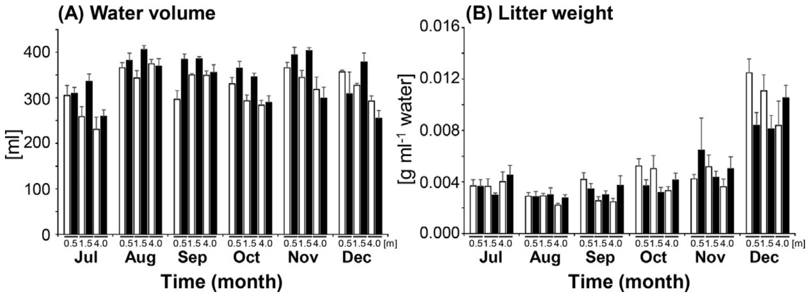

Fig. 1. Temporal changes in (A) water volume and (B) litter weight in the artificial treeholes at three different heights in dense (black bar) and sparse (open bar) understory vegetation. Vertical lines indicate standard errors.

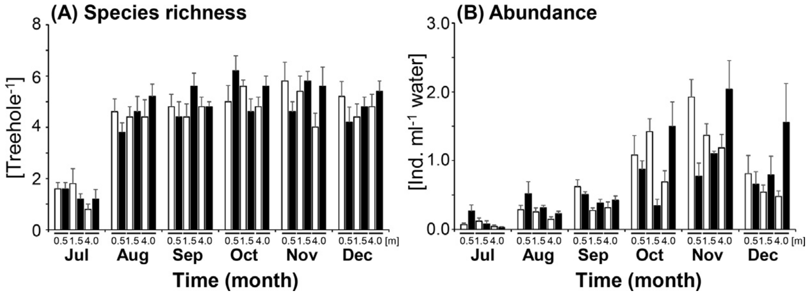

Fig. 2. Temporal changes in (A) species richness (per treehole) and (B) abundance (number of individuals per ml water) of aquatic invertebrates in the artificial treeholes at three different heights in dense (black bar) and sparse (open bar) understory vegetation. Vertical lines indicate standard errors.

between 183 and 74 cm in dense and sparse understory vegetation habitats, respectively. We randomly allocated nine trees to each of three height classes (0.5 m, 1.5 m, and 4.0 m above the ground) in the dense understory habitat (i.e., 9 trees × 3 height classes = 27 trees), and eight trees in the sparse understory habitat.

Cylindrical polyethylene cups (approximately 500 cm^3 in volume, 53 cm^2 in open area, and 9.3 cm in depth) were used as artificial treeholes. We put 1.0 g of dried *Q. serrata* leaf-litter and 200 cm^3 of distilled water in each artificial treehole. The artificial treehole size, water volume, and the leaf-litter weight were determined based on a preliminary study of natural treeholes in three broad-leaved forests in the Kanto Plain (see Supplementary Appendix B), where we observed an average water volume of 395 cm^3 (±66 m^3 standard errors, $n = 105$), a treehole depth of 12 cm (±0.7 cm, $n = 105$), and a leaf-litter weight of 3.3 g (±0.7 g, $n = 55$). We used black soft polyethylene pots (12 cm diameter, 10 cm height), which were fixed to tree trunks, as artificial treehole holders. We set each artificial treehole into a polyethylene pot and then fastened it with aluminum wire to the tree trunk in order to ensure good contact between artificial treeholes and tree trunks. On each tree, a total of four treeholes were fixed at a given height (9 or 8 trees per habitat × 4 treeholes = 36 or 32 treeholes per height class). These treeholes were placed approximately 5 cm apart. Sampling was conducted at monthly intervals from July to December of 2011. On each sampling occasion, we randomly selected five trees for each height class in each habitat (5 trees × 3 heights × 2 habitats) and took one of the four treeholes on each tree. We replaced the collected artificial treehole with a fresh container containing distilled water and litter, so that the same habitat conditions were retained on each tree. The replaced treeholes were, however, not sampled.

Sampling of internal components and aquatic invertebrates

Before collecting samples from tree trunks, we measured water electric conductivity (EC) and water temperature in the samples using a digital conductivity meter (EC/TDS, HI98312, Hanna Instruments, Woonsocket, RI, USA) in the field. We also measured pH and dissolved oxygen (DO) in the samples using a portable pH meter (pHTestr30, Eutech Instruments, Singapore) and a DO meter (OM-51, Horiba, Japan) in the laboratory on the day of sampling. Dissolved oxygen was, however, not measured in October due to device failure. Collected samples were weighed to determine the total weight, including the container. We then used forceps to remove large litter such as dead leaves and terrestrial insect carcasses (insects often dropped into the containers and subsequently drowned), and a pipette was used to collect aquatic invertebrates from the water. Where possible, we identified aquatic invertebrates to genus and species levels, using Sasa, Kurihara, and Uemura (1976) and Shimura (2005). However, some invertebrates were only sorted to higher taxonomic levels, and were then subjected to morphospecies coding. While some Culicidae larvae were identified to species or genus levels, other unidentified specimens were classified as “other Culicidae”

Table 1. AICc values of the linear mixed effect models (LMMs) based on indirect and direct models that explain invertebrate species richness, total abundance, and abundances of individual species. Lowest AICc values are highlighted in bold.

	Indirect	Direct				
		Water + litter	+Water temperature	+EC	+pH	+DO
Total abundance	364.3	377.2	372.9	380.7	379.1	381.9
Species richness	52.0	**−26.3**	−21.5	−17.6	−17.5	−19.1
Uranotaenia novobscura	396.6	**370.0**	371.7	375.8	376.0	375.0
Tripteroides bambusa	**454.3**	492.9	483.9	486.1	489.5	496.5
Orthopodomyia anopheloides	234.9	**216.1**	219.0	223.1	223.3	220.7
Aedes spp.	**387.3**[a]	**387.4**[a]	388.9	393.0	392.7	392.0
Other Culicidae	29.1	**−36.3**	−31.5	−27.4	−27.0	−28.5
Ceratopogonidae sp. A	**444.3**	466.4	455.3	468.9	471.8	467.1
Ceratopogonidae sp. B	337.2	332.0	**330.8**	338.0	338.5	337.0
Nematoda sp. A	**532.7**[a]	**532.3**[a]	**532.5**[a]	536.4	537.2	536.0
Nematoda sp. B	9.7	**−38.1**	−33.8	−29.4	−29.0	−30.5

[a] AIC values were similar between the indirect and direct models.

Remaining residue in a sample was strained through a 0.2 mm nylon mesh, and strained materials were regarded as fine particle organic matter (FPOM). The collected litter and FPOM were dried at 60 °C for two days, and were then weighed. The insect carcasses were identified to order level and preserved in 60% ethanol. We did not count live terrestrial insects such as beetles, ants, and spiders, because they were not part of the detrital community. Litter weight was the sum of the weight of large litter and FPOM, and water weight was calculated by subtracting the combined litter and container weight from the total sample weight. We assumed that 1 g water equalled 1 ml water volume in our study.

Statistical analysis

We first generated two competing models: a direct-effect model that reflected the physical and chemical properties of treeholes (e.g., water volume and litter weight, water temperature, pH, dissolved oxygen, and electrical conductivity); and an indirect-effect model that reflected the differences in vertical position and surrounding understory vegetation *per se*. Regarding the direct-effect model, we constructed a number of models that included water volume and litter weight (and their interaction) plus one of the other measured properties. Only one additional property was added for each model, because the addition of more than two properties inflated the Akaike Information Criterion (AIC) values.

The direct and indirect effect models were tested using the linear mixed effect model (LMM) analysis available in the *lme4* package of *R* software version 3.2.2 (R Development Team 2016). For both direct and indirect effect models, we incorporated time since deployment as fixed factors. We also incorporated the presence of drowned insects (e.g., stick insects and cicadas) as a random factor, because preliminary analyses indicated that both species richness and abundance of aquatic invertebrates increased in treeholes containing dead insects (ANOVA results: species richness, $F_{(1,148)} = 8.07$, $P = 0.005$; abundance, $F_{(1,148)} = 19.46$, $P < 0.001$). Although our study design was analogous to a repeated measures analysis, we did not correct the degrees of freedom, because the artificial treeholes on each tree were generally independent of each other. We used a modified AIC (AICc), which is suitable for a small number of samples relative to the number of treatments, and compared the performance of competing models when explaining patterns of total abundance, species richness, and the abundances of individual species.

We further analyzed the relative importance of treehole height, habitat type (presence/absence of understory vegetation), time since deployment, and associated interactions on species richness, total abundance, and the abundances of individual species. The presence of drowned insects was again incorporated as a random factor. We also tested how water volume, litter weights and other physicochemical properties changed in response to different treatments of the three factors.

For abundance data, we used densities (abundance per ml of water) instead of absolute values to control for the influence of the water volume, which reflected the habitat size. In order to improve linearity, all abundance data (i.e. densities) as well as species richness were log-transformed before analysis. For the univariate analyses described above, samples collected in October were excluded as DO was not measured in this month.

To test the effects of the three factors (height, habitat type, and time) on treehole invertebrate community composition, we used non-metric multi-dimensional scaling (NMDS) and permutation-based analysis of variance (PERMANOVA), which were available within the PERMANOVA+ add-on package in PRIMER6 statistical software (Clarke & Gorley 2006). Before multivariate analyses were conducted, abundances were converted to density by dividing them by the

Table 2. Summary results of linear mixed effect models (LMMs) that incorporated time since deployment, habitat type (dense vs. sparse understory vegetation), treehole height, and associated interactions. Numerator degrees of freedom are shown in brackets (denominator degrees of freedom varied depending on the response variables as Kenward–Roger approximation was used to calculate denominator degrees of freedom).

	Time (T) (*df* = 4)		Habitat (Hab) (*df* = 1)		Height (Ht) (*df* = 2)		Hab × Ht (*df* = 2)		T × Hab (*df* = 4)		T × Ht (*df* = 8)		T × Hab × Ht (*df* = 8)	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Water volume	21.00	**<0.001**	12.39	**0.001**	16.75	**<0.001**	5.32	0.07	2.16	0.08	2.65	**0.01**	1.48	0.17
Litter weight	65.80	**<0.001**	0.01	0.90	1.30	0.28	3.12	**0.05**	1.80	0.13	0.44	0.89	1.57	0.14
Water temperature	10414.1	**<0.001**	20.90	**<0.001**	151.23	**<0.001**	13.96	**<0.001**	17.62	**<0.001**	44.11	**<0.001**	3.56	**0.001**
EC	16.48	**<0.001**	6.06	**0.02**	8.60	**<0.001**	2.00	0.14	1.03	0.40	1.21	0.30	1.23	0.29
pH	5.92	**<0.001**	0.02	0.88	7.14	**0.001**	0.17	0.84	1.99	0.10	0.44	0.89	1.22	0.30
DO	70.93	**<0.001**	0.23	0.63	6.67	**0.002**	0.51	0.60	3.79	**0.006**	3.29	**0.002**	1.29	0.25
Total abundance	35.84	**<0.001**	1.61	0.21	1.55	0.22	6.46	**0.003**	2.01	0.10	1.17	0.32	3.29	**0.002**
Species richness	25.68	**<0.001**	0.03	0.87	1.42	0.26	2.47	0.11	0.83	0.51	1.23	0.29	0.94	0.49
Uranotaenia novobscura	21.68	**<0.001**	0.10	0.75	0.66	0.52	2.02	0.14	0.29	0.89	0.65	0.73	0.41	0.91
Tripteroides bambusa	0.74	0.57	8.53	**0.004**	21.98	**<0.001**	6.04	**0.004**	0.39	0.82	0.63	0.76	0.31	0.96
Orthopodomyia anopheloides	2.25	0.07	0.22	0.64	4.30	**0.02**	0.14	0.87	0.26	0.90	1.99	**0.05**	0.48	0.87
Aedes spp.	20.49	**<0.001**	3.76	**0.05**	0.93	0.40	0.74	0.48	1.18	0.32	1.38	0.21	0.73	0.66
Other Culicidae	11.62	**<0.001**	0.73	0.40	0.77	0.46	0.35	0.70	0.20	0.94	0.29	0.97	0.45	0.89
Ceratopogonidae sp. A	22.05	**<0.001**	0.36	0.55	22.32	**<0.001**	8.29	**<0.001**	0.15	0.96	4.55	**<0.001**	3.83	**<0.001**
Ceratopogonidae sp. B	4.53	**0.002**	2.98	0.09	1.73	0.18	1.67	0.19	0.40	0.81	0.54	0.83	1.62	0.13
Nematoda sp. A	19.39	**<0.001**	0.00	0.97	0.27	0.76	5.44	**0.006**	2.08	0.09	0.12	0.99	1.80	0.08
Nematoda sp. B	3.44	**0.01**	0.02	0.89	1.65	0.20	0.96	0.39	0.90	0.47	1.17	0.33	0.34	0.95

water volume. A similarity matrix was generated using Bray–Curtis indices. A dummy variable of very small abundance (0.0001) was added to all samples, so that similarity values could be calculated for samples, including those with few or no invertebrates collected. An NMDS ordination was generated with 25 random restarts to find the lowest stress values, and the PERMANOVA included the main three factors and associated interactions. Individual trees and the presence of dead insects were incorporated as random factors. Type III sums of squares were used, and *p*-values were calculated using 4999 permutations. When the main factors (or their interactions) were significant, we conducted a pairwise post-hoc analysis (a multivariate analysis analogous to *t*-tests) using 4999 permutations to calculate *p*-values.

Table 3. Summary of PERMANOVA results with pseudo-*F* values and degrees of freedom (*df*) associated with time since deployment, habitat type, treehole height, and associated interaction effects on aquatic invertebrate assemblages.

	df	Pseudo-*F*	*P*
Time (T)	5	23.84	**<0.001**
Habitat (Hab)	1	1.60	0.121
Height (Ht)	2	6.70	**<0.001**
Hab × Ht	2	3.04	**<0.001**
T × Hab	5	1.06	0.380
T × Ht	10	1.00	0.463
T × Hab × Ht	10	1.41	**0.021**

Results

In total, we collected 39 468 individual invertebrates that represented 10 species and morphospecies (hereafter referred to as species) from the artificial treeholes. The aquatic fauna collected from the artificial treeholes was similar to that found in the natural treeholes, except for Scirtidae and nematodes, which were very abundant in natural and artificial treeholes, respectively (see Supplementary Appendix B: Table 1). Some of the most abundant species in the artificial treeholes included *Uranotaenia novobscura*, *Tripteroides bambusa* (Diptera: Culicidae), Ceratopogonidae sp. A, and Nematoda sp. A (see Supplementary Appendix B: Table 1). One species (Coleoptera spp.) was removed from the univariate analysis, because only a few individuals were collected during the entire study.

Regarding species richness, the lowest AICc value was observed in the direct effect model, suggesting that this model best explained variation in species richness of treehole invertebrates (Table 1). In contrast, total abundance was best explained by the indirect effect model. Abundances of individual species were best explained by different models: direct effect model (*U. novobscura*, *Orthopodomyia anopheloides*, other Culicidae, Ceratopogonidae sp. B, Nematoda sp. B); indirect effect model (*T. bambusa*, Ceratopogonidae sp. A); and both models (*Aedes* spp., Nematoda sp. A).

We conducted further analyses for investigating the influences of the three main factors (height, habitat type, and time since deployment) on physicochemical properties and aquatic invertebrates. Water volume in artificial treeholes was significantly influenced by the understory vegetation, treehole height, and time (Table 2). Water volume was greater in the lower treeholes, with the exception of August and September when precipitation was higher (Fig. 1A). Water volume at lower treeholes was even greater within dense understory vegetation (Fig. 1A). Water-related factors (water volume, water temperature, pH, and DO) were significantly affected by the understory vegetation and treehole height, suggesting that the indirect effect model encompasses some factors of the direct effect model (Table 2). Litter weight increased towards December (Table 2, Fig. 1B). Litter weight was generally higher in lower treeholes within the sparse vegetation, whereas the opposite was found within the dense vegetation (Fig. 1B).

Time was the only significant factor with regard to both total abundance and species richness. Species richness appeared to be saturated within two months after deployment (August) (Fig. 2A). When the LMM analysis was re-run on species richness without data from July, time was not significant. In contrast, the total abundance continued to increase until November, and it then decreased in December (Fig. 2B). There were significant interaction effects in total abundance (habitat type and height), where the total abundance was greater in the upper treeholes of the dense vegetation, and in the lower treeholes of the sparse vegetation towards the end of the sampling period (Fig. 2B).

Time was a significant factor for all but two species (Table 2). *U. novobscura*, Ceratopogonidae sp. A, Ceratopogonidae sp. B, Nematoda sp. A, and Nematoda sp. B increased towards the end of the study period, whereas other species (*Aedes* spp. and other Culicidae) peaked in August (Fig. 3). Significant effects of height were found for *T. bambusa*, Ceratopogonidae sp. A and *O. anopheloides*. Higher abundances of *T. bambusa* were found in the lower treeholes throughout the study period. However, Ceratopogonidae sp. A was abundant only in the upper treeholes at the dense vegetation, as suggested by significant interaction effect of habitat and height (Table 2, Fig. 3). *Aedes* spp. was more abundant in the dense vegetation than in the sparse vegetation (Table 2, Fig. 3).

The community composition of aquatic invertebrates, visualized with NMDS ordination, gradually changed from high (4.0 m) to mid (1.5 m) and to low (0.5 m) vertical positions (Fig. 4). The PERMANOVA results also indicated significant effects of height, and time, though there were significant interaction effects between height and habitat type (Table 3). Subsequent post-hoc tests showed that all pairwise comparisons of height within dense vegetation were significant, whereas non-significant differences between 0.5 m and 1.5 m and between 1.5 m and 4.0 m were found within sparse vegetation.

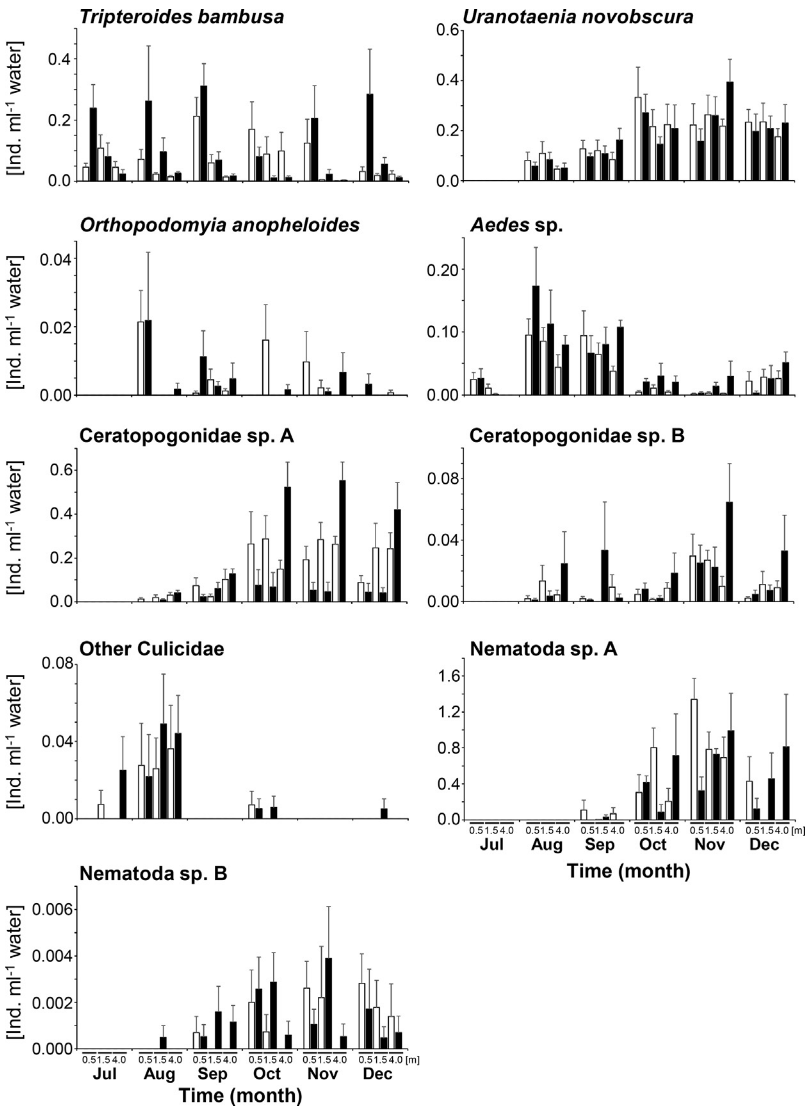

Fig. 3. Temporal changes in abundance of individual species in the artificial treeholes at three different heights in dense (black bar) and sparse (open bar) understory vegetation. Vertical lines indicate standard errors.

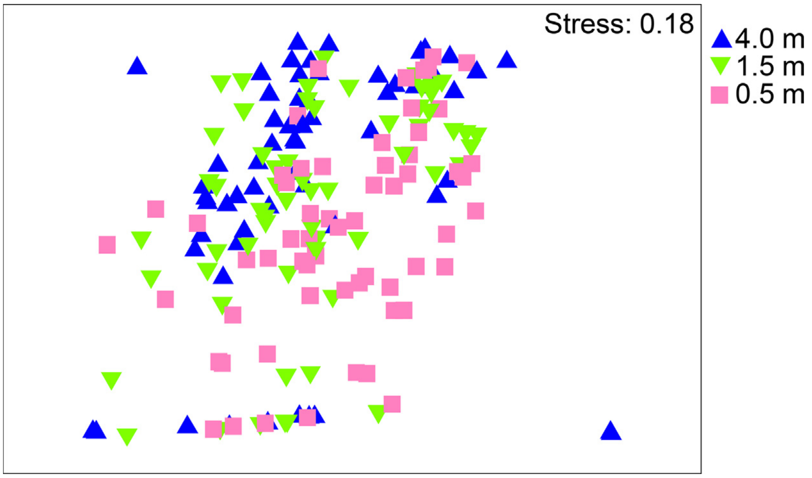

Fig. 4. Non-metric multi-dimensional scaling (NMDS) ordination of artificial treehole samples based on Bray–Curtis similarity values using the square root transformed abundances of species.

Discussion

We found that species richness was best explained by the direct effect model, whereas the differences in invertebrate abundances were best explained by the indirect effect model. The direct effect model suggests that increased water volume and litter weight create heterogeneous resource conditions, which potentially provide niche space diversity that leads to coexistence among species in the container habitats (Srivastava & Lawton 1998). Increasing water volume, for example, leads to increased water depth, resulting in spatial niche segregation between species within container habitats (Yee, Kesavaraju, & Juliano 2004; Gilbert, Srivastava, & Kirby 2008). However, litter weight may not necessarily be linked to species richness. For instance, Paradise (2004) reported that the effects of water volume on species richness override the effects of litter quantity in treeholes.

In contrast, the strong influence of indirect effect models on total abundance and the abundances of several species may be explained by species behavior and unmeasured treehole properties. Treehole height, for example, may have determined oviposition site of adult mosquitoes, potentially influencing the vertical distribution patterns of mosquito larvae (Ellis 2008). Additionally, diurnal and seasonal temperature fluctuations at different heights and habitat types may have influenced offspring survival rates (Gilbert et al. 2008), thus leading to changes in the abundance of specific species.

The more individuals hypothesis (MIH) was supported by an artificial treehole experiment performed by Yee and Juliano (2007), who concluded that the indirect effect of productivity through abundance on richness was important. In the present study, we found that both abundance and species richness were positively influenced by the presence of dead insects, and were correlated with a correlation coefficient of 0.60. This is not a surprising result as abundance and richness are not independent properties. However, the correlation coefficient was not as high, suggesting that species richness is not a simple function of an increase in abundance *per se*. This is also supported by our results showing that species richness and abundance did not follow the same trend with time after deployment (Fig. 2). The present study demonstrates that the MIH is likely to explain some correlation between species richness and abundance, whereas different factors are also likely to explain variations in species richness and abundance.

In our study, litter weight and the total abundances of aquatic invertebrates decreased in lower treeholes with dense vegetation. Contrary to the expectation, understory vegetation did not act as a source of litter supply but as litterfall interception. Litterfall interception by dense understory vegetation is known to affect the spatial distribution of litterfall on the forest floor and litter decomposition at the ecosystem level (Dearden & Wardle 2008; He, Lin, Han, & Ma 2013; Yang, Wang, Huang, Hui, & Wen 2014). Shading by understory vegetation not only has a negative effect on litter supply, but it also has a positive effect on water retention in container habitats because of decreased evaporation due to shading (Aubin et al. 2000; Messier et al. 1998). In the present study, water volume was indeed found to be greater in lower treeholes than in higher ones located in the dense understory vegetation.

Some species showed varying spatio-temporal patterns in the artificial treeholes. At the site with dense understory vegetation, *T. bambusa* utilized lower treeholes, while Ceratopogonidae sp. A was more abundant in the higher treeholes near the end of the study period. *T. bambusa* prefer persistent water-filled habitats (Sunahara & Mogi 1997b) because of their slow growth (Sunahara & Mogi 1997a). Ceratopogonidae sp. A did not have different densities between treehole heights in the sparse understory vegetation, but densities significantly increased at higher treeholes in the dense understory vegetation. These results may have been primarily caused by two factors. First, understory vegetation may provide a vertical gradient of microclimatic conditions that alter habitat suitability of this species. Second, fitness of Ceratopogonidae sp. A may be negatively affected by other competing species (e.g., *T. bambusa*), which are abundant in the understory vegetation. In the dense understory vegetation, *Aedes* spp. was also found to be abundant. This species may utilize dense understory vegetation as a feeding habitat (targeting small mammals and birds that are likely to use understory vegetation for shelter), as a resting site to digest blood meals, and/or as a breeding habitat. In addition, larval survival rates may also be responsible (Ellis 2008; Gilbert et al. 2008).

The saturation of species richness after two months could be attributed to the emergence of species in the summer and their persistence throughout the study period. Abundances of individual species, however, fluctuated significantly during the study period. Species that increased their abundances in summer (e.g., *Aedes* spp. and other Culicidae) may prefer higher humidity or stemflow flushing, which dilutes nutrients, reduces bacterial abundance, and alleviates density-dependent competition in the treeholes (Walker, Lawson, Merritt, Morgan, & Klug 1991). On the other hand, the species that flourished in autumn and early winter (*U. novobscura*, Ceratopogonidae sp. A, Ceratopogonidae sp. B, Nematoda sp. A, and Nematoda sp. B) may prefer stable conditions, or they may require longer periods for growth and development because they grew less during the rainy season.

In conclusion, our study demonstrates that treehole invertebrate species richness, abundance, and composition can be explained by the physicochemical properties of treeholes (direct effects) and other ambient conditions (indirect effects). However, the underlying mechanisms of these patterns are highly complex as suggested by the variable response patterns of individual species. Our study focused on the understory habitat, but the overstory canopy strata generate different environmental characteristics such as vertical foliage complexity (Ulyshen 2011) and more intensive sunlight (Parker 1995). Additional studies that examine suspended aquatic habitats across all aboveground strata of forests are necessary to comprehensively understand the vertical distribution patterns of aquatic invertebrates.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.baae.2017.11.002.

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